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EDITORIAL

Update on the treatment of metastatic renal cell carcinoma

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Abstract

Metastatic renal cell cancer (mRCC) management has undergone a paradigm shift in recent decades. The first revolution came with the emergence of vascular endothelial growth factor inhibitors; there was a second wave with the unprecedented success of checkpoint inhibitors, and then the latest approach, which is becoming the new care standard in mRCC, of combining these two strategies in different ways. Updated results of Checkmate-214 after 42 mo of follow-up were consistent with previously published results showing the superiority of nivolumab/ipilimumab over sunitinib in progression free survival (PFS), overall survival (OS), and objective response rate (ORR) in intermediate and high-risk patients. However, several studies presented at the American Society of Clinical Oncology 2020 suggested that the best place, and so far, the only one for nivolumab/ipilimumab is the frontline setting. The update on Keynote-426 after 23 mo of follow-up showed no superiority of pembrolizumab/axitinib over sunitinib in favorable-risk mRCC, suggesting that it should no longer be the first line of choice in low-risk patients. Finally, the phase III Checkmate 9ER trial results revealed the superiority of nivolumab/cabozantinib *vs* sunitinib in PFS, OS, and ORR, providing a new first-line option among all International Metastatic RCC Database Consortium risk patients. Some phase II clinical trials also presented this year showed promising results with new combination therapies such as nivolumab/sitravatinib, cabozantinib/atezolizumab, and lenvatinib/pembrolizumab, providing promising grounds upon which to start phase III studies. In addition, other works are using novel therapeutic agents with different mechanisms of action, including telaglenastat (a glutaminase inhibitor), entinostat [an inhibitor of histone deacetylases (HDACs)],

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and olaparib and talazoparib, poly(ADP-ribose) polymerase inhibitors widely used in other tumors. However, some questions regarding mRCC management still need to be addressed, such as head-to-head comparisons between the current options, treatment sequencing, non-clear cell mRCC, and the role of biomarkers to ascertain the best treatment choice.

Key Words: Metastatic renal cell carcinoma; Systemic treatment; Immune checkpoint inhibitors; Antiangiogenic; Update; Biomarkers

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Core Tip: Kidney cancer therapeutics is a fast-changing field, and the outcome of metastatic renal cell carcinoma (mRCC) has thus improved considerably in recent years with the introduction of different combinations of immune checkpoint and vascular endothelial growth factors inhibitors. State-of-the-art systemic therapy regimens must be addressed to be in a position to offer patients the best options. The aim of this editorial is to provide an update and insight on future directions on mRCC management.

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INTRODUCTION

Historically, the therapeutic strategy for metastatic renal cell carcinoma (mRCC) relied on cytokines. These drugs had a moderate response rate and were associated with substantial side effects[1].

Since then, the treatment of mRCC has improved considerably with the introduction and regulatory approval of agents that block vascular endothelial growth factor (VEGF) or mechanistic target of rapamycin (mTOR) pathways and significantly improve objective response rates (ORR) and/or median progression free survival (PFS) compared to previous treatment approaches. Since 2005, the United States Food and Drug Administration and the European Medicines Agency have approved VEGF receptors; tyrosine kinase inhibitors (TKIs) sorafenib, sunitinib, pazopanib, axitinib, cabozantinib, and lenvatinib; the anti-VEGF antibody bevacizumab (in combination with interferon); and mTOR inhibitors everolimus and temsirolimus to treat mRCC[2].

Despite the notable efficacy of these targeted therapies, which changed the treatment landscape, tumor resistance to TKI made it necessary to investigate different treatment mechanisms. In this context, stimulating the immune system through drugs targeting the so-called checkpoint pathways through the blockage of programmed cell death 1 (PD1), programmed cell death ligand 1 (PDL1), and the cytotoxic T-lymphocyte antigen 4 have been tested in RCC with successful results. As a result, nivolumab was the first immune checkpoint inhibitor (ICI) approved based on data from the phase III Checkmate 025 study of nivolumab *vs* everolimus in patients who had received prior antiangiogenic therapy for mRCC[3].

Combination therapies, based on the rationale that using drugs that work by different mechanisms may decrease the likelihood of cancer resistance, emerged in an effort to improve outcomes. The treatment landscape for first-line therapy has thus changed dramatically in recent years with the publication of a phase III clinical trial (CT) that showed three combinations' advantage over sunitinib: (1) Nivolumab/ipilimumab (Checkmate-214), which proved a higher overall survival (OS), PFS, and ORR in intermediate and high-risk patients[4,5]; (2) Avelumab/axitinib, which showed longer PFS (JAVELIN Renal 101)[6]; and (3) Pembrolizumab/axitinib, which proved higher in OS, PFS, and ORR among all International Metastatic RCC Database Consortium (IMDC) risk patients (KEYNOTE-426)[7]. This work has led to the current standard practice recommendations set in the European Association of Urology[8],

Table 1 Results from clinical trials

Checkmate 214	Nivolumab/ipilimumab (*n* = 425)	Sunitinib (*n* = 422)
Minimum follow-up	42 mo	42 mo
OS IP	52%; 47 (35.6-NE) mo	39% 26.6 (22.1-33.5) mo
ORR IP	42% (37-47)	26% (22-31)
CR IP	10%	1%
Checkmate 025	Nivolumab (*n* = 410)	Everolimus (*n* = 411)
Minimum follow-up	5 yr	5 yr
OS	26% (22.2-29.8)	18% (17.6-22.1)
ORR	23% (19-27)	4% (2-7)
mDOR	18.2 (12.9-25.8) mo	14 (8.3-19.2) mo
Keynote 426	Pembrolizumab/axitinib (*n* = 432)	Sunitinib (*n* = 429)
Minimum follow-up	23 mo	23 mo
OS	74%	66%
	HR: 0.68; 95%CI: 0.55-0.85; $P < 0.001$	
PFS favorable risk	20.8 (15.4-28.8) mo	18 (12.5-20.8) mo
Checkmate 9ER	Nivolumab/cabozantinib (*n* = 323)	Sunitinib (*n* = 328)
Minimum follow-up	10.6 mo	10.6 mo
PFS	16.6 (12.5-24.9)	8.3 (7-9.7)
OS	NR (NE)	NR (22.6-NE)
ORR	55.7% (50.1-61.1)	27% (22.4-32.3)
CR	8%	4.6%
Adverse events grades 3-5	60.6%	50.9%

PFS: Progression free survival; OS: Overall survival; ORR: Objective response rate; CR: Complete response; mDOR: Median duration of objective response; NR: No results; NE: Not ended; HR: Hazard ratio; CI: Confidence interval; IP: Poor risk.

ESMO[9], and National Comprehensive Cancer Network guidelines[10].

UPDATES IN AMERICAN SOCIETY OF CLINICAL ONCOLOGY GENITOURINARY, AMERICAN SOCIETY OF CLINICAL ONCOLOGY, AND ESMO CONGRESSES

American Society of Clinical Oncology genitourinary 2020

Updated results of Checkmate 214 after 42 mo of follow-up have been presented[11]. These results were consistent with the superior performance of nivolumab/ipilimumab *vs* sunitinib in intermediate and poor-risk patients. An OS of 52% with nivolumab/ipilimumab *vs* 39% with sunitinib [hazard ratio (HR): 0.66; 95% confidence interval (CI): 0.55-0.80]; ORR of 42% with nivolumab/ipilimumab *vs* 26% with sunitinib ($P = 0.0001$); and complete response (CR) of 10% with nivolumab/ipilimumab *vs* 1% with sunitinib have been observed (Table 1).

The final analysis of Checkmate 025 after 5 years of follow-up was also presented [12]. An OS of 26% with nivolumab *vs* 18% with everolimus; ORR of 23% with nivolumab *vs* 4% with everolimus; and median duration of objective response (mDOR) of 18.2 mo with nivolumab *vs* 14 mo with everolimus were presented (Table 1).

The first phase II in-human study of the hypoxia-inducible factor (HIF)-2α inhibitor Midkine (MK)-6482 was also presented[13]. This is an oral agent with antiangiogenic activity. Preliminary results on 55 patients treated in the second, third, and fourth line settings revealed a disease control of 80%, ORR of 24%, and tumor reduction of 67%. The median PFS was 11 mo. After 1 year, 30% continued under treatment, which was

well tolerated. These results provided promising grounds upon which to start the phase III trial (MK-6482 005 against everolimus).

Finally, another interesting approach was the combination of nivolumab/sitravatinib, a novel TKI that modulates the tumor microenvironment in order to render it more responsive to immunotherapy[14]. Administration in the first, second, and third line settings (n = 40) demonstrated a tumor reduction of 92%, disease control of 90%, ORR of 39%, and PFS of 10.5 mo. Again, this is promising data for the next phase III trial.

American Society of Clinical Oncology 2020

Updated data for Keynote-426 after a minimum follow-up of 23 mo were presented [15]. OS was 74% with pembrolizumab/axitinib *vs* 66% with sunitinib. Patients with favorable-risk disease no longer presented a significant difference in OS or PFS, with a median PFS of 20.8 mo with pembrolizumab/axitinib and 18 mo with sunitinib. However, patients with IMDC intermediate or poor-risk disease showed significant differences in OS and PFS with an HR of 0.63 for OS and 0.69 for PFS. The CR rate increased from 6% at 12 mo of follow-up[16] to 9% after 23 mo. A new *post hoc* analysis of the relationship between depth of response and OS showed that in patients receiving pembrolizumab/axitinib, deeper responses, as measured by percent shrinkage of target lesions, correlated to better OS (See Table 1).

Two studies, the OMNIVORE study[17] (n = 83) and the HCRN GU16-260 study[18] (n = 123), were presented to investigate whether treating mRCC patients with nivolumab initially and later adding ipilimumab in patients with either stable or progressive disease would be as effective as an upfront combination therapy. The results showed only 4% and 11% additional partial responses, respectively, suggesting that delaying treatment with ipilimumab decreased the overall efficacy of upfront combination treatment.

The results of the phase II FRACTION-RCC CT[19] to assess nivolumab/ipilimumab after progression to an ICI (PD-1) were also presented (n = 46). The ORR was 15.2%, which suggests that this combination should ideally be administered as first-line therapy.

However, one study showed the results of a phase II bevacizumab/erlotinib study in 83 patients, of which 50% had hereditary leiomyomatosis (HLRCC) and 50% had sporadic (PSRCC) advanced papillary RCC[20]. This combination proved to be very active in papillary RCC, especially in HLRCC, with an ORR of 64%, tumor shrinkage of 95%, and PFS of 21.1 mo.

Finally, a phase III study (SAVOIR) with savolitinib *vs* sunitinib for papillary RCC with abnormal *MET* gene was presented (n = 60)[21]. The results showed a PFS of 7.0 and 5.6 mo in the savolitinib and sunitinib groups, respectively, with better tolerability in the savolitinib group. Initial data look promising, despite the small cohort study.

ESMO 2020

The results of Checkmate 9ER[22], a phase III study of nivolumab/cabozantinib *vs* sunitinib in previously untreated mRCC with a clear cell component, were presented. Patients were stratified by IMDC, PD-L1, and region (n = 651). At a median follow-up of 18.1 mo, nivolumab/cabozantinib led to higher rates of PFS, OS, and ORR *vs* sunitinib (Table 1), with consistent improvements observed across all pre-specified subgroups according to IMDC risk and PD-L1 expression. The combination was generally well tolerated, and patients had significantly better quality of life than those treated with sunitinib. These results support nivolumab/cabozantinib as a potential first-line option for patients with advanced renal cell carcinoma in every IMDC risk (Table 1).

The results of COSMIC 021, a phase II study that tested an escalation dose of cabozantinib from 40 mg to 60 mg with atezolizumab in first-line treatment, was also reported[23]. Data of 70 mRCC patients were presented, showing encouraging clinical efficacy with reasonable safety profiles. The findings suggested that PD-L1+ tumors with high CD8+ T cell infiltrates were more likely to respond to therapy. There is a phase III study (CONTACT-03) currently underway to confirm this combination's efficacy.

A phase II trial of lenvatinib plus pembrolizumab in 104 mRCC patients that were not responding to treatment with immunotherapy was also presented[24]. The ORR was 51%, PFS 11.7 mo, and mDOR 12.2 mo. These results are currently being studied in the phase III CLEAR trial [(lenvatinib + pembrolizumab) *vs* (lenvatinib + everolimus) *vs* sunitinib].

FUTURE DIRECTIONS AND BIOMARKERS

Updates and new trials presented in conferences this year may establish new care standards for mRCC. The update of Keynote 426 presented during American Society of Clinical Oncology (ASCO) 2020[15] suggested that pembrolizumab/axitinib should no longer be offered as the first line of choice of treatment in favorable risk mRCC. Moreover, the results of Checkmate 9ER presented at ESMO[22] showed some advantages of nivolumab/cabozantinib over sunitinib in first-line treatment among all IMDC subgroups and proposed it as a potential first-line option for mRCC.

At this point, there are multiple combination options for first-line treatment and the medical community is divided over which choice is better - two immunotherapies or immunotherapy plus an antiangiogenic drug - considering that the different combinations appear to have similar rates of efficacy, and there are no clear recommendations as to which is the most appropriate for each patient. More data and longer follow-up are needed to clarify the issue and learn whether there are certain populations who would benefit more from one of these combinations, as well as head-to-head comparisons between the combination therapies approved for first-line treatment. Additionally, biomarker-based studies are advisable when several approaches are available and clinical criteria are insufficient to guide treatment strategies.

Until then, taking into account the usual caveats pertaining to this practice, some insight may be gleaned from comparing CTs. At ASCO 2020, for example, the current first-line treatments in intermediate and high-risk mRCC patients (Checkmate 214[11] and Keynote-426[15]) were compared and discussed. Regarding OS data, outcomes in KEYNOTE426 appear to be slightly better at 2 years, and the ORR appears to be slightly higher with pembrolizumab/axitinib in KEYNOTE-426 (55%) than with nivolumab/ipilimumab in Checkmate 214 (42%). However, the percentage of patients who experienced primary progression with tumor growth while on treatment is more striking: 27% for nivolumab/ipilimumab and approximately half that, 15%, for pembrolizumab/axitinib. In clinical practice, pembrolizumab/axitinib appears to be the better choice, compared with nivolumab/ipilimumab, for a patient who needs a response to a rapidly progressing disease or to ameliorate symptoms, based on this cross-study comparison. For other patients, the adverse event profile of each combination would likely help to choose the most appropriate treatment.

An additional consideration is that the choice of first-line treatment may impact selection of second-line therapy. Starting with a combination of immune therapy only forces an automatic choice to use an antiangiogenic drug in the second line. However, starting with a combination of immune therapy and an antiangiogenic makes the second-line choice less clear. For this reason, more data are needed on the most suitable order of therapy for the population at large and specific groups, such as high *vs* slow-growing disease. Indeed, some ongoing CTs are trying to find the best alternative in second and third lines: Atezolizumab/cabozantinib *vs* cabozantinib (CONTACT-03)[25]; MK-6482 *vs* everolimus[26].

Also noteworthy is the recent trend toward three-part strategies, with various ongoing CTs, which have so far provided only preliminary results, including nivolumab + ipilimumab +/- cabozantinib (COSMIC 313)[27]; and the PDIGREE study [28], which proposes the use of nivolumab and ipilimumab followed by nivolumab or nivolumab with cabozantinib.

Conversely, other trials, such as the Checkmate 209-8Y8[29] and the KEYNOTE-427, are looking at maintaining monotherapies. The former proposes the use of nivolumab alone after nivolumab/ipilimumab in intermediate to poor-risk mRCC, while the latter studies the use of pembrolizumab in the frontline setting, showing promising activity (ORR of 36.4%, and disease control of 57.3%)[30].

Another field of study pertains to neoadjuvants and adjuvants, where either nivolumab or pembrolizumab is being evaluated in treatment before surgery (NCT02595918 and NCT02212730, respectively). The PROSPER trial (NCT03055013) assesses nivolumab in neoadjuvant and adjuvant use in node-positive or stage T2-T4 patients compared to observation[31].

Generally speaking, ongoing trials are moving away from sunitinib as the control arm and focus their research on triple therapies or novel therapeutic agents. PIVOT-9, a phase III randomized study, compares NKTR-214 plus nivolumab *vs* sunitinib or cabozantinib in previously untreated mRCC (NCT03729245). A phase II CT (NCT03634540) is studying the combination of HIF-2α inhibitor (PT2977) and cabozantinib.

Telaglenastat, a glutaminase inhibitor, is being studied in previously treated mRCC in combination with cabozantinib and everolimus in two phase II trials (CANTATA and ENTRATA, respectively), and entinostat, an orally available inhibitor of HDACs,

is being considered in several combination therapies[32].

Poly(ADP-ribose) polymerase inhibitors, widely used in other tumors, have been proposed for RCC: Olaparib for patients with DNA repair gene mutations and talazoparib with avelumab.

Finally, the great challenge in mRCC treatment remains to find predictive and prognosis biomarkers. Interesting data are emerging from mRCC patients enrolled in CTs. PD-L1 expression, for example, was associated with poor outcomes in a meta-analysis[33]; but as a predictive marker, the results have been varied[4,34]. Genes have also been studied, including BRCA1-associated protein, which correlates with a poor survival[35], and PBRM1 mutation, which was associated with a longer PFS in the sunitinib and atezolizumab/bevacizumab group in IMotion150[36]. Another attempt to find a gene expression signature tool was made in IMmotion 151[34], where tumors characterized by angiogenesis-high signatures had better PFS with sunitinib and tumors with T effector/interferon-γ-high or angiogenesis-low signatures exhibited better outcomes with atezolizumab/bevacizumab. However, to date, the only predictive biomarker likely to be validated in a phase III randomized controlled trial is the IMDC risk model.

CONCLUSION

In conclusion, we are hopeful that in the coming years, patients and oncologists will continue to move away from a "one-size-fits-all" approach to treatment sequencing and instead move toward a more personalized treatment paradigm in mRCC.

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REVIEW

New approaches for patients with advanced radioiodine-refractory thyroid cancer

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Abstract

The cumulative evidence over the past decades has shown that the incidence of differentiated thyroid carcinoma (DTC) has exponentially increased. Approximately 10% of patients with DTC exhibit recurrent or metastatic disease, and about two-thirds of the latter will be defined as refractory to radioactive iodine (RAIR) treatment. Since this condition implies 10-year survival rates less than 10% after detection, using available treatments, such as systemic and targeted therapies, have become increasingly relevant. The initiation of these treatments aims to reach stabilization, tumor volume reduction, and/or symptom improvement and it should be decided by highly specialized endocrinologists/oncologists on the basis of patient's features. Considering that despite enlarged progression-free survival was proven, multikinase inhibitors remain non-curative, their benefits last for a limited time and the side effects potentially cause harm and quality of life reduction. In this context, molecular testing of cancer cells provides a promising spectrum of targeted therapies that offer increased compatibility with individual patient needs by improving efficacy, progression free survival, overall survival and adverse events profile. This review article aims to provide a summary of the current therapeutic strategies in advanced RAIR-DTC, including approved target therapies as well as those for *off-label* use, RAI resensitization agents, and immunotherapy.

Key Words: Advanced differentiated thyroid cancer; Radioactive iodine refractory thyroid cancer; Multikinase inhibitors; Systemic therapy; Target therapy

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Core Tip: The incidence of differentiated thyroid carcinoma has increased due to the rising detection of low-risk small carcinomas. Nevertheless, approximately 10% of patients exhibit advanced disease and two-thirds of the latter will be defined as radioactive iodine (RAI) refractory. After detection, 10-year survival rates are less than 10%, therefore the role of systemic and targeted therapy in these patients has become increasingly relevant in recent years. This review article aims to provide a summary of the current therapeutic strategies in iodine-refractory thyroid cancer, including approved target therapies as well as those for off-label use, RAI resensitization agents, and immunotherapy.

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INTRODUCTION

The cumulative evidence over the past decades has shown that the incidence of differentiated thyroid carcinoma (DTC) has increased exponentially, probably due to the rising detection of low-risk small carcinomas[1]. Nevertheless, approximately 10% of patients with DTC exhibit a more aggressive behavior in which persistent or recurrent distant metastatic disease is developed, and about two-thirds of them will be defined as refractory to radioactive iodine (RAI) treatment[2]. This condition cannot be defined by a single criterion, but it rather comprises a spectrum of scenarios included into any of the following: (1) Lack of initial RAI uptake in all or some of the metastatic foci in a whole-body scan (diagnostic or following a therapeutic dose) or lose of the ability to take up RAI after previous evidence of uptake; (2) Disease progression in a patient who has received RAI; (3) Disease progression in a patient who has received 600 mCi of ^{131}I of cumulative activity; and/or (4) Locally advanced disease for whom surgical resection is not feasible and RAI uptake status cannot be assessed[2]. After the detection of radioiodine refractory (RAIR) disease, 10-year survival rates may decrease to less than 10%[2]. Therefore, using second-choice treatments, such as systemic and targeted therapy, in these patients has become increasingly relevant in recent years. This review article aims to provide a summary of the current therapeutic strategies for patients with RAIR thyroid cancer, including approved target therapies as well as those prescribed for *off-label* use, RAI resensitization agents, and immunotherapy.

MOLECULAR PATHWAYS OF THYROID CANCER

The underlying carcinogenic molecular pathways of differentiated thyroid cancer have been well defined. The MAPK signaling pathway is one of the most extensively studied[3]. Driver mutations such as in *BRAF* and *RAS* oncogenes, as well as fusions involving tyrosine kinase receptors, lead towards a constitutive activation of the downstream events resulting in cell proliferation, dedifferentiation, and cancer cell survival. These mutations could be targeted with specific therapies which result in cell growth inhibition[3,4]. Meanwhile, multikinase inhibitors (MKIs) confer their anti-tumor effect in radioiodine-refractory metastatic thyroid cancer by other effects, mainly through their anti-angiogenic activity[4]. The main molecular signaling pathways involved in thyroid carcinogenesis and the most significant inhibitors are summarized in Figure 1.

INITIATION OF SYSTEMIC THERAPY

The initiation of health agencies approved systemic therapy or the enrollment of a patient in a clinical trial should be managed by highly specialized endocrinologists/oncologists. The aim of this treatment will be to reach stabilization, tumor volume reduction, and/or symptom improvement[2,4]. Nevertheless, it should be

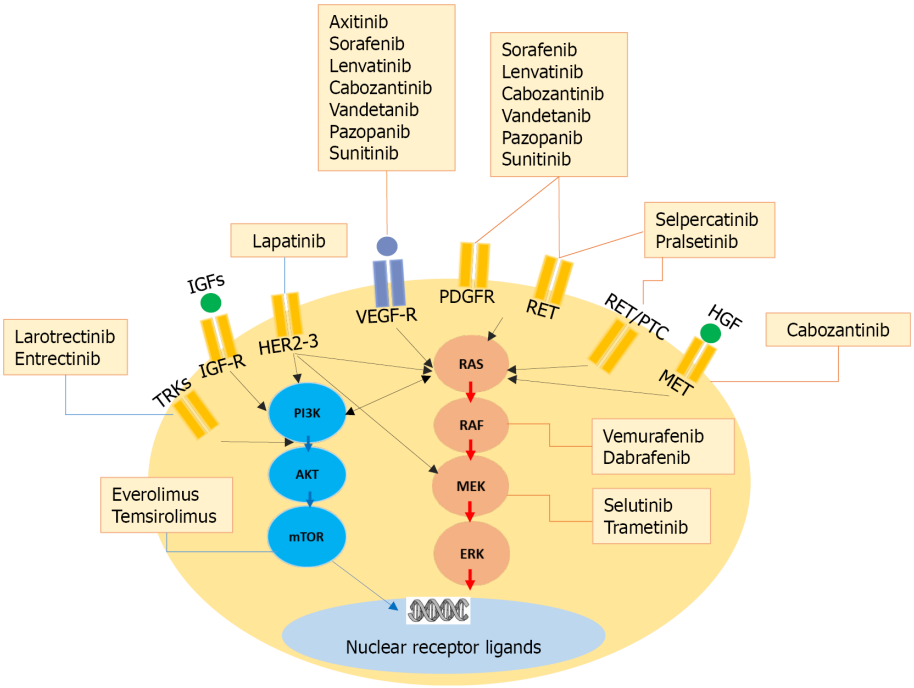


Figure 1 Major molecular signaling pathways involved in thyroid carcinogenesis and its most significant inhibitors.

decided on an individualized basis and under a coordinated decision taken together with patients, considering that target and multikinase inhibitor-based therapies remain non-curative and their benefits in terms of extending progression-free survival last for a limited time. Furthermore, the side effects of these therapies may have the potential to cause harm and significantly reduce the patient's quality of life[4]. Thus, the assessment of tumor burden, disease progression, symptoms, or a high risk of local complications is essential[2]. If available, genetic interrogation should be granted in order to initiate a selective TKI (either an approved drug or from clinical trials) in a patient with a progressive advanced RAIR-DTC that carries a specific target mutation [5]. If not genetic alterations are found, RAIR-DTC patients and those having tumor lesions in which the sum of diameter is larger than 2 cm and showing < 12-mo progression should be considered for multikinase inhibitors[2]. Additionally, all patients with DTC-related imminent symptoms and potentially symptomatic disease should be guaranteed treatment initiation[2]. This is a simplistic view but may help to decide the correct moment of treatment initiation when no other therapies are no longer amenable. A proposed decision-making algorithm for systemic therapy initiation in RAIR-DTC is shown in Figure 2. The main available agents studied in the treatment of RAIR-DTC are summarized in Table 1.

MULTIKINASE INHIBITORS (SORAFENIB AND LENVATINIB)

Multikinase inhibitors block several signaling pathways responsible for tumor proliferation and survival, with varying degrees of potency[2]. However, the main target for MKIs is the vascular endothelial growth factor receptor (VEGFR) and therefore the inhibition of tumor angiogenesis[3]. That is why they are also called antiangiogenic MKIs. These MKIs have demonstrated in phase III trials, an increase in the median progression free-survival (mPFS) from 11 to 18 mo, and objective responses of 12% to 64%[6,7]. We should consider that these drugs are usually tumoristatic and will eventually lose their effect due to *on-target* or *off-target* resistance, after which, another therapy will be needed. To date, only one *post-hoc* analysis of the SELECT study has demonstrated improved overall survival in a subgroup of patients receiving an Lenvatinib *vs* placebo[8].

Sorafenib

Sorafenib inhibits the VEGFR 1, 2, and 3, platelet-derived growth factor, RET, c-kit, and less potently, BRAF kinases[9]. In the phase III DECISION trial, patients treated with sorafenib (n = 207) had a significantly longer PFS over patients receiving placebo

Table 1 Available agents studied in the treatment of radioiodine refractory-differentiated thyroid carcinoma

Agent and national clinical trial number[1]	Molecular target	Phase	Dosage	Enrolled patients (*n*)	PR (%)	mPFS (mo)	Common AEs	Serious AEs (grade ≥ 3)	Withdrawal due to AEs
Sorafenib[6]; NCT00984282	VEGFR1–3, PDGFR, RET, c-kit, BRAF	III	400 mg orally twice daily	207	10.8	12.2	Hand– foot skin reaction (76%), diarrhea (69%), alopecia (67%), rash (50%)	Hand-foot skin reaction (20%), hypertension (10%), weight loss (6%)	19%
Lenvatinib[7]; NCT01321554	VEGFR1–3, FGFR1–4, PDGFR, RET, c-kit	III	24 mg per d in 28-d cycles	261	63.2; 65 (4 complete response + 165 partial response)	18.3	Hypertension (68%), diarrhea (59%), fatigue (59%), decreased appetite (50%), decreased weight (46%), nausea (41%)	Hypertension (42%), proteinuria (10%), decreased weight (10%), fatigue (9%), diarrhea (8%)	14%
Cabozantinib [28]; NCT01811212	VEGFR2, MET, FLT3, RET, c-kit	II	60 mg/d orally	25	40	12.7	Fatigue (44%), weight loss (36%), diarrhea (36%), hand– foot skin reaction (32%), hypertension (24%)	Hypophosphatemia (16%), lipase/amylase increase, neutropenia, fatigue, weight loss (12%)	
Axitinib[71]; NCT00094055	VEGFR, PDGFR, c-kit	II	5 mg twice daily	60	30	18.1	Fatigue (50%), diarrhea (48%), nausea (33%), anorexia (30%), hypertension (28%), stomatitis (25%), weight loss (25%), and headache (22%)	Hypertension (12%), proteinuria (5%), fatigue (5%)	
Vandetanib [72]; NCT00537095	VEGFR2/3, EGFR, RET	II	300 mg/d	72	8.3	11.1	Diarrhea (74%), hypertension (34%), acne (27%), asthenia, anorexia (26%), nausea, rash (25%), fatigue, QTc prolongation (23%)	QTc prolongation (14%), diarrhea (10%), asthenia (7%), fatigue (5%)	33%
Sunitinib[73]; NCT00381641	PDGFR, FLT3, c-kit, VEGFR, RET	II	37.5 mg/d orally	35	31	12.8	Neutropenia (34%), leukopenia (31%), fatigue (26%), HFS (26%), diarrhea (26%)	Neutropenia (34%), leukopenia (31%), diarrhea, hand/foot syndrome (17%), fatigue (11%)	11%
Pazopanib [74]; NCT00625846	VEGFR, PDGFR, c-kit	II	800 mg/d orally in 4-wk cycle	37	49	11.7	Fatigue (78%), skin and hair hypopigmentation (75%), diarrhea (73%), nausea (73%)	Raised alanine aminotransferase level (11%)	5%
Dovitinib[75]; NCT02964144	FGFR, VEGFR	II	500 mg/d orally for five days, followed by a 2-d rest every week	40	20.5	5.4	Diarrhea (54%), anorexia (36%), vomiting (26%), fatigue (23%), and nausea (21%)	Neutropenia (13%)	20%
Apatinib[31]; NCT03167385	VEGFR2, c-Kit, c-SRC	II	750 mg/d orally (*n* = 10, group I) - 500 mg/d orally (*n* = 10, group II)	20	90 (I); 70 (II)	18.4	Hand– foot skin reaction (95%), proteinuria (90%) and hypertension (80%)		
Lapatinib[76]; NCT01947023	HER2/3	I	750 mg initial dose, escalated to 500 mg daily; + Dabrafenib 150 mg twice daily	13	60	15		Lymphocytic toxicity (7%)	
Vemurafenib [58]; NCT01286753	BRAF V600E	II	960 mg orally twice daily	51	VEGFR naive: 39%; Previous VEGFR:	VEGFR naive: 18.8; Previous VEGFR:	Rash (73%), fatigue (69%), alopecia, dysgeusia (54%), creatinine increase, weight decrease	Skin squamous cell carcinoma (23.5%), lymphopenia, and increased γ-glutamyl-transferase (8%)	27%

					27%	8.9	(50%), arthralgia, anorexia, nausea, skin papilloma (46%)		
Dabrafenib [57]; NCT00880321	BRAF V600E	I	150 mg twice daily	13	29	11.3	Skin papillomas (57%), hyperkeratosis (36%), alopecia (29%)	Elevated lipase, elevated amylase, fatigue, febrile neutropenia and squamous cell carcinoma (7%)	0%
Selumetinib [66]; NCT00559949	MEK-1/2, RAS, BRAF V600E	II	100 mg twice daily for 28-d cycles	39	3	8	Rash (77%), fatigue (49%), diarrhea (49%), peripheral edema (36%)	Rash (18%), fatigue (8%)	15%
Larotrectinib [33]; NCT02122913	NTRK fusions	II	100 mg twice daily	153	129 (95%); 24 (16%) complete response	28.3	Fatigue (30%), cough, constipation (27%), dizziness, alanine aminotransferase increase (25%)	Anemia (10%), decreased neutrophil count (5%)	2%
Entrectinib [36]; NCT02097810 (STARTRK-1) NCT02568267 (STARTRK-2)	NTRK fusions	II	600 mg/d orally	54	50	10	Dysgeusia (47%), fatigue, constipation (28%), diarrhea (27%), edema peripheral, dizziness (24%)	Anemia (12%), weight gain (10%)	4%
Everolimus [62]; NCT01118065	mTOR	II	10 mg/d orally	33	3	12.9	Mucositis, acneiform rash, fatigue, cough	Fatigue (8%), weight loss, infection (6%)	
Temsirolimus [63]; NCT01025453	mTOR	II	Temsirolimus (25 mg IV weekly) + sorafenib (200 mg twice daily)	36	22	12		Hyperglycemia (19%), fatigue (13%), anemia (11%), oral mucositis, alanine aminotransferase increased (8%)	14%

[1]from ClinicalTrials.gov.

PR: Partial response; AE: Adverse event; VEGFR: Vascular endothelial growth factor receptor; NTRK: Neurotrophic tropomyosin receptor kinase.

(*n* = 209) (10.8 *vs* 5.8 mo, respectively; HR, 0.587; 95%CI: 0.45–0.76; *P* < 0.0001)[6]. The clinical benefit rate (CR + PR + SD > 6 mo) was 54%, with a PR rate of 12.2% and an SD > 6 mo of 41.8%[6]. The median duration of PR was 10.2 mo. An improvement in OS could not be demonstrated, probably because a large proportion of patients in the placebo arm (71%) crossed over to treatment[6]. In the last metanalysis that included 636 patients from 15 studies receiving sorafenib, 26% of patients (95%CI: 0.19-0.34) achieved a PR, and 44% (98%CI: 0.39-0.48) an SD[10]. PFS time ranged from 9 to 21.3 mo and OS ranged from 10 to 56 mo[10]. In an exploratory analysis of the phase III trial, patients who received open-label sorafenib after progression under the placebo arm achieved a comparable PFS to those receiving sorafenib from the beginning of the trial (9.6 *vs* 10.8 mo)[11]. This could suggest that delaying the initiation of sorafenib could not have a significant impact on the effectiveness. Also, in the same analysis, patients who continued receiving sorafenib after progression had a still longer PFS in comparison to patients who initially received placebo (6.7 *vs* 5.8)[11], meaning that sorafenib could still be an option in patients when an alternative drug is not available or not possible. In our real-life experience with sorafenib (*n* = 18), 72% had SD ≥ 6 mo and 11% demonstrated PR with a PFS of 16.5 mo[12].

The most frequent adverse events during sorafenib treatment were hand-foot skin reaction, diarrhea, fatigue, alopecia, weight loss, and rash[6,10,12]. HFS reaction and hypertension were the most frequent grade 3-4 AEs, reported from to and from to, respectively[6,10,12]. As reported with other MKIs, dose reductions and interruptions were frequent, however, drug withdrawal was uncommon[6,10,12]. The recommended initial dose of sorafenib is 400 mg twice a day[13]. In an exposure-response model, initial lower doses of sorafenib (600 or 400 mg/d) were associated with improved tolerability but reduced PFS. However, a strategy of 800 mg/d for an initial two cycles followed by dose reductions seemed likely to maintain efficacy while possibly mitigating some AEs[14]. The summary of the efficacy and safety of sorafenib in patients with thyroid cancer reported by clinical trials is shown in Table 2.

Table 2 Summary of the efficacy and safety of sorafenib in patients with thyroid cancer reported by clinical trials

Ref.	*n*	Type	PR, %	SD, %	Median PFS (mo)	Median OS (mo)	Most frequent AE	Most frequent grade 3-4 AE
Gupta-Abramson *et al* [77], 2008	27	DTC	26	59	19	-	HFS, 93%	Hypertension, 13%
Kloos *et al*[78], 2009	33	PTC	15	57	16	23	Fatigue, 85%	Fatigue, 16%
Hoftijzer *et al*[79], 2009	31	DTC	25	34	14.5	-	HFS, 66%	HFS, 18%
Cabanilas *et al*[59], 2010	13	DTC	20	60	19		HFS, 60%	-
Keefe *et al*[80], 2011	47	DTC/PD	38	47	22	32.4	-	-
Ahmed *et al*[81], 2011	19	DTC	16	-	-	-	Dermatology (other than HFS), 88%	HFS, 44%
Chen *et al*[82], 2011	9	DTC	33	44	10.5	-	Alopecia, 100%	-
Marotta *et al*[83], 2012	17	DTC	30	41	9	10	HFS, 88%	
Schneider *et al*[84], 2012	31	DTC	31	42	18	34.5	HFS, 71%	HFS, 22%
Capdevilla *et al*[85], 2012	16	DTC	19	50	13.3	23.6	HFS and diarrhea, 62%	HFS, 23%
Brose *et al*[6], 2014	207	DTC	12	42	10.8	.	HFS, 73.6%	HFS, 20.3%
Benekli *et al*[86], 2014	14	DTC	-	43	21.3	-	-	HFS, 22%
Dadu *et al*[87], 2008	51	DTC	-	-	-	56	-	-
Luo *et al*[88], 2014	8	DTC	50	37	9.4	12.8	Alopecia, 75%	Hypocalcemia and serum amylase increased, 12.5%
Gallo *et al*[89], 2015	20	DTC	25	40	8.2	28.4	Fatigue, 95%	Gastrointestinal symptoms, 15%
Kim *et al*[90], 2018	98	DTC	25	37	9.7	-	HFS, 76%	HFS, 41%
Jerkovich *et al*[12], 2019	18	DTC	11	72	16.5	-	HFS, 67%	HFS, 14%

DTC: Differentiated thyroid carcinoma. PR: Partial response; PFS: Progression free survival; SD: Stable disease; OS: Overall survival; AE: Adverse event.

Lenvatinib

Lenvatinib inhibits FGFR1, -2, -3, -4, PDGFR, VEGFR1, -2, -3, RET, and KIT kinases [15]. In phase III clinical trial SELECT, median PFS was significantly longer in patients treated with lenvatinib in comparison to those receiving placebo (18.3 *vs* 3.6 mo, respectively; HR, 0.21; 99%CI: 0.14-0.31; $P < 0.001$)[7]. The response rate was 64.8% (CR 1.5% and PR 63.2%), with a median time to response of only 2 mo[7]. Real-life studies published afterward had reported PR from 31% to 69%, SD from 20% to 60%, and PFS from 10 to 13.8 mo[16-23]. This apparent lower efficiency of lenvatinib in observational data could be explained by the fact that these studies included patients with more than one prior MKI treatment, ECOG PS ≥ 3, more comorbidities, and patients who did not start with a full dose (24 mg per day). In fact, in our experience with lenvatinib (n = 22), when we excluded patients that would have not met the SELECT inclusion criteria, PR increased from 31.8% to 50% and PFS from 13.7 to 22 mo[23]. Hypertension was the most common adverse event (63%-83%) in almost all studies[7,16-19,21,23] and the most frequent grade 3-4 adverse event, occurring in 31%-42% of cases[7,23]. Other adverse effects include diarrhea, fatigue, decreased appetite, and decreased weight[7,16-23]. The recommended initial dose is 24 mg per day[13]. A lower initial dose and longer dose interruptions led to lower response rates and shorter progression-free survival[24,25]. A summary of the efficacy and safety of lenvatinib in patients with thyroid cancer reported by phase III clinical trial and real-life studies is shown in Table 3.

Cabozantinib

Cabozantinib is a RET, vascular endothelial growth factor receptor-2 (VEGFR2), and MET kinases inhibitor agent currently approved for the treatment of advanced medullary thyroid cancer[26]. However, it has also been studied in 15 patients with RAI-refractory DTC in a phase I clinical trial, with promising efficacy[27]. Ten of the

Table 3 Summary of the efficacy and safety of lenvatinib in patients with thyroid cancer reported by phase III clinical trial and real-life studies

Ref.	*n*	Patients with prior TKIs %	CR %	PR %	SD %	Median PFS (mo)	Median OS (mo)	Most frequent AE	Most frequent grade 3-4 AE
Schlumberger *et al*[7] 2015	261	25	1	63	23	18.3	-	Hypertension, 68%	Hypertension, 42%
Berdelou *et al*[16], 2017	75	68	0	31	51	10	-	Fatigue, 75%	Hypertension 35%
Jasim *et al*[17], 2017	25	31	0	50	28	-	-	Hypertension 64%	Hypertension 40%
Sugino *et al*[18], 2018	29	13	0	69	21	-	-	Hypertension 76%	-
Locati *et al*[19], 2019	94	64	0	36	41	10.8	23.8	Fatigue, 13%	Fatigue, 8%
Lee *et al*[20], 2019	57	89	0	38	60	5.1	19.3	General weakness 43%	
Masaki *et al*[21], 2019	42	10	0	62	24	13.8	-	Hypertension, 83%	Proteinuria, 36%
Aydemirli *et al*[22], 2020	39	77	2	33	37	9.7	18.3	Hypertension and fatigue, 64%	Hypertension, 28%
Jerkovich *et al*[23], 2020	22	59	4	32	32	13.7	-	Hypertension, 64%	Hypertension, 23%

TKIs: Tyrosine kinase inhibitors; CR: Complete response; PR: Partial response; SD: Stable disease; PFS: Progression free survival; OS: Overall survival; AE: Adverse event.

included patients were previously treated with VEGF inhibitors, mostly sorafenib. Cabozantinib was administered at a starting dose of 140 mg daily. A partial response was observed in 8 (53%) patients, 5 with prior VEGF inhibitors treatment. On the other hand, a phase II trial is currently ongoing, which involves a cabozantinib therapy in RAIR-DTC patients who experienced disease progression after second- or third-line VEGFR-targeted therapy[28]. Partial response was reached in 10 (40%) of the 25 enrolled patients, with a starting dose of 60-80 mg daily. The median PFS and OS were 12.7 and 34.7 mo, respectively[28].

Exelixis announced by the end of 2020 that, at a planned interim analysis, the phase III COSMIC-311 pivotal trial met the co-primary endpoint, demonstrating a significant reduction in the risk of disease progression or death of 78% of patients receiving cabozantinib compared to placebo (HR, 0.22, 96%CI: 0.13-0.36; $P < 0.0001$) in patients with RAIR differentiated thyroid cancer who have progressed after up to two prior VEGFR-targeted therapies. The safety profile was consistent with that previously observed for cabozantinib. In 2021, Exelixis® announced that the United States Food and Drug Administration (FDA) approved cabozantinib as a second/third line additional treatment for patients with RAIR thyroid cancer[29]. With this third MKI approved, there will surely be a change in defining first and second line of treatment according to the drug potency.

Apatinib

Apatinib, also known as rivoceranib, is a tyrosine kinase inhibitor that selectively inhibits the *VEGFR2*. Apatinib inhibits VEGF-mediated endothelial cell migration and proliferation thus blocking new blood vessel formation in tumor tissue. This agent also mildly inhibits c-Kit and c-SRC tyrosine kinases[30]. A recent phase II study performed in 20 patients with advanced thyroid cancer showed promising results with an objective response rate (ORR) of 80%, a median PFS of 18.4 mo (95%CI: 9.2-36.8 mo) and a median OS of 51.6 mo (95%CI: 29.2-not reached). The most common adverse events included palmar-plantar erythrodysaesthesia syndrome (19/20), proteinuria (18/20) and hypertension (16/20)[31].

SELECTIVE KINASE INHIBITORS

NTRK inhibitors (larotrectinib and entrectinib)

Neurotrophic tropomyosin receptor kinase (*NTRK*) fusions have been reported in

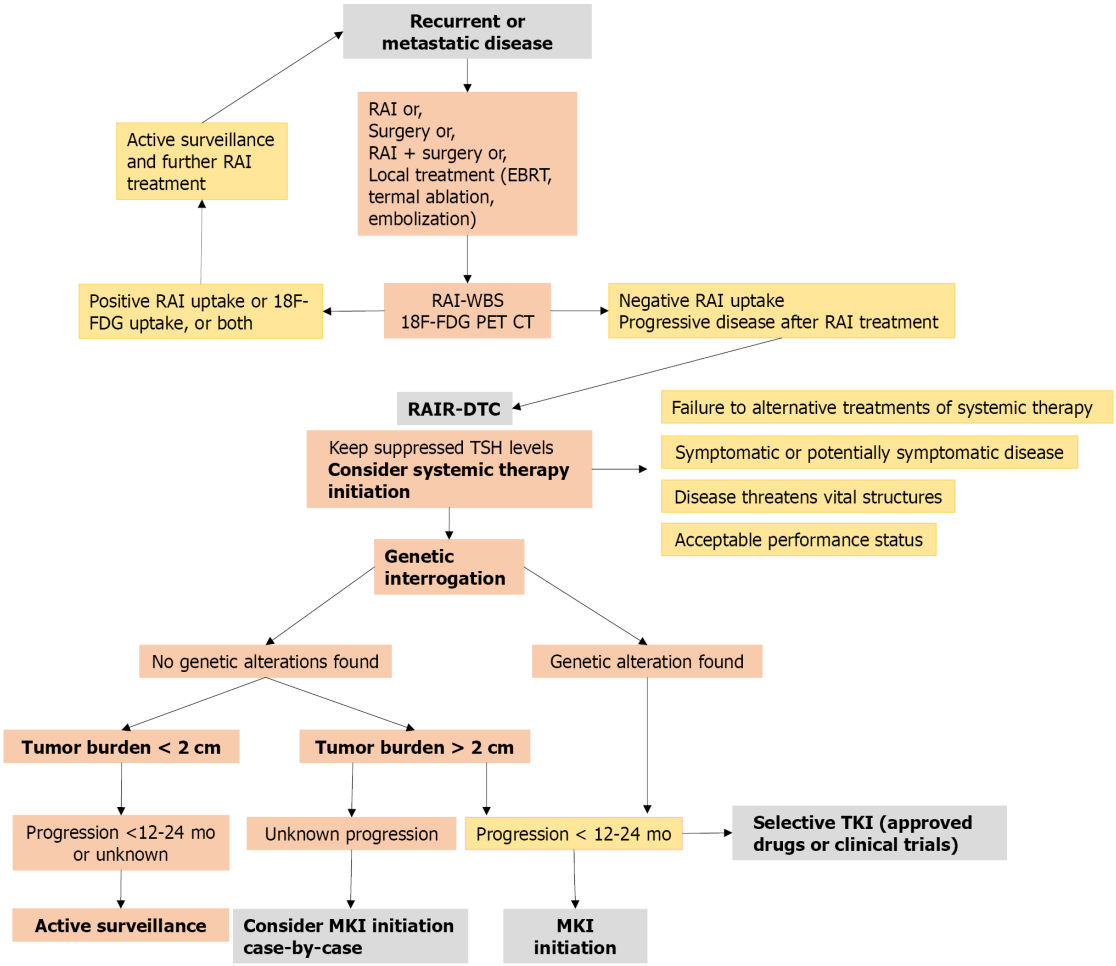


Figure 2 Proposed decision-making algorithm for systemic therapy initiation in radioiodine refractory-differentiated thyroid carcinoma.

variable percentages of patients with DTC (2%-25%)[32]. Larotrectinib and Entrectinib are highly selective inhibitors of *TRK* receptors and have been approved by the FDA for the treatment of any solid tumor-bearing an *NTRK1-3* fusion mutation (tumor-agnostic indication). Entrectinib also inhibit altered oncogenic expression of *ALK* and *ROS1*, which are much less frequent in DTC[32].

In a pooled analysis of three-phase 1/2 clinical trials, out of 24 patients with DTC bearing an *NTRK* fusion who received larotrectinib, 79% experienced an objective response[33]. This drug showed durable responses with a median time of 35 mo in the overall group of patients with solid tumors[33]. Also, larotrectinib seems to be active within the central nervous system (CNS)[33], which makes it an indispensable option when brain metastases are present in patients harboring this fusion, knowing that they have a worse outcome in patients with differentiated thyroid cancer[34]. Most frequent adverse events were primarily grade 1 and 2 and included fatigue (30%), cough, constipation (27%), dizziness (25%), and alanine aminotransferase increase (25%). The most common grade 3 or worse treatment-emergent adverse events (regardless of attribution) were anemia (10%) and decreased neutrophil count (5%)[33]. We recently showed our experience with Larotrectinib in a patient with RAIR DTC who had a rapid progression on MKI therapy (sorafenib and lenvatinib), and who had a complete response to treatment including the disappearance of multiple CNS metastasis[35].

Entrectinib also blocks *ROS1* and *ALK* and was specifically designed to have systemic activity and cross the blood–brain barrier. In an analysis of three-phase I or II trials, two out of four patients had a PR with entrectinib[36]. Most AE were grade 1-2 and included dysgeusia (47%), fatigue (28%), and constipation (28%). The most common grade 3 or 4 treatment-related AE were anemia (12%) and weight gain (10%)[36].

Resistance to larotrectinib and entrectinib

TRK fusion-positive cancers can develop resistance to *TRK* inhibition[37]. This resistance can be classified into *off-target* (new additional mutations that may occur in

the tumor) or *on-target* (within the same altered receptor, due point mutations that lead to amino acid substitutions in the solvent front, the gatekeeper residue or the *xDFG* motif)[38]. Mutations in the *NTRK* kinase domain cause resistance to *TRK* inhibitors by interfering with binding of the inhibitor, altering the kinase domain conformation or altering ATP-binding affinity[38].

New drugs are currently in development for those patients who develop *on-target* resistance, among them, selitrectinib and repotrectinib. Due to their small size, these low molecular weight molecules are able to engage the ATP-binding pocket while avoiding the steric penalties of kinase domain substitutions[39,40]. Selitrectinib is currently the drug with which the most experience has been gained. Thirty-one patients with solid tumors with *NTRK* fusions, previously treated with a *TRK* inhibitor (larotrectinib, entrectinib or PLX7486) with a median duration of prior therapy of 11 mo (range 2-30 mo) received treatment with selitrectinib. In patients with *TRK* kinase domain mutations (the majority of which involved the solvent front), the ORR was 45%[41].

Selective RET inhibitors (selpercatinib and pralsetinib)

RET/PTC rearrangements are present in 5%-25% of papillary thyroid carcinomas[42], although the occurrence of these mutations may be less frequent in advanced DTC [43]. Selpercatinib and pralsetinib, are kinase inhibitors that selectively target *RET* kinase, and were approved by the FDA for the treatment of advanced or metastatic *RET* fusion-positive thyroid cancer. In the phase 1/2 trial LIBRETTO-001, among 19 *RET* fusion-positive, non-medullary thyroid cancer patients, objective response was reported in 79%[44]. At 1 year, 71% of responses were ongoing, and 64% of the patients were free of progression[44]. The most common grade 3 or 4 adverse events included hypertension (21%), increased alanine aminotransferase (11%), increased aspartate aminotransferase (9%), hyponatremia (8%), and diarrhea (6%)[44].

In the phase 1/2 ARROW trial, praseltinib demonstrated objective responses in 75% (9/12), with a median duration of response of 14.5 mo, and 67% of responding patients continuing treatment[45]. Most treatment-related adverse events were grade 1-2, and included increased aspartate aminotransferase (31%), anemia (22%), increased alanine aminotransferase (21%), constipation (21%) and hypertension (20%)[45].

Mutation-specific kinase inhibitors -*RET* and *NTRK* inhibitors, as well as *BRAF* inhibitors-produced higher and durable objective responses[32,33,42,43]. Although prolongation of progression-free survival has not yet been demonstrated in phase III clinical trials, they seem to be promising options for RAIR thyroid cancer patients. In line with this, the implementation of molecular screening strategies seems to be necessary to improve the clinical course of these patients.

Resistance to selpercatinib and pralsetinib

Evidence on acquired resistance mechanisms to *RET*, both *on target* and *off-target*, is recently arising. Selpercatinib and pralsetinib were oriented to target gatekeeper mutations, such as *RET V804* and *S904F* which was associated with resistance to *RET*-targeted kinase inhibitors, as vandetanib[46]. Nevertheless, five *RET* kinase domain mutations at three non-gatekeeper residues were identified from selpercatinib and pralsetinib-resistant medullary thyroid cancer cell lines in a recent experimental study [47]. Information on acquired resistance to these drugs obtained from studies on non-small cell lung cancer (NSCLC) is slightly more extensive. For example, it was found acquired *RET G810R/C/S/V* mutations in *RET* fusion-positive tumors from patients who developed resistance to selpercatinib[48] and pralsetinib treatments[49]. Other reports of acquired selpercatinib resistance with *MET* amplification were demonstrated, in which probably this could be overcome by combing selpercatinib with crizotinib[50]. On the other hand, it was postulated that the combination of pralsetinib or selpercatinib with a selective *MET* inhibitor -such as capmatinib, savolitinib, or tepotinib- could offer acceptable tolerability and efficacy in NSCLC patients[51].

These experimental findings have shown the imperative need to develop next-generation targeted *RET* agents focused on both gatekeeper and non-gate keeper mutations for on- and off-target resistance in order to develop and validate combination therapies.

OFF-LABEL DRUGS FOR DIFFERENTIATED THYROID CARCINOMA

Considering the time-limited benefits of *FDA*-approved kinase inhibitors treatment in RAI-refractory thyroid cancer, it became necessary to develop additional new

therapeutic lines that would enhance compatibility with individual patient needs by improving efficacy and adverse events profile. Several targeting agents are being studied in advanced differentiated thyroid cancers, but none of them have been approved yet (Table 1). A summary of some relevant ongoing clinical trials for the treatment of advanced RAIR-DTC are shown in Table 4.

SELECTIVE BRAF INHIBITORS

Combination of dabrafenib-trametinib

BRAF oncogene mutations are present in approximately 50% of PTCs, while it has been observed that it rises to over 90% when an anaplastic transformation emerges from a prior history of PTC[52]. Under this premise, a clinical study using the combination dabrafenib 150 mg twice daily + trametinib 2 mg daily (selective inhibitors of *BRAF V600E* kinase and *MEK1-2* kinase, respectively) in 23 patients with locally advanced, unresectable, or metastatic ATC[53], prompted the rapidly FDA approval for these patients. This study showed an overall response rate of 61%, with complete and partial response rates of 4% and 57%, respectively. Progression free survival for at least 6 mo was seen in 64% of these patients and overall survival was 80% at 1 year. The most common adverse events were fatigue (44%), fevers (31%), and nausea (31%), and the most common grade 3 and 4 adverse event was anemia (13%) [53].

In our setting, where access to molecular tests and target therapies is not widely available yet, we have reported the cases of two patients with metastatic and locally unresectable ATC, in whom the use of dabrafenib-trametinib (D-T) provided a dramatic reduction of the cervical mass with a minimal residual loco-regional disease, and even allowed surgical resection on one of them. Besides, a partial and complete response to the pulmonary metastatic disease was also observed[54,55].

The combination of D-T was studied in a phase II clinical trial that included 53 patients with *BRAF* mutated RAIR-PTC with disease progression within the last year [56]. The participants were randomized to Arm A (dabrafenib 300 mg daily, n = 26) or Arm B (dabrafenib 150 mg daily + trametinib 2 mg daily, n = 27). Cross-over to Arm B was allowed at the time of progression. Out of 25% of patients had prior therapy with multi-kinase inhibitors. Preliminary results exhibited partial responses in 10 (38%) and 9 (33%) patients from Arm A and B, respectively. Progression-Free Survival for patients who received D-T was 11.4 mo, with a median follow-up of 13 mo. The treatment-related adverse events were similar to previously reported trials[56].

Dabrafenib and vemurafenib in RAIR-DTC

Dabrafenib and vemurafenib have been approved as single agents for the treatment of advanced melanoma, but they also have been evaluated in phase 2 trials in patients with *BRAF V600E*–mutated PTC[57,58]. Both *BRAF* inhibitors are effective also in papillary carcinoma, although the outcomes have not been as robust as for ATC, so neither are currently approved for this use. In general terms, objective responses were seen for up to half of patients treated with either vemurafenib or dabrafenib in different trials and clinical experiences[57-59]. Among them, a randomized, multi-institutional, open-label phase 2 trial was conducted over two arms of patients with *BRAF V600E*–mutated PTC[57]. Arm A employed dabrafenib as a single agent and arm B, the combination of dabrafenib with trametinib. Partial responses were reached in 10 of 26 patients (38%) from arm A, and 9 of 17 (33%) from arm B, with median PFS of 11.4 and 15.1 mo, respectively. Common adverse events included fever, diarrhea, anemia, fatigue, nausea, alopecia and skin reactions[57]. Meanwhile, a non-randomized, open-label, multicenter phase 2 vemurafenib trial was conducted in two cohorts of patients with *BRAF V600E*–mutated PTC. Cohort 1 was comprised of 26 patients who had never received multikinase VEGFR inhibitors, in which the best overall response (partial response) was reached in 12 patients (38%), with a median duration of PFS of 18.8 mo (14.2–26), and the median OS had yet to be reached. In cohort 2 were included 25 patients who previously received MKIs treatment. Partial response rates were seen in 27.3%, with a median PFS of 8.9 mo. The most common adverse events reported were rash, fatigue, weight loss, dysgeusia, and alopecia. Serious adverse events were seen in 62% and 68% of the patients in cohort 1 and 2, respectively, including benign and malignant skin lesions and cerebrovascular accidents, among others[58].

Table 4 Some relevant ongoing clinical trials for the treatment of advanced radioiodine refractory-differentiated thyroid carcinoma (thru March 11, 2021, from clinicaltrials.gov)

NCT number	Title	Status	Interventions	Characteristics	Population	Dates	Locations
NCT04554680	Clinical Trial in RAI-Refractory Thyroid Carcinoma Evaluating BRAF & MEK Blockade for Redifferentiation Therapy	Recruiting	Drug: Dabrafenib and trametinib	Study type: Interventional Phase: Phase 2 Study design: Allocation: N/A; Intervention model: Single group assignment; Masking: None (open label); Primary purpose: Treatment outcome; Measures: The proportion of participants attaining at least one tumor lesion with lesional dosimetry of ≥ 2000 cGy with I-131 dose of =	Enrollment: *n* = 5 Age: 21-99 yr Sex: All	Study start: December 30, 2020 Study completion: April 2022	National University Hospital, Singapore, Singapore
NCT01709292	Vemurafenib Neoadjuvant Trial in Locally Advanced Thyroid Cancer	Active, not recruiting	Drug: Vemurafenib (all groups) Drug: Vemurafenib (Post Surgery) - Group A + C Other: Post Surgery - Group B	Study type: Interventional Phase: Phase 2 Study design: Allocation: NonRandomized intervention; Model: Parallel assignment; Masking: None (open label); Primary purpose: Treatment outcome; Measures: Percent change in ERK (extracellular-signal regulated kinase) phosphorylation and tumor size, objective response rate	Enrollment: *n* = 24 Age: 18 yr and older Sex: All	Study start: November 7, 2012 Study completion: November 30, 2020	University of Texas MD Anderson Cancer Center, Houston, Texas, United States
NCT03167385	Phase 2 Trial of Apatinib Mesylate in Locally Advanced/ Metastatic Differentiated Thyroid Carcinoma	Unknown	Drug: Apatinib mesylate	Study type: Interventional Phase: Phase 2 Study design: Allocation: N/A; Intervention model: Single group; assignment; Masking: None (open label); Primary purpose: Treatment outcome; Measures: Disease control rate, progression free survival, overall survival, objective response rate	Enrollment: *n* = 20 Age: 18 to 75 yr Sex: All	Study start: March 22, 2017 Study completion: December 31, 2020	Tianjin Medical University Cancer Institute and Hospital, Tianjin, Tianjin, China
NCT03753919	Durvalumab Plus Tremelimumab for the Treatment of Patients With Progressive, Refractory Advanced Thyroid Carcinoma - The DUTHY Trial	Recruiting	Drug: Durvalumab Drug: Tremelimumab	Study type: Interventional Phase: Phase 2 study Design: Allocation: N/A; Intervention model: Single group assignment; Masking: None (open label); Primary purpose: Treatment outcome; Measures: Progression-free survival rate at 6 mo, overall survival rate at 6 mo, overall response rate,	Enrollment: 46 Age: 18 yr and older Sex: All	Study start: April 2 Study completion: July 2021	Instituto Catalán de Oncología de Hospitalet, L'Hospitalet de Llobregat, Barcelona, Spain; Hospital Provincial de Castellón, Castelló, Valencia, Spain; Hospital Clínic Barcelona, Barcelona, Spain; Hospital Universitari Vall d'Hebron, Barcelona, Spain; MD Anderson Cancer Center, Madrid, Spain; Hospital Clínico San Carlos, Madrid, Spain; Hospital Universitario 12 de

				duration of response, median progression-free survival, incidence of treatment, emergent adverse events (safety and tolerability), median overall survival, response status after start of study treatment			Octubre, Madrid, Spain; Hospital Universitario HM Sanchinarro, Madrid, Spain; Hospital Universitario La Paz, Madrid, Spain; Hospital Universitario Ramón y Cajal, Madrid, Spain; and 5 more
NCT00537095	Efficacy and Safety of Vandetanib (ZD6474) in Patients With Metastatic Papillary or Follicular Thyroid Cancer	Active, not recruiting	Drug: Vandetanib Other: Placebo	Study type: Interventional Phase: Phase 2 Study design: Allocation: Randomized; Intervention model: Parallel assignment; Masking: Double (participant, investigator); Primary purpose: Treatment outcome; Measures: Time to tumor progression, disease control rate at 6 mo, objective response rate, time to death	Enrollment: *n* = 165 Age: 18 yr and older Sex: All	Study start: September 29, 2007 Study completion: December 2021	Research Site, Brussels, Belgium; Research Site, Odense, Denmark; Research Site, Angers Cedex 9, France Research Site, Angers Cedex, France; Research Site, Bordeaux Cedex, France; Research Site, Caen Cedex 5, France; Research Site, Caen Cedex, France; Research Site, Lyon Cedex, France; Research Site, Lyon, France; Research Site, Marseille Cedex 9, France; and 12 more
NCT03602495	Donafenib in 131I-Refractory Differentiated Thyroid Cancer	Recruiting	Drug: Donafenib Drug: Placebo	Study type: Interventional Phase: Phase 3 Study design: Allocation: Randomized; Intervention model: Parallel assignment; Masking: Double (participant, investigator); Primary purpose: Treatment outcome; Measures: Progression-free survival, overall survival, objective response rate, disease control rate, time to disease progression	Enrollment: *n* = 204 Age: 18 yr and older Sex: All	Study start: August 29, 2018 Study completion: December 2021	Peking Union Medical College Hospital, Beijing, Beijing, China

Selective mTOR inhibitors (everolimus, temsirolimus)

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that exerts as an essential regulator of cell growth-related processes[60]. Everolimus and temsirolimus are two *mTOR* inhibitors that demonstrated clinical benefits in other cancers like advanced renal carcinoma, metastatic breast cancer, and pancreatic neuroendocrine tumors, in which they were approved by the FDA[61]. Since the mTOR pathway is over-activated in thyroid cancer, some studies have tested these agents' effects on advanced thyroid cancer, with promising outcomes[62,63].

Everolimus was evaluated in a single-arm, multicentric phase II study that included 31 patients with aggressive RAIR-DTC, among other thyroid tumor histologies. There was one PR (3%) but 27 patients (82%) had SD, for a clinical benefit rate of 84.8% and a median PFS for 12.9 mo. Median OS was not reached and 2-year OS was 73.5%[62]. For its part, a phase 2 study that enrolled 36 patients with metastatic RAIR-DTC evaluated the efficacy of the combination of oral sorafenib (200 mg twice daily) and intravenous temsirolimus (25 mg weekly)[63]. A partial response was reached in 8 patients (22%), while stable and progression disease was seen in 21 (58%) and 1 (2%) patients, respectively. The mPFS at one year was 30.5% and the most common toxicities included hyperglycemia, fatigue, anemia, and oral mucositis. The authors concluded that this combination appears to have better response rates in patients with RAI-refractory thyroid cancer who received no prior treatment, regardless of whether *RAS* or *RAF* mutation was present[63].

Redifferentiation agents

It has been well described that activating *BRAF* mutations induce loss of differentiated

features required for response to radioiodine treatment, while its blockade would restore radioiodine uptake in experimental models[64]. In patients with radioiodine-refractory differentiated carcinoma with somatic *BRAF* or *RAS* mutations, treatment with the specific targeted inhibitors may restore radioiodine responsiveness in up to two-thirds of patients, permitting iodine therapeutic administration leading to tumor shrinkage in up to one-third[57-59]. On the other hand, constitutive activation of MAPK pathway causes inhibition of a variety of thyroid genes, including NIS, leading to the investigation of selective MAPK blocking agents as Selumetinib, as redifferentiation agent[64,65].

Selumetinib

Selumetinib is a *MEK1–2*, *RAS* and *BRAF V600E* inhibitor which efficacy was evaluated in 32 RAIR-DTC patients enrolled in a multicenter, open-label, phase II trial [66]. There were 1 partial response (3%), 21 stable disease (54%), and 11 progressive diseases (28%). Median PFS was 32 wk, and it was seen that *BRAF V600E* mutants had a longer median PFS compared with patients with BRAF wild-type cancer (33 *vs* 11 wk, respectively). This suggest a potential beneficence of Selumetinib based on underlying genetic disorders. The most common adverse events included rash, fatigue, diarrhea, and peripheral edema[66]. A phase III trial is currently in progress which continues to explore selumetinib's redifferentiation benefits in a larger number of participants[66] (Table 2).

Immunotherapy

In recent years, there has been significant progress in the field of oncological immunotherapy. Several immunotherapeutic agents have now been approved by the FDA for the treatment of a variety of malignancies, including melanoma, non-small cell lung cancer, renal and breast carcinomas, among others[67]. In this line, several phase I studies research the use of immunotherapy in the treatment of advanced differentiated thyroid cancer focuses on restoring immune surveillance[68]. The recent identification of blocking antibodies of CTLA-4 and PD-1 to their corresponding ligands (CD80/86 and PD-L1/PD-L2 respectively) enhances the effector T cells and inhibits the regulatory suppressor cells. Thus, the evidence of PD-1 (+) T cell in thyroid tumors involved lymph nodes in PTC patients suggests the potential utility of immune checkpoint inhibitors like pembrolizumab (as a single agent or in combination with MKIs) for advanced thyroid cancers[68]. Only a few immunotherapy trials in patients with thyroid cancer have been published to date, but several trials are ongoing.

Pembrolizumab

Pembrolizumab is an anti–PD-1 monoclonal antibody that exhibits antitumor activity by blocking interaction between PD-1 and its ligands[68]. Patients with advanced thyroid cancer were enrolled in the nonrandomized, phase Ib KEYNOTE-028 trial conducted to evaluate its safety and antitumor activity in 22 patients with advanced papillary or follicular thyroid cancer. Pembrolizumab 10 mg/kg was administered every 2 wk up to 24 mo or until confirmed progression or intolerable toxicity. SD was achieved by 57% (4/7) of patients with follicular histology and 60% (9/15) of patients with papillary histology and two patients reached partial response for 8 and 20 mo. Median PFS was 7 mo and median overall survival was not reached. Diarrhea and fatigue were the most common adverse events[69]. This study suggests that pembrolizumab may be effective and have a favorable safety profile in PD-L1–positive thyroid cancer, providing a baseline for future research[69].

Other ongoing single-arm multicenter phase II study combine lenvatinib and pembrolizumab in patients with RAIR-DTC[70]. Patients were excluded if they had received previous VEGFR-directed multikinase therapy. The lenvatinib starting dose was 20 mg/d orally and pembrolizumab was 200 mg IV every 3 wk. The preliminary results showed that out of 29 evaluable patients, 18 (62%) had a partial response, 10 (35%) had stable disease and the clinical benefit rate was 97%. The PFS at 12 mo was 74%, and median PFS was not yet reached. The most common adverse events were hypertension (47%), weight loss (13%), maculopapular rash (13%), leukopenia (7%), diarrhea (7%), and oral mucositis (7%)[70]. While the results are promising, the continuation of this study will help determine the magnitude of the responses.

CONCLUSION

In conclusion, therapeutic options for patients with advanced radioiodine-refractory differentiated thyroid carcinoma have been increasingly evolving and fine-tuned. While the introduction of new therapies for multiple molecular targets has made it possible to extend progression-free survival, their impact on overall survival is still unclear. Based on the improving knowledge of the underlying molecular mechanisms in these patients, novel agents under study bring us a new scope for the near future. Thus, increasingly tailored therapy focused on critical molecular pathways will be offered, allowing to overcome drug evasion mechanisms, enhance efficacy, minimize adverse events, and finally achieve an overall survival improvement in these patients.

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MINIREVIEWS

Immunotherapy: A new standard in the treatment of metastatic clear cell renal cell carcinoma

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Abstract

Renal cell cancer (RCC) represents 2%-3% of all adulthood cancers and is the most common malignant neoplasm of the kidney (90%). In the mid-nineties of the last century, the standard of treatment for patients with metastatic RCC was cytokines. Sunititib and pazopanib were registered in 2007 and 2009, respectively, and have since been the standard first-line treatment for metastatic clear cell RCC (mccRCC). Renal cell cancer is a highly immunogenic tumor with tumor infiltrating cells, including CD8+ T lymphocytes, dendritic cells, natural killer cells (NK) and macrophages. This observation led to the design of new clinical trials in which patients were treated with immunotherapy. With the growing evidence that proangiogenic factors can have immunomodulatory effects on the host's immune system, the idea of combining angiogenic drugs with immunotherapy has emerged, and new clinical trials have been designed. In the last few years, several therapeutic options have been approved [immunotherapy and immunotherapy/tyrosine kinase inhibitors (TKI)] for the first-line treatment of mccRCC. Nivolumab/ipilimumab is approved for the treatment of patients with intermediate and poor prognoses. Several checkpoint inhibitors (pembrolizumab, nivolumab, avelumab) in combination with TKI (axitinib, lenvatinib, cabozantinib) are approved for the treatment of patients regardless of their International mRCC Database Consortium prognostic group and PD-L1 expression. There is no specific and ideal biomarker that could help in selecting the ideal patient for the appropriate first-line treatment.

Key Words: Renal cell carcinoma; Immunotherapy; Checkpoint inhibitors; Biomarkers; Tumor microenvironment; Programmed cell death 1 receptor

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Core Tip: Renal cell cancer is a highly immunogenic tumor infiltrated by cells, including CD8+ T lymphocytes, dendritic cells, natural killer cells and macrophages. This observation led to the design of new clinical trials in which patients were treated with immunotherapy. With the growing evidence that proangiogenic factors can have immunomodulatory effects on the host's immune system, the idea of combining angiogenic drugs with immunotherapy has emerged, and new clinical trials have been designed. In the last few years, several therapeutic options have been approved (immunotherapy and immunotherapy/tyrosine kinase inhibitors) as first-line treatment for metastatic clear cell renal cell cancer.

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INTRODUCTION

Renal cell cancer (RCC) represents 2%-3% of all adulthood cancers and is the most common malignant neoplasm of the kidney (90%)[1]. Clear cell cancer (75%) is the most prevalent histological subtype of RCC, followed by papillary (10%), chromofobe (5%), collecting ducts (0.4%-1.8%) and unclassified (4%-6%)[2]. RCC typically occurs in the fifth and sixth decade of life and is twice as frequent in men than in women[3]. At the time of diagnosis, one-third of all patients have metastatic disease, while a quarter of all patients, with initially localized disease, relapse after nephrectomy[4]. According to two prognostic models, Memorial Sloan Kettering Cancer Center (MSKCC) and International mRCC Database Consortium (IMDC), metastatic RCC (mRCC) patients can be divided into 3 prognostic categories: favorable, intermediate and poor risk[5,6] (Table 1).

In the mid-nineties of the last century, the standard of treatment for patients with metastatic RCC was cytokines, typically interferon-alpha and interleukin 2. Beside the high toxicity profile of cytokines, patients who were treated achieved an objective response rate (ORR) of 10-20%, while the median overall survival (OS) was 11-14 mo [7-9].

Renal clear cell carcinoma is commonly associated with Von Hippel-Lindau (VHL) gene mutations (70% of patients) located on chromosome 3p and mediates cell apoptosis in response to hypoxia[10,11]. If this mutation is present, apoptosis does not occur, hypoxia-induced factor (HIF) accumulates and activates vascular endothelial growth factor (VEGF), and platelet growth factor (PDGF) and others engage in the angiogenesis process, which is one of the key promoters of cell growth in RCC[12]. This knowledge leads to the development of new antiangiogenetic drugs. Other mutations, such as PBRM1 (40%), SETD28 (15%) and BAP1 (15%), have recently been discovered. Sunititib and pazopanib were registered in 2007 and 2009, respectively and have been the standard first-line treatment for mRCC ever since. The median survival of patients treated with these drugs is 24-29 mo, while the objective response rate (ORR) is 30%-33%[13,14].

RCC is a highly immunogenic tumor with infiltrating cells, including CD8+ T lymphocytes, dendritic cells, natural killer cells (NK) and macrophages. This observation led to the design of new clinical trials in which patients were treated with immunotherapy[15]. Checkpoint inhibitors are monoclonal antibodies targeting the link between programmed cell death protein 1 (PD-1) and its ligands PD-L1 and PD-L2[16]. The PD-1 receptor is located on T cells, while PD-L1 and PD-L2 are present on other immune cells. The ligand can be found on both tumor cells and immune infiltrate cells, allowing them to bind to the PD-1 receptor of T-cells and escape the host immune response[17,18]. Checkpoint inhibitors block this interaction and permit the host's immune response to the tumor[16].

Nivolumab is humanized PD-1 monoclonal antibody. The first data on nivolumab in mRCC were the results of the phase I Checkmate 033 trial, where nivolumab was

Table 1 Poor prognostic factor

Poor prognostic factor	MSKCC	IMDC
Time from diagnosis to treatment	< 12 mo	< 12 mo
Hemoglobin	< lower limit of normal	< lower limit of normal
Corrected serum calcium	> 10 mg/dl (2.5 mmol/L)	> upper limit of normal
Karnofsky performance score	< 80%	< 80%
Neutrophil count	/	> upper limit of normal
Platelet count	/	> upper limit of normal
Lactate dehydrogenase	> 1.5 x upper limit of normal	/
Good risk	0 risk factor	0 risk factor
Intermediate risk	1 or 2 risk factors	1 or 2 risk factors
Poor risk	3, 4 or 5 risk factors	3, 4, 5 or 6 risk factors

MSKCC: Memorial Sloan Kettering Cancer Center; IMDC: International mRCC Database Consortium.

investigated in pretreated patients. The objective response rate was 24%; after a median follow-up of 63.9 mo, the ORR was 29%, the median duration of response (DOR) was 12.9 mo, and the median OS was 22.4 mo[19]. In the phase 2 trial, nivolumab was again investigated in pretreated mRCC patients. Patients received 0.3 mg/kg, 2 mg/kg or 10 mg/kg nivolumab. There was no difference in PFS in these subgroups. At 3 years, ORR was 21% while OS was 41%[20]. The phase 3 trial, CheckMate 025, investigated nivolumab in comparison to everolimus in pretreated patients. The primary endpoint was OS, while the secondary endpoints were response rates and safety profile. The median OS in patients treated with nivolumab was 25 mo, compared to 1.6 mo with everolimus (HR 0.73). Differences in OS were recorded across all subgroups of patients regardless of PD-L1 expression. The objective response rate was 25% in the nivolumab cohort and 5% in the everolimus cohort. There was no significant difference in PFS of 4.6 *vs* 4.4 mo for nivolumab and everolimus, respectively. Grade 3 and 4 adverse events were reported in 19% of patients in the nivolumab group and 37% of patients in the everolimus group[21]. The results of this trial led to FDA approval of nivolumab as a second-line treatment of mccRCC.

In April 2018, nivolumab and ipilimumab combination therapy was approved by the FDA for the first-line treatment of intermediate- and poor-risk mRCC patients. This approval was a result of the phase 3 trial, CheckMate 214, which compared nivolumab and ipilimumab *vs* sunitinib in treatment-naïve patients. The trial included 1096 patients, 847 of whom were intermediate- and poor-risk IMDC risk groups. Patients were randomized 1:1. The primary endpoints were OS, PFS and ORR in intermediate- and poor-risk patients, while the secondary endpoints were OS, PFS and ORR in the intended-to-treat (ITT) population. Intermediate- and poor-risk patients in the nivolumab/ipilimumab group had significantly longer PFS than those in the sunitinib group. The favorable-risk prognostic group had longer PFS when treated with sunitinib. Patients with PD-L1 expression > 1% had significantly longer PFS when treated with nivolumab/ipilimumab *vs* sunitinib, while the treatment groups did not differ in patients with PD-L1 < 1%. Nivolumab/ipilimumab significantly prolonged patient OS compared to sunitinib. There were 46% grade 3-4 adverse events in the nivolumab/ipilimumab group *vs* 63% in the sunitinib group[22]. After 48 mo of follow-up, patients in the intermediate- and poor-risk groups treated with nivolumab/ipilimumab achieved significantly longer overall survival[23].

Nivolumab also proved efficacious in patients with brain metastasis: the ORR was 12%, and the PFS was 2.7 mo[24]. When nivolumab was combined with ipilimumab, the ORR and PFS were 29% and 9 mo, respectively[25].

Angiogenesis is one of the key initiators of disease in RCC, which itself is an immunogenic tumor. In patients with VHL gene mutations, instead of apoptosis, HIF accumulates and activates VEGF and PDGF, which mediate the activation of the angiogenesis process[10-12]. It has been shown that accumulation of VEGF leads to suppression of the host's immune response. It also interferes with monocyte differentiation into mature dendritic cells that are essential for the activation of the host's immune system. VEGF increases the number of myeloid suppressing cells present in

the tumor infiltrates that disable the activity of tumor infiltrating lymphocytes, the expression of PD-L1 in dendritic cells, as well as PD-1 and CTLA-4 on immune cells. It inhibits the differentiation of progenitor cells into CD4+ and CD8+ cells. Proangiogenic factors also modify the expression of proteins on endothelial cells, blocking the infiltration of the tumor by immune cells[26,27]. With the growing evidence that proangiogenic factors can have immunomodulatory effects on the host's immune system, the idea of combining angiogenic drugs with immunotherapy emerged, and new clinical trials have been designed[28].

Atezolizumab is a humanized monoclonal PD-L1 antibody investigated in combination with bevacizumab *vs* sunitinib. After the phase I study reported a 40% ORR , a phase II study was conducted (atezolizumab or atezolizumab/bevacizumab or sunitinib), and ORRs of 32%, 25% and 29%, respectively, were observed. In the ITT population, the PFS difference was not significant, while in the PD-L1-positive patients, a significant difference was noticed in the cohort treated with atezolizumab/bevacizumab *vs* sunitinib. PFS was not significant when atezolizumab alone was compared with sunitinib in PD-L1-positive patients[29]. The phase 3 trial, IMmotion 151, followed these results and compared atezolizumab/bevacizumab *vs* sunitinib in treatment-naïve patients. Patients were randomized 1:1 according to the MSKCC score, PD-L1 expression (< 1% *vs* > 1%), and presence of liver metastases. Patients with sarcomatoid tumor features were also included. The co-primary endpoints were PFS in the PD-L1-positive population and OS in the ITT population. Secondary endpoints were PFS, ORR and duration of response in the ITT population. In the PD-L1-positive patients, PFS was 11.2 mo (atezolizumab/bevacizumab) in comparison to 7.7 mo (sunitinib), HR 0.74. In the ITT population, PFS was 11.2 mo (atezolizumab/bevacizumab) *vs* 8.4 mo (sunitinib), HR 0.83. The ORR in the PD-L1+ population was 43% (atezolizumab/bevacizumab) *vs* 35% (sunitinib), while the ORR in the ITT population was 37% *vs* 33% (atezolizumab/bevacizumab *vs* sunitinib)[30]. After 24 mo of follow-up, there were no differences in survival (HR 0.93) in the ITT population[31]. Considering the results of IMmotion 150 and 151, data subanalysis was performed according to the molecular profile of tumor tissue. IMmotion 150 patients were classified into angio-high, T effector-high and myeloid-high. The subanalysis showed that angio-high patients had a higher benefit from TKIs and were in the favorable prognostic group, while T effector-high patients had a greater benefit from immunotherapy and were in the intermediate and poor prognostic groups. It was also observed that patients with BAP1 mutations had a worse prognosis and shorter PFS when treated with sunitinib, while patients with PBRM1 mutations had a worse prognosis and shorter PFS when treated with immunotherapy. IMmotion 151 included patients with sarcomatoid features, who generally had a worse prognosis. The results of this subanalysis showed that half of these patients were T effector-high, had higher PD-L1 expression and achieved the highest benefit from immunotherapy[29,32].

Cosmic-021 was a phase 1b trial that investigated the efficacy of atezolizumab in combination with cabozantinib in different solid tumors. One of the cohorts consisted of mccRCC patients. Seventy patients were included in the study: 34 patients were treated with cabozantinib 40 mg, and 36 patients were treated with cabozantinib 60 mg and 1200 mg atezolizumab. Most of the patients were in the intermediate prognostic group. After a median follow-up of 11.5 mo (cabozantinib 60 mg) *vs* 22 mo for cabozantinib 40 mg, the ORR in the cabozantinib 60 mg group was 58% *vs* 47% in the cabozantinib 40 mg group. The median PFS was 19.5 mo (cabozantinib 40 mg) and 20.4 mo (cabozantinib 60 mg). Two years PFS was 67% (cabozantinib 40 mg) and 71% (cabozantinib 60 mg). Treatment-related grade 3-4 adverse events were reported in 71% (cabozantinib 40 mg) and 67% (cabozantinib 60 mg) of the patients. The most common adverse events were hypertension, hypophosphatemia, diarrhea and elevation of liver enzymes[33].

The Contact-03 trial investigating atezolizumab in combination with cabozantinib in patients with mRCC who have progressed on previous immunotherapy is underway [34].

Pembrolizumab is a humanized monoclonal PD-L1 antibody studied in combination with axitinib in a phase 1b trial. The response rate was 73%[35]. In the randomized phase 3 clinical trial (Keynote-426), pembrolizumab/axitinib was compared to sunitinib. Patients were randomized 1:1. The primary endpoints were OS and PFS in the ITT population, while the secondary endpoint was ORR. After 12.8 mo of follow-up, the one-year OS was 89.9% (pembrolizumab/axitinib) *vs* 78.3% (sunitinib), HR 0.53, $P < 0.0001$. PFS was 15.1 mo (pembrolizumab/axitinib) *vs* 11.1 mo (sunitinib), HR 0.69, $P < 0.0001$. The ORR was 59.3% and 37.5% in the pembrolizumab/axitinib *vs* sunitinib group, respectively. Treatment-related grade 3 adverse events accounted for 75.85% of the patients in the combination cohort. Benefit was observed across all

subgroups analyzed regardless of the IMDC risk score or PD-L1 expression[36]. At 27 mo, PFS and OS were significantly longer in all subgroups of patients[37]. Pembrolizumab was investigated in combination with levantinib in a phase 2 trial (Keynote 146) in patients with mccRCC who were previously treated with immunotherapy. The primary endpoint of the trial was an ORR of 51%, a median PFS of 11.7 mo, and a median DOR of 9.9 mo[38]. The phase 3 trial, CLEAR/Keynote 581, investigated pembrolizumab/Lenvatinib *vs* everolimus/Lenvatinib *vs* sunitinib in patients with mccRCC. The primary endpoint was PFS, while the secondary endpoints were ORR and OS in the ITT population. All three prognostic MSKCC and IMDC risk score groups were included in the trial. After 26.6 mo of follow-up, PFS in the group of patients treated with pembrolizumab/Lenvatinib *vs* those treated with sunitinib was 23.9 *vs* 9.2 mo (HR 0.39, $P < 0.0001$). In patients treated with everolimus/Lenvatinib *vs* sunitinib, PFS was 14.7 and 9.2 mo, respectively, HR 0.65, $P < 0.0001$. Median overall survival was not reached; however, OS was longer with pembrolizumab/Lenvatinib than with sunitinib, HR 0.66, $P = 0.005$. There was no significant OS difference in patients treated with everolimus/Lenvatinib and patients treated with sunitinib, HR 1.15, $P = 0.30$. The objective response rate in the pembrolizumab/Lenvatinib cohort *vs* the everolimus/Lenvatinib *vs* sunitinib cohort was 71%, 53.5%, and 36.1%, respectively. Median DOR in the pembrolizumab/Lenvatinib cohort *vs* everolimus/Lenvatinib *vs* sunitinib was 25.8, 16.6 and 14.6 mo, respectively. All subgroups of patients had a benefit in PFS when treated with pembrolizumab/Lenvatinib. Grade 3 or higher toxicity was observed in 82.4% *vs* 83.1% and 71.8% of the patients treated with pembrolizumab/Lenvatinib, everolimus/Lenvatinib and sunitinib, respectively. The most common grade 3 toxicities were diarrhea, hypertension, and elevated lipase and triglyceride levels[39].

Avelumab is a humanized PD-L1 monoclonal antibody. It was investigated in a phase 1b trial in combination with axitinib in treatment-naïve patients with mccRCC. The objective response rate was 58%[40]. The phase 3 trial, JAVELIN Renal 101, compared avelumab/axitinib with sunitinib in patients who were not previously treated. The co-primary endpoints were PFS and OS in PD-L1-positive patients, while the secondary endpoint was PFS in the ITT population. In PD-L1-positive patients, PFS was 13.8 mo for avelumab/axitinib in comparison to 7.2 mo for patients treated with sunitinib, HR 0.61, $P < 0.0001$. In the ITT population, PFS was 13.8 mo for avelumab/axitinib in comparison to 8.4 mo for patients treated with sunitinib, HR 0.69, $P < 0.0001$. In the PD-L1-positive population, the ORR was 55.2% in the avelimab/axitinib group and 25.5% in the sunitinib group. Adverse grade 3 or higher events were reported in 71.2% of patients treated with avelumab/axitinib and 71.5% of patients treated with sunitinib[41]. At 13 mo PFS was significantly longer for the patients treated with avelumab/axitinib *vs* sunitinib in both PD-L1 positive (HR 0.62, $P < 0.0001$, 13.8 *vs* 7 mo) and ITT populations (HR 0.69, $P < 0.0001$, 13.3 *vs* 8 mo). Data for OS are still pending[42]. In May 2019, this combination was approved for the first-line treatment of mccRCC patients, regardless of the IMDC score prognostic subgroup.

In January 2021, nivolumab in combination with cabozantinib was approved by the FDA for the first-line treatment of patients with mRCC based on the results of the CheckMate 9ER trial. The trial included treatment-naïve patients, regardless of the PD-L1 expression or IMDC prognostic score. Patients were randomized into two cohorts: nivolumab/cabozantinib and sunitinib. The primary endpoint was PFS, and the secondary endpoints were OS and ORR. At 18.1 mo, PFS and OS were both significantly longer in the nivolumab/cabozantinib *vs* the sunitinib cohort in all patient subgroups analyzed[43] in Table 2.

Most of the trials that examined the efficacy of immunotherapy or immunotherapy/TKI combinations did not include mnccRCC. Some of the retrospective trials with immunotherapy reported ORRs of 9-20%. The greatest benefit occurred in patients with the papillary histology subtype[44,45]. In the phase 2 trial, Keynote 427, pembrolizumab was investigated in previously untreated mnccRCC patients. Most of the patients had papillary subtype (72%). In the ITT population, the ORR was 24.8%, while the ORRs of papillary, chromofobe and nonclassified subtypes patients were 25.4%, 9.5% and 34.6%, respectively. The twelve-month PFS and OS were 22.8% and 72%. After 11 mo of follow-up, the median DOR was not reached in either subgroup of patients[46].

Nivolumab was investigated in the phase 3b/4 trial, Checkmate 374, which included treatment-naïve patients as well as patients previously treated with a maximum of 3 Lines of therapy. Most of the patients (66%) were treatment naïve. After 11 mo of follow-up, the median OS was 16.3 mo, with no difference in OS between patients regardless of PD-L1 expression. The median PFS was 2.2 mo. At one year, PFS was 14%. The median DOR was 10.2 mo, and ORR was 13.6%[47]. The Cosmic-021

Table 2 Results of phase 3 studies in first line treatment of patients with metastatic clear cell renal cell cancer

Drug/Study	No. of patients	Follow-up (mo)	PFS (mo)	OS (mo)	ORR, %	Ref.
Nivolumab/Ipilimumab *vs* Sunitinib (Checkmate 214)	1096	48	ITT 12.2 *vs* 12.3; HR 0.89; I/P risk 11.2 *vs* 8.3; HR 0.74	ITT NR *vs* 38.4; HR 0.69; I/P risk 48.1 *vs* 26.6; HR 0.65; F risk; HR 0.93	ITT 39.1 *vs* 32.4; I/P risk 41.9 *vs* 26.8; F risk 29.6 *vs* 51.6	[24]
Pembrolizumab/Axitinib *vs* Sunitinib (Keynote 426)	861	27	ITT 15.4 *vs* 11.1; HR 0.71; $P < 0.0001$	ITT NR *vs* 35.7; HR 0.68; $P = 0.0003$	ITT 60 *vs* 40	[39]
Avelumab/Axitinib *vs* Sunitinib (Javelin 101)	560	13	ITT 13.3 *vs* 8; HR 0.69; $P < 0.0001$; PD-L1 + 13.8 *vs* 7; HR 0.62; $P < 0.0001$	ITT NR; HR 0.80; $P = 0.0392$; PD-L1 + NR; HR 0.83; $P = 0.1301$	ITT 52.5 *vs* 27.3; PD-L1 + 55.9 *vs* 27.3	[44]
Nivolumab/Cabozantinib *vs* Sunitinib (Checkmate 9ER)	651	18.1	ITT 16.6 *vs* 8.3; HR 0.51; $P < 0.0001$	ITT NR *vs* NR; HR 0.60; $P = 0.0010$	ITT 55.7 *vs* 27.1	[45]
Pembrolizumab/Lenvatinib *vs* Everolimus/Lenvatinib *vs* Sunitinib (Clear/Keynote 581)	1069	26.6	ITT Pembro/lenva *vs* sunitinib 23.9 *vs* 9.2; HR 0.39; $P < 0.000$; Everolimus/lenva *vs* sunitinib; 14.7 *vs* 9.2; HR 0.65; $P < 0.0001$	ITT Pembro/lenva *vs* sunitinib NR *vs* NR; HR 0.66; $P = 0.005$; Evero/lenva *vs* sunitinib NR *vs* NR; HR 1.15; $P = 0.30$	ITT Pembro/lenva *vs* Evero/lenva *vs* sunitinib; 71% *vs* 53.5% *vs* 36.1%	[41]

phase 1b trial analyzed the efficacy of atezolizumab in various solid tumors. One of the cohorts was patients with mnccRCC. These patients were treated with cabozantinib 40 mg and 1200 mg of atezolizumab.

According to the IMCD, all three prognostic subgroups were included in the trial, and most of them were in the intermediate prognostic group. After a median follow-up of 9.2 mo, the ORR was 33%, with no difference between subgroups. The median DOR was 7.9 mo. Grade 3-44 adverse events were reported in 30% of the patients, and a low phosphorus level was the most common adverse event[48]. The Calypso trial, phase 1b/2, examined the combination of durvalumab and savolitinib in patients with papillary mnccRCC previously treated, as well as treatment naïve. The primary endpoint was ORR, while the secondary endpoints were PFS, OS and safety. The trial included all IMDC score prognostic groups. Most of the patients (63%) were in the intermediate prognostic group. Median follow up was 8.9 mo. In the ITT population, the ORR was 27%, while the median PFS was 3.3 mo. In the subgroup of patients who were treatment naïve, the ORR was 27%, and the median PFS was 12.2 mo. Fifteen out of 42 patients included had grade 3-4 toxicities[49] (Table 3).

Predictive biomarkers

Is there a biomarker that can predict the response to either immunotherapy or tyrosine kinase inhibitors (TKIs)? One of the essential promoters of cell growth in RCC is angiogenesis. Patients in the favorable prognostic group had abundant tumor infiltrates with blood vessels. However, RCC is also an immunogenic tumor with inflammatory tumor infiltrates and is a characteristic in patients with intermediate and poor prognoses. The Bionikk trial assessed the response to immunotherapy and TKI therapy relative to the molecular tumor profile (35 genes). Patients were classified into four subgroups: group 1 (immune-low), group 2 (angio-high), group 3 (normal-like), and group 4 (immune-high). They were randomized so that groups 1 and 4 were treated with either nivolumab or nivolumab/ipilimumab, while patients in groups 2 and 3 received either sunitinib or nivolumab/ipilimumab. Primary endpoint was ORR. In group 1, the ORR was 33.3% and 20.7% for patients treated with nivolumab/ipilimumab or nivolumab, respectively. There was no difference in ORR between patients in group 4 who were treated with nivolumab *vs* nivolumab/ipilimumab 42.9% *vs* 41.2%. In group 2, the ORR was 58.3% *vs* 34.5% in patients treated with sunitinib *vs* nivolumab/ipilimumab. A very small number of patients were included in group 3, and responses were only achieved in patients treated with the nivolumab/ipilimumab combination[50]. PD-L1 is the most commonly analyzed biomarker that predicts the response to immunotherapy. Several trials pointed out that high expression of PD-L1 in patients with RCC is a predictor of poor prognosis[21,23]. Checkmate 025 reported that nivolumab is superior to everolimus in previously treated patients. Higher expression of PD-L1 was related to worse prognosis regardless of whether patients were treated with nivolumab or everolimus. The median OS was longer in PD-L1-negative patients regardless of the treatment[21].

Table 3 Results of checkpoint inhibitors in treatment of patients with metastatic clear cell renal cell cancer

Drug/Study	Phase	Indication	Follow-up	Results	Ref.
Pembrolizumab (Keynote 427)	II	mnccRR	11	ITT ORR 24.8%; ORR Papillary *vs* phromophobe *vs* unclasified 25.4% *vs* 9.5% *vs* 34.6%; 12 mo PFS 22.8%; 12 mo OS 72%	[50]
Nivolumab (Checkmate 374)	IIIb/IV	mnccRR	11	ITT; OS 16,3 mo; PFS 2,2 mo; ORR 13,6%	[51]
Atezolizumab/Cabozantinib (Cosmic 021)	Ib	mnccRR	9,2	ITT ORR 33%	[52]
Durvalumab/Savolitinib (Calypso)	Ib/II	mnccRCC-papillaryuntreated or previously treated	8,9	ITT; ORR 27%; PFS 3,3 mo; Untreated ORR 29%; PFS 12,2	[53]

In the Checkmate 214 trial, intermediate- and poor-risk patients who were also PD-L1 positive had longer PFS when treated with nivolumab/ipilimumab *vs* sunitinib. There was no PFS difference in the PD-L1-negative population[22]. According to the JAVELIN Renal 101 and Keynote 426 trials, all subgroups of patients had significantly longer PFS regardless of the prognostic group or PD-L1 expression[37,42]. Different histology subtypes of RCC have different TMB values. The lowest TMB is found in chromofobic subtype, and the highest was found in the papillary and clear cell histology subtypes[51]. In other malignancies, such as lung cancer and melanoma, TMB is a predictor of a favorable response to treatment. Although they have relatively low TBM, patients with RCC have higher rates of response to immunotherapy[52,53]. The results of trials that analyzed the prognostic value of TBM in RCC are inconclusive [54,55]. A retrospective analysis showed that TMB values do not correlate with either survival or PD-L1 expression[56]. Subanalysis of the IMmotion 150 trial showed that TMB did not influence the response to nivolumab[31]. Tumor infiltrates in RCC consist of CD8+ T lymphocytes, dendritic cells, NK cells and macrophages[15]. Some trials have shown that if tumor infiltrates are abundant with CD8+ cells and M1 macrophages, patients have a better prognosis, while infiltrates rich in regulatory T cells and M2 macrophages predict poor prognosis[57-60]. Other trials indicated that if tumor infiltrate is abundant with CD8+, patients will have a better response to immunotherapy[61]. IMmotion 150 and IMmotion 151 confirmed these results[29,31].

To date, there are no biomarkers that can predict the response to immunotherapy. Some drugs approved for first-line treatment may benefit many patients regardless of prognostic group or PD-L1 expression[36,39,42]. Further investigations are warranted to improve the selection of patients for the best possible choice of first-line therapy.

CONCLUSION

We are witnessing the evolution of mccRCC treatment. Starting with interferon-alpha and interleukin 2 in the late twentieth century, the first TKI was administered in 2007. In the last few years, several therapeutic options have been approved (immunotherapy and immunotherapy/TKI) as first-line treatment options. Nivolumab/ipilimumab is approved for the treatment of patients with intermediate and poor prognoses. Several checkpoint inhibitors (pembrolizumab, nivolumab, avelumab) in combination with TKIs (axitinib, lenvatinib, cabozantinib) are approved for the treatment of patients regardless of their IMDC prognostic group and PD-L1 expression. There is no specific and ideal biomarker that could help select the ideal patient for the appropriate first-line treatment. If patients are symptomatic, have visceral metastasis and require prompt response, then checkpoint inhibitors/TKIs are deemed most beneficial. If the patient is asymptomatic, then other factors, such as toxicity profile, may influence the first-line treatment option.

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MINIREVIEWS

Role of radiotherapy in oligometastatic breast cancer: Review of the literature

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Abstract

Metastatic breast cancer has been historically considered as an incurable disease. Radiotherapy (RT) has been traditionally used for only palliation of the symptoms caused by metastatic lesions. However, in recent years the concept of oligometastatic disease has been introduced in Cancer Medicine as a clinical scenario with a limited number of metastases (≤ 5) and involved organs (≤ 2) with controlled primary tumor. The main hypothesis in oligometastatic disease is that locoregional treatment of primary tumor site and metastasis-directed therapies with surgery and/or RT may improve outcomes. Recent studies have shown that not all metastatic breast cancer patients have the same prognosis, and selected patients with good prognostic features as those younger than 55 years, hormone receptor-positive, limited bone or liver metastases, a low-grade tumor, good performance status, long disease-free interval (> 12 mo), and good response to systemic therapy may provide maximum benefit from definitive treatment procedures to all disease sites. While retrospective and prospective studies on locoregional treatment in oligometastatic breast cancer demonstrated conflicting results, there is an increasing trend in favor of locoregional treatment. Currently, available data also demonstrated the improvements in survival with metastasis-directed therapy in oligometastatic breast cancer. The current review will discuss the concept of oligometastases and provide up-to-date information about the role of RT in oligometastatic breast cancer.

Key Words: Breast cancer; Oligometastatic; Radiotherapy; Locoregional treatment; Ablative therapy; Metastasis-directed therapy

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Core Tip: Radiotherapy (RT) has been traditionally used for only palliation of the

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INTRODUCTION

Breast cancer is the most common cancer in females worldwide, with an estimated 276480 new cases, and the second most common cause of cancer death with an estimated 42170 deaths in 2020[1]. Metastasis at the time of diagnosis has been observed in 3%-10% of breast cancer patients and has been considered in the past unlikely to be cured[2]. However, the metastatic disease has a broad spectrum ranging from a single metastasis to widespread dissemination, and it has been observed that not all metastatic patients have the same prognosis.

The concept of oligometastases was first described by Hellman and Weichselbaum [3] in 1995, and they hypothesized that patients with oligometastases should be considered as candidates for curative therapeutic strategies. Oligometastases was described as a clinical scenario with a limited number of metastases (1 to 5) and involved organs (≤ 2) with controlled primary tumors[3]. The exact number of metastasis for the concept of oligometastases has not been clearly defined yet; however, most studies evaluating oligometastatic disease included patients with five or less metastasis[4,5].

While the standard treatment for metastatic disease includes systemic therapy with or without palliative radiotherapy (RT), recent studies are evaluating the role of ablative therapies to metastases and locoregional treatment to the primary tumor site in oligometastatic breast cancer[6-9]. In recent years, the prognosis of breast cancer has improved with the introduction of novel systemic therapies, even in patients with metastatic disease[10,11]. Some patients with good prognostic features may achieve complete response for more than 5 years after systemic therapy[12]. Several factors affect the prognosis in breast cancer patients with oligometastatic disease as the disease-free interval between primary cancer and metastasis formation, number of metastatic lesions, metastatic sites, hormone receptor status, human epidermal growth factor 2 (Her2) status, and pN stage[13-15]. Systemic therapy and local treatment to both primary and metastatic lesions may improve outcomes in such selected patients with metastatic breast cancer. Herein, we will review the impact of RT in oligometastatic breast cancer for both metastatic and primary tumor sites.

SHOULD WE PERFORM LOCOREGIONAL TREATMENT IN OLIGOMETASTATIC BREAST CANCER PATIENTS?

In the past, the locoregional treatment in metastatic breast cancer was believed to have a role only for palliation of the symptoms caused by the local progression of the tumor. However, beginning from the early 2000s, with the advent of novel systemic therapies as new chemotherapeutic agents, anti-HER2 agents, hormonal therapies, immunotherapies, and cyclin-dependent kinase inhibitors, the destiny of patients with metastatic breast cancer have changed. A significant number of patients showed at least good partial response both in the primary and metastatic sites, which led to questioning the idea of treating these patients with some form of locoregional treatment based on the idea that the primary tumor could be a source of reseeding of cancer outside the breast. The National Cancer Database study revealed that surgery

to the primary site when added to systemic therapy in patients with stage IV breast cancer significantly improved survival[16]. A similar retrospective study using the Surveillance, Epidemiology, and End Results database also showed that median survival was longer in metastatic breast cancer patients who had surgery to the primary site than patients who did not (36 mo *vs* 21 mo, $P < 0.001$)[17]. However, the prospective phase III ABCSG-28 POSYTIVE trial that randomized metastatic breast cancer patients to surgery followed by systemic therapy or systemic therapy alone could not demonstrate an overall survival (OS) benefit for the surgery arm[18]. Another study from India randomized 350 patients with *de novo* metastatic breast cancer who had an objective tumor response after 6-8 courses of chemotherapy to locoregional treatment to primary or no locoregional treatment arms[19]. At a median follow-up of 23 mo, no statistically significant difference in OS was observed between treatment arms (19.2 mo *vs* 20.5 mo, $P = 0.79$). However, locoregional treatment was associated with improved locoregional progression-free survival (PFS) but shorter distant PFS[19]. In another study by Soran *et al*[20], 274 treatment naïve metastatic breast cancer patients were randomized to receive locoregional treatment followed by systemic therapy *vs* systemic therapy alone. With a median follow-up of 55 mo, median survival was significantly longer in the locoregional treatment arm compared to patients with systemic therapy alone arm (46 mo *vs* 37 mo, $P = 0.005$). Unplanned subgroup analysis of this study showed that improvement in survival was observed in patients with estrogen receptor/progesterone receptor positive, Her2 negative disease, younger than 55 years, and with solitary bone-only metastasis[20].

Several ongoing trials are evaluating the impact of locoregional treatment on survival in metastatic breast cancer. Early results of the ECOG E2108 trial that randomized 256 patients whose disease responded to initial systemic therapy, or stayed stable, to systemic therapy plus locoregional treatment or systemic therapy alone, showed that there was no significant difference in 3-year OS (68.4% *vs* 67.9%, $P = 0.63$); however, the locoregional recurrence or progression was significantly higher in the systemic therapy alone arm (3-year rate 25.6% *vs* 10.2%, Gray test $P = 0.003$)[21]. Preliminary results of another multicentric prospective ongoing trial (TBCRC 013) evaluating the impact of surgery on OS in metastatic breast cancer patients who responded to first-line systemic therapy showed that the addition of surgery to systemic therapy had no impact on OS even in responders to first-line systemic therapy[22]. JCOG1017 PRIM-BC trial comparing surgery to primary plus systemic therapy with systemic therapy alone has completed accrual, and results of this trial are being expected[23]. Ongoing SUBMIT (NCT01392586) trial is also investigating whether upfront surgery in patients with metastatic breast cancer will result in an improvement of the 2-year survival compared to the survival achieved by systemic therapy and delayed local treatment or systemic therapy alone[24]. The details of prospective randomized trials investigating the role of locoregional treatment in metastatic breast cancer are given in Table 1. The final results of these prospective randomized studies will hopefully clarify the exact role of locoregional treatment in metastatic breast cancer patients.

There is no prospective randomized study comparing surgery with surgery plus RT or RT alone as a locoregional treatment in metastatic breast cancer. A retrospective study by Gultekin *et al*[7] evaluating the impact of locoregional treatment in 227 oligometastatic breast cancer patients showed that locoregional treatment per se did not affect OS and PFS, however, surgery and RT when used together improved OS and PFS. The authors also observed that patients with solitary metastasis had longer PFS than patients with multiple metastases. In another retrospective study, Le Scodan *et al*[8] compared 320 metastatic breast cancer patients who received exclusive locoregional RT with or without surgery with 261 metastatic breast cancer patients who did not receive locoregional treatment. In this study, 78% of patients had exclusive locoregional RT, and patients with locoregional treatment had longer 3-year OS rates (43.4% *vs* 26.7%, $P < 0.001$). Although there was no statistically significant difference in locoregional treatment modalities regarding survival outcomes, multifactorial analysis in the Le Scodan *et al*[8] study showed that age at diagnosis, visceral metastases, involvement of multiple sites, endocrine treatment, and locoregional treatment were independent prognostic factors for OS[8]. Retrospective studies published within the last decade investigating the impact of locoregional treatment on primary tumor sites are detailed in Table 2.

There is still no consensus about the efficacy of locoregional treatment in metastatic breast cancer. There is again no consensus about the optimal treatment strategy as surgery alone or surgery plus RT or RT alone when locoregional treatment is indicated. Based on the available data, locoregional treatment may be offered to patients who have a long-life expectancy, such as those younger than 55 years, have

Table 1 Prospective randomized phase III trials investigating the role of locoregional treatment in *de novo* metastatic breast cancer

Ref.	*n*	Treatment	Patients	Median follow-up (mo)	Outcomes
Khan *et al*[21] (ECOG-ACRIN E2108)	256	Primary systemic therapy: LRT (n = 125); No LRT (n = 131)	NR	59	3-yr OS: 68.4% *vs* 67.9%, P = 0.63 3-yr locoregional recurrence/progression: 10.2% *vs* 25.6%, P = 0.003
Fitzal *et al*[18] (ABCSG-28 POSYTIVE)	90	Arm A: Primary surgery + systemic therapy (n = 45) Arm B: Primary systemic therapy (n = 45)	Arm A: More cT3 and cN2 tumors	37.5	Stopped early Median OS (mo): 34.6 *vs* 54.8, P = 0.267 Time to distant progression (mo): 13.9 *vs* 29.0, P = 0.0668
Soran *et al*[20] (MF07-01)	274	LRT + systemic therapy (n = 138) Systemic therapy (n = 136)	LRT arm: More ER/PR (+), less triple negative tumors	54.5 *vs* 55	Median OS (mo): 46 *vs* 37, P = 0.005 Unplanned subgroup analysis: Improvement in survival: ER/PR (+), HER2 (-), < 55 yr, solitary bone-only metastasis
Badwe *et al*[19] (NCT00193778)	350	Primary systemic therapy: LRT (n = 173); No LRT (n = 177)	Similar patient and tumor characteristics	23	Median OS (mo): 19.2 *vs* 20.5, P = 0.79 Median LR-PFS (mo): not attained *vs* 18.2, P < 0.0001 Median distant-PFS (mo): 11.3 *vs* 19.8, P = 0.012

NR: Not reported; LRT: Locoregional treatment; c: Clinic; T: Tumor; N: Node; ER: Estrogen receptor; PR: Progesterone receptor; OS: Overall survival; HER2: Human epidermal growth factor receptor 2; LR: Locoregional; PFS: Progression-free survival.

hormone receptor-positive, HER2 positive, bone or limited liver metastases, presence of a low-grade tumor, good performance status, good response to systemic therapy, and a limited number of metastases[8,25].

SHOULD WE TREAT METASTASES IN OLIGOMETASTATIC BREAST CANCER PATIENTS?

Traditionally, the standard treatment is systemic therapy for metastatic breast cancer patients; however, long-term complete response with systemic therapy alone is rare [12]. Given that progression in metastatic breast cancer patients frequently occurs at sites of known metastases rather than new metastatic lesions, local ablative therapies to metastatic sites may provide therapeutic benefit[9,26]. In addition to surgery and radiofrequency ablation, stereotactic body RT (SBRT) or stereotactic ablative RT (SABR), which allows highly conformal dose distribution using high dose per fraction with a low number of fractions, may be used as local ablative therapies to metastases to prevent progression[26,27].

Response to systemic therapy is a significant prognostic factor in metastatic breast cancer[4]. In a retrospective study by Weykamp *et al*[28], the 2-year local control and OS rates were reported as 89% and 62%, respectively, in patients with oligometastatic disease. The authors also observed that solitary metastasis and young age were independent factors for PFS and OS, respectively[28]. In another study by Kobayashi *et al*[29], 75 oligometastatic breast cancer patients who had a complete or partial response after systemic therapy and treated with local therapy were retrospectively evaluated, and it was demonstrated that complete response or no evidence of disease rates were significantly better in patients with a single organ metastasis than with two organ metastases (P = 0.002)[29]. In this retrospective study, the multidisciplinary treatment improved OS compared to systemic therapy alone[29].

Few studies have investigated the role of SBRT as a local treatment of metastases in oligometastatic disease including metastatic breast cancer patients (Table 3)[4-6,9,26,28,30,31]. Studies in the literature showed that the maximum benefit from SBRT to all metastatic sites was provided in young patients whose primary breast cancer was controlled, with a limited number of metastases, low tumor volume, only bone metastases, good response to systemic therapy, long disease-free interval (> 12 mo), and hormone receptor-positivity[4,29,30].

Table 2 Retrospective studies published within the last decade investigating the impact of locoregional treatment to the primary tumor site in de novo metastatic breast cancer

Ref.	*n*	Treatment	Patient	Median follow-up (mo)	Outcomes
Ma *et al*[35]	987	Surgery (*n* = 463) No surgery (*n* = 524)	Surgery arm: More T1-2, HR-positive, solitary metastasis, bone only metastasis	NR	Median survival (mo): 45 *vs* 28, *P* < 0.001 Better survival in surgery after systemic therapy than primary surgery Triple negative, brain metastases: No benefit of surgery
Lane *et al*[16] (NCDB)	24015	Systemic therapy alone (*n* = 13505) Surgery before systemic therapy (*n* = 4552) Systemic therapy before surgery (*n* = 5958)	Surgery after systemic therapy arm: Younger, more T3-4 and HR-negative	NR	Median OS (mo): 37.5 *vs* 49.4 *vs* 52.8, *P* < 0.001 RT: No impact on OS
Li *et al*[36] (SEER database)	20870	Surgery (*n* = 5779) No surgery (*n* = 15091)	Surgery arm: Younger, more T1-3, N+, Gr III, and less HR+ More chemo and RT received	NR	Surgery arm (± RT): Improved BCSS and OS (*P* < 0.001)
Pons-Tostivint *et al*[37]	4276	LRT (*n* = 1706): Surgery, RT or both No LRT (*n* = 2570)	LRT arm: Younger, more solitary or bone-only metastases	45.3	Median OS (mo): HR-positive, HER2- negative: 61.6 *vs* 45.9, *P* < 0.001 HR-positive, HER2-positive: 77.2 *vs* 52.6, *P* = 0.008 Triple negative: 19 *vs* 18.6, *P* = 0.54 Bone only metastases: 70.4 *vs* 62, *P* < 0.001 Visceral metastases: 83 *vs* 52.7, *P* < 0.001
Choi *et al*[38]	245	LRT (*n* = 82): Surgery, RT or both No LRT (*n* = 163)	LRT arm: < T4, no liver or brain metastasis, and < 5 metastatic sites	40	5-yr LRFS: 62% *vs* 20%, *P* < 0.001 5-yr OS:73% *vs* 45%, *P* = 0.02
Gultekin *et al*[7]	227	LRT (*n* = 188): Surgery, RT or both No LRT (*n* = 39)	LRT arm: Less T3-4 and more solitary metastases	35	5-yr OS: 56% *vs* 24%, *P* < 0.001 5-yr PFS: 27% *vs* 7%, *P* < 0.0001
Nguyen *et al*[39]	733	LRT (*n* = 378): Surgery, RT or both No LRT (*n* = 355)	LRT arm: Younger, more T1-2, N0-1, limited M1 disease	21	5-yr OS: 21% *vs* 14%, *P* < 0.001 5-yr PFS 72% *vs* 46%, *P* < 0.001
Neuman *et al*[25]	186	Surgery (*n* = 69): 13% RT No surgery (*n* = 117)	Surgery arm: More HER2-negative, smaller tumors, more solitary metastasis	52	No difference in OS (*P* = 0.10)

NCDB: National Cancer Database; SEER: Surveillance, Epidemiology, and End Results, RT: Radiotherapy; LRT: Locoregional treatment; T: Tumor; HR: Hormone receptor; HER2: Human epidermal growth factor receptor 2; N: Node, Gr: Grade; M: Metastasis; NR: Not reported; OS: Overall survival; PFS: Progression-free survival; BCSS: Breast cancer-specific survival; LRFS: Local recurrence-free survival.

The prospective studies exploring the role of SBRT to metastatic sites in oligometastatic breast cancer are limited (Table 4)[5,6,9,26,30,31]. Milano *et al*[9] performed hypofractionated stereotactic radiation (50 Gy in 10 fractions) to all sites of disease in 48 breast cancer patients with 1-5 extracranial metastases. The authors observed that some patients who have only bone metastases rather than visceral metastases and with low tumor burden (volume and number of lesions) survived longer than 10 years[9]. Five- and ten-year OS rates after hypofractionated stereotactic radiation was 83% and 75%, for patients with only bone metastases *vs* 31% and 17%, respectively, for patients with not only bone metastases[9]. Trovo *et al*[6] in a prospective phase II trial

Table 3 Retrospective studies investigating the role of radiotherapy as a local treatment of metastases in oligometastatic disease

Ref.	*n*	Treatment	Patients	Median follow-up (mo)	Outcomes
Weykamp *et al* [28]	46	SBRT: Bone, lung, liver, adrenal gland Median 3 frx (1-10)/28 Gy (24-60 Gy)	Inclusion criteria: breast cancer, oligometastatic (≤ 3) or oligoprogressive (1) disease 58 lesions	21	2-yr LC, DC, PFS and OS: 89%, 44%, 17%, and 62%, respectively Solitary metastasis: Poor prognostic factor for DC and PFS Higher age: Poor prognostic factor for OS
Kobayashi *et al* [29]	75	Primary systemic chemotherapy: CR/PR Surgery or RT	Inclusion criteria: breast cancer, ≤ 2 metastatic organs, < 5 metastases, < 5 cm lesions	103	10-yr and 20-yr OS: 59.2% and 34.1%, respectively 10-yr and 20-yr RFS: 27.4% Single organ metastasis, local treatment and shorter DFI: Better RFS
Hong *et al*[4]	361	SBRT 10 frx/50-60 Gy or 3 frx/24-48 Gy	Extracranial oligometastases (≤ 5) Breast cancer (16%)	26.2	3-yr OS, PFS and TMC were 56%, 24%, and 72%, respectively Primary tumor type, interval to metastasis, number of treated metastasis, and mediastinal/hilar LN, liver, or adrenal metastases: Associated with OS All breast cancer patients: RPA class 1 (3-yr OS 75%)
Cha *et al*[40]	49	Endocrine therapy plus LRT (n = 33) 82% RT: Bone, LN Endocrine therapy alone (n = 16)	Inclusion criteria: HR-positive, HER2-negative Similar patient and tumor characteristics	101.6 *vs* 105.6	Median OS (mo): 72.3 *vs* 91, P = 0.272 Median PFS (mo): 30 *vs* 18, P = 0.049

SBRT: Stereotactic body radiation therapy; frx: Fraction, Gy: Gray, CR: Complete response; PR: Partial response; RT: Radiotherapy; LN: Lymph node; LRT: Locoregional treatment; HR: Hormone receptor; HER2: Human epidermal growth factor receptor 2; LC: Local control; DC: Distant control; OS: Overall survival; PFS: Progression-free survival; DFI: Disease-free interval; TMC: Treated metastasis control; RPA: Recursive partitioning analysis.

administered SBRT (30-45 Gy in 3 fractions) or intensity-modulated RT (60 Gy in 25 fractions) to all metastatic sites in 54 oligometastatic breast cancer patients whose primary tumor was controlled. The authors reported that 2-year local control, OS, and PFS rates with a median follow-up of 30 mo were 97%, 95%, and 53%, respectively, and no ≥ grade 3 toxicity was documented[6]. The first randomized phase II study in metastatic cancer is the SABR-COMET study in which 99 patients with the oligometastatic disease were randomized to receive systemic therapy plus palliative RT (8 Gy in 1 fraction or 30 Gy in 10 fractions) or systemic therapy plus SABR to all metastatic sites. Only 18% of the patients were with breast cancer in this study. There was a significant improvement in terms of 5-year OS (17.7% *vs* 42.3%, P = 0.006), 4-year PFS (3.2% *vs* 21.6%, P = 0.001), and local control rates (46% *vs* 63%, P = 0.039) in patients treated with SABR without any significant adverse events[5]. Results of three ongoing prospective randomized studies (SABR-COMET 10, STEREO-SEIN, and NRG-BR002) are being expected to clarify the role of SBRT to all metastatic sites in oligometastatic breast cancer[32-34].

CONCLUSION

Metastatic breast cancer includes a wide spectrum of disease ranging from oligometastatic to disseminated disease. There has been growing interest during the last 20 years in the curative treatment of oligometastatic breast cancer with the advances in systemic therapy. Aggressive local treatment of primary tumor and metastases-directed therapies may improve survival in selected patients, and should especially be suggested to young patients with limited number of metastases. The results of ongoing trials specific to breast cancer will be more helpful in the future.

Table 4 Prospective studies exploring the role of radiotherapy to metastatic sites in oligometastatic disease including primary breast cancer

Ref.	*n*	Treatment	Patients	Median follow-up (mo)	Outcomes
Palma *et al*[5] (SABR-COMET)	99	Palliative RT ± systemic therapy (*n* = 33) 1 frx/8 Gy or 10 frx/30 Gy SABR ± systemic therapy (*n* = 66) Different regimens according to tumor size and location	Inclusion criteria: 1-5 metastases, life expectancy ≥ 6 mo, controlled primary tumor Primary breast cancer (*n*): 5 *vs* 13	51	5-yr OS: 17.7% *vs* 42.3%, *P* = 0.006 4-yr PFS 3.2% *vs* 21.6%, *P* = 0.001 LC 46% *vs* 63%, *P* = 0.039 ≥ Gr 2 toxicity: 9% *vs* 29%, *P* = 0.026 SABR: Gr 5 toxicity (*n* = 3)
Milano *et al*[9]	48	HSRT: ≥ 50 Gy in 10 frx	Inclusion criteria: breast cancer, 1-5 extracranial metastases, primary controlled	52	5- and 10-yr OS: Bone-only oligometastases: 83% and 75% Non-bone-only oligometastases: 31% and 17% (*P* = 0.002) GTV > 25 cc: Poor prognostic factor for LC
Trovo *et al*[6]	54	SBRT: 3 frx/30-45 Gy (*n* = 44) IMRT: 25 frx/60 Gy (*n* = 10)	Inclusion criteria: breast cancer, ≤ 5 extracranial metastases, primary controlled 92 lesions	30	2-y LC: 97% 2-y OS: 95% 1- and 2-yr PFS: 75% and 53%, respectively No ≥ Gr 3 toxicity
Salama *et al* [26]	61	SBRT: Lung, LN, liver, bone, adrenal, soft tissue, pancreas 3 frx/24-48 Gy	Inclusion criteria: 1-5 metastatic sites, life expectancy > 3 mo Breast cancer (11.3%)	20.9	1-yr and 2-yr OS: 81.5% and 56.7%, respectively 1-yr and 2-yr PFS: 33.3% and 22.0%, respectively
Scorsetti *et al* [31]	33	SBRT: 3-4 frx/48-75 Gy	Inclusion criteria: breast cancer, < 5 lung or liver metastases, other metastatic sites stable or responding after chemotherapy 43 lesions	24	1- and 2-yr LC: 98% and 90%, respectively 1- and 2-yr OS: 93% and 66%, respectively 1- and 2-yr PFS: 48% and 27%, respectively No grade 3-4 toxicities
Milano *et al* [30]	40	SBRT doses and fractionation was not mentioned	Inclusion criteria: breast cancer, ≤ 5 metastases	NR	4-yr OS: 59% 4-yr PFS: 38% 4-yr LC: 89% Favorable prognosis: Solitary metastasis, smaller tumor volume, bone-only disease, and stable or regressing lesions

RT: Radiotherapy; frx: Fraction; Gy: Gray; SBRT: Stereotactic body radiation therapy; HSRT: Hypofractionated stereotactic radiotherapy; IMRT: Intensity-modulated radiation therapy; NR: Not reported; OS: Overall survival; PFS: Progression-free survival; LC: Local control; LN: Lymph node; Gr: Grade; GTV: Gross tumor volume.

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ORIGINAL ARTICLE

Retrospective Study

Association of cancer with comorbid inflammatory conditions and treatment in patients with Lynch syndrome

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Abstract

BACKGROUND

Individuals with Lynch syndrome (LS) and hereditary non-polyposis colorectal cancer (HNPCC) are at increased risk of both colorectal cancer and other cancers. The interplay between immunosuppression, a comorbid inflammatory condition (CID), and HNPCC on cancer risk is unclear.

AIM

To evaluate the impact of CIDs, and exposure to monoclonal antibodies and immunomodulators, on cancer risk in individuals with HNPCC.

Informed consent statement: This study was exempt from signed consent form requirements.

Conflict-of-interest statement: Authors have no conflicts of interest to disclose.

Data sharing statement: Consent was not obtained but the presented data are anonymized and risk of identification is low.

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METHODS

Individuals prospectively followed in a hereditary cancer registry with LS/HNPCC with the diagnosis of inflammatory bowel disease or rheumatic disease were identified. We compared the proportion of patients with cancer in LS/HNPCC group with and without a CID. We also compared the proportion of patients who developed cancer following a CID diagnosis based upon exposure to immunosuppressive medications.

RESULTS

A total of 21 patients with LS/HNPCC and a CID were compared to 43 patients with LS/HNPCC but no CID. Cancer occurred in 84.2% with a CID compared to 76.7% without a CID (P = 0.74) with no difference in age at first cancer diagnosis 45.5 ± 14.6 *vs* 43.8 ± 7.1 years (P = 0.67). LS specific cancers were diagnosed in 52.4% with a CID *vs* 44.2% without a CID (P = 0.54). Nine of 21 (42.9%) patients were exposed to biologics or immunomodulators for the treatment of their CID. Cancer after diagnosis of CID was seen in 7 (77.8%) of exposed individuals *vs* 5 (41.7%) individuals unexposed to biologics/immunomodulators (P = 0.18). All 7 exposed compared to 3/5 unexposed developed a LS specific cancer. The exposed and unexposed groups were followed for a median 10 years and 8.5 years, respectively. The hazard ratio for cancer with medication exposure was 1.59 (P = 0.43, 95%CI: 0.5-5.1).

CONCLUSION

In patients with LS/HNPCC, the presence of a concurrent inflammatory condition, or use of immunosuppressive medication to treat the inflammatory condition, might not increase the rate of cancer occurrence in this limited study.

Key Words: Lynch syndrome; Hereditary non-polyposis colorectal cancer; Inflammatory bowel disease; Immunosuppression; Biologics

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Core Tip: Individuals with hereditary non-polyposis colorectal cancer (HNPCC) are at increased risk of both colorectal cancer and other cancers. When they have a comorbid inflammatory condition (CID) that requires immunosuppression, clinicians may be hesitant to prescribe these medications due to concern of an elevated cancer risk. We show that individuals with HNPCC and CID have a similar cancer risk to those with HNPCC alone, and that the addition of immunosuppression does not increase overall cancer risk, but may increase the risk of LS-specific cancers.

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INTRODUCTION

Lynch syndrome (LS) is the most common hereditary cancer syndrome. Patients with LS have an increased cumulative lifetime risk of developing colorectal, endometrial, ovarian, stomach, small bowel, hepatobiliary, urothelial, and brain cancers[1]. This is explained by a germline pathogenic variant (PV) in one of the DNA mismatch repair (MMR) genes resulting in the translation of a defective enzyme unable to correct errors in base pairing during DNA replication[2]. The defective system leads to the accumulation of mutations, regulatory escape from the cell division cycle, and ultimately cancer[3]. LS accounts for 3% of all newly diagnosed colorectal and endometrial cancers[4]. Individuals who meet Amsterdam II criteria, have MSI-H colorectal cancers, but do not have a MMRPV are also have an elevated risk of colorectal cancer

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[5]. Hereditary non-polyposis colorectal cancer (HNPCC) is an umbrella term that, regardless of MMRPV status, includes individuals with MSI-H cancers and a suggestive family history.

Individuals with HNPCC may have co-existent systemic inflammatory conditions (CID) such as inflammatory bowel disease (IBD) and rheumatic diseases. A deregulated immune system contributes to the pathogenesis of these diseases and various inflammatory mediators and pathways have been implicated[6]. Treatment of moderate to severe inflammatory disease generally involves modulating the immune system with systemic immunosuppressive medications, in particular monoclonal antibodies and immunomodulators alone or in combination[7]. Because the immune system is known to protect against cancer by detecting neoantigens presented by cancer cells[8], of particular importance in HNPCC individuals with immunogenic MSI-H cancers[9,10], clinicians may be hesitant to prescribe these medications in patients with HNPCC due to concern of an elevated cancer risk. Limited data exists regarding the impact of CID and immunosuppressive medication exposure on the cancer risk in HNPCC.

Our primary aim was to evaluate the impact of CID on the cancer risk in HNPCC by comparing HNPCC individuals with and without a CID. Our secondary aim was to assess the effect of monoclonal antibody and/or immunomodulator exposure on cancer risk in HNPCC patients with concurrent CID.

MATERIALS AND METHODS

This study was approved by Cleveland Clinic Institutional Review Board (IRB 2884). HNPCC individuals enrolled in the David G. Jagelman Hereditary Colorectal Cancer Registries at the Sanford R. M.D. Center for Hereditary Colorectal Neoplasia at the Cleveland Clinic from 1979 to 2019 who met inclusion criteria were included in the study. HNPCC was defined as individuals with an MSI-H tumor and belonging to a family fulfilling Amsterdam II criteria. Individuals with comorbid IBD including ulcerative colitis (UC) and Crohn's disease (CD), and rheumatic diseases including rheumatoid arthritis and other inflammatory arthritides, psoriasis, ankylosing spondylitis, spondyloarthritis, systemic sclerosis, scleroderma, dermatomyositis, polymyositis, lupus, sarcoidosis, mixed connective tissue disease and undifferentiated connective tissue disease were included.

Variables extracted from the medical record included demographics, age at first cancer diagnosis and last follow up, MMRPV, sex, race, smoking history, personal and family history of LS-specific (colorectal, endometrial, urothelial, and small bowel) and other cancers, cancer stage and comorbid disease history (age at diagnosis, presenting signs and symptoms, treatments, exposure to biologics and/or immunomodulators with type, dose and duration of treatment noted for each medication).

The primary aim was to compare the proportion of individuals with HNPCC who develop cancer based on CID status. Cases (HNPCC with CID) were matched to controls (HNPCC without CID) in a 1:2 ratio. Controls were randomly chosen from the registry after matching for presence and type of MMRPV, age at last follow up, and gender. We compared the proportion of patients who had developed any cancer up to last follow up or death between the two groups. In a subgroup analysis, we then compared proportion of patients who developed colorectal cancer in HNPCC patients with and without IBD.

Our secondary aim was to compare the proportion of CID patients (n = 21) who developed cancer with and without exposure to a monoclonal antibody and/or immunomodulator therapy. Patients were divided into two groups based on any exposure to these medications. Duration of exposure was determined through the electronic medical record or paper chart review by duration of prescription length and provider notes. Immunosuppressive medications included anti-tumor necrosis factors (TNF) (infliximab, adalimumab, certolizumab pegol, etanercept and golimumab), anti-integrins (natalizumab and vedolizumab), anti-interleukins [anakinra, tocilizumab, sarilumab, ixekizumab, guselkumab, ustekimumab), janus kinase inhibitor (tofacitinib), and immunomodulators (methotrexate, azathioprine and 6-mercaptopurine (6MP)]. The proportion of individuals who developed a cancer was calculated from the year of diagnosis of comorbid disease until last follow up or death.

Continuous variables are presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Categorical variables are presented as number and percentage. Student *t*-test or Mann-Whitney-*U* test was used to compare continuous variables. Categorical variables were compared using *chi*-square test or Fisher exact

test. For the primary aim, the proportion of individuals in each group with cancer was compared. For the secondary aim, we also carried out single variable cox proportional survival analysis to calculate hazard ratio (HR) for cancer and Kaplan Meier curve was constructed for the comparison between exposed and unexposed groups. Time to event started from the year of comorbid disease diagnosis to cancer diagnosis. Individuals who were lost to follow up are included until that time in analysis. All statistical work was done using SPSS v26.0.

RESULTS

Part 1: Presence of a comorbid inflammatory condition and proportion of patients who develop cancer in HNPCC

64 HNPCC patients including 21 cases with a CID and 43 controls without CID were analyzed. Of the 14 patients with LS, MMRPV included MLH1 (23.8%), MSH2 (14.3%), MSH6 (9.5%) and PMS2 (19.6%). Seven (33%) did not have a MMRPV. Age at last follow up, gender, race, smoking history and family history of cancer did not differ between cases and controls (Table 1). CID in the 21 patients included CD (23.8%), UC (9.5%), inflammatory/rheumatoid arthritis (33.3%), psoriasis (14.3%), and one case each of psoriatic arthritis, dermatomyositis, ankylosing spondylitis, and sarcoidosis. The mean age at CID diagnosis was 39 ± 13 years.

The proportion of patients who had a history of cancer diagnosis at the time of their last follow up was 84.2% in cases and 76.7% in controls (P = 0.74). Age at first cancer diagnosis was 45.5 ± 14.6 years for cases and 43.8 ± 7.1 years for controls (P = 0.67). The proportion of patients who had developed cancer after diagnosis of CID in cases was 57.1% with a 10 year (6.0-16.5) median duration of follow-up and 46.5% in controls (P = 0.42) when also followed for 10 years prior to last follow up or death. Approximately half of the cancers were HNPCC-specific: 52.4% of cases *vs* 44.2% of controls (P = 0.54) (Table 2). The distribution of cancers based on MMRPV is presented in Table 3.

Median total surveillance colonoscopies after HNPCC diagnosis were 5 (IQR 3.0-6.0) for cases and 4 (IQR 2.0-6.0, P = 0.19) for controls with a median 1 year interval for both groups. 42.9% of cases *vs* 46.5% of controls had partial colectomy and 33.3% of cases compared to 7.0% controls had total proctocolectomy. Overall, 83% of female cases and 76% of female controls had a history of total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH-BSO[MSF1]). 50.0% of cases and 28.0% of controls had the procedure prophylactically while the others had TAH-BSO for endometrial cancer. 1 (4.7%) female in case group had prophylactic TAH-BSO prior to the diagnosis of CID.

In a sub-group analysis, we compared the 7 individuals with IBD (5 CD and 2 UC) to the control group (n = 43) for the proportion of patients who developed specifically CRC. One CD individual had stricturing ileitis and no colonic disease or prior colon surgery and another developed CD in an ileal pouch following proctocolectomy and ileal pouch anal anastomosis for resection of a cecal adenocarcinoma. Both individuals were exposed to monoclonal antibodies/immunomodulators for treatment of CD. Of the other three CD patients, two had ileitis and one had mild segmental colitis. They did not receive monoclonal antibodies/immunomodulators for treatment. One UC patient had moderate pancolitis and received monoclonal antibodies/immunomodulator. The other UC patient had moderate left sided colitis and did not receive above mentioned immunosuppressive treatment. Mean duration of follow up for IBD in the exposed group was 19.2 ± 12.1 years compared to the unexposed group 16.0 ± 12.6 years, P = 0.64. All cases had intact colons at the time of CID diagnosis except the one CD individual mentioned above.

Four (57.1%) cases developed colorectal (CRC) cancer (P = 0.86) over a median 10-years (IQR 5-18) from IBD diagnosis compared to 53.5% of controls when followed for 10 years. Both UC and 2/5 CD (40%) patients developed CRC at a median age of 41.8 years (IQR 19-57) compared to 46.0 year (IQR 41-52) in the control group. Median stage of CRC cancer at the time of diagnosis was II in both groups.

Part 2: Monoclonal antibody and/or immunomodulator exposure and the proportion of patients who develop cancer in HNPCC

For the secondary aim, we compared the proportion of individuals who developed cancer after diagnosis of a CID in the 9/21 (39.5%) individuals exposed to monoclonal antibodies and/or immunomodulators to the 12/21 (61.5%) unexposed. Mean age at end of follow-up for the exposed group was 54.2 ± 20.0 years compared to 53.7 ± 12.4

Table 1 Demographics, genetic diagnosis, surveillance, surgical history, and cancer in hereditary non-polyposis colorectal cancer

	Inflammatory disease present, *n* = 21	Controls, *n* = 43	*P* value
Current age (yr), mean ± SD	53.9 ± 15.7	53.8 ± 7.8	0.97
Gender, *n* (%)			
Female	12 (57.1)	25 (58.1)	0.94
Race, *n* (%)			
White	17 (81.0)	40 (93.0)	0.34
Black	1 (4.8)	1 (2.3)	
Others	3 (14.3)	2 (4.6)	
Smoking Status, *n* (%)			
Former/current	9 (42.9)	20 (46.5)	
Never Smoker	12 (57.1)	23 (53.5)	0.72
Family History of Cancer, *n* (%)			
Colon	13 (61.9)	33 (78.6)	0.16
LS Cancer	11 (52.4)	24 (57.1)	0.72
Other cancers	12 (57.1)	25 (58.1)	0.94
LS MMRPV, *n* (%)	14 (66.7)	21 (67.4)	1
MLH1	5 (23.8)	10 (23.3)	
MSH2	3 (14.3)	7 (16.3)	
MSH6	2 (9.5)	4 (9.3)	
PMS2	4 (19.0)	8 (18.6)	
No MMRPV	7 (33.3)	14 (32.6)	
Age of HNPCC diagnosis, mean ± SD			
LS	43.6 ± 14.0	45.4 ± 7.6	0.66
No MMRPV	49.0 ± 5.4	46.3 ± 2.2	0.59
Screening colonoscopies			
Median total number	5 (IQR 3.0-6.0)	4 (IQR 2.0-6.0)	0.19
Median years between colonoscopies	1.0 (IQR 1.0-1.5)	1.0 (IQR 1.0-1.6)	0.87
History of complete or partial colectomy	15 (76.2)	23 (53.5)	0.08
TAH-BSO (% of females in each group)	10 (83.3)	19 (76.0)	0.8
History of Prophylactic TAH-BSO (% of females in each group)	6 (50.0)	7 (28.0)	0.27
Proportion of patients with any cancer, *n* (%)	16 (84.2)	33 (76.7)	0.74
Cancer Incidence-10 yr follow up	12 (57.1)	20 (46.5)	0.42
Age at Diagnosis of first cancer (yr), mean ± SD	45.5 ± 14.6	43.8 ± 7.1	0.67

HNPCC: Hereditary non-polyposis colorectal cancer; LS: Lynch syndrome; MMRPV: Mismatch repair pathogenic variant; TAH- BSO: Total abdominal hysterectomy-bilateral salpingo-oophorectomy.

years for the unexposed group (P = 0.70). The exposed and unexposed groups were followed for a median 10 and 8.5 years, respectively.

No significant difference in age, gender, race, smoking, or family cancer history was observed between the groups (Table 4). 22.2% (2/9) of exposed and 16.7% (2/12) of the unexposed group had a history of cancer prior to CID diagnosis. One individual in the exposed group had a history of breast cancer while the other had a history of both pancreatic and colon cancer. In the unexposed group, one individual had history of colon cancer while the other had a history of both endometrial and colon cancer. Median duration of exposure to monoclonal antibodies and immunomodulators was

Table 2 Cancers observed based on presence or absence of a comorbid inflammatory condition over 10 yr of follow up, *n* (%)

Number of patients who developed a cancer	CID present, *n* = 21	No CID, *n* = 43
	12 (57.1)	20 (46.5)
Lynch syndrome specific cancers		
Colorectal	9 (42.9)	12 (27.9)
Endometrial	2 (9.5)	8 (18.6)
Small Bowel	0 (0)	1 (2.3)
Urothelial	1 (4.8)	1 (2.3)
Non-Lynch Syndrome-specific cancers		
Breast	1 (4.8)	0 (0)
Nasopharynx	1 (4.8)	0 (0)
Prostate	1 (4.8)	0 (0)
Ovarian	0 (0)	1 (2.3)
B-Cell Lymphoma	1 (4.8)	0 (0)

CID: Comorbid inflammatory condition.

Table 3 Distribution of cancers based on genetic diagnosis by comorbid inflammatory condition status

	CID present			CID non present		
	CRC	LS specific[1]	All other	CRC	LS specific	All other
MLH1	2 (40.0)	2 (40.0)	1 (20.0)	2 (20.0)	2 (20.0)	0 (0)
MSH2	2 (66.7)	0 (0)	1 (33.3)	2 (28.6)	4 (57.1)	1 (14.3)
MSH6	0 (0)	0 (0)	0 (0)	2 (50.0)	1 (25.0)	0 (0)
PMS2	2 (50.0)	0 (0)	1 (25.0)	0 (0)	2 (25.0)	0 (0)
No MMRPV	3 (42.9)	1 (14.3)	1 (14.3)	6 (42.9)	1 (7.1)	0 (0)

[1]LS Specific cancers other than colorectal cancer. These include endometrial, small bowel and urothelial.
CRC: Colorectal cancer; LS: Lynch syndrome; CID: Comorbid inflammatory condition.

5.7 (3.4-8.3) years and 2.5 (0.8-8.0) years, respectively. Four patients on biologics also received combination therapy with an immunomodulator (Table 5).

Seven of nine (77.8%) exposed compared to 5/12 (41.7%) unexposed patients developed any cancer after diagnosis of a CID (P = 0.18). The hazard ratio for cancer with medication exposure was calculated to be 1.59 (P = 0.43, 95%CI: 0.5-5.1). Figure 1 shows the Kaplan-Meier curve for cancer after diagnosis of CID. Median time to cancer after IBD or rheumatic disease diagnosis was 5.0 years (P = 0.64) in both groups. All 7 (77.8%) exposed individuals developed a LS-specific cancer compared to 3 (25%) unexposed. 9 total cancers developed in the 7 exposed individuals, including CRC (n = 6), and one each of breast, renal and endometrial cancer. The five unexposed individuals developed seven cancers in total, including CRC (n = 3) and one each of prostate, endometrial, nasopharynx and B cell lymphoma.

In individuals with IBD, 71.4% developed malignancy. All three (60.0%) exposed individuals with IBD developed cancer compared to two (40.0%) unexposed (P = 0.43). Fifty percent of rheumatic disease patients developed cancer. Four (57.1%) were exposed to immunosuppressive medications compared to three unexposed (42.9%). We found no significant difference in the proportion of individuals with cancer based on type of CID[MSF2] (IBD *vs* rheumatic disease) (P = 0.64). Mean age of diagnosis of CID was also similar in individuals who developed cancer (37.8 ± 14 years) compared to those who did not (40.3 ± 13.4 years, P = 0.39). The duration of CID was not associated with cancer incidence, 10.0 years (IQR 6.5-18.0) in those who developed cancer compared to 10.5 years (IQR 4-14.5) in those who did not develop cancer (P =

Table 4 Characteristics of individuals with a comorbid inflammatory condition based on medication exposure

Characteristic	Exposed	Unexposed	*P* value
	n = 9 (39.5)	*n* = 12 (60.5)	
Current age (yr), mean ± SD	54.2 ± 20	53.6 ± 12.4	0.7
Gender, *n* (%)			
Female	4 (44.4)	8 (66.7)	0.4
Race, *n* (%)			
Caucasian	7 (77.8)	10 (83.3)	1
Others	2 (22.2)	2(16.7)	
Smoking Status, *n* (%)			
Former/current	4 (44.4)	5 (41.7)	1
Never smoked	4 (55.6)	7 (58.3)	
Previous history of cancer, *n* (%)	2 (22.2)	2 (16.7)	1
Family history of cancer, *n* (%)			
LS specific cancer	6 (66.7)	5 (41.7)	0.39
All other cancers	6 (44.4)	8 (66.7)	0.4
Duration of Follow up, (yr), median (IQR)	10.0 (9.0-22.0)	8.5 (5.3-17.3)	0.38
Comorbid disease, *n* (%)	3 (33.3)	4 (33.3)	1
Crohn's disease	2 (22.2)	3 (25.0)	
Ulcerative colitis	1 (11.1)	1 (8.3)	
Rheumatic disease, *n* (%)	6 (66.7)	8 (66.7)	1
Pathogenic variant			
MLH1	2 (22.2)	3 (25.0)	
MSH2	2 (22.2)	1 (8.3)	
MSH6	0 (0)	2 (16.7)	0.68
PMS2	2 (22.2)	2 (16.7)	
MSI-H	3 (33.3)	4 (33.3)	
Cancer after CID diagnosis	7 (77.8)	5 (41.7)	0.18
Time to Cancer After Diagnosis of CID, (yr), median (IQR)	5.0 (2.0-16.0)	5.0 (1.0-10.5)	0.64
Age at Diagnosis of first cancer (yr), median (IQR)	49 (23.0-54.0)	48 (44.0-50.0)	0.99

HNPCC: Hereditary non-polyposis colorectal cancer; LS: Lynch syndrome; MMRPV: Mismatch repair pathogenic variant; CID: Comorbid inflammatory disease.

0.66).

DISCUSSION

When an inflammatory condition coexists with HNPCC, the clinician must evaluate the risk of cancer associated with the inflammatory disease itself, and any immunosuppressive medications used to treat the inflammatory condition, since the immune system detects and destroys tumor specific antigens produced by MSI-H cancers[11,12]. Our objective was to first evaluate the impact of a CID on the proportion of patients who develop cancer in HNPCC and additionally the risk based on immunosuppressive medication exposure for treatment of the CID. In patients prospectively followed in a hereditary cancer registry, we found no difference in the proportion of HNPCC individuals with cancer based on CID status. The age at comorbid disease diagnosis, type of disease, and disease duration did not correlate

Table 5 Details of exposure for each patient with type of cancer and age at diagnosis of cancer

Disease	Genetic Diagnosis	Medication	Duration /mo	Dose/mg	Cancer type and stage	Age at diagnosis of cancer
Ulcerative colitis	PMS2	Ustekinumab,	12		Colon II	19
		Golimumab	6			
		Vedolizumab	24			
Crohn's disease	MLH1	6MP	24	50	Colon I	57
Crohn's disease	PMS2	Adalimumab	18		Colon III	17
		Golimumab	6			
		Vedolizumab	12			
Sarcoidosis	MLH1	MTX	60	15	Renal I	49
Rheumatoid arthritis	LLS	Etanercept	120		Breast I, Colon IV	76
		Tofacitinib	9			
		MTX	35	15		
		Azathioprine	72	50		
Rheumatoid arthritis	MSH2	MTX	4	20	Colon II	51
		Rituximab	72			
Psoriatic arthritis	MSI-H	Adalimumab	36		Colon III	44
		Ustekinumab	10			
		MTX	36	15		
Dermatomyositis	MSH2	MTX	12	15	NA	NA
Rheumatoid arthritis	MSI-H	MTX	120	10	NA	NA

MSI-H: High microsatellite instability; MTX: Methotrexate; 6MP: 6-mercaptopurine, NA: Not applicable (no cancer seen during duration of the study).

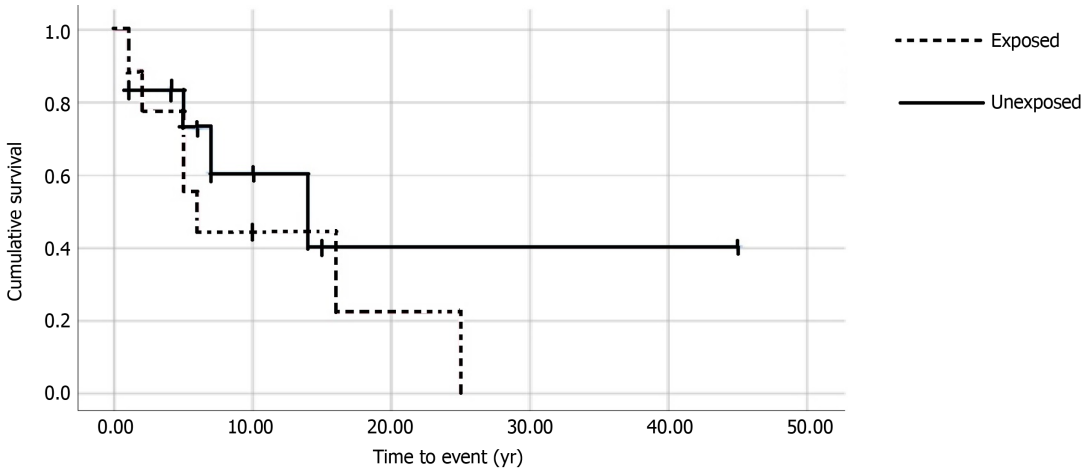


Figure 1 Kaplan Meier curve for cancer free survival between individuals exposed and unexposed to immunosuppressive medications.

with cancer development.

Epidemiological studies in the general population demonstrate that inflammatory diseases are associated with increased cancer risks. The standardized incidence ratio for CRC ranges from 1.1 to 7.0 in IBD and correlates with disease duration, anatomical extent, and severity[13]. A 10%-15% increased risk of cancer is observed in rheumatoid arthritis, particularly for lung cancer and lymphom[14]. Derikx *et al*[15] found that the incidence rate (4/15) of cancer in LS individuals with IBD was similar to LS without IBD, though they developed cancer at a younger age. Individuals were followed for 7 years and the impact of treatment was not evaluated. Another case series of 12 LS individuals with IBD found that 4 developed CRC between the ages of 32-47 years

[16]. We also found a younger median age of cancer diagnosis at 42 years in the four of seven (57.1%) IBD patients but did not observe a difference in the overall cancer risk between those with IBD compared to controls. Interestingly, in the above quoted studies, most CRC cases occurred in those with UC, as both UC patients developed CRC in our study. However, we also found that two of five with colon-predominant CD developed CRC. We hypothesize that a colon-predominant inflammatory process in HNPCC individuals further compounds the risk of colon cancer as observed in IBD. This is further supported by the observation that MLH1 and MSH2 deficient mice who have experimentally induced colonic inflammation develop CRC at faster rates and younger ages than MMR-proficient mice[17,18].

Inflammatory diseases are associated with significant morbidity and poor quality of life if left untreated[19,20]. Therefore, professional societies recommend the use monoclonal antibodies and immunomodulators to treat moderate to severe disease[21-23]. A logical conclusion is that the treatment of an inflammatory disease decreases any associated cancer incidence. However, monoclonal antibodies and immunomodulators have been associated with increased cancer risk in the general population. Wolfe demonstrated increased risks of lymphoma OR 1.7 (95%CI: 1.3-2.2), melanoma OR 2.3 (95%CI: 0.9-5.4), and non-melanotic skin cancer (OR 1.5, 95%CI: 1.2-1.8) in individuals exposed to an anti-TNFs compared to the general population[24]. A meta-analysis from nine clinical trials that compared the cancer risk in rheumatoid arthritis patients exposed to TNF-α inhibitors *vs* placebo found an elevated OR for cancer of 3.3 (95%CI: 1.2-9.1) in the exposed group[25]. This risk was dose dependent. Similarly, a US Food and Drug Administration analysis from 1998 to 2008 demonstrated higher incidence rates of lymphomas and solid tumors in children and adolescents who received TNF-alpha inhibitors for IBD and juvenile rheumatoid arthritis[26]. The combination of a TNF-alpha inhibitor with a thiopurine immunomodulator further increased this cancer risk and was associated with an increased risk of non-melanoma skin cancer as well [27,28]. In addition, methotrexate has been implicated in lymphoproliferative disorders[29], and azathioprine and 6-mercaptopurine have been implicated in skin cancers and lymphomas[30].

Given the lack of difference in the proportion of cancer cases between HNPCC with and without CID, our secondary aim was to evaluate if medication exposure had any effect on the proportion of individuals who develop cancer. It has been described that TNF-alpha inhibitors play a major role by regulating the TNF Related Apoptosis Inducing Ligand, an important mediator in immune surveillance[31]. In addition, murine models demonstrate that immune suppression results in an increased risk of sporadic breast, lung, small intestine and colon cancers[32]. Suppression of the immune system's antineoplastic role in MSI-H cancers could further compound these risks. Interestingly, we found that, though non-significant, there was an increased proportion of LS-specific cancers in the exposed group. The near doubling of LS-specific cancers (78% *vs* 42%) was primarily attributed to CRC cancer (66.6% *vs* 25%). Individuals with a colon-predominant IBD may benefit from medication sparing therapies and colonic resection may mitigate the elevated cancer risk as well.

The risk of CRC in IBD has historically been associated with severity and duration of the disease[33]. From this standpoint, immunosuppressive medications can potentially decrease CRC incidence in patients with IBD. In a metanalysis, Lu *et al*[34] describe an antineoplastic effect of thiopurines on colorectal neoplasia in patients with IBD, particularly amongst the patients with ulcerative colitis. However, these studies did not directly address individuals with LS. Genetic susceptibility to malignancy in LS adds another layer of complexity given the intricacy of balancing immunosuppression which decreases malignancy risk in inflammation but may also theoretically decrease immune surveillance.

There are limitations to our series worth mentioning, most of which are related to the inherent limitations seen in a retrospective study. Given the small subset of HNPCC patients with CID, we may not have captured a difference in cancer incidence between the exposed and unexposed groups when indeed there is one. However, this is the largest group of patients described with a length of follow up of at least 10 years. Furthermore, we are not able to specifically comment on the impact of each inflammatory disease based on severity and duration of illness. Moreover, the degree of immunosuppression was highly variable based on type, dose and duration of treatment which is described but not accounted for in the results. In our clinical experience, medications may be prescribed based on the severity of the inflammatory disease and personal history of malignancy. Though there is no data to guide specific management, the average duration of medication exposure exceeded one year. Drug trials also exclude patients with hereditary cancer syndromes, and it is unlikely that there will be a large enough population to evaluate each medication for each inflam-

matory condition. This study is the first to evaluate the impact of biologics and immunomodulators on cancer risk in HNPCC. Our study population was primarily Caucasian, however the malignancy risk associated with these medications have not been correlated with race. We also included individuals with HNPCC but without a MMRPV potentially masquerading a significant difference between exposed and unexposed groups, if any. However, these patients had MSI-H tumors and were followed in a similar fashion as those with LS due to an increased incidence of malignancy. The number of individuals without a MMRPV was also similar in both groups. Future studies should report cancer specific survival rates.

In our small cohort, CID does not appear to add any additional cancer risk to patients with HNPCC regardless of MMRPV status. The decision to start a biologic or immunomodulator in this cohort is understandably complex and should be individualized with consideration given to any colonic inflammatory disease.

CONCLUSION

In our small cohort, CID does not appear to add any additional cancer risk to patients with HNPCC regardless of MMRPV status. The decision to start a biologic or immunomodulator in this cohort is understandably complex and should be individualized with consideration given to any colonic inflammatory disease.

ARTICLE HIGHLIGHTS

Research background

Patients with Lynch Syndrome and hereditary non polyposis colorectal cancer (HNPCC) have an increased cumulative lifetime risk of developing colorectal, endometrial, ovarian, stomach, small bowel, hepatobiliary, urothelial, and brain cancers. These individuals may have co-existent systemic inflammatory conditions such as inflammatory bowel disease (IBD) and rheumatic diseases. Treatment of moderate to severe inflammatory disease generally involves modulating the immune system with systemic immunosuppressive medications, in particular monoclonal antibodies and immunomodulators alone or in combination. Interaction of the inflammatory disease and immunosuppressive medications in individuals at increased risk of malignancy due to baseline genetic diagnosis is unknown.

Research motivation

The immune system is known to protect against cancer by detecting neoantigens presented by cancer cells, so clinicians may be hesitant to prescribe these medications in patients with HNPCC due to concern of an elevated cancer risk. This leads to significant morbidity for these individuals. Moreover, treatment with immunosuppressive medications might theretically place them at higher risk for cancer. There is limited existing data to guide clinicians in this regards.

Research objectives

The primary aim was to compare the proportion of individuals with Lynch syndrome and HNPCC who develop cancer based on comorbid inflammatory disease status. Lynch syndrome and HNPCC individuals with comorbid inflammatory disease (cases) were matched to controls (Lynch syndrome and HNPCC without comorbid inflammatory disease) in a 1:2 ratio. Our secondary aim was to compare the proportion of comorbid inflammatory disease patients (n = 21) who developed cancer with and without exposure to a monoclonal antibody and/or immunomodulator therapy in Lynch syndrome and HNPCC population.

Research methods

Lynch Syndrome and HNPCC individuals enrolled in the David G. Jagelman Hereditary Colorectal Cancer Registries at the Sanford R. M.D. Center for Hereditary Colorectal Neoplasia at the Cleveland Clinic from 1979 to 2019 who met inclusion criteria were included in the study. Individuals with comorbid IBD including ulcerative colitis (UC) and Crohn's disease (CD), and rheumatic diseases were included. For our primary aim, controls were randomly chosen from the registry after matching for presence and type of mismatch repair gene pathogenic variant, age at last

follow up, and gender. We compared the proportion of patients who had developed any cancer up to last follow up or death between the two groups. For our secondary aim, patients were divided into two groups based on any exposure to these medications. Duration of exposure was determined through the electronic medical record or paper chart review by duration of prescription length and provider notes. The proportion of individuals who developed a cancer was calculated from the year of diagnosis of comorbid disease until last follow up or death.

Research results

64 HNPCC patients including 21 cases with a comorbid inflammatory disease and 43 controls without comorbid inflammatory disease were analyzed. The proportion of patients who had developed cancer after diagnosis of comorbid inflammatory disease in cases was 57.1% with a 10 year (6.0-16.5) median duration of follow-up and 46.5% in controls (P = 0.42) when also followed for 10 years prior to last follow up or death. Approximately half of the cancers were HNPCC-specific: 52.4% of cases *vs* 44.2% of controls (P = 0.54). For the secondary aim, we compared the proportion of individuals who developed cancer after diagnosis of a comorbid inflammatory disease in the 9/21 (39.5%) individuals exposed to monoclonal antibodies and/or immunomodulators to the 12/21 (61.5%) unexposed. Seven of nine (77.8%) exposed compared to 5/12 (41.7%) unexposed patients developed any cancer after diagnosis of a CID (P = 0.18). The hazard ratio for cancer with medication exposure was calculated to be 1.59 (P = 0.43, 95%CI: 0.5-5.1). This is the first study of its kind, attempting to address the interaction between genetic predisposition to cancer, inflammatory disease and immunosuppression. It remains to be seen whether these results are reproduced in larger multicenter studies.

Research conclusions

In our small cohort, comorbid inflammatory disease does not appear to add any additional cancer risk to patients with HNPCC regardless of MMRPV status. The decision to start a biologic or immunomodulator in this cohort is understandably complex and should be individualized with consideration given to any colonic inflammatory disease.

Research perspectives

We propose collaborative research to assess the risk of malignancy in lynch syndrome and HNPCC individuals on immunosuppressive medications. The risk of colorectal cancer in IBD has historically been associated with severity and duration of the disease. From this standpoint, immunosuppressive medications can potentially decrease colorectal incidence in patients with IBD. However, there are no studies that directly address individuals with genetic predisposition to cancer. Genetic susceptibility to malignancy adds another layer of complexity given the intricacy of balancing immunosuppression which decreases malignancy risk in inflammation but may also theoretically decrease immune surveillance.

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CASE REPORT

Late recurrence of localized pure seminoma in prostate gland: A case report

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Abstract

BACKGROUND

Late relapses of early-stage germ cell tumors are rare. Most patients (~85%) with stage I seminoma are cured by radical orchiectomy. The detection of late relapse is challenging given the relative rarity of this phenomenon, and the fact that patients who have completed surveillance are usually not undergoing regular oncologic workup nor imaging. While many treatment options do exist for a patient with late relapse of seminoma, surgery is typically the mainstay as these tumors are generally thought to be more chemo-resistant.

CASE SUMMARY

In this article, we describe the management of a patient with an early-stage pure seminoma who was subsequently identified to have a recurrence two decades later. We provide a review of the literature not only focused on clinical factors and biology, but also the management of late recurrences specifically in pure seminoma and in prostate gland.

CONCLUSION

There is a paucity of data and treatment recommendations for this clinical entity, and a multidisciplinary approach emphasizing subspecialty expert consultation and patient education is imperative.

Key Words: Late recurrence of pure seminoma; Pure seminoma; Seminoma; Primary prostatic seminoma; Germ cell tumor relapse; Treatment of relapsed seminoma; Biology of pure seminoma; Seminoma tumor markers; Case report

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Grade C (Good): C
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Core Tip: This case report describes of a patient with early-stage pure seminoma subsequently identified to have a recurrence within the prostate over two decades later. We provide a robust review of the literature focused on clinical factors, biology, and management of late recurrences specifically in pure seminoma and in prostate gland. There remains a lack of consensus data on treatment recommendations for this clinical disease. As such, a multidisciplinary approach emphasizing subspecialty expert consultation and patient education is imperative.

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INTRODUCTION

Late relapses of early-stage germ cell tumors (GCTs) [both seminomatous and non-seminomatous GCTs (NSGCTs)] are rare and are typically defined as recurrence at least two years after completion of successful primary treatment[1]. Notably, most patients (-85%) with stage I seminoma are cured by radical orchiectomy[2]. As such, the detection of late relapse is challenging given the relative rarity of this phenomenon, and the fact that patients who have completed surveillance are usually not undergoing regular oncological workup nor imaging. While many treatment options do exist for a patient with late relapse of seminoma, surgery is typically the mainstay as these tumors are generally thought to be more chemo-resistant[1,11].

The purpose of this report is to describe the management of a patient with an early-stage pure seminoma who was subsequently identified to have a recurrence two decades later. A majority of the reports studying late relapse combine a mixed patient population of seminoma and NSGCTs, and focus more on clinical characteristics that may be associated with the late relapse. As such, we aim to provide a brief review of the literature not only focused on clinical factors and biology, but also the management of late recurrences specifically in pure seminoma. There is a paucity of data and treatment recommendations for this clinical entity, and a multidisciplinary approach emphasizing subspecialty expert consultation and patient education is imperative.

CASE PRESENTATION

Chief complaints

The patient complained of urinary frequency, nocturia and intermittent urinary stream 20 years after his initial diagnosis of stage I pure seminoma.

History of present illness

The patient is a 60 years old man who originally presented in 1998 to a different facility and underwent a right radical orchiectomy for a testicular mass. Pathology showed a stage I (pT1 N0 M0) pure seminoma. He received adjuvant radiation therapy to the periaortic fields and subsequently did well with no evidence of disease recurrence.

Twenty years later, he developed frequency of urination, nocturia, and intermittent urinary stream. He was found to have an abnormal digital rectal exam and underwent a trans-rectal ultrasound which showed an irregular prostate gland. He ultimately underwent a prostate biopsy which revealed pure seminoma in 10 out of 12 cores bilaterally (Figures 1-3).

History of past illness

Tumor markers after diagnosis of recurrence were as follows: Alpha fetoprotein (AFP) 3.4 and human chorionic gonadotropin (HCG) < 2. A left testicular ultrasound showed a spermatocele, but no obvious masses. Staging computed tomography (CT) scans of the chest, abdomen and pelvis demonstrated an enlarged prostate with extracapsular

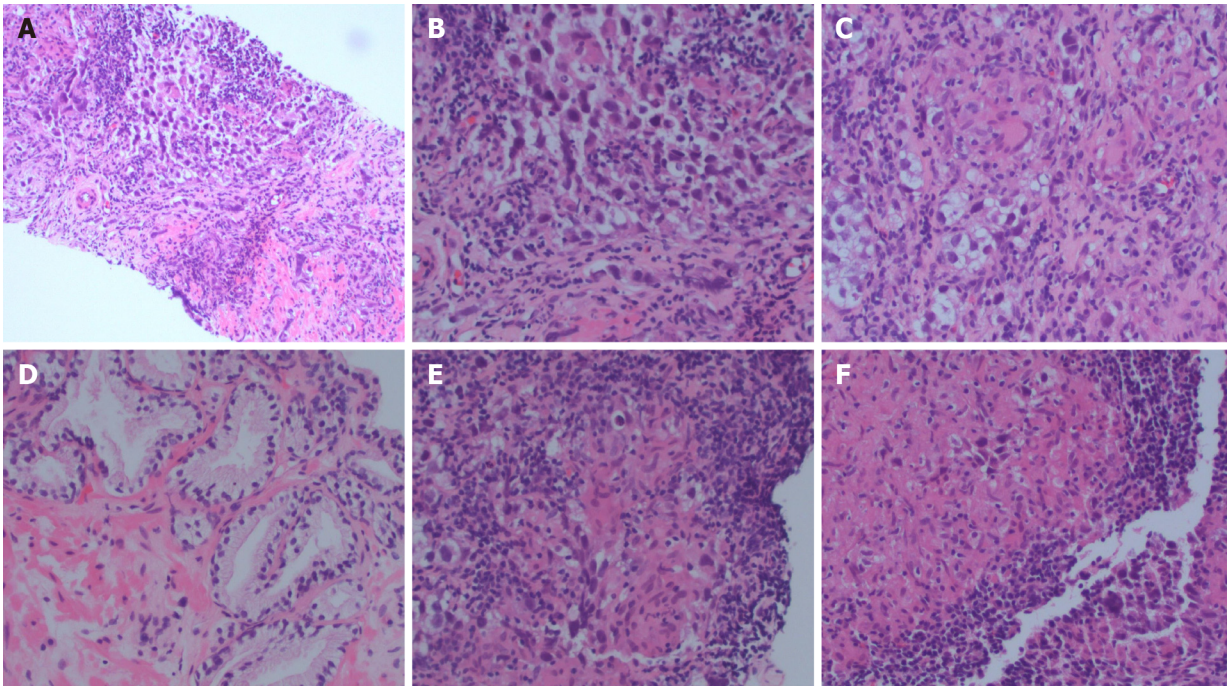


Figure 1 Representative hematoxylin and eosin sections. A: Tumor section; B: Tumor section; C: Granuloma; D: Benign prostate; E: Granuloma and lymphocytes; F: Granuloma.

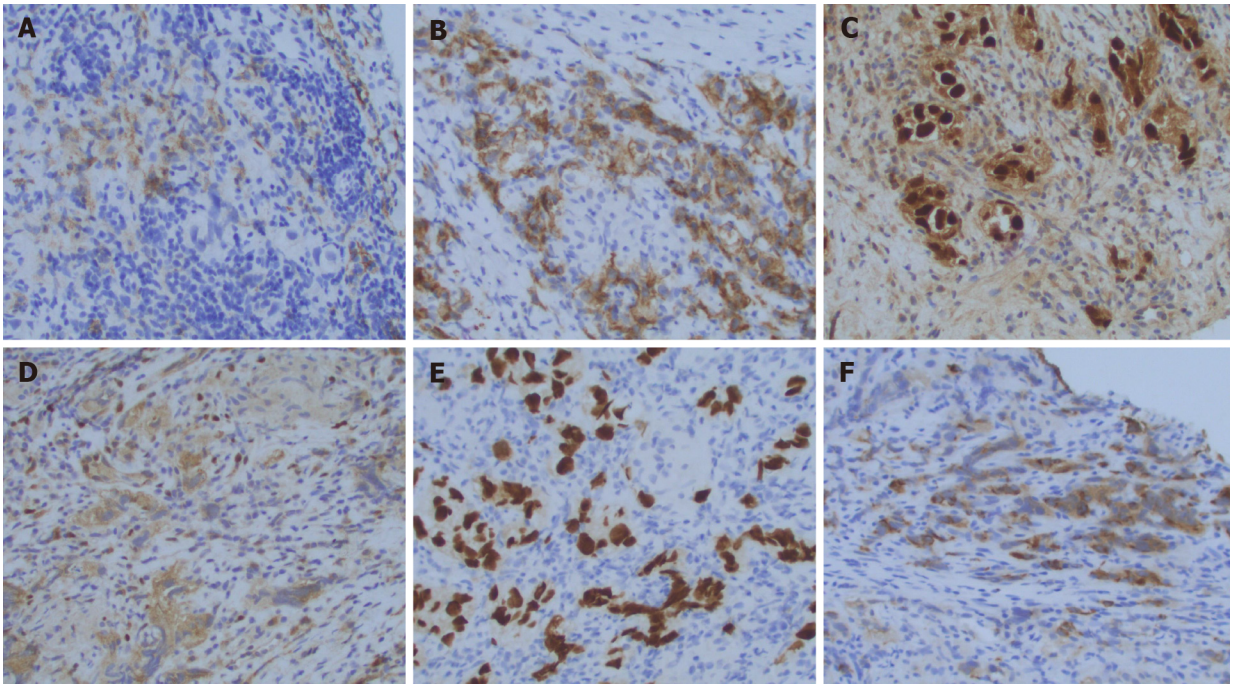


Figure 2 Representative immuno-stains. A: OCT4; B: PLAP; C: SALL4; D: EMA; E: Keratin; F: EMA.

extension, seminal vesicle invasion, possible tumor invasion into the rectal wall, an enhancing 1 cm periprostatic lymph node, and a 6 mm right pelvic sidewall lymph node. He was then referred to our tertiary care center for further management. His case was presented at a multidisciplinary genitourinary tumor board where further imaging was recommended.

He underwent a positron emission tomography (PET) scan which showed an fluorodeoxyglucose (FDG)- avid mass replacing the prostate gland with invasion into the right seminal vesicle, loss of the normal fat planes between the prostate, bladder and rectum concerning for extracapsular invasion, and an FDG-avid prominent right mesenteric lymph node consistent with nodal metastasis (Figure 4A).

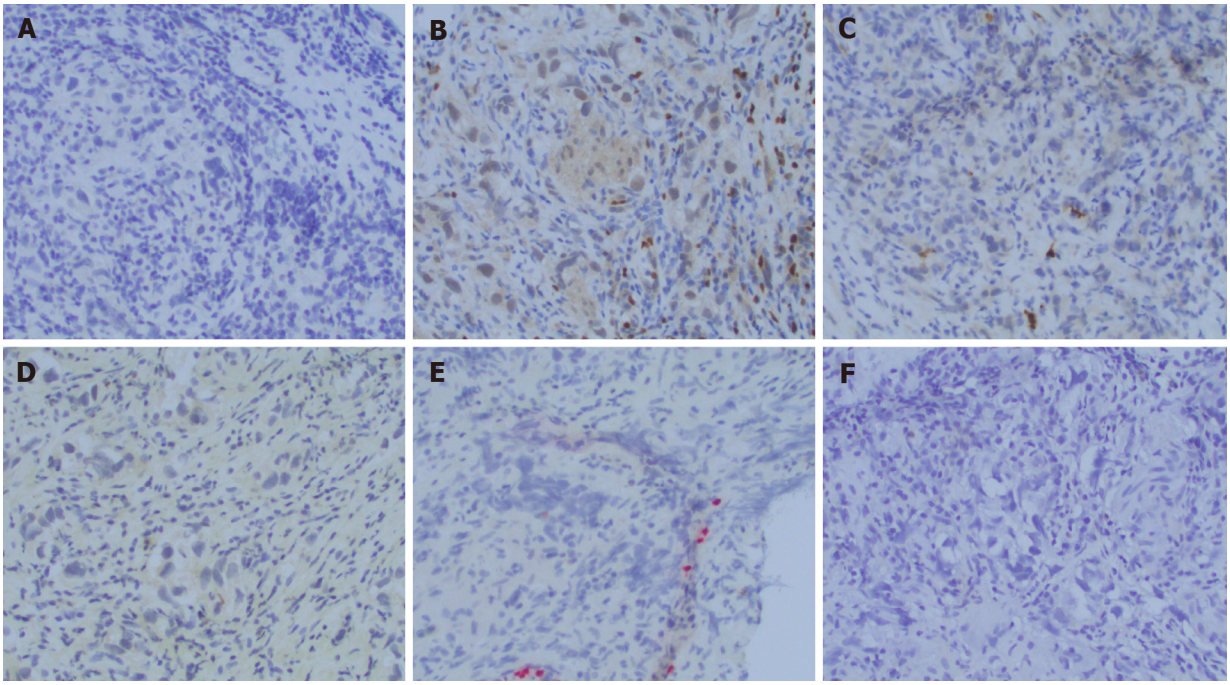


Figure 3 Representative immuno-stains. A: PAX8; B: PIN4; C: PSA; D: PSAP; E: S100; F: SOX10.

Personal and family history

Past medical history: Gastroesophageal reflux disease; post-nasal drip/recurrent sinusitis; hyperlipidemia - diet controlled; low testosterone; B12 deficiency.

Past surgical history: Left inguinal hernia repair - 2014; right orchiectomy - 1998; tonsillectomy - age 3.

Allergies: No known drug allergies.

Family history: Mother - lymphoma at age 94; maternal uncle - prostate cancer in his 80 s.

Social history: Patient denied history of smoking, alcohol or drug use. He currently lives with his wife. He has 3 children. He is a pilot.

Physical examination

Electrocorticography performance status: 0; pain score: 0/10; general: Alert and oriented to place, self, time, in no acute distress well-appearing; head, ears, eyes, nose, and throat: Normocephalic, atraumatic, anicteric sclera, moist mucous membranes, no oral lesions or thrush; chest: Chemotherapy port site clean; cardiovascular: Normal S1, S2, regular rate and rhythm, no murmurs/rubs/gallops; pulmonary: Clear to auscultation bilaterally, no crackles/wheezing/rales; abdomen: Soft, non-distended, non-tender, normal bowel sounds, no rebound/guarding, no hepatosplenomegaly; back: No costovertebral angle tenderness. No tenderness along the spine. Extremities: Warm, well perfused, no joint deformities. No cyanosis, clubbing, or edema.

Laboratory examinations

Tumor markers after diagnosis of recurrence were as follows: AFP 3.4 and HCG < 2.

Imaging examinations

Staging CT scans of the chest, abdomen and pelvis demonstrated an enlarged prostate with extracapsular extension, seminal vesicle invasion, possible tumor invasion into the rectal wall, an enhancing 1 cm periprostatic lymph node, and a 6 mm right pelvic sidewall lymph node.

Literature review: Late recurrences of early stage GCTs remains a rare clinical entity. This applies to both pure seminoma and NSGCTs. Studies detailed below have shed light on additional clinical factors associated with late recurrences aside from the clinical stage. In particular, there is an observation that NSGCTs have a higher

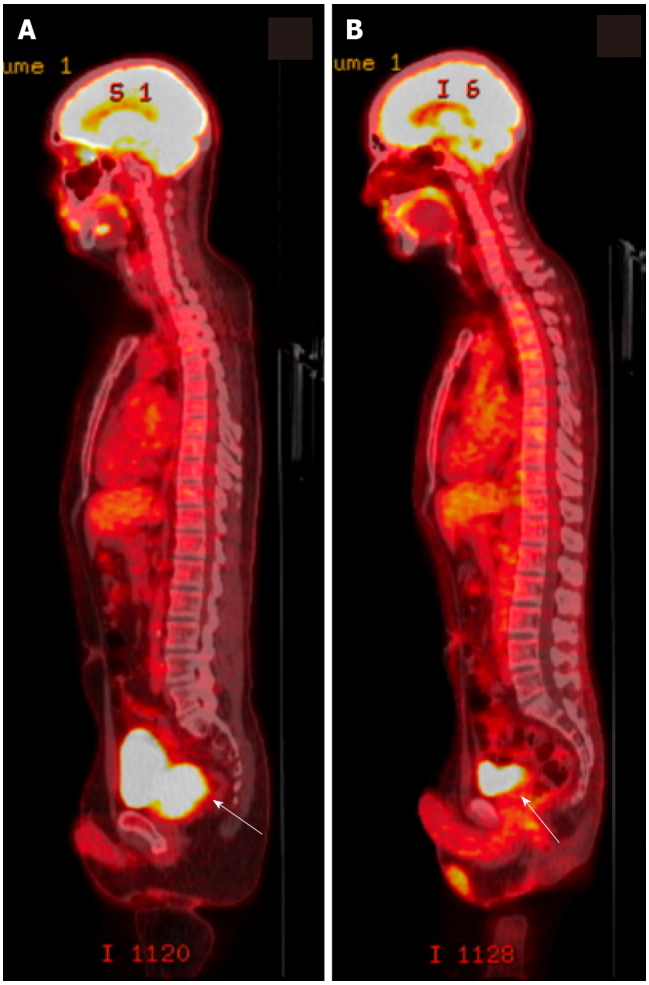


Figure 4 Representative positron emission tomography/computed tomography scans. A: Positron emission tomography/computed tomography (PET/CT) scan at diagnosis of relapse of pure seminoma. An fluorodeoxyglucose (FDG)-avid mass is shown (white arrow) replacing the prostate gland with invasion of the right seminal vesicle. Loss of the normal fat planes between the prostate, bladder and rectum is concerning for extracapsular invasion. An FDG-avid prominent right mesenteric lymph node consistent with nodal metastasis. B: PET/CT scan after completion of 4 cycles of etoposide and cisplatin. Significant decrease in size and activity of prostate mass and resolution of FDG-avid right mesenteric lymph node consistent with response to therapy (white arrow).

propensity to recur late than do pure seminomatous tumors[3]. While the treatment of a germ cell tumor recurrence can generally involve surgery or chemotherapy[2,3], a thorough assessment of tumor locality, patient functional status and prior treatments must be taken into account in detailing a personalized approach especially for curative intent.

A study of 1263 patients with late recurrence of GCTs has demonstrated that positive tumor markers at initial presentation and the presence of differentiated teratoma in post-chemotherapy surgical specimens are predictors of late recurrence. Additionally, late recurrences more than 5 years from initial therapy occurred mainly in patients with metastatic NSGCTs, whereas late recurrence was only seen in one case of metastatic seminoma and in one case of stage I NSGCT managed by surveillance. In patients with stage I seminoma treated with adjuvant radiation, the latest recurrence in this study was seen at 21 mo[3]. Interestingly, very late recurrences after 5 years in stage I and II seminoma treated with post- operative radiotherapy have been reported and were more common in bulky stage II disease[4].

The possibility of a new extra-gonadal primary tumor rather than a recurrence of the initial primary tumor for these patients must be seriously considered. Although ionizing radiation can have late effects mediating new tumorigenesis, the recurrence we have described has occurred outside of the radiated field, or "landing zone". As such, it is important to note that there have been very few cases reported of primary seminoma of the prostate. To our knowledge, this entity has only been described in at least 5 circumstances. Hashimoto *et al*[12] recently reported the case of a 54-year old man with difficulty urinating who was found to have an enlarged and irregular prostate, as in our patient. Core biopsy demonstrated cells positive for placental

alkaline phosphatase, CD117, periodic acid-Schiff, and negative for cytokeratin 7, leukocyte common antigen, vimentin, S100 protein, CD30, and prostate-specific antigen. The pathological exam was therefore consistent with seminoma. Notably, this patient had completely normal testes on ultrasound and physical exam with no other distant disease on imaging by chest and abdominal CT scan. He was treated with three cycles of bleomycin, etoposide, and cisplatin (BEP) chemotherapy and has responded well with complete remission of disease. Hence, although rarely described, the notion of an extra-gonadal germ cell tumor is certainly plausible and data does point towards platinum-sensitivity of these particular tumors[12,13].

A population based study by Oldenburg *et al*[5] evaluated 1123 patients with seminoma and 826 patients with non-seminoma, identifying twenty-five patients who developed a late relapse. Of note, four of ten initial seminoma patients relapsed with non-seminomatous pathology, one non-seminoma patient relapsed as a seminoma, and three relapses were noted to have undifferentiated carcinomas. This observation implies the significance of biological heterogeneity within these relapses, which necessitates a comprehensive multidisciplinary approach to their management. All ten of the seminoma patients in this study and seven of the eight non-seminoma patients received salvage chemotherapy regimens. The authors caution regarding the use of salvage chemotherapy in patients considered to have a late germ cell tumor relapse. Indeed, the role for surgical resection can lead to cure, as it did for eight of their non-seminoma patients. A suggested treatment strategy includes cisplatin-based chemotherapy followed by a complete resection of residual masses, if needed.

A descriptive analysis of 122 cases of malignant GCTs assessed the characteristics of late relapse in 50 patients with pure seminoma and 72 patients with non-seminoma. The time course to late relapse was 42 mo (range 25 to 276 mo) in seminoma and 64.5 mo (range 28 to 216 mo) in non-seminoma[6]. The wide ranges seen for both disease states are intriguing and implies that specific biological factors are at play to mediate relapses such as the one presented above. Another intriguing observation from the authors was that for late recurring seminoma, 80% of cases developed from stage 1 disease while for non-seminoma, 75% of late relapses developed from primary systemic disease.

Hence, in regards to pure seminoma relapse we postulate that cellular and molecular mechanisms governing self-renewal, senescence and even extracellular contacts could play a viable role. Factors that may be at play in the evolution of a late relapse include a derangement in genes regulating these processes, thereby resulting in a persistence of seminoma cancer stem cells. Gene expression studies have clearly documented that the human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in seminoma, supporting the role for such a mechanistic evolution of late relapse[7]. Whether these cancer progenitor cells can maintain a long-term quiescence followed by transformation to a more mitogenic phenotype is a matter of debate, but is certainly possible given rare but notable reports of pure seminoma late relapses.

Importantly, in comparing the pattern of relapses in pure seminoma, it is noted that both early (< 2 years) and late (> 2 years) relapses harbor similar anatomical distributions with few distinctions. In one retrospective review of 1060 stage I seminoma patients, it was observed that a single site of relapse with isolated para-aortic or pelvic nodes were the most predominant distributions. However, all late relapses after adjuvant radiotherapy occurred in the mediastinum, whereas early relapse sites were inguinal, supraclavicular and lung. There were no significant differences between the times to late relapse between patients who were managed with adjuvant radiation *vs* active surveillance[1]. This supports, to some degree, a similar biology in both early and late seminoma patients. While studies may be limited into understanding these mechanistic factors due to the small number of relapses, treatment decisions for late relapse can be made in similar fashion to early relapse. Guidance is provided in the National Comprehensive Cancer Network guidelines, with surgical excision preferred for solitary localized relapses as well as for early (< 2 years) relapses and systemic chemotherapy preferred for late (> 2 years) relapses where surgery is not feasible.

It is not well established what the ideal modality of therapy is for relapse after adjuvant radiotherapy, but as these tumors may be chemo-sensitive it is reasonable to consider systemic and localized approaches with a preference for surgery where possible. In the study previously mentioned, among 294 stage I pure seminoma patients treated with adjuvant radiotherapy after orchiectomy, 14 (5%) had a relapse after a median time of 15 mo (range 5-72 mo). Specifically, later relapses were noted after adjuvant radiotherapy in three patients, representing 1% of the entire adjuvant radiotherapy group (3/294) and 21% of the relapsed patients after adjuvant radiation (3/14). To date, our case is the only known report of relapsed stage I pure seminoma

occurring approximately 20 years after adjuvant radiation.

Nine of the patients in the review by Hosni *et al*[1] who relapsed after adjuvant radiation went on to receive salvage chemotherapy with platinum based regimens such as etoposide and cisplatin (EP). One patient with late relapse in the mediastinum received both chemotherapy and salvage radiation, whereas four patients with isolated inguinal early relapse either received salvage radiation (3 patients) or inguinal lymph node dissection (1 patient). Importantly, none of the patients in the adjuvant radiotherapy group developed a second relapse. Similarly, among patients with relapse of seminoma managed with active surveillance, none of the patients in the late relapse group developed a second relapse after either salvage radiotherapy or chemotherapy. Although not conclusive, this suggests a similar chemo-sensitivity profile for relapsed seminoma originally managed with active surveillance or adjuvant radiation.

While treatment of these relapses has curative potential, the outcomes for seminoma tend to be better than for non-seminoma. In the retrospective study by Dieckmann *et al* [6], thirty-seven out of 72 (51.3%) patients with non-seminoma failed to be cured in contrast to only 6 out of 48 (12.5%) patients with seminoma who failed to be cured. The use of surgery increased the chance of cure for these patients. This again supports a distinct and perhaps more chemo-resistant tumor biology for late relapses, more-so for patients who have previously received chemotherapy. Hence, while seminomas and other chemotherapy-naive cases may respond to chemotherapy, inclusion of experienced urological surgeons in care of these patients is crucial to determine the appropriate intervention for best possible outcomes.

FINAL DIAGNOSIS

Pure seminoma localized to prostate gland.

TREATMENT

The patient mentioned in this review subsequently was consented for treatment with EP and completed 4 cycles of therapy.

OUTCOME AND FOLLOW-UP

Response assessment with CT chest, abdomen and pelvis scans showed reduction in the size of the dominant prostate mass and resolution of the pelvic adenopathy. A restaging PET scan showed no FDG-avid lesions (Figure 4B). His case was again reviewed at the multidisciplinary tumor board, where review of imaging confirmed response to chemotherapy.

Subsequent scans continued to show complete response to therapy. At this point, the patient is over two years from completion of cancer-directed treatment for his recurrence of pure seminoma. Clinically, he is doing well without major functional limitations.

DISCUSSION

Our case represents an anomaly of late relapsed stage I pure seminoma due to its discovery after approximately 20 years since completion of adjuvant postoperative radiation therapy. He has maintained remission successfully after receipt of platinum-based chemotherapy. This observation sparks an intriguing discussion into the clinical factors, biological mechanisms, and treatment modalities surrounding this rare clinical entity. While a lack of concrete evidence for management is marred by the small number of late relapsed cases in pure seminoma, a few retrospective studies have shed light on patient outcomes after varied approaches inclusive of surgery, platinum-based chemotherapy and radiation.

The clinical vignette presented in our paper is also unique in that he has achieved a complete response to treatment with a less intensive chemotherapy regimen. Although the standard of therapy for non- pulmonary metastasis from testicular cancer generally

includes 4 cycles of BEP or etoposide, ifosfamide and cisplatin, this patient has received less than that and has been cured. Factors that may help explain this include the fact that his disease was managed with adjuvant radiation alone after orchiectomy, implying chemo-sensitive disease at relapse. Nonetheless, it remains unknown whether there are chemosensitivity differences between early and late relapsed seminomas managed with adjuvant radiation.

Studies suggested that serum levels of microRNA (miR)-371a-3p (the so-called M371 test) have a much higher sensitivity and specificity than the classic markers of testicular GCTs and are applicable toward both seminoma and non-seminoma. The M371 test outperforms the classic markers of GCT with both a sensitivity and a specificity greater than 90%. All histologic subgroups, except teratoma, express this marker. If validated, M371 can be used for detection of late recurrence without concern for excess radiation exposure over time[14]. Furthermore, in a case like ours, M371 can help to detect residual disease after completion of chemotherapy and offer early salvage surgery.

CONCLUSION

Whether his recurrence was a manifestation of true relapse from his original seminoma or a completely new primary is also unclear and not likely to be proven with the present scope of our knowledge. We have presented a literature review that details findings by other groups in regards to primary seminoma of the prostate. This clinical entity has demonstrated chemosensitivity to platinum-containing regimens and remains a plausible diagnosis to consider. Whether pure seminoma can manifest with a late relapse into the prostate is not known, but the theory of an extra-gonadal second primary tumor should be considered in this regard. Given our patient's pure seminomatous pathology and the fact that he has responded well to chemotherapy, the biology of his initial and relapsed disease states may share a common evolutionary tumorigenesis.

In summary, further research will be required to identify the ideal follow-up strategy to detect late relapses in pure seminoma and prognostic factors including more specific biological characteristics. The identification of patients at risk for late relapse is limited by its rarity and a lack of large-scale data[1,2]. The surveillance schedule for stage I seminoma patients has generated some controversy. While some experts suggest lifelong follow-up in contrast to 5-10 years of follow-up[8-10], a more personalized approach taking into account patient characteristics with risk-adapted projections can be considered.

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REVIEW

Management of genitourinary syndrome of menopause in breast cancer survivors: An update

Daniel María Lubián López

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Abstract

There is increasing attention about managing the adverse effects of adjuvant therapy (Chemotherapy and anti-estrogen treatment) for breast cancer survivors (BCSs). Vulvovaginal atrophy (VVA), caused by decreased levels of circulating estrogen to urogenital receptors, is commonly experienced by this patients. Women receiving antiestrogen therapy, specifically aromatase inhibitors, often suffer from vaginal dryness, itching, irritation, dyspareunia, and dysuria, collectively known as genitourinary syndrome of menopause (GSM), that it can in turn lead to pain, discomfort, impairment of sexual function and negatively impact on multiple domains of quality of life (QoL). The worsening of QoL in these patients due to GSM symptoms can lead to discontinuation of hormone adjuvant therapies and therefore must be addressed properly. The diagnosis of VVA is confirmed through patient-reported symptoms and gynecological examination of external structures, introitus, and vaginal mucosa. Systemic estrogen treatment is contraindicated in BCSs. In these patients, GSM may be prevented, reduced and managed in most cases but this requires early recognition and appropriate treatment, but it is normally undertreated by oncologists because of fear of cancer recurrence, specifically when considering treatment with vaginal estrogen therapy (VET) because of unknown levels of systemic absorption of estradiol. Lifestyle modifications and nonhormonal treatments (vaginal moisturizers, lubricants, and gels) are the first-line treatment for GSM both in healthy women as BCSs, but when these are not effective for symptom relief,

other options can be considered, such as VET, ospemifene, local androgens, intravaginal dehydroepiandrosterone (prasterone), or laser therapy (erbium or CO2 Laser). The present data suggest that these therapies are effective for VVA in BCSs; however, safety remains controversial and a there is a major concern with all of these treatments. We review current evidence for various nonpharmacologic and pharmacologic therapeutic modalities for GSM in BCSs and highlight the substantial gaps in the evidence for safe and effective therapies and the need for future research. We include recommendations for an approach to the management of GSM in women at high risk for breast cancer, women with estrogen-receptor positive breast cancers, women with triple-negative breast cancers, and women with metastatic disease.

Key Words: Genitourinary syndrome of menopause; Breast cancer survivors; Aromatase inhibitors; Vaginal moisturizers and lubricants; Vaginal estrogens; Laser

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Core Tip: Genitourinary syndrome of menopause (GSM) is commonly experienced by breast cancer survivors (BCSs) receiving antiestrogen therapy, specifically aromatase inhibitors. Vaginal dryness, itching, irritation and dyspareunia produce impairment of sexual function and negatively impact on the quality of life. Healthy women, and even more so BCSs, are reluctant to discuss this problem with their general practitioner or oncologist. Safety of vaginal estrogen therapy for management of GSM refractory to other nonhormonal treatment in BCSs has not been definitively established, and recommendations for use remain controversial. This review aims to summarize the clinical approach and emerging therapeutic alternatives, considering the efficacy and potencial adverse effects in this population.

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INTRODUCTION

Genitourinary syndrome of menopause

Vulvovaginal atrophy (VVA) (also referred to as vaginal atrophy, urogenital atrophy, or atrophic vaginitis) results from estrogen loss and is often associated with vulvovaginal complaints (*e.g.*, dryness, burning, dyspareunia) and less often with urinary frequency and recurrent bladder infections in menopausal patients[1].

In 2014, the new term Genitourinary Syndrome of Menopause (GSM) was introduced by the International Society for the Study of Women´s Sexual Health and the North American Menopause Society[2]. This term encompasses all of the atrophic symptoms patients may have in the vulvovaginal and bladder-urethral areas from loss of estrogen that occurs with menopause. The spectrum of adverse consequences makes long-term treatment essential in many patients, not only for relief of symptoms, but also for the more troublesome problems that may occur, such as, postcoital bleeding and recurrent urinary tract infections. This in turn can complicate the process of sexual arousal and achievement of orgasm, thus, leading to sexual dysfunction[3].

The prevalence of VVA, as confirmed by physical examination or pH measurement, has been described as between 69% and 98% in postmenopausal women[4,5], but it is even more frequent in young patients receiving anti-estrogenic or antineoplastic drugs for breast cancer[6]. These symptoms are often underdiagnosed and undertreated due to underreporting by the patients and limited awareness by professionals[7].

Genitourinary syndrome of menopause in breast cancer survivors

Improved treatment and screening for female breast cancer in developed countries has resulted in higher survival rates over the past two decades, with five-year survival rates currently as high as 90% (99% for women free of lymph node metastases in comparison to 84% if lymph nodes are positive)[8]. As a result, there are many millions of BCSs living in Western countries. In these countries, approximately 43% are ≥ 65 years old and 25% are ≤ 50 at diagnosis[9].

There are many definitions and phases of cancer survivorship. A cancer survivor is defined as any person with cancer, starting from the moment of diagnosis[10]. This is consistent with definitions from the National Coalition for Cancer Survivorship[11] and the National Cancer Institute[12]. The majority

of women with hormone receptor-positive early breast cancer are offered adjuvant endocrine therapy, including tamoxifen (TAM) or aromatase inhibitors (AIs), for at least 5 years to reduce the risk of recurrence and death. Practice guidelines now recommend up to 10 years of endocrine therapy and this has significant implications for compliance with treatment and ensuring that the adverse effects of treatment are adequately managed[13]. Many BCSs are still of premenopausal age and have the potential risk of receiving antineoplastic treatments that may affect ovarian function or anti-estrogenic treatments that mimic a postmenopausal state[14]. This hypoestrogenic state can lead to climacteric symptoms inducing significant alterations in their quality of life[15]. Many BCSs are already in a postmenopausal state at diagnosis, and the treatments used to treat BC worsens their basal hypoestrogenic state, which enhances associated problems. Due to dependence on estrogen, the vaginal epithelium can progress to VVA because of antiestrogenic treatments or natural menopause. Data suggest that long-term BCSs often report normalization of physical and emotional functioning but experience continued difficulty with sexual functioning and satisfaction for 5 or more years after treatment[16]. Women may be reluctant to bring up the topic of vaginal and sexual health and are often relieved when their clinicians begin a conversation. Many clinicians are uncertain about how to treat these symptoms in BCSs[17,18], and lack of treatment usually leads to a worsening of VVA over time [19].

Therefore, the management of breast cancer, the most common cancer in women, can lead to a variety of symptoms that can impair the quality of life (QoL) of many survivors. Although GSM affects more than 50% of the general population of postmenopausal women, it is even more prevalent in survivors of breast cancer (over 70%)[20-27], most of whom are undiagnosed and untreated[28-32]. This wide range of symptoms is a consequence of the decreased levels of circulating estrogen caused by ovarian failure induced by chemotherapy, bilateral oophorectomy performed in some patients, or by the use of endocrine therapies with AIs and selective estrogen receptor modulators (SERMs), such as TAM, in estrogen-receptor-positive BCs (ER+BCs), resulting in a faster transition to menopause[14,15].

Postmenopausal women treated with AIs may experience a severe form of vulvovaginal atrophy (VVA) with significantly higher rates of vaginal dryness (16.3%) and dyspareunia (17.8%) than women taking TAM (8.4% and 7.5%, respectively), as reported by The Arimidex, Tamoxifen, Alone or in Combination (ATAC) Adjuvant Breast Cancer Trial[33]. Data originating from a follow-up study of women with breast cancer no longer on therapy and 6 years on average after diagnosis showed that in the group of women aged 50 to 59, 72.8% reported vasomotor symptoms (VMS), and 80.8% reported sexual symptoms[20]. Another study with 97 BCSs reported moderate or severe symptoms of vaginal atrophy in 58% of patients on AIs and in 32% of those on TAM[34].

Several studies have suggested a deterioration of quality-of-life scores due to GSM in BCSs[35,36].

In a recent study, Lubián *et al*(2020)[37], observed a high prevalence of sexual inactivity among BCSs (47.6%) regardless of AI use. Patients with AI use presented a significantly higher prevalence of female sexual dysfunction (FSD), worse QoL, and greater anxiety.

We can conclude that AI users usually report more negative effects on sexual life than TAM users. These differences could be explained by some estrogenic effect of TAM over vaginal tissues in postmenopausal women, whereas AI can dramatically reduce plasma estradiol levels to less than 3 pmol/L[38].

Therefore, the aim of this review was to provide an update and overview of the most relevant and recent literature on therapeutic interventions with demonstrated efficacy in BCSs presenting GSM and the current evidence of their safety profiles. In addition, we provide recommendations for an approach to the management of GSM in women at high risk of breast cancer, women with estrogen receptor-positive breast cancers (ER+BCs), women with triple-negative breast cancers (TN BCs), and women with metastatic disease.

BIOLOGICAL CHANGES ASSOCIATED WITH GENITOURINARY SYNDROME OF MENOPAUSE IN BREAST CANCER SURVIVORS

Systemic loss of estrogen results in physiological and structural modifications within the genital structures and vaginal mucosa. Postmenopausal estrogen depletion induces changes that include a reduction in cervical gland secretions, deterioration of tissue, decrease in blood flow, loss of elasticity, thinning of tissue and epithelium, and an increase in pH[39-41]. The vaginal mucosa has reduced glycogen content and lack lactobacilli which convert glycogen into lactic acid to maintain a healthy vaginal pH in the range of 3.5-4.5. A reduction in lactic acid increases vaginal pH to the range of 5.0-7.5 [39,42]. Such atrophic changes predispose women to symptoms and vaginal infections, as the more basic pH environment is conducive for infection from pathogenic bacteria such as staphylococci and Group B streptococci[39]. In summary, atrophic vaginitis is a result of multiple changes in the external genitalia and internal mucosa with inflammation, overgrowth of pathogens, and a resultant acidic environment [41].

EFFECTS OF BREAST CANCER TREATMENT IN GENITOURINARY SYNDROME OF MENOPAUSE

The majority of women with BC receive systemic treatment (chemo-, hormonal- or biologic therapies) to reduce their risk of systemic disease. These therapies have significantly improved clinical outcomes but they can lead to biological changes that affect long-term vaginal health and impact quality of life in survivors. Pre- and postmenopausal women can experience symptoms of estrogen deprivation, including VVA[43], at higher rates than age-matched women without BC.

Chemotherapy

In a cohort of premenopausal BCSs receiving chemotherapy (CTx), vaginal dryness was reported by 23.4% of women[44]. CTx can promote a chemotherapy-induced ovarian failure (CIOF). The use of chemotherapy during the first year after the diagnosis of breast cancer significantly increases the risk of CIOF[45-47]. CIOF occurs secondary to CTx agents, which cause follicular destruction[45,47]. Consequently, decreases in the levels of estrogen and progesterone are observed. Forty- and fifty-year-old women undergoing CTx were found to have an increased risk of developing CIOF (40% and 90%, respectively) *vs* an increased risk of CIOF in healthy age-matched forty- and fifty-year-old women (< 5% and 20%, respectively)[47]. Postmenopausal women can also experience increased or recurrent symptoms of estrogen deprivation, depending on the amount of endogenous estrogen circulating in their system, including estrogen produced by the adrenal glands and estrogen stores in body fat.

Endocrine therapy

A total of 70%-80% of all BCSs are estrogen receptor-positive[48]. Endocrine therapy is extremely successful in suppressing circulating estrogen, an effect desired for efficacy. Endocrine therapies for the management of breast cancer include aromatase inhibitors (AIs), tamoxifen (TAM) (a selective estrogen receptor modulator-SERM-) and fulvestrant. These drugs can trigger the onset of VVA or exacerbate existing symptoms[49].

Aromatase inhibitors: AIs are frequently prescribed for postmenopausal breast cancer patients[50,51]. Multiple clinical trials have shown that AIs have better clinical outcomes in these patients than SERM; thus, they have become the standard of care[50,51]. These drugs inhibit the activity of the enzyme aromatase, which is utilized to convert androgens to estrogens[52], and significantly reduce plasma concentrations of estrogen from 20 pmol/L to 3 pmol/L or less[35]. These changes explain the commonly reported side effects as vaginal dryness and decreased libido[35]. The increasing use of AIs over SERMs (including for premenopausal women in conjunction with a gonadotropin-releasing hormone agonist- GnRH-a-) suggests that more women may experience new or increased atrophic vaginitis[35,50] than when TAM alone was used. Additionally, the recommended duration of these therapies has been extended from 5 to 10 years[53]. The severity of menopausal side effects, including VVA, may compromise compliance with AIs over time[54].

Tamoxifen: Tamoxifen (TAM) has been the most widely used traditional SERM and continues to be prescribed for premenopausal women with ER+BC[55]. TAM acts as an antagonist to estrogen positive breast cancer cells, although it often acts as an agonist to alfa estrogen receptors in the vagina. Hence TAM provides a quasi-estrogenic effect on the vulva and vagina and increases vaginal secretions without the presence of estrogen[35]. Due to its estrogenic effect, the incidence rate of vaginal dryness with TAM is only 8%, compared to 18% with AIs[35]. Therefore, this effect may inhibit the onset of atrophic vaginitis and actually improve existing vaginal dryness induced by CTx or menopause.

Fulvestrant: Fulvestrant is a competitive estrogen receptor antagonist that acts as an estrogen receptor downregulator, and is used in patients with metastatic BC[56]. Overall, six studies reported gynaecological toxicity (urinary tract infection, vulvovaginal dryness, vaginal haemorrhage, vaginitis, and pelvic pain) and no difference was observed between fulvestrant and control arms (RR 1.22, 95%CI 0.94 to 1.57; 2848 women; I^2 = 66%; P = 0.01; high□quality evidence)[57]. Because of its mechanism of action, rates of GSM may be less when compared with aromatase inhibitors[58] but higher than with tamoxifen therapy.

SYMPTOMS OF GENITOURINARY SYNDROME OF MENOPAUSE IN BREAST CANCER SURVIVORS

Vulvar and vaginal atrophy (VVA) are major components of the genitourinary syndrome of menopause (GSM). Symptoms of atrophic vaginitis include vaginal dryness, dyspareunia, irritation of genital skin, pruritus, burning, vaginal discharge, and soreness[19,41,59,60].

The related vaginal dryness and dyspareunia are chronic and progressively worsening conditions that affect quality of life (QoL) and intimate relationships in both healthy women and BCSs[36,61]. Atrophic vaginitis can disrupt sexual activity, and lead to problems such as pain with vaginal penetration (dyspareunia), decreased lubrication, and fear of pain with sexual activity[41]. Typical symptoms of atrophic vaginitis usually occur within 4-5 years after a woman's last menstrual cycle[62], but women who undergo menopause at an accelerated rate (CTx, surgical removal or radiation therapy of the ovaries or anti-estrogen therapy) can experience earlier onset of GSM[31,32].

Atrophic vaginitis as a survivorship issue impacts women of all ages. Premature menopause with associated symptoms in young breast cancer survivors may have a profound negative impact on quality of life secondary to sexuality and intimacy changes[58]. Regardless, women of all ages seek to preserve their sexual function and improve their sexual quality of life[63,64]. Many young women are at increased risk for premature menopause following adjuvant treatment for BC. These women must deal with consequences of menopause, including loss of fertility and physiologic symptoms such as night sweats, hot flashes, vaginal dryness, and weight gain. These symptoms can be particularly distressing for young women and can adversely affect both health-related and psychosocial quality of life (QoL). BSC patients in Eastern countries are younger and more likely to have related problems. While there are a wide range of pharmacologic and non-pharmacologic interventions available to help with these symptoms and in turn, improve QoL, there is little data available about the use and efficacy of these interventions in younger women who become menopausal as a result of their breast cancer treatment. Consequently, it is suggested that future studies should focus on this vulnerable population, with the goal of identifying effective strategies to relieve symptoms and improve QoL in young BCSs.

Atrophic vaginitis is prevalent in women with and without breast cancer. Complaints of vaginal dryness were 67% *vs* 49%, fear of pain with vaginal penetration 31% *vs* 19% and irritation from toilet tissue 21% *vs* 9%, respectively[42].

DIAGNOSIS AND ASSESSMENT OF GENITOURINARY SYNDROME OF MENOPAUSE IN BREAST CANCER SURVIVORS

Validating the effect of GSM on BCSs and the importance of seeking treatment for relieving symptoms and improving quality of life (QoL) is critical. Clinicians should explain the pathophysiology of GSM and review the potential genitourinary effects of breast cancer treatment[65,66]. Despite these bothersome symptoms, few women discuss them with their health-care professional or seek gynecological care[65], in part due to embarrassment, lack of knowledge, and an awareness of menopausal changes. The underdiagnosis and undertreatment of the condition lead to chronicity, disease progression and a considerable impact on women's daily living, despite the currently available therapeutic options. Therefore, it is imperative that clinicians ask both partnered and unpartnered patients about potential physical changes and alterations that can be associated with atrophic vaginitis [5,66,67].

The simplest approach for clinicians to detect sexual problems related to GSM is to start a conversation with the woman when it feels relevant during the encounter. Clinicians can also ask a direct screening question such as, 'Do you have any problems or concerns related to sex or pain with sexual activity?

There are readily available, simple, and effective tools for the identification of symptoms and assessment of the effect on QoL, including the Day-to-Day Impact of Vaginal Aging questionnaire[68] and the Sexual Symptom Checklist for Women After Cancer[69]. The structured approach to incorporating sexuality into clinical practice, devised specifically for patients with cancer, is The BETTER model (B - bringing up the topic, E - explaining the importance of sexuality, T - telling the patient about resources, T - addressing timing, E - educating about sexual side effects of treatment, and R - recording the discussion)[70].

It is important to gain a clear understanding of a woman's genitourinary symptoms and how they affect her QoL and intimate relationship. In addition to a complete history, which includes review of potential medications that might cause vaginal dryness, women with genitourinary complaints should undergo a physical examination before starting treatment. The examination should include visual external inspection, speculum, and bimanual pelvic examination as clinically relevant and to exclude other conditions that might mimic GSM, such as vaginitis, lichen sclerosus, or other dermatopathology.

During an examination, the woman and clinician can review areas of concern, and women can be educated regarding anatomy and instructed in the application of local therapies, using a hand mirror as needed[65].

When assessing women with GSM with a history of breast cancer, it is important for the clinician to identify factors that may affect decision-making[71]. These factors include balancing the risk of recurrence, which is influenced by the stage and grade of the cancer; presence of lymphovascular invasion; hormone-receptor status; use of endocrine therapy; and the time since diagnosis, with the severity of genitourinary symptoms, QoL, and efficacy of conservative therapies. Although data are lacking, based on the consensus recommendations from The North American Menopause Society and

The International Society for the Study of Women's Sexual Health, women with an overall lower risk of recurrence *vs* higher risk; with receptor-negative *vs* receptor-positive disease; using TAM *vs* AIs; and with severe symptoms and greater concerns about quality of life *vs* fewer symptoms and concerns may be better candidates for local hormone therapy[65].

Counseling patients with or at high risk of breast cancer about treatment options for GSM should include a shared decision-making approach employing the principles of informed consent[67]. The discussion about treatment options should include the mechanism of action, if known; potential adverse effects; current data regarding efficacy and safety; as well as the benefits and risks of each treatment option[72]. Clinicians should evaluate the woman's perceived need for treatment *vs* fears regarding breast cancer risk or recurrence risk. Additionally, consultation with a woman's oncology team is suggested[65,73,74]. A recent study found that 41% of breast oncologists refer BCSs to gynecologists for treatment of vulvovaginal atrophy, and 35% manage it independently. Seventy-one percent of oncologists mentioned that the main reason not to prescribe vaginal estrogen therapy is the probability of increased cancer recurrence[31].

Finally, when therapy is initiated, follow-up care should be arranged to ensure improvement in or resolution of symptoms and to assess compliance and barriers to treatment.

CURRENT TREATMENT OPTIONS FOR GENITOURINARY SYNDROME OF MENOPAUSE IN BREAST CANCER SURVIVORS

The growing awareness of quality-of-life issues in BCSs has done that management of GSM has been increasingly emphasized as a major problem that oncologist should know. The key is to determine the severity of the signs and symptoms of VVA and the degree of discomfort, tailoring the treatment to the individual needs of the patient.

The primary goal for the treatment of genitourinary symptoms is to improve or alleviate symptoms and to reverse the atrophic changes arising from estrogen deprivation[64,75]. Currently available treatments for GSM include both over-the-counter treatments (OTCs), such as nonhormonal vaginal moisturizers and lubricants, and prescription drugs, including local estrogen therapy (LET), intravaginal dehydroepiandrosterone (DHEA), or systemic therapies. These prescription drugs aim to treat the underlying condition of GSM, while OTC drugs only treat the symptoms, such as vaginal dryness, itching, burning and dyspareunia. Ideally, the optimal therapy for estrogendeficiency symptoms is systemic or topical estrogen administration[76]. However, estrogen may be contraindicated in women with a history of hormone receptor-positive breast cancer[77]. As a result, patients and their clinicians are sometimes reluctant to use topical estrogens[78], and effective alternative approaches with nonhormonal lubricants and moisturizers are needed.

Clinician reluctance to treat may reflect the paucity of evidence regarding the safety of currently available therapies for GSM in women with or at high risk of breast cancer[79]. The unintended consequence is that women are driven to untested and non-FDA-approved therapies. In women with a history of breast cancer, the decision of how to treat GSM depends on many factors, including receptor status, genetic characteristics, extent of disease time interval since diagnosis, and response to prior therapies. Care for women with or at high risk of breast cancer would be enhanced by an evidence-based compilation of available GSM treatment options, along with a discussion of the limitations in the science concerning risks specific to this population[16,29,80].

According to international guidelines, nonhormonal therapies are the first-line treatment for mild-moderate VVA. Therefore, survivorship guidelines from the American Society of Clinical Oncology (ASCO)/American Cancer Society (ACS)[81] and the North American Menopause Society[82] recommend the use of nonhormonal therapies, specifically water-or silicone-based lubricants and vaginal moisturizers, as first-line therapy for dyspareunia and vaginal dryness in BCSs. Severe signs or symptoms usually require pharmacological management (local hormonal therapy)[83].

Treatment of GSM in BCSs remains an area of unmet need. Vaginal estrogen is not generally advised, particularly for those on AIs, because it is absorbed in small amounts and raises blood levels within the normal postmenopausal period and could potentially stimulate occult breast cancer cells. The safety of intravaginal dehydroepiandrosterone and oral ospemiphene after breast cancer has not been established. Vaginal laser therapy is being used for VVA in BCSs, but efficacy and safety data from clinical trials are lacking. Therapies such as lasofoxifene, neurokinin B inhibitors and stellate ganglion blockade are undergoing development[84]. To date, there is no consensus on how to treat moderate-severe GSM in BCSs.

Lifestyle modifications

As a first-line therapy for mild symptoms, lifestyle modifications (healthy diet, smoking cessation, losing weight, maintaining adequate vitamin D and calcium levels, limiting alcohol and regular physical activity) may be sufficient[85,86]. Smoking cessation may decrease the atrophic effects due to increased capillary refill[87], while weight loss of 5-10% of total body weight has been shown to improve urinary incontinence (UI)[88].

Sexual activity maintenance should also be encouraged. Regular coitus and masturbation can increase blood flow to the genital area, helping to keep this tissue healthy and maintaining normal vaginal pH [18,39,89]. Vaginal penetration with lubricated fingers or vaginal dilators may prevent fibrotic changes. Scented hygiene products should be avoided as they may reduce normal vaginal flora[18].

Control of underlying medical conditions

Women with pre-existing comorbidities (diabetes, obesity or hypertension) are more likely to develop VVA, UI and sexual dysfunction[90]. Thus, optimal management of these comorbidities may help to improve genitourinary and sexual health[90,91]. Furthermore, underlying depression treatment has been shown to improve both sexual functioning and quality of life (QoL) in breast cancer patients[92]. If an antidepressant is prescribed, an option that may be appropriate for TAM users is the SNRI venlafaxine, which increased libido in women with early breast cancer without interfering with the metabolism of TAM[93], or the SNRI desvenlafaxine[94]. Other options, given their minimal effect on sexual function and no appreciable inhibitory effect on CYP2D6, are mirtazapine and agomelatine[95, 96].

Complementary and alternative therapies

Between 48%-83% of BCSs use at least one type of complementary or alternative therapy following diagnosis[97,98] despite limited evidence of the effectiveness/toxicity of these therapies in managing GSM in these patients[98-101]. This is important because at least half of breast cancer patients do not discuss their use of an alternative therapy with their clinicians[98,101]. Patients who use an alternative or complementary treatment should check with the manufacturer regarding whether the product contains estrogen or other hormones.

'Natural products': BCSs are very attracted to 'natural' products and generally have the impression that they are less toxic than conventional medicine[102]. In a clinical trial, dietary supplements with soy, black cohosh, and some other herbs did not show superiority over placebo in relieving a range of genitourinary symptoms[103].

Also, the safety of many of these products is unknown and there may be possible interactions with TAM and unknown effects on breast cancer cells[102]. Indeed, there is increasing concern about the lack of rigorous quality-control measures with regard to purity and levels of 'active compound' by some manufactures of herbal medicines as pointed out by the North American Menopause Society (NAMS) [103]. Clearly, there is the need in the long-term to investigate adequately designed RCTs to determine whether these products are of any help to breast cancer patients experiencing GSM-related symptoms. Most importantly, a risk assessment should be performed to help define their safety. Until such evidence- based data are available, their use merits caution[101].

Acupuncture and cognitive behavioral therapies: Stress management may be helpful in decreasing the anxiety associated with fear of painful intercourse[104,105]. There is very limited clinical data on the efficacy of acupuncture and behavioral interventions in the management of GSM in healthy women and none in BCSs. Acupuncture can decrease the urogenital subscale scores on the Menopause Rating Scale [106] and improve bladder capacity, urgency and frequency[107], but there are no evidence-based data [108]. In an RCT, cognitive behavioral therapy, physical exercise and a combination of both significantly decreased urinary symptoms and increased sexual activity in BCSs with treatment-induced menopausal symptoms compared to controls[109]. However, further studies are needed to recommend these treatments in BCSs.

Nonhormonal vaginal treatments: Lifestyle modification measures alone are usually insufficient to significantly improve atrophic vaginitis in BCSs. Nonhomonal vaginal therapies may provide additional treatment options to alleviate or improve vaginal dryness, irritation and itching by increasing vaginal moisture[104,105,110]. Local nonhormonal therapies include vaginal moisturizers, vaginal lubricants, vaginal pH-Balance Gel, vaginal autologous platelet-rich plasma (A-PRP) and avoidance of perfumed soaps and toilet tissue, rubber products, synthetic garments including panties, and certain fabric softeners[59]. Clinicians treating BCSs need to inquire about type and severity of their symptoms and the individual women's expectations of treatment. So, if the most important concern for a woman is pain during intercourse, lubricants during sexual intimacy[110] may be recommended[78]. Additionally, adding vaginal moisturizers on a regular basis may promote hydration of the epithelium, providing more long-term (a few d) relief of symptoms such as itching, irritation and dyspareunia[111]. However, these therapies are not able to reverse atrophy once it occurs, and they may not completely solve the problem, especially in women with severe symptoms. Nevertheless, the evidence to support the efficacy of these formulations is limited (level II)[111]. Carter *et al*[112] (2011) developed a patient handout that summarizes how to best use vaginal lubricants, moisturizers and pelvic floor exercises.

According to international guidelines, nonhormonal therapies are the first-line treatment for mild-moderate VVA in both healthy women and BCSs[113]. According to a systematic review carried out this year (2021) by Mension *et al*[114] about current treatment options for genitourinary syndrome of menopause in BCSs, there are 10 studies related to nonhormonal options (excluding laser therapy) (4 prospective studies and 6 randomized controlled trials (RCTs)) (Table 1).

Table 1 Non-hormonal treatments (Classic moisturizers and lubricants and innovative preparations) in breast cancer survivors: Summary of studies and their outcomes

Ref.	Yr	n[1]	Design	Treatment	Conclusion
Loprinzi *et al* [119]	1997	45	A double-blind, crossover, randomized clinical trial	Vaginal lubricating preparation, (Replens®)	Both Replens and the placebo appear to substantially ameliorate vaginal dryness and dyspareunia in breast cancer survivors
Lee *et al*[129]	2011	44 *vs* 42	Randomised controlled trial, double blinded	pH balanced gel *vs* placebo for 12 wk	Vaginal pH balanced gel could relieve vaginal symptoms
Juraskova *et al* [137]	2013	25	Prospective, observational study	polycarbophil-based vaginal moisturizer + olive oil as a lubricant during intercourse	Significant improvements in dyspareunia, sexual function, and quality of life over time
Goetsch *et al* [130,131]	2014 2015	46	Double-blind rct	4% aqueous lidocaine *vs* saline	Significative and safe reduction in dyspareunia
Hickey *et al* [128]	2016		In a single-center, randomized, double-blind, ab/ba crossover design	Water- *vs* silicone-based lubricants	Total sexual discomfort was lower after use of silicone-based lubricant than water-based
Juliato *et al* [126]	2017	25 *vs* 25	Randomised trial	Polyacrylic acid *vs* lubricant	Polyacrylic acid was superior to lubricant
Marschalek *et al*[136]	2017	11 *vs* 11	Randomised controlled trial, double blinded pilot study	Vaginal lactobacillus capsules *vs* placebo	Lactobacillus improves microbiota in BCSs
Hersant *et al* [139]	2018	20	Prospective, comparative (before/after) pilot study	A-PRP and evaluated at 0,1,3 and 6 mo	A-PRP improves vaginal mucosa in 6 mo treatment according VHI criteria
Chatsiproios *et al*[125]	2019	128	Open, prospective, multicentre, observational study.	oil-in-water emulsion during 28 d	This treatment seems to improve VVA symptoms with a short treatment
Carter *et al*[122]	2021	101	Single-arm, prospective longitudinal trial	Hyaluronic acid (HLA) vaginal gel for 12 wk	HLA moisturization improved vulvovaginal health/sexual function of cancer survivors

[1]Cases *vs* control/placebo/other treatment. BC: Breast Cancer, BCSs: Breast cancer survivors; A-PRP: Autologous platelet-rich plasma; VHI: Vaginal Health Index.

Vaginal moisturizers: Vaginal moisturizers intend to replace normal vaginal secretions and maintain tissue integrity, elasticity, and pliability and should be used on a regular basis independent of sexual activity[105].

Although there is limited data to support the efficacy of over-the-counter products[113,114], vaginal moisturizers and lubricants are considered the initial and mainstay treatment options for GSM in women with breast cancer[40], and they are widely used[18], particularly for women with mild symptoms and those who want to avoid local estrogens[18,40]. However, these products are poorly differentiated and characterized[112].

In GSM induced by oncology treatment and with menopausal hormone treatment (MHT) contraindications, everyday use of a paraben-free with acidic pH and low osmolality vaginal moisturizer is indicated[115].

A 12-wk multicenter RCT compared vaginal estradiol tablets *vs* vaginal moisturizers *vs* placebo. All three groups demonstrated similar reductions in the most bothersome symptoms, with no evidence for the superiority of vaginal moisturizers or 10-mcg vaginal estradiol tablets over placebo gel[116].

In some studies, polycarbophil-based nonhormonal moisturizers (Replens©) were demonstrated to be more effective than lubricants and even as effective as vaginal estrogen creams in improving vaginal moisture, fluid volume, pH, and elasticity, as well as reducing dryness, itching, and dyspareunia[117,118]. This effect is not sustained over time unless the moisturizer is used on a regular basis[117,118]. However, in a double-blind, crossover randomized controlled trial (RCT) assessing 45 BCSs with a history of vaginal dryness or itching, a polycarbophil-based vaginal moisturizer was no more effective than placebo in relieving vaginal dryness and dyspareunia[119].

Vaginal hyaluronic acid: Another non-hormonal option is hyaluronic acid vaginal gel. Hyaluronic acid (HLA) releases water molecules into the tissue, thus alleviating the dry state of the vagina and also plays a role in tissue repair. RCTs comparing hyaluronic acid with estrogen cream in postmenopausal women found that both significantly improved clinical symptoms of vaginal dryness in women without breast cancer[120]. Moreover, Jokar *et al*[121], in an RCT, found that improvement in urinary

incontinence, dryness, the maturation index, and composite score of vaginal symptoms was better in the HLA group than in the estrogen cream group.

In a very recent prospective study (2021) including 101 postmenopausal patients with hormone receptor-positive breast and endometrial cancer, treatment with a hyaluronic acid vaginal gel for 12 wk. improved the vulvovaginal health/sexual function of cancer survivors. While HLA administration at 1-2×/week is recommended for women in natural menopause, a 3-5×/week schedule appeared to be more effective for symptom relief in cancer survivors[122]. One year earlier, these authors demonstrated that the HLA-based gel improved vulvovaginal health and sexual function in 43 endometrial cancer survivors in their perceived symptoms and clinical exam outcomes[123].

Vaginal lubricants: Lubricants (water-, glycerin- or silicone-based products) are designed to be applied during sexual activity, with direct application to the external genitalia, vaginal introitus, and vaginal mucosa to reduce friction and discomfort. Lubricants are shorter acting than moisturizers and have no effect on vaginal pH or underlying moisture content due to the ingredients and manufacturing of the product. The World Health Organization (WHO) suggests the use of lubricants with an osmolality of < 380 mOsm/kg, but most available lubricants do not list osmolality on the product label and have higher osmolality associated with mucosal irritation[117]. Lubricants with pH levels ≤ 3.0 are considered unacceptable for human use, given their association with vaginal irritation in animal models[124]. Additives (parabens, glycerin, flavors, and spermicides) should be avoided because they may irritate vaginal and vulvar tissues.

In dyspareunia induced by an oncology treatment and with MHT contraindicate, use of a paraben-free vaginal with acid pH and low osmolality lubricant during sexual intercourse is indicated[117].

In a prospective multicenter observational study by Chatsiproios *et al*[125], who evaluated the effect of an oil-in-water emulsion for 28 d in 128 patients diagnosed with BC and managed with chemotherapy or hormonal therapy, the authors concluded that there were improvements in symptom frequency after treatment and that the cream was an effective and safe nonhormonal topical option in the treatment of vulvovaginal dryness symptoms in patients undergoing breast cancer treatment. However, the study duration and follow-up time during 4 wk. as well as the non-randomized trial design are limitations of the study. The quality assessment (Qa) was fair.

Polyacrylic acid appeared to be superior to lubricants according to a randomized trial conducted by Juliato *et al*[126]. Fifty-two women (25 polyacrylic acid *vs* 25 Lubricant) with breast cancer who were being treated with TAM and who complained of vaginal dryness were evaluated. There was improvement in the female sexual function index (FSFI) after both treatments.

The polyacrylic acid group showed a decrease in sexual dysfunction from 96% to 24% ($P < 0.0001$) and the lubricant group showed a decrease from 88.9% to 55.6% ($P = 0.0027$). Polyacrylic acid was superior to the lubricant in treating sexual dysfunction [Qa = Good]. Products that contain glycerin may provide improved comfort during sexual activity as compared to water-based products. Silicone-based products may last longer than either water- or glycerin-based products. The ideal combination is to insert polycarbophil gels intravaginally 4-7 times per week, and utilize generous amounts of a glycerin-based vaginal lubricant before and during sexual activity[43]. This combination not reverse vaginal atrophy, but may provide additional short-term comfort during sexual activity.

Although the use of water-based lubricants are advised in cancer survivors[127], recent findings suggested that silicone-based lubricants may be more effective in treating discomfort during sexual activity in postmenopausal women with breast cancer, although both therapies were unlikely to reduce sexually-related distress[128].

Vaginal pH-balanced gel: A double-blinded RCT using vaginal pH-balanced gel in postmenopausal BCSs suffering from atrophic vaginitis was conducted in 2011. A total of 88 BCSs were randomly assigned to receive either pH-balanced gel (with lactic acid, pH 4 to 7.2) or placebo. The treatment was used three times per week for 12 wk. The pH-balanced gel provided significant ($P = 0.001$) improvements in vaginal dryness and dyspareunia compared to placebo and was effective in reducing the vaginal pH ($P < 0.001$). In addition, the pH-balanced gel enhanced vaginal maturation index ($P < 0.001$) and vaginal health index ($P = 0.002$). No significant difference in adverse events between the two gels was noted with minimal side effects (mild irritation during the first four wk. of therapy administration). These findings suggest that vaginal pH-balanced gel is an alternative option to alleviate vulvovaginal symptoms in symptomatic patients and can ultimately protect against vaginal colonization by nonvaginal microflora, which predisposes women to vaginal infections and UTIs[129].

Vulvar lidocaine: For women with pain isolated at the vulvar vestibule with penetration, topical lidocaine may provide relief[130]. A double-blinded RCT evaluating 4% aqueous lidocaine *vs* saline applied with a cotton ball to the vestibule for 3 minutes before vaginal penetration for insertional dyspareunia in 46 postmenopausal survivors of breast cancer with severe GSM for 4 wk. showed a significant reduction in dyspareunia of 88% *vs* 33% with saline ($P = 0.007$) and may be considered a safe option for painful intercourse in BCSs[131].

Vitamins E and D: Vaginal application of vitamin E capsules before intercourse increases vaginal

lubrication and provides some atrophic-related symptom relief[100,132]. Oral vitamin D supplementation may help squamous maturation of the vaginal epithelium [133], but there were no significant improvements in vulvovaginal symptoms or pH[134]. The available evidence does not support the use of vitamins for relief of genitourinary symptoms[3].

Vaginal/oral probiotics: Oral and vaginal probiotics to change the vaginal microbiota could possibly be beneficial for the treatment of symptoms of GSM, but comprehensive trials are needed for validation [135]. A prospective, randomized, double-blinded trial (2017) evaluating new options, such as capsules including Lactobacillus, for the maintenance of the vaginal microbiota in women with breast cancer during chemotherapy was shown to be useful. The quality assessment (Qa) was good[136].

Olive oil, vaginal exercise, and moisturizer: The OVERcome study (Olive Oil, Vaginal Exercise, and Moisturizer) resulted in significant improvements in quality of life, sexual function, and dyspareunia *(P* < 0.001). Maximal benefits were noted after 12 wk. of intervention. However, the quality of this study was very poor, with only 25 breast cancer patients recruited[137]. There is concern regarding the use of natural oils (*e.g.*, olive and coconut) for lubrication because these products are associated with vaginal infections[138].

Vaginal autologous platelet-rich plasma: Other recent options (2018) include autologous platelet-rich plasma (A-PRP), which was demonstrated in 20 patients with diagnosed BC, with a median age of 60.8 years, to improve vaginal mucosa following 6 mo. of treatment according to the Vaginal Health Index (10.7 to 20.75; $P < 0.0001$) in a prospective, comparative (before/after) pilot study [Qa = Fair][139].

Vaginal dilators: In addition to the use of vaginal moisturizers and lubricants, regular use of vaginal dilators has been recommended for symptomatic vaginal atrophy[40] and has been found to reduce pain with vaginal penetration by improving vaginal elasticity[140].

Patients should be counseled regarding the use of vaginal dilators of graduated sizes (either by themselves or with their partners) to promote stretching of vaginal tissues. Vibratory stimulation, applied either to the vagina or directly to the clitoris, has also been studied as a modality to reduce pain with vaginal penetration[141]. Finally, pelvic floor therapy under the care of a physical therapist trained in the management of pelvic floor disorders is recommended to reduce pain with vaginal penetration; physical therapists may also be helpful in the education of vaginal dilator therapy[142,143].

In summary, among nonhormonal therapies there are multiple options to treat symptoms of dyspareunia and daily wellbeing. However, these compounds do not reverse atrophy, and neither do they improve vaginal epithelium characteristics, and hence, the improvement observed is temporary and short term. These therapies are usually lubricants and moisturizer agents composed by non-hormonal substances, mainly based on water, silicone or vegetable oil. Water-based agents have fewer side effects compared to oil-based products[29].

The main limitation of non-hormonal therapies is the short-term efficacy. Among the trials included in this systematic review, 85% described efficacy with a 30-day follow-up or less. Further studies evaluating longer follow-up periods would be of interest.

The lack of data on hormonal receptor status and adjuvant treatments in the studies reviewed, as well as the absence of hormone levels and information about BC recurrence after treatment did not allow these trials to make conclusions in relation to safety. Nonetheless, from general population trials, it can be extrapolated that there is a low risk of potential side effects from nonhormonal therapies used for climacteric symptoms[144].

Hormonal treatment

When nonhormonal methods fail in symptomatic survivors, local short-term hormonal therapy may be considered, following appropriate counseling and assessment of riskbenefits balance[145].

Whether the antiestrogen effect is induced by SERMs (tamoxifen or raloxifene) or by lowering endogenous estrogen production (e.g., bilateral oophorectomy, ovarian suppression with GnRH agonists, use of AIs), the goal of reducing the estrogen environment to lower breast cancer risk has remained the same. Therefore, both systemic and local estrogen-based treatments are controversial or discouraged for women with a history of or at high risk of breast cancer[81].

Systemic estrogen or estrogen/progestogen treatment: Healthy women can expect up to a 75% reduction in frequency and 87% reduction in severity of symptoms of GSM when prescribed systemic estrogen[146].

To date, there is a consensus in the literature that estrogen administration in BCSs or in women at high risk of BC should only be prescribed topically, since systemic administration has been shown to increase the risk of BC occurrence or recurrence[147] and is formally contraindicated by international guidelines (International Menopause Society-IMS)[148]. This consensus is supported by the results of two Swedish RCTs of systemic hormone therapy (HT) in survivors of early breast cancer. In 2001, the pivotal HABITS study (Hormonal Replacement Therapy After Breast Cancer—Is It Safe?) was conducted by Holmberg *et al*[149]. The authors studied the effects of systemic hormone replacement

therapy on breast cancer recurrence among Scandinavian BCSs. A total of 434 women who had completed treatment of stage 0 to II breast cancer with symptoms of menopause were randomly assigned to receiving cyclic or continuous combination hormonal therapy (HT) with estradiol hemihydrates and norethisterone acetate. In 2003, it was prematurely stopped after a median follow-up of 2.1 years because of a statistically significant increased breast cancer recurrence in the HT group *vs* non- HT group (HR of 3.5 (95%CI, 1.5 to 8.1). The HABITS study showed that BCSs who received HT not only had a higher risk of breast cancer recurrence but also a higher risk of adverse events than BCS patients receiving the best symptomatic treatment without hormones. A four-year follow-up of the study sample found that women in the hormone replacement therapy group had twice the rate of a breast cancer event as compared to the control group (HR = 2.4).

Another RCT (The Stockholm trial)[150] also studied BCSs (n = 378) randomized to hormonal therapy or nonhormonal therapy for symptoms related to lack of estrogen. Hormonal therapy included cyclic estradiol and medroxyprogesterone acetate or estradiol valerate alone or non-HT. The trial, similar to HABITS, prematurely ceased due to safety concerns of breast cancer recurrence. In contrast to the HABITS trial, the Stockholm trial did not actually find an increase in breast cancer recurrence after a median follow-up period of 4.1 years in the hormone replacement study arm (HR = 0.82; 95%CI, 0.35 to 1.9). However, there was statistically significant (P = 0.02) heterogeneity in the rate of recurrence between the two studies, and the Stockholm trial investigators concluded that HT may be associated with the recurrence of breast cancer. On the basis of these studies, HT is currently contraindicated in BCSs because of an increased risk of breast cancer recurrence or new primary development.

Tibolone: Tibolone is a synthetic steroid that, after absorption, is rapidly converted to three active metabolites (with weak estrogenic, progesterogenic, and androgenic properties) that bind to estrogen receptors in the vagina. In a nonrandomized, open-label study of healthy postmenopausal women (n = 113), the use of tibolone over six years reversed vaginal atrophy and improved symptoms[151]. Later, tibolone was shown to improve vaginal dryness and may have a favorable effect on sexual function [152].

The Livial Intervention Following Breast Cancer; Efficacy, Recurrence and Tolerability Endpoints (LIBERATE) trial has been the most important study of the relationship between tibolone and BCSs[153, 154]. This prospective randomized placebo controlled study was conducted to evaluate the safety of tibolone in BCSs (n = 3098) (1556 in the tibolone group and 1542 in the placebo group). The study showed that tibolone (2.5 mg) was effective in improving menopausal symptoms, including vaginal dryness and enhanced QoL in BCSs, but the trial was terminated early due to an increase in breast cancer-related events in the tibolone arm. After a median follow-up of 3.1 years, 237 of 1556 (15.2%) women on tibolone had a significantly increased risk of breast cancer recurrence compared with 165 of 1542 (10.7%) on placebo (HR 1.40 [95%CI 1.14-1.70]; P = 0.001). Therefore, the use of tibolone was contraindicated after breast cancer, with the authors warning that any off-label use incurred a now proven risk[154]. The IMS has supported this recommendation[155].

Bazedoxifene: Two RCTs provided data for consideration of bazedoxifene, a Selective Estrogen Receptor Modulators (SERM), combined with conjugated equine estrogens (BZA/CE) to treat symptoms of postmenopausal vulvovaginal atrophy. In the first study, healthy, postmenopausal, nonhysterectomized women (n = 652) with symptoms of moderate to severe vulvar/vaginal atrophy were randomized to different doses with BZA/CE or placebo. Treatment with BZA/CE for 12 wk. was shown to significantly improve sexual function and quality-of-life measures in symptomatic healthy postmenopausal women. As a single agent, bazedoxifene alone was not effective in relieving vulvovaginal symptoms. It remains unknown whether this combination will be safe and well tolerated in women with breast cancer[156].

Kagan *et al*[157] showed similar results in another RCT in which they concluded that BZA/CE was effective in treating moderate to severe VVA and vaginal symptoms. Because no studies have investigated drug safety in BCSs, it should not be recommended in these women.

Ospemifene: Ospemifene is a systemically administered SERM with therapeutic options for women with moderate-severe VVA and estrogen contraindications[82]. Comprehensive studies of ospemifene demonstrated an improvement in the vaginal maturation index and relief of most VVA symptoms in healthy women, as well as improvement in measures of sexual wellbeing[158]. At a dose of 60 mg per day, ospemifene significantly reduced the severity of dyspareunia, had a beneficial effect on vaginal dryness[159], improved vulvar vestibular symptoms and normalized the vulvar vestibular innervation sensitivity[160], and improved bone mineral density. The levels of estradiol remained within the normal postmenopausal range, with mean estradiol levels similar to baseline at week 12, and seemed to have an anti-estrogenic effect at the endometrial and breast levels[161]. It is approved by The US Food and Drug Administration (FDA) and by The European Medicine Agency (EMA). The NAMS recognize ospemifene as a nonestrogen therapy to improve vaginal symptoms of GSM and sexual dysfunction due to dyspareunia[82].

Coadministration of ospemifene with drugs that inhibit CYP3A4, CYP2C9 CYP2C19 may increase the risk of adverse reactions. Ospemifene has a good safety profile. The most common treatment-emergent adverse events in clinical trials were hot flushes (7.5% *vs* 2.6% for ospemifene *vs* placebo), vaginal

discharge (3.7% *vs* 0.3%) and headache (3.1% *vs* 2.4%)[162]. The safety of using ospemifene concomitantly with estrogens or other SERMs, such as TAM (indicated for BC patients), has not been studied, and its concurrent use is not recommended. Therefore, ospemifene could be used for the treatment of VVA only once BC treatment, including adjuvant therapy, has been completed[82].

The most important property of ospemifene is that preclinical and clinical data demonstrated its antiestrogenic effect on breast tissue[163,164]. There are no clinical data showing that ospemifene would increase the risk of BC, and similar to other SERM, the data suggest that ospemifene acts as an antiestrogen in breast tissue and is more likely to have beneficial than detrimental effects[164]. However, the follow-up periods of these trials were too short to conclude the long-term effects of ospemifene[59,164].

Despite antiestrogenic effects on the breast in preclinical trials, the effects of ospemifene on breast density or breast cancer risk have not been systematically established in healthy women, nor has ospemifene been studied in women with breast cancer. Although it is not contraindicated for women in Europe with a history of breast cancer who have completed treatment[165], the United States Food and Drug Administration (FDA) does not recommend ospemifene for women at risk or with a history of BC or those with known or suspected estrogen-dependent neoplasia[82].

There are no differences in ospemifene-related improvements in symptoms of vulvar and vaginal atrophy in women with and without a history of BC[166], but there was a very small posthoc analysis in which is had an efficacy comparable to that of estrogenic treatments[167]. Bin Cai *et al* (2020)[168], in a retrospective matched cohort study, reported similar BC incidence rates per 1000 person-years of 2.03 (95%CI: 1.06-3.91) for treated patients and 3.53 (95%CI: 2.49-4.99) for controls (RR = 0.58, 95%CI: 0.28-1.21). Moreover, no difference in recurrence was observed between ospemifene-treated and matched untreated patients: 10 (32.3%) treated *vs* 25 (40.3%) controls in the 1:2 matched analysis.

According to the available evidence, ospemifene seems safer from the perspective of breast tissue. Therefore, it is a first-choice treatment in these cases at the end of adjuvant treatment until breast safety studies are conducted in which ospemifene will be directly compared to vaginal or systemic estrogens [167].

Information from three RCTs and one retrospective matched cohort study regarding systemic hormone treatment in BCSs is shown in Table 2.

Vaginal estrogens: The use of local hormone therapies (LHT) may be an option for some women who fail to resolve symptoms with nonpharmacologic and nonhormonal treatments after a discussion of risks and benefits plus review with an oncologist (Table 3).

Vaginal estrogen products are the most effective and sole intervention for menopausal symptoms limited to vaginal atrophy[19,50,64] compared to oral hormone menopausal therapy(HMT)[147].

A Cochrane review[169] of 30 randomized controlled trials of low to moderate quality including 6235 postmenopausal women showed that the local estrogenic preparations, in the form of creams, tablets and the estradiol-releasing vaginal ring, appeared to be equally effective in relieving the symptoms of VVA but there was a very small posthoc analysis in which is had an efficacy comparable, and a higher proportion of these women reported improvement in symptoms compared to those who received placebo. Also, adequate estrogen therapy act on the vaginal mucosa increasing its thickness, revascularizing the epithelium and increasing the number of superficial cells, thereby decreasing vaginal pH and restoring the vaginal microflora, increasing vaginal secretions, and decreasing vaginal dryness and resultant dyspareunia[76]. The evidence to support a role of systemic estrogen therapy in the management of urinary tract symptoms was conflicting according to a 2012 Cochrane review[170]. However, the authors suggested that the use of local (vaginal) estrogen therapy for incontinence may be beneficial (RR 0.74; 95%CI: 0.64-1.48), and less frequency and urgency were also reported[170].

Local therapies include estradiol-releasing intravaginal tablets, low-dose estrogen vaginal inserts, estrogen-based vaginal creams, and estradiol-releasing vaginal rings. All forms of vaginal estrogen therapies have similar rates of effectiveness but different levels of systemic absorption[171]. All preparations result in a minor degree of systemic absorption but do not exceed normal postmenopausal levels [172]. Vaginal estrogen absorption is variable and largely depends of potency of estrogenic ingredient, frequency and duration of use and also varies according to the condition of the vagina (atrophic *vs* estrogenized)[171]. The thin, atrophic vagina is highly absorptive, and this diminishes when the epithelium thickens in response to estrogenization of the vaginal mucosa[173]. In an atrophic mucosa, there is increased absorption, decreasing the level of estrogen absorbed once there is improvement in epithelium quality[174]. Whether a very small increase in estradiol exposure will stimulate quiescent, occult breast cancer cells or contribute to the development of breast cancer is not known. Preclinical data have shown that long-term estrogen deprivation can result in a state of estradiol hypersensitivity, to both proliferation and apoptosis[171], but it is not clear which effect would predominate.

The estrogen most commonly used in these preparations is estriol, which is a weak action estrogen. However, while clearance is more rapid, if used in a manner in which serum levels are consistently elevated, estriol can act as a systemic estrogen; therefore, the same cautions as with vaginal estradiol use are applied. Estriol vaginal preparations (gels, creams, and suppositories) are available in many countries but not in the United States. RCTs have found benefits for vaginal symptoms in healthy postmenopausal women[175]. Limited evidence reported in a small RCT suggested that 0.5 mg of

Table 2 Systemic hormonal treatments in breast cancer survivors: summary of studies and their outcomes

Ref.	Yr	n[1]	Design	Treatment	Conclusion
Holmberg *et al*[147,149]	2004 2008	221 *vs* 221	Randomized, non-placebo-controlled noninferiority trial	Oral estradiol hemihydrate and Norethisterone (cyclic or continuous) *vs* control	In BCSs, an increased risk of new breast cancer events and adverse events were observed after 2 yr of therapy (HR = 2.4)
von Schoultz *et al*[150]	2005	188 *vs* 190	Randomized, non-placebo-controlled noninferiority trial	2 mg estradiol for 21 d with addition of 10 mg medroxyprogesterone acetate for last 10 d; or 2 mg estradiol for 84 d with 20 mg medroxyprogesterone acetate for last 10 d; or 2 mg estradiol valerate daily	No increased risk of breast cancer recurrence; trial was closed early. So, HT doses of estrogen and progestogen and treatment regimens for menopausal hormone therapy may be associated with the recurrence of breast cancer
Kenemans *et al*[153]	2009	1556 *vs* 1542	Prospective randomized placebo controlled	Tibolone 2.5 mg daily or placebo	Trial was closed early. Tibolone had a significantly increased risk of breast cancer recurrence
Cai *et al*[168]	2020	1728 *vs* 3456	Retrospective matched cohort study	Incidence rate in ospemifene users *vs* untreated patients	No differences were observed in the BC incidence and recurrence rates in ospemifene users compared with matched controls

[1]Cases *vs* control. BC: Breast cancer, BCSs: Breast cancer survivors.

vaginal estriol cream may also prevent recurrent urinary tract infection (UTIs)[176].

The use of estriol rather than estradiol has been suggested for BCSs since its metabolic clearance is more rapid[177]. Dew *et al*[178] in a retrospective cohort study with a follow-up of 5.5 years, administered estriol 0.5 mg cream and pessaries or estradiol 25 μg tablets in a study group of confirmed BC patients with VVA or without VVA as a control, among whom 48% were using TAM, and found that vaginal estrogen therapy does not seem to be associated with an increased relative risk (HR = 0.57; 95%CI: 0.20-1.58, *P* = 0.28) [Qa = Poor]. Biglia *et al*[179] in a prospective study (12 wk) with 31 postmenopausal BCSs not using AIs (TAM or Gn-RH analogs were permitted) and 18 receiving vaginal estrogen therapy (VET) (estriol 0.25 mg, estradiol 12.5 ng or 2.5 g Replens), concluded that VET was effective in improving symptoms and objective evaluations in BCSs, but they did not describe any results on safety [Qa = Good]. In a prospective, randomized study of 10 postmenopausal women with breast cancer who were taking AIs, a two-wk span of daily 0.5 mg vaginal estriol did not increase serum estrogen or estradiol levels but significantly decreased gonadotropin levels, indicating that the systemic effects have to be kept in mind when offering vaginal estriol to BCSs receiving an AI[180]. In one 12-week, open-label pilot study of 16 women with a history of breast cancer taking AIs, a 0.03 mg estriol tablet in combination with lactobacilli improved vaginal symptoms in 100% of patients (effective), and no changes in estradiol or estrona with a small transient increase in estradiol levels were found (safe) [Qa = poor][181,182]. Estriol is not FDA approved for any indication and must be used as an off-label hormone option.

There were no associations between the use of local low potency estrogen therapy and different breast cancer histologies, ductal or lobular, in a population-based case-control study. Only the estimates for tubular cancer were not significantly above unity, with no trend of increased estimates for longer vaginal estrogen use[183].

According to the current recommendations of the North American Menopause Society, the use of low-dose vaginal estrogen treatment is accepted if there is no improvement when using nonhormonal treatments in BCSs with VVA. The lowest effective dose must be administered, starting with the so-called "ultra-low-dose", which has shown efficacy in healthy postmenopausal women[184]. However, the use of low-dose vaginal estrogen in BCSs receiving AIs has been discouraged by the American Cancer Society/American Society of Clinical Oncology[81]. Therefore, currently there is some reluctance to use local estrogen therapy in BCSs because of its potential adverse effects, with up to 70% of oncologists managing BCSs not prescribing hormone therapies. There is fear of interferences with adjuvant treatments, such as TAM or AIs, which may result in an increased risk of BC recurrence[31].

Observational studies have suggested the relative safety of local estrogen treatment, although definitive placebo-controlled RCT data are lacking. A large Finnish observational study identified no elevated risk of de novo breast cancer associated with the use of vaginal ET[185]. Crandall *et al*, in 2018, reported no increased breast cancer risk in healthy participants in the Women's Health Initiative (WHI) observational study despite a very large sample size and duration of follow-up[186].

The results of observational studies are reassuring, at least when vaginal estrogen was administered concurrently with TAM[178,187,188]. Therefore, vaginal estrogens may be appropriate for women with severe urogenital symptoms who use TAM because competitive interaction with the estrogen receptor prevents mild serum estradiol elevations from increasing the risk of breast cancer[189]. Le Ray *et al*[187] conducted a retrospective, nested case-control study of women with breast cancer (*n* = 13479) who used concomitant TAM (*n* = 1 0806) or AIs (*n* = 2673) and local estrogen. Overall, the risk of recurrence in cases treated with local estrogen was not increased compared to the control group (RR: 0.78, 95%CI,0.48-

Table 3 Local hormonal treatments in breast cancer survivors: summary of studies and their outcomes

Ref.	Yr	*n*[1]	Design	Treatment	Conclusion
Dew *et al* [178]	2003	69	Retrospective Cohort study	Estriol 0.5 mg cream and pessaries (33); Estradiol 25 µgtablets (*n* = 33)	VET does not seem to be associated with increased RR of BC
Kendall *et al*[190]	2005	7	Prospective before-after analysis	Estradiol 25 mg daily for 2 wk	Vaginal estradiol tablet significantly raises systemic estradiol levels. This reverses the estradiol suppression achieved by AIs in women with BC and is contraindicated
Biglia N *et al*[179]	2010	26	Prospective study	Estriol cream 0.25 mg (*n* = 10) or estradiol tablets 12.5 microg (*n* = 8) polycarbophil-based moisturizer 2.5 g (Replens®) (*n* = 8)	VET is effective in improving symptoms and objective evaluations in BCSs
Pfeifer *et al* [180]	2011	10	Prospective before-after analysis	0.5 mg vaginal estriol daily for 2 wk	Increase in FHS and LH may indicate systemic estradiol effects
Whiterby *et al*[201]	2011	21	Phase I/II pilot Before-After study	Testosterone cream daily for 28 d. 300/ 150 µg	Vaginal testosterone was associated with improved signs and symptoms of vaginal atrophy related to AI therapy without increasing estradiol or testosterone levels
Wills *et al* [49]	2012	24 *vs* 24	Prospective clinical trial	25 mcg estradiol vaginal tablet or ring *vs* control	VET treatment increases E2 levels. Should be used with caution
Le Ray *et al* [187]	2012	13479TAM (*n* = 10806) or AIs (*n* = 2673)	Retrospective, nested case-control study	Vaginal cream and tablets containing estrogen	Use of VET is not associated with increase in BC recurrence in those treated with TMX or AI
Dahir *et al* [202]	2014	13	Pilot before-after study	Testosterone cream daily for 28 d, 300 µg	Improvement in FSFI scores
Donders *et al*[181]	2014	16	Open label bicentric phase I pharma-cokinetic study	0.03 mg Estriol + Lactobacillus	Estriol + Lactobacillusis safe in BCpatients andimprovessymptoms
Melisko *et al*[204]	2016	69	Randomised non-comparative study	Estradiol ring 7.5 ng *vs* Testosterone cream at 1% concentration: 1.5 mg/wk	Transient increase in E2 that finally reached normal levels. Meets the primary safety endpoint
Davis *et al* [203]	2018	44	Double-blind, randomised, placebo-controlled trial	Testosterone cream daily for 26 week/ 300 µg *vs* placebo	Testosterone improves sexual test items compared to placebo

[1]Cases *vs* control. BC: Breast cancer, BCSs: Breast cancer survivors; TAM: Tamoxifen; AIs: Aromatase inhibitors; VET: Vaginal estrogen treatment; FSFI: Female Sexual Function Index.

1.25). In stratified analyses, the risk was likewise not increased in those women on TAM (RR: 0.83, 95%CI,0.51-1.34). In women taking AIs, the risk was not estimable as no women experienced a recurrence. It is important to highlight the retrospective design and the short follow-up of 3.5 years of this trial, which may be too short to show survival outcomes, and thus, lead to uncertainty regarding the data.

Regarding the use of low-dose vaginal estradiol in BCSs receiving AIs, Kendall *et al*[190] in a prospective study, measured serum estrogen levels in patients on adjuvant AIs therapy for BC (*n* = 7) and using 25 mcg estradiol vaginal tablets for severe symptoms of atrophic vaginitis daily for 2 wk. At 2 wk of analysis, estradiol increased 83%, and at 10 wk., it increased by 66%. The authors concluded that vaginal estradiol tablets significantly raised systemic estradiol levels, at least in the short term. This effect would reverse the estradiol suppression achieved by AIs in women with breast cancer and is contraindicated [Qa = Fair]. Similarly, Wills *et al*[49], conducted a prospective clinical trial of postmenopausal women with estrogen receptor-positive breast cancer or at high risk of breast cancer (*n* = 24) who were taking AIs or SERM and VET (25 mcg estradiol vaginal tablet or ring) for ≥ 90 d for atrophic vaginitis and 24 controls taking AIs only. They concluded that VET, regardless of type, resulted in elevated circulating E2 Levels in this population, even with cornification of tissue, and should be used with caution [Qa = Fair]. Therefore, these studies do not provide robust evidence regarding the safety of vaginal estrogens in BCSs taking AIs, whose efficacy is due to markedly suppressed estrogen levels[49, 190]. Nevertheless, Santen *et al*[191] reported that the increased levels of serum estradiol resulting from vaginal estrogen use may not exceed the normal range of postmenopausal serum estradiol. But, there is a lack of clarity regarding whether higher levels within a narrow postmenopausal range associate with increased risk for breast cancer recurrence, and similarly, whether lower levels are reassuring[49]. In addition, unmeasurable levels by commercially available estrogen assays can still mediate changes in distant tissues (*i.e.*, bone or liver).

Conversely, Hirschberg *et al*[192], in sixty-one BCS patients receiving AIs (50 received estriol vaginal gel and 11 received placebo), found that ultra-low-dose 0.005% estriol vaginal gel showed efficacy in improving the symptoms and signs of vulvovaginal atrophy and that estriol levels increased initially and normalized by week 12, while estradiol and estrone remained mostly undetectable throughout the study. They concluded that the negligible impact of the product on the levels of estrogens, FSH, and LH supported the safe use of this ultra-low-dose estriol vaginal gel as a treatment option for vulvovaginal atrophy in BCSs receiving AIs [Qa = Good].

This year (2021), Streff *et al*[193] in a prospective trial to measure the change in blood estradiol levels in only 8 postmenopausal women with ER(+)-BC undergoing treatment with AIs when treated with vaginal estrogen preparation for their urogenital symptoms, found that there was no significant difference between the baseline and week 16 estradiol levels (P = 0.81). In addition, patients in the prospective group reported subjective improvement in their vaginal dryness symptoms questionnaires. Therefore, VET did not cause persistent elevations in serum estradiol levels and might be a safer option for women with hormone receptor-positive breast cancer who have persistent urogenital symptoms [Qa = Poor].

In estrogen/progesterone negative tumors (ER-/PR-), the North American Menopause Society 2013 Position Statement[40] supports that topical vaginal estrogen can be prescribed. To date, there is no data that specifically separates groups of ER+PR+ or ER-PR- tumors in studies of the effectiveness, feasibility, or safety of estrogen in these groups. Based on the results of this review there is clear controversy on this topic, with some studies reporting no recurrence of BC, while others suggest caution due to a possible increase of serum estrogen levels that could lead to an increased risk of BC recurrence, specially in AIs users. Further studies are needed to evaluate these results.

In summary, taking into account the controversy, it is recommended that the risks and benefits be explained, individualizing each case with oncologists before using local estrogen therapies in BCSs. Without evidence to support value in clinical decision making, clinicians should be discouraged from measuring serum estrogen levels to assess systemic absorption of local estrogens as an indirect measure of risk for breast cancer recurrence[65].

BCS should use the lowest effective dose of vaginal estrogen as recommended by American College of Obstetricians and Gynecologists[73], American Cancer Society/American Society of Clinical Oncology[81], the Endocrine Society [72] and North American Menopause Society[82].

Vaginal promestriene: Promestriene (3-propyl ethyl, 17β-methyl estradiol) is a synthetic estrogen analog with reported minimal systemic absorption that has been suggested for topical treatment of vaginal atrophy. Low doses of topical vaginal estrogen therapy, because of its limited systemic absorption, are believed to have little or no effect on the breasts[194]. Therefore, as promestriene does not alter hormone levels, it should not modify the risk of breast cancer. Promestriene is an effective treatment for relieving the symptoms of VVA in BCSs with very poor vaginal absorption[195]. Furthermore, the absence of a systemic effect of promestriene has been confirmed with accurate and sensitive mass spectrometry and even after up to 4-6 mo. of therapeutic doses in clinical studies that included women with estrogen-sensitive malignancies[196].

Thus, it could be a first-line option for those who need minimal or ideally no vaginal absorption, particularly in symptomatic cancer patients. There are little data available in the literature, mostly consisting of small, open-label, short duration studies, and few RCTs. After a long-term market experience (almost 40 years), in 34 countries, and millions of pieces prescribed, the side effects were very rarely reported in pharmacovigilance data, whereas the effectiveness to relieve atrophy was good. To further improve the safety of promestriene, especially in estrogen-sensitive cancer patients, a very low dose is used from the beginning, starting with half or less of the usual dose, and then gradually increased until the minimum effective dose, which could further reduce its already minimal vaginal absorption[196]. However, *in vitro* studies[197] concluded that the potential estrogen-like properties of promestriene to stimulate the growth of estrogen receptor-responsive breast cancer cell lines, especially in estrogen-deprived conditions, suggest caution when prescribing for vaginal atrophy in postmenopausal BCSs on AIs. Its ability to activate growth and gene expression in ER-BC cells warrants further study.

Vaginal testosterone: Other options, such as intravaginal androgens, are gaining attention as a potential treatment for VVA in BCSs, since androgen receptors have been identified in the vaginal mucosa[198].

In one trial, treatment of 80 healthy postmenopausal women for 12 wk with a compounded vaginal cream containing 300 mg of testosterone propionate improved vaginal signs and symptoms[199].

Testosterone administration at the vaginal level seemed to trigger the activation of estrogen and androgen receptors in the vaginal epithelium layers without activating estrogen receptors in other tissues due to the lack of aromatase at this level[200].

Testosterone can induce proliferation of the vaginal epithelium, but testosterone's conversion to estrogen is blocked by AIs and therefore may be effective in reversing atrophic changes without raising circulating estrogen levels and compromising aromatase inhibitor therapy[201].

Up to 2020, three clinical trials evaluating the safety and efficacy of intravaginal testosterone (IVT) in BCSs were found[201-203] (Table 3). All were conducted in patients on adjuvant AI therapy for BC. The

longest follow-up was 26 wk. Only one clinical trial by Witherby *et al*[201], with 21 BCSs, measured serum estradiol levels. They assessed the use of daily vaginal testosterone to treat vaginal atrophy in women with breast cancer receiving AIs. Testosterone cream in one of two dosages, 150 mcg (n = 10) or 300 mcg (n = 10) was applied to the inner labia minora, introitus, and internal vaginal mucosa for 28 d. Both dosages of testosterone improved symptoms of vaginal atrophy including dyspareunia (P = 0.001) and vaginal dryness (P < 0.001), although only the 300 mcg decreased vaginal pH (*e.g.*, 5.5-5.0), and improved the vaginal maturation index (*e.g.*, 20%-40%). This study did not show any significant elevation (P = 0.91) in serum estradiol levels (remained less than 8 pg/mL) at either dose of testosterone at 4 wk. of therapy. They concluded that a 4-wk course of vaginal testosterone was associated with improved signs and symptoms of vaginal atrophy related to AI therapy without increasing estradiol or testosterone levels, but longer-term trials are warranted.

Melisko *et al*[204] in a randomized, noncomparative trial, analyzed 69 patients on adjuvant AI therapy for BC who completed 12 wk. of estradiol ring 7.5 ng *vs* intravaginal testosterone cream at a 1% concentration 1.5 mg/week treatment. They found a persistent estradiol elevation in no women with vaginal estradiol ring and in 12% with IVT. Vaginal atrophy and sexual interest and dysfunction improved for all patients. This study supported the efficacy and safety of using intravaginal testosterone or estradiol-releasing vaginal rings in patients with breast cancer receiving AI therapy to treat vulvovaginal atrophy. However, persistent estradiol elevation was seen in the intravaginal testosterone group, suggesting that a lower dose of testosterone cream can be used. Therefore, the International Society for the Study of Women's Sexual Health (ISSWSH) concluded that open-label studies that have used high doses of intravaginal testosterone in the presence of AIs for breast cancer have resulted in supraphysiological serum testosterone levels and have been reported to lower vaginal pH, improve the vaginal maturation index, and reduce dyspareunia[205].

Clinical use of vaginal testosterone therapies is limited because no currently available local (or systemic) testosterone formulations are FDA-approved for administration to women.

Vaginal dehydroepiandrosterone: Vaginal dehydroepiandrosterone (DHEA) (prasterone®) 6.5 mg/d, a steroid prohormone with the ability to transform into testosterone and estradiol, is currently FDA-approved for the treatment of GSM. It induces local effects in tissues due to its intracrine or intracellular transformation to reproductive steroids and theoretically provides a nonsystemic hormonal approach. Two 12-week, randomized, double-blinded, placebo-controlled efficacy trials in women using 6.5 mg of DHEA nightly showed significant improvement *vs* placebo in vaginal cell maturation, pH, and dyspareunia because of GSM[206,207]. Intravaginal DHEA tested for 52 wk. showed improvement in all domains of sexual function on the Female Sexual Function Index (FSFI)[208,209].

Martel *et al*[210], with highly sensitive and specific mass spectrometry assays, suggested a slight but statistically significant increase in plasma estradiol and testosterone when using intravaginal DHEA, although they concluded that this rise was within the normal range of estradiol concentrations for postmenopausal women. Therefore, longer studies are required to evaluate the safety of this treatment.

Prasterone has been studied as a treatment for GSM in cancer survivors. Barton *et al*[209], in 2018, conducted a phase III randomized clinical trial that evaluated two doses (3.25 and 6.5 mg/d) of vaginal DHEA gel compared to plain moisturizer (PM) for the improvement of vaginal symptoms (dryness or dyspareunia) in postmenopausal women (n = 464) with a history of breast (97%) or gynecologic cancer who could be receiving endocrine therapy. In peripheral blood analyses (n = 345), estradiol was significantly increased in those on 6.5 mg/d DHEA but not in those on 3.25 mg/d DHEA (P < .05 and P = .05, respectively) and not in those on AIs. They concluded that DHEA resulted in increased hormone concentrations, although the levels were still within the lowest half or quartile of the postmenopausal range, and provided more favorable effects on vaginal cytology than PM.

Prasterone label includes a warning against this use in BCS. There are no studies directly comparing vaginal DHEA to vaginal estrogen in efficacy or hormone levels, and for this reason, there can be no recommendation of one over the other in BCS.

Pilocarpine

Pilocarpine, a cholinergic agonist used to treat Sjögren´s syndrome, was investigated for this indication. Two hundred and one postmenopausal women with a history of breast cancer (currently without evidence of active breast cancer) or did not want to take vaginal estrogen for a fear of an increased risk of breast cancer were randomized to receive a target oral pilocarpine hydrochloride dose of 5 mg two times a day, or a target pilocarpine dose of 5 mg four times a day or identical appearing placebos (half with a target dose of two times a day while the other half with a target dose of four times a day). The authors did not find improvements invaginal dryness compared with placebo. Thus, pilocarpine cannot be recommended for use in the treatment of vaginal dryness, despite the preliminary pilot information that suggested that it might have been beneficial[211].

Vaginal laser (Fractional CO2 laser/erbium laser)

Laser and other energy-based devices have been marketed for the treatment of vulvovaginal atrophy, but the safety and efficacy of these devices remain uncertain[212]. Laser therapy typically consists of three laser treatment sessions over a specified time period (usually one session every four to six weeks).

Table 4 Vaginal laser therapy in breast cancer survivors: Summary of studies and their outcomes.

Ref.	Yr	*n*[1]	Design	Treatment	Conclusion
Pieralli *et al*[223]	2016	50	Prospective Before-after study	3 sessions of Fractional Microablative CO2 Laser every 30 d	The treatment seems to be feasible and effective
Pagano *et al*[221]	2016	26	Observational retrospective study	3 sessions of Fractional Microablative CO2 Laser every 30 d	The treatment seems to be effective and with good tolerance
Gambacciani *et al*[218]	2017	43	Pilot before-after study	3 sessions of Vaginal Erbium Laser every 30 d	The treatment seems to be effective
Pagano *et al*[214]	2018	82	Observational retrospective study	3 sessions of Fractional Microablative CO2 Laser every 30 d	The treatment seems to be effective
Mothes *et al*[225]	2018	16	Retrospective study	1 session of Vaginal Erbium YAG Laser	The treatment seems to be effective
Pearson *et al*[222]	2019	26	Single-arm pilot study Before-After study	3 sessions of Fractional Microablative CO2 Laser every 30 d	The treatment seems to improve sexual function and vaginal atrophy
Areas *et al*[224]	2019	24	Open, prospective study	3 sessions of Vaginal Erbium YAG Laser every 30 d	The treatment seems to improve sexual function and vaginal atrophy

[1]Cases.

The first studies evaluating the effectiveness of vaginal lasers were performed in 2015 using a fractional microablative carbon dioxide laser approved by the FDA as a therapy for GSM in healthy women[213-215]. In recent years, another laser, the nonablative vaginal Erbium YAG laser (VEL), has also been tested in these women[216-218]. Although the CO2 Laser appears to target more superficial tissue, VEL appears to remodel deep collagen and promote collagen synthesis. This effect may promote the production of new collagen that ultimately could result in improved tissue integrity and elasticity. This therapy improves the vascularization of vaginal mucosa by stimulating remodeling of the underlying connective tissue, thereby enlarging the vaginal epithelium and allowing it to accumulate glycogen. The accumulation of glycogen allows restoration of the vaginal flora, a reduction in vaginal pH and improvement in GSM symptoms caused by estrogen deficit[219]. In addition, vaginal lasers (CO2 or VEL) have been shown to improve stress urinary incontinence and vaginal prolapse and to improve vaginal dryness and dyspareunia. Nonetheless, the available data are short term, and the efficacy and safety of repeated applications are not clear[212]. Also, CO2 Laser treatment is very expensive ($1800 to $3000) and it is a procedure that is not yet widely performed by gynecologists, and thus access may be limited by a patient's geographic location.

When treating GSM in women with or at high risk of breast cancer, CO2 or VEL lasers are options that avoid hormone interventions, which is a potential advantage over pharmacologic therapies. Bercopi *et al*, 2018[220], found nonsignificant changes in the vaginal microbiome in BCSs and a high remodeling status in the vaginal epithelium after CO2 vaginal laser, mediated by significant changes in inflammatory and modulatory cytokine patterns. Eight recent studies[214,216,218,221-225] were found on the use of vaginal lasers in BCSs (3 single-arm pilot studies; 2 prospective, open, cohort studies; and 3 retrospective cohort studies) (Table 4). All the studies concluded that laser therapy improves VVA symptoms (FSFI, the Vaginal Health Index (VHI) and the Visual Analog Scale (VAS)) and reported no side effects in short-term follow-up. However, clinical assays evaluating the efficacy and safety of the use of erbium laser are needed, since all the studies included in this review were observational; some were prospective while others were retrospective, with a short follow-up and assessed only subjective variables regarding VVA improvement. There is a lack of data regarding safety and BC relapse, since no study provided information about recurrence during follow up, and serum estradiol levels were not measured.

Pagano *et al*[214] published a retrospective case series of 82 BCS patients who failed to achieve adequate relief of their GSM symptoms with nonestrogenic local treatments. These women were treated with three cycles of a CO2 Laser at 30- to 40-day intervals and demonstrated significant improvements in genital sensitivity during intercourse and vaginal dryness, as well as decreased itching/stinging, dyspareunia, dysuria, bleeding, and movement-related pain when assessed after the three treatments. These benefits were significant regardless of the woman's age or type of adjuvant breast cancer therapy. The authors noted that the optimal number of treatment cycles, as well as the need for and number of retreatments remained to be defined and have called for randomized, prospective comparative trials [216].

The longest follow-up period was that of a pilot study by Gambacciani *et al*[218] in which BCSs were followed for up to 18 mo. Pieralli *et al*[223] evaluated 50 cases of BCSs presenting VVA who were treated with an erbium laser. Of these, 52% were satisfied with the results after an average 11-month follow-up time. A very current (2021) RCT with only 18 gynecologic cancer survivors concluded that fractional CO2 Laser therapy is feasible in these patients, with preliminary evidence of safety and improvement in

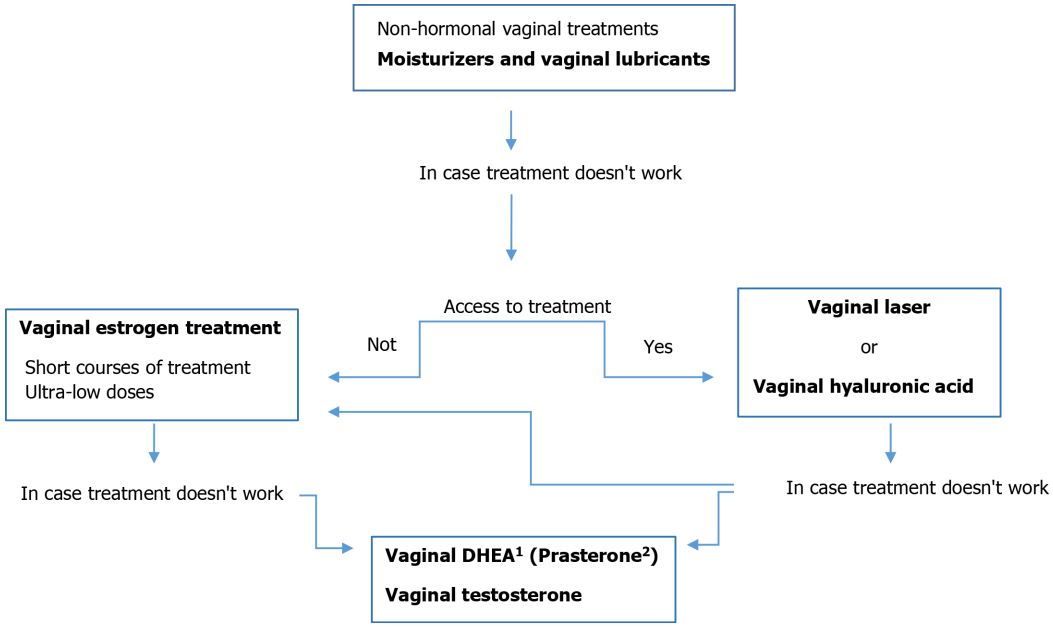


Figure 1 Treatment of genitourinary syndrome of menopause in women undergoing adjuvant treatment for breast cancer. [1]Dehydroepiandrosterone; [2]Prasterone label includes a warning against this use in breast cancer survivors.

sexual function compared with those of sham treatment[226]. Although large, sham-controlled RCTs have not been completed to date in women with breast cancer, one ongoing Greek clinical trial with 50 BCSs with GSM and with microablative fractional CO2 Laser therapy (Vaginal Laser Therapy for the Management of Genitourinary Syndrome of Menopause of Breast Cancer Survivors: NCT03738605) had an estimated study completion date of August 2020,but the conclusions are not yet available[227].

Laser or energy-based devices have not been cleared or approved by the FDA for the treatment of VVA. In 2018, the FDA issued safety communication warning patients about the risks associated with the use of these devices, which include vaginal burns, scarring, pain during sexual intercourse, and recurring/chronic pain[228]. In 2020, the American College of Obstetricians and Gynecologists advised that additional data from randomized trials are needed to further assess the efficacy and safety of this procedure[229]. In the same year, a clinical consensus statement by the American Urogynecologic Society concluded that while energy-based therapies had shown treatment promise, long-term outcomes were not yet understood[230]. Therefore, additional large clinical trials are needed to determine the benefits, risks, and cost-effectiveness of laser therapy for vulvovaginal atrophy in healthy women and in BCSs.

MANAGEMENT OF GENITOURINARY SYNDROME OF MENOPAUSE IN SPECIFIC PATIENT POPULATIONS

In Table 5, we provide consensus recommendations of The North American Menopause Society (NAMS) and The International Society for the Study of Women's Sexual Health (ISSWSH) Expert Consensus Panel[65] for an approach to the management of GSM in specific patient populations, including women at high risk of breast cancer, women with estrogen receptor-positive breast cancers, women with triple-negative breast cancers, and women with metastatic disease.

SUMMARY OF THE RECOMMENDATIONS ON THE TREATMENT OF GENITOURINARY SYNDROME OF MENOPAUSE IN BREAST CANCER SURVIVORS

A diagram for the treatment of GSM in women undergoing adjuvant treatment for breast cancer is shown in Figure 1. Figure 2 shows a schematic diagram for the treatment of GSM in women who have completed their adjuvant treatment for breast cancer.

Table 5 Treatment options for management of genitourinary syndrome of menopause in specific patient populations: Consensus recommendations of the The North American Menopause Society[65]

General guidelines
Individualize treatment, taking into account risk of recurrence, severity of symptoms, effect on QoL, and personal preferences
Moisturizers and lubricants, pelvic floor physical therapy, and dilator therapy are firstline treatments
Involve treating oncologist in decision making when considering the use of local hormone therapies[1]
Ospemifene, an oral SERM, has not been studied in women at risk for breast cancer and is not FDAapproved for use in women with or at high risk for breast cancer
Offlabel use of compounded vaginal testosterone or estriol is not recommended
Laser therapy may be considered in women who prefer a nonhormonal approach; women must be counseled regarding lack of longterm safety and efficacy data
Women at high risk for breast cancer[2]
Local hormone therapies are a reasonable option for women who have failed nonhormonal treatment
Observational data do not suggest increased risk of breast cancer with systemic or local estrogen therapies beyond baseline risk
Women with ERpositive breast cancers on tamoxifen
Tamoxifen is a SERM that acts as an ER antagonist in breast tissue; small transient elevations in serum hormone levels noted with local hormone therapies in women on tamoxifen are less concerning than in women on AIs
Women with persistent, severe symptoms who have failed nonhormonal treatments and who have factors suggesting a low risk of recurrence may be candidates for local hormone therapy
Women with ERpositive breast cancers on AI
AIs block conversion of androgen to estrogen, resulting in undetectable serum estradiol levels; transient elevations in estradiol levels may be of concern
GSM symptoms are often more severe
Women with severe symptoms who have failed nonhormonal treatments may still be candidates for local hormone therapies after review with the woman's oncologist vs consider switching to tamoxifen
Women with triplenegative breast cancers
Theoretically, the use of local hormone therapy in women with a history of triplenegative disease is reasonable, but data are lacking
Women with metastatic disease
QoL, comfort, and intimacy may be a priority for many women with metastatic disease
Use of local hormone therapy in women with metastatic disease and probable extended survival may be viewed differently than in women with limited survival when QOL may be a priority

[1]Local hormone therapies are vaginal estrogen and intravaginal DHEA (prasterone).
[2]Lifetime risk > 20%, carriers of the BRCA mutation, atypical ductal hyperplasia, lobular carcinoma in situ, or ductal carcinoma in situ. AI: Aromatase inhibitor; ER: Estrogen receptor; GSM: Genitourinary syndrome of menopause; QoL: Quality of life; SERM: Selective estrogen-receptor modulator.

CONCLUSION

GSM in BCSs is the leading cause of sexual dysfunction and severely limits the QoL of these patients. The current recommendations are that nonhormonal approaches are the first-line choices for managing mild-moderate urogenital symptoms experienced by women during or after treatment of breast cancer, which seem to be safe but present limited efficacy and short-term effects. Despite current evidence and the cautious support of multiple medical societies of the use of local ET for the management of GSM refractory to other nonpharmacologic and nonhormonal treatments, the safety of these therapies in women with or at high risk of breast cancer has not been definitively established, and recommendations for use remain controversial. Only vaginal estrogen administration is approved for BCSs and always with the lowest possible dose. Current data do not show an increase in cancer recurrence with VET; however, some studies have revealed concerns regarding elevated serum estradiol levels with estradiol vaginal rings and creams, which may reverse the effects of AIs. There is confusion about what specific estradiol or estrone levels should raise concern for postmenopausal BCSs. Due to several contradictions in published studies, a large, randomized, placebo-controlled study investigating the changes in serum levels of estrogen from varying doses and forms of topical vaginal estrogen therapies is warranted.

The decision to use vaginal hormonal therapy must be made on an individual basis with discussions between the treating physician and the patient, but we should keep in mind that the beneficial effects of VET for BCSs with severe GSM without response to nonhormonal therapies could outweigh the risks

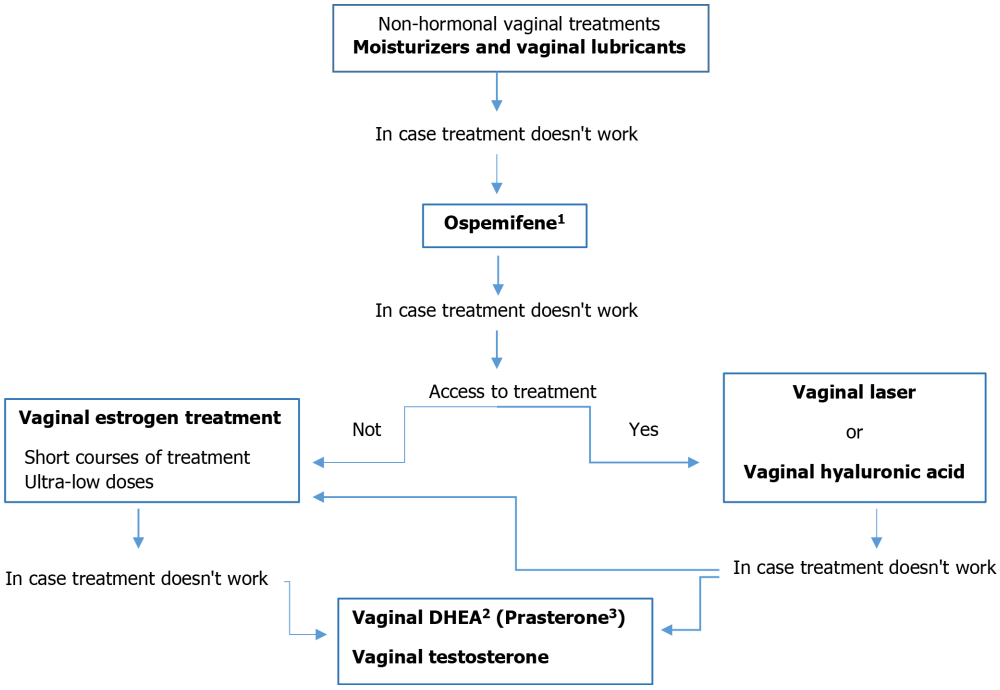


Figure 2 Treatment of genitourinary syndrome of menopause in women who have completed their adjuvant treatment for breast cancer. [1]Food and Drug Administration does not recommend ospemifene for women at risk or with history of breast cancer or those with known or suspected estrogen-dependent neoplasia[84]; [2]Dehydroepiandrosterone; [3]Prasterone label includes a warning against this use in breast cancer survivors.

and provide an overall improvement in QoL. Engaging clinicians caring for this population to ask about and treat GSM is important for QoL and requires consensus about treatment because clinical data are lacking.

The duration of therapy with all of these therapies is currently unknown, and at this time, we recommend individualizing the duration of therapy based on symptom improvement and quality of life. We suggest avoiding routine use of VET for women who are on AIs for adjuvant treatment of breast cancer. However, given the emerging data, laser or use of low-dose vaginal prasterone in select women with breast cancer who are at low risk of recurrence is reasonable if they are on AIs. Promestriene and ospemifene must be considered as alternative options. Ospefimine is a nonhormonal estrogen agonist/antagonist with promising results for BCSs when adjuvant treatment has been concluded; however, additional investigation is warranted to ensure the safety in this population. Finally, further research to define the safety and efficacy of intravaginal DHEA and different types of vaginal lasers, as well as to develop new therapies is critical.

FOOTNOTES

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MINIREVIEWS

Single-fraction stereotactic ablative body radiation therapy for primary and metastasic lung tumor: A new paradigm?

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Abstract

Stereotactic ablative body radiotherapy (SABR) is an effective technique comparable to surgery in terms of local control and efficacy in early stages of non-

small cell lung cancer (NSCLC) and pulmonary metastasis. Several fractionation schemes have proven to be safe and effective, including the single fraction (SF) scheme. SF is an option cost-effectiveness, more convenience and comfortable for the patient and flexible in terms of its management combined with systemic treatments. The outbreak of the severe acute respiratory syndrome coronavirus 2 pandemic has driven this not new but underutilized paradigm, recommending this option to minimize patients' visits to hospital. SF SABR already has a long experience, strong evidence and sufficient maturity to reliably evaluate outcomes in peripheral primary NSCLC and there are promising outcomes in pulmonary metastases, making it a valid treatment option; although its use in central locations, synchronous and recurrencies tumors requires more prospective safety and efficacy studies. The SABR radiobiology study, together with the combination with systemic therapies, (targeted therapies and immunotherapy) is a direction of research in both advanced disease and early stages whose future includes SF.

Key Words: Stereotactic body radiotherapy; Sterotactic ablative body radiotherapy; Radiosurgery; Non-small cell lung cancer; Lung cancer; Lung metastases

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Core Tip: There is strong evidence (two phase II prospective studies) to support using single-fraction stereotactic ablative body radiotherapy schemes in early stage peripheral non-small cell lung cancer. In pulmonary oligometastatic disease, there are promising outcomes and publication of one randomized prospective phase II study is pending. The association of this scheme with new systemic therapies looks promising for the future.

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INTRODUCTION

Stereotactic ablative body radiotherapy (SABR) is an important development in the early stages of non-small cell lung cancer (NSCLC). An effective, non-invasive and well-tolerated treatment which, by delivering high and precise doses over several sessions, improves the survival outcomes of these medically inoperable patients compared with conventional fractionation schemes[1,2]. It achieves a high level of local control in stages I and IIA, and a similar survival to surgery[3] in both primary tumors and lung metastases[4].

In spite of an increasingly widespread use of SABR over the past two decades, no consensus has been reached about the most suitable fractionation schemes, as several have proven to be safe and effective[5-7], including a single fraction scheme (SF)[8].

SF SABR was first utilized in intracranial stereotactic radiosurgery (SRS)[9,10] and showed promising efficacy that was comparable to surgery. Pioneering extracranial developments of SF included the treatment of thoracic malignancies[11], although in clinical practice or research it has been adopted much less than fractionated SABR[12].

The outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic in 2019 has resulted in an urgent need to reduce the number of patients' face-to-face visits. There is, therefore, renewed interest in SF as a viable treatment option for primary and metastatic lung cancer[13,14], as it delivers a radical treatment dose in just one hospital visit. In fact, oncological guidelines[15-17] now recommend using SF in these patients.

Moreover, recent studies reporting the use of SABR in carefully-selected patients with sustained metachronic extracranial oligometastases[18-22], will increase the number of indications for this approach, and SF could also be an attractive option in this setting.

Other possible applications for SF could include synchronous or oligorecurrent tumors, after a first treatment.

However, SF differs from other treatments in two important ways. One concerns the radiobiological principles and the other refers to a possible different immunogenicity than with multifraction SABR.

The clinical efficacy of SABR is greater than would be expected by the linear-quadratic model. This is because, in addition to the directly ablative effect of SABR, it also has indirect effects that induce

vascular endothelial lesion and immune activation[23,24].

Apart from reoxygenation, perhaps, the other radiobiological principles of the 4Rs are not applicable to SABR. Tumoral hypoxia can persist after vascular lesions caused by SABR[25]. Moreover, it is unlikely that switching from 1 to 5 fractions will permit the initially hypoxic tumor cells to become sufficiently reoxygenated, and could explain the small number of local failures observed in fractionated SABR[8]. To date, no clinical study has measured the effect of SABR on tumoral hypoxia in patients with NSCLC, to determine whether the efficacy of SABR depends on reoxygenation[25].

In the present review, we examine current evidence for the safety and efficacy of SF, its benefits and limitations to use. We also examine possible future directions for new systemic treatments and immunotherapies.

EARLY-STAGE PERIPHERAL NSCLC

In 2005[26,27], the first published experiences began to appear using single fraction radiation therapy in lung, in which doses of 30-34G achieved local response rates at one-year of 93%, with a G3 toxicity of 2%.

The first prospective studies in dose-escalation (Table 1) were published by Stanford University[28] in 2003. Later, the authors presented the outcomes for different doses[29]. This was a Phase I trial in dose-escalation with a study design with 4 doses of SF increasing from 15 Gy to 30 Gy, by increments of 5 Gy. The primary end-point was to identify the maximum tolerated dose (MTD) three months after dose delivery by SF. A total of 32 inoperable patients were recruited, of whom 20 had NSCLC and 12 were metastatic with lesions smaller than 5 cm. After 5-6 mo, the patients with central tumors and with a PTV > 50 cc presented pneumonitis G2-3. On the other hand, delivery of 25 Gy to patients with prior radiotherapy (RT) produced a significant increase in toxicity effects. Therefore, an addendum was applied for the 30 Gy dose to exclude the population with PTV > 50 cc who had received prior RT. The three G5 toxicities reported all corresponded to centrally located tumors, in patients with prior chemotherapy, one before SABR and two as adjuvant therapy to SABR, and two patients had a PTV > 50 cc. Local control (LC) at one-year was 91% for those delivered a dose higher than 20 Gy and 54% for doses lower than 20 Gy. Local control was significantly less in metastatic lesions than in primary tumors. The authors conclude that SF SABR of 25 Gy is well-tolerated in patients with prior thoracic radiotherapy with a PTV < 50 cc. However, central lesions and the population receiving prior chemotherapy, before or after SABR, could be at greater risk.

After this experience in dose-escalation, two prospective studies were published. The first was the Radiation Therapy Oncology Group (RTOG) 0915 study, published by Videtic *et al*[30] in 2015. This is a phase II study that analyzed 84 patients with a median follow up of 30.2 mo. Patients had T1-T2 N0 peripheral NSCLC and were randomized into one of two arms: SF 34 Gy (Arm A) *vs* 48 Gy delivered in 4F (Arm B). The aim of the study design was to identify the schedule that produced the least G3 adverse events in one year. The results showed a Grade 3 toxicity of 10.3% in Arm A and of 13.3% in Arm B. One G toxicity was recorded in each Arm. This was not related to SABR in Arm A, whereas it was related to treatment in Arm B. Local control at one-year was 97% in Arm A and 92.7% in Arm B with a tendency towards increased overall survival (OS), although this was not statistically significant with the 34 Gy dose.

The study conducted by Singh *et al*[31] at the Roswell Park Cancer Institute and published in 2019 was a phase II study that recruited 98 patients with T1/T2 primary peripheral lung cancer, randomized to receive a SF of 30 Gy (Arm 1) *vs* 60Gy delivered in 3 fractions, not correcting for heterogeneity (Arm 2). The primary endpoint of the study was to determine the incidence of toxicity of G3 or higher, with secondary endpoints of LC, survival and quality-of-life. With a mean follow-up of 53.8 mo, no significant differences were found between the two arms in G3 toxicity, LC at two years, which was 94.9% in Arm 1 and 97.1% in Arm 2, or in OS or progression free survival (PFS). A statistically significant improvement was only observed in social functioning in the 30Gy arm.

The results show that SF presents a comparable toxicity profile to multi-fraction radiotherapy without differences in LC. It would, therefore, seem pertinent to propose a phase III trial[8] that compares SF with Timmerman's classical fractionation schedules of 54 Gy in 3 fractions[32]. Possible limitations to this phase III study would be the problem of obtaining a sufficient sample size, and a possible excess toxicity to the ribs in extreme peripheral lesions.

Because of the SARS-CoV-2 pandemic, the use of hypofractionated schedules are being considered and, specifically, for NSCLC the use of a SF of SABR with a 30 Gy or 34 Gy dose, as both these doses have been reported to produce similar outcomes[33].

CENTRAL TUMORS

Central tumors are defined in the clinical trial protocol of the cooperative group of the RTOG 0236 as those located less than 2cm away from the proximal bronchial tree (PBT)[34]. The initial experience in

Table 1 Prospective data, single fraction stereotactic ablative body radiotherapy early-stage peripheral non-small cell lung cancer

Ref.	Design	Arms	*n*	Toxicity rates > GIII	LC	PFS	OS	FU[3]	SABR technique/prescription
Le *et al* [29], 2006	Phase I, *n* = 32	15 Gy (1fr)	9	0	54	NSCLC[1]: 67%	NSCLC[1]: 85%	18	Cyberknife Gold fiducials
		20 Gy (1fr)	1	0					Breathold or Synchrony (Accuray) respiratory tracking system/Isodose coverage: 95% of PTV
		25 Gy (1fr)	20	1p (GIII)3p (GV)	91	Metastatic[1]: 25%	Metastatic[1]: 56%		
		30 Gy (1fr)	2	0					
Videtic *et al*[30], 2015	Phase II, *n* = 84	48 Gy (4fr)	45	6 (13.3%)	92.7%[1]	71.1%[2]	77.7%[2]	30.2	Abdominal compression, gating with the respiratory cycle, tumor tracking, and active breath-holding techniques were allowed. Image guidance was required/prescription isodose surface ≥ 60% and < 90% of the maximun dose.
RTOG 0915		34 Gy (1fr)	39	4 (10.3%)	97%[1]	56.4%[2]	62.3%[2]		
Singh *et al*[31], 2019	Phase II, *n* = 98	60 Gy (3fr)	49	6 (15%)	97.1%[1]	50%[2]	62%[2]	53.8	Body Fix (Elekta) immobilizer. Real-Time Position Management by Varían Medical System or abdominal compression. 3D-CRT was preferred. Image guidance was required/tumor coverage and normal tissue dose constraints followed RTOG 0915
		30 Gy (1fr)	49	8 (17%)	94.9%[2]	65%[2]	73%[2]		

[1]At 1 yr.
[2]At 2 yr.
[3]Median follow-up months.
Fr: Fraction; RTOG: Radiation Therapy Oncology Group.

centrally located tumors treated with SABR but following protocols for non-central targets, showed a high toxicity, including deaths from complications[35,36]. Ultra-central tumors[37], in which the margin is reduced to within 1 cm of the boundary of the PBT, and the lesion touches or immediately invades one of the organs at risk, such as the mediastinum, trachea, bronchus or esophagus, are at greater risk of treatment-related death with SABR, associated with necrosis of the respiratory tract. However, for small (T1, T2) ultra-central lesions, SABR has been used quite safely.

There is growing interest in the treatment of central and ultra-centrally located targets by SABR, and in identifying the optimum dosing schedule that is both effective and safe to administer[38].

The Phase I/II RTOG 0813 study[39] evaluated the maximum tolerated dose, efficacy and safety in 120 patients with NSCLC cT1-2N0 of central location. The maximum tolerated dose was 12 Gy/fraction, and was associated with 7.2% of dose-limiting toxicity and high control rates. Local control rates at two years for the 71 evaluable patients in cohorts of 11.5 and 12.0 Gy/fraction were 89% and 88%per cent, respectively. Analysis of ultra-central *vs* central locations is still pending.

The fractionation schedules most used in centrally-located tumors are: 45-50 Gy delivered in 4-5 fractions following recommendations of the American Society for Radiation Oncology[6] or 60 Gy in 8 fractions according to the UK SBRT consortium[40].

Ongoing radiation studies in ultra-central tumors are attempting to elucidate the ideal dosing schedule. These include the Canadian trial SUNSET[41] and the EORTC LungTech study[42], which use the 60 Gy scheme delivered in 8 fractions.

SF SABR schedules of 30-34 Gy in phase II trials have been validated in peripheral tumors[30,31] but not in central targets. The Phase I dose-escalation study of Standford[29], which did include centrally-located tumors, reported a greater toxicity in these patients, as mentioned in the previous section. The remaining studies focusing on the use of SF on central targets are retrospective.

One retrospective study published by the Roswell Park Cancer Institute analyzed 42 patients with central tumors, and compared treatment outcomes in 11 patients delivered a single fraction of 26-30 Gy *vs* 31 patients treated with 52.6-60 Gy in 5 fractions. They found no significant differences in OS, PFS or in local, lymph node, or distant failure at 18 mo[43]. In spite of the higher rate of grade ≥ 3 toxicity (P = 0.28) in the cohort of patients treated with the single fraction, in the univariant analysis dose had no significant effect on risk of toxicity ≥ 3. Local control at one year was 100% in patients treated with SF and 96% in the multiple fraction group.

A retrospective review by Siva *et al*[44] that included 65 patients with 1-3 pulmonary metastases compared SF (26 Gy to peripheral lesions and 18 Gy to central lesions) *vs* delivery of multiple fractions (48 Gy in 4 fractions and 50 Gy in 5 fractions). With a mean follow-up of 25 mo they found no significant

differences in OS, local or distant progression, or toxicity. There were no cases of grade ≥ 3 toxicity.

To conclude, the treatment of centrally-located pulmonary tumors with SABR is still controversial owing to greater toxicity risks associated with central compared with peripheral locations. Evidence from published studies for SF in central or ultra-central tumors shows a higher toxicity risk, as doses exceed tolerance doses for central structures. Therefore, until prospective studies can establish SF as an alternative to multifraction SABR in this location, it cannot be recommended.

SYNCHRONOUS TUMORS AND RECURRENT DISEASE

There is little solid evidence about the use of SABR, and even less for SF, in synchronous lung tumors. This setting is particularly complex as there is often no anatomical pathology thus complicating therapeutic planning.

A study at Stanford[45] describes the results of a dosing strategy for SABR adapted to tumor volume, in primary and metastatic pulmonary tumors. In one of the groups, patients with a tumoral volume < 12 mL received a SF of 18-30 Gy. This group studied 48 patients with a total of 62 tumors, so an important proportion of patients treated with SF SABR had more than one tumor (between 2 and 4).

With a median follow up of 13 mo, patients with one or more small tumors treated by SF with a BED < 100 Gy, had a high rate of local control and a low toxicity, equivalent to rates recorded in patients treated with multiple fractions of BED >100 Gy.

In 2014, Kumar *et al*[46], of the Cleveland Clinic published the data of their updated series of 445 patients with early stage NSCLC treated with SABR, including 26 patients (5.8%) with synchronous pulmonary tumors confirmed by biopsy and/or PET-CT. Both the group of synchronous and of single pulmonary tumors included patients who had received SF (30 Gy in both groups and 34 Gy in the group of single tumors). At one-year of follow-up, there were no differences in survival or progression between the groups.

In a retrospective analysis by Tekatli *et al*[47] on SABR in both primary and metastatic synchronous pulmonary tumors, out of a total of 84 patients and 188 pulmonary lesions treated, only 7 Lesions (3.7%) were delivered a single session of 34 Gy by multicentric VMAT to simultaneously treat lesions some distance apart. A toxicity ≥ G3 was only recorded in 2% of the patients.

Another setting for which few studies have been published is rescue therapy by SABR, either in cases of tumoral recurrence, or persistence after a first oncological treatment. Most of the published studies of SABR used in tumoral recurrence deliver from 3 to 8 fractions[48-50]. A retrospective series published by Pennathur *et al*[51] analyzed 100 patients receiving SABR treatment for tumoral recurrence using the following regimens: surgery ± radio/chemotherapy, chemo-or radiochemotherapy, RT or radiofrequency. Of these, 31% were given 20 Gy as SF SABR, whereas the remaining patients were given 45 to 60 Gy in 3-5 fractions. With an important median follow up of 51 mo, the OS estimated for the whole sample at 1, 2 and 5 years was 74%, 49% and 31%, respectively. Although these data are from a retrospective study, they are the best data published to date in the setting of SABR for oligorecurrent or persistent lung cancer after a first treatment. No severe toxicity was reported.

Ultimately, there are few published experiences of SABR delivered in a single session for synchronous tumors, or for tumoral recurrence. Further studies are required to establish the viability of this treatment modality in these settings.

PULMONARY METASTASES

The lung is the second most frequent location of metastases[52]. Although metastectomy is the standard treatment[4], not all patients are candidates for pulmonary resection.

The efficacy and safety of SF SABR in primary or metastatic pulmonary tumors has been known since 2000[11].

Studies and data on SF SABR for the treatment of pulmonary metastases are summarized in Table 2. For series that also include patients treated with fractionated SABR, only data referring to the single fraction treatment are included. Nakagawa *et al*[11] also include pleural and costal metastases. Hara *et al*[26], Fritz *et al*[53], Le *et al*[29] and Wulf *et al*[54] combine patients with primary and those with metastatic pulmonary tumors, although only two studies assess local control and distinguish between that achieved in primary *vs* metastatic tumors. The phase I trial at Standford of Le *et al*[29] , also mentioned previously, found a lower LC rate at one-year in metastases of 58%, compared to 78% with primary tumors, whereas the prospective series by Fritz *et al*[53], found no significant differences in LC (80% 5 years) or OS (mean survival between 20 and 26 mo) in metastases *vs* primary tumors.

Treatment with SF SABR has been used for pulmonary metastases for over 20 years, in over 1000 cases. In seven publications the lesions included for analysis exceed 90[54-60].

In these series, the mean dose delivered to peripheral lesions was 27.03 Gy (range: 12-30 Gy), and for centrally-located tumors was 18.75 Gy (range: 16-23 Gy). The lowest doses are found in publications of the first exploratory studies of SABR and dose-escalation. In more recent publications (from 2010

Table 2 Single fraction stereotactic ablative body radiotherapy for pulmonary metastases

Ref.	Study design	Total lesions (*n*)/LM (*n*)	Mean, Dose Gy (range)/Location	SABR technique/prescription	Mean GTV (cc) (range) failing this, cm	FU (mo), median	LC	Toxicity ≥ GIII	Comments
Nakagawa *et al* [11]	P	22/12	22.8 (18-25)[1]/NR	Rotational or StaticTherapy 3D-CRT. Abdominal compression/PTV enclosing isodose.	4.8 (0.8-13)	10	100%[1]	0	Non actuarial LC
Hara *et al*[26]	P	59/48	30(20-34)/Periph	Static 3D-CRT. Gating/Minimal dose to GTV	5 (1-19)	12(mean)	1-yr 93% 2-yr 78%	1 GIII	LC 52% < 30 Gy LC 83% ≥ 30 Gy *P* = 0.068
Wulf *et al*[54]	R	92/31	26/Central	Static 3D-CRT. Abdominal compression/65-80%-isodose enclosing PTV	NR	14	100%	NR	SF data are shown
Fritz *et al*[53]	P	64/31	30/Periph	Static 3D-CRT. Abdominal compression/Isocenter, 90% isodose enclosing GTV, 80% isodose enclosing PTV	Median: 6 (2.8-55.8)[1]	22[1]	5-yr 80%[1]	0	No difference LC and OS LM *vs* primary lung cancer
Le *et al* [29]	Phase I	32/11	22.34 (15-30)/Periph	Cyberknife. Gold fiducials.Breathold or Synchrony (Accuray) respiratory tracking system / Isodose coverage: 95% of PTV	Median: 17.1 (2-103)	18	1-yr 91% (≥ 20 Gy) 1-yr 54% (< 20 Gy)	1 GIII (pn) 3 GV (central)	LC primary *vs* LM: 78% *vs* 58% And OS (85% *vs* 56%) Higher toxicity in central tumors
Hof *et al* [63]	P	0/71	24.35 (12-30)/NR	Static 3D-CRT. Abdominal compression/Isocenter: 80% isodose enclosing PTV	10 (1-53)	14	1-yr 88.6% 2-yr 73.7% 3-yr 63.1%	3 GIII (pn)	LC 3 yr 78% 26-30 Gy
Gandhidasan *et al* [56]	R	186/95	18/Central26 or 28/Periph	Static 3D-CRT or IMRT/80% isodose enclosing PTV	NR	22	2yr 84%	0	
Osti *et al* [57]	P	0/103	23Gy/Central30 Gy/Periph	Static 3D-CRT. 4DCT. 80% isodose enclosing PTV	NR	15	Central *vs* peripheral:1-yr 79.4% *vs* 94.7% Global: 1-yr 89.1%, 2-yr 82.1%	2 GIII (pn)	Prognostic factors for LC: sex and histology
Filippi *et al*[58]	R	0/90	26Gy/Periph	Static 3D-CRT or IMRT or VMAT. Abdominal compression/80% isodose enclosing PTV	< 5 cm	24	1-yr 93.4% 2-yr 88.1%	8 GII-IIIlate radiological toxicity 6 GII-IIIchest wall toxicity	They suggest not to use a SF in lesions close to the chest wall
Siva *et al* [44]	R	0/41	18/Central26/Periph	Static 3D-CRT or IMRT or VMAT. /70-80% isodose enclosing PTV	< 5 cm	25	2-yr 93%	0	LC, OS and toxicity rates between SF and multi-fraction SABR

Osti *et al* [59]	R	0/166	30/Periph	Static 3D-CRT. 4DCT/95% isodose enclosing PTV	3.46 (0.03-47.48)	38	3-yr 80.1% 5-yr 79.2%	6 GIII (pn) 11 GIIIlung fibrosis 1 GV at 15 mm PBT	Lesions ≤ 15 mm from mediastinum were not included in the study
Sharma *et al* [61]	R	32	30/Periph	Cyberknife. Radiopaque markers Tumor traking.70-90% isodose enclosing PTV	< 3 cm	22	2-yr 68% 3-yr 63% 4-yr 59%	No details for SF	BED_{10} < 100, delivery of pre-SBRT chemo. and synchronous metastasis: independently < LC
Sogono *et al* [60]	R	167 (95% peripher)	16-18/Central26-28/Periph	Static 3D-CRT or IMRT or VMAT. 4DCT/99% isodose enclosing PTV	NR	37	1-yr 96% 2-yr 92% 5-yr 92%	NR	Several locations
Siva *et al*[55]	Phase II	133	28/NR	Static 3D-CRT or IMRT or VMAT. Abdominalo compression/70-80% isodose enclosing PTV	2.2 cm (mean)	12	1-yr 93%	2 GIII	Preliminary results (TROG 13.01 SAFRON II) 1-3 metastases non-central targets < 5 cm

[1]Data refer to subgroup pulmonary metastases; when not specified otherwise, data refer to the whole series.

FU: Follow up; LC: Local control; OS: Overall survival; G: Grade; LM: Lung metastases; P: Prospective; R: Retrospective; NR: No reported; pn: Pneumonitis; periph: Peripheral; 3D-CRT: Tridimensional conformal radiotherapy. 4DCT: Four-dimensional CT; IMRT: Intensity-modulated radiotherapy; VMAT: Volumetric-modulated arc therapy.

onwards), the most frequently used dosing interval in peripheral tumors is from 26 to 30 Gy. Recently, central tumors have also been included in SF SABR protocols[44,54,56,57,60].

With an estimated mean follow up of 22 mo, the mean local control at one- and two-years is 87.1% and 84.2%, respectively; only Osti *et al*[59] and Sogono *et al*[60] provide data for LC at 5 years, of 79% and 92%, respectively.

Sharma *et al*[61] obtained particularly poor results (LC at 2 years of 68%, at 3 years of 63% and at 4 years of 59%). They attribute this to the dose calculation algorithm used[62], and suggest that the real dose delivered was lower than the theoretical dose. Although Filippi *et al*[58] advise against using SF schemes in tumors close to the chest wall , Sogono *et al*[60] and Siva *et al*[44] report no toxicities when dosing limits are of organ at risk are respected.

As shown in Table 2, grade 3 toxicity is very rare in all the cited studies. A total of 4 deaths were reported, all in patients with centrally-located tumors treated with non-adapted regimens. Three of these were reported in the phase I dose-escalation trial at Stanford[29] mentioned in previous sections, and one of them located at 15mm PBT in the retrospective series of Osti *et al*[59].

As mentioned in the section on centrally-located tumors, in the retrospectrive review of Siva *et al*[44], with appropriate dose constraints, no significant differences are observed in LC or toxicity compared with peripheral pulmonary tumors in retrospective series.

The phase II multicentric prospective study TROG 13.01 SAFRON II of Siva *et al*[55] is currently addressing the equivalence of a SF SABR schedule of 28 Gy and a schedule of 48 Gy delivered in four fractions for peripheral pulmonary oligometastases smaller than 5 cm. The prespecified primary evaluation criterion relating to safety was satisfied. Preliminary results point to an equivalence of both schedules for LC, OS and DFS (disease free survival), although more time is required to verify these, and other secondary endpoints such as quality-of-life and cost-effectiveness.

BENEFITS AND CONSTRAINTS TO IMPLEMENTATION

SF SABR schemes are an attractive option in terms of more convenience for the patient, reduced costs (direct and indirect) and greater flexibility for combinations with systemic treatments[58]. These features have become even more critical during the pandemic, to minimize patients´ visits to hospital, and hospital stay.

However, some constraints have prevented the widespread implementation of this technique such as: a fear of severe toxicity (especially in early studies and central tumors[29]), the scarcity of long-term studies compared with fractionated SABR (a scheme the specialist is already familiar with), and the possibility of errors in geographical positioning (which can be fatal in SF-SABR), requiring a high quality SABR control and appropriate technical capability[8,17] , among others (Table 3).

As in fractionated SABR, motion management methods should be used in SF, at planning and/or treatment; depending on available technology and previous experience (Figure 1). The treatment and prescription techniques of the studies mentioned in this article are shown in (Tables 1 and 2).

Regarding the benefits of this therapy, there is now strong evidence (two phase II prospective studies), and of sufficient maturity to reliably evaluate outcomes (5 years), to support using SF SABR schemes in early stage peripheral NSCLC[30,31].

In pulmonary oligometastatic disease, prospective and retrospective studies (Table 2) describe promising outcomes for LC, acceptable toxicity and emphasize benefits for patient adherence, more convenience and less associated costs. Publication of the randomized prospective phase II study, which is also studying cost-effectiveness, is pending[55,63].

Published data on SF-SABR in centrally-located and large-volumed primary tumors (> 50 cc), are controversial, and its use in these settings is not recommended [29,35].

There is some uncertainty about the combined use of some systemic therapies (especially with gemcitabine) due to a possible rise in cases of *recall pneumonitis*, more associated with higher doses per fraction[29].

Radiobiologically, single fraction schemes use a similar biologically effective dose (BED) to fractionated schemes (BED > 100 Gy). Therefore, theoretically both should have the same effect on the tumor (> 90%). The BED for healthy tissues suggests a possible rise in toxicity, although this has not been demonstrated in prospective studies (Table 4)[30,31,64]. Moreover, toxicity could be mitigated by increasing the precision of the irradiation, with a high dose gradient between the tumor and healthy tissues, adequate inmolibilization to minimize motion and account for intra-fraction movement[17].

The use of single fraction schedules has been found to be 40% less costly than 3 fraction schedules, according to Medicare 2009 rates, approximately 9000$ *vs* 150000$), although the reimbursement per fraction scheme has been a barrier in countries that implement it (US)[65]. The combination of greater patient comfort, with fewer hospital visits and shorter duration of treatments (one day *vs* one week), less burden on accelerators, and guarantees of acceptable toxicity and effectiveness[8,66], have all contributed to making SF SABR a valid treatment option to consider.

RECOMMENDATIONS OF SF SABR DURING THE COVID-19 PANDEMIC

The SARS-CoV-2 has posed a major challenge for the practice of radiation oncology, especially in lung cancer patients that represents one of the greatest risk groups[67].

The first results of the Thoracic Cancers International COVID-19 Collaboration (TERAVOLT), the first registry created to establish the effects of SARS-CoV-2 infection in patients with thoracic cancers, report a higher mortality in this group of patients and less access to intensive care units, although 74% of cases corresponded to Stage I patients[68].

During the pandemic, we have had to evaluate alternative dose fractionation schemes and RT techniques, with two main goals: (1) To reduce the number of hospital visits and limit exposure to SARS-CoV-2 of patients receiving RT of curative intent for lung cancer; and (2) To make room, in the radiotherapeutic oncology services, to treat operable lung cancer patients who cannot receive surgery during the pandemic.

Several guidelines have been published (Table 5) in order to provide an objective and transparent framework with which to classify patients according to the stage of the pandemic and the healthcare resources available.

Table 3 Benefits and constraints to using single fraction stereotactic ablative body radiotherapy schemes

Benefits	Constraints
Low medium-long term toxicity	Fear of severe toxicity in initial studies
Prospective efficacy and toxicity data	Insufficient long-term data
Convenience for patient, fewer hospital visits (indirect costs), shorter treatment times	
Less occupation of accelerators	
Reduced positioning errors between fractions	Greater risk of positioning errors
Peripheral tumors	Central tumors
Reduction in direct costs	
Less interference with systemic therapies	Cases of *Neumonitis recall* with some systemic therapies
Convenience for COVID-19 pandemic	

Table 4 Biologically effective dose

	Early tumor effects $\alpha/\beta = 10$	Late tumor effects $\alpha/\beta = 3$
28 Gy in 1 fraction	106 Gy	289 Gy
48 Gy in 4 fractions	105 Gy	240 Gy

Table 5 Summary of indications for stereotactic ablative body radiotherapy in pandemic COVID-19 in patients with early stage non-small cell lung cancer

ESTRO-ASTRO	UK	GOECP/SEOR
45-54 Gy in 3 fx, 48 Gy in 4 fx; Maximum hypofractionation supported, 30-34 Gy in 1 fx (90% support if choosing hypofractionation)	Safe zone: 34 Gy in 1 fx Tumours within 2.5 cm of the Chest Wall: 48-54 Gy in 3 fx Moderately central: 50 Gy in 5 fx Ultra-central: 45-50 Gy in 4-5 fx, 60 Gy in 8 fx Central/ultra-central early stage tumours not suitable for stereotactic ablative radiotherapy: 50-60 Gy in 15 fx	Safe zone: 30-34 Gy, 1 fx (first option); 54 Gy in 3 fx Peripheral lesions: 48 Gy in 4 fx (first option) Central tumour: 50-60 Gy in 5 fx, 60 Gy in 8 fx

ESTRO: European Society for Radiotherapy and Oncology; ASTRO: American Society for Radiation Oncology; GOECP: Oncologic Group for the Study of Lung Cancer; SEOR: Spanish Society of Radiation Oncology; UK: United Kingdom.

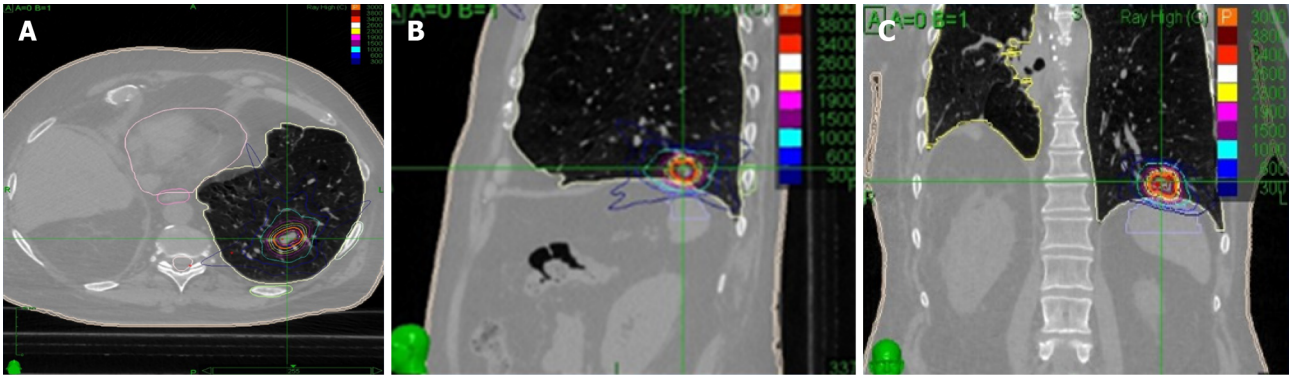
There is unanimous agreement about recommending SABR in operable patients with early stage NSCLC or oligometastatic lesions, owing to closed operating theatres or delayed surgical interventions [15-17].

Some teams have adopted an approach called SABR-BRIDGE (Stereotactic ABlative Radiotherapy Before Resection to avoId Delay for early-stage lunG cancer or oligomEts) in which SABR is used as a bridge to provide a radical treatment based on a combination of immediate SABR followed by programmed surgery 3 to 6 mo later[69].

Of the different SABR schemes available, the guidelines support administration of a SF treatment to reduce the number of visits during the pandemic. The preferred option is SF of 30-34 Gy for tumors ≤ 2 cm and > 1 cm distant from the chest wall that are outside the no-fly zone. However, the timing and the ability to implement changes in doses/fractionation schedules will depend upon the healthcare resources and technology available (for example, daily CBCT, 4 DCT *etc.*), and previous experience in SABR is preferable if SF dosing schemes are to be implemented.

FUTURE DIRECTIONS

New systemic treatments have changed the paradigm for lung cancer, benefitting both OS and DFS.



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Figure 1 Stereotactic ablative body radiotherapy dose distribution for an oligometastasis from non-small cell lung cancer. Treatment was delivered by means of the CyberKnife. A: Axial view; B: Sagittal view; C: Coronal view.

Immune checkpoint inhibitors (ICI) are a standard procedure of locally-advanced and metastatic NSCLC[24]. The use of targeted therapies in carriers of actionable mutations (EGFR, ALK, ROS1, BRAF, TRK, RET and MET) has also changed the course of advanced disease[70]. Unfortunately, platinum-based chemotherapy doublets produce low response rates[71]. Moreover, new treatments can present primary and/or secondary resistances that limit their efficacy in most patients. The exclusive use of ICI in monotherapy produces a clinical benefit in fewer than 30% of patients, and 20% of those receiving targeted therapies develop acquired resistance during the first year[24,72].

Several studies have shown that systemic treatment is effective at controlling the microscopic disease, but largely ineffective macroscopically[20]. This has led to studies exploring combinations of systemic treatments with local therapies such as SABR. The clinical benefit of this combination has been demonstrated in two randomized phase II studies in oligometastatic patients with NSCLC[21,22]. In both of these, the patients received induction ChT and were then randomized to receive SABR or surgery *vs* systemic treatment exclusively. Similar outcomes were obtained, and PFS was three times greater in the group receiving local consolidation therapy.

Randomized retrospective and prospective studies on the combined use of SABR and targeted therapies in stage IV NSCLC have also reported a clinical benefit for LC, PFS and OS[73,74]. This was confirmed in the Phase III randomized study (SINDAS), which showed an increase in PFS and OS[75].

The combination of ICI and SABR (I-SABR) is attracting even more interest. This is because SABR can induce an effective immunogenic death that can reactivate the antitumoral immune response[24]. A systematic review of stage IV NSCLC found that I-SABR increased the objective response rate (ORR) in 40% and also the PFS[76]. The PEMBRO-RT study (phase II randomized) used SABR (24 Gy in 3 fractions) prior to starting pembrolizumab, and reported an ORR, PFS and OS of 36%, 6.6 mo and 15.9 mo in favor of the combination[77]. Bauml *et al*[78] (phase II study of one arm) evaluated the combination of local ablative therapies with pembrolizumab and found a PFS of 19 mo and an OS of 77.5% at two years. A pooled analysis of the PEMBRO-RT and MDACC trials reported better outcomes for I-SABR with a median PFS and OS of 9 mo and 19.2 mo, respectively[79].

In the light of these promising results for I-SABR in advanced disease, current research is focusing on its benefits in early stages. One example is the PACIFIC-004 study, a multicentric phase III trial that combines SABR with durvalumab in stage I-II[80].

Although current evidence tends to favor hypofractionation, there is still controversy regarding the optimum fractionation schedule. Some ongoing studies are attempting to evaluate the role of single fraction SABR combined with immunotherapy. One example is the NCT03217071 trial that uses induction SABR at 12 Gy associated with pembrolizumab in stages I-IIIA. In advanced disease, the NCT02639026 trial evaluates the 17Gy scheme associated with durvalumab + tremelimumab[81].

Carbon ion radiotherapy (CIRT) has also proven effective in NSCLC. The use of CIRT in single fraction has achieved LC rates of 95% at 5 years with doses higher than 48 GyE[82]. Several possible synergistic mechanisms have been proposed for combinations with immunotherapy, but this research is still ongoing[83].

CONCLUSION

SF SABR is a valid treatment option in patients with lung cancer owing to an increased convenience of this approach, its lower costs and greater flexibility for combining with systemic therapy. During the SARS-CoV-2 pandemic, there has been renewed interest in hypofractionated and ultra-short schedules including SF, which has transformed the paradigm of radiation oncology.

Results reported in the literature reveal comparable local control, PFS and OS, late onset toxicity and quality-of-life for both SF SABR and multifraction SABR in primary NSCLC and there are promising outcomes in lung metastases.

However, there are some settings in which SF could entail too high a toxicity risk such as: patients who have received prior RT, when PTV > 50 cc, or in peripheral locations where noncompliance of SF with dosing limits for healthy tissues could endanger structures such as the chest wall. Moreover, the use of this scheme in centrally-located tumors with SABR is still controversial owing to toxicity risks and the current evidence so should be used in a clinical trial scenario.

The radiobiology of SF and combinations of this technique with immunotherapy are still under investigation, and studies focusing on high dose ablative regimens will continue.

Combining SABR with systemic treatments is safe and effective. Preclinical trials have reported an immune effect for SABR in a SF, and this is also easier to deliver between one systemic treatment and the next. However, the clinical application of SF with immunotherapy to trigger synergistic effects is still being investigated.

FOOTNOTES

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MINIREVIEWS

Optimal timing of thoracic irradiation for limited stage small cell lung cancer: Current evidence and future prospects

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Abstract

Lung cancer is a global health concern as the leading cause of cancer related mortality worldwide. Small cell lung cancer (SCLC) poses a formidable challenge to the treating physicians with the worst prognosis among all lung cancers. However, limited stage SCLC (LS-SCLC) has a relatively better outcome with multimodality management. Efforts have been focused on optimal integration of treatment modalities to achieve an improved therapeutic ratio for patients with LS-SCLC. While chemotherapy and thoracic radiation therapy (TRT) are primary components of initial management for LS-SCLC, there is no consensus on optimal timing of TRT. Within this context, we herein provide a concise overview of current evidence and future prospects regarding the optimal timing of thoracic irradiation for LS-SCLC in light of the literature.

Key Words: Small cell lung cancer; Thoracic irradiation; Limited stage small cell lung cancer; Timing of thoracic radiation therapy; Thoracic radiation therapy

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Core Tip: There has been extensive effort to establish optimal timing of thoracic radiation therapy (TRT) in limited stage small cell lung cancer (LS-SCLC) management. While late TRT may have utility for management of LS-SCLC patients who may not tolerate curative-intent upfront chest irradiation due to excessive tumor burden at the outset, early TRT allows for exploiting the synergistic effect of chemoradiotherapy to eradicate as many tumor cells as possible in a shorter timeframe. Admittedly, differences in trial designs, definition of early and late TRT, patient selection criteria, administered chemotherapy regimens, treatment compliance, TRT dose and fractionation may affect treatment outcomes.

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INTRODUCTION

Lung cancer presents a major and global health concern as a leading cause of cancer related mortality worldwide[1,2]. The 2 major histological types of lung cancer include small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). While NSCLC is the most common type, SCLC is typically associated with worse prognosis due to short doubling time and high growth fraction[3-7]. SCLC has been traditionally staged as limited stage SCLC (LS-SCLC) and extensive stage SCLC (ES-SCLC) by the Veterans' Administration Lung Study Group (VALSG) two-stage classification scheme, however, International Association for the Study of Lung Cancer staging with tumor, node, metastasis classification may also be used[8-10]. According to VALSG system, LS-SCLC is defined as disease confined to one hemithorax which can be adequately encompassed in a reasonable radiation portal. ES-SCLC refers to disease extending beyond one hemithorax which can not be encompassed within a tolerable radiation portal and may include presence of malignant pleural or pericardial effusions, contralateral hilar or supraclavicular lymph nodes, and hematogenous metastases[9,10]. While the overall prognosis of SCLC is typically poor, a subgroup of patients with LS-SCLC may have relatively more favorable treatment outcomes[11]. In light of high level evidence from systematic reviews, guidelines, and metaanalyses, current standard management of LS-SCLC includes combination of chemotherapy and thoracic radiation therapy (TRT)[12-16]. While there is consensus on combined modality management for LS-SCLC, controversies remain regarding target volumes, dose fractionation regimens and optimal sequencing of chemotherapy and TRT[17,18]. Herein, we provide a concise overview of current evidence and future prospects regarding the optimal timing of thoracic irradiation for LS-SCLC in light of the literature.

COMBINED MODALITY MANAGEMENT FOR LS-SCLC

Addition of TRT to chemotherapy has been shown to improve survival of patients with LS-SCLC as demonstrated by high level evidence[15,16]. In this context, combined modality management with chemotherapy and TRT has been standard treatment for LS-SCLC. Since the mechanism of action is different between these 2 modalities, there is potential for additive and synergistic effects which may lead to improved therapeutic outcomes[18]. SCLC is well known for its propensity to disseminate early in the course of the disease. From this standpoint, it may be feasible to consider the potential of combining 2 different therapeutic modalities for eradication of tumor clonogens to achieve both local and systemic control. In addition to eradication of tumor cells by different mechanisms, synchronous administration of chemotherapy may also play a radiosensitizer role which may enhance the overall effect of combined modality management[18,19].

Main rationale of combined modality management is to eradicate as many tumor cells as possible in a shorter timeframe by exploiting the synergistic effect of chemoradiotherapy. SCLC has tendency for early systemic dissemination, however, there is great potential for achieving good response from chemoradiotherapy given the radiosensitivity and chemosensitivity of tumor cells. Combined modality management may also offer a judicious strategy to overcome accelerated repopulation which is an important cause of treatment failures[18].

OPTIMAL TIMING OF THORACIC IRRADIATION FOR LS-SCLC

Optimal TRT timing in LS-SCLC management has been the subject of several studies, systematic reviews and metaanalyses over the years[20-41]. Selected studies of early and late TRT for LS-SCLC management are summarized in Table 1.

Murray *et al*[20] reported outcomes of a randomized National Cancer Institute of Canada Clinical Trials Group study including 308 eligible patients with LS-SCLC. The study included 155 patients in the early TRT arm (starting in day 22) and 153 patients in the late TRT arm (starting in day 106). Administered chemotherapy regimen included cyclophosphamide, doxorubicin, and vincristine alternating with etoposide and cisplatin, delivered for 3 cycles each every 3 wk. Dose of TRT was 40 Gy delivered in 15 daily fractions over 3 wk. Median progression free survival (PFS) was 15.4 mo in early TRT arm and 11.8 mo in late TRT arm. There was statistically significant improvement in 3 year PFS (26% *vs* 19%, $P = 0.036$), 3 year overall survival (OS) (29.7% *vs* 21.5%, $P = 0.006$), and median survival (21.2 mo *vs* 16 mo, $P = 0.008$) in favor of early TRT[20].

Work *et al*[21] conducted a randomized study of initial *vs* late chest irradiation combined with chemotherapy in LS-SCLC on behalf of the Aarhus Lung Cancer Group from Denmark. A total of 199 consecutive patients were randomly assigned to receive initial chest irradiation or late chest irradiation given 18 wk delayed. There were 99 patients in early TRT arm and 100 patients in late TRT arm all receiving the same 9 cycles of combination chemotherapy including 3 cycles of cisplatin and etoposide and 6 cycles of cyclophosphamide, doxorubicin, and vincristine. Median survival was 10.5 mo in early TRT arm and 12 mo in late TRT arm. Timing of TRT was not found to affect on the incidence of in field recurrences, CNS recurrences, or OS in the study[21]. Inferior outcomes in this study may be partly explained by the reduced chemotherapy doses in the concurrent chemoradiation arm and changing of the TRT schedule from 40 Gy in 20 fractions to 45 Gy in 22 fractions during the study period. Admittedly, initially delivered TRT doses of 40 Gy in 20 fractions may be considered low in comparison with current management standards and may have contributed to inferior outcomes in the study.

Jeremic *et al*[22] reported outcomes of a randomized study on initial *vs* delayed accelerated hyperfractionated TRT and concurrent chemotherapy for LS-SCLC. The study was conducted at the Department of Oncology, University of Kragujevac, Yugoslavia. Out of the total 103 eligible patients, 52 patients were allocated to receive early TRT (starting on day 1) and 51 patients were allocated to receive late TRT (starting on day 43). All patients received a total TRT dose of 54 Gy delivered twice daily fractions of 1.5 Gy. Chemotherapy schedule consisted of concurrent daily carboplatin/etoposide (C/E) (30 mg each) and 4 sequential cycles of cisplatin/etoposide (PE) (30 mg/m^2 and 120 mg/m^2, respectively, on days 1 to 3). Median survival was 34 mo in early TRT arm and 26 mo in late TRT arm, and the Kaplan-Meier 5-year survival rates were 30% *vs* 15%, in favor of early TRT[22].

Gregor *et al*[23] conducted the European Organization for Research and Treatment of Cancer Lung Cancer Cooperative Group randomized trial of alternating *vs* sequential radiotherapy/chemotherapy in LS-SCLC. A total of 335 eligible patients were randomized to 5 courses of cyclophosphamide, doxorubicin, and etoposide (CDE) chemotherapy followed by TRT and same total dose of chemotherapy and TRT split into 4 courses of 5 daily fractions delivered on days 14 to 21 of the second and subsequent chemotherapy courses. No significant difference was found between the 2 arms in terms of median survival (14 *vs* 15 mo in early *vs* late TRT arms), 1-year survival (60% *vs* 64% in early *vs* late TRT arms), 2 year survival (26% *vs* 23% in early *vs* late TRT arms), and 3-year survival (12% *vs* 15% in early *vs* late TRT arms)[23]. The study failed to confirm the superiority of an alternating schedule of delivery which may be partly explained by hematologic toxicity with this combination of chemotherapy and TRT.

Perry *et al*[24] reported the 10 year update of the experience of the Cancer and Leukemia Group B 8083 study assessing addition of TRT to chemotherapy in LS-SCLC. In the study, a total of 399 patients with LS-SCLC were randomized to receive TRT starting on day 1 (arm I) or day 64 of chemotherapy treatment (arm II), or chemotherapy alone with cyclophosphamide, vincristine, and etoposide (later, doxorubicin). Total TRT dose was 50 Gy delivered in daily fractions of 2 Gy over 5 wk. Median survival was 13.04 mo, 14.54 mo, and 13.58 mo in arm I, arm II, and arm III, respectively with statistical significance ($P = 0.0072$). The authors concluded that the 2 arms including TRT remained to be superior to chemotherapy alone with 10 years of follow-up[24].

Skarlos *et al*[25] conducted the randomized phase II Hellenic Cooperative Oncology Group (HeCOG) study assessing the timing of hyperfractionated TRT (early *vs* late) when given concurrently with chemotherapy. A total of 81 eligible patients with LS-SCLC were randomized to receive hyperfractionated TRT either concurrently with the first cycle of chemotherapy (early TRT group) or with the fourth cycle of chemotherapy (late TRT group). Chemotherapy included carboplatin delivered at an area under the curve of 6 as intravenous infusion followed by etoposide at a dose of 100 mg/m^2 intravenously for 3 consecutive days every 3 wk up to a total of 6 cycles. Overall response rate was 76% in early TRT group and 92.5% in late TRT group. Complete response rate was 40.5% and 56.5% in early and late TRT groups, respectively. Overall median survival was 17.5 and 17 mo, 2 year survival was 36% and 29%, 3 year survival was 22% and 13% in early and late TRT groups, respectively without statistical significance[25].

Table 1 Selected randomized studies of early and late thoracic radiation therapy for limited stage small cell lung cancer management

Ref.	Period	Number of patients	Age	Performance status	Timing of RT (start of TRT) (d)	RT dose fractionation schedule	Chemotherapy Schedule	PCI	Survival	*P* value
Murray *et al*[20], 1993, National Cancer Institute of Canada Clinical Trials Group Study	1985-1988	Total number of patients 308; 155 patients in early TRT group; 153 patients in late TRT group	Median age 61.8 yr in early TRT group; Median age 61.6 yr in late TRT group	ECOG 0-1 87% in early TRT group; ECOG 0-1 90% in late TRT group	Day 22 in early TRT group; Day 106 in late TRT group	40 Gy/2.67 Gy daily RT (hypofractionation)	Platinum based chemotherapy	86% of patients received PCI (2.5 Gy × 10 fractions)	Median survival 21.2 mo in early TRT group; 2-yr survival 40% in early TRT group; 3-yr survival 29.7% in early TRT group; Median survival 16 mo in late TRT group; 2-yr survival 34% in late TRT group; 3-yr survival 21.6% in late TRT group	0.006 in favor of early TRT
Work *et al*[21], 1997, Aarhus Lung Cancer Group Study	1981-1989	Total number of patients 199; 99 patients in early TRT group; 100 patients in late TRT group	Age range 36-70 yr in early TRT group; Age range 36-69 yr in late TRT group	KPS 80-100 82% in early TRT group; KPS 80-100 80% in late TRT group	Day 1 in early TRT group; Day 120 in late TRT group	40-45 Gy/2 Gy daily (conventional fractionation) split course RT over 7 wk	Platinum based chemotherapy	All early RT patients received PCI; 58% of late RT patients received PCI	Median survival 10.5 mo in early TRT group; 2-yr survival 20.2% in early TRT group; 3-yr survival 13.1% in early TRT group; Median survival 12 mo in late TRT group; 2-yr survival 19% in late TRT group; 3-yr survival 12% in late TRT group	Not statistically significant
Jeremic *et al*[22], 1997, University of Kragujevac, Yugoslavia study	1988-1992	Total number of patients 103; 52 patients in early TRT group; 51 patients in late TRT group	Age range 40-67 yr in early TRT group; Age range 44-66 yr in lateTRT group	KPS 90-100 52% in early TRT group; KPS 90-100 47% in late TRT group	Day 1 in early TRT group; Day 43 in late TRT group	54 Gy 1.5 Gy BID (hyperfractionation)	Platinum based chemotherapy	All patients with complete or partial response received PCI (2.5 Gy × 10 fractions)	Median survival 34 mo in early TRT group; 2-yr survival 71.2% in early TRT group; 3-yr survival 48.1% in early TRT group; Median survival 26 mo in late TRT group; 2-yr survival 52.9% in late TRT group; 3-yr survival 39.2% in late TRT group	0.027 in favor of early TRT
Gregor *et al*[23], 1997, EORTC Lung Cancer Co-operative Group Study	1989-1995	Total number of patients 335	Median age 61 yr (range: 33-75 yr)	ECOG 0-1 in 311 patients	Day 42 in early TRT group; Day 91 in late TRT group	12.5 Gy/2.5 Gy daily (1 wk on, 3 wk off) × 4 in early TRT group (hypofractionation); 50 Gy/2.5 Gy daily in late TRT group (hypofractionation)	No platinum based chemotherapy	PCI was not a formal part of the study, however, all patients with complete response were eligible	Median survival 14 mo in early TRT group; 2-yr survival 26% in early TRT group; 3-yr survival 12% in early TRT group; Median survival 15 mo in late TRT group; 2-yr survival 23% in late TRT group; 3-yr survival 15% in late TRT group	Not statistically significant
Perry *et al*[24], 1998, Cancer and Leukemia Group B (CALGB) study	1981-1984	Total number of patients 270; 125 patients in early TRT group; 145 patients in late TRT group	Age range 32-79	ECOG 0-1 86% in early TRT group; ECOG 0-1 87% in late TRT group	Day 1 in early TRT group; Day 64 in late TRT group	50 Gy/2 Gy daily conventionally fractionated RT over 5 wk	No platinum based chemotherapy	All patients received PCI (3 Gy × 10 fractions)	Median survival 13.04 mo in early TRT group; 2-yr survival 24% in early TRT group; 3-yr survival 7.2% in early TRT group; Median survival 14.54 mo in late TRT group; 2-yr survival 31.7% in late TRT group; 3-yr survival 13.8% in late TRT group	0.0072 in favor of lateTRT
Skarlos *et al*[25], 2001, Hellenic	1993-1999	Total number of patients 81; 42	Age range 40-76 yr in early	ECOG 0-1 76% in early TRT group;	Day 1 in early TRT	45 Gy 1.5 Gy BID (hyperfractionation)	Platinum based chemotherapy	All patients with complete or near	Median survival 17.5 mo in early TRT group; 2-yr survival 36% in	Not statistically significant

Cooperative Oncology Group (HeCOG) study		patients in early TRT group; 39 patients in late TRT group	TRT group; Age range 38-79 yr in late TRT group	ECOG 0-1 85% in late TRT group	group; Day 56 in late TRT group			complete response received PCI (1.5 Gy BID × 6)	early TRT group; 3-yr survival 22% in early TRT group; Median survival 17 mo in late TRT group; 2-yr survival 29% in late TRT group; 3-yr survival 13 % in late TRT group	
Takada *et al*[26], 2002, Japan Clinical Oncology Group (JCOG) Study	1991-1995	Total number of patients 228; 114 patients in early TRT group; 114 patients in late TRT group	Age range 39-74 yr in early TRT group; Age range 30-74 yr in late TRT group	ECOG 0-1 95% in both early and late TRT groups	Day 2 in early TRT group; Day 85 in late TRT group	45 Gy 1.5 Gy BID (hyperfractionation)	Platinum based chemotherapy	All patients with complete response received PCI (4 Gy × 6)	Median survival 27.2 mo in early TRT group; 2-yr survival 54.4% in early TRT group; 3-yr survival 29.8% in early TRT group; Median survival 19.7 mo in late TRT group; 2-yr survival 35.1% in late TRT group; 3-yr survival 20.2% in late TRT group	0.097 in favor of early concurrent TRT but not statistically significant

TRT: Thoracic radiation therapy; PCI: Prophylactic cranial irradiation.

Takada *et al*[26] reported the results of the Japan Clinical Oncology Group phase III study 9104 assessing concurrent *vs* sequential TRT in combination with cisplatin and etoposide for LS-SCLC. Total dose of TRT was 45 Gy delivered in twice daily fractions of 1.5 Gy over 3 wk. Patients were randomized to sequential or concurrent TRT arms. All patients received 4 cycles of cisplatin plus etoposide every 3 wk in the sequential arm or 4 wk in the concurrent arm. TRT was started on day 2 of the first cycle of chemotherapy in concurrent arm and after the fourth cycle in the sequential arm. Concurrent TRT conferred improved survival compared to sequential TRT (P = 0.097, not statistically significant). Median survival was 19.7 mo *vs* 27.2 mo in the in the sequential and concurrent TRT arms, respectively. The 2, 3, and 5 year survival rates were were 35.1%, 20.2%, and 18.3% *vs* 54.4%, 29.8% and 23.7% for patients receiving sequential *vs* concurrent TRT, respectively[26].

While all these randomized studies have addressed sequencing of chemotherapy and TRT in multimodality management of LS-SCLC, there are several critical points to consider in interpretation of the results from the perspective of TRT timing. Trial designs and protocols, patient selection criteria, definition of early and late TRT, administered chemotherapy regimens and compliance, TRT doses and fractionation schemes, follow up durations and outcome measures show significant diversities which emphasizes the need for vigilance in interpretation of the results. Administered TRT dose and treatment delivery techniques, dose and content of chemotherapy regimens in some of these studies may be considered inadequate and outdated as compared to current treatment standards.

There have also been more recent studies and metaanalyses addressing timing of TRT in LS-SCLC. Huncharek and McGarry[27] conducted a metaanalysis of timing of chest irradiation in combined modality treatment of LS-SCLC. Eight randomized trials including 1574 patients were analyzed, and 60% relative benefit was found in 2 year OS for early TRT which increased to 81% when only trials using cisplatin/etoposide based chemotherapy were included[27].

Bayman *et al*[28] assessed impact of early *vs* late TRT on survival for patients with LS-SCLC. A total of 70 consecutive patients receiving chemoradiotherapy for LS-SCLC were retrospectively analyzed. Administration of TRT was either after 1 to 2 cycles of chemotherapy (early TRT) or after 3 to 6 cycles of chemotherapy (late TRT). At a median follow-up duration of 2 years, late TRT was found to provide

improved response rate[28].

A metaanalysis by Pijls-Johannesma *et al*[29] used a different definition for early TRT as starting within 30 days of chemotherapy initiation. Seven randomized trials were included in the metaanalysis, and no statistically significant difference was found in 2-year OS rates (P = 0.18). However, a statistically significant survival improvement was observed in favor of early TRT when the only trial using non-platinum based chemotherapy was excluded (P = 0.01). The authors emphasized that the results should be interpreted with caution given the potential influence of patient selection, systemic treatment, and compliance rates[29].

American Radium Society Thoracic Appropriate Use Criteria Committee reported consensus recommendations for LS-SCLC[30]. The panel reaffirmed that early delivery of TRT was supported by high level evidence and suggested that it was appropriate for TRT to be incorporated in combined modality management of LS-SCLC no later than second cycle of chemotherapy[30].

Sun *et al*[35] conducted aphase III trial of concurrent TRT with either first or third cycle chemotherapy for LS-SCLC. TRT dose was 52.5 Gy in 25 daily fractions of 2.1 Gy delivered over 5 wk. Chemotherapy consisted of 4 cycles of etoposide/cisplatin which was delivered every 21 d. Median OS and PFS did not significantly differ between early and late TRT arms. Also, significantly lower rate of neutropenic fever was observed in the late TRT arm which could be partly explained by relatively smaller postchemotherapy TRT treatment volumes[35].

De Ruysscher *et al*[36] assessed impact of earlier or later TRT and shorter or longer TRT in LS-SCLC by conducting an individual patient data metaanalysis on behalf of the RadioTherapy Timing in SCLC (RTT-SCLC) Collaborative Group. Importance of using individual patient data was emphasized. Data from 9 trials including 2305 patients were available for the analysis. Median follow-up duration was 10 years. OS was not significantly effected by earlier or shorter *vs* later or longer TRT when all trials were analyzed together. Nevertheless, earlier or shorter TRT resulted in improved OS when trials including similar proportion of patients in both arms with respect to chemotherapy compliance were analyzed. Absolute gain in 5-year OS was 7.7% with earlier or shorter TRT when trials with similar chemotherapy compliance in both arms were analyzed, albeit with a higher incidence of severe acute esophagitis[36].

Wong *et al*[37] examined the National Cancer Data Base to evaluate practice patterns and survival for TRT timing in association with chemotherapy for non metastatic SCLC. A total of 8391 patients were included, and early TRT was found to improve survival compared to late TRT particularly when hyperfractionated TRT was used. Multivariate analysis revealed that hyperfractionated TRT was associated with reduced mortality[37].

Zhao *et al*[38] assessed effects of TRT timing and duration on PFS in LS-SCLC. A total of 197 patients receiving chemoradiotherapy for LS-SCLC were retrospectively analyzed. Early and short TRT was found to be correlated with longer PFS on univarite analysis. The study confirmed that early and short TRT had a positive prognostic role in LS-SCLC particularly for patients receiving hyperfactionated TRT and etoposide/cisplatin chemotherapy[38].

Results of a survey among 309 US Radiation Oncologists on timing of TRT with chemotherapy in LS-SCLC by Farrell *et al*[39] revealed that adherence to guidelines was excellent. When delivering TRT concurrently with chemotherapy, 71%, 25%, and 4% of participants preferred beginning TRT in cycle 1, cycle 2, cycle 3 or later of chemotherapy, respectively[39].

Hu *et al*[40] compared standard hyperfractionated TRT with hypofractionated TRT in combination with concurrent chemotherapy for LS-SCLC in a retrospective study. Analysis of patients enrolled in 2 independent prospective studies revealed that both hyperfractionated and hypofractionated TRT delivered with concurrent EP chemotherapy may confer good locoregional control and OS. The authors concluded that early commencement of TRT and utilization of a short course TRT schedule should be considered[40].

Hasan *et al*[41] evaluated optimal timing of TRT in LS-SCLC with daily fractionation using the National Cancer Database (NCDB). Trends in timing of TRT in LS-SCLC treated with daily fractionation, the significance of 30 day window to start TRT in this patient population, as well as optimal duration and completion times of TRT were assessed. Three and 5-year actuarial survival rates were 32.7% and 22.9% *vs* 28% and 18.4% when TRT was initiated within 30 d and beyond 30 d of chemotherapy, respectively (P < 0.001). Multivariable analysis revealed that commencement of TRT beyond 30 d of chemotherapy was associated with reduced survival[41].

To summarize, the literature includes conflicting results regarding the optimal timing of TRT in multimodality management of LS-SCLC. Different results between the studies may be partly explained by differences in trial designs, definition of early and late TRT, patient selection criteria, administered chemotherapy regimens, treatment compliance, TRT dose and fractionation.

CONCLUSION

There has been extensive effort to establish optimal timing of TRT in LS-SCLC management. The debate continues despite the accumulating data from randomized studies, systematic reviews, and metaanalyses. While late TRT may have utility for management of LS-SCLC patients who may not

tolerate curative-intent upfront chest irradiation due to excessive tumor burden at the outset, early TRT allows for exploiting the synergistic effect of chemoradiotherapy to eradicate as many tumor cells as possible in a shorter timeframe. Admittedly, differences in trial designs, definition of early and late TRT, patient selection criteria, administered chemotherapy regimens, treatment compliance, TRT dose and fractionation may affect treatment outcomes.

It appears that thorough individualized patient assessment gains critical importance in decision making for optimal TRT timing in combined modality management of LS-SCLC. Nevertheless, early TRT may be suggested to overcome accelerated repopulation and improve treatment outcomes particularly when compliance with chemotherapy is high. Future trials should focus on optimal TRT dose and fractionation, incorporation of immunotherapy approaches, adaptive TRT strategies and contemporary image guidance techniques to improve the toxicity profile of radiation delivery.

Article highlights

Lung cancer presents a major and global health concern as a leading cause of cancer related mortality worldwide. While chemotherapy and TRT are primary components of initial management for LS-SCLC, there is no consensus on optimal timing of TRT.

While several randomized studies have addressed sequencing of chemotherapy and TRT in multimodality management of LS-SCLC, there are several critical points to consider in interpretation of the results from the perspective of TRT timing. Trial designs and protocols, patient selection criteria, definition of early and late TRT, administered chemotherapy regimens and compliance, TRT doses and fractionation schemes, follow up durations and outcome measures show significant diversities which emphasizes the need for vigilance in interpretation of the results.

While late TRT may have utility for management of LS-SCLC patients who may not tolerate curative-intent upfront chest irradiation due to excessive tumor burden at the outset, early TRT allows for exploiting the synergistic effect of chemoradiotherapy to eradicate as many tumor cells as possible in a shorter timeframe. It appears that thorough individualized patient assessment gains critical importance in decision making for optimal TRT timing in combined modality management of LS-SCLC. Nevertheless, early TRT may be suggested to overcome accelerated repopulation and improve treatment outcomes particularly when compliance with chemotherapy is high.

Future trials should focus on optimal TRT dose and fractionation, incorporation of immunotherapy approaches, adaptive TRT strategies and contemporary image guidance techniques to improve the toxicity profile of radiation delivery.

FOOTNOTES

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MINIREVIEWS

Artificial intelligence and cholangiocarcinoma: Updates and prospects

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Abstract

Artificial intelligence (AI) is the timeliest field of computer science and attempts to mimic cognitive function of humans to solve problems. In the era of "Big data", there is an ever-increasing need for AI in all aspects of medicine. Cholangiocarcinoma (CCA) is the second most common primary malignancy of liver that has shown an increase in incidence in the last years. CCA has high mortality as it is diagnosed in later stages that decreases effect of surgery, chemotherapy, and other modalities. With technological advancement there is an immense amount of clinicopathologic, genetic, serologic, histologic, and radiologic data that can be assimilated together by modern AI tools for diagnosis, treatment, and prognosis of CCA. The literature shows that in almost all cases AI models have the capacity to increase accuracy in diagnosis, treatment, and prognosis of CCA. Most studies however are retrospective, and one study failed to show AI benefit in practice. There is immense potential for AI in diagnosis, treatment, and prognosis of CCA however limitations such as relative lack of studies in use by human operators in improvement of survival remains to be seen.

Key Words: Artificial intelligence; Machine learning; Cholangiocarcinoma; Diagnosis; Treatment; Prognosis

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Core Tip: The wide array of modalities available to treat cholangiocarcinoma (CCA) in addition to the diversity of the tumor urges us to use individualized therapy. To establish the proper approach to diagnose, treat, and prognose CCA, analysis of available data is the key to achieve the individualized care. Artificial intelligence can be a potential modality for achieving this goal.

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INTRODUCTION

The ever-growing rate of technological advancement in medicine has resulted in the era of "Big data". Artificial intelligence (AI) and its various techniques are used to harness the infinite potential of Big data in medical field[1]. AI, the timeliest field of computer science, involves development of computer algorithms attempting to mimic cognitive function of humans in order to learn and solve problems[2]. Since invention of the first operational computer by Alan Turing in 1940s, we have seen a prodigious rise in AI advancement. Machine learning (ML) is a very practical area of AI that enables computers to learn without direct programming. ML helps machines learn from previous data and improve their learning behavior by gaining experience from data patterns, thereby establishing ever improving predictive models[3]. Various AI techniques including representation learning, natural language processing, and different ML techniques, such as regression trees, support-vector machines (SVM), artificial neural networks (ANN) and more recently, deep learning (DL), have been used in medical field [4]. ML and DL have vastly increased the scope of AI and enabled individualized medicine rather than algorithm-only-based care and has resulted in improved accuracy, efficiency, and outcomes[4].

Despite all the benefits of AI, one should be wary of the drawbacks[5]. The field of AI brings enormous potential however it concurrently brings big ethical problems. ML algorithms, to some extent, function as "black-boxes" where there is difficulty in finding the logic behind the decision by the machine. This will have medicolegal consequences which will be more pronounced as the models become more sophisticated and companies behind ML software reluctant to reveal the details of their software. Moreover, AI poses threats to privacy, data security, and patient autonomy. Lastly, ML algorithms do make mistakes and may not provide accurate results across race, gender, and socioeconomic status spectrum[5].

Cholangiocarcinoma (CCA) is the second most common primary malignancy of the liver. CCA originates from the epithelial cells of the bile ducts exclusive of gallbladder and ampulla of Vater. CCA is an aggressive tumor diagnosed sporadically in advanced stages with high mortality[6]. The incidence of CCA is increasing; therefore, there is increased interest in diagnosis, prognosis, and treatment of this malignancy[7]. Both serum markers and radiologic imaging are used for diagnosis of CCA. A combination of serum markers like liver function tests, carbohydrate antigen (CA) 19-9, and carcinoembryonic antigen (CEA) are utilized to diagnose the disease[8]. The presence of the vast array of serum markers has led to utilization of the markers in novel AI tools in combination with imaging. Positron emission tomography with fluorodeoxyglucose (FDG-PET) integrated with computed tomography (CT) and Magnetic resonance imaging (MRI) in combination with magnetic resonance cholangiopancreatography (MRCP) are valuable tools harnessed by AI to assess the extent of tumor and stage the disease[9, 10]. Treatment includes surgical management, neoadjuvant/adjuvant chemotherapy and chemoradiotherapy, hepatic artery radioembolization, and orthotopic liver transplant in selected patients[11-14]. Endoscopic retrograde cholangiopancreatography (ERCP) has two roles of diagnosis and treatment of CCA. Its diagnostic role includes inspecting and providing samples from the biliary system. As palliative treatment, stent placement provides increased quality of life especially in most unresectable cases[15]. Novel AI tools have been able to help in individualizing candidates for each treatment modality.

Increased mortality from CCA in the last decade has coincided with development of AI technology. Figure 1 illustrates how AI can be used to diagnose, treat, and prognose patients with CCA. This review depicts how AI can analyze the radiologic, serologic, and histologic markers of CCA to diagnose, stage, and aid with an individualized treatment plan in addition to giving a prognostic estimate with or without treatment modalities.

AI has shown promise to aid in diagnosis of CCA. AI is particularly helpful in CCA diagnosis as the condition is not common and there is heterogeneity in anatomical location of the tumor and risk factors of the tumor[16]. This makes the traditional algorithms inferior compared to AI. Many AI tools in the field of ML have been utilized for diagnosis of CCA (Table 1). LR is a linear regression model used for binary classification of problems[17]. SVM is an appropriate model for small samples, high-dimensional,

Table 1 Advantages and disadvantages of artificial intelligence models used for cholangiocarcinoma diagnosis in radiology

AI technology	Imaging modalities used in	Advantages	Disadvantages
Logistic regression	US/CT	Interpretable	Low precision
Support-vector machine	US/CT/MRI	Avoids overlearning and dimension disaster problems	Prone to missing data
Extreme learning machine	CT	Does not need high amount of data for training	Slow processing speed
Artificial neural network	CT/MRI	High generalization power	Needs long training time
Convolutional neural network	US/CT/MRI	Higher efficacy and speed as there is no need to compute features as first step	Needs large training data

AI: Artificial intelligence; CT: Computed tomography; MRI: Magnetic resonance imaging; US: Ultrasound.

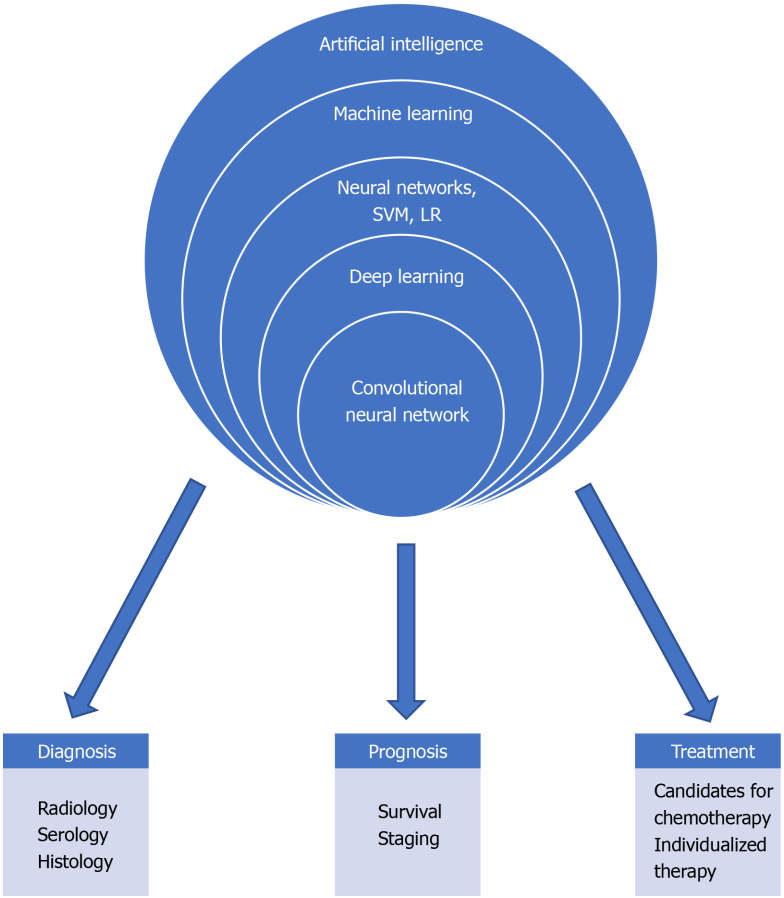


Figure 1 Application of artificial intelligence in addressing cholangiocarcinoma. LR: Logistic regression; SVM: Support-vector machine.

and non-linear patterns assigning labels to objects and has advantage of avoiding "over learning" problem[18]. ANN or multilayer perceptron is an attempt to simulate the biologic nervous system with neurons interconnected able to do parallel processing[17]. Developed by Huang *et al*, Extreme Learning Machines (ELM) are a type of feedforward neural network models that have shown superiority over SVMs and traditional feedforward neural networks[19]. Convoluted neural network (CNN), a type of DL consists of multilayer of ANN that results in a superior learning ability of complex tasks and has been used in radiology and imaging of the malignancy and associating the radiological data to the clinicopathologic data[20,21]. Every method has their advantages and drawbacks illustrated in Table 1.

AI IN THE DIAGNOSIS OF CCA

Serum markers

Evaluation of serum markers is amongst the least invasive and most available data that is present in many patients even before there is a suspicion for diagnosis of CCA. Due to wide availability, these tests are used in adjunct with radiological and other clinical factors in diagnosis of CCA. Sometimes serological models are enough to diagnose the malignancy; for example, Negrini *et al*[22] developed a ML model that analyzed 15 bile acids of the serum and was able to diagnose CCA with good sensitivity of 79% and excellent accuracy, Area Under Curve (AUC), and specificity of 86.4%, 95%, and 100%, respectively. ANN based model using combination CCA associated carbohydrate antigen and alkaline phosphatase showed promise in diagnosing CCA with a sensitivity and specificity of more than 95% [23].

Cytology

ERCP and Cytology of brushings is a valuable tool for diagnosis of CCA. As a common malignant cause of biliary stricture is CCA, cytology can be crucial in early stages of the malignancy when radiology may have limited roles. Urman *et al*[24], using a neural network model studying metabolomic and proteomic profile of bile from 36 CCA patients, was able to satisfactorily distinguish CCA from benign stricture with AUC, sensitivity, and specificity of 98.4%, 94.1% and 92.3%, respectively.

Histology

Histology remains the gold standard for diagnosis of malignancies including CCA. From their Shanghai laboratory, Sun *et al*[25] developed a CNN model for diagnosis of CCA from microscopic hyperspectral pathological slides with promising results. After setting up the first benchmark based on microscopic pathological images consisting of 880 images with pixels manually labeled as tumor or non-tumor for the AI learning, the CNN model was able to diagnose CCA with 88.3% accuracy[25]. AI assistance in histology has not always shown benefits. Stanford University researchers developed an AI diagnostic assistant using DL model to assist pathologists in differentiating hepatocellular carcinoma (HCC) from CCA (26). The model had a good accuracy rate of 84.2% on a set of 80 slides however it failed to improve performance among pathologists [Odds ratio (OR) 1.287, 95%CI: 0.886-1.871]. For all case difficulty levels, the model highly biased the decision of pathologists which led them to wrong diagnosis[26]. The authors concluded that this would question the use of current AI technology for difficult subspecialty tasks[26]. Sometimes CCA can manifest as cancer of unknown primary site (CUP) as it metastasizes to other organs. AI has been used to delineate source of CUP, consisting of 3 to 5% of tumors[27]. CUP-AI-Dx is a CNN model that was trained on more than 18,000 tumors including CCA and has achieved an accuracy of 98.54% in finding the primary site of tumor from the human body system in cross-validation[28].

CT

To elucidate the lesion detected by ultrasound, further workup is required with CT, MRI, and MRCP. As CNN is a DL technique that consists of multilayers of ANN, it has shown great potential especially once it comes to radiology image analysis of pixels. Human yield in diagnosing CCA is limited. Nakai *et al*[29] have developed CNN models factoring in a combination of CT with serum tumor markers including CEA and CA 19-9. Their CNN model was superior to human radiologists in detecting CCA (0.68 *vs* 0.45; $P = 0.04$) [29]. One challenge in diagnosing CCA is differentiating intrahepatic CCA from other intrahepatic malignancies. Xu *et al*[30] have developed an AI model on 28 intrahepatic lymphomas and 101 CCAs. Their model was able to differentiate between the two tumors with AUC and accuracy both more than 85%. Pannoprat *et al*[31] have developed CNN model that can differentiate between CCA and hepatocellular carcinoma (the most common primary liver malignancy) with an 88% accuracy. Zhang *et al*[32] performed a retrospective analysis of contrast enhanced CT of 86 patients with CCA and 46 with combined CCA/HCC tumors, which are difficult to differentiate from CCA necessitating biopsy and surgery. Using ML techniques to classify the lesions as CCA or combined CCA/HCC achieved an AUC of 94.2%[32].

MRI and MRCP

MRI and MRCP have a superior function to diagnose CCA than CT due to ability to illustrate soft tissue, vasculature, and biliary system better than that of CT. ML has been widely utilized in MRI and MRCP. Xu *et al*[33] and Yu *et al*[34] each studied MRI of more than 100 patients with CCA and developed SVM models that showed superiority (validation group AUC 87.0% and 90%, respectively). Logeswaran *et al* [35] in a 2009 study showed 88 to 94% detection rate of Multilayer Perceptron ANN in diagnosis of CCA in MRCP. Yang *et al*[36] developed an AI model for MRI diagnosis and evaluation of extent of lymph node metastasis of CCA patients. After training the model on 100 CCA patients, the model was able to differentiate high *vs* low risk CCA groups and lymph node metastasis with AUCs of 80% and 90% in testing cohorts, respectively[36]. Table 2 lists the studies using AI models to diagnose CCA.

Table 2 Studies utilizing artificial intelligence in the diagnosis of cholangiocarcinoma

Ref.	Year of publication	Title of study	Diagnostic modality	AI model
Chu *et al*[44]	2021	Radiomics using CT images for preoperative prediction of futile resection in intrahepatic cholangiocarcinoma	CT	LR
Ibragimov *et al*[45]	2020	Deep learning for identification of critical regions associated with toxicities after liver stereotactic body radiation therapy	CT	CNN
Liu *et al*[46]	2021	Can machine learning radiomics provide pre-operative differentiation of combined hepatocellular cholangiocarcinoma from hepatocellular carcinoma and cholangiocarcinoma to inform optimal treatment planning?	MRI, CT	SVM
Logeswaran[35]	2009	Cholangiocarcinoma--an automated preliminary detection system using MLP	MRCP	ANN
Midya *et al*[47]	2018	Deep convolutional neural network for the classification of hepatocellular carcinoma and intrahepatic cholangiocarcinoma	CT	CNN
Nakai *et al*[29]	2021	Convolutional neural network for classifying primary liver cancer based on triple-phase CT and tumor marker information: a pilot study	CT, tumor markers	CNN
Negrini *et al*[22]	2020	Machine Learning Model Comparison in the Screening of Cholangiocarcinoma Using Plasma Bile Acids Profiles	Serum bile acids	ML
Pattanapairoj *et al*[23]	2015	Improve discrimination power of serum markers for diagnosis of cholangiocarcinoma using data mining-based approach	Tumor markers	ANN
Peng *et al*[48]	2020	Preoperative Ultrasound Radiomics Signatures for Noninvasive Evaluation of Biological Characteristics of Intrahepatic Cholangiocarcinoma	US	SVM
Peng *et al*[49]	2020	Ultrasound-Based Radiomics Analysis for Preoperatively Predicting Different Histopathological Subtypes of Primary Liver Cancer	US	Radiomics
Ponnoprat *et al*[31]	2020	Classification of hepatocellular carcinoma and intrahepatic cholangiocarcinoma based on multi-phase CT scans	CT	CNN
Selvathi *et al*[50]	2013	Automatic segmentation and classification of liver tumor in CT images using adaptive hybrid technique and Contourlet based ELM classifier	CT	ELM
Sun *et al*[25]	2021	Diagnosis of cholangiocarcinoma from microscopic hyperspectral pathological dataset by deep convolution neural networks	Histology	CNN
Urman *et al*[24]	2020	Pilot Multi-Omic Analysis of Human Bile from Benign and Malignant Biliary Strictures: A Machine-Learning Approach	Bile acids, lipids	ANN
Uyumazturk *et al*[26]	2019	Deep learning for the digital pathologic diagnosis of cholangiocarcinoma and hepatocellular carcinoma: evaluating the impact of a web-based diagnostic assistant	Histology	DL
Wang *et al*[51]	2020	SCCNN: A Diagnosis Method for Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma Based on Siamese Cross Contrast Neural Network	CT	ANN
Wang *et al*[52]	2019	Deep learning for liver tumor diagnosis part II: convolutional neural network interpretation using radiologic imaging features	MRI	DL
Xu *et al*[33]	2019	A radiomics approach based on support vector machine using MR images for preoperative lymph node status evaluation in intrahepatic cholangiocarcinoma	MRI	SVM
Xu *et al*[30]	2021	Differentiation of Intrahepatic Cholangiocarcinoma and Hepatic Lymphoma Based on Radiomics and Machine Learning in Contrast-Enhanced Computer Tomography	Contrast enhanced CT	ML
Yang *et al*[36]	2020	Radiomics model of magnetic resonance imaging for predicting pathological grading and lymph node metastases of extrahepatic cholangiocarcinoma	MRI	Radiomics
Yao *et al*[34]	2020	A Novel Approach to Assessing Differentiation Degree and Lymph Node Metastasis of Extrahepatic Cholangiocarcinoma: Prediction Using a Radiomics-Based Particle Swarm Optimization and Support Vector Machine Model	MRI	SVM
Yasaka *et al*[53]	2018	Deep Learning with Convolutional Neural Network for Differentiation of Liver Masses at Dynamic Contrast-enhanced CT: A Preliminary Study	CT	CNN
Zhang *et al*[32]	2020	Differentiation combined hepatocellular and cholangiocarcinoma from intrahepatic cholangiocarcinoma based on radiomics machine learning	CT	Radiomics
Zhao *et al*[28]	2020	CUP-AI-Dx: A tool for inferring cancer tissue of origin and molecular	Tissue biopsy	CNN

		subtype using RNA gene-expression data and artificial intelligence		
Zhou *et al*[54]	2021	Automatic Detection and Classification of Focal Liver Lesions Based on Deep Convolutional Neural Networks: A Preliminary Study	Multiphasic CT	CNN

AI: Artificial intelligence; ANN: Artificial Neural Network; CCA: Cholangiocarcinoma; CNN: Convolutional neural network; CT: Computed tomography; DL: deep learning; ML: machine learning; ELM: Extreme learning machine; LR: Logistic regression; MRCP: Magnetic resonance cholangiopancreatography; MRI: Magnetic resonance imaging; SVM: Support-vector machine, US: Ultrasound.

TREATMENT AND PROGNOSIS OF CCA

ML techniques have also been used for treatment and prognosis of CCA. Almost all studies use a combination of radiological, histological, serological, and clinical data for the best results in predicting the survival of the patients and their response to treatment. Table 3 illustrates the studies using AI models to treat and prognose CCA. The fact that such sophisticated models are needed is proof to the complexity of the CCA pathophysiology and ever developing variety of treatment protocols that makes decision making impossible without help of AI technology. One example of such potential is studied by Tsilimigras *et al*[37]. They constructed a ML model that predicted survival of CCA patients after surgery based on preop serological and radiological data[37]. They conducted an international multi-institutional study on 826 CCA patients, clustering them into groups based on CA 19-9, neutrophil-to-lymphocyte ratio, and tumor size. Their machine learning model showed an excellent agreement with cluster (k = 0.93, 95%CI: 0.90-0.96). This study shows that ML models detect patterns and clusters not detectable to humans using traditional statistical techniques[38]. In this study, AI was able to detect a group of high-risk patients otherwise undetectable. These groups benefit the most from additional neoadjuvant therapy prior to resection as they have a high recurrence[37,38].

CT imaging

Another example of tight interrelation between prognosis and treatment is by Jeong *et al*[39] who elaborated a ML algorithm using the combination of serology, patient characteristics, and CT images of 1421 CCA patients to classify patients to stable and latent risk group. The model was able to predict the disease-free survival between latent and stable groups and response to adjuvant therapy in latent group with excellent ability proven by hazard ratios (HR) of 3.56 and 0.46, respectively ($P < 0.001$ for both) [39]. Tang *et al*[40] drew up a predictive model of CCA survival after studying 101 patients with CCA. Their AI model analyzed radiologic characteristics of the CT scan, tumor markers, and past clinical history like cirrhosis with AUC of 78% and 75% for 3-year and 5-year overall survival, respectively[40].

CA 19-9

CA 19-9 as a tumor marker has shown promise in prognosis of CCA. Li *et al*[41] and Müller *et al*[42] each validated an AI model to prognosticate the CCA tumors based on clinical, tumor markers such as CA 19-9, serologic like albumin level, and clinical data like nodal metastasis. Li *et al*[41] model retrospectively studied a total of 1390 patients and achieved a Concordance Index (C-index) superior to the staging system proposed by the 8th edition of the American Joint Committee on Cancer (C-index: 0.693, 95%CI: 0.663-0.723). Müller *et al*[42] model was able to predict the 1-year survival of patients with an AUC of 89% and 80% for the training and validation sets, respectively.

Palliative measures

Palliative measures like stent placement recommended for inoperable hilar CCAs, are also analyzed by AI models. Shao *et al* developed an ANN model based on data of 288 CCA patients requiring stent placement that can predict stent occlusion with high AUC of 96% (95%CI: 94-99%)[43].

FUTURE DIRECTIONS

The literature review showed a wealth of studies utilizing AI in CCA, however there is room for much improvement. First, there is need for larger prospective studies including different races, nationalities, and socioeconomic statuses to validate role of AI in diagnosis, treatment, and prognosis of CCA. As study from Stanford showed the AI may not prove to be beneficial in all cases in real life; therefore, in some cases there is need for prospective studies showing AI effectiveness in practice[26]. This precaution is accentuated since there was a lack of negative studies in our review of the literature which can potentially bias toward increased efficacy of AI. Furthermore, the prognostic data should be validated by implementing the data into treatment strategies and seeing an increase in not only survival but also quality of life in CCA patients. One last recommendation for medical field is that healthcare professionals' education should be improved to prepare them for the ever-increasing role of AI in daily diagnosis, treatment, and prognosis of CCA and at the same time informing them of the current limits

Table 3 Studies utilizing artificial intelligence in the treatment and prognostication of cholangiocarcinoma

Ref.	Year of publication	Title of study	AI variables	AI model
Jeong *et al*[39]	2020	Latent Risk Intrahepatic Cholangiocarcinoma Susceptible to Adjuvant Treatment After Resection: A Clinical Deep Learning Approach	CT, albumin, platelets, Diabetes, CA 19-9	ML
Ji *et al*[55]	2019	Biliary Tract Cancer at CT: A Radiomics-based Model to Predict Lymph Node Metastasis and Survival Outcomes	CT reported LN features	ANN
Li *et al*[41]	2020	A Novel Prognostic Scoring System of Intrahepatic Cholangiocarcinoma With Machine Learning Basing on Real-World Data	CEA, CA 19-9, tumor stage	ML
Muller *et al*[42]	2021	Survival Prediction in Intrahepatic Cholangiocarcinoma: A Proof-of-Concept Study Using Artificial Intelligence for Risk Assessment	Tumor size, tumor boundary, serology	ANN
Shao *et al*[43]	2018	Artificial Neural Networking Model for the Prediction of Early Occlusion of Bilateral Plastic Stent Placement for Inoperable Hilar Cholangiocarcinoma	Tumor size, nodal involvement	ANN
Tang *et al*[40]	2021	The preoperative prognostic value of the radiomics nomogram based on CT combined with machine learning in patients with intrahepatic cholangiocarcinoma	Tumor size, cirrhosis in CT	Radiomics
Tsilimigras *et al*[37]	2020	A Novel Classification of Intrahepatic Cholangiocarcinoma Phenotypes Using Machine Learning Techniques: An International Multi-Institutional Analysis	Tumor size, nodal involvement, serology	ML

AI: Artificial intelligence; ANN: Artificial Neural Network; CA 19-9: Carbohydrate antigen 19-9; CCA: Cholangiocarcinoma; CEA: Carcinoembryonic antigen; CT: Computed tomography; ML: Machine learning.

and future potentials of the AI technology.

CONCLUSION

In the recent years, we have seen an increase in CCA incidence and, in parallel, a more exponential rise in AI utilization in medicine. AI will be able to utilize the vast amount of data to assist healthcare professionals in addressing CCA. Currently the AI models are showing potential in diagnosis, treatment, and prognosis of CCA. Nonetheless, AI has limits that should be considered; further research is needed to validate use of AI models in real life in use by medical professional to determine their effectiveness and acceptance as auxiliary tools to augment human intelligence. Finally, ethical issues regarding AI including equity and transparency will also need to be addressed to improve acceptance of the technologies by healthcare industry and, more importantly, the patients.

FOOTNOTES

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ORIGINAL ARTICLE

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Neurotrophic receptor tyrosine kinase family members in secretory and non-secretory breast carcinomas

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Abstract

BACKGROUND

Breast cancer is the most common female cancer and a major cause of morbidity and mortality. Progress in breast cancer therapeutics has been attained with the introduction of targeted therapies for specific sub-sets. However, other subsets lack targeted interventions and thus there is persisting need for identification and characterization of molecular targets in order to advance breast cancer therapeutics.

AIM

To analyze the role of lesions in neurotrophic receptor tyrosine kinase (NTRK) genes in breast cancers.

METHODS

Analysis of publicly available genomic breast cancer datasets was performed for identification and characterization of cases with fusions and other molecular abnormalities involving *NTRK1*, *NTRK2* and *NTRK3* genes.

RESULTS

NTRK fusions are present in a small number of breast cancers at the extensive GENIE project data set which contains more than 10000 breast cancers. These cases are not identified as secretory in the database, suggesting that the histologic characterization is not always evident. In the breast cancer The Cancer Genome Atlas (TCGA) cohort the more common molecular lesion in NTRK genes is amplification of *NTRK1* observed in 7.9% of breast cancers.

CONCLUSION

Neurotrophin receptors molecular lesions other than fusions are observed more often than fusions. However, currently available NTRK inhibitors are effective

mainly for fusion lesions. Amplifications of *NTRK1*, being more frequent in breast cancers, could be a viable therapeutic target if inhibitors efficacious for them become available.

Key Words: Neurotrophic receptor tyrosine kinases; Breast cancer; Amplifications; Fusions; Tropomyosin related kinases

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Core Tip: Molecular lesions in neurotrophic receptor tyrosine kinase (NTRK) receptors have been brought to the forefront of cancer therapy with the introduction of specific inhibitors which are effective in cancers with fusions involving the family of receptors. In breast cancer fusions involving the NTRK receptors are rarely seen and concern exclusively the secretory sub-type. In non-secretory breast carcinomas amplifications are observed in a minority of cases.

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INTRODUCTION

Breast cancer is the most common neoplasm in women and a significant cause of morbidity and mortality. In United States alone, an estimated 284000 cases of breast cancer will occur in 2021, with more than 44000 resulting deaths[1]. Breast cancers represent about 30% of all cancers diagnosed in women. Despite recent advances in breast cancer therapies produced by an improved understanding of molecular pathogenesis, the disease remains difficult to treat when metastatic. It is currently well established that breast cancer does not represent a single entity, but several sub-types exist with implications for prognosis and treatment[2]. The most frequent sub-type is estrogen receptor (ER) positive and negative for the epidermal growth factor receptor (EGFR) family receptor 2, known as human EGFR receptor 2 (HER2) and is further divided in a more indolent disease corresponding with the genomic luminal A sub-type and a more aggressive, less estrogen-dependent disease aligning with the genomic luminal B classification[3]. Another breast cancer sub-type is positive for the HER2 receptor, while the triple negative sub-type is negative for both ER and HER2, as well as the receptor for Progesterone, PR, which is positive in most ER positive cases. Treatments for each sub-type has evolved to differ significantly and therapeutic decisions in breast cancer depend on the sub-type. HER2 positive cancers, for example, are treated with monoclonal antibodies, small molecule kinase inhibitors and antibody drug conjugates targeting HER2[4].

Several other receptor tyrosine kinases have been implicated in cancer pathogenesis and progression and treatments targeting some of them exist. These include the angiogenesis kinase receptor vascular endothelial growth factor receptor (VEGFR) and its ligand VEGF, EGFR, fibroblast growth factor receptor (FGFR) and c-Met, the receptor for hepatocyte growth factor (HGF). In breast cancer, besides HER2, no receptor tyrosine kinase inhibitors have been approved. Recently, inhibitors of the neurotrophic kinase family of tyrosine kinase receptors have become available and confirmed to be effective in cancers with fusions involving these receptors[5]. These fusions are observed in the majority of rare histologic types of cancers such as mammary analogue secretory carcinomas of the salivary glands and secretory breast cancers but are exceedingly rare in more common histologic subtypes of cancers[6]. The current analysis examines fusions and other molecular lesions of neurotrophin receptors in secretory and non-secretory carcinomas of the breast using publicly available genomic data.

MATERIALS AND METHODS

Study design and data collection

Published genomic studies of molecular lesions in neurotrophic receptor tyrosine kinases (NTRKs) in breast cancer were interrogated in the cBioportal platform (http://www.cbioportal.org). cBioportal is a platform freely available to investigators containing molecular studies and corresponding clinical data [7,8]. The platform allows for a multi-dimensional interrogation of genomic data from publicly available studies. cBioportal also provides the opportunity to associate data of molecular lesions (mutations, fusions, copy number alterations and mRNA hyperexpression or hypo-expression) of any gene of

interest in studies from the Cancer Genome Atlas (TCGA) and other studies with patient clinical characteristics and survival outcomes[7]. The current analysis is based on the TCGA breast cancer study and on the project GENIE study, both included in the cBioportal and providing open access to their data[9, 10]. The analysis of copy number alterations (CNAs) in TCGA is performed with the algorithm Genomic Identification of Significant Targets in Cancer (GISTIC) [11]. In GISTIC, putative amplification of a given gene is defined as a score of 2 or above. TCGA provides an aneuploidy score (AS) as a measure of chromosomal instability of each sample. AS is calculated as the sum of the number of chromosome arms in each sample that have copy number alterations (gains or losses). A chromosome arm is considered copy number altered, either gained or lost, if there is a somatic copy number alteration in more than 80% of the length of the arm as calculated by an algorithm called ABSOLUTE based on Affymetrix 6.0 SNP arrays[12]. Chromosomal arms with somatic copy number alterations in 20% to 80% of the arm length are considered not evaluable and chromosomal arms with somatic copy number alterations in less than 20% of the arm length are considered not altered. mRNA expression grids in cBioportal are constructed and normalized using the RSEM (RNA-Seq by Expectation Maximization) algorithm[13].

Expression of NTRK proteins in breast cancer were evaluated using publicly available data from the Human Protein Atlas (www.proteinatlas.org), a database of protein expressions in human normal and neoplastic tissues[14]. The Human Protein Atlas contains a semi-quantitative immunohistochemistry-based evaluation of the expression of proteins of interest.

The effect of mRNA expression level of *NTRK1* gene on survival of breast cancer patients was examined with data derived from the online publicly available platform Kaplan Meier Plotter (www.kmplot.com)[15]. The cut-off of amplified and non-amplified samples for each gene was set at the higher quartile of amplification, which is the closer cut-off provided by the platform to the percentage of breast cancer cases with *NTRK1* amplifications.

Statistical analysis

The Fisher's exact test or the χ^2 test and the *t* test, respectively, are used to compare categorical and continuous data. Kaplan-Meier survival curves were compared using the Log Rank test. All statistical comparisons were considered significant if $P < 0.05$.

RESULTS

Among 11,886 patients with breast cancer in the project GENIE cohort, 27 patients (0.22%) had fusions in one of the three NTRK genes. Two male patients were included among the 27 NTRK fusion positive breast cancer patients. Most patients with information for race were White, while 2 patients were Black and one was Asian. Histologically, 25 patients were diagnosed with ductal carcinomas or breast carcinomas not otherwise specified and one patient had an invasive lobular carcinoma. Interestingly, only one case was diagnosed as juvenile secretory carcinoma, in a male patient. A total of 32 fusions involving the 3 NTRK genes were present in the 27 patients (5 patients had more than one different fusions). The most frequent fusions involved *NTRK3*, in 16 samples, followed by *NTRK1* in 13 samples and *NTRK2* in 3 samples. Fusions in seven cases were intragenic and a variety of partners were involved in other fusions. Recurring partner genes include *ETV6* with *NTRK3* and *LMNA* with *NTRK1*. Frequent mutations observed in breast cancers such as in *TP53*, *PIK3CA* and *GATA3* genes are also observed in cases with NTRK gene fusions. with a frequency not statistically significant different compared with cases without NTRK fusions. Similarly, common amplifications in breast cancers of *CCND1* at 11q13.3, *ERBB2* at 17q12, *NSD3* at 8p11.23 and *c-MYC* at 8q24.21 are encountered in cases with NTRK fusions in frequencies comparable to cancers without NTRK fusions.

In TCGA breast cancer cohort, no fusions involving the 3 NTRK genes were observed. The most common molecular lesions were amplifications that were observed in 9.8% of patients, most commonly in NTRK1 (7.9%) and more rarely in NTRK3 (1.9%) and in NTRK2 (0.2%). NTRK amplified cancers have a distribution of histologic types (ductal, lobular, mixed, other) that is similar to non-amplified cases. NTRK amplified cancers are basal more commonly than NTRK non-amplified cancers (31.1% *vs* 14.1%, χ^2 $P < 0.001$) but less commonly of the luminal B phenotype (11.3% *vs* 18.9% in non-amplified cancers, χ^2 $P = 0.05$) (Figure 1). Total tumor mutation burden (TMB) was not different between NTRK amplified and non-amplified breast cancers with about 10% of cases in each group displaying a TMB above 120. In contrast, NTRK amplified cancers had more commonly chromosomal instability as measured by an AS of 4 or greater (χ^2 $P < 0.001$) (Figure 2). Among cancer-associated genes frequently mutated in breast cancer, tumor suppressor *TP53* was mutated in 41.5% of NTRK amplified cancers and in 31.6% of non-amplified cancers (χ^2 $P = 0.03$) (Figure 3). In contrast, oncogene *PIK3CA* was more often mutated in NTRK non-amplified cases (33.5% *vs* 23.6% in NTRK amplified cases), although the difference did not reach statistical significance (χ^2 $P = 0.06$). Amplifications of oncogene *c-MYC* were more common in NTRK amplified cases (24.5% *vs* 14% in NTRK non-amplified cases, χ^2 $P = 0.005$).

In breast cancer cases with amplifications of NTRK genes, expression of the respective mRNAs is not increased, except in rare cases. However, breast cancers with increased NTRK1 mRNA expression at the upper quartile tend to have a better overall survival (OS) than cancers with low NTRK1 mRNA

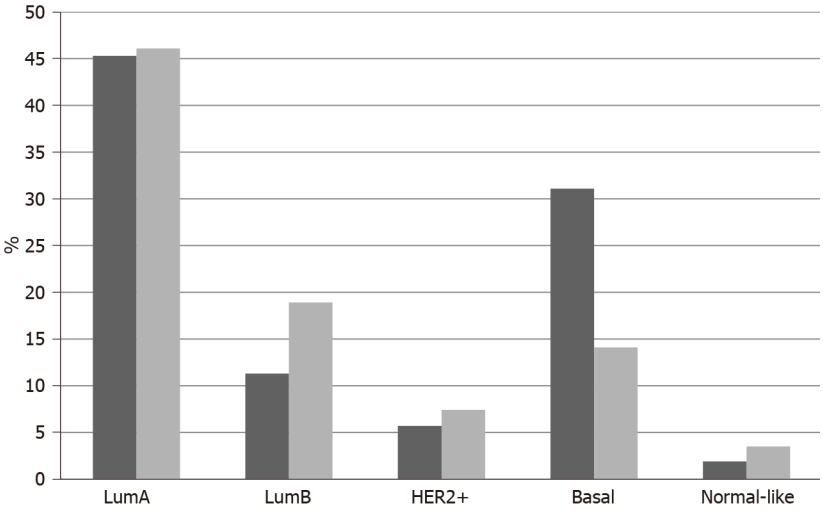


Figure 1 Percentage of patients with different breast cancer molecular sub-types among the neurotrophic receptor tyrosine kinase amplified and non-amplified groups. Data are from TCGA breast cancer cohort. Black columns: Neurotrophic receptor tyrosine kinase amplified; Grey columns: Neurotrophic receptor tyrosine kinase non-amplified.

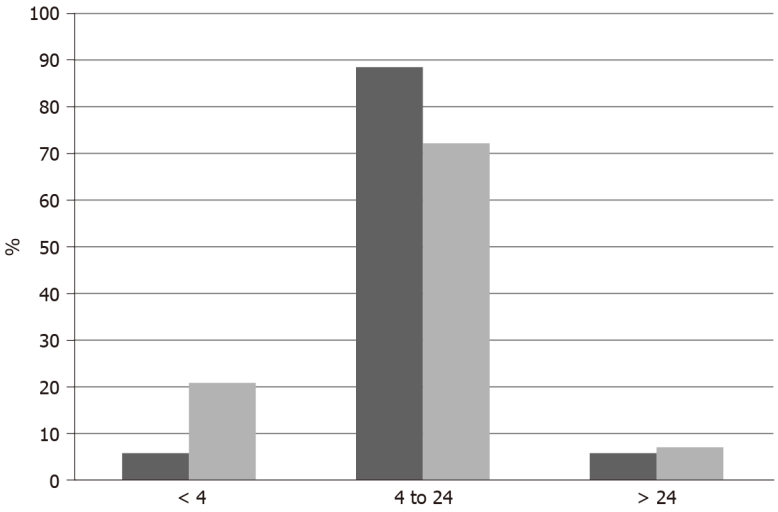


Figure 2 Percentage of patients with different aneuploidy score levels in breast cancer groups with and without neurotrophic receptor tyrosine kinase amplifications. Data are from TCGA breast cancer cohort. Black columns: Neurotrophic receptor tyrosine kinase amplified; Grey columns: Neurotrophic receptor tyrosine kinase non-amplified.

expression, falling in the three lower quartiles (Figure 4A). This holds true also for an analysis restricted to basal breast cancers (Figure 4B).

Protein expression of TrkA is present in most breast cancers as evaluated by immunohistochemistry. The Human Protein Atlas has found moderate to high expression of the protein in 72.7% (8 of 11 samples) and 63.6% (7 of 11 samples) using two different antibodies (HPA035799 rabbit polyclonal antibody from Sigma-Aldrich and CAB004606 mouse monoclonal antibody from Santa Cruz Biotechnology). One and three additional cases have shown a low intensity of staining. In contrast, almost all cases of breast cancer examined showed low or absent staining for TrkB and TrkC in the Human Protein Atlas.

DISCUSSION

The human neurotrophin system consists of four ligands, nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5), and three Trk receptors, TrkA, TrkB, TrkC, as well as two additional non-Trk receptors, $p75^{NTR}$ and sortilin[16]. NGF is the ligand for receptor TrkA, encoded by gene *NTRK1*, BDNF and NT-4/5 are the ligands for TrkB encoded by gene *NTRK2* and NT-5 is the ligand for TrkC, encoded by gene *NTRK3*. NT-5 can also ligate

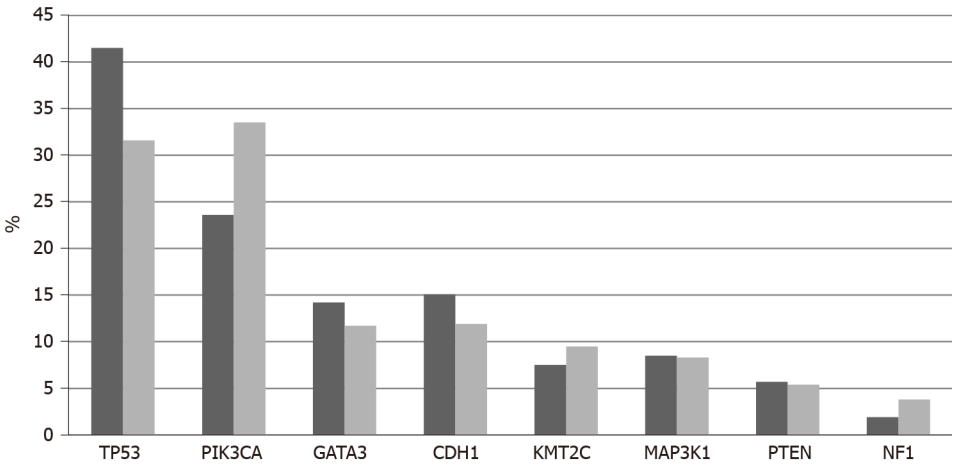


Figure 3 Percentage of mutations in the most common breast cancer associated genes in breast cancers with and without neurotrophic receptor tyrosine kinase amplifications. Data are from TCGA breast cancer cohort. Black columns: Neurotrophic receptor tyrosine kinase amplified; Grey columns: Neurotrophic receptor tyrosine kinase non-amplified.

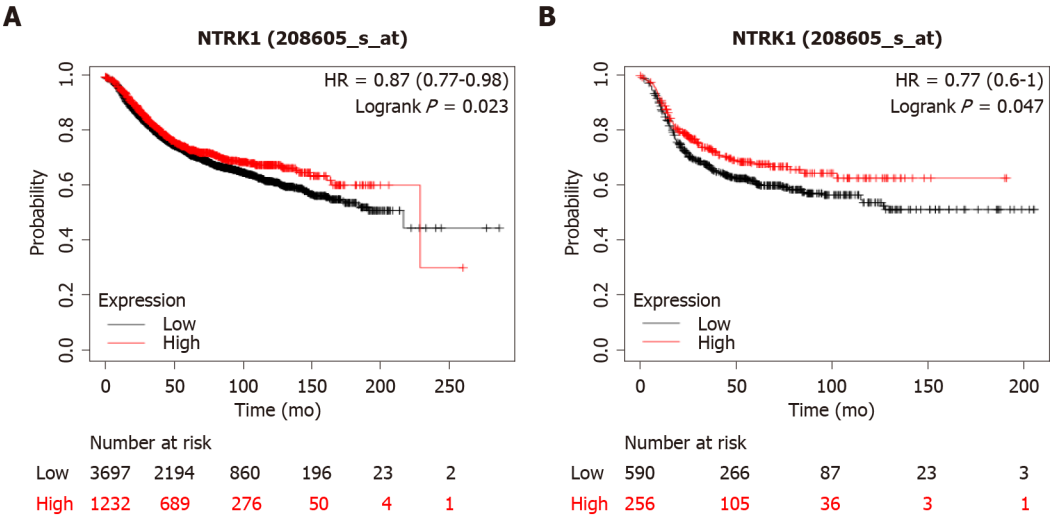


Figure 4 Overall survival of breast cancers according to *NTRK1* mRNA expression. A: Overall survival curves of all breast cancer patients with high *NTRK1* mRNA expression *vs* low *NTRK1* mRNA expression, across sub-types. Log-rank *P* = 0.02; B: Overall survival curves of basal sub-type patients with high *NTRK1* mRNA expression *vs* low *NTRK1* mRNA expression. Log-rank *P* = 0.04.

the two other Trk receptors. All four ligands ligate the common neurotrophin receptor p75^{NTR}, which also serves as a receptor for the precursor forms of the four ligands pro-NGF, pro-BDNF, pro-NT-3 and pro-NT-4/5 and possesses an intracellular death domain[17]. All precursor forms ligate, additionally, sortilin, also known as neurotensin receptor 3 (NTSR3). The three Trk kinase receptors have a common structure with an extracellular part consisting of two cysteine-rich domains separated by three leucine-rich repeats. Closer to the cell membrane, the three Trk receptors possess two immunoglobulin-like domains. The intracellular part of the receptors holds the tyrosine kinase domain with five conserved tyrosines, three of which are phosphorylated when the receptors are activated by ligand binding. Downstream of receptors activation, the MAPK and PI3K/Akt kinase cascades are activated transmitting signals of proliferation and apoptosis inhibition. The physiologic role of neurotrophins signaling is best described in the nervous system, both in peripheral nervous system and the brain[18]. In peripheral nervous system, NGF signaling stimulates neurite growth and prevents apoptosis. NGF is also functional in brain where BDNF is also present and involved in signal transduction through binding and induction of phosphorylation of TrkB resulting in neuron survival and differentiation[18,19].

Similarities of downstream signaling between the neurotrophin receptors and the EGFR family of tyrosine kinase receptors, which are well-known oncogenes, have led to the exploration of the role of Trk receptors in cancer[18,20]. Expression of receptor TrkA is observed in breast cancer cell lines and in breast cancer tissues, where it is more prominent than surrounding normal breast epithelium[21]. TrkA signaling enhances breast cancer cell proliferation and anchorage independent growth. Moreover, TrkA

inhibits apoptosis and anoikis in metastatic sites. In breast cancer cells, activation of TrkA transmits signals through the MAPK pathway consistent with the physiologic circuit of the receptor[18]. Pro-NGF derived from cancer cells also binds TrkA and signals in an autocrine manner[22]. TrkA ligation promotes interaction with another receptor kinase EphA2 and downstream activation of Src and Akt kinases, resulting in phosphorylation and activation of these kinases and stimulation of cell growth[22]. In contrast, pharmacologic or small interfering RNA inhibition of TrkA or EphA2 decreases tumor growth and metastases of breast cancer in an *in vivo* mouse model. Immunohistochemical studies showed that Pro-NGF is expressed at higher levels in breast cancer tissues compared with normal breast tissues and benign lesions[23]. Receptor $p75^{NTR}$ is also expressed in breast cancer cells and leads to increased proliferation and inhibition of apoptosis through activation of transcription factor NF-κB[24]. $p75^{NTR}$ interacts with NF-κB through mediator TRADD (TNFR Associated Death Domain) when MCF-7 breast cancer cells are exposed to NGF and absence of TRADD promotes apoptosis in the same conditions[24].

BDNF and its receptor TrkB are expressed in breast cancer cells and promote cell survival and proliferation[25,26]. Knock down of BDNF or pharmacologic inhibition of TrkB reduced the viability of breast cancer cells *in vitro*. TrkB is expressed in cancer stem cells with the CD44 positive phenotype derived from triple negative breast cancers and participates in a paracrine circuit ligated by BDNF derived from differentiated breast cancer cells[27]. BDNF stimulation induces stemness core factor KLF4 and promotes stemness phenotype and stem cell renewal. In addition, TrkB expression predicts relapse of localized triple negative breast cancers[27]. Related to stemness, epithelial to mesenchymal transition (EMT) is a process that promotes cancer cell metastasis[28]. TrkB is a target of micro-RNA miR-200c, which protects against EMT and preserves breast cancer cell epithelial phenotype, further supporting the role of TrkB in breast cancer promotion[29].

Further interest in neurotrophin family receptors in cancer was stimulated by the discovery of rare fusions involving the receptor genes *NTRK1*, *NTRK2* and *NTRK3* that act as oncogenic drivers and can be neutralized by NTRK inhibitors[30]. Fusions involving most frequently NTRK3 were confirmed to be the underlying molecular mechanism of secretory mammary carcinomas but were absent in other more common breast cancer histologies[30,31]. The carcinogenic mechanism of the fusion involves deregulated activation of the RAS-MAPK and PI3K-Akt pathways that are the physiologic targets of neurotrophic kinases signaling[32]. In contrast, these fusions do not affect the differentiation programs of cells, as suggested by their presence in diverse cancers, in addition to secretory breast carcinomas, including nephromas, infant fibrosarcomas, mammary analogue secretory carcinomas of the salivary glands and acute myeloblastic leukemias[33,34].

Breast secretory carcinoma is a rare type of breast cancer malignancy, accounting for 0.15% to 0.2% of all breast cancers[35]. It has generally an indolent clinical behavior, with late local recurrence and prolonged survival, even with lymph node involvement, that occurs at nearly 30% of cases[36]. It was initially described by Mc Divitt and Stewart as a variation of mammary carcinoma in the pediatric population, also called "juvenile carcinoma"[37]. In 1980, Tavassoli and Norris[38] reported several cases presenting at variable ages and recommended to replace the previous definition with the term "secretory carcinoma". Secretory breast cancer displays a particular phenotype related to the presence of large amounts of intracellular and extracellular secretory material. Although it has been described as a sub-family of basal-like triple negative breast cancers with cytokeratin 5/6 expression and no expression of either hormone receptors or HER2, this tumor has a unique immunophenotypic profile among triple negative breast carcinomas. It stains diffusely for mammaglobin and gross cystic disease fluid protein (GCDFP) and displays strong and diffuse SOX10 and S-100 Labeling[39,40]. The hallmark genetic alteration of this cancer is a t(12;15) translocation, resulting in the ETV6-NTRK3 gene fusion which is pathognomonic, detected in more than 90% of secretory breast carcinomas. The same translocation is also identified in the mammary analog secretory carcinoma, its salivary gland counterpart[30,39,41]. Given that NTRK3 protein expression is not detected in breast epithelial cells physiologically, immunohistochemical analysis using pan-Trk antibodies has been shown to constitute a sensitive and specific approach for the detection of NTRK3 rearrangements in breast secretory carcinomas[42]. Absence of Trk receptor expression in non-secretory breast cancer was also observed in a micro-array study of 339 breast cancer patients, using a pan-Trk rabbit monoclonal antibody[43]. However, since TrkA is expressed in a sub-set of non-secretory breast cancers, as shown in the human Protein Atlas, the intensity of staining should be taken into consideration and confirmatory molecular detection of fusions should be standard practice.

Patients with tumors that harbor NTRK fusions as a specific molecular alteration may benefit from treatment with selective tyrosine kinase inhibitors or antagonistic monoclonal antibodies. There is accumulating evidence for the efficacy of Trk inhibitors in the control of the disease in these patients. Two Trk inhibitors, larotrectinib and entrectinib, are both approved by the American Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Larotrectinib is a selective inhibitor of the Trk proteins (including TrkA, TrkB, and TrkC) approved for the treatment of locally advanced and metastatic solid tumors with NTRK fusions, in both adult and pediatric cancer patients. Its efficacy and safety have been studied in three multicenter, phase 1/2, open-label, single-arm clinical trials[44-46]. An objective response rate (ORR) of 78% [95% confidence interval (CI): 71%-84%] regardless of histology, age, and type of NTRK fusion was shown in the joint analysis of these studies. The median

progression-free survival (PFS) was 36.8 months [95%CI: 25.7 mo - not estimated (NE)] with 90% (95%CI: 75%-90%) of patients being alive at 1 year. A specific adult population analysis, that included 5% with breast cancers, has showed an ORR of 71% (95%CI: 62%-79%), a median PFS of 25.8 mo (15.2 mo to NE), and 87% of patients alive at 1 year. The most frequent fusion transcripts were NTRK3 (54%) and NTRK1 (43%), and only 3% had NTRK2[47]. Impressive responses have also been observed in locally advanced disease highlighting the potential utility of Trk inhibition as neoadjuvant therapy in the non-metastatic setting. The most common side effects were fatigue (30%), constipation (27%), cough (27%), elevation of liver enzymes (24%), dizziness (25%) and nausea (24%), mainly grade 1-2. Only 2% of patients had to discontinue treatment and 16% had grade 3-4 toxicity related to treatment, predominantly elevation of transaminases (3%), decreased neutrophil count (2%) and anemia (2%).

Entrectinib, a multikinase inhibitor of the TrkA, TrkB, TrkC, ROS1, and ALK proteins was tested in three clinical trials, two phase 1 and one "basket" phase 2 trial in the adult population with tumors harboring NTRK fusions[48]. Breast cancer represented the 11% of the tumors in these trials. In the analysis of the first 54 patients included, an ORR of 59% (95%CI: 45%-72%), a PFS of 11.2 mo (95%CI: 8.0-14.9 mo), and an OS of 23.9 mo (95%CI: 16.8 mo to NE) were observed. Entrectinib crosses the blood-brain barrier and has showed activity at the CNS level. In the 22% of patients with brain metastases the intracranial ORR was 55% (95%CI: 23%-83%)[48,49]. The activity of Trk inhibitors in the CNS is crucial because cancers that harbor NTRK fusions can present with primary or metastatic intracranial disease. The tolerability of the inhibitors was acceptable with the most frequent adverse events being anemia (10%), weight gain (14%), dyspnea, and asthenia (25%)[50,51]. The majority of adverse events encountered were grade 1-2. These side effects are manageable with drug dose modifications.

Despite the marked efficacy of Trk inhibitors and, in many cases, long-lasting responses, resistance may occur leading to progression. Several potential mechanisms of resistance have been described, including through the development of mutations in the NTRK genes, mutations of mitogen-activated protein kinase (MAPK) pathway genes such as BRAF (V600E) and KRAS (G12D), and the amplification of MET[52]. Razavi *et al*[53] identified lesions in the MAPK pathway that were responsible for resistance to endocrine therapy. The same findings were supported by Ross *et al*[54], who aimed at characterizing kinase fusions within a large cohort of 4857 patients with advanced breast cancer. In total, 56% with fusion-positive breast cancers had a history of previous endocrine therapy and none of the fusion-positive breast cancer samples harbored *ESR1* hotspot mutations. Two patients with acquired LMNA-NTRK1 fusions and metastatic disease received larotrectinib and demonstrated clinical benefit[54]. The kinase fusions, even if they are rare in breast cancer, they are enriched in hormone-resistant, metastatic carcinomas and mutually exclusive with *ESR1* mutations. These data expand the spectrum of genetic alterations activating MAPK signaling that can substitute for *ESR1* mutations in this setting. Thus, molecular testing in metastatic breast cancer at progression after endocrine therapy should include fusion testing, especially in case of absence of *ESR1* hotspot alterations. Specific mutations in NTRK1 producing substitutions at position p.G595R and p.G667C have been described as associated with resistance to entrectinib in a patient with colon cancer bearing the LMNA-NTRK1 rearrangement[55].

Second-generation Trk inhibitors such as selitrectinib and repotrectinib, have been developed, and have shown activity in these patients, in both adult and pediatric populations, in the first in human dose escalation clinical trials[56,57]. Currently there are ongoing trials evaluation the safety and efficacy of these drugs in solid tumors. Inspired by the resistance of other kinases, dual blocking TRKs or combination inhibitors could be a new treatment approach. The inhibitor foretinib was able to inhibit xenografts with the LMNA-NTRK1 fusion that had become resistance to entrectinib after acquiring the above mentioned mutation p.G667C, suggesting that mutations producing resistance to first generation inhibitors may still be sensitive to second generation inhibitors in development[58].

Regarding NTRK gene amplification, it was shown to result in TRK overexpression in a sub-set of breast cancers[59]. Among the 1250 analyzed tumor specimens, NRTK amplification was detected in 2.2% cases, representing 14.8% of cases with TrkA protein overexpression. The efficacy of TRK inhibitors in tumors harboring NTRK gene amplification is not well characterized. These patients were not included in the trials of TRK inhibitors. Interestingly, two patients with NTRK gene amplification have been described in whom larotrectinib has marked a durable antitumor activity[60,61] suggesting that these therapies may have a role in a sub-set of amplified patients, who remain to be identified. Regarding patients with mutations as a primary abnormality in Trk proteins (as opposed to the discussed above secondary mutations in patients with fusions), TRK inhibitors have not shown activity [61]. It has been suggested that these mutations are not driver lesions in these cancers. However, it is possible that a subset of these mutations are indeed oncogenic[62]. In various hematologic malignancies including AML, ALL, CML and myelofibrosis, NTRK2 and NTRK3 mutations were observed in about 5% of patients and cells bearing some of them were inhibited by entrectinib *in vitro*[63].

CONCLUSION

The current study confirms the rarity of NTRK fusions in breast cancer with a prevalence rate of less than 1%. Amplifications of NTRK1 are more common and are associated with basal cancers and *TP53*

mutations which are most common in this breast cancer type. Although amplification of the NTRK1 gene does not always lead to mRNA over-expression, TrkA protein is expressed in many breast cancers and thus can be a therapeutic target. In contrast to our results, another study that examined expression of TrkA at the protein level suggested that the receptor is more often expressed in HER2 positive cancers compared to luminal and basal carcinomas[64]. This study also confirmed discrepancies between gene dosage and protein expression. Clearly, transcription and post-transcription regulations are critical for TrkA expression in breast cancer.

ARTICLE HIGHLIGHTS

Research background

Breast cancer is a common female cancer. It constitutes a major cause of morbidity and mortality. Progress in breast cancer therapeutics has been attained with the introduction of targeted therapies for specific sub-sets. However, other breast cancer subsets lack targeted therapeutics.

Research motivation

Personalized therapies for breast cancer have the potential to improve outcomes. These therapies take consideration of specific molecular abnormalities that could be targeted for optimal results.

Research objectives

This article aims to analyze the landscape of neurotrophic receptor tyrosine kinase (NTRK) abnormalities in breast cancer. These are molecular lesions in a family of receptor tyrosine kinases. Specific drug inhibitors have been developed and have obtained regulatory approval, paving the way for effective patient treatment.

Research methods

An analysis of publicly available genomic breast cancer datasets was performed for identification and characterization of cases with fusions and other molecular abnormalities involving *NTRK1*, *NTRK2* and *NTRK3* genes.

Research results

NTRK fusions are rare in breast cancers as a whole. They are present in a small number of breast cancers at the extensive GENIE project data set which contains more than 10000 breast cancers. These cases are not identified as secretory in the database.

Research conclusions

NTRK lesions other than fusions are more common than fusions in breast cancers. However, confirmation of efficacy for the currently available inhibitors exist only for NTRK fusions.

Research perspectives

Amplifications of NTRK receptor genes are more common than fusions in breast cancers. Inhibitors effective for interfering with the activity of amplified NTRK receptors could advance therapeutics in this subset of breast cancers.

FOOTNOTES

Author contributions: Both authors contributed to the conception of the study, literature review, data analysis, writing of the article and revising the article.

Institutional review board statement: No institutional review board approval was required or obtained as the study was not a clinical trial.

Clinical trial registration statement: This study was not registered as a clinical trial as it includes only data from publicly available previously published studies and no new patient information.

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Abstract

BACKGROUND

The targeted therapy cetuximab [directed at the epidermal growth factor receptor (EGFR)] in combination with 5-fluorouracil and platinum-based chemotherapy (the EXTREME regimen) has shown substantial efficacy for patients with recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN). Thus, this scheme has been established as the preferred first-line option for these patients. However, more recently, a new strategy combining platinum, taxanes, and cetuximab (the TPEx regimen) has demonstrated similar efficacy with a more favorable toxicity profile in clinical trials.

AIM
To evaluate the safety and efficacy of the TPEx scheme as first-line therapy in advanced SCCHN in a multicenter cohort study.

METHODS
This retrospective multicenter cohort study included patients with histologically confirmed recurrent or metastatic SCCHN treated with first-line TPEx at five medical centers in Argentina between January 1, 2017 and April 31, 2020. Chemotherapy consisted of four cycles of docetaxel, cisplatin, and cetuximab followed by cetuximab maintenance therapy. Clinical outcomes and toxicity profiles were collected from medical charts. Treatment response was assessed by the investigator in accordance with Response Evaluation Criteria in Solid Tumors (version 1.1). Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0).

RESULTS
Twenty-four patients were included. The median age at diagnosis was 58 years (range: 36-77 years). The majority of patients (83.3%) received at least four chemotherapy cycles in the initial phase. In the included group, the overall response rate was 62.5%, and 3 patients achieved a complete response (12.5%). The median time to response was 2.4 mo [95% confidence interval (CI): 1.3-3.5]. With a median follow-up of 12.7 mo (95%CI: 8.8-16.6), the median progression-free survival (PFS) was 6.9 mo (95%CI: 6.5-7.3), and the overall survival rate at 12 mo was 82.4%. Patients with documented tumor response showed a better PFS than those with disease stabilization or progression [8.5 mo (95%CI: 5.5-11.5) and 4.5 mo (95%CI: 2.5-6.6), respectively; P = 0.042]. Regarding the safety analysis, two-thirds of patients reported at least one treatment-related adverse event, and 25% presented grade 3 toxicities. Of note, no patient experienced grade 4 adverse events.

CONCLUSION
TPEx was an adequately tolerated regimen in our population, with low incidence of grade 3-4 adverse events. The median PFS were consistent with those in recent reports of clinical trials evaluating this treatment combination. This regimen may be considered an attractive therapeutic strategy due to its simplified administration, decreased total number of chemotherapy cycles, and treatment tolerability.

Key Words: Recurrent and/or metastatic head and neck cancer; TPEx schema; Cetuximab; Docetaxel; Cisplatin; First-line

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Core Tip: We evaluated the safety and efficacy of the combination platinum, taxanes, and cetuximab scheme as a first-line therapy for patients with recurrent or metastatic squamous cell carcinoma of the head and neck in a real-world setting. Among the 24 patients included, the median progression-free survival was 6.9 mo (95% confidence interval: 6.5-7.3), and the overall survival rate at 12 mo was 82.4%, which was consistent with previous clinical trials. Patients with documented tumor response showed statistically better progression-free survival than those with disease stabilization or progression (P = 0.034). The combination platinum, taxanes, and cetuximab regimen was adequately tolerated by most of the analyzed patients, as the incidence of grade 3-4 adverse events was surprisingly lower than expected (25%).

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INTRODUCTION

Squamous cell carcinoma of the head and neck (SCCHN) cases represent 5% of all newly diagnosed cancer cases, leading to over 300000 deaths per year[1]. Despite appropriate primary treatments, in

approximately 50% to 60% of patients with stage III to IV disease locoregional relapse occurs[2]. Given that a significant proportion of these patients are not suitable for surgery or radiotherapy, systemic treatments and best supportive care are the preferred therapeutic options.

Up to the early 2000s, the median overall survival (OS) of patients with metastatic disease was only 6 mo[3,4]. This poor prognosis encouraged significant research efforts to develop novel drugs in the last 15 years. In this setting, the targeted therapy cetuximab [directed at the epidermal growth factor receptor (EGFR)] has shown substantial efficacy for recurrent or metastatic (R/M) SCCHN treatment in combination with 5-fluorouracil and platinum-based chemotherapy (the EXTREME regimen)[3]. More recently, a new strategy using the immune checkpoint inhibitor pembrolizumab alone or in combination with 5-fluorouracil and platinum has become an appropriate first-line treatment for R/M SCCHN patients[5-7].

Currently, the EXTREME regimen still represents a recommended first-line treatment option in selected scenarios, such as cases with programmed death-ligand 1-negative tumors or when immunotherapy is contraindicated. Notably, this treatment regimen may represent an attractive approach for patients with disease progression after first-line immune checkpoint inhibitors are given as monotherapy[8].

Taxanes have maintained widespread clinical use, particularly in solid tumors since their discovery in the early 1970s, and several clinical trials have shown their antineoplastic activity against SCCHN[9-11]. The addition of fluorouracil to a taxane seeks to take advantage of the potential immunogenic and proapoptotic synergy between cetuximab and docetaxel or paclitaxel[12,13]. Cetuximab-, platinum-, and taxane-based schedules have been associated with promising survival results and cytoreductive properties in clinical studies[14-18]. TPExtreme was the first large, phase 3, randomized trial comparing the TPEx regimen (cetuximab, taxane, and platinum) with the EXTREME scheme in a first-line setting [19]. This trial demonstrated similar efficacy outcomes in 539 R/M HNSCC patients, showing a median OS of 14.5 and 13.4 mo using the TPEx and EXTREME regimens, respectively. Furthermore, the TPEx arm had a more favorable toxicity profile, leading to better compliance of the planned treatment (72% *vs* 44%) and fewer dose interruptions (10% *vs* 27%).

Based on these considerations and given the scarce real-world studies including patients treated with this scheme, we retrospectively evaluated the efficacy and safety of the TPEx regimen as first-line therapy in patients with R/M SCCHN.

MATERIALS AND METHODS

Study population and treatment characteristics

This retrospective multicenter cohort study included patients seen between January 1, 2017 and April 31, 2020 with a histologically confirmed diagnosis of R/M SCCHN who received TPEx as first-line treatment at five medical centers in Argentina. Chemotherapy consisted of four cycles of docetaxel 75 mg/m^2 and cisplatin 75 mg/m^2 every 3 wk and cetuximab (400 mg/m^2 on day 1 of cycle 1 and then 250 mg/m^2 weekly), with systemic granulocyte colony-stimulating factor support during each cycle. Patients with controlled disease continued with weekly cetuximab 250 mg/m^2 or cetuximab 500 mg/m^2 every 2 wk as maintenance until disease progression or unacceptable toxicity. Demographic and clinicopathological characteristics, including age, Eastern Cooperative Oncology Group performance status, smoking status, alcohol consumption, primary tumor site, and previous treatments, were collected from medical charts and entered into a predefined centralized database. Efficacy and safety information was also retrieved, and treatment strategies, responses, adverse events, and discontinuation were also documented.

Disease progression and treatment response were collected from medical charts. Treatment response was assessed by the investigator using computed tomography or magnetic resonance imaging scans in accordance with Response Evaluation Criteria in Solid Tumors (RECIST version 1.1). Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). The study was reviewed by our expert biostatistician Santiago Duarte, MD.

Statistical analysis

Data are summarized as frequencies and percentages for categorical variables and as medians, ranges, and interquartile ranges for continuous variables. The progression-free survival (PFS) and OS of patients treated with TPEx as first-line treatment were calculated from the date of therapy initiation to first documented relapse (PFS) or death due to any cause (OS). Data were censored at the last follow-up if the patient was alive. The duration of response (DOR) was defined as the time from the first complete response (CR) or partial response to progressive disease or death. Survival curves were generated using the Kaplan-Meier method, and differences between groups were calculated using the log-rank test. Relevant prognostic factors were stratified by univariate Cox regression models for PFS. All statistical analyses were performed using SPSS software version 23.0 (SPSS, Inc., Armonk, NY, United States).

RESULTS

Patients characteristics

In this multicenter retrospective study, 24 patients with R/M SCCHN were included from five Argentinian medical centers. All patients received first-line chemotherapy with TPEx. The median age at diagnosis was 58 years (range: 36-77), males made up 62.5% of the population (n = 15), and the majority of patients had an Eastern Cooperative Oncology Group score of 0-1 (22, 91.7%) (Table 1). A smoking history was reported in 13 patients (54.2%), and approximately one-third of the patients reported alcohol consumption. Of note, only 2 patients (8.3%) had a body mass index < 18.5.

Previous treatments included definitive concomitant chemoradiotherapy (33.3%), surgery (20.8%), surgery plus radiotherapy (12.5%), chemoradiotherapy (20.8%), and definitive radiotherapy alone (4.2%). Approximately half of the population had previously received cisplatin (n = 13, 54.2%), and only 2 patients (8.3%) had metastatic disease at diagnosis. The most common reason for treatment discontinuation was disease progression (58.3%), and only 2 patients (8.3%) discontinued treatment prematurely due to unacceptable toxicity. Notably, most patients (83.3%) received at least four chemotherapy cycles during induction therapy.

Efficacy

A total of 3 patients achieved a complete response (12.5%), and in half of the patients, a partial response was documented (Table 2). Remarkably, most of the patients benefited from TPEx therapy since the overall response rate (ORR) and disease control rate (DCR) were 62.5% and 87.5%, respectively. The median time to response was 2.4 mo [95% confidence interval (CI): 1.3-3.5).

No statistical differences were observed in terms of ORR or DCR between patients with only locoregional recurrence prior to TEPx initiation and the rest of the included patients [ORR 50% (7/14), DCR 85.7% (12/14) and ORR 80% (8/10), DCR 90% (9/10), respectively; P = 0.21 and P = 1.0].

After a median follow-up of 12.7 mo (95%CI: 8.8-16.6), 14 progression events occurred. The median PFS and DOR were 6.9 mo (95%CI: 6.5-7.3) (Figure 1A) and 5.1 mo (95%CI: 3.0-7.2), respectively (Figure 1B). Univariate relevant prognostic factor analyses for first-line TPEx PFS are reported in Table 3. As expected, patients with documented tumor response showed a better PFS than those with disease stabilization or progression [8.5 mo (95%CI: 5.5-11.5) and 4.5 mo (95%CI: 2.5-6.5), respectively; P = 0.042] (Figure 1C). Notably, in 2 out of the 3 patients with documented CR, substantially longer PFS (22.3 and 18.8 mo) and DOR (16.6 and 16.9) were observed. Patients with hypo/oropharyngeal tumors had a better PFS compared to those with other primary sites [22 mo (95%CI: 19.9-25.1) and 6.7 mo (95%CI: 4.7-8.9), respectively; P = 0.038] (Figure 1D). No difference was observed when comparing patients with advanced and metastatic disease (P = 0.953) (Figure 1E). The OS rate at 12 mo was 82.4% (Figure 1F). Remarkably, among the 14 patients who experienced disease progression on TPEx, 13 received second-line treatment with immunotherapy [pembrolizumab (n = 9) and nivolumab (n = 4)].

Safety and adverse events

Two-thirds of the patients reported at least one treatment-related adverse event, and 25% reported at least one grade 3 adverse event. Of note, no patient experienced grade 4 toxicity. A summary of the safety profile is listed in Table 4. The most commonly reported hematological adverse events were febrile neutropenia (12.5%), anemia (12.5%), and hyponatremia/hypokalemia (12.5%). Among nonhematological events, acne-like rash was the most frequent (33.3%) related adverse event. Grade 3 nausea-vomiting, asthenia, and renal failure were noted in 4.2% of the patients. Only 1 patient experienced a grade 1 hypersensitivity reaction during taxane infusion.

Overall, serious adverse events were reported in 5 patients (20.8%). Three of the patients developed febrile neutropenia, 1 developed acute renal failure, and the remaining patient was hospitalized due to grade 3 vomiting that required intravenous hydration. All patients continued treatment after the toxicity resolved. The median duration of hospitalization among patients with severe adverse events was 6 d (range: 2-22). Additionally, no fatal events were reported. Globally, TPEx was associated with a low rate of adverse events leading to treatment interruption (12.5%), discontinuation (8.3%), or dose reduction (8.3%).

DISCUSSION

Despite substantial advances in the last decade, R/M SCCHN remains a significant clinical challenge because of its associated high mortality rate. As such, increasing the tumor response rate is an important goal in these patients given its association with symptom improvement and better quality of life.

Over the past years, the EXTREME regimen has become a preferred first-line strategy for R/M SCCHN patients[3]. While significant improvements in OS, PFS, and ORR were demonstrated in the cetuximab plus platinum–fluorouracil arm of a pivotal phase 3 trial, 82% of the included patients experienced grade 3-4 adverse events, mostly related to 5-fluorouracil continuous infusion. Of note, all these findings were observed in fit patients; hence, treatment decisions in this setting should be

Table 1 Clinicopathological characteristics

Characteristics	Number of patients (%)
Total	24
Median age (range), yr	58 (36-77)
Sex	
Male	15 (62.5)
Female	9 (37.5)
ECOG at TPEx treatment initiation	
0-1	22 (91.7)
2	2 (8.3)
Smoking history	
Never	6 (25)
Current or former	13 (54.2)
NS	5 (20.8)
Alcohol consumption	
Occasional or regular	8 (33.3)
None	7 (29.2)
NS	9 (37.5)
Primary tumor site	
Larynx	7 (29.2)
Oropharynx	6 (25)
Oral cavity	5 (20.8)
Hypopharynx	1 (4.2)
Other	5 (20.8)
Previous treatment	
Concomitant chemoradiotherapy only	8 (33.3)
Surgery only	5 (20.8)
Surgery + concomitant chemoradiotherapy	5 (20.8)
Surgery + radiotherapy	3 (12.5)
Radiotherapy only	1 (4.2)
No	2 (8.3)
Extent of disease at TPEx treatment initiation	
Locoregional recurrence only	14 (58.3)
Locoregional recurrence + distant metastasis	5 (20.8)
Metastatic disease	5 (20.8)
Time from initial diagnosis to recurrence(Median, IQR), mo	16.2 (5.4-37.5)
Metastatic or unresectable disease at diagnosis	11 (45.8)

NS: Not specified; ECOG: Eastern Cooperative Oncology Group; HPV: Human papilloma virus; IQR: Interquartile range.

analyzed on a case-by-case basis. Clinical comorbidities, performance status, nutritional assessment results, access to infusion pumps, or even availability for patient hospitalization are some of the considerations made in clinical practice before treatment decisions are made.

Given that not all patients can tolerate the EXTREME regimen, alternative treatment protocols have been developed, mostly replacing 5-fluorouracil with taxanes (Table 5). The phase 2 GORTEC study evaluated cisplatin, docetaxel, and cetuximab as a first-line treatment in 54 patients with R/M SCCHN

Table 2 Summary of treatment response

	TPEx (*n* = 24)
Type of response, *n* (%)	
Complete	3 (12.5)
Partial	12 (50)
Stable disease	6 (25)
Progression	1 (4.2)
Nonassessable	2 (8.3)
Objective response rate - % of patients (95%CI)[1]	62.5
Disease-control rate - % of patients (95%CI)[2]	87.5
Time to response – mo[3]	
Median (95%CI)	2.4 (1.3-3.5)
Duration of response – mo[4]	
Median (95%CI)	5.1 (3.0-7.2)

[1]An objective response was considered to be a confirmed complete or partial response, as assessed by the investigator.
[2]The disease-control rate was calculated considering patients with a confirmed complete response, partial response, or stable disease as assessed by the investigator.
[3]The time to response was calculated with the use of the Kaplan-Meier method from the date of TPEx initiation to the date of the first documented partial or complete response.
[4]The duration of response was calculated with the use of the Kaplan-Meier method from the date of the first documented response until the date of documented disease progression, death, or the last response assessment in the absence of disease progression.
CI: Confidence interval.

Table 3 Univariate prognostic factor analyses for TPEx progression-free survival

Variable	HR (95%CI)	*P* value	Median PFS (95%CI)
ECOG(0 *vs* 1-2)	0.91 (0.30-2.80)	0.87	6.9 mo (5.1-8.8) *vs* 6.8 mo (4.7-8.9)
Primary tumor site (Hypo/oropharyngeal *vs* Others)	0.15 (0.02-1.17)	0.04	22 mo (19.9-25.1) *vs* 6.7 mo (4.7-8.9)
Response (Responders *vs* Nonresponders)	0.34 (0.12–0.97)	0.04	8.5 mo (5.5-11.5) *vs* 4.5 mo (2.5-6.5)
Extent of disease at TPEx initiation (Locoregionally advanced *vs* Metastatic)	0.95 (0.33-2.85)	0.95	6.9 mo (4.2-9.7) *vs* 6.8 mo (5.6-7.8)
Relapse-free survival of the primary treatment (≤ 24 *vs* > 24 mo)[1]	0.37 (0.11-1.21)	0.09	6.1 mo (3.6-8.6) *vs* 8.5 mo (4.5-12.5)
Previous treatment[2](Multimodality *vs* Unimodality)	0.44 (0.14–1.41)	0.17	7.5 mo (6.3-8.7) *vs* 6.1 mo (2.7-9.4)
Treatment interruption, discontinuation, or dose reduction (Yes *vs* No)	1.15 (0.39-3.41)	0.80	6.9 mo (6.4-7.4) *vs* 6.8 mo (5.0-8.5)
Adverse events (Grade 1-2 *vs* 3-4)	0.74 (0.23-2.44)	0.62	6.9 mo (5.2-8.6) *vs* 6.7 mo (0.3-13.9)

[1]Time from primary definitive treatment to advanced disease and first-line TPEx initiation.
[2]Treatment received as primary intention.
Unimodality included surgery or radiotherapy only. Multimodality included surgery and/or radiotherapy +/- chemotherapy. ECOG: Eastern Cooperative Oncology Group; CI: Confidence interval.

[14]. The median OS, PFS, and ORR were 14 mo, 6.2 mo, and 44.4%, respectively. In this selected population, only 12 patients (22.2%) experienced grade 4 adverse events. In another phase 2 trial, Bossi *et al*[15] randomized 201 patients with R/M SCCHN to receive first-line cetuximab plus cisplatin with or without paclitaxel. The authors reported a median PFS of 7 mo and an ORR of 51.7% in the cetuximab, cisplatin, and paclitaxel arm. With this regimen, 72.5% and 33% of the included patients presented grade ≥ 3 and 4 adverse events, respectively.

Guigay and collaborators[19] have recently published the results of a phase 2 trial that compared TPEx with EXTREME as first-line treatment for 541 patients. PFS and ORR values were 14.5 *vs* 13.4 mo, 6.0 *vs* 6.2 mo, and 57.6% *vs* 57%, respectively, and there were no significant differences between the two

Table 4 Adverse events of any cause during TPEx treatment

Event, *n* (%)	TPEx (*n* = 24)		
	Any grade	Grade 3	Grade 4
Any treatment-related adverse event[1]	18 (75)	6 (25)	0
Hematological			
Febrile neutropenia	3 (12.5)	3 (12.5)	0
Anemia	3 (12.5)	0	
Hyponatremia and/or hypokalemia	3 (12.5)	2 (8.3)	0
Hypomagnesemia	2 (8.3)	1 (4.2)	0
Thrombocytopenia	1 (4.2)	0	0
Nonhematological			
Acne-like rash	8 (33.3)	0	0
Nausea - vomiting	4 (16.7)	1 (4.2)	0
Asthenia	4 (16.7)	1 (4.2)	0
Diarrhea	2 (8.3)	0	0
Renal failure	1 (4.2)	1 (4.2)	0
Hypersensitivity	1 (4.2)	0	
Oral mucositis	1 (4.2)	0	0
Any serious adverse event[2]	-	5 (20.8)	0
Treatment-related death	0	-	-
Event leading to interruption of any treatment component[3]	3 (12.5)	-	-
Chemotherapy	2 (8.3)	-	-
Cetuximab	1 (4.2)	-	-
Event leading to discontinuation of any treatment component[3]	2 (8.3)	-	-
Chemotherapy	2 (8.3)	-	-
Cetuximab	0		
Event leading to dose reduction	2 (8.3)	-	-

[1]The investigators determined whether adverse events were related to the treatment.
[2]Adverse events that lead to hospitalization.
[3]This category includes patients who experienced cisplatin, docetaxel, or cetuximab treatment interruption or discontinuation because of an adverse event at any time and patients who experienced cetuximab maintenance therapy interruption or discontinuation for an adverse event after completing the chemotherapy cycles.
Events were attributed to the specific treatment by the investigator.

arms. The TPEx regimen was associated with a grade 4 adverse event incidence of 33%, which was significantly lower than the 46% incidence reported with the EXTREME scheme. Furthermore, an exploratory analysis for this trial showed a better quality of life in patients who received TPEx, mainly in terms of global health status, physical functioning, role functioning, and scores of appetite[20].

Remarkably, real-world data in this setting are scarce. Before the GORTEC trial, Even and collaborators[21] presented the results of 30 patients treated with TPEx at Gustave Roussy Institute between 2011 and 2013. In this group of patients, the median PFS and OS were 6.0 and 13.6 mo, respectively. A total of eight grade 3-4 adverse events were documented, including vomiting, mucositis, skin rash, diarrhea, hypersensitivity, and neutropenia. Additionally, Fuchs *et al*[22] reported similar results in a retrospective single-institution study, including 38 R/M SCCHN patients treated with TPEx at the Medical University of Vienna. In this study, the median OS, PFS, and ORR values were 10.8 mo, 6.3 mo, and 50%, respectively.

To the best of our knowledge, our study presents the first multicenter cohort (including data from South America) of patients treated with the TPEx schema. Notably, the PFS and ORR were consistent with those reported in previous clinical trials. Intriguingly, 2 patients with complete responses had

Table 5 Selected studies that assessed first-line TPEx schema in patients with recurrent or metastatic head and neck cancer

Ref.	Study type	*n*	ORR (%)	PFS (mo)	OS (mo)	Grade 3, adverse events (%)	Grade 4, adverse events (%)
Guigay *et al*[19]	Phase 2	541	57.6	6.0	14.5	73	33
Guigay *et al*[14]	Phase 2	54	44.4	6.2	14	93	22
Bossi *et al*[15]	Phase 2[1]	201	51.7	7.0	11	73	33
Even *et al*[21]	Retrospective	30	87	6.0	13.6	17	10
Fuchs *et al*[22]	Retrospective	38	50	6.3	10.8	100	

[1]This trial used paclitaxel.

ORR: Overall response rate; PFS: Progression-free survival; OS: Overall survival.

longer PFS, which may support that depth of response could be studied as a prognostic factor in patients with R/M HNSCC.

In our study, the TPEx regimen was adequately tolerated by most of the analyzed patients. The incidence of grade 3-4 adverse events was surprisingly lower than expected (25%), but it should be noted that 5 patients had treatment-related hospitalizations. Fortunately, no fatal toxicities were experienced.

Our experience confirms that the replacement of 5-fluorouracil with docetaxel may be a reasonable treatment strategy for R/M SCCHN patients. TPEx has been incorporated as a standard regimen in our centers, considering that this regimen is associated with a lower duration of treatment infusions and lower total number of cycles and the recent reports of safety and quality of life outcomes. These particular characteristics are essential in low- and middle-income countries with limited access to infusion pumps. Furthermore, the instauration of simplified regimens has become extremely important during the coronavirus disease 2019 pandemic[23].

Our results should be interpreted with caution considering the study limitations. This observational study was conducted in five private care centers, which may have been responsible for the high proportion of patients with access to immunotherapy after disease progression (92.9%). The low number of included patients and the retrospective nature of the study may also hamper the extrapolation of our results to Hispanic and Latino American populations. Additionally, our follow-up was not long enough to analyze adequately OS in our sample. Accordingly, the high response rate and the low incidence of grade 3-4 adverse events and serious toxicity may also be explained by a patient selection bias. Although public and private care centers were invited to register their experience with the TPEX regimen, only private-care physicians reported patients that received this treatment strategy.

Finally, it should also be highlighted that the landscape in R/M SCCHN is evolving. First-line treatment strategies currently include immunotherapy given alone or in combination with chemotherapy[7]. Nevertheless, TPEx represents an adequate alternative for patients with R/M HNSCC without programmed death-ligand 1 expression or as a subsequent treatment after disease progression on immune checkpoint inhibitors given as monotherapy. It should be emphasized that drug combination regimens, such as TPEx, have proven to be associated with a higher ORR, which is particularly beneficial in patients with a high tumor burden.

CONCLUSION

TPEx was a well-tolerated regimen in our population, showing a lower incidence of grade 3-4 adverse events than previously reported. PFS was comparable to those of recently reported clinical trials using the same treatment scheme. We observed a higher ORR compared to the previous results in phase 2 trials. This regimen may be considered an attractive therapeutic strategy due to its simplified administration, decreased total number of chemotherapy cycles, and treatment tolerability. Overall, quality of life, cost of hospitalization, and adverse event management should be carefully analyzed before deciding the best therapeutic plan for patients with R/M SCCHN.

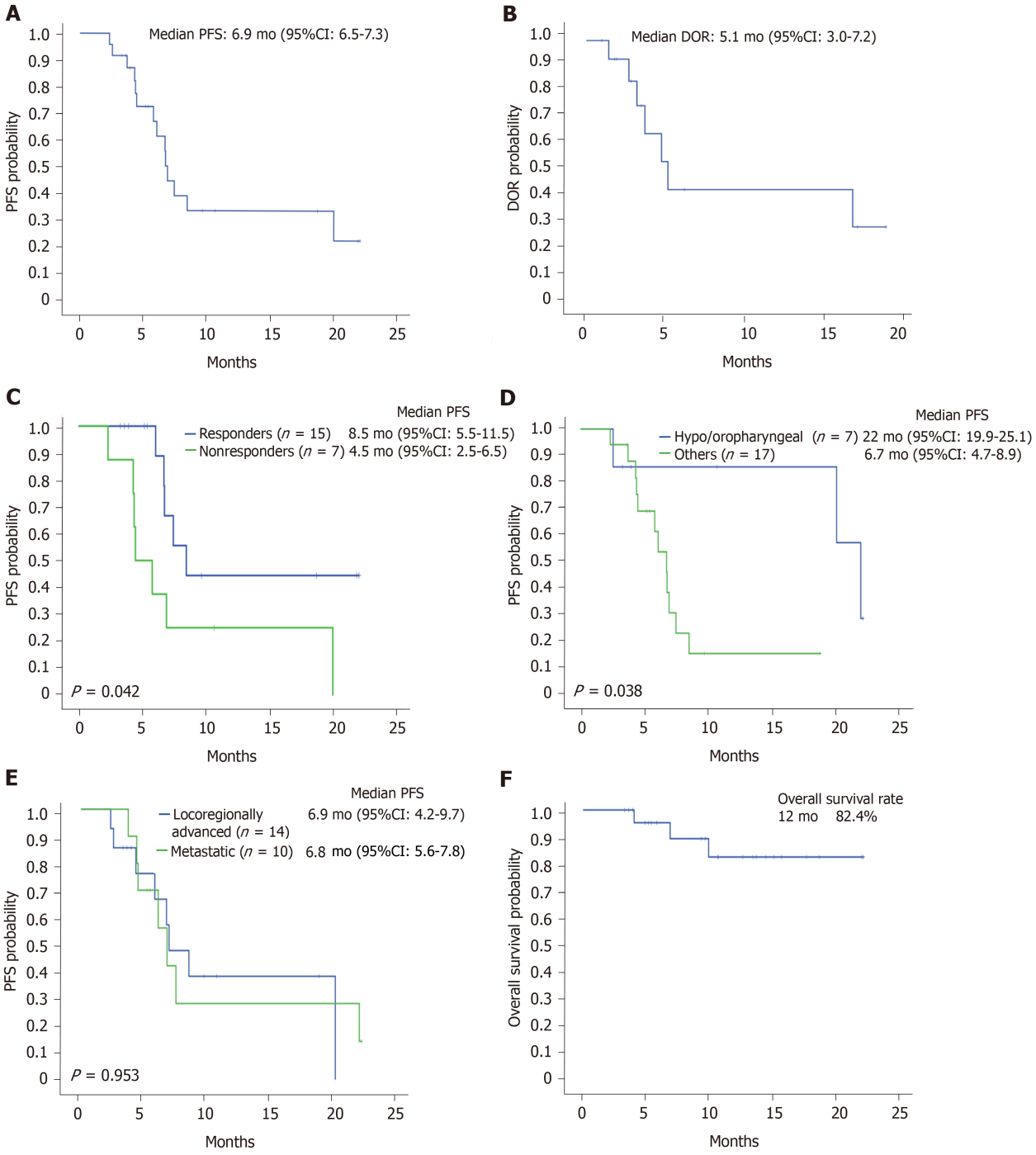


Figure 1 Kaplan–Meier curves. A: For progression-free survival (PFS); B: For duration of response (DOR); C: For PFS according to response; D: For PFS according to primary tumor site; E: For PFS according to extent of disease at TPEx initiation; F: For overall survival. PFS: Progression-free survival.

ARTICLE HIGHLIGHTS

Research background

The targeted therapy cetuximab in combination with 5-fluorouracil and platinum-based chemotherapy (the EXTREME regimen) has shown substantial efficacy for patients with recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN). However, a new strategy combining platinum, taxanes, and cetuximab (the TPEx regimen) has demonstrated similar efficacy with a more favorable toxicity profile in clinical trials.

Research motivation

There is scarce evidence outside randomized clinical trials including patients treated with TPEx scheme.

Research objectives

To evaluate the safety and efficacy of the TPEx scheme as first-line therapy in advanced SCCHN in a multicenter cohort study.

Research methods

This retrospective multicenter cohort study included patients with histologically confirmed recurrent or metastatic SCCHN treated with first-line TPEx at five medical centers in Argentina between January 1, 2017, and April 31, 2020. Chemotherapy consisted of four cycles of docetaxel, cisplatin, and cetuximab followed by cetuximab maintenance therapy. Clinical outcomes and toxicity profiles were collected from medical charts. Treatment response was assessed by the investigator in accordance with Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1). Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0).

Research results

Among the 24 patients included, the majority of patients (83.3%) received at least four chemotherapy cycles in the initial phase. The overall response rate was 62.5%, and 3 patients achieved a complete response (12.5%). The median time to response was 2.4 mo (95%CI: 1.3-3.5). With a median follow-up of 12.7 mo [95% confidence interval (CI): 8.8-16.6), the median progression-free survival (PFS) was 6.9 mo (95%CI: 6.5-7.3), and the overall survival rate at 12 mo was 82.4%. Patients with documented tumor response showed a better PFS than those with disease stabilization or progression [8.5 mo (95% CI: 5.5-11.5) and 4.5 mo (95%CI: 2.5-6.6), respectively; $P = 0.042$]. Regarding the safety analysis, two-thirds of patients reported at least one treatment-related adverse event, and 25% presented grade 3 toxicities. Of note, no patient experienced grade 4 adverse events.

Research conclusions

TPEx was a well-tolerated regimen in our population, showing a lower incidence of grade 3-4 adverse events than previously reported. PFS was comparable to those of recently reported clinical trials using the same treatment scheme. We observed a higher overall response rate compared to the previous results in phase 2 trials.

Research perspectives

This regimen may be considered an attractive therapeutic strategy due to its simplified administration, decreased total number of chemotherapy cycles, and treatment tolerability.

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FOOTNOTES

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EVIDENCE REVIEW

Evidence-based approach to the treatment of esophagogastric junction tumors

Francisco Schlottmann, María A Casas, Daniela Molena

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Abstract

The incidence of esophagogastric junction (EGJ) adenocarcinoma is increasing in developed nations due to the rising prevalence of obesity and gastroesophageal reflux disease. Due to the peculiar location in a histological transition zone between the esophagus and the stomach, the management of EGJ tumors is controversial. Two main surgical approaches exist: total gastrectomy with distal esophagectomy or esophagectomy by either transhiatal or transthoracic approach. These operations differ significantly in the extent of lymphadenectomy. In addition, patients with locally advanced disease can receive either preoperative chemoradiation or perioperative chemotherapy. This evidence-based review analyzes current evidence regarding the management of EGJ tumors in order to help defining the best surgical and systemic treatment of these patients.

Key Words: Esophagogastric junction tumors; Esophagectomy; Gastrectomy; Esophageal adenocarcinoma; Chemotherapy; Chemoradiation

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Core Tip: Management of patients with esophagogastric junction tumors is challenging. Several surgical approaches and systemic therapies are currently available to treat these patients. This evidence-based review will help determining the optimal treatment for this complex disease.

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INTRODUCTION

Adenocarcinoma of the esophagogastric junction (EGJ) remains a major global health problem associated with poor prognosis[1]. The majority of patients are diagnosed at an advanced stage, and only half of the patients undergo curative treatment[2]. EGJ tumors arise in the histological transition area between the esophagus and the stomach. This zone is vulnerable to gastric acid reflux and consequently has an increased risk of malignant transformation[3]. The incidence of EGJ tumors vary among countries, but it has been increasing in the past years due to the rising prevalence of obesity and gastroesophageal reflux disease in developed nations[4,5].

Siewert described three types of EGJ tumors based on the relationship of the epicenter of the tumor and the endoscopic location of the Z line (*i.e.*, squamocolumnar junction): type I (distal esophageal tumors) when the epicenter is 1-5 cm above Z line, type II (true EGJ tumors) from 1 over to 2 cm below the Z line, and type III (subcardial tumors) when the epicenter is 2-5 cm distal to the Z line[6]. The Nishi classification is also based on where the center of the tumor is located; there are 5 types depending on the relative extent of the esophageal or gastric involvement (E, EG, E=G, GE, and G) and true EGJ tumors are represented by EG, E=G or GE[7].

Both Siewert and Nishi classifications describe the location of the center of the lesion, but do not consider the proximal or distal extent of the tumor, which is more relevant to guide the extent of surgical resection. In addition, the lymphatic drainage of EGJ cancers is variable. Specifically, type II tumors can metastasize to either paraoesophageal nodes in the lower mediastinum or upper abdominal lymph nodes[8].

Two main surgical approaches for EGJ tumors exist: total gastrectomy with distal esophagectomy and esophagectomy by either transhiatal or transthoracic approach[9,10].

Both operations allow for adequate dissection of para-celiac and para-aortic lymph nodes. However, better mediastinal lymph node dissection and larger proximal resection margins can be achieved with an esophagectomy[11,12].

Some oncological and surgical principles that are well-established for esophageal and gastric tumors cannot be simply applied to junctional cancers due to their specific location and pathological features. The aim of this study was to review the available evidence in an attempt to determine the optimal treatment for patients with EGJ tumors.

SURGICAL TREATMENT OF EGJ TUMORS

Esophagectomy remains the cornerstone of curative treatment of esophageal cancer. The goals of the operation are to achieve a resection with clear margins, with an adequate lymphadenectomy, and with acceptable morbidity and mortality rates in order to offer better long-term survival.

Surgical approach

R0 resection remains one of the most important prognostic factors for survival irrespectively of the tumor type or surgical approach. Consequently, technical considerations regarding proximal margin should influence surgical strategy. Most experts base their surgical approach on Siewert classification, recommending an esophagogastrectomy for type I tumors and a total gastrectomy for type III tumors. However, the main debate arises for type II lesions: esophagectomy or gastrectomy?

Different approaches are proposed for true cardia tumors. Some authors support esophagectomy because it allows an extensive mediastinal lymph node resection along with a longer proximal resection margin that may decrease the likelihood of microscopically positive margins. On the other hand, a total gastrectomy with distal esophagectomy may be preferred because it avoids entering the chest, and an adequate abdominal lymph node dissection can be achieved (potentially the most important nodes in these patients).

Esophagectomy and gastrectomy are significantly different in terms of invasiveness, type of reconstruction, and, more importantly, extent of gastric and esophageal resection. After analyzing 1002 consecutive patients undergoing surgery for EGJ cancers, Siewert *et al*[13] concluded that in patients with type II EGJ tumors an esophagectomy offers no advantage over an extended gastrectomy if a complete tumor resection can be achieved. No differences were observed in R0 resection rates or number of lymph nodes removed. In addition, esophagectomy was associated with higher 30-d mortality when compared with total gastrectomy[13].

Barbour *et al*[11] evaluated whether the length of esophageal resection or the operative approach influences the outcomes in patients with EGJ tumors. They analyzed 153 patients undergoing gastrectomy and 352 esophagectomy. No differences were found regarding lymph nodes harvested, R0 resection rates, or mortality between groups. Gastrectomy was indeed associated with shorter proximal margins than those undergoing esophagectomy for each Siewert type. Improved outcomes were seen with an esophageal margin > 3.8 cm. The authors concluded that if an adequate proximal margin is achieved, the operative approach might not modify overall survival[11]. Another study, which included 266 patients with surgically resected type II EGJ tumors, found that gastrectomy was more frequently associated with a positive circumferential resection margin than esophagectomy (29% *vs* 11%; $P = 0.025$) [14]. Considering how critical is to achieve adequate proximal margins in these patients, we strongly believe that a gastrectomy should only be considered if a large proximal margin is feasible.

Another matter of debate are the morbidity and mortality rates associated with the different surgical approaches proposed for EGJ tumors. A previous study compared patients undergoing thoracoabdominal esophagectomy ($n = 56$) with transhiatal extended gastrectomy ($n = 186$); this study did not find significant differences regarding perioperative morbidity, anastomotic leak rates, pulmonary complications, or mortality[15]. Another study analyzed two large databases, including 4996 patients with type II EGJ tumors, which found similar major postoperative morbidity (34% *vs* 33%; $P = 0.84$) and 30-d mortality (1.9% *vs* 3.4%; $P = 0.24$) with the esophageal and gastric approach. In addition, the surgical approach was not an independent predictor of overall survival[16]. These findings were supported by other authors[17-19]. Nevertheless, postoperative morbidity after an esophagectomy remains high[20]. In an effort to decrease morbidity, minimally invasive esophagectomy (MIE) has been widely adopted in the last decades[21]. For instance, the TIME trial was the first randomized trial comparing patients undergoing MIE or open esophagectomy and showed that postoperative pulmonary infections rates significantly decrease after MIE. Also, shorter length of stay and better quality of life were achieved in the MIE group[22].

Lymphadenectomy

Lymph node metastasis is another critical prognostic factor in patients with esophageal adenocarcinoma. Therefore, another major goal of the operation is to perform an adequate lymphadenectomy. As an increased number of metastatic lymph nodes is predictive of poor survival, an extensive lymphadenectomy is recommended by the American Joint Committee on Cancer in order to achieve accurate N staging[23]. However, whether extensive lymphadenectomy can improve overall survival because of better control of locoregional disease or better staging remains unclear. In addition, an extensive lymphadenectomy may potentially increase surgical morbidity.

Many studies have tried to determine how many nodes should be removed in patients with EGJ tumors for achieving optimal oncological outcomes[24-27]. For instance, Samson *et al*[26] found that sampling 15 or more lymph nodes was independently associated with lower overall mortality. Moreover, overall survival was improved when more than 20-25 lymph nodes were sampled even in patients with negative nodes, probably due to an increased staging accuracy[26]. This finding was also supported by other authors[25,28]. Greenstein *et al*[29] found that in patients with T2/T3 tumors, better survival rates were observed when more than 10 lymph nodes retrieved, and for T1 tumors, more than 18 lymph nodes were needed for superior survival rates. A recent study recommends the removal of at least 15 lymph nodes in both primary surgery and after induction therapy[24]. Sihag *et al*[28] analyzed 778 patients with locally advanced esophageal adenocarcinoma and found that overall and disease-free survival improved when harvesting up to 20-25 lymph nodes. A lower number of lymph node resection was independently associated with worse overall and disease-free survival[28].

Overall nodal metastasis rate in EGJ tumors varies among the literature between 40% and 80%[30-32]. The EGJ has two main lymphatic drainage pathways: abdominal and mediastinal. Mediastinal lymph nodes involvement varies between 15%-45% in the literature[31,32]. Siewert *et al*[13] evaluated the pattern of lymphatic spread specifically in type II EGJ cancers and showed that almost 70% of the tumors spread towards paracardial, lesser curvature, and left gastric artery nodes while only 15% towards lymphatic nodes in lower posterior mediastinum. However, as all patients underwent a gastrectomy, upper mediastinal nodes were not evaluated in these patients.

Leers *et al*[30] analyzed patients with distal esophageal and EGJ tumors undergoing an esophagectomy with systematic mediastinal and upper abdominal lymphadenectomy. The authors found that 26% of the distal esophageal tumors and 25% of the EGJ tumors had positive mediastinal nodes. Moreover, in 9% and 8% of the patients, respectively, this location was the only site of nodal involvement, concluding that mediastinal node dissection was essential in the surgical therapy for EGJ tumors[30].

Yamashita *et al*[33] recently showed that nodal metastasis in EGJ tumors more frequently involve abdominal nodes, especially those at the right and left cardia, lesser curvature, and along the left gastric artery.

A recent study showed higher incidence of metastasis or recurrence in the upper and middle mediastinal zones when the esophageal invasion length was more than 25 mm[34]. These findings were supported by a Japanese prospective study that included 363 patients undergoing either gastrectomy by a transhiatal approach or distal esophagectomy by a right transthoracic approach. The authors

concluded that upper and lower mediastinal station dissections should be performed in cases of more than 4 cm or 2 cm of esophageal involvement, respectively[35]. Conversely, routine dissection of lymph nodes at the lesser curvature and along the left gastric artery for any EGJ tumor was recommended.

A randomized trial was conducted to compare extended transthoracic resection with limited transhiatal resection for Siewert type I and II. Although a higher number of lymph nodes were harvested through the transthoracic approach (31 *vs* 16), the 5-year survival was similar between groups (34% *vs* 36%). A subgroup analysis was also performed for type I tumors, and a survival benefit of 14% was achieved with the transthoracic approach (51% *vs* 37%). The authors concluded that in type I tumors the transthoracic approach might have survival advantages, especially in those with 1 to 8 positive nodes in the resection specimen[36]. Parry *et al*[14] also showed that a better mediastinal lymph node resection was achieved with an esophagectomy, and these results were supported by other authors [11,37,38].

Advanced techniques to optimize intraoperative lymphadenectomy have been developed in the last decades. For instance, the indocyanine green (ICG) fluorescence imaging for the evaluation of lymph node involvement has increasingly been used, and it might help guiding lymphadenectomy. The goal of this technology is to sample specific tumor-associated lymph nodes and increase pathological evaluation of more likely affected nodes. A targeted lymphadenectomy might provide more accurate and relevant prognostic information and may potentially decrease operative time and reduce postoperative complications. For instance, a previous study evaluated the lymphatic drainage pattern in patients with distal esophageal or EGJ cancer and found that in 89% of the cases, the first nodal station was along the left gastric artery. Interestingly, all patients with nodal involvement had positive nodes in the first nodal station identified with ICG[39]. Therefore, histopathological examination of the first nodal station might avoid unnecessary extensive lymphadenectomy. Further studies are needed to determine how fluorescence imaging can guide lymphadenectomy during an esophagectomy.

Expert commentary

Current evidence shows that surgical resection of an EGJ tumor can be achieved by either an esophagectomy or gastrectomy.

Type I tumors should probably be resected with an esophagectomy due to the higher risk of mediastinal lymph nodes involvement and the impossibility to achieve adequate margins with a gastrectomy. Type III tumors are adequately treated with a gastrectomy and abdominal lymphadenectomy.

Conflicting data exist regarding the optimal approach and the extent of lymphadenectomy for type II tumors. Although both approaches have shown similar oncological and clinical outcomes in these patients, we prefer an esophagectomy in order to obtain safe proximal margins and achieve adequate mediastinal lymphadenectomy.

Overall, tumor extension, lymph node involvement in preoperative imaging, patient's comorbidities and frailty, and experience of the surgical team should all be considered when deciding the surgical approach.

Table 1 describes potential advantages and disadvantages of the "esophageal" and "gastric" approach for the treatment of EGJ tumors.

NEOADJUVANT THERAPY

The optimal systemic therapy for EGJ tumor is also a debatable topic. It is clear that neoadjuvant therapy is required for locally advanced EGJ tumors to increase overall survival[40]. For this purpose, neoadjuvant chemoradiation and perioperative chemotherapy are both valid treatment modalities. However, which is the best approach for patients with EGJ tumors remains controversial.

Neoadjuvant chemoradiation

In 2012, the benefits of neoadjuvant therapy in patients with esophageal cancer were revealed by the results of the CROSS trial. This study randomized patients with esophageal or esophagogastric junction tumors to surgery alone (n = 188) or preoperative chemoradiotherapy (carboplatin and paclitaxel + concurrent radiotherapy) followed by surgery (n = 178). Patients receiving preoperative chemoradiotherapy had higher rates of R0 resections (92% *vs* 69%; $P < 0.001$) and better overall survival (49.4 mo *vs* 24 mo). In addition, 29% of the patients with chemoradiotherapy had complete pathological response [40]. The long-term results of the trial confirmed the benefits of neoadjuvant chemoradiotherapy. It is worth to mention, however, that patients with squamous cell carcinoma (ESCC) (23% of the included patients in the trial) had greater overall survival benefit than patients with adenocarcinoma[41].

Perioperative chemotherapy

In 2006, the MAGIC trial evaluated the role of perioperative chemotherapy for patients with gastric and EGJ tumors, comparing those receiving 3 cycles of Epirubicin – Cisplatin - Fluorouracil (ECF) before and after the operation against those undergoing surgery alone. The study showed significantly improved

Table 1 Potential advantages (+) and disadvantages (-) of total gastrectomy and esophagectomy for the treatment of esophagogastric junction tumors

Gastrectomy	Esophagectomy
+ Only abdominal approach, avoiding thoracotomy/thoracoscopic associated morbidity	+ Better proximal and circumferential resection margins
+ Adequate abdominal lymph node dissection	+ Extensive mediastinal lymph node dissection
+ No GERD/No PPI	+ Preservation of ¾ of stomach
- Inadequate mediastinal lymph node dissection	- Abdominal and thoracic approach
- Shorter proximal margins	- Hiatal herniation risk
- Vitamin B12 malabsorption	- Gastroesophageal reflux (necessity of PPI)
- Dumping	- Pylorospasm

GERD: Gastroesophageal reflux disease; PPI: Proton pump inhibitor.

overall and progression-free survival in patients receiving chemotherapy[42]. Two things, however, should be highlighted: only 11% of the patients had EGJ adenocarcinoma, and only 42% were able to complete the full six-cycle regimen.

In 2011, the ACCORD-07 trial compared patients receiving 2 or 3 cycles of cisplatin and fluorouracil before and after surgery with patients undergoing surgery alone. In this study, 64% of the patients had EGJ tumors. The trial showed better overall survival (38% *vs* 24%), 5-year disease-free survival (34% *vs* 19%), and higher rates of R0 resections in patients receiving perioperative chemotherapy[43].

In 2019, the FLOT trial (fluorouracil, leucovorin, oxaliplatin, and docetaxel) compared the use of perioperative FLOT (n = 356) or ECF (n = 360) plus surgery in patients with locally advanced gastric and EGJ tumors. The study demonstrated an overall survival benefit with the use of FLOT (50 *vs* 35 mo). Remarkably, only 50% of the patients completed the entire perioperative FLOT treatment[44]. These results have motivated the adoption of FLOT as the standard perioperative chemotherapy for patients with EGJ tumors.

CROSS vs FLOT

Few studies have compared the efficacy of both approaches. A propensity score-matched analysis of patients with esophageal and EGJ adenocarcinoma compared the outcomes of CROSS (n = 40) against FLOT (n = 40). Patient receiving CROSS had higher rates of complete pathological response (97% *vs* 85%; P = 0.049) and higher rates of negative lymph node metastases (68% *vs* 40%; P = 0.014). However, overall survival was similar in both groups[45]. A recent study using the National Cancer database investigated whether preoperative chemoradiation offers an advantage over chemotherapy alone in patients with lower esophageal or gastric cardia adenocarcinoma. The authors found that although patients undergoing chemoradiation had higher rates of complete pathological response (2.7 times), overall survival was similar with both treatment modalities[46]. Similar survival outcomes with CROSS and FLOT were also seen in other studies[47-49].

Expert commentary

Current evidence is weak and scarce but shows that patients with locally advanced EGJ tumors have similar survival with either preoperative chemoradiation or perioperative chemotherapy. We believe that both location and burden of disease (*i.e.* ability to obtain R0 resection) are key determinants.

For patients with Siewert type III tumors, perioperative chemotherapy is undoubtedly more reasonable due to the multiple trials supporting this approach (*i.e.* MAGIC, ACCORD, and FLOT). FLOT has shown to be the most effective regimen, and thereby should be chosen whenever possible.

Although for patients with Siewert type I and II the debate is still open, we think that avoiding the morbidity of radiation (whenever possible) is a better strategy. Patients with squamous cell carcinoma of the distal esophagus might still benefit from neoadjuvant chemoradiation. EGJ adenocarcinomas are probably better treated with perioperative chemotherapy.

Future directions: Immunotherapy

Overall survival of patients with locally advanced EGJ tumors remains low. Moreover, recurrence rates after neoadjuvant chemoradiotherapy plus surgery are high, especially among patients who do not have a pathological complete response[50-52]. Therefore, there is special interest in developing novel treatment modalities to improve outcomes. Multiples targeted therapies and immunotherapies are currently being investigated. Immunotherapy utilizes monoclonal antibodies directed against immune checkpoints proteins (*e.g.*, PD-1, PD-L1, CTLA-4). Multiples trials have shown clinical benefits with the use of immunotherapy in patients with metastatic or recurrent esophageal cancer[53-56]. The

KEYNOTE-590 study showed that adding pembrolizumab to cisplatin-fluoropyrimidine chemotherapy improved overall survival in patients with ESCC[53]. The ATTRACTION-3 trial, which included patients who had a previously treated advanced gastroesophageal cancer, showed a 2.5-mo difference in median overall survival in favor of nivolumab in comparison with chemotherapy[54]. The ATTRACTION-4 trial, on the other hand, did not show overall survival benefit, despite improvements in progression-free survival[55]. Recently, the Checkmate-577 phase III trial was conducted to compare postoperative nivolumab monotherapy against placebo in patients with locally advanced tumors who underwent resection and did not achieve complete pathologic response. Nivolumab monotherapy improved significantly disease-free survival in compared with placebo (median disease-free survival: 22.4 mo *vs* 11.0 mo; $P = 0.0003$). Interestingly, in the subgroup analysis according to histopathological type, the median disease-free survival period of patients with ESCC treated with nivolumab was better than for EAC patients. Despite this encouraging data, the trial was discontinued because of adverse events[56]. The trials PALACE-1 and PERFECT have also investigated the use of neoadjuvant chemoradiotherapy combined with immunotherapy in an effort to achieve higher rates of complete pathologic response[57,58]. However, phase 3 trials evaluating immunotherapy as neoadjuvant therapy are still warranted.

Overall, although immunotherapy has shown promising results, additional studies are needed to define safety and efficacy of this novel treatment modality.

CONCLUSION

Management of patients with EGJ tumors is challenging. Several surgical approaches and systemic therapies are currently available to treat these patients. Appropriate surgical margins and adequate lymphadenectomy should be the main goals of surgical treatment. Patients with locally advanced disease should also receive preoperative chemoradiation or perioperative chemotherapy. Tumor size and extension, nodal involvement in preoperative imaging and patient's comorbidities should all be considered for choosing the optimal treatment in these patients.

FOOTNOTES

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REVIEW

Intestinal Wnt in the transition from physiology to oncology

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Abstract

Adult stem cells are necessary for self-renewal tissues and regeneration after damage. Especially in the intestine, which self-renews every few days, they play a key role in tissue homeostasis. Therefore, complex regulatory mechanisms are needed to prevent hyperproliferation, which can lead in the worst case to carcinogenesis or under-activation of stem cells, which can result in dysfunctional epithelial. One main regulatory signaling pathway is the Wnt/β-catenin signaling pathway. It is a highly conserved pathway, with β-catenin, a transcription factor, as target protein. Translocation of β-catenin from cytoplasm to nucleus activates the transcription of numerous genes involved in regulating stem cell pluripotency, proliferation, cell differentiation and regulation of cell death. This review presents a brief overview of the Wnt/β-catenin signaling pathway, the regulatory mechanism of this pathway and its role in intestinal homeostasis. Additionally, this review highlights the molecular mechanisms and the histomorphological features of Wnt hyperactivation. Furthermore, the central role of the Wnt signaling pathway in intestinal carcinogenesis as well as its clinical relevance in colorectal carcinoma are discussed.

Key Words: Wnt signaling; Beta-catenin; Intestine; Colorectal cancer; Cell signaling; Intestinal stem cells

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Core Tip: Wnt signaling pathway is a key regulator of intestinal stem cells. Mutations in this pathway are frequently found in adenomas and carcinomas of the colorectum. Therefore, it represents a potential target for anticancer therapy. This review sums up the physiological role and the regulatory mechanism of Wnt signaling in the human intestine, and moreover, discusses the central role of the Wnt signaling pathway in intestinal carcinogenesis, the morphological features associated with Wnt hyperactivation and clinical relevance of Wnt in the colorectal carcinoma.

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INTRODUCTION

The gastrointestinal epithelia are tissues that self-renew every few days. Therefore, pluripotent stem cells are needed, which have the potential to develop into different epithelial cells. These highly complex mechanisms need complex fine-tuning. An overactivation of pluripotent stem cells could lead to hyperproliferation and in the worst case to cancer development. Conversely, under-activation could lead to insufficient development of the epithelia with dysfunction of the epithelia. One main regulatory signaling responsible for intestinal epithelial development is Wnt signaling.

Since 1976 it has been known that the Wingless (*WNT*) gene in Drosophila not only influences development, but also provokes abnormalities of the mesothorax[1]. In recent decades, other genes of the Wnt family have been found and the signaling pathways around Wnt in humans have also become more and more clear. Today 19 *WNT* genes in humans are known and the Wnt pathway is known to play a critical role in embryonic development and tissue homeostasis[2]. An imbalance in Wnt signaling can lead to several diseases including carcinogenesis, neurodegenerative, metabolic and cardiovascular diseases[3]. In addition to the canonical Wnt/β-catenin pathway, which is the main focus of this review, there is also the noncanonical pathway and the noncanonical Wnt/calcium pathway[4].

This work focuses on the regulation and the role of the canonical Wnt/β-catenin signaling pathway in physiological epithelial differentiation and the molecular activities of Wnt contributing to autonomous hyperproliferation and injured cell death as hallmarks of carcinogenesis.

WNT/β-CATENIN SIGNALING PATHWAY

The most common Wnt pathway and evolutionarily conserved pathway is the canonical Wnt/β-catenin signaling (Figure 1). It consists of the transmembrane complex (Lrp5/6 and Frizzled), a destruction complex [Axin, Adenomatous polyposis coli (APC), glycogen synthase kinase-3 (GSK3), casein kinase 1 (CK1), protein phosphatase 2A (PP2A)] and β-catenin[5-7]. In the absence of the Wnt ligand, β-catenin is phosphorylated by the kinases CK1 and GSK3[8]. The phosphorylation leads to the ubiquitination and degradation of β-catenin. If Wnt binds to the transmembrane complex, the protein Disheveled is activated and turns down the destruction complex, resulting in accumulation of β-catenin in the cytoplasm[9,10]. Then, β-catenin is translocated into the nucleus and acts there as a transcription factor together with P300, B-cell CLL/lymphoma 9, pygo and T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) as cofactors[11-13]. Moreover, there are inhibitors of this pathway like Dickkopf 1 (Dkk1), which binds to Lrp5 and inhibits the binding of Wnt at the transmembrane complex[14,15].

The role of Wnt/β-catenin signaling in the development of the gastrointestinal tract becomes clear when we look at the main genes which are regulated by the Wnt signaling pathway. Nuclear β-catenin activates genes which code for proteins involved in important pathways as well as processes including embryogenesis, proliferation, cell differentiation and the regulation of cell death (Table 1)[16-18].

THE NECESSITY OF WNT SIGNALING IN INTESTINAL MUCOSAL PHYSIOLOGY

In the intestinal tract, the canonical Wnt is an essential and fundamental molecular cascade to establish and constitute the mucosal barrier. However, in the different segments of the intestinal tract, the Wnt shows different cellular and molecular players as well as facets that are characteristic for each compartment. Wnt signaling is required in all parts for stem cell renewal, while Wnt overactivation in the stomach can lead to intestinal shift. Mutations in the Wnt ligands affect all parts of the intestine[19, 20]. These points are addressed further in the following paragraphs.

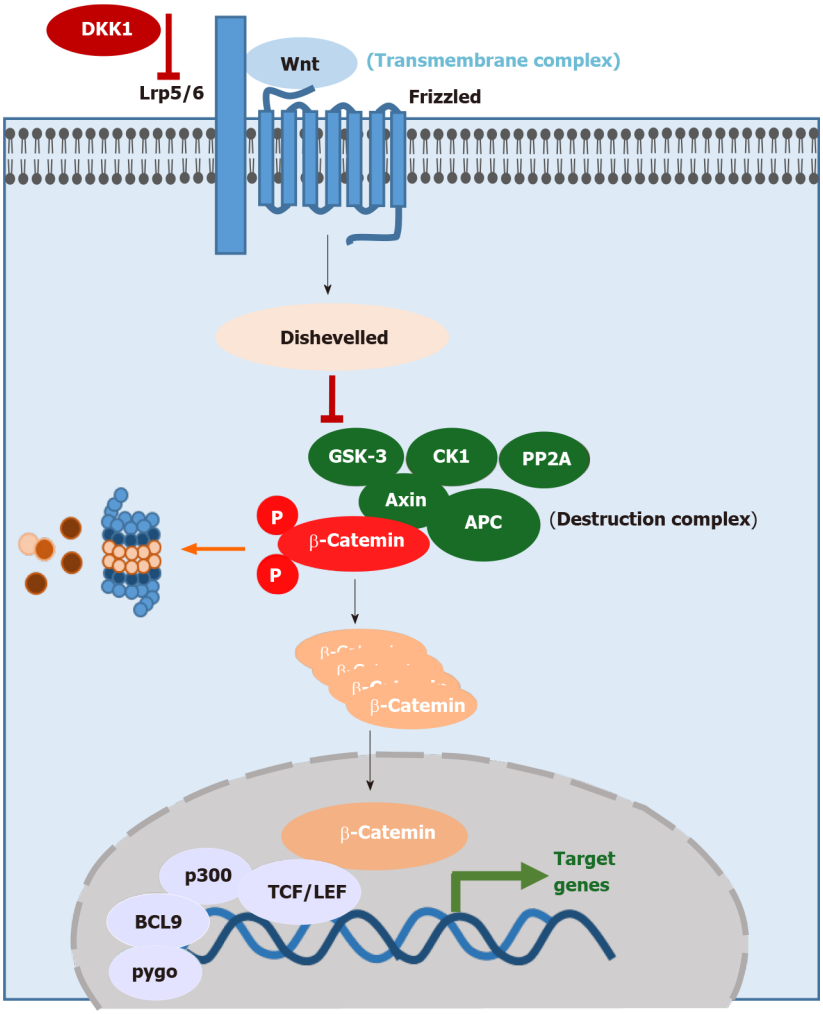
Table 1 Selection of assumed target genes of β-catenin

Gene	Function of the protein	Ref.
ATOH1	Transcription factor, secretory cell line differentiation	[137,138]
AXIN2	Part of destruction complex Wnt signaling	[139]
BCL2	Antiapoptotic	[140]
BIRC5	Apoptosis inhibitor	[141]
BMP4	Possible Wnt inhibitor	[142]
CCND1	Cell proliferation	[143]
CDKN2A	Cell cycle inhibitor	[144]
CDX1	Transcription factor, intestinal cell differentiation	[145]
CDX2	Transcription factor, intestinal cell differentiation	[146]
DKK1/4	Inhibitor of Wnt signaling	[147,148]
EPHB2/3	Migration and proliferation in intestine epithelial	[149]
HD5/6	Defensine, microbial defense	[150]
HEF1	Supports activation of oncogenic signaling pathways	[151]
HES1	Regulation of Notch signaling	[152]
JAG1	Ligand of Notch signaling	[153]
JUN	Cell cycle progression, apoptosis inhibitor	[154,155]
LGR5	Part of Wnt signaling	[156]
MDR1	Plasma membrane protein involved in the drug resistance	[123,124]
MET	Differentiation of intestinal epithelium	[157]
MYC	Protooncogene	[158]
MYCBP	Control of transcriptional activity of c-MYC	[159]
NOTCH2	Notch receptor	[160]
SGK1	Inhibits pro-apoptotic transcription factors	[161]
SOX9	Paneth cell differentiation	[32,162]
YAP	Transcription factor (Hippo signaling) activates genes involved in cell proliferation, suppresses apoptotic genes	[163]

ATOH1: Atonal BHLH transcription factor 1; BCL2: B-cell lymphoma 2 ; BIRC5: Baculoviral IAP repeat containing 5; BMP4: Bone morphogenetic protein 4; CCND1: Cyclin D1; CDKN2A: Cyclin dependent kinase inhibitor 2A; CDX1: Caudal type homeobox 1; CDX2: Caudal type homeobox 2; DKK: Dickkopf; EPHB2/3: EPH receptor B2/3; HD5/6: Human alpha defensin 5/6; HEF1: Human enhancer of filamentation 1; HES1: Hairy and enhancer of split-1; JAG1: Jagged Canonical Notch Ligand 1; JUN: C-Jun N-terminal kinase; LGR5: Leucine-rich repeat-containing G-protein coupled receptor 5; MDR1: Multidrug-Resistance-1; MET: Tyrosine-protein kinase Met; MYC: Myc proto-oncogene, bHLH transcription factor; MYCBP: MYC binding protein; NOTCH2: Notch Receptor 2; SGK1: Serum/glucocorticoid regulated kinase 1; SOX9: SRY-Box transcription factor 9; YAP: Yes-associated protein.

Stomach

The stomach can be divided, based on its local glands, into two main parts: The corpus/fundus and the antrum. The corpus and fundus contain oxyntic glands with chief cells, parietal cells and endocrine cells, while the antrum glands mainly contain mucous and endocrine cells[21]. Wnt/β-catenin signaling was required for the development of the embryonic fundus and in the β-catenin-deficient epithelium, parietal cells were absent[22]. In the antrum glands, $Lgr5^+$ and $Axin2^+$ stem cells were found[23]. Both proteins are regulated throughout Wnt signaling. Wnts are necessary for the maintenance of $Lgr5^+$ cells and are necessary for the zymogenic cell line from $Lgr5^+$ cells[24]. Moreover, they suppress the differentiation along the pit cell lineage. The Wnt ligands in the stomach will be secreted by pericyte-like stromal cells[25]. These cells are conserved and exist in the colon as well as in the stomach. Besides, activation of Wnt signaling in the stomach can lead to an intestinal fate in the stomach. Therefore, the mesenchymal transcription factor Barx1 represses the Wnt signaling and inhibits an intestinal shift of the stomach epithelium[26].



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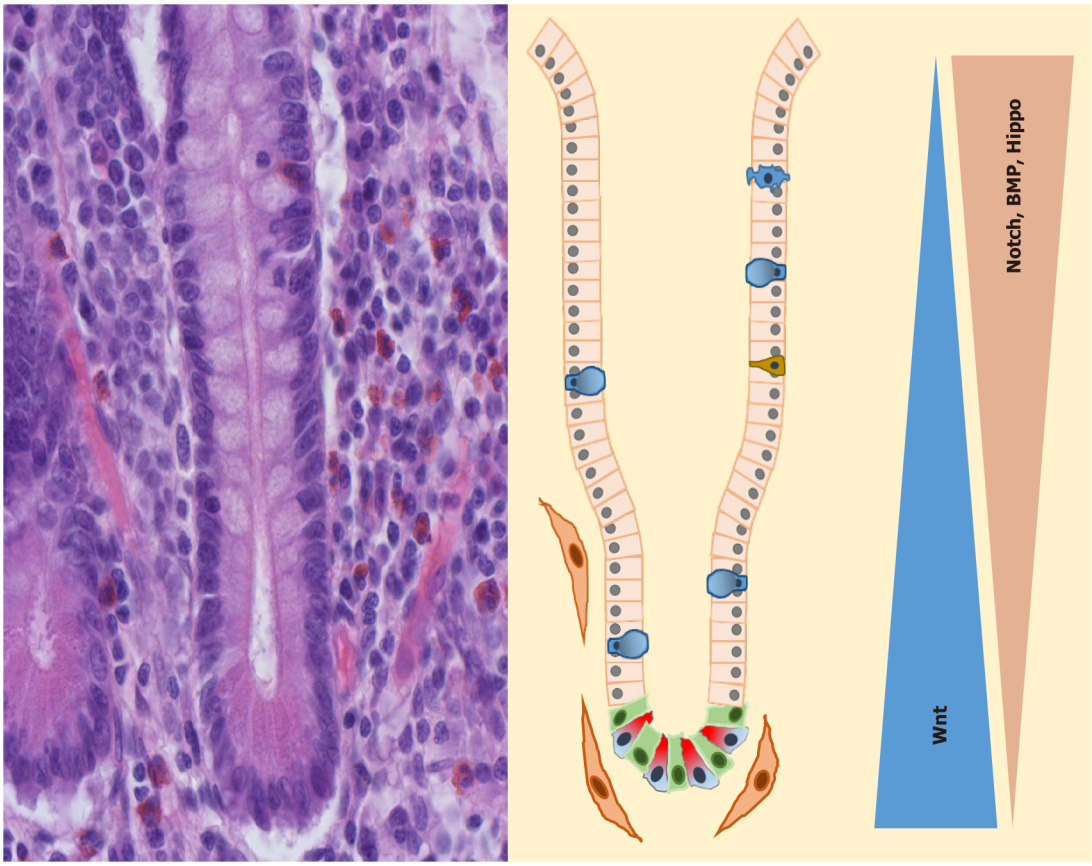
Figure 1 Wnt signaling pathway. Activated Wnt signaling pathway: Wnt ligand binds to the transmembrane complex and activates Disheveled, which turns down the destruction complex. β-catenin accumulates in the cytoplasm and translocates in the nucleus, where it acts with several cofactors as a transcription factor. Inactivated Wnt signaling pathway: β-catenin is phosphorylated by the destruction complex and gets degraded. Dkk1: Dickkopf 1; GSK-3: Glycogen synthase kinase-3; APC: Adenomatous polyposis coli; PP2A: Protein phosphatase 2A; TCF/LEF: T-cell factor/lymphoid enhancer-binding factor; BCL9: B-cell lymphoma 9.

Small intestine

The small intestine consists of finger-like villi with an absorptive function and crypts of Lieberkühn (Figure 2). In the crypts, two different populations of intestinal stem cells (ISC) are located[27]. At the bottom of the crypts are columnar ISCs which express Lgr5, have a high division rate and are preferred for the renewal of the intestinal epithelia[28]. These cells can be activated throughout Wnt. On the other hand, there are quiescent ISCs that have a slow division rate, are less vulnerable to radiation and Wnt signaling is not activated. These cells are located above the Paneth cells and are also called +4 cells[29]. The role of these cells has not been fully investigated yet. But in the absence of columnar ISCs, quiescent ISCs can be activated and assume the tasks of columnar ISCs[30]. The localization of the subpopulation of ISC in the crypt is controlled by the surrounding mesenchymal cells through bone morphogenetic protein (BMP) signaling[27]. The regulation of the ISC occurs through Wnt3A which is secreted by Paneth cells[31].

Paneth cells are located in the base of the crypt of the small intestine next to Lgr5^{+} cells. Their differentiation is induced by SOX9, a transcriptional target and a critical regulator of Wnt signaling[32]. In contrast to other differentiated intestine cells, they do not migrate upwards to the top of the villus tip and their lifetime is, at 30 d, much longer[33]. Their main role is to synthesize and secrete defensins, anti-microbial peptides and trophic factors. Nevertheless, they seem to have an impact on crypt homeostasis.

Above the Paneth cells and stem cells is the transit-amplifying zone. The progenitor cells of the differentiated enterocytes are settled here, which can divide themselves two to five times[34,35]. All differentiated cells with the exception of Paneth cells migrate from the crypts upwards to the villi. The main parts of differentiated cells are enterocytes, which make up 80%-90% and have an absorptive function. In addition to them, there are tuft cells, goblet cells, enteroendocrine cells and microfold cells that are



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Figure 2 Small intestinal crypt of Lieberkühn with signaling pathway gradients. On the left sight histology of a small intestinal crypt (400 × Hematoxylin eosin) and on the right a schematic drawing of a small intestinal crypt with intestinal stem cells (green), Paneth cells (red), goblet cells (light blue), tuft cell (blue) and neuroendocrine cell (yellow). BMP: Bone morphogenetic protein.

also termed M cells[35,36].

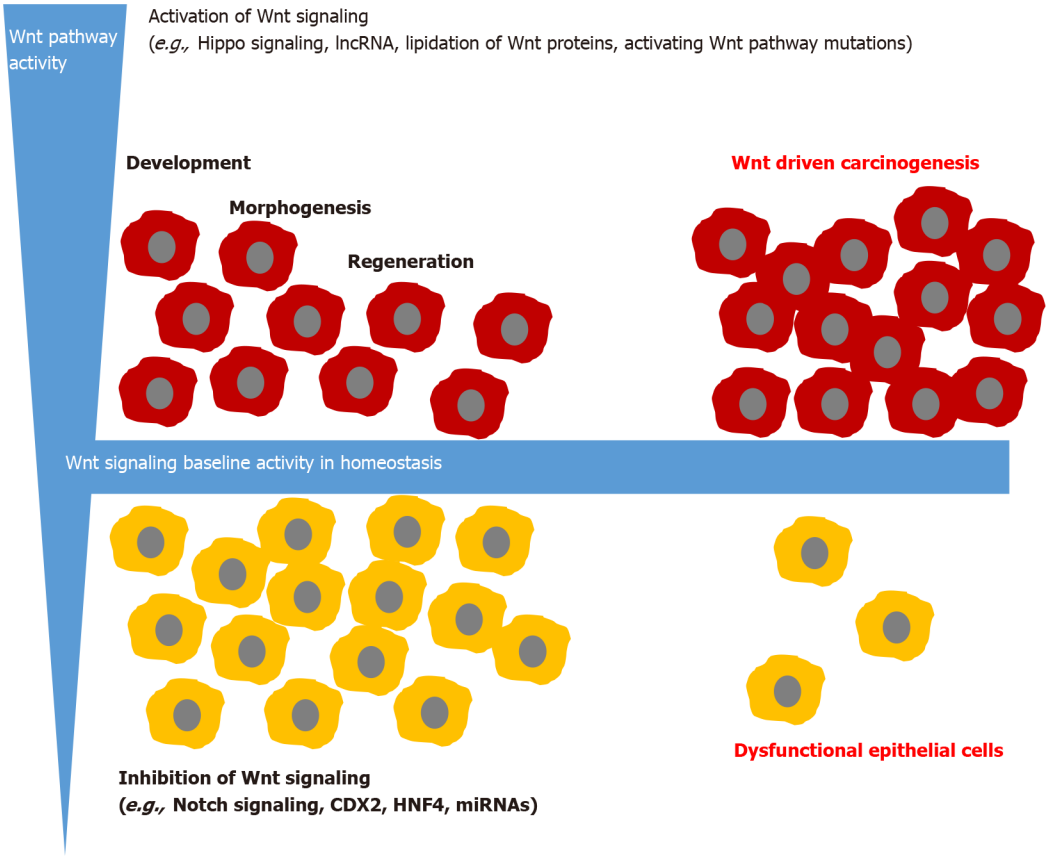
That Wnt signaling is essential for intestinal development has been already shown in the work of Pinto *et al*[37]. Overexpression of the Wnt inhibitor Dkk1 leads to a loss of crypts and reduced epithelial proliferation[37]. Furthermore, inhibition of Dkk leads to a reduced rate of fission of crypts in postnatal growth[38]. A negative autoregulatory feedback loop of Wnt signaling prevents a hyperactivation of Wnt signaling[28,39].

Colon

The colon has, in contrast to the small intestine, crypts, but no villi. The so-called colonocytes are functionally equivalent to the enterocytes[35]. Like the small intestine, the colon epithelia renew themselves through crypt-based columnar ISCs[35]. The work of Davies *et al*[40] revealed that Wnt activity is lower in the colon than in the small intestine. This may be influenced by the fact that instead of Paneth cells the colon epithelia have deep secretory cells with similar functions to Paneth cells, but in contrast to Paneth cells, they do not secrete Wnt ligands[35,41]. Furthermore, *in vitro* studies show that the reaction of Wnt-signaling activation also differs between the left and the right colon[42]. In embryonic development, a Wnt3A gradient plays an important role in hindgut extension and colon formation[43]. Like the small intestine, the colon epithelia include goblet cells, tuft cells and enteroendocrine cells[35].

THE COMPLEX REGULATION NETWORK OF WNT SIGNALING

As mentioned above, the Wnt signaling pathway is a highly conserved pathway and essential for intestinal homeostasis. To preserve this homeostasis, precise fine-tuning is absolutely necessary. The regulation of Wnt ligands occurs on different pathway levels. The mechanisms involved in this regulation are explained below and summed up in Figure 3.



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Figure 3 Wnt signaling regulatory mechanisms in intestinal cell development. Wnt signaling balances intestinal development, morphogenesis and regeneration due to a gradient of Wnt pathway activity in epithelial layers with major activated cells (red) and minor activated cells (yellow). In Wnt-driven carcinogenesis, the gradient of Wnt pathway activity is lost and major activated, neoplastic cells (red) dominate. lncRNA: Long non-coding RNA; miRNAs: MicroRNAs.

Notch signaling pathway

Notch signaling is one of the most important signaling pathways in terms of adjacent cellular communication and regulation of gastrointestinal stem cells[44]. It plays a crucial role in determining whether a cell develops into a secretory or an absorptive cell[44]. Deletion of NOTCH1 and NOTCH2 leads to hyperplasia of secretory cells[45]. It is not surprising that Wnt and Notch signaling act closely together and regulate each other[46,47]. The amount of Notch correlates here inversely with the amount of β-catenin[48,49]. On the other hand, Disheveled, which is part of the Wnt signaling, inhibits Notch signaling[50,51]. As Notch signaling requires cell-cell contact, Paneth cells are important for controlling the Notch signaling of small ISC[52]. In conclusion, Notch signaling determines cell fate to absorptive cell lines, while Wnt signaling drives cells to secretory cell lines[35,53].

Caudal-related homeobox transcription factor 2

Caudal-related homeobox transcription factor 2 (CDX2) is essential for human development. In the gastrointestinal tract, it determines gastric and intestinal development[54]. In adult mice, the absence of CDX2 leads to a cessation of intestinal differentiation[54]. In various works it has been shown that CDX2 activates Axin 2, which is part of the destruction complex in Wnt/β-catenin signaling[55,56]. Yu *et al*[56] showed in their work that CDX2 upregulates not only Axin 2 but also GSK-3β, which is also part of the destruction complex. The absence of CDX2, which in colorectal cancer is directly correlated with a higher tumor grade, leads to an activation of Wnt signaling[57].

BMPs

BMPs belong to the transforming growth factor-β (TGF-β) family. They are produced by mesenchymal cells especially at the tip of the villus and generate a contrary gradient with Wnt through the crypt-villus axis[58]. At the crypt base, BMP signaling is repressed by BMP inhibitors like gremlin and chordin-like 1 secreted by smooth muscle cells or myofibroblasts[59]. BMP represses ISC proliferation, while the influence of BMP on Wnt signaling is the subject of controversial debate. The work of He *et al*[60] postulates that BMP inhibits Wnt signaling, while the work of Qi *et al*[61] describes a direct suppression of $Lgr5^+$ cells through BMP without changes in the Wnt target genes.

Hippo signaling pathway

Hippo signaling is a highly conserved pathway and important for intestinal homeostasis and regeneration. Inactivation of Hippo signaling leads to an activation of the transcription factor Yes-associated protein 1 (YAP1), which has the highest activity at the bottom of the crypts[62]. YAP1 is an oncogene that is a facultative regulator of stem cell homeostasis and an essential regulator for the regeneration of the intestinal epithelial after injury[62]. Hippo and Wnt signaling are closely linked to each other[63]. YAP1 increases the transcriptional activity of β-catenin, while active Hippo signaling leads to the formation of the destruction complex of Wnt signaling[64,65].

Hepatocyte nuclear factor 4

Hepatocyte nuclear factor 4 (HNF4) is a transcription factor family that mainly regulates metabolism in cells. Especially fatty acids have a high impact on ISC homeostasis[66]. Chen *et al*[67] show in *in vitro* studies that HNF4α and HNF4γ activate genes involved in fatty acid oxidation and that HNF4 is necessary for stem cell renewal in the intestine. Studies about the interaction of HNF4 and Wnt are rare, few studies indicate that HNF4 may regulate Wnt signaling. The study by Yao *et al*[68] demonstrated that HNF4α is downregulated in human colon carcinoma and showed in *in vitro* experiments that HNF4α suppresses Wnt/β-catenin signaling. These results coincide with the data shown in hepatocellular carcinoma[69].

Posttranslational modification of Wnt ligands

Wnt ligands need posttranslational modifications before they can activate Wnt signaling. In the endoplasmic reticulum, Wnt ligands were glycosylated and lipidated[70]. These modifications are essential for intracellular transport, secretion of Wnt ligands and signaling[71,72].

Wnt signaling could also be inhibited by posttranslational palmitoylation. Acyl-CoA synthetase 5 (ACSL5), a mitochondrial enzyme, activates long-chain fatty acids, while binding a thioester. ACSL5-dependent palmitoylation of Wnt2β leads to an accumulation of Wnt2β in the mitochondrion and a decrease in Wnt signaling activity[73].

Furthermore, the degradation of Wnt components by the proteasome can be regulated *via* ubiquitination through ligases. For example a phosphor switch in the E3 ubiquitin ligase RNF43 leads to a lack of degradation of Frizzled and therefore to Wnt activation[74]. The ligase RNF43 itself is inhibited by receptor Lgr4[75]. Park *et al*[76] summed up the different regulation possibilities of Wnt signaling throughout ubiquitination and deubiquitination. The ubiquitination is done by E3 Ligases while deubiquitination is done by deubiquitinating enzymes. In Wnt signaling, every protein component is targeted by ubiquitination or deubiquitination[76]. Therefore, it is an important regulator of Wnt signaling.

Non-coding RNAs

Long non-coding RNAs are over 200 nt long non-coding RNA molecules. As reviewed in Zarkou *et al*[77], they can act as a Wnt enhancer by transcriptional activation of genes coding for Wnt proteins or by interaction with transcription factors regulating Wnt signaling.

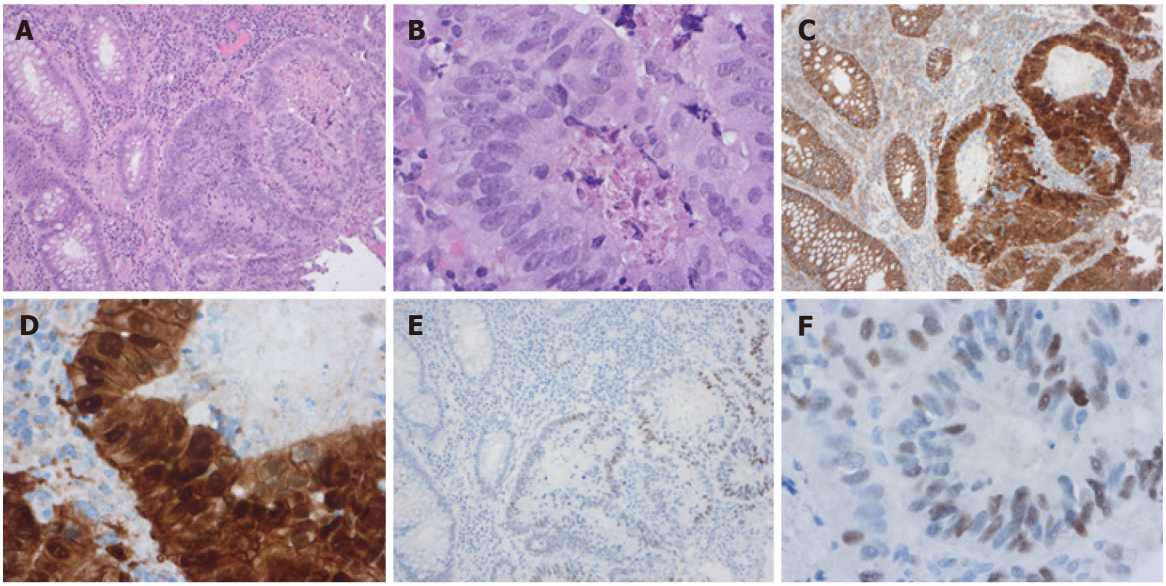
MicroRNAs (miRNAs) are small 18-25 nt long non-coding RNA molecules and can bind on their target messenger-RNA (mRNA) and suppress translation. Rahmani *et al*[78] summed up about 17 miRNAs that target mRNAs encoding for proteins of Wnt signaling. Here, they can act as an activator of Wnt signaling by suppressing translation of mRNA encoding for the destruction complex or as a suppressor of Wnt signaling, by inhibiting translation of mRNAs encoding for transmembrane complex or β-catenin. Kim *et al*[79] examined the crosstalk between stress-driven ribosome dysfunction and Wnt signaling. A proteinkinase R-activating ribosomal insult leads to changes in the Wnt and connective tissue growth factor crosstalk, which leads to progression in cancer stemness.

Other pathways

Despite the above-described pathways, growing evidence demonstrates that other pathways including the mitogen-activated protein kinase (MAPK) pathway, TGF-β signaling, and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathways involved in cell proliferation and survival have an influence on Wnt signaling[80]. It is reported that MAPK signaling regulates Wnt activity on stemness phenotypes in colorectal carcinoma cells[80,81]. Moreover, it has been found that Wnt and TGF-β pathways interact with each other to regulate genes participating in epithelial to mesenchymal transition (EMT)[82]. Hu *et al*[83] depict that epidermal growth factor receptor mediated PI3K/AKT activation enhances Wnt signaling activity through promoting β-catenin translocation, leading to increased tumor cell invasiveness.

HYPERACTIVATION OF WNT SIGNALING DRIVES PATHOPHYSIOLOGY

In spite of these regulatory mechanisms, Wnt hyperactivation is not always avoidable. In this context,



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Figure 4 Colorectal carcinoma. A: Invasive growth and loss of polarity [100 × Hematoxylin eosin (HE)]; B: Cellular atypies (400 × HE); C: β-catenin staining (100 ×) membranous in normal epithelial, nuclear staining in dysplastic cells; D: β-catenin staining (400 ×) with partly extensive accumulation of β-catenin in the nucleus; E: Positive staining of c-myc (a target of β-catenin) in the dysplastic cells (100 ×); F: Positive nuclear staining of c-myc (400 ×).

controlled activation must be distinguished from autonomous activation. Controlled activation is triggered by a stimulus outside the cell and determined through the presence of the stimulus, while autonomous activation is mainly triggered through modifications of proteins involved in the pathway and independently of the regulatory mechanism. The detailed mechanisms which lead to hyper-activation of Wnt signaling and the histomorphological correlation will be discussed hereafter.

Molecular mechanisms resulting in Wnt hyperactivation

As mentioned above, Wnt signaling is a complex regulated signaling pathway and many possibilities lead to hyperactivation of Wnt signaling in the intestine. Especially Wnt activation, while the loss of *APC* gene is well-studied *in vitro* and *in vivo*. In Drosophila, *APC* loss induced intestinal tumorigenesis [84]. A germline mutation in the *APC* gene with a loss-of-function mutation leads to familial adenomatous polyposis, representing a hereditary disease characterized by hundreds of colorectal adenomas[85]. But hyperactivation is not always accompanied by pathological tissue growth. In intestinal epithelial after injury, Wnt is also hyperactivated and enables regeneration[86]. Nevertheless, there is a fine line between Wnt activation for tissue regeneration and tissue hyperplasia. Ahmed *et al* [87] show in mice that Wnt and Notch signaling balance transmissible murine colonic hyperplasia and colitis induced by *citrobacter rodentium*. In the chronically inflamed intestine such as bowel disease, Wnt signaling is activated[88]. These patients had an increased risk of developing dysplasia and colorectal carcinoma[89]. Abnormal β-catenin expression was more closely linked to E-cadherin alterations in inflammatory bowel disease-related cancers than in sporadic cancers suggesting that specific alterations in this pathway may differ in these two cancer groups[90].

As long as Wnt signaling is controlled by other pathways, hyperproliferation of epithelial is stoppable. Problematic is uncontrolled Wnt activation, which leads to a permanent-growth stimulus. This could be caused by loss-of-function mutations in the genes encoding for the destruction complex. As mentioned above, familial adenomatous polyposis is a good example of this. But growth stimulation alone is not sufficient for carcinoma development. Fearon and Vogelstein generate the model of the adenoma-carcinoma-sequence[91]. They postulate that stepwise genetic alterations in oncogenes and tumor suppressor genes lead to hyperproliferative epithelial, low-grade and high-grade adenoma to carcinoma development. Besides *APC* mutations, which are hypothesized as a key event in adenoma development, gain-of-function mutations in *KRAS* and loss of functions in *P16-INK4*, *TP53* and *Smad4* are described in the model of multiple step carcinogenesis[92]. It is assumed that this model applies to 80% of colorectal carcinoma[93]. Nonetheless, not only *APC* mutations but also mutations in *KRAS* influence Wnt/β-catenin signaling[84]. In cell culture, KRAS stabilizes β-catenin through inhibition of GSK-3β, while others postulate that KRAS mutations activate Wnt signaling through DNA demethylation[93,94]. Interestingly, *APC* mutation and Wnt activation is a common finding in colorectal cancer, but not in carcinoma of the small intestine, even though Wnt activity in the small intestine is higher than in the colon[40,95]. That suggests that in colorectal carcinogenesis the Wnt activation is not triggered by a regulatory activation of Wnt signaling, but through an autonomous, uncontrolled activation of the Wnt signaling pathway.

In the stomach, bile acid reflux leads to an epigenetic downregulation of Dkk1, an inhibitor of Wnt signaling[96]. The bile acid-induced downregulation of Dkk1 is correlated with gastric intestinal metaplasia and might be triggered by Wnt activation. Other studies have demonstrated high expression of Dkk1 in gastric carcinomas[97].

Morphological changes caused by mutations associated with Wnt activation

The genotypic changes in colorectal adenomas lead to phenotypic changes (Figure 4). Adenoma with the classical adenoma-carcinoma-sequence often present macroscopically or endoscopically as polypoid lesions, while tumors with CpG island hypermethylation and *BRAF* mutations often present as flat mucosal lesions[92]. *APC* mutations are more often in adenomas with villous or tubulovillous formation, which are reminiscent of small intestinal villi, but *APC* mutation is also found in tubular adenomas which had elongated crypts[98]. Furthermore, Paneth cell metaplasia is also a common finding in conventional adenoma, following the adenoma-carcinoma-sequence. Joo *et al*[99] examined colonic epithelial neoplasms for Paneth cell metaplasia and Paneth cells were found in 38.5% of the conventional adenoma. This Paneth cell metaplasia was always associated with positive nuclear β-catenin staining[99]. The adenoma cells also show, depending on their grading, enlarged, hyperchromatic nuclei and loss of polarity and decreased numbers of goblet and absorptive cell lines[100]. In conclusion, hyperactivation of Wnt in the colon shifts the phenotype to a small intestinal-like phenotype.

As in the intestine, APC downregulation occurs in gastric adenomas[101]. In the stomach, the downregulation of APC is mostly caused by hypermethylation of the APC promoter and might be triggered by *Helicobacter pylori* infection[102]. Koushyar *et al*[103] summed up the parts of Wnt signaling which are deregulated in gastric cancer. In gastric cancer organoids, Wnt inactivation leads to a shift from morphological poorly carcinoma not other specified to signet-ring cell carcinoma[104].

CLINICAL RELEVANCE OF WNT ACTIVATION IN THE INTESTINE

Clinical relevance of Wnt activation in gastric cancer

In studies, Wnt signaling was upregulated in more than 80% of the examined gastric cancers and may mark Lgr5 stem cells[105]. The detailed mechanism which leads to Wnt activation is similar to colorectal cancer and is reviewed in detail by Chiurillo[106]. Mao *et al*[107] examined that Wnt1 overexpression accelerated the growth of gastric cancer. Wnt/β-catenin signaling inhibitors suppress gastric tumor growth in a mice model[108].

Clinical relevance of Wnt activation in the small intestine

Chen *et al*[109] showed cells of the Paneth cell lineage are present in intestinal adenomas. They secrete Wnt 3 and a deletion of Paneth cells leads to reduced growth of adenomas in the small intestine in APC^{min} mice. The authors concluded that Wnt3 is required for early tumorigenesis in the small bowel.

Clinical relevance of Wnt activation in colorectal cancer

In recent decades, the role of genetic aberration as a prognostic value has moved increasingly to the fore. It is therefore evident that APC mutations, which occur in the majority of microsatellite stable colorectal cancers, are examined to determine whether they had a prognostic value of colorectal cancer. Jorissen *et al*[110] analyzed over seven hundred patients with sporadic colorectal cancer and found that wild-type *APC* correlates with poor prognosis (5-year survival) in microsatellite stable proximal colon cancer. On the other hand, some studies indicate that nuclear β-catenin promotes metastasis of colon cancer, which usually display poor prognosis, by EMT[111,112].

As mentioned above, mutations that activate Wnt/β-catenin signaling are common genetic events in colorectal cancer and usually occur in an early state of carcinogenesis. Therefore, Wnt inactivation is a possible target for preventing tumor progression and as a potential treatment of colorectal cancer. 5-aminosalicylic acid (5-ASA) is a well-established treatment against inflammatory bowel disease, especially in ulcerative colitis. Therefore, it has not only anti-inflammatory but also anti-proliferative effects[113]. Several cohort studies and case-control studies have demonstrated that 5-ASA treatment is associated with a reduced colorectal cancer risk in patients with ulcerative colitis[114-116]. Therefore, guidelines recommend 5-ASA treatment for ulcerative colitis patients also under the aspect of cancer prevention. The anti-proliferative effect is forced by PP2A-dependent accumulation of nuclear β-catenin [117]. Munding *et al*[118] examined the role of the chemopreventive effects of 5-ASA *in vivo*. After three years, there were no significant differences regarding the progression of adenomas between the patients treated with 5-ASA and the placebo group. But in the group treated with 5-ASA, a significant decrease in nuclear β-catenin expression was found[118]. Further studies with a longer treatment time were necessary because the development of carcinoma through the adenoma-carcinoma sequence takes about ten to fifteen years[119]. Serafino *et al*[120] examined in their study the β-catenin expression and the expression of the β-catenin regulated proteins c-Myc and Cyclin D1 in bowel disease and found elevated

Table 2 Selection of potential target opportunities to inhibit Wnt/β-catenin signaling

Target	Effect	Ref.
Ligand-dependent Wnt signaling activation		
Wnt ligands	Wnt inhibitors	[164]
	Posttranslational modification	[165]
Dkk1	Stabilization, increase of Dkk1	[128,166]
Transmembrane complex	Inhibition of Lgr5/6	[167]
	Inhibition of Frizzled	[168,169]
Dishevelled	Inhibition	[170]
Ligand independent Wnt signaling activation		
Destruction complex	Stabilization of the destruction complex	[171,172]
β-catenin	Increase of degradation	[130,131]
	Inhibition of translocation to the nucleolus	[173]
β-catenin cofactors		[174]
Ribosome biogenesis		[134]
Oncolytic viruses		[132,133]

Dkk1: Dickkopf 1.

expression levels of these proteins especially in low-grade and high-grade dysplasia. These results emphasize the potential benefit of Wnt signaling inactivation as a predictive cancer therapy.

As reviewed by Zhu *et al*[121], Wnt activation has an impact on the resistance to chemotherapy in colorectal adenocarcinoma. Hu *et al*[122] determined that Wnt activation through exosomal Wnt secretion of fibroblasts leads to an increase in chemoresistance of cancer stem cells. Zhang *et al*[123] also identified the tumor microenvironment as a crucial factor in Wnt-induced chemoresistance. The increased chemoresistance in Wnt upregulated cancers is not only caused by enhancing the expression of antiapoptotic proteins, but also by enhancing the expression of multidrug resistance proteins[123, 124]. Zhong *et al*[125] summarized different studies where chemoresistance is associated with Wnt activation in conventional radiochemotherapy, but also in targeted and immunotherapy. Wnt signaling seems to have a big impact on the response to cancer therapy. Hence, the development of a personalized therapy targeting components of the Wnt signaling pathway in treatment of cancer is required.

WNT/β-CATENIN SIGNALING AS A POTENTIAL TARGET IN THE PREVENTION AND TREATMENT OF INTESTINAL CANCER

Application of Wnt inhibitors might be a possible therapeutic strategy to inactivate the Wnt pathway in cancer, for example obviation of binding of Wnt to Frizzled, stabilization of Dkk or destruction complex, inhibition of the transmembrane complex or Disheveled, application of β-catenin antagonist and antagonist of β-catenin cofactors, *etc*. Different drugs targeting Wnt pathway are currently in clinical trials, as reviewed in detail in Caspi *et al*[126]. Kleeman *et al*[127] postulate that there may be a difference in the therapeutic approach in ligand-dependent and ligand-independent tumors. Therefore, the localization of the mutation should be taken into account in the choice of Wnt signaling-targeting therapy. Ligand-dependent tumors should be targeted to the ligands or the transmembrane complex. In ligand-independent tumors, such as APC mutated tumors, targeting transmembrane complex is useless. A therapeutic option in these tumors is increased degradation of β-catenin. This is achieved by a stabilization of the destruction complex or directly by an increase of β-catenin degradation. One way to stabilize the destruction complex is an increased polymerization of conductin/axin2[128]. *In vitro* it represses the growth of colorectal cancer cells[128]. An opportunity to strengthen the degradation of β-catenin is *via* the proteasome through binding of molecules, which induces proteolysis. Kessler *et al*[129] examined potential binding sites of β-catenin proteolysis targeting chimeras (PROTACs). The first PROTACs are tested in mice and showed, in $APC^{min/+}$ mice, prevention and regression of colorectal cancer[130]. The E3 Ligase, TRIM58 enhances β-catenin degradation in gastric cancer and is a potential therapeutic target[131]. A different approach would be oncolytic viruses. *In vitro* and in a mice model, the adenovirus CD55-Smad4 represses tumor proliferation in metastasis by, inter alia, suppression of

Wnt signaling[132]. Adenoviruses that inhibit tumor growth by repressing the Wnt pathway have also been developed for other carcinomas such as hepatocellular carcinoma[133]. Another possible therapeutic approach in Wnt-activated tumors would be the inhibition of the ribosome biogenesis. Raveux *et al*[134] show that ribosome biogenesis dysfunction alleviates Wnt-driven tumor initiation and reduces cancer cell proliferation. In a study, kinase inhibitors in gastric cancer were screened for Wnt pathway inhibition and 34 kinases inhibit Wnt signaling more than 50%[135]. Potential targets to inhibit Wnt/β-catenin signaling are summarized in Table 2.

However, it must be noted that there could be a YAP/TAZ-dependent transcriptional reprogramming which leads to a lineage reversion and a Wnt-independent tumor growth, which can lead to failure of Wnt signaling inhibitors[136].

Development of therapeutic approaches by targeting Wnt signaling main players is challenging though it brings new hope for the management of colorectal cancer in the future.

CONCLUSION

The Wnt/β-catenin signaling pathway is a highly regulated pathway and essential for intestinal homeostasis. Disruption of this homeostasis with Wnt signaling hyperactivation can lead to tumor development and indeed Wnt activation is common in human colorectal cancer. The prognostic value of Wnt activation in colorectal cancer has not been fully elucidated yet. Furthermore, components of the Wnt signaling pathway have been brought into focus as possible targets in anti-cancer therapy and as possible adjuvant treatment for chemoresistant cancers.

FOOTNOTES

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REVIEW

From *Helicobacter pylori* infection to gastric cancer: Current evidence on the immune response

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Abstract

Gastric cancer (GC) is the result of a multifactorial process whose main components are infection by *Helicobacter pylori* (*H. pylori*), bacterial virulence factors, host immune response and environmental factors. The development of the neoplastic microenvironment also depends on genetic and epigenetic changes in oncogenes and tumor suppressor genes, which results in deregulation of cell signaling pathways and apoptosis process. This review summarizes the main aspects of the pathogenesis of GC and the immune response involved in chronic inflammation generated by *H. pylori*.

Key Words: Gastric cancer; *Helicobacter pylori*; Chronic inflammation; Host immune response

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Core Tip: Understanding the factors related to the host, infection by *Helicobacter pylori* and the mechanisms of tumor evasion are fundamental to understand the development of gastric cancer (GC). However, in the face of a complex immune environment, there are still many questions to be answered. Thus, we highlight in this work the main aspects related to GC, from infection and gastric microenvironment to immune response.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is acquired mainly in early childhood and if not treated properly can remain for life. Because of this, the infection is highly present around the world and has been linked to a wide spectrum of gastrointestinal diseases[1]. The prevalence of the bacteria varies according to geographic regions, age of the patient, socioeconomic status, education, living environment and profession. In developing countries, such as those in Latin America, they can have a prevalence of up to 80% of infected adults[2]. Recent work by Elzouki *et al*[3] evaluated 114 patients with gastric cancer (GC) and indicated that the infection rate per *H. pylori* was 63.2%. Through its virulence factors, it damages the gastric mucosa and the hormonal release, changing the stomach environment, often asymptomatically[4].

Thus, after a chronic inflammatory process, the host may develop GC in the long term, with adenocarcinoma as the most common type. GC is a worldwide public health problem since approximately one million new people are diagnosed each year with this pathology. Among the types of neoplasms, gastric adenocarcinoma is the fifth most common and is the third cause of cancer-related death worldwide. Although there is a strong association between *H. pylori* infection and gastric neoplasms, only 1%-3% of those infected end up developing GC[5]. Also in this sense, in 1994, the World Health Organization identified *H. pylori* as a group 1 carcinogen, which means a certain relationship to carcinogenesis, confirming the role of this bacterium in the process of GC development.

The mechanisms involved in this process are complex and not well known, but it is known that the tumor evasion mechanism of the immune response has a fundamental role in this process[5]. The development of this neoplastic microenvironment results from genetic and epigenetic changes in oncogenes and tumor suppressor genes, which results in deregulation of cell signaling pathways and the process of apoptosis[6]. It is known that the prognosis of patients with GC is not good, with an average 5-year survival rate of less than 20%[7]. The prognosis of patients with GC can also vary according to their classification, which can be based from anatomical location to recent molecular discoveries.

This work aims to provide an updated review on the main characteristics that lead to the development of gastric neoplasms from gastric infection by *H. pylori* in order to provide solid data that help in the knowledge about GC.

VIRULENCE FACTORS OF *H. PYLORI*

H. pylori has a lot of virulence factors that favor its maintenance in the hostile gastric environment. The recognition of these factors can even determine how serious the infection with the bacteria can be. Here, the main factors will be listed.

Flagella

Of primary importance for the pathogenic action of *H. pylori*, the flagella play a crucial role in colonization by this pathogen[8]. About four to eight flagella make up the *H. pylori* flagellar group, and each of these unitary flagella is made up of three structures: the basal body, the hook and the filament [9]. Such composition allows this bacterium not only the motility through gastrointestinal fluids, known as "swimming" but also in solid or semi-solid media, known as "spreading" and "swarming" movements, which are crucial for entry into gastrointestinal epithelial cells[8].

Chemotaxis

In addition, still in the mobility and fixation of this bacterium in the gastric environment, the chemotaxis process guarantees the interaction with molecules such as mucins, sodium bicarbonate, urea and sodium chloride, facilitating the effectiveness of the infection[10,11]. Another factor of paramount importance in the role of chemotaxis occurs in the process of continuity of infection, causing it to become chronic. In this sense, the chemotaxis ability allows *H. pylori* to circumvent the host's immune responses, achieving chronic infection[12].

Adhesion molecules

Various adhesion molecules are described as important for the colonization process by *H. pylori*, in addition to helping to protect this pathogen against mucin activity and contributing to access to important nutrients, such as nickel, which are essential for effective infection[13]. An important adherence factor that has been described in the literature is BabA, responsible for the connection with Lewis H-1 type antigens. In addition to this adhesion function, this molecule also appears to be related to the type of clinical manifestation presented by the host in the face of infection[14-16].

Another molecule of importance for the adhesion of this pathogen to gastric tissue is the outer inflammatory protein, which is also linked to the production of interleukin (IL)-8, mucosal damage and duodenal ulcer[17]. In addition, studies also argue that there is a possible relationship between outer inflammatory protein expression and greater chances of developing GC[18]. Still on the adhesion molecules of this pathogen, 33 proteins form the *H. pylori* outer membrane proteins (Hop)[19,20]. Even though most of them still do not have their activity well described or understood, some of them already have their role in the pathogenicity of *H. pylori* highlighted, such as BabA (HopS), SabA (HopP), HopQ and HopZ. BabA is related to the specific link to the b and H-1 Lewis antigens from the surface of the gastric epithelial cells, and SabA is associated with binding to Le^x and the adherence of the bacterium to laminin[21-23]. Meanwhile, the HopQ and HopZ proteins are relatively consolidated as to their importance for pathogen adherence, and the former also appears to be related to gene A associated with cytotoxins (*CagA*) gene expression[24].

Pathogenicity

Several molecules are listed as important for the pathogenesis of *H. pylori*. Among them, the role of urease stands out, which is activated even before the bacterium adheres to the gastric tissue, making an adequate acclimation of the pH of the gastric environment, regulating it to protect this bacterium[25]. In addition, urease is related to the production process of ammonia derived from urea, due to the urea channels that allow the entry of this substance in the pathogen and the intrabacterial action of this enzyme[26]. Furthermore, in addition to its role in colonization, urease seems to be important for regulating the immune response, controlling a macrophage-pathogen interaction, modulating the pH of the phagosome and ensuring the survival of *H. pylori*[27].

One of the proteins most expressed by *H. pylori* is the catalase that converts hydrogen peroxide into water and molecular oxygen[28]. Of paramount importance for the protection of the pathogen against the host's immune responses, prevention of death mediated by the complement system and avoidance against the action of phagocytes, catalase seems to be related to the clinic of gastric tumors and cancers [29,30]. Apparently, this process occurs through chronic inflammation, prevention of apoptosis and induction of mutagenesis (processes related to the action of this enzyme)[31].

H. pylori strains can be classified as *CagA* positive or *CagA* negative. Apparently, *CagA* is the main virulence factor of this pathogen, and its greatest expression seems to be directly related to more aggressive clinical manifestations, such as acute gastritis, gastric ulcer and GC[32-35]. This process is related to its ability to affect cellular motility, proliferation and apoptosis, affecting the entire conformation of gastric tissue and predisposing inflammatory pathways that facilitate these clinical presentations[35]. Still on the *CagA* gene, different forms of phosphorylation that occur (EPIYA A, B, C or D) are related to different results, with types C and D (Western and Eastern strains) more related to the outcome of GC[36].

Vacuolating cytotoxin A is an essential cytotoxin for the pathogenesis of *H. pylori*, promoting autophagic processes during the acute phase of infection, in addition to promoting the appearance of impaired autophagosomes and unbalancing cell proliferation and death during a chronic phase of infection[30]. Present in all strains of this pathogen, vacuolating cytotoxin A can be encoded by different genopatterns, being the strains s1 and m1 more related to higher levels of inflammation and consequently less indolent clinical presentations, such as gastric atrophy and carcinoma[37-40].

Another determinant factor in the pathogenesis of *H. pylori* is the *cag*-pathogenicity island, which is composed of approximately 32 genes[41]. The *cag*-pathogenicity island is responsible for the encoding of a type 4 secretion system that helps modulate the cellular metabolism of the host cell, translocate virulence factors such as CagA to the gastric epithelial cells and upregulate proinflammatory cytokine secretion[42,43]. Also, strains that present *cag*-pathogenicity island are more related to peptic ulcer and GC[44,45]. Moreover, the interaction of CagA with the SH2 containing protein tyrosine phosphatase-2 is extremely relevant. The CagA/SH2 containing protein tyrosine phosphatase-2 link happens through a tyrosine phosphorylation-dependent process, which promotes activation of the SH2 containing protein tyrosine phosphatase-2/extracellular signal-regulated kinase/mitogen-activated protein kinase pathway and consequently causes cytoskeleton alterations known as the "hummingbird" phenotype[46, 47]. These changes interfere in cellular growth and motility, which may predispose the host to genetic mutations and further GC[48]. In addition, *H. pylori* lipopolysaccharides also play an important role in the pathogenesis of this bacterium. The lipopolysaccharides are capable of binding laminin and as a consequence promote a gastric leakiness and further cellular apoptosis[49]. Furthermore, *H. pylori* lipopolysaccharides might be related to the development of GC, given that it upregulates toll-like receptor 4 and enhances cell proliferation, both *via* activation of the mitogen-activated protein kinase

kinase 1/2-extracellular signal-regulated kinase 1/2-mitogen-activated protein kinase pathway[50].

Still in the virulence factors of *H. pylori*, several others can be mentioned, such as heat shock proteins, superoxide dismutase and degrading enzymes (proteases and phospholipases), being listed in this article.

HOST IMMUNE RESPONSE TO INFECTION

H. pylori induces a significant immune response in the gastric environment of infected individuals. The onset of the inflammatory processes related to the infection occurs with the promotion of innate immunity mechanisms, involving the triggering of pattern recognition receptors of gastric epithelial cells by bacterial components such as lipopolysaccharide, NapA and nucleic acids[51]. The aforementioned recognition of foreign antigens by immune system receptors leads to the activation of intracellular signaling pathways that culminate in the release of proinflammatory cytokines, which promote the activation and recruitment of CD4+ and CD8+ T cells to the gastric environment[52]. Subsequently, a chronic inflammation against *H. pylori* infection is established, being characterized by a polarization of T helper (Th) 1/Th17 responses, which is followed by the action of regulatory T (Treg) cells responsible for controlling the inflammatory process.

The inflammatory pattern varies between groups of patients and seems to be strongly influenced by age[53]. Figure 1 summarizes the main changes in the immune response to infection by *H. pylori* according to age. In general, a predominance of the Th1 response is commonly observed in adults, along with high levels of interferon γ (IFN-γ), tumor necrosis factor α, IL-1β and IL-8[54-56], which is mostly responsible for the recruitment of neutrophils and further setup of an inflammatory environment[57]. However, when it comes to children, this pattern of cytokine release and consequent responses are not presented in the same way as adults. In a previous investigation with *H. pylori*-positive adults and children evaluating Treg and Th17 responses in the gastric mucosa, our group observed that children have higher expression of Treg-related cytokines such as IL-10 and transforming growth factor beta 1 (TGF-β1) than adults. On the other hand, adults had a prominent expression of cytokines associated with Th17 responses (IL-1β, IL-17A and IL-23) compared to children. Moreover, that study found that the expression of FoxP3+ Treg cells in the gastric mucosa was significantly higher in infants than in adults, and more intense infiltration of mononuclear and polymorphonuclear cells was observed in the latter group[58]. In another investigation evaluating the levels of cytokines associated with innate and Th1 responses in the gastric mucosa of infected individuals, we demonstrated that children express significantly higher concentrations of tumor necrosis factor α and IL-1α than adults, whereas the contrary was observed with regard to the expression of IL-2, IL-12p70 and IFN-γ. In addition, a progressive reduction in the levels of IFN-γ and IL-12p70 was observed with aging among adults, including elderly individuals, whereas a similar process was observed with the expression of IL-1, IL-2, IL-12p70 and IFN-γ in children[59].

H. pylori-induced Th1 responses have been associated with the development of corpus gastritis, which can result in gastric atrophy and intestinal metaplasia, important in precancerous lesions[60]. Moreover, Treg cells have been associated with various relevant protumor mechanisms in the setting of GC. Enhanced tumor infiltration of FoxP3+ Treg cells have been positively correlated with poor outcomes among patients with gastric adenocarcinoma[61].

Although the aforementioned immune profiles play important roles in the GC onset and progression, growing evidence have emphasized the remarkable protumoral activities associated with Th17 cells. High levels of IL-17 in the tumor environment have been related to increased concentrations of vascular endothelial growth factor and enhanced tumor vascularization. In addition, cytokines promote IL-6 production in tumor cells, and it is a protein that induces vascular endothelial growth factor release as well but also stimulates STAT3, which suppresses apoptosis and prolongs the survival of malignant cells[62]. In a recent study enrolling patients with *H. pylori*-related diseases, our group demonstrated that GC patients lack IL-27 production both in the gastric environment and peripheral blood[63]. This cytokine is an important inhibitor of Th17 responses by impairing the expression of RORγT, the main IL-17A transcription factor[64].

GC CLASSIFICATION

The classification of GC can be useful for determining a more effective diagnosis as well as a more targeted treatment and better prognosis for cancer patients. The classification system can use anatomical location, degree of invasion, lymphatic involvement, histological type and molecular subtypes[65,66]. Among several classification options, there are older ones that have fallen out of use or continue to be used today (such as Lauren's), and there are recent updates from renowned institutions such as the World Health Organization.

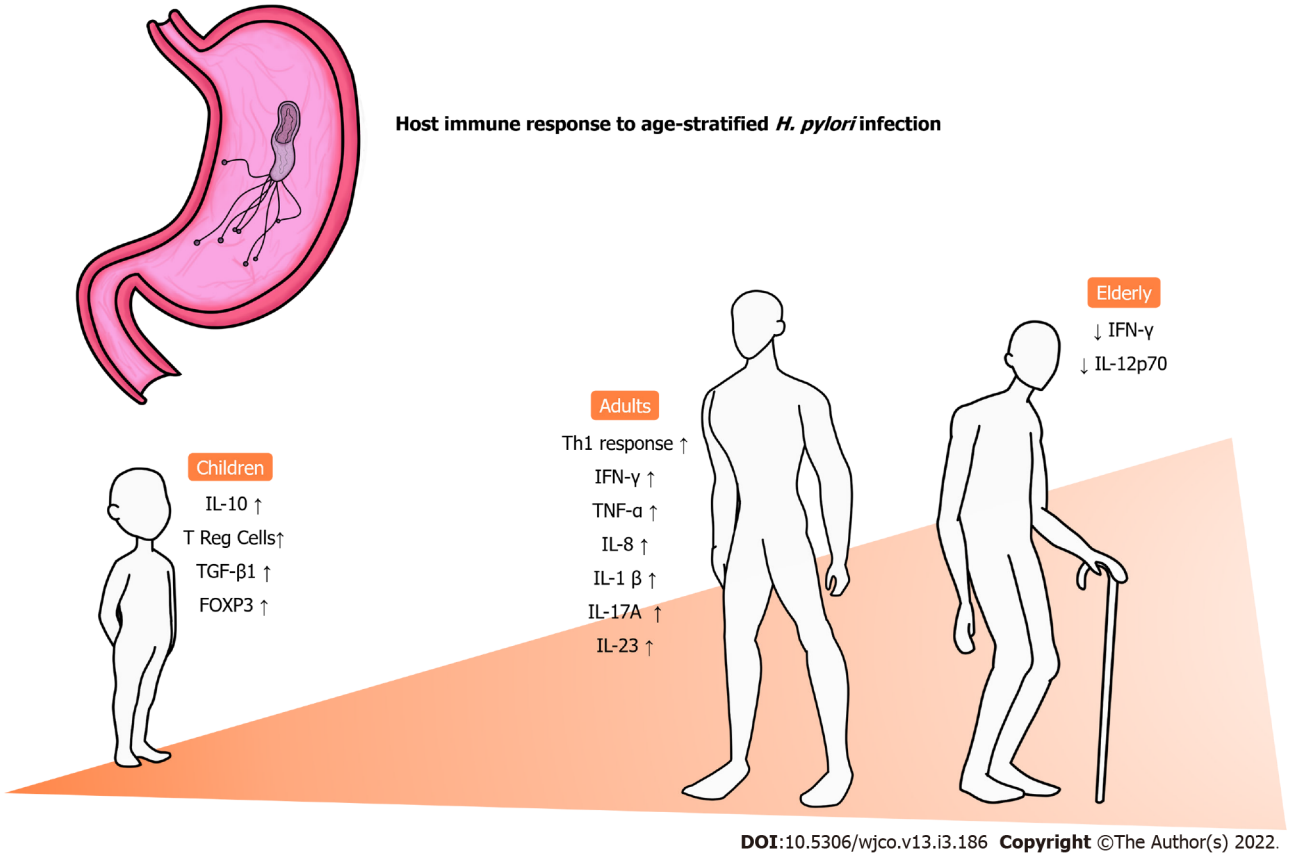


Figure 1 Host immune response to age-stratified *Helicobacter pylori* infection. *H. pylori*: *Helicobacter pylori*; IL: Interleukin; TNF-α: Tumor necrosis factor α; IFN-γ: Interferon γ; T Reg Cells: Regulatory T cells.

The anatomical classification can be divided into: (1) Cardial; and (2) Distal. The location of tumors of origin at the gastroesophageal junction, whether esophageal or gastric, may not be identified until the tumor has already reached an expressive size. The literature has shown that the tumor originating in the cardia usually presents a more aggressive behavior in relation to the distal ones, frequently invading the gastric walls[67]. In addition, the occurrence of tumors in the distal region has decreased to the detriment of those in the proximal region[68].

Classification according to the degree of invasion can be done in early or advanced cancer. The early type, limited to the mucosa and submucosa, has a lower degree of development and injury and has a 5-year survival rate of 85% to 90%, while patients with the advanced type have a 5% to 20% survival rate. Furthermore, the advanced type can be evaluated by the Borrmann classification: polypoid (type 1), ulcerated with defined edges (type 2), ulcerated with ill-defined edges (type 3) and plastic linitis, characterized by diffuse infiltrate without evidence of ulceration (type 4)[69].

Lauren's Histological Classification, widely used since its publication in 1965, has been useful in the discussion of GC. This classification divides gastric adenocarcinoma into two histomorphologic types, intestinal (well, moderately or poorly differentiated) and diffuse (undifferentiated, with or without signet ring cells). The intestinal type is more common in males and older patients, with a better prognosis. It is characterized by tumor cells that unite and organize into glandular formations, just as it occurs in intestinal adenocarcinomas. In addition, the intestinal type usually develops in an environment of atrophic gastritis and presents greater expression of the e-cadherin adhesion molecule [70]. The diffuse type, more prevalent in young individuals and more easily identified in early stages, is characterized by tumor cells that invade neighboring tissues, with little cohesion, loss of e-cadherin expression, without gland formation and with marked fibrosis. In addition, this type presents endocrine markers more frequently and has a higher production of basic fibroblast growth factor[71-73].

Among the most recent classifications, in 2019 the World Health Organization updated the classification of tumors of the digestive system, including GC. In this new approach, histogenesis and the degree of differentiation were not considered, but it recognizes several types of malignant epithelial tumors (tubular, papillary, poorly cohesive signet ring phenotype, another type of poorly cohesive, mucinous, mixed cell) as well as rare variants[74].

The National Institute of Health Cancer Genome Atlas project helped to redefine the molecular classification of GC into four subtypes: (1) GC with Epstein-Barr virus infection; (2) Microsatellite unstable tumors; (3) Genetically stable tumors; and (4) Chromosomally unstable GCs[75,76]. The first constitutes about 9% of GCs and is more common in males, has lesions in the bottom and gastric body with a lower

mortality rate and has hypermethylated DNA[77]. The second type represents 22% of GC cases and has a high mutation rate (with high frequency in the KRAS pathway), generally related to an epigenetic event[75,78]. The third type is usually aneuploid and diagnosed early, representing about 20% of GC cases, in addition to having a predominance of diffuse histology and located in the distal region of the stomach[75]. The latter type represents 50% of CG and has histology of the intestinal type, and its frequency is high in cancers of the esophagogastric junction. Chromosomal instability is the result of DNA aneuploidy and mutations in various proto-oncogenes and tumor suppressor genes[75,79].

PATHOGENESIS

Precancerous lesions

Atrophic gastritis: In atrophic gastritis (AG), there is an inflammatory process that promotes gland loss and decreased secretory function, modifying the gastric environment, which may be associated with a state of achlorhydria or hypochlorhydria[80]. A recent study showed that the relative risk for GC was 1.7 in moderate AG and 4.9 in severe AG compared to none or mild AG (control)[81]. However, it seems to be possible to identify the evolution of this risk early. In the study by Miki *et al*[82], it was reported that the progression of AG is closely linked with progressive reductions in the levels of pepsinogen I and II. Therefore, measuring these levels can be an opportunity to assess the progression of gastritis [82]. Another way to assess the risk of progression is through the location and extent of atrophy. A staging system based on the degree of atrophy and the topography of atrophy, called Operative Link for Gastritis Assessment was created for this purpose. In this system, stages 3 and 4 are strongly associated with GC development[83].

Gastric intestinal metaplasia: This lesion is characterized by the replacement of the gastric epithelium by two types of intestinal epithelium. This replacement is generally considered a condition that predisposes to malignancy and an increased risk for GC, especially type III (incomplete)[84,85]. Although the presence of this lesion is considered by many authors as a mild form of dysplasia[86], it is still controversial whether gastric intestinal metaplasia is really a precancerous lesion. After all, several studies have shown that gastric intestinal metaplasia is not always seen in patients who progress to GC [87]. However, further studies are needed to define this question.

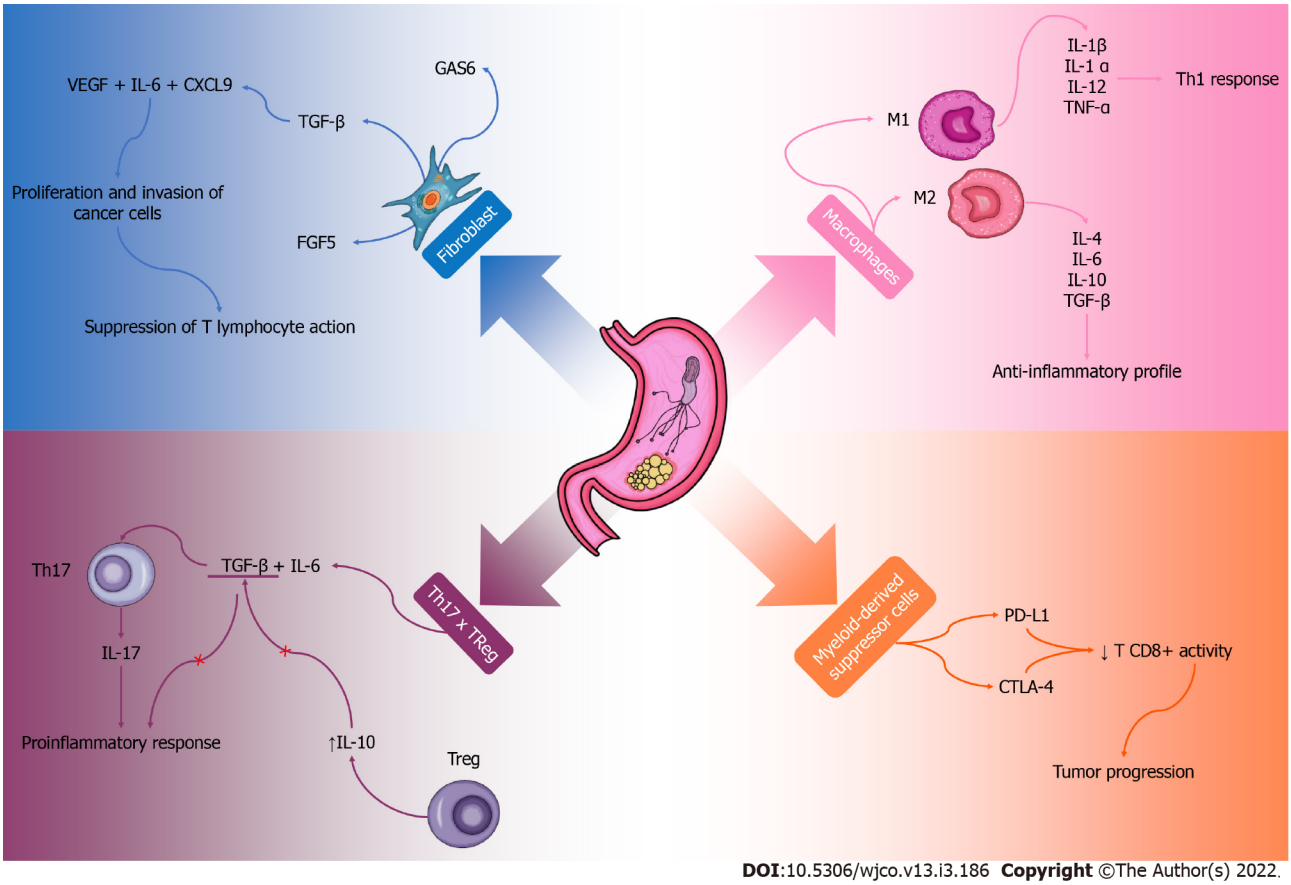
Along with this questioning, it remains uncertain whether the eradication of *H. pylori* promotes the improvement of these precancerous lesions, as there are recent works that have not found any change [88]. However, most studies that assess patients for more than 5 years after the eradication of the bacteria, demonstrate improvements in these lesions. Some possible reasons for these discrepancies are ethnic variations, disease stage, follow-up period, medications used and resistance to the drugs used[89, 90].

GC MICROENVIRONMENT

The GC microenvironment has a complex local immune response, and factors that promote the growth and expansion of cancer cells can be observed. Although the microenvironment is still poorly elucidated, some components have already been recognized. Inflammatory cells, fibroblasts and macrophages associated with cancer, endothelial cells and other infiltrating immune components play an important role in this process[91,92] (Figure 2).

Cancer-associated fibroblasts

Cancer-associated fibroblasts are involved in the synthesis and remodeling of the extracellular matrix and are directly related to angiogenesis, mechanical factors of the tumor and metastatic modulation[93]. The secretome of these cells produce TGF-β, FGF5 and specific growth arrest protein 6 that contribute to the proliferation and invasion of cancer cells[94]. In addition, the presence of vascular endothelial growth factor, IL-6 and chemokine ligand (CXCL9) can be observed, which together with TGF-β reduce the immune response of T lymphocytes[95]. Cancer-associated fibroblasts contribute to increase the stiffness of tumor tissue, compress blood vessels generating a hypoxemic process and contribute to a more aggressive cancer, providing immune evasion and less effective therapeutic response[96,97]. Interestingly, a study using mice found that these cells contribute to the progression of GC, but their functions are not fully understood[98]. This whole process contributes to an immunosuppression in the tumor microenvironment and creates an ideal environment for the development and progression of tumor cells in the affected tissue and possibly other tissues. These cells are probably important in the process of malignant or benign evolution of GC and should be better explored so that their knowledge is directed to therapeutic contexts.



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Figure 2 Summary scheme on the microenvironment of gastric cancer. TGF-β: Transforming growth factor beta; FGF5: Fibroblast growth factor 5; GAS6: Specific growth arrest protein 6; VEGF: Vascular endothelial growth factor; CXCL9: Chemokine ligand; PD-L1: Programmed death ligand 1; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; IL: Interleukin; Th: T helper cell; TNF: Tumor necrosis factor; Treg: Regulatory T cell.

Tumor-associated macrophages

Tumor-associated macrophages are also abundant components of the immune system that play an important role in the microenvironment of GC[99,100]. They can be M1 type and produce proinflammatory cytokines such as IL-1β, IL-1α, IL-12, tumor necrosis factor α, IL-12 and CXCL9, which polarize and recruit components of a Th1 response profile[93,101]. This process culminates in the inhibition of tumor growth[102]. In contrast, type M2 stimulates a Th2 profile secreting cytokines such as IL-4, IL-6, IL-10, IL-13, IL-33, TGF-β and IL-10, which have an anti-inflammatory profile and important tumor activity[103]. They act on tumor progression, metastasis and angiogenesis, which are important factors for the tumor formation microenvironment[104]. The polarization of M1/M2 macrophages is conducted according to the inflammatory profile of the tumor microenvironment or therapeutic intervention. More studies would be interesting in order to observe possible therapies in order to reverse the polarization of M2 macrophages in M1 so that with a more proinflammatory profile, the immune system is stimulated, and the fight against the tumor is more effective.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells are part of the cell population involved in immune responses and are observed in the tumor microenvironment. They suppress the cytotoxic function of CD8+T cell activity and antitumor, as they have a high expression of programmed death ligand 1 and cytotoxic T-lymphocyte-associated protein 4[93,105]. An interesting study in mice observed that the expression of IL1-β is associated with a greater recruitment of these suppressor cells in the tumor microenvironment [106]. Baumann *et al*[107] observed that the neutralization of the activities of these cells, concomitant with the inhibition of the checkpoint, obtained a greater efficacy in cancer therapy.

Relationship between Th17 /Treg

Regarding the expression of cytokines in the microenvironment of GC, there is an important increase in Th17 and Treg cells causing an imbalance in the relationship between these cytokines. This phenomenon is observed gradually with the progression of cancer[108]. The increase in Th17 cells, stimulated by TGF-β and IL-6, promote tumor progression due to increased expression of IL-17 and consequently greater local inflammation[109]. However, Treg cells provide an immunosuppressed environment,

stimulating a high production of IL-10 and inhibiting the production of TGF-β. Thus, these Treg cells stimulate the progression of the GC, decreasing the host's immune surveillance in the tumor microenvironment[110]. Apparently, the balance of the Th17 and Treg relationship in the gastrointestinal tract reflects the integrity of the mucosal immune response and plays an important role in the mechanisms of tumor progression and metastasis.

Most studies are *in vitro* and murine models. Despite few studies on human models with GC, it is important to highlight the role of immunosuppression in the tumor microenvironment, which is essential for the development of cancer and for possible metastases. The immune response in the tumor microenvironment has a direct link to the host's general immune response to cancer. These responses are dependent on factors such as genetics, polymorphisms, chronic diseases and the use of drug therapies. In addition, the lack of studies in different stages of human life is a problem since the immunological profile is different throughout life. More studies on the microenvironment of GC are needed, so it would be possible to better understand the mechanism of the immune response and possibly find even more effective therapies in the treatment of this cancer.

TUMOR EVASION MECHANISMS TO THE IMMUNE RESPONSE OF THE HOST

During the initial development of tumors, including GC, their cells use several mechanisms to resist innate immune response and prevail in the organism, and when the tumor achieves more advanced stages it evades from the action of T effector cells[111]. Of note, the tumor microenvironment is a protagonist in that context[112]. The immunosuppression promoted by the programmed cell death protein 1 is strongly related to the immune evasion and worse outcomes in GC[113]. In that context, the podoplanina is an immune checkpoint molecule that has been associated with the immune response evasion in various malignancies[114].

Liu *et al*[115] reported in their study that high levels of podoplanina-expressing cells infiltrating gastric tumors were associated with an increased recruitment of protumoral macrophages and T effector cell dysfunction. Moreover, the infiltrating podoplanina cells contributed to the reduction of INF-γ, granzyme B and perforin-1 levels as well as with an increased expression of programmed cell death protein 1, T cell immunoglobulin and immunoglobulin and mucin-domain containing-3. These findings suggest that the expression of those cells is a tumor evasion mechanism by gastric tumor cells[115].

Another study observed that the macrophage-derived chemokine CXCL8 plays a crucial role in tumor progression in GC patients by mediating immune response evasion and metastasis. CXCL8 acts by reducing the infiltration of Ki67 CD8+ T cells and inhibiting them through the expression of programmed death ligand 1 in macrophages[116]. Interestingly, a study evaluated a GC-derived extracellular compound and concluded that it presents immunosuppressive activities through selective inhibition of CD161CD3 natural killer cells, proliferative stimulus and reduction of the intracellular levels of IFN-γ, granzyme B and perforin[117]. Complementarily, Zhang *et al*[118] investigated the importance of IL-10-related tumor-associated macrophages in the GC immune response evasion and observed that a tumor microenvironment with high levels of IL-10 tumor-associated macrophages was characterized by the infiltration of Treg cells and dysfunction of CD8+ T cells.

Shi *et al*[119] demonstrated in a study that the density of Foxp3+ Treg cells and A2aR/CD8+ T cells were highly expressed in the tumor microenvironment and were able to avoid immune responses against GC. The mechanism used by the FoxP3+ Treg cells was the cell apoptosis induction through the ATP decomposition into adenosine as well as the inhibition of CD8+ T cells through the A2aR pathway, leading to an immunosuppressive effect[119]. Notably, a study showed that IL-10-producing regulatory B cells have the potential to avoid the immune surveillance in patients with GC and predict poorer outcomes since the population of CD19CD24+hiCD27 B cells included cells that are able to suppress CD4+ T cells and the production of IFN-γ by autologous CD4+ T cells[120].

Another protein, the costimulatory molecule B7-H4, is a member of the B7 inhibitors family expressed in tumor-related monocytes and macrophages. It is considered an important component of GC immune system evasion, being that it is correlated to invasion depth and to the presence of venous and lymphatic invasion as well as to the expression of HLA-DR[121]. Interestingly, circulating tumor cells undergo an epithelial-mesenchymal transition that allows them to survive in several metastatic environments. In this sense, an assay demonstrated that patients with GC had subtypes of epithelial-mesenchymal transition markers able to regulate ULBP1 (a major member of the natural killer group 2 member D ligand family) in the circulating tumor cells, which aid in immune response evasion[122].

Considering that the tumor purity consists of the proportion of cancer cells in the tumor and is intimately related to the tumor microenvironment characteristics, it is important to emphasize that the low tumor purity implies an unfavorable prognosis, accentuated infiltration of Treg, M1 and M2 macrophages, high expression of immune checkpoints and recruitment of immunosuppressor molecules [123]. A computational study used the interface mimicry technology in order to predict host-pathogen interactions in the context of *H. pylori* infection and their repercussions in GC. This study found that the *H. pylori* infection interferes with the apoptosis of host cells through proteins such as HP0231, which is able to impair the CASP6 homodimerization, a crucial step for apoptotic signaling.

The aforementioned interaction might explain the *H. pylori*-induced resistance to death of host cells, a well-known characteristic associated with carcinogenesis[124]. The studies mentioned here highlight the importance of understanding the mechanisms that lead to the immune system evasion of GC in order to better understand the pathophysiology of the disease as well as to improve prognosis assessments and therapeutic tools for affected individuals. Finally, it is evident that the *H. pylori* virulence factors are closely related to the gastric carcinogenesis by means of adaptative mechanisms that not only contribute to the infection persistence but also unleash premalignant changes in the gastric microenvironment. These variations progress and perpetuate along with tumor development, aiding in its evasion from the immune response.

FUTURE PERSPECTIVES

Faced with such complex pathways to be understood, future work needs to detail the immune response to bacterial infection, especially to help with early intervention in patients who may develop CG. This may enhance the discovery of new pharmacological therapies that interfere with precancerous lesions and even in advanced stages of cancer. In addition, they should also help in the discovery of new, non-conventional, non-invasive, highly specific biomarkers capable of providing early detection of GC.

CONCLUSION

Infection by *H. pylori*, its relationship with the host's immune system and oncogenesis as well as the tumor evasion mechanisms is crucial for the understanding of the mechanisms involved in the appearance and progression of GC. In view of the chronic inflammation process, a variety of factors can affect the patient's prognosis, especially according to the individual's age. The gastric microenvironment has not been well established, and some fundamental components for the progression of GC have been recognized to be related to the host's immune response.

FOOTNOTES

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Abstract

Celiac disease (CeD) is a chronic autoimmune disorder that is triggered by gluten in genetically susceptible individuals, and that is characterized by CeD-specific antibodies, HLA-DQ2 and/or HLA-DQ8 haplotypes, enteropathy and different clinical pictures related to many organs. Intestinal lymphoma may develop as a result of refractory CeD. If a patient diagnosed with CeD is symptomatic despite a strict gluten-free diet for at least 12 months, and does not improve with severe villous atrophy, refractory CeD can be considered present. The second of the two types of refractory CeD has abnormal monoclonal intraepithelial lymphocytes and can be considered as pre-lymphoma, and the next picture that will emerge is enteropathy-associated T-cell lymphoma. This manuscript addresses "CeD and malignancies" through a review of current literature and guidelines.

Key Words: Refractory celiac disease; Enteropathy-associated T-cell lymphoma; Pre-lymphoma; Low grade lymphoma

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Core Tip: Malignancies are among the leading consequence of celiac disease (CeD), and intestinal lymphoma and adenocarcinomas in particular. Enteropathy-associated T-cell lymphoma type 1 has been shown to develop from refractory CeD type 2, while the association of CeD with other cancer types is controversial. Decades of reported studies suggest that a non-delayed diagnosis of CeD and strict adherence to a gluten-free diet significantly reduces the rate of cancer development associated with CeD.

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INTRODUCTION

Celiac disease (CeD) is a chronic autoimmune disorder that is triggered by gluten in genetically susceptible persons with HLA-DQ2 and/or HLA-DQ8 haplotypes, and is characterized by CeD-specific antibodies and enteropathy[1-3]. The prevalence of CeD in the general population is approximately 1% on serological screening, and 0.6% as histologically confirmed[3].

CeD can affect many organs, and can cause or trigger, or be associated with different clinical pictures, including growth retardation, short stature, chronic diarrhea, constipation[1], iron deficiency anemia[4], dermatitis herpetiformis[5], dental enemal defects[6], aphthous stomatitis[7], rickets, osteoporosis[8,9], arthralgia, arthritis[10], idiopathic epilepsy[11], peripheral neuropathy[12], ataxia[13], abnormal liver tests, autoimmune hepatitis[14], type1 diabetes mellitus[15], IgA deficiency[16], psychiatric comorbidities[17], intestinal lymphoma[3], etc. It is not known exactly why these clinical pictures emerge as different manifestations in different patients, as there are complex underlying mechanisms. Although the relationship between CeD and intestinal lymphoma is known, there have been many studies and case reports suggesting its association with other malignancies. For the present manuscript, a systematic literature search of PubMed/MEDLINE was carried out using the search terms "Celiac disease AND guideline, and Celiac disease AND malignancy" and a review was made on the subject of "CeD and malignancies" in current literature and guidelines in line with the following structure: (1) Pathogenesis of CeD; (2) Refractory CeD; (3) Enteropathy-associated T-cell lymphoma (EATL); (4) CeD and malignancies; and (5) Conclusion.

PATHOGENESIS OF CELIAC DISEASE

Although the pathogenesis of CeD is not fully understood, it is considered to be attributable to the coaction of genetic, environmental and immunologic factors. The HLA-DQ2 and/or HLA-DQ8 haplotypes are necessary for CeD development. Studies have shown that around 4% of HLA-DQ2 + cases develop CeD, and that HLA-DQ2 and HLA-DQ8 negative CeD development is extremely rare [18]. It is evident that environmental factors are at the core of the CeD pathogenesis, of which gluten is the *sine qua non* trigger. The gliadin proteins found in gluten are composed of glutamine and prolamine residues, and cannot be fully digested, even in a healthy person. HLA-DQ2 and HLA-DQ8 proteins are located on the surface of intestinal antigen-presenting cells. Undigested gliadin peptides in the intestinal lumen pass through the intestinal epithelium and undergo cross-linking and deamination through tissue transglutaminase (tTG) in the lamina propria. The glutamine contained within gliadin is converted to glutamic acid, bound to HLA-DQ2 and HLA-DQ8 and presented to CD4+ T cells. The cross-linking of gliadin and tTG results in the formation of tTG antibodies that impair the function of tTG. Activated CD4+ T cells cause the production of pro-inflammatory cytokines like interferon-γ that contain T-helper cells that worsen the inflammatory effect in the process. Matrix metalloproteinases cause the degradation of the extracellular matrix and damage to the basement membranes, resulting in an increase in natural killer (NK) T lymphocytes within the epithelial cell. Gliadins also upregulate the expression of the zonulin protein by increasing intestinal permeability in both CeD patients and healthy people. Increased anti-tTG levels are also known to inhibit tTG and make gliadin harder to digest, which in turn increases tTG activity, resulting in a vicious cycle. Intraepithelial lymphocytes (IELs) include T cell receptor (TCR)αβ+ and -γδ+ T cells, and NK cells. Most of these TCR+ IELs express a variety of NK cell receptors, and in addition, the number of CD8+ TCRαβ+ and TCRγδ+ increases. Consequently, characteristic lesions of CeD develop by apoptosis[2,18-22].

CeD is in general similar to other autoimmune diseases, but has a very clear and indispensable trigger: gluten. Gluten-induced intestinal lesions and autoantibodies begin to improve in the absence of gluten. Anti-tTG antibodies increase to protect against the disease, and are at the center of the pathogenesis. They may appear before villous atrophy develops and can induce CeD[21].

REFRACTORY CeD

Refractory CeD (RCeD) patients are those with a pre-existing diagnosis of CeD whose CeD-related symptoms fail to improve, and in whom villous atrophy develops despite a strict gluten-free diet for more than 12 months[23-25]. RCeD is mostly diagnosed after the age of 50 years, but younger cases have

been identified. The incidence for both types of RCeD is in the 0.04%-1.5% range[3].

When RCeD is suspected, a second endoscopy and several biopsies are mandatory. Duodenal biopsies show Marsh type III, and sometimes Marsh type II[3]. The presence of subepithelial collagen extending to the lamina propria in the duodenal second part, chronic inflammation and crypt hypoplasia (not hyperplasia) with villous atrophy are common microscopic findings of RCeD[23].

Refractory CeD is divided histologically into two subgroups according to the immunophenotype of IELs: type 1 (RCeD-1) and type 2 (RCeD-2). RCeD-1 has a normal intraepithelial lymphocyte phenotype while RCeD2 has an abnormal clonal lymphocyte population[25]. In RCeD-1, the symptoms are less severe, and the endoscopic and histological features are similar to active uncomplicated CeD. RCeD-1 shows the same normal immunophenotype as CeD, often leading to difficulties in differential diagnosis from CeD, although differentiating between RCeD-1 and RCeD-2 is mandatory due to the different treatment strategies and prognosis[3].

The immunophenotype of abnormal IELs in RCeD-2 is different to that of RCeD-1. It has been reported that interleukin-15 and somatic mutations in JAK1 or STAT3 in the proliferation of aberrant T cells play an important role in the formation of RCeD-2[24]. Cording *et al*[26] identified a complex mutational profile of JAK1 and STAT3 that activated the NF-κB pathway in CeD-associated lymphomagenesis.

While most lymphocytes express CD3, CD8 and polyclonal TCRβ, RCeD-2 is characterized by abnormal T cells that do not express surface CD3 or CD8, but instead express intracellular CD3 by a TCR gamma rearrangement[23-25], and these cells also express NK surface markers[24,27]. RCeD-1 becomes involved when abnormal T cells account for less than 20%, and RCeD-2 for more than 20%. RCeD-2 may be referred to as pre-lymphoma or low grade lymphoma due to the high risk of conversion to EATL[3,28]. Verbeek *et al*[29] suggest that the quantification of abnormal T cells using flow cytometry is preferable to T cell clonality analyses in differentiating RCeD patients. The use of a cut-off value of 20% for the classification of patients can also support the selection of long-term follow-up and treatment.

Figure 1 summarizes the properties of RCeD-1, RCeD-2 and EATL.

The goal of treatment is to prevent RCeD-1 patients from converting to RCeD-2, and then to EATL, in that a total of 52% of RceD-2 patients have been reported to develop EATL within 4–6 years of diagnosis of RCEeD-2[30]. Immunosuppressive drugs are used together with nutritional support for the treatment of RCeD-1. Although similar therapies have been applied for RCeD-2, their usefulness is limited. In such patients, autologous hematopoietic stem cell transplantation following high-dose chemotherapy is an alternative treatment[3,31].

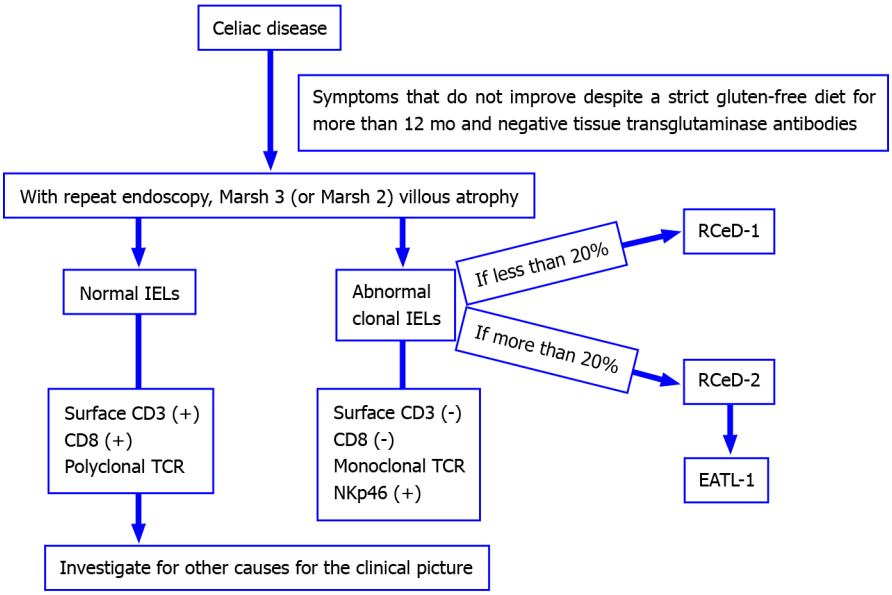
ENTEROPATHY-ASSOCIATED T-CELL LYMPHOMA

Enteropathy-associated T-cell lymphoma accounts for less than 1% of all non-Hodgkin lymphomas, and as such is considered a rare GI lymphoma[3]. Approximately 50% of RCeD-2 patients are thought to develop overt lymphoma within 5 years of diagnosis[18]. EATL occurs predominantly in patients in the sixth and seventh decades, and usually develops in those diagnosed with CeD[25,32,33]. EATL is thought to be derived from IELs, and the abnormal immune phenotype of IELs seen in RCeD-2 indicates early-stage lymphoma development. To date, two histologically subtypes of EATL have been described [23].

A microscopic examination of type I EATL (EATL-1) reveals transmural infiltration including pleomorphic medium- to large-size neoplastic lymphocytes, histiocytes and eosinophils. Mitotic figures and necrosis are common, and enteropathic changes such as villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis may be seen in the non-tumor gastrointestinal tract mucosa[25,33]. Tumor cells in EATL-1 have a pattern of CD2+, CD3+, CD5-, CD4-, CD7+, CD8-, CD56-, TCR- (usually), CD103+ and CD30+ (often), and a high Ki-67 proliferative index and p53 expression. Epstein-Barr virus is negative[33]. In some cases, tumor cells may show pronounced pleomorphism reminiscent of anaplastic large cell lymphoma or Hodgkin lymphoma[23]. The IELs in the non-neoplastic mucosa have the same immunophenotype as in RCeD-2. Type 2 EATL (EATL-2) is rare, and is generally not associated with a previous diagnosis of CeD[3]. While the features of non-tumoral mucosa resemble those of CeD, the tumor cells in EATL-2 have a CD3+, CD8+, CD56+ or CD4- pattern. NKp46, indicating progression from RCeD-2, has also been reported in EATL[23].

CeD AND MALIGNANCIES

The increased risk of malignant lymphomas in CeD is correlated to small bowel histopathology, and so no increased risk of lymphoma is expected in CeD patients with improved intestinal mucosal changes and with a gluten-free diet, or in potential CeD patients with an already normal intestinal mucosa[34]. Goerres *et al*[35] found intestinal UDP-glucuronosyltransferases, which are involved in the detoxification of ingested toxins and carcinogens, to be decreased in CeD, and suggested that this could



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Figure 1 Properties of refractory celiac disease type 1, type 2 and enteropathy-associated T-cell lymphoma. RCeD: Refractory celiac disease; EATL: Enteropathy-associated T-cell lymphoma; IEL: Intraepithelial lymphocytes; TCR: T cell receptor.

potentially pose a risk of cancer. Kamycheva *et al*[36] reported the leukocyte telomere length to be shorter in CeD seropositive patients, which may indicate genomic instability – a well-known predisposing factor of genetic changes and eventual carcinogenesis.

Ferguson *et al*[37] reported a 1.9 times greater risk of mortality in 653 CeD patients after a mean follow-up of 13.5 years, with the most common causes of death being lymphoproliferative disease and esophageal cancer. Freeman[38] identified 8.4% lymphoma, 1.4% small bowel carcinoma and 0.5% hypopharyngeal carcinoma in 214 patients with CeD, and reported the risk of lymphoma and small bowel adenocarcinoma to be increased especially in patients diagnosed with CeD after the age of 60 years, suggesting that risk increases the longer the diagnosis of CeD is delayed. Howdle *et al*[32] reported 13% of adenocarcinoma cases and 39% of lymphomas to have CeD.

Grainge *et al*[39] reported in their cohort study that the risk of any malignancy in CeD patients was 40% greater than in the general population, with an average follow-up of 25 years. They reported the highest risk in those with non-Hodgkin's lymphomas, with an overall incidence of 1.3 per 1000 person-years, but that the overall malignancy risk did not increase significantly 15 years after the diagnosis of CeD. Eigner *et al*[40] identified RCeD in 2.6% of 1,138 CeD patients, and reported that in 29 RCeD patients followed for 25 years, RCeD-1 developed in 1.3%, RCeD- 0.6%, EATL in 0.6% and small intestine adenocarcinoma in 0.4%, with a mortality rate of 48%. They noted further that in the preceding five years, there had been no patients diagnosed with RCeD-2, EATL or small bowel adenocarcinoma, which could be related to the increased awareness of CeD and strict adherence to a gluten-free diet.

Green *et al*[41] reported detecting small bowel adenocarcinoma in two (0.2%) and non-Hodgkin's lymphoma in five (0.4%) of 1,612 CeD patients, with EATL being found in three patients (relative risk was 300). In a meta-analysis Han *et al*[42] reported a pooled odds ratio (OR) for the risk of all malignancies of 1.25, and 1.60 for GI malignancy in CeD patients. Of the GI malignancies, esophageal cancer (pooled OR= 3.72) and small intestinal carcinoma (pooled OR = 14.41) were associated with a greater risk. Ilus *et al*[43] reported that the standardized incidence ratio (SIR) did not increase for the series as a whole in 32,439 CeD patients, but reported a decrease in breast and lung cancers, and an increase in NHL (SIR: 1.94) and small bowel cancers (SIR: 4.29) 5 years after the CeD diagnosis. In a recent study, Koskinen *et al*[44] reported that although the overall mortality in adult CeD diagnosed in 2005–2014 had not increased, mortality associated with lymphoproliferative diseases had increased, but to a lesser degree than previously reported.

Table 1 provides details of studies of malignancies in CeD patients, including those identifying and not identifying an increased risk. The malignancies associated with CeD in the case reports are presented in Table 2.

CONCLUSION

A causal relationship between CeD and EATL2 has been proven. Although its relationship with other cancer types is controversial, considering the pathogenesis of CeD, such a possibility can be considered.

Table 1 Malignancies with increased risk, or not reported in studies of patients with celiac disease

Ref.	Study design	Increased risk	No increased risk
Eigner *et al*[40]	Retrospective cohort	EATL	-
		Small bowel adenocarcinoma	
Freeman[38]	Retrospective cohort	Lymphoma	-
		Small bowel carcinoma	
		Hypopharyngeal carcinoma	
Grainge *et al*[39]	Cohort	All malignancies	-
		Non-Hodgkin's lymphoma	
Howdle *et al*[32]	Survey	Small bowel adenocarcinoma	-
		Small bowel lymphoma	
van Gils *et al*[47]	Case-control	T-cell lymphoma, predominantly EATL	Other types of lymphomas
		Small bowel adenocarcinoma	GI carcinomas
		Esophageal squamous cell carcinoma	
Anderson *et al* [48]	Retrospective cohort	Non-Hodgkin's lymphoma (but not statistically significant)	-
Green *et al*[41]	National survey	Small bowel adenocarcinoma	-
		Non-Hodgkin's lymphoma	
Han *et al*[42]	Meta-analysis	All malignancies	Other GI cancers
		Small intestinal cancers	
		Esophageal cancer	
Ilus *et al*[43]	Retrospective cohort	Non-Hodgkin lymphoma	Decreased risk of lung, pancreatic, bladder, renal and breast cancer
		Small intestinal cancer	
		Colon cancer	
		Basal cell carcinoma of the skin	
Kent *et al*[49]	Cohort	Papillary thyroid cancer	-
Lebwohl *et al* [50]	Population-based setting	-	Cutaneous malignant melanoma
Volta *et al*[51]	Cohort	-	Colon carcinoma

EATL: Enteropathy-associated T-cell lymphoma; GI: Gastrointestinal; OR: Odds ratio; RR: Relative risk.

Studies have suggested that this risk is gradually decreasing[38,39] due to the increased awareness of CeD over the years, and the widespread use of diagnostic tests and endoscopy, which have made diagnosis easier and more common. Furthermore, the increase in the availability of commercial gluten-free products has facilitated stricter compliance with gluten-free diets. Today, the follow-up of CeD patients at certain periods is recommended in CeD guidelines[1,45]. In the event of suspected non-compliance with a gluten-free diet, or when presented with symptoms, the patient is re-evaluated with CeD-specific antibodies and the presence of RCeD is investigated. The major limitation of most of the above-mentioned studies is the lack of reporting on the compliance of CeD patients with the diet "assessed from year to year" based on CeD-specific tests. Indeed, in some of the studies, the CeD diagnosis was made either together or recently in some of the patients diagnosed with lymphoma at elderly ages. For this reason, objective evaluations (monitoring with CeD-specific antibodies or measurement of gluten immunogenic peptides in urine and feces[46]) of CeD patients diagnosed in childhood will yield better results. In addition to the above, since intestinal villous atrophy improves with a gluten-free diet, an early diagnosis of CeD and a lifelong gluten-free diet are very important in preventing the formation of intestinal lymphoma and adenocarcinoma. Regular follow-ups can support patients in their compliance with a gluten-free diet.

Table 2 Malignancies associated with celiac disease in case reports

Ref.	Diagnosis of malignancies (age in years)
Ahluwalia *et al*[52]	Burkitt-like lymphoma of colon (75)
Buess *et al*[53]	EATL causing obstructive jaundice (54)
Cankurtaran *et al*[54]	Plasma cell dyscrasia (65)
Cereda *et al*[55]	1st patient: Burkitt lymphoma of the small bowel (5)
	2nd patient: Ependymoma (4)
	3rd patient: Ewing sarcoma (6)
Zunguo *et al*[56]	Large B-cell lymphoma and enteropathy-type T-cell lymphoma (65)
Fallah *et al*[57]	Adenocarcinoma of the small intestine (89)
Jafroodi *et al*[58]	Hodgkin's lymphoma (11)
Naderi *et al*[59]	Two patients: germ cell tumor (3.5 and 5)
	3rd patient: Wilm's tumor (6)
	4th patient: Acute lymphobolastic lymphoma (4.5)
	5th patient: Astrocytoma (8)
Sahin *et al*[60]	Intestinal adenocarcinoma (58)
Zullo *et al*[61]	Intestinal adenocarcinoma (77)

FOOTNOTES

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MINIREVIEWS

Breast cancer in India: Present scenario and the challenges ahead

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Abstract

Breast cancer is the commonest malignancy among women globally. From being fourth in the list of most common cancers in India during the 1990s, it has now become the first. In this review, we examine the available literature to understand the factors that contributed to the high burden of breast cancer in the country. We also provide the landscape of changes in the field of early diagnosis and the treatment modalities as well as the limitations of the Indian healthcare delivery systems (*e.g.*, delayed diagnosis, human resources and funding for treatment). This review also sheds light on the newer interventions and the future of breast cancer management keeping in mind the coronavirus disease 2019 imposed limitations.

Key Words: Breast cancer; Challenges; Prospects; India; Treatment

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Core Tip: This review highlights the progress that has been made in the field of breast cancer management in India over the past few decades, in terms of addressing the various challenges of breast cancer control including diagnostic methods and treatment options. It also highlights the future of breast cancer control strategies with a focus on the coronavirus disease 2019 situation.

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INTRODUCTION

Introduction and epidemiology

Breast cancer (BC) is the commonest malignancy among women globally. It has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases[1]. Epidemiological studies have shown that the global burden of BC is expected to cross almost 2 million by the year 2030[2]. In India, the incidence has increased significantly, almost by 50%, between 1965 and 1985[3]. The estimated number of incident cases in India in 2016 was 118000 (95% uncertainty interval, 107000 to 130000), 98.1% of which were females, and the prevalent cases were 526000 (474000 to 574000). Over the last 26 years, the age-standardised incidence rate of BC in females increased by 39.1% (95% uncertainty interval, 5.1 to 85.5) from 1990 to 2016, with the increase observed in every state of the country[4]. As per the Globocan data 2020, in India, BC accounted for 13.5% (178361) of all cancer cases and 10.6% (90408) of all deaths (Figure 1 and Figure 2) with a cumulative risk of 2.81[5].

Current trends point out that a higher proportion of the disease is occurring at a younger age in Indian women, as compared to the West. The National Cancer Registry Program analysed data from cancer registries for the period from 1988 to 2013 for changes in the incidence of cancer. All population-based cancer registries have shown a significant increase in the trend of BC[6]. In India in 1990, the cervix was the leading site of cancer followed by BC in the registries of Bangalore (23.0% *vs* 15.9%), Bhopal (23.2% *vs* 21.4%), Chennai (28.9% *vs* 17.7%) and Delhi (21.6% *vs* 20.3%), while in Mumbai, the breast was the leading site of cancer (24.1% *vs* 16.0%). By the years 2000-2003, the scenario had changed, and breast had overtaken as the leading site of cancer in all the registries except in the rural registry of Barshi (16.9% *vs* 36.8%). In the case of BC, a significant increasing trend was observed in Bhopal, Chennai and Delhi registries[7].

When it comes to the 5-year overall survival, a study reported it to be 95% for stage I patients, 92% for stage II, 70% for stage III and only 21% for stage IV patients[8]. The survival rate of patients with breast cancer is poor in India as compared to Western countries due to earlier age at onset, late stage of disease at presentation, delayed initiation of definitive management and inadequate/fragmented treatment[9]. According to the World Cancer Report 2020, the most efficient intervention for BC control is early detection and rapid treatment[10]. A 2018 systematic review of 20 studies reported that BC treatment costs increased with a higher stage of cancer at diagnosis. Consequently, earlier diagnosis of BC can lower treatment costs[11].

EARLY DETECTION AND SCREENING PROGRAMMES

Success and failure of screening programs depend on several factors ranging from the presence of proper guidance manuals, development and usage of an appropriate instrument for diagnosis to proper implementation and availability of adequate human resources. Another factor is the efficacy of the screening test in avoiding the risk of false positives and unnecessary biopsies and surgeries[12]. Organised screening programmes provide screening to an identifiable target population and use multidisciplinary delivery teams, coordinated clinical oversight committees and regular review by a multi-speciality evaluation board to maximise the benefit to the target population[13]. Screening strategies are moving towards a risk-based approach rather than a broad age-based and sex-based recommendation. To use this risk-based approach, India needs to assess risk factors and incorporate this information into BC screening[14].

A recent study from Mumbai has reported that clinical breast examination conducted every 2 years by primary health workers significantly downstaged breast cancer at diagnosis and led to a nonsignificant 15% reduction in breast cancer mortality overall (but a significant reduction of nearly 30% in mortality in women aged ≥ 50)[15]. Mammography sensitivity has been reported to vary from 64% to 90% and specificity from 82% to 93%[16]. Indian women have more dense breasts, and there is a lack of adequate mammography machines and trained manpower. This may result in false positives and over-diagnosis. Digital mammography uses computer-aided detection software but remains costly. It is due to these reasons that mass-scale routine mammography screening is not a favoured option for a transitioning country like India.

Ultrasonography has an overall sensitivity of 53% to 67% and specificity of 89% to 99%[17,18] and might be particularly helpful in younger women (aged 40 to 49 years). However, the requirement of trained professionals to perform and interpret ultrasound is a major hurdle. Though breast self-examination is not accepted as an early detection method for BC, this technique, if used diligently and skilfully, can serve as a useful adjunct to making the woman aware of her normal breast[19].

Understanding India-specific differences by utilising genomics may enable the identification of women at high risk of developing cancer, where targeted screening may be cost-effective. There is an urgent need to identify Indian-specific genetic/epigenetic biomarkers. These may have the potential to be used as biomarkers for early detection at the screening stage[20].

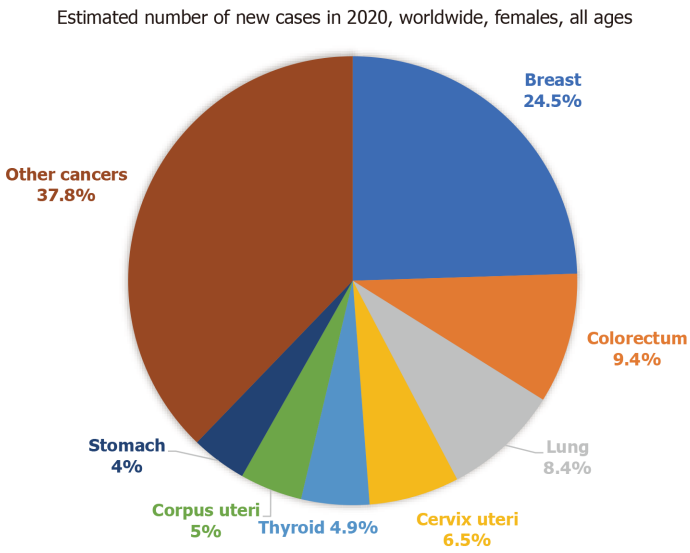


Figure 1 World Health Organization: Estimated number of new cancer cases in 2020, worldwide. Image available under Common Creative License [68] (Available at https://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf).

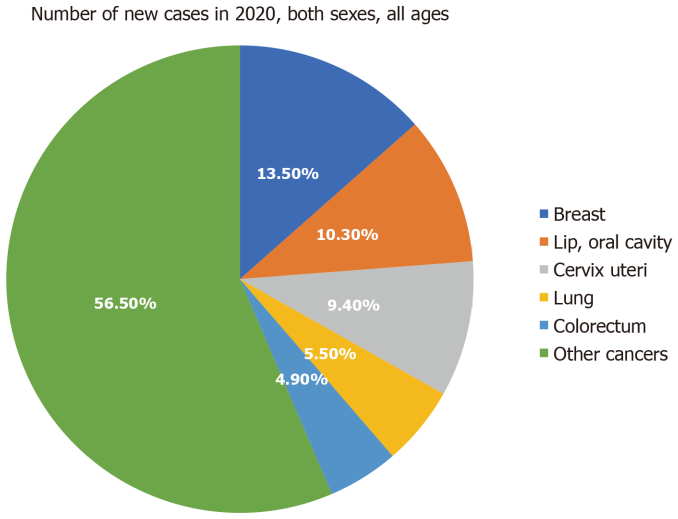


Figure 2 World Health Organization Globocan 2020 India. Image available under Common Creative License[68] (Available at https://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf).

TREATMENT OPTIONS IN PAST AND PRESENT

Management of BC is multidisciplinary and has come a long way. In the past, the widely used treatment option was mastectomy followed by adjuvant chemotherapy for locally advanced BC, triple-negative breast cancer and HER2neu expressing tumours (human epidermal growth factor receptor 2). At present, it includes a loco-regional approach (targeting only the tumour with the help of surgery and radiation therapy) and a systemic therapy approach that targets the entire body. The systemic therapy includes endocrine therapy for hormone receptor-positive disease, chemotherapy, anti-HER2 therapy for HER2 positive disease, bone stabilising agents, polymerase inhibitors for BRCA (breast cancer gene) mutation carriers and, recently, immunotherapy. However, the majority of patients still undergo primary ablative surgical procedures. Gene expression profiling in hormone receptor-positive disease is also a promising option but has financial implications.

From using drugs like cyclophosphamide, methotrexate, *etc.* in the 1970s for chemotherapy to using their modifications like anthracycline-based combination chemotherapy protocols in the 1980s and 1990s, we have come a long way. Taxanes are the newer additions that show a promising future. The radiation treatment of BC has evolved from 2D to 3D conformal radiotherapy and accelerated partial breast irradiation, aiming to reduce normal tissue toxicity and overall treatment time[21]. The newer additions, viz. intensity-modulated radiation therapy and deep inspiration breath-hold, are still inaccessible to many. The same is the case with brachytherapy.

The outcomes with triple-negative breast cancer are poor, and the treatment options are mainly restricted to systemic chemotherapy. Immunotherapy, poly adenosine diphosphate-ribose polymerase inhibitors (poly(adenosine diphosphate-ribose) polymerase) and antibody-drug conjugates have the potential to change the current scenario of BC treatment. One important point that should be considered while planning the treatment is that there is a lot of hype regarding the newer drugs that flood the market, however with little or no difference in the survival benefit. Hence it is important to choose wisely.

The field of breast surgery has also evolved from total mastectomy to breast conservation therapy to oncoplastic breast surgery. The rapidly advancing field of oncoplastic breast surgery offers a pragmatic alternative to total mastectomy and breast conservation therapy. It is currently nascent but expected to attain mainstream status in the near future as oncoplastic breast surgery has economic feasibility and cost-effectiveness and is well suited for a low-resource setting such as India[22].

CHALLENGES IN BC CONTROL

Delay in seeking healthcare

Continuously increasing BC prevalence is just the tip of the iceberg. There are many underlying problems that contribute to this mounting burden. In India, nearly 60% of BC cases are diagnosed at stage III or IV of the disease[23]. Most of the patients present to the healthcare facility only when there is a large palpable mass or secondary changes like local skin/chest wall changes are visible. Women tend to ignore the minor symptoms and do not show up at the hospital until it is unbearable, owing to their household responsibilities. Other factors that may contribute to the late presentation include a lack of awareness about the disease, especially in rural areas. This also leads to fewer women performing a self-breast examination, opting for a periodic examination by a healthcare worker or mammography for BC screening, despite it being available for free in a few government hospitals. For those willing to pay for it, mammography is available in private hospitals. This lack of awareness regarding the risk factors and early detection methods of BC is unfortunately even prevalent in 49% of healthcare workers[24].

The initial manifestation of BC, *i.e.* a lump, is generally not associated with pain, which further adds to the delay in seeking treatment in 50% to 70% of the cases in rural areas[25]. Other factors that may influence the early detection and treatment of BC are the presence of a diagnostic/treatment facility in the nearby area, patient's preference and trust in the healthcare provider, amount of time required for travelling to the service centre and amount and availability of money that can be spent on the treatment. However, this grim situation has slowly started to change due to various awareness campaigns, and now women have slowly started to understand and value their health. Another deterring factor in seeking early care is the stigma of social embarrassment and isolation. Women not only fear death and contagion by cancer but also fear that their and their family's reputation would suffer if people knew of their cancer diagnosis, including potential difficulties in their daughter's wedding. It is also a widespread assumption that cancer, especially in the private parts (breast and genitals) is linked to "bad" and "immoral behaviour"[26]. The issue of social stigma urgently needs to be addressed through awareness campaigns, as it not only jeopardises early diagnosis but also the treatment-seeking behaviour of women with symptoms of BC.

Delay from the healthcare provider's side

On average, more than 12 wk of delay is seen in diagnosis and treatment in 23% of patients[27]. A study examined provider's delay (defined as the period between the first consultation and diagnosis) and observed that the mean provider delay was 80 d in rural areas and 66 d in urban areas[28]. More than half of the women were observed to have a delay of more than 90 d in seeking care. The patient-related delay was observed to be 6.1 wk, and the system-related delay was 24.6 wk with a mean total delay of 29.4 wk in treatment. This led to a poor prognosis[29]. These delays may be attributed partly to the ever-increasing patient load on the health care system and competing priorities.

High attrition rates

After crossing all these hurdles, once the diagnosis is done and treatment is started, there are further hazards. In 1990, India's facilities for the diagnosis and treatment of cancer were far behind recommendations[30]. Even today, due to a large variation in the health care standards between regions, the quality of treatment for BC patients varies from pathetic to world-class. Few patients are treated at well-equipped centres in a protocol-based manner, while some are subjected to numerous compromises. Fortunately, BC is curable if detected early, but due to various underlying factors, improper treatment provided locally by non-oncologists without standard oncology expertise may lead to the mismanagement of BC cases. At the same time, patients with advanced, metastatic incurable cancer may require only palliative care are referred to tertiary cancer centres. This leads to the improper use of limited, valuable resources.

Another issue that adds to the high attrition rates/loss to follow-up of BC treatment is an unacceptable out-of-pocket expenditure, which is three times higher for private inpatient cancer care in India[30]. More than half the patients from low-income households spend > 20% of their annual household expenditure on BC treatment, leading to catastrophic results[31]. An analysis of three public insurance schemes for anticancer treatment in India published in 2018 revealed inconsistencies in the selection of reimbursed treatments. The reimbursed amount was usually found to be insufficient to cover the total cancer chemotherapy costs, leading to an average budget shortage of up to 43% for BC treatment[32]. Cancer insurance policies can significantly reduce the financial burden caused by out-of-pocket expenditure and prevent catastrophic health expenditure, distress financing and even bankruptcy. However, a study by Singh *et al*[33] to understand the use of health insurance in India reported a lack of awareness regarding the use of these schemes as the key reason for the low penetration of health insurance policies.

Shortage of resources/skewed distribution of available limited resources

Another problem is a shortage of manpower. India has just over 2000 oncologists for 10 million patients, and the number of oncologists is unevenly spread, being lower in semi-urban and rural areas. Although nearly 70% of the Indian population lives in rural areas, about 95% of facilities for cancer treatment exist in the urban areas of the country. There are also regional variations. About 60% of specialist facilities are in Southern and Western India, whereas more than 50% of the population lives in the Northern, Central and Eastern regions, distorting service provision[34].

There are currently 57 courses for radiotherapy technologists and about 2200 certified radiation technologists in practice in India[35]. As per a recent study, India has just 10% of the total requirement of 5000 radiation therapy units indicating a shortfall of > 4500 machines. The World Health Organization recommends at least one teletherapy unit per million population, and there is a shortfall of 700 teletherapy units[35]. If we look at the treatment infrastructure, at least half of patients with cancer will be judged to need radiotherapy at some point. Yet only 26% of the population, living in the Eastern region of India, have immediate access to only 11% of radiotherapy facilities[36]. Nearly 40% of hospitals in India are not adequately equipped with advanced cancer care equipment. Very few centres in the country provide integrated surgical and chemoradiation for BC. Nearly 75% of the patients in the public sector do not have access to timely radiotherapy[37].

All of these factors aid in raising the overall cost of BC treatment for the common person. They are forced to make out-of-pocket cancer care payments, as most of the patients have to bear the cost of therapy. The government facilities are inadequate in number to cater to a large number of patients. Thus, patients are required to go for treatment in major cities along with their attendants, resulting in loss of livelihood of both the patient and her attendants. There is an urgent need to establish a larger number of cancer care facilities accessible to those living in rural areas so that the gap of cancer detection and treatment services may be bridged.

RAYS OF HOPE/WAY FORWARD

The integrative approach

The rising human cost, both social and economic, of BC underscores the need for more holistic, multidimensional approaches that encompass the cancer care continuum including prevention, early detection, treatment, palliation and survivorship. A balanced approach is necessary to integrate traditional medical practices into mainstream oncology practice, starting with a meaningful discussion among all the relevant stakeholders and identifying the areas where the benefits of a complementary approach are beyond doubt[38]. Exercise has been proven to be an effective, safe and feasible tool in combating the adverse effects of treatment, prevents complications and decreases the risk of BC-specific mortality[39]. A recent review has reported evidence that diet-related and physical activity-related interventions for the primary prevention of BC are cost-effective[40]. Such lifestyle modifications need to be included in mainstream treatment planning.

The internet era

Screening programmes in high-income countries that have increased patient participation have done so with high-quality and periodic education programmes with campaigns tailored to the specific cultural context of a community[41]. This can only be achieved by creating and sustaining the level of awareness among the general population. One effective way of doing that is providing information on BC to the relatives/patients in the hospital setting. However, due to the ever-increasing patient burden, it is not always possible for healthcare providers to give appropriate time and counselling to the people. The distribution of pamphlets with valuable information may be the cause. In recent times, the Internet is being increasingly used as a reliable source to seek health-related information[42,43]. A lot of people search for information online. Having a credible source of information that can be revisited as and when required by the people is always a plus. Keeping these things in mind a website 'Cancer India' was developed by the National Institute of Cancer Prevention and Research providing comprehensive

information on common cancers in India, including BC, in layman's language (available in English and Hindi). A recent evaluation by this group reported that the website managed to serve the intended purpose of improving cancer awareness with reasonable success[44].

Involvement of community health workers

Community participation with the engagement of the health system and local self-government are required for implementing a comprehensive cancer screening strategy. A BC screening program using local volunteers for early detection is feasible in low-income settings, thereby improving survival[45]. Community health workers can play an important role in the early detection of BC in low- and middle-income countries, with responsibilities including awareness-raising, conducting clinical breast examinations, making referrals and supporting subsequent patient navigation. However, this promise can only be turned into genuine progress if these activities are appropriately supported and sustained. This will involve adopting contextually appropriate early detection initiatives that are embedded within the broader health system where community health workers are appropriately trained, equipped, paid and supported with appropriate links to specialist oncology services. A recent study reported the effectiveness of the Extension for Community Healthcare Outcomes model training program in reaching primary care physicians across the country and improving their knowledge and skills related to screening for breast, oral and cervical cancer[46].

Early diagnosis

With advancements in molecular diagnostics and therapeutics, newer non-invasive prognostic biomarker tests to detect BC at a very early stage, such as digital breast tomosynthesis and breast biopsy techniques, are becoming available[47]. Above all, early detection programmes in low- and middle-income countries must make provisions for every individual at risk of BC. This will mean considering the needs of the hardest individual to reach first, so that no woman is left behind in the goal to end unjust and untimely deaths attributable to the leading cause of female mortality in low- and middle-income countries[48].

Out of pocket expenditure

To address the issue of out-of-pocket expenditure and to reach out to the poorer section of the society, a scheme called Pradhan Mantri Jan Arogya Yojana under the new universal healthcare programme named "Ayushman Bharat" has been recently launched in India. It is the largest health assurance scheme in the world that aims at providing a health cover of Rs. 5 Lakhs (6814 USD) per family per year for secondary and tertiary care hospitalisation to over 10.74 crores (approximately 107 million) poor and vulnerable families (approximately 500 million beneficiaries) that form the bottom 40% of the Indian population[49]. The Indian government's efforts to bring anti–HER2 drug under-pricing regulations have enhanced access and improved outcomes. The launch of low-cost T-DM1 (the antibody-drug conjugate trastuzumab emtansine) and anti–HER2 therapy biosimilars is keenly awaited. Linking cancer registry data with Ayushman Bharat, mortality databases and the Hospital Information System could improve cancer registration, follow-up and outcome data[50].

Newer initiatives

There are several health-tech start-ups that aid in different stages of cancer care. For example, Niramai® uses machine learning and big data analytics to develop low-cost diagnostics for BC[51]. Oncostem® uses multi-marker prognostics tests that aid in personalised treatment[52], and UE Lifesciences® uses contactless and radiation-free handheld screening devices[53] that may come in handy during coronavirus disease 2019 (COVID-19) times. Panacea® and Mitra Biotech®[54] are start-up companies that deal with providing precision therapies. Tumourboard® and Navya Network®[55] provides affordable and precise consultations. There are several drug-patient assistance programs from pharmaceutical companies like Roche®, Novartis®, Dr Reddys®, *etc.* that help in following patient-centric care from beginning to end. The BC Initiative 2.5 is a global campaign to reduce disparities in BC outcomes and improve access to breast health care worldwide. It is a self-assessment toolkit that can help countries conduct a comprehensive breast health care situational analysis[56] Apart from these, patient navigation strategies are also rapidly growing and evolving concepts. 'Kevat' is the first initiative in India to create a trained task force to facilitate the cancer patient's journey from entry to the hospital to follow-up. It is a nascent area of speciality in cancer care that is set to target the pressing need of well-trained patient navigators in onco-care[57].

Apart from these, the World Health Organization, on March 9, 2021, introduced a Global Breast Cancer Initiative to reduce global breast mortality by 2.5% by 2040. The aim is to reduce 2.5 million global deaths, particularly in low-income countries, where the progress to tackle the disease has been relatively slow. An evidence-based technical package will be provided to countries as part of the initiative[58]. Such initiatives instil faith in the future for better breast cancer management.

COVID-19 challenge

The COVID-19 pandemic has challenged the prioritisation of various diseases in healthcare systems

across the globe, and BC patients are no exception. It impacted their access to physicians, medication and surgeries. Nearly 70% of patients could not access life-saving surgeries and treatment. Chemotherapy treatments and follow-ups were postponed due to lockdowns[59,60]. A recent study reported that the average monthly expenditure of cancer patients had increased by 32% during the COVID-19 period while the mean monthly household income was reduced by a quarter. More than two-thirds of the patients had no income during the lockdown, and more than half of the patients met their expenditure by borrowing money. The incremental expenditure coupled with reduced or no income due to the closure of economic activities in the country imposed severe financial stress on patients with BC[61].

It became of utmost importance to balance between the benefits and risks associated with BC treatment[62]. There are different treatment modalities and each case is different. The shorter duration of radiotherapy, transient spacing of chemotherapy in metastatic BC setting, if deemed feasible, oral hormone therapy to delay surgery and judicious use of immunomodulators are some recent guidelines in evidence-based practice in this unprecedented crisis[63]. The core idea is to delay the surgery until the pandemic is over whenever feasible. Additionally, apart from healthcare management, one area that has suffered tremendously due to the infectious outbreak is cancer research[64]. The estimated decrease in cancer funding in India ranges from 5% to 100%, as many funding agencies have cancelled calls for funding. The private/charity sector is the worst hit, with an estimated decrease of more than 60% of its funding[65]. A robust health system is a prerequisite for providing the facilities for the treatment of BC that is diagnosed through the early detection programmes, whether through screening or the presence of symptoms[66]. Of course, it goes without saying that a proper evaluation of the programmes will not only allow improvement of quality of services but also generate valuable evidence on the effectiveness of screening and early diagnosis in the countries "in transition"[67].

CONCLUSION

To conclude, the BC burden is rising at a rate much faster than it was a decade ago. Acknowledging that BC is one of the foremost cancers in India now would be the first step towards making people cognizant of the disease. It is fast developing into a public health crisis, and society's discomfort to talk about women's bodies has made the situation even worse. To combat the consequences as a country, better preparedness is essential. A robust awareness campaign and effective implementation of a national cancer screening program are the need of the hour. We also need to stand up and deliver on the healthcare front. The shortage of skilled manpower and infrastructural requirements need to be met, and for this, the total healthcare budget of the country needs to be increased. In the jargon of the challenges of BC control, prioritising the adoption of a preventive approach and early detection would go a long way. Another important aspect is the country's preparedness for unprecedented events like COVID-19, for which there should be a separate provision to deal with public health disasters. Creating a cadre of trained medical and paramedical professionals, efficient utilisation and timely upgrading of skills of the existing healthcare workforce along with adopting newer technologies would further the cause of BC control.

FOOTNOTES

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MINIREVIEWS

Immunotherapy in triple-negative breast cancer: A literature review and new advances

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Abstract

Triple-negative breast cancer (TNBC) is a highly complex, heterogeneous disease and historically has limited treatment options. It has a high probability of disease recurrence and rapid disease progression despite adequate systemic treatment. Immunotherapy has emerged as an important alternative in the management of this malignancy, showing an impact on progression-free survival and overall survival in selected populations. In this review we focused on immunotherapy and its current relevance in the management of TNBC, including various scenarios (metastatic and early -neoadjuvant, adjuvant-), new advances in this subtype and the research of potential predictive biomarkers of response to treatment.

Key Words: Triple-negative breast cancer; Early disease; Immunotherapy; Biomarkers; Metastatic disease

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Core Tip: Triple-negative breast cancer (TNBC) is an exceptionally heterogeneous disease and historically a cancer with limited treatment options other than chemotherapy. Recent advances in immunotherapy has changed the standard of care in selected groups, especially in metastatic TNBC. This article review continues the detailed, updated and comprehensive literature review regarding immunotherapy in TNBC, including the discussion of clinical trials in different scenarios (metastatic, neoadjuvant, adjuvant) and potential biomarkers to provide useful knowledge for medical oncologists and the medical community. Our goal is sharing updated information for TNBC which is considered an overlooked population with an enormous necessity of novel treatments and biomarkers.

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INTRODUCTION

Triple-negative breast cancer (TNBC) which effects approximately 15 - 20% of all patients, is a heterogeneous, complex disease with a more aggressive behavior than other subtypes of breast cancer. It is associated with a high incidence of visceral metastasis (predominance of hepatic, pulmonary and central nervous system metastasis), a high risk of early recurrence and a worse prognosis[1]. Unlike other subtypes, historically, TNBC has had no other systemic treatment options other than chemotherapy which has been the cornerstone of treatment for many years. However, this has recently changed with the introduction of immunotherapy in patients with programmed death ligand 1 (PD-L1) expressing tumors, both in unresectable locally advanced/metastatic disease. In the neoadjuvant setting, the use of immunotherapy has recently been approved[1].

Based on efforts in genetic studies, breast cancer was divided into molecular subtypes. Perou *et al*[2] proposed a classification based on expression patterns, subdivided into 4 clinical molecular subtypes (luminal A, luminal B, HER2 enriched and basal-like). Most basal-like tumors are included in TNBCs (they represent 70%-80% of the TNBCs)[3]. Lehmann *et al*[4] identified 6 different subtypes using DNA and RNA profiles in TNBC ["basal-like 1" (BL1), "basal-like 2" (BL2), "immunomodulatory" (IM), "mesenchymal" (M), "mesenchymal stem-like" (MSL) and "luminal androgen receptor" (LAR)] each with particular characteristics. BL1 and IM tumors have a higher sensitivity to DNA-damaging agents such as platinum and are associated with a young age at diagnosis. They are also the subtype with the highest pathological complete response (pCR) rate (65.6%) followed by BL2 (36.4%) in a cohort of patients treated with platinum-based neoadjuvant therapy (n = 97). The LAR subtype has the lowest pCR rate (21.4%)[4].

Although breast cancer has traditionally been considered a non-immunogenic tumor, multiple studies have shown that TNBC can stimulate the immune system. Compared with luminal breast cancer, TNBC has a higher tumor mutational burden (TMB), elevated levels of PD-L1 expression and increased levels of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment which are associated with higher rates of pCR to neoadjuvant chemotherapy and efficacy to immunotherapy which justifies the use of immunotherapy in this subtype[5].

Due to advances in the molecular characterization of TNBC, with addition of immunotherapy, new therapeutic agents including poly ADP-ribose polymerase-1 (PARP) inhibitors, tyrosine kinase inhibitors (TKI), checkpoint inhibitors, antiandrogens, antibody-drug conjugates (ADC) and other targeted therapies are being researched. Moreover, ongoing trials are evaluating immunotherapy (immune checkpoint inhibitors) in combination with PARP inhibitors in a series of cancers including BC [6].

IMMUNOTHERAPY AGENTS APPROVED IN TNBC

The high mutational burden of the TNBC was determined to lead to the synthesis of abnormal proteins, acting as "neoantigens" which will be recognized by the antigen presenting cells and would initiate an antitumor immune response[7].

Early-stage TNBC has a high TIL infiltrate but breast cancer has not traditionally been considered immunogenic. Recent trials demonstrate TIL infiltrate has a high expression of PD-1 (and other inhibitory checkpoint molecules). TNBC has potential therapeutic targets such as immune checkpoint inhibitors (ICIs) (anti-PD-1/PD-L1 agents) in metastatic and the early-stage scenario[8] (Table 1).

Table 1 Randomized phase II/III immunotherapy trials en triple-negative breast cancer

Scenario	Trial	Phase	*n*	Intervention	Recruitment Status	Magnitude of clinical benefit		
						PFS (mo)	OS (mo)	Additional information
Neoadjuvant	NCT03639948	II	100	Carboplatin + Docetaxel + Pembrolizumab	Recruiting			
	NCT03289819	II	50	Pembrolizumab + Nab-paclitaxel → Pembrolizumab + Epirubicin and Cyclophosphamide	Recruiting			
	NCT03356860 (B-IMMUNE)	II	57	Paclitaxel + Epirubicin + Cyclophosphamide + Durvalumab	Recruiting			
	NCT02685059 (GeparNuevo) (June 2018)	II	174	Epirubicin + Nab-paclitaxel + Cyclophosphamide + Durvalumab	Active, no recruiting	-	-	
				Population: Early TNBC				pCR was increased to 53.4% with Durvalumab *vs* 44.2% with chemotherapy alone, not being statistically significant ($P = 0.048$) In the PD-L1 (+) subgroup: pCR 58% *vs* 50.7% ($P = 0.363$) pCR was increased in patients with high levels of TILs y TMB-H ($P < 0.01$) 3-yr iDFS was 84.9% with durvalumab *vs* 76.9% with placebo (HR: 0.54, 0.27-1.09, $P = 0.0559$); 3-yr DDFS 91.4% *vs* 79.5% (HR: 0.37, 0.15-0.87, $P = 0.0148$); 3-yr OS: 95.1% *vs* 83.1% (HR: 0.26, 0.09-0.79, $P = 0.0076$)
Neoadjuvant/Adjuvant	NCT03036488 (KEYNOTE-522) (August 2020)	III	1174	Carboplatin + Paclitaxel + AC (anthracycline + cyclophosphamide) +/- Pembrolizumab → Adjuvant Pembrolizumab	Active, no recruiting	-	-	Co-primary endpoints were pCR and EFS
				Population: Early TNBC				pCR: 64.8% in Pembro group *vs* 51.2% with placebo ($P < 0.001$) The benefit of Pembro in pCR was consistent in all subgroups, including PD-L1 (+): pCR 68.9% *vs* 54.9% ($P < 0.001$) A statistically benefit was observed in EFS (HR: 0.63, 0.48-0.82) Pembro showed a favorable trend in OS (HR: 0.72, 0.52-1.02)
	NCT02620280 (NeoTRIPaPDL1)	III	280	Carboplatin/nab-paclitaxel +/- Atezolizumab → anthracycline (AC/EC)	Active, no recruiting	-	-	Primary endpoint was pCR

	(December 2019)			Population: Early TNBC		The pCR rates were not statistically significant between both groups: 43.5% with atezolizumab *vs* 40.8% with chemotherapy alone A multivariate analysis showed that the only variable associated with pCR was the PD-L1 (+) status: pCR 51.9% *vs* 48% ($P < 0.0001$) These results differ from KEYNOTE-522, where pembrolizumab achieved significant rates of pCR in a similar population
	NCT03281954	III	1520	Doxorubicin + Cyclophosphamide + Paclitaxel + Carboplatin +/- Atezolizumab → Atezolizumab	Recruiting	
	NCT03197935 (IMpassion031) (September 2020)	III	204	AC (doxorubicin + cyclophosphamide) + Nab-paclitaxel +/- Atezolizumab → Adjuvant Atezolizumab Population: Early TNBC	Active, no recruiting	pCR was 58% in Atezolizumab group *vs* 41% in placebo group ($P = 0.0044$) In the PD-L1 (+) population, pCR was 68.8% in the Atezolizumab group *vs* 49.3% in the placebo group ($P = 0.021$) A favorable trend was obtained in EFS (immature data) (HR: 0.76, 0.40 -1.44) In patients with early TNBC, neoadjuvant treatment of Atezolizumab + nab-paclitaxel and an anthracycline-based regimen achieve higher rates of pCR, with an acceptable safety profile
Adjuvant (for patients with residual disease after neoadjuvant chemotherapy)	NCT02954874	III	1000	Pembrolizumab *vs* observation	Recruiting	
	NCT03756298	II	284	Capecitabine +/- Atezolizumab	Recruiting	
Adjuvant	NCT03498716 (IMpassion030)	III	2300	Paclitaxel → dd Doxorubicin/Epirubicin + Cyclophosphamide +/- Atezolizumab	Recruiting	Primary endpoint was iDFS Secondary endpoints were iDFS according to PD-L1 status and nodal affectation, OS, safety, y health related to a QoL
	NCT02926196 (A-Brave)	III	335	Avelumab *vs* observation	Recruiting	This trial evaluates patients in two groups: (1) Primary TNBC patients who completed surgery followed by adjuvant therapy; and (2) Primary TNBC patients with residual disease after neoadjuvant chemotherapy (did not achieve pCR) The first and second co-primary endpoints are DFS in all patients and DFS in B group
Locally advanced or mTNBC	NCT02768701	II	40	Cyclophosphamide + Pembrolizumab	Active, no recruiting	

	NCT03121352	II	30	Carboplatin, Nab-paclitaxel y Pembrolizumab	Recruiting			
	NCT02499367 (TONIC)	II	67	Control or irradiation 3 x 8 Gy or oral cyclophosphamide or Cisplatin or Doxorubicin → anti-PD-1 (Nivolumab)	Active, no recruiting			Five cohorts were included in the randomization, all followed by nivolumab Overall, the ORR was 20% Most responses were observed with cisplatin (ORR: 23%) and doxorubicin (ORR: 35%)
	NCT02819518 (KEYNOTE-355) (December 2020)	III	858	Nab-paclitaxel or Paclitaxel or Carboplatin/Gemcitabine +/- Pembrolizumab Population: First-line mTNBC	Active, no recruiting	9.7 *vs* 5.6 (HR: 0.82) in CPS ≥ 10	-	Co-primary endpoints were PFS and OS (this latter is pending outcome) Pembro treatment was statistically significant only for patients with high levels of PD-L1 (expressed in CPS ≥ 10) Pembro + chemotherapy showed a significant increase in PFS among mTNBC patients A recent update showed that KEYNOTE-355 trial met primary endpoint of OS in patients with mTNBC whose tumors expressed PD-L1 (CPS ≥ 10)
	NCT02555657 (KEYNOTE-119) (September 2019)	III	600	Capecitabine, Eribulin, Gemcitabine, or Vinorelbine *vs* Pembrolizumab Population: Second and third-line mTNBC	Active, no recruiting	2.1 *vs* 2.1 (HR: 1.14)	12.7 *vs* 10.7 (HR: 0.78)	Pembro did not show improvement in OS or PFS as 2L/3L of treatment for mTNBC *vs* chemotherapy (OS: 9.9 mo *vs* 10.8 mo, HR: 0.97, 0.82- 1.15) OS in tumors with CPS > 10: 12.7 mo *vs* 11.6 mo (HR: 0.78, 0.57-1.06) A greater benefit was obtained in OS/PFS in tumors with high levels of PD-L1 (expressed in the CPS score) Pembro was well tolerated and had less adverse events compared with chemotherapy
	NCT02447003 (KEYNOTE-086) (March 2019)	II	285	Pembrolizumab monotherapy	Active, no recruiting	-	-	Primary endpoint: ORR in the total population and PD-L1 (+) ORR was 5.3% in the total population, and 5.7% in the PD-L1 (+) population Pembro demonstrated antitumor activity in patients previously treated with mTNBC (≥ 1 systemic treatments)
	NCT02425891 (IMpassion130) (November 2018)	III	902	Atezolizumab + Nab-paclitaxel (comparator: placebo + Nab-paclitaxel)	Active, no recruiting	7.5 *vs* 5.5 (HR: 0.62, $P < 0.001$)	25.0 *vs* 15.5 (HR: 0.62)	In the analysis of the ITT population, the median PFS was 7.2 mo *vs* 5.5 mo (HR: 0.80, $P = 0.002$). In PD-L1 (+) patients, the median PFS was 7.5 mo *vs* 5.5 mo (HR: 0.62, $P < 0.001$)

				Population: First-line mTNBC				In the analysis of the ITT population, the median OS was 21.3 mo *vs* 17.6 mo (HR: 0.84, P = 0.08). In PD-L1 (+) patients, the median OS was 25.0 mo *vs* 15.5 mo (HR: 0.62) Final analysis showed that OS benefit with atezolizumab + nab-paclitaxel in the ITT population was not statistically significant, but a clinically meaningful OS benefit was observed in PD-L1 IC-(+) patients
	NCT03125902 (IMpassion131) (September 2020)	III	600	Paclitaxel +/- Atezolizumab (comparator: placebo + paclitaxel) Population: First-line mTNBC	Active, no recruiting	5.7 *vs* 6.0 (HR: 0.82, P = .20) in PD-L1 (+) population	22.1 *vs* 28.3 (HR: 1.12) in PD-L1 (+) population	Primary endpoint was PFS In the ITT population, the median PFS was 5.7 mo in atezolizumab group *vs* 5.6 mo in placebo group (HR: 0.86) OS: 19.2 mo *vs* 22.8 mo (HR: 1.11, 0.87-1.42) The 2-yr OS rates were 51% and 49% in placebo and atezolizumab groups, respectively
	NCT03371017 (IMpassion132) (early recurrence)	III	350	Carboplatin + Gemcitabine or Capecitabine +/- Atezolizumab	Recruiting			Primary endpoint was OS Estimated completion date: July 2023

CPS: Combined positive score; dd: Dense dose; DDFS: Distant-disease free survival; DFS: Disease-free survival; EFS: Event-free survival; HR: Hazard ratio; IC: immune cells; iDFS: Invasive disease-free survival; ITT: Intention to treat; mTNBC: Metastatic triple-negative breast cancer; pCR: Pathological complete response; PFS: Progression-free survival; ORR: Objective response rate; OS: Overall survival; QoL: Quality of life; TNBC: Triple-negative breast cancer.

In the early stage scenario there are considerations for the addition of immunotherapy to chemotherapy in the neoadjuvant setting: the benefit of improving the pCR rates (KEYNOTE-522, IMpassio031), and the risks regarding toxicities (immune related adverse events in a potentially curable setting) and costs.

Atezolizumab

Atezolizumab is a humanized anti-PD-L1 monoclonal antibody, non-glycosylated IgG1 that binds to PD-L1 and blocks interaction with PD-1 and B7.1 (a co-stimulatory protein on the cell surface) that induces a reactivation of the antitumor immune response without antibody-induced cellular cytotoxicity [9].

Atezolizumab monotherapy in mTNBC: A phase I study (Schmid *et al*[10], 2017) that evaluated the safety and tolerability of atezolizumab single-drug (primary endpoints), demonstrated an antitumor activity and safety with the use of atezolizumab in patients with mTNBC (n = 116). It was also observed that the greatest benefit was in patients who received atezolizumab in the first line and among those with high levels of TILs and PD-L1 immune cells (IC)[10].

Other measured endpoints were overall survival (OS) (41% at 1 year, 19% at 2 years, and 16% at 3 years) and the PD-L1 IC ≥ 1% was associated with a higher objective response rate (ORR) (12% *vs* 0%) and higher OS (10.1 mo *vs* 6 mo, respectively). Atezolizumab was well tolerated and provides clinical

benefit in patients with mTNBC. 100% of the patients who responded to atezolizumab were alive at 1 year *vs* 38% of non-responders[10].

Atezolizumab + nab-paclitaxel in mTNBC (IMpassion130): The IMpassion130 (November 2018), phase III, randomized trial evaluated patients with mTNBC or unresectable locally advanced disease without previous treatment (n = 902) and regardless of PD-L1 expression, who were randomized (in a 1:1 ratio) to receive nab-paclitaxel (100 mg/m^2 on days 1, 8 and 15 every 28 d) in association with atezolizumab (840 mg IV on days 1 and 15 every 28 d) or with placebo until disease progression or limiting toxicity [11]. The two primary end points were progression-free survival (PFS) [in the intention-to-treat (ITT) population and PD-L1 positive subgroups] and OS (tested in the ITT population; if the finding was significant, it would be tested in the PD-L1 (+) subgroup). Stratification factors were: receipt or nonreceipt neoadjuvant or adjuvant taxane therapy, presence or absence of liver metastases at baseline, and PD-L1 expression at baseline (positive *vs* negative) according to immunohistochemical testing (Ventana SP142). The trial was initially designed to assign 350 patients for the evaluation of primary end point (PFS), but during the course of trial, enrollment was expanded to about 900 patients to accommodate the addition of OS as a second primary end point. 41% of the patients were PD-L1 (+) [11]. The possible rationale for using taxane-based chemotherapy is that it can enhance tumor antigen release and antitumor response to checkpoint inhibitors. Furthermore, nab-paclitaxel can promote dendritic cell activity and was used to avoid the interaction between atezolizumab and corticosteroids (under the rationale that the use of corticosteroids could decrease the immune response of anti-PD-L1 therapy). In addition, nab-paclitaxel has a decreased risk of hypersensitivity reactions and does not require corticosteroid treatment[12].

After a median follow-up of 12.9 mo in the ITT population, the addition of atezolizumab to nab-paclitaxel increased the median PFS (7.2 mo with atezolizumab + nab-paclitaxel *vs* 5.5 mo with placebo + nab-paclitaxel, hazard ratio [HR]: 0.80, 95% confidence interval [CI]: 0.69-0.92, P = 0.002), although this did not increase OS (21.3 mo with atezolizumab + nab-paclitaxel *vs* 17.6 mo with placebo + nab-paclitaxel, HR: 0.84, 95%CI: 0.69-1.02, P = 0.08). However, in the subgroup of PD-L1 (+) patients (defined as PD-L1 expression in tumor infiltrating immune cells ≥ 1% of the tumor area), the median PFS (7.5 mo *vs* 5.0 mo, HR: 0.62, 95%CI: 0.49-0.78, P < 0.001) and OS (25 mo *vs* 15.5 mo, HR: 0.62, 95%CI: 0.45-0.86) was improved with the combination of atezolizumab + nab-paclitaxel compared to placebo + nab-paclitaxel[11].

Regarding adverse events, the frequency of grade ≥ 3 adverse events (AEs) was 48.7% in the atezolizumab + nab-paclitaxel group and 42.2% in the placebo + nab-paclitaxel group, with neutropenia (8%), peripheral neuropathy (6%), fatigue (4%) and anemia (3%) being the most common events in both groups. Grade ≥ 3 immune-related events (irAEs) occurred in 7.5% and 4.5% of the atezolizumab + nab-paclitaxel and placebo + nab-paclitaxel groups, respectively. Authors conclude that atezolizumab + nab-paclitaxel prolonged PFS among patients with mTNBC in both ITT population and PD-L1 (+) subgroup [11].

An OS data update from a second interim analysis of a median follow-up of 18 mo showed an OS of 21.0 mo in the atezolizumab + nab-paclitaxel group *vs* 18.7 mo in the placebo + nab-paclitaxel group (P = 0.0777) on ITT. In the PD-L1 (+) subgroup, OS was 25.0 mo *vs* 18.0 mo (HR: 0.71). This update confirms the benefit in OS of the population with PD-L1 (+)[13]. Very recently, a final OS analysis from the IMpassion130 trial was published: final OS data from IMpassion130 agree with prior interim analysis. The OS benefit in the ITT population was not statistically significant (21.0 mo *vs* 18.7 mo, HR: 0.87, 95%CI: 0.75-1.02, P = 0.077). Data showed clinically meaningful OS benefit with the combination of atezolizumab + nab-paclitaxel in the PD-L1 IC-positive population (25.4 mo *vs* 17.9 mo, HR: 0.67, 95%CI: 0.53-0.86), 3-year OS rates in the PD-L1 group were 35.8% using atezolizumab + nab-paclitaxel *vs* 22.2% in the placebo group and no new safety events were reported with longer follow-up. The authors conclude that although OS benefit in the ITT population was not statistically significant, a clinical meaningful OS benefit was reported in PD-L1 IC-positive patients with atezolizumab + nab-paclitaxel. The statistical results of this trial (ITT population) were negative[14].

In conclusion, the combination of atezolizumab plus nab-paclitaxel prolongs PFS and OS in the mTNBC subgroup with PD-L1 (+) but not in the intention-to-treat (ITT) population, changing the treatment paradigm with patients in the metastatic setting. This combination has been initially included in international clinical practice guidelines (currently NCCN guidelines removed this option)[15] (IB, ESMO guidelines)[16] and the FDA (Food and Drug Administration) accelerated approval in March 2019 for its use in the treatment of patients with mTNBC or unresectable locally advanced disease with PD-L1 positive using a validated test[7]. This was the first approval of atezolizumab and of an immunotherapy regimen for the treatment of breast cancer[17]. It is important to note that the FDA has granted accelerated approvals to oncology medicines on the basis of evidence that suggests a benefit to patients, however many immunotherapies (atezolizumab, pembrolizumab, nivolumab, durvalumab) approval are under evaluation since the approval is based on a surrogate endpoint and it requires a confirmatory trial with a clear benefit. In addition, four indications were voluntarily withdrawn by manufacturers (nivolumab in metastatic small cell lung cancer, durvalumab in locally advanced or metastatic urothelial carcinoma, pembrolizumab for metastatic small cell lung cancer and atezolizumab for metastatic urothelial carcinoma)[18]. Although in April 2021 the FDA Oncologic Drugs Advisory Committee

(ODAC) voted 7 to 2 in favour of maintaining accelerated approval of atezolizumab in combination with nab-paclitaxel for the treatment of adults with unresectable locally advanced or mTNBC whose tumours express PD-L1. In August 2021, the manufacturer announced that it was voluntarily withdrawing atezolizumab indication for BC in United States. Due to recent changes in the treatment landscape (including IMpassion131 results) the FDA will no longer consider it appropriate to maintain the accelerated approval for atezolizumab in BC. The indication received accelerated approval based in benefit in PFS and OS of IMpassion130, but there was no difference in survival advantage in PD-L1 (+) nor ITT population of IMpassion131[19,20].

Ventana SP142: In the IMpassion130 study, not only was the approval of atezolizumab in combination with chemotherapy achieved, but the FDA also approved the antibody diagnostic measurement test "Ventana PD-L1 SP142 assay", to select TNBC patients to receive treatment with atezolizumab, and perhaps it could be considered a predictive biomarker[21]. Tumor samples were evaluated by immunohistochemistry to evaluate the expression of PD-L1 (Ventana SP142) in tumor infiltrating immune cells (PD-L1 IC), using a 2-level system: "a percentage of tumor area" < 1% (= PD-L1 negative) or > 1% (= PD-L1 positive). The study revealed that patients whose tumors were positive for PD-L1 (approximately 41%) and received atezolizumab + nab-paclitaxel had a better median PFS compared to placebo + nab-paclitaxel (7.2 mo *vs* 5.5 mo)[11]. In the PD-L1 (+) subgroup, the ORR was 59% with atezolizumab + nab-paclitaxel compared to 43% in the placebo + nab-paclitaxel group. Furthermore, 10% of the patients in the atezolizumab group achieved complete response (CR) compared to only 1% in the placebo group [17] (Table 2).

Atezolizumab + paclitaxel in TNBC (IMpassion131): IMpassion131, a phase III randomized trial, evaluated the combination of atezolizumab + paclitaxel compared with placebo + paclitaxel in patients with unresectable locally advanced disease or mTNBC who had not received prior therapy or ≥ 12 mo since neoadjuvant chemotherapy) (n = 651). Forty-five percent of patients were PD-L1 (+), 48% were treated with taxanes, 31% had mTNBC, and 27% had liver metastases. The primary endpoint of IMpassion131 was PFS, and there was no significant difference in PFS between the atezolizumab group *vs* placebo in PD-L1 (+) patients: 5.7 mo *vs* 6.0 mo, respectively (HR: 0.82, P = 0.20) or in the ITT population: median PFS was 5.6 *vs* 5.7 in the atezolizumab and placebo groups, respectively (HR: 0.86). Even in the OS analysis, no benefit was demonstrated with atezolizumab in the ITT population or in the PD-L1 (+) population. Regarding AEs, grades 3-4 were similar in both groups (43% *vs* 49%)[22].

In IMpassion130 trial, atezolizumab + nab-paclitaxel did not improve OS in ITT but resulted in a "clinically significant" improvement in OS in PD-L1 (+) patients. The results of the IMpassion130 trial demonstrated the benefit of atezolizumab in combination with nab-paclitaxel. However, the results were divergent in the IMpassion131. Potential reasons for the divergent results between the two studies are under investigation. Tumor heterogeneity could be a reason. Other reasons could be the use of concomitant corticosteroids (necessary for paclitaxel infusion) may have a negative effect on the immunotherapy activity (checkpoint inhibitors); likewise, the differences in the study populations may have a role, as well as the cremophor associated with paclitaxel.

In July 2021, primary results from IMpassion131 have been published. Neither PFS or OS were improved with the combination of atezolizumab + paclitaxel in PD-L1 (+) nor ITT population. The baseline characteristics of the populations in both trials were similar, including median PFS in control groups (5.6 mo with paclitaxel alone *vs* 5.5 mo with nab-paclitaxel alone). Ongoing research may be valuable to explain possible reasons for the IMpassion131 results; authors said the lack of information on BRCA status could be a limitation, as imbalances between treatment arms for this prognostic biomarker may not be detected. In addition, findings from IMpassion131 differ with KEYNOTE-355 results, which evaluated pembrolizumab and more chemotherapy backbones (nab-paclitaxel, paclitaxel, gemcitabine/carboplatin). Despite the main goal of KEYNOTE-355 was similar to that of IMpassion131 and there were important differences regarding eligibility, statistical design, PD-L1 testing and chemotherapy regimens[22].

Atezolizumab + adjuvant chemotherapy (Impassion 030): A pending question is to determine the effectiveness of anti-PD-1/PD-L1 in the adjuvant setting. Several studies are underway including IMpassion030, a phase II study evaluating atezolizumab + adjuvant chemotherapy *vs* placebo + chemotherapy[23].

Pembrolizumab

Pembrolizumab monotherapy in mTNBC (KEYNOTE-119): Pembrolizumab showed antitumor activity and a manageable toxicity profile in TNBC in the umbrella study KEYNOTE-012 (June 2017), a phase Ib study that evaluated the use of immunotherapy in advanced solid tumors. In the subgroup of patients with TNBC, an ORR of 18.5%, a stable disease rate (SD): 25.9%, partial response (PR): 14.8% and complete response (CR): 3.7% rates were obtained[24].

Then, the KEYNOTE-086 (March 2019) phase II study, which evaluated the use of pembrolizumab for up to 2 years as a second or subsequent line of treatment in patients with mTNBC (that previously received anthracyclines and taxanes). The primary endpoint was ORR in the subgroup of patients with PD-L1 (+). As results, an ORR of 4.7%, SD of 20.6%, PR of 4.1% and CR of 0.6% were obtained. In the

Table 2 Common commercially monoclonal programmed death ligand 1 antibodies for immunohistochemical analysis to assess the expression of programmed death ligand 1 (considering Food and Drug Administration approvals)

PD-L1 antibody	Immunotherapy	IHC assay	Cut-off	Line
22C3	Pembrolizumab	DAKO	TPS ≥ 1%	1L
			TC ≥ 1%	2L
28-8	Nivolumab	DAKO	TC ≥ 1%	2L
SP142	Atezolizumab	Ventana	TC ≥ 50% and/or IC ≥ 10%	1L
			TC ≥ 1% and/or IC ≥ 1%	2L
SP263	Durvalumab	Ventana	TC ≥ 1%	1L maintenance, in unresectable stage III after chemoradiation therapy
	Nivolumab		TC ≥ 1%	2L
	Pembrolizumab		TC ≥ 50%	1L
73-10	Avelumab	DAKO	TC ≥ 1%	2L (not approved)

Notes: (1) Atezolizumab in combination with nab-paclitaxel is approved as 1L of treatment for patients with unresectable locally advanced or metastatic triple-negative breast cancer (mTNBC) whose tumors express programmed death ligand 1 (PD-L1) immune cells (IC) (PD-L1 stained tumor-infiltrating IC of any intensity covering ≥ 1% of the tumor area), as determined by a Food and Drug Administration (FDA) approved test (Ventana SP142); and (2) Pembrolizumab with chemotherapy is approved as 1L of treatment for patients with locally recurrent unresectable or mTNBC whose tumors express PD-L1 CPS ≥ 10, as determined by an FDA approved test (PD-L1 IHC 22C3 PharmDx). CPS: Combined positive score; IC: Immune cell; IHC: Immunohistochemistry; TC: Tumor cell; TPS: Tumor proportion score; 1L: First-line; 2L: Second-line; PD-L1: Programmed death ligand 1.

latter, the response was independent of PD-L1 expression [4.8% in patients with PD-L1 (+) *vs* 4.7% PD-L1 (-)][25].

Subsequently, the KEYNOTE-119 (September 2019), phase III, open-label, randomized study was presented which used pembrolizumab monotherapy (n = 312) *vs* single agent chemotherapy (n = 310) in previously treated mTNBC patients (1-2 prior systemic treatments). The patients were stratified in PD-L1 (+) and (-). The primary endpoint was OS in patients with a combined positive score (CPS) ≥ 10, patients with CPS ≥ 1, and all patients. Secondary endpoints were PFS, ORR and safety. As results, pembrolizumab did not improve OS in patients with CPS ≥ 10 or CPS ≥ 1. In an exploratory analysis of patients with CPS ≥ 20, the median OS was 14.9 mo *vs* 12.5 with chemotherapy (HR: 0.58, 95%CI: 0.38-0.88), no improvement in PFS was observed. Grade 3-5 AEs were 14% *vs* 36% with chemotherapy. In conclusion, this monotherapy treatment did not improve significantly as a second or third line of treatment for mTNBC *vs* chemotherapy, but it was well tolerated and had a lower toxicity than chemotherapy[26].

Pembrolizumab + chemotherapy in mTNBC (KEYNOTE-355): Since pembrolizumab monotherapy showed antitumor activity in mTNBC patients, the KEYNOTE-355 (December 2020), phase III, randomized study evaluated the addition of pembrolizumab to chemotherapy in previously untreated patients with inoperable disease or mTNBC (n = 847), in two groups: pembrolizumab (200 mg IV every 21 d) plus nab-paclitaxel (100 mg/m^2 on days 1, 8 and 15 of a 28-d cycle), paclitaxel (90 mg/m^2 on days 1, 8 and 15 of a 28-d cycle), or gemcitabine (1000 mg/m^2) with carboplatin (AUC 2 on days 1 and 8 of a 21-d cycle) *vs* placebo plus chemotherapy. The co-primary endpoints were PFS and OS, evaluated in the PD-L1 subgroup with CPS ≥ 10, CPS ≥ 1, and in the ITT population[27]. As results, among patients with CPS ≥ 10, the median PFS was 9.7 mo in the pembrolizumab group *vs* 5.6 mo in the placebo group (statistically significant) (HR: 0.65, 0.49-0.86, P = 0.0012). Among patients with CPS ≥ 1, median PFS was 7.6 mo *vs* 5.6 mo (HR: 0.74, 0.61-0.90, P = 0.0014) (not significant) and in the ITT population, median PFS was 7.5 mo *vs* 5.6 mo (HR: 0.82, 0.67-0.97). The effect of pembrolizumab was increased in the enriched PD-L1 population (CPS ≥ 10). In the subgroup analysis, in the ITT population there was more benefit when pembrolizumab is used with paclitaxel, followed by nab-paclitaxel and gemcitabine/carboplatin, showing an asymmetry of chemotherapy regimens used with anti-PD-1 therapy. Similar results were observed in the population with CPS ≥ 1. Regarding AEs, grades 3-5 were 68% in the pembrolizumab group *vs* 67% in the placebo group, including death in < 1% in the pembrolizumab group *vs* 0% in the placebo group. In conclusion, pembrolizumab associated with chemotherapy showed a significant clinical improvement in PFS *vs* placebo in mTNBC patients with CPS of 10 or more[27].

The authors suggest a role in adding pembrolizumab to standard first-line chemotherapy in mTNBC. In fact, NCCN guidelines recommend pembrolizumab (associated to chemotherapy) as first-line treatment options in mTNBC (category 1, preferred as first-line therapy)[15].

It should be noted that, to date, ESMO guidelines do not recommend the use of immunotherapy in subsequent lines for mTNBC due to its low response rates (IB, ESMO)[16].

In the San Antonio Breast Cancer Symposium (SABCS) 2020, new findings from the KEYNOTE-355 trial were presented. Pembrolizumab plus chemotherapy improved PFS, ORR, durable CR and duration of response for patients with locally recurrent, unresectable or mTNBC with tumors expressing PD-L1 and a CPS ≥ 10. This additional endpoint results showed the PFS benefit for the addition of pembrolizumab to chemotherapy, regardless of which chemotherapy partner was chosen, particularly in PD-L1 enriched (CPS ≥ 10) patients[28].

In the ITT population, the median PFS in the pembrolizumab and placebo groups was 7.5 mo *vs* 5.4 mo when given with nab-paclitaxel, 8.0 mo *vs* 3.8 mo with paclitaxel, and 7.4 mo *vs* 7.4 mo with gemcitabine plus carboplatin. The hazard ratios (HRs) favored pembrolizumab over placebo, at a significant HR: 0.69 and HR: 0.57 for nab-paclitaxel and paclitaxel, respectively, and a nonsignificant HR: 0.93 for gemcitabine plus carboplatin. When stratified by PD-L1 expression, patients with a CPS ≥ 10 or CPS ≥ 1 had longer PFS with pembrolizumab. The trial was not powered to compare efficacy among treatment groups by different chemotherapy regimens[28].

In patients with CPS ≥ 10, secondary endpoints favored pembrolizumab plus chemotherapy compared with chemotherapy alone (ORR: 53.2% *vs* 39.8%, disease control rate: 65% *vs* 54.4%). The authors conclude these findings support a role of addition of pembrolizumab to standard chemotherapy for the first-line treatment of mTNBC[28].

In ESMO Congress 2021 (September 2021) final results from the KEYNOTE-355 confirmed pembrolizumab + chemotherapy met dual primary endpoints (PFS and OS) in patients with mTNBC whose tumors expressed PD-L1 (CPS ≥ 10). For all endpoints, the pembrolizumab effect increased with PD-L1 enrichment. No new safety signals were identified[29].

Recently, in SABCS 2021 (December 7-10th, 2021), final results of pembrolizumab plus chemotherapy in mTNBC were presented and demonstrated that the addition of pembrolizumab yielded significant survival over placebo. The authors suggested that a CPS ≥ 10 is considered a "reasonable" cutoff to determine expected treatment benefit[30].

PD-L1 IHC 22C3 pharmDx: The determination of PD-L1 status in the KEYNOTE-355 trial was assessed the PD-L1 IHC 22C3 pharmDx assay and characterized by the CPS, defined as the number of PD-L1 positive cell (tumour cells, lymphocytes and macrophages) divided by total number of tumour cells x 100. PD-L1 (+) tumours are classified as CPS ≥ 10 and CPS ≥ 1, and PD-L1 (-) tumours are classified as CPS < 1. The PFS and OS analysis in the KEYNOTE-355 trial was stratified using CPS ≥ 10, CPS ≥ 1 and the ITT population[27].

Based on KEYNOTE-355 results, in November 2020, the FDA granted accelerated approval to pembrolizumab (200 mg IV every 3 wk or 400 mg every 6 wk prior to chemotherapy) in combination with chemotherapy for the treatment of patients with locally recurrent unresectable or mTNBC whose tumors express PD-L1 (CPS ≥ 10) as determined by an FDA approved test. The FDA also approved the use of PD-L1 IHC 22C3 pharmDx (Dako North America Inc.) as a companion diagnostic test for selecting patients with TNBC who may be appropriate for treatment with pembrolizumab[31].

Pembrolizumab + neoadjuvant chemotherapy (KEYNOTE-522): Pembrolizumab associated to neoadjuvant chemotherapy demonstrated antitumor activity and safety in patients with early TNBC in the I-SPY 2 and the KEYNOTE-173 studies. The I-SPY 2 (September 2017), phase II randomized study designed to test new treatments by identifying therapies based on molecular characteristics included patients with HER2 negative, stage II - III breast cancer who were randomized to receive weekly neoadjuvant paclitaxel with or without pembrolizumab (200 mg IV every 3 wk x 4 cycles) followed by AC (every 3 wk x 4 cycles). In the TNBC subgroup (n = 118), it was demonstrated that the combination in the neoadjuvant setting increases pCR up to 3 times more (62.4% *vs* 22.3%, respectively) compared to the control[32].

Subsequently, the results of the KEYNOTE-522 (August 2020), phase III study, which included patients with non-metastatic TNBC, without previous treatment (n = 1174), were randomized 2:1 to receive pembrolizumab (200 mg every 3 wk) or placebo, both given with 4 cycles of paclitaxel + carboplatin, followed by 4 cycles of doxorubicin or epirubicin + cyclophosphamide (neoadjuvant phase). After surgery, patients received either pembrolizumab or placebo for 9 cycles until recurrence or unacceptable toxicity (adjuvant phase). The co-primary endpoints were pCR and event-free survival (EFS). As results, a pCR was achieved in 64.8% of the pembro group *vs* 51.2% with placebo (P < 0.001). The benefit in pCR with pembrolizumab was consistent across all subgroups, including those with PD-L1 (+). After a median of 15.5 mo, 7.4% of the pembro group and 11.8% of the placebo group had disease progression, local or distant recurrence, or death from any cause (HR: 0.63). The safety of pembrolizumab was consistent with previous studies. In conclusion, pCR was higher in patients receiving pembro + neoadjuvant chemotherapy compared with placebo[33]. A post-hoc analysis showed a better pCR difference in pembrolizumab group *vs* placebo group in clinical stages (CS) IIIA (66.7% *vs* 42.1%, Δ 24.6) and IIIB (48.6% *vs* 23.1%, Δ 25.6), also a better pCR difference by lymph node involvement: positive (64.8% *vs* 44.1%, Δ 20.6) *vs* negative (64.9% *vs* 58.5%, Δ 6.3).

An update of the KEYNOTE-522 trial (presented in ESMO virtual plenary, 15-16 July 2021) showed that at the median follow-up of 39 mo, pembrolizumab had a statistically and clinically significant EFS benefit (HR: 0.63, 95%CI: 0.48-0.82, P = 0.0003) compared with chemotherapy alone. At a 3-year follow-up, EFS was 84.5% in the pembrolizumab group compared with 76.8% in the placebo group. The most common event was distance recurrence (7.7% with pembrolizumab group *vs* 13.1% with placebo group). Moreover, pembrolizumab showed a favorable trend in overall survival (OS) (HR: 0.72, 95%CI: 0.51-1.02). Regarding the adverse events (AEs), the immune-mediated AEs (IMAEs) of any grade were found in 43.6% of pembrolizumab group *vs* 21.9% in the placebo group. The most common AEs reported with pembrolizumab were infusion reactions and hypothyroidism[34]. Based on results of the KEYNOTE-522, on July 2021, the FDA approved pembrolizumab for high-risk early-stage TNBC in combination with chemotherapy as neoadjuvant treatment. This is the first immunotherapy approved for early-stage TNBC[35].

DIFFERENCES BETWEEN IMPASSION130 AND KEYNOTE-355 TRIALS IN mTNBC

To clarify, the IMpassion130 and KEYNOTE-355 trials have similar designs and results are consistent. The overall survival results are expected to be similar and the benefit was in PD-L1 (+) patients, suggesting that the direction is identifying the presence of a biomarker (PD-L1 status).

One difference is the way the authors define PD-L1 (+) by immunohistochemistry at a central laboratory and the companion diagnostic methods: in IMpassion130 trial they look at PD-L1 expression of ICs (IC score: greater than 1% of the area of tumor, using SP142 assay), meanwhile, in the KEYNOTE-355, the authors used a different antibody (22C3) to look at PD-L1 expression (CPS: a combination of PD-L1 staining on ICs and the tumor cells, looking for immune cell staining and tumor cell staining greater than 10% of the area). SP142 was seen in 41% of PD-L1 (+) patients but the 22C3 is much higher (is close to 80%)[13,29]. Diagnostic companions can link or homogenize cut-off points of validated tests in order to obtain similar results.

Another difference is that the KEYNOTE-355 includes several standard chemotherapy regimens as taxanes (paclitaxel, nab-paclitaxel) or gemcitabine-carboplatin (IMpassion130 only used nab-paclitaxel as chemotherapy regimen) and patients with early recurrences, thereby offering more treatment options to a population with a high unmet medical need. It is important to note that the KEYNOTE-355 trial was not designed to compare chemotherapy regimens but the last update shows a trend of benefit using taxanes instead of gemcitabine-carboplatin in addition to pembrolizumab[29].

EMERGING BIOMARKERS OF RESPONSE TO IMMUNOTHERAPY IN TNBC

The results obtained in the PD-L1 (+) subgroup of the IMpassion130 trial confirm the benefit of immunotherapy in mTNBC. However, PD-L1 is not the ideal biomarker to select patients for anti-PD-1/anti-PD-L1 therapies as it has been shown in other cancers. Therefore, there is an urgent need for identification and implementation of emerging biomarkers that can predict response to immunotherapy.

TILs

High levels of TILs have been shown to have a prognostic value in patients with HER2 (+) breast cancer and TNBC, as a predicting factor of pathological complete response (pCR) to chemotherapy and its high expression seems to be linked to a better prognosis after adjuvant therapy as well as a reduction in the risk of recurrence[36].

TILs are frequently present in TNBC (around 20%) and they are associated with a good prognosis[37,38]. The characterization of the immune lymphocytic infiltrates, with the presence of a high number of T lymphocytes (CD8+ TILs), defines a better prognosis for neoadjuvant (higher pCR) and adjuvant chemotherapy (higher DFS and OS). The evidence indicates that in the neoadjuvant setting of TNBC, intratumoral TILs, as well as stromal ones, are predictive of pathological response to platinum-based chemotherapy[39]. However, currently, TILs score should not be used to make treatment decisions nor to escalate or de-escalate. TILs score can be used as a prognostic marker, providing a relative improvement of 15% to 20% in survival due to a 10% increase in TILs, and its use as a prognostic factor is supported by the 2019 St. Gallen Consensus[40,41].

Various studies on neoadjuvant and adjuvant therapies have measured TILs both at the intratumoral and stromal levels[42]. Some studies used immunohistochemistry while others evaluated molecular markers using immunohistochemistry and gene expression. At present, there is no specific cut-off point for TILs (+) established[43,44].

Stromal TIL score

A biomarker of interest is the stromal TIL score which is known to be prognostic and predictive in the

neoadjuvant setting. In the IMpassion130 analysis, the stromal TIL score or CD8+ cell count (T cells) did not predict the benefit of the use of atezolizumab. It also appears that a dearth of stroma in metastatic breast cancer samples could contribute to an inability to detect an association between stromal TILs and the benefit of atezolizumab[45]. Another study that compared the number of TILs in primary and metastatic tumors showed that TILs decrease in metastasis compared to primary breast tumors[46].

PD-L1

PD-L1, which can be expressed in tumor cells and/or in tumor infiltrating immune cells, contributes to the inhibition of the antitumor immune response in the tumor microenvironment[47].

TNBC can present a higher expression of PD-L1 (in a range of 21-56%) compared to the other subtypes, predominantly in inflammatory immune cells and occasionally in neoplastic cells[48].

PD-L1 expression is considered a useful biomarker of response to treatment pf anti-PD-1 or anti-PD-L1 therapies[49]. PD-L1 expression in immune cells (IC) has been estimated in a range from 40%-65% in TNBC patients[50,51]. In the IMpassion130 trial, the expression of PD-L1 IC ≥ 1% was used to define PD-L1 (+)[11].

It has recently been shown that the expression of PD-L1 IC along with TILs influence the prognosis of TNBC and can predict the response to immunotherapy with pembrolizumab and atezolizumab in breast cancer[52]. In the KEYNOTE-086 study, TNBC patients with PD-L1 (+) IC and high TILs had a better response to immunotherapy[53]. Furthermore, an exploratory analysis of the KEYNOTE-173 study investigating the combination of pembrolizumab and neoadjuvant chemotherapy in TNBC, shows that high levels of stromal TILs prior to treatment and the expression of PD-L1, reported in a combined score, were significantly associated with a higher pCR and overall response rates in TNBC patients who received chemotherapy and immunotherapy combined[54,55].

PD-L1 detection in tumor cells and immune cells (IC) varied by antibody clone and is easily evaluated using IHC. The most common commercially available monoclonal PD-L1 antibodies for immunohistochemical analysis to assess the expression of PD-L1 are the following: 22C3, 28-8, SP142, SP263 and 73-10. While many PD-L1 assays are available, only Ventana SP142 and PD-L1 IHC 22C3 pharmDx are licensed companion diagnostic tests for selecting patients with mTNBC who are candidates for treatment with atezolizumab and pembrolizumab, respectively[56].

Other emerging biomarkers in TNBC: PD-L1 has been mentioned as a biomarker to select patients to receive anti-PD1/PD-L1 therapies, being an imperfect marker as has been demonstrated in trials (in IMpassion031 and KEYNOTE-522 the benefit not confined to PD-L1 group). PD-L1 has some limitations: the difficult and subjective scoring (tissue types, cell types, antibodies), the expense for 22C3 validation for independent laboratories, the dependence on immune content of biopsy (number of immune cells), also it is not considered a great marker in most disease types. There is a great need for better predictive biomarkers for response to immunotherapy and many of them are under investigation, including: TILs, genetic signatures, TMB, microsatellite instability [microsatellite instability-high (MSI-H)/mismatch repair (MMR) deficiency], major histocompatibility complex (MHC) class I and II, *etc*.

TMB: The mutational burden of the tumor has been correlated with response to immunotherapy in various types of neoplasms; however, a high mutational burden is rare in breast cancer. In the study only 3.1% of breast cancers had high TMB (TMB-H) (≥ 10 mutations/Mb) when compared to 39.7% of melanomas and 24.3% of lung cancer[25]. TMB could be a potential biomarker in TNBC with TMB-H, but this could exclude patients that can benefit from immunotherapy[57,58].

TMB has an indication but clinically is not a great marker and is probably mostly driven by MSI.

MSI-H or deficient MMR: MSI-H or deficient MMR (dMMR) could be a predictive marker of response or benefit with anti-PD-1 therapy, taking into consideration that pembrolizumab is FDA approved for adult and pediatric patients with unresectable or metastatic, MSI-H or dMMR solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options. However, MSI is infrequent in TNBC with an approximate frequency of 0.7%-2%[59].

Other potential biomarkers in TNBC: In view of the above, the research of new predictive biomarkers or risk factors (*e.g*., LDH levels, visceral liver disease) are underway to identify a group of patients that could benefit from atezolizumab as monotherapy or in combination, and thus optimize the treatment of mTNBC[60]. In the KEYNOTE-086 study, it was observed that patients with elevated levels of lactate dehydrogenase and visceral liver disease had little or no response to immunotherapy. Another study reports that patients with liver metastases derive limited benefit from immunotherapy independent of other established biomarkers of response: liver metastases create a systemic immune desert in preclinical models (apoptosis of CD8 T cells) and reduction of peripheral T cell numbers and diminished tumoral T cell diversity and function[61].

MHC-I and II are new potential biomarkers under analysis: most tumor cells (including BC) express MHC-I, whereas MHC-II is expressed by only a fraction of tumor/tumor cells (MHC-II is considered a professional antigen-presenting cell). A previous trial showed tumors which express high levels of MHC-I or II have high counts of CD4 and CD8 T lymphocytes ($P < 0.001$). Positive expression of MHC-II in tumor cells is associated with better disease-free survival (DFS) in patients who have lymph node

metastases (P = 0.009). Also, the expression of MHC-II in tumor cells was associated with an increased level of TILs[62]. A recent study reported MHC-II predicts early-stage HER2-negative breast cancer response to immunotherapy + neoadjuvant chemotherapy[63].

In general, the evolution of treatment with immunotherapy can be divided into "three waves":

The "first wave" includes the use of immunotherapy as monotherapy, which has shown antitumor activity and modest results in advanced disease.

The "second wave" used immunotherapy in combination with chemotherapy. Cytotoxic therapy can induce increased antigen release from tumor cells, change in tumor microenvironment, upregulation of PD-L1 and increased expression of cell surface markers (*e.g.*, MHC I). All of these effects can increase immunotherapy effectiveness. Despite evaluations of which would be the ideal (safest or most effective) chemotherapy for combination therapy with immunotherapy, several questions remain. Nab-paclitaxel was used in the IMpassion130 because it facilitates the reduction of corticosteroid use. However, other chemotherapy agents have also been evaluated to improve the immunogenicity of breast cancer, including anthracyclines, taxanes, platinum salts, among others[64].

The TONIC, phase II trial compared the effects of induction chemotherapy associated with immunotherapy (nivolumab). Objective response rate (ORR) was 20%, and the highest ORR rates were observed in the cisplatin (ORR: 23%) and doxorubicin (ORR: 35%) cohorts. Initial and post-induction biopsies analysis showed an upregulation of immune-related genes in PD-1/PD-L1 and T-cell cytotoxicity in the cisplatin and doxorubicin cohorts[65].

The lymphocyte depleting effect of combination therapy should also be considered. A comparison of chemotherapy (capecitabine or paclitaxel) associated with pembrolizumab showed a profound and significant depletion of T cells (including CD4+ and CD8+). This could explain the decrease in efficacy of anti-PD-1/PD-L1 in later lines of chemotherapy in TNBC[66].

The "third wave" includes immunotherapy in combination with targeted therapies (as PARP inhibitors). Currently, a phase II/III trial (KEYLYNK-009) of olaparib + pembrolizumab compared with chemotherapy (carboplatin/gemcitabine) + pembrolizumab after initial treatment with chemotherapy + pembrolizumab in TNBC (n = 932) is ongoing. The aim is evaluating if combination of olaparib and pembrolizumab is effective and safe. Co-primary endpoints are PFS and OS and results are ongoing[67].

In this setting, the use of antibody-drug conjugates (ADCs) can be included. Sacituzumab govitecan (a new ADC) is approved by the FDA for treatment of adult patients with mTNBC who received at least two prior therapies for metastatic disease based in results of ASCENT trial[68].

CONCLUSION

The treatment of TNBC has evolved in the last decade with the application of immunotherapy, which has become the new standard of treatment and is changing the management paradigm, mainly in advanced disease, where there were only limited treatment options such as systemic chemotherapy. Knowledge of the molecular profile of TNBC and immunogenicity has made it possible to identify characteristics that differentiate them from other subtypes. Likewise, immunotherapy was evaluated and approved for more TNBC scenarios (metastatic, neoadjuvant).

TNBC is considered a more immunogenic subtype compared to the other subtypes of breast cancer due to the higher expression of TILs and PD-L1. According to the analysis of IMpassion130, PD-L1 has been shown to be a discussible predictive biomarker of response in selected patients [subgroup with PD-L1 (+)]. Other potential biomarkers are under investigation (LDH levels, presence of visceral disease, TMB, MSI-H) to identify and select patients who may benefit from immunotherapy alone or in combination in the different scenarios of TNBC.

New advances have been made with immunotherapy in mTNBC. First, progression-free survival (PFS) and overall survival (OS) benefit have been demonstrated in selected populations (PD-L1 positive subgroups) with immunotherapy + chemotherapy (nab-paclitaxel) in metastatic stage (mTNBC), locally advanced or unresectable disease (IMpassion130 trial). Furthermore, the approval of anti-PD-1 also led to the approval of a companion diagnostic test (Ventana SP142) for selecting patients who are candidates for atezolizumab. However, the benefit of atezolizumab (PFS and OS) could not be demonstrated in combination with paclitaxel (study IMpassion 131). The reasons for the divergent results between IMpassion130 and IMpassion131 trials are currently under investigation. Second, the KEYNOTE-355 trial results are consistent with Impassion130 trial and pembrolizumab is considered as a first-line option of treatment in mTNBC. Moreover, there is another companion diagnostic test approved (PD-L1 IHC 22C3 PharmDx) as an aid to identify patients with TNBC who are candidates for pembrolizumab.

In the neoadjuvant setting of TNBC, pembrolizumab has achieved the 2 co-primary endpoints evaluated (KEYNOTE-522): a higher pCR when combined with chemotherapy and a statistically significant event-free survival (EFS) benefit compared with chemotherapy alone. In the metastatic setting, benefit has been shown with the use of pembrolizumab + chemotherapy (KEYNOTE-355 study) as the first-line of treatment in those patients with enriched expression of PD-L1 (CPS ≥ 10).

Finally, in adjuvant disease, ongoing studies (such as IMpassion030) are evaluating the benefit of immunotherapy. It should be noted that, for TNBC in early disease, the standard of treatment continues to be neoadjuvant chemotherapy, as this is considered a systemic disease.

The evolution of immunotherapy in TNBC began with immunotherapy as monotherapy ("first wave"), followed by combination of immunotherapy + chemotherapy ("second wave") that is considered the new standard of care as first line in selected mTNBC PD-L1 (+). Currently, there are ongoing trials evaluating the combination of immunotherapy (immune checkpoint inhibitors) plus targeted therapies (as PARP inhibitors) for several cancers including TNBC and the development of antibody-drug conjugates (as sacituzumab govitecan) which had demonstrated benefit in refractory mTNBC ("third wave").

FOOTNOTES

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GUIDELINES

GOECP/SEOR radiotheraphy guidelines for non-small-cell lung cancer

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Abstract

Non-small cell lung cancer (NSCLC) is a heterogeneous disease accounting for approximately 85% of all lung cancers. Only 17% of patients are diagnosed at an early stage. Treatment is multidisciplinary and radiotherapy plays a key role in all stages of the disease. More than 50% of patients with NSCLC are treated with radiotherapy (curative-intent or palliative). Technological advances-including highly conformal radiotherapy techniques, new immobilization and respiratory control systems, and precision image verification systems-allow clinicians to individualize treatment to maximize tumor control while minimizing treatment-related toxicity. Novel therapeutic regimens such as moderate hypofractionation and advanced techniques such as stereotactic body radiotherapy (SBRT) have reduced the number of radiotherapy sessions. The integration of SBRT into routine clinical practice has radically altered treatment of early-stage disease. SBRT also plays an increasingly important role in oligometastatic disease. The aim of the present guidelines is to review the role of radiotherapy in the treatment of localized, locally-advanced, and metastatic NSCLC. We review the main radiotherapy techniques and clarify the role of radiotherapy in routine clinical practice. These guidelines are based on the best available evidence. The level and grade of evidence supporting each recommendation is provided.

Key Words: Radiotherapy; Non-small cell lung cancer; Guidelines; Stereotactic radiation therapy; Hypofractionated radiation; Oligometastasis

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Core Tip: Radiotherapy is a critical component of multi-modality treatment of non-small-cell lung cancer (NSCLC). This guideline provides recommendations on the use of radiation therapy to treat patients with different stages of NSCLC. Our goal is to promote medical knowledge among physicians and improve health-care quality on these patients. These guidelines are based on the best available evidence. The level and grade of evidence supporting each recommendation is provided.

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INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in both men and women. In 2018, there were more than 1.7 million cancer-related deaths worldwide. In that same year, more than 2 million people were newly-diagnosed with lung cancer. At diagnosis, approximately 57% of lung cancers are metastatic, 22% present lymph node involvement, and only 17% of cases are diagnosed at early stages [1]. Various environmental and lifestyle factors have been associated with the development of lung cancer. The main risk factor is tobacco use, accounting for 85%-90% of cases[2]. Non-small cell cancer (NSCLC) comprises more than 85% of all lung cancer diagnoses. Despite important treatment advances in recent years, 5-year overall survival (OS) rates remain low, ranging from to 0%-10% in stage IVA-IVB disease to as high as 68% in early stage[3,4].

Advances in treatment and diagnosis include minimally-invasive diagnostic/therapeutic techniques such as endobronchial ultrasound (EBUS) and video-assisted thoracic surgery[5]. In addition, determination of the histological subtypes has become standard practice to assess eligibility-based on tumor

histology and molecular status-for systemic therapy[6,7].

Radiotherapy (RT) is one of the three pillars of the multidisciplinary treatment of lung cancer. In recent years, technological advances have greatly improved this treatment modality. It is estimated that more than half of all cancer patients will require curative or palliative-intent RT at some point in the course of the disease[8]. A series of important advances-including simulation with four-dimensional computed tomography (4D-CT), three-dimensional conformal RT (3D-CRT), intensity-modulated RT (IMRT), volumetric modulated arc therapy (VMAT), cone beam CT (CBCT) image verification systems, and control of respiratory movement-have made it possible to maximize tumor control while minimizing toxicity to adjacent healthy organs and tissues[9]. As a result, the radiation dose can be precisely delivered to the target and adapted to the patient's individual characteristics [anatomy and tumor location, TNM stage, comorbidities, and general performance status (PS)].

In the present guidelines, we review the clinical indications for RT in NSCLC according to disease stage, with a discussion of fractionation schedules, treatment volumes, and organs at risk (OAR). We also discuss the management of the main clinical scenarios seen in routine practice, establishing the grades of recommendation for each treatment according to the strength of evidence.

METHODS

These guidelines are based on the most relevant studies published in peer reviewed journals. A comprehensive review of the clinical literature in the following databases was performed: MEDLINE (Pubmed), EMBASE (Ovid), Web of science (Web of Knowledge). Article selection was undertaken by the expert authors. The Infectious Diseases Society of America grading system[10] was used to assign levels of evidence and grades of recommendation (Table 1). Statements without grading were considered justified standard clinical practice by the authors.

Diagnosis

The clinical manifestations of lung cancer are frequently nonspecific. If NSCLC is suspected, the patient should be referred to the pulmonologist and/or the rapid diagnosis unit and be evaluated by a multidisciplinary team (II, C)[11,12]. The evaluation begins with CT[13,14] and positron emission tomography (PET), which are essential for diagnosis, staging, and treatment planning (I, A). Brain magnetic resonance imaging (MRI) is also essential[15]. All nodes > 1.5 cm on the CT scan should be biopsied, even if the PET scan is negative (I, C). A positive PET scan should be further evaluated, regardless of lesion size[16,17], through EBUS or digestive endoscopic ultrasonography[18-20] (I, A). In uncertain cases, conventional mediastinoscopy or video-assisted mediastinoscopy and video-assisted thoracoscopy are surgical alternatives to obtain samples for subsequent analysis[21,22]. Peripheral lesions can be evaluated by CT-guided transthoracic fine-needle aspiration biopsy[14,23]. Pathologic confirmation is required in patients with a single metastatic lesion and uptake on PET[24].

Pathologic diagnosis of NSCLC should be based on the criteria established in the World Health Organization classification system[6]. It is important to differentiate between the histological subtype: Squamous cell carcinoma, adenocarcinoma (the most common), large cell carcinoma, and neuroendocrine tumors (I, B). The International Association for the Study of Lung Cancer (IASLC) has developed a classification system for adenocarcinoma with prognostic implications[25]. Immunohistochemical studies and determination of molecular alterations such as epidermal growth factor receptor (EGFR), Kirsten rat sarcoma, and anaplastic lymphoma kinase (ALK) mutations should be performed, as these alterations can predict sensitivity to certain drugs and/or targeted therapies[26] (I, B). Classification of NSCLC or not otherwise specified histology should be avoided. Staging is based on the IASLC TNM classification system (8th edition), which is used to classify patients according to disease stage to determine the prognosis and appropriate treatment[27].

CLINICAL INDICATIONS BY TNM STAGE

Early stages: Stereotactic body radiation therapy

Indications: Stereotactic body radiation therapy (SBRT), also known as stereotactic ablative body radiation, consists of delivery of high dose radiation to a very specific target volume, with a high dose gradient in all directions[28]. The indication for this technique is based on the patient's surgical risk category: Inoperable, high-risk, or standard-risk[29]. As follows: (1) Inoperable: Approximately 25% of patients with early-stage NSCLC (ES-NSCLC) are inoperable due to age or comorbidities[30]. In this population, prospective studies of SBRT have reported local control (LC) rates of 90% at 5 years[31] and 91.9% at 7 years[32] and, with a ≥ grade (G)3 toxicity rate under 10%. The well-designed phase II TROG 09.02 CHISEL trial[33] compared SBRT to conventional 3D-RT. SBRT was superior to conventional 3D-RT in terms of LC [hazard ratio (HR) = 0.32, 95% confidence interval (CI): 0.13-0.77, P = 0.0077] with no increase in treatment-related adverse events (AEs). SBRT is therefore the treatment of choice in

Table 1 Level of evidence and grades of recommendation

Level of evidence	
I	Evidence from at least one large randomised controlled trial of good methodological quality (low potential for bias) or meta-analyses of well-conducted randomised trials without heterogeneity
II	Small randomised trials or large randomised trials with a suspicion of bias (lower methodological quality) or meta-analyses of such trials or of trials with demonstrated heterogeneity
III	Prospective cohort studies
IV	Retrospective cohort studies or case-control studies
V	Studies without control groups; case reports; expert opinions
Grades of recommendation	
A	Strong evidence for efficacy with a substantial clinical benefit, strongly recommended
B	Strong or moderate evidence for efficacy but with a limited clinical benefit, generally recommended
C	Insufficient evidence for efficacy or benefit does not outweigh the risk or the disadvantages (adverse events, costs, *etc.*), optional
D	Moderate evidence against efficacy or for adverse outcomes, generally not recommended
E	Strong evidence against efficacy or for adverse outcome, never recommended

inoperable patients (III, A); (2) Operable NSCLC: Only one prospective study (a pooled analysis of the ROSEL and STARS trials) has (indirectly) compared SBRT to 3D-CRT in operable patients[34,36]. The findings of that study, published in 2015, were criticised for the underpowered statistical analyses and the poor surgical quality in the two trials[35]. Several non-comparative prospective studies of SBRT have been conducted, most notably the phase II RTOG 0618 trial[36]. In that trial, 33 operable patients received SBRT, with a 4-year LC rate of 96% and ≥ G3 toxicity of only 8%. The findings of retrospective series comparing SBRT to surgery through matched pair analysis are inconclusive[37,38]. However, a recent meta-analysis[39] suggested that surgery may provide better outcomes on various survival parameters, including OS, cancer-specific survival, and disease-free survival (DFS). Prospective phase III trials are needed to confirm these findings. Currently, four prospective trials are underway to compare surgery to SBRT. Of these trials, the only non-randomised study is the Canadian RAXSIA trial (NCT03431415). The POSTILV trial (NCT01753414) is comparing SBRT to surgery in operable patients while the STABLE-MATES trial (NCT02468024) is comparing sublobar resection to SBRT. Although the VALUE trial (NCT02984761) was activated in 2016, they are still recruiting patients as of the last update (December 2020). Therefore, at present, there is no evidence to support SBRT *vs* surgery in operable patients, unless the patient refuses surgery (III, C); and (3) High-risk patients or patients > 75 years of age. The American Society of Clinical Oncology and the American Society of Radiation Oncology (ASTRO) recommend offering SBRT as an alternative treatment in high-risk patients[40,41] (III, A).

Fractionation: In order to select the appropriate fractionation schedule in SBRT, it is essential to carefully weigh the risks and benefits. LC is poor when the biological equivalent dose (BED) is < 100 Gy [42]. Consequently, the dose should be determined according to the location of the target lesion and, therefore, to the tolerance of adjacent organs. Tumor are classified as central, peripheral, or "safe" (> 2 cm from mediastinal structures and > 1 cm from the chest wall) depending on their location within the thoracic cavity.

Central tumors: Central tumor fractionations as defined by the IASLC[42]: The most important prospective phase I/II trial for central tumors was the RTOG 0813 trial[43], a dose escalation study comparing 50 Gy to 60 Gy, both administered in 5 daily fractions (fx), with an escalation schedule of 0.5 Gy per fraction/arm. The maximum tolerated dose was 12.0 Gy/fx, with a ≥ G3 toxicity rate of 7.2%. Two-year LC rates in patients who received the lowest dose fraction (10 Gy/fx) was 87.5% *vs* 87.9% in the 12 Gy/fx regimen, with 2-year progression free survival (PFS) rates of 50% *vs* 54.5%, respectively.

The dose-escalated SUNSET[44] trial (dose level 1:60 Gy/7 fr-dose level 3 60 Gy/5 fr) and the Hilus [45] trial (56 Gy/8 fr to 65%-70% isodose line) were both performed to assess high-dose SBRT in central/ultracentral tumors. The recently published, Hilus trial showed that this fraccionation regimen in tumors located ≤ 1 cm from the main bronchus and trachea had a high risk of G3 to G5 toxicity (33.8%) with 10 patients experiencing G5 toxicity. These regimens contrast with the more conservative Dutch regimen (60 Gy/8 fx)[46], which obtained a 3-year LC rate of 92.6% and ≥ G3 toxicity of 7.9%.

Based on the available evidence, the optimal fractionation in central tumors appears to be 50 to 60 Gy delivered in five fractions. The dose per fraction should be adjusted to OAR tolerances, and can range from 10-12 Gy/fraction with a total dose of 50-60 Gy administered in 5 daily fractions or 8 fx of 7.5 Gy each to a total of 60 Gy.

Lesions adjacent to the chest wall: In patients with tumors located adjacent to or in contact with the chest wall, European guidelines[47] recommend a total dose of 48 Gy in four fractions. Prospective studies[48] have shown that this fractionation schedule yields 3-year LC rates ranging from 85.4% to 87.3%, with a ≥ G3 toxicity rate (rib fracture) of 3%. Other fractionation schedules have been proposed in this location. For example, Haasbeek *et al*[46] proposed 60 Gy in five fractions, with a 3-year LC rate of 89.3% and a late ≥ G3 toxicity rate (chest wall pain) of 2.1%[46]. Nyman *et al*[49] proposed 45 Gy in three fractions, which achieved a LC rate of 80% with late toxicity (rib fracture) in 4%.

Tumors located in the "safe" zone: Lesions located in the "safe" zone can be considered non-central tumors located > 2 cm from the chest wall. Evidence from two prospective phase II trials - Singh *et al*[50] and RTOG 0915[31]-support extreme hypofractionation (single 30-34 Gy fraction). Singh *et al*[50] found that a single 30 Gy fraction yielded a 2-year LC rate of 94.9%, with G3 toxicity in 17%, and no ≥ G4 toxicity. In RTOG 0915, which evaluated a single 34 Gy dose, the one-year LC rate was 97.0%, with ≥ G3 toxicity rate of 10.3%. Timmerman *et al*[51] conducted a prospective phase II trial to evaluate SBRT in inoperable ES-NSCLC, the findings of that trial supported the classical Timmerman fractionation scheme, with a 3-year LC rate in peripheral tumors ranging from 90.6%-94% and ≥ G3 AEs ranging from 10% to 16.3% (Table 2).

Locally-advanced, inoperable disease

Radical chemoradiotherapy: Concomitant *vs* sequential: At diagnosis, approximately 35% of patients with NSCLC present locally-advanced disease, for which the standard treatment is CRT. The recommended RT dose is 60-66 Gy (I, A). Increasing the radiation dose in combination with chemotherapy (ChT) does not improve outcomes but does increase toxicity rates[52].

In patients with good PS, the recommended treatment is concomitant chemoradiotherapy, which has been shown to improve OS *vs* sequential chemoradiotherapy by 5.7% at 3 years and 4.5% at 5 years, with a mean survival time of 22-25 mo and 5-year OS of 20%[53], probably due to better locoregional control (2.9% at 3 years and 2.2% at 5 years) (I, A). However, concomitant chemoradiotherapy also has a higher incidence of acute non-hematological toxicity[54], mainly G3-G4 esophagitis (range: 4%-18%), but no effect on acute pulmonary toxicity[55]. To date, no differences in treatment outcomes have been observed for the following variables: Type or ChT scheme, age, sex, PS, histology, or disease stage. Neither induction nor consolidation ChT are indicated, although data from the phase III PACIFIC trial showed that consolidation therapy with durvalumab improves both PFS and OS in patients with programmed death ligand 1 > 1% who do not progress after concomitant chemoradiotherapy[56].

Neoadjuvant chemoradiotherapy: Several studies, including the SAKK Lung Cancer Project Group trial [57] and the Lung Intergroup Trial 0139[58], have evaluated the role of neoadjuvant chemoradiotherapy, finding this approach improves PFS in patients who receive trimodal treatment, but without any benefit for OS. This lack of benefit in the surgical arm may be due to higher early mortality rates, especially in patients undergoing right pneumonectomy. A subanalysis found a significant improvement in survival in patients treated with induction chemoradiotherapy followed by lobectomy *vs* those who received concomitant chemoradiotherapy [58].

Induction chemoradiotherapy has been shown to achieve a greater reduction in nodal downstaging than ChT alone, but with no benefit in OS[53] except for potentially resectable superior sulcus tumors, for which the treatment of choice is concomitant chemoradiotherapy (45-54 Gy, 1.8-2 Gy/d) (III, A). However, it is important to plan radical dose RT in case surgery is ultimately not performed[59].

Adjuvant RT: Adjuvant RT is indicated when complete resection (R0) has not been achieved and salvage surgery is not feasible (I, A). In these cases, sequential chemoradiotherapy (ChT followed by RT) should be offered, although with a less aggressive ChT scheme. Concomitant chemoradiotherapy should be limited to patients with macroscopic residual disease (V, C).

The role of adjuvant RT has long been controversial, especially after a meta-analysis published in 1998 showed higher mortality rates after postoperative RT (PORT) in patients with N0-N1 disease[60]. However, the increased mortality was probably due to the excessive toxicity associated with older radiation therapy techniques. By contrast, no deleterious effects of adjuvant RT have been observed in N2 disease. A recent meta-analysis concluded that adjuvant RT is associated with better OS and PFS rates in these patients[61].

The role of PORT was evaluated in two recent phase III trials. In the phase III Lung-ART trial (definitive results still pending publication) patients with N2 involvement were randomized to receive PORT (54 Gy) or observation after complete tumor resection. The initial results showed that PORT did not improve DFS or OS, although fewer thoracic relapses were observed in the group treated with PORT (25% *vs* 46.1%). The lack of improvement in survival outcomes (DFS and OS) could be due to higher rates of cardiopulmonary toxicity ≥ G3 (10.8% *vs* 4.9%). In this regard, it should be noted that 89% of the patients were treated with 3D-CRT and only 11% with IMRT[62].

The second recently-published phase III trial was a single-center study involving 394 patients with stage IIIA-pN2 disease randomized to receive PORT (50 Gy) or observation after complete resection and four cycles of adjuvant chemotherapy. Most of the patients (89.3%) were treated with IMRT (10.7%

Table 2 Recommended stereotactic body radiation therapy dose in early-stage disease

Localization	Dose	Ref.	Evidence level
Central tumour	50/5 fx-60/5 fx	Bezjak *et al*[43], 2019	II, B
	60 Gy/8 fx	Haasbeek *et al*[46], 2011	
Chest wall	48 Gy/4 fx	Guckenberger *et al*[47], 2017	II, B
	60 Gy/5 fx	Nagata *et al*[48], 2015	
	45 Gy/3 fx	Nyman *et al*[49], 2006	
Safe zone	30 Gy/1 fx	Singh *et al*[50], 2019	II, B
	34 Gy/1 fx	Videtic *et al*[31], 2019	
	54 Gy/3 fx	Timmerman *et al*[36], 2018	

SBRT: Stereotactic body radiation therapy; fx: Fractions.

received 3D-CRT). In the intention-to-treat analysis, PORT did not improve DFS, although it did improve DFS in the per-protocol analysis. The results of a pre-planned exploratory analysis in which patients were stratified according to the number of resected nodes (20 *vs* > 20) and involved nodes [(1-3) *vs* ≥ 4] revealed a significant improvement in DFS (HR = 0.75; 95%CI: 0.58-0.98; *P* = 0.04). PORT had no impact on OS. Toxicity rates were lower than observed in the Lung-ART trial, probably due to the high proportion of patients treated with IMRT, stricter dose limits to the OARs, and the exclusion of the contralateral mediastinal nodes from the treatment volume[63].

Despite the significant improvement in LC achieved in both trials, this did not lead to better survival outcomes. For this reason, PORT is not currently recommended as part of standard treatment in patients with N2 involvement and R0 resection. However, stage IIIA-N2 patients are a heterogeneous group and some of these patients could benefit from PORT, as suggested by data from previous studies as well as the recently published findings of the Lung-ART trial (ESMO 2021), which show that a mediastinal nodal involvement ratio (nodes involved/nodes evaluated) ≥ 25% is a prognostic factor for DFS, suggesting that the extent of nodal involvement could help to select patients who may benefit from PORT. Nonetheless, more studies are needed to better determine, through a comprehensive analysis of clinical and molecular characteristics, the patients most likely to benefit from PORT. Based on the postoperative pathologic findings, the recommended PORT doses are as follows: (1) R0: 50-54 Gy, 1.8-2 Gy/fx; (2) Involved margins or microscopic disease: 54-60 Gy; and (3) Macroscopic residual disease: ≥ 60 Gy[59,64].

Altered fractionation schemes: Various dose-intensification strategies have been explored, including accelerated hyperfractionation and other hypofractionated schemes.

Accelerated fractionation and hyperfractionation: Three phase III trials compared different hyperfractionated schemes to conventional RT, demonstrating that hyperfractionated RT yields positive results when administered alone or after induction ChT (I, A). Those trials include the Continuous Hyperfractionated Accelerated Radiotherapy Trial (CHART)[65,66], HART[67], and Continuous Hyperfractionated Accelerated Radiotherapy Weekend Less (CHARTWEL)[68]. The findings of these trials were recently confirmed in a large retrospective series[69].

A meta-analysis evaluated the results of nine trials (2000 patients) - including the CHART, HART, and CHARTWEL trials - comparing conventional RT to various hyperfractionated and accelerated RT schemes. All of the altered fractionation schemes improved OS, although without any significant between-group differences in PFS. The administration or not of ChT did not impact OS. The modified fractionation schemes, particularly very accelerated RT, increased the risk of acute severe esophagitis in Table 3[70].

Moderate hypofractionation: Some patients - due to advanced age, the presence of comorbidities, and/or travel-related difficulties - are poor candidates for conventional (60-66 Gy, 30-36 daily fractions) or hyperfractionated RT. In recent months, due to the coronavirus disease 2019 (COVID-19) pandemic and the consequent need to reduce the number of hospital visits, the use of moderately hypofractionated RT has become more common in patients eligible for radical RT.

The available evidence suggests that dose escalation with standard fractionation techniques (achieved by extending treatment duration) does not improve outcomes[52]. However, radiobiological models show that each 1% increase in the radiation dose improves LC by 1% to 2%[71]. A systematic review of clinical data from dose escalation studies[72] found a BED_{10} dose-response relationship for NSCLC. That review evaluated studies that applied various fractionation schemes, including standard fractionation, hyperfractionation, and hypofractionation. Although the best results were obtained with hypofrac-

Table 3 Accelerated fractionation-hyperfractionation studies

Ref.	Study type	Number of patients	Radiotherapy	Chemotherapy	Results	Toxicity
[65, 66]	Phase III RCT	*n* = 563: Stage I (29%), II (7%), IIIA (38%), IIIB (23%). Similar in both arms	[cRT: 60 Gy, 2 Gy/d (6 wk). INP 44 Gy + boost 16 Gy tumour and involved nodes] *vs* (CHART: 54 Gy, 1.5 Gy/3 times/d, 6 h apart, on 12 consecutive days). INP 37.5 Gy in 25 fx + boost 16.5 Gy in 11 Gy to tumour and involved nodes	No	Absolute 2-yr survival improvement of 9%: 20% cRT *vs* 29% CHART. 21% relative risk reduction for PL. Major improvement in squamous cell disease: 13% 2-yr survival: 20% cRT *vs* 33% CHART. 25% relative risk reduction of PL	Clinical pneumonitis 19% cRT and 10% CHART
[67]	Phase III RCT	*n* = 141: Stage III A-B unresectable. ECOG 0-1	[cRT: 64 Gy, 2 Gy/d (6 ½ wk)] *vs* [HART: 57.6 Gy, 1.5 Gy 2 times/d (2.5 wk)]	Induction: Carboplatin AUC 6 + paclitaxel 225 mg/m^2 2 cycles prior to RT	2-yr OS: 44% HART *vs* 24% cRT; 3 yr: 34% *vs* 14%. Non-significant trend towards better survival with HART. Feasible treatment. Trial close early due to slow recruitment	Esophagitis ≥ G3: 23% HART *vs* 15% cRT. Pneumonitis ≥ G3: 0 HART *vs* 10% cRT
[68]	Phase III RCT	*n* = 406: Stage I 10%, II 5%, IIIA 38%, IIIB 46%. Similar in both arms	(CHARTWEL: 60 Gy, 1.5 Gy 2 times/d in 2.5 wk) *vs* (cRT: 66 Gy, 2 Gy/d, 6.5 wk)	Neoadjuvant 27%. Similar in both arms	Better LC in CHARTWEL. No difference between arms in OS at 2, 3, 5 yr. Better LC CHARTWEL trend in advanced stages and after neoadjuvant ChT	Greater acute dysphagia CHARTWEL. Greater radiological pneumonitis CHARTWEL, no differences in clinical pneumonitis
[69]	Retrospective	*n* = 849, 9 United Kingdom centres. Stage I 33%, II 13%, IIIA 24%, IIIB 24%, IV 1%	CHART: 54 Gy, 1.5 Gy/3 times/d, 6 h apart, in 12 d	Induction: 27% patients, 82% stage III (96% platinum doublets: Cisplatin or carboplatin with vinorelbine, gemcitabine or paclitaxel)	OS 2 and 3 yr: 47% and 32%. OS 3 yr: 38% stage I and 27% stage III. Tendency to better survival in stage III after ChT	Esophagitis, pneumonitis ≥ G3 5%

RCT: Randomised controlled trial; cRT: Conventional radiotherapy; ENI: Elective nodal irradiation; CHART: Continuous Hyperfractionated Accelerated Radiotherapy Trial; OS: Overall survival; RT: Radiotherapy; LC: Local control; CHARTWEL: Continuous Hyperfractionated Accelerated Radiotherapy Weekend Less.

tionated RT, the differences were not significant.

Phase I dose escalation trials of hypofractionated RT have evaluated various regimens[73-75]. Prospective and retrospective series[76-80] have found that accelerated RT is both feasible and well-tolerated when administered alone or concurrently/sequentially with ChT, a finding that was also confirmed in the interim analysis of a phase III trial (Iyengar *et al*[81]) comparing accelerated hypofractionated RT to conventional RT.

The phase III EORTC 08972-22973 trial[82] and the randomised phase II SOCCAR trial[83] compared concurrent to sequential CRT in patients receiving hypofractionated RT. Based on the excellent results obtained with concomitant CRT in the SOCCAR trial[83], this scheme is now widely used in routine practice in the United Kingdom. Iqbal *et al*[84] showed that modifying the ChT dose, incorporating advanced imaging techniques such as PET-CT for staging, and the use of IMRT and VMAT improved survival outcomes at 2-years (58%), with acceptable rates of acute toxicity (Table 4).

A systematic review evaluated 33 studies (1902 patients) involving radical-intent hypofractionated RT for the treatment of stage III NSCLC. The number of fractions in those studies ranged from 15 to 35, with dose fractions ranging from 2.3 Gy to 3.5 Gy, and total doses from 45.0 to 85.5 Gy. Nearly half of those studies (15/33) included concurrent ChT with radiation schemes ranging from 52.5 to 75 Gy at 2.24-3.5 Gy/dose in 15-30 fx. The other studies included neoadjuvant, adjuvant, or no ChT, at RT doses ranging from 45-85.5 Gy (2.25-3.42 Gy/fx, 15-35 fx). There was a linear relationship between BED_{10} and OS: Every 1 Gy increase in BED_{10} yielded an absolute survival benefit of 0.36% to 0.70%. Compared to non-concurrent schemes, concurrent CRT was associated with better OS, albeit with higher - but still acceptable - rates of esophageal toxicity[85].

A single-centre study evaluated 563 patients; 43% received CHART and 57% hypofractionated RT (55 Gy in 20 fx of 2.75 Gy). Both treatment regimens yielded comparable results in terms of survival and treatment-related AEs[86]. Based on their findings, the authors concluded that moderately hypofractionated RT with concurrent ChT is safe when delivered with modern RT techniques and may improve treatment outcomes. However, these findings need to be confirmed in phase III trials.

The ongoing COVID-19 pandemic has led to an increase in the use of hypofractionated RT. To address the challenges presented by the pandemic, a group in the United Kingdom[87] and the ESTRO-

Table 4 Studies of moderate hypofractionated radiotherapy

Ref.	Type of study	Number of patients	Radiotherapy	Chemotherapy	Results	Toxicity
[76]	Prospective	30, stage III-IVA. ECOG ≥ 2	60 Gy (20 fx 3 Gy); (BED_{10} 79.4 Gy)	Sequential (80% patients)	LR 37%. OS 2-yr 38.1%. LR 37%. Distant relapse 57%	Acute esophagitis G3 7%. Acute pneumonitis G3 3%. No chronic toxicity
[77]	Prospective	83 (32 stage III)	66 Gy (24 fx 2.75 Gy); (BED_{10} 84 Gy)	Sequential 90.6% stage III (platinum + vinorelbine)	OS 2 yr 37.5%. SCE 2 yr 41.5%	No toxicity ≥ G3
[78]	Retrospective	300, stage III, inoperable, MEG	3 arms: 45 Gy (15 fx 3 Gy); 60-63 Gy (6 wk); > 63 Gy (6 wk)		No significant differences in LC, distant control, or OS. > DFS in 60-63 Gy	Lower in hypofractionated arm
[79]	Retrospective	609 (9 centres). Stage IA (18%), IB (30.7%), II (14.8%), IIIA (16.4%), IIIB (19.2%). Unresectable or inoperable	55 Gy (20 fx 2.75 Gy)	ChT 28% (83% stage III). Platinum doublets. Most neoadjuvant	OS at 2, 3 and 5 yr: 50%, 36% and 20%. 2 yr OS: stage IA, 72%, stage Ib 51%, stage IIIA 40%. Adenocarcinoma better median survival (31 m) *vs* squamous (20.4 m). No difference in OS between ChT *vs* no ChT. Stage III, trend towards better OS with ChT	No toxicity ≥ G3. Pneumonitis G1-2, 15%
[80, 82]	Retrospective	31, stage I (15), II (15), IIIA (57), IIIB (43). Medically inoperable or unresectable	3 arms: 66 Gy (24 fx 2.75 Gy) + daily cisplatin (6 mg/m^2); same sequential RT after 2 cycles cisplatin/gemcitabine; RT alone 66 Gy (24 fx 2.75 Gy) or 60 Gy (20 fx 3 Gy)	Concurrent: Cisplatin daily (6 mg/m^2). Sequential: (2 cycles cisplatin/gemcitabine) prior to RT	LR 36%, DM 46%. Better RT + ChT than RT alone. 5 yr OS: Concurrent CRT, 23%. No significant difference between concurrent and sequential CRT. LR 36%, DM 46%	Severe late toxicity greater in CRT (27% concurrent, 23% sequential) than in RT alone (8%)
[81]	Phase III RCT[1]	60, stage II/III (11.6%/88.3%). ECOG ≥ 2. Not candidates for ChT/RT	cRT 60-66 Gy/30-33 fx *vs* accelerated hypofx 60 Gy/15 fx 4 Gy	Non-concurrent ChT. Possible neoadjuvant or adjuvant	OS and PFS without significant differences between cRT and hypofx	No G4 toxicity. G3 toxicity: 35% cRT and 18.75% hypofx
[82]	Phase III RCT	158, stage I (3% sequential, 1% concurrent), II (4% sequential, 5% concurrent), IIIA (45% sequential, 30% concurrent), IIIB (47% sequential, 64% concurrent). Inoperable ECOG 0-1	66 Gy (24 fx 2.75 Gy)	Concurrent: Daily cisplatin (6 mg/m^2) + RT 66 Gy (24 fx 2.75 Gy) *vs* sequential: 2 cycles gemcitabine 1250 mg/m^2 days 1, 8 and cisplatin (75 mg/m^2 day 2, prior to RT 66 Gy (24 fx 2.75 Gy)	No significant differences between the 2 groups in DM, OS, PFS. OS 2 and 3 yr: 39%-34% concurrent and 34%-22% sequential. Both schemes well tolerated. Due to early closure, no conclusions drawn	Acute esophagitis G3/4 more common in concurrent (14% *vs* 5%). Late esophagitis G3 = 4% in both arms. Pneumonitis G3/4 = 18% concurrent and 14% sequential
[83]	Phase II RCT	130, stage III inoperable. ECOG 0-1	55 Gy (20 fx 2.75 Gy)	Concurrent: Cisplatin 20 mg/m^2 days 1-4 and 16-19 and vinorelbine 15 mg/m^2 days 1, 6, 15 and 20 RT and 1 or 2 post ChT cycles (CDDP) 80 mg/m^2 day 1 and vinorelbine 25 mg/m^2 days 1 and 8). Sequential: Cisplatin 80 mg/m^2 day 1 and vinorelbine 25 mg/m^2 days 1 and 8, x 3-4 cycles before RT	No significant differences. OS 1 yr: 70% concurrent *vs* 83% sequential and 2 yr: 50% concurrent *vs* 46% sequential. PFS 1 yr: 74% concurrent *vs* 85% sequential; 2 yr: 47% concurrent *vs* 45% sequential. Both safe and effective treatments. Non-significant trend towards better survival with concurrent RT/ChT	Similar esophagitis ≥ G3 in both arms (8.8% concurrent and 8.5% sequential. Pneumonitis ≥ G3: 3.1% concurrent *vs* 5.2% sequential. No grade 4/5 esophagitis. G3 neutropenia lower in concurrent (37%) *vs* sequential (55%)
[84]	Retrospective	100, stages IIIA-B 95%, II 5%. ECOG 0/1	55 Gy (20 fx 2.75 Gy)	Concurrent: Cisplatin 20 mg/m^2 days 1-4 and 16-19 RT and vinorelbine 15 mg/m^2 days 1, 6, 15, 20 and 2 cycles post RT/ChT	OS 2 yr 58%. PFS 2 yr 49%	Esophagitis G3/4 14%. Pneumonitis G3/4 4%

[1]Interim analysis of NCT01459497 with 226 patients: Arm A (experimental), 60 Gy in 15 fractions (3 wk) with image-guided radiotherapy versus arm B,

conventional radiotherapy 60-66 Gy in 30-33 fractions (6 wk) with optional concurrent carboplatin/taxol. Final data expected in December 2021 and December 2022.

RT: Radiotherapy; cRT: Conventional radiotherapy; LR: Local recurrence; DM: Distant metastases; LC: Local control; G: Grade; ChT: Chemotherapy; PFS: Progression-free survival; MFS: Metastasis-free survival; OS: Overall survival; CDDP: Concurrent cisplatin.

ASTRO[88] have both published recommendations for hypofractionated schemes during this period. The United Kingdom group recommends 55 Gy in 20 fx of 2.75 Gy with concurrent ChT in patients with good PS. In patients unable to tolerate concurrent CRT, those guidelines recommend either sequential CRT or RT alone. If ChT is not administered, then hypofractionated RT schemes (*e.g.*, 50-58 Gy in 15 fx) can be considered[87]. The ESTRO-ASTRO practice guidelines, developed through a modified Delphi consensus process, proposed recommendations for two different scenarios: (1) Early pandemic phase, focused on risk mitigation; and (2) A later phase (severe pandemic scenario) in which RT resources may be limited. In the first scenario, there was strong support (97% of the expert panel) for hypofractionated RT (60 Gy in 15 fx, 60 Gy in 20 fx, 60-66 Gy in 24-30 fx, or 55 Gy in 20 fx) if treatment was limited to RT alone. For sequential CRT, there was also strong support (97%) for the same fractionation and dose schemes, although with a clear preference for the 55 Gy (20 fx) or 60-66 Gy (24-30 fx at 2.2-2.75 Gy/d) schemes (II, A). There was no consensus to support concomitant hypofractionated CRT. An alternative would be 55-60 Gy in 20 fx[88] (II, B).

RT in advanced NSCLC

RT in oligometastatic patients: Approximately two-thirds (60%-70%) of patients with NSCLC are diagnosed with stage IV disease. Of these, 20% - or more if PET-CT imaging is used for staging - are oligometastatic at diagnosis[89]. Oligometastasis may present in one of two ways: (1) "*De novo*" oligometastasis: Patient with 3-5 lesions at diagnosis (synchronous) or after 3-6 mo of treatment of the primary tumor (metachronous); and (2) Induced oligometastatic: Polymetastatic patient with metastatic disease in 3-5 locations after systemic therapy.

This recently described concept of oligometastatic disease[90,91] can be further subdivided as follows: (1) Oligopersistence: Persistent disease that is stable on imaging studies, with < 5 lesions after systemic treatment; (2) Oligoprogression: Progression (new lesions or growth of known lesions) in 3 to 5 sites after systemic treatment; and (3) Oligorecurrence: Recurrent disease in 3-5 sites in patients not receiving active systemic therapy.

In these patients, a prior with disseminated disease, the use of local treatments has been shown to improve OS[92] (II, B). In this regard, three prospective[93-95] studies involving patients with oligometastasis at diagnosis have been published (Table 5). Those trials demonstrated that the patients most likely to benefit from local treatments are those whose disease remains stable or responds to systemic therapy, which is why the National Comprehensive Cancer Network guidelines for oligoprogression recommend mutation-directed therapies (EGFR, ALK). However, it is important to keep in mind that patients in the experimental arms of those trials did not receive immunotherapy, an approach that has altered the treatment paradigm in metastatic disease. In this regard, several studies are currently evaluating radioimmunotherapy, which combines local RT with immunotherapy[96].

Multiple studies have sought to identify the characteristics of the "true" oligometastatic patient and those with the best prognosis based on predictors identified in retrospective series (Table 6), as well as other predictive variables currently under investigation[97,98]. These patients are candidates for radical RT, with the dose adjusted for the lesion location and size. The most common metastatic sites in patients with stage IV NSCLC are the brain, lungs, liver, bone, and adrenal glands.

RT in metastatic patients: In metastatic disease, the main objective of RT is symptom relief and better quality of life (QoL). Prior to RT, it is important to assess the patient's functional status, social and family situation, and systemic treatment. Thanks for the important advances in targeted therapies and immunotherapy in recent years, survival in this subgroup has substantially improved[99]. The specific symptoms will depend on the tumor location; symptom relief is the main indication for RT in this setting.

Based on currently available data[100], symptom control appears to be similar regardless of the specific palliative RT scheme (I, A). Short course RT is associated with a higher risk of reirradiation, which is why it is recommended only in patients with poor PS or short life expectancy[101,102] (II, A). Higher doses (20-30 Gy in 5-10 fx) have been shown to improve OS by 5% in selected patients[103], which is why this RT scheme is recommended for thoracic lesions (II, B). Another option is endobronchial brachytherapy, which until recently was reserved for the treatment of airway obstruction in previously-irradiated patients. However, a systematic review published in 2012 comparing endobronchial brachytherapy + external beam RT (EBRT) to EBRT alone reported better symptom control in the EBRT group[104] (II, B).

The optimal management of brain metastases is increasingly controversial. In patients ineligible for stereotactic radiosurgery (SRS) and patients with multiple diffuse brain metastases, the treatment of choice is whole-brain RT (WBRT). However, the findings of the QUARTZ trial, a randomised phase III

Table 5 Radiation therapy in patients with oligometastatic non-small cell lung cancer

Ref.	Type	Design	Palliative treatment	Histology	Presentation	No. of metastases/location	RT type	Follow-up (mo)	PFS (mo)	MFS (mo)	OS (mo)
Gomez *et al* [93], 2019	Phase II RCT. Multicentre	Induct. ChT: (RT + MT) *vs* MT	49	NSCLC (No EGFR, ALK)	Synchronous. Metachronous	≤ 3 (1%:65%)/lung, CNS, bone, liver SSRR, nodes	SABR/SBRT (MTX) hypofra. RT (primary)	38.8	14.2 (SABR/SBRT + MT) *vs* 4.4 (MT)	11.9 (SABR/SBRT + MT) *vs* 5.7	41 (SABR/SBRT + MT) *vs* 17
Iyengar *et al*[95], 2018	Phase II RCT. Multicentre	Induct. ChT: (SBRT + mChT) *vs* mChT	29	NSCLC (No GFR, ALK)	Synchronous	≤ 5 (1%:21%, 2%-3%:76%)/lung, lymph, bone, SSRR	SABR/SBRT (MTX) hypofra. RT (primary)	9.6[1]	9.7 (SABR/SBRT + MT) *vs* 3.5 (MT)	NR	NR (SABR/SBRT + MT) *vs* 17
Palma *et al* [94], 2020	Phase II RCT. Multicentre	(ChT + PT) *vs* (ChT + SABR/SBRT)	99	Lung (18/99)	Synchronous. Metachronous	≤ 5 (1%-3%:93%)/lung, bone, CNS, liver, SSRR	SABR/SBRT	51	11.6 (SABR/SBRT + MT) *vs* 5.4 (TP-MT)	NR	50 (SABR/SBRT + MT) *vs* 22

[1]Study stopped early due to significant difference in progression-free survival between the two arms.

RT: Radiotherapy; MT: Maintenance treatment; mChT: Maintenance chemotherapy; SABR: Stereotactic ablative radiotherapy; SBRT: Stereotactic body radiotherapy; EGFR: Epidermal growth factor receptor; ALK: Anaplastic lymphoma kinase; MTX: Metastasis; NR: Not reported; PFS: Progression-free survival; MFS: Metastasis-free survival; OS: Overall survival; PT: Palliative treatment; NSCLC: Non-small cell lung cancer.

trial comparing WBRT to supportive treatment in patients unsuitable for SRS, which found no benefit for WBRT in terms of OS or QoL, called this indication into question[105] (I, A).

In patients with asymptomatic brain metastases who have not yet started systemic therapy - and could potentially benefit from targeted therapy due to the presence of oncogenic driver mutations (*e.g.*, ALK mutation) - the start of RT can be considered given the intra- and extra-cranial effects of RT[106] (III, B).

At present, there are no clear recommendations on how to best combine RT and immunotherapy. However, two phase II studies (one randomised)[107,108] found that combined treatment was safe and provided adequate symptom control without negatively affecting QoL (III, B).

DEFINITION OF VOLUMES AND RISK ORGANS, CONSTRAINTS

Definition of tumour volumes

Systematic errors (inaccurate contouring of the target volume, OARs, and/or margins) reduce the likelihood of LC while increasing treatment-related toxicity. In 2018, the ESTRO published consensus guidelines for target volume definition in the treatment (radical and PORT) of locally-advanced NSCLC, with four grades of recommendation[109].

According to those guidelines, contrast-enhanced CT should be used for treatment planning. If possible, a recent PET-CT scan in the treatment position is recommended[110]. Respiratory motion should be quantified by 4D-CT, particularly in lower lobe tumours or treatments involving SBRT (IV, A). Treatment volumes[111]: (1) Gross tumour volume (GTV): The primary tumor GTV (GTV-P) and lymph nodes (GTV-N) should be delineated separately. It is important to select the correct window on CT (lung window: W = 1600, l = 600 for lesions surrounded by the lung; mediastinum window: W = 400,

Table 6 Prognostic factors associated with better survival in oligometastatic patients with non-small cell lung cancer

Factor	Comments
Gender	Female > male
Histology	Adenocarcinoma > squamous cell carcinoma
Presentation	Metachronous > synchronous
Karnofsky index - ECOG	80% < - ≤ 100%
Number of lesions	1 > 2-3 > 4-10
Size	< 3 cm
Location	Lung, bone > adrenal glands, lymph nodes > liver, brain

ECOG: Eastern Cooperative Oncology Group.

l = 20, for lymph nodes and tumors invading the mediastinum/chest wall) (III, A); (2) GTV-P: Areas of atelectasis should be excluded[112], which is why PET-CT imaging is particular valuable. If neoadjuvant ChT is administered, the initial volume based on the current CT scan should be used for contouring (III, B); and (3) GTV-N[113-115]: Lymph nodes that are positive on biopsy or pathologic by PET-CT or CT (≥ 1 cm) should be included. Nodes that are highly suspicious on PET-CT imaging but with negative findings on EBUS should be included due to the risk of false negatives (III, A).

If neoadjuvant ChT has been performed, include the lymph nodes or nodal stations involved prior to ChT, regardless of the response. Contouring atlases should be used for nodal station delineation[116-118]. Clinical target volume (CTV): The CTV includes the GTV plus adjacent subclinical disease. It is generally not contoured in SBRT (III, B). CTV-P[119]: For the CTV-P, the GTV should be expanded by 5-8 mm and manually edited to account for surrounding anatomy. CTV-N: The CTV-N can be created in two ways: Either by including the involved nodal station with a margin ≥ 5 mm around the GTV-N[109] or through geometric expansion of the GTV-N (5-8 mm), adapted to anatomical barriers. Elective or prophylactic nodal radiation is not recommended since it does not improve locoregional control but does increase toxicity. PORT[120]: The following areas should be irradiated: Involved lymph nodes, bronchial stump, ipsilateral hilum, and lymph node stations 4 and 7. In left lung cancers, levels 5 and 6 should also be irradiated.

Internal target volume: The internal target volume (ITV) takes into account the internal motion of the tumor. Various systems are available to estimate this motion, which can be limited to reduce the ITV, or monitored with 4D-CT or target lesion tracking[121,122]. One of the most widely used and recommended systems is 4D-CT. The CTV-GTV is contoured in each respiratory phase, or directly in the maximum intensity projection reconstruction. If this is not possible, a slow acquisition CT, or CT on inspiration, expiration and free breathing can be acquired, contouring the CTV-GTV at each point (III, B).

Planning target volume: This is generated by expanding the ITV to account for geometric uncertainties. The planning target volume (PTV) will vary according to the RT centre since differences between centres (*e.g.*, the immobilization system, the method used to compensate for respiratory motion, the specific image-guided technique, *etc.*) can affect the PTV (III, A).

OARs in SBRT and 3D/4D-RT

In many cases, the radiation dose is limited by OARs in the chest cavity. Accurate contouring of these organs is essential, especially for extreme hypofractionated schemes. In 2003, Collier *et al*[123] described the intra- and inter-observer uncertainty in manual contouring of thoracic OARs, thus making it possible to determine the dosimetric impact of these uncertainties. In the last decade, several different contouring atlases have been published to assist in contouring tissues in this anatomic region[124,125].

Lung (lung window settings): Although each lung should be contoured separately, the dosimetric evaluation should be based on the sum of doses to both lungs, excluding the main bronchial tree, the trachea, areas of atelectasis, and the primary GTV (IV, A).

Esophagus (mediastinal window): All layers (mucosa, submucosa and muscular) from the cricoid cartilage to the gastroesophageal junction should be included (IV, A). Oral contrast can be used to ensure correct visualization. For SBRT, contouring of the esophagus should start ≥ 10 cm above the upper limit of the PTV to ≥ 10 cm below the lower limit.

Heart (mediastinal window): There are various approaches to contouring this organ, although the most common approach is to contour the entire heart, including the pericardium and cardiac base, from the lower limit of the pulmonary artery below the aortic arch to the cardiac apex at the level of the diaphragm (IV, A). The pulmonary artery, aorta, and superior vena cava should be excluded. In some

Table 7 Dose constraints in normofractionated radiotherapy

Organ	Volume	Endpoint	Dose (Gy), dose/volume	Rate, %	Ref.
Spinal cord	Partial	Myelopathy	Dmax 50, Dmax 60, Dmax 69	0.2%, 6%, 50%	
Lung	Whole organ, both lungs	Pneumonitis	V20 ≤ 30%, MD = 7, MD = 13, MD = 20, MD = 24, MD = 27	< 20%, 5%, 10%, 20%, 30%, 40%	Palma *et al*[133], 2013 Marks *et al*[130], 2010
Esophagus	Whole organ	≥ Grade 3 acute esophagitis, ≥ grade 2 acute esophagitis	MD < 34, V60 ≤ 17%, V35 < 50%, V50 < 40%, V70 < 20%	5%-20%, < 30%, < 30%, < 30%	Al-Halabi *et al*[135], 2015
Heart	Pericardium. Whole organ	Pericarditis. Cardiac mortality long term	MD < 26, V30 < 46%, V25 < 10%, V50 ≤ 25%	< 15%, < 15%, < 1%	Speirs *et al*[136], 2017
Brachial plexus	Whole organ	Brachial plexopathy	MD > 69 Gy. Dosis maximum 75 Gy to 2 cc of the brachial plexus		Amini *et al*[137], 2012

Dmax: Maximum dose; MD: Median dose.

cases, other subvolumes, such as the coronary arteries (IV, C), can be included[125].

Spinal cord (mediastinal window): Generally, for EBRT, the spinal canal is delineated on the planning CT, corresponding to the planning risk volume (PRV) for the spinal cord (IV, B). For SBRT, the GTV should be contoured if it is located close to the spinal cord; MRI images are useful in these cases. Next, a PRV of the area of interest should be created.

Brachial plexus[126]: Tumors located at the lung apex should be contoured to avoid neurotoxicity (IV, B). A contrast-enhanced CT (or fusion MRI/CT) should be performed to ensure contouring accuracy. The brachial plexus is located between the anterior and middle scalene muscles. There are 5 roots (C5-T1), as follows: (1) Upper limit: The exit point between C4-C5; (2) Lower limit: Subclavian artery and vein; (3) Internal limit: The neural foramina extending from the lateral aspect of the spinal canal to the small space between the two scalene muscles; and (4) Outer limit: The space between the two scalene muscles. For tumors located in the right lung base, the liver should also be contoured (IV, C).

SBRT OARs[127]: Chest wall[128] (mediastinal window): The involved hemithorax should be contoured from the sternal border to the vertebral body, including the ribs and intercostal muscles, excluding other muscles and skin (IV, B). In peripheral tumors, the ribs closest to the tumor should be contoured separately in a bone window setting (IV, C). Trachea (mediastinal window): Include the mucosa, submucosa, and tracheal rings from the lower edge of the cricoid to the upper limit of the proximal bronchial tree (2 cm above the carina). This can also be delineated starting 10 cm above the PTV extension or 5 cm above the carina (whichever is more superior). The lower border is the upper limit of the proximal bronchial tree (IV, B). Proximal bronchial tree (mediastinal window for the trachea and carina and lung window for the bronchi). This includes the area 2 cm distal from the trachea, right and left (R/L) main bronchi, upper lobe (R/L), intermediate bronchus, middle lobe bronchus, lingula, and lower lobe (R/L) (IV, B). Aorta and great vessels[129] (mediastinal window): The aorta and superior vena cava should be included. The vascular wall and all muscle layers must be included (IV, B), and contoured starting ≥ 10 cm above the upper limit of the PTV continuing to at least 10 cm below the lower limit. Skin (mediastinal window): This is a hollow organ. Automatically contour the body and subtract 5 mm (IV, B).

Constraints in normofractionated RT, hypofractionated RT, and SBRT

Normofractionated radiation therapy: The Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) study was published in 2010[130]. The aim of this study was to review the available data on the effects of radiation on normal tissue. QUANTEC updated and further refined the tolerance doses for normal tissues described by Emami *et al*[131] in 1991. QUANTEC provides normal tissue complication probability (NTCP) models, with summary tables of specific results for each organ. However, as the authors indicate, these limitations are not intended to replace comprehensive data provided by organ-specific reviews, and they apply primarily to adult patients. The NTCP according to organ and dose is summarised in Table 7.

The specific limits are as follows: (1) Lung: With conventional fractionation (2 Gy/fx), the recommended V20 limit for both lungs is ≤ 30%-35% and MD ≤ 20-23 Gy to minimize the risk of symptomatic pneumonitis to < 20%[132]. However, several different factors must be considered, included the patient's age and any concurrent systemic treatments. A meta-analysis of data from 836 patients treated with concurrent CRT (60 Gy; cisplatin-etoposide in 38%, carboplatin-paclitaxel in 26%, other schemes in 36%)[133] found that two variables - the lung volume receiving ≥ 20 Gy (V20) and carboplatin/paclitaxel ChT - were predictors of pneumonitis. The highest risk was observed in patients > 65 years receiving carboplatin/paclitaxel-based chemotherapy. The probability of fatal pneumonitis was greater if the daily dose was > 2 Gy and the tumor was located in the lower lobe. Although the latest results presented at ESMO 2020 have called into question the role of PORT in the absence of a

Table 8 Dose constraints for moderate hypofractionation

Organ	Concurrent RT/ChT (55 Gy/20 fx)	Sequential RT/ChT (55 Gy/20 fx)	RT (50-58 Gy/15 fx)	RT (50-60 Gy/15 fx) [138]
Spinal cord	MD 44 Gy (0.1 cc)	Dmax ≤ 36	MD 42 Gy (0.1 cc)	MD < 38 Gy
Esophagus[1]	MD < 55 Gy (1 cc)	V42 < 32%	MD < 52 Gy (1 cc)	MD < 50 Gy (1 cc), V45 < 10 cc
Lungs-GTV	V20 < 35%, MD < 18 Gy	V20 < 25%-30%, MD ≤ 15 Gy	V19 < 35%, MD < 16 Gy	V20 < 30%, V5 < 60%, MD < 20 Gy
Heart	V30 < 36%	V33 < 25%	D100% < 33 Gy, D67% < 40 Gy, D33% < 52 Gy	MD 63 Gy, V57 < 10 cc
Great vessels	NA	NA	MD 58 Gy	MD 63 Gy, V57 < 10 cc
Trachea, carina and main bronchus	NA	NA	MD 58 Gy	MD 63 Gy, V57 < 10cc
Rib	MD < 63 Gy	NA	V30 < 30 cc	MD 63 Gy; V30 < 30cc

[1]Esophagus within the planning target volume ≤ 12 cm.
MD: Median dose; Dmax: Maximum dose; Fx: Fraction; ChT: Chemotherapy; NA: Not available.

definitive analysis, in patients with involved margins PORT is still indicated. A recent study published by a group from the Memorial Sloan Kettering Cancer Center[134] compared dosimetric parameters in 285 patients with NSCLC treated with PORT between 2004 and 2017. The incidence of pneumonitis ≥ G2 was 12.6%. The following factors were associated with pneumonitis: Lung and heart dose, age, and carboplatin-based ChT. These data suggest that elderly patients may be more susceptible to lower lung doses. To limit the risk of pneumonitis ≥ G2 to less than 5% in patients receiving PORT, the authors recommended the following limits: (1) Lung V5 ≤ 65% in patients < 65 years of age and V5 ≤ 36% in patients ≥ age 65. After pneumonectomy, the recommended limits are lung V5 < 60%, V20 < 4%-10%, and median lung dose < 8 Gy[132]; (2) Esophagus: In a study published in 2015, Al-Halabi *et al*[135] evaluated 20 patients who underwent CRT for tumours located < 1 cm from the esophagus. The median radiation dose was 70.2 Gy (range: 63-72.15 Gy). Due to measures taken to protect the contralateral esophagus, there were no cases of esophagitis ≥ G3. The proposed dose contraints to the contralateral esophagus were: V45 < 2.5 cc and V55 < 0.5 cc. IMRT and VMAT allow for dose reduction to the esophagus, thus reducing the incidence of esophagitis; (3) Heart: A subanalysis of the RTOG 0617 dose escalation trial[136] evaluated the association between heart dosimetric parameters and OS. Heart V_{50} < 25% *vs* ≥ 25% was associated with a significant improvement in OS at both one and two years: 70.2% *vs* 46.8% and 45.9% *vs* 26.7% (P < 0.0001), respectively. The median heart V_{50} was significantly higher (20.8% *vs* 13.9%, P < 0.0001) in patients with ≥ G1 cardiac toxicity; and (4) Plexus: An analysis of 90 patients with apical lung cancer treated with CRT found an association between brachial plexopathy and the mean dose to the brachial plexus > 69 Gy (60% of doses > 69 Gy *vs* 13% ≤ 69 Gy) and maximum dose > 75 Gy at 2 cc of the brachial plexus (43% *vs* 13%)[137].

Hypofractionated radiation therapy: Several different total and fractional dose schedules have been used for moderate hypofractionation, including concurrent CRT with various ChT schemes and sequential RT after ChT, or EBRT alone. The dose constraints were not reported in all studies. Table 8 summarises the recommended dose constraints for the most common moderately hypofractionated schemes published by Faivre-Finn *et al*[87].

SBRT: Several reviews have described the constarints to OARs in SBRT base on the studies shown in Table 9[19,31,36,43,139-145].

RT TECHNIQUES (3D-RT, IMRT, VMAT, RESPIRATORY CONTROL, PROTONS, ADAPTIVE RT)

Technological advances in recent years have led to significant changes in the radiotherapeutic treatment of NSCLC, which has progressed from 3D-CRT to IMRT and VMAT, together with advances in image-guided RT (IGRT) and the introduction of proton RT.

Based on data from non-randomised studies, these more sophisticated techniques reduce toxicity to OARs and improve tumor control, thereby leader to better survival outcomes when compared to 3D-CRT[147,148]. The phase III RTOG 0617 trial comparing IMRT to 3D-CRT in advanced stage disease showed that IMRT reduced lung doses (V20), leading to lower rates of severe (≥ G3) pneumonitis and

Table 9 The constraints to organs at risk s in stereotactic body radiotherapy based on the studies

Organ	Single fraction (30-34 Gy)		Three fractions (54-60 Gy)		Four fractions (48 Gy)		Five fractions (50-60 Gy)		Eight fractions (60 Gy)		Ref.
	Optimal	Mandatory	Optimal	Mandatory	Optimal	Mandatory	Optimal	Mandatory	Optimal	Mandatory	
Brachial plexus	14 Gy < 3 cc	17.5 Gy ≤ 0.035 cc	20.4 Gy < 3cc	24 Gy ≤ 0.035 cc			27 Gy < 3 cc	30.5 Gy ≤ 0.035 cc			Benedict *et al*[139], Grimm *et al*[140]
	14.4 Gy < 3cc	17.5 Gy Dmax	22.5 Gy < 3 cc	24 Gy			30 Gy < 3 cc	32 Gy			Bezjak *et al*[43]
					23.6 Gy < 3 cc, 30 Gy < 10 cc, 35 Gy < 1 cc	27.2 Gy Dmax, 40 Gy Dmax					Videtic *et al*[31], Chang *et al*[146]
			24 Gy ≤ 0.5 cc	26 Gy ≤ 0.5 cc			27 Gy ≤ 0.5 cc	29 Gy ≤ 0.5 cc	27 Gy ≤ 0.5 cc	38 Gy ≤ 0.5 cc	Hanna *et al*[141]
Spinal cord	10 Gy < 0.35 cc, 7 Gy < 1.2 cc	14 Gy ≤ 0.035 cc	14 Gy < 0.35 cc, 12.3 Gy < 1.2 cc	18 Gy ≤ 0.035 cc			23 Gy < 0.35 cc, 14.5 Gy < 1.2 cc	30 Gy ≤ 0.035 cc			Benedict *et al*[139]
	7 Gy < 1.2 cc	7 Gy < 1.2 cc	18 Gy < 0.25 cc, 11.1 Gy < 1.2 cc	18 Gy	20.8 Gy < 0.35 cc, 13.6 Gy < 1.2 cc	26 Gy Dmax	22.5 Gy < 0.25 cc, 13.5 Gy < 1.2 cc, 13.5 Gy < 0.5 cc				Bezjak *et al*[43], Videtic *et al*[31], Timmerman *et al*[36]
			18 Gy < 0.1 cc	21.9 Gy < 0.1 cc			23 Gy < 0.1 cc	30 Gy < 0.1 cc	25 Gy < 0.1 cc	32 Gy < 0.1 cc	Hanna *et al*[141]
Esophagus	11.9 Gy < 5 cc, 14.5 Gy < 5 cc	15.4 Gy Dmax	17.7 Gy < 5 cc	25.2 Gy			19.5 Gy < 5 cc	35 Gy			Videtic *et al*[31]
			21 Gy < 5 cc	27 Gy	18.8 Gy < 5 cc, 30 Gy < 10 cc, 35 Gy < 1 cc	30 Gy Dmax, 50 Gy Dmax	27.5 Gy < 5 cc	35 Gy, 52.5 Gy			Timmerman *et al*[36], Bezjak *et al*[43], Chang *et al*[146]
				25.2 Gy < 0.5 cc			32 Gy < 0.5 cc	34 Gy < 0.5 cc		40 Gy < 0.5 cc	Hanna *et al*[141]
Heart	16 Gy < 15 cc, 16 Gy < 15 cc	22 Gy Dmax, 22 Gy Dmax	24 Gy < 15 cc, 24 Gy < 15 cc	30 Gy Dmax, 30 Gy Dmax	28 Gy < 15 cc, 35 Gy < 10 cc, 40 Gy < 1 cc	34 Gy Dmax, 50 Gy Dmax	32 Gy < 15 cc, 32 Gy < 15 cc	38 Gy Dmax, 38 Gy Dmax, 52.5 Gy Dmax			Benedict *et al*[139], Timmerman *et al*[36], Bezjak *et al*[43], Chang *et al*[146]
			24 Gy < 0.5 cc	26 Gy < 0.5 cc			27 Gy < 0.5 cc	29 Gy < 0.5 cc	50 Gy < 0.5 cc	60 Gy	Hanna *et al*[141]
Great Vessels	31 Gy < 10 cc	37 Gy Dmax	39 Gy < 10 cc	45 Gy Dmax			47 Gy < 10 cc	53 Gy Dmax			Benedict *et al*[139]
	31 Gy < 10 cc	37 Gy < 0.035 cc	39 Gy < 10 cc	45 Gy Dmax	43 Gy < 10 cc, 35 Gy < 10 cc, 40 Gy < 1 cc	49 Gy Dmax	47 Gy < 10 cc	52.5 Gy Dmax			Bezjak *et al*[43], Videtic *et al*[31], Chang *et al*[146]
				45 Gy < 0.5 cc		53 Gy < 5 cc					Hanna *et al*[141]

Trachea and bronchus	10.5 Gy < 4 cc	20.2 Gy Dmax	15 Gy < 4 cc	30 Gy Dmax			16.5 Gy < 4 cc	40 Gy Dmax			Benedict *et al*[139]
	8.8 Gy < 4 cc, 10.5 Gy < 4 cc	22 Gy Dmax, 20.2 Gy < 0.035 cc	21 Gy < 5 cc	30 Gy Dmax	30 Gy < 10 cc, 35 Gy < 1 cc, 15.6 Gy < 4 cc	50 Gy Dmax, 34.8 Gy Dmax					Bezjak *et al*[43], Videtic *et al*[31], Timmerman *et al* [36], Chang *et al*[146]
			30 Gy < 0.5 cc	32 Gy < 0.5 cc			32 Gy < 0.5 cc	35 Gy < 0.5 cc	32 Gy < 0.5 cc	44 Gy < 0.5 cc	Hanna *et al*[141]
Skin	23 Gy < 10 cc, 14.4 Gy < 10 cc	26 Gy Dmax, 16 Gy Dmax	30 Gy < 10 cc, 22.5 Gy < 10 cc	33 Gy Dmax, 24 Gy Dmax	35 Gy < 10 cc, 40 Gy < 1 cc, 33.2 Gy < 10 cc	36 Gy Dmax	36.5 Gy < 10 cc, 30 Gy < 10 cc	39.5 Gy Dmax, 32 Gy Dmax			Benedict *et al*[139], Chang *et al*[146], Videtic *et al*[31]
Chest wall	22 Gy < 1 cc	30 Gy Dmax	28.8 Gy < 1 cc, 30 Gy < 30 cc	36.9 Gy Dmax			35 Gy < 1 cc	43 Gy Dmax			Benedict *et al*[139]
	22 Gy < 1 cc	30 Gy Dmax	30 Gy < 30 cc, 50 Gy < 2.3 cc		35 Gy < 10 cc, 32 Gy < 1 cc	40 Gy Dmax	30 Gy < 30 cc, 50 Gy < 2.3 cc, 60 Gy < 1.4 cc				Videtic *et al*[31], Kong *et al* [145], Liao *et al*[147]
			37 Gy < 0.5 cc, 30 Gy < 30 cc				39 Gy < 0.5 cc, 32 Gy < 30 cc		39 Gy < 0.5 cc, 35 Gy < 30 cc		Hanna *et al*[141], Dunlap *et al*[142], Ma *et al*[143]
			40 Gy < 5 cc, 60 Gy < 0.5 cc				V30 < 30 cc, V30 < 70 cc				Herth *et al*[19]
Normal lungs	Minimal critical volume under threshold. 1500 cc, 1000 cc	Threshold dose: 7 Gy, 7.4 Gy		Threshold dose: 11.6 Gy, 12.4 Gy			Threshold dose: 12.5 Gy, 13.5 Gy				Benedict *et al*[139]
	Minimal critical volume under threshold. 1500 cc, 1000 cc, 1500 cc, 1000 cc	7 Gy, 7.4 Gy	20 Gy < 10%, 20 Gy < 15%	10.5 Gy, 11.4 Gy	11.6 Gy, 12.4 Gy, 20 Gy < 20%, 30 Gy < 10%		12.5 Gy, 13.5 Gy, 20 Gy < 20%, 30 Gy < 10%				Bezjak *et al*[43], Videtic *et al*[31], Chang *et al*[146]
				V20 < 10%, V12.5 < 15%			V20 < 10%, V12.5 < 15%		V20 < 10%, V12.5 < 15%		Hanna *et al*[141]
	Treatment on lesion: V20 < 10%; treatment 2-3 lesions: V20 < 12.5% (optimal); V20 < 15% (acceptable); V20 < 20% (selected cases) 3-8 fractions on alternating days. If the lesions are not included in the treatment field, alternate the treatment days for the different lesions										Hanna *et al*[141]
	In 3-5 fraction Dmean ≤ 8 Gy and V20 ≤ 10%-15%										Kong *et al*[145]

lower heart doses, which is a predictor of survival[149,150]. VMAT offers many of the same advantages as IMRT, including a reduction in the number of treatment sessions, similar lung doses and PTV coverage, but with lower heart doses; as a result, VMAT is becoming more common in the treatment of NSCLC[151].

Intrathoracic motion of lung tumor and healthy tissues is a major challenge that can significantly influence treatment delivery. Breathing control techniques can help reduce PTV margins and allow for more precise treatment delivery based on the unique motion of a given tumor, thus providing better tumor control and lower doses to OARs. During planning, several techniques can be used to quantify

tumor motion, including "slow" CT, inspiration-expiration CT, or 4D-CT, as well as techniques to control movement, such as abdominal compression, deep-inspiration breath hold, and breath synchronization techniques such as "gating" in which CT acquisition and treatment are performed in specific phases of the respiratory cycle, and "real-time" tumor tracking-used mainly in SBRT[152]. A useful resource for the implementation of respiratory control is the AAPM Task Group 76 report, which can be used to develop institutional guidelines based on the technical resources available at each centre[153].

The incorporation of CBCT has improved IGRT. CBCT allows for more accurate positioning and reduces inter- and intrafraction errors, thus resulting in smaller PTV margins and lower OAR doses. In addition, CBCT can measure changes in location, morphology, and physiology, thus permitting changes in the initial treatment plan[154-156]. This capacity to adjust the treatment plan, known as adaptive RT, permits administration of higher radiation doses to the tumor with lower doses to the OARs[155,157,158]. Data from small studies suggest that adaptive RT improves LC[159]. This technique is currently being evaluated in the phase II RTOG 1106 trial (NCT01507428) comparing standard concomitant CRT (60 Gy) to adaptive RT based on PET-CT imaging.

Data from both retrospective and prospective studies suggest that proton radiation therapy (PRT) may be superior to photon RT in the treatment of NSCLC[160-162]. However, only one randomised study has compared SBRT to PRT in stage I disease and that trial was closed early[163]. In patients with stage III disease, prospective and retrospective studies have shown acceptable locoregional control with PRT combined with ChT[164]. PRT has the potential to reduce toxicity to OARs such as the lung, heart, and esophagus, especially in unresectable central tumors[165-167]. However, to date, only one randomised phase II trial has compared IMRT to PRT, finding no significant advantages for PRT, nor any significant differences between these modalities in terms of pneumonitis or LC[168]. Consequently, the theoretical advantages of PRT need to be validated in randomised trials, such as RTOG 1308, which is currently recruiting patients[169].

REIRRADIATION

Approximately 20%-40% of patients with early stage or locally-advanced NSCLC develop locoregional progression or metachronous disease at 2 years[170]. Most of these recurrences or second primaries are unresectable, which explains the growing interest in reirradiation. Due to technological advances in radiation therapy delivery - IMRT, SBRT, proton therapy, and IGRT - it is now possible to consider reirradiating certain tumors. However, there is no consensus on the optimal approach to RT for local recurrences in previously-irradiated patients[171].

Reirradiation with photons

The two most common techniques in the radical dose reirradiation setting are IMRT and SBRT. To select the technique that provides the best local disease control with acceptable toxicity, it is important to consider the following parameters: Type of prior RT, anatomic location of the recurrence, and whether the lesion is located in or outside of the original RT field. Several factors - good PS, lung function, small PTV, and a BED dose > 100 Gy - are predictive of better LC and survival. Consequently, these factors should be considered when determining suitability for reirradiation.

SBRT is the technique of choice for peripheral recurrences located far from the mediastinum[172] because SBRT-related toxicity can be severe when the tumor is located near the bronchial tree and/or esophagus. Vyfhuis *et al*[173] reported a 92% LC rate in patients treated with 50 Gy in four fractions (SBRT) while Kilburn *et al*[174] reported a 2-year LC rate of 67% for recurrences located within the prior treatment field, with an acceptable toxicity profile (G2 = 30%, only case of G3 toxicity). The findings of the MD Anderson studies[175] show that IMRT is the most appropriate technique for reirradiation in central tumors, as high doses are required to achieve better LC. IMRT also reduces the dose to healthy tissues, thus limiting toxicity.

Reirradiation with particle therapy (protons and carbon ions)

Particle therapy (protons/carbon ions) is another option to consider for re-irradiation, mainly to reduce toxicity to OARs, as the physical characteristics of these particles reduces the integral dose (low-dose bath of photons at the beam exit point). However, these patients have a high rate of metastases. Some studys reported a significant decrease in OAR toxicity in patients reirradiated with PRT[176,177].

Proton therapy is increasingly being used as a primary treatment for NSCLC and may also have an important role in the reirradiation setting, mainly due to the lack of exit doses. Although carbon ion radiation therapy (CIRT) appears to be superior to proton therapy, due to greater linear energy transfer and relative biological effectiveness, its use is currently very limited[178].

The ROCOCO dosimetric comparison study[179] showed that PRT reduced the integral dose and doses to OARs, even with dose escalation. Chao *et al*[180] found that patients treated with PRT had a high rate of toxicity, with 39% of patients developing ≥ G3 toxicity. In that study, the one-year OS and DFS rates were 59% and 58%, respectively. However, given the toxicity findings, the authors recommended careful selection of patients.

Several studies are currently evaluating reirradiation in NSCLC. Some of these trials have completed patient recruitment and results are pending. One trial (NCT01808677) is evaluating reirradiation with IMRT or PRT; the main endpoint is severe toxicity (≥ G3) and survival is a secondary endpoint.

Reirradiation with CIRT has shown moderate efficacy and acceptable toxicity, suggesting that this modality could be an effective treatment option in selected patients[181]; however, large multicentre trials are required to confirm these findings.

To conclude this section, the best candidates for reirradiation have the following characteristics: Good PS, small volume recurrences, non-central locations, and the capacity to tolerate high dose radiation (SBRT, IMRT, or particle therapy)[175,182].

MANAGEMENT OF TREATMENT INTERRUPTIONS

Management of the overall treatment time (OTT) is especially important in NSCLC. Depending on the fractionation scheme, the effects of prolonging the OTT may vary, and different strategies can be employed to minimize these deleterious effects. In normofractionated schemes, extending the OTT will negatively impact locoregional control and OS[183-186]. One report suggested that OS rates may decrease by up to 1.8% for each day of treatment prolongation[187]. In hyperfractionated regimens, interruptions that increased the OTT by ≥ 5 d in high dose schemes (≥ 69.6 Gy) negatively impact OS, especially in patients with good prognostic factors, such as Karnofsky Performance Status 90%-100%, weight loss < 5%, and ≤ N2[185].

Compensation for treatment interruption

In the year 2000, the Royal College of Radiologists in the United Kingdom published recommendations for the management of unscheduled treatment interruptions, which were updated in 2019[188]. These recommendations divide the treatment type into three categories: Radical (categories 1 and 2) and palliative (category 3) treatment, as follows: (1) Category 1: Patients whose tumors have a high repopulation rate (*e.g.*, squamous cell tumors) who are being treated with radical curative-intent RT. The United Kingdom recommendations include both NSCLC and SCLC in this group. Treatment prolongation in these patients should be no more than two days beyond the prescribed time in 95% of patients; (2) Category 2: Patients with slow growing cancers (mainly adenocarcinomas) receiving radical-intent RT. This group includes breast, transitional bladder carcinoma, and prostate cancer; and (3) Category 3: Patients undergoing palliative-intent RT. OTT prolongation is less critical in these cases. However, it is advisable to compensate for prolonged (> 7 d) interruptions.

Compensation methods

Some authors have suggested that modern RT techniques such as IMRT reduce the incidence of treatment interruptions[189]. Nevertheless, the general principle is to ensure that interruptions are kept as short as possible and to anticipate interruptions whenever possible.

In general, treatment delays can be classified into two main groups: Planned and unplanned interruptions. Two types of measures - universal and specific - can be applied to address these scenarios. Universal measures are useful in both groups, while specific measures will depend on whether the interruption is programmed or not.

There are two main types of universal compensation measures, as follows: (1) Compensation on weekends and holidays; and (2) The use of compatible linear accelerators, which allow for treatment delivery on either machine. Although this is a "planned" measure, it also allows for compensation in the event of unexpected equipment malfunction.

Specific measures can be classified according to whether the interruption was planned or unplanned, as follows: (1) Unplanned: Option 1: Administer two sessions on the same day, 6 h apart, to compensate for the delay. Option 2: Compensate for the dose in the remaining fraction based on the BED, taking into account the a/b for healthy tissue or tumor according to the following formula[190].

$$N.d\left[1+\frac{d}{\alpha/\beta}\right]$$

Where N is the number of fractions, d is the dose per fraction, and α/β is the repair coefficient between lethal and sub-lethal damage. If we take into account the accelerated repopulation time, assuming a tumor α/β ratio of 10, the formula would be as follows.

$$N.d\left[1+\frac{d}{\alpha/\beta}\right]-K\,(T-T\kappa)=\;30.2\left[1+\frac{2}{10}\right]-0.45\,(39-28)=\;67.05\ \text{Gy}$$

Where: + K (esti-mated loss of biological efficacy in Gy per day of delay that would need to be added to compensate)[183]: (1) Stages T1-3, N0-1: 0.27 Gy/d; stages T1-3 N2-3 or T4: 0.75 Gy/d; all stages: Mean 0.45 Gy/d. + T: Total treatment time. In the example, the T is 39 d and treatments assumed to start on a Monday. + Tκ (time from the start of RT at which accelerated repopulation begins) reported: 3-4 wk [187]: 28 d. Therefore, to calculate the dose per remaining fraction, we need to consider the remaining BED needed to reach 67.05 Gy, and the remaining fractions not to exceed two days of treatment

Table 10 Summary of recommendations

Diagnosis	Level of evidence, grade of recommendation
If lung cancer is suspected, refer patient to a rapid diagnostic service for evaluation by a multidisciplinary team	II, C
PET-CT is recommended for initial staging in patients with stage I-III disease who are candidates for radical treatment	I, A
EBUS/EUS is recommended for clinical staging in patients with enlarged lymph nodes without distant metastases, with or without PET uptake	I, C
EBUS/EUS is recommended for stating in patients with positive PET-CT scans and normal-sized lymph nodes without distant metastases	I, A
Histological confirmation of the mediastinum by EBUS/EUS is recommended in central tumours, tumours > 3 cm, and N1 cases	I, C
Histological confirmation is required in cases with a single metastatic lesion and positive PET-CT	II, A
Brain MRI is recommended in candidates for curative-intent treatment	II, A
VAMS should be performed when EBUS/EUS findings are not evaluable	I, B
Differentiation between adenocarcinomas and squamous cell carcinomas is recommended even for small biopsies or cytology	I, B
EGFR mutations and ALK rearrangements should be assessed in patients with stage IV, non-squamous cell carcinomas. This determination should be performed in all cases (regardless of smoking status) and in all non-smokers independently of tumour histology	I, B
Early stage NSCLC - SBRT	
Inoperable	II, A
Operable	III, C
High surgical risk	III, A
Locally-advanced disease	
Concomitant radiotherapy: This is the treatment of choice for unresectable stage IIIA/IIIB with ECOG 0-1 and weight loss < 5% in 3 mo	I, A
60-66 Gy in 30-33 daily fractions of 2 Gy/fx and 2-4 ChT cycles	I, A
Platinum-based ChT	I, A
Treatment should be completed in < 7 wk	III, B
Sequential radiotherapy	
If concomitant treatment is not possible, the alternative is sequential CRT	I, A
Treatment should be completed in a short period of time	I, A
Neoadjuvant radiotherapy	
Assessment by a multidisciplinary team is recommended	IV, C
In potentially-resectable upper sulcus tumours, the recommended approach is neoadjuvant CRT followed by surgery	III, A
This approach can be considered in potentially-resectable T3/T4 tumours, but only in well-selected cases at experienced centres	III, B
Surgery must be performed within 4 wk after completion of RT	III, B
Adjuvant radiotherapy	
Not recommended in early stage disease with complete resection (R0)	I, A
It should be considered if resection is incomplete or margins are involved (R1)	IV, B
Not recommended as standard in R0 cases with N2 involvement	I, A
In N2 disease, adjuvant RT could be considered based on risk factors for local recurrence	IV, C
If adjuvant ChT and RT are both administered, the recommended sequence is ChT followed by RT	V, C
Altered fractionation schemes	
Accelerated hyperfractionation schemes provide better disease control than conventional RT	I, A

Recommended fractionation schemes for RT administered alone or sequentially after ChT: 55 Gy (20 fx, 2.75 Gy), 60 Gy (20 fx, 3 Gy), 60 Gy (15 fx, 4 Gy), 45-50 Gy (15 fx, 3-3.33 Gy)	II, A
If RT administered concurrently with ChT in patients with good performance status: 55 Gy (20 fx 2.75 Gy)	II, B
General considerations: There is no evidence to support prophylactic WBRT in stage III disease	II, A

PET-CT: Positron emission tomography-computed tomography; fx: Fractions; MRI: Magnetic resonance imaging; EGFR: Epidermal growth factor receptor; ALK: Anaplastic lymphoma kinase; NSCLC: Non-small cell lung cancer; ECOG: Eastern Cooperative Oncology Group; ChT: Chemotherapy; CRT: Conformal radiotherapy; RT: Radiotherapy; WBRT: Whole-brain radiotherapy.

extension. Using this equation, we calculate the *d* (dose per fraction); and (2) Planned: Option 1: Compensate on a holiday. Option 2: Perform the dose calculation per fraction to compensate for the missed treatment days using the formulas described above, provided that the dose is ≤ 3.5 Gy/fx and the OAR dose tolerance is within the stipulated limits, after adjusting for the relevant biological calculation.

Recommendations: Prioritise patients with squamous cell tumors. Use IMRT whenever possible, especially in locoregionally-advanced cases**.** Conventional fractionation: Keep delays to a minimum. Compensate if the OTT is > 45-50 d and/or the interruption is ≥ 4-5 d. Adjuvant RT: Although there are no published data in this scenario, as a precautionary measure, avoid delays ≥ 5-10 d, especially in patients without signs of poor prognosis or squamous cell tumors. In hyperfractionated schemes, compensation strategies are more complex, which is why treatment on holidays is preferred. However, if the treatment delay is ≥ 10 d, full compensation is not recommended due to the risk of excess toxicity [188]. The number of indications for moderately hypofractionated RT and SBRT has increased substantially during the COVID-19 pandemic. Specific guidelines for these cases have been published[191].

FOLLOW-UP (AFTER SBRT, EARLY STAGE AND LOCALLY ADVANCED)

Approximately 40% of patients with lung cancer will develop a distant recurrence from 3 to 5 years after treatment completion. At 3-years, approximately 30% of patients will develop a locoregional recurrence (potentially-curable)[55]. After SBRT, approximately 12% of patients develop locoregional recurrence at 4 years[36].

The risk for development of a second primary lung cancer after treatment ranges from 1% to 6% per patient per year and this risk does not decrease over time. The mean interval from the first to the second primary tumor ranges from 59 to 62 mo[192]. Early management of these relapses, whether curative or palliative intent, is associated with better survival and QoL, which underscores the importance of close follow-up[193,194]. For the assessment of treatment-related toxicity and recurrence, we recommend the following follow-up measures.

Patients treated with SBRT

Most recurrences occur more than 6 mo after treatment. Based on recommendations from the ESTRO [47], the United Kingdom SBRT consensus statement[195], and updates on high-risk CT features[196] , the following follow-up procedures are recommended.

First year post-treatment: The first clinical follow-up visit (complete medical history and physical examination) should take place within 4-6 wk of treatment completion. The first CT scan should be performed at least 3 mo after treatment. Clinical evaluation, including contrast-enhanced CT, should be performed every 3 mo for at least one year.

Second to third year after treatment: After the first year, follow-up should be performed every 3-6 mo for three years. CT images performed every 3 mo should be compared to previous CTs.

Third to fifth year after treatment: CT imaging should be performed every 6 mo from year three to year five. Low-dose CT should be performed annually from that time if risk factors are present. If the CT scan reveals risk factors[197,198], then a PET scan (III, B) should be ordered. If salvage therapy is feasible, then a biopsy should be performed to confirm the PET findings (III, B). Lung function testing should be performed annually.

CONVENTIONAL FRACTIONATION AND LOCALLY-ADVANCED DISEASE

Based on recommendations from ESMO[59], the Italian Association of Medical Oncology[199], and SEPAR[15], we recommend the following: (1) Unsalvageable patients. Perform clinical evaluations

(complete medical history, physical examination, and blood tests) every 6 mo for two years. A chest CT should be performed at months 12 and 24, with annual follow-up thereafter (III, B); and (2) Salvageable patients. First three years: CT IV contrast every 3-6 mo (III, B). Years four and five: Follow-up every 6 mo; thereafter, annual low-dose CT without contrast. If pathologic findings are detected on CT, perform PET-CT and brain MRI. Obtain histopathologic confirmation of PET findings in accordance with the therapeutic option (III, B). Maintain follow-up for at least 5 years.

General recommendations: The treating physician should actively participate in follow-up (I, C). In patients unlikely to benefit from salvage therapy, the frequency of follow-up should be adapted to the patient's individual needs (V, B). Follow-up with PET-CT or abdominal ultrasound is not recommended (I, C). Smoking cessation[200] (III, A). Behavioral therapy combined with pharmacological intervention (I, A). Influenza and pneumococcal vaccination should be offered if not contraindicated.

RECOMMENDATIONS

Summary of recommendations is provided in Table 10.

CONCLUSION

Radiotherapy is a critical component of multi-modality treatment of NSCLC. This GOECP/SEOR guidelines, can help physicians to improve medical knowledge and find better ways to treat their NSCLC patients. Following the level of evidence this guidelines are summarized in Table 10.

FOOTNOTES

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MINIREVIEWS

Tsunami of immunotherapy reaches mesothelioma

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Abstract

Malignant pleural mesothelioma (MPM) is the most common type of malignant mesothelioma. It is a rare tumor linked to asbestos exposure and is associated with a poor prognosis. Until very recently, patients with advanced or unresectable disease had limited treatment options, primarily based on doublet chemotherapy with cisplatin and pemetrexed. In 2020 and 2021, after more than a decade with no major advances or new drugs, two phase III clinical trials published results positioning immunotherapy as a promising option for the first- and second-line treatment of MPM. Immunotherapy has revolutionized the treatment of many cancers and is also showing encouraging results in malignant mesothelioma. Both immune checkpoint inhibition and dual cytotoxic T-lymphocyte–associated antigen 4 and programmed death-ligand 1 pathway blockade resulted in

significantly improved overall survival in randomized phase III trials. In the CheckMate 743 trial, first-line therapy with nivolumab plus ipilimumab outperformed standard chemotherapy, while in the CONFIRM trial, nivolumab outperformed placebo in patients previously treated with chemotherapy. These two trials represent a major milestone in the treatment of MPM and are set to position immunotherapy as a viable alternative for treatment-naïve patients and patients with progressive disease after chemotherapy.

Key Words: Mesothelioma; Malignant pleural mesothelioma; Immunotherapy; Immune checkpoint inhibitors; Cytotoxic T-lymphocyte–associated antigen 4; Programmed cell death protein 1; Nivolumab; Ipilimumab; Immunotherapy combo; CheckMate 743; CONFIRM

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Core Tip: Malignant pleural mesothelioma (MPM) is the most common type of malignant mesothelioma and is associated with a poor prognosis. The treatment options for advanced MPM were limited until very recently, when the results from two phase III trials showed improved survival in patients treated with immunotherapy. In the first trial, CheckMate 743, nivolumab plus ipilimumab as first-line therapy achieved better overall survival than standard chemotherapy, while in the second trial, CONFIRM, nivolumab *vs* placebo significantly improved overall survival in patients previously treated with chemotherapy. In this article, we discuss recent advances and highlights in the treatment of MPM.

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INTRODUCTION

Malignant mesothelioma (MM) is a rare tumor, with just 30870 cases diagnosed in 2020. The annual incidence is 0.3 cases per 100000 inhabitants worldwide, but rates vary depending on the region. In more developed areas, such as Europe, the annual incidence of MM is > 1 case per 100000 population [1]. MM arises from the mesothelial cells of serous membranes such as the pleura, peritoneum, pericardium, and tunica vaginalis of the testes. Malignant pleural mesothelioma (MPM) accounts for approximately 80% of all cases and carries a poor prognosis, with an overall 5-year survival rate of just 10%. There is a clear causal link between MM and a history of asbestos exposure, although the latency period between exposure and tumor development is between 20 years and 50 years. MPM mainly affects men (male to female ratio, 3:1) and is considered an occupational disease. The mean age at presentation is 74 years[2]. MPM has three subtypes with distinct histologic, biologic, and prognostic features: The epithelioid subtype, which accounts for 50%-70% of cases; the sarcomatoid subtype, which accounts for 7%-20% of cases and carries the worst prognosis; and the biphasic subtype, which carries a moderate prognosis[3].

The standard treatment for MM up to 2020 was doublet chemotherapy with cisplatin and pemetrexed, and no relevant advances had been made in this area for over a decade. As has occurred in many cancers, the advent of immunotherapy is changing the landscape of MM treatment and has already shown promising results[4].

In this article, we review the history of treatment options for MPM, including attempts to add immunotherapy-based strategies to the existing armamentarium. We then analyze the recent results from two phase III clinical trials set to position immune checkpoint inhibitors as effective first- and second-line treatments for MPM.

HISTORICAL HIGHLIGHTS IN THE TREATMENT OF MESOTHELIOMA IN THE PRE-IMMUNOTHERAPY ERA

Polychemotherapy, with or without antiangiogenic therapy, was the only option for treating MPM until the recent approval of nivolumab plus ipilimumab. The standard first-line treatment, based on the results of a phase III trial of 456 patients published in 2003, is pemetrexed 500 mg/m^2 plus cisplatin 75

mg/m^2 every 21 d. In the trial, this combination significantly outperformed cisplatin alone in terms of overall survival (OS) [12.1 mo *vs* 9.3 mo; hazard ratio (HR) = 0.77; P = 0.02], progression-free survival (PFS) (5.7 mo *vs* 3.9 mo; HR = 0.68; P = 0.001), and response rates (41.3% *vs* 16.7%; P < 0.001). The most common adverse effect was hematologic toxicity (grade 3/4 neutropenia, 27.9%; grade 3/4 leukopenia, 17.7%)[5].

Contrasting with the situation for non-small cell lung cancer, it has not been confirmed that maintenance treatment with antifolates improves survival in patients with MM after four to six cycles of chemotherapy with cisplatin plus pemetrexed[6]. In 2019, the results of a phase II trial of patients who had achieved at least stable disease with cisplatin plus pemetrexed showed no significant differences for PFS [3.4 mo *vs* 3.0 mo; HR = 0.99; 95% confidence interval (CI): 0.51-1.9; P = 0.9733] or OS (11.8 mo *vs* 16.3 mo; HR = 0.86; 95%CI: 0.44-1.71; P = 0.6737) between patients randomized to maintenance treatment with pemetrexed and those randomized to placebo[7]. In the same year, however, another phase II trial showed a survival benefit for maintenance gemcitabine *vs* palliative treatment only (median DFS, 6.2 mo *vs* 3.2 mo; HR = 0.42; 95%CI: 0.28-0.63)[8], but the improvement was not considered important enough for this option to be included in clinical guidelines.

Carboplatin plus pemetrexed can be used in patients unfit for cisplatin, as several phase II trials have shown that it has comparable efficacy to the cisplatin-pemetrexed doublet[9-11].

Attempts to improve survival outcomes in patients treated with standard chemotherapy include the addition of antiangiogenic therapy (bevacizumab or nintedanib). The rationale is that vascular endothelial growth factor (VEGF) is a key mitogen for MM cells[8]. The open-label phase III MAPS trial showed that adding bevacizumab 15 mg/kg to first-line cisplatin plus pemetrexed chemotherapy improved median OS (18.8 mo *vs* 16.1 mo; HR = 0.77; 95%CI: 0.62-0.95; P = 0.0167). It also allowed the use of bevacizumab as maintenance therapy. Patients treated with bevacizumab plus chemotherapy, however, showed higher rates of hypertension (26% *vs* 0%, grade 3/4) and thrombotic events (6% *vs* 1%, grade 3/4)[12]. The addition of bevacizumab to cisplatin and pemetrexed chemotherapy is recommended in clinical guidelines but has not yet received regulatory approval. The phase III LUME-Meso trial found no significant improvements in PFS following the addition of nintedanib, a tyrosine kinase inhibitor, to the combination of cisplatin and pemetrexed. Other studies of second-line vascular endothelial growth factor receptor tyrosine kinase inhibitors used as second-line treatments have also reported no significant benefits, but their findings may have been influenced by the profile of patients studied[13].

Chemotherapy combining cisplatin and gemcitabine showed promising activity against MM in two phase II multicenter trials conducted before the approval of pemetrexed in this setting[14,15]. This combination thus would be the treatment of choice for previously treated patients, unless, of course, they had not received first-line treatment with pemetrexed[16]. Poor results have been reported for other second- and third-line treatments investigated. The only drugs that have shown a slight survival benefit to date are weekly vinorelbine (median PFS, 2.3 mo and median OS, 6.2 mo)[17] and weekly gemcitabine[18]. The use of these drugs is supported by data from small phase II trials, subgroup analyses from first-line studies, and retrospective analyses. Nonetheless, the phase II trial, RAMES, whose results were published in 2020, showed a significant OS benefit for gemcitabine plus ramucirumab *vs* gemcitabine only in previously treated patients (13.8 mo *vs* 7.5 mo; HR = 0.71; 95%CI: 0.59-0.85; P = 0.057), positioning this combination as a promising second-line option[19].

IMMUNOTHERAPY-BASED TREATMENT STRATEGIES FOR MESOTHELIOMA

MM is considered to be an inflamed tumor. High programmed death-ligand 1 (PD-L1) expression is associated with a worse prognosis and increased immune infiltration[20,21]. Immunotherapy is thus an attractive option for this tumor and has attracted increasing attention from researchers in recent years. Numerous types of immunomodulatory treatments have been investigated, including interferon, interleukin 2, tumor necrosis factor-α, granulocyte/macrophage colony-stimulating factor, oncolytic viruses, dendritic cell immunotherapy, and, currently at the forefront of efforts, immune checkpoint inhibitors[4,22]. Currently, most developed ICIs in the treatment of solid tumors are anti-cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and anti-programmed cell death protein 1 (PD-1)/PD-L1 monoclonal antibodies (mabs), each of which acts at a different level of activation of immune response. Anti-CTLA-4 mabs promote T cell proliferation and trigger antitumor response acting in the priming of immune response in peripheral lymphoid organs. On the other hand, anti-PD-1/PD-L1 mabs make their action in the tumor restoring the antitumor function of T cells, avoiding to become exhausted T lymphocytes. Attempts to find an effective immunotherapy-based treatment, however, were largely unsuccessful, until the phase III CheckMate 743 and CONFIRM trials, whose results were released in 2020 and 2021.

Tremelimumab, a CTLA-4 inhibitor, was investigated as an option for progressive disease after chemotherapy in two open-label single-arm trials - MESOT-TREM-2008[23] and MESOT-TREM-2012[24] - and a randomized, placebo-controlled, phase IIb trial - DETERMINE[25]. The two single-arm trials evaluated different dosages of tremelimumab, but only MESOT-TREM-2012 met the primary endpoint,

with an objective response rate (ORR) of 52%. The results for the secondary endpoints, OS and PFS, were promising and the drug also showed a favorable safety profile. The larger DETERMINE trial, which compared tremelimumab and placebo in patients who progressed after chemotherapy, did not demonstrate any significant differences in OS, PFS, or ORR.

Anti-PD-1/PD-L1 monotherapy as both a first- and second-line option has also been studied but mostly in phase Ib and II trials. The multicenter phase II DREAM trial evaluated the combination of durvalumab and standard first-line chemotherapy[26]. Its results were encouraging, with a median OS of 6.9 mo, a median PFS of 18.4 mo, an ORR of 48%, and an acceptable safety profile. They have not, however, been validated in comparative study or phase III trial. In a phase Ib trial, avelumab, an anti-PD-L1 drug, showed a good ORR in previously treated patients, with a complete response rate of 2% and a partial response rate of 8%[27]. Nonetheless, although the adverse events reported were to be expected, 8% of patients had an event that resulted in death[27].

The ETOP-PROMISE-Meso-Trial is the only phase III trial conducted in the setting of previously treated MM. It compared pembrolizumab and chemotherapy (gemcitabine or vinorelbine) in patients with MM that had progressed after at least one treatment but found no significant differences for PFS (primary endpoint) or OS[28]. While ORR was significantly higher in the pembrolizumab group (22% *vs* 6%; $P = 0.004$), responses were mostly short lived. Nivolumab, another anti-PD-1 drug, was studied in patients with pretreated MM in two single-arm phase II trials. The results for ORR, disease control rate, and OS were promising and were further investigated in the phase III placebo-controlled CONFIRM trial, whose results were recently published. The results for the two primary endpoints - OS and PFS - were positive, with an OS of 9.2 mo (*vs* 6.6 mo in the placebo group) (HR = 0.72; 95%CI: 0.55-0.94; $P = 0.02$) and a PFS of 3 mo (*vs* 1.8 mo) (HR = 0.6; 95%CI, 0.48-0.77; $P < 0.001$). These results undoubtedly represent a milestone in the management of previously treated mesothelioma, but as the comparator was placebo, it remains unclear whether nivolumab is truly a better option than chemotherapy or gemcitabine plus ramucirumab in this setting[29-31].

Combination immunotherapy with the immune checkpoint inhibitors CTLA-4 (ipilimumab) and PD-1 (nivolumab) showed promising results in two phase II trials - MAPS2[32] and INITIATE[33], leading to further investigation in the phase III CheckMate 743 trial. Combined tremelimumab and durvalumab therapy also showed activity against mesothelioma and an acceptable safety profile in the phase II NIBIT-MESO-1 trial[34] (Table 1).

NIVOLUMAB AS NEW SALVAGE THERAPY OPTION

Stand-Up-To-Cancer Cancer Research United Kingdom CONFIRM trial is a double blind phase 3 randomized study evaluating nivolumab (3 mg/kg/q2w) *vs* placebo with 2:1 ratio in patients with previously treated unresectable MM (pleural or peritoneal) until disease progression or a maximum of 12 mo. Co-primary objectives were investigator-assessed PFS and OS. 221 patients were randomized to nivolumab and 111 to placebo. Preliminary data were presented in World Conference of Lung Cancer 2020, and although OS was not mature, longer survival was achieved with nivolumab (9.2 mo *vs* 6.6 mo; HR = 0.72; 95%CI: 0.55-0.94; $P = 0002$), and PFS was also better for nivolumab arm (3.0 mo *vs* 1.8 mo; HR = 0.62; 95%CI: 0.49-0.78; $P < 0.001$). In the subgroup analysis of OS by histologic subtype, significant benefit was found in the epithelioid subtype but not significant benefit in non-epithelioid one. Grade 3-4 treatment-related adverse effects were reported in 19% on nivolumab *vs* 6.3% on placebo arm[29] (Table 2).

NIVOLUMAB AND IPILIMUMAB AS NEW FRONTLINE OPTION

The pivotal open-label, multicenter CheckMate 743 trial represented a major step forward in the treatment of mesothelioma, as it was the first phase III trial to publish results on the use of immunotherapy as first-line therapy. It compared nivolumab plus ipilimumab against standard chemotherapy in previously untreated patients with unresectable MPM[35]. In total, 605 patients were randomly assigned (1:1) to receive nivolumab 3 mg/kg every 2 wk plus ipilimumab 1 mg/kg every 6 wk for 2 years or standard chemotherapy with six cycles of cisplatin 75 mg/m^2 or carboplatin with an area under the curve value of 5 plus pemetrexed 500 mg/m^2. Patients in both arms continued to receive treatment until disease progression or unacceptable toxicity; the maximum time established for the experimental arm was 24 mo. The characteristics of the two groups were comparable; 77% of the participants were men and 75% had an epithelioid subtype. The results of the first prespecified interim analysis, at 29.7 mo, showed higher median OS (the primary endpoint) in the immunotherapy group (18.1 mo *vs* 14.1 mo; HR: 0.74; $P = 0.002$). OS in the immunotherapy *vs* chemotherapy group was 68% *vs* 58% at 1 year and 41% *vs* 27% at 2 years. Median duration of response was 11.0 *vs* 6.7 mo. All the subgroup analyses showed trends that favored nivolumab plus ipilimumab over chemotherapy. On stratifying the results by MPM subtype and PD-L1 expression, the survival benefit was higher for patients in the immunotherapy group, with a median OS of 18.1 mo *vs* 8.8 mo for patients with non-epithelioid MPM and 18

Table 1 Main pre-phase III clinical trials of immunotherapy-based strategies for the treatment of mesothelioma

Clinical trial (Phase): Drug analyzed	Setting	Primary endpoint
MESOT-TREM 2008 (Phase II): Tremelimumab 15 mg/kg every 90 d[23]	Salvage setting	ORR: 6.9%
MESOT-TREM 2012 (Phase II): Tremelimumab 10 mg/kg every 4 wk[24]	Salvage setting	ORR: 13.7%
DETERMINE (Phase IIb): Tremelimumab 10 mg/kg every 4 wk *vs* Placebo[25]	Salvage setting	OS: 7.7 mo *vs* 7.3 mo (HR = 0.92; P = 0.41)
DREAM (Phase II): Durvalumab 1125 mg + Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Pemexetrad 500 mg/m^2 every 3 wk[26]	Front-line setting	6-mo PFS: 57%
JAVELIN Solid (Phase Ib): Avelumab 10 mg/kg every 2 wk[27]	Salvage setting	ORR: 9%

AEs: Adverse events; AUC 5: Area under the curve value of 5; HR: Hazard ratio; ORR: Overall response rate; OS: Overall survival; PFS: Progression free survival.

Table 2 Recently published practice changing phase 3 studies in Malignant Pleural Mesothelioma

Clinical trial (Phase)	Population	Treatment arms	mOS		mPFS		AEs G3
CheckMate 743 (Phase III)[35]	Untreated MPM	Nivolumab 3 mg/kg every 2 wk + ipilimumab 1 mg/kg every 6 wk	18.1 mo	HR: 0.74, P = 0.002	6.8 mo	HR: 1.00	30%
		Cisplatin + pemetrexed	14.1 mo		7.6 mo		32%
CONFIRM (Phase III)[29]	Relapsed MPM	Nivolumab 3 mg/kg every 2 wk	9.2 mo	HR: 0.72, P = 0.002	3 mo	HR: 0.61; P < 0.001	19%
		Placebo	6.6 mo		1.8 mo		6.3%

MPM: Malignant pleural mesothelioma; MM: Malignant mesothelioma (pleural or peritoneal); AEs: Adverse events; mOS: Median overall survival; mPFS: Median progression free survival; G: Grade; HR: Hazard ratio.

mo *vs* 13.3 mo for those with PD-L1 expression > 1%. In the nivolumab plus ipilimumab group, the survival outcomes were similar across the different subtypes and were independent of PD-L1 expression. The incidence of grade 3-4 adverse events was similar in both groups: 30.3% in the immunotherapy group and 32% in the chemotherapy group. Adverse events led to treatment discontinuation in 15% of the patients treated with immunotherapy and 7.4% of those treated with chemotherapy. The most common adverse effect of any grade in immunotherapy arm was diarrhea (21%), and nausea in the chemotherapy group (37%). Most commonly reported any-grade immunotherapy-related adverse effects were skin (36%), gastrointestinal (22%), endocrine (17.3%), hepatic (12%), hypersensitivity/infusion reaction (12%), pulmonary (6.7%), and renal (5%).

The safety profile observed for the combined use of nivolumab and ipilimumab was comparable to that reported elsewhere[36]. Based on these results, the United States Food and Drug Administration approved nivolumab plus ipilimumab as a first-line treatment for MPM in October 2020 (Table 3).

IMMUNOTHERAPY BIOMARKERS IN MESOTHELIOMA

Numerous biomarkers of response to immunotherapy have been investigated in recent years, but the results have varied widely, precluding any definitive conclusions. In this section, we review the most promising results reported to date.

Approximately 38%-75% of MMs express PD-1/PD-L1, and this variability is partly due to the immune microenvironment that characterizes this tumor. PD-1/PD-L1 expression has been linked to significantly worse OS, suggesting that it might be a marker of poor prognosis, especially at values > 30%[22,37]. PD-1/PD-L1 Levels are higher in sarcomatoid tumors, which have a worse prognosis than epithelioid subtypes. Nonetheless, contradictory findings have been reported for the relationship between PD-1/PD-L1 expression and response to different forms of immunotherapy. The CONFIRM trial performed subgroup analyses according to PD-L1 expression but found no significant differences supporting the predictive value of this marker. In the PD-L1 ≥ 1% subgroup, patients treated with nivolumab had a median OS of 8 mo *vs* 8.7 mo for those treated with placebo (HR = 0.95; 95%CI: 0.51-1.76; P = 0.864), while in the < 1% PD-LI group, they had a median OS of 9 mo *vs* 6.4 mo for those in the placebo group (HR = 0.74; 95%CI: 0.51-1.08; P = 0.115)[29]. The predictive value of PD-L1 expression was a secondary endpoint in the CheckMate 743 trial, and the data showed a significant OS benefit for immunotherapy *vs* chemotherapy in patients with PD-L1 ≥ 1% (HR = 0.69; 95% CI 0.55-0.87). By

Table 3 Comparison of safety and efficacy of frontline Nivolumab + Ipilimumab *vs* chemotherapy in malignant pleural mesothelioma

Clinical trial	Phase	Treatment arm	mOS		mPFS		ORR	AEs G3
CheckMate-743 [35]	III (open-label)	Nivolumab 3 mg/kg every 2 wk + Ipilimumab 1 mg/kg every 6 wk	18.1 mo	HR: 0.74, P = 0.002	6.8 mo	HR: 1.00	32%	30%
		Cisplatin + Pemetrexed	14.1 mo		7.6 mo		8%	32%
EMPHACIS[5]	III (single blind)	Pemetrexed 500 mg/m^2 and Cisplatin 75 mg/m^2	12.1 mo	HR: 0.77, P = 0.002	5.7 mo	HR: 0.68, P = 0.001	41.3% *vs* 16.7% (P < 0.001)	
		Cisplatin 75 mg/m^2	9.3 mo		3.9 mo		16.7%	
MAPS[12]	III (open-label)	Pemetrexed 500 mg/m^2 and Cisplatin 75 mg/m^2 with 15 mg/kg Bevacizumab in	18.8 mo	HR: 0.77, P = 0.0167	9.2 mo	HR: 0.61, P < 0.0001	NR	71%
		Pemetrexed 500 mg/m^2 + Cisplatin 75 mg/m^2	16.1 mo		7.3 mo			62%

AEs: Adverse events; mOS: Median overall survival; mPFS: Median progression free survival; ORR: Overall response rate; G: Grade; HR: Hazard ratio.

contrast, OS rates were similar in the two groups with < 1% PD-L1 expression (HR = 0.94; 95%CI: 0.62-1.40)[35].

The V-domain Ig-containing suppressor of T-cell activation (*VISTA*) gene has also shown promise as an immunotherapy biomarker in MM. It has been detected in > 85% of patients with MPM, and in two-thirds of cases, it was present in > 50% of cells. Unlike PD-1/PD-L1, it was primarily detected in epithelioid tumors and was associated with significantly improved OS, especially at an expression level > 40%[38]. The *VISTA* gene is thus a promising immunotherapy target and is currently being analyzed in prospective studies.

Tumor mutational burden (TMB) is another potential target, but expression levels vary considerably according to tumor type and are low in mesothelioma. Nonetheless, a recent study of pembrolizumab in the treatment of advanced solid tumors, including MM, showed that high tumor mutational burden expression (> 10 mutations) could identify patients with a better response to pembrolizumab monotherapy[39].

FUTURE PERSPECTIVES IN MESOTHELIOMA

Further advances in immunotherapy for MM in the near future will probably involve combinations of strategies with proven efficacy drugs and continued investigation of new targets and approaches, such as immune checkpoint inhibition combined with chemotherapy and/or antiangiogenic drugs (BEAT-Meso, PrE0506/DREAM3R, PEMBIB)[40]; targeted therapy with AXL inhibitors[41]; other checkpoint inhibitors such as VISTA (NCT02812875), BH73, lymphocyte activation gene-3 (LAG-3), and T cell immunoglobulin and mucin-domain containing-3 (TIM-3); radiotherapy; vaccine-based strategies (MESOVAX); and mesothelin-targeted and metabolism-based therapies.

Other immunomodulatory strategies under investigation are vaccination, T-cell transduction pathway therapies, dendritic cell immunotherapy, adoptive cell therapy (chimeric antigen receptor T-cell) (MesoCancerVa, DENIM)[42], and oncolytic viruses.

Vaccination with Wilms Tumor antigen (WT1) combined with chemotherapy (MESODEC, NCT02649829) and autologous tumor-infiltrating lymphocytes plus interleukin-2 is also being investigated.

Apart from exploring different treatment combinations in advanced MM, researchers should also analyze the benefits of immunotherapy in earlier-stage disease and its perioperative use with multimodal treatment approaches.

CONCLUSION

The treatment options for patients with MPM were very limited until recently and had remained largely unchanged for more than a decade. Recent years, however, have witnessed dramatic improvements in our understanding of this disease and a surge in new research and treatments. From a practical perspective, the main breakthrough has been made in the field of immunotherapy, with two phase III trials set to mark a paradigm shift positioning immune checkpoint inhibitors as first- and second-line

treatment options for MPM. CheckMate 743 is the first phase III trial in over a decade to show a survival benefit for a new treatment—combined CTLA-4 and PD-L1 inhibition–over standard chemotherapy in MPM. The data showed that nivolumab plus ipilimumab significantly improved OS and, as was to be expected based on data from other settings, had an acceptable safety profile. This new strategy is set to become a priority alternative for the frontline treatment of unresectable MPM. The results of the CONFIRM trial signaled another major milestone. In this double-blind randomized phase III trial, intravenous nivolumab 240 mg every 2 wk achieved a significant improvement in OS compared with placebo in patients with previously treated MPM, positioning it as a very likely alternative for the second-line treatment of patients with progressive disease after chemotherapy. Efforts to identify reliable biomarkers to help select the best candidates for immunotherapy must be intensified in the coming years. The evolving landscape will also drive further research into treatment combinations that will hopefully continue to improve OS in this population.

FOOTNOTES

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MINIREVIEWS

New horizons for uncommon mutations in non-small cell lung cancer: *BRAF*, *KRAS*, *RET*, *MET*, *NTRK*, *HER2*

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Abstract

The 2004 discovery of *EGFR* mutations, followed by *ALK* rearrangements, ushered in a targeted therapy era for advanced non-small cell lung cancer (NSCLC). Tyrosine kinase inhibitors targeting gene alterations have substantially improved survival and quality of life for patients with NSCLC. In the last decade, rearrangements of the ROS1 oncogene have been incorporated into healthcare

practice that are applicable to another small subgroup of patients who benefit from similar targeted strategies. Recent genome studies of lung adenocarcinoma have identified other possible therapeutic targets, including *RET*, *NTRK* fusions, *c-MET* alterations, and activating mutations in *KRAS*, *BRAF*, and *HER2,* all with frequencies greater than 1%. Lung cancers harbouring these genome changes can potentially be treated with agents approved for other indications or under clinical development. This review updates the therapeutic arsenal that especially targets those genes.

Key Words: *BRAF*; *NTRK*; *KRAS*; *MET*; *RET*; *HER2*; Non-small cell lung cancer; Targeted therapy; Uncommon mutations

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Core Tip: Compared to other types of cancer, non-small cell lung cancer (NSCLC) is highly genetically altered. Outside of *EGFR, ALK,* and *ROS1*, reflecting 15%-20% of clinical practice, other molecular alterations with important recent advances in their therapeutic arsenal and already in phase II/III trials are *BRAF, KRAS, RET, MET, NTRK,* and *HER2*. The goal is to achieve, compared to conventional treatments such as chemotherapy, better symptom control, better response rates, and improved progression-free survival and overall survival in patients with NSCLC.

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INTRODUCTION

Approximately 60% of lung adenocarcinomas harbour molecular alterations in driver oncogenes, with incidence, which varies according to ethnic origin and alteration, as follows: epidermal growth factor receptor (EGFR) mutation, 15%-20%[1]; anaplastic lymphoma kinase (*ALK*) rearrangement, 5%-7%[2]; and c-ros 1 (*ROS1*) rearrangement, approximately 1%[3]. There has been an impressive improvement in survival in response to tyrosine kinase inhibitors (TKIs), which also have a better toxicity profile compared to standard chemotherapy.

The consequent improvement in molecular understanding of non-small cell lung carcinoma (NSCLC) has allowed increasingly exhaustive molecular classification as well as identification of a subset of patients susceptible to specifically targeted therapy. The outcome of massive gene-sequencing platforms with higher throughput than gene-to-gene determinations is that patients can be offered more treatments that more specifically impact on their quality of life and survival. The current recommendation is to carry out a comprehensive molecular analysis using multiplex platforms – next-generation sequencing (NGS) – if available, considering advantages in terms of coverage, time, and a favorable economic profile[4]. NGS is capable of detecting less common or difficult-to-identify oncogenes, such as Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations (30%-35%), V-raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations (4%-5%), mesenchymal-epithelial transition factor (*c-MET*) alterations, exon 14 insertions and/or amplifications (5%-9%), rearrangements during transfection (*RET*) (1%-2%), human epidermal growth factor receptor 2 (*HER2*) mutations (2%), and neurotrophic receptor tyrosine kinase (*NTRK*) fusions (< 1%)[5]. Identifying these alterations is increasingly important, as new specific drugs in clinical development show promise in terms of modifying the natural history of NSCLC. We focus on direct inhibitors of pathways and their practice-changing results.

BRAF

Present in 2%-3% of NSCLC cases, the *BRAF* mutation is mostly encountered in patients diagnosed with adenocarcinoma[6]. The most common variant is *V600E*, found in 50%-60% of patients with *BRAF*-mutated (*BRAF*m) NSCLC. Not clear is the prognostic value of *BRAF-V600E* compared with non-*V600E* or with the rest of patients with NSCLC[7].

The drugs used to date for this molecular alteration are the same TKIs that have proven to be effective in treating melanoma, a tumour with high *BRAF*m frequency.

Table 1 summarizes the efficacy of the main drugs used to date. The best results have been reported for dabrafenib combined with trametinib, which attempt to block the MAPK pathway at two different sites (*BRAF* and *MEK*), thus overcoming possible tumour resistance to TKIs. The BRF113928 study in patients who received 2-4 Lines of therapy reported an objective response rate (ORR) of 63.2%, and a first-line ORR of 64%[8-12].

However, the absence of comparative data for first and subsequent lines of therapy as currently used for this group of patients means that it is not possible to confirm significant clinical benefit and efficacy over alternative therapies. Dabrafenib and trametinib may therefore be of use for patients for whom standard therapies are not possible or have failed.

Phase II studies are also currently recruiting for the encorafenib + binimetinib (NCT04526782) and cobimetinib + vemurafenib (NCT03178552) combinations.

KRAS

KRAS is the most common mutation in NSCLC, present in up to 30% of adenocarcinomas[13]. In 80% of cases it is located at codon 12, and the most frequent mutation is *KRAS-G12C*, reflected in 13% of all lung adenocarcinomas. It is considered practically exclusive in relation to any other clinical practice drivers, although co-occurrences have been found with alterations in *TP53*, cyclin dependent kinase inhibitor 2A/B (*CDKN2A/B*), *STK11*, and *KEAP1 (*Kelch Like ECH Associated Protein 1)[14].

While *KRAS* has been a therapeutic target for decades, no direct therapeutic option has been established. In recent years, new direct inhibitors of *KRAS-G12C* have emerged. Phase II trial results for sotorasib, an irreversible and highly selective *KRAS-G12C* inhibitor, have positioned it as a major lung cancer milestone for the *KRAS* mutation[15,16]; for 126 included patients, the ORR was 37.1%, there were three complete responses (CRs) and 43 partial responses (PRs), and the disease control rate was 80.6%, for a median progression-free survival (PFS) of 6.8 mo and a good tolerability profile. Based on those data, an application for marketing authorization has been submitted to the FDA and EMA.

In two presentations at the 32nd Symposium on Cancer Therapeutics and Molecular Targets EORTC-NCI-AACR[17,18], investigators from the KRYSTAL-1 phase I and II clinical trial reported that adagrasib clinical activity has been demonstrated in previously treated patients with NSCLC and the *KRAS-G12C* mutation. Promising preliminary data for this drug are to be further evaluated in trials, along with combinations, including with pembrolizumab in the KRYSTAL-7 phase 2 trial (NCT04613596) of untreated patients[19].

RET

RET gene fusions and activating point mutations are primary oncogenic drivers that are usually mutually exclusive with other oncogenic driver alterations[20]. Among the various oncogene drivers in NSCLC, the *RET* gene is involved in various chromosomal rearrangements, found in 1%-2% of all NSCLC patients[21].

Most of the drugs active against *RET* are TKIs. Multikinase inhibitors initially studied in phase II clinical trials include cabozantinib, nintedanib, lenvatinib, vandetanib, and sorafenib, each with a different ORR (Table 2)[22-25].

Selpercatinib (LOXO-292) is a highly selective, potent, central nervous system (CNS)-active, small-molecule *RET* kinase inhibitor. Selpercatinib has nanomolar potency against wild-type *RET* and other *RET* alterations, including the *KIF5B-RET* fusion and *V804M* gatekeeper mutation, in both enzyme and cellular assays, with minimal activity against other kinase and non-kinase targets[26].

In the LIBRETTO-001 phase I/II trial, selpercatinib treatment demonstrated clinically meaningful responses and sustained antitumour activity, for a manageable toxicity profile, in both heavily pre-treated and treatment-naive patients, and including patients with brain metastases and with *RET* fusion-positive NSCLC (intracranial CNS (n = 10/11): ORR 91%). In May 2020, selpercatinib was approved by the FDA under the Accelerated Approval programme for the treatment of *RET*-altered cancers (NSCLC and thyroid cancer)[27].

Pralsetinib (BLU-667) is a novel small-molecule *RET* inhibitor, designed for high potency and selectivity against oncogenic *RET* alterations, including the most frequent *RET* rearrangements (*e.g.*, *KIF5B–RET* and *CCDC6–RET*). The global phase I/II ARROW study has demonstrated broad and durable antitumour activity for pralsetinib in a variety of advanced *RET*-altered solid tumours, including *RET* fusion+ NSCLC. For 354 patients with advanced solid tumours who received pralsetinib as first-line treatment, the ORR was 73%, for a 12% CR rate (n = 26). Treatment-related adverse events were most frequently grade 1-2[28]. Table 2 summarizes the activity of the different TKIs against *RET*.

Table 1 Phase II trials with BRAF inhibitors

Drug	*n*	ORR (%)	PFS (mo)	OS (mo)
Vemurafenib BRAF V600E[8]	62	37.1	6.51	15.38
Vemurafenib V600E[9]	101	0	5.2	10
Vemurafenib non-V600E[9]	17	44.9	NR	NR
Dabrafenib in 2nd line or beyond[10]	78	33.3	5.5	12.7
Dabrafenib + trametinib in 2nd line or beyond[11]	57	63.2	10.2	18.2
Dabrafenib + trametinib en 1st line[12]	36	64	10.9	24.6

ORR: Overall response rate; PFS: Progression free survival; OS: Overall survival; NR: Not reported.

Table 2 Phase II trials with multikinase RET inhibitors

Drug	*n*	ORR	PFS	OS
Cabozantinib[22]	25	28%	5.5 mo	9.9 mo
Vandetanib[23]	18	18%	4.5 mo	11.6 mo
Lenvatinib[24]	25	16%	7.3 mo	NR
Sorafenib[25]	3	0	NR	NR
Selpercatinib[26]	105	64% in platinum chemotherapy pretreated 85% in platinum chemotherapy naïve	90% in response at 6 mo	NR
Pralsetinib[27]	106	61% in platinum chemotherapy pretreated 73% in platinum chemotherapy naïve	NR	NR

ORR: Overall response rate; PFS: Progression free survival; OS: Overall survival; NR: Not reported.

RXDX-105 differs from the other multi-targeted TKIs because it has *RET* activity but limited activity against the vascular endothelial growth factor (*VEGF*) receptors. In *RET* TKI-naive patients, the drug showed modest activity. Subset analysis revealed that the ORR varied by fusion partner. ORRs were 0% (0/20) in the *RET-KIF5B* rearrangement subset (the most common rearrangement) and 67% (6/9) in the *RET*-non-*KIF5B* rearrangement subset[29].

MET

c-MET is an oncogene that encodes a tyrosine kinase receptor whose ligand is hepatocyte growth factor (*HGF*). Alterations in *c-MET* (mutation, amplification, or overexpression) cause abnormal receptor activity that is associated with rapid tumour growth, greater tumour aggressiveness, and resistance to cancer treatments[30].

c-MET amplification is present in 1%-6% of patients with NSCLC. Skipping mutation of exon 14 occurs in 3%-4% of cases, most frequently for non-squamous and sarcomatoid histologies (20%-30%). This alteration occurs most frequently in older patients and in smokers.

Selective and non-selective *c-MET* inhibitors (Tables 3 and 4) are currently available that can impact on survival in patients with NSCLC. The first drug to demonstrate efficacy with this tumour subtype was crizotinib: In the PROFILE 1001 study, the ORR was 32% and PFS was 7.3 mo[31].

Capmatinib is another drug that has been shown to be active: in the GEOMETRY MONO-1 study, the ORR was 41% and PFS was 5.4 mo in previously treated patients; in first-line patients, the ORR was 68% and PFS was 12.4 mo, while ORR was 54% for intracranial activity[32]. In the VISION study, tepotinib achieved an ORR greater than 40%, irrespective of the therapy line, PFS of 8.5 mo, and an ORR of 55% for intracranial activity[33]. Regarding *MET* amplification, TKIs have only significantly benefited tumours with a high level of amplification (*MET/CEP7* > 5), for an ORR of 40% with crizotinib and of 47% with capmatinib.

Amplification, which may appear de novo or as a mechanism of resistance to the targeted treatment of *EGFR* tumours, is present in 4% of cases of progression to first/second generation inhibitors, and in 15% of cases of progression to osimertinib. Being explored, therefore, is the combination of *EGFR*

Table 3 Mesenchymal-epithelial transition factor inhibitors

Drug	MET-specific	Type	Other targets	IC50 (nmol/L)
Crizotinib	No	Ia	ALK, ROS1	22.5
Capmatinib	Yes	Ib	--	0.6
Tepotinib	Yes	Ib	--	3
Salovitinib	Yes	Ib	--	2.1
Bozitinib	Yes	I	--	0.51
Cabozantinib	No	II	RET, ROS1, VEGFR2, KIT	7.8
Merestinib	No	II	TIE-1, AXL, ROS1, DDR1/2, FLT3, MERTK, RON	8.1
Glesatinib	No	II	MET, VEGFR, RON, TIE-2	21.1

IC50: Half maximal inhibitory concentration; MET: Mesenchymal-epithelial transition factor.

Table 4 Clinical trials of mesenchymal-epithelial transition factor inhibitors

Drug	Clinical trial	Phase	Treatment	Objective	Status
Glesatinib	NCT02954991	2	Glesatinib + Nivolumab	ORR	Active, not recruiting
Multi-TKI					
Glesatinib	NCT02544633	2	Glesatinib	ORR	Completed
Multi-TKI					
Merestinib	NCT02920996	2	Merestinib	ORR	Active, not recruiting
Multi-TKI					
Savolitinib	NCT02897479	2	Savolitinib	ORR	Active, not recruiting
Selective-TKI					
Telisotuzumab (ABBV 399)	NCT03574753	2	ABBV-399	ORR	Completed
MET-mab					
JNJ-61186372	NCT02609776	1	JNJ-61186372	ORR, security	Recruiting
EGFR and MET mab					

TKI: Tyrosine kinase inhibitor; mab: Monoclonal antibody; ORR: Overall response rate; MET: Mesenchymal-epithelial transition factor; EGFR: Epidermal growth factor receptor.

inhibitors and *MET* inhibitors.

The TATTON study explored osimertinib combined with savolitinib in patients with NSCLC and mutated *EGFR*. In the group that received initial treatment with a first/second generation inhibitor, the ORR was 52%, while in the group that received osimertinib, the ORR was 25%, for an acceptable toxicity profile[34].

As for immunotherapy, despite the fact that the tumours may present with elevated *PD-L1* expression, the benefit reported for retrospective studies by a French group was limited, at an ORR of 16% and PFS of 3.4 mo[35].

NTRK

The tropomyosin receptor kinase (*TRK*) family consists of three tyrosine kinase receptors – *TRKA, TRKB,* and *TRKC* isoforms, encoded by the *NTRK1, NTRK2,* and *NTRK3* genes, respectively – that are mainly expressed in the nervous system. Their fusions involve some 80 associated genes and they are known oncogenic drivers[35-38]. The incidence of *NTRK* fusions in NSCLC is estimated to be 0.1%-0.2%, affecting a population that is unselected in terms of sex, age, or smoking[37].

Currently, two first-generation TKIs targeting *NTRK* fusions have been approved by the FDA and the EMA: entrectinib (multikinase *ALK, ROS1,* and pan-*TRK* inhibitor) and larotrectinib (selective pan-*TRK* inhibitor). Both have demonstrated great efficacy (irrespective of histology or fusion gene) and intracranial activity, as well as good toxicity profiles[38-41].

Larotrectinib efficacy and safety in patients with solid tumours and *NTRK* fusions have been evaluated in two registrational phase I/II studies (NCT02122913 and NCT02576431). By July 2020, 20 patients with TRK fusion-positive lung cancer had been treated. Joint analysis of those studies, yielded an ORR of 73% and a CR rate of 7% for patients with lung cancer. The median PFS and OS in lung cancer patients was 35.4 and 40.7 mo. Among patients with baseline central nervous system metastases, the ORR was 63%. Reported adverse events were mostly grade 1-2[38].

Entrectinib was evaluated in the phase I ALKA-372-001 trial, phase I STARTRK-1 trial and phase II STARTRK-2 basket trial. For the 10 patients with NSCLC, the ORR was 70%, the CR rate was 10%, and PFS was 14.9 mo. Entrectinib showed a good toxicity profile; most adverse events were grade 1 or 2 and reversible, *e.g.*, dysgeusia, constipation, fatigue, diarrhoea, oedema, and dizziness[39].

Selitrectinib (LOXO 195), repotrectinib (TPX-0005), and taletrectinib (DS-6051b/AB-106) are second-generation drugs capable of inhibiting on-target resistance of *NTRK*[37,40]. They are currently being evaluated in phase I/II clinical trials in patients with *NTRK*-positive tumours who have progressed to first-generation inhibitors (NCT03215511, EudraCT 2017-004246-20, NCT04094610, TRIDENT-1: NCT03093116, NCT02279433).

HER2

HER2 is a cell growth promoting protein, a member of the *ERBB* family of tyrosine kinase receptors expressed on the surface of many types of tumours.

Overexpression, which occurs in 2%-20% of cases depending on the immunohistochemistry (IHC) level (IHC2+/3+), is associated with a poor prognosis. *HER2* amplification occurs, especially in adenocarcinomas, in around 3% of cases without prior treatment and in approximately 10% of cases of *EGFR* resistance to TKIs[42].

HER2 mutations *(HER2*m) – usually consisting of insertions in exon 20, especially in codon 776 – appear mainly in women, in adenocarcinoma cases, and in the Asian population, and never in smokers. The insertions cause constitutive activation of the receptor, making it sensitive to dual TKI action against *EGFR* and *HER2*, but not exclusively to *EGFR* inhibition[43].

The therapies commonly used to target *HER2* in breast cancer have not had the same results for NSCLC. The emergence of new TKIs and conjugated antibodies have given a new boost to therapies for this molecular alteration in NSCLC (Table 5). Reported for the largest retrospective EUHER2 study, which included patients with *HER2* exon 20 insertions, was an ORR of 7.4% for treatment with the TKIs afatinib, lapatinib, and neratinib; for the trastuzumab antibody and the trastuzumab emtansine (T-DM1) antibody-drug conjugate, the ORR was a more effective 50.9%, but that treatment was in most cases combined with chemotherapy[44,45].

Two phase II studies, of neratinib combined with trastuzumab in *HER2*m patients in first or successive therapy lines (NCT01953926) and of neratinib with temsirolimus (NCT01827267), have reported ORRs of 17% and 19%, respectively[46]. Zhou *et al*[47] explored the efficacy of pyrotinib in monotherapy, reporting an ORR of 30%, median PFS of 6.9 mo, and overall survival (OS) of 14.4 mo; the main toxicity, as with other *HER2*-targeting TKIs such as neratinib and lapatinib, was diarrhoea. In the phase II ZENITH20 trial of poziotinib, another pan-*HER* TKI, for the *HER2*m treatment the ORR was 28%, PFS was 5.5 mo, and the toxicity profile was similar to that for pyrotinib[48].

In addition to the *HER2* TKIs, also being evaluated in this setting are antibody-drug conjugates such as T-DM1 and trastuzumab deruxtecan (DS-8201, T-Dxd). Peters *et al*[49]. explored responses to TDM-1 in 49 patients with IHC2+/3+ overexpression, reporting no response for the IHC2+ cohort and 4 PRs for the IHC3+ cohort (20%). Better data is available for trastuzumab deruxtecan. For 42 patients with *HER2* m in the DESTINY-Lung01 cohort, the ORR was 62%, PFS was 14 mo; median OS was not achieved, while OS was 24.5% in the IHC2+/3+ overexpression cohorts[50].

To confirm the PFS benefit, a phase III trial of pyrotinib *vs* docetaxel called PYRAMID-1 (NCT04447118) is ongoing.

CONCLUSION

Compared to traditional chemotherapy, the improved TKI targeting of *EGFR* mutations and *ALK/ROS1* translocations has led to significant efficacy and quality of life improvements in the management of patients with NSCLC. While this subgroup of patients inevitably develops resistance to TKIs, this can be overcome by developing new next-generation TKIs or drugs aimed at overcoming resistance from the outset or from the time of discovery[51,52].

Table 5 Phase II trials with HER2 inhibitors

Drug	Molecular alteration	*n*	ORR%	PFS (mo)	OS (mo)
Dacomitinib[44]	HER2 mutant	26	12	NR	NR
	HER2-amplified	4	0	NR	NR
Neratinib + Trastuzumab[46]	HER2 mutant	52	17	4	10.2
Neratinib + Temsirolimus[46]	HER2 mutant	43	19	4	15.1
Pyrotinib[47]	HER2 mutant	60	30	6.9	14.4
Poziotinib[48]	HER2 mutant	90	28	5.5	NR
Trastuzumab emtansine[49]	IHC 2+	29	0	2.6	12.2
	IHC 3+	20	20	2.7	15.3
Trastuzumab deruxtecan[49]	HER-2 mutant	42	61.9	NR	NR
Trastuzumab deruxtecan[49]	IHC 2+	39	25.6	5.4	11.3
	IHC 3+	10	20		

ORR: Overall response rate; PFS: Progression free survival; OS: Overall survival; NR: Not reported.

These developments may also be transferable to the treatment of patients with other molecular alterations of *BRAF, KRAS, RET, MET, NTRK* and *HER2*. As can be seen above, a growing number of drugs and combinations are becoming available that target these alterations, often producing a significant improvement in response and survival rates.

Given the many common and rare molecular alterations in NSCLC, full-panel multigene NGS is recommended rather than gene-by-gene sequencing, as not only is it more cost-effective, it allows patients with a target to be easily identified and treated, whether with an approved drug or in a clinical trial of a promising drug[53-55].

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Is there utility for fluorine-18-fluorodeoxyglucose positron-emission tomography scan before surgery in breast cancer? A 15-year overall survival analysis

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Abstract

BACKGROUND

The prognostic value of preoperative fluorine-18-fluorodeoxyglucose positron-emission tomography (^{18}F-FDG PET) scan for determining overall survival (OS) in breast cancer (BC) patients is controversial.

AIM

To evaluate the OS predictive value of preoperative PET positivity after 15 years.

METHODS

We performed a retrospective search of the Universitair Ziekenhuis Brussel patient database for nonmetastatic patients who underwent preoperative PET between 2002-2008. PET positivity was determined by anatomical region of interest (AROI) findings for breast and axillary, sternal, and distant sites. The prognostic role of PET was examined as a qualitative binary factor (positive *vs* negative status) and as a continuous variable [maximum standard uptake value (SUVmax)] in multivariate survival analyses using Cox proportional hazards models. Among the 104 identified patients who received PET, 36 were further analyzed for the SUVmax in the AROI.

RESULTS

Poor OS within the 15-year study period was predicted by PET-positive status for axillary (P = 0.033), sternal (P = 0.033), and combined PET-axillary/sternal (P = 0.008) nodes. Poor disease-free survival was associated with PET-positive axillary status (P = 0.040) and combined axillary/sternal status (P = 0.023). Cox models confirmed the long-term prognostic value of combined PET-axillary/sternal status [hazard ratio (HR): 3.08, 95% confidence interval: 1.42-6.69]. SUVmax of ipsilateral breast and axilla as continuous covariates were significant predictors of long-term OS with HRs of 1.25 (P = 0.048) and 1.54 (P = 0.029), corresponding to relative increase in the risk of death of 25% and 54% per SUVmax unit, respectively. In addition, the ratio of the ipsilateral axillary SUVmax over the contralateral axillary SUVmax was the most significant OS predictor (P = 0.027), with 1.94 HR, indicating a two-fold relative increase of mortality risk.

CONCLUSION

Preoperative PET is valuable for prediction of long-term survival. Ipsilateral axillary SUVmax ratio over the uninvolved side represents a new prognostic finding that warrants further investigation.

Key Words: Restricted mean survival time; Long-term prognosis; Overall survival; Preoperative workup; Breast surgery; Positron-emission tomography scan

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Core Tip: In our study population of nonmetastatic breast cancer patients, preoperative fluorine-18-fluorodeoxyglucose positron-emission tomography (^{18}F-FDG PET) scan provided valuable overall survival prognostic information. This retrospective study included the longest (15-year) follow-up observation period to date in a series of these patients. Data from anatomical regions of interest and statistical analyses determined that the ipsilateral axillary maximum standard uptake value (SUVmax) with reference to the contralateral uninvolved axilla was the strongest predictor of survival.

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INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed cancer in the female population worldwide, as well as the most common cause of cancer deaths among women[1]. For staging and disease assessment, current guidelines acknowledge the use of fluorine-18-fluorodeoxyglucose (National Library of Medicine Medical Subject Headings [MeSH] entry term: ^{18}F-FDG) positron-emission tomography [MeSH entry term: positron-emission tomography (PET) scan] before surgery for patients with a IIB or higher stage of BC, and reservedly for patients in stage IIA[2,3]. The potential staging advantage of PET combined with computed tomography (PET/CT) over CT scan alone or even bone scintigraphy is that PET/CT can more efficiently detect lymph node invasion and distant metastases[4].

Unfortunately, the published evidence to support the use of PET/CT to predict disease prognosis has been contradictory or of insufficient quality. Some studies have indicated that the specific survival rate is significantly shorter when metastases have been detected on PET[5]. One study determined PET-positive status for axillary nodes to be the foremost preoperative prognostic factor for disease-free survival (DFS) at 5 years[6]. Additionally, a few studies have suggested that the maximum standard uptake value (SUVmax) of the primary tumor could be predictive of survival; however, the threshold values have differed among studies[7,8].

The lack of consensus on the prognostic value of PET may be due to short follow-up times in the previous studies. Additionally, in a study that compared PET with various prognostic markers, the value of PET was overshadowed by assessment of lymph nodes obtained *via* axillary lymph node dissection (authors' reference cited in Data Sharing Statement). However, sentinel node biopsy has taken precedence over axillary dissection, and analyzing the data without axillary dissection would be more relevant to current practice. Furthermore, the potential role for SUVmax in prognosis has not yet been fully considered, and no consideration has been given to the restricted mean survival time (RMST), although it enjoys a growing acceptance rate among clinical practitioners as a preferred survival measurement[9]. Based on all these factors, renewed analyses into the prognostic role of preoperative PET positivity on the long-term outcome of BC needs to be addressed.

MATERIALS AND METHODS

Study design, patients and data

Patients with primary BC who underwent a PET scan prior to their originally recommended surgery as part of the multidisciplinary team management at the Universitair Ziekenhuis Brussel (UZ Brussel; https://www.uzbrussel.be/) between 2002-2008 were identified retrospectively. Patients who met any one or more of the following criteria were excluded: noncarcinoma histology; palliative surgery; or clinically-detected metastatic disease. Follow-up data was collected for each included patient, with the last update having occurred on January 31, 2020.

This study was approved by the institution's ethics committee. All diagnostic and therapeutic procedures had been performed in accordance with the local national guidelines and the Declaration of Helsinki 1964. All patients had received appropriate information and provided informed consent to undergo the procedures. All clinical data were described previously[6], and the steps used in data acquisition are detailed at https://dx.doi.org/10.17504/protocols.io.bf7jjrkn. The study's collective data are available at https://doi.org/10.17632/sfvtmrd8z9.2.

^{18}F-FDG PET scan image acquisition

Patients had fasted for + 6 h, and the PET scan had been rescheduled if hyperglycemia was detected. For all, the tracer activity was in the range of 370-536 MBq (mean: 464 ± 56 MBq). Sixty minutes after tracer perfusion, each patient had been placed in a supine position with arms extended above the head. Whole-body images had then been acquired using attenuation correction and an interleaved protocol with an LSO PET camera (ECAT Accel; Siemens, Hoffman Estates, IL, United States). The parameters of emission data, 2-dimensional mode, and reconstruction-segmentation used were identical to the procedure described elsewhere[6]. Synchronous computed tomography had not been available at the time.

Image analyses

We abstracted the PET image data according to the anatomical regions of interest (AROIs) and according to the source of the available image media storage.

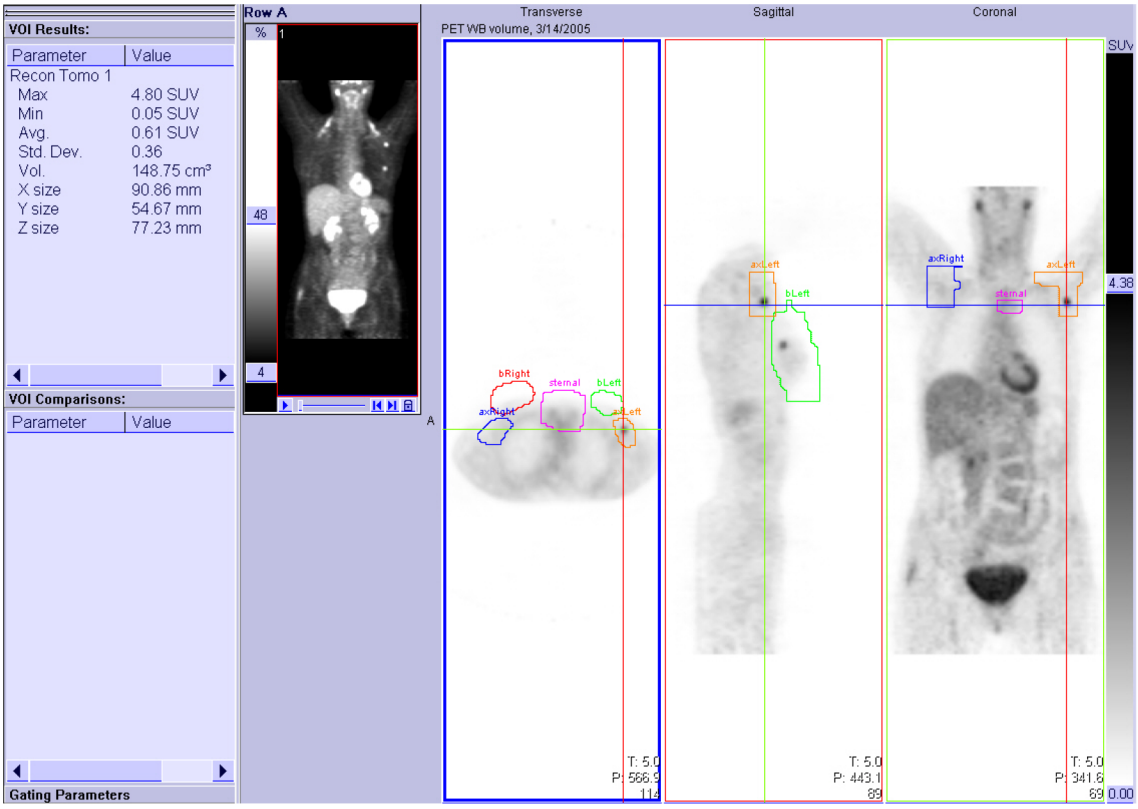
The AROI selected for this study conformed to anatomical regions considered in the radiotherapy of the breast[10], including the whole ipsilateral breast, whole contralateral breast, ipsilateral axillary-supraclavicular lymph node region (shortened to "axillary" in the remainder of the present report), contralateral axillary-supraclavicular lymph node region, sternal-mediastinal area (internal mammary chain), and the remaining body volume (*i.e.* distant, outside the breasts, axillary and sternal areas).

The image media storage comprised two forms: a medical FDG-PET diagnostic report with a screen image printout (for all patients) and image files archived on the PET server (for some patients). First, using the medical diagnostic report, the image in each AROI was assigned a binary score of positive or negative according to visibly increased activity or lack thereof in the AROI. Next, using the PET server workstation and accompanying free-hand volumetric drawing tool, the 3-dimensional AROIs were delineated on all available patient PET scan datasets. Ultimately, the SUVmax was abstracted for each patient from their AROIs (Figure 1).

Repeated PET scans were excluded. For bilaterally involved breasts, only the scan from the first side involved was retained, or from the side with more extensive axillary surgery if there was doubt about the diagnostic precedence.

Statistical analysis

Missing data were imputed using multivariate imputation by chained equations[11] for first histopathology finding (1 missing), HER2-neu score (1 missing), estrogen receptor (ER) and progesterone receptor (PR) status (1 missing), grade (4 missing), tumor size (1 missing), and number of examined and positive nodes (5 missing). Lymphovascular invasion was dichotomized as present *vs* no invasion or unknown (20 missing). HER2 fluorescence *in situ* hybridization (FISH) status was considered as nonimputable and was therefore excluded from the analysis (51 missing).



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Figure 1 Anatomical regions of interest. Free-hand three-dimensional non-overlapping anatomical regions of interest (AROI) volumes drawn on the PET scan workstation for the right breast (red), right axilla-supraclavicular nodal area (blue), left breast (green), left axilla-supraclavicular (orange), and sternal-mediastinal area (purple). Viewing planes are indicated by crosshairs.

Survival analyses used times from the date of first pathological diagnosis to the date of last known status. The outcome event for overall survival (OS) was death from any cause. The outcome events for DFS were any local-regional or distant recurrence, secondary tumor, or death from any cause. Survival rates were computed using the Kaplan-Meier method[12]. The expectation of remaining years of life, or the RMST, up to a time horizon of 15 years was used to summarize the patients' survival[9]. This value was calculated as the area below the survival curve. The area between survival curves measures the difference between RMSTs (Δ), but the precision and statistical significance are affected by the amount of censoring. As in other tests comparing survival curves, the difference and the area between the two curves can appear large and still might be nonsignificant when there are few events. Δ is expressed in years, in the same unit of time as the survival time, and indicates the impact of prognostic factors in terms of difference in life expectancy according to the prognostic levels.

The prognostic role of PET was examined as a qualitative binary factor (positive *vs* negative status) and as a continuous variable (SUVmax) in multivariate survival analyses using Cox proportional hazards models[13]. The models excluded pathological lymph node involvement, which is a strong predictor of survival that would otherwise confound and mask the significance of other factors (authors' reference cited in Data Sharing Statement). Long-term follow-up with OS and DFS at 15 years was used to validate models previously reported elsewhere[6]. The Nagelkerke index of explained variation (*R2N*) and the Royston-Sauerbrei index of prognostic separation (*R2D*) were computed to evaluate the Cox models[14].

All computations was carried out with the R statistical software, version 3.6.3[15], with the *mice* package for missing data imputation, and the *survRM2* package for the RMSTs.

RESULTS

A total of 157 consecutive records were identified, out of which 53 cases were excluded for postsurgical PET performance (n = 17), breast surgery not specified in the original multidisciplinary team's recommended management of the case (n = 12), history of previous cancer (n = 8), nonunique records (n = 5), no diagnosis of cancer (n = 2), axillary surgery not performed (n = 2), no histology data (n = 2), noncarcinoma (n = 2 sarcoma, n = 1 noninvasive tumor), and no information on the primary therapy (n = 2). Among the remaining 104 patients representing the study population, surgery had been performed

at a median of 6 d after the PET imaging for the 85 (81.7%) patients who did not receive neoadjuvant therapy and at a median of 83 d after the PET imaging (range: 5-201 d, n = 1 date unknown) for the 19 (18.3%) patients who received neoadjuvant therapy. The type of breast surgery performed was lumpectomy in 26 (25.0%) patients, mastectomy in 77 (74.0%), and exclusive radiotherapy assimilated to mastectomy in 1 (1.0%). All lymph node surgeries were limited to the axilla, without exploration of the internal mammary chain. Table 1 summarizes the main characteristics of the study's patient population.

The patterns of PET observed among the total 104 patients were abstracted as binary score, and the SUV measurements of 36 patients for whom imaging could be retrieved are listed in Table 2 and Table 3, respectively. PET status of axillary, combined PET axillary/sternal, and combined PET all-sites were significantly related to tumor size (Table 2). SUVs were measured on the AROIs regardless of visual enhancement, making them available in all breast, axillary, and sternal sites. Ipsilateral local-regional uptake also correlated with tumor size (Table 3). For distant sites, the SUV measurements were carried out on visually hypermetabolic areas only, and therefore, the information was limited to 8 non-analyzable cases.

Surviving patients (no OS event) had a median follow-up of 15.1 years. The OS was significantly worse for those who had a PET-positive axillary nodal status (Δ = 2.2 years, P = 0.033); thus, at a time horizon of 15 years, patients with a PET-positive axillary status had a life expectancy that was 2.2 years shorter than patients with a PET-negative axillary status. Patients with a PET-positive sternal nodal status also had a shorter life expectancy, with Δ of 5.1 years (P = 0.033) (Figure 2). When patients were considered as a group defined by PET-positive axillary status and/or sternal status but without distant positivity (*i.e.*, regional positivity without distant positivity, regardless of breast local status), life expectancy was decreased, with Δ of 2.6 years (P = 0.008). When patients were considered as a group defined by any PET-positive status for axillary, sternal or distant (*i.e.* any regional or distant positivity, regardless of breast local status), life expectancy was also decreased, with Δ of 2.1 years (P = 0.025). Survival differences were not evident when comparing the PET-positive groups for breast or distant separately (Figure 2). Survival differences were also not evident on subgroup analyses for PET-positive axillary status for tumors ≤ 20 mm (TNM T1 classification[16]). For tumors > 20 mm, survival differences were evident when axillary and/or sternal status were PET-positive (*i.e.*, regional positivity), with Δ of 3.0 years (P = 0.015).

DFS was also worse for patients with a PET-positive axillary nodal status (Δ = 2.5 years, P = 0.040) and with a PET-positive status in any of the axillary and sternal regions (Δ = 2.6 years, P = 0.023) (Figure 3). DFS was not statistically different for patients with tumors ≤ 20 mm; however, in patients with tumors > 20 mm, DFS was significantly worse for those with PET-positive axillary status (Δ = 3.4 years, P = 0.017), sternal status (Δ = 5.9 years, P = 0.048), and combined axillary and sternal status (Δ = 3.9 years, P = 0.005). The pattern of the DFS survival curves according to tumor size ≤ 20 mm or > 20 mm (data not shown) were comparable to the global DFS's shown in Figure 3.

Multivariate OS and DFS analyses used PET axillary | sternal status, age at diagnosis, and adjuvant hormone therapy covariates, which were the same parameters used previously in our short-term follow-up study[6]. In the earlier study, OS was inconclusive and was not reported, and DFS at 5 years of follow-up indicated significance for PET-positive axillary status only[6]. Table 4 shows a comparison of the previous short-term follow-up DFS hazard ratios (HRs) with the current OS and DFS HRs for the longer follow-up period. The DFS HRs for age and adjuvant hormone therapy at 15 years of follow-up were 1.03 and 0.51, respectively, comparable to the previous DFS HRs of 1.05 and 0.43, respectively, at 5 years of follow-up. That is, despite patients becoming older, from diagnosis at 58.9-years-old (Table 1) to near 75-years-old in the present study, age and adjuvant hormone therapy retained their prognostic value. Of note, however, the DFS HR for PET was 1.74 at 15 years of follow-up, as compared with 2.81 at 5 years of follow-up, suggesting some loss of prognostic value regarding DFS. In contrast, regarding the OS, the *R2N* of 0.077 and *R2D* of 0.159 for PET positivity at 15 years of follow-up (Table 4) indicate that the PET covariate provided prognostic information almost half that of the *R2N* of 0.189 and *R2D* of 0.396 from a full model that was computed by taking into account all three covariates together.

The impact of SUVmax on OS is shown in Table 5. The regions of interest were the tumor and ipsilateral breast, the ipsilateral axillary lymph node region, and the internal mammary chain (sternal). In addition to the absolute SUVmax, the ratios of ipsilateral breast SUVmax relative to the contralateral breast and ipsilateral axillary relative to the contralateral axillary SUVmax were computed.

The absolute SUVmax of the uninvolved contralateral breast and contralateral axilla did not correlate with OS (P = 0.528). Likewise, SUVmax of the contralateral axillary site also did not correlate with OS (P = 0.969). In contrast, the SUVmax of the involved breast and the ipsilateral axillary were significantly associated with OS (P = 0.048 and P = 0.029, respectively). The SUVmax on all combined local-regional sites was also significant (P = 0.032). However, the ratio of SUVmax ipsilateral axilla over SUVmax contralateral was the most significant of all factors examined (P = 0.027), with HR of 1.94, indicating a two-fold relative increased risk of death (Table 5).

Table 1 Patient characteristics

Characteristic	*n*	%
Sex		
Male	2	2.0
Female	102	98.0
Age at diagnosis (yr)		
Median (range)	58.9	(32.5–83.0)
< 40	8	7.7
40-59	51	49.0
≥ 60	45	43.3
Histology		
Invasive ductal carcinoma	84	80.8
Lobular carcinoma	14	13.4
Other	6	5.8
Tumor laterality		
Bilateral	5	4.8
Left	45	43.3
Right	54	51.9
Tumor quadrant		
Inner	16	15.4
Central	14	13.5
Outer	64	61.5
Other	10	9.6
Clinical T4 stage	8	7.7
Tumor size		
0-20 mm (TNM T1 classification[16])	37	35.6
> 20 mm (one imputed as 30 mm)	67	63.5
Stage		
I	18	17.6
IIA	28	27.5
IIB	18	17.6
III	34	33.3
IV	4	3.9
Unknown	2	–
Grade		
1	29	29.0
2	42	42.0
3	29	29.0
Unknown	4	–
Hormone receptor status		
ER+/PR+	67	64.4
ER–/PR–	20	19.2
Other	17	16.3

Events		
Loco-regional recurrence	4 of 104	3.8
Distant metastases	31 of 104	29.8
Death from any cause	28 of 104	26.9

ER+: Estrogen receptor-positive; ER-: Estrogen receptor-negative; PR+: Progesterone receptor-positive; PR-: Progesterone receptor-negative.

Table 2 Positron-emission tomography negative or positive status according to anatomical region of interest and according to tumor size, *n* (%)

Anatomical region of interest	Tumor ≤ 20 mm	Tumor > 20 mm	*P*
	n = 37	*n* = 67	
Breast ipsilateral			< 0.001
PET negative	12 (32.4)	5 (7.5)	
PET positive	25 (67.6)	62 (92.5)	
Axillary ipsilateral			0.006
PET negative	29 (78.4)	34 (50.7)	
PET positive	8 (21.6)	33 (49.3)	
Sternal			0.906
PET negative	35 (94.6)	63 (94.0)	
PET positive	2 (5.4)	4 (6.0)	
Distant			0.447
PET negative	32 (86.5)	54 (80.6)	
PET positive	5 (13.5)	13 (19.4)	
Any of axillary or sternal			0.019
PET negative	27 (73.0)	33 (49.3)	
PET positive	10 (27.0)	34 (50.7)	
Any of axillary, sternal, or distant			0.005
PET negative	26 (70.3)	28 (41.8)	
PET positive	11 (29.7)	39 (58.2)	

PET: Positron-emission tomography.

DISCUSSION

PET-positive axillary nodal and sternal status were found to be the predominant preoperative prognostic factor for OS, providing a stronger prognostic perspective than previously found[6]. Although the present study is limited by its retrospective design, it represents the longest observation period published for the prognostic role of PET scan status in OS of BC patients[7].

To place the present study in context, it started 20 years ago with the first patient diagnosed and receiving preoperative PET in 2002, while the principal investigator was working at his alma mater the UZ Brussel, a university hospital in Belgium. The first case series was presented at the San Antonio Breast Cancer Symposium in 2004 (Poster 2010, *Breast Cancer Res Treat* 2004; 88: S90, reference omitted). Among the patients who had received adjuvant radiotherapy, 32 had previously undergone a PET scan and we found an association between the PET nodal positivity and more extensive nodal involvement. We hypothesized that the larger number of nodes retrieved in PET-positive cases suggested lymphangiogenesis factors associated with an increased tumor metabolic activity. Obviously, preoperative PET would have served as an important tool to tailor radiation treatment fields in BC, as reported by Bral *et al*, who showed how ignorance of PET imaging could lead to failed targeting of radiation for hypermetabolic lymph nodes (*Strahlenther Onkol* 2008; 184(2): 100-4, reference omitted). It was, however, impossible to implement preoperative PET scan in our daily practice at the time of treatment of our

Table 3 Maximum standardized uptake value according to anatomical region of interest and according to tumor size

Anatomical region of interest	Tumor ≤ 20 mm	Tumor > 20 mm	*P*
	n = 18	*n* = 18	
Breast ipsilateral			
SUVmax, mean (SD)	2.3 (1.4)	4.4 (3.1)	0.012
Breast contralateral			
SUVmax, mean (SD)	1.7 (1.3)	1.7 (0.6)	0.918
Axillary ipsilateral			
SUVmax, mean (SD)	1.8 (0.9)	2.8 (1.8)	0.063
Axillary contralateral			
SUVmax, mean (SD)	1.5 (0.5)	1.8 (0.5)	0.140
Sternal			
SUVmax, mean (SD)	1.8 (0.5)	2.1 (0.6)	0.061
All breast, axillary and sternal regions combined			
SUVmax, mean (SD)	2.7 (1.5)	4.8 (3.0)	0.014

SUVmax: Maximum standardized uptake value; SD: Standard deviation.

Table 4 Multivariate models, original model with 5 years follow-up, *vs* current models with 15 yr follow-up

	Disease-free survival 5 yr of follow-up[6]		Disease-free survival 15 yr of follow-up, present study			Overall survival 15 yr of follow-up, present study		
Variable	HR (95%CI)	R2N	HR (95%CI)	R2N	R2D	HR (95%CI)	R2N	R2D
PET axillary/sternal (positive *vs* negative)	**2.81** (1.17, 6.74)	0.059	**1.74** (0.96, 3.14)	0.030	0.053	**3.08** (1.42, 6.69)	0.077	0.159
Age at diagnosis (yr)	**1.05** (1.01, 1.09)	0.046	**1.03** (1.00, 1.06)	0.047	0.075	**1.06** (1.02, 1.10)	0.109	0.227
Adjuvant hormone therapy (yes *vs* no)	**0.43** (0.16, 1.13)	0.030	**0.51** (0.24, 1.08)	0.025	0.049	**0.46** (0.18, 1.18)	0.020	0.083
Full model		NA		0.091	0.142		0.189	0.396

R2N and R2D values shown are the differences between the full model (computed with all three PET, age, adjuvant hormone therapy variables together) and the same full model without the variable. Higher values indicate higher importance of the variable in the model. Hazard ratios > 1 indicate increased risk of mortality. CI: Confidence interval; NA: Not available; HR: Hazard ratio; R2D: Royston-Sauerbrei index of prognostic separation; R2N: Nagelkerke proportion of explained variation.

study's cases because of a Belgium healthcare restriction on PET scan. Only 13 facilities nationwide were allowed to implement PET and our cases fell beyond the defined population restrictions; moreover, BC was not a recognized indication for PET scan[17].

Despite these precluding logistical healthcare restrictions, the need for PET did not abate and we continued to observe a trickle of patients who had received PET. The 2004 study reported only on nodal pathology but had no follow-up. By simple logic, if PET correlates with lymph node involvement, then it would also correlate with survival. The study was reconducted in 2010 without funding, by investigators devoting volunteer time, accruing the present cohort of 104 patients. That study confirmed the nodal correlation and, indeed, provided evidence for the expected impact on early DFS. The analysis was published 2 years later, in 2012[6]. Meanwhile, guidelines did not change and still considered PET inappropriate for the early assessment of BC. To address that issue, we established a quasi-prospective protocol in 2015, with the intent to increase the number of observations with a second cohort of patients. The protocol is available at https://www.isrctn.com/ISRCTN17962845 through the linked file https://www.isrctn.com/editorial/retrieveFile/b3691bca-5277-4025-bd3e-ebea2701d143/38272.

As detailed in the protocol, the number of patients needed was 162. We expected 210 cases to be retrieved in 2015 to allow for comparison of patterns of practice between the cohorts from 2002-2008 (PET without CT) and 2009-2015 (PET with CT). The analysis was intended to be completed in 2020, with the intent to give precedence to explore the innovative concept of SUV ratios (protocol, link in

Table 5 Mortality hazard ratio of the maximum standard uptake value according to the anatomical region of interest, single or combined, where the maximum standard uptake value was measured

Region of interest	HR	*P*
Sternal	3.50	0.080
Breast ipsilateral	1.25	0.048
Breast contralateral	0.72	0.528
Axillary ipsilateral	1.54	0.029
Axillary contralateral	1.03	0.969
Combined breast/axillary/sternal	1.27	0.032
Ratio SUVmax breast ipsilateral/ SUVmax breast contralateral	1.34	0.101
Ratio SUVmax axillary ipsilateral/ SUVmax axillary contralateral	1.94	0.027
Ratio SUVmax in any of breast/axillary/sternal/lowest of SUVmax breast or axillary contralateral	1.50	0.036

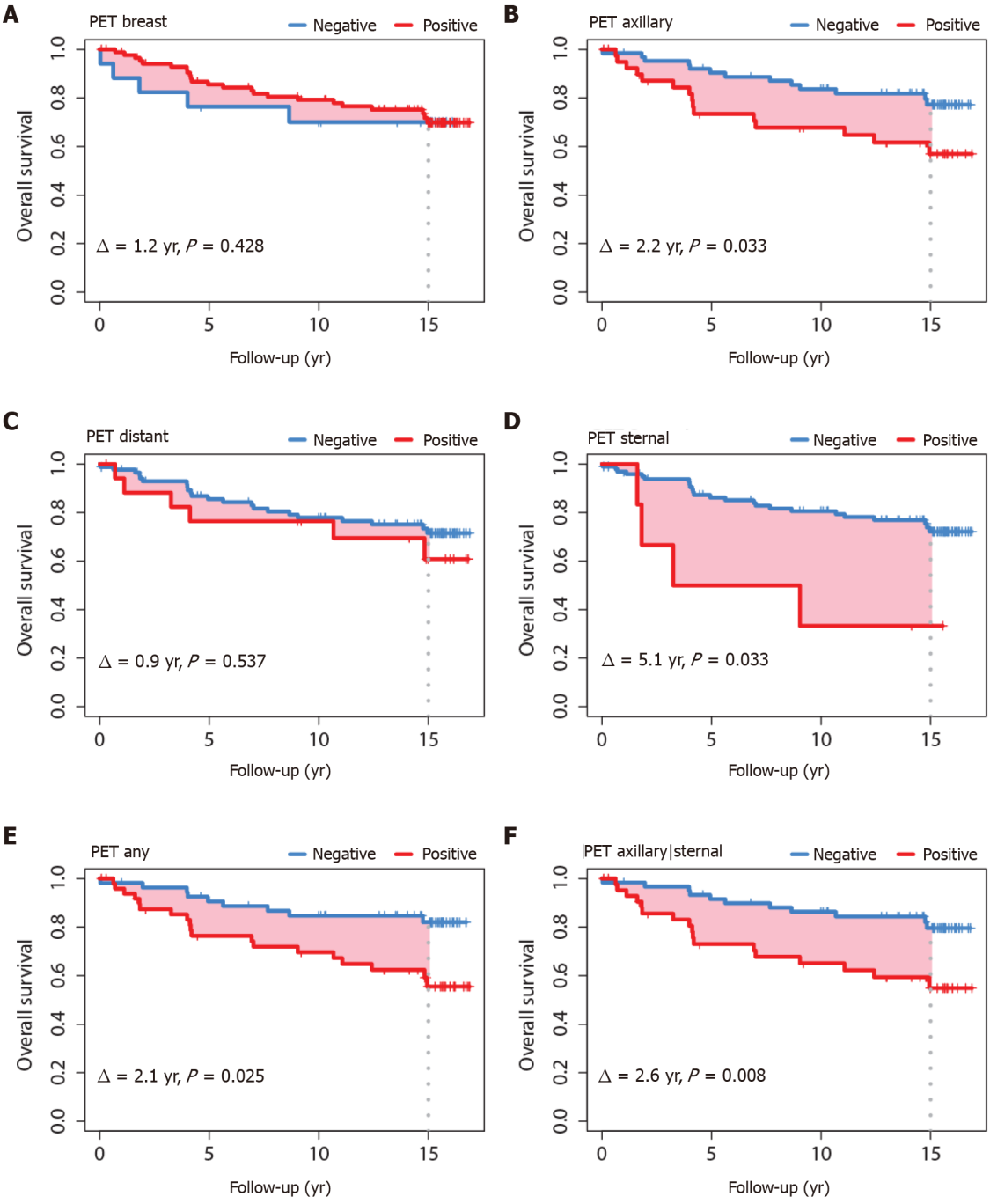
HR: Hazard ratio; SUVmax: Maximum standard uptake value.

ISRCTN17962845). As planned and reported herein, we updated the follow-up of the present cohort on January 31, 2020. Unexpectedly, however, when we returned to the PET server workstation, the 3-dimensional irregular free-hand volumetric measurement tool (Figure 1) had been wiped out by an upgrade to the system. As such, we could not verify the consistency of our earlier AROI delineations and SUVmax measurements. Repeated delineation measurement and extending the present study to a larger cohort have had to be deferred.

The focus of this study was on prognosis, rather than diagnostic accuracy. Nevertheless, a note on the latter is warranted. Out of the 63 patients who had PET-negative axillary status (Table 2, axillary ipsilateral), 29 (imputed) had histopathologically identified involved lymph nodes, and out of the 41 PET-positive axillary patients, 5 had no histopathologically identified node involvement. These data yield a sensitivity of 55% [95% confidence interval (CI): 43%-68%] and a specificity of 87% (95%CI: 73%-96%) which indicate that PET, like other imaging modalities, cannot replace pathology to determine microscopic involvement. This is concordant with findings from a United States' multicentric prospective study for the detection of axillary nodal metastasis; the sensitivity and the specificity of PET were 61% (95%CI: 54%-67%) and 80% (95%CI: 79%-81%) respectively[18]. A meta-analysis of 19 studies including 1729 patients evaluated the performances of PET (with or without CT) for axillary detection and found that the sensitivity was 66% (95%CI: 50%-79%) and the specificity was 96% (95%CI: 90%-99%) [19]. Several tumor characteristics are known to correlate with higher rates of false negatives, such as low tumor grade or proliferation index, lobular histology, estrogen receptor- or progesterone receptor-positive status[20,21], in contrast to higher FDG uptake being less likely to cause false negativity in tumors exhibiting high proliferation rate and enhanced microvasculature[22].

In a previous study that focused on metastatic disease, the OS for 47 out of 189 patients with stage IIB and higher classified M1 by PET in a 3-year follow-up was significatively shorter (57% *vs* 88%, $P < 0.0001$)[23]. Other authors also noted that at stage IIB and higher, survival of patients was shorter when a distant metastasis was detected on PET-CT regardless of tumor phenotype[5]. However, several studies found that positive PET status is predictive of recurrence and survival, irrespective of metastatic status, most likely because of the correlation with poor clinicopathologic factors[24]. Our study was not designed to correlate between distant metastasis and survival. Metastatic patients were excluded from the selection, hence, there were few remaining cases detected afterwards with additional distant localizations (8 in this series).

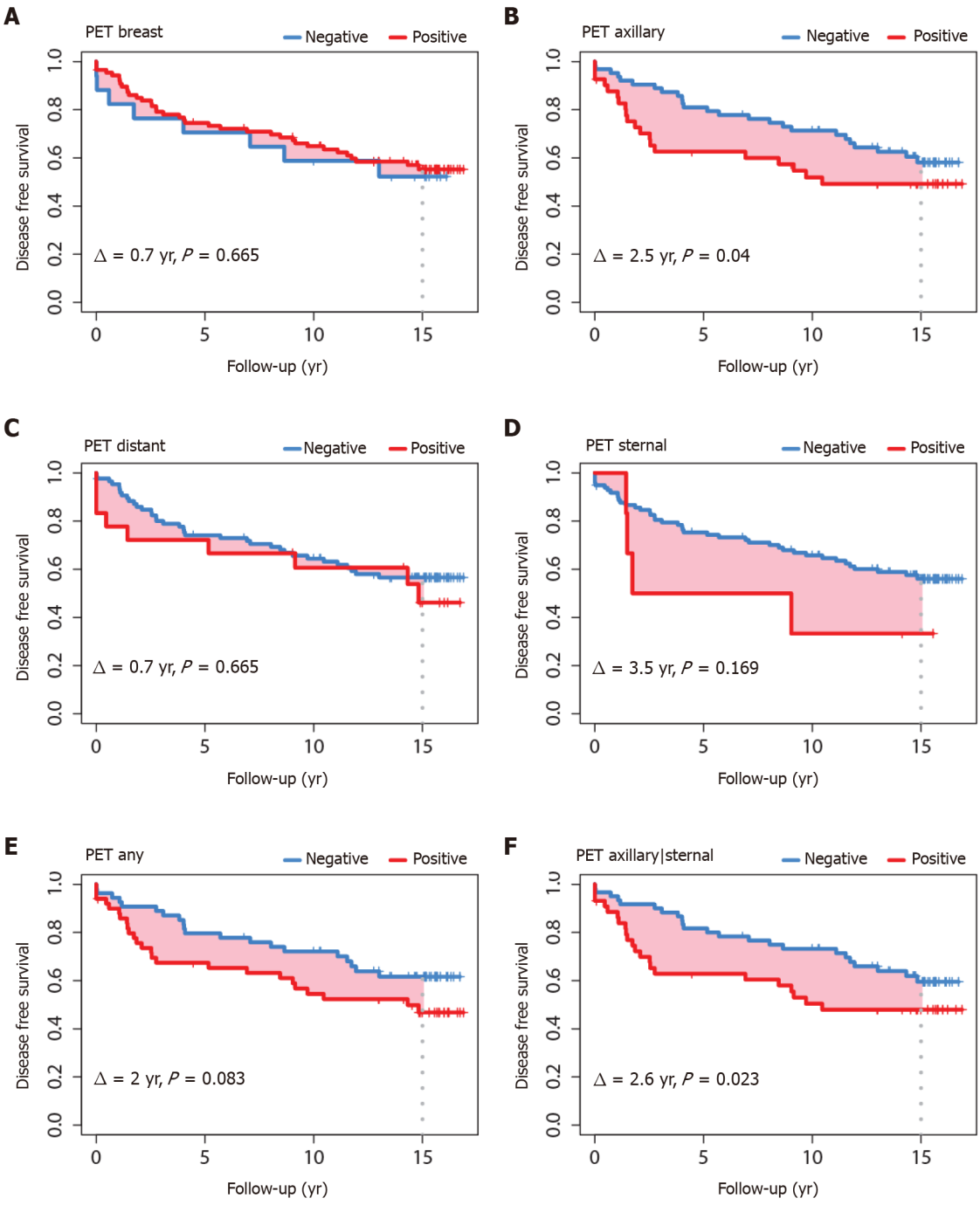
PET-positive status was found to not be significantly predictive of OS and DFS for patients with tumors ≤ 20 mm, which can be explained by the small number of patients and the lower mortality in this subgroup. This could also be explained by the spatial resolution of PET for small tumors and small axillary node, especially data obtained in 2002 during which PET performance was lower than that of sentinel lymph node biopsy[19,25]. In the subgroup of tumors ≤ 20 mm, corresponding to stage I, a multicentric study concluded that out of the 325 women with a BC, only 13 had a PET-positive status, and of those, only 3 were confirmed and 10 were deemed false positives[25]. Considering the whole series, PET status in the breast was not prognostic. The contribution of primary breast tumor size, which affects PET detection, is a long-standing debate[26] (see also Claire Verschraegen, on the effect of tumor size in breast cancer, *Ann Surg* 2005; 241: 309-318, reference omitted). The issue, however, is beyond the scope of this report. We can only remark that most patients in our study (74%) received mastectomy.



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Figure 2 Overall survival according to fluorine-18-fluorodeoxyglucose positron-emission tomography status in anatomical regions of interest. A: Breast; B: Axillary; C: Distant; D: Sternal; E: Any axillary, sternal or distant; F: Any axillary or sternal.

Compared to the previous study involving 5 years of follow-up, PET-positive axillary and combined axillary and sternal status remained significant predictors of DFS at the extended follow-up of 15 years, although to a lesser extent (Figure 3 and Table 4). A diagnostic check of the DFS model revealed a violation of the proportional hazards assumption of the Cox model. The assumption requires that the HRs between two treatment groups be independent of time[27]. The assumption fails if the survival curves cross over or overlap for a long time, or when the treatment has an early effect but the initial separation gets smaller over time[27]. This latter pattern of violation is evidenced in Figure 3, where the differences between the pairs of DFS curves show a tendency to narrow with longer follow-up, in contrast to Figure 2 where the OS curves remain proportionally distinct. OS is the gold standard; several studies have shown that DFS is not always predictive of OS[28]. Also, OS has the advantage of being unambiguously defined, in contrast to DFS, which has multiple definitions depending on the type of study and cancer involved. DFS is frequently used because it requires less observation time and fewer patients. However, with longer survival, patients advance in age. They present with increasing



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Figure 3 Disease-free survival according to fluorine-18-fluorodeoxyglucose positron-emission tomography status in anatomical regions of interest. A: Breast; B: Axillary; C: Distant; D: Sternal; E: Any axillary, sternal or distant; F: Any axillary or sternal.

comorbidities or with physical function deterioration and they are no longer willing or are unable to attend oncology consultations (typically when we call to enquire, patients would report a neurological, cardiac, respiratory, or joint and mobility problem) or in case they remain fit, the follow-up consultation is often discontinued after 10 years. Severe comorbidities can mask recurrence, and over time cancer surveillance loses priority for attending physicians. Consequently, less information on recurrence is available over time, whereas information on living or dead status can be obtained through national registries and is more reliable than disease status. These reasons likely explain why prognostication is better with DFS outcome in the short-term and better with OS outcome in the long-term.

Limitations of the study include bias inherent to its retrospective design. Beyond that, the investigators were not blinded to patients' outcomes, which could have affected the scoring of the PET images; moreover, the scoring itself depended upon the visual appreciation of screen printouts and predefined rules to abstract images were not established. There was also no assessment of inter-observer agreement on the scores. The patients had been treated 15 years prior, which represented both a

strength and a weakness, with the latter being related to medical management and treatment changes over that time. Most of the patients had presented with advanced tumors, for which a high prevalence of lymph node involvement and increased likelihood of PET positivity could be expected. Few patients presented with T1 tumors (Table 2); no conclusion could be drawn for these smaller tumors.

Molecular subtypes are known to affect PET positivity, as already mentioned[20-22]. However, the small study size (with only 28 events for OS; Table 1) precluded extensive analyses. By the one-in-ten rule of thumb (*i.e.* one variable for ten events[29]), it was decided to retain only the three-variables parsimonious model of Table 4 in the multivariate analysis, as built onto the precursor study[6]. Interestingly, despite the small study size, these three variables illustrate distinct facets relevant to BC management, specifically: PET as an indicator of disease aggressivity; age as a potential surrogate of increased risk of co-morbidity; and adjuvant hormone therapy as a surrogate of tumor subtype reflecting that hormone therapy is normally given only when the breast tumor expresses hormone receptors.

The present study innovates measurement of SUVmax based on a full-anatomical region of interest. The AROI's were defined regardless of SUV pattern, avoiding the potential arbitrary selection of small presumably pathological areas; although, without dual acquisition of CT, delineation of the anatomical boundaries was uncertain.

Surprisingly, few studies have implemented PET image analysis using AROIs. Yoo *et al*[30] delineated the nipple-areolar complex on the ipsilateral index breast and the contralateral normal breast. The ratio of the ipsilateral over contralateral SUVmax in the delayed image phase PET was then found to be an independent predictor of nipple-areolar involvement. Other than study of PET for nononcological cognitive symptoms and dementia, where the interest was in the whole brain and subregions [31], we are not aware of any other BC study implementing full-AROI-based PET measurements. Reflecting on PET studies in the peer-reviewed literature, the European Association of Nuclear Medicine Guidelines define volumes of interest only as relative to tumor areas, without allowance to full organs or anatomic regions[32].

The prognostic value of FDG-PET in particular has been demonstrated in numerous disease conditions[33-38]. The present study shows that the prognostic role in BC is no different than that with other cancers, serving as an indicator of increased metabolism and therefore adverse survival outcome. The SUVmax in different anatomical regions was related to the overall risk of death. There is a growing recognition that quantitative continuous SUVmax and other SUV metrics have an important prognostic role[39]. Our study adds to the evidence that SUVmax as a continuous variable improves the power of the analysis to optimize research yield, which can be particularly important when the resources and number of patients are restricted. The study also contributes a new intuitive finding, in that using the uninvolved side as a reference for SUV measurements can improve PET prognostication; the observation, however, deserves further study.

CONCLUSION

This study confirms that PET-positive axillary status in preoperative BC is a significant predictor of OS after 15 years of follow-up, and more so with breast tumors > 20 mm. In view of the long-term survival impact, the finding argues that preoperative PET should be considered as a standard in all BC cases whenever the primary tumor size exceeds 20 mm. The role of preoperative PET in tumors ≤ 20 mm is less clear and warrants further investigation.

ARTICLE HIGHLIGHTS

Research background

The role of preoperative fluorine-18-fluorodeoxyglucose positron-emission tomography (^{18}F-FDG PET) scan (referred to hereafter as FDG-PET) in early operable breast cancer (BC) is considered controversial and is even discouraged by clinical guidelines.

Research motivation

In dissension with guidelines, the evidence indicates that FDG-PET is a metabolic indicator of aggressive disease, warranting reconsideration of its role in the preoperative evaluation of BC.

Research objectives

Long-term follow-up is needed to address the importance of any marker. The study evaluates the very long-term (15-year) prognostic role of preoperative FDG-PET.

Research methods

The medical records of clinically nonmetastatic BC patients receiving preoperative FDG-PET were retrieved. Survivals were compared according to FDG-PET positive/negative status using the restricted mean survival time at a time horizon of 15 years. Multivariate analyses was performed with Cox proportional hazard models. In addition, the survival impact of absolute maximum standard uptake value (SUVmax) and ratios of SUVmax relative to the contralateral uninvolved side were evaluated.

Research results

Among 104 patients, regional FDG-PET positivity in the axillary or the sternal region was found to be a strong predictor of 15-year overall survival (P = 0.008). Patients with a positive regional PET status had an expected survival that was 2.6 years shorter than patients with negative regional PET status. Statistical significance was maintained for tumors > 20 mm, though not for tumors ≤ 20 mm. Cox models demonstrated the independent prognostic role. In addition, in a subgroup of 36 patients for whom quantitative SUV was available, representing 36 × 15 years = 540 patient-years follow-up and hence no lesser importance than a study of 189 patients but with only 3 years of follow-up, the ratio of ipsilateral axillary SUVmax *vs* uninvolved contralateral axillary SUVmax was the most significant among other SUV measures (P = 0.027).

Research conclusions

This study involved the longest known follow-up of preoperative FDG-PET in early operable BC. It provides survival information heretofore unavailable. Predicting an expected survival difference of 2.6 years out of a time horizon of 15 years can be a major consideration in the initial management of BC. In addition, the SUVmax ratio of ipsilateral over uninvolved side might represent a new finding that warrants investigation.

Research perspectives

FDG-PET might have a predominant role in the workup of BC. The present research did not have sufficient power to address the role of preoperative FDG-PET in tumors ≤ 20 mm. Future studies should consider accruing patients presenting with small tumors.

FOOTNOTES

Author contributions: Vinh-Hung V and Everaert H were responsible for conception and design of the study; Vinh-Hung V, Everaert H and Van Parijs H were responsible for acquisition of the data; Vinh-Hung V, Hendrik Everaert, Gorobets O, Perrin J and De Ridder M were responsible for drafting of the manuscript; Perrin J, Vinh-Hung V, Nguyen NP and Djassemi N were responsible for reviewing the literature; Vinh-Hung V was responsible for analysis of the data; Perrin J, Farid K, Djassemi N, De Ridder M, Nguyen NP and Everaert H were responsible for further writing of the manuscript; Vinh-Hung V, Gorobets O and Perrin J were responsible for generating the tables and figures; all authors performed critical review and gave final approval of the version to be published.

Institutional review board statement: The study was reviewed and approved by the UZ Brussel Ethics Committee. All diagnostic and therapeutic procedures were performed in accordance with the local national guidelines and the Declaration of Helsinki 1964. The study registration occurred on 12 May 2020, No. ISRCTN17962845 (https://www.isrctn.com/ISRCTN17962845).

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CASE REPORT

Mesentery solitary fibrous tumor with postoperative recurrence and sarcomatosis: A case report and review of literature

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Abstract

BACKGROUND

Solitary fibrous tumors are rare neoplasms of mesenchymal origin. They are often of low malignant potential and rarely metastasize. They frequently arise from the pleura and can occur at any soft tissue site in the body. However, these tumors rarely develop in the mesentery, peritoneal cavity or peritoneum.

CASE SUMMARY

We report on a scarce case of solitary fibrous tumor of the rectal mesentery showing sarcomatosis about 4 years after previous tumor resection. This 69-year-old male had no clinical symptoms but was transferred to our hospital because of a suspected tumor recurrence from follow-up abdominal computed tomography.

Tumor markers (CEA, CA 19-9 and CA 125) were within the normal range. Open laparotomy showed sarcomatosis, and pathology confirmed its mesenchymal origin and diagnosis as the solitary fibrous tumor. Our case may be the second recurrent mesentery solitary fibrous tumor reported to date, and the only one with progression to sarcomatosis. There has been no evidence of recurrence in follow-up at the 28th mo after extensive intra-operative peritoneal lavage and cytoreductive surgery.

CONCLUSION
Although there are few risk factors of cancer recurrence in this patient, careful long-term follow-up after cytoreductive surgery is necessary.

Key Words: Solitary fibrous tumor of rectum mesentery; Recurrence; Sarcomatosis; Extensive intra-operative peritoneal lavage; Cytoreductive surgery; Case report

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Core Tip: Solitary fibrous tumors (SFTs) are mostly benign and they rarely develop in the mesentery and cause sarcomatosis. The favored treatment strategy is whole-tumor excision with continued follow-up. According to a literature review, our patient is the first case report of mesentery SFT with the presentation of postoperative intraperitoneal recurrence and sarcomatosis.

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INTRODUCTION

Solitary fibrous tumors (SFTs) are mostly of mesenchymal origin and were first documented by Klemperer and Rabin[1]. SFTs growing from the mesentery are very rare[2]. In this paper, we report a patient with SFT of mesentery origin with postoperative recurrence and sarcomatosis, and subsequently we provide a review of the literature.

CASE PRESENTATION

Chief complaints

According to regular computed tomography (CT) follow-up, a 69-year-old Japanese man was noted to have a suspected SFT recurrence.

History of present illness

This patient received surgical resection of a rectal mesenteric tumor on 13 January 2016. Pathology confirmed a pedunculated rectal mesenteric tumor of mesenchymal origin to be SFT with malignant potential in terms of mildly positive p53 immunoreactivity. In addition to the primary tumor, a white nodule found on the peritoneal cavity was simultaneously resected and was noted with the same pathologic characteristics. He underwent regular follow-up without any symptoms at the hospital, but according to CT images on 1 March 2020, SFT recurrence was suspected. For further management he was referred to Kishiwada Tokushukai Hospital in Osaka Prefecture, Japan.

History of past illness

He had no medical history except for the surgical resection of a rectal mesenteric SFT with malignant potential on 13 January 2016.

Personal and family history

The patient had no family history.

Physical examination

Physical examination revealed no evident abnormalities on admission except for the previous abdominal operation scar.

Laboratory examinations

Routine blood results were normal, including the levels of the tumor marker cancer antigen 19-9 (CA 19-9; < 2.0 U/mL; normal level < 37.0 U/mL), carcinoembryonic antigen (CEA; 1.1 ng/mL; normal level < 5.0 ng/mL), and cancer antigen 125 (CA 125; 9.1 U/mL; normal level < 35.0 U/mL).

Imaging examinations

Contrast-enhanced CT images of the abdomen and pelvis revealed large and small nodules in the peritoneal cavity around the pelvic floor as well as suspected disseminated lesions. No prominent abnormal findings were noted in the liver, biliary system, pancreas, spleen or adrenal glands with no evident ascites (Figure 1).

FINAL DIAGNOSIS

Pathological findings

This patient received cytoreductive surgery and the specimen was examined. Macroscopic examination revealed more than 100 whitish tumor nodules (each measuring 1-3 mm) seeded over the parietal peritoneum and visceral peritoneum of the partial ileum and colon as well as the urinary bladder. The specimens were noted to have relatively clear boundaries, and some were noted to have fat infiltration (Figures 2 and 3).

Histopathologically, this tumor had a heterogeneous cell population comprising mainly spindle cells with proliferated fibrous collagen and diverse groups of cells exhibiting patternless or storiform growth. No tumor necrosis, nuclear polymorphism or cellular atypia was noted. The nuclear mitotic index was 4 mitoses/50 high-power fields (Figure 4A and B).

Immunohistochemically, the tumor showed diffuse strong immunoreactivity against CD34 (Figure 5A), CD99 (Figure 5B) and Bcl-2 (Figure 5C). The patient was diagnosed with SFT. Immunoreactivity for p53 was mildly positive (Figure 5D). The mitotic proliferative index for Ki-67 immunostaining was fewer than 4 mitoses/HP, representing a Ki-67 mitotic proliferative index of approximately 5% (Figure 5E). However, the tumor stained negatively for S100, HMB45, actin, desmin, CD10, cytokeratin, cytokeratin 7, CD31, EMA and CD68.

Grossly, pathology examination indicated that our patient's tumor fitted both the histopathological and immunohistochemical characteristics of SFT. Moreover, the washing cytology of the pelvis did not reveal any suspicious malignant cells.

TREATMENT

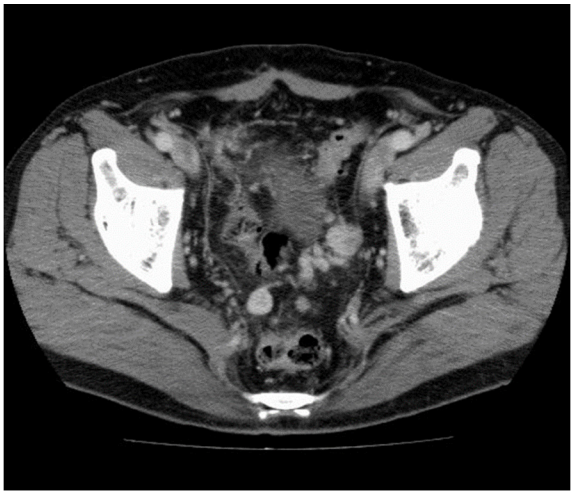
Operative intervention

A midline laparotomy was performed from the xiphoid to the pubis. The old abdominal incision was also excised. Nearly 100 whitish tumor nodules (each measuring 1–3 mm) were seeded over the parietal peritoneum and visceral peritoneum of the partial ileum and colon as well as the urinary bladder, with no ascites in the pelvis (Figure 6). The intraoperative peritoneal carcinomatosis index was 19 (2-0-0-0-2-2-2-2-2-1-2-2-2).

The laparotomy procedure was initiated after extensive intraoperative peritoneal lavage (EIPL) with 10 L of saline in the abdominopelvic cavity. Cytoreductive surgery (CRS) with electroevaporation was performed, which included adhesiolysis, umbilical and falciform ligament resection, total anterior parietal peritonectomy, bilateral subphrenic peritonectomy, complete pelvic peritonectomy (Figure 7), greater and lesser omentectomy, cholecystectomy, stripping of the tumor from Glisson's capsule and hepatoduodenal ligament, stripping of the floor of the omental bursa, circumferential resection of hepatogastric ligaments and extended radical right hemicolectomy. Subsequently, EIPL was performed with 10 L of saline in the abdominopelvic cavity. At the end of the surgery, primary ileo-transverse colon anastomosis was performed without stoma establishment. The overall cancer resection score was 0 and the operative duration was 285 minutes. Total blood loss was 1180 mL, and blood component transfusion (total packed red blood cells = 4 units, fresh frozen plasma = 8 units) was required.

OUTCOME AND FOLLOW-UP

The patient experienced an uneventful recovery with no postoperative complications and was



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Figure 1 Image of abdominal computed tomography. Suspected carcinomatosis or sarcomatosis was noted in the pelvis with no evident ascites.



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Figure 2 Intestine specimen after extended radical right hemicolectomy. Multiple whitish tumor nodules seeding over the visceral peritoneum of the partial ileum and colon.

discharged 13 d after surgery. Neither chemotherapy nor radiation therapy was administered. Repeat CT imaging was performed for every 4 mo during the first 2 years of follow-up. He has been in good general condition without evidence of recurrence or metastasis 28 mo after our surgical management. Subsequent CT imaging every 6 mo through the subsequent 5 years is planned.

DISCUSSION

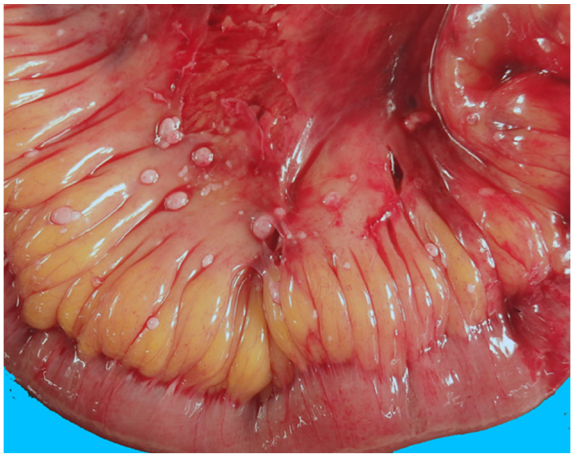
SFT was first described by Klemperer and Rabin in 1931[1] and was originally an exclusive diagnosis of pleural neoplasms[3]. Later, some SFTs were recognized as originating in various extra-thoracic sites[4]. They are predominantly localized in the pleura, followed by the head and neck, and are seldom present in the abdomen or pelvis[1,5-7]. To date, fewer than 1,000 cases of SFT in the pleura[8], as well as fewer than 100 cases of SFT in the abdomen or pelvis[9] have been reported. SFT of mesentery origin is extremely rare. We searched the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) and reviewed the relevant papers published. Of these reports of SFTs originating from the mesentery retrieved from the literature, we only found 13 cases (Table 1)[2,10-20].

SFT is predominant in the sixth and seventh decades of life with no difference in sex distribution[5, 21]. To date, no definite causative/contributing etiology or genetic predilection exists for this tumor[8].

Table 1 Patients with mesentery solitary fibrous tumors

Patient	Age	Sex	Symptom	Tumor location	Tumor size in cm	Management	Follow-up (F/U) in mo	Recurrence during F/U	Ref.
1	33	M	NM	Mesentery	NM	Operation	NM	NM	[10]
2	68	M	Abdominal pain	Sigmoid mesentery	18	Operation	NM	NM	[11]
3	53	M	Abdominal pain	Ileum mesentery	22	Operation	1	NM	[12]
4	73	M	Abdominal pain	Mesentery	25	Operation	NM	NM	[13]
5	71	M	Abdominal mass	Mesentery	16	Operation	12	No	[14]
6	41	M	Abdominal pain	Mesentery	23	Operation	7	No	[15]
7	26	M	Abdominal distension	Ileum mesentery	12	Operation	18	No	[16]
8	36	M	Abdominal pain	Rectum mesentery	16	Pre-op radiotherapy → operation	NM	NM	[17]
9	59	F	Abdominal pain	Mesentery	21	Operation	9	Yes	[18]
10	61	M	No symptom	Jejunum mesentery	3	Operation	2	NM	[19]
11	32	M	Abdominal mass	Sigmoid mesentery	13	Operation	252	No	[19]
12	41	M	Abdominal mass	Ileum mesentery	10	Operation	NM	NM	[20]
13	65	M	Abdominal pain	Ileum mesentery	26	Operation	12	No	[2]
14	69	M	No symptom	Sarcomatosis (rectum mesentery recurrence related)	Multiple	Operation (cytoreductive)	28	No	Our patient

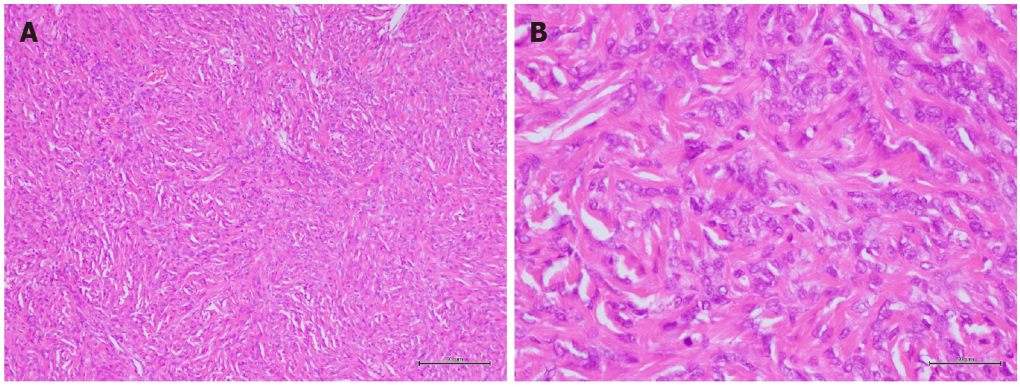
F: Female; NM: Not mentioned; M: Male.



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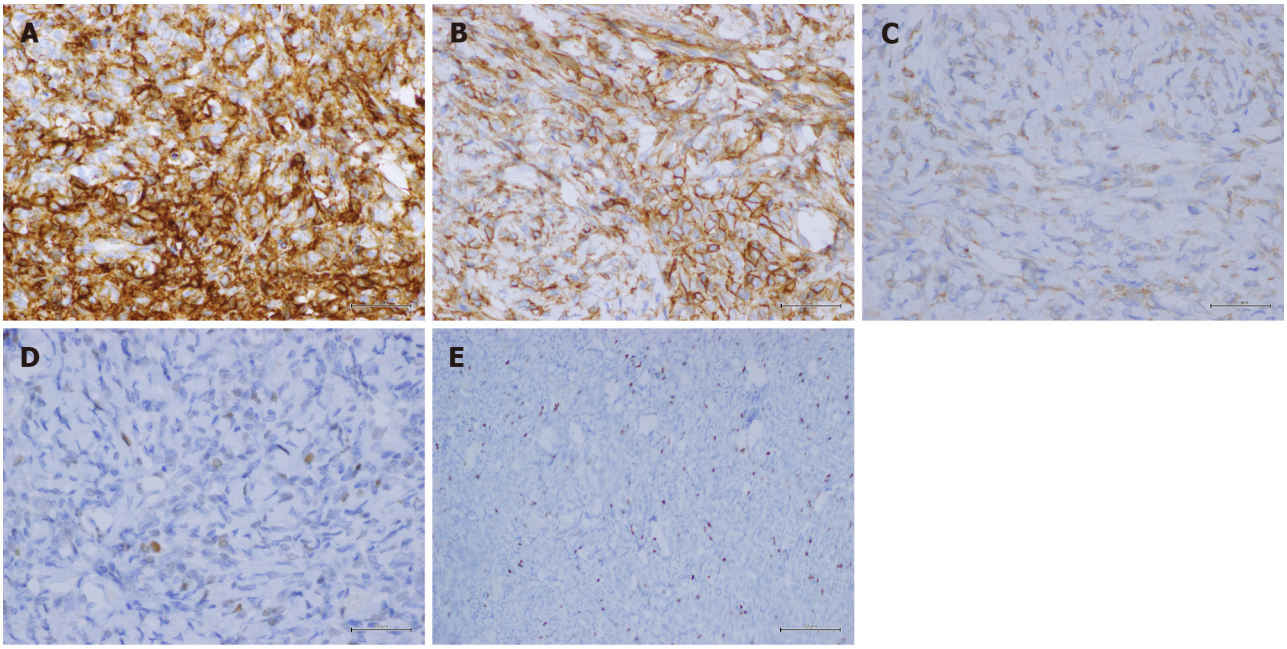
Figure 3 Image of an ileum specimen. Multiple whitish tumor nodules seeding over the visceral peritoneum of the distal ileum.

Patients with SFT may be asymptomatic at presentation[8]. However, extra-pleural lesions may cause clinically related symptoms to the tumor site. Systemic symptoms such as hypoglycemia (caused by insulin-like growth factor II secretion from the tumor[22]), arthralgia, osteoarthritis and clubbing have been documented[21]. These symptoms usually resolve upon tumor removal. In the present case, recurrent SFT was incidentally detected during regular follow-up and the patient did not exhibit any



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Figure 4 Microscopic features. The heterogeneous cell population comprised of mostly spindle cells with fibrous collagen proliferation as well as various other cell populations exhibiting patternless or storiform growth. No tumor necrosis, nuclear polymorphism, or cellular atypia was noted. The nuclear mitotic index was 4 mitoses/50 high-power fields (A: × 100 original magnification; B: × 400 original magnification).



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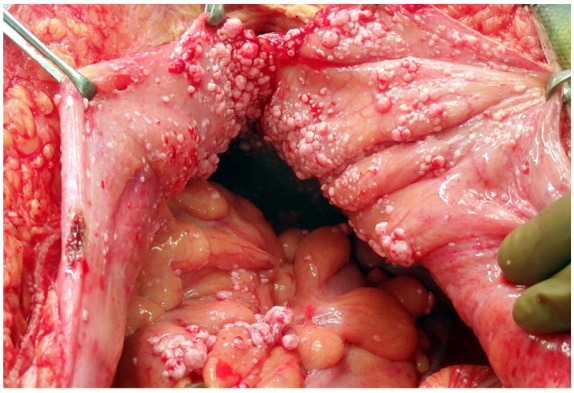
Figure 5 Immunohistochemical staining. A-E: The lesion showed diffuse strong staining for CD34 (× 400 original magnification) (A); CD99 (× 400 original magnification) (B); Bcl-2 (× 400 original magnification) (C); mildly positive immunoreactivity for p53 (× 400 original magnification) (D); and a Ki-67 mitotic proliferative index of approximately 5% (× 100 original magnification) (E).

symptoms.

SFTs do not display any tumor markers. Fluorodeoxyglucose positron emission tomography (FDG-PET) manifestations of SFT have been sparsely reported in the literature and clinical use of FDG-PET imaging for SFT detection remains unclear[23,24]. Cardillo *et al*[24] showed a weak association between malignancy and FDG-PET uptake in their eight-case study. However, we could acquire important information about the lesions or detect postoperative recurrence according to the radiological examinations, such as type-B ultrasonic, CT and magnetic resonance imaging scans[25,26].

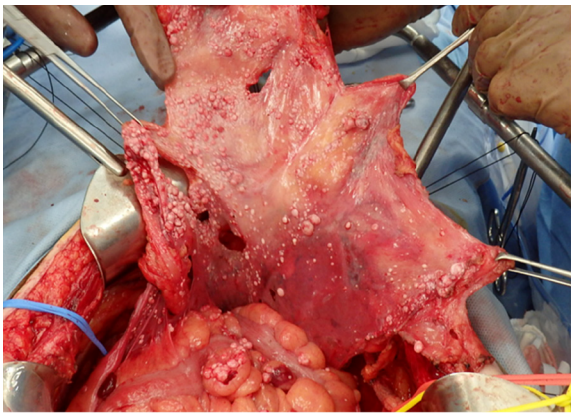
Type-B ultrasonic examination could show intraperitoneal SFT as a hypoechoic but sometimes as a heterogeneous lesion. Besides, this lesion might exhibit flow during Doppler imaging due to its characteristic of high vascularity[23].

On CT imaging, we could note that some well-circumscribed SFT lesions compress adjacent tissues and organs and even cause colon obstruction, urinary retention or bilateral hydronephrosis[27,28]. After contrast use, larger lesions would present with scattered intratumoral foci of hypoenhancement or non-enhancement in the necrosis, hemorrhage or cystic change regions. On the contrary, the homogeneous enhancement would be typically demonstrated in the smaller lesions[29]. Besides, it could provide information on the local extent of disease and the presence of distant metastases. However, the malignancy potential could not be decided according to this radiological distinction.



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Figure 6 Image of the pelvis during operation. Multiple whitish nodules were noted to be seeded over the parietal peritoneum and visceral peritoneum of the partial ileum and colon as well as the urinary bladder, with no ascites in the pelvis.



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Figure 7 Peritonectomy during cytoreductive surgery. Total anterior parietal peritonectomy, bilateral subphrenic peritonectomy, and complete pelvic peritonectomy (including the visceral peritoneum covering the urinary bladder) were performed.

T1 weighted signal enhancement of MRI imaging could identify subacute hemorrhage of the lesion with the characteristic of intermediate heterogeneous signal intensity. On T2 weighted images, flow voids could also be noticed as areas of heterogeneous low-signal intensity[30]. Besides, intense heterogeneous enhancement of the lesion is noticed in the arterial phase of Gadolinium-enhanced, fat-suppressed T1 weighted images and progressive enhancement in the venous phase[29].

Definite diagnosis of SFT is confirmed through histopathology and immunohistochemical staining. Histopathologically, most SFTs have a patternless or storiform architecture characterized by the coexistence of hypo- and hypercellular areas separated by fibrous stroma, with hemangiopericytoma-like branching blood vessels[31]. Studies of immunohistochemical and electron microscopic aspects have proven that SFTs grow from fibroblastic or myofibroblastic cells of the mesothelium[32,33]. Although differential diagnosis includes other spindle cell tumors, SFT has a unique staining pattern (positive for STAT6, CD34, CD99 and Bcl-2). Among these immunohistochemical markers, STAT6 is probably the most sensitive and specific marker of SFT, because most SFTs have an NAB2-STAT6 fusion gene, which is specific to this tumor type[34]. By contrast, SFTs generally exhibit negative reactivity to cytokeratin, alpha-SMA, S-100, CD31 and c-kit[35]. In our patient, intense diffuse strong staining for CD34, CD99 and Bcl-2 was detected, and SFT was confirmed, although the STAT6 marker was not examined.

SFTs have historically been considered indolent tumors that rarely metastasize in the literature. However, their behavior is unpredictable, with a broad spectrum of biologic behavior. Most SFTs behave benignly after complete surgical resection; however, some have been reported to behave aggressively either through local recurrence or distant metastasis[35]. The 2013 World Health Organization classification of SFT defines malignant forms as having a large tumor size (longer than 5 or 10 cm), sessile lesions, hypercellularity, and increased mitotic index (> 4 mitoses/10 high-power fields) [36,37], with cytological atypia, nuclear pleomorphism, tumor necrosis, infiltrative margins or hemorrhage[38]. However, discrepancies of morphological malignancy and clinical malignancy have been observed for SFT[3]. Some experts have advocated that malignant transformation with dedifferen-

tiation of tumor cells might contribute to such discrepancies[39]. Malignant transformation is of two types: malignant or high-grade SFT and the *de novo* occurrence of malignancy[21]. In our case, malignancy may be the recurrence of the previous tumor.

Immunohistochemistry for some proteins may clinically provide hints of SFT malignancy[3]. Takizawa *et al*[40] noted no tumor recurrence in cases with positive immunostaining for both CD34 and Bcl-2. Deprivation of CD34 and Bcl-2 immunoreactivity was observed in the component with malignant transformation[39,41]. In benign SFTs, p53 immunoreactivity was not detected. However, p53 immunoreactivity has been confirmed in morphologically and clinically malignant SFT[3,41]. According to these criteria, our present case had borderline benign (diffuse strong immunostaining for CD34, CD99 and Bcl-2) and malignant (mildly positive p53 immunoreactivity) pathologic characteristics. However, due to the clinical presentation of intraperitoneal sarcomatosis, recurrence remains a concern despite the complete removal of the seeding tumors achieved for our patient.

No consensus has been reached for the treatment guidelines for SFTs in this particular location (rectal mesentery) because of their scarcity and the confusion regarding their pathological confirmation[14]. However, complete surgical resection is the standard and mainstay treatment for most SFTs, including abdominopelvic SFTs and those that arise in other organs, regardless of histologic subtype[38]. The most essential prognostic factor is surgical resectability because complete resection of the tumor is curative in more than 90% of cases[1,42]. Poor prognosis is observed in patients with incomplete resection[43]. However, SFTs are hypervascular, complicating surgical resection[9] regardless of the tumor location.

Local recurrence or metastasis develops in 12%-22% of cases[44]. Patients with extra-thoracic SFTs are statistically more likely to develop the metastatic disease than those with thoracic SFTs[45]. Adjuvant chemoradiotherapy is not widely practiced or accepted as the standard of care[46]. Some experts have suggested radiotherapy or adjuvant chemotherapy, although poor outcomes have been reported for these treatments[43]. Some experts have used antiangiogenic agents as a therapeutic strategy in recent years. However, similar to the finding of the low response rate in standard chemotherapy, progression-free survival appeared similar between cytotoxic chemotherapy and antiangiogenic agents[47]. Thus, improved systemic therapies are required for metastatic or unresectable diseases.

To date, no standard therapy has been established for inoperable SFTs[4]. SFTs are generally regarded as chemoresistant tumors[48]. Our patient's Ki-67 mitotic proliferative index was only approximately 5%, suggesting that it was a type of low-grade sarcoma; we proposed that chemotherapy would have little effect. Although radiotherapy was another option, radiotherapy was not conducted for our patient because of the side effect of radiation injury on the small bowel related to the proposed large area of radiation and adhesion after two episodes of laparotomy. Instead, we sincerely believed that EIPL could effectively lower the amount of intra-abdominal free cancer cells, and it is a preventative strategy for further peritoneal recurrence. Moreover, we performed complete *en bloc* surgical resection with negative margins through CRS for this "inoperable" patient, which is of paramount importance.

Predicting the aggressive clinical behavior of SFTs is difficult[21,35]. Late recurrence or metastasis may develop even when the SFT has been identified as benign[49]. While surgical resection continues to be the initial modality for treating malignant SFTs, the utility of adjuvant chemotherapy or radiation is still unknown given that most data is based on small case series. Thus, no guidelines exist for determining the modality and frequency of post-treatment surveillance[31]. Long-term and regular surveillance is mandatory[50]. Some experts have suggested that long follow-up periods (≥ 15 years) should be maintained with closer follow-ups during the first 2 years[49], particularly for patients with high-risk features.

CONCLUSION

In conclusion, this article reported the first case of rectal mesentery SFT with postoperative recurrence and sarcomatosis. Instead of adjuvant chemotherapy or radiotherapy, we performed EIPL and CRS, and no evidence of recurrence was found during the 28-mo follow-up period.

FOOTNOTES

Author contributions: Chiu CC collected clinical records, wrote, revised and submitted the draft; Ishibashi H, Wakama S and Liu Y assisted with the operation and cared for the patient; Hao Y and Liu Y assisted with photo management; Hung CM provided critical opinion; Lee PH revised the manuscript; Rau KM and Lee HM searched the related references during the preparation process; Yonemura Y performed the operation, provided critical feedback, supervision, and opinion of this study.

Informed consent statement: Written informed consent for the publication of this case report was obtained from the patient.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

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MINIREVIEWS

Neoadjuvant treatment in non-small cell lung cancer: New perspectives with the incorporation of immunotherapy

Carlos Aguado, Luis Chara, Mónica Antoñanzas, Jose Maria Matilla Gonzalez, Unai Jiménez, Raul Hernanz, Xabier Mielgo-Rubio, Juan Carlos Trujillo-Reyes, Felipe Couñago

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Abstract

The aim of neoadjuvant treatment in non-small cell lung cancer (NSCLC) is to eliminate micrometastatic disease to facilitate surgical resection. Neoadjuvant

chemotherapy (ChT) in localised NSCLC has numerous advantages over other therapeutic modalities and is considered standard treatment in resectable disease. Treatment with immune checkpoint inhibitors (ICI) improves long-term survival in advanced disease and has a better toxicity profile than conventional therapies. These immunotherapy agents (anti-PD1/PD-L1), administered with or without ChT, are currently being evaluated in the preoperative setting, with initial results showing better pathological response rates and more long-term benefits. Importantly, these drugs do not appear to increase the rate of severe adverse effects and/or postoperative complications. However, several questions still need to be resolved, including the identification of predictive biomarkers; comparative studies of immunotherapy alone *vs* combined treatment with ChT and/or radiotherapy; the optimal duration of treatment; the timing of surgery; the need for adjuvant treatment; appropriate radiologic evaluation and mediastinal staging; and the correlation between pathological response and survival outcomes. Here we review the current evidence for immunotherapy from a multidisciplinary perspective and discuss current and future controversies.

Key Words: Non-small cell lung cancer; Neoadjuvant; Immune checkpoint inhibitors; Immunotherapy; Anti-PD1; Anti-PD-L1; Complete pathological response

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Core Tip: Studies evaluating neoadjuvant immunotherapy in non-small cell lung cancer have reported extraordinary pathological response rates without any increase in postoperative complications. However, before immunotherapy is implemented in routine clinical practice, several issues still need to be resolved. This review analyses the current evidence for immunotherapy from a multidisciplinary perspective and discusses current and future controversies.

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INTRODUCTION

Approximately 30% of patients with non-small cell lung cancer (NSCLC) are diagnosed with early-stage disease and most will undergo curative intent surgery. However, a substantial proportion of these patients will develop distant metastases, leading to a poor 5-year overall survival (OS) rate (< 35%) in patients with stage IIIA disease. Platinum-based adjuvant chemotherapy (ChT) has shown a marginal benefit in these patients, increasing 5-year survival rates by an additional 5%[1].

Multiple studies have directly compared adjuvant to neoadjuvant (preoperative) treatment, but have failed to demonstrate differences in efficacy between these two strategies. Nonetheless, neoadjuvant treatment has several advantages over adjuvant therapy, including: (1) A reduction in tumour volume and disease stage (thus increasing the potential for complete surgical resection); (2) early treatment of micrometastatic disease; (3) assessment of *in vivo* response to systemic therapy; and (4) improvement in the patient's preoperative performance status, which may increase adherence to the therapeutic plan.

The introduction of immune checkpoint inhibitors (ICI), which have been shown to substantially prolong survival in many patients, has radically altered the therapeutic landscape in advanced NSCLC. By contrast, the role of ICIs in localised disease is poorly understood. In this context, the aim of this article is to provide a detailed review, from a multidisciplinary perspective, of the current status of neoadjuvant therapy and the future of immunotherapy in locally-advanced NSCLC.

CONTRIBUTION OF NEOADJUVANT TREATMENT TO SURGERY IN STAGE III NSCLC

Numerous studies have evaluated the role of neoadjuvant therapy–mainly ChT–in surgically-treated patients with stage IIIA NSCLC. However, this approach remains controversial, in part due to the contradictory findings. Randomised studies have failed to demonstrate a clear advantage for neoadjuvant ChT followed by surgery *vs* definitive chemoradiotherapy (CRT). It seems likely that these

conflicting results are due to the wide heterogeneity in study designs (patient selection, treatment regimens, and treatment duration periods). Moreover, the type of surgery can also have a large influence on the outcomes. For example, in the Intergroup 0139 trial[2], neoadjuvant therapy significantly improved 5-year survival compared to CRT, but only in the lobectomy arm, mainly due to the high postoperative mortality rate (26%) in the pneumonectomy arm. Similarly, a subgroup analysis of the EORTC 08941 trial[3] also found that lobectomy was a predictor of better survival. That trial also included patients with unresectable disease, many of whom were treated with sequential CRT. By contrast, the ESPATUE trial failed to confirm these differences in survival outcomes according to type of treatment or surgical procedure, finding no significant differences in 5-year OS between the neoadjuvant and CRT arms (44% *vs* 40%)[4].

CRT has also been compared to induction ChT alone in the neoadjuvant setting[5], with no clear differences between these approaches in stage IIIA disease. Several studies have found that CRT does not significantly increase mortality or postoperative complications, even in patients undergoing pneumonectomy[6,7]. A major limitation of neoadjuvant treatment is the increased surgical complexity caused by the presence of thoracic adhesions and fibrosis, although complications associated with these treatments have decreased in recent years[8].

NEW HORIZONS FOR PREOPERATIVE RADIOTHERAPY

Radiotherapy (RT) continues to play a fundamental role in the management of localised NSCLC, either as radical-intent monotherapy [*e.g.*, stereotactic body radiation therapy (SBRT)] or combined (pre- or postoperatively) with ChT. In advanced disease, palliative RT can help manage symptoms such as hemoptysis, pain, and dyspnea. For this reason, it is crucial to determine the optimal timing and treatment modality.

Although the immune system will trigger an effective innate response when it detects the presence of cancer cells, in some cases tumours may become resistant to this immune response[9,10]. Exposure to ionising radiation induces changes in the tumour microenvironment, triggering the release of antigens that stimulate the immune system through a "vaccine" effect. In this clinical scenario, immunotherapy can trigger both a local response as well as a systemic response against tumour cells located outside the irradiation field, known as the "abscopal effect"[11,12]. However, several studies have shown that the real incidence of these responses in clinical practice is low.

Based on the results reported to date, the combination of radiotherapy and immunotherapy in NSCLC appears to be a promising strategy, but more robust data are needed to definitively establish the most appropriate treatment regimen for this combined approach, especially in localised disease. More specifically, studies are needed to evaluate this combination in the neoadjuvant setting in NSCLC.

REINVENTING SYSTEMIC TREATMENT: ROLE OF IMMUNOTHERAPY

General aspects

The main advantage of neoadjuvant immunotherapy is its capacity to stimulate the production and activation of T cells. In this therapeutic approach, the primary tumour cells are used as a source of antigen production, thus activating different types or clones of effector T cells, which may then act against tumour cells throughout the body (primary tumour, metastatic sites, circulation, *etc.*), thus allowing systemic elimination of micrometastases[13]. Compared to adjuvant therapy, the structure of the pulmonary lymphatic system before surgery remains intact, which enhances the potential for tumour cell-immune interaction. This ability to maximize antigen exposure to T cells not only permits a stronger initial response, but a longer lasting one. However, neoadjuvant immunotherapy also has several possible disadvantages, including the lack of long-term survival and safety data, and the potential impact on the timing of surgery and surgical complications.

Clinical evidence for neoadjuvant immunotherapy in NSCLC

Neoadjuvant therapy with ICI monotherapy: The first study to prospectively assess the role of neoadjuvant immunotherapy in NSCLC was a pilot study by Forde *et al*[8], who evaluated 21 patients with stage I-IIIA NSCLC treated preoperatively with two cycles of the anti-PD-1 agent nivolumab. Of these, nine patients (45%) achieved a major pathological response (MPR) and two patients (10%) a pathological complete response (pCR). By RECIST criteria, most patients (85%) had stable disease and 10% showed a partial response. A stage reduction was observed in eight patients (40%). At a median follow-up of 18 mo, the disease-free survival (DFS) rate was 73%.

The phase II LCMC3 trial[14] was performed to evaluate the effects of two cycles of atezolizumab followed by surgery in stage IB-IIIB disease. Of the 181 patients included, 159 underwent surgery. In the surgically-treated patients without a known EGFR/ALK mutation, MPR was observed in 20% (30/147) and pCR in 7% (10/147). In 43% of patients (66/155), the tumour was downstaged. At 18 mo of follow-

up, the DFS and OS rates in patients with stage I-II disease were 79% and 91%, respectively, *vs* 77% and 87% in stage III patients.

Other anti-PD-1 or anti-PD-L1 agents have also been investigated in recent years. One study evaluated sintilimab in 40 patients with stage IA-IIIB NSCLC, with 40% of patients achieving MPR and 16% pCR[15]. Most patients (70%) in that study had stable disease on radiologic assessment. In contrast to many studies, the tumour histology in most patients (80%) was squamous cell carcinoma. Another study evaluated the effects of two cycles of pembrolizumab, another anti-PD-1 agent, in stage II-IIIA NSCLC, with similar results (MPR, 27% and pCR, 13%)[16].

In the phase II IONESCO trial[17], patients with stage IB-IIIA NSCLC received three cycles of durvalumab. The preliminary results (n = 46) showed an MPR and pCR of 18% and 7%, respectively, with an objective response rate (ORR) of 8%. Despite promising 12-mo DFS and OS (78% and 89%, respectively), the trial was closed early due to high postoperative mortality (9%).

The combination of nivolumab and ipilimumab was evaluated in the phase II NEOSTAR trial[18]. In the 37 surgically-treated patients, combined therapy achieved higher MPR (50% *vs* 24%) and pCR (38% *vs* 10%) rates than nivolumab alone. There were no significant between-group differences in severe (≥ grade 3) toxicity (13% *vs* 10%).

Neoadjuvant therapy: immune checkpoint inhibitors combined with chemotherapy: Several studies have been performed (or are currently underway) to evaluate immunotherapy combined with ChT in an attempt to further improve the survival and pathologic response rates observed with ICI monotherapy. In a single-arm open label trial, Shu *et al*[19] preoperatively administered four cycles of atezolizumab plus carboplatin + nab-paclitaxel in patients with stage IB-IIIA NSCLC (77% stage IIIA). The MPR, pCR, and ORR rates were 57%, 33%, and 63%, respectively, all of which are higher than typically achieved with monotherapy. Median OS has not yet been reached due to the short follow-up.

The phase II NADIM trial[20] evaluated the combination of carboplatin + paclitaxel + nivolumab for three cycles in 46 patients with stage IIIA disease followed by adjuvant nivolumab for six months. In the 41 patients who underwent surgery, the MPR, pCR, and ORR were 83%, 63%, and 76%, respectively. No cases of disease progression were observed during neoadjuvant treatment. At 2-years of follow-up, DFS and OS were 77% and 90%, respectively. Adverse events ≥ grade 3 were observed in 30% of patients, but not associated with delays in surgery or death.

The findings of the phase II SAKK 16/14 trial in patients (n = 62) with stage IIIa NSCLC[21] were recently reported. In that study, patients received three cycles of cisplatin + docetaxel followed by two cycles of durvalumab and one-year of postoperative durvalumab maintenance therapy. The MPR, pCR, and overall response rates were 60%, 18%, and 58%, respectively. At 12 mo, the DFS was 73.4% (Table 1).

Unresolved questions

Assessment of response to immunotherapy: The ORR is a key indicator for evaluating the antitumour activity of neoadjuvant therapy; however, postoperative pathological findings are not always consistent with the radiologic response[22]. For this reason, fluorodeoxyglucose-positron emission tomography-computed tomography (FDG-PET-CT)[23] remains the gold standard for assessing response to neoadjuvant therapy. FDG-PET-CT imaging measures tumour metabolic activity to assess response and rule out distant disease. However, in some cases, neoadjuvant immunotherapy modifies the peritumoral inflammatory environment, and it can be difficult to determine whether there is a tumour response (increase or decrease) due to the presence of lymphocytic infiltrates. This phenomenon was described in the NEOSTAR trial[24] as "nodal immune flare", which was observed in 11% of cases with proven histological pCR after surgical resection. Several of the aforementioned studies have reported this phenomenon.

Correlation with long-term survival: One of the most striking results of immunotherapy is the marked increase in the MPR and/or pCR rates; in fact, some authors[25] have proposed using these parameters as surrogates for OS. For this reason, the systematic, standardised evaluation of surgical specimens should be prioritised. Various algorithms have been proposed[26] and several groups have also published consensus statements aimed at standardising assessment of pathological response after systemic therapy (including immunotherapy)[27,28]. Given the higher pathological response rates observed in phase II trials[20], it seems highly likely that, when long-term data become available, OS rates should increase; however, this expected benefit needs to be confirmed in prospective randomised trials, many of which are still ongoing.

Biomarkers: The neoadjuvant scenario is an excellent context in which to explore biomarkers that may predict the benefit of immunotherapy. As in metastatic disease, PD-L1 expression and tumour mutational burden are the two most well-documented biomarkers in clinical trials of ICI[29]. Higher pretreatment PD-L1 expression levels have been associated with a greater probability of achieving MPR [18] or pCR[20]. However, no association has been observed between elevated PD-L1 expression and longer survival, and a substantial proportion of patients without PD-L1 expression also achieve MPR.

Table 1 Clinical evidence for neoadjuvant immunotherapy in non-small cell lung cancer

Study	Phase	Stages	Treatment	Cycles	Patients included	Main endpoint	ORR	MPR	pCR
Forde *et al*[8]	I	I-IIIA	Nivolumab	2	21	Safety and feasibility	10%	45%	10%
LCMC3[14]	II	IB-IIIB	Atezolizumab	2	181	MPR	7%	20%	7%
NEOSTAR[18]	II	I-IIIA	Nivolumab *vs* nivolumab + ipilimumab[1]	3	44	MPR	22% *vs* 19%	24% *vs* 50%	10% *vs* 38%
Gao *et al*[15]	IB	IA-IIIB	Sintilimab	2	40	Safety	20%	40%	16%
NEOMUN[16]	II	II-IIIA	Pembrolizumab	2	15	Safety and feasibility	28%	27%	13%
IONESCO[17]	II	IB-IIIA	Durvalumab	3	46	% R0	8%	18%	7%
Shu *et al*[19]	II	IB-IIIA	Atezolizumab + carboplatin + nab-paclitaxel	4	30	MPR	63%	57%	33%
NADIM[20]	II	IIIA	Nivolumab + carboplatin + paclitaxel	3	46	PFS 24 mo	76%	83%	63%
SAK 16/14[21]	II	IIIA	Cisplatin + docetaxel followed by durvalumab[2]	2	62	DFS 12 mo	58%	60%	18%

[1]Nivolumab x3 cycles with or without a single dose of ipilimumab.
[2]Cisplatin + docetaxel x3 cycles followed by 2 cycles of durvalumab. ORR: Objective response rate; MPR: Major pathological response; pCR: Pathological complete response; % R0: % complete resection; PFS 24 mo: Progression-free survival at 24 mo; DFS 12 mo: Disease-free survival at 12 mo.

A higher density of tumour infiltrating lymphocytes–especially CD3+, CD8+, and CD103+–has been described as a prognostic factor associated with longer survival. The NEOSTAR and LCMC3 trials both assessed the influence of these lymphocytes[30], finding that resected tumours in patients with MPR presented a higher level of infiltration by effector-memory T-cells (CD3+, CD8+, CD45RO+) compared to those without MPR, suggesting a possible predictive capacity.

Other predictive biomarkers in peripheral blood are being evaluated: T-cell receptor, circulating tumour DNA, and somatic mutations (KEAP, STK11, RB1)[20], although these all need to be validated in prospective trials.

Beyond immunotherapy: the role of targeted therapy

In patients with metastatic NSCLC with certain molecular alterations (EGFR mutations, ALK rearrangements), treatment with tyrosine kinase inhibitors has shown a large benefit. Preoperative administration of drugs such as erlotinib[31] and crizotinib[32] improves ORR, but not OS, and postoperative recurrence rate after treatment discontinuation is high[33]. In this regard, prolonged treatment after surgery will probably be needed to reduce the likelihood of recurrence. Several studies are currently exploring this strategy, including the phase III NeoADAURA trial (NCT04351555), which is evaluating neoadjuvant osimertinib as monotherapy or combined with ChT.

NEW CHALLENGES: CHANGES FROM THE SURGICAL PERSPECTIVE

The high MPR and pCR rates obtained in clinical trials with neoadjuvant immunotherapy, with or without ChT, suggest that more patients with stage II-III disease will be candidates for surgery, even with the same operability and resectability criteria.

However, immunotherapy can induce atypical radiologic response patterns (*i.e.*, pseudoprogression, hyperprogression), which can make it more challenging to identify patients with negativization of the mediastinal nodes and therefore ideal candidates for surgical resection. Traditional response assessment criteria may not be optimal to adequately classify patients after immunotherapy, especially with regard to mediastinal evaluation. For this reason, new protocols with specific restaging criteria need to be developed and validated.

In current treatment algorithms, the indication for surgery depends on the presence or absence of contrast uptake on the PET-CT scan after neoadjuvant therapy, considered together with the findings of invasive diagnostic tests. However, high mediastinal uptake on PET-CT images should not immediately rule out surgery in these patients, since this finding is more common after immunotherapy than induction ChT or radiotherapy. For this reason, the introduction of new PET-CT response criteria[34] is expected to lead to an increase in invasive testing. However, the diagnostic efficacy of these invasive

Table 2 Ongoing clinical trials of neoadjuvant therapy

Treatment strategy	Study number (name)	Phase	Treatment
Anti-PD-1 + chemotherapy	NCT03838159 (NADIM II)	Phase 2 randomised	3 cycles of carboplatin + paclitaxel +/- nivolumab → surgery → 6 mo of adjuvant nivolumab (experimental arm)
	NCT04728724	Phase 2	Grupo A: sintilimab 2-4 cycles → surgery; Group B: sintilimab + chemotherapy (carboplatin + pemetrexed/gemcitabine/paclitaxel) 2-4 cycles → surgery
	NCT04326153	Phase 2	2 cycles of sintilimab + carboplatin + nab-paclitaxel → surgery → 8 cycles of sintilimab
	NCT04379739	Phase 2	2-4 cycles of camrelizumab + apatinib or camrelizumab + chemotherapy (carboplatin + pemetrexed/ gemcitabine) → surgery
	NCT04061590	Phase 2	2 cycles of pembrolizumab + chemotherapy (cisplatin + pemetrexed) → surgery
	NCT04638582	Phase 2	3 cycles of pembrolizumab +/- chemotherapy (carboplatin + pemetrexed/paclitaxel) → surgery
	NCT04025879	Phase 3	chemotherapy +/- nivolumab → surgery → adjuvant nivolumab (experimental arm)
	NCT02998528 (CheckMate 816)	Phase 3	3 cycles of chemotherapy (platinum doublet) + nivolumab → surgery +/- adjuvant chemotherapy (one experimental arm)
Anti-PD-L1 + chemotherapy	NCT04646837	Phase 2	2 cycles of chemotherapy (platinum-based + nab-paclitaxel) + durvalumab → surgery → durvalumab 1 yr
Anti-PD-L1 + anti-CTLA-4	NCT02998528 (CheckMate 816)	Phase 3	3 cycles of nivolumab + 1 cycle of ipilimumab → surgery +/- adjuvant chemotherapy (one experimental arm)
Anti-PD-1	NCT03197467 (NEOMUN)	Phase 2	2 cycles of pembrolizumab → surgery
Anti-PD-1 + anti-LAG3	NCT04205552 (NEOpredict)	Phase 2	2 cycles of nivolumab +/- relatlimab → surgery
Anti-PD-L1 + radiotherapy	NCT04245514	Phase 2	3 cycles of chemotherapy → 1 cycle durvalumab + radiotherapy → surgery → durvalumab 1 yr
	NCT03237377	Phase 2	2 cycles of durvalumab +/- tremelimumab (antiCTLA-4) + radiotherapy → surgery → adjuvant chemotherapy
	NCT03871153	Phase 2	Carboplatin + paclitaxel + radiotherapy + durvalumab → surgery → durvalumab 1 yr

tests in this clinical context are not known, and there is little data on the utility of EBUS-TBNA after immunotherapy[35].

Another question surrounding immunotherapy is the potential interference with the timing of surgery. In patients treated with monotherapy, surgery can be performed earlier (1-2 wk after treatment); by contrast, after combined treatment (immunotherapy and ChT), surgery will need to be delayed by 4-6 wk. Nevertheless, major changes in the timing of surgery are not expected.

Another issue is that the surgical procedure may be more technically challenging due to the possible presence of multiple inflamed lymph nodes induced by neoadjuvant immunotherapy. While thoracotomy is the most common route of access, minimally invasive surgery is generally indicated when an optimal resection is considered feasible. Nonetheless, several studies have reported a high conversion rate to open surgery (23%-54%)[36,37]. Minimally-invasive techniques are expected to become more standardised and reproducible as surgical teams gain more experience.

CONCLUSION

The emergence of immunotherapy with ICIs has radically altered the course of disease in advanced NSCLC. The results reported to date for neoadjuvant immunotherapy–demonstrating significant increases in major and complete pathological response rates–suggest that patients with localised disease could also benefit from ICIs, potentially increasing cure rates and prolonging survival in these patients.

The currently available pre- and postoperative safety data support the use of this therapeutic strategy. However, many open questions remain: (1) Does combined chemo-immunotherapy provide greater long-term benefits than immunotherapy alone? (2) Are there any predictive biomarkers of response? (3) What is optimal treatment duration and timing of surgery? (4) Is adjuvant treatment necessary in all patients? and (5) Are new protocols needed for re-evaluation and restaging?

Several ongoing studies are evaluating different therapeutic strategies (Table 2), and will allow us to answer these and other questions that may emerge in the future.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Tumor specifically internalizing peptide 'HN-1': Targeting the putative receptor retinoblastoma-regulated discoidin domain receptor 1 involved in metastasis

Frank-Un Hong, Miguel Castro, Klaus Linse

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Abstract

BACKGROUND

Less than 0.5% of intravenously injected drugs reach tumors, contributing to side effects. To limit damage to healthy cells, various delivery vectors have been formulated; yet, previously developed vectors suffer from poor penetration into solid tumors. This issue was resolved by the discovery of HN-1 peptide isolated *via* biopanning a phage-display library. HN-1 targets human head and neck squamous cell carcinoma (HNSCC) (breast, thyroid; potentially lung, cervix, uterine, colon cancer), translocates across the cell membrane, and efficiently infiltrates solid tumors. HN-1 peptide has been conjugated to various anticancer drugs and imaging agents though the identity of its receptor remained enigmatic.

AIM

To decipher the clues that pointed to retinoblastoma (Rb)-regulated discoidin-domain receptor 1 as the putative receptor for HN-1 is described.

METHODS

HN-1 peptide was synthesized and purified using reverse-phase high-performance liquid chromatography and gel electrophoresis. The predicted mass was confirmed by mass spectroscopy. To image the 3-dimensional structure of HN-1 peptide, PyMOL was used. Molecular modeling was also performed with PEP-FOLD3 software *via* RPBS bioinformatics web portal (INSERM, France). The immunohistochemistry results of discoidin domain receptor 1 (DDR1) protein were obtained from the publicly accessible database in the Human Protein Atlas portal, which contained the images of immunohistochemically labeled human cancers and the corresponding normal tissues.

RESULTS

The clues that led to DDR1 involved in metastasis as the putative receptor

mediating HN-1 endocytosis are the following: (1) HN-1 is internalized in phosphate-buffered saline and its uptake is competitively inhibited; (2) HN-1 (TSPLNIHNGQKL) exhibits similarity with a stretch of amino acids in alpha5 beta3 integrin (KLLITIHDRKEF). Aside from two identical residues (Ile-His) in the middle, the overall distribution of polar and nonpolar residues throughout the sequences is nearly identical. As HN-1 sequence lacks the Arg-Gly-Asp motif recognized by integrins, HN-1 may interact with an "integrin-like" molecule. The tertiary structure of both peptides showed similarity at the 3-dimensional level; (3) HN-1 is internalized by attached cells but not by suspended cells. As culture plates are typically coated with collagen, collagen-binding receptor (expressed by adherent but not suspended cells) may represent the receptor for HN-1; (4) DDR1 is highly expressed in head and neck cancer (or breast cancer) targeted by HN-1; (5) Upon activation by collagen, DDR1 becomes internalized and compartmentalized in endosomes consistent with the determination of 'energy-dependent clathrin-mediated endocytosis' as the HN-1 entry route and the identification of HN-1 entrapped vesicles as endosomes; and (6) DDR1 is essential for the development of mammary glands consistent with the common embryonic lineage rationale used to identify breast cancer as an additional target of HN-1. In summary, collagen-activated tyrosine kinase receptor DDR1 overexpressed in HNSCC assumes a critical role in metastasis. Further studies are warranted to assess HN-1 peptide's interaction with DDR1 and the therapeutic potential of treating metastatic cancer. Additionally, advances in delivery (conformation, endocytic mechanism, repertoire of targeted cancers of HN-1 peptide), tracking (HN-1 conjugated imaging agents), and activity (HN-1 conjugated therapeutic agents) are described.

CONCLUSION

The discovery of DDR1 as HN-1 peptide's putative receptor represents a significant advance as it enables identification of metastatic cancers or clinical application of previously developed therapeutics to block metastasis.

Key Words: HN-1 peptide; Solid tumor; Targeted drug delivery; Discoidin domain receptor 1; Tyrosine kinase; Metastasis

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Core Tip: The side effects associated with current drugs are exacerbated by the accumulation of administered drugs in non-tumor tissues. To guide, various tumor-homing vectors have been developed though their delivery efficacy is limited by poor penetration into solid tumors. To resolve, the 'tumor specifically internalizing peptide' HN-1 was isolated *via* biopanning a phage-display library. HN-1 peptide targets human head and neck squamous cell carcinoma (breast, thyroid, potentially cervical, lung, uterine, colon cancer), translocates across the cell membrane and effectively penetrates solid tumors. Here, deciphering of the clues that pointed to discoidin domain receptor 1 as the putative receptor for HN-1 is described.

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INTRODUCTION

The genetic analysis of head and neck cancer has uncovered novel therapeutic targets. Head and neck cancer represents ~5% of all cancers diagnosed in the United States and the affected regions include the oral cavity, paranasal sinuses, pharynx, nasal cavity, larynx, thyroid gland, parathyroid gland, salivary gland, skin or cervical lymph nodes. Aesthetic loss or functional impairment (ex. difficulties with swallowing, speech, chewing) frequently accompany the disorder. Squamous cells constitute the mucosal membranes lining the lips, mouth, throat, breathing tubes, nose, eyelids, *etc.* and head and neck squamous cell carcinoma (HNSCC) (the second most common type of skin cancer) affects more men than women. Its mortality rate (~50%) has not changed significantly over the past several decades although early diagnosis may increase survival. Most HNSCC-associated deaths are caused by loco-regional recurrence or distant metastasis[1].

Epithelial carcinogenesis leading to head and neck cancer involves progression through multiple stages (from normal to hyperplasia to dysplasia to carcinoma to invasive carcinoma) accompanied by distinct genetic changes. Among the alterations are the loss of heterozygosity at chromosomal region 9p21 or 3p12-14 (squamous dysplasia, carcinoma *in situ*, invasive carcinoma), loss of heterozygosity at 9p21 or 3p14 (oral leukoplakia), and translocation between chromosomes 11 and 19 (mucoepidermoid carcinoma of the minor salivary gland). Microsatellite instability is observed in a subset of dysplastic, invasive, and aggressive lesions while aneuploidy is a frequent occurrence amongst HNSCC tumors[2].

The inactivation of retinoblastoma (Rb) function represents a critical step in HNSCC development. Briefly, following the identification of the prototypic tumor suppressor *RB* gene, its role in mammalian DNA damage checkpoint (in G1 or S phase) was elucidated[3]. Rb's ability to arrest at G1 is negatively regulated by distinct cyclin-dependent kinases (Cdks) through phosphorylation by cyclin D-Cdk4/6 at early G1 phase and cyclin E-Cdk2 late G1 phase[4,5]. Cdks are, in turn, negatively regulated by $p14^{Art}$, $p16^{INK4A}$, or other factors including $p21^{Cip1}$, which is transactivated by p53. The above 'Rb pathway' is dysregulated in nearly all human cancers and is also targeted by oncogenic viruses (ex. papillomavirus, adenovirus) to transform human cells. In HNSCC, the loss of $p16^{INK4A}$ is characteristic of hyperplasia and the loss of 3p21 and 17p13 (inactivates p53) is observed during progression to dysplasia. The amplification of the *CCND1* gene (encodes cyclin D) and the loss of 11q13, 14q32, or 13q21 is associated with carcinoma. The loss of 6p, 4q27, 8 or 10q23 as well as the inactivation of phosphatase and tensin homolog (PTEN) has been documented in invasive carcinoma[6]. In a significant fraction of HNSCC patients with poor prognosis, human papillomavirus DNA is found in tumors, whose gene product E7 inactivates Rb while E6 inactivates p53. Additionally, FAT1, NOTCH1, KMT2D, NSD1, TGFBR2, PIK3CA, and HER-2/neu are frequently upregulated in a subset of HNSCC.

The above advances have led to the development of targeted therapies, *i.e.*, Cdk4/6 inhibitor, epidermal growth factor receptor inhibitor, *etc*. However, even with targeted drugs, the problem of side effects persists. Furthermore, less than 0.5% of systemically administered drugs reach tumors, requiring a higher dose for treatment which exacerbates side effects[7]. To resolve, tumor-homing vectors are increasingly sought for targeted delivery of existing drugs. In the case of HNSCC, 'sac'-like structures contain tumor cells enclosed within the basement membrane. The parenchyma is comprised of tumor cells with intercellular spaces. A solid tumor may contain multiple such 'sacs' interspersed within the stroma. Stroma is a complex structure comprised of cytokeratin and normal cells, ex. fibroblasts, endothelial cells, immune cells. As such, an ideal delivery vector should be capable of penetrating these barriers to reach tumor cells located at the interior of the sac-like structures.

For targeted delivery, antibodies recognizing tumor-specific antigens such as CD20 (B-cell lymphoma), HER-2 (breast cancer), CD33 (acute myeloid leukemia), epidermal growth factor receptor have been developed. Anticancer agents conjugated to these antibodies include radioactive isotopes (non-Hodgkin's lymphoma) and chemotherapeutics, ex. doxorubicin, maytansine, and calicheamicin (acute myelogenous leukemia). Alternatively, larger molecules such as polyethylene glycol have been used as they can escape through leaky tumor vasculature to deposit on a tumor. The leakage occurs primarily at venules and small veins that are lined by a continuous endothelium. Their exiting route consists of a system of vesiculo-vacuolar organelles, cell junction, or endothelial fenestrae. Previously, polyethylene glycol has been conjugated to taxol or tumor-specific antibodies. Also, liposomes have been utilized as they can accumulate at the tumor due to enhanced permeability and retention. Liposomes deliver drugs *via* fusion, destabilization of the membrane or endocytosis. Liposomes conjugated to polyethylene glycol exhibit greater stability due to lesser removal by the reticuloendothelial system[8]. Pegylated liposomes containing doxorubicin or cisplatin or liposomes coupled to tumor-targeting molecules have been developed.

However, the above delivery vectors face several limitations. For antibody vectors, its preparation or purification is complex due to the relative ease with which antibodies can denature. Other potential issues are immunogenicity (requires humanization) and the lack of internalization by the cancer cells. Poor penetration of solid tumors by antibodies further limits their use and may contribute to a higher level of drugs in circulation contributing to toxicity, ex. Herceptin antibody penetrates mere 38 micrometers after exiting blood vessels[9]. For these reasons, its use has been limited to lymphocytic cancers. For minute micrometastasized tumors that rely on nutrients obtained from adjacent blood vessels *via* diffusion, nanoparticles such as polyethylene glycol or liposome-based vectors that escape through leaky tumor vasculature may find little utility. The use of liposomes is delimited by the inefficient release of the enclosed drugs[10]. Overall, inefficient penetration of solid tumors has been a major issue with larger nanoparticles. As the interior of solid tumors may harbor hypoxic regions that give rise to refractory metastatic cancer cells to which patients ultimately succumb, the need to develop delivery vectors with greater infiltrating potential remains a priority[11].

The above issues have been largely resolved through the discovery of the prototypic 'tumor specifically internalizing peptide' HN-1, which was reported in Cancer Research in 2000[12]. HN-1 peptide is unique as it provided multiple advantages including tumor-selectivity for HNSCC, the capacity to translocate across the cell membrane, and the ability to penetrate solid tumors to reach cancer cells located within the sac-like structures. Since its discovery, HN-1 has been conjugated to various agents for both cancer therapy (taxol, doxorubicin, protein kinase C inhibiting peptide, ribonucleotide reductase inhibiting siRNA, diphtheria toxin, polyethylene glycol linked to doxorubicin,

graphene oxide nanoparticle-containing doxorubicin) and imaging (gamma-ray emitting isotopes for radiotherapy, near-infrared fluorescent dyes for surgical navigation) (see below). Recently, the target of HN-1 has been extended to breast cancer, thyroid cancer, and potentially cervical, lung, uterine, or colon cancer. Here, we describe the deciphering of clues that helped to unravel a 20 year-old enigma regarding the identity of the receptor mediating HN-1 endocytosis. Discoidin domain receptor 1 (DDR1) is a collagen tyrosine kinase overexpressed in head and neck cancer (also breast cancer), and the transcription of *DDR1* gene is regulated by the RB-interacting protein E2F[13]. DDR1 differs from conventional tyrosine kinases as it is activated by collagen, and plays a critical role in metastasis by facilitating invasion or migration, promoting epithelial-to-mesenchymal transition, reactivating previously disseminated metastasis-initiating cancer cells, *etc.* As such, it has become a novel therapeutic target of high importance for the pharmacological management of metastatic cancers (see below).

MATERIALS AND METHODS

Peptide

HN-1 peptide was synthesized and purified using reverse-phase high-performance liquid chromatography to attain ~95% purity. For *in vivo* application, it was further purified using gel electrophoresis. The predicted mass was confirmed by mass spectroscopy.

Molecular modeling

PyMOL (Python-enhanced molecular graphics) was used to image the 3-dimensional structure of the HN-1 peptide. Additional molecular modeling was performed using PEP-FOLD3 software *via* RPBS (Ressource Parisienne en Bioinformatique Structurale) bioinformatics web portal (INSERM, France) (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/)[14,15].

Immunohistochemistry

The immunohistochemistry images of DDR1 protein in a panel of human head and neck cancer specimens *vs* normal human oral mucosa were obtained from the publicly accessible database in the Human Protein Atlas portal (http://www.proteinatlas.org/). It contained the images of immunohistochemically labeled human cancers and the corresponding normal tissues.

RESULTS

Structure of HN-1 peptide

F. Hong (a.k.a. Frank Un, Frank D. Hong)'s contributions to the *RB* gene field was previously described [16]. Briefly, for cancer biology and genetics, his works include the identification of the human *RB* gene and its sequence containing cyclin-dependent kinase recognition motifs[3], determination of RB genome structure including the promoter[17] and its mutant in prostate cancer[18], the discovery of the DNA binding property of Rb protein indicative of its function as a transcription factor[19], uncovering of RB-to-RB self-interaction to form higher-ordered structures implicating its role in epigenetics, DNA replication, histone modification, heterochromatin, DNA condensation, *etc*[20-23]. For cancer therapy, his works concerned elucidating the cytotoxic mechanism of anticancer drugs to solve the side effects or drug resistance problem. He discovered tumor-specific lytic path 'hyperploid progression mediated death' targeting G1 DNA damage checkpoint-defective RB or p53 mutants induced by antimicrotubule drugs (ex. Taxol)[24], RB's role as the mediator of DNA crosslinking drug cytotoxicity (ex. cisplatin) in G1 checkpoint retaining human cancers[25], and the reversal of drug resistance to antimetabolite drugs (ex. hydroxyurea) *via* attenuating the Rb-associating protein ICBP90 (UHRF1)[26].

Following the identification of the human *RB* gene, W. Lee (University of California at San Diego, La Jolla, United States) collaborated with T. Friedmann (University of California at San Diego, La Jolla, United States) to develop a recombinant retrovirus expressing human *RB* or *p53* gene during 1988-1993. The retrovirally expressed RB or p53 suppressed the formation of tumors derived from human retinoblastoma, breast, or prostate cancer cells in murine xenograft models[27-29]. These works led to gene therapy clinical trials testing the efficacy of p53 expressing retrovirus or adenovirus in non-small cell lung cancer patients by J. Roth (University of Texas M. D. Anderson Cancer Center, Houston, United States) in 1996 and HNSCC patients by G. Clayman (University of Texas M. D. Anderson Cancer Center, Houston, United States) in 1998[30,31]. The clinical trial was conducted in collaboration with W. K. Hong (University of Texas M. D. Anderson Cancer Center, Houston, United States), who pioneered the chemoprevention of cancer[32]. Despite the therapeutic gains made in gene transduction, gene expression, and clinical response, further improvement was necessary regarding tumor specificity, solid tumor penetration, and *in vivo* stability.

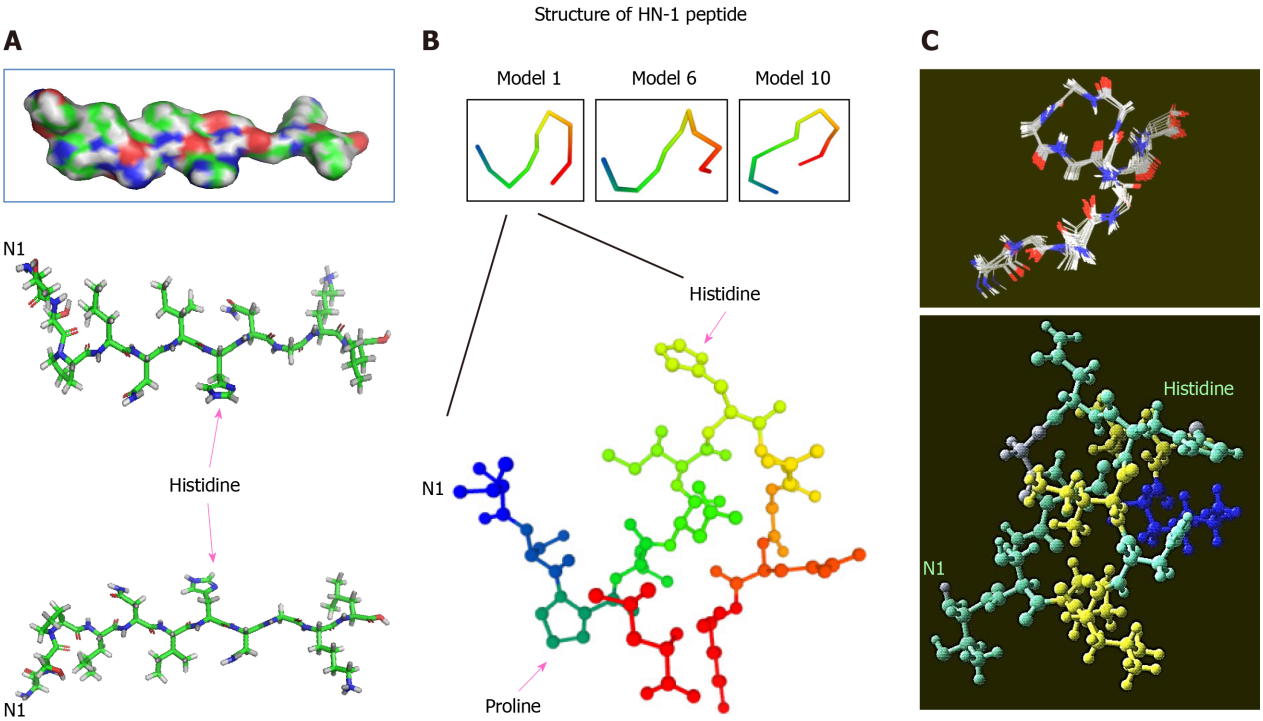
The side effects occurred as the RB or p53 expressing recombinant virus indiscriminately infects both cancer and normal cells. To provide tumor specificity, F. Hong sought to identify a human HNSCC-specific peptide to be displayed on the surface of viral vectors. Earlier In 1984-1985, he used M13 single-stranded bacteriophage to perform site-directed mutagenesis at the Salk Institute (Molecular Biology & Virology Laboratory). Later, in 1993-1995, he worked with normal human fibroblasts while studying the mechanism of aging at the Salk Institute (Neurobiology Laboratory). During 1998-2000, F. Hong screened M13 bacteriophage-displayed random peptide libraries (2.5×10^{12} random peptides) using live cancer cells at the University of Texas M. D. Anderson Cancer Center[12]. Filamentous phages displaying peptides fused to coat proteins were designed by G. Smith (Nobel prize, 2018). The biopanning involved 5 successive rounds of selection using human HNSCC cells, followed by 3 cycles of subtraction using normal human fibroblasts in the presence of serum to ensure stability *in vivo*. It led to the discovery of HN-1 peptide (TSPLNIHNGQKL; ~1.2 kDa) that meets multiple criteria for targeted drug delivery into solid tumors: (1) Translocates drugs across the cell membrane into the cytosol; (2) Tumor specifically internalized; and (3) Capable of penetrating solid tumors. Further, HN-1 peptide is nontoxic, nonimmunogenic, stable *in vivo*, transports payload efficiently within 48 h, and does not trigger biological responses.

Recent researches increasingly indicate the dynamic nature of protein structures, *i.e.*, 'intrinsically disordered protein'[33]. Despite the conformational flexibility, they can adopt a fixed or rigid structure upon recognizing the interacting target. Such properties have been harnessed by peptides, which provide further advantages over antibodies or small molecules for pharmaceutical treatment, resulting in the approval of > 60 peptide drugs worldwide in the last two decades. In the case of HN-1, computational modeling predicted several structural conformations. A 3-dimensional model depicting HN-1 peptide in a beta-sheet configuration is shown (Figure 1A). Alternate conformations of HN-1 peptide predicted by the PEP-FOLD modeling program are also shown (Figure 1B). Finally, a structural model consisting of a gamma-turn is shown (Figure 1C).

DDR1 may mediate HN-1 endocytosis

The treatment issue of recurrent metastatic cancer refractory to current drugs remains unresolved for HNSCC as well as breast cancer. The dysregulation of the extracellular matrix dynamics is thought to play a significant role in metastasis. The extracellular matrix is comprised of glycoproteins, proteoglycans, and other proteins that function in biomechanics, cell motility, growth factor reservoir, cell-to-cell communication, *etc.* An early event in tumor development involves neovascularization (for nutrient, oxygen, excretion), which requires the outgrowth of blood vessels *via* endothelial branching mediated by tip cells and stalk cells that depend on the extracellular matrix. The extracellular matrix also attracts immune cells and activates them to release cytokines and proteases (causes tumor-associated inflammation) while suppressing the activation of macrophages that lyse cancer cells. The dysregulated extracellular matrix exhibits altered activities of remodeling enzymes (ex. collagenase or metalloprotease secreted by stroma), excess deposition of the matrix components (ex. collagen types I, IV or XVII by tumor cells, cancer-associated fibroblasts or tumor-associated macrophages in colorectal cancer; heparan sulfate proteoglycan), stiffness through the crosslinking by lysyl oxidase, *etc.* Distinct collagen types found in the microenvironment of various cancers have been compiled, *e.g.*, type I (head and neck squamous cell carcinoma, non-small cell lung cancer, breast cancer), type III (breast cancer), type IV (oral squamous cell cancer, colorectal cancer)[34]. Other changes include the altered orientation (linearization) of collagen fibers, which facilitates tumor cell migration (for invasion into adjacent tissues following the breakdown of basement membrane by matrix metalloproteinase)[35]. Further, the extracellular matrix is involved in the development of lymphatic vessels that serve as a conduit (in addition to the leaky tumor vasculature) for metastasis.

Discoidin domain receptors DDR1 and DDR2 are cell membrane-associated receptors with tyrosine kinase activity, whose extracellular domain resembles discoidin of *Dictyostelium discoideum*. DDR1 is activated by most collagen types including I and IV (abundant in the basement membrane) whereas DDR2 is activated by fibrillar collagen types I, III, and X but not II or IV[36]. Discoidin domain receptors regulate cell adhesion, growth, polarity, and migration through sensing extracellular matrix and interacting with TGF-beta, Notch, or adhesive receptors for signaling. The stromal-epithelial interaction mediated by DDR1 is essential for the normal development of mammary glands in mice[37]. DDR1 promotes cancer progression by facilitating the migration of squamous cell carcinoma cells[38], bone metastasis by lung cancer[39], lung metastasis by breast cancer[40], stroma-induced peritoneal metastasis of gastric cancer[41], and tissue invasion by metastatic colorectal cancer cells[42]. Mechanistically, activation of DDR1 by collagen induces matrix metalloproteinase to degrade extracellular matrix [43]. DDR1 overexpressed in oral squamous carcinoma is involved in angiolymphatic invasion[44]. DDR1 is involved in invadosome formation *via* collagen-mediated activation of Rho-GTPase Cdc42[45]. DDR1 facilitates the invasion of a collective group of tumor cells by modulating actomyosin contractility at the cell-cell contacts[38]. Collagen type IV was shown to activate DDR1 to induce migration of breast cancer cells[46]. The epithelial-to-mesenchymal transition represents a critical step for metastasis and is triggered by extracellular matrix molecules or growth factors. The triggering by collagen type I is mediated by DDR1, whose signaling is transduced by proline-rich tyrosine kinase 2 to upregulate N-cadherin[47]. The DDR1 expression positively correlates with epithelial-to-mesenchymal transition in



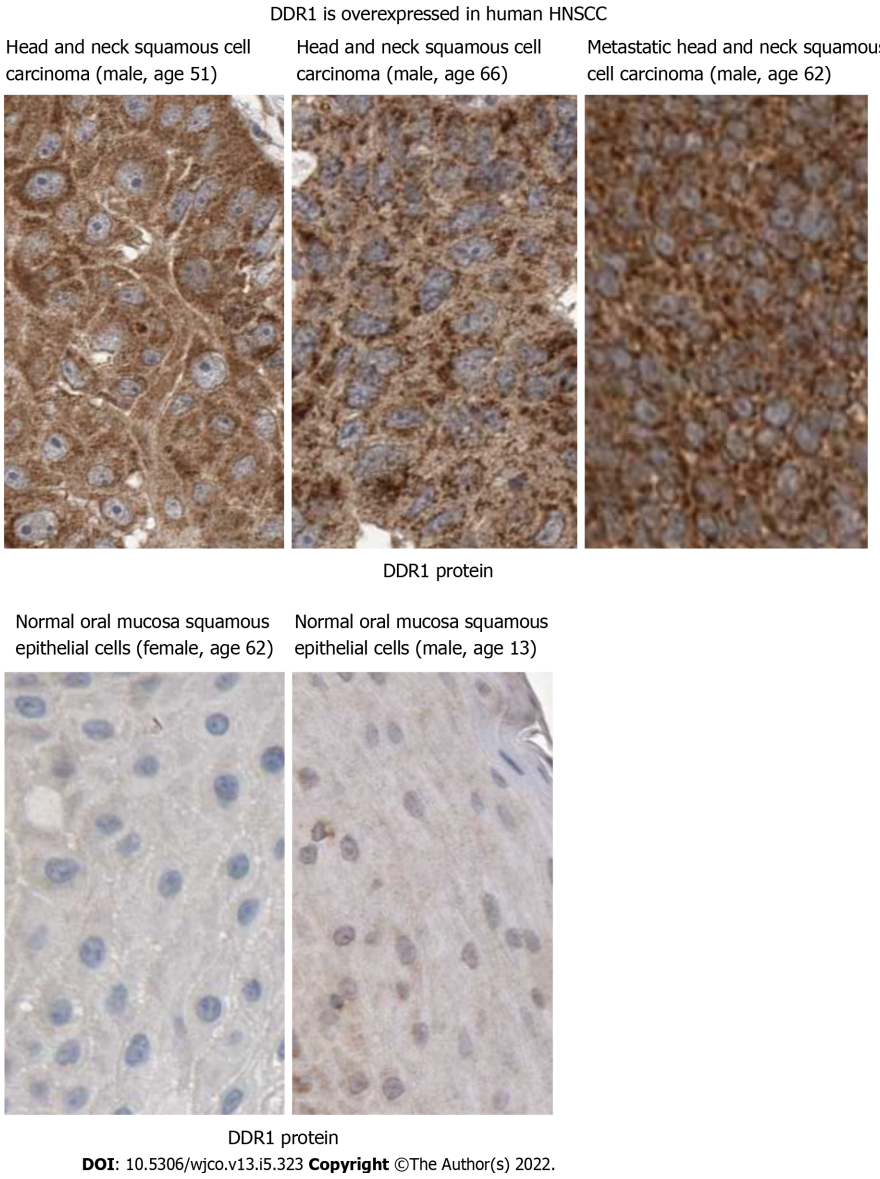
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Figure 1 Three-dimensional structure of HN-1 peptide. A: A 3D model of HN-1 peptide generated using PyMol molecular graphics system, version 1.2r3pre, Schrödinger, LLC. All graphics depict an identical configuration with the bottom two panels in the opposite orientation; B: An ensemble of *de novo* conformations generated by PEP-FOLD (INSERM, France) in RPBS bioinformatics web portal: https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/; C: A 3-dimensional profile of the lowest energy structure obtained for HN-1 peptide was viewed using Raswin computer modeling software. All structures were generated using TSPLNIHNGQKL as the raw input peptide sequence. N1: The N-terminal residue.

squamous cell carcinoma, breast cancer, ovarian cancer, and hepatoma[48]. Additionally, DDR1 (activated by collagen type I) functions in the reactivation of previously disseminated metastasis-initiating cancer cells after dormancy[13,40].

Rb's tumor-suppressing function extends to the genetic regulation of DDR1 to inhibit metastasis. Through associating with E2F, Rb inhibits the transactivation of DDR1 by E2F[49]. The transcription of DDR1 is also regulated by p53 of the 'Rb pathway'[50]. The human *DDR1* gene consists of 17 exons, which encode an extracellular domain (discoidin domain for ligand binding), a transmembrane domain, and an intracellular domain (tyrosine kinase). Alternative splicing generates multiple isoforms, which include DDR1c encoding the full-length receptor, DDR1b lacking 6 residues (between exon 13 and 14), DDR1a lacking these and additional 37 residues (juxtamembrane region), and DDR1d plus DDR1e (both tyrosine kinase-deficient due to C-terminal truncation)[48,51]. DDR1 remains a dimer (N-glycosylated) without the bound ligand. Binding to collagen activates DDR1 *via* clustering, causing autophosphorylation to initiate signaling[43,52]. DDR1 is overexpressed in head and neck, esophagus, lung, breast, ovarian, prostate, and brain cancers in addition to leukemia and lymphoma. DDR1 is also upregulated in osteosarcoma, endometrial cancer, primary central nervous system lymphoma, and liver cancer[13]. It correlated with a reduced overall survival (*e.g.*, metastatic colorectal cancer)[36] and a poor prognosis (*e.g.*, non-small cell lung cancer) for most cancers. Figure 2 shows the overexpression of DDR1 in human head and neck squamous cell carcinoma.

Multiple data indicate that the entry of HN-1 peptide is mediated by DDR1. First, HN-1 is internalized in phosphate-buffered saline (lacking the components of fetal bovine solution); plus, its uptake is competitively inhibited, indicating that specific interaction with cell surface receptor is necessary for its uptake[12]. Second, a high degree of similarity was discovered between HN-1 (TSPLNIHNGQKL) and a stretch of amino acids in alpha5 beta3 integrin (KLLITIHDRKEF). Aside from two identical residues (Ile-His) in the middle, the overall distribution of polar and nonpolar residues throughout the sequences was nearly identical (Figure 3A). The tertiary structure of both peptides exhibited similarity at the 3-dimensional level (Figure 3B). The found motif represents the membrane-proximal sequence of the beta subunit's cytoplasmic tail that interacts with the alpha subunit's cytoplasmic tail, whose disruption changes the conformation of the extracellular domain to engage the ligand[53,54]. As the HN-1 sequence lacks the Arg-Gly-Asp motif recognized by integrins, HN-1 may interact with an "integrin-like" molecule (instead of integrin). Third, HN-1 is uptaken by attached cells but not by suspended cells[55]. As tissue culture plates are typically coated with collagen (type I or IV), it suggests that collagen-binding receptor (expressed by adherent but not suspended cells) may represent the receptor for HN-1. Fourth, DDR1 is overexpressed by head and neck cancer or breast cancer targeted by HN-1. Fifth, upon



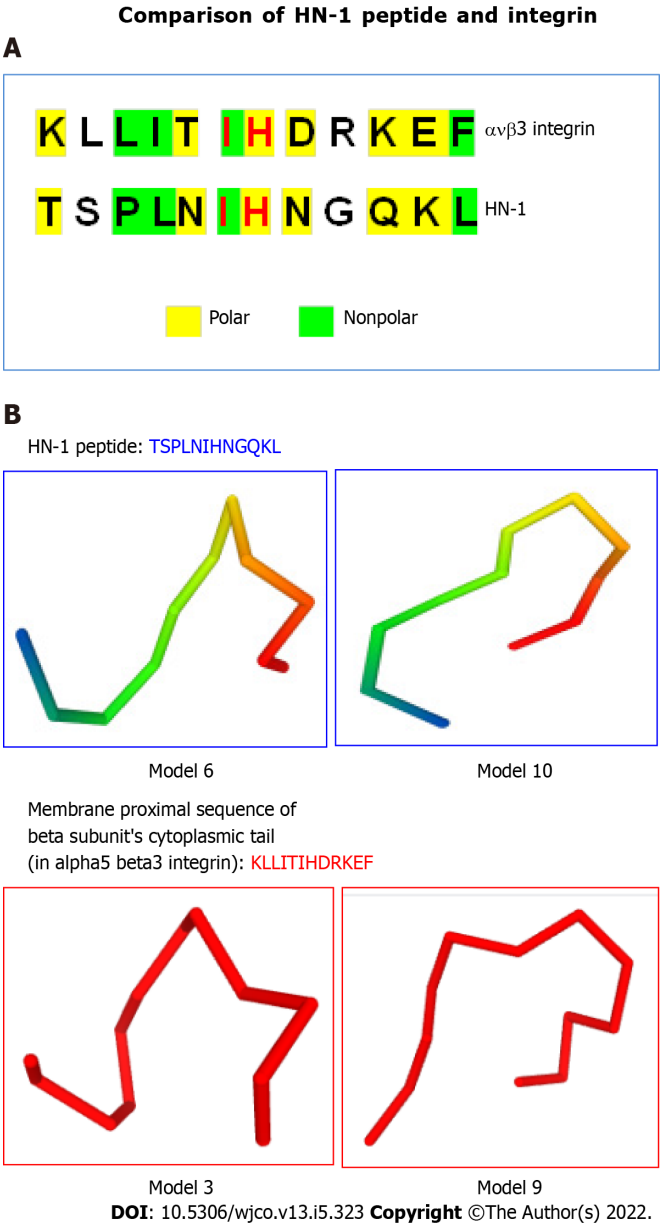
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Figure 2 Discoidin domain receptor 1 may mediate HN-1 endocytosis. Discoidin domain receptor 1 (DDR1) protein is upregulated in human head and neck squamous cell carcinoma. Immunohistochemical analysis of DDR1 was conducted by comparing tumor *vs* normal tissues in Human Protein Atlas database (http://www.proteinatlas.org/). The results showed positive cytoplasmic and membranous staining. Bar: 25 micrometers; DDR1: Discoidin domain receptor 1.

activation by collagen, DDR1 becomes internalized and compartmentalized in endosomes[56], which is consistent with the identification of energy-dependent clathrin-mediated endocytosis as the entry route of HN-1[57] and the determination of HN-1 entrapped vesicles as endosomes[38]. Sixth, DDR1 is essential for the development of mammary glands[37] in keeping with the common embryonic lineage rationale used to identify breast cancer as an additional cancer target of HN-1[58]. Taken together, these results suggest that DDR1 may mediate the endocytosis of the HN-1 peptide.

DISCUSSION

HN-1 peptide is preferentially internalized by cancer cells. HN-1 was internalized by almost all human HNSCC cell lines examined to date (MDA177Tu, MDA138Tu, MDA59Tu, MDA167Tu, MDA686Tu, MDA1986Tu, UMSCC1, UMSCC36)[12,59]. UMB-SCC-745, UT-SCC-36, UT-SCC-38[60], SCC-25, Detroit 562[61], CAL-27, and SCC-25 human HNSCC cells[62] also internalized HN-1. Recently, the uptake of HN-1 by human oral squamous cell carcinoma SCC-25 and CAL-27 cells was reported[63]. Further, HN-1 was selectively internalized by human head and neck squamous cell carcinoma derived SCC4, SCC9, and CAL27[57]. Little uptake of HN-1 was observed with MDA182Tu cells. Additionally, HN-1 peptide was internalized by human pharynx squamous cell carcinoma FaDu cells used in the mouse xenograft model study[64]. *In vivo,* intravenously administered HN-1 selectively localized to human HNSCC-

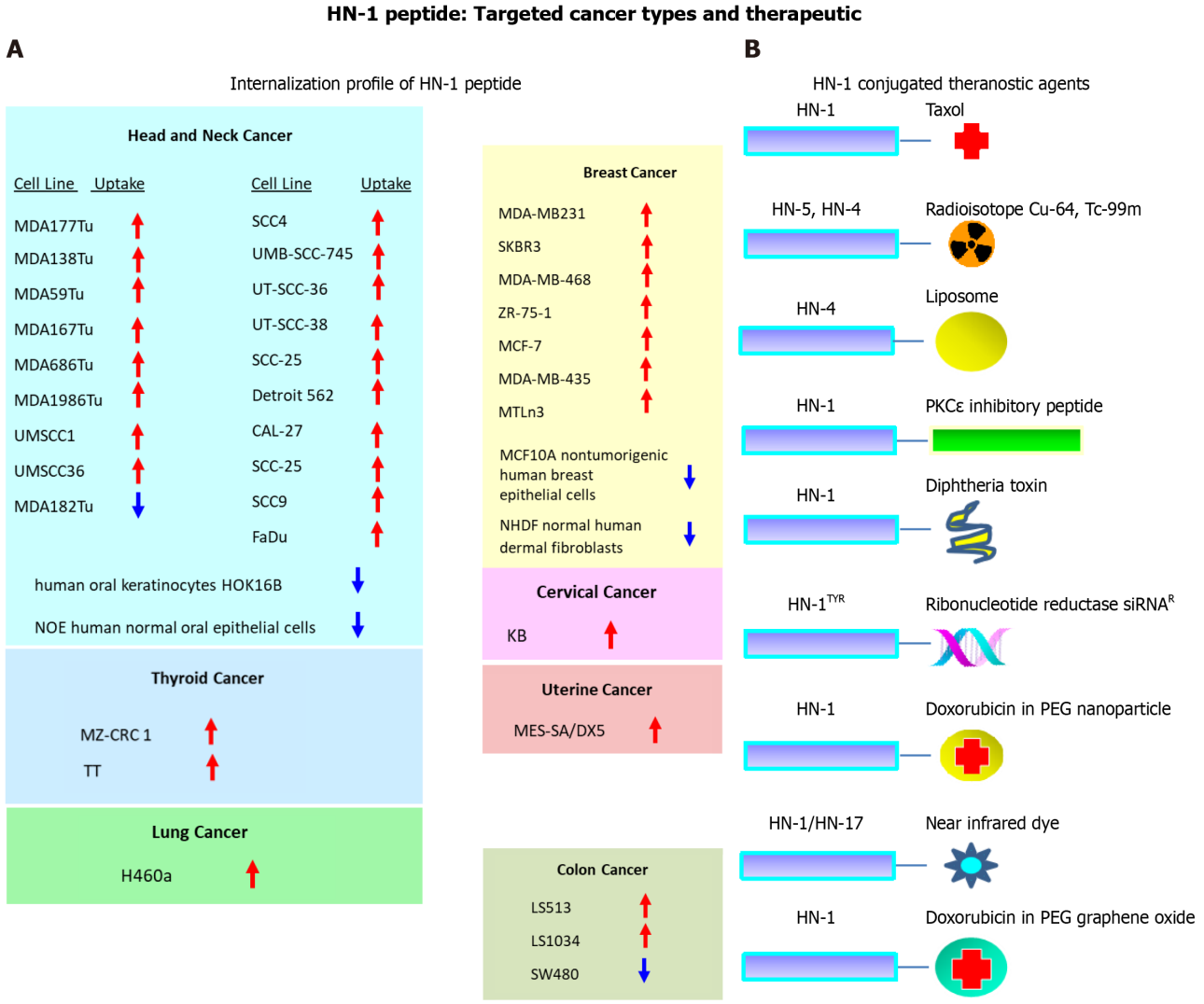


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Figure 3 HN-1 peptide exhibits similarity to integrin peptide. A: The similarities between the HN-1 sequence (TSPLNIHNGQKL) and a stretch of amino acids in alpha5 beta3 integrin (KLLITIHDRKEF) are highlighted. As HN-1 peptide lacks the recognition motif (RGD) of integrin, HN-1 may interact with an "integrin-like" molecule. HN-1 is internalized by attached cells but not by suspended cells. As tissue culture plates are typically coated with collagen, collagen-binding DDR1 receptor (expressed by adherent but not suspended cells) may represent the receptor for HN-1 consistent with that DDR1 is overexpressed in human head and neck cancer (also breast cancer) targeted by HN-1. avb3: alpha5 beta3; B: Comparison of 3D models generated by PEP-FOLD (INSERM, France) in RPBS bioinformatics web portal: https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/ TSPLNIHNGQKL: HN-1 peptide (top panels); KLLITIHDRKEF, membrane proximal sequence of beta subunit's cytoplasmic tail in alpha5 beta3 integrin (bottom panels); TSPLNIHNGQKL: Thr-Ser-Pro-Leu-Asn-Ile-His-Asn-Gly-Gln-Lys-Leu; KLLITIHDRKEF: Lys-Leu-Leu-Ile-Thr-Ile-His-Asp-Arg-Lys-Glu-Phe.

derived tumors in a mouse xenograft model[12,57,59,62,65]. In contrast, HN-1 was poorly uptaken by their normal counterpart (human oral keratinocytes HOK16B, NOE human normal oral epithelial cells, NHDF normal human dermal fibroblasts)[12,58,59].

Recent works have uncovered additional cancer types targeted by HN-1 peptide (Figure 4A). HN-1 uptake was also observed with human large cell lung carcinoma H460a cells[64]. Also, the HN-1 derived peptide HN17 (contains rearranged HN-1 sequence) was internalized by MZ-CRC 1 and TT human thyroid cancer cells[66]. HN-1 was also shown to target breast cancer irrespective of their 'triple status'. HN-1 or HN-1^{TYR} (HN-1 with two extra tyrosine residues added N-terminally) was internalized by MDA-MB231, SKBR3, MDA-MB-468, ZR-75-1, or MCF-7 breast cancer cells while MCF10A nontumorigenic mammary epithelial cells exhibited little HN-1 uptake[58,59]. Additionally, HN-1 was internalized by MDA-MB-435, MDA-MB-231, and MTLn3 breast cancer cells[64]. Further, HN-1 was internalized by KB cells[58] (originally human oral epidermoid carcinoma cells but subsequently found to contain HeLa human cervical adenocarcinoma cells)[67], raising the prospect that HN-1 may target



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Figure 4 The repertoire of targeted cancers and therapeutic application of HN-1 peptide. A: HN-1 uptake profile. The internalization of HN-1 peptide by various human cancer cells *vs* the corresponding normal human cells was compared. HN-1 uptake: red arrow (internalized), blue arrow (undetectable); B: Previously developed HN-1 based conjugates for therapy or diagnosis. Note: whether HN-17 peptide (consisting of a permutated version of HN-1 sequence) enters cells *via* the same route as HN-1 peptide or through a distinct route is not known. HOK: Human oral keratinocyte; NOE: Normal oral epithelial; NHDF: Normal human dermal fibroblasts; Cu: Copper; Tc: Technetium; PKCe: Protein kinase C epsilon; siRNA: Small interfering RNA; PEG: Polyethylene glycol.

cervical cancer. For uterine cancer, HN-1 uptake by MES-SA/Dx5, a multi-drug resistant cell-line derived from the human uterine sarcoma cell line MES-SA, was documented[64]. For gastrointestinal cancer, HN-1 uptake was observed with human colorectal carcinoma LS513 and LS1034 cells[64].

HN-1 peptide translocates across the cell membrane to facilitate drug delivery. Upon entry, HN-1 is localized in punctate particles, which were subsequently identified as endosomes[57,60]. Multiple reports suggest that HN-1 is internalized *via* the 'receptor-mediated endocytosis': (1) The internalization of HN-1 is 'specific' (based on competition assay); (2) HN-1 can be uptaken in a serum-free medium (*i.e.*, its receptor is cell-associated); (3) HN-1 is compartmentalized in endosomes after entry; and (4) Fusing the translocation domain of diphtheria toxin allows HN-1 to escape from the endosome into the cytosol. Using inhibitors of various entry routes (receptor-dependent endocytosis, pinocytosis, simple transmembrane diffusion, caveolae-mediated pathway), HN-1 uptake was shown to be mediated by 'energy-dependent clathrin-mediated endocytosis'[57].

HN-1 efficiently penetrates tumor mass, which is significant as > 90% of human cancers are comprised of solid tumors. The intravenously injected HN-1 was localized at the interior of solid tumors derived from MDA177Tu or MDA167Tu human HNSCC cells in a mouse xenograft model[12]. An independent report showed that the intravenously injected HN1 penetrates solid tumors derived from UMSCC1 human HNSCC cells *in vivo*[59]. Using radiolabeled HN-5 peptide, a derivative of HN-1, its penetration into solid tumors formed by SCC4 human HNSCC cells was documented using a nude rat model[57]. Further, nanoparticles displaying HN-1 peptide effectively penetrated solid tumors derived from human oral squamous cell carcinoma SCC-25 cells in a murine xenograft model[62]. Through these

works, the pharmacological properties of HN-1 have been confirmed globally.

The unique properties of HN-1 have been exploited to develop novel anticancer therapeutics (Figure 4B). In 2009, Henri *et al*[64] (Tapestry Pharmaceuticals, Boulder, United States) developed targeted chemotherapy comprised of HN-1 peptide and taxol. Taxol is an antimicrotubule drug that induces lethality by triggering 'hyperploid progression mediated death' in G1 DNA damage checkpoint defective human cancer cells due to mutant *Rb* or *p53* gene[16,24,68]. The authors delineated a series of complex organic chemistry synthesis steps to conjugate HN-1 to taxol without affecting the latter's antimicrotubule property. Using a murine xenograft model derived from human head and neck cancer FaDu cells, the authors demonstrated that the HN-1 peptide-to-taxol conjugate completely suppresses the growth of tumors. Further, it exhibited synergy with a second chemotherapeutic agent against breast, lung, uterine, or colon cancer cells. Of significance, the combined regimen increased growth inhibition from < 15% (by a single agent) to ~80% against breast cancer cells. For drug resistance, the conjugate showed activity against multi-drug resistant human uterine sarcoma cells.

In 2007, Zheng (University of Texas Health Science Center, San Antonio, United States) developed HN-5 (CYTSPLNIHNGQKL), a derivative of HN-1 peptide containing one cysteine and one tyrosine residue at the N terminus[57]. Using HN-5 peptide radiolabeled with Cu-64 gamma-ray emitter, human HNSCC cell-derived tumor xenograft in a nude rat model was successfully imaged *via* positron emission tomography. HN-5 peptide exhibited specificity and affinity to HNSCC and exhibited a high diffusion capacity within a solid tumor. More significantly, by conjugating to HN-5, a significant reduction in the accumulation of unconjugated Cu-64 radioisotopes in the liver, heart, kidney, lung, and small intestine was observed. Subsequently, in 2013, Nordquist (University of Texas Health Science Center, San Antonio, United States) quantitatively documented selective accumulation of the radiolabeled HN-5 peptide in HNSCC-derived tumor xenografts. Of relevance, HN-4 peptide linked to the radioisotope Tc-99m has been presented at the 2007 American Association for Advancement of Science annual meeting.

In 2008, the HN-4 peptide was used to develop a human HNSCC-targeting liposome for targeted drug delivery. The components used to construct the liposomes included the lipids DPPC, DMPG, and PEG. The synthesis involved the preparation of lipid film, reconstitution in sucrose solution, and extrusion to limit particle size to 100 nm, which was presented at the 2008 Texas Science & Engineering Fair.

In 2010, Bao *et al*[59] (Ohio State University Medical, Center, Columbus, United States) developed HN1-PKC(epsilon), a capped bi-functional peptide comprised of HN-1 peptide and PKC(epsilon)-inhibitory peptide connected through a linker. The bifunctional inhibitory peptide was selectively internalized by human HNSCC cells and suppressed the growth of HNSCC xenograft in nude mice. The report also extended the targets of HN1 peptide to breast cancer. Of clinical significance, whereas cisplatin alone inhibited the growth of HNSCC cells by 49%, combining cisplatin with the above bi-functional peptide suppressed growth by 72%.

In 2011, Potala *et al*[60] (Indian Institute of Technology Madras, Chennai, India) developed a novel fusion toxin comprised of HN-1 peptide and diphtheria toxin. The fusion toxin displayed a high degree of selectivity towards HNSCC and exhibited IC_{50} of 1-5 nM (nanomolar). They characterized 'energy-dependent clathrin-mediated endocytosis' as the HN-1 entry route and identified the intracellular punctate particles containing internalized HN-1 peptides as endosomes. The authors also found that the internalized fusion toxin utilized the translocation domain (of diphtheria toxin) to gain entry into the cytosol from endosomes.

In 2011, Dudas *et al*[55] (Medical University Innsbruck, Innsbruck, Austria) reported that the pharmacological properties of HN-1 peptide could be recapitulated using distinct human HNSCC cells. Further, the authors reported a seminal finding that the binding of HN-1 peptide to human HNSCC cells occurred in attached but not suspended cells[55]. It suggested that the expression level of the cognate cell surface receptor for HN-1 is governed by the presence of an extracellular matrix to which it adheres, providing an important clue for the discovery of DDR1 as the putative receptor for HN-1 peptide (see above).

In 2012, for gene therapy, Un *et al*[58] (Beckman Research Institute of City of Hope National Medical Center, Duarte, United States) developed a therapeutic conjugate composed of HN-1(Tyr) and a siRNA targeting human ribonucleotide reductase subunit M2. HN-1(Tyr) contains two tyrosine residues added N-terminally to the HN-1 peptide[58]. The siRNA(R) resistant to RNase degradation was designed by J. Rossi (City of Hope National Medical Center, Duarte, United States)[69] and was able to suppress endogenously expressed ribonucleotide reductase *in vivo* after delivering in a nanoparticle constructed by M. Davis (California Institute of Technology, Pasadena, United States)[70]. The clinical trial involving melanoma patients was conducted by Y. Yen (City of Hope National Medical Center, Duarte, United States), a former colleague of J. Doroshow (National Cancer Institute, Bethesda, United States). For RNA interference therapy, the HN-1(TYR)-anti-hRRM2 siRNA(R) construct moderatly suppressed the endogenously expressed ribonucleotide reductase M2 subunit in breast cancer cells.

In 2017, Wang *et al*[62] (Tianjin Medical University, Tianjin, China) constructed a nanoparticle comprised of polyethylene glycol *via* self-assembly, which contained the chemotherapeutic doxorubicin. Doxorubicin's primary anticancer activity is associated with its propensity to intercalate with double-stranded DNA and inhibit topoisomerase II, and it has been used to treat leukemia, breast, lung,

bladder, and other cancers. The nanoparticle was further modified to display HN-1 peptide at the exterior[62,71]. The spherical construct (~150 nanometers) exhibited HNSCC-specific uptake and cytotoxicity. *In vivo,* HN-1 peptide endowed greater HNSCC targeting capacity and tumor penetrating potential to the PEGylated nanoparticle. Whereas unconjugated doxorubicin remained at the periphery, HN-1 Linked nanoparticle readily infiltrated to deeper regions of the tumor. The clinical use of doxorubicin is hampered by side effects-especially, cardiac damage. Intriguingly, a significantly lower amount (~1/15 fold) of doxorubicin accumulated in the heart and other organs (ex. liver, kidney, spleen, lung) after administering the construct in a murine xenograft model.

In 2017, Rossfeld *et al*[66] (Arthur G. James Comprehensive Cancer Center and Ohio State University, Columbus, United States) developed a conjugate comprised of the near-infrared dye IRdye800 and HN-17 (TLPNSNHI KQGL), a derivative of HN-1 peptide, for intraoperative fluorescence imaging of tumors for optical surgical navigation. HN-17 (also called Compound-17) differs significantly from HN-1 at the primary structural level as it consists of a differentially permutated version of the HN-1 sequence. Though the sequence of HN-17 is comprised of identical amino acids (or their representation) as HN-1, it may not necessarily utilize the same entry mechanism as HN-1 (as indicated by its considerably faster rate of uptake). Further, whether HN-17 targets the same types of cancer as HN-1 is not known. Nevertheless, the study demonstrated that HN-1 peptide or its derivatives target medullary thyroid cancer. Subsequently, in 2019, Ding *et al*[65] (Ohio State University, Columbus, United States) demonstrated that IRdye800 conjugated HN-1 or HN-17 peptide could be used for fluorescence image guided resection of HNSCC during surgery using an animal model harboring an HNSCC-derived tumor xenograft.

In 2021, Li *et al*[63] (Shanxi Medical University School and Hospital of Stomatology, Taiyuan, China) developed a pH-sensitive (for drug release) delivery vector comprised of HN-1 peptide and nanoscale graphene oxide. After introducing polyethylene glycol to stabilize nanoscale graphene oxide, the construct was further modified to display HN-1 peptide to provide specificity for oral squamous cell cancer and achieve greater penetration into solid tumors. Doxorubicin was then loaded through the formation of hydrogen bond and pie-bond, which is weakened in an acidic milieu to allow drug release. Nearly 70% release of loaded drugs occurred at pH 5.6 compared to a significantly lower percentage at pH 7.4. As the tumor tissue exhibits an acidic environment, lower toxicity of the doxorubicin-containing nanoparticle to normal tissues (displaying higher pH) is expected.

CONCLUSION

In closing, we would like to reiterate that the accumulation of systemically administered cytotoxic anticancer drugs at various normal tissues following the intravenous injection can be debilitating for many cancer patients. To a large extent, the failure to guide drugs to tumors (resulting in < 0.5% of drugs reaching tumors) represents one of the major causes of side effects. Given the number of obstacles that the microenvironment of solid tumors poses (ex. stroma, basement membrane, interstitial pressure), overcoming these hindrances remains a major challenge in drug delivery. To reduce side effects, tumor-homing vectors are increasingly sought for targeted delivery. The use of previously developed vectors has been limited by inefficient penetration into solid tumors. Through the isolation of tumor specifically internalizing peptides such as HN-1, we have begun to chip away at the formidable problem of eliminating side effects associated with current drugs--hence, providing a molecular biological solution to the drug delivery problem. Following its discovery, multiple laboratories located globally have developed HN-1 conjugated therapeutics to mitigate side effects[61]. The international effort has been extended to developing imaging agents for tumor diagnosis as well as surgical navigation. Taken together, these works confirm the tumor specificity of the HN-1 peptide and underscore the expanding repertoire of its therapeutic application. Finally, the deciphering of clues that pointed to DDR1 tyrosine kinase as the putative receptor of HN1 may prove to be critical in moving toward clinical application. Further studies are planned to assess HN-1 peptide's interaction with DDR1 and the therapeutic potential of treating metastatic cancer.

ARTICLE HIGHLIGHTS

Research background

The genetic basis of human cancers was elucidated *via* the identification of the prototypic human tumor suppressor retinoblastoma (*Rb*) gene by F. Hong (previously worked on phosphate transferase system governing diauxie at the Johns Hopkins University, whose alternate interpretation inspired operon concept) at the University of California at San Diego. His determination of the *Rb* gene sequence helped to uncover the central role of Rb in regulating the cell cycle as a component of DNA damage checkpoint at the G1 or S phase, which is regulated by cyclin-dependent kinase (Cdk) resulting in FDA-approved Cdk4/6 inhibitors for treating advanced-stage breast cancer. His discovery of Rb's intrinsic properties to

interact with DNA as well as to form oligomers like the breast cancer type 1 susceptibility protein C-terminus (BRCT) laid the foundation for understanding Rb's function in regulating DNA replication, transcription (ex. E2F), epigenetics (histone modification), heterochromatin, and condensation. These works culminated in his discovery of the tumor-specific lytic path 'hyperploid progression mediated death' targeting Rb or p53 mutant cancers.

Research motivation

Metastatic cancer diagnosed in late-stage remains a formidable challenge, often resulting in mortality. Combinatorial regimens consisting of multiple chemotherapeutic agents administered to treat metastatic cancer incur an unacceptably high level of morbidity.

Research objectives

There is a great unmet need to direct or guide the intravenously injected drugs to tumors as less than 0.5% reach tumors currently, contributing to severe side effects.

Research methods

Harnessing the power of molecular biology, random peptide displaying M13 bacteriophage-based library was screened by F. Hong, who previously utilized the recombinant phages to determine the genomic sequence of avian infectious bronchitis virus' spike protein for vaccine development at the Salk Institute, which predated the emergence of COVID-19 coronavirus. The screening was conducted at the University of Texas M. D. Anderson Cancer Center using live surgically derived human head and neck squamous cell carcinoma cells. After screening 2.5×10^{12} random peptides, a single peptide TSPLNIHNGQKL (HN-1) was isolated, which is tumor-specific, translocates across the cell membrane, and capable of penetrating solid tumors for targeted drug delivery.

Research results

Through global participation, the above properties of the HN-1 peptide have been confirmed. The international endeavor also led to the development of numerous HN-1 peptide conjugated agents for therapy (taxol, doxorubicin, protein kinase C inhibiting peptide, ribonucleotide reductase inhibiting siRNA, diphtheria toxin, polyethylene glycol linked to doxorubicin, graphene oxide nanoparticle-containing doxorubicin) as well as imaging (gamma-ray emitting isotopes for radiotherapy, near-infrared fluorescent dyes for surgical navigation) of cancer. More significantly, we now know that HN-1 peptide also targets breast and thyroid (potentially cervical, lung, uterine, colon) cancers.

Research conclusions

While analyzing its amino acid content, an important clue was obtained that pointed to discoidin domain receptor 1 (DDR1) as the HN-1 peptide's cognate receptor. The finding is in alignment with previously accrued experimental data globally concerning the uptake route of HN-1. The identification of Rb-regulated DDR1 as the putative receptor for HN-1 opens unexpected opportunities to block cancer progression *via* targeting the very protein mediating metastasis.

Research perspectives

Through abrogating metastasis, it may preempt the recurrence of refractory metastatic cancers, which inevitably arise due to the acquiring of drug resistance.

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FOOTNOTES

Author contributions: Hong FU has worked on cancer genetics and molecular pharmacology; Castro M participated in therapeutics research; Linse K performed molecular modeling to predict the structure of peptides; Hong FU, Castro M, and Linse K meet the criteria for authorship established by the International Committee of Medical Journal Editors and verify the validity of the results reported; Hong FU wrote the manuscript; all authors approved the final version of the article.

Conflict-of-interest statement: Dr. Hong has received royalties from University of Texas M. D. Anderson Cancer Center patent covering materials related to HN-1 peptide. All other authors have nothing to disclose.

Data sharing statement: No additional data available.

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ORIGINAL ARTICLE

Retrospective Study

Co-relation of SARS-CoV-2 related 30-d mortality with HRCT score and RT-PCR Ct value-based viral load in patients with solid malignancy

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Abstract

BACKGROUND

Coronavirus disease 2019 (COVID-19) patients with malignancy are published worldwide but are lacking in data from India.

AIM

To characterize COVID-19 related mortality outcomes within 30 d of diagnosis with HRCT score and RT-PCR Ct value-based viral load in various solid malignancies.

METHODS

Patients included in this study were with an active or previous malignancy and with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection from the institute database. We collected data on demographic details, baseline clinical conditions, medications, cancer diagnosis, treatment and the COVID-19 disease course. The primary endpoint was the association between the mortality outcome and the potential prognostic variables, specially, HRCT score, RT-PCR Ct value-based viral load, *etc.* using logistic regression analyses treatment received in 30 d.

RESULTS

Out of 131 patients, 123 met inclusion criteria for our analysis. The median age was 57 years (interquartile range = 19-82) while 7 (5.7%) were aged 75 years or older. The most prevalent malignancies were of GUT origin 49 (39.8%), hepato-

pancreatobiliary (HPB) 40 (32.5%). 109 (88.6%) patients were on active anticancer treatment, 115 (93.5%) had active (measurable) cancer. At analysis on May 20, 2021, 26 (21.1%) patients had died. In logistic regression analysis, independent factors associated with an increased 30-d mortality were in patients with the symptomatic presentation. Chemotherapy in the last 4 wk, number of comorbidities (≥ 2 *vs* none: 3.43, 1.08-8.56). The univariate analysis showed that the risk of death was significantly associated with the HRCT score: for moderate (8-15) [odds ratio (OR): 3.44; 95% confidence interval (CI): 1.3-9.12; P = 0.0132], severe (> 15) (OR: 7.44; 95%CI: 1.58-35.1; P = 0.0112).

CONCLUSION

To the best of our knowledge, this is the first study from India reporting the association of HRCT score and RT-PCR Ct value-based 30-d mortality outcomes in SARS-CoV-2 infected cancer patients.

Key Words: SARS-CoV-2; COVID-19; Cancer; HRCT; Viral load

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Core Tip: There is a higher fatality rate in cancer patients as compared to non-cancer patients. Also, a higher incidence of serious clinical events and intensive care unit (ICU) admissions in cancer patients. Analysis suggests patients have increased morbidity and mortality from recent cytotoxic chemotherapy. Patients with active untreated cancer, metastatic disease, progressive disease with multiple co-morbidity as well as getting palliative treatment are at a higher risk of mortality. Mortality rates are higher in patients with high baseline HRCT values at presentation and need longer ICU stays. Mortality rates are not a statistically significant co-relation with higher baseline RT-PCR based viral load values at presentation. Mortality rates are not higher in older cancer patients as compared to younger counterparts with cancer.

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INTRODUCTION

After initially being identified in December 2019 in the Chinese city of Wuhan, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated sickness of coronavirus disease 2019 (COVID-19) has become a global pandemic[1]. The novel enveloped beta-coronavirus was immediately recognized as the infecting agent[2,3]. Coronaviruses are non-segmented enveloped positive-sense RNA viruses that belong to the Coronaviridae family[4]. This is the 3rd large-scale health crisis caused by beta-coronaviruses. The novel coronavirus pandemic (2019-nCoV) was designated a public health emergency of international concern by the World Health Organization (WHO) on January 30 and COVID19 was classified as a pandemic by the WHO on March 11, 2020[5]. As of May 31, 2021, there have been 171 million cases reported worldwide in 222 countries with 3.55 million deaths. In India alone, there are 28 million cases and 0.32 million fatalities. India represents an approximately 16.3% share of worldwide coronavirus cases and a 9.1% share of worldwide mortality[6]. Patients with a history of active malignancy may be at a higher risk of getting COVID-19 and having COVID-19-related problems according to various reports[7-9]. Initial reports, however, are limited by sample size, geographic region and the inability to generalize findings to the entire community of cancer patients. The impact of antineoplastic therapy and supportive care for cancer patients may impair their immune system. We conducted a retrospective study on cancer patients with COVID-19 infection comparing different demographic and clinical parameters with treatment-related mortality.

MATERIALS AND METHODS

Study design and settings

This is a single-center, retrospective study conducted at a tertiary cancer care hospital. Patients with active cancer presented to the hospital between April 2020 to April 2021 with a confirmed SARS-CoV-2 infection. The inclusion criteria were the patients with confirmed COVID-19 in a diagnosed case of solid

malignancy.

The study has been approved by our Institutional Review Board (RGCIRC/Res/SCM/46 2021/95) and was conducted according to the Declaration of Helsinki.

Variables and outcomes

The primary endpoint was to measure mortality within 30 d of diagnosis of COVID-19 with HRCT score and RT-PCR Ct value-based viral load. Secondary endpoints were measuring mortality compared with demographic variables (*i.e.* age, sex, obesity, smoking status) and clinic variables such as HRCT scoring including baseline laboratory values for D dimer, C-reactive protein (CRP), number of comorbidities, Eastern Cooperative Oncology Group (ECOG) performance status, requiring active treatment, recent surgery (including, but not limited to cancer surgeries, within 4 wk of COVID-19 diagnosis), type of malignancy, cancer status (remission *vs* active disease), with active further need as stable *vs* responding to treatment *vs* progressing disease), anticancer therapy and COVID-19 treatment with azithromycin, hydroxychloroquine, ivermectin or in combination *vs* various other treatment options used, *i.e.* Steroid alone or in combination with Remdesivir, Tocilizumab, Plasma therapy during infection. As it is a retrospective cohort study, selection bias occurred due to the unavailability of data for a few patients.

Statistical analysis

Descriptive statistics such as age and sex was used to show the baseline demographic information of the participants included in our analyses. All quantitative data are expressed as a mean ± SD. Categorical variables are expressed as numbers and their respective percentage. Univariate analysis was conducted to determine the risk factor of death in all the admitted patients by using Logistic regression. All data entries and statistical analyses will be performed by using SPSS® Version 23.0 software. All these statistics will be accompanied by 95% confidence intervals (CI). All the reported p-values will be two-sided and *P*-values < 0.05 shall be considered to indicate statistical significance.

RESULTS

Out of 131 patients, 123 met the inclusion criteria for our analysis. The clinical features are shown in Table 1. The median age was 57 years (interquartile range [IQR] 19-82), 7 (5.7%) were aged ≥ 75 and 64 (52%) of the patients were female. The most common malignancies were of GUT origin 49 (39.8%) and hepato-pancreaticobiliary 40 (32.5%). 109 (88.6%) patients were receiving active anticancer therapy with 115 (93.5%) patients having active (measurable) cancer. At analysis on May 20, 2021, 26 (21.1%) of the patients had died.

Patients with mild or moderate disease were given symptomatic treatment, and most mild disease patients were treated with home-based care. Hydroxychloroquine, ivermectin and/or dexamethasone were administered in moderate disease cases. Corticosteroids, hydroxychloroquine, ivermectin, remdesivir, tocilizumab and convalescent plasma therapy were used to treat severe COVID-19-infected cancer patients. Assisted ventilation was given to 18 patients (6.45%) but all of these patients later experienced COVID-19-related complications such as pneumonitis and subsequent respiratory failure, septic shock, or sudden cardiac arrest and succumbed to their illness.

The univariate logistic regression analysis for mortality has been shown in Table 2. The risk of death was statistically significant with the presence of symptomatic presentation (odds ratio [OR] = 11.1, P = 0.0211), number of comorbidities ≥ 2 *vs* none (OR = 3.43, P = 0.0303), Eastern Cooperative Oncology Group performance status of 0/1 v/s ≥ 2 (OR = 3.88, P = 0.047). The odds of mortality were significantly higher in patients presenting with moderate OR = 3.44, P = 0.0132) and severe HRCT score (OR = 7.44, P = 0.0112) as compared to patients with mild HRCT score (Table 3). Similarly, patients with smoking habits were at a high risk of 30-d mortality (OR = 5.54, P < 0.001). Progressive disease was also found to be a significant risk factor for mortality with OR = 25.5) (Figure 1).

No statistically significant association of 30-d mortality was found concerning age, sex, type of malignancy, type of anticancer therapy obesity status, recent surgery and active cancer (progressing *vs* remission). Also, no significant effect on mortality was noted for the patients with RT PCR based on different viral load levels.

DISCUSSION

Cancer patients are a particularly vulnerable group in the current COVID-19 pandemic. They are at a higher probability of severe illness and increased mortality once diagnosed with COVID-19. This article analyzes previously known cancer patients and COVID-19 prognostic factors provide information on clinical management and outcomes of cancer and COVID-19 patients.

In a few studies, men were found to have a higher mortality risk than women. In addition to sex disparities and smoking rates, this is due to the difference in immunological and endocrine systems

Table 1 Patient demographic, clinical, baseline laboratory parameter and tumour characteristics, *n* (%)

Characteristics	Analysable population (*n* = 123)
Age, yr	
Median	59
Range	19-82
< 65	91 (74)
65-74	25 (20.3)
≥ 75	7 (5.7)
Sex	
Male	59 (48)
Female	64 (52)
Smoking status	
Never smoked	69 (58.9)
Smoker (former/current)	31 (26.3)
Unknown	18 (15.2)
Obesity status	
Not specified	106 (86.2)
Obese	13 (10.4)
Data missing	4 (3.4)
Number of comorbidities	
0	54 (43.9)
1	32 (26)
≥ 2	37 (30.1)
Type of malignancy	
Thorax	10 (8.1)
GIT	9 (7.3)
HPB	40 (32.5)
GUT	49 (39.8)
Others	15 (12.2)
Cancer status	
Remission or no evidence of disease	8 (6.5)
Present, stable, or responding to treatment	66 (53.7)
Present, progressive disease unknown	49 (39.8)
ECOG performance status	
0 or 1	90 (73.2)
2	18 (14.6)
≥ 3	15 (12.2)
Type of anticancer therapy	
None in the 4 wk before COVID-19 diagnosis	14 (11.4)
Non-cytotoxic therapy targeted therapy/endocrine therapy	9 (7.3)
Immunotherapy	3 (2.4)
Cytotoxic systemic therapy	90 (73.2)
External beam radiotherapy	7 (5.7)

Recent surgery	
None in the 4 wk before COVID-19 diagnosis	113 (91.9)
Yes	10 (8.9)
Baseline laboratory parameters	
C Reactive protein	44 (35.8)
D-Dimer	11 (8.9)
IL-6	5 (4.1)
RT-PCR test (viral load)	
Mild (17-24)	49 (39.8)
Moderate (25-31)	30 (24.4)
High (> 31)	44 (35.8)
HRCT score	
Mild	77 (62.6)
Moderate	38 (30.8)
Severe	8 (6.5)
Treatment of COVID-19	
Hydroxychloroquine (HCQ) alone, azithromycin alone or with combination HCQ/azithromycin/ivermectin	22 (17.9)
+ Steroids	36 (293)
+ Remdesivir	29 (23.6)
+ Tocilizumab	3 (2.4)
+ Plasma therapy	11 (8.9)
Neither	22 (17.9)

GIT: Gastrointestinal tract; HPB: Hepatopancreatobiliary; GUT: Genitourinary tract; COVID-19: Coronavirus disease 2019; ECOG: Eastern Cooperative Oncology Group.

between men and women which may result in differential responses to the SARS-CoV-2 infection. The present study has no similar difference related to the sex of the patient. In reports from Europe, the United States and China, non-malignant populations are consistent with COVID-19 outcome data reported for overall mortality and was associated with comorbidities such as obesity and advanced population age[10,11].

Moreover, case-fatality rates for patients with COVID-19 who had breast, thyroid, or cervical cancer were low in the previously published study. As reported, 62 (57%) of 109 women had one of these three types of cancers[12-14]. The United Kingdom Coronavirus Cancer Monitoring project (UKCCMP) with a database of 800 patients with the most common cancers were the gastrointestinal, respiratory, breast, male genital and hematological cancers. In our study, 43% of our patients had metastatic disease and 89 (72.3%) of these patients having the metastatic disease were in the GIT and HPB malignancy cohorts. Sixty-five patients had cancer treatment in the previous 4 wk for the UKCCMP while in our study 88.6% of patients had received some form of treatment in the last 4 wk. In the UKCCMP study and in our study ≥ 50% of the patients receiving treatment had received cytotoxic chemotherapy. 45 patients had a severe form of infection. The mortality rates were high at 28% (226 out of 800 in the UKCCMP) while in our present study 21.1% (26 out of 123). Disease fatality is the function of pathogen virulence, host tolerance and pathogen load[15]. Pathogenicity is often the consequence of an overactive immune or inflammatory response[15,16]. Cancer patients normally have a compromised immunity due to their existing cancer and associated treatment[17]. So, cancer patients may have persistent SARS-CoV-2 viral infection which cannot be cleared by their compromised immune system in a short time, but their COVID-19 disease is not severe and some of them may still recover from the COVID-19 disease. The majority of patients exhibited COVID-19-like symptoms and the overall rate of complications were higher. The patients who died had higher co-morbidities and were older than those who recovered. Patients who got chemotherapy within the last 4 wk of COVID-19 did not have a higher mortality rate than those who did not get chemotherapy. Patients who received non-chemotherapy treatments (radiation, hormone therapy, immunotherapy, and targeted therapy) did not have an increased risk of death[18]. COVID-19 has been associated to a greater fatality rate in cancer patients but cancer treatments have not been associated with an increased risk of mortality as found in this study.

Table 2 Primary and Secondary outcomes by potential prognostic variables (*n* = 123), *n* (%)

	Admitted to ICU	Met composite endpoint	Required mechanical ventilation	Died
Total (*n* = 123)	20 (16.2)	30 (24.3)	10 (8.1)	18 (14.6)
Age, yr				
< 65 (*n* = 91)	18 (19.7)	22 (22.2)	9 (9.9)	16 (17.6)
65-74 (*n* = 25)	1 (4)	5 (20)	1 (4)	1 (4)
≥ 75 (*n* = 7)	1 (14.3)	3 (42.8)	0	1 (14.3)
Sex				
Male (*n* = 59)	6 (10.2)	11 (18.6)	3 (5)	5 (8.5)
Female (*n* = 64)	14 (21.8)	19 (29.7)	7 (10.9)	13 (20.3)
Number of comorbidities				
0 (*n* = 54)	6 (11.1)	9 (16.7)	0	4 (7.4)
1 (*n* = 32)	5 (15.6)	11 (34.4)	4 (12.5)	5 (15.6)
≥ 2 (*n* = 37)	9 (24.3)	10 (27)	6 (16.2)	9 (24.3)
Type of malignancy				
Thorax (*n* = 10)	1 (10)	2 (20)	1 (10)	1 (10)
GIT (*n* = 9)	5 (55.5)	6 (66.6)	3 (33.3)	4 (44.5)
HPB (*n* = 40)	8 (20)	14 (35)	4 (10)	7 (17.3)
GUT (*n* = 49)	5 (10)	7 (14.2)	2 (4)	5 (10)
Others (*n* = 15)	1 (6.7)	1 (6.7)	0	1 (6.7)
Cancer status				
Remission or no evidence of disease (*n* = 8)	0	2 (25)	0	0
Present, stable, or responding to treatment (*n* = 66)	6 (9.9)	9 (13.6)	5 (7.6)	5 (7.6)
Present, progressive disease unknown (*n* = 49)	14 (28.6)	19 (38.7)	5 (10)	13 (26.5)
ECOG performance status				
0 or 1 (*n* = 90)	0	10 (11.1)	0	0
2 (*n* = 18)	2 (11.1)	5 (27.7)	2 (11.1)	4 (22.2)
≥ 3 (*n* = 15)	15 (100)	15 (100)	8 (53.3)	14 (93.3)
Type of anticancer therapy				
None in the 4 wk before COVID-19 diagnosis (*n* = 14)	6 (42.5)	8 (57.1)	3 (21.4)	5 (35.7)
Non-cytotoxic therapy targeted therapy/endocrine therapy (*n* = 9)	2 (22.2)	5 (55.5)	1 (11.1)	2 (22.2)
Immunotherapy (*n* = 3)	0	0	0	0
Cytotoxic systemic therapy (*n* = 90)	11 (12.2)	16 (17.7)	6 (6.7)	10 (11.1)
External beam radiotherapy (*n* = 7)	1 (14.3)	1 (14.3)	0	1 (14.3)

Recent surgery				
None in the 4 wk before COVID-19 diagnosis (n = 113)	19 (16.8)	28 (24.8)	9 (8)	17 (15)
Yes (n = 10)	1 (10)	2 (20)	1 (10)	1 (10)
RT-PCR Test (viral load)				
Mild (n = 49)	5 (10.2)	8 (16.3)	3 (6.1)	5 (10.2)
Moderate (n = 30)	5 (16.7)	9 (30)	4 (13.3)	5 (16.7)
High (n = 44)	10 (22.7)	13 (29.5)	3 (6.8)	8 (18.1)
HRCT Score				
Mild (n = 77)	7 (9)	13 (16.9)	0	5 (6.5)
Moderate (n = 38)	7 (18.4)	11 (28.9)	4 (10.5)	7 (18.4)
Severe (n = 8)	6 (75)	6 (75)	6 (75)	6 (75)
Treatment of COVID-19				
Hydroxychloroquine (HCQ) alone, azithromycin alone or with combination HCQ/azithromycin/ivermectin (n = 22)	1 (4.5)	3 (13.6)	0	1 (4.5)
+ Steroids (n = 36)	4 (11.1)	8 (22.2)	2 (5.6)	4 (11.1)
+ Remdesivir (n = 29)	8 (27.6)	11 (37.9)	4 (13.8)	7 (24.1)
+ Tocilizumab (n = 3)	1 (33.3)	1 (33.3)	0	1 (33.3)
+ Plasma therapy (n = 11)	6 (54.4)	7 (63.6)	4 (36.6)	4 (36.6)
Neither (n = 22)	0	0	0	0

GIT: Gastrointestinal tract; HPB: Hepatopancreatobiliary; GUT: Genitourinary tract; COVID-19: Coronavirus disease 2019; ECOG: Eastern Cooperative Oncology Group; ICU: Intensive care unit.

The COVID-19 and Cancer Consortium (CCC19) published the results of 928 cancer patients from the United States, Canada and Spain who had COVID-19 infection. 654 patients with solid tumors, hematological malignancies diagnosed in 167 patients and 107 patients with multiple malignancies. In this study, 73.2% of the patients got cytotoxic chemotherapy in the previous 4 wk, whereas 160 patients received chemotherapy treatment and 206 patients received alternative forms of cancer therapy. The mortality rate was 13% within 30 d of COVID-19 diagnosis. Interestingly, 59% (n = 71) of the patients who died were never admitted to the intensive care unit (ICU); while in this study 30.8% (n = 8) died who were never admitted to the ICU. Outside the ICU, patients with active cancer have a higher death rate than those in remission. There was no association between 30-d all-cause mortality and non-cytotoxic treatments, recent surgery and cytotoxic treatment[19].

Mehta *et al*[20] have reported outcomes on 218 cancer patients with COVID-19. Seventy-five patients had solid tumors and 25% had hematological malignancies. The most common tumor types were genitourinary, breast and colorectal cancer, respectively. A total of 61 (28%) patients died. The mortality rate was 55% in patients with lung cancer and 67% with pancreatic cancer. Breast (14%) and genitourinary cancer (15%) were associated with a relatively lower mortality rate. Active chemotherapy and radiation therapy were not associated with increased mortality. Active disease (< 1 year) and metastatic disease were associated with higher numerical mortality values but without statistical significance[20].

Studies that report Ct values of RT-PCR to quantify SARS-CoV-2 RNA in clinical material is limited. Patients with severe disease had significantly higher viral loads and the viral load was higher during the early stages of the disease according to Zheng *et al*[21]. Karahasan Yagci *et al*[22] reported that higher viral load was linked with increased age, comorbidities, smoking status and recent chemotherapy. SARS-CoV-2 RNA had a median Ct value of 28.16 (IQR: 24.5–31.6) in hospitalized patients and 26.77 (IQR: 23.1-29.7) in outpatients in the study. The number of comorbidities were higher in hospitalized patients (P < 0.01). In COVID-19, Huang *et al*[23], in 2020, reported that elevated CRP was associated with higher composite poor outcome and disease severity.

Table 3 Univariate regression models of potential variables associated with 30 d all-cause mortality (*n* = 123)

	Odds ratio	*P* value
Age, yr		
< 65	1	
65-74	0.48 (0.13-1.78)	0.2756
≥ 75	1.42 (0.26-7.88)	0.6883
Sex		
Male	1	
Female	1.22 (0.51-2.96)	0.6567
Smoking status		
Never smoked	1	
Smoker (former/current)	5.54 (2.05-14.99)	0.0008
Unknown	2.19 (0.39-12.21)	0.3711
Obesity status		
Not specified	1	
Obese	0.69 (0.14-3.32)	0.6414
Number of comorbidities		
0	1	
1	2.67 (0.83-8.56)	0.0993
≥ 2	3.38 (1.12-10.20)	0.0303
Type of malignancy		
Thorax	1	
GIT	2.57 (0.19-34.48)	0.4758
HPB	4.5 (0.51-39.44)	0.1744
GUT	1.5 (0.16-13.75)	0.7198
Others	1.38 (0.11-17.67)	0.8022
Cancer status		
Remission or no evidence of disease	1	
Present, stable, or responding to treatment	1.02 (0.16-6.43)	0.9832
Present, progressive disease unknown	25.5 (5.14-126.59)	0.0001
Type of anticancer therapy		
None in the 4 wk before COVID-19 diagnosis	1	
Non-cytotoxic therapy targeted therapy/endocrine therapy	0.13 (0.01-1.28)	0.08
Immunotherapy	0	0.9981
Cytotoxic systemic therapy	0.19 (0.06-0.63)	0.0067
External beam radiotherapy	0.2 (0.02-2.18)	0.1867
Recent surgery		
None in the 4 wk before COVID-19 diagnosis	1	
Yes	0.41 (0.05-3.38)	0.4053
Baseline laboratory parameters		
C reactive protein	1	
D-dimer	4.44 (1.76-11.23)	0.0016
IL-6	18.48 (1.96-173.82)	0.0108

HRCT score		
Mild	1	
Moderate	3.44 (1.3-9.12)	0.0132
Severe	7.44 (1.58-35.1)	0.0112
RT-PCR Test (viral load)		
Mild (49)	1	
Moderate (30)	1.28 (0.40-4.14)	0.6786
High (44)	1.71 (0.62-4.76)	0.3933
Treatment of COVID-19		
Hydroxychloroquine (HCQ) alone, azithromycin alone or with combination HCQ/azithromycin/ivermectin	1	
+ Steroids	3.91 (0.39-39.31)	
+ Remdesivir	31.06 (3.79-255.41)	0.2470
+ Tocilizumab		0.0014
+ Plasma therapy	75.25 (7.30-775.28)	0.0003
Presentation		
Asymptomatic	1	
Symptomatic	11.1 (1.43-85.85)	0.0211
Managed at		
Home-based care	1	
Ward admission	121.6 (21.8-677.7)	< 0.0001
ICU admission	0.4 (0.04-3.57)	0.4119

GIT: Gastrointestinal tract; HPB: Hepatopancreatobiliary; GUT: Genitourinary tract; COVID-19: Coronavirus disease 2019; ECOG: Eastern Cooperative Oncology Group; ICU: Intensive care unit.

The CRP levels available at the time of the PCR request were largely for hospitalized patients, hence a statistical comparison could not be established in this study. In a study of 76 patients, it was found that the Ct values of severe cases remained considerably lower for the first 12 d following commencement as compared to moderate instances[24].

Early in the disease course, Pan *et al*[25] observed a lot of ground-glass opacity abnormalities followed by the development of crazy paving patterns on chest CT and finally increasing consolidation later on. According to study outcomes, chest CT has a high specificity but a low sensitivity, particularly in patients who appear within the first 4 d of the sickness. The clinical value of chest CT was observed to be limited in a review article, particularly for individuals who have no symptoms and are screened early in the disease progression[26]. The inverse relationship between viral load and chest CT TSS was the most striking finding. In hospitalized patients and outpatients with extensive lesions on CT, the viral load of nasopharyngeal samples was considerably lower. The severity of a CT scan was related to the patient's age and older patients having higher severity scores ($P < 0.01$). Hospitalization was related to the presence of any kind and number of comorbidities, but not to CT severity. Patients with obesity or other metabolic syndromes like diabetes mellitus still have a competent immunity which may be malfunctioning due to overnutrition. When these patients are infected by the SARS-CoV-2 virus, the infection may trigger hyper inflammation which makes a lot of collateral damage to all organs (those who were not infected by the virus) in the body. So, the COVID-19 disease can be very severe or even the quick demise of the patient, even if their competent immune system is able to clear the SARS-CoV-2 viruses effectively.

Even if the viral load of SARS CoV-2 in nasopharyngeal swab specimens is high in the early stages of COVID-19, it is not always related to changes in chest CT. The viral load of nasopharyngeal swab specimens decreases as SARS CoV-2 progresses but the viral load of lower respiratory tract samples increases and chest CT changes become more visible. It's thought that viral load is vital for recognizing early stages of Covid-19 infection and limiting transmission but CT can only help identify cases that require substantial medical care.

Patients who died had lower average Ct values across multiple time points during the disease course than those who recovered or were still hospitalized at the end of the study [recovered: median 37.43

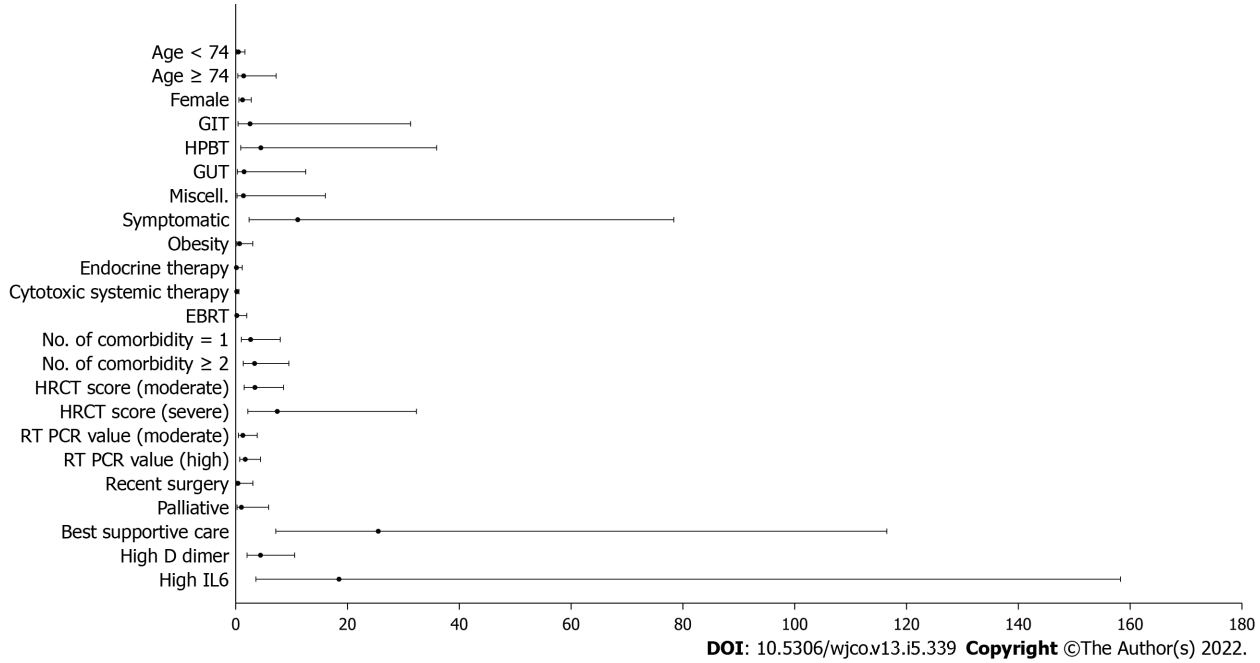


Figure 1 Forest plot for different parameters (demographic, clinical and laboratory) and 30-d mortality outcomes. GIT: Gastrointestinal tract; HPB: Hepatopancreatobiliary; GUT: Genitourinary tract; HPBT: Hepato-pancreato-biliary tract; EBRT: External beam radiotherapy.

(IQR 34.94-38.67); still hospitalized: median 36.97 (IQR 34.33-38.70); deceased: median 34.79 (IQR 24.46-37.65); P = 0.001] in a study of 308 patients from China. A study reported on the link between mortality and SARS-Cov-2 Ct values and found that lower Ct values were associated with a higher risk of death which is consistent with previous results regarding epidemic-causing coronaviruses[27,28]. C-reactive protein levels were shown to be adversely linked with Ct value in a study of 12 patients (r = -0.584; P = 0.03), but not in a study of 25 patients (P = 0.07)[29].

Several studies have reported the relationship between viral load as determined by Ct values and disease severity, and one of them (including 96 patients) found that higher viral loads were significantly related with more severe disease (Table 2)[30]. Mean viral loads were not significantly different between patients with pneumonia, severe pneumonia and those without pneumonia in a study by Shi *et al*[31]. Patients with severe pneumonia had a significantly higher viral load than those without pneumonia but severity outcomes were not statistically significant in this study. Shah *et al*[32] reported similar results in that there is no correlation between Ct values and severity of the disease.

CONCLUSION

With a CFR of 21.1%, this study reveals the significant rates of mortality in COVID-19 cancer patients. When comparing older cancer patients to younger cancer patients, mortality rates are not higher. Patients who had high baseline HRCT values at presentation and required ICU care had a higher mortality.

ARTICLE HIGHLIGHTS

Research background

The lack of correlation data between coronavirus disease 2019 (COVID-19) and Solid malignancy limits the understanding of the true mortality impact of the COVID-19 in the Indian settings.

Research motivation

Higher incidence of serious clinical events and intensive care unit (ICU) admissions in cancer patients. Analysis suggests patients have increased morbidity and mortality from recent cytotoxic chemotherapy. Patients with active untreated cancer, metastatic disease, progressive disease with multiple co-morbidity and getting palliative treatment are at a higher risk of mortality.

Research objectives
The primary objectives of the study include COVID-19 related mortality outcomes within 30 d of diagnosis with HRCT score and RT-PCR Ct value-based viral load in various solid malignancies.

Research methods
This is a single-center, retrospective study conducted at a tertiary cancer care hospital including confirmed COVID-19 in a diagnosed case of solid malignancy. The primary endpoint was to measure mortality within 30 d of diagnosis of COVID-19 with HRCT score and RT-PCR Ct value-based viral load.

Research results
The risk of death was statistically significant with the presence of symptomatic presentation, number of comorbidities ≥ 2 *vs* none, Eastern Cooperative Oncology Group performance status of 0/1 v/s ≥ 2. The odds ratio of mortality were significantly higher in patients presented with moderate and severe HRCT scores as compared to patients with mild HRCT scores. No statistically significant association of 30-d mortality was found concerning age, sex, type of malignancy, type of anticancer therapy, obesity status, recent surgery and active cancer (progressing *vs* remission). Also, no significant effect on mortality was noted for the patients with RT PCR based on different viral load levels.

Research conclusions
Mortality rates are higher in patients with high baseline HRCT values at presentation and who need longer ICU stays. Mortality rates are not higher in older cancer patients as compared to younger counterparts with cancer. Mortality rates are not statistically significant in co-relation with high baseline RT-PCR based viral load values at presentation.

Research perspectives
If there are further COVID-19 outbreaks, the findings of these studies will be helpful for clinical practice to categorize the patients on the basis of various demographic and clinical parameters for prognostication of patients.

FOOTNOTES

Author contributions: Narayan S and Talwar V were involved in design of study and the acquisition of data; Goel V, Chaudhary K, Redhu P, Soni S, Jain A were involved in the drafting and revision of the manuscript; Narayan S and Sharma A were involved in the statistical calculation; All authors discussed and contributed to the final manuscript.

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ORIGINAL ARTICLE

Retrospective Study

Survival characteristics of fibrolamellar hepatocellular carcinoma: A Surveillance, Epidemiology, and End Results database study

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Abstract

BACKGROUND

Fibrolamellar hepatocellular carcinoma (FL-HCC) is a rare and distinct type of hepatocellular carcinoma that frequently presents in an advanced stage in younger patients with no underlying liver disease. Currently, there is a limited understanding of factors that impact outcomes in FL-HCC.

AIM

To characterize the survival of FL-HCC by age, race, and surgical intervention.

METHODS

This is a retrospective study of The Surveillance, Epidemiology, and End Results database. We identified patients with FL-HCC between 2000-2018 by using an ICD-O-3 site code C22.0 and a histology code 8171/3: Hepatocellular carcinoma, fibrolamellar. In addition, demographics, tumor characteristics, types of surgical procedure, stages, and survival data were obtained. We conducted three separate survival analyses by age groups; ≤ 19, 20-59, and ≥ 60-year-old, and race; White,

Black, Hispanic, Asian and Pacific islanders (API), and surgical types; Wedge resection or segmental resection, lobectomy, extended lobectomy (lobectomy + locoregional therapy or resection of the other lobe), and transplant. The Chi-Square test analyzed categorical variables, and continuous variables were examined using the Mann-Whitney U test. The Kaplan-Meier survival curve was used to compare survival. Multivariate analysis was done with Cox regression analysis.

RESULTS

We identified 225 FL-HCC patients with a mean age of 36.9. Overall median survival was 34 (95%CI: 27-41) mo. Patients ≤ 19-years-old had more advanced disease with positive lymph nodes status. However, they received more surgical interventions such as a wedge, segmental resection, lobectomy, extended lobectomy, and transplant. Survival for ≤ 19 was 85 (95%CI: 37-137) mo, age 20-59 was 29 (95%CI: 18-41) mo, and age ≥ 60 years was 12 (95%CI: 7-31) mo ($P < 0.001$). There were no differences in stage, lymph node status, metastasis status, and surgical treatment among races. The median survival were; Whites had 39 (95%CI: 29-63), Blacks 26 (95%CI: 5-92), Hispanics 31 (95%CI: 11-54), and APIs 28 (95%CI: 5-39) mo ($P = 0.28$). Of 225 patients, 111 FL-HCC patients had surgical procedures. Median survivals for a wedge or segmental resection was 112 (95%CI: 78-NA), lobectomy was 92 (95%CI: 57-NA), extended lobectomy was 54 (95%CI: 23-NA), and a transplant was 63 (95%CI: 20-NA) mo ($P < 0.001$). The median survival was better in patients who had surgical treatments regardless of lymph nodes or metastasis status ($P < 0.001$).

CONCLUSION

FL-HCC occurs in a primarily younger population, but survival can be prolonged despite the aggressive disease. There were no racial differences in the survival of FL-HCC; however, Asians with FL-HCC tended to be older than in other races. Surgical treatment provided better survival even in those patients with nodal disease or metastases. Although future studies are needed to explore other therapies for FL-HCC, surgical options should be considered in all cases of FL-HCC unless contraindicated.

Key Words: Fibrolamellar hepatocellular carcinoma; Transplant; Race; Age; Survival

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Core Tip: Fibrolamellar hepatocellular carcinoma (FL-HCC) is a rare and distinct type of hepatocellular carcinoma. Currently, there is limited data on survival associated with FL-HCC. This retrospective study based on the Surveillance, Epidemiology, and End Results database suggests a better survival of younger patients with FL-HCC, although they had aggressive diseases. This trend may be because they received more surgical interventions. There were no racial differences in survival for FL-HCC, which is seen in HCC. The patient who had wedge or segmental resection or lobectomy had better survival.

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INTRODUCTION

Fibrolamellar hepatocellular carcinoma (FL-HCC) is a rare and distinct type of hepatocellular carcinoma (HCC) with an estimated incidence of 0.02 per 100000 in the U.S., and it accounts for < 1% of all primary liver tumors[1-3]. It is often found in the younger patients without known underlying cirrhosis or hepatic dysfunction and may present with advanced stages[4,5]. The pathogenesis of FL-HCC remains unclear, and it has not been associated with alcohol intake or viral hepatitis infections[6]. HCC was thought to originate from mature hepatocytes[7]. However, recent studies have suggested that FL-HCC may be derived from neuroendocrine progenitors[1]. FL-HCC is a vascular tumor with significant fibrosis and a well-differentiated tumor[1,5]. On computed tomography scan, FL-HCC appears as a hypodense mass with arterial enhancement and calcifications[8]. FL-HCC patients may present with non-specific symptoms such as abdominal pain and fullness, weight loss, fatigue, nausea, and vomiting [5], or asymptomatic with tumors found incidentally[9].

As FL-HCC is a rare type of cancer, there are no specific guidelines from the National Comprehensive Cancer Network in the United States to direct therapy. The majority of HCC clinical trials have excluded FL-HCC patients due to distinct disease progressions[10]. Therefore, treatment outcome with systemic therapy or immunotherapy has yet to be elucidated. A small study with 5-fluorouracil based chemotherapy demonstrated an incomplete response to treatment[11]. In general, FL-HCC has better survival than the common type of HCC, likely because these patients are younger and in the absence of underlying liver disease, they are more likely to qualify for curative surgical procedures[12]. While treatment modalities vary based on the tumor stages and resectability, complete resection with regional lymphadenectomy has the longest survival[13]. In a study by Stipa *et al*[9], 28 patients with FL-HCC who underwent complete resection had a 5-year overall survival of 76%. However, advanced stage FL-HCC has poor prognosis with a median survival of fewer than 12 mo[7].

There is a limited understanding of disease characteristics and factors affecting the survival outcomes on FL-HCC due to disease rarity and lack of randomized control trials. Single institution or multicenter studies do not have enough cases to characterize FL-HCC survival. This study aimed to characterize the survival of FL-HCC by age, race, and surgical intervention using a larger population-based database.

MATERIALS AND METHODS

Study design

The National Cancer Institute publishes population data on the Surveillance, Epidemiology, and End Results (SEER) database, and research data was obtained through Surveillance Research Program, National Cancer Institute SEER*Stat software (https://seer.cancer.gov/seerstat/) version 8.3.6[14]. SEER Registries contains population-based data on cancer incidence, characteristics, treatment, and mortality in select states in United States since 1973. Approximately 34.6% of all cancer cases in the United States population are included[15]. The SEER dataset utilized in this study is based on 18 states in United States and regions available to conduct survival analysis: Alaska Native Tumor Registry, California (San Francisco-Oakland, San Jose-Monterey, Los Angeles, Greater California), Connecticut, Georgia (Atlanta, Greater Georgia, Rural Georgia), Hawaii, Iowa, Kentucky, Louisiana, Michigan (Detroit), New Jersey, New Mexico, Utah and Washington (Seattle-Puget Sound) (more details are available at https://seer.cancer.gov/registries/terms.html). This study followed the SEER Research Data Use Agreement. As we utilized a publicly available, de-identified database, approval from an Institutional Review Board was not required to conduct this study.

Patients

We initially identified patients with FL-HCC between 2000-2018 by using an ICD-O-3 site code C22.0 and a histology code 8171/3: Hepatocellular carcinoma, fibrolamellar. Subsequently, we excluded patients from 2000-2003 and 2016-2018 due to the high number of missing data on demographic and disease-specific variables. Therefore, the years between 2004 and 2015 were included in this study. Additionally, one patient had duplicate data, so we used the variables with initial disease onset. Variables related to demographics data [age at the time of diagnosis, sex, race (Whites, Blacks, API, and Hispanics), living settings (population > 1 million and other), household income (< $55000, $55000-70000, and > $70000)], staging by American Joint Committee on Cancer Staging Manual, 6th edition[16] (categorized into stage I, II, III, IV, and unknown), tumor characteristics (size, metastasis status, lymph node status), surgical treatment modality (wedge or segmental resection, lobectomy, extended lobectomy (lobectomy + locoregional therapy or resection of the other lobe), transplant, and None (Including a small number of patients who had locoregional therapy and unspecified surgery), and survival data were obtained. Data on chemotherapy and interventional therapy was not included due to limitations acknowledged by the SEER database to avoid data inaccuracy.

Statistical analysis

Statistical analysis was performed with SAS version 9.4 (SAS Institute Inc., Cary, NC, United States). The Chi-Square test was used to compare categorical variables. The Mann-Whitney U test compared continuous variables without normal distributions of two groups and Druska-Wallis for more than two groups. Survival analysis was done by using the Kaplan-Meier survival curve. Three separate analyses were done: Analysis by age groups, race, and surgical procedures. For age group analysis, patients were divided into three groups: ≤ 19, 20-59, and ≥ 60-year-old. For race analysis, we divided into four racial groups: White, Black, API, and Hispanic. For analysis of surgical procedures, we separated into five groups: Wedge resection or segmental resection, lobectomy, extended lobectomy, transplant, and none. For additional survival analysis, we separate the patients based on positive lymph nodes or metastasis status (N1M0, N1M1, N0M1, NxM1: NM+) *vs* negative (N0M0, NxM0, N0Mx, NxMx: NM-). We then stratified by surgical status vs. no surgery or locoregional therapy. We conducted multivariate Cox regression analysis. $P < 0.05$ was considered significant. The statistical methods of this study were reviewed by Jihyun Ma from the Department of Biostatistics, College of Public Health, University of Nebraska Medical Center.

RESULTS

Overall

We initially identified 339 FL-HCC patients between 2000 and 2018. After excluding 114 patients due to missing data, 225 FL-HCC patients were included in our study. Baseline characteristics are shown in Table 1. The mean age was 36.9 years, with a median age of 27 years. (Interquartile range: 19-56). One hundred fourteen (62.7%) patients were male. Sixty-five (28.9%) had stage I, 18 (8.0%) had stage II, 61 (27.1%) had stage III, 61 (27.1%) had stage IV, and 20 (89%) had unknown stages. Thirty-nine patients (17.8%) had a wedge or segmental resection, 42 (19.2%) had a lobectomy, 11 (5.0%) had an extended lobectomy, 19 (8.7%) underwent live transplant, 108 (49.3) did not have any surgical intervention. Overall median survival was 34 (95%CI: 27-41) mo (Figure 1). Overall 5-years survival rate was 37.3 ± 3.3%.

Age

Table 1 summarizes the characteristics by age group. Sixty-two (27.5%) patients were age ≤ 19 years, 114 (50.7%) patients were between 20 to 59 years, and 49 (21.8%) patients were age ≥ 60 years. Patients ≤ 19-year-old had more nodal involvement and higher stages. A higher proportion of patients ≤ 19-year-old received the surgical intervention and none of the patients ≥ 60-year-old received extended lobectomy or transplant. There were no differences in sex, race, living settings, household income, and metastasis status. Detailed characteristics are listed in Table 1. The median survival for ≤ 19 years was 85 mo (95%CI: 37-137), 20-59 years was 29 mo (95%CI: 18-41), and ≥ 60 years was 12 (95%CI: 7-31) mo (P < 0.001) (Figure 2). The five-year survival rate for patients ≤ 19-year-old was 55.3 (6.5%), 20-59-year-old was 35.8 (4.6%), and ≥ 60-year-old was 14.6 (5.9%).

Race

There were 124 (55.1%) Whites, 52 (23.1%) Hispanics, 27 (12.0%) Blacks, and 22 (9.8%) APIs. Mean ages were 35.9, 37.2, 33.6, and 49.5 years, respectively. There were no differences in the distribution between the age groups or sex. APIs lived in the area with a higher median household income, and Blacks lived in the area with a lower median household income. A higher proportion of Whites lives in areas with a population < 1 million. There were no differences in stages, lymph node status, metastasis status, and surgical treatment. Detailed characteristics are listed in Table 2. The median survival differences by race were not significant: Whites had 39 (95%CI: 29-63), Blacks 26 (95%CI: 5-92), APIs 28 (95%CI: 6-39), and Hispanics 31 (95%CI: 11-54) mo (P = 0.28) (Figure 3). Furthermore, Whites had similar median survival compared to all other non-White races combined (39 *vs* 29 mo, P = 0.11) (Figure 4).

Surgery

After excluding six patients with missing data on surgery status, one hundred eleven FL-HCC patients had surgical intervention. One hundred and eight patients did not have a surgical intervention or received life review therapy, 42 underwent liver transplantation, 19 had wedge or segmental liver resection, 39 had a lobectomy, and 11 had extended lobectomy. Age groups, stages, lymph nodes status, metastasis status, and tumor size had a significant difference among types of surgical intervention. Detailed characteristics are listed in Table 3. The median survivals for a wedge or segmental resection were 112 (78-NA), lobectomy was 92 (57-NA), extended lobectomy was 54 (23-NA), the transplant was 63 (20-NA), and none was 10 (6-13) mo (P < 0.001) (Figure 5). The median survival for NM+/no surgery was 9 (4-14) mo, NM+/surgery was 54 (34-85) mo, NM-/no surgery was 11 (6-17) mo, and NM-/surgery was 142 (92-NA) mo (P < 0.001) (Figure 6).

Stages

Median survival months for stage I was 97 (95%CI: 34-NA), stage II was 87 (95%CI: 35-NA), stage III was 45 (95%CI: 29-63), stage IV was 14 (95%CI: 8-21), and the unknown stage was 18 (95%CI: 7-75) mo. The five-year survival rate for stage I was 53.6 ± 6.4%, stage II was 70.1 ± 11.2%, stage III was 37.6 ± 6.7%, stage IV was 13.1 ± 4.3%, and the unknown stage was 31.3 ± 10.9%.

Cox regression analysis

Multivariate Cox regression analysis included the following variables: age groups (compared to age ≤ 19), sex (compared to female), race (compared to White), income (compared to < $55000), surgery (compared to no surgery or locoregional therapy), population (compared to other), tumor size (compared to ≤ 50 mm), lymph node status (compared to N0), and metastasis status (compared to M0). This model excluded two patients with unknown surgical status. Due to the interaction with tumor size, lymph node status, and metastasis status, the stage was excluded from the model. This model showed that only Nx had a significant hazard ratio of 0.11 ± 1.37. Table 4 summarizes the result.

Table 1 Baseline characteristics and age groups (total 225 patients)

Factor	Group		Alive (%)	*P* value	< 20	20-59	60+	*P* value
n (%)		225	67 (29.8)		62 (27.5)	114 (50.7)	49 (21.8)	
Median age (IQR)		27 (19-56)			15.5 (13-17)	28.5 (23-44)	69.0 (65-76)	
Male (%)		141 (62.7)	42 (29.8)	0.99	33 (23.4)	74 (52.5)	34 (24.1)	0.17
Race (%)	White	124 (55.1)	39 (31.5)	0.66	34 (27.4)	67 (54.3)	23 (18.6)	0.07
	Black	27 (12.0)	8 (29.6)		6 (22.2)	16 (59.3)	5 (18.5)	
	API	22 (9.8)	4 (18.2)		3 (13.6)	9 (40.9)	10 (45.5)	
	Hispanic	52 (23.1)	16 (30.8)		19 (36.5)	22 (42.3)	11 (21.2)	
Household income (%)	< $55000	55 (24.4)	20 (36.4)	0.47	16 (29.1)	32 (58.2)	7 (12.7)	0.37
	$55000-70000	88 (39.1)	24 (27.8)		26 (29.6)	40 (45.5)	22 (25.0)	
	> $70,000	82 (36.4)	23 (28.0)		20 (24.4)	42 (51.2)	20 (24.4)	
Living settings (%)	Population > 1 million	144 (61.0)	36 (25.0)	0.04	38 (26.4)	71 (49.3)	35 (24.3)	0.47
	Other	81 (36.0)	31 (38.3)		24 (29.6)	43 (53.1)	14 (17.3)	
Stages (%)	I	65 (28.9)	32 (49.2)	< 0.001	13 (20)	29 (44.6)	23 (35.4)	0.02
	II	18 (8.0)	8 (44.4)		7 (38.9)	7 (38.9)	4 (22.2)	
	III	61 (27.1)	19 (31.1)		20 (32.8)	32 (25.5)	9 (14.8)	
	IV	61 (27.1)	2 (3.3)		20 (32.8)	34 (55.7)	7 (11.5)	
	Unknown	20 (8.9)	6 (30.0)		2 (10.0)	12 (60.0)	6 (30.0)	
Lymph Node status (%)	N0	143 (63.6)	52 (36.4)	0.009	35 (24.5)	68 (47.6)	40 (28.0)	0.004
	N1	60 (26.7)	13 (21.7)		24 (40.0)	32 (53.3)	4 (6.7)	
	Nx	22 (9.78)	2 (9.1)		3 (13.6)	14 (63.6)	5 (22.7)	
Metastasis status (%)	M0	154 (68.4)	64 (41.6)	< 0.001	41 (26.6)	74 (48.05)	39 (25.3)	0.16
	M1	61 (27.1)	2 (3.3)		20 (32.8)	34 (55.7)	7 (11.5)	
	Mx	10 (4.4)	1 (10.0)		1 (10.0)	6 (60.0)	3 (30.0)	
Surgery (%)	Wedge/segmental resection	39 (17.8)	23 (59.0)	< 0.001	15 (38.5)	17 (43.6)	7 (18.0)	< 0.001
	Lobectomy	42 (19.2)	21 (50.0)		23 (43.4)	23 (54.8)	3 (7.1)	
	Extended lobectomy	11 (5.0)	6 (54.5)		7 (63.6)	4 (36.4)	0 (0)	
	Transplant	19 (8.7)	7 (36.8)		6 (31.6)	13 (68.4)	0 (0)	
	None	108 (49.3)	10 (9.3)		18 (16.7)	54 (50.0)	36 (33.3)	

DISCUSSION

FL-HCC is a rare and unique type of HCC, and it commonly affects younger patients without underlying cirrhosis. Due to the rare nature of FL-HCC, disease characteristics and survival of FL-HCC by age, race, and surgical intervention remain scarce. To our knowledge, this is one of the larger population-based studies on FL-HCC obtained from a nationwide cancer registry, including detailed tumor characteristics. The study highlights and provides a better understanding of FL-HCC survival based on age, race, and surgical interventions at the population level. Overall median survival of patients with FL-HCC in this study was 34 mo, similar to that of other studies[2,17]. Our median survival was lower than the 75 mo obtained by Mayo and colleagues in 2014, analyzing SEER data from 1986 to 2008[12]. This difference is due to the fact that they only included surgically managed FL-HCC. Besides, their patients were younger (mean age: 25 *vs* 36 years) than ours, and they had a smaller sample size of FL-HCC (90 patients) compared to ours. In effect, younger age at diagnosis has been associated with better survival[18,19]. Also, FL-HCC was only established as a separate diagnosis in 1986, and there may have been a few years of transition before providers consistently coded FL-HCC as a distinct entity[12].

Table 2 Summary of Characteristics by Race (Total 225 patients)

Factor	Group	Total	White	Black	API	Hispanic	*P* value
n (%)		225	124 (55.1)	27 (12.0)	22 (9.8)	52 (23.1)	
Median age (IQR)		27 (19-56)	27.0 (19-54.5)	32.0 (21-55)	52.0 (27-70)	23.0 (17-53.5)	0.04
Age groups (%)	≤ 19	62 (27.5)	34 (54.8)	6 (9.7)	3 (4.8)	19 (30.7)	0.07
	20-59	114 (50.7)	67 (58.8)	16 (14.0)	9 (7.9)	22 (19.3)	
	≥ 60	49 (21.8)	23 (46.9)	5 (10.2)	10 (20.4)	11 (22.5)	
Male (%)		141 (62.7)	75 (53.2)	18 (12.8)	14 (9.9)	34 (24.1)	0.89
Household income (%)	< $55000	55 (24.4)	34 (61.8)	10 (18.2)	1 (1.8)	10 (18.2)	0.02
	$55000-70000	88 (39.1)	42 (47.7)	6 (6.8)	12 (13.6)	28 (31.8)	
	> $70000	82 (36.4)	48 (58.5)	11 (13.4)	9 (11.0)	14 (17.1)	
Living settings (%)	Population > 1 million	144 (61.0)	68 (47.2)	21 (14.6)	18 (12.5)	37 (25.7)	0.01
	Other	81 (36.0)	56 (69.1)	6 (7.4)	4 (4.9)	15 (18.5)	
Stages (%)	I	65 (28.9)	38 (58.5)	7 (10.8)	7 (10.8)	13 (20.0)	0.71
	II	18 (8.0)	11 (61.1)	2 (11.1)	2 (11.1)	3 (16.7)	
	III	61 (27.1)	30 (49.2)	7 (11.5)	3 (4.9)	21 (34.4)	
	IV	61 (27.1)	34 (55.7)	7 (11.5)	8 (13.1)	12 (19.7)	
	Unknown	20 (8.9)	11 (55.0)	4 (20.0)	2 (10.0)	3 (15.0)	
Lymph Node status (%)	N0	143 (63.6)	74 (51.8)	16 (11.2)	17 (11.9)	36 (25.2)	0.11
	N1	60 (26.7)	38 (63.3)	5 (8.3)	3 (5.0)	14 (23.3)	
	Nx	22 (9.78)	12 (54.6)	6 (27.3)	2 (9.1)	2 (9.1)	
Metastasis status (%)	M0	154 (68.4)	85 (55.2)	18 (11.7)	12 (7.8)	39 (25.3)	0.63
	M1	61 (27.1)	34 (55.7)	7 (11.5)	8 (13.1)	12 (19.7)	
	Mx	10 (4.4)	5 (50.0)	2 (20.0)	2 (20.0)	1 (10.0)	
Surgery (%)	Wedge/segmental resection	39 (17.8)	28 (71.8)	2 (5.1)	3 (7.7)	6 (15.4)	0.28
	Lobectomy	42 (19.2)	25 (59.5)	6 (14.3)	3 (7.1)	8 (19.1)	
	Extended lobectomy	11 (5.0)	5 (45.5)	0 (0)	1 (9.1)	5 (45.5)	
	Transplant	19 (8.7)	12 (63.2)	2 (10.5)	1 (5.3)	4 (21.1)	
	None	108 (49.3)	49 (45.4)	17 (15.7)	13 (12.0)	29 (26.9)	

Age, comparison to the previous study, possible explanations and implications

The median age of patients in our study was 36 years, similar to that of other studies[18,20]. FL-HCC is known to have a predilection to develop in young patients[18]; hence it was no surprise that the majority of patients with FL-HCC in our study were younger. Also, younger patients (≤ 19 years) in our study with FL-HCC were more likely to have advanced stags with positive lymph nodes status and were more often treated with resection or transplantation as described in previous studies[12,18]. These studies have suggested FL-HCC has a better prognosis because it primarily affects children and teens and because of the many surgical therapies available (wedge, segmental resection, lobectomy, and transplant) for this patient population, unlike with HCC[12]. Likewise, young patients with FL-HCC are usually otherwise healthy, lack liver cirrhosis, and have high resectability rates with low rates of surgical complications[21,22]. Pinna *et al*[22] in 1997 reported survival of 66% at five years despite 90% of FL-HCC patients presenting with stage IV disease.

Race, comparison to the previous study, possible explanations and implications

Patients with FL-HCC in our study were overwhelmingly non-Hispanic whites, findings similar to the study by El-Serag *et al*[18] in 2004. The rates of FL-HCC were not significantly different among Whites, Blacks, APIs, and Hispanics in our study, unlike with HCC patients, where incidence rates and prognosis vary with racial backgrounds[18,23]. APIs were older than others races in our study. Notably, there were no differences in tumor characteristics and surgical treatment by race. Also, survival rates of FL-HCC were similar across racial groups in our study; although there was a non-significant trend for

Table 3 Summary characteristics of by surgery types (total 219 patients)

Factor	Group	None	Transplant	Wedge or segmental resection	Lobectomy	Extended lobectomy	*P* value
n	Total 219	108	19	39	42	11	
Alive	Total 67	10 (9.3)	7 (36.8)	23 (59.0)	21 (50.0)	6 (54.6)	< 0.001
Age group (%)	≤ 19	18 (16.7)	6 (31.6)	15 (38.5)	16 (38.1)	7 (63.6)	< 0.001
	20-59	54 (50.0)	13 (68.4)	17 (43.6)	23 (54.8)	4 (36.4)	
	≥ 60	36 (33.3)	0 (0)	7 (18.0)	3 (7.1)	0 (0)	
Sex (%)	Male	70 (64.8)	12 (63.2)	27 (69.2)	21 (50.0)	7 (63.6)	0.43
Stages (%)	I	22 (20.4)	4 (21.1)	19 (48.7)	15 (35.7)	2 (18.2)	< 0.001
	II	3 (2.8)	0 (0)	8 (20.5)	5 (11.9)	2 (18.2)	
	III	24 (22.2)	10 (52.6)	9 (23.1)	13 (31.0)	4 (36.4)	
	IV	43 (39.8)	4 (21.1)	3 (7.7)	8 (19.1)	2 (18.2)	
	Unknown	16 (14.8)	1 (5.3)	0 (0)	1 (2.4)	1 (9.1)	
Race (%)	API	13 (12.0)	1 (5.3)	3 (7.7)	3 (7.1)	1 (9.1)	0.28
	Black	17 (15.7)	2 (10.5)	2 (5.1)	6 (14.3)	0 (0)	
	Hispanic	29 (26.9)	4 (21.1)	6 (15.4)	8 (19.1)	5 (45.5)	
	White	49 (45.4)	12 (63.2)	28 (71.8)	25 (59.5)	5 (45.5)	
Income (%)	< $55000	29 (26.9)	3 (15.8)	12 (30.8)	8 (19.1)	1 (9.1)	0.22
	$55000-70000	41 (38.0)	8 (42.1)	15 (38.5)	13 (31.0)	8 (72.7)	
	> $70000	38 (35.2)	8 (42.1)	12 (30.8)	21 (50.0)	2 (18.2)	
Lymph nodes status (%)	N0	64 (59.3)	12 (63.2)	32 (82.1)	24 (57.1)	6 (54.6)	0.007
	N1	25 (23.2)	6 (31.6)	7 (18.0)	17 (40.5)	4 (36.4)	
	Nx	19 (17.6)	1 (5.3)	0(0)	1 (2.4)	1 (9.1)	
Metastasis status (%)	M0	56 (52.9)	15 (79.0)	36 (92.3)	34 (81.0)	8 (72.7)	< 0.001
	M1	43 (39.8)	4 (21.1)	3 (7.7)	8 (19.1)	2 (18.2)	
	Mx	9 (8.3)	0 (0)	0 (0)	0 (0)	1 (9.1)	
Population (%)	≥ 1 million	76 (70.4)	13 (68.4)	21 (53.9)	24 (57.1)	7 (63.6)	0.32
	Others	32 (29.7)	6 (31.6)	18 (46.2)	18 (42.9)	4 (36.4)	
Size (%)	≤ 50 mm	18 (16.7)	1 (5.3)	12 (30.8)	6 (14.3)	1 (9.1)	< 0.001
	51-100 mm	25 (23.2)	5 (26.3)	15 (38.5)	14 (33.3)	3 (27.3)	
	≥ 100 mm	37 (34.3)	11 (57.9)	10 (25.6)	21 (50.0)	7 (63.6)	
	Unknown	28 (25.9)	2 (10.5)	2 (5.1)	1 (2.4)	0 (0)	

survival in Whites to be higher than in non-Whites, behaving like findings seen with HCC patients, where non-Whites have lower survival rates[23]. On the other hand, some studies have found the Whites and female gender to be negative prognostic factors after surgery[24]. However, these findings have remained controversial[18,25]. Racial disparities observed in HCC patients are thought to be related to etiological and socio-demographic factors[26]. Our findings suggest that these factors may not play a role in FL-HCC survival as we found no significant racial disparities.

Surgery, comparison to the previous study, possible explanations and implications

Surgical resection has remained the treatment of choice for FL-HCC given the younger age of these patients, localized disease, and lack of underlying liver cirrhosis[24,27]. Most of these surgeries were segmental surgeries and lobectomies, with fewer cases of liver transplantation. In our study, the majority of FL-HCC patients who had surgical intervention were young and had earlier stages. Nineteen (17.1%) patients underwent liver transplantation, and 11 (9.9%) had extended lobectomy, with many having an advanced disease. Our findings are similar to those of Eggert *et al*[25], 2013 and Assi *et al*[20], 2020. The survival in our study increased with surgery and was highest in FL-HCC patients who

Table 4 Multivariate Cox regression analysis

		Hazard ratio	Standard error	*P* value
Age group (%)	20-59	0.931	0.327	0.83
	≥ 60	2.554	0.557	0.09
Sex (%)	Male	1.169	0.288	0.59
Race (%)	API	0.624	0.581	0.42
	Black	2.296	0.461	0.07
	Hispanic	0.9	0.368	0.78
Income (%)	$55000-70000	1.044	0.368	0.91
	> $70000	0.644	0.345	0.2
Sugery	Surgery	1.465	0.472	0.42
Population (%)	≥ 1 million	1.209	0.287	0.51
Size (%)	≥ 100 mm	1.104	0.44	0.82
	51-100 mm	1.233	0.426	0.62
	Unknown	3.196	0.646	0.07
Lymph nodes (%)	N1	1.503	0.39	0.3
	Nx	0.107	0.993	0.02
Metastasis status (%)	M1	0.301	0.782	0.13
	Mx	7.503	1.366	0.14

had a wedge or segmental hepatic resection with 112 mo. Previous studies have demonstrated age and tumor resectability to be independent predictors of survival in FL-HCC patients[2,19,20]. In effect, having normal underlying liver parenchyma may allow for more aggressive and complete resections, decreasing the risk for recurrence. Currently, there is a paucity of data on aggressive surgical intervention in the setting of extended disease. Our study also highlights the importance of surgical treatment regardless of lymph nodes or metastasis status. As we do not have randomized clinical trials to see the effectiveness of systemic chemotherapy or immunotherapy to treat FL-HCC, surgical treatment may reduce tumor burden for curative or palliative intent.

Limitation

Although this study included a large number of FL-HCC patients, there are several limitations to this study. Due to the nature of the SEER database, there is no information about comorbid medical conditions, laboratory data, or underlying liver disease, which may affect survival. One-third of the cases needed to be excluded due to inadequate information. As this is a registry study, we cannot account for errors and variations in reporting from the many coders required to acquire this data. Furthermore, the generalizability of this study may be limited as the SEER database includes select states and regions in United States. Finally, as FL-HCC is often diagnosed at an advanced stage in young, otherwise healthy patients, there may be a lead time bias when interpreting survival.

CONCLUSION

This study demonstrated a better survival of younger patients with FL-HCC despite the presence of aggressive diseases. There were no racial differences in survival for FL-HCC, which is typically seen in HCC. Surgical treatment provided better survival even in the face of nodal disease or metastases. Until more definitive data on locoregional therapy and systemic therapy can be elucidated, all patients with FL-HCC should be strongly considered for surgical intervention. Future studies will also be necessary to identify genetic markers for the population at risk for FL-HCC to enhance earlier detection.

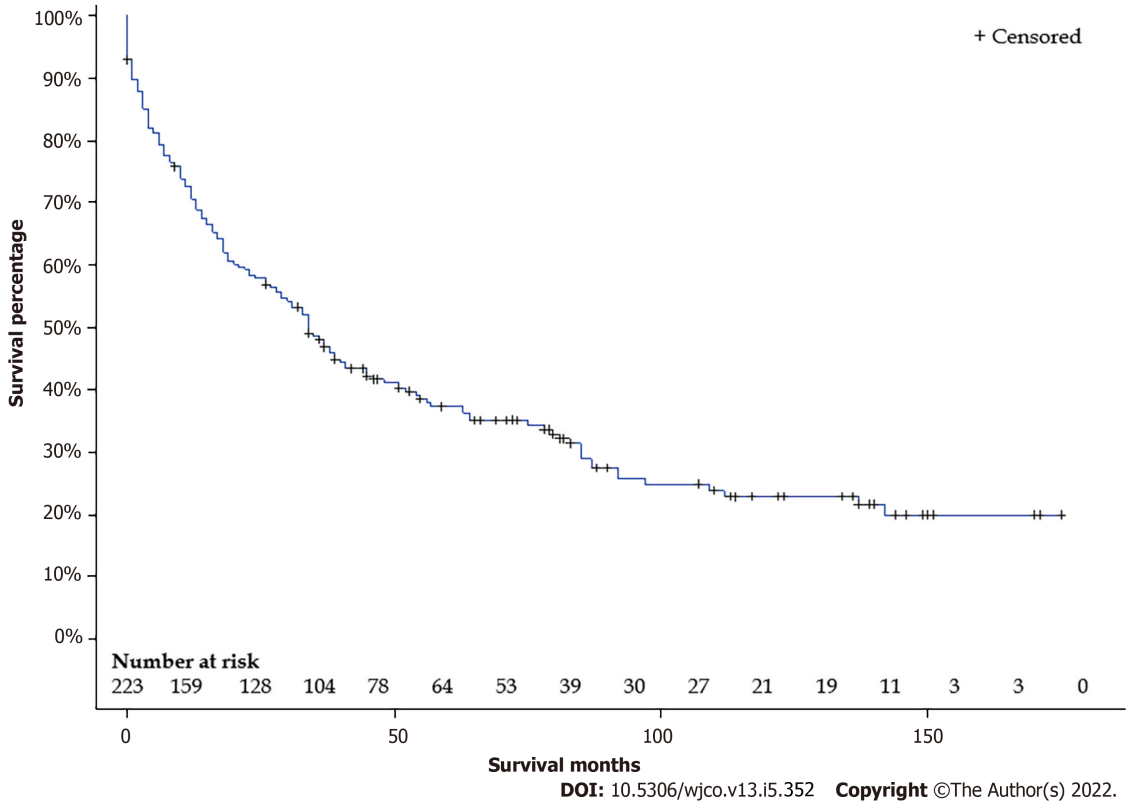


Figure 1 Overall Kaplan-Meier survival curve. Median survival for all patients is 34 (95%CI: 27-41) mo.

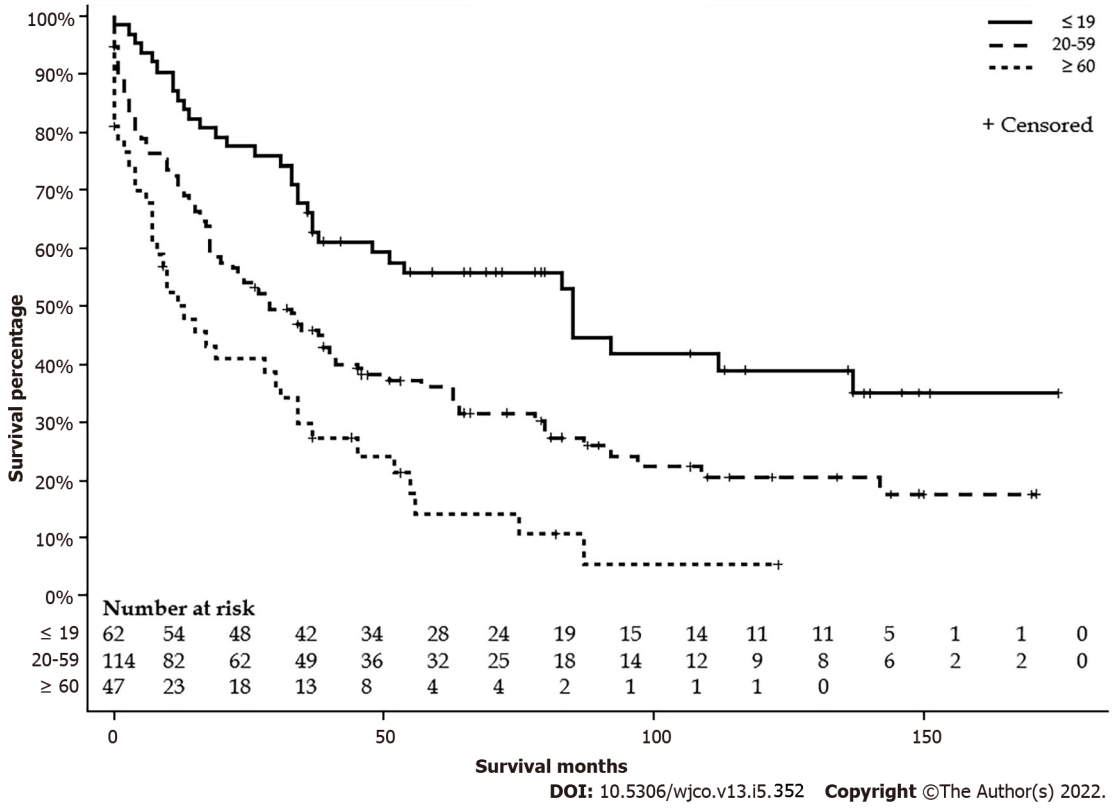


Figure 2 Kaplan-Meier survival curve by age groups. Median survival for patients ≤ 19-years-old is 85 (95%CI: 37-137) mo, patients between 20 and 59-years-old are 29 (18-41) mo, and patients ≥ 60-years-old is 12 (95%CI: 7-31) mo ($P < 0.001$).

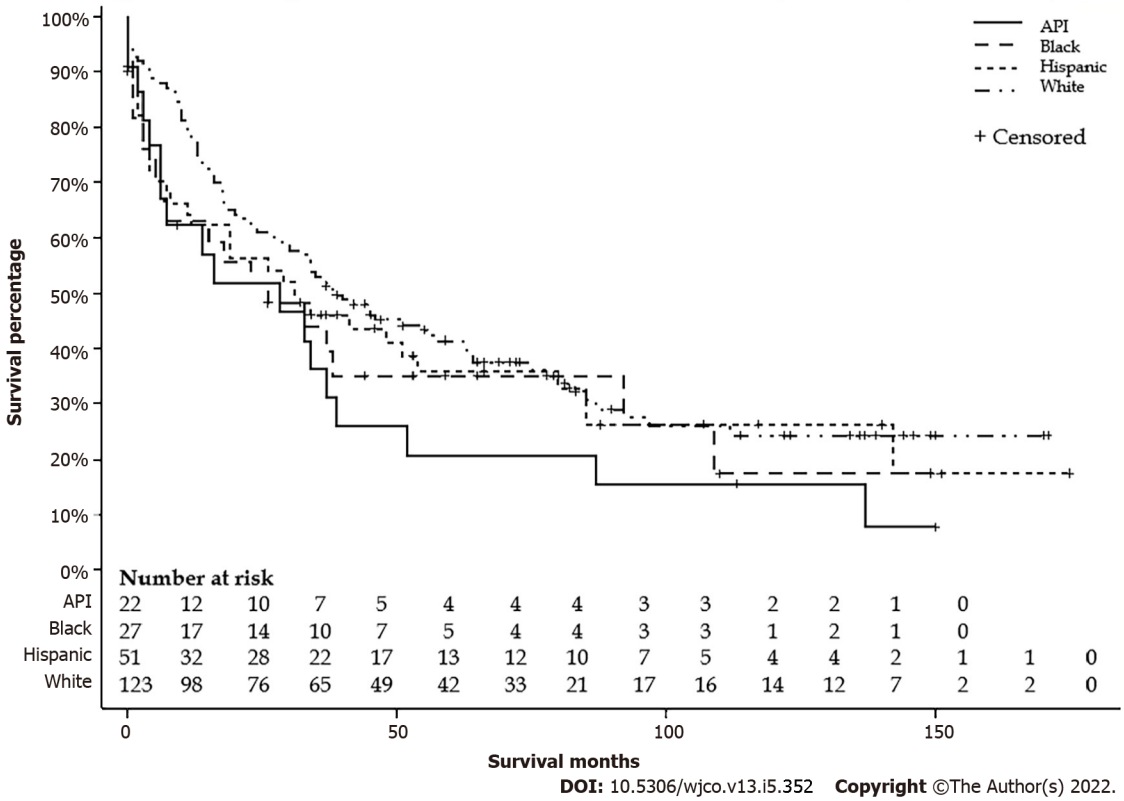


Figure 3 Kaplan-Meier survival curve by race (all race). Median survival for White 39 (95%CI: 29-63) mo, Black 26 (95%CI: 5-92) mo, Asian and Pacific Islander is 28 (95%CI: 6-39) mo, and Hispanic 31 (95%CI: 11-54) mo (P = 0.28).

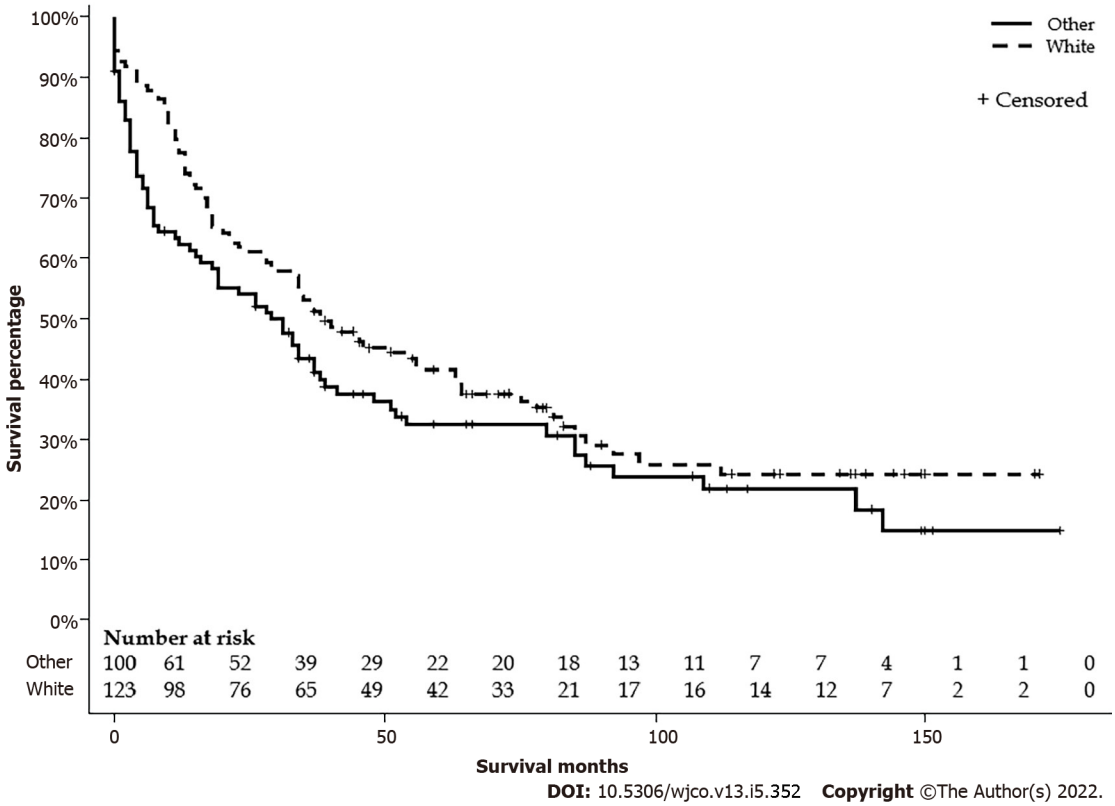


Figure 4 Kaplan-Meier survival curve by race (White *vs* non-White). Median survival for White 39 (95%CI: 29-63) mo, and Non-White is 29 (95%CI: 15-38) mo (P = 0.11).

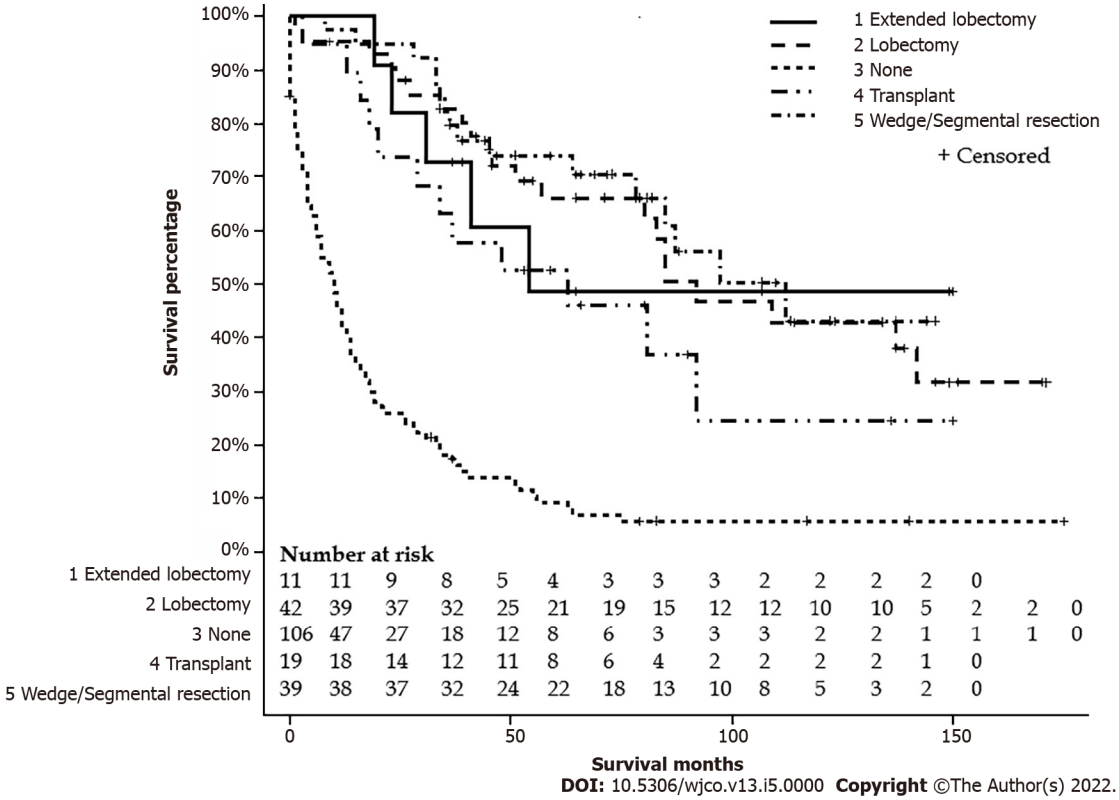


Figure 5 Kaplan-Meier survival curve by surgery types. Median survival for a wedge or segmental resection was 112 (95%CI: 78-NA) mo, lobectomy was 92 (95%CI: 57-NA) mo, extended lobectomy was 54 (95%CI: 23-NA) mo, none had 10 (95%CI: 6-13) mo, and the transplant was 63 (95%CI: 20-NA) mo ($P < 0.001$).

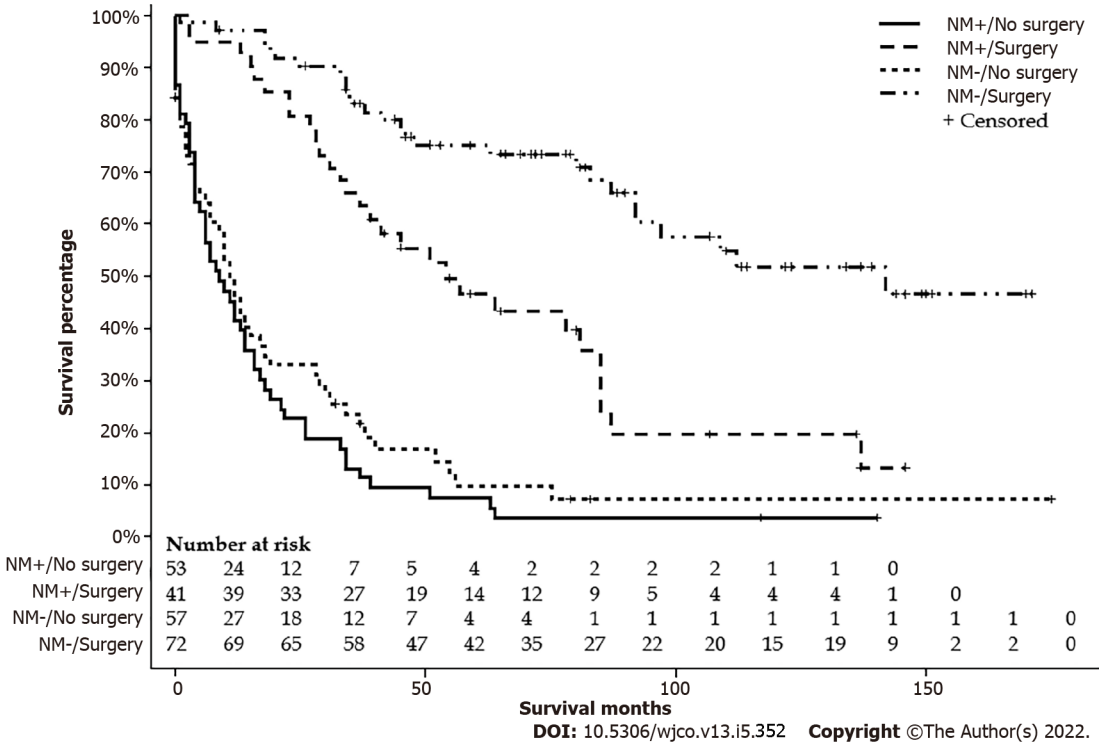


Figure 6 Kaplan-Meier survival curve by surgical status and metastasis status. Median survival for NM+/no surgery was 9 (95%CI: 4-14) mo, NM+/surgery was 54 (95%CI: 34-85) mo, NM-/no surgery was 11 (95%CI: 6-17) mo, and NM-/surgery was 142 (95%CI: 92-NA) mo ($P < 0.001$).

ARTICLE HIGHLIGHTS

Research background

Fibrolamellar hepatocellular carcinoma (FL-HCC) is a rare and distinct type of hepatocellular carcinoma.

Research motivation

Due to its rare nature, there is a limited understanding of factors affecting the survival outcomes.

Research objectives

This study aims to characterize the survival of FL-HCC by age, race, and surgical intervention.

Research methods

FL-HCC patients were retrospectively identified with The Surveillance, Epidemiology, and End Results database. We conducted three separate survival analyses by age groups; ≤ 19, 20-59, and ≥ 60-year-old, and race; White, Black, Asian and Pacific Islanders (API), and Hispanic and surgical types; Wedge resection or segmental resection, lobectomy, extended lobectomy (lobectomy + locoregional therapy or resection of the other lobe), and transplant.

Research results

We identified 225 FL-HCC patients. Overall median survival was 34 (95%CI: 27-41) mo. Patients ≤ 19-year-old had more advanced disease with positive lymph nodes status. However, they received more surgical interventions. Survival months for ≤ 19 was 85 (95%CI: 37-137), 20-59 was 29 (95%CI: 18-41), and ≥ 60 was 12 (95%CI: 7-31) mo ($P < 0.001$). APIs lived in the area with a higher median household income, and Blacks lived in the area with a lower median household income. There were no differences in stages, lymph node status, metastasis status, and surgical treatment. Whites had 39 (95%CI: 29-63), Blacks 26 (95%CI: 5-92), Hispanics 31 (95%CI: 11-54), and APIs 28 (95%CI: 5-39) mo ($P = 0.28$). Of 225 patients, 111 FL-HCC patients had surgical procedures. Median survivals for a wedge or segmental resection was 112 (95%CI: 78-NA), lobectomy was 92 (95%CI: 57-NA), extended lobectomy was 54 (95%CI: 23-NA), and a transplant was 63 (95%CI: 20-NA) mo ($P < 0.001$). The median survival was better in patients who had surgical treatments regardless of lymph nodes or metastasis status ($P < 0.001$).

Research conclusions

This study demonstrated a better survival of younger patients with FL-HCC, although they had aggressive diseases. There were no racial differences in survival for FL-HCC, which is seen in HCC. Surgical treatment provided better survival regardless of advanced disease.

Research perspectives

This study can help healthcare professionals to guide FL-HCC patients about the outcome, especially after the surgical intervention. Further prospective studies are needed to elucidate in the era of personalized cancer therapy.

FOOTNOTES

Author contributions: Sempokuya T contributed to study design, data collection, statistical analysis; Sempokuya T, Forlemu A, Silangcruz K, Azawi M, Khoury N contributed to the literature review, manuscript drafting, and editing; Ma J contributed to study design and statistical analysis; Wong LL contributed to study supervision, manuscript drafting, and editing; all of the authors have approved the final version of the manuscript.

Institutional review board statement: Due to the utilization of a publicly available, de-identified database, review by our institutional review board was not required.

Informed consent statement: Informed consent was not required to conduct this study.

Conflict-of-interest statement: Wong LL is a speaker bureau for Eisai. All other authors do not have any conflicts of interest.

Data sharing statement: All of the data used in this analysis is available from the SEER database.

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ORIGINAL ARTICLE

Retrospective Study

Modified binding pancreaticogastrostomy *vs* modified Blumgart pancreaticojejunostomy after laparoscopic pancreaticoduodenectomy for pancreatic or periampullary tumors

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Abstract

BACKGROUND

Laparoscopic pancreaticoenteric anastomosis is one of the technically challenging steps of minimally invasive pancreaticoduodenectomy (PD), especially during the learning curve. Despite multiple randomized controlled trials and meta-analyses, the type of pancreatico-enteric anastomosis as a risk factor for post-pancreatectomy complications is debatable. Also, the ideal technique of pancreatic reconstruction during the learning curve of laparoscopic PD has not been well studied.

AIM

To compare the short-term outcomes of modified binding pancreaticogastrostomy (PG) and Blumgart pancreaticojejunostomy (PJ) during learning curve of laparoscopic PD.

METHODS

The first 25 patients with resectable pancreatic or periampullary tumors who underwent laparoscopic PD with modified binding PG or modified Blumgart PJ between January 2015 and May 2020 were retrospectively analyzed to compare perioperative outcomes during the same learning curve. A single layer of the full-thickness purse-string suture was placed around the posterior gastrotomy in the modified binding PG. In the modified Blumgart technique, only a single transpancreatic horizontal mattress suture was placed on either side of the pancreatic duct (total two sutures) to secure the pancreatic parenchyma to the jejunum. Also, on the ventral surface, the knot is tied on the jejunal wall without going through the pancreatic parenchyma. Post pancreatectomy complications are graded as per the

International Study Group for Pancreatic Surgery criteria.

RESULTS

During the study period, modified binding PG was performed in 27 patients and modified Blumgart PJ in 29 patients. The demographic and clinical parameters of the first 25 patients included in both groups were comparable. Lower end cholangiocarcinoma and ampullary adenocarcinoma were the primary indications for laparoscopic PD in both groups (32/50, 64%). The median operative time for pancreatic reconstruction was significantly lower in the binding PG group (42 *vs* 58 min, $P = 0.01$). The clinically relevant (Grade B/C) postoperative pancreatic fistula (POPF) was significantly more in the modified PJ group (28% *vs* 4%, $P = 0.04$). In contrast, intraluminal postpancreatectomy hemorrhage (PPH) was more in the binding PG group (32% *vs* 4%, $P = 0.02$). There was no significant difference in the incidence of delayed gastric emptying between the two groups.

CONCLUSION

During the learning curve of laparoscopic PD, modified binding PG reduces POPF but is associated with increased intraluminal PPH compared to PJ using the modified Blumgart technique.

Key Words: Pancreaticoduodenectomy; Laparoscopy; Pancreatic cancer; Pancreaticojejunostomy; Neoplasms; Tumors

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Core Tip: During the learning curve of laparoscopic pancreaticoduodenectomy, modified binding pancreaticogastrostomy reduces the operative time for pancreatic reconstruction. Also, modified binding pancreaticogastrostomy reduces clinically relevant postoperative pancreatic fistula compared to modified Blumgart pancreaticojejunostomy. However, modified binding pancreaticogastrostomy is associated with increased intraluminal postpancreatectomy hemorrhage. The present study results could guide surgeons to tailor the pancreatic reconstruction during the learning curve of laparoscopic pancreaticoduodenectomy.

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INTRODUCTION

Laparoscopic pancreaticoduodenectomy (PD) is considered one of the most complex minimal access surgical procedures, requiring proficiency in advanced laparoscopic surgery. With advancements in laparoscopic skills and technology, multiple studies have reported the feasibility, safety, and oncological equivalence of Laparoscopic PD compared to open PD[1-3]. Despite improved surgical techniques and perioperative management, PD remains a morbid procedure with a 30%-50% estimated morbidity rate [4]. As in open PD, pancreatico-enteric anastomosis remains the Achilles' heel in laparoscopic PD, and postoperative pancreatic fistula (POPF) is the critical cause of morbidity in these patients. The type of pancreatico-enteric anastomosis as a risk factor for POPF is still debatable. Multiple retrospective studies, some randomized controlled trials (RCTs), and meta-analyses have reported that pancreaticogastrostomy (PG) is associated with less incidence of POPF compared to pancreaticojejunostomy (PJ)[5, 6]. However, other RCTs and meta-analyses did not report any difference between the two anastomotic techniques concerning clinically relevant POPF rates[7,8].

In laparoscopic PD, in addition to conventional risk factors for POPF, laparoscopic instruments' restricted range of motion poses an additional risk, especially during the learning curve. A review of various techniques of laparoscopic pancreatic reconstruction following laparoscopic PD reported that PJ was more commonly used than PG like open PD[9]. However, to date, no RCT has compared different techniques of pancreatic reconstruction in laparoscopic PD, precluding a definite conclusion. The ideal method of managing remnant pancreas following laparoscopic PD should be safe and easy to perform, especially during the learning curve. In open PD, binding PG using two layers of purse-string sutures has been described as a safe and technically simpler method of pancreatic reconstruction[10,11]. Of the

many techniques of PJ, the Blumgart method of PJ is a popular one, and its safety has been established in multiple open PD series[12-14]. However, the outcomes of these techniques of pancreatic reconstruction during the learning curve of Laparoscopic PD have not been previously studied. We used the binding PG and Blumgart method of PJ that was modified to suit the laparoscopic pancreatic reconstruction[12,15]. The present study compares the short-term outcomes of modified binding PG and Blumgart technique of PJ for pancreatic reconstruction in laparoscopic PD during the learning curve.

MATERIALS AND METHODS

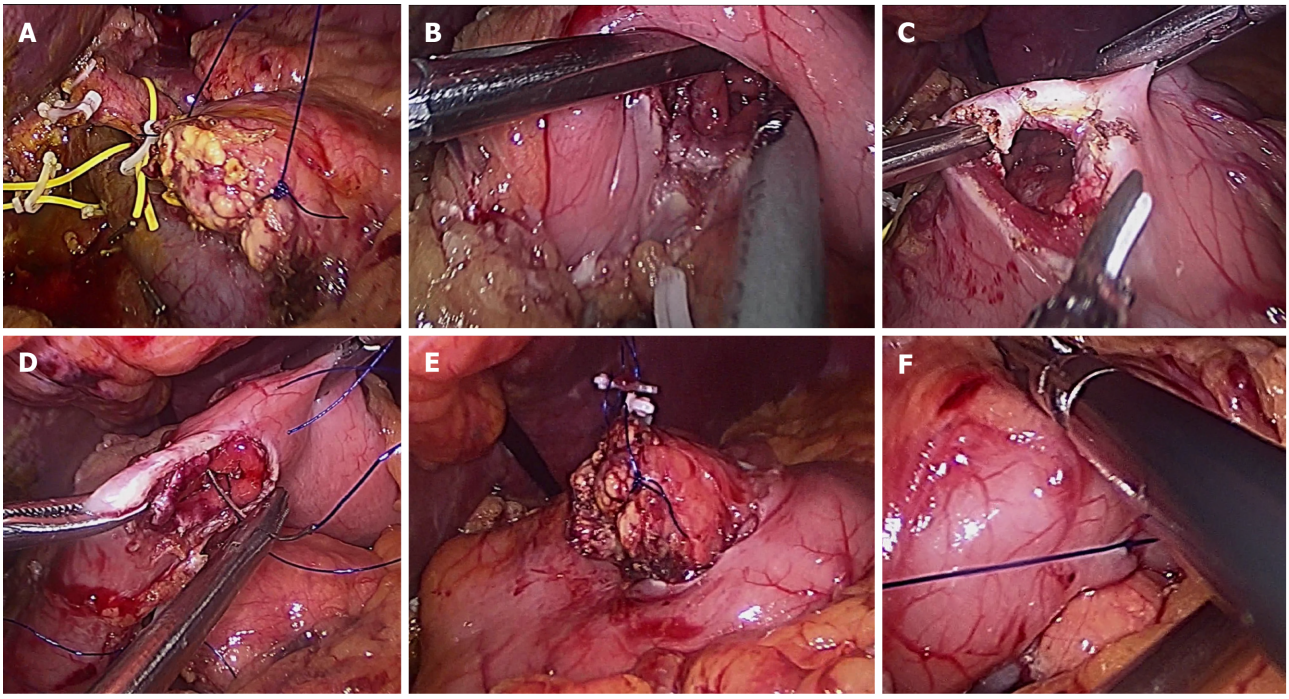
Patient selection

Laparoscopic PD was started in the institute in January 2015. Until October 2017, modified binding PG was used for pancreatic reconstruction in laparoscopic PD. Subsequently, the modified Blumgart technique was mainly used for pancreatic reconstruction, except in patients whose pancreatic duct could not be identified after pancreatic transection, where invagination PJ or binding PG was used. Clinical data of the first 25 patients with resectable pancreatic and periampullary tumors who underwent laparoscopic PD with modified binding PG or modified Blumgart PJ between January 2015 and May 2020 were retrospectively analyzed to evaluate the outcomes during the same learning curve. Pancreatic cancer patients with suspected vascular involvement and those with contraindications for laparoscopic surgery were not considered for laparoscopic PD. Patients who underwent laparoscopic PD with different techniques of pancreatic reconstruction and those who underwent robotic PD were excluded from the analysis. Also, patients who underwent laparoscopic PD for chronic pancreatitis or other nonmalignant etiology were not included in the study. All surgeries were performed by a single surgeon (RK) with sufficient experience in advanced minimally invasive gastrointestinal surgery. The study was approved by the institute scientific advisory committee (PGRMC 19.04.2021-18) and the institute ethics committee (JIP/IEC/2021/0194).

Operative technique

The procedure was performed using six laparoscopic ports: One infra umbilical 12 mm port, two 12 mm pararectal ports, one left subcostal 12 mm port, one right subcostal 5 mm port, and one 5 mm epigastric port with the patient in French position (supine with leg split). The infraumbilical port is used for laparoscopic camera except during uncinate dissection when the camera is moved to the right pararectal port. For ligation and division of gastrocolic trunk, division of stomach, lymph node dissection in hepatoduodenal ligament, and bile duct division, the two 12 mm ports on the left side are used as primary working ports with the surgeon standing on the left side of the patient. The primary surgeon moves to the patient's right side for the remaining dissection. The two right-sided ports are used as a primary working port for the pancreatic reconstruction using modified binding PG. Two full-thickness stay sutures are taken at the corners of the pancreatic cut surface using 3-0 polypropylene to facilitate pancreatic mobilization and invagination into the stomach (Figure 1). The pancreas is carefully mobilized from the splenic vein and artery after sealing and dividing small vessels for approximately 3-4 cm. The left gastric vein that usually drains to the splenic portal vein junction should be identified during pancreatic mobilization to avoid inadvertent injury and troublesome bleeding. Anterior gastrotomy of length approximately 4-5 cm was made proximal to the stapled end of the stomach. A posterior gastrotomy was made at a site where the pancreas can be invaginated without undue tension for a length approximately equivalent to the width of the pancreatic cut surface. In contrast to the original technique of binding PG that used two layers (inner mucosal and outer seromuscular) of purse-string sutures, the modified binding PG technique utilizes only a single layer of a full-thickness purse-string suture[10,15]. The modified binding PG technique used in the current series was adapted from the publication by Hong *et al*[15] that reported the feasibility of binding PG using a single layer of the full thickness purse-string suture in 10 patients undergoing laparoscopic central pancreatectomy. The placement of the purse-string suture using 3-0 polypropylene should start from the superior edge of the posterior gastrotomy to ensure adequate visualization of knots after invagination of the pancreas. The pancreas was lifted using the stay sutures and invaginated into the stomach through posterior gastrotomy. The stay sutures are held with a laparoscopic grasper advanced through anterior gastrotomy. Once the invagination of at least 2 cm of the pancreas into the stomach was confirmed, the stay suture is tied to bind the gastric wall to the pancreatic stump. The position of the pancreas inside the stomach was rechecked after completion of the hepaticojejunostomy to ensure a tension-free anastomosis. An anterior gastrotomy was used for hand sewn gastrojejunostomy.

For PJ using the modified Blumgart technique, the surgeon stands between the patient's legs and uses the infraumbilical and right subcostal ports as working ports. The laparoscopic camera was inserted through the right pararectal port. In the original Blumgart technique, two to three transpancreatic full-thickness U-shaped sutures were placed on either side of the pancreatic duct[16]. In the modified technique, a single transpancreatic horizontal mattress suture was placed on either side of the pancreatic duct (total two sutures) to secure the pancreatic parenchyma to the jejunum (Figure 2). The modified Blumgart PJ used in the present series was based on the previous studies in open PD that reported the



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Figure 1 Steps of modified binding pancreaticogastrostomy. A: Two full-thickness stay sutures are taken at the corners of the pancreatic cut surface; B: A posterior gastrotomy is made at a site where the pancreas can be invaginated without undue tension; C: Anterior gastrotomy of approximately 4-5 cm is made proximal to the stapled end of the stomach; D: A full-thickness purse-string suture is placed around the posterior gastrotomy using 2-0 polypropylene; E: The pancreas lifted using the stay sutures and invaginated into the stomach through posterior gastrotomy; F: At least 2 cm of the pancreas invaginated into the stomach. Purse-string suture tied to bind the gastric wall to the pancreatic stump.

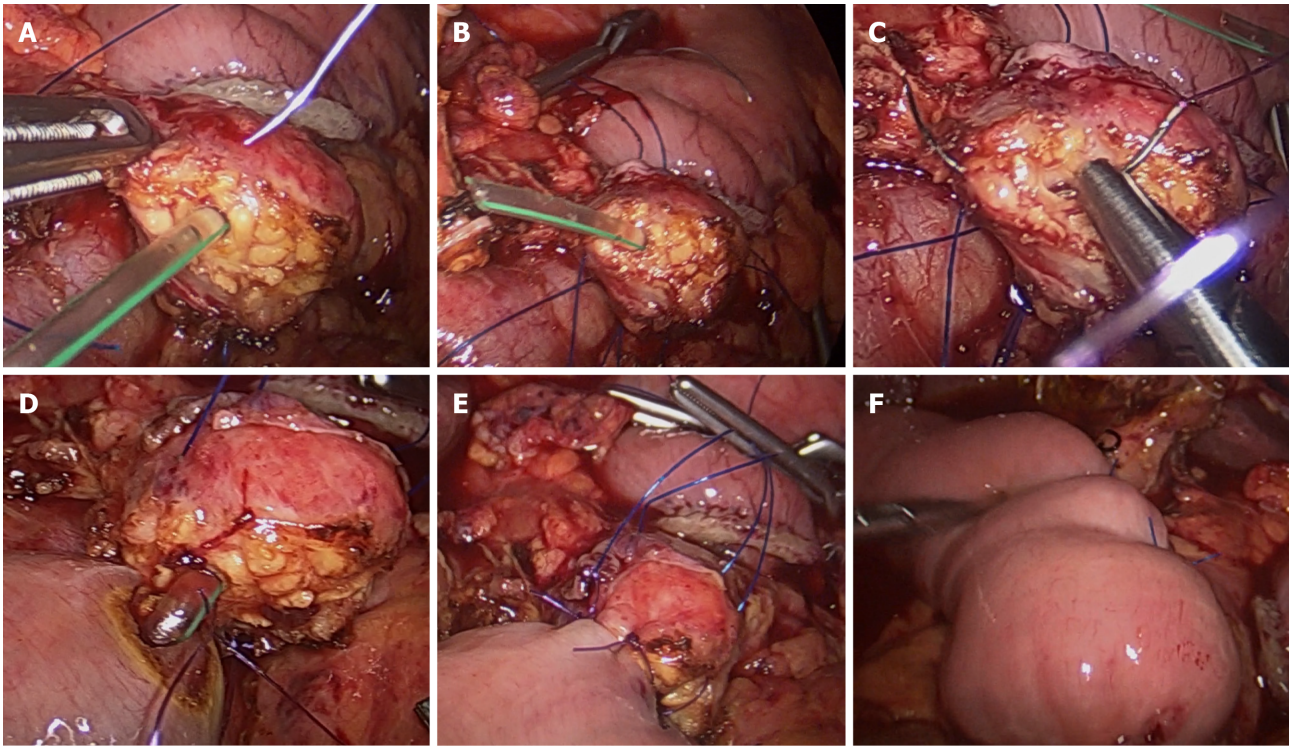
advantages of using fewer transpancreatic sutures to minimize the risk of pancreatic juice leakage[12, 14]. The 26 mm ½ circle round body needle of 3-0 polypropylene suture was straightened to facilitate the placement of transpancreatic suture. For duct to mucosa anastomosis, six interrupted 4-0 PDS sutures are placed at 4, 6, 8, 10, 12, and 2o'clock position. The needle moves in-out direction in the ductal end to ensure accurate placement of pancreatic duct sutures. In-out needle movement was facilitated by taking the initial bite in the pancreatic duct for 4, 6, and 8 o'clock sutures. For the remaining sutures, the initial bite was taken in the jejunal end. The pancreatic duct stent was placed after knotting the 6 and 8o'clock sutures. However, the stent was not fixed with sutures. After knotting the remaining duct to mucosa sutures, the transpancreatic suture needle was used to take a seromuscular bite on the antimesenteric edge of the jejunum. Ligation of these sutures wraps the ventral portion of the pancreatic cut edge with the jejunum. In contrast to the original Blumgart technique, no suture was taken on the anterior surface of the pancreas. A feeding jejunostomy was routinely performed in all patients undergoing laparoscopic PD.

Outcome measures

The patients' demographic and clinical data, including age, sex, body mass index, bilirubin level, preoperative biliary drainage, total operative time, time taken for pancreatic reconstruction, estimated blood loss, need for blood transfusions, fistula risk score, and tumor type, were reviewed and compared between the two groups[17]. Postoperative morbidity was graded as per Clavien-Dindo classification [18]. Delayed gastric emptying [DGE], postpancreatectomy hemorrhage (PPH), and postoperative pancreatic fistula [POPF] were graded as per the International Study Group for Pancreatic Surgery [ISGPS] definition[19-21]. Postoperative mortality is defined as any death, regardless of cause, occurring within 90 d after surgery in or out of the hospital.

Statistical analysis

Continuous variables were expressed as the median with range. Categorical variables were expressed as proportions. Continuous variables were analyzed using the Mann-Whitney U test. Categorical variables were analyzed using the chi-square test or Fisher's exact test. A P value of less than 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics for Windows, Version 28.0. (Armonk, NY: IBM Corp).



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Figure 2 Steps of modified Blumgart pancreaticojejunostomy. A: The 26 mm ½ circle round body needle of 3-0 polypropylene suture is straightened to facilitate the placement of transpancreatic suture; B: One transpancreatic U suture is taken on either side of the pancreatic duct, and the sutures were held with bulldog clamps; C: 8 o'clock duct to mucosa suture taken with the needle moving in-out direction in the ductal end; D: The pancreatic duct stent is placed after ligating the 6 and 8 o'clock sutures; E: Completion of duct to mucosa sutures; F: Transpancreatic U suture is tied to wrap the pancreatic cut edge with the jejunum.

RESULTS

During the study period, 78 patients underwent minimally invasive PD. Of these, 22 patients [Robotic PD (n = 18), nonmalignant etiology (n = 2), invagination PJ (n = 2)] who did not meet the inclusion criteria were excluded from the analysis. Overall, modified binding PG was performed in 27 patients and modified Blumgart PJ in 29 patients. To evaluate the short-term outcomes during the learning curve of laparoscopic PD, the first 25 consecutive patients who underwent modified binding PG and modified Blumgart PJ were included in the study.

The demographic and clinical parameters between the two groups were comparable (Table 1). Both groups had lower end cholangiocarcinoma and ampullary adenocarcinoma as the primary indications for laparoscopic PD (32/50, 64%). Hence, most patients had jaundice (43/50, 86%) at presentation. All 3 patients with intraductal papillary mucinous neoplasm had the main duct type of tumor. Of the 3 patients with neuroendocrine tumor, 1 patient had an ampullary tumor, and the other 2 had cancer in the head and uncinate process of the pancreas.

There was no significant difference in the total operative time and estimated blood loss between the two groups (Table 2). However, the median time to perform modified binding PG was significantly less than modified Blumgart PJ. While most patients had intermediate or high fistula risk scores (38/50, 76%), the proportion was not significantly different between the two groups. However, the modified binding PG group had a significantly lesser number of patients with Grade B/C POPF. None of the patients required reoperation for POPF. Overall, 9 patients had PPH (Grade A-3, Grade B-5, Grade C-1). The proportion of patients with PPH was significantly more in the modified binding PG group. On the fifth postoperative day, 1 patient in the binding PG group was reoperated in an emergency due to severe upper gastrointestinal bleeding that manifested as hematemesis. To visualize the pancreatic stump, an anterior gastrotomy was made away from the gastrojejunostomy site. After evacuating the clots in the gastric lumen, an arterial bleeder in the inferior edge of the pancreatic stump was suture ligated. DGE was present in 13 patients (Grade A-7, Grade B-4, Grade C-2). However, there was no significant difference in the rate of DGE between the two groups. There was no postoperative mortality in both groups.

Table 1 Comparison of demographic and clinical parameters of patients who underwent laparoscopic pancreatoduodenectomy with binding pancreaticogastrostomy and modified Blumgart pancreaticojejunostomy

Variable	Binding PG group, *n* = 25	Modified Blumgart PJ group, *n* = 25	*P* value
Age in yr, median (range)	53.7 (37-75)	58.2 (31-79)	0.12
Sex, Male:Female	14:11	15:10	> 0.99
BMI in kg/m^2, median (range)	23.8 (17.6-41.6)	24.6 (18.2-40.0)	0.69
Jaundice, *n* (%)	22 (88)	21 (84)	> 0.99
Cholangitis, *n* (%)	8 (32)	5 (20)	0.52
Peak total bilirubin levels in mg/dL, median (range)	12.8 (1.2-28.3)	10.6 (1.1-31.2)	0.59
Preoperative biliary drainage, *n* (%)	14 (56)	12 (48)	0.78
CA 19-9 (U/mL), median (range)	55 (1-5682)	84 (2-3318)	0.12
Diagnosis, *n* (%)			
Cholangiocarcinoma	9 (36)	7 (28)	0.76
Pancreatic adenocarcinoma	3 (12)	2 (8)	> 0.99
Ampullary adenocarcinoma	7 (28)	9 (36)	0.76
Duodenal adenocarcinoma	3 (12)	4 (16)	> 0.99
Intraductal papillary mucinous	2 (8)	1 (4)	> 0.99
Neoplasm pancreas			
Neuroendocrine tumor	1 (4)	2 (8)	> 0.99

PG: Pancreaticogastrostomy; PJ: Pancreaticojejunostomy.

DISCUSSION

The present study results suggest that during the learning curve of laparoscopic PD, modified binding PG reduces POPF but is associated with increased intraluminal PPH compared to PJ using the modified Blumgart technique. The feasibility, safety, and oncological outcomes of laparoscopic PD have been documented in multiple retrospective series and a few single-center prospective trials[1-3]. However, the multicenter randomized trial (LEOPARD-2) comparing laparoscopic with open PD was prematurely terminated because of higher complication-related mortality in the laparoscopic group[22]. As in open PD, pancreatico-enteric anastomosis is the critical cause of morbidity and mortality in patients undergoing laparoscopic PD, especially during the learning curve in low and medium volume centers[4, 22]. While the learning curve for laparoscopic PD has not been well studied, a few single-center studies have suggested that operative time and complications stabilize after 30-42 procedures[23-25]. Hence, in the present study, the perioperative outcomes of the first 25 laparoscopic procedures are compared.

The type of pancreatico-enteric anastomosis as a risk factor for POPF is still controversial. While a few RCTs and meta-analyses have documented the benefits of PG in reducing POPF, others did not find any difference between the two anastomotic techniques[5-8]. The ideal pancreatic reconstruction technique during the learning curve of laparoscopic PD should be safe and easy to perform. The binding technique for pancreatoenteric anastomosis was described by Peng *et al*[26] based on the hypothesis that avoiding pancreatic sutures at the level of the anastomosis can minimize POPF. Initially, he described binding PJ with an excellent postoperative outcome[26]. However, binding PJ cannot be used when the pancreatic stump is too large to be invaginated into the jejunum. Hence, binding PG was developed in which the pancreatic stump was invaginated into the stomach and held in place by two purse-string sutures: an outer seromuscular and inner mucosal purse-string suture[10]. Despite encouraging outcomes with binding PG in open PD, its safety and feasibility have not been well studied in laparoscopic PD. Wakabayashi *et al*[27] reported the feasibility of double purse-string suture PG in robotic PD as a technical report. In the present study, only a single layer of the full-thickness purse-string suture was used that was adapted from the previous report on the feasibility of binding PG using a single layer of the full thickness purse-string suture in patients undergoing laparoscopic central pancreatectomy[28]. The efficacy of the Blumgart technique in reducing the POPF rate has been documented in multiple open PD series[29,30]. The transpancreatic, full-thickness, mattress U-sutures used in the Blumgart technique reduce the tangential tension and shear force at the pancreatic stump. However, more sutures on the pancreas increase the POPF risk[31]. Another potential risk with the original Blumgart technique

Table 2 Comparison of perioperative outcomes of patients who underwent laparoscopic pancreatoduodenectomy with binding pancreaticogastrostomy and modified Blumgart pancreaticojejunostomy

Variable	Binding PG group, *n* = 25	Modified Blumgart PJ group, *n* = 25	*P* value
Total operative time in min, median (range)	445 (390-710)	405 (330-670)	0.06
Operative time for pancreatic reconstruction in min, median (range)	42 (26-65)	58 (44-81)	0.01
Estimated blood loss in mL, median (range)	320 (210-740)	310 (175-950)	0.09
Blood Transfusion, *n* (%)	6 (24)	7 (28)	> 0.99
Gland texture, *n* (%)			
Soft	17 (68)	19 (76)	0.75
Firm	8 (32)	6 (24)	
Pancreatic duct diameter in mm, median (range)	3 (1-9)	3 (2-10)	> 0.99
Fistula risk score, *n* (%)			
Low	5 (20)	7 (28)	0.74
Intermediate	12 (48)	13 (52)	> 0.99
High	8 (32)	5 (20)	0.52
Postoperative morbidity, Clavien-Dindo classification IIIa or more, *n* (%)	8 (32)	9 (36)	> 0.99
Pancreatic fistula as Grade B/C, *n* (%)	1 (4)	7 (28)	0.04
Delayed gastric emptying, *n* (%)	7(28)	6 (24)	> 0.99
Post pancreatectomy hemorrhage, *n* (%)	8 (32)	1 (4)	0.02
Bile leak, *n* (%)	0	1 (4)	> 0.99
Postoperative hospital stay in days, median (range)	9 (6-38)	8 (5-56)	0.72

PG: Pancreaticogastrostomy; PJ: Pancreaticojejunostomy.

is excessive compression on the pancreas while tying the transpancreatic sutures. Hence, only two transpancreatic U sutures were used in the present technique. Also, on the ventral surface, only a seromuscular bite was taken on the jejunum without taking any suture on the anterior surface of the pancreas to reduce shear force and excessive compression of the pancreatic parenchyma.

The perioperative outcomes of the modified binding PG and modified Blumgart technique of PJ have not been previously compared in the laparoscopic approach. As documented in the present study, modified binding PG can minimize the pancreatic reconstruction time as it requires only a single layer of the full-thickness purse-string suture. Also, only 1 patient developed clinically relevant POPF in the binding PG group despite the high fistula risk score of the included patients. In Binding PG, no sutures are taken to fix the pancreas with the stomach, which precludes the risk of suture cut through in the soft pancreas. Also, the portion of the pancreas through which stay sutures are taken is invaginated into the stomach. It ensures that a minor pancreatic leak from the needle entry site enters the gastric lumen rather than the peritoneal cavity. The clinically relevant POPF rate with the modified Blumgart technique was 28% in the present study. The grade B/C POPF rate with the Blumgart technique in open PD ranges from 2.5% to 20.5%[12-14,29,30]. Nagakawa *et al*[31] reported a Grade B/C POPF rate of 20% in their laparoscopic series using the modified Blumgart technique. The relatively high POPF rate in the present series could be due to the learning curve effect and inclusion of high fistula risk score patients.

In contrast to POPF, modified binding PG is associated with an increased incidence of intraluminal PPH. While most patients had Grade A or B PPH, surgical intervention was required in 1 patient. Also, seeing blood through the nasogastric gastric tube makes the patient anxious. Raw pancreatic stump lying freely in the gastric lumen without any compression effect of jejunum may be the reason for an increased incidence of intraluminal PPH. Hong *et al*[27] suggested that full-thickness suture closure of pancreatic stump can reduce the incidence of intraluminal PPH with binding PG. It is recommended to stent the pancreatic duct to avoid including it while taking the hemostatic sutures.

The choice of pancreatic reconstruction in both open and laparoscopic PD is determined by surgeon preference and familiarity with a particular technique. As binding PG is a technically more straightforward procedure, we used it in our initial patients who underwent PD. The increased incidence of

intraluminal PPH was the primary reason for changing to modified Blumgart PJ. The present study results suggest that it may be preferable to start with a simpler technique of pancreatic reconstruction to reduce the POPF rate. Modified Binding PG with hemostatic pancreatic sutures on either side of the pancreatic duct may achieve the goal without increasing PPH. Alternatively, tailored pancreatic reconstruction with modified binding PG for patients with a high fistula risk score and modified Blumgart PJ for patients with low fistula risk score may be a reasonable approach during the learning curve of laparoscopic PD. While retrospective study design is the primary limitation of the current series, it is the first study to compare the perioperative outcomes of modified binding PG and modified Blumgart technique of PJ.

CONCLUSION

Modified Binding PG reduces the pancreatic reconstruction time and POPF rate during the learning curve of laparoscopic PD but is associated with increased intraluminal PPH compared to PJ using the modified Blumgart technique.

ARTICLE HIGHLIGHTS

Research background

Complications related to pancreatico-enteric anastomosis are a significant cause of morbidity, especially during the learning curve in laparoscopic pancreaticoduodenectomy (PD). Despite multiple randomized controlled trials and meta-analyses, the type of pancreatico-enteric anastomosis [pancreaticojejunostomy (PJ) *vs* pancreaticogastrostomy(PG)] as a risk factor for post-pancreatectomy complications is debatable.

Research motivation

The ideal technique of pancreatic reconstruction during the learning curve of laparoscopic PD has not been well studied.

Research objectives

To compare the short-term outcomes of modified binding PG and Blumgart technique of PJ for pancreatic reconstruction in laparoscopic PD during the learning curve.

Research methods

The first 25 patients with resectable pancreatic or periampullary tumors who underwent laparoscopic PD and pancreatic reconstruction with modified binding PG or Blumgart PJ between January 2015 and May 2020 were retrospectively analyzed. A single layer of the full-thickness purse-string suture was placed around the posterior gastrotomy in the modified binding PG. In the modified Blumgart technique, a total of two transpancreatic horizontal mattress sutures were placed on either side of the pancreatic duct to secure the pancreatic parenchyma to the jejunum. Also, on the ventral surface, the knot is tied to the jejunal wall without going through the pancreatic parenchyma. Post pancreatectomy complications are graded as per the International Study Group for Pancreatic Surgery criteria and compared to evaluate perioperative outcomes during the same learning curve.

Research results

The demographic and clinical parameters of the patients included in both groups were comparable. The median operative time for pancreatic reconstruction was significantly lower in the binding PG group (42 *vs* 58 min, $P = 0.01$). The clinically relevant (Grade B/C) postoperative pancreatic fistula (POPF) was significantly more in the modified PJ group (28% *vs* 4%, $P = 0.04$). In contrast, intraluminal postpancreatectomy hemorrhage (PPH) was more in the binding PG group (32% *vs* 4%, $P = 0.02$). There was no significant difference in the incidence of delayed gastric emptying between the two groups.

Research conclusions

Modified binding PG reduces the pancreatic reconstruction time and POPF rate during the learning curve of laparoscopic PD but is associated with increased intraluminal PPH compared to PJ using the modified Blumgart technique.

Research perspectives

Modified Binding PG combined with techniques to reduce PPH like hemostatic pancreatic sutures on either side of the pancreatic duct may reduce POPF without increasing PPH during the learning curve of laparoscopic PD. A tailored pancreatic reconstruction with modified binding PG for patients with a high fistula risk score and modified Blumgart PJ for patients with low fistula risk score may be a

reasonable approach during the learning curve of laparoscopic PD.

FOOTNOTES

Author contributions: Kalayarasan R conceptualized the study and performed the surgical procedures; Choudhury SR and Gnanasekaran S performed the data acquisition and wrote the first draft of the manuscript; Kalayarasan R and Pottakkat B supervised the writing, gave intellectual inputs, and critically revised the manuscript.

Institutional review board statement: The study was approved by the Institute scientific advisory committee (PGRMC 19.04.2021-18) and the Institute ethics committee (JIP/IEC/2021/0194).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent. For full disclosure, the details of the study are published on the home page of JIPMER (http://www.jipmer.edu.in study ID - JIP/IEC/2021/0194).

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ORIGINAL ARTICLE

Observational Study

Assessing optimal Roux-en-Y reconstruction technique after total gastrectomy using the Postgastrectomy Syndrome Assessment Scale-45

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Abstract

BACKGROUND

Following a total gastrectomy, patients suffer the most severe form of postgastrectomy syndrome. This is a significant clinical problem as it reduces quality of life (QOL). Roux-en-Y reconstruction, which is regarded as the gold standard for post-total gastrectomy reconstruction, can be performed using various techniques. Although the technique used could affect postoperative QOL, there are no previous reports regarding the same.

AIM

To investigate the effect of different techniques on postoperative QOL. The data was collected from the registry of the postgastrectomy syndrome assessment study (PGSAS).

METHODS

In the present study, we analyzed 393 total gastrectomy patients from those enrolled in PGSAS. Patients were divided into groups depending on whether antecolic or retrocolic jejunal elevation was performed, whether the Roux limb was "40 cm", "shorter" (≤ 39 cm), or "longer" (≥ 41 cm), and whether the device used for esophageal and jejunal anastomosis was a circular or linear stapler. Subsequently, we comparatively investigated postoperative QOL of the patients.

RESULTS

Reconstruction route: Esophageal reflux subscale (SS) occurred significantly less frequently in patients who underwent antecolic reconstruction. Roux limb length: "Shorter" Roux limb did not facilitate esophageal reflux SS and somewhat attenuated indigestion SS and abdominal pain SS. Anastomosis technique: In terms of esophagojejunostomy techniques, no differences were observed.

CONCLUSION

The techniques used for total gastrectomy with Roux-en-Y reconstruction significantly affected postoperative symptoms. Our results suggest that elevating the Roux limb, which is not overly long, through an antecolic route may improve patients' QOL.

Key Words: Total gastrectomy; Roux-en-Y; Postgastrectomy syndrome; Quality of life; Postgastrectomy Syndrome Assessment Scale-45

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Core Tip: Following a total gastrectomy using various techniques, patients suffer the severe form of postgastrectomy syndrome. We investigated the effect of different techniques in Roux-en-Y reconstruction on postoperative quality of life (QOL) using the Postgastrectomy Syndrome Assessment Scale-45. We analyzed 393 total gastrectomy patients. Esophageal reflux subscale (SS) occurred significantly less frequently in patients who underwent antecolic reconstruction. Shorter Roux limb did not facilitate esophageal reflux SS and somewhat attenuated indigestion SS and abdominal pain SS. Our results suggest that elevating the Roux limb which is not overly long, through an antecolic route may improve patients' QOL.

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INTRODUCTION

Postgastrectomy syndrome is a serious clinical problem that decreases quality of life (QOL) of patients following gastrectomy[1-5]. As postgastrectomy syndrome is the severest form of the side effect following total gastrectomy[1,2,4,5], reducing the incidence of syndrome should be deliberated while choosing the surgical technique. Post-total gastrectomy Roux-en-Y reconstruction (TGRY) is a simple and robust form of reconstruction performed following a total gastrectomy, and it is widely performed and regarded as the gold standard. As laparoscopic surgery is more widely used in recent years, TGRY

techniques have become more diverse now than when open surgery was used[6-12]. Although the differences in techniques appear to affect postoperative QOL, the reasons remain unclear due to lack of sufficient investigation. Therefore, we used Postgastrectomy Syndrome Assessment Scale-45 (PGSAS-45), which has developed for postgastrectomy evaluation, to investigate how TGRY surgical techniques affect postoperative QOL[13].

MATERIALS AND METHODS

Retrieving the questionnaire

A total of 52 institutions participated in this study. A questionnaire was distributed to 2922 patients between July 2009 and December 2010 (Figure 1). Eligibility criteria for patients were as follows: (1) Diagnosis of pathologically-confirmed stage IA or IB gastric cancer[14]; (2) first-time gastrectomy; (3) aged 20-75 years; (4) no history of chemotherapy; (5) no recurrence or distant metastasis indicated; (6) gastrectomy conducted one or more years prior to the enrollment date; (7) performance status (PS) ≤ 1 on the Eastern Cooperative Oncology Group scale[15-17]; (8) full capacity to understand and respond to the questionnaire; (9) no history of other diseases or surgeries which might influence responses to the questionnaire; (10) absence of organ failure or mental illness; and (11) written informed consent. Patients with dual malignancy or concomitant resection of other organs (with co-resection equivalent to cholecystectomy being the exception) were excluded. Of the distributed questionnaires, 2520 (86%) were retrieved; 152 questionnaires were excluded. A total of 2368 questionnaires were analyzed and it was observed that total gastrectomy was performed in 393 patients; all underwent reconstruction using Roux-en-Y method. Questionnaires of these 393 patients were selected for examination in this study.

QOL assessment

PGSAS-45 consists of 45 items, including all eight items of the Short Form General Health Survey (SF-8) [18], all 15 items from the Gastrointestinal Symptom Rating Scale[19], and 22 newly-added items that cover various factors reflecting the postgastrectomy patient's well-being (Table 1)[13].

The following 18 outcome measures were evaluated, each consisting of a single item or an integration of related items from the PGSAS-45: esophageal reflux subscale (SS), abdominal pain SS, meal-related distress SS, indigestion SS, diarrhea SS, constipation SS, dumping SS, total symptom score, ingested amount of food per meal, necessity for additional meals, quality of ingestion SS, ability for working, dissatisfaction with symptoms, dissatisfaction at the meal, dissatisfaction at working and dissatisfaction for daily life SS, and the physical component summary (PCS) and mental component summary (MCS) of SF-8. Percentage changes in body weight (decrease in body weight/preoperative weight) were also determined as an outcome measure. These 19 main outcome measures were scored and classified into three domains: symptoms, living status, and QOL. Higher scores denote better outcomes for the items of PCS, MCS, ingested amount of food per meal, quality of ingestion SS, and changes in body weight, whereas lower scores denote better outcomes for the other 14 outcome measures.

Postoperative follow-up with PGSAS-45

The gastrectomy patients were provided with a PGSAS-45 questionnaire by the surgeon during an outpatient visit. Each patient was asked to complete the questionnaire and mail it to the data center. The clinical data were reported to the data center by the responsible surgeons using case report form and matched to PGSAS-45 responses. All the data were analyzed at the data center. Postgastrectomy daily living was compared among: (1) Elevated route of Roux limb: antecolic *vs* retrocolic; (2) length of the Roux limb (defined as the distance from esophagojejunostomy to jejunojejunostomy): "shorter (≤ 39 cm)" *vs* "40 cm" *vs* "longer (≥ 41 cm)"; and (3) anastomotic procedure for esophagojejunostomy: circular stapler (CS) *vs* linear stapler (LS) (Figure 2). The study protocol was approved by the institutional review board of each participating institution and registered with the University Hospital Medical Information Network's Clinical Trials Registry (registration number, 000002116). All patients provided their written informed consent for the confidential use of their information in the data analysis, in compliance with institutional guidelines.

Statistics

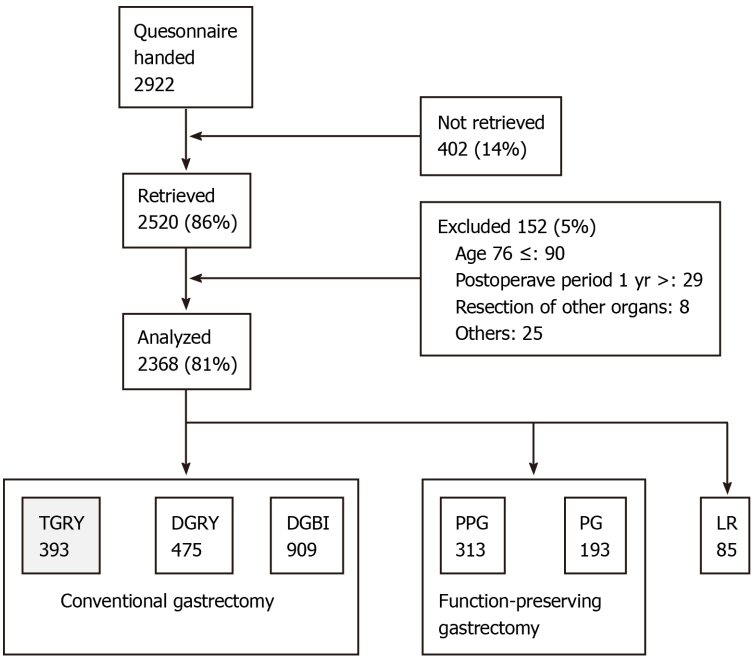
The values are shown as the mean ± SD. Two-group differences in the mean values were analyzed using an unpaired *t*-test and multiple-group differences were analyzed using one-way analysis of variance (ANOVA). Tukey multiple comparisons test was used when the ANOVA yielded a P value of < 0.1. Generally, a P value of < 0.05 was considered statistically significant. When the P values were < 0.1 in the *t*-test or Tukey-test, the effect size (Cohen's d) was calculated. The value of Cohen's d reflects the impact of each causal variable: values between 0.2 and < 0.5 denote a small but clinically meaningful difference between the groups; values between 0.5 and < 0.8 denote a medium effect; and values ≥ 0.8 indicate a large effect. All statistical analyses were performed using JMP12.0.1 software (SAS Institute Inc., Cary, NC, United States).

Table 1 Structure of postgastrectomy syndrome assessment scale 45 (domains/subdomains/items/subscales)

Domains	Subdomains	Items	Subscales
QOL	SF-8 (QOL)	1 Physical functioning*	Physical component summary* (items 1-8)
		2 Role physical*	
		3 Bodily pain*	Mental component summary* (items 1-8)
		4 General health*	
		5 Vitality*	
		6 Social functioning*	
		7 Role emotional*	
		8 Mental health*	
Symptoms	GSRS (symptoms)	9 Abdominal pains	Esophageal reflux subscale (items 10, 11, 13, 24)
		10 Heartburn	
		11 Acid regurgitation	
		12 Sucking sensations in the epigastrium	Abdominal pain subscale (items 9, 12, 28)
		13 Nausea and vomiting	
		14 Borborygmus	Meal-related distress subscale (items 25-27)
		15 Abdominal distension	
		16 Eructation	Indigestion subscale (items 14-17)
		17 Increased flatus	Diarrhea subscale (items 19, 20, 22)
		18 Decreased passage of stool	
		19 Increased passage of stool	Constipation subscale (items 18, 21, 23)
		20 Loose stool	
		21 Hard stool	Dumping subscale (items 30, 31, 33)
		22 Urgent need for defecation	
		23 Feeling of incomplete evacuation	Total symptom score (above seven subscales)
	Symptoms	24 Bile regurgitation	
		25 Sense of food sticking	
		26 Postprandial fullness	
		27 Early satiation	
		28 Lower abdominal pain	
		29 Number and type of early dumping symptoms	
		30 Early dumping general symptoms	
		31 Early dumping abdominal symptoms	
		32 Number and type of late dumping symptoms	
		33 Late dumping symptoms	
Living status	Meals (amount) 1	34 Ingested amount of food per meal*	Quality of ingestion subscale* (items 38-40)
		35 Ingested amount of food per day*	
		36 Frequency of main meals	
		37 Frequency of additional meals	
	Meals (quality)	38 Appetite*	

		39 Hunger feeling*	
		40 Satiety feeling*	
	Meals (amount) 2	41 Necessity for additional meals	
	Social activity	42 Ability to work	
QOL	Dissatisfaction (QOL)	43 Dissatisfaction with symptoms	Dissatisfaction for daily life subscale (items 43-45)
		44 Dissatisfaction at the meals	
		45 Dissatisfaction at working	

In items or subscales with *; higher score indicating better condition. In items or subscales without *; higher score indicating worse condition. Each subscale is calculated as the mean of composed items or subscales except PCS and MCS of SF-8. Item 29 and 32 don't have score. Then, they were analyzed separately. Citation: Nakada K, Ikeda M, Takahashi M, Kinami S, Yoshida M, Uenosono Y, Kawashima Y, Oshio A, Suzukamo Y, Terashima M, Kodera Y. Characteristics and clinical relevance of postgastrectomy syndrome assessment scale (PGSAS)-45: newly developed integrated questionnaires for assessment of living status and quality of life in postgastrectomy patients. *Gastric Cancer* 2015; 18: 147-158. QOL: Quality of life.



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Figure 1 Outline of the study. TGRY: Total gastrectomy with Roux-en-Y reconstruction; DGRY: Distal gastrectomy with Roux-en-Y reconstruction; DGBI: Distal gastrectomy with Billroth I reconstruction; PPG: Pylorus-preserving gastrectomy; PG: Proximal gastrectomy; LR: Local resection.

RESULTS

Patient characteristics

Characteristics of the 393 patients are listed in Table 2. The mean age was 63.4 years and the mean postoperative follow-up period was approximately 35 mo. It was observed that the number of male patients was more than the number of female patients and open surgery was more commonly used than laparoscopic surgery. The combined resection of another organ was performed for the gall bladder (83 patients) and spleen (52 patients). Dissection of the lymph node was over D1b in most of the patients. Conversely, celiac branch of the vagus nerve was not preserved in most patients.

Route of the Roux limb

The jejunum elevation route during Roux-en-Y reconstruction was described for 385 (98.0%) patients (Table 3). Retrocolic elevation (206 patients) was performed more commonly than antecolic elevation (179 patients). Among the 19 main outcome measures, scores for the esophageal reflux SS were significantly superior in antecolic elevation group compared to retrocolic elevation group with small but clinically meaningful effect (P = 0.028, Cohen's = 0.23).

Table 2 Patients' characteristics (393 cases are listed)

Characteristics	Values
Number of patients	393
Postoperative period (mo), mean ± SD	35.0 ± 24.6
Preoperative BMI, mean ± SD	23.0 ± 3.3
Postoperative BMI, mean ± SD	19.8 ± 2.5
Age, mean ± SD	63.4 ± 9.2
Gender (male/female)	276/113
Approach (laparoscopic/open)	97/293
Extent of lymph node dissection[1]	
D2	164
D1b	192
D1a	28
D1	4
D1>	0
None	0
Celiac branch of the vagal nerve (preserved/divided)	12/371
Combined resection	
Gallbladder	83
Spleen	52
Miscellaneous	2
None	246

[1]According to the Japanese gastric cancer treatment guideline. BMI: Body mass index.

Length of the Roux limb

Of the 393 patients, the length of the Roux limb was described in 373 (94.9%) patients (Table 4). The most common Roux limb length was "40 cm" (238 patients), followed by "longer (≥ 41 cm)" (119 patients) and "shorter (≤ 39 cm)" (16 patients) Roux limb length (Figure 3). "Shorter" Roux limb length had not worsen the esophageal reflux SS, and rather reduced the indigestion SS compared to both the "40 cm" and "longer" Roux limb groups with medium effect size in terms of Cohen's *d* values (shorter *vs* 40 cm: $P = 0.020$, Cohen's $d = 0.69$; "shorter" *vs* "longer": $P = 0.030$, Cohen's $d = 0.68$, respectively). In addition, "shorter" Roux limb attenuated abdominal pain SS with marginal significance ($P = 0.081$).

Anastomotic procedure for esophagojejunostomy

Of the 393 patients, the device used for anastomosis between the esophagus and jejunum was described in 388 (98.7%) patients (Table 5). The CS was used in 348 patients, while the LS was used in 40 patients. Among the 19 main outcome measures of PGSAS-45, there was no difference between the two procedures.

DISCUSSION

Postgastrectomy syndrome is the severest following total gastrectomy and persists in the long-term; thereby, lowering patients' QOL[1,2,4,5]. Therefore improvement of surgical techniques to reduce the onset of this syndrome is important. TGRY is a simple and robust technique that is performed widely and regarded as the gold standard for post-total gastrectomy reconstruction. While the increased use of laparoscopic surgery and anastomotic devices has resulted in the diversification of TGRY surgical techniques[6-12], the effects of different TGRY techniques on patients' QOL remains unknown. Our results indicate that elevation of the Roux limb *via* antecolic route resulted in fewer esophageal reflux SS, and the relatively "shorter" Roux limb length accompanied by fewer indigestion SS without increasing esophageal reflux SS. In terms of device selection for esophagojejunostomy, no difference was

Table 3 The effect of the reconstruction route (antecolic or retrocolic) of Roux–limb on postoperative quality of life after total gastrectomy

Reconstruction route of Roux limb	Retro-colica (*n* = 206)		Ante-colica (*n* = 179)		*P* value	Cohens *d*
	mean	SD	mean	SD		
Esophageal reflux SS	2.1	1.1	1.8	0.9	0.028	0.229
Abdominal pain SS	1.8	0.8	1.7	0.8	NS	
Meal-related distress SS	2.7	1.1	2.6	1.1	NS	
Indigestion SS	2.3	0.98	2.3	0.9	NS	
Diarrhea SS	2.4	1.3	2.2	1.1	NS	
Constipation SS	2.1	1.0	2.0	0.8	NS	
Dumping SS	2.4	1.1	2.3	1.1	NS	
Total symptom score	2.2	0.8	2.1	0.7	NS	
Change in Body weight	-13.6%	7.8%	-14.0%	8.1%	NS	
Ingested amount of food per meal	6.5	1.9	6.4	1.8	NS	
Necessity for additional meals	2.3	0.8	2.4	0.7	NS	
Quality of ingestion SS	3.7	1.0	3.8	0.9	NS	
Ability to work	2.1	0.9	2.0	0.8	NS	
Dissatisfaction with symptoms	2.1	1.0	2.0	1.0	NS	
Dissatisfaction at the meal	2.8	1.1	2.8	1.1	NS	
Dissatisfaction at working	2.1	1.1	2.2	1.0	NS	
Dissatisfaction for daily life SS	2.4	0.9	2.3	0.9	NS	
Physical component summary	49.2	5.8	50.1	5.4	NS	
Mental component summary	49.1	6.1	49.2	5.9	NS	

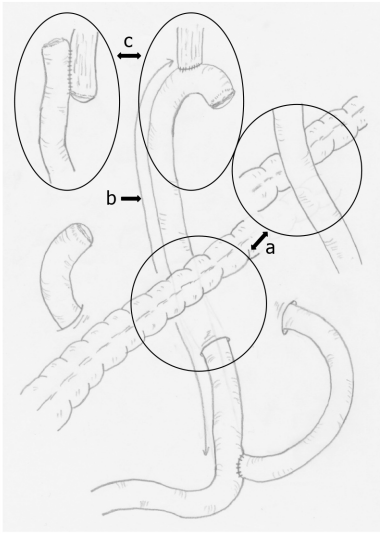
SS: Subscale; NS: Not significant.

Table 4 The effect of the length of Roux-limb (shorter, 40 cm, longer) on postoperative quality of life after total gastrectomy

Length of Roux limb	Shorter (*n* = 16)		40 cm (*n* = 238)		Longer (*n* = 119)		ANOVA	Multiple comparisons	*P* value	Cohens *d*
	mean	SD	mean	SD	mean	SD	*P* value			
Esophageal reflux SS	1.8	0.9	2.0	1.1	2.0	1.0	NS			
Abdominal pain SS	1.4	0.4	1.8	0.8	1.7	0.7	0.081	Shorter *vs* 40 cm	0.053	0.52
Meal-related distress SS	2.2	0.9	2.7	1.2	2.7	1.0	NS			
Indigestion SS	1.7	0.7	2.3	0.9	2.3	0.9	0.026	Shorter *vs* 40 cm	0.020	0.69
								Shorter *vs* longer	0.030	0.68
Diarrhea SS	2.0	1.2	2.3	1.2	2.3	1.2	NS			
Constipation SS	2.3	0.9	2.1	0.9	2.1	0.9	NS			
Dumping SS	1.8	0.9	2.4	1.1	2.3	1.1	NS			
Total symptom score	1.9	0.6	2.2	0.8	2.2	0.7	NS			
Change in Body weight	-14.1%	8.6%	-13.8%	8.2%	-13.5%	7.5%	NS			
Ingested amount of food per meal	5.5	2.6	6.4	1.9	6.5	1.7	NS			
Necessity for additional meals	2.4	0.8	2.4	0.8	2.3	0.7	NS			
Quality of ingestion SS	3.3	1.2	3.8	0.9	3.8	1.0	NS			
Ability to work	2.4	1.2	2.0	0.9	2.1	0.9	NS			

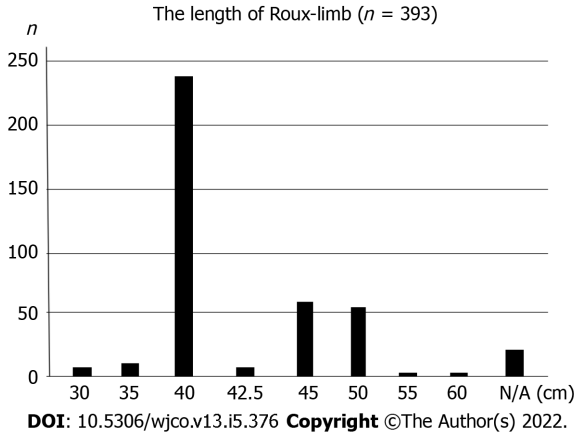
Dissatisfaction with symptoms	1.8	1.0	2.0	1.1	2.2	1.0	NS
Dissatisfaction at the meal	3.3	1.2	2.8	1.2	2.8	1.0	NS
Dissatisfaction at working	2.5	1.3	2.2	1.1	2.1	1.0	NS
Dissatisfaction for daily life SS	2.5	1.0	2.3	0.9	2.4	0.8	NS
Physical component summary	49.2	6.7	49.4	5.7	50.1	5.5	NS
Mental component summary	48.1	5.9	48.7	6.3	49.9	5.5	NS

SS: Subscale; NS: Not significant.



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Figure 2 Schema of Roux-en-Y reconstruction after total gastrectomy. a: Route of the Roux limb (antecolic or retrocolic); b: Length of the Roux limb defined as the distance from the esophago-jejunostomy to the jejunojejunostomy [shorter (≤ 39 cm), average (40 cm) or longer (≥ 41 cm)]; c: Anastomotic procedure for esophagojejunostomy (reconstruction using a circular or linear stapler).



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Figure 3 The distribution of the length of Roux-limb after total gastrectomy. N/A: Not answered group indicated.

observed between the CS and LS procedures. To the best of our knowledge, this is the first report to demonstrate that differences in surgical techniques in TGRY affect postoperative QOL.

The Roux limb reconstruction in TGRY has often been performed *via* retrocolic route in open surgeries, as it applies slight tension to the anastomosis due to the short distance to the esophageal stump. With the increased use of laparoscopic surgery, surgeons began elevating the Roux limb *via* antecolic route due to its technical simplicity[7]. And then, the antecolic elevation became more common even for open total gastrectomy. Our investigation into the effects of different Roux limb reconstruction routes in TGRY on postoperative QOL indicate that esophageal reflux SS was significantly attenuated in

Table 5 The effect of anastomotic procedure for esophagojejunostomy (circular stapler, linear stapler) on postoperative quality of life after total gastrectomy

Anastomotic method	Circular stapler(*n* = 348)		Liner stapler (*n* = 40)		*P* value
	mean	SD	mean	SD	
Esophageal reflux SS	2.0	1.0	1.9	0.8	NS
Abdominal pain SS	1.8	0.8	1.7	0.8	NS
Meal-related distress SS	2.6	1.1	2.8	1.2	NS
Indigestion SS	2.3	0.9	2.2	0.8	NS
Diarrhea SS	2.3	1.2	2.2	1.3	NS
Constipation SS	2.1	0.9	2.1	1.0	NS
Dumping SS	2.3	1.1	2.4	1.1	NS
Total symptom score	2.2	0.7	2.1	0.7	NS
Change in Body weight	-13.9%	7.9%	-12.8%	7.9%	NS
Ingested amount of food per meal	6.5	1.9	6.2	1.8	NS
Necessity for additional meals	2.3	0.8	2.4	0.8	NS
Quality of ingestion SS	3.8	1.0	3.8	0.9	NS
Ability to work	2.0	0.9	2.1	0.9	NS
Dissatisfaction with symptoms	2.1	1.0	2.1	0.9	NS
Dissatisfaction at the meal	2.8	1.1	3.0	1.0	NS
Dissatisfaction at working	2.1	1.1	2.2	1.0	NS
Dissatisfaction for daily life SS	2.3	0.9	2.5	0.8	NS
Physical component summary	49.6	5.7	50.2	4.9	NS
Mental component summary	49.2	6.0	49.2	5.9	NS

SS: Subscale; NS: Not significant.

the antecolic route group than the retrocolic route group. One of the possible explanation is that in the antecolic reconstruction, duodenal fluid hardly flow back into the esophagus unless it passes over the height of the transverse colon when the patient took the lying-down position. As a result, this physical barrier of gravity could attenuate the esophageal reflux SS in addition to the preventive effect of the peristalsis of the Roux limb. Based on these, the antecolic route may be a suitable surgical procedure when performing TGRY. Although the caution is needed for the occurrence of the internal hernia through Petersen's defect especially when the gastrectomy underwent laparoscopically, and the implementing preventive methods such as the closure of these defects with sutures[20,21] should be performed.

Many surgeons concern that the insufficient length of Roux limb likely to increase the esophageal regurgitation. However, in the present study, the esophageal reflux SS did not worsened in the "shorter" Roux limb length group compared to the other groups, therefore, even relatively short Roux limbs of 30-35 cm may have produced the sufficient intestinal peristalsis to prevent esophageal regurgitation. Interestingly, significantly more indigestion SS was observed in the "40 cm" and "longer" Roux limb length groups compared to the "shorter" group. This may be, in part, explained by the previous report[22] showing that relatively long Roux limbs could be a cause of Roux-en-Y syndrome. The Roux limb length should be adjusted as an appropriate length, and not too long[22].

Although esophagojejunostomy in TGRY had mainly performed using the CS, the increase in laparoscopic surgery has resulted in the diversification of anastomotic techniques and the esophagojejunostomy using the LS is increasing[9-11]. Comparison of the CS and LS procedures in terms of the effect of the esophagojejunostomy technique on postoperative QOL revealed no differences in any of the main outcome measures of PGSAS-45, therefore, either of the CS or LS procedures can be selected depending on the clinical situation to achieve a safe and simple anastomosis procedure.

Many surgeons had chosen the retrocolic route as that of the Roux limb from the problems concerned with the distance of Roux limb and occurrence of internal hernia, and enough length of the Roux limb preventing the regurgitation to esophagus. The result of this PGSAS study may provide a hint for the optimal surgical procedures after total gastrectomy. A limitation of the present study is its retrospective

nature and the unbalanced number of patients in each group. A well-designed prospective study should be conducted in the future.

CONCLUSION

Our results revealed that the specific surgical technique used for TGRY affects postoperative QOL to some extent. Since postgastrectomy syndrome is the severest following total gastrectomy, a technique that could maintain a favorable postoperative QOL should be selected. The findings of this study suggest that some of the postgastrectomy symptoms following TGRY could be attenuated by elevating Roux limb through antecolic route with not too long Roux limb length.

ARTICLE HIGHLIGHTS

Research background

Following a total gastrectomy using various techniques, some patients suffer the severe form of postgastrectomy syndrome.

Research motivation

Although the differences in techniques of Roux-en-Y reconstruction appear to affect postoperative quality of life (QOL), the reasons remain unclear due to lack of sufficient investigation.

Research objectives

We investigated the effect of different techniques on postoperative QOL.

Research methods

Using the Postgastrectomy Syndrome Assessment Scale-45, we investigated the effect of different techniques in Roux-en-Y reconstruction on postoperative QOL. We analyzed 393 total gastrectomy patients.

Research results

Esophageal reflux subscale (SS) occurred significantly less frequently in patients who underwent antecolic reconstruction. Shorter Roux limb did not facilitate esophageal reflux SS and somewhat attenuated indigestion SS and abdominal pain SS.

Research conclusions

Our results suggest that elevating the Roux limb which is not overly long, through an antecolic route may attenuate some of the postgastrectomy symptoms.

Research perspectives

Patients' QOL after total gastrectomy may be improved by this study.

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FOOTNOTES

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SYSTEMATIC REVIEWS

Immune checkpoint inhibitors in head and neck squamous cell carcinoma: A systematic review of phase-3 clinical trials

Jissy Vijo Poulose, Cessal Thommachan Kainickal

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Abstract

BACKGROUND

The outcomes of patients diagnosed with head and neck squamous cell carcinoma (HNSCC) who are not candidates for local salvage therapy and of those diagnosed with recurrent or metastatic disease are dismal. A relatively new systemic therapy option that emerged in recent years in the treatment of advanced HNSCC is immunotherapy using immune checkpoint inhibitors (ICIs). The safety profile and anti-tumor activity of these agents demonstrated in early phase clinical trials paved the way to the initiation of several promising phase-3 trials in the field.

AIM

To evaluate the evidence on the effectiveness of ICIs in HNSCC, based on published phase-3 clinical trials.

METHODS

We searched PubMed, Cochrane Library, Embase, and Scopus to identify published literature evaluating immunotherapy using ICIs in recurrent or metastatic HNSCC (R/M HNSCC) and locally advanced head and neck squamous cell carcinoma (LAHNSCC). We used a combination of standardized search terms and keywords including head and neck squamous cell carcinoma, recurrent, metastatic, locally advanced, immunotherapy, immune checkpoint inhibitors, monoclonal antibodies, programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), cytotoxic T- lymphocyte associated protein-4 (CTLA-4), and phase-3 clinical trial. A sensitive search filter was used to limit our results to randomized controlled trials.

RESULTS

Five phase-3 clinical trials have reported the data on the effectiveness of immuno-

therapy in HNSCC so far: Four in R/M HNSCC and one in LAHNSCC. In patients with R/M HNSCC, anti-PD-1 agents nivolumab and pembrolizumab demonstrated improved survival benefits in the second-line treatment setting compared to the standard of care (standard single-agent systemic therapy). While the net gain in overall survival (OS) with nivolumab was 2.4 mo [hazard ratio (HR) = 0.69, P = 0.01], that with pembrolizumab was 1.5 mo (HR = 0.80 nominal P = 0.0161). The anti-PD-L1 agent durvalumab with or without the anti-cytotoxic T- lymphocyte associated protein-4 agent tremelimumab did not result in any beneficial outcomes. In the first-line setting, in R/M HNSCC, pembrolizumab plus platinum-based chemotherapy resulted in significant improvement in survival with a net gain in OS of 2.3 mo (HR = 0.77, P = 0.0034) in the overall population and a net gain in OS of 4.2 mo in the PD-L1 positive (combined positive score > 20) population compared to standard of care (EXTREME regime). In patients with PD-L1 positive R/M HNSCC, monotherapy with pembrolizumab also demonstrated statistically significant improvement in survival compared to EXTREME. In LAHNSCC, immunotherapy using avelumab (an anti-PD-L1 agent) along with standard chemoradiation therapy did not result in improved outcomes compared to placebo plus chemoradiation therapy.

CONCLUSION

Anti-PD-1 agents provide survival benefits in R/M HNSCC in the first and second-line settings, with acceptable toxicity profiles compared to standard therapy. There is no proven efficacy in the curative setting to date.

Key Words: Head and neck squamous cell carcinoma; Recurrent/metastatic head and neck squamous cell carcinoma; Locally advanced head and neck squamous cell carcinoma; Immune checkpoint inhibitors; Immunotherapy; Monoclonal antibody

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Core Tip: Immune checkpoint inhibitors have demonstrated better survival outcomes and acceptable toxicity profiles in recurrent/metastatic head and neck squamous cell carcinoma in the first and second-line treatment settings. While anti- programmed cell death protein-1 agents demonstrated efficacy, evidence on the effectiveness of anti-programmed death ligand-1 and anti-cytotoxic T lymphocyte-associated antigen-4 agents is lacking. There is no proven efficacy in the curative setting to date. Gaps in knowledge were found in terms of predictive biomarkers and identification of patients who would benefit from immuno-therapy based on biomarker assessment. Several promising trials are currently ongoing to fill this knowledge gap. Novel combination strategies to potentiate and prolong the anti-tumor activity of immune checkpoint inhibitors are also being evaluated currently.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is one of the major causes of cancer-associated morbidity and mortality globally[1-3]. Treatment approaches for HNSCC vary according to the stage of the disease at presentation. Around 40% of HNSCCs present at an early stage and are treated by a single treatment modality, either radical radiotherapy or surgery. The remaining 60% of cases present as locally advanced disease, and treatment options include chemoradiation or surgery followed by adjuvant therapy. However, within 3 years, over 50% of these patients relapse locally or at distant sites. Salvage approaches for the locally recurrent disease include surgery, surgery followed by re-irradiation, or re-irradiation with or without concurrent chemotherapy[4,5]. For a recurrent disease that is not amenable to salvage approach and for metastatic disease, platinum-based chemotherapy was the only available treatment option until recently. While the median survival of recurrent/metastatic HNSCC (R/M HNSCC) patients receiving platinum-based chemotherapy is 7.4 mo, some patients become refractory to platinum and die within a period of 4 mo[6-12]. Subsequently, the addition of the anti-epidermal growth factor receptor (EGFR) targeted agent cetuximab to platinum-based chemotherapy showed improvement in survival compared to platinum-based chemotherapy alone, as demonstrated in a landmark phase-3 trial in 2008[12-16].

A relatively new systemic therapy option that emerged in recent years in the treatment of advanced HNSCC is immunotherapy using immune checkpoint inhibitors (ICIs). The checkpoint pathways in the tumor microenvironment are responsible for immune escape and T cell exhaustion related to the survival of the cancer cells. ICIs are monoclonal antibodies that can block these pathways by inhibiting the binding of checkpoint proteins on the T cells to similar proteins on the tumor cells. Thus, these agents act by reinvigorating the immune cells and re-establishing the anti-tumor immune responses that promote the elimination of cancer cells. Programmed cell death protein-1 (PD-1) receptors, programmed death-ligand 1 (PD-L1) receptors, and cytotoxic T- lymphocyte associated protein-4 (CTLA-4) are the major established targets for cancer immunotherapy with ICIs, and the therapeutic effects of ICIs result from blockade of these receptors[17-20].

In recent years, many interventional studies have evaluated ICI therapy for the treatment of HNSCC. The objective of this systematic review is to gather the evidence from published phase-3 randomized controlled trials (RCTs) comparing immunotherapy with the standard of care (SOC), among patients with R/M HNSCC or locally advanced HNSCC (LAHNSCC). We aimed to evaluate and synthesize the evidence from the published phase-3 studies investigating immunotherapy in advanced head and neck cancer using checkpoint inhibitors, either alone or in combination with chemotherapy, radiation therapy, or another checkpoint inhibitor.

MATERIALS AND METHODS

Data sources and literature search

The study followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines[21]. We systematically searched PubMed, SCOPUS, EMBASE, and COCHRANE Library without any language limit. We used a combination of standardized search terms and keywords including head and neck squamous cell carcinoma, recurrent, metastatic, locally advanced, immunotherapy, checkpoint inhibitors, monoclonal antibodies, PD-1, PD-L1, CTLA-4, and phase-3 clinical trial. A sensitive search filter was used to limit our results to RCTs reported from January 2000 till February 2021. The initial search was conducted in February 2021. We also looked for any updates on the selected studies till April 2021. The search syntax is given in the Supplementary file.

Inclusion/exclusion criteria and study selection

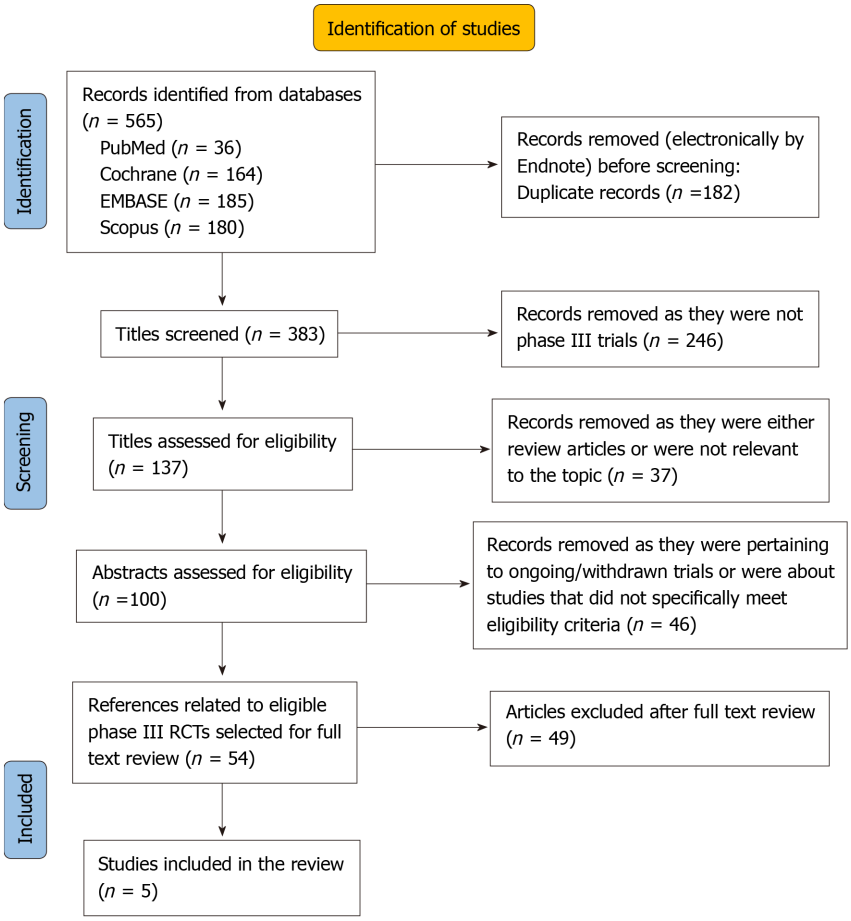
Studies were included if they were completed phase-3 RCTs conducted among patients with R/M HNSCC or LAHNSCC, in which the intervention patients received ICI either alone or in combination with chemotherapy, radiation therapy, or with another IO and the control patients received SOC. Anatomical sites of primary tumors were oral cavity, oropharynx, hypopharynx, and larynx in the included studies. Early phase trials and observational studies were excluded. Studies involving patients with nasopharyngeal carcinoma were also excluded.

Titles generated from the initial search results were exported to EndNote. Duplicates were removed, and the remaining titles were scanned for relevance. Abstracts of articles pertaining to potentially eligible studies were independently reviewed by both authors and uncertainties were resolved through discussion. Potentially eligible studies were further evaluated for relevance, trial status (completed/ongoing/withdrawn), and availability of results.

The following descriptive data were extracted from the included studies: Study design, population, details of the intervention, details of treatment received by the control arm, and the primary and secondary endpoints. Information on adverse events and statistical data on the outcomes were also extracted, which included, overall survival (OS), progression-free survival (PFS), overall response rate (ORR), biomarker effect, and patient-reported outcomes. The flow chart of study selection (PRISMA) is given in Figure 1.

RESULTS

The original literature search generated 565 titles altogether, of which 100 titles were eventually selected for abstract review for identification of potentially eligible studies. Others were excluded as they were related to phase-1 or phase-2 studies or not precisely relevant to the topic of the review. Through the abstract review, we identified 56 references (including one conference abstract) pertaining to potentially eligible studies. Through full-text review of these references, we selected five original phase-3 RCTs to be included in the systematic review[22-26]. In four of the trials[22-25], participants were patients with R/M HNSCC, while in one trial, participants were patients diagnosed with LAHNSCC[26,27]. All four studies among patients with R/M HNSCC were open-label RCTs; three of them investigated the effectiveness of ICI as second-line treatment[22-24], while in one study[25], ICI was evaluated as first-line treatment. The study among LAHSCC patients was a double-blinded placebo-controlled RCT[26,27].



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Figure 1 Article selection flow diagram. RCTs: Randomized controlled trials.

ICIs assessed in these studies were nivolumab, pembrolizumab, durvalumab, tremelimumab, and avelumab. While nivolumab and pembrolizumab are anti-PD-1 monoclonal antibodies, durvalumab and avelumab are anti-PD-L1 antibodies. The monoclonal antibody tremelimumab is an anti-CTLA-4 agent[28-31].

We classified the studies into three groups based on the disease status and the treatment setting. The details of these studies in terms of the study population, intervention, comparator, outcomes, and adverse events are given in Table 1.

Phase-3 studies evaluating ICI as second-line treatment in R/M HNSCC (three RCTs: CheckMate 141, KEYNOTE 040, and EAGLE)

So far, three phase-3 RCTs have compared the effectiveness of ICI against the existing SOC (single-agent systemic therapy with methotrexate, docetaxel, or cetuximab) in the second-line treatment setting[22-24] (Table 1).

CheckMate 141 (nivolumab vs standard single-agent systemic therapy)

Ferris *et al*[22] conducted a randomized, open-label, phase-3 study (n = 361) among patients with platinum-refractory recurrent HNSCC (recurrence within 6 mo after platinum-based chemotherapy) to investigate the effectiveness of the anti-PD-1 checkpoint inhibitor agent nivolumab. The intervention arm (n = 240) received nivolumab at a dose of 3 mg/kg body weight every 2 wk, while the control patients (n = 121) received SOC in the form of standard single-agent systemic therapy with methotrexate [40 mg/m^2 intravenously (IV) weekly], docetaxel (30 mg/m^2 IV weekly), or cetuximab (400 mg/m^2 IV once followed by 250 mg/m^2 weekly). OS was the primary endpoint of the study. Secondary endpoints included PFS, ORR, and biomarker effects on survival, safety, and quality of life assessments. The median duration of follow-up was 5.1 mo (range, 0 to 16.8).

Outcomes

OS: The median OS was 7.5 mo [95% confidence interval (CI): 5.5-9.1] with nivolumab *vs* 5.1 mo (95%CI: 4.0-6.0) with SOC [hazard ratio (HR) = 0.69; 97.73%CI: 0.53-0.91; P = 0.01]. The estimated 1-year survival rate was 36.0% in the nivolumab group *vs* 16.6% in the control group.

Table 1 Studies included in the systematic review

Ref.	Design	Population	Intervention (I)	Control (C)	OS	PFS	ORR	QOL measures/symptom burden	Biomarker effect	AE Grade 3 or more
Phase-3 clinical trials evaluating ICI as second line therapy in R/M HNSCC										
Ferris *et al* [22], 2016	RCT (2:1), open-label phase-3 trial	Patients with R/M HNSCC not amenable to curative therapy	Nivolumab 3 mg/kg IV Q2W	SOC: Investigator's, choice of methotrexate 40 mg/m^2 IV weekly, docetaxel 30 mg/m^2 IV weekly, or cetuximab 400 mg/m^2 IV once followed by 250 mg/m^2 weekly	Nivolumab: 7.5 mo (95%CI: 5.5-9.1)	Nivolumab: 2.0 mo, 95%CI: 1.9-2.1	Nivolumab: 13.3%, 95%CI: 9.3-18.3	Between group differences in favor of Nivolumab group	**OS**	Nivolumab: 13.1%
Checkmate 141		*n* = 361	MoA: PD-1 inhibition	*n* = 121	SOC: 5.1 mo, 95%CI: 4.0-6.0; HR 0.69, 95%CI: 0.53-0.91, *P* = 0.01	SOC: 2.3 mo, 95%CI: 1.9-3.1; HR 0.89, 95%CI: 0.70-1.13, *P* = 0.32	SOC: 5.8%, 95%CI: 2.4-11.6	Physical functioning: at 9 wk *P* = 0.01; at 15 wk, *P* < 0.001	PD-L1 ≥ 1%: Nivolumab 8.7mo; SOC: 4.6 mo, HR for death 0.55 (95%CI: 0.36-0.83)	Two treatment related deaths
			n = 240, median follow up = 5.1 mo (range: 0 to 16.8)		Estimated 1-yr survival rate 36.0% in the nivolumab group *vs* 16.6% in the control group			Role functioning: at 9 wk, *P* = 0.003; at 15 wk, *P* < 0.001	PD-L1 < 1%: Nivolumab, 5.7 mo; SOC: 5.8 mo, HR for death 0.89 (95%CI: 0.54-1.45) *P* for int. = 0.17	SOC: 35.0%
								Social functioning: at 9 wk *P* = 0.002; at 15 wk *P* < 0.001	P16 + ve tumors: Nivolumab 9.1 mo; SOC: 4.4 mo, HR for death 0.56 (95%CI: 0.32-0.99)	One treatment related death
								Symptom burden pain: at 9 wk, *P* < 0.001; at 15 wk, *P* = 0.02 Sensory problems: at 9 wk, *P* = 0.01; at 15 wk, *P* < 0.001	P16 -ve tumours: Nivolumab 7.5 mo; SOC: 5.8 mo, HR 0.73 (95%CI: 0.42-1.25), *P* for Interaction = 0.55	
								Social contact problems: at 9 wk, *P* = 0.26; at 15 wk, *P* < 0.001		
Cohen *et al* [23], 2019	RCT (1:1), open-label phase-3	Patients with R/M HNSCC	Pembrolizumab: 200 mg IV Q3W	SOC: methotrexate 40 mg/m^2 weekly (in absence of	Pembrolizumab: 8.4 mo, 95%CI: 6.4-9.4	Pembrolizumab: 2.1 mo 95% CI: 2.1-2.3	Pembrolizumab: 14.6%, 95%CI: 10.4-19.6	Exploratory HRQOL analysis (published separately) by means of	**OS**	Pembrolizumab: 13%, treatment related death in

	trial			toxicity could increase to 60 mg/m^2), docetaxel 75 mg/m^2 Q3W, or cetuximab loading dose of 400 mg/m^2 followed by 250 mg/m^2 weekly				EORTC QOLQ-C30, EORTC QOLQ- H&N35, and EuroQOL-5 dimensions questionnaires		four patients
KEYNOTE 040		3-6 mo after multimodal treatment with platinum or progression after platinum-based treatment	MoA: PD-1 inhibition	*n* = 248, median follow-up 7.1 mo (IQR 3.7-12.4)	SOC: 6.9 mo, 95%CI: 5.9-8.0; HR 0.80, 95%CI: 0.65-0.98, nominal *p* = 0.0161	SOC: 2.3 mo, 95%CI: 2.1-2.8; HR 0.96, 95%CI: 0.79-1.16, nominal *P* = 0.325	SOC: 10.1%, 95%CI: 6.6-14.5, nominal *P* = 0.061	At 15 wk, GHS/QOL scores were stable with pembrolizumab: least square mean (LSM) 0.39; 95%CI: -3.00 to 3.78	TPS ≥ 50%	SOC: 36.1%, treatment related death in two patients
		n = 495	*n* = 247, median follow up = 7.5 mo (IQR 3.4-13.3) until data cut-off /8.4 mo (IQR 3.3-14.5) until death			PFS based on modified RECIST 1.1		At 15 wk, GHS/QOL scores declined with SOC; (LSM -5.86; 95%CI: -9.68 to -2.04)	Pembrolizumab 11.6 mo (95%CI: 8.3-19.5); SOC: 6.6 mo (95%CI: 4.8-9.2), HR 0.53 (95%CI: 0.35-0.81; nominal *P* = 00014)	
						Pembrolizumab: 3.5 mo SOC: 4.8 mo		LSM between-group difference was 6.25 points (95%CI: 1.32-11.18: nominal 2-sided *P* = 0.01)	TPS < 50% Pembrolizumab: 6.5 mo (95% CI 5.6-8.8); SOC: 7.1 mo (95%CI: 5.7-8.1), HR for death 0.93 (95%CI: 0.73-1.17; nominal *P* = 0.2675), *P* for int. = 0.015 CPS ≥ 1 Pembrolizumab: 8.7 mo (95%CI: 6.9-11.4); SOC: 7.1 mo (95%CI: 5.7-8.3), HR for death = 0.74 (95%CI: 0.58-0.93) nominal *P* = 0.0049) CPS < 1 Pembrolizumab: 6.3 mo (95% CI 3.9-8.9); SOC: 7 mo (95%CI: 5.1-9.0), HR for death 1.28 (95%CI: 0.8-2.07; *P* = 08476) *P*	

									for int.= 0.07 **PFS** Based on modified RECIST1.1 TPS ≥ 50%: PFS longer with Pembrolizumab than with SOC CPS ≥ 1: PFS almost equal to that in the overall population for both Pembrolizumab and SOC (3.6 mo *vs* 4.8 mo) CPS < 1, & TPS < 50%: PFS longer with SOC than with Pembrolizumab	
Ferris *et al* [24], 2020	RCT (1:1:1), open-label phase-3 trial	R/M HNSCC not amenable to curative therapy	Arm 1	SoC	Durvalumab: 7.6 mo 95%CI: 6.1-9.8	Durvalumab: 2.1 mo, 95%CI: 1.9-3.0	Durvalumab: 17.9%, 95%CI: 13.3-23.3	Not assessed	**OS**	Durvalumab: 10.1%, four treatment related deaths
EAGLE		*n* = 736	Durvalumab MoA: PD-L1 inhibition 10 mg/kg every 2 wk	Single-agent systemic therapy using one of the following: cetuximab paclitaxel, docetaxel, methotrexate, 5 FU, TS-1, or capecitabine	Durvalumab + Tremelimumab: 6.5 mo, 95%CI: 5.5-8.2	Durvalumab + Tremelimumab: 2.0 mo, 95%CI: 1.9-2.3	Durvalumab + Tremelimumab: 18.2%, 95%CI: 13.6-23.6		TC ≥ 25%	Durvalumab + Tremelimumab, 16.3 %, two treatment related deaths
			n = 240, median follow-up: 7.6 mo	*n* = 249, median follow-up = 7.8 mo	SoC: 8.3 mo, 95%CI: 7.3-9.2	SoC: 3.7 mo, 95%CI: 3.1-3.7	SoC: 17.3%, 95%CI: 12.8-22.5		Durvalumab: 9.8 mo (95%CI: 4.3-14.1); Durvalumab + Tremelimumab: 4.8 mo (95%CI: 3.3-6.4); SoC: 9 mo (95%CI: 6.8-11.0)	SoC: 24.2%, No treatment related deaths
			Arm 2		Durvalumab *vs* SoC: HR = 0.88, 95%CI: 0.72-1.08, *P* = 0.20	Durvalumab *vs* SoC: HR = 1.02, 95%CI: 0.84-1.25, *P* = 0.75			TC < 25%	
			Durvalumab plus Tremelimumab MoA: CTLA-4 blockade		Durvalumab + Tremelimumab *vs* SoC.: HR = 1.04, 95%CI: 0.85-	Durvalumab + Tremelimumab *vs* SoC: HR = 1.09,			Durvalumab: 7.6 mo (95%CI: 6.2-9.5); Durvalumab +	

					1.26, P = 0.76	95%CI: 0.90-1.33, P = 0.54				Tremelimumab: 7.8 mo (95%CI: 5.9-10.3); SoC: 8 mo (95%CI: 6.7-8.9)	
			Durvalumab: 20 mg/kg plus Tremelimumab 1 mg/kg every 4 wk-4 times, then Durvalumab: 10 mg /kg every 2 wk							TC ≥ 1%: Both treatment arms *vs* SoC had no difference in OS	
			n = 247, median follow-up: 6.3 mo							TC < 1%: OS was longer for Durvalumab *vs* SoC; but no difference for Durvalumab + Tremelimumab *vs* SOC	
Phase-3 clinical trials evaluating ICI as first line therapy in R/M HNSCC											
Burtness *et al*[25], 2019	RCT (1:1:1), open-label phase-3 trial	Patients with R/M HNSCC	Arm 1: Pembrolizumab (MoA: PD-1 inhibition), monotherapy; Pembrolizumab 200 mg once every 3 wk	EXTREME regime: cetuximab 400 mg/m² loading dose, then 250 mg/m², per week plus, carboplatin (AUC 5 mg/m^2) or cisplatin (100 mg/m^2) and 5-FU (1000 mg/m^2 for 4 consecutive days) every 3 wk	Arm 1: Pembrolizumabalone, 11.6 mo, 95%CI: 10.5-13.6	Arm 1: Pembrolizumab alone, 2.3 mo (95%CI: 2.2-3.3)	Arm 1: Pembrolizumab, 17%	NA		**OS**	Pembrolizumab alone: 55% (all cause), 17% (TRAE)AE led to death in 8% of pts
KEYNOTE 048		Three arms	n = 301, median follow-up: 11.5 mo	n = 300, median follow-up: 10.7 mo	Arm 2: Pembrolizumab + CT, 13.0 mo, 95%CI: 10.9-14.7	Control arm: Cetuximab + CT 5.2 mo (95%CI: 4.9-6)	Arm 2: Pembrolizumab + CT, 36%			CPS of ≥ 20: Pembrolizumab alone *vs* EXTREME: 14.9 mo *vs* 10.7 mo, HR 0.61; 95%CI: 0.45-0.83, P = 0.0007	Pembrolizumab + CT: 85% (all cause), 72%(TRAE), AE led to death in 12% of pts
		n = 882	Arm 2: Pembrolizumab + CT (platinum-FU), Pembrolizumab 200 mg once every 3 wk plus carboplatin (AUC 5 mg/m^2) or cisplatin (100 mg/m^2) and 5-FU (1000 mg/m^2 for 4 consecutive days) every 3 wk		Control arm: Cetuximab + CT, 10.7 mo, 95%CI: 9.3-11.7	Arm 2: Pembrolizumab + CT, 4.9 mo (95%CI: 4.7-6)	Control arm: Cetuximab + CT, 36%			Pembrolizumab + CT *vs* EXTREME: 14.7 mo *vs* 11.0 mo, HR 0.60; 95%CI: 0.45-0.82, P = 0.0004	Cetuximab + CT: 83% (all cause), 69%(TRAE), AE led to death in 10% of pts

			n = 281, median follow-up: 13.0 mo		Pembrolizumab alone *vs* EXTREMEHR 0.85, 95%CI: 0.71-1.03, *P* = 0.0456	Control arm: Cetuximab + CT, 5.1 mo (95%CI:4.9-6)			CPS of ≥ 1: Pembrolizumab alone *vs* EXTREME: 12.3 mo *vs* 10.3 mo, HR 0.78 [0.64-0.96], *P* = 0.0086	
					Pembrolizumab + CT *vs* EXTREME, HR 0.77, 95%CI: 0.63-0.93, *P* = 0.0034	Pembrolizumab alone *vs* EXTREME: HR = 1.34; 95%CI: 1.13-1.59			Pembrolizumab + CT *vs* EXTREME: 13.6 mo *vs* 10.4 moHR 0.65; 95%CI: 0.53-0.80, *P* < 0.0001	
						Pembrolizumab + CT *vs* EXTREME: HR = 0.92, 95%CI: 0.77-1.10, *P* = 0.169			**PFS** CPS of ≥ 20: Pembrolizumab alone *vs* EXTREME, 3.4 mo *vs* 5.0 mo, HR 0.99; 95%CI: 0.75-1.29, *P* = 0.456 Pembrolizumab + CT *vs* EXTREME: 5.8 mo *vs* 5.2 mo, HR 0.73; 95%CI: 0.55-0.97, *P* = 0.0162 CPS of ≥ 1: Pembrolizumab alone *vs* EXTREME, 3.2 mo *vs* 5.0 mo, HR 1.16; 95%CI: 0.96-1.39 Pembrolizumab + CT *vs* EXTREME: 5.0 mo *vs* 5.0 mo, HR 0.82; 95% CI: 0.67-1.00	
Phase-3 clinical trials evaluating ICI for treatment of LAHNSCC										
Cohen *et al* [26], 2020	RCT (1:1) double blind placebo-controlled	Patients with pathologically confirmed previously untreated LA HNSCC who were eligible for definitive CRT with curative intent	Avelumab (PD-L1 inhibitor) 10 mg/kg iv every 2 wk plus CRT with cisplatin 100 mg/m^2 every 3 wk plus standard fractionation of 70 Gy in 35 fractions over 7 wk	Placebo plus CRT with cisplatin 100 mg/m^2 every 3 wk plus standard fractionation of 70 Gy in 35 fractions over 7 wk	OS: not reached, HR: 1.31, 95%CI: 0.93-1.85; one sided *P* = 0.94	PFS: not reached, HR: 1.21, 95%CI: 0.93-1.57; one sided *P* = 0.92	Avelumab + CRT: 74%, 95%CI: 69-79; based on modified RECIST 1.1	NA	PFS	Intervention: 80 %, serious AEs in 36% pts, treatment related death 1%, 7% pts discontinued due to TRAEs
Lee *et al*	Phase-3	*n* = 697	*n* = 350, median	*n* = 347, median	Favors control arm	Favors control	Placebo + CRT:		Avelumab + CRT *vs*	Control: 74%,

[31], 2021 JAVELIN head and neck 100 trial	trial	follow-up for PFS = 14.6 mo (IQR 8.5-19.6) for OS =16.7 mo (IQR 12.8-21.2)	follow-up for: PFS = 14.8 mo (11.6-18.8), OS =16.8 mo (IQR 13.1-20.8)	arm	75%; 95%CI: 70-79; based on modified RECIST 1.1 OR = 0.95; 95%CI: 0.66-1.35, *P* = 0.62	Placebo + CRT, PD-L1 ≥ 25%: HR 0.59 (95%CI: 0.28-1.22); PD-L1 < 25%, HR: 1.37 (95%CI: 1.00-1.88), *P* for int. = 0.03	serious AEs in 32% pts, treatment related death < 1%, 3% pts discontinued due to TRAEs

QOL: Quality of life; HRQOL: Health-related QOL; CRT: Chemoradiation therapy; OS: Overall survival; PFS: Progression-free survival; ORR: Objective response rate; ICI: Immune checkpoint inhibitors; R/M HNSCC: Recurrent or metastatic head and neck squamous cell carcinoma; LAHNSCC: Locally advanced head and neck squamous cell carcinoma; RCT: Randomized controlled trial; PD-1: Programmed cell death protein-1; PD-L1: Programmed death-ligand 1; CTLA-4: Cytotoxic T- lymphocyte associated protein-4; AE: Adverse event; TRAE: Treatment-related AEs; SOC: Standard of care; CPS: Combined positive score; CI: Confidence interval; IV: Intravenously; MoA: Mechanism of action; HR: Hazard ratio; IQR: Interquartile range; LSM: Least mean square; TPS: Tumor proportion score; Mab: Monoclonal antibody; CT: Chemotherapy.

PFS: PFS was reported as 2 mo (95%CI: 1.9-2.1) with nivolumab *vs* 2.3 mo (95%CI: 1.9-3.1) with SOC (HR = 0.89; 95%CI: 0.70-1.13; *P* = 0.32).

ORR: ORR was 13.3% (95%CI: 9.3-18.3) in the intervention arm with nivolumab, whereas it was 5.8% (95%CI: 2.4-11.6) in the control arm (SOC).

Patient-reported outcomes (quality of life): Physical, role, and social functioning (assessed by means of EORTC QOLQ-C30) as well as symptom burden (assessed using EORTC QLQ-H&N35) remained stable or slightly improved with nivolumab, while SOC patients had a decline in QOL. Statistical analysis showed significant between-group differences in physical functioning (*P* = 0.01 at 9 wk; *P* < 0.001 at 15 wk), role functioning (*P* = 0.003 at 9 wk; *P* < 0.001 at 15 wk), social functioning (*P* = 0.002 at 9 wk; *P* < 0.001 at 15 wk), pain (*P* < 0.001 at 9 wk; *P* = 0.02 at 15 wk), sensory problems (*P* = 0.01 at 9 wk; *P* < 0.001 at 15 wk), and social contact problems (*P* = 0.26 at 9 wk; *P* < 0.001 at 15 wk).

Biomarker effect: Biomarker effect on OS was evaluated after stratifying patients based on their PD-L1 expression status (≥ 1% *vs* < 1%). Among patients with PD-L1 ≥ 1%, median OS was 8.7 mo with nivolumab *vs* 4.6 mo with SOC (HR = 0.55; 95%CI: 0.36-0.83), whereas in patients with PD-L1 < 1%, median OS was 5.7 mo with nivolumab *vs* 5.8 mo with SOC (HR for death = 0.89; 95%CI: 0.54-1.45; *P* for interaction = 0.17). Post-hoc exploratory subgroup analysis based on p16 status was also done in this study. Among patients with p16 positive tumors, the median OS was 9.1 mo with nivolumab *vs* 4.4 mo with SOC (HR for death 0.56; 95%CI: 0.32-0.99), whereas, among patients with p16 negative tumors, the median OS was 7.5 mo with nivolumab *vs* 5.8 mo with SOC (HR =0.73; 95%CI: 0.42-1.25; *P* for interaction = 0.55).

Adverse events: In CheckMate 141, adverse events of grade 3 or more occurred in 13.1% of patients with nivolumab *vs* 35% with SOC. Two patients in the nivolumab arm and 1 patient in the control arm had treatment-related death. The most common adverse events (of any grade) with nivolumab were fatigue, nausea, decreased appetite, pruritis, and rash. Gastrointestinal side effects (primarily diarrhea) were less in the nivolumab group (6.8%) compared to SOC patients (14.4%), whereas adverse events of

skin (rash and pruritus) were more common in the nivolumab group (15.7%) than in the SOC patients (12.6%). Endocrine system-related side effects (hypothyroidism) were also more with nivolumab (7.6%) compared to SOC (0.9%)[22].

KEYNOTE 040 (Pembrolizumab vs standard single-agent systemic therapy)

In this open-label phase-3 RCT, the investigators tested the efficacy and safety of the immune checkpoint inhibitor pembrolizumab (an anti-PD-1 monoclonal antibody) compared to standard therapy for the treatment of metastatic/recurrent head and neck cancer[23]. This was a multi-center study involving 97 medical centers across 20 countries. There were 247 patients in the intervention arm, while the control arm included 248 patients. Patients with platinum-refractory recurrent or metastatic (or both) HNSCC were included in this study. PD-L1 expression was assessed and categorized according to the tumor proportion score (≥ 50% *vs* < 50%) as well as the combined positive score (≥ 1 *vs* < 1) The intervention arm received pembrolizumab 200 mg every 3 wk, while the control arm received investigator's choice of standard doses of methotrexate (40 mg/m^2 IV weekly), docetaxel (75 mg/m^2 IV every 3 wk) or cetuximab (250 mg/m^2 IV weekly following a loading dose of 400 mg/m^2).

Outcomes

OS: Primary outcome of the study was OS. The median OS was 8.4 mo (95%CI: 6.4-9.4) with pembrolizumab *vs* 6.9 mo (95%CI: 5.9-8.0) with SOC (HR = 0.80; 95%CI: 0.65-0.98; nominal *P* = 0.0161).

PFS: PFS was 2.1 mo (95%CI: 5.9-8.0) with pembrolizumab *vs* 2.3 mo (95%CI: 2.1-2.8) with SOC (HR = 0.96; 95%CI: 0.79-1.16; nominal *P* = 0.325).

ORR: ORR was 14.6% (95%CI: 10.4-19.6) with pembrolizumab *vs* 10.1% (95%CI: 6.6-14.5) with SOC (nominal *P* = 0.061).

Patient-reported outcomes: Results (published separately in another article) of an exploratory health-related quality of life analysis showed that at 15 wk, global health status/quality of life (GHS/QOL) scores were stable with pembrolizumab with a least square mean (LSM) of 0.39; 95%CI: -3.00-3.78), while GHS/QOL scores declined with SOC (LSM -5.86; 95%CI: -9.68 to -2.04). LSM between-group difference was 6.25 points (95%CI: 1.32-11.18: nominal 2-sided *P* = 0.01)[32].

Biomarker effect: Cohen *et al*[23] found statistically significant interaction between PD-L1 expression [in terms of tumor proportion score (TPS) and combined positive score (CPS)] and treatment effect in KEYNOTE 040. Among patients with TPS ≥ 50%, median OS was 11.6 mo (95%CI: 8.3-19.5) with pembrolizumab *vs* 6.6 mo (95%CI: 4.8-9.2) with SOC (HR = 0.53;95%CI: 0.35-0.81; nominal *P* = 00014). Among patients with TPS < 50%, OS was 6.5 mo (95%CI: 5.6-8.8) with pembrolizumab *vs* 7.1 mo (95%CI: 5.7-8.1) with SOC (HR = 0.93;95%CI: 0.73-1.17; nominal *P* = 0.2675; *P* for interaction = 0.015). Similarly, among patients with CPS ≥ 1, median OS was 8.7 mo (95%CI: 6.9-11.4) with pembrolizumab *vs* 7.1 mo (95%CI: 5.7-8.3) with SOC (HR = 0.74; 95%CI: 0.58-0.93; nominal *P* = 0.0049). Among patients with CPS < 1, OS was 6.3 mo (95%CI: 3.9-8.9) with pembrolizumab *vs* 7.0 mo (95%CI: 5.1-9.0) with SOC (HR = 1.28; 95%CI: 0.8-2.07; *P* = 08476; *P* for interaction = 0.07). In terms of PFS, based on the modified RECIST1.1, for patients with TPS ≥ 50%, PFS was longer with pembrolizumab than with SOC, whereas for patients with CPS ≥ 1, PFS was slightly lower (3.6 mo) with pembrolizumab compared to SOC (4.8 mo). Among patients with CPS < 1 and those with TPS < 50%, PFS was longer for SOC compared to pembrolizumab [23].

Adverse events: In KEYNOTE 040, adverse events of grade 3 or more occurred in 13% of patients with pembrolizumab *vs* 36.1% with SOC. Four patients in the pembrolizumab arm and 2 patients in the control arm had treatment-related death. While hypothyroidism was the most common treatment-related adverse event with pembrolizumab (13%), fatigue was the most common adverse event with SOC (18%)[23].

EAGLE study (durvalumab with or without tremelimumab vs standard single-agent systemic therapy)

Ferris *et al*[24] conducted an open-label phase-3 RCT among 736 patients with R/M HNSCC not amenable to curative therapy[24]. In this three-arm study (1:1:1), one of the intervention arms (*n* = 240, median follow-up 7.6 mo) received the anti PD-L1 agent durvalumab (10mg/kg every 2 wk), and the other intervention arm (*n* = 247, median follow-up 6.3 mo) received durvalumab (20 mg/kg every 4 wk-4 times followed by 10 mg /kg every 2 wk) plus the anti CTLA-4 agent tremelimumab (1 mg/kg every 4 wk-4 times). The control arm (*n* = 240 median follow-up 7.8 mo) received investigator's choice of a standard single-agent [cetuximab, paclitaxel, docetaxel, methotrexate, 5-fluorouracil (5-FU), TS-1, or capecitabine] systemic therapy (SOC) dosed and administered according to local regulations.

Outcomes

OS: Primary outcome of the EAGLE study was OS. The median OS was reported as 7.6 mo (95%CI: 6.1-9.8) with durvalumab *vs* 8.3 mo (95%CI: 7.3-9.2) with SOC (HR = 0.88; 95%CI: 0.72-1.08, *P* = 0.20),

whereas it was 6.5 mo (95%CI: 5.5-8.2) with durvalumab plus tremelimumab *vs* 8.3 mo with SOC (HR = 1.04; 95%CI: 0.85-1.26, *P* = 0.76).

PFS: PFS was 2.1 mo with durvalumab (95%CI: 1.9-3.0) *vs* 3.7 mo (95%CI: 3.1-3.7) with SOC (HR = 1.02; 95%CI: 0.84-1.25, *P* = 0.75). PFS with durvalumab plus tremelimumab was 2.0 mo (95%CI: 1.9-2.3) *vs* 3.7 mo (95%CI: 3.1-3.7) with SOC (HR = 1.09; 95%CI: 0.90-1.33, *P* = 0.54).

ORR: ORRs were 17.9% (95%CI: 13.3-23.3) with durvalumab monotherapy, 18.2% (95%CI: 13.6-23.6) with durvalumab plus tremelimumab, and 17.3% (95%CI: 12.8-22.5) with SOC.

Patient-reported outcomes: QOL measures were not assessed in the study.

Biomarker effect: In the EAGLE study, investigators measured PD-L1 expression in terms of percentage of tumor cell (TC). Among patients with TC ≥ 25%, the median OS was 9.8 mo (95%CI: 4.3-14.1) with durvalumab and 4.8 mo (95%CI: 3.3-6.4) with durvalumab plus tremelimumab, while SOC patients had an OS of 9.0 mo (95%CI: 6.8-11.0). Among patients with TC < 25%, the median OS with SOC was 8.0 mo (95%CI: 6.7-8.9), whereas it was 7.6 mo (95%CI: 6.2-9.5) with durvalumab and 7.8 mo (95%CI: 5.9-10.3) with durvalumab plus tremelimumab. In patients with TC ≥ 1%, both intervention groups had no difference in OS compared to SOC. In patients with TC < 1%, OS was higher with durvalumab compared to SOC, but no difference in OS was found between the durvalumab plus tremelimumab arm and the SOC arm.

Adverse events: In the EAGLE study, 10.1% of patients in the durvalumab arm, 16.3% patients in the durvalumab plus tremelimumab arm, and 24.2% patients in the control arm developed adverse events of grade 3 or more. Six patients died due to treatment-related issues: 4 with durvalumab, 2 with durvalumab plus tremelimumab, and 0 with SOC. Hypothyroidism was the most common treatment-related adverse event (of any grade) in the durvalumab (11.4%) arm as well as in the durvalumab plus tremelimumab arm (12.2%). Anemia was the most common treatment-related adverse event in the SOC arm (17.5%)[24].

Phase-3 studies evaluating ICI as first-line treatment in R/M HNSCC (I RCT: Keynote 048)

Prior to immunotherapy, the standard first-line treatment option for R/M HNSCC was the EXTREME regime, a combination of cetuximab, platinum (carboplatin or cisplatin), and 5-FU[13]. So far, one phase-3 trial has evaluated immunotherapy against the EXTREME regime in the first-line treatment setting for patients diagnosed with R/M HNSCC.

KEYNOTE 048 (pembrolizumab monotherapy vs EXTREME, pembrolizumab plus platinum-based CT vs EXTREME)

In this large three-arm RCT (*n* = 882), one of the intervention arms (*n* = 301, median follow-up: 11.5 mo) received pembrolizumab as monotherapy (pembrolizumab 200 mg once every 3 wk), while the second intervention arm (*n* = 281, median follow-up: 13.0 mo) received pembrolizumab (200 mg once every 3 wk) along with platinum-based chemotherapy {carboplatin [area under the curve (AUC) 5 mg/m^2] or cisplatin (100 mg/m^2) and 5-FU (1000 mg/m^2 for 4 consecutive d) every 3 wk}. The control arm (*n* = 300, median follow-up: 10.7 mo) received the EXTREME regime [cetuximab 400 mg/m^2 loading dose, then 250 mg/m^2 per week plus carboplatin (AUC 5 mg/m^2) or cisplatin (100 mg/m^2) and 5-FU (1000 mg/m^2 for 4 consecutive days) every 3 wk][25] (Table 1).

Outcomes

OS: The median OS (primary end point) was 11.6 mo (95%CI: 10.5-13.6) with pembrolizumab monotherapy *vs* 10.7 mo (95%CI: 9.3-11.7) with EXTREME (HR = 0.85; 95%CI: 0.71-1.03; *P* = 0.0456). In the pembrolizumab plus chemotherapy arm, median OS was 13.0 mo *vs* 10.7 mo (95%CI: 9.3-11.7) in the EXTREME arm (HR = 0.77; 95%CI: 0.63-0.93; *P* = 0.0034).

PFS: PFS was assessed as a primary outcome and was reported as 2.3 mo (95%CI: 2.2-3.3) with pembrolizumab monotherapy *vs* 5.2 mo (95%CI: 4.9-6.0) with EXTREME (HR = 1.34; 95%CI: 1.13-1.59). In the pembrolizumab plus chemotherapy arm PFS was 4.9 mo (95%CI: 4.7-6.0) *vs* 5.1 mo (95%CI: 4.9-6) in the EXTREME arm (HR = 0.92; 95%CI: 0.77-1.10; *P* = 0.169).

ORR: The pembrolizumab monotherapy arm had an ORR of 17% compared to 36% in the EXTREME arm. With pembrolizumab plus chemotherapy, ORR was similar to that with EXTREME (36%).

Biomarker effect: In KEYNOTE 048, PD-L1 expression was measured as CPS. For patients with CPS ≥ 20, median OS with pembrolizumab monotherapy was 14.9 mo *vs* 10.7 mo with EXTREME (HR = 0.61; 95%CI: 0.45-0.83; *P* = 0.0007), while median OS with pembrolizumab plus chemotherapy was 14.7 mo *vs* 11.0 mo with EXTREME (HR = 0.60; 95%CI: 0.45-0.82; *P* = 0.0004). Similarly, for patients with CPS ≥ 1, median OS with pembrolizumab monotherapy was 12.3 mo *vs* 10.3 mo with EXTREME (HR = 0.78; 95%CI: 0.64-0.96; *P* = 0.0086), whereas OS was 13.6 mo in the pembrolizumab plus chemotherapy arm *vs*

10.4 mo with EXTREME (HR = 0.65; 95%CI: 0.53-0.80; $P < 0.0001$).

For patients with CPS ≥ 20, median PFS with pembrolizumab monotherapy was 3.4 mo *vs* 5.0 mo with EXTREME (HR = 0.99; 95%CI: 0.75-1.29; $P = 0.456$). Median PFS with pembrolizumab plus chemotherapy was 5.8 mo *vs* 5.2 mo with EXTREME (HR = 0.73; 95%CI: 0.55-0.97; $P = 0.0162$). Similarly, for patients with CPS ≥ 1, median PFS with pembrolizumab monotherapy was 3.2 mo *vs* 5.0 mo with EXTREME (HR = 1.16; 95%CI: 0.96-1.39), whereas PFS was 5.0 mo with pembrolizumab plus chemotherapy *vs* 5.0 mo with EXTREME (HR = 0.82; 95%CI: 0.67-1.00).

Adverse events: In KEYNOTE 048, 55% patients in the pembrolizumab arm, 85% patients in the pembrolizumab plus chemotherapy arm, and 83% patients in the control arm developed grade 3 or more adverse events of any cause. Of these, treatment-related adverse events consisted of 17% in the pembrolizumab alone group, 72% in the pembrolizumab plus chemotherapy group, and 69% in the control group. While adverse events led to death in 8% of patients in the pembrolizumab arm and 12% of patients in the pembrolizumab plus chemotherapy arm, 10% in the control arm also died of adverse events. Major adverse events (of any grade) in the intervention groups were anemia, fatigue, hypothyroidism, and nausea[25].

Phase-3 studies evaluating ICI for treatment of LAHNSCC (I RCT: JAVELIN head and neck 100 trial)

The current SOC for the treatment of LAHNSCC is concurrent chemoradiation therapy (CRT)[33]. So far, only one phase-3 trial has investigated the usefulness of adding an ICI to concurrent CRT.

JAVELIN head and neck 100 trial (avelumab plus CRT vs placebo plus CRT)

The preliminary results of the study were presented in the 2020 European Society for Medical Oncology annual meeting by Cohen *et al*[26] followed by a recent journal publication[27].

This study ($n = 697$) was conducted among patients with previously untreated LA HNSCC who were eligible for definitive CRT with curative intent. The intervention arm ($n = 350$; median follow-up for PFS 14.6 mo, for OS 16.7 mo) received the PD-L1 inhibitor avelumab (10 mg/kg IV every 2 wk) plus CRT, which consisted of cisplatin (100 mg/m^2 every 3 wk) concurrently with intensity-modulated radiotherapy (standard fractionation of 70 Gy in 35 fractions over 7 wk). The control arm ($n = 347$; median follow-up for PFS 14.8 mo, for OS 16.8 mo) received placebo plus CRT (Table 1).

Outcomes

PFS: Median PFS (primary endpoint) was not reached in the intervention group or the control group. Statistical reports showed that hazard ratio (HR= 1.21; 95%CI: 0.93-1.5; one-sided $P = 0.92$) did not favor the avelumab plus CRT arm.

OS: OS was one of the secondary endpoints in this trial. Median OS was not reached in either study group. Statistical reports showed that the hazard ratio for death (HR = 1.31; 95%CI: 0.93-1.85; one-sided $P = 0.937$) did not favor the avelumab plus CRT arm.

ORR: Based on modified RECIST 1.1, ORR in the intervention arm was 74% (95%CI: 69-79) and that in the control arm was 75% (95%CI: 70-79) with an OR of 0.95 (95%CI: 0.66-1.35, $P = 0.62$).

Biomarker: Exploratory subgroup analysis of PFS based on PD-L1 expression showed that patients with PD-L1 ≥ 25% had an HR of 0.59 (95%CI: 0.28-1.22), while patients with PD-L1 < 25% had an HR of 1.37 (95%CI: 1.00-1.88) with avelumab plus CRT compared to placebo plus CRT (P for interaction = 0.03).

Adverse events: Treatment-related adverse events of grade 3 or more occurred in 80% of patients in the avelumab arm and in 74% of patients in the control arm. Serious adverse events occurred in 36% of patients in the intervention arm and in 32% of patients in the control arm. In the intervention arm, 7% of patients discontinued due to treatment-related adverse events *vs* 3% in the control arm[27].

DISCUSSION

ICIs have emerged as a novel treatment strategy for HNSCC in recent years. The safety profile and anti-tumor activity of these agents demonstrated in early phase clinical trials paved the way for the initiation of several promising phase-3 trials in the field. Safety profile and clinical activity of pembrolizumab were first reported in KEYNOTE 012, an open-label phase 1b trial among patients with R/M HNSCC [34]. KEYNOTE 055, a phase-2 trial conducted among patients with platinum-resistant R/M HNSCC also reported manageable toxicity and an acceptable safety profile of pembrolizumab[35]. The study demonstrated a clinically meaningful anti-tumor activity of the agent in terms of ORRs and survival. These findings led to the initiation of KEYNOTE 040, the phase-3 trial investigating pembrolizumab for treating patients with platinum-refractory R/M HNSCC, and KEYNOTE 048, the phase-3 trial investigating pembrolizumab as first-line therapy in R/M HNSCC[23,25]. Similarly, two phase-2 trials, the

HAWK study (a single-arm study investigating durvalumab monotherapy in R/M HNSCC with > 25% tumor PD-L1 expression) and the CONDOR phase-2 trial (an RCT investigating durvalumab with or without tremelimumab in PD-L1 Low/negative R/M HNSCC) served as the rationale for investigating combination immunotherapy regimens in platinum-refractory R/M HNSCC and to initiate the EAGLE study[24,36,37]. Studies on the effectiveness of nivolumab in other solid tumors supported the initiation of CheckMate 141 trial, the first phase-3 trial of nivolumab among patients with platinum-resistant R/M HNSCC[22,38]. Chemotherapy and radiotherapy, alone or in combination, have demonstrated potential synergetic effects when combined with immunotherapy in early phase studies. This phenomenon and the proven effectiveness of the anti-PD-L1 agent avelumab in other advanced solid tumors paved the way to the JAVELIN head and neck 100 trial, the first phase-3 RCT to investigate the effectiveness of combining ICI with chemoradiation in locally advanced head and neck cancer[27,39,40].

In this systematic review, we included the published phase-3 clinical trials evaluating the effectiveness of ICIs in HNSCC. Five studies met our eligibility criteria. Three studies (CheckMate 141, KEYNOTE 040, and EAGLE study) evaluated ICI as second-line treatment for R/M HSCC, one study (KEYNOTE 048) evaluated ICI as first-line treatment for R/M HSCC, while one phase-3 trial (JAVELIN head and neck 100 trial) evaluated the effectiveness of immunotherapy in LAHNSCC[22-27].

Effectiveness of ICI for R/M HNSCC in the second-line treatment setting

In the second-line treatment setting, nivolumab in CheckMate 141 and pembrolizumab in KEYNOTE 040 demonstrated promising outcomes among patients with platinum-refractory R/M HNSCC[22,23]. In CheckMate 141, the anti-PD-1 agent nivolumab showed a statistically significant 31% reduction in risk of death (HR = 0.69, P = 0.01) and a net gain of 2.4 mo in terms of OS. A 2.3-fold increase in ORR was also reported with nivolumab compared to SOC. A favorable toxicity profile was another finding with nivolumab, with lower rates of treatment-related adverse events of grade 3 or more compared to SOC (13.1% *vs* 35%). Patient-reported QOL measures remained stable with nivolumab, while a decline in QOL occurred among the control patients. However, the study did not demonstrate any significant PFS benefits with nivolumab (HR = 0.89, 95%CI: 0.70-1.13; P = 0.32). Regarding the impact of biomarkers, survival benefit with nivolumab was found to be irrespective of PD-L1 expression (P for int. = 0.17) in the subgroup analyses based on PD-L1 status, although patients with PD-L1 ≥ 1% had a better magnitude of effect (HR = 0.55) than those with PD-L1 < 1 (HR = 0.89)[22,41,42]. Similarly, based on the post-hoc exploratory subgroup analysis according to p16 status, the investigators concluded that the longer median OS with nivolumab was irrespective of the p16 status (P for interaction = 0.55).

In KEYNOTE 040, the anti-PD-1 agent pembrolizumab demonstrated statistically significant improvement in OS with a 20% reduction in risk of death (HR = 0.80, P = 0.016) compared to SOC in the overall study population[23]. Higher ORR (14.6% *vs* 10.1%, nominal P = 0.061) and lower rates of adverse events of grade 3 or more (13% *vs* 36.1%) were also demonstrated with pembrolizumab compared to SOC. At 15 wk, stable GHS/QOL scores were reported with pembrolizumab, while the control patients had a decline in QOL. The study did not, however, demonstrate any PFS benefits with pembrolizumab (HR = 0.96, nominal P = 0.325) compared to SOC. Exploratory subgroup analyses based on PD-L1 expression demonstrated statistically significant interactions between treatment effects and PD-L1 status. For patients with TPS ≥ 50% and CPS > 1, the treatment effects of pembrolizumab *vs* SOC were found to be higher than in those with TPS < 50% and CPS < 1[23]. For instance, in terms of OS, patients with TPS ≥ 50% had a net gain of 5 mo with a 47% reduction in risk of death with pembrolizumab compared to SOC (HR = 0.53, nominal P = 00014), suggesting PD-L1 expression may be explored as a predictive biomarker while selecting patients for pembrolizumab therapy. Based on the findings of CheckMate 141 and KEYNOTE 040, nivolumab and pembrolizumab were approved as standard second-line treatment options for platinum-resistant R/M HNSCC[22,23,43].

The EAGLE study did not detect any statistically significant improvements in OS with durvalumab (HR = 0.88, P = 0.20) or with durvalumab plus tremelimumab (HR = 1.04, P = 0.76) compared to SOC. Again, there were no significant benefits in terms of PFS with durvalumab or with durvalumab plus tremelimumab, compared to SOC. However, investigators of EAGLE have postulated that control patients in the study had an unexpectedly high OS as the data were confounded by discrepancies in performance status favoring the control arm. Option of using paclitaxel as SOC (paclitaxel was not an option in the other two studies in the second-line setting), and subsequent immunotherapy after discontinuation of SOC treatment by control patients were also mentioned as reasons for this finding[24]. Although the primary objectives were not met, one positive finding was that the rates of adverse events of grade 3 or more were lower with immunotherapy compared to SOC.

Effectiveness of ICI for R/M HNSCC in the first-line treatment setting

In the first-line treatment setting, in KEYNOTE 048, pembrolizumab with platinum-based chemotherapy demonstrated statistically significant improvements in OS (13.0 *vs* 10.7 mo) with a 23% reduction in risk of death (HR = 0.77, P = 0.0034) compared to cetuximab plus platinum-based chemotherapy (EXTREME) in the total population. Pembrolizumab monotherapy was found to be non-inferior to EXTREME (HR = 0.85; 95%CI: 0.71-1.03; P = 0.0456) in terms of OS (11.6 mo *vs* 10.7 mo) in the total population. No significant impact on PFS was detected with pembrolizumab alone or pembrolizumab with chemotherapy compared to EXTREME in the overall population. Pembrolizumab alone

had a lower ORR (17%) compared to EXTREME (36%), while pembrolizumab plus chemotherapy had an ORR (36%) like that of EXTREME. Interestingly, biomarker (PD-L1) based stratified analysis demonstrated superiority in terms of OS in the CPS ≥ 20 and CPS ≥ subgroups with pembrolizumab alone as well as with pembrolizumab plus chemotherapy compared to EXTREME. For instance, within the CPS ≥ 20 population, pembrolizumab monotherapy compared to EXTREME resulted in a net gain of 4.2 mo in terms of OS (14.9 mo *vs* 10.7 mo) with a highly significant 39% reduction in risk of death (HR = 0.61, $P = 0.0007$). In the CPS ≥ subgroup pembrolizumab monotherapy also demonstrated superiority in terms of OS (12.3 mo *vs* 10.3 mo) compared to EXTREME (HR = 0.78, $P = 0.0086$), indicating that pembrolizumab monotherapy is a suitable treatment option for PD-L1 positive R/M HNSCC. Similarly, in both subgroups, pembrolizumab with chemotherapy resulted in statistically significant improvements in OS compared to EXTREME. For instance, R/M HNSCC patients with CPS ≥ 20 had a highly significant 40% reduction in risk of death with pembrolizumab plus chemotherapy compared to EXTREME (HR = 0.60, $P = 0.0004$). Patients with CPS ≥ 1 also had a significant reduction in risk of death with pembrolizumab plus chemotherapy compared to EXTREME (HR = 0.65, $P < 0.0001$). These findings indicate that tumor PD-L1 expression can be a predictive biomarker for identifying patients who will benefit from pembrolizumab[25,44].

Based on the findings from KEYNOTE 048, pembrolizumab monotherapy was approved as an appropriate SOC for PD-L1 positive R/M HNSCC, and pembrolizumab plus platinum-based chemotherapy became the new SOC for the treatment of R/M HNSCC in the first-line setting[25,43]. In this study, rates of treatment-related adverse events of grade 3 or more were lower with pembrolizumab monotherapy (17%) compared to EXTREME (69%). However, rates of treatment-related adverse events of grade 3 or more were noticeably high (72%) in the combination therapy arm[25]. This finding highlights the importance of weighing up the survival benefits of the pembrolizumab plus chemotherapy regime against its adverse events profile while making treatment decisions for patients with R/M HNSCC.

Effectiveness of ICI in LAHNSCC

Regarding immunotherapy in LAHNSCC, there is no definite evidence of benefit according to the primary results of the JAVELIN study[26,27]. The combination of avelumab and CRT did not demonstrate any beneficial outcomes in terms of PFS or OS over placebo plus CRT, and based on the modified RECIST 1.1, there were no ORR benefits (74% *vs* 75%) either. Moreover, avelumab plus CRT resulted in slightly higher rates of adverse events of grade 3 or more compared to CRT plus placebo (80% *vs* 74%). As an explanation for the absence of PFS benefits, the investigators postulated that the dysfunction of T cells or changes in the tumor microenvironment after radiotherapy might have reduced the ability of the immune system to eliminate the microscopic disease. A recent phase-2 randomized trial of pembrolizumab with radiation therapy against cetuximab with radiotherapy in LAHNSCC also failed to demonstrate significant treatment benefits, although the combination therapy had a favorable toxicity profile[45]. Similarly, a previous randomized phase-2 trial of nivolumab with stereotactic body radiotherapy compared to nivolumab alone did not result in tumor shrinkage in R/M HNSCC[46]. Interestingly, an exploratory subgroup analysis of patients with high PD-L1 expression in the JAVELIN study indicated a potential PFS benefit with avelumab plus CRT compared to placebo plus CRT. Although definite conclusions cannot be made based on this small subgroup analysis, this is a finding that should be explored further to understand the role of biomarker analysis to select patients for immunotherapy.

In terms of PFS, none of the studies included in this review demonstrated any beneficial outcomes. A recent meta-analysis by Gyawali *et al*[47] found no correlation between median OS and median PFS in studies evaluating anti-PD-1 agents. Defining PFS based on the traditional RECIST criteria (developed in the pre-immunotherapy era) that do not properly capture the concept of disease progression with immunotherapy was hypothesized as a probable reason for the finding.

While immunotherapy involving anti-PD-1 checkpoint inhibitors resulted in significant improvements in survival, PD-L1 and CTLA-4 blockade did not demonstrate any encouraging outcomes. More studies are needed to build evidence on the role of anti-PD-L1 and CTLA-4 blocking agents in the treatment of advanced HNSCC. Again, in the first-line setting, the evidence on the effectiveness of immunotherapy for R/M HNSCC is based on one single phase-3 trial (KEYNOTE 048), and currently, pembrolizumab is the only ICI approved for treating this group of patients[25]. During our literature search, we identified some of the ongoing phase-3 clinical trials investigating various checkpoint inhibitor agents either alone or as part of combination therapy. Subsequently, we searched the 'clinical trials.org' database and identified the major ongoing clinical trials and confirmed the status of those trials.

Major ongoing clinical trials

Studies investigating the combination of two different ICI agents or ICI in combination with another immunomodulatory agent in R/M HNSCC in the first-line treatment setting[48-51]: An ongoing open-label phase-3 trial (KESTREL) is currently evaluating anti-PDL-1 agent durvalumab alone and in combination with the anti-CTLA-4 agent tremelimumab for R/M HNSCC against the EXTREME regime in the first-line treatment setting[48]. Checkmate 651, another ongoing phase-3 study, is currently

evaluating the anti-PD-1 agent nivolumab in combination with the CTLA-4 blocking agent ipilimumab for R/M HNSCC against the EXTREME regime in the first-line setting[49]. In a phase-3 trial among R/M HNSCC, patients with a PD-L1 biomarker expression of CPS ≥ 1, the combination of pembrolizumab and lenvatinib, an anti-vascular endothelial growth factor-multiple kinase inhibitor, is being investigated as first-line treatment against pembrolizumab plus placebo[50]. Similarly, ICI in combination with another immunomodulatory agent is being investigated in the ECHO-304/KEYNOTE 669 study[51]. In this phase-3 trial, the combination of pembrolizumab and epacadostat, an indoleamine 2,3-dioxygenase 1, inhibitor agent is being investigated against pembrolizumab monotherapy, and the EXTREME regime, in R/M HNSCC as first-line treatment[51].

Studies investigating ICI plus CRT *vs* CRT alone in LAHNSCC[52,53]: In KEYNOTE 412, the effectiveness of pembrolizumab given concurrently with CRT and as maintenance therapy is being evaluated against placebo plus standard CRT for the treatment of LAHNSCC[52]. In REACH, the superiority of avelumab in combination with RT-cetuximab compared to cisplatin -RT and/or to RT-cetuximab alone is being evaluated[53].

Studies investigating ICI plus RT *vs* cetuximab plus RT in platinum ineligible LAHNSCC[54,55]: In HN004, durvalumab plus RT is being compared to cetuximab plus RT in platinum ineligible patients [54]. In a recently completed phase-3 trial with no published results (CheckMate 9TM), cisplatin-ineligible patients received nivolumab plus RT as intervention while control patients received cetuximab plus RT[55].

Studies investigating ICI as neoadjuvant/adjuvant therapy[56-59]: In KEYNOTE 689, pembrolizumab with RT (with or without cisplatin) before and after surgery is compared to RT (with or without cisplatin) given after surgery[56]. Atezolizumab, an anti-PD-L1 agent, is being evaluated as an adjuvant therapy against placebo in the ongoing trial iMvoke010[57]. In IMSTAR-HN, nivolumab alone or in combination with the anti-CTLA-4 agent ipilimumab is evaluated as follow-up after adjuvant therapy against standard follow-up in surgically resectable LAHNSCC[58]. In NIVOPOSTOP, the efficacy of postoperative adjuvant nivolumab along with CRT is compared to post-operative CRT alone[59].

The details of these ongoing phase-3 studies are given in Table 2.

Future directions

Novel combination strategies to potentiate and prolong the anti-tumor activity of ICI are being evaluated currently. Thus, several early phase clinical trials (phase 1/2) investigating combination strategies of ICIs and other novel immunomodulatory agents are in the pipeline[60,61]. For example, a randomized phase-2 trial to study the safety and tolerability of nivolumab administered alone or in combination with relatlimab (antibody targeting the novel immunomodulatory receptor lymphocyte activation gene-3) or the anti-CTLA-4 agent ipilimumab is currently ongoing among patients with locally advanced surgically resectable HNSCC[62]. Immune biomarker modulation in response to nivolumab given along with Toll-like receptor 8 agonist motolimod is being analyzed in an ongoing phase-1b pre-operative biomarker trial[63]. Combination of pembrolizumab and the vascular endothelial growth factor-multiple kinase inhibitor lenvatinib demonstrated good anti-tumor activity and manageable toxicity among R/M HNSCC patients in a phase-1b/2 trial, and LEAP 010, a phase-3 trial of this combination strategy is currently ongoing[50,64]. The combination of pembrolizumab and the anti-EGFR agent cetuximab had demonstrated encouraging outcomes in the interim analysis of an ongoing multi-arm phase-2 trial[65,66]. A recently completed study among R/M HNSCC patients investigating pembrolizumab in combination with epacadostat has shown clinically meaningful results, and a larger phase-3 trial (ECHO 304/KEYNOTE 669) of this combination strategy is ongoing currently [51,67]. Combination therapy of pembrolizumab with the EGFR-tyrosine kinase inhibitor afatinib, which also included predictive biomarker analysis, had been evaluated recently in a phase-2 clinical trial (the ALPHA study) in R/M HNSC[68]. The study demonstrated augmentation of the anti-tumor activity of pembrolizumab by afatinib, and the results of biomarker analysis suggested that PD-L1 and EGFR amplification could be predictive biomarkers for cancer immunotherapy. EACH, a randomized phase-2 trial among R/M HNSCC is investigating the superiority of avelumab and cetuximab combination compared to avelumab monotherapy[69]. Another recently completed early phase study on the combination of pembrolizumab with the therapeutic vaccine talimogene laherparepvec demonstrated a tolerable safety profile among patients with R/M HNSCC. However, this investigation did not progress into a phase-3 trial as the efficacy of the combination was found to be similar to pembrolizumab monotherapy[70].

Immunotherapy trials among patients with p16-positive head and neck cancer (oropharyngeal squamous cell carcinoma) are also currently underway. In this group of patients, p16 positivity is a known independent predictive biomarker for survival[71]. The efficacy and tolerability of the combination of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) along with RT in locoregionally advanced human papilloma virus-positive oropharyngeal squamous cell carcinoma are being evaluated in an ongoing phase-2 single-arm trial[72]. Another phase-2 randomized study (KEYCHAIN trial) is investigating RT along with concurrent and adjuvant pembrolizumab against concurrent chemoradiation among p16-positive HNSCC[73,74].

Table 2 Major ongoing phase-3 studies investigating immunotherapy in head and neck squamous cell carcinoma

Study	Status/trial ID	Population	Intervention	Control	No of participants	Target receptor
KESTREL[47]	Active, not recruiting/NCT02551159	R/M HNSCC	Arm 1: Durvalumab Arm 2: Durvalumab with Tremelimumab	EXTREMEregime	823	PDL-1, CTLA-4
Checkmate 651[48]	Active, not recruiting/NCT02741570	R/M HNSCC	Nivolumab with Ipilimumab	EXTREME regime	947	PD-1, CTLA-4
LEAP-10[49]	Active, recruiting/NCT04199104	R/M HNSCC	Pembrolizumab with Lenvatinib	Pembrolizumabwith placebo	500	PD-1, VEGF-multiple kinase
ECHO-304/KEYNOTE 669[50]	Active, not recruiting/NCT03358472	R/M HNSCC	Arm1: Pembrolizumab with Epacadostat Arm 2: Pembrolizumab alone	EXTREME	625	PD-1,IDO1
KEYNOTE 412[51]	Active, not recruiting/NCT03040999	LAHNSCC	Pembrolizumab with CRT concurrently and as maintenance	Standard CRT plus placebo	780	PD-1
REACH[52]	Active, not recruiting/NCT02999087	LAHNSCC	Avelumab in combination with RT-cetuximab	Cisplatin-RT and/or RT-cetuximab alone	707	PD-L1
HN004[53]	Active, recruiting/NCT03258554	LAHNSCC Platinum in eligible patients	Durvalumab plus RT	Cetuximab plus RT	474	PD-L1
CheckMate 9TM [54]	Completed awaiting results/NCT03349710	LAHNSCC Platinum ineligible cohort	Nivolumab plus RT	Cetuximab plus RT	68	PD-1
		LAHNSCC Platinum eligible cohort	Nivolumab pluscisplatin plus RT	Cisplatin plus RT		
KEYNOTE 689[55]	Active, recruiting/NCT03765918	LAHNSCC	Pembrolizumab with RT (with or without cisplatin) before and after surgery	RT (with or without cisplatin) given after surgery	704	PD-1
iMvoke010[56]	Active, recruiting/NCT03452137	LAHNSCC	Atezolizumab as adjuvant therapy after definitive local therapy	Placebo	400	PD-L1
IMSTAR-HN[57]	Active, not recruiting/NCT03700905	Surgically resectable LAHNSCC	Nivolumab alone or in combination Ipilimumab as follow up after adjuvant therapy	Standard follow-up after adjuvant therapy	276	PD-1, CTLA-4
NIVOPOSTOP[58]	Active, recruiting/NCT03576417	LAHNSCC	Adjuvant Nivolumab with CRT postoperatively	CRT alone post operatively	680	PD-1

CRT: Chemoradiation therapy; R/M HNSCC: Recurrent or metastatic head and neck squamous cell carcinoma; LAHNSCC: Locally advanced head and neck squamous cell carcinoma; PD-1: Programmed cell death protein-1; PD-L1: Programmed death-ligand 1; CTLA-4: Cytotoxic T- lymphocyte associated protein-4; IDO1: Indoleamine 2,3-dioxygenase 1; VEGF: Vascular endothelial growth factor.

Regarding biomarkers, in addition to p-16 positivity and PD-L1 expression, other biomarkers like microsatellite instability (MSI) and tumor mutation burden were also found to be associated with favorable outcomes with ICI therapy in HNSCC[75]. Tardy *et al*[76] recently reported a case of complete response to anti-PD-L1 therapy in HNSCC in a patient with high tumor MSI (MSI-H) and a negative PD-L1 histochemical status. Similarly, Hanna *et al*[77] reported that higher tumor mutation burden predicted response to ICI and better treatment outcomes in virus-negative head and neck cancer. Again, some subtypes of tumor-infiltrating lymphocytes (TILs) such as PD-1$^+$TIM-3$^+$CD8$^+$ TILs and PD-1$^+$LAG-3$^+$ CD8$^+$ TILs have also predicted treatment response to ICIs[75,77]. The data on these emerging predictive biomarkers is still not conclusive; therefore, further research is essential. PRECISION 01, an ongoing prospective observational study is currently evaluating biomarker signatures in tissue samples

of platinum-refractory HNSCC patients who received nivolumab monotherapy; the findings may contribute to the knowledge on predictive biomarkers for ICIs[78].

In future studies, patient-reported outcomes like QOL should be evaluated meticulously since such outcomes are very crucial for advanced HNSCC patients and their families[79,80]. Cost-effectiveness is another issue to be considered before including ICIs in the routine treatment guidelines for patients from developing countries and resource-poor settings[81,82]. The impact of factors like age, comorbidities, and performance status on outcomes of patients receiving immunotherapy also needs to be determined[83].

Limitations/strengths

There are very few published phase-3 clinical trials evaluating checkpoint inhibitor immunotherapy among patients diagnosed with HNSCC, and the evidence we gathered in this review is based on the five phase-3 RCTs published so far. A previous systematic review on this topic included eight studies, of which two were phase-3 RCTs[84]. Wang *et al*[85] conducted a systematic review and meta-analysis of nine studies on the effectiveness of checkpoint inhibitors in HNSCC, of which two were phase-3 trials.

To our knowledge, this is the first systematic review conducted on the effectiveness of ICIs in HNSCC incorporating phase-3 trials alone. The evidence we presented based on the five studies in this review will help the practicing clinicians to make informed decisions. We further explored the literature and identified a variety of promising clinical studies that are ongoing currently focusing on combination strategies in enhancing and prolonging the anti-tumor effects of ICIs. We also identified the gaps in knowledge on some important issues such as predictive biomarkers and about the identification of patients who will benefit from immunotherapy based on biomarker assessment[86,87].

CONCLUSION

ICIs have shown improved survival outcomes with acceptable toxicity profile in R/MHNSCC in the first and second-line treatment settings. The marginal improvement in survival should be weighed against the cost of these therapeutic agents and the QOL of patients. While anti-PD-1 agents demonstrated efficacy, evidence on the effectiveness of anti-PD-L1 and anti-CTLA-4 agents is lacking. There is no proven efficacy in the curative setting to date. The ongoing clinical trials may better define the role of ICI in R/M HNSCC and LAHNSCC in the future.

ARTICLE HIGHLIGHTS

Research background

Head and neck squamous cell carcinoma (HNSCC) is one of the major causes of cancer-associated morbidity and mortality globally, especially in developing countries. Treatment approaches for HNSCC vary according to the stage of the disease at presentation. For recurrent/metastatic HNSCC (R/M HNSCC), platinum-based chemotherapy was the only available treatment option until recently. A relatively new systemic therapy option that emerged in recent years in the treatment of advanced HNSCC is immunotherapy using immune checkpoint inhibitors (ICI).

Research motivation

Advanced HNSCCs are often associated with significant functional limitations, and aggressive treatment may adversely affect the quality of life of these patients who are already suffering from the effect of advanced cancer. The median survival of R/M HNSCC patients receiving platinum-based chemotherapy is 7.4 mo. Some patients become refractory to platinum and die within a period of 4 mo. The safety profile and anti-tumor activity of ICIs demonstrated in early phase clinical trials paved the way to the initiation of several promising phase-3 trials in the field. Therefore, we decided to gather the current evidence on the effectiveness of these agents in advanced head and neck cancer based on the findings from phase-3 clinical trials of ICI published so far. We also wanted to examine the feasibility of incorporating these agents into routine clinical practice in resource-poor settings.

Research objectives

The objective of this systematic review was to gather the evidence from phase-3 randomized controlled trials (RCTs) evaluating the effectiveness of immunotherapy among patients with advanced HNSCC. We aimed to synthesize the evidence from the published phase-3 studies that investigated the efficacy and toxicity profile of ICIs administered either alone or in combination with chemotherapy, radiation therapy, or with another checkpoint inhibitor, in advanced HNSCC.

Research methods

We conducted this systematic review according to the PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines. We searched four major databases including PubMed, Scopus, Embase, and COCHRANE library, without any language limit. A combination of standardized search terms and keywords including head and neck squamous cell carcinoma, recurrent, metastatic, locally advanced, immunotherapy, checkpoint inhibitors, monoclonal antibodies, programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), cytotoxic T- lymphocyte associated protein-4 (CTLA-4), and phase-3 clinical trial were used for searching the literature. Studies were included if they were completed phase-3 RCTs conducted among patients with R/M HNSCC or LAHNSCC, in which the intervention patients received ICI either alone or in combination with chemotherapy, radiation therapy, or with another ICI and the control patients received the standard of care treatment (SOC). Anatomical sites of primary tumors were oral cavity, oropharynx, hypopharynx, and larynx in the included studies.

Research results

Five phase-3 clinical trials have reported the data on the effectiveness of immunotherapy in HNSCC so far: Four in R/M HNSCC and one in LAHNSCC. In patients with R/M HNSCC, anti-PD-1 agents nivolumab and pembrolizumab demonstrated improvement in overall survival (OS) in the second-line treatment setting compared to the SOC. While the net gain in OS with nivolumab was 2.4 mo, that with pembrolizumab was 1.5 mo. However, the study that investigated the anti-PD-L1 agent durvalumab with or without the anti-CTLA-4 agent tremelimumab in the second-line treatment setting did not demonstrate any beneficial outcomes.

In the first-line setting, pembrolizumab together with platinum-based chemotherapy demonstrated statistically significant improvement in survival with a net gain in OS of 2.3 mo in the overall population and a net gain in OS of 4.2 mo in the population with a combined positive score of > 20 compared to the SOC treatment. Pembrolizumab monotherapy was found to be non-inferior to EXTREME in terms of OS (11.6 mo *vs* 10.7 mo) in the total population. In patients with PD-L1 positive R/M HNSCC, monotherapy with pembrolizumab also demonstrated statistically significant improvement in survival compared to SOC. In LAHNSCC, immunotherapy using the anti-PD-L1 agent avelumab along with standard chemoradiation therapy did not result in improved outcomes compared to placebo plus chemoradiation therapy.

Research conclusions

This systematic review helped us to conclude that anti-PD-1 agents provide survival benefits in R/M HNSCC in the first and second-line settings with manageable toxicity profiles. However, it is important to weigh the marginal survival benefits provided by these therapeutic agents against their cost, especially in resource-poor settings. The review showed that the evidence on the effectiveness of anti-PD-L1 and anti-CTLA-4 agents in advanced head and neck cancer is lacking. To date, there is no evidence on the effectiveness of ICIs in the curative setting either. We believe that the ongoing clinical trials (discussed in the article) will help to define better the role of ICI in R/M HNSCC and LAHNSCC in the future.

Research perspectives

Novel combination strategies to potentiate and prolong the anti-tumor activity of ICI are being evaluated currently. Gaps in knowledge exist on some important issues such as predictive biomarkers, and about the identification of patients who will benefit from immunotherapy based on biomarker assessment. Future studies should focus on these issues.

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FOOTNOTES

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LETTER TO THE EDITOR

Commentary: Evaluating potential glioma serum biomarkers, with future applications

Michael Goutnik, Brandon Lucke-Wold

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Abstract

Systemic inflammation within malignant glioma is a topic of ongoing significance. In this commentary, we highlight recent findings from Gandhi *et al* and discuss alternative approaches. We present a counter argument with findings that IL-6 markers are controversial. We highlight the potential benefit of looking at microRNAs and other biomarkers. Finally, we present ideas for future application involving differentiation between radiation necrosis and recurrence. The commentary is intended to serve as a catalyst for further scientific discovery.

Key Words: Systemic inflammation; Malignant glioma; Neutrophil-lymphocyte ratio; Interleukin-6

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Core Tip: Systemic inflammation in malignant glioma, along with the potential for blood-based biomarkers, is an exciting field of ongoing research. We have discussed supporting and contrasting evidence for glioma blood-based biomarkers, along with future research proposals.

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TO THE EDITOR

The paper titled "Novel molecular panel for evaluating systemic inflammation and survival in therapy naïve glioma patients" by Gandhi *et al*[1] highlights the use of a

non-invasive panel consisting of four inflammatory markers to distinguish between histological grades of glioma and IDH-mutant/wildtype glioma, as well as predicting overall survival. The premise behind the potential effectiveness of such a panel is the chronic inflammatory state that results from various stimuli like tumor antigens and oncogenes that promote abnormal growth and leakage of markers into the peripheral circulation. The inflammatory environment of gliomas is not a new finding, as Morimura *et al*[2] previously found. 20%-30% of cells in glioma samples were recognizable by various macrophage/microglia markers and that tumor proliferation correlates with macrophage infiltration[2]. Parney *et al*[3] similarly demonstrated the infiltration of gliomas by macrophages. However, there is conflicting evidence as to whether these infiltrating macrophages are capable of secreting cytokines and promoting an effective immune response[4,5].

Nonetheless, other studies have found similar results with respect to the markers that Gandhi *et al*[1] focused upon within their paper. For example, Adams *et al*[6] found the kynurenine pathway to be significantly activated in plasma samples from glioblastoma (GBM) patients, an effect that is hypothesized to inhibit anti-tumor immunity by depleting tryptophan from the tumor microenvironment and thus suppressing T-cell proliferation. Du *et al*[7] also demonstrated that the serum Kyn/Trp ratio in patients with high grade gliomas was significantly higher than in those with lower grade gliomas. Similarly, Juhász *et al*[8] used dynamic PET imaging of patients with gliomas to demonstrate shunting of tryptophan (Trp) toward kynurenine (Kyn) metabolism. Mitsuka *et al*[9] evaluated the expression of indoleaine 2,3-dioxygenase (IDO), an important enzyme in tryptophan metabolism that yields catabolites including kynurenine, in 75 surgical specimens including diffuse astrocytomas, anaplastic astrocytomas, and GBMs. The authors found IDO expression correlated with glioma grade, expression increased in secondary glioblastoma relative to the initial lower-grade glioma, and stronger expression was associated with worse survival in GBM patients[9]. Zhai *et al*[10] also found GBM patients with high kynurenine/tryptophan ratios to have worse survival compared to those with lower values. However, no other studies were found that replicated Gandhi *et al*[1]'s findings of tryptophan metabolites distinguishing between IDH-wildtype and mutant gliomas.

The neutrophil-lymphocyte ratio was another significant marker in Gandhi *et al*[1]'s study, which has been shown to be effective in distinguishing between different grades of glioma and predicting overall survival and progression-free survival in a variety of gliomas[11-19]. Concurrent with Gandhi *et al*[1]'s results, NLR has also been shown to distinguish between IDH-mutant and wildtype gliomas, with mutant IDH1 gliomas featuring lower levels of NLR[17]. Furthermore, telomerase activity has also been associated with glioma grade and overall survival, which Gandhi *et al*[22] also demonstrated[20-22]. However, IDH mutant cell lines appear to indirectly reactivate hTERT, which contrasts with Gandhi *et al*[22]'s finding of higher hTERT in IDH-wildtype tumors[23].

Gandhi *et al*[1] highlighted positive correlations between median marker values and tumor grade, as well as significantly higher molecular marker values for IDH-wildtype compared to IDH-mutant gliomas. Furthermore, they found that IL-6 had a strong correlation with tumor grade, which has been replicated by immunohistochemistry, gene expression studies and CSF and serum analysis[24,25]. Some of these findings have been challenged in the literature, however. Cytokines interact with receptors, antibodies, binding proteins, and also often have short half-lives, so total concentrations may not reflect production and/or secretion levels[26]. Samaras *et al*[26] thus used the ELISPOT method (a cell-based cytokine measuring system) to demonstrate greater IL-6 secretion from peripheral monocytes and greater IL-10 secretion from peripheral mononuclear and tumor cells in glioma patients compared to controls. However, there was only a marginal increase in significance in median IL-6 secretion between glioma grades, but this may be due to small sample size[26]. Holst *et al*[27] studied 158 patients and found no difference in serum IL-6 between GBM and lower grade gliomas once age was accounted for, and that IL-6 was significant for worse survival only in univariate analysis. However, Holst *et al*[27] and Jiang *et al*[28] did find *IL6* RNA expression to differ between IDH-mutant and wild type gliomas, which parallels the finding of Gandhi *et al*[1]. Other studies have not found a relationship between IL-6 Levels and survival in GBM[29,30]. In one study involving 38 glioma patients, serum IL-6 decreased in glioma patients and inversely correlated with grade, while serum IL-17A was specific to gliomas (compared to meningiomas and schwannomas) and positively correlated with grade[31]. However, serum IL-6 has been associated with a negative prognosis in other cancers[32].

Divergent results regarding IL-6 may reflect confounding bias and/or differential treatment, as corticosteroid treatment may decrease plasma IL-6[27,33]. Similarly, brain surgery may increase serum inflammatory markers, suggesting that these proteins reflect brain injury and disruption of the blood-brain barrier rather than tumor burden[34]. There may also be false positives in patients with other inflammatory or malignant processes[12]. Furthermore, there are a variety of other circulating biomarkers that may influence survival, such as circulating tumor cells and microRNAs[35,36]. In addition, other non-serum based noninvasive biomarkers like urinary 2-hydroxyglutarate (2-HG), a product of mutant IDH acting on α-ketoglutarate, may distinguish between IDH-mutant and IDH-wild type glioma[37,38]. This metabolite may also be detected by magnetic resonance spectroscopy, and correlates with IDH mutation status[39]. Nonetheless, Gandhi *et al*[1]'s panel is promising with a 94.4% sensitivity and 96.7% specificity, suggesting potential therapeutic targets. More prospective work with larger cohorts is needed to evaluate the efficacy of Gandhi *et al*[1]'s proposed immune marker panel in predicting tumor grade and survival, and whether adding, removing, and/or combining other

circulating and non-circulating biomarkers may be more effective in terms of accuracy and cost.

An interesting application of Gandhi *et al*[1]'s work would involve testing the ability of their panel to differentiate tumor progression from radiation necrosis[40]. Inflammation, including the pro-inflammatory IL-6 cytokine, likely contributes to the pathophysiology of radiation necrosis[41,42]. It is feasible that a different set of thresholds for the four molecular markers, or the inclusion of other markers like miR-21[43], predicts radiation necrosis compared to tumor progression. Furthermore, a different choice of patient controls could be useful in further evaluating the panel's specificity. Instead of forty-five healthy controls without a history of inflammation or autoimmune disease, patients with non-glial brain tumors and/or other inflammatory conditions may serve as controls.

Further testing of the panel may include other potentially important molecules like IL-33. IL-33 has been shown to induce a pro-inflammatory environment within gliomas and inversely correlates with survival[44-46]. De Boeck *et al*[44] also demonstrated IL-33 induced upregulation of inflammatory gene expression, including IL-6, and proposed that IL-33 secretion from glioma cells recruits monocytic cells from the circulation. Thus, IL-33 may be more specific to glioma than Gandhi *et al*[1]'s markers, and may also be sufficient alone as a marker. Differentiating the markers that distinguish high grade verse low grade gliomas early will be valuable and can be validated in preclinical studies.

FOOTNOTES

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LETTER TO THE EDITOR

How to improve metastatic pancreatic ductal adenocarcinoma patients' selection: Between clinical trials and the real-world

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Abstract

As underlined in the minireview by Blomstrand *et al*, given the poor prognosis and the paucity of data on a therapeutic sequence in pancreatic ductal adenocarcinoma (PDAC), additional randomized controlled trials and real-world evidence studies addressing current and novel regimens are needed. The real-world outcomes of first-line chemotherapy regimens such as FOLFIRINOX and gemcitabine/nab-paclitaxel are thoroughly reviewed and seem to be largely generalizable in a real-world context. Regarding second-line chemotherapy, the key question about the optimal sequence of regimens remains uncertain. Precisely in this setting, it is therefore useful to encourage the implementation of clinical studies that may contribute to the scarcity of data available up to now. We report our experience with a small group of patients treated with second-line liposomal irinotecan (nal-IRI) plus 5-fluorouracil and leucovorin. To improve the treatment of patients affected by PDAC, it is useful to identify subgroups of patients who may benefit from target treatments (*e.g.*, BRCA mutant) and it is also important to focus on any prognostic factors that may affect the survival and treatment of these patients.

Key Words: Metastatic pancreatic ductal adenocarcinoma; Palliative chemotherapy; Real-world data; Molecular selection; Biomarkers; Second-line treatment

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Core Tip: The present letter is intended to contribute to the collection of data on pancreatic ductal adenocarcinoma (PDAC) treatments in second-line settings through our experience with the promising data of efficacy and safety of a small group of study patients treated with second-line liposomal irinotecan (nal-IRI) plus 5-fluorouracil and leucovorin. We also focused on highlighting the subgroups of PDAC patients who might benefit from target treatments, such as a small proportion of mutated BRCAs, and to identify comorbidities or characteristics that impact the prognosis of PDAC patients through our retrospective analysis that demonstrate a correlation between type II diabetes mellitus and improved overall survival.

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TO THE EDITOR

We read with great interest the review by Pretta *et al*[1], entitled "Real-world evidence on first- and second-line palliative chemotherapy in advanced pancreatic cancer". This review provides a comprehensive overview on real-life data of metastatic pancreatic ductal adenocarcinoma (PDAC) patients treated in the first- and second-line setting. The authors critically compare the results obtained in a real-world population with those provided by randomised clinical trials (RCT), highlighting that the outcomes are consistent and similar, especially on first-line with FOLFIRINOX and gemcitabine -nab-paclitaxel.

We agree with the authors that PDAC patients enrolled in RCT represent a highly selected population and that despite real-world outcomes derive from different countries, with various regulatory agencies and health care systems, all data available in the literature seems to confirm the effectiveness and safety of chemotherapy regimens in real-life settings. Moreover, since no strong data on second-line settings are available, we greatly appreciate the authors' effort to analyse this topic.

At our Centre, we conducted a retrospective analysis in a real-world population of metastatic PDAC patients treated with second-line liposomal irinotecan (nal-IRI) plus 5-fluorouracil and leucovorin (5FU/LV) in a compassionate use program, in order to assess clinical outcome, tolerability and potential prognostic factors. Statistical analysis was performed with the MedCalc package. The association between categorical variables was estimated by chi-square test. Survival distribution was estimated by the Kaplan-Meier method and survival curves comparison was evaluated with the log-rank test. The cut off value for laboratory parameters was identified with ROC curves. The study was approved by the Ethical Committee of the Cagliari University Hospital (Prot. PG /2018/7339, June 1, 2018) and was performed in accordance with the ethical principles stated in the Declaration of Helsinki and in the International Conference on Harmonization (ICH) Note for Guidance on Good Clinical Practice (GCP, ICH E6, 1995) and all applicable regulatory requirements.

Globally, 14 patients treated with nal-IRI and 5FU/LV from June 2016 to November 2018 were included in our analysis. Baseline characteristics are shown in Table 1. Median overall survival (OS) was 9.1 (95%CI: 5.8-13.1) mo. Median progression free survival (PFS) was 7.2 mo (95%CI: 3.0-33.8). Three patients achieved a partial response, two had stable disease, and nine developed progression. Median duration of treatment with nal-IRI plus 5FU/LV was 6 mo; treatment longer than 6 mo was related to improved OS [13.1 mo (95%CI: 9-17) *vs* 7.1 mo (95%CI: 2.9-13.1), $P = 0.0127$, HR = 0.18] and PFS [9.1 mo (95%CI: 7.2-33.8) *vs* 3.1 mo (95%CI: 2.2-7.7), $P = 0.0011$, HR = 0.08]. Baseline haemoglobin levels equal or lower than 10 g/dL were associated with worse prognosis [OS: 11 mo (95%CI: 1-15.9) *vs* 3 mo, $P = 0.0384$, HR = 0.005; PFS: 7.6 mo (95%CI: 3.1-33.8) *vs* 3 mo, $P = 0.0384$, HR = 0.005]. 64% of patients developed grade > 2 toxicity. The occurrence of grade > 2 anaemia was related to shorter OS [3 mo *vs* 11 mo (95%CI: 7.1-15.9), $P = 0.0384$, HR = 0.005] and PFS [3 mo *vs* 7.6 mo (95%CI: 3.1-33.8), $P = 0.0384$, HR = 0.005]. As shown in Figure 1, the need for erythropoietin administration was associated with poorer OS [3 mo *vs* 10.7 mo (95%CI: 7.1-15.9), $P = 0.0287$, HR = 0.003] and PFS [3 mo *vs* 7.6 mo (95%CI 3.1-33.8), $P = 0.0287$, HR = 0.003]. In our real-world retrospective analysis, nal-IRI plus 5FU/LV confirmed its efficacy and tolerability, consistently with NAPOLI-1 RCT[2,3]. Longer duration of treatment was related to improved survival, whereas lower baseline haemoglobin levels, anaemia occurrence and the necessity of erythropoietin were negative prognostic factors. These results are consistent with some findings of a previous Italian large real-world analysis[4].

As underlined by Blomstrand *et al*[1], there is an urgent need for prognostic and predictive biomarkers to improve the therapeutic management of PDAC patients and to prolong survival as well as more effective treatment strategies. Indeed, intensive research efforts and substantial progress in the

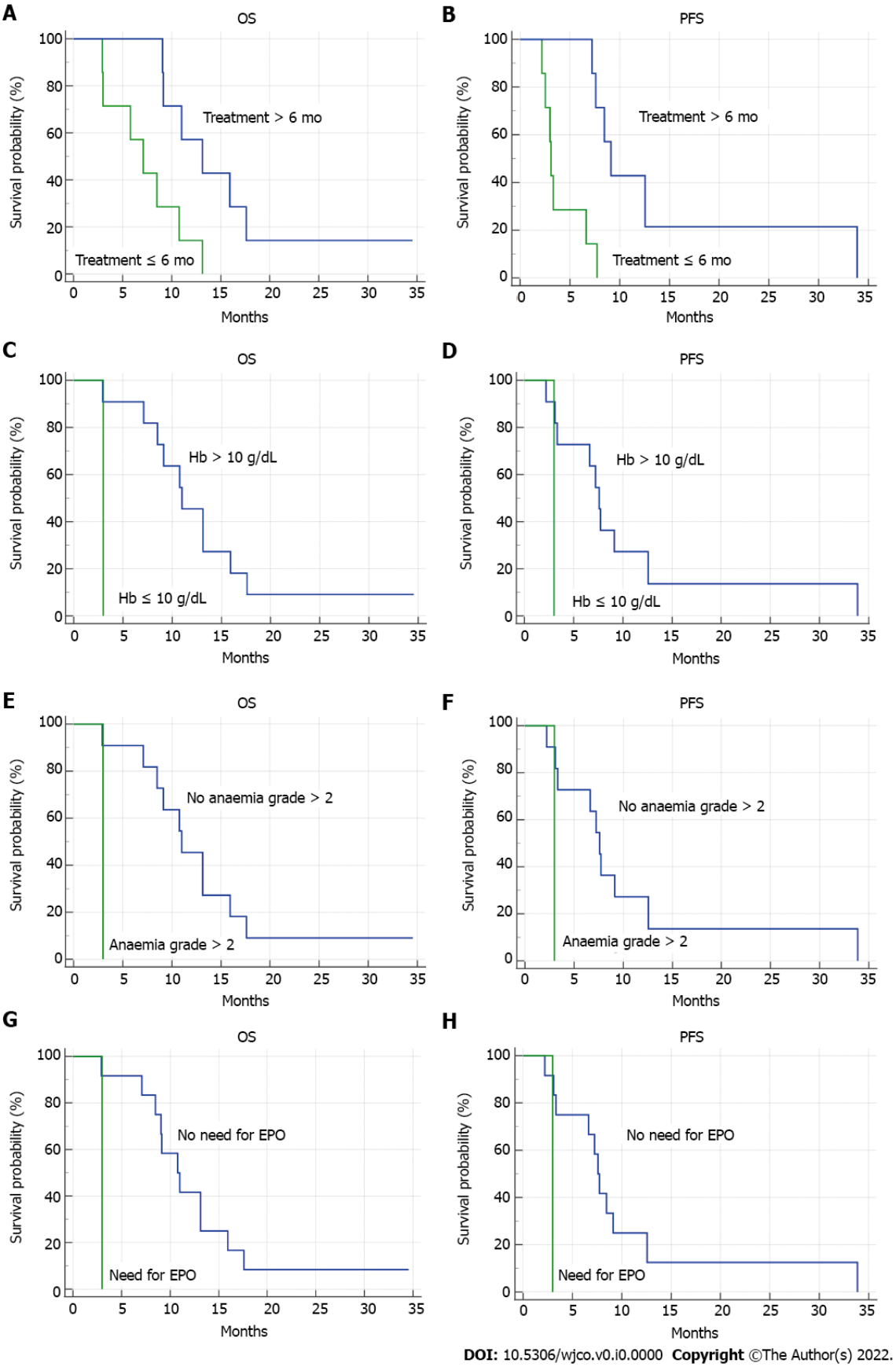
Table 1 Baseline characteristics of patients treated with second-line Nal-iri at the Medical Oncology Unit, University Hospital and University of Cagliari, Cagliari, Italy

Baseline characteristics	Patients, %
Sex	
Male	71
Female	29
Age, yr	
< 65	36
≥ 65	64
Location of primary tumour	
Head-uncinate process	57
Body	21.5
Tail	21.5
Previous surgery	
Yes	28.5
No	71.5
Number of metastatic sites	
Single site	36
Multiple sites	64
Location of metastatic sites	
Lymph nodes	78.5
Liver	57
Peritoneum	42.8
Lung	35.7
First-line chemotherapy regimen	
Gemcitabine-nab-paclitaxel	92.8
Other	7.2

understanding of the PDAC genetic background and molecular biology have not yet been matched either by the successful development of novel agents or by the identification of predictive biomarkers that could increase the effectiveness of existing therapies[5]. In contrast to other solid tumours, immunotherapy strategies have failed to yield any notable impact in PDAC. This is likely related to the critical role of the tumour microenvironment as a physical barrier and its inhibitory immune signalling. The most promising therapeutic strategy seems to be combination of immunotherapeutics with other targeted treatments[6].

To our knowledge, the only successful biomarker-driven phase III RCT so far is the POLO trial, which showed improved PFS with maintenance Olaparib, a PARP-inhibitor, *vs* placebo in germline BRCA-mutant PDAC not progressing after first-line platinum-based chemotherapy[7]. Notably, BRCA-mutant PDAC represents a unique entity with specific disease features that are still to be fully understood and BRCA1/2 alterations are the most explored targetable mutations. Moreover, in this patients' setting, the sensitivity to platinum chemotherapy requires further research and confirmations. Recently, the concept of "BRCAness" has gained increasing importance. This term refers to high-grade genomic instability of non-BRCA-mutant cancers and represents a phenotype of defective homologous recombination to which somatic mutations in genes like *BRCA1/2*, *ATM*, *PALB2*, *CHEK1*, *RAD51*, and *FANCA*, can contribute. For these reasons, "BRCAness" is under evaluation as a biomarker for DNA-damaging agents and PARP inhibitors[8].

Finally, we believe that in a real-life focusing-approach, a particular focus should be deserved to the assessment of the impact of comorbidities on the PDAC patients' prognosis. In a retrospective analysis that we conducted on 164 advanced PDAC patients, we demonstrated a correlation between type II diabetes mellitus and improved OS, both in the exploratory and in the validation cohort at univariate analysis (16 *vs* 10 mo; P = 0.004 and 11 *vs* 6 mo; P = 0.01, respectively). Moreover, in multivariate



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Figure 1 Kaplan-Meier overall survival and progression free survival curves according to clinical variables. A: Overall survival (OS) Kaplan-Meier curve according to nal-IRI treatment duration; B: Progression free survival (PFS) Kaplan-Meier curve according to nal-IRI treatment duration; C: OS Kaplan-Meier curve according to baseline haemoglobin levels; D: PFS Kaplan-Meier curve according to baseline haemoglobin levels; E: OS Kaplan-Meier curve according to occurrence of grade > 2 anaemia; F: OS Kaplan-Meier curve according to occurrence of grade > 2 anaemia; G: OS Kaplan-Meier curve according to the need for

erythropoietin administration; H: PFS Kaplan-Meier curve according to the need for erythropoietin administration.

analysis, insulin-treated patients compared with non-diabetic patients had a significantly increased survival of 4.6 mo (P = 0.03). Surely, the correlation between OS and insulin-treated type II diabetes mellitus should be confirmed in prospective clinical trials[9].

In conclusion, in the era of precision medicine, larger and prospective studies in the real-world population, with the focus on specific PDAC subtypes (*e.g.*, BRCA-mutant), would further clarify the impact of available and innovative treatment strategies on PDAC patients and help identify potential biomarkers to improve patients' selection and prognosis.

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FOOTNOTES

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OPINION REVIEW

Implementation of a virtual multicenter gastrointestinal tumor board to reduce cancer disparities in Argentina

Federico Esteso, Natalia Soledad Tissera, Juan Manuel O'Connor, Romina Luca, Eduardo Huertas, Fernando Sánchez Loria, Ivana Pedraza, Alejandro Pairola, Fernando Brancato, Lisandro Paganini, Mariana Kucharczyk, Mora Amat, Maria Teresa Pombo, Mariana Galli, Luisina Bruno, Luis Caro, Andrés Rodriguez, Diego Enrico, Federico Waisberg, Matías Chacón

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Abstract

Given the increasing complexity of cancer care, multidisciplinary tumor boards have become essential in daily clinical oncology practice. The Project Extension for Community Healthcare Outcomes (ECHO) initiative developed an innovative telementoring model using a "hub and spoke" design consisting of a team of experts (hub) that offers a full service to multiple participants (the spokes) during regularly scheduled sessions discussing patients' clinical cases. The Alexander Fleming Cancer Institute in Buenos Aires was the first hub in Latin America to implement Project ECHO for gastrointestinal tumors. In our 3-year experience, 80 patients from 37 centers were evaluated within Project ECHO and a range of three

to five cases were discussed in each meeting. From our perspective, the impact of this novel approach was a remarkable strategy to reduce care disparities by equalizing access to high-quality medical knowledge in a multidisciplinary environment for medical discussions. Additionally, it was shown to have a cost-effective impact directly on the patients and the local health system, since relevant costs were saved after unnecessary treatments, studies and travel expenses were avoided.

Key Words: Tumor board; Virtual; Gastrointestinal; Cancer disparities; Oncology; Extension for Community Healthcare Outcomes project

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Core Tip: Tumor boards (TBs) have existed for the last 50 years, and at the present time, represent an essential strategy in daily clinical oncology practice. We reported our positive experience and perspective with a novel approach of multidisciplinary virtual TBs using an innovative telementoring model called Project Extension for Community Healthcare Outcomes. This first experience in Latin America for gastrointestinal tumors has shown to reduce care disparities by equalizing access to high-quality medical knowledge in a context of a multidisciplinary environment for medical discussions.

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INTRODUCTION

During recent decades, cancer care has become increasingly complex mainly due to the personalized approach for every single patient. Each patient requires a careful assessment which often involves a multidisciplinary effort and experienced physicians. However, many countries experience the centralization of tertiary referral cancer units in the most populated cities. In many urban centers in Argentina, medical oncologists provide care for patients with multiple types of cancer which challenges practitioners to stay current with the evidence that is necessary to deliver high-quality care. However, given the increasing knowledge of cancer, and the observed trend of subspecialization among oncologists, tumor boards (TBs) have become a widely accepted and successful strategy to promote discussion and evidence-based decisions in a scenario with unequal health access[1,2].

A TB is a multidisciplinary treatment planning approach in which health professionals with different specialties review and discuss the diagnosis and treatment strategies using an integrative approach[3]. In the past few years, TBs have incorporated virtual modalities to make them accessible in remote locations, promoting timely diagnostic and treatment planning for patients in different regions and socioeconomic settings.

In this context, Project Extension for Community Healthcare Outcomes (ECHO) created by Dr. Sanjeev Arora in 2003 in New Mexico, EEUU, uses video conferencing technology to discuss cases and treat rural cases of hepatitis-C[4]. It aims to reduce health disparities by allowing clinicians to share current medical knowledge in underserved and remote areas to cooperate with the care of patients with hepatitis C through innovative telementoring. The ECHO model uses a hub-and-spoke knowledge-sharing approach where expert teams lead virtual meetings amplifying the capacity for providers to deliver best-in-practice care to underserved areas in their communities (Figure 1)[5]. This "hub and spoke" design consists of a model that arranges a network consisting of a team of experts (the hub) that offers a full service to multiple participants (the spokes) during regularly scheduled sessions where patients with clinical cases that need a more accurate treatment are discussed[6,7]. The use of this design also provides the capability to facilitate clinical mentoring and the implementation of regular educational sessions for medical training. Thus, the ECHO approach represents a completely different model than "telemedicine", wherein a specialist assumes the care of a patient in a typical consultation by using remote technology.

Currently, over 373 academic centers serve as ECHO hubs for multiple severe medical conditions such as infectious diseases, rheumatologic diseases, chronic pain, addiction, human immunodeficiency virus, diabetes, complex multisystem diseases and cancer[8,9].

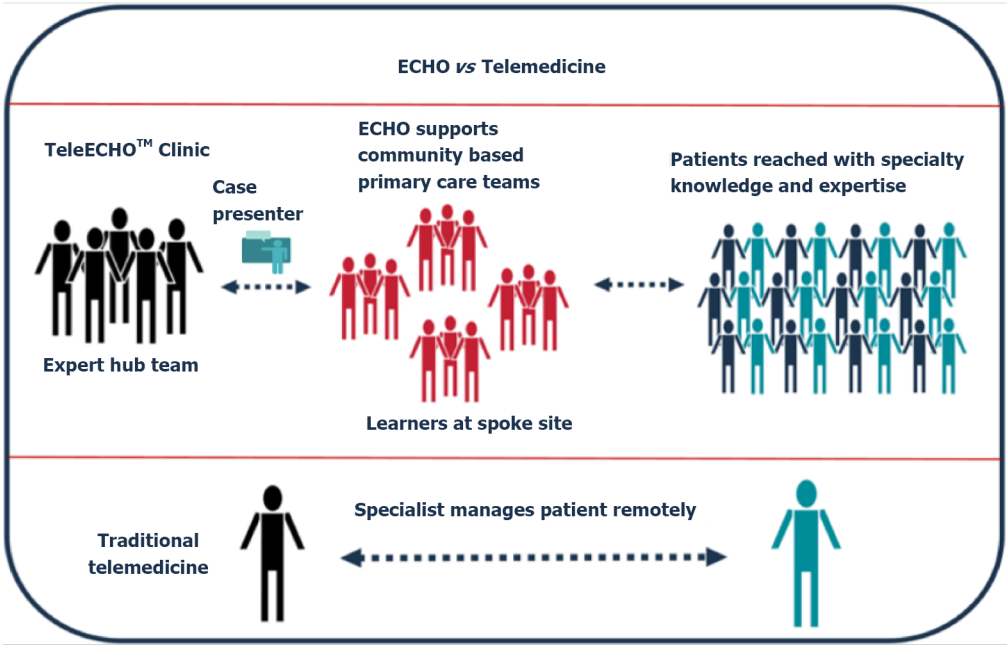


Figure 1 Extension for Community Healthcare Outcomes hub and spoke model. Citation: ECHO. Project ECHO. [cited 11 August 2021]. Available from: https://hsc.unm.edu/echo/. Copyright© Project ECHO 2022. Published by ECHO.

Multidisciplinary virtual TBs represent an opportunity to reduce the existing care disparities by information equality. Under this premise, the Alexander Fleming Cancer Institute in Buenos Aires was the first hub in Latin America to implement Project ECHO for gastrointestinal tumors. Since December 2017, monthly virtual meetings (an hour and a half long) using videoconferencing software have been performed to discuss cases of gastrointestinal cancer. The participating physicians had primary practices in academic and community general hospitals in at least 8 provinces of Argentina. The central hub staff was composed of surgeons, radiotherapists, imaging specialists, pathologists, genetic counselors, gastroenterologists and medical oncologists specifically dedicated to gastrointestinal cancer care. The modality included a case-based discussion followed by a moderated discussion with a final medical recommendation taking into account local available resources. The first 15 min of each meeting were dedicated to an educational lecture.

In our 3-year experience, 80 patients were evaluated within the Project ECHO, and a range of three to five cases was discussed in each meeting (Table 1). During the first 2 years, 15 health centers regularly participated in the virtual meetings. Notably, exponential growth was observed concurrently with the coronavirus disease 2019 (COVID-19) quarantine. Since June 2020, professionals from an average of 37 centers have habitually participated in these monthly meetings. Of note, each participating institution decided and proposed to the expert hub team the most relevant clinical cases that required a multidisciplinary discussion to the expert hub team. The median time from the first oncology visit until the ECHO referral was 16 d (range 12-19).

Most patients included in the program were diagnosed with colorectal cancer (n = 43, 53.75%), followed by neuroendocrine (n = 14, 17.5%), esophagogastric (n = 12, 15%), biliodigestive (n = 7, 8.75%), anal (n = 3, 3.75%) and appendix (n = 1, 1.25%) tumors. Most patients had advanced disease at the time of presentation to the TB (n = 28, 40%). Case discussions included systemic treatment for the advanced scenario, surgical approaches, and adjuvant decisions. Of note, the suggested strategies were mostly managed at local places (n = 60, 75%); other patients were referred to the Alexander Fleming Cancer Institute or tertiary health care centers (n = 8, 10%) for surgery or chemotherapy (n = 10, 12.5%) and a minority of cases were referred for radiotherapy (n = 2, 2.5%). Notably, during the COVID-19 pandemic period, only 6% of the patients were suggested to receive centralized treatment at a tertiary center. In addition, participant satisfaction was evaluated by a centralized digital survey provided to 30 professionals showing the highest level of satisfaction in 25 (83%) participants.

TBs have existed for the last 50 years and have been proven to improve medical training and, in the long run, patient care[10]. Under current conditions, Project ECHO emerges as a collaborative and integrative networking environment for cancer management in remote locations. The impact of novel virtual TB approaches in Argentina is a remarkable strategy to reduce care disparities by equalizing access to a multidisciplinary environment for medical discussions. Furthermore, these models have proven to be consistently cost-effective. Available evidence has highlighted that relevant costs were saved after unnecessary treatments, studies and travel expenses were avoided. The latter is particularly relevant, considering the vast extensions of Argentina[11-14]. As a typical example, a patient with a specific gastrointestinal tumor who would need to travel and have a consultation at a reference cancer

Table 1 Characteristics

Characteristics	*n* = 80
Age (range)	57 yr (48-68)
Sex	
Male	45 (56.25%)
Female	35 (43.75%)
Tumor type	
Colorectal cancer	43 (53.75%)
Neuroendocrine	14 (17.5%)
Esophagogastric	12 (15%)
Biliodigestive	7 (8.75%)
Anal	3 (3.75%)
Appendix	1 (1.25%)
Stage	
Locally/locally advanced	52 (65%)
Metastatic	28 (35%)
Treatment strategy management	
Local institution	60 (75%)
Referred to specialized cancer center	20 (25%)
Reference areas of Argentina (Province)	
North	40 (50%)
Center	24 (30%)
South	16 (20%)

center in Argentina would have to spend approximately 500 USD regardless of the study and treatment. Additionally, in terms of saving time, this strategy could normally take approximately 3 more weeks in delaying the treatment plan decision in Argentina.

Our heal system is heterogeneous, including the private and public sub-systems. Under this circumstance, some patients have to be referred to tertiary or local centers for coverage of treatment and studies to become effective. We believe that the discussion of the clinical cases in a context such as the ECHO initiative represents one of the better chances for high-quality cancer care considering that the referral does not cause a significant delay in the treatment. In our health system context, the virtual ECHO initiative would be more accessible, accurate, affordable and properly developable than the strategy of extending more sub-specialized oncologists in urban and suburban areas.

Our Project ECHO experience has led us to address some important factors that should be improved upon in the future. Internet access, low-quality video-conferencing devices and protected time availability are some of the key areas to expand in the future. The participation of professionals with non-oncology medical specialties should also be promoted to facilitate a comprehensive discussion of the multiple dimensions that are involved in cancer care.

One of the potential limitations of the virtual TB approach could be the lack of complete information on the clinical case for proper and personalized clinical decision-making, given that the medical opinions of the board are based on the case presentation and not on the direct evaluation of the patient. Additionally, monthly meetings may not meet the demand, mainly in situations when a medical decision is urgent.

Although limited, our experience was extremely positive. We are convinced that this strong professional network also creates a unique opportunity to promote national evidence-based recommendations, academic collaborations and clinical cancer research, as well as continuing medical education programs.

CONCLUSION

In our view, multidisciplinary virtual experiences, such as the Project ECHO, should be carefully addressed by health care decision-makers given their popularity and their demonstrated cost-effectiveness. Many of the evaluated barriers require government participation to improve budget and technology access in health care facilities. The COVID-19 pandemic has led to a tremendous need to incorporate modern technology into different work scenarios. Under these circumstances, the implementation of virtual educational and medical activities may be one of the key elements that cannot be excluded in the design and execution of National Cancer Control Programs.

FOOTNOTES

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REVIEW

New perspectives in the management of small cell lung cancer

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Abstract

The treatment of small cell lung cancer (SCLC) is a challenge for all specialists involved. New treatments have been added to the therapeutic armamentarium in recent months, but efforts must continue to improve both survival and quality of life. Advances in surgery and radiotherapy have resulted in prolonged survival times and fewer complications, while more careful patient selection has led to increased staging accuracy. Developments in the field of systemic therapy have resulted in changes to clinical guidelines and the management of patients with advanced disease, mainly with the introduction of immunotherapy. In this article, we describe recent improvements in the management of patients with SCLC,

review current treatments, and discuss future lines of research.

Key Words: Small cell lung cancer; Whole-brain radiotherapy; Prophylactic cranial irradiation; Stereotactic body radiotherapy; Immunotherapy; Atezolizumab; Durvalumab

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Core Tip: The treatment of small cell lung cancer (SCLC) continues to be a challenge. Recent studies have described survival benefits achieved by new treatments or combinations of treatments that are both safe and effective. Immunotherapy has a new role in SCLC. Nevertheless, continued research efforts are needed. Here, we review the current management of SCLC and discuss recent improvements and future lines of research.

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INTRODUCTION

Small cell lung cancer (SCLC) accounts for 14% of all lung cancers[1,2], and most cases are associated with tobacco use[3]. Although the global incidence of SCLC is falling, the ratio of male to female cases is currently 1:1[1,2]. SCLC is a fast-growing cancer, and most patients have extensive disease when diagnosed. In approximately one-third of cases, the cancer is limited to the thorax and can be treated with concurrent chemotherapy and radiotherapy. Just a small percentage of patients are amenable to surgery and adjuvant therapy. The goal of treatment in patients with extensive disease is to alleviate symptoms and prolong survival, although long-term survivorship in this setting is rare[4].

LIMITED-STAGE DISEASE

Surgical treatment of SCLC

Early-stage SCLC, stage I and IIA (T1-2N0M0) SCLC in the American Joint Committee on Cancer/International Union Against Cancer classification[5-7], accounts for 7% of all SCLCs and 0.29% of all lung cancers[8]. Numerous studies have shown excellent survival rates in patients with SCLC cT1-2N0M0 treated with surgery as part of a multimodal approach[6,9-28] (Table 1).

Surgical resection followed by adjuvant therapy is currently recommended by most clinical guidelines for operable stage I and IIA SCLC. Choice of adjuvant treatment varies according to pathologic tumor-node-metastasis stage: Chemotherapy for pN0, chemotherapy ± radiotherapy for pN1 and chemoradiotherapy for pN2[29-32] (Figure 1). The indications for the surgical treatment of SCLC can be summarized as follows: (1) Intraoperative diagnosis of a pulmonary SCLC nodule. Between 3% and 5% of SCLCs present as a pulmonary nodule. Multidisciplinary treatment involving surgical resection, systematic nodal dissection, and adjuvant chemotherapy or chemoradiotherapy can achieve survival rates comparable to those seen in non-SCLC[8]; (2) Diagnosis of stage I or IIA SCLC. Local or regional recurrence[33-39] (tumor and/or hilar-mediastinal lymph nodes) is the most common form of disease in patients who relapse after complete remission with chemoradiotherapy[40-45]. Surgery as part of a multimodal approach achieves better local disease control[46-50] than chemoradiotherapy[51-54]; (3) Mixed histology (SCLC with a non-SCLC component). Between 2% and 28% of patients have mixed SCLC/non-SCLC[55-59]. Recurrence or failure to respond to first-line chemotherapy is likely to be due to the non-SCLC component; and (4) Salvage surgery for local chemo-resistant SCLC or exclusively local recurrence after response to chemoradiotherapy. Selected patients in this setting might benefit from surgical resection[60-62].

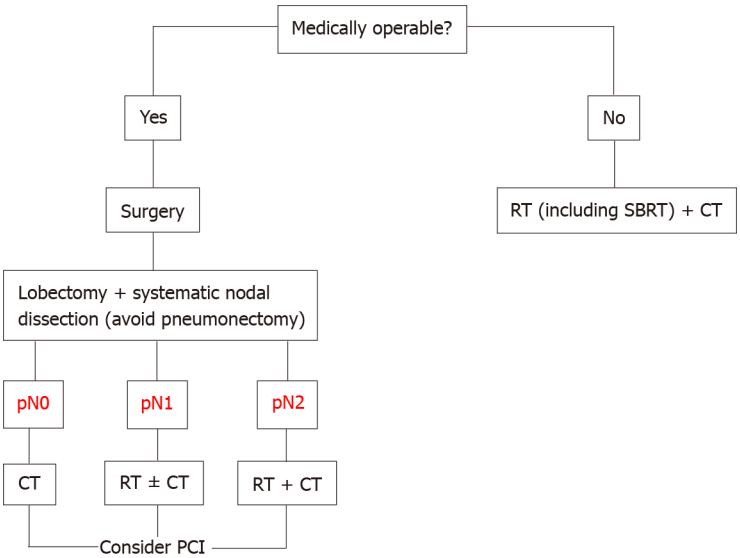
Lobectomy is the preferred procedure for surgical resection, as it is associated with significantly better survival than sublobar resection[40,45,49,54,63]. The significant discrepancies observed between clinical and pathologic stages (mainly due to undetected lymph node metastasis before surgery) highlight the importance of accurate clinical nodal staging and systematic lymph node dissection[47,64]. The recommendations for ruling out hilar and mediastinal lymph node involvement are very similar across the different guidelines. Ideally, clinical staging should be performed using semi-invasive

Table 1 Surgical and survival rates for patients with small cell lung cancer (period time revised 1999-2020) - (dash), lack of information or details

Ref.	Study type & time period. LoE	Inclusion criteria	Number of patients	Neoadjuvant/adjuvant treatments	PCI	Survival data
Jin *et al*[9], 2018	RS; SEER 2004-2013; 3A	cI-II	*n* = 2129; S: 387; RT 1032; S + RT: 154; No S or RT: 556		-	5-yr OS T1N0: 46.0% S *vs* 23.8% RT; 5-yr OS T2N0: 42.6% S *vs* 24.7% RT; T3N0 or T1-2N1 (stage IIB) patients treated with S did not have higher 5-yr OS rates than those treated with RT
Yang *et al*[10], 2018	RS; NCDB 2003-2011; Propensity score match S + AC *vs* CRT; 3A	cT1-2N0M0	S + AC: 501; CRT: 501	S + AC: 501	-	5-yr OS: 47.6% S + AC *vs* 29.8% CRT ($P < 0.01$)
Ahmed *et al*[11], 2017	RS; SEER 2007-2013; 3A	Stage I SCLC	*n* = 1902; S: 427; S + RT: 115	-	-	MST: 50 mo (S); MST: 60 + mo (S + RT)
Wakeam *et al*[12], 2017	RS; NCDB 2004-2013; 3A	cT1-2N0M0	*n* = 5079	-		MST: 25.3 mo
Wakeam *et al*[13], 2017	RS; NCDB 2004-2013; Stage-specific propensity score match S *vs* NST; 3A	cI-III	*n* = 2619	No AD treatment 24% NC or NR 4%; AC 27%; AR 1%; ACR 32%; NC or NR and AC or AR 2%; Other 10%	-	MST cI 38.6 *vs* 22.9 mo S *vs* NST; MST cII 23.4 *vs* 20.7 mo S *vs* NST; MST cIIIA 21.7 *vs* 16.0 mo S *vs* NST
Combs *et al*[14], 2015	RS; NCDB 1998-2011; 3A	cT1-3N0-2 SCLC	*n* = 2476; S 841 cIA, 168 cIB	All; S: 68%	-	5-yr OS: 54% (cIA); 36% (cIB)
Ogawa *et al*[15], 2012	RS; Institutional 1995-2008; 4	cI-III; pI-III SCLC	*n* = 28 (23 SCLC before S); S 21 cI, 5 cII, 7 cIII2	NC 8; AC 19, ACR 2	-	5-yr OS 47%
Ju *et al*[16], 2012	RS; Institutional 1990-2009; 4	pI-III	*n* = 34	NC 3; AC 1, AR 19, 10 CRT	-	5-yr OS 66%
Vallières *et al*[6], 2009	RS; IASLC 1990-2000; 3A	Resected SCLC	*n* = 349 (68 pIA, 91 pIB)	-	-	5-yr OS: 53% (pIA); 44% (pIB)
Lim *et al*[17], 2008	RS; Institutional 1980-2007; 4	cI-cIIIB	*n* = 59	AC 13; AR 2; ACR 1	-	5-yr OS for all patients 52%; No difference in 5-yr survival across; cT and cN categories; No difference in 5-yr survival across; cI to cIII stages
Wang *et al*[18], 2007	RS; Institutional; 4	pI-III	*n* = 122	QT & CRT (not specified)	-	MST 50 mo; 5-yr OS 66%
Veronesi *et al*[19], 2007	RS; Institutional; 4	cI-IIIA	*n* = 23	AC all	-	MST 24 mo
Tsuchiya *et al*[20], 2005	Prospective phase II trial; 1991-1996; 2B	cI-IIIA	*n* = 62	AC 42 (69%)	-	MST not reached in pI; MST 449 d for pII; MST 712 d for pIIIA; 3-yr OS 61%; 3-yr survival rate cI, cII, cIIIA 68%, 56% and 13% respectively
Brock *et al*[21], 2005	RS; Institutional 1976-2002; 4	Resected SCLC	*n* = 82 (24 stage I, S + AC)	AC 55%	23%	5-yr OS: 86% (platinum AC); 42% (non-platinum AC)
Nakamura *et al*[22], 2004	RS; Institutional; 4	cI-III SCLC	*n* = 69	S 37, NC 32, AC 41, ACR 7	-	5-yr survival 48.9 % cI, 33.3 % cII, 20.2 % cIIIA, 0 % cIIIB
Badzio *et al*[23], 2004	Comparative RS; Institutional 1984-1996; 4	cI-III balanced in both, S and NST groups	*n* = 134	S 67 (all AC); NST 67 (all QT)	34% only S group	MST 22 mo (S); MST 11 mo (NST); 5-yr OS S 27%, NST 4%
Lewiński *et al*[24], 2001	R; Institutional 1976-2002; 4	cI-IIIA SCLC	*n* = 75	NC all	If CR to NC	MST N0+1 25 mo; MST N2 14 mo; MST resected 18 mo; 5-yr OS resected 29%
Cataldo *et al*[25], 2000	RS; Institutional 1982-1992; 4	cI-III SCLC	*n* = 60	AC 88%; pII AR (11%); pIII AR (21%)	41%	5-yr survival rate 40% pI, 36% pII and 15% pIII

Inoue *et al*[26], 2000	RS; Institutional 1975-1994; 4	Resected SCLC	*n* = 91 (32 cIA, 30 cIB)	All 78%	5.5%	MST 53 mo, 5-yr OS 49% (cIA); MST 25 mo, 5-yr OS 47% (cIB)
Kobayashi *et al* [27], 2000	RS; Institutional 1982-1992; 4	cI-III SCLC	*n* = 59	NC 71%	-	5-yr survival rate 55% pI, 33% pII, 23% pIII
Eberhardt *et al* [28], 1999	Prospective phase II trial; Institutional 1991-1995; 2B	cIB-cIIIB	*n* = 46	IB/IIA had NC + S; IIB/IIIA had NCR + S	-	MST all patients 36 mo; MST R0 patients 68 mo; 5-yr survival rate all patients 46%; 5-yr survival rate R0 patients 63%

ACR: Adjuvant chemoradiotherapy; AD: Adjuvant; AC: Adjuvant chemotherapy; cIA: Clinical stage IA; cIB: Clinical stage IB; CR: Complete response; CRT: Chemoradiotherapy; IASLC: International Association for the Study of Lung Cancer; ISC-LCSG: The Lung Cancer Study Group of the International Society of Chemotherapy; LoE: Level of evidence; MST: Median survival time; NC: Neoadjuvant chemotherapy; NST: Non-surgical treatment; NCDB: National Cancer Data Base; OS: Overall survival; PCI: Prophylactic cranial irradiation; pIA: Pathologic stage IA; pIB: Pathologic stage IB; pII: Pathologic stage II; pIIIA: Pathologic stage IIIA; pIIIB: Pathologic stage IIIB; QT: Chemotherapy; R0: Complete resection; RS: Retrospective study; RT: Radiotherapy; S: Surgery; SCLC: Small cell lung cancer; SEER: Surveillance, Epidemiology, and End Results database.



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Figure 1 Proposed algorithm for the treatment of early-stage small cell lung cancer focused on surgical treatment. CT: Chemotherapy; PCI: Prophylactic cranial irradiation; RT: Radiotherapy; SBRT: Stereotactic body radiation therapy.

techniques that enable biopsy and the pathologic study of lymph nodes (*e.g.*, transbronchial ultrasound and esophageal echoendoscopy) and invasive techniques such as video mediastinoscopy, anterior mediastinotomy, and videothoracoscopy.

Radiotherapy in limited-stage SCLC

Thoracic radiotherapy and stereotactic body radiotherapy in early-stage SCLC: SCLC is usually classified as limited-stage (LS) or extensive-stage (ES) disease[65]. With adequate treatment, overall survival (OS) is 16-22 mo in patients with LS-SCLC and 8-13 mo in those with ES-SCLC. The corresponding 5-year survival rates are < 20% and < 2%[66]. Radiotherapy is associated with better OS when given in the first few weeks after the start of chemotherapy (ideally during cycle 1 and never later than cycle 3), and the shorter the duration the better[67].

Hypofractionated radiotherapy is well tolerated and produces similar response rates to standard fractionation. Proposed schedules include 40 Gy in 16 fractions with chemotherapy and prophylactic cranial irradiation (PCI)[68] and 55 Gy in 25 once-daily fractions, also with chemotherapy and PCI[69]. Higher complete response rates and longer OS have been observed for hyperfractionated *vs* hypofractionated radiotherapy (45 Gy in 30 fractions twice daily *vs* 42 Gy in 15 fractions twice daily), but the differences were not statistically significant[70].

Treatment must be individualized. Some clinical guidelines recommend surgery and adjuvant chemotherapy for stage I and IIA disease[30,71]. This combination has achieved OS rates of 50%-70%[20,21,72,73]. Nonetheless, stereotactic body radiotherapy (SBRT) should be considered in patients who are unfit for or refuse surgery, as it is not inferior to conventional treatment and has an acceptable safety

profile (toxicity < grade 3)[74-80]. Although the evidence is based on small series, SBRT can achieve local control rates > 85%. No clear benefit, however, has been observed for OS (63%-83% at 1 year, 35%-76% at 2 years, and 21%-26% at 3 years) (Table 2). This could have several explanations. First, SCLC is a fast-spreading tumor (associated with distant metastases in 50% of cases), requiring clinicians to consider neoadjuvant or adjuvant chemotherapy (preferably adjuvant in the case of SBRT due to its short treatment time), particularly in the case of tumors > 2 cm[77-81]. Adjuvant chemotherapy can improve OS by up to 25%[82]. Second, the disease may have been initially understaged. Thus, staging with positron emission tomography-computed tomography (CT) and mediastinoscopy/endobronchial ultrasound is recommended before proposing surgery or SBRT. SBRT should be planned using intensity-modulated techniques (*e.g.*, intensity-modulated radiotherapy, volumetric modulated arc therapy) and delivered with image-guided inter- and/or intrafraction monitoring (*e.g.*, Conebeam, ExacTrac) and respiratory control (*e.g.*, four-dimensional CT, deep inspiration breath hold, active breathing control, gating). The number of fractions can vary, but a biologically effective dose of >100 Gy must be delivered to the isocenter of the tumor. Because SCLC is highly radiosensitive, some groups have suggested using a lower dose, particularly in patients with ultracentral tumors[83].

PCI

Patients with SCLC are at high risk of brain metastases (BM)[84,85]. Research into the potential of PCI began in the late 1970s[86]. Brain magnetic resonance imaging (MRI) should be performed after chemoradiotherapy or systemic therapy[87], as 21.8%-32.5% of patients who achieve complete response subsequently develop BM[88,89]. A meta-analysis published by Aupérin *et al*[90] in 1999 showed that PCI was associated with a reduced incidence of BM at 3 years (59% *vs* 33%) and a 5.4% increase in OS. Subsequent meta-analyses have shown similarly favorable results for PCI in patients who had responded to treatment[91-94]. Most of these studies, however, were published before the introduction of restaging with brain MRI, and therefore the true benefit of PCI in LS-SCLC is not so clear[95,96]. Nonetheless, retrospective studies have described beneficial effects for PCI in patients with a previous negative brain MRI scan[97,98]. Patients who have undergone complete resection should benefit from PCI, except patients with stage I disease, who have a low risk of BM[99-101]. There is a growing interest in the use of brain MRI and stereotactic irradiation rather than PCI in patients with LS-SCLC[102], but prospective randomized trials are needed.

Concomitant treatment in locally advanced disease

Radical treatment with chemotherapy and concomitant radiotherapy are recommended for patients with stage IIB-IIIC disease in good general health[4,103]. Eighty percent of patients with mediastinal involvement treated exclusively with chemotherapy experience local recurrence[104], but the addition of radiotherapy lowers this rate and increases survival[104,105]. The CONVERT trial, which compared fractionated and unfractionated radiotherapy in patients treated with cisplatin-etoposide, reported an overall response rate (ORR) of 70%-90%, an OS of 24-30 mo, and a 5-year OS rate of 25%-30%[106]. Another two trials investigated the combination of bevacizumab, an angiogenic, with conventional chemoradiotherapy, but had to be discontinued because of a relatively high incidence of severe adverse events (tracheoesophageal fistulae)[107].

Perspectives for radiotherapy in LS-SCLC

Radiotherapy with immunotherapy in LS-SCLC: Three trials are currently analyzing the combined use of radiotherapy and immunotherapy in LS-SCLC: The NRG Oncology and Alliance trial (ClinicalTrials.gov Identifier: NCT03811002) investigating chemoradiotherapy with and without atezolizumab; the phase II STIMULI trial (NCT02046733) analyzing nivolumab and ipilimumab after chemoradiotherapy and PCI; and the phase III ADRIATIC trial (NCT03703297) comparing durvalumab, durvalumab plus tremelimumab, and placebo in patients without progression after chemoradiotherapy.

Hippocampal avoidance to reduce the neurotoxicity of PCI: The role of PCI with hippocampal avoidance (HA) in patients with LS- or ES-SCLS without BM is being investigated in three phase III trials: The Dutch NKI/AVL trial (NCT01780675), the NRG Oncology CC003 trial (NCT02635009), and the Spanish PREMER-TRIAL (NCT02397733)[108]. The Dutch group found no significant differences in recall assessed using the revised version of the Hopkins Verbal Learning Test between patients who received PCI and those who received HA-PCI[109]. Using the Free and Cued Selecting Reminding Test, the Spanish group found a significant decline in 3-mo delayed recall [22.22% *vs* 5.08%; odds ratio (OR) = 5.33; 95% confidence interval (CI): 1.44-19.65; P = 0.006) and total recall (20.63% *vs* 6.78%; OR = 3.57; 95%CI: 1.09-11.68; P = 0.02] in the PCI *vs* HA-PCI group[110]. Another potentially interesting line of research is the use of Alzheimer disease drugs to preserve cognition in patients treated with PCI[111].

Proton beam radiation therapy: In non-SCLC, proton therapy has been used to reduce doses to the heart while maintaining high doses to the tumor[112]. Proton beam radiation therapy (PBRT) is potentially beneficial in SCLC, as patients tend to have bulky central disease at diagnosis. In a study of 30 patients at the University of Pennsylvania, PBRT at a median dose of 63.9 cobalt Gy equivalents achieved a promising median OS of 28.2 mo with low toxicity[113]. These results need to be validated in

Table 2 Thoracic radiotherapy and stereotactic body radiotherapy in early-stage small cell lung cancer

Ref.	Sample size	Fractionation	QT	Prophylactic cranial irradiation	Local control	Overall survival	Disease-free survival
Videtic *et al*[76], 2013	*n* = 6	60 Gy (3 fx); 50 Gy (5 fx); 30 Gy (1 fx)	4/6	4/6	100% (1 yr)	63% (1 yr)	75% (1 yr)
Shioyama *et al*[77], 2015	*n* = 64	48 Gy (4 fx)	36/64	10/64	89% (2 yr)	76% (2 yr)	
Stahl *et al*[79], 2017	*n* = 285	48-60 Gy (3-5 fx)	130/285		35% (3 yr). 21.5% (5 yr)		
Verma *et al*[75], 2017	*n* = 74	50 Gy (5 fx)	45/74	17/74	96% (3 yr)		
Shioyama *et al*[78], 2018	*n* = 43	36-60 Gy (3-10 fx)	8/43	8/43	80.2% (2 yr)	72.3% (2 yr)	44.6% (2 yr)
Verma *et al*[74], 2019	*n* = 149	45-60 Gy (3-8 fx)	149/149		83.8% (29.2 mo)		
Newman *et al*[81], 2019	*n* = 239	BED > 100 Gy (max 8 fx)	84/239		27% (5 yr); 36% (5 yr, with QT)		
Singh *et al*[80], 2019	*n* = 21	BED 105.6 Gy (3-5 fx)	4/21		100% (1, 2, 3 yr)	73.1% (1 yr); 36.6% (2 yr)	85.7% (1 yr); 42.9% (2 yr)

BED: Biologically equivalent dose; fx: Fraction; QT: Chemotherapy.

further studies.

ES SCLC

Initial management

Chemotherapy with platinum compounds and etoposide has been the standard treatment for ES-SCLC for many decades. The COCIS meta-analysis showed that cisplatin- and carboplatin-based chemotherapy produced comparable results in terms of OS (9.6 *vs* 9.4 mo), progression free survival (PFS) (5.5 *vs* 5.3 mo), and ORR (67% *vs* 66% mo)[114]. Other strategies attempted, including maintenance treatments and combinations with antiangiogenics, have produced disappointing results[115-117]. The recently published results of the IMpower 133[118] and CASPIAN[119] trials comparing combinations of chemotherapy and immunotherapy followed by immunotherapy with standard platinum and etoposide chemotherapy in ES-SCLC have shown that the combined use of chemotherapy and immunotherapy prolongs OS.

IMpower133 is a phase III trial in which patients received four cycles of carboplatin and etoposide and either atezolizumab or placebo followed by maintenance atezolizumab[118]. The response rates in both arms were similar, but patients in the atezolizumab arm survived for a median of 2.3 mo longer [hazard ratio (HR) = 0.7; 95%CI: 0.54-0.91; P = 0.007]. The updated trial data presented at the 2019 European Society for Medical Oncology congress showed an increase in OS at both 12 mo (39% to 51.9%) and 18 mo (21% to 34%)[120,121].

The phase III CASPIAN trial has three treatment arms. Treatment with durvalumab plus chemotherapy (4-6 cycles of cisplatin or carboplatin plus etoposide) followed by durvalumab maintenance achieved an OS of 12.9 mo (*vs* 10.5 mo for standard chemotherapy) (HR = 0.75; 95%CI: 0.62-0.9; P = 0.0032), a 2-year PFS of 11% (*vs* 2.9%), and a 2-year response rate of 13.5% (*vs* 3.9%)[119,122]. In the third arm, tremelimumab plus durvalumab *vs* chemotherapy showed no benefit in antitumor activity and was associated with increased toxicity[123].

Results from other studies evaluating combinations of anti-programmed death 1 (PD-1) antibodies have been disappointing. While the combined use of pembrolizumab and chemotherapy increased PFS, it did not provide any significant improvements in OS[124]. In the phase II ECOG-ACRIN EA5161 trial, chemotherapy plus nivolumab followed by maintenance treatment achieved a non-significant improvement in PFS (5.5 *vs* 4.7 mo) and OS (11.3 *vs* 8.5 mo)[125] (Table 3). A systematic review and two meta-analyses published in 2020 concluded that a combination of chemotherapy with atezolizumab or durvalumab was the best first-line treatment for ES-SCLC[126,127]. Other options that have been explored include combinations of ipilimumab and chemotherapy (no benefit and greater toxicity)[128,129] and combinations of different chemotherapy agents, such as irinotecan plus etoposide and cisplatin plus irinotecan (also without benefits)[130-132].

Table 3 Combined first-line immunotherapy options for extensive-stage small cell lung cancer

Study	*n*	Design	Treatment	RR	PFS	OS
NCT01450761	1132	Phase III; Randomized, double-blind; Drug: Ipilimumab	Arm A: PE × 4C + ipilimumab × 4C; Control: PE × 4C + placebo × 4C	PR 62% *vs* 62%; SD 26% *vs* 27%; PD 6% *vs* 9%	4.6 *vs* 4.4 mo; HR = 0.85, *P* = 0.0161	11.0 *vs* 10.9 mo; HR = 0.94, *P* = 0.3775
Impower 133	403	Phase III. Randomized, double-blind; Drug: Atezolizumab	Arm A: PE + atezolizumab × 4C/atezolizumab; Control: PE + placebo × 4C/placebo	60% *vs* 64%	5.2 *vs* 4.3 mo; HR = 0.77, *P* = 0.02	12.3 *vs* 10.3 mo; HR = 0.70, *P* = 0.007
CASPIAN	805	Phase III. Randomized, open-label; Drug: Durvalumab	Arm B (*n* = 268): Durvalumab + PE × 4C/durvalumab; Control: PE × 4C	68% *vs* 58%	5.1 *vs* 5.4 mo; HR = 0.78, *P* not tested	13.0 *vs* 10.3 mo; HR = 0.73, *P* = 0.0047
CASPIAN	805	Phase III. Randomized, open-label; Drug: Durvalumab + tremelimumab	Arm A (*n* = 268): Durvalumab + tremelimumab + PE × 4C/durvalumab + tremelimumab. Control: PE × 4C	58% both arms	4.9 *vs* 5.4 mo; HR = 0.84	10.4 *vs* 10.5 mo; HR = 0.82, *P* = 0.045
KEYNOTE 604	453	Phase III; Randomized, double-blind; Drug: Pembrolizumab	Arm A: Pembrolizumab + PE; Control: PE + placebo	71% *vs* 62%	4.5 *vs* 4.3 mo; HR = 0.75, *P* = 0.0023	10.8 *vs* 9.7 mo; HR = 0.80, *P* = 0.0164
ECOG-ACRIN	160	Phase I. Randomized, open-label; Drug: Nivolumab	Arm A: PE + nivolumab × 4C/nivolumab; Control: PE × 4C	52.29% *vs* 47.71%	5.5 *vs* 4.6 mo; HR = 0.65, *P* = 0.012	11.3 *vs* 8.5 mo; HR = 0.67, *P* = 0.038

4C: 4 cycles; OS: Overall survival; PD: Progressive disease; PE: Platinum and etoposide; PFS: Progression free survival; PR: Partial response; RR: Response rate; SD: Stable disease.

PCI in ES-SCLC

The results of the first randomized trial to demonstrate a reduction in the risk of symptomatic BM (14.6% *vs* 40.4% at 1 year) and an improvement in OS (27.1% *vs* 13.3%) in chemotherapy responders who underwent PCI were published in 2007[133]. The results are supported by data from several meta-analyses[134-136], although as a shortcoming of the trial, pre-PCI brain imaging was not performed [133]. The results of a randomized trial conducted in Japan comparing PCI with close MRI follow-up in patients with ES-SCLC who had responded to chemotherapy and had a negative brain MRI were published in 2017. While they did not show an increase in OS (11.6 mo for PCI *vs* 13.7 mo for MRI follow-up; HR = 1.27; 95%CI: 0.96-1.68; *P* = 0.094), they did show a significant decrease in the incidence of BM[137].

A recent meta-analysis showed that PCI was only associated with prolonged OS in studies where brain imaging was not performed between chemotherapy and irradiation (HR = 0.70; 95%CI: 0.57-0.85). In other words, PCI did not offer any significant benefits when preceded by MRI or CT to test for BM (HR = 0.94; 95%CI: 0.74-1.18)[138]. Considering the above results and the neurotoxic effects of PCI[139], it would seem reasonable to consider periodic MRI examination as an alternative to PCI in patients with ES-SCLC. In such cases, a joint evaluation should be made by the medical and radiation oncologists[30]. The recommended dose for PCI is 25 Gy in 10 fractions, as higher doses do not appear to reduce the incidence of BM at 2 years and are associated with higher mortality and chronic neurotoxicity[140].

Treatment of refractory and relapsed SCLC

Relapsed SCLC tends to be resistant to treatment and is associated with an OS of 4-5 mo. Response to second-line treatment varies according to PFS and is 10% in patients with a PFS < 3 mo (refractory SCLC) and 25% in those with a PFS of 3-6 mo (sensitive SCLC)[141-143].

Relapse after PFS > 3 mo: Rechallenge treatment with combinations of platinum-based chemotherapy has been investigated in patients with sensitive SCLC. Patients treated with carboplatin and etoposide had a longer PFS than those treated with topotecan, and the greatest benefits were observed for those who relapsed after 6 mo[144,145].

Relapse after PFS of < 3 mo: Until recently, topotecan was the only drug authorized by the US and Food and Drug Administration (FDA) to treat relapsed SCLC. In the 2006 phase III trial that led to its approval, it significantly improved survival compared with best supportive care only[146]. Another phase III trial comparing topotecan and CAV (cyclophosphamide, doxorubicin, and vincristine) reported similar survival and response rates for the two treatments, but found topotecan to be associated with better symptom control and lower toxicity[147]. An additional study evaluating topotecan plus aflibercept, an antiangiogenic, reported an OS of 5 mo[148].

One recent advance in this setting is the recent approval by the FDA of lurbinectedin as a second-line treatment for SCLC. In a study of patients with SCLC without BM, lurbinectedin achieved an ORR of 35%, and a median response duration of 5.1 mo (> 6 mo in 25% of patients)[149]. The combination of

lurbinectedin and doxorubicin was investigated in two cohorts in a phase I trial and showed disease control rates of 81% and 70% and a median response duration of 4.5 and 5.2 mo[150]. These findings led to the design of the phase III ATLANTIS trial comparing lurbinectedin plus doxorubicin with topotecan and with CAV; a press release, however, announced no improvement in OS[151] (Figure 2).

Amrubicin is available for the treatment of relapsed SCLC in Japan, but it has not been approved by the FDA. A phase III trial comparing amrubicin with topotecan showed superior symptom control for topotecan but no significant differences in OS[152]. Immune checkpoint inhibitors have also been tested. The CheckMate 032 trial comparing nivolumab alone with nivolumab plus ipilimumab in recurrent SCLC reported improved ORR and OS in both treatment arms regardless of prior treatment or PD-L1 expression[153,154]. With these data, the FDA approved nivolumab for use in previously treated patients.

The phase III CheckMate 331 trial showed similar OS for nivolumab *vs* standard chemotherapy in the second-line treatment of SCLC[155]. Pembrolizumab has also been tested in SCLC. A pooled analysis of the KEYNOTE-028 (phase Ib)[156] and KEYNOTE-158 (II)[157,158] trials found an ORR of 19.3%, leading to FDA approval. Atezolizumab was also tested in a phase II trial, but the primary endpoint was not met[159]. Paclitaxel every 3 wk for 6 cycles plus pembrolizumab after the second cycle until disease progression achieved a disease control rate of 80% and a median OS of 9.2 mo[160]. Other drugs tested in the setting of relapsed SCLC are temozolomide[161,162], irinotecan[163], paclitaxel[164,165], docetaxel[166], gemcitabine[167,168], and vinorelbine[169]. Finally, a recent phase IIb study showed that belotecan was associated with better OS and disease control than topotecan in patients with sensitive SCLC[170].

Recent advances in systemic therapy

New drugs linked to targets with a role in cell proliferation have been developed. These include poly (ADP-ribose) polymerase (PARP) inhibitors, delta-like ligand 3 inhibitors (DLL3), and drugs that selectively inhibit oncogenic transcription. The expression of DNA damage response proteins [especially PARP1/checkpoint kinase 1 (CHK1)] is elevated in SCLC, and *in vitro* studies have shown an antitumor effect for PARP inhibitors[171]. Monotherapy with PARP inhibitors has also been investigated in different clinical trials, but the results have been disappointing. In an early study, talazoparib showed an ORR of 8.7%[172]. No benefit was observed for maintenance treatment with olaparib after first-line chemotherapy with cisplatin and etoposide[173] or for the addition of veliparib *vs* placebo to first-line cisplatin and etoposide, with findings showing no significant differences in PFS (6.1 *vs* 5.5 mo) or OS (10.3 *vs* 8.9 mo)[174,175].

Discordant results have been reported for combinations of chemotherapy and PARP inhibitors in successive treatment lines. No significant differences were found for PFS or OS in a study comparing temozolomide plus veliparib *vs* temozolomide only[176]. Temozolomide combined with olaparib, however, was associated with a response rate of 41.7%, a PFS of 4.2 mo, and an OS of 8.5 mo in a phase I/II clinical trial[177]. No benefits have been observed for the combined use of PARP inhibitors and immunotherapy (durvalumab with olaparib, among others)[178]. Future actions targeting this actionable molecular pathway in SCLC will probably involve combinations of PARP inhibitors and chemotherapy agents and immunotherapy, or new molecules. Promising results have been reported for CHK1 (SRA737) combined with low-dose gemcitabine and anti-PD-1/programmed death ligand 1 (PD-L1) immune checkpoint inhibitors[179] and for PARP inhibitors combined with WEE1 inhibitors, which act at the cell-cycle level[180].

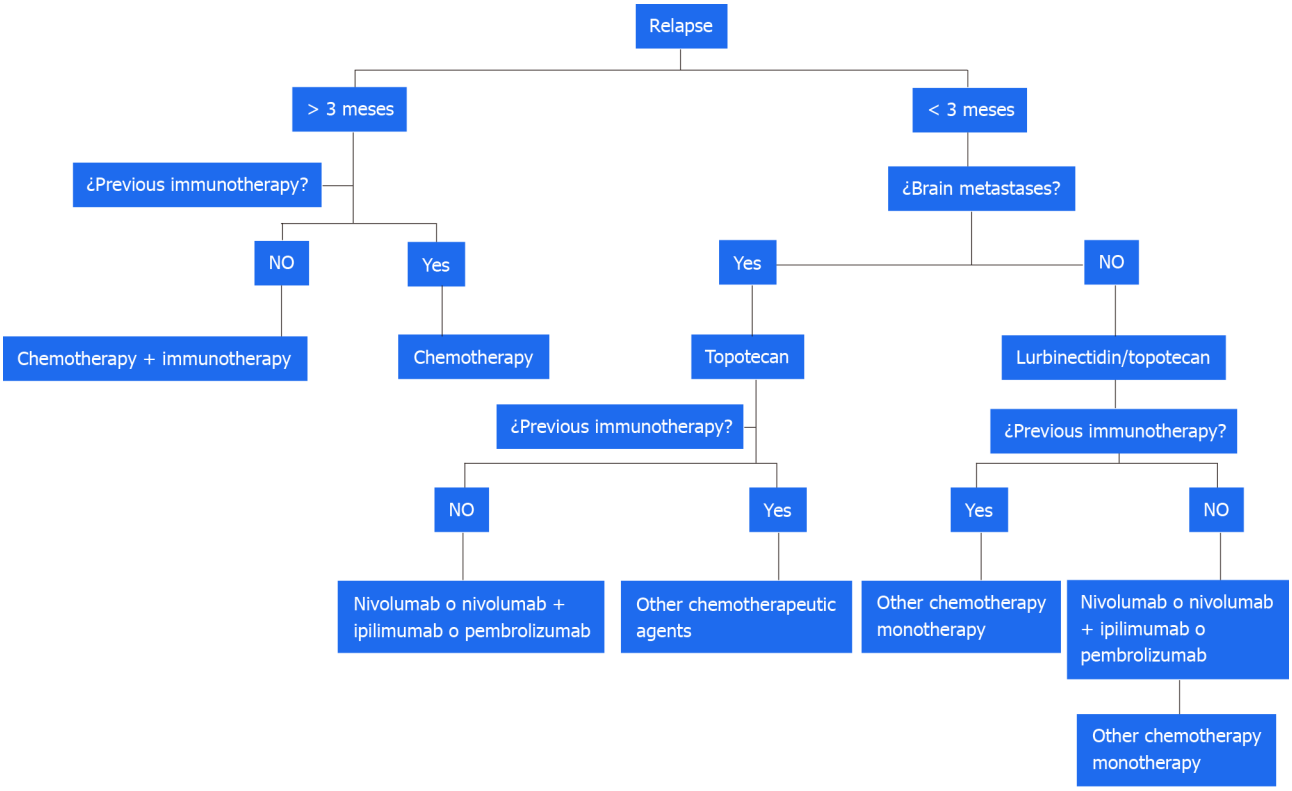
Other treatments have also yielded positive results. Lurbinectedin, a selective oncogenic transcription inhibitor, was recently evaluated in combination with irinotecan in pretreated patients in a phase Ib/II basket trial. The results for the SCLC cohort showed an ORR of 62%, a clinical benefit rate of 81%, a disease control rate of 90%, and a PFS of 6.1 mo[181]. Other new molecules with different ligands under investigation include DLL3 inhibitors, such as rovalpituzumab-tesirine. This is a promising drug in pretreated patients expressing DLL3, although recent reports have described greater toxicity and little benefit compared with topotecan[182-184]. AMG 757, a half-life extended DLL3 bispecific T-cell engager, has also shown promising results in pretreated patients in an ongoing phase I trial, with an ORR of 14%, a disease control rate of 37%, and a very promising median duration of 6.2 mo[185].

Perspectives for radiotherapy in ES-SCLC

Numerous questions remain to be answered regarding the role of radiotherapy in ES-SCLC.

Consolidation radiotherapy in extensive SCLC: What is the optimal radiation dose or indication for patients with complete thoracic response or partial distant response? The Chinese phase III trial (NCT02675088) is comparing 45 Gy at 3 Gy/d in 15 fractions *vs* 10 fractions (CREST trial schedule) with a primary endpoint of OS at 2 years[186]. How can radiotherapy be best combined with immunotherapy? The RAPTOR phase II/III trial (NCT04402788) is evaluating the use of radiotherapy to the chest and distant lesions after 4-6 cycles of carboplatin and etoposide plus atezolizumab.

Stereotactic radiosurgery to treat BM: Stereotactic radiosurgery has not traditionally been investigated in SCLC due to the high incidence of BM and poor prognosis. Nonetheless, there is growing evidence



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Figure 2 Proposed algorithm for the treatment of relapsed small cell lung cancer.

that it may be appropriate[187]. ENCEPHALON, a phase II trial (NCT03297788) is currently comparing stereotactic radiosurgery with whole-brain radiotherapy in patients with SCLC and 1-10 BM.

CONCLUSION

The treatment of SCLC will continue to be a challenge. Immunotherapy has a new role lung cancer and will be the future treatment standard alone or in combination, as well as the new radiotherapy techniques. As has been occurred in non-SCLC, the future of treatments in both early and advanced stages is through immunotherapy and targeted treatments. Furthermore, the use of different combinations of chemoimmunotherapy in recent months has improved the prognosis of patients with advanced SCLC. Nevertheless, continued research efforts are needed. Different lines of investigation are open and we hope that their findings will continue to improve prognosis and quality of life in this setting.

FOOTNOTES

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REVIEW

Immunotherapy for advanced hepatocellular carcinoma: From clinical trials to real-world data and future advances

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Abstract

Hepatocellular carcinoma (HCC) is a leading cause of cancer-associated mortality worldwide. HCC is an inflammation-associated immunogenic cancer that frequently arises in chronically inflamed livers. Advanced HCC is managed with systemic therapies; the tyrosine kinase inhibitor (TKI) sorafenib has been used in 1st-line setting since 2007. Immunotherapies have emerged as promising treatments across solid tumors including HCC for which immune checkpoint inhibitors (ICIs) are licensed in 1st- and 2nd-line treatment setting. The treatment field of advanced HCC is continuously evolving. Several clinical trials are investigating novel ICI candidates as well as new ICI regimens in combination with other therapeutic modalities including systemic agents, such as other ICIs, TKIs, and anti-angiogenics. Novel immunotherapies including adoptive cell transfer, vaccine-based approaches, and virotherapy are also being brought to the fore. Yet, despite advances, several challenges persist. Lack of real-world data on the use of immunotherapy for advanced HCC in patients outside of clinical trials constitutes a main limitation hindering the breadth of application and generalizability of data to this larger and more diverse patient cohort. Consequently, issues encountered in real-world practice include patient ineligibly for immunotherapy because of contraindications, comorbidities, or poor performance status; lack of response,

efficacy, and safety data; and cost-effectiveness. Further real-world data from high-quality large prospective cohort studies of immunotherapy in patients with advanced HCC is mandated to aid evidence-based clinical decision-making. This review provides a critical and comprehensive overview of clinical trials and real-world data of immunotherapy for HCC, with a focus on ICIs, as well as novel immunotherapy strategies underway.

Key Words: Hepatocellular carcinoma; Liver cancer; Immunotherapy; Immune checkpoint inhibitors; Clinical trials; Real-world data

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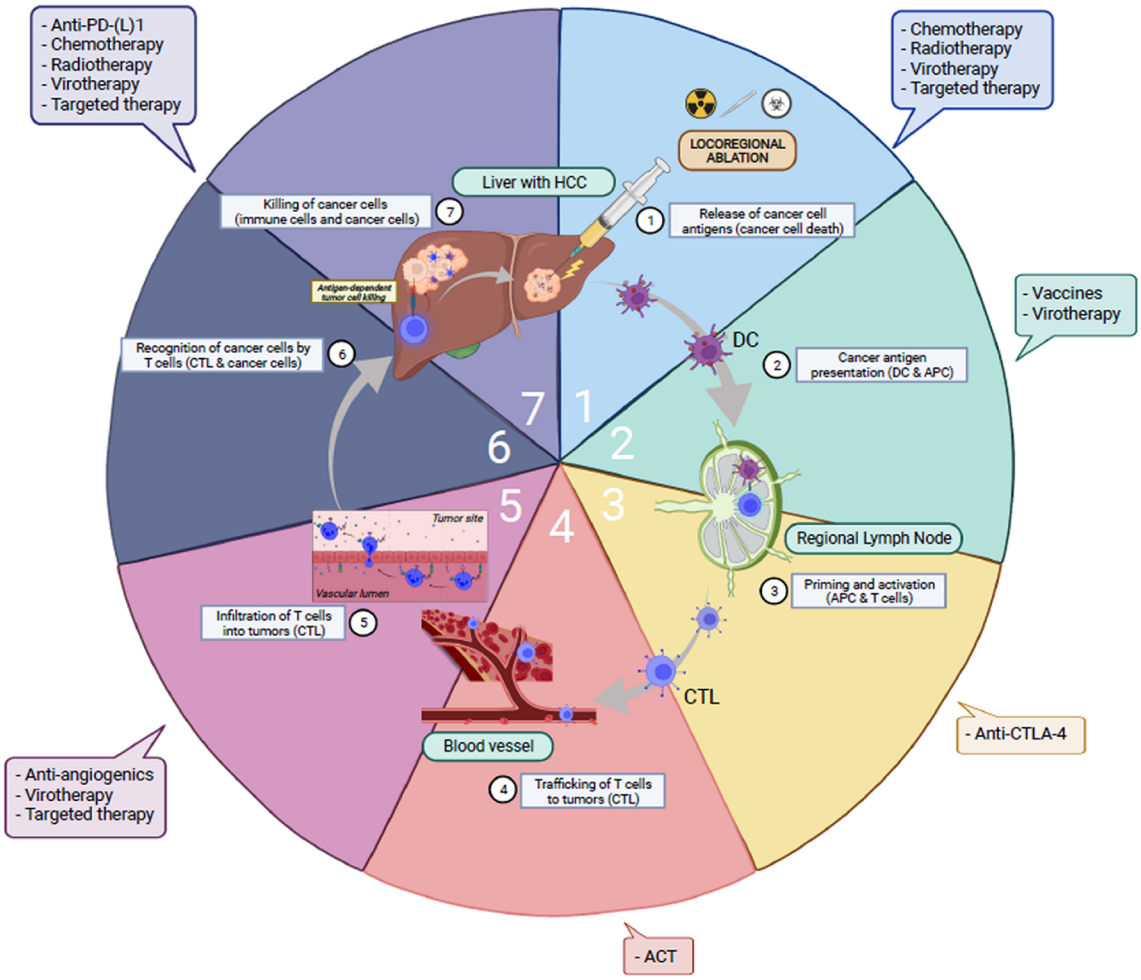
Core Tip: In the last five years, immune checkpoint inhibitors (ICIs) have entered the treatment landscape of hepatocellular carcinoma (HCC) in the 1st and 2nd line setting. However, due to restrictions in clinical trial inclusion and exclusion criteria, there remains a need for further real-world data on the efficacy, toxicity, and cost-effectiveness of ICIs in a broader cohort of HCC patients. New trials are underway investigating further ICI regimens, including combination therapy strategies, while novel immunotherapies are also being brought to the fore. This review discusses key clinical trials, real-world data, and future advances of immunotherapy for HCC, with a focus on ICIs.

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INTRODUCTION

Liver cancer is the third leading cause of cancer-related mortality worldwide and sixth in terms of incidence accounting for 830180 deaths and 905677 cases in 2020[1]. Hepatocellular carcinoma (HCC) is the leading type of primary liver cancer representing 85%-90% of cases[2]. The incidence of HCC is expected to continue to increase in countries, including the United States, until 2030. Asia and Africa feature the highest incidence of disease due to the endemic prevalence of hepatitis B or C virus (HBV or HCV) which, when untreated, lead to chronic liver disease and subsequent development of HCC. Global vaccination efforts against HBV and HCV are expected to lower the incidence of HCC, with effects becoming apparent after a latency period of 20-30 years correlating to the time required from liver damage to cancer development[3]. Second to viral hepatitis, alcohol abuse is another main cause of HCC development[4]. Diabetes, aflatoxin-B1 exposure, obesity, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease, and metabolic syndrome represent other leading contributors to HCC development[5]. NASH typically develops in patients with obesity, type 2 diabetes, dyslipidemia, and hypertension, therefore being a leading risk factor for HCC in rich developed countries such as the United States[3]. Both incidence and mortality of liver cancer are expected to double in the next two decades[6]. With a 5-year survival rate of less than 20%, liver cancer carries one of the worst cancer prognoses after pancreatic cancer[7]. Although this figure represents a significant improvement compared to the 3% 5-year survival observed in the 1970s[7], further research is warranted to improve treatments, especially for individuals with distant and regional metastatic disease which feature a 3% and 12% 5-year survival, respectively[8].

The current treatment landscape of HCC depends on disease stage (Figure 1). Surgical resection, liver transplantation (LT), and locoregional ablation therapies (2nd-line) are used with curative intent in early and intermediate disease. Yet, recurrence rates are high, while only 30%-40% of patients qualify for the above treatments[9,10]. Advanced HCC (aHCC) is managed with systemic therapies. Historically, systemic chemotherapies have largely been ineffective in HCC due to high rates of chemoresistance and liver impairment with associated susceptibility to toxicities[9]. Starting with the Food and Drug Administration (FDA) approval of sorafenib, a multiple tyrosine kinase inhibitor (TKI) with antiangiogenic and antiproliferative action, as a frontline systemic therapy for HCC in 2007, systemic therapies for HCC have evolved remarkably[11]. In 2018, following several randomized controlled trials exploring systemic therapies, which failed to surpass sorafenib, the multikinase inhibitor, lenvatinib, gained FDA approval as another 1st-line therapy in HCC following results of a phase III non-inferiority trial[12]. Subsequently, the TKIs regorafenib[13], cabozantinib[14], and ramucirumab[15] received approval in refractory HCC.



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Figure 1 Schematic of the cancer-immunity cycle and strategies to overcome mechanisms of resistance in each step by enhancing necessary immune stages *via* different anti-cancer therapeutic modalities in advanced hepatocellular carcinoma. ACT: Adoptive cell transfer; APC: Antigen presenting cell; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell.

In the last decade, the field of cancer immunotherapy has evolved tremendously, largely owing to the success of monoclonal antibodies (mAbs) directed against negative regulator molecules of T-cell activation, namely cytotoxic T-lymphocyte protein-4 (CTLA-4), programmed cell death protein 1 (PD-1), and its ligand, PD-L1. Immune checkpoint inhibitors (ICIs) reverse the immunosuppressive cancer phenotype by binding to and blocking co-inhibitory immune signalling molecules that are upregulated in cancer providing a means of systemic immune recognition and targeting of malignant cells. Following the approval of ipilimumab (anti-CTLA-4) for metastatic melanoma in 2011, ICIs have gradually been trialled and expanded across solid tumors. To date, four ICI regimens have been approved for HCC: nivolumab (anti-PD-1), approved as 2nd-line in 2017[16]; pembrolizumab (anti-PD-1), approved as 2nd-line in 2018[17]; nivolumab plus ipilimumab, approved as 2nd-line in 2020[18]; and atezolizumab (anti-PD-L1) plus bevacizumab (anti-vascular endothelial growth factor [VEGF] mAb), approved as 1st-line in 2020[19].

The complex interdependent relationship between chronic inflammation and anticancer immunity in HCC represents a possible opportunity and challenge for immunotherapy. Intelligent therapeutic strategy design that balances enhancing anti-tumor immunity whilst minimizing pro-tumorigenic inflammation and immunosuppressive adaptations lies at the center of successful immunotherapeutic regimens for HCC. Furthermore, effective anti-cancer immunity to overcome cancer immune escape involves multiple steps. Hence, new immunotherapies continue to be investigated for HCC, with novel adoptive cell transfer (ACT), therapeutic cancer vaccines, and virotherapy being developed as monotherapies or in combination strategies. This review summarizes updates and future directions for immunotherapies and their combinations in HCC.

IMMUNOGENICITY IN HCC

Immunotherapies are potentially promising therapeutic strategies in HCC. A complex interdependent relationship exists between chronic inflammation and anticancer immunity in the normal liver and in HCC, representing an opportunity and challenge for immunotherapy in HCC.

The liver itself is an immune organ with rich and unique immune cell populations (*e.g.*, Kupffer cells), functional anatomy, and immune functions. Under normal conditions, the liver finetunes immune tolerance, systemic inflammation and immunity, and anti-tumor immunity (*reviewed in*[20]). The tolerogenic potential of the liver – required for the modulation of host response to gut flora – underlies its capacity to generate potent immune tolerance to tumors when liver metastases occur from other primary cancers[21]. This same tolerogenic potential of the liver also underlies its ability to fully accept allograft LT and safely discontinue immunosuppressants in some LT patients[22]. Immune tolerance within the liver develops through complex interactions between liver-resident cells and peripheral leukocytes involving poor or incomplete activation of CD4+ and CD8+ T cells, elevated expression of immune checkpoints, and an immunosuppressive environment mediated by IL-10 and TGFβ[23,24]. Indeed, through new technologies and machine learning algorithms tumor immune microenvironment features have been correlated with patient prognostication to classify patients into separate groups based on response to immunotherapy and other treatments[25-28].

HCC represents a typical inflammation-associated immunogenic cancer as it often arises in chronically inflamed livers (necroinflammation)[29]. It is well known that chronic inflammation causes local and systemic immunosuppression of innate and adaptive immunity due to chronically elevated pro-inflammatory stimuli[20], while scar tissue itself impedes immunosurveillance[30]. Chronic antigen stimulation results in T-cell exhaustion, immune inhibitory receptor upregulation (*e.g.*, PD-1), and progressive loss of polyfunctional cytokine production[20]. Moreover, cirrhotic patients are systemically immunocompromised, due to loss of synthetic liver functions, and are susceptible to life-threatening infections[31]. Locally, both tumor cells and surrounding stroma orchestrate tissue remodeling with concurrent functional and phenotypical immunobiology adaptations resulting in a dysfunctional and immunosuppressive tumor milieu[32]. Simultaneously, successive chronic inflammatory stresses cause hepatocellular DNA damage, whereby genetic and epigenetic mutations give rise to immunogenic pathogen-associated proteins (abnormal amino acid sequences) through transcription and translation of mutated genetic sequences. In turn, tumor associated antigens (TAA) and neo-antigens may result that act as recognizable epitope targets to facilitate effector T-cell recognition of a non-self antigen against which to mount an immune response, so long as strong human leukocyte antigen binding and immunological synapse is possible against the new abnormal peptide sequence[32].

ICIs and other emerging forms of immunotherapy display high efficacy in cancers expressing targetable TAAs and neo-antigens. Indeed, some forms of immunotherapy incorporate molecular recognition of specific TAAs and neo-antigens in their mechanistic design[33]. Tumor mutational burden (TMB) is regarded as a surrogate marker for the expression of TAA and neo-antigens, and hence immunotherapy efficacy, as seen in the case of melanoma[34]. HCC has been shown to feature a low-to-moderate TMB compared to other tumors[35]. Although this theoretically corresponds to lower probability of immunotherapy efficacy, the antigenicity and immunogenicity of any resultant TAAs and neo-antigens in HCC is not well characterized and these may still be sufficiently potent targets for immunotherapies[36].

Intelligent therapeutic strategies that achieve an acceptable balance between enhancing anti-tumor immune surveillance and destruction whilst minimizing pro-tumorigenic inflammation and immunosuppression lie at the center of successful immunotherapy regimen design for HCC.

IMMUNE CHECKPOINT INHIBITORS IN HCC

ICI monotherapy in aHCC

The first study to investigate the efficacy of ICIs in HCC was CheckMate 040, a phase I/II clinical trial of nivolumab with or without ipilimumab in aHCC. Patients treated in the nivolumab monotherapy arm demonstrated an objective response rate (ORR) of 20% [95% confidence interval (CI): 15-26%] and a manageable toxicity profile; 25% of patients experienced grade 3-4 treatment-related adverse events (AEs). Of interest, 68% (n = 145 of 216) of patients in the expansion phase had previously received sorafenib in the 1^{s}-line setting. Analysis was stratified by PD-L1 expression but not by receipt of previous treatment[16]. Nivolumab received accelerated approval in the 2nd-line setting for the treatment of aHCC following results from this trial.

Another phase I/II study of ICI therapy for aHCC was NCT01693562, a trial assessing the efficacy of durvalumab in advanced solid tumors, including aHCC. In this trial, 93% of patients had been previously treated with sorafenib. ORR for the whole cohort was 10% (95%CI: 2.9%-24.2%) with a median overall survival (mOS) of 13.2 mo (95%CI: 6.3-21.1). Grade 3-4 AEs were noted in 20% of patients, with elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) among the most common (7.5% and 5.0%, respectively). Overall, durvalumab was shown to exert promising

activity over aHCC with an acceptable toxicity profile. The above studies established ICIs as tolerable and effective alternative (2nd-line) options to sorafenib in patients with aHCC. ICIs were subsequently trialed in 1s-line setting against sorafenib.

CheckMate 459 was a phase III study of nivolumab *vs* sorafenib as a 1st-line therapy in patients with aHCC. No statistically significant difference in mOS was found between treatment arms (nivolumab: 15.2 mo *vs* sorafenib: 14.7 mo); however, treatment-related AEs were more favorable with nivolumab [37]. Although results from CheckMate 495 have not yet been strong enough to justify approval of nivolumab as a 1st-line therapy for aHCC over sorafenib – due to the prespecified significance boundary for superior OS compared to sorafenib not being met – conclusions from this trial are significant as they indicate nivolumab as an alternative treatment option that should be offered to patients who cannot receive anti-angiogenics and TKIs because of contraindications or AE severity. This is reflected in the National Comprehensive Cancer Network Clinical Practice Guidelines in the USA. However, it has not been adopted in the European or Asian guidelines to date. Following this trial, the accelerated FDA approval of nivolumab monotherapy was withdrawn as it did not meet the post-marketing requirements. Importantly, to date this is the only phase III clinical trial completed to investigate single-agent anti-PD-1 or anti-PD-L1 monotherapy against single agent TKI monotherapy in the 1st-line setting for aHCC.

In the 2nd-line setting for the treatment of aHCC, the use of ICIs has been investigated with more success leading to clinical approvals. Keynote 224 was a phase II study of 2nd-line pembrolizumab in patients with aHCC that had previously been treated with, or were intolerant to, sorafenib. ORR for pembrolizumab was 17% (95%CI: 11%-26%) while treatment-related AEs were noted in 73% of patients, with 25% experiencing grade 3 AEs. Of note, most common AEs were increased AST and AL in 7% and 4% of patients, respectively. Based on these results, pembrolizumab has been granted FDA approval in the 2nd-line setting for patients with aHCC who previously received treatment with sorafenib.

In another study investigating the anti-PD1 agent camrelizumab as 2nd-line therapy in patients with aHCC previously treated with sorafenib ORR was 14.7% (95%CI: 10.3%-20.2%). Grade 3-4 AEs were encountered in 22% of patients, with increased AST being the most common AE (5%). This study demonstrated that camrelizumab also had a manageable toxicity profile[38].

Following the successful results of Keynote 224, Keynote 240, a randomized phase III study of 2nd-line pembrolizumab *vs* placebo in patients treated with 1st-line sorafenib, was initiated. The study showed a benefit in both mOS [13.9 *vs* 10.6 mo, hazard ratio (HR): 0.78, 95%CI: 0.61-0.99] and median progression-free survival (mPFS) (3.0 *vs* 2.8 mo, HR: 0.71, 95%CI: 0.57-0.90) in favor of pembrolizumab. The trial did not meet the prespecified criteria for mOS and mPFS despite the superior results observed with pembrolizumab. However, pembrolizumab had a favorable risk-to-benefit ratio and received accelerated FDA approval as a 2nd-line treatment for aHCC[39]. These results were similar to those of Keynote 224, suggesting that ICIs could become the preferred treatment of choice for patients who are at high risk for AEs.

More recently, the anti-PD-L1 agent avelumab showed moderate efficacy in a phase II trial in 30 patients previously treated with sorafenib (NCT03389126)[40]. The mOS and mPFS were 14.2 mo (95%CI: 9.5-18.9) and 3.5 mo (95%CI, 2.0–5.1), respectively. Treatment was well-tolerated, with 23% of patients exhibiting grade 3 AEs – commonest being increased AST/ALT (13%)—and none experiencing grade 4 AEs.

Clinical trials of ICI monotherapy in HCC attest to their efficacy and tolerability; however, these failed to demonstrate clear superiority over sorafenib, prompting investigators towards combination strategies to increase efficacy.

ICI combination therapies

ICI duplet therapy: The use of ICI combinations has gained attention in the last few years across a wide spectrum of solid tumors. Combinations of ICIs usually include an anti-PD-L1/PD-1 and an anti-CTLA-4 agent and demonstrate better responses compared to single-agent therapy, but also higher rates of AEs, especially serious and life-threatening ones[41]. Several clinical trials have assessed the efficacy and safety of anti-CTLA-4 and anti-PD-L1 combination regimens for aHCC.

A phase II trial (NCT02519348) assessed the efficacy of 2nd-line tremelimumab, an anti-CTLA-4 antibody, combined with durvalumab in patients with aHCC that were intolerant to, progressed to, or refused sorafenib. Patients were randomized into 4 different arms (tremelimumab 300 mg with durvalumab for the 1st cycle followed by durvalumab; durvalumab monotherapy; tremelimumab monotherapy; and tremelimumab 75mg with durvalumab for 4 cycles followed by durvalumab alone). Grade 3-4 AEs were observed in 35.1%, 17.8%, 42.0% and 24.4% of patients, respectively. ORRs were 22.7%, 9.6%, 7.2% and 9.5%, respectively, while mOS was 18.7 [95%CI: 10.8-not reached (NR)], 11.7 (95%CI: 8.5-16.9), 17.1 (95%CI: 10.9-NR) and 11.3 (95%CI: 8.4-14.6) months, respectively. Between treatment arms, tremelimumab 300 mg with durvalumab demonstrated the best benefit-to-risk profile [42]. After these results, the combination of tremelimumab-durvalumab was subsequently investigated in 1st-line setting in HIMALAYA (NCT03298451), a phase III trial that showed better outcomes with tremelimumab-durvalumab compared to sorafenib; mOS was 16.4 *vs* 3.8 mo, respectively (HR: 0.78%, 95%CI: 0.65-0.92), and ORR was 20.1% *vs* 17.0%, respectively[43].

In the nivolumab-ipilimumab combination part of the Checkmate 040 trial of patients with aHCC pre-treated with sorafenib, participants were randomized 1:1:1 into 3 different groups: Group A patients received nivolumab 1 mg/Kg every 2 wk & ipilimumab 3 mg/Kg every 3 wk for the first 3 mo, followed by nivolumab 1 mg/Kg every 2 wk; those in group B received nivolumab 3 mg/Kg every 2 wk and ipilimumab 1 mg/Kg every 3 wk for the first 3 mo, followed by nivolumab 3 mg/Kg every 2 wk; and those in group C were treated with nivolumab 1 mg/kg every 2 wk and ipilimumab 3 mg/Kg every 6 wk. ORR was 32% (95%CI, 20%-47%) for group A, 27% (95%CI: 15%-41%) for group B, and 29% (95%CI: 17%-43%) for group C. Median duration of response was not reached for patients in group A and was 15.2 and 21.7 mo for groups B and C, respectively. Although the total number of patients that experienced AEs of any grade was high (94% for group A, 71% for B, and 76% for C), serious AEs were not very common; in group A, 10% of patients reported grade 3-4 AEs, compared to 4% for group B, and 2% for group C. Overall, the study showed promising results on the efficacy and tolerability of ICI combinations for advanced HCC[18]. Following results from this trial, the combination of nivolumab-ipilimumab received FDA approval for aHCC becoming the new standard of care in the 2nd-line setting for patients who progress on prior TKI therapy and who do not have a contraindication to ICIs and are fit and able tolerate the higher toxicity observed in double ICI combination. Consequently ICI monotherapy with pembrolizumab (or nivolumab off-license) in the 2nd-line setting was reserved for less fit patients. The success of Checkmate 040 in establishing the efficacy of nivolumab-ipilimumab combination therapy for aHCC lead to Checkmate 9DW, another ongoing phase III trial comparing nivolumab-ipilimumab combination *vs* sorafenib or lenvatinib monotherapy.

ICI+TKI: The combination of ICIs with other factors with proven efficacy for aHCC has been investigated extensively through several trials subsequent to the negative results reported from Checkmate 459. TKIs have been among the most commonly tested agents. A recent phase Ib study investigated the use of pembrolizumab-lenvatinib combination therapy for patients with unresectable HCC. ORR was 36% (95%CI: 26.6%-46.2%), with a 12.6-mo duration of response, a mOS of 22 mo, and a mPFS of 8.6 mo. Grade ≥ 3 AEs were observed in 67% of patients[44].

The RESCUE trial was a phase II study of patients with aHCC treated with camrelizumab, a PD-1 inhibitor, combined with apatinib – another VEGFR-2 TKI that has demonstrated activity as 1st- and 2nd-line therapy for aHCC[45,46]. Patients were enrolled into the study irrespective of previous treatment status and analyses were stratified by line of therapy. For patients treated with 1st-line camrelizumab-apatinib combination, ORR was 34% (95%CI: 23.3%-46.6%) and mPFS was 5.7 mo. For 2nd-line therapy patients, ORR was 23% (95%CI: 15.4%-31.0%) and mPFS was 5.5 mo. Interestingly, grade ≥ 3 AEs were experienced by 77% of patients, while serious AEs were witnessed in 28.9%[47].

Another trial, COSMIC-132, is a randomized phase III trial comparing atezolizumab-cabozantinib combination *vs* cabozantinib *vs* sorafenib as a 1st-line therapy for patients with aHCC. mPFS for the combination therapy group was significantly improved over sorafenib monotherapy (6.8 *vs* 4.2 mo, HR: 0.63, 95%CI: 0.44-0.91). As expected, reported grade ≥ 3 AEs were much higher in the combination group (54%) as opposed to the sorafenib monotherapy group (32%)[48].

Results of ICI and TKI combination therapies have been promising; however, combination regimens have also been associated with much higher rates of AEs – especially grade ≥ 3 AEs – compared to ICI monotherapy or double ICI combinations. Several ongoing clinical trials are investigating combinations of ICIs, such as nivolumab, with TKIs; such studies will provide more information on the efficacy and tolerability of ICI-TKI combinations (Table 1). In the 2nd-line treatment setting of aHCC, clinicians may reserve TKI-ICI combinations for patients who are fitter, and offer double ICI combinations and ICI monotherapy options for patients who are less fit and least fit, respectively.

ICI+VEGF: Anti-VEGF agents are another popular category of therapeutic factors used in combination with ICIs. Existing evidence points towards a synergistic effect of anti-VEGF factors and ICIs through reversal of VEGF-mediated immunosuppression, and promotion of T-cell tumor infiltration[49,50]. IMbrave150, a phase III clinical trial of atezolizumab-bevacizumab combination *vs* sorafenib in treatment-naïve patients with aHCC, was the first study to demonstrate a benefit with such a combination. Six- and 12-mo OS was significantly better for the combination arm (85% and 67% respectively) compared to sorafenib (72% and 55% respectively), while mOS was not reached in the combination arm after 17 mo compared to a mOS of 13.2 mo for sorafenib. mPFS was also longer with atezolizumab-bevacizumab combination (6.8 *vs* 4.3 mo for sorafenib, HR: 0.59, 95%CI: 0.47-076), while ORR was also better in the combination arm (27%, 95%CI: 22.5-32.5 *vs* 12%, 95%CI: 7.4-18.0 for sorafenib). The toxicity profile of the combination therapy was manageable. As expected, rates of serious AEs were slightly higher[19]. Following results from this trial, atezolizumab-bevacizumab combination received FDA approval for aHCC becoming the new standard of care in the 1st-line setting for patients without contraindication to ICIs or anti-angiogenics; TKIs sorafenib or lenvatinib may be reserved as 1st-line treatment for patients who: (1) are less fit, and thus unlikely to tolerate atezolizumab-bevacizumab combination; and (2) those with contraindications to ICIs or anti-angiogenics[51].

NCT04393220 is another phase II clinical trial comparing nivolumab-bevacizumab combination as 1st-line therapy in aHCC. This trial was recently completed with results pending. Several other trials at various stages of completion are currently investigating combinations of ICIs with anti-VEGF agents

Table 1 Ongoing clinical trials investigating immune checkpoint inhibitor - oral tyrosine kinase inhibitor combinations

NCT	Phase	Study drugs	Treatment line	Endpoint	Estimated End of Trial
NCT04194775	3	CS1003 +LENVATINIB *vs* LENVATINIB	1	OS, PFS	June 2023
NCT04344158	3	PENPULIMAB + ANLOTINIB *vs* SORAFENIB	1	OS	December 2024
NCT03713593	3	PEMBROLIZUMAB + LENVATINIB *vs* LENVATINIB	1	OS, PFS	May 2022
NCT04411706	2	SINTILIMAB + APATINIB + CAPECITABINE	1	ORR	June 2022
NCT04042805	2	SINTILIMAB + LENVATINIB	1	ORR	August 2024
NCT04444167	2	BISPECIFIC AK104 + LENVATINIB	1	ORR	March 2022
NCT04183088	2	TISLELIZUMAB + REGORAFENIB	1	ORR, PFS, Safety	March 2025
NCT04310709	2	NIVOLUMAB + REGORAFENIB	1	ORR	May 2023
NCT04442581	2	PEMBROLIZUMAB + CABOZANTINIB	1	ORR	September 2024
NCT03439891	2	NIVOLUMAB + SORAFENIB	1	MTD, ORR	May 2022
NCT04170556	2	NIVOLUMAB + REGORAFENIB	2	Safety	December 2022
NCT04401800	1b/2	TISLELIZUMAB + LENVATINIB	1	ORR	December 2022
NCT04443309	1b/2	CAMRELIZUMAB + LENVATINIB	1	ORR	August 2024
NCT03347292	1	PEMBROLIZUMAB + REGORAFENIB	1	DLT, Safety	October 2022

NCT: Number of the clinical trial (Clinicaltrials.gov); OS: Overall survival; PFS: Progression-free survival; ORR: Overall response rate; MTD: Maximum tolerated dose; DLT: Dose-limiting toxicities.

(Table 2).

Immunotherapy with locoregional ablation: Another means of enhancing the immune response is through stress-induced tissue damage which stimulates inflammation and immunogenicity. Chemotherapy and radiotherapy cause immunogenic cell death, enhance T-cell activation and priming, induce tumor T-cell trafficking and infiltration, and enhance effector T-cell function whilst depleting tolerogenic T-cells[52-54]. Early and intermediate HCC is routinely treated with percutaneous and intraarterial locoregional therapies, including radiofrequency, thermal, and non-thermal ablation, and TACE[55,56]. These approaches may be ideal candidates in sequential or simultaneous combination therapy with immune-based treatments to enhance efficacy through immune modulation[57]. Aside from local immune effects, locoregional ablation methods produce systemic immune effects in innate and adaptive immune cells stimulating immunological tumor regression in tumor sites distant to the primary site of ablation through the abscopal effect. Upregulation of local and systemic immune checkpoint expression and cytokine production are also observed (*reviewed in*[58]).

Trials have investigated the combination of ablation with immunotherapy. In a proof-of-concept study (NCT03939975), radiofrequency or microwave ablation successfully increased response rates from 10% to 24% in patients undergoing therapy with nivolumab or pembrolizumab who exhibited stable disease or atypical progressive disease; toxicity was tolerated and there was a relative improvement in median survival[59]. Vice versa, immunotherapy may also be used as an adjunct to radiofrequency ablation, with one study demonstrating superior survival from anti-PD-1 (camrelizumab) immunotherapy and radiofrequency ablation compared to radiofrequency ablation monotherapy[60]. Evidence from a phase II trial (NCT01853618) in patients receiving anti-CTLA-4 immunotherapy with tremelimumab supports the added benefit from combination with radiofrequency ablation or TACE [61]. Partial response rate was 26% (95%CI: 9.1%-51.2%) and mOS was 12.3 mo (95%CI: 9.3-15.4 mo). Tumor biopsies taken at 6 wk exhibited a clear increase in CD8+ T cells in the patients who observed a clinical benefit, and 86% of patients with active HCV infection experienced a marked reduction in viral load demonstrating positive clinical activity. Additionally, phase I and II studies (NCT02837029, NCT03380130) demonstrated the safety and tolerability of nivolumab in combination or sequential therapy with selective internal radiation therapy containing yttrium-90 resin in patients who were ineligible for TACE, offering good disease control without increasing the adverse event rate in patients with advanced Child-Pugh scores[62]. Promising results from these trials have encouraged further clinical trials to evaluate ICI combinations with ablation methods (Table 3).

ICIs in the adjuvant/neoadjuvant setting

The use of ICIs may not be exclusive only for advanced stage disease as per BLBC criteria; they have also been investigated in adjuvant and neoadjuvant setting. In the neoadjuvant setting, preliminary

Table 2 Ongoing clinical trials investigating combinations of immune checkpoint inhibitors and anti-vascular endothelial growth factors factors

NCT	Phase	Study drugs	Treatment line	Endpoint	Estimated End of Trial
NCT03794440	2/3	SINTILIMAB + BEVACIZUMABBIOSIMILAR	1	OS, ORR	December 2022
NCT03970616	1b/2	DURVALUMAB + TIVOZANIB	1	Safety	August 2022
NCT03973112	2	HLX-10+BEVACIZUMAB BIOSIMILAR	1	ORR	June 2022

NCT: Number of the clinical trial (Clinicaltrials.gov); OS: Overall survival; ORR: Overall response rate.

results from a phase II study of camrelizumab-apatinib combination for systemic treatment-naïve, resectable HCC, showed a major pathologic response rate of 29% and a pathologic complete response rate of 6%, while demonstrating a manageable toxicity profile with 30% of patients experiencing grade 3 treatment-related AEs. No grade 4-5 AEs were observed[63]. Plenty of ongoing trials are investigating the use of ICIs with or without other agents in the perioperative setting for HCC (Table 4).

Safety of ICIs and HCC

In recent years, ICIs have demonstrated efficacy across a broad spectrum of tumors including HCC, prompting significant interest into their therapeutic value. Due to their involvement in the immune response, ICIs have been linked to immune-related AEs (IRAEs) of varying significance, from mild to life-threatening conditions such as myocarditis, colitis, pneumonitis and hepatitis[64]. Although the precise mechanisms by which ICIs exert these AEs is not known, evidence suggests that ICI administration leads to changes in T-cell population with emergence of autoreactive T-cells, along with increased B-cell clonality and germinal center activation, and display of autoantibodies against thyroid antigens[65,66] and pancreatic islet cells[67,68]. Expression or upregulation of target molecules such as CTLA-4 or PD-1/PD-L1 in normal tissues has also been associated with risk of IRAEs targeting the respective cells[69,70]. The composition of gut microbiota is implicated in the risk for IRAEs development[71]; the former is known to be associated with response to ICIs[72] and active modification through probiotic supplementation has been shown to enhance ICI activity and responses[73]. Systemic administration of antibiotics is known to affect gut microbiota composition[74] and has been associated with worse responses to ICIs[75,76]. The risk for IRAEs also includes reactivation of pre-existing autoimmune conditions, and other complications in patient populations where IRAEs have not been extensively studied, such as transplant patients and those with chronic viral infections[77].

In patients with HCC, the most common IRAEs observed with single-agent therapy include rash (up to 23% of patients), pruritus (up to 19% of patients), and diarrhea (up to 17%). For anti-CTLA-4 and anti-PD-L1 combination treatment, incidence rates are up to 29%, 45%, and 24%, respectively. These results align with the evidence of higher risk of IRAEs with double ICI therapy[78]. Higher rates of AEs observed with combination therapy regimens are a limiting factor that should be considered in clinicians' therapeutic decision-making. Patient eligibility for combination regimens much be considered on a case-by-case basis. Hepatic-related IRAEs such as AST/ALT elevation, defined as an increase of either AST or ALT 1-2.5 times the Upper Normal Limit (UNL)[79] are more common in patients with HCC compared to other tumors. Transaminitis of any grade has been observed in up to 14% of patients with HCC compared to 3% among patients with other tumor types[78], while there are reports that estimate incidence as high as 30%[80]. The association of IRAEs and viral infections is particularly important in HCC as 50%-60% of patients with HCC in the United States are infected with HCV, while 10%-15% are infected with HBV[81]. Recent evidence suggests that ICI therapy for advanced cancer in HBV/HCV positive patients is associated with an increased risk for reactivation of hepatitis. Interestingly, the risk for hepatitis was not significantly different for patients with HCC compared to other malignancies[82]. Although IRAEs can complicate treatment with ICIs, evidence suggests that IRAE incidence positively correlates with better response to ICIs. In a study of patients with HCC treated with ICIs, patients with history of IRAEs had longer PFS, OS and higher Disease Control Rate compared to those who did not experience IRAEs[83].

Treatment of immune-related hepatitis/transaminitis is dependent upon AST/ALT levels. Temporary hold of treatment is indicated for enzyme level elevations between 2 to 5 times the UNL, while permanent discontinuation of the associated checkpoint inhibitor is indicated for elevations greater than 5 times the UNL[84-86]. For patients with an elevation 5- to 10-times the UNL, a course of 1-2 mg/kg/d prednisone is indicated with possible escalation to IV methylprednisolone if no improvement is seen in 3-5 d. Further treatment escalation to mycophenolate mofetil (1000 mg twice daily) should be considered in patients that do not improve after maximum steroid treatment[87].

Table 3 Ongoing clinical trials investigating combinations of locoregional therapy and immune checkpoint inhibitors

NCT	Phase	Locoregional Therapy	Systemic Therapy	Endpoint	Estimated End of Trial
NCT03817736	2	TACE + SBRT	ICI	Sequential	February 2024
NCT03638141	2	DEB-TACE	DURVALUMAB + TREMELIMUMAB	Sequential	November 2023
NCT03143270	1	TACE	NIVOLUMAB	Combination	April 2022
NCT03572582	2	TACE	NIVOLUMAB	Combination	June 2023
NCT03397654	1/2	TACE	PEMBROLIZUMAB	Sequential	December 2021 (*results awaited*)
NCT03383458	3	Curative resection or ablation	NIVOLUMAB	Adjuvant	June 2025
NCT02821754	2	TACE, RFA, Cryo	DURVALUMAB, TREMELIMUMAB	Combination	December 2022
NCT03033446	2	Y90-Radioembolization	NIVOLUMAB	Combination	December 2021 (*results awaited*)
NCT03099564	1	Y90-Radioembolization	PEMBROLIZUMAB	Combination	July 2022
NCT03259867	2	TATE	NIVOLUMAB OR PEMBROLIZUMAB	Combination	December 2022
NCT03937830	2	TACE	DURVALUMAB + TREMELIMUMAB + BEVACIZUMAB	Combination	December 2023
NCT03778957	3	TACE	DURVALUMAB or DURVALUMAB + BEVACIZUMAB	Combination	August 2024
NCT04340193	3	TACE	NIVOLUMAB + IPILIMUMAB or NIVOLUMAB MONOTHERAPY or DOUBLE PLACEBO	Combination	January 2024
NCT04246177	3	TACE	PEMBROLIZUMAB + LENVATINIB	Combination	December 2029
NCT04268888	2/3	TACE/TAE	NIVOLUMAB	Combination	June 2026
NCT05162898	N/A	RFA	TORIPALIMAB + LENVATINIB	Combination	December 2025
NCT05057845	2	Cryo	TISLELIZUMAB + LENVATINIB	Combination	September 2024
NCT04988945	2	TACE + SBRT	DURVALUMAB + TREMELIMUMAB	Sequential (for downstaging)	December 2026
NCT04727307	2	RFA	ATEZOLIZUMAB (neoadjuvant) + ATEZOLIZUMAB-BEVACIZUMAB (adjuvant)	Combination	July 2027
NCT04663035	2	Ablation	TISLELIZUMAB	Combination	December 2025
NCT04652440	1/2	RFA	TISLELIZUMAB	Combination	November 2023
NCT04639180	3	Curative resection or ablation	CAMRELIZUMAB + APATINIB	Adjuvant	July 2024
NCT04220944	1	MWA+TACE	SINTILIMAB	Combination	September 2022
NCT04102098	3	Surgical resection or ablation	ATEZOLIZUMAB+ BEVACIZUMAB	Adjuvant	July 2027
NCT03867084	3	Surgical resection or local ablation	PEMBROLIZUMAB	Adjuvant	June 2025
NCT03864211	1/2	Thermal ablation (MWA or RFA)	TORIPALIMAB	Combination	June 2023
NCT03753659	2	MWA or RFA or Brachy-therapy or TACE	PEMBROLIZUMAB	Combination	June 2024
NCT03630640	2	Electroporation	NIVOLUMAB (neoadjuvant & adjuvant)	Combination	November 2023

NCT: Number of the clinical trial (Clinicaltrials.gov); TACE: Transarterial chemoembolization; SBRT: Stereotactic body radiotherapy; DEB: Drug eluting bead; RFA: Radiofrequency ablation; Cryo: Cryoablation; TATE: Transarterial tirapazamine embolization; TAE: Transarterial embolization; MWA: Microwave ablation.

REAL-WORLD DATA FOR IMMUNOTHERAPY IN HCC

Despite the advances in ICI therapies for HCC in recent years, phase II/III studies are generally limited by strict inclusion and exclusion criteria, thus lacking ecological validity and generalizability to real-life clinical practice outside of clinical trial setting[88]. Real-world data describe health-related information gathered outside of clinical trials. Gathering and reporting real-world data through cohort and observational studies is important for clinicians who aim to apply approved clinical trial regimens to a broader patient group.

Immunotherapy ineligibility

In clinical trials of systemic therapies in HCC, patients who have aHCC or intermediate disease and are not suitable for locoregional therapies are enrolled; however, in real-world clinical practice, a large proportion of such patients are ineligible to receive immunotherapy due to contraindications.

In unselected HCC patients in the general population, no more than a third are amenable to ICIs as a 1st-line approach and this figure decreases considerably in combination therapy with anti-VEGF or TKI agents according to an analysis of the Italian Liver Cancer (ITA.LI.CA) database involving 2483 patients across liver dysfunction stages[55,89]. When considering only aHCC and intermediate HCC that was unresponsive to locoregional ablation (n = 1514), eligibility increased from 21% with nivolumab and 11% with pembrolizumab to 35% and 18%, respectively. Overall, the main contraindications to frontline ICI were Child-Pugh class > A (24%, n = 601), uncontrolled ascites (15%, n = 380), performance status > 1 (13%, n = 343), active alcohol intake (13%, n = 323), thrombocytopenia (12%, n = 299), hepatic encephalopathy (6%, n = 155), aminotransferase levels > 5 times the UNL (5%, n = 123), and concurrent autoimmune diseases (2%, n = 57)[89]. In the 2nd-line, ICI eligibility was substantially lower with 5% and 8% of patients amenable to nivolumab and pembrolizumab, respectively[89]. When repeating this analysis to take into account anti-VEGF and TKI combination therapy with ICIs, atezolizumab-bevacizumab eligibility drops to 18% in the whole HCC population and 29% in aHCC or intermediate HCC patients who are not eligible for surgery or locoregional procedures[55]. Reasons for the exclusion of these additional patients were clinically significant heart disease (n = 52), chronic non-healing skin ulcerations (n = 15), uncontrolled hypertension (n = 10), and non-liver-related coagulative abnormalities increasing the risk of bleeding (n = 1).

The expert opinion panel of ASCO has acknowledged the role of ICIs in the treatment of patients with aHCC and especially patients with contraindications, or intolerance to, TKIs who may derive immense benefit from immune therapies[51]. However, they also highlight that patients and clinicians should be aware of life-threatening toxicities that may occur with ICIs. Future research may provide additional information on specific patient subpopulations within this subgroup that may have a favorable risk to benefit ratio.

Immunotherapy in patients with liver dysfunction (Child-Pugh class B and above, hepatitis, NASH)

Due to the lack of Child-Pugh class B and above patients in HCC trials, which often specify Child-Pugh class A in the inclusion criteria, there is a large unmet need for data to support treatment efficacy and toxicity profiles in this cohort[51]. As a result, published recommendations and guidelines for systemic therapy in HCC are often limited to patients with Child-Pugh class A[51]. Experts recommend cautious consideration of systemic therapies for Child-Pugh class B HCC patients with good performance status, taking into account their liver function, bleeding risk, presence of portal hypertension, extent of extrahepatic spread, tumor burden, and major vascular invasion[51]. A handful of studies have compared explorative primary outcomes in Child-Pugh class B patients treated with immunotherapy with different outcomes. Use of the Barcelona Clinic Liver Cancer (BCLC) staging criteria helps avoid the unselect exclusion of all Child-Pugh class B and above patients by allowing for holistic patient scoring and selection based on other performance status criteria.

One retrospective case series of 18 Child-Pugh class B HCC patients treated with nivolumab monotherapy reported a higher rate of AEs compared to those observed in Child-Pugh A patients in CheckMate 040; however, the majority of serious AEs and other AEs were associated with complications of comorbid liver dysfunction and advanced tumor burden, including 11% *vs* 4% of serious treatment-related AEs, and 28% *vs* 19% treatment-related AEs grade ≥ 3. The ORR was comparable in both studies (17% *vs* 20%)[90]. Notably, the mOS in this case series was 5.9 mo, which is lower compared to the 7.6-mo mOS reported in the analogous CheckMate 040 cohort, though higher compared to the limited mOS data reported for analogous patients treated with sorafenib (3-5 mo). Comparable safety and efficacy of nivolumab and pembrolizumab across Child-Pugh class and line of therapy has been confirmed in other real-world data studies with no significant difference observed in ORR and toxicity in terms of AEs; however, mOS and OS tends to be shorter in Child□Pugh B and above patients[91-93].

In a study of 34 HCC patients (5/29 BLBC B/C; 19/14/1 Child–Pugh A/B/C) including sorafenib pre-treated individuals, nivolumab was safe and efficacious with reported 6% (n = 2) grade 3 toxicity, 12% (n = 4) partial response, and 24% (n = 8) stable disease[94]. However, mOS was only 7.5 wk as 59% (n = 20) of patients had died on assessment due to tumor progression (80%, n = 16), acute liver failure (15%, n = 3), and variceal bleeding (5%, n = 1)[94]. On analysis, 24% of patients (n = 8) were still on

Table 4 Ongoing clinical trials investigating immune checkpoint inhibitor - based clinical trials in the adjuvant and neoadjuvant setting

NCT	Phase	Study drugs	Treatment setting	Endpoint	Estimated End of Trial
NCT03383458	3	NIVOLUMAB *vs* PLACEBO	Adjuvant	RFS	June 2025
NCT03867084	3	PEMBROLIZUMAB *vs* PLACEBO	Adjuvant	RFS, OS	June 2025
NCT03847428	3	DURVALUMAB + BEVACIZUMAB *vs* PLACEBO	Adjuvant	RFS	September 2023
NCT04102098	3	ATEZOLIZUMAB + BEVACIZUMAB *vs* PLACEBO	Adjuvant	RFS	July 2027
NCT03859128	2/3	TORIPALIMAB *vs* PLACEBO	Adjuvant	RFS	April 2024
NCT03839550	2	CAMRELIZUMAB + APATINIB	Adjuvant	RFS	February 2023
NCT04418401	2	ANTI-PD1 + DONAFINIB	Adjuvant	RFS	June 2023
NCT03510871	2	NIVOLUMAB + IPILIMUMAB	Neoadjuvant	ORR, downstaging rate	December 2022
NCT04123379	2	NIVOLUMAB + CCR2/5-inhibitor *vs* NIVOLUMAB +ANTI-IL8	Neoadjuvant	Safety	October 2024
NCT03222076	2	NIVOLUMAB	Neoadjuvant	Safety	September 2022
NCT03682276	1/2	NIVOLUMAB + IPILIMUMAB	Neoadjuvant	Safety, Delay to surgery	September 2022
NCT03383458	1	NIVOLUMAB *vs* PLACEBO	Adjuvant	RFS	June 2025
NCT04425226	N/A	PEMBROLIZUMAB + LENVATINIB	Neoadjuvant	RFS, ORR	December 2025

NCT: Number of the clinical trial (Clinicaltrials.gov); RFS: Recurrence-free survival; OS: Overall survival; RFS: Recurrence-free survival; ORR: Overall response rate.

nivolumab treatment and 18% (n = 6) had stopped treatment for other reasons [patients wish (n = 5), toxicity (n = 1)]. On multivariate analysis, Child–Pugh stage was the only significant independent risk factor for survival (HR 7.72, 95%CI: 2.62-22.78, P < 0.001). Although safe and efficacious, the study concluded that patients with advanced liver disease require further prospective evaluation due to probable limited efficacy of nivolumab. Overall, efficacy was approximately half of that observed in Checkmate 040. Results from this study are in agreement with latest evidence indicating that the survival of patients with aHCC treated with nivolumab is correlated to the Child-Pugh liver function score at baseline[95], as reported in aforementioned studies as well[92-94]. Equally, the finding that unselected Child-Pugh B and above patients exhibit an unsatisfactory response to and survival with nivolumab has been echoed in another retrospective study of 203 HCC patients; (ORR 3% *vs* 16% in Child–Pugh class B/A, P = 0.01; mOS 11.3 *vs* 42.9 wk in Child–Pugh class B/A, adjusted HR, 2.10, P < 0.001)[96].

Aside from Child-Pugh class, other liver dysfunction causes have also been examined in real-world studies. Safety and antitumor activity had been demonstrated in hepatitis-induced cirrhosis and in patients with active hepatitis viral load, even those on anti-viral treatment, while viral hepatitis status has been suggested as a possible predictive biomarker for response since it is clearly not a contradiction against the use of ICIs, yet further prospective studies are mandated[93,97,98]. Conversely, the same cannot be said for patients with underlying NASH and those with HCCs with activated Wnt/β-catenin signaling which observe reduced efficacy from ICI therapy according to pre-clinical and clinical data (*reviewed in*[99]).

Given that the level of liver dysfunction in this cohort may have significant implications on guidelines regarding the optimal selection of drugs in each line of therapy, further data is needed to guide the evidence-based use of systemic immune therapies in Child-Pugh class B HCC, as supported by the above limited data.

Macrovascular invasion

Another indicator of poor performance status is macrovascular invasion (MVI). Approximately 10%-40% of HCC patients present with MVI at diagnosis[100], and as such are not amenable to curative treatment and exhibit very poor prognosis[101]. Despite MVI being common, patients are often excluded from clinical trials. Thus, real-world data are needed to demonstrate the relative efficacy of ICIs in this cohort.

Tsai *et al*[102] retrospectively compared the efficacy of PD-1 inhibitors in 34 HCC patients with vascular metastases in the portal vein and inferior vena cava *vs* 34 patients without tumor thrombi; ORR and survival were comparable between both cohorts. The response rate of vascular tumor thrombosis

was 52.9%, and responders exhibited a superior survival benefit than non-responders. MVI responsiveness closely correlated with the maintenance of optimal liver function and a lower occurrence of distal metastases. These findings are in agreement with those from an earlier study showing that the magnitude of treatment response is significantly more intense in vascular invasion compared to hepatic tumors, and vascular response is also an independent prognostic factor that is significantly associated with PFS, while ECOG (Eastern Cooperative Oncology Group) performance status was a significant independent predictor of OS[103]. Moreover, similar findings have been observed in renal cell carcinoma displaying inferior vena cava thrombus treated with ICIs that proposed the response of vascular thrombi to ICIs is stronger in a high T-cell inflamed tumor microenvironment[104]. ICIs markedly decrease or stabilize tumor thrombus volume, and this response may be affected by the diversity of tumor microenvironments[102,103,105,106]. Vascular metastasis regression helps preserve organ function while the use of ICIs in these patients also delays distant metastases[102]. Therefore, ICIs should be prioritized in patients with MVI in an attempt to prevent further progression as well as mediate vascular tumor response, to hopefully improve outcomes.

Autoimmune disease

The incidence of autoimmune diseases in cancer patients has been reported at 13%-30% with hypothyroidism, rheumatoid arthritis, type 1 diabetes and psoriasis representing the most common conditions[107,108]. Hepatobiliary autoimmune diseases, such as autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are known risk factors for HCC[109,110]. The risk of HCC is lower with PSC compared to AIH[110], especially when the latter co-exists with cirrhosis[111]. Yet, patients with underlying autoimmune disease are typically excluded from immunotherapy trials because of the risk of immune-mediated flares of their underlying autoimmune disease [99].

In an observational, retrospective study including 15 patients with pre-existing autoimmune diseases, including 4 HCC patients treated with nivolumab, only 4 (27%) patients experienced an autoimmune disease exacerbation with ICIs, including 1 of the HCC patients[112]. Moreover, the most frequent cause of treatment discontinuation was disease progression rather than toxicity. Studies in other cancers (mostly melanoma and non-small cell lung cancer) report a wide range of incidence of flare-ups and IRAEs in patients with underlying autoimmune disease that receive ICI treatment[113-117]. One systematic review of 123 cancer patients from 49 publications reported incidences of 41% exacerbation of previous autoimmune disease, 25% de novo IRAEs, and 11% of both; no difference was observed between those with active *vs* inactive disease[118]. Patients receiving immunosuppressive therapy at initiation of ICI therapy appeared to experience fewer AEs than those not receiving treatment. AEs improved in over half of patients without ICI discontinuation while 3 patients died. The incidence of IRAEs is higher in patients with autoimmune disease treated with ICIs compared to incidences quoted in studies and trials of patients without autoimmune conditions. In terms of efficacy, studies show no difference in ORR, PFS, and OS in patients with underlying autoimmunity compared to those without [113,116]. However, the evidence is conflicting regarding the response rate depending on concomitant immunosuppressive therapy at the time of ICI initiation with some studies quoting lower response rates [114] and others quoting no association[117]. Recently, the use of selective immunosuppressive drugs over non-selective immunosuppressants in patients with underlying autoimmune disease for ICI therapy has been recommended, as the former may be less likely to adversely affect ICI efficacy[119].

Overall, although limited, these data support the administration of immunotherapy in cancer patients with a pre-existing (controlled) autoimmune disease with adequate follow-up and early management if flare ups or IRAEs occur; immune exacerbations can usually be management with steroids or other immunosuppressants without treatment discontinuation. Therefore, every cancer patient with underlying autoimmune disease should be considered for ICI therapy with a decision on management achieved through multidisciplinary team discussion that weighs up the risks and benefits[120]. Recommendations state that ICIs should be avoided: (1) whenever autoimmune disease reactivation may be life threatening, (2) in patients with neurological or neuromuscular disorders, and (3) in patients with poorly controlled autoimmune disease or on high doses of immunosuppression[120]; TKIs should be considered 1st-line in these cases[101].

In the case of HCC particularly, there is a lack of data. Further large prospective studies are needed to establish the incidence of IRAEs and autoimmune disease exacerbations in patients with pre-existing autoimmune conditions treated with immunotherapy to evaluate the overall risk-to-benefit ratio and generate practical evidence-based management guidelines for this subpopulation.

Therapeutic decisions: Radiological progression

The concurrent availability of several systemic therapy regimens in the 1st- and 2nd-line settings offers clinicians and patients a wider selection of drugs to choose from. Decisions regarding the selection of a specific agent over another is not only determined by the availability and accessibility to a specific drug, but also, more importantly, by the efficacy and tolerability that is expected or indeed observed in a patient. Thus, the decision of which agent to choose, or switch to, is largely dependent on individual patient characteristics. As previously mentioned, there are inherent difficulties regarding the lack of wide representation of patients of poor performance status in immunotherapy clinical trials that affect a

clinician's ability to triage toxicity risk in these patients. Additionally, assessing tumor progression and response to immunotherapy, which also governs treatment selection and switching, is challenging for several reasons both inside and outside of clinical trials.

Immunotherapies are known to produce an atypical response patten featuring pseudo-progression (PP) and hyper-progressive disease (HPD). Therefore, multiple variations of Response Evaluation Criteria in Solid Tumors (RECIST) have been proposed, with RECIST 1.1 recommended for primary endpoints and immune RECIST (iRECIST) for exploratory analyses. Importantly, patients in clinical trials undergo thorough radiological assessment from specialized radiologists to a more robust standard than what is available outside of trial setting. Decisions about switching or continuing past progression (assumed PP) are usually made based on the trial radiologist report in conjunction with the opinion of experienced oncologists involved in the trial; usually, treatment is stopped in trials when patients progress on immunotherapy whereas in real life a decision may be made to continue treatment if the patient reports benefits and if the drug is well-tolerated. A retrospective multicenter analysis of 31 HCC patients treated with nivolumab in real-life practice assessed radiological response to treatment using both RECIST 1.1 and iRECIST and found that response rates were similar to those reported in prospective clinical trials[122]. However, authors highlight the heterogeneity in response and progression patters, and emphasize the risk of misinterpretation of results in terms of endpoints as well as the difficulty of deciding when to stop treatment past progression. Additional real-life studies such as the above are warranted to support these findings.

In terms of HPD – which remains a controversial concept that is doubted by some clinicians due to lack of robust data to differentiate it from natural cancer progression in non-responders – the above study reported an occurrence of this phenomenon in four cases (13%). All of these patients presented at baseline with massive tumor burden involving different anatomical regions (burden 76-159 mm; 6-15 measurable lesions per patient) before nivolumab initiation[121]. These findings are consistent with those reported in a separate case series of 47 patients in which 3 exhibited HPD (including 1 aHCC patient) when treated with nivolumab; the main characteristics in the hyper-progressors were age < 75 years, ≥2 metastatic sites, PD-L1 < 50%, neutrophil-to-lymphocyte ratio > 3, and elevated lactate dehydrogenase[122]. This and other studies support the notion that high metastatic burden at baseline may be a clinical predictor of HPD during ICI therapy. Other predictors of HPD have been proposed with contradictory data (*reviewed in*[122]).

Cost-effectiveness

In 1st-line setting, the combination of atezolizumab-bevacizumab *vs* sorafenib for aHCC has been shown to lack cost-effectiveness from a US payer perspective despite offering a significant clinical survival benefit, according to data from the IMBRAVE150 clinical trial; an incremental cost-effectiveness ratio of $322500 per quality-adjusted life-year (QALY) gained was observed[123]. In a threshold analysis, prices for atezolizumab and bevacizumab would have to be reduced by 37% and 47%, respectively, to be considered a cost-effective alternative at common willingness-to-pay thresholds of $150000 or $100000 compared to sorafenib[123]. However, it should be noted that patients in the IMBRAVE150 disproportionately represented patients with well-preserved liver function (Child-Pugh class A) and good performance status (ECOG score 0-1), meaning that it is unclear how generalizable the benefits and/or risks, and therefore the cost-effectiveness, of these drugs are in clinical practice, where patients present with more severe disease[124]. IMBRAVE150 also disproportionately included fewer patients of Black and Hispanic ethnicity. Higher age specified mortality and incidence of aHCC are observed in Asian, Black, and Hispanics compared to non-Hispanic White individuals, while Black and Hispanic patients also tend to present with more advanced tumor burden and have worse survival compared with non-Hispanic White patients[125]. Lack of such ethnic representation in IMBRAVE150 means that the above cost-effectiveness analysis model does not accurately represent disease demographics across ethnicity and is thus likely to lack in generalizability[124]. Moreover, racial and ethnic disparities are known to occur in immunotherapy receipt; atezolizumab-bevacizumab regimes are likely to widen existing disparities in HCC mortality, especially given lack of ethnic representation in the aforementioned cost-effectiveness analysis model[124]. Aforementioned results are echoed in a separate study which indicated that 1st-line TKI followed by 2nd-line immunotherapy was the most cost-effective strategy for aHCC[125].

Lack of ICI cost-effectiveness for aHCC also stands true in the 2nd-line setting in the US, where results from a separate cost-effectiveness analysis of pembrolizumab for aHCC based on data from the KEYNOTE-240 trial reported an incremental cost-effectiveness ratio of $340409 per QALY gained[126]. The price of pembrolizumab would need to be reduced by 58% to achieve cost-effectiveness, with a willingness-to-pay threshold of $150000 per QALY. These results are likely to be very similar for nivolumab monotherapy as an alternative anti-PD-1 agent for aHCC in 2nd-line setting; however, further cost-effectiveness analyses are warrant especially for the combination of nivolumab-ipilimumab in the 2nd-line setting for aHCC[126]. It should be noted that other 2nd-line non-immunotherapy alternatives for aHCC including regorafenib, cabozantinib, and ramucirumab have also been shown to have an incremental cost-effectiveness ratio with $224362 and over $1 million per QALY for regorafenib and cabozantinib, respectively, while no cost-effectiveness data has been published for ramucirumab which is also unlikely to be cost-effective[126]. The lack of robust head-to-head trials comparing different 2nd-

line therapies means that it is difficult to undertake a robust cost-effectiveness comparison of agents in this setting[126].

From a patients' perspective, sadly, differences in drug costs are often an important factor that impact patient decision as some cancer therapies pose a more significant financial burden than others. Additionally, potential burdens—financial or otherwise—associated with regular travel to a treatment center for IV infusion therapy may render a patient more likely to opt for a treatment regimen composed of oral medications which they can take at home. In the case of the latter, the importance of medication compliance, even in the presence of adverse events, as well as patient safety-netting must be stressed. Occasionally, providers themselves may have financial biases for supporting some regimens over others when there is no significant difference in treatment effectiveness.

Immunotherapy in LT

One of the questions yet to be answered is the role of immunotherapy in LT. Immunotherapy post-LT may prevent or be useful in the management of recurrent HCC as well as other post-transplant secondary malignancies. Current guidelines state that immunotherapy approaches should be avoided in patients who recur following LT because of the high rates (40%) of allograft rejection and mortality, owing to stimulation of the host immune response by these agents; however, aside from rejection, anti-tumor efficacy and tolerability are promising[127,128]. A review of 25 patients receiving immunotherapy post-transplant identified immunotherapy initiation after short duration from transplant and graft PD-L1 positivity as potential risk factors for rejection[129]. Despite the limited amount of data, in the current era it is not safe to advise for the use of immunotherapy to prevent or treat disease recurrence after transplant. Elucidating better predictors of patients that are at higher risk of experiencing transplant rejection may help identify a subset of patients who are more likely to observe a favorable benefit-to-risk ratio from immunotherapy after transplant, and may thus be eligible for this approach.

In the pre-LT setting, the role of neoadjuvant immunotherapy is even less clear. Immunotherapy pre-LT may facilitate downstaging of unresectable HCC bridging to subsequent surgical eligibility, thus offering these patients their only chance of disease cure; however, this potentially comes at a higher risk of donor graft rejection[127]. The latest study published on this topic identified seven patients from their center and three from the literature who received anti-PD-1 ICIs pre-LT[130]. Eight patients (80%) observed partial response, and the disease control rate was 100%. Acute rejection occurred in 30% of patients with two patients dying as a result, despite treatment with immunosuppressive medications. Despite this growing body of evidence, further research is warranted. Currently, a phase II multicenter clinical trial is underway to investigate the role of durvalumab and tremelimumab for patients with HCC listed for LT (NCT05027425). Such trials are needed to determine the safety and efficacy of immunotherapy as a potential bridging strategy to LT.

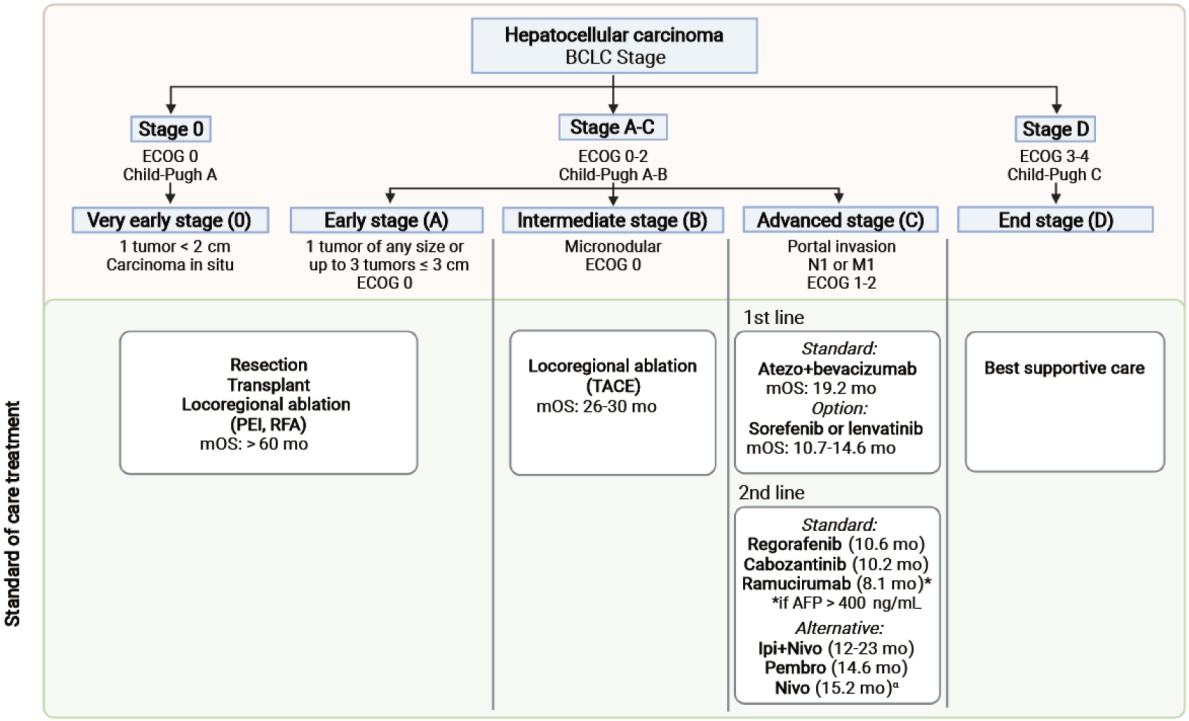
FUTURE DIRECTIONS

Immunotherapy beyond ICIs: ACT, vaccination, and virotherapy

Other promising forms of immunotherapy for HCC aside from ICIs include ACT strategies, anti-tumor cancer vaccines, and transgenic therapy applied through viral vectors. These strategies have begun to be investigated clinically as monotherapy options in HCC and as combination therapies, to enhance the efficacy of other treatments. The potential of such immunotherapies as combination treatments is highly promising, particularly in the context of combination with other immunotherapies, such as ICIs, as they improve immunogenicity. Combining immunotherapeutics with different mechanisms of action and primary immune effects is a well-recognized approach to counteract the multiplicity of tumor immune evasion mechanisms and ensure all necessary steps for the successful mounting of an anti-tumor immune response are met, as described in the *cancer immunity cycle* theory[31,131,132]. The same principle stands true as the underlying biological rationale to justify combination therapy of ICIs with different mechanisms of action – an example being combination with anti-PD-1 and anti-CTLA-4 agents which has been shown to significantly improve ORR, at the expense of increased but tolerable toxicity (Figure 2).

ACT: Following the success and approval of a plethora of ACT strategies in hematologic cancers[133], researchers have explored various forms of ACT in solid tumors. ACT involves the autologous or allogeneic transplant of tumor-infiltrating lymphocytes (TILs), or genetically modified T-cells engineered to express novel T-cell receptors (TCR) or chimeric antigen receptors (CAR)[134]. Cytokine-induced killer (CIK) cells represent another form of ACT wherein T-cells are co-cultured *in vitro* under cytokine manipulation to express natural killer (NK) cell-surface markers in addition to TCR. Cytotoxic cells with this double T/NK phenotype are capable of lysing a broad array of tumor cell targets in a non-MHC-restricted manner[135].

The primary immune effects of ACT result in supplementation of immune effector cells[31]. Compared to other immunotherapies, one of the advantages of ACT is that it is considered a "living"



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Figure 2 Treatment algorithm for immunotherapy in hepatocellular carcinoma according to American Society of Clinical Oncology guidelines. Atezo: Atezolizumab; Ipi: Ipilimumab; Nivo: Nivolumab; Pembro: Pembrolizumab; mOS: Median overall survival; mo: Months; PEI: Percutaneous ethanol injection; RFA: Radiofrequency ablation; TACE: Transarterial chemoembolization; ECOG: Eastern Cooperative Oncology Group; BCLC: Barcelona Clinic Liver Cancer; AFP: Alpha fetoprotein; cm: centimeter; N1: Regional nodal spread; M1: Metastatic spread.

treatment method as it exhibits the capability to become active and replicate *in vivo* for long lasting anti-tumor effect[136]. In theory, this grants the possibility of disease cure due to the accrual of long-term immunological memory; however, in practice this is often not achieved due to multifactorial lack of cell persistence[137]. Currently, most novel ACT strategies, such as CARs, are bespoke to each patient and are manufactured specifically for them. Off-the-shelf ACT strategies are being investigated as a means to improve costs, time and ease of manufacturing, and allow for universal applicability across patients [133]. Due to the presence of TAAs with an acceptable specificity, HCC in one of the most promising organs for ACT in solid tumors, as TAA specificity decreases chances of on-target off-tumor recognition and subsequent toxicity due to target antigen expression on normal cells which are then destroyed[138].

Combinations of ACT with ICIs have not yet been trialed in HCC, while in other solid malignancies such combinations have been shown to be feasible and safe[139]. Conversely, ACT in combination with ablative therapies in HCC is undergoing investigation in phase I/II setting[58]. Regarding ACT monotherapy for HCC, early phase I/II studies have shown feasibility and tolerability with TILs, TCR, and CAR cell variants while several other phase I/II trials are ongoing (*reviewed in*[35,138,140]). CIK is the only ACT that has been investigated for HCC in a phase III setting (NCT00699816). This multicenter trial involved 230 HCC patients who had undergone curative surgical resection, radiofrequency ablation, or percutaneous ethanol injection that were randomized to receive adjuvant CIK immunotherapy (injection of 6.4 × 10^9 autologous CIK cells, 16 times over 60 wk) or no adjuvant therapy (controls)[141]. mPFS was significantly prolonged in the CIK arm *vs* control (44 mo *vs* 30 mo; HR: 0.63, 95%CI: 0.06-0.75). All-cause death and cancer-related death were also significantly reduced in the experimental arm. AE occurrence was higher in the experimental arm but SAEs did not differ significantly. In combination with transarterial chemoembolization (TACE), an international registry analysis of 106 clinical trials including 10225 patients of which 4889 patients in over 30 distinct tumor entities were treated with CIK cells alone or in combination with conventional or novel therapies, CIK has been shown to significantly improve mPFS and mOS (27 trials), and 5-year survival rate (9 trials) with mild AEs and graft-versus-host diseases[142]. Additionally, a systematic review and meta-analysis of 6 randomized controlled trials including 844 HCC patients concluded that adjuvant autologous CIK after curative resection significantly improved 1-year, 2-year, and 3-year disease-free survival and OS but did not significantly extend these at 4 and 5 years; AEs were comparable in CIK and control patients [143]. Furthermore, combination of CIK with ICIs has been trialed in solid and hematologic cancer with promising results and potential to be trialed in HCC in the future[142]. Despite these positive results, ACT including CIK is still not used in most centers as an adjuvant therapy, probably due to the limitations of in-house cell therapy facilities[35].

Vaccination and virotherapy: Following results of several early phase trials involving relatively small numbers of HCC patients in the past decade, vaccines and virotherapy are currently being investigated as enhancer strategies in combination with other forms of therapy as opposed to a viable monotherapy option[31,35].

Vaccines: The underlying primary immune effect of vaccines lies in their ability to enhance T-cell priming and expansion[31]. Cancer vaccines are being constructed to enhance presentation of tumor-associated epitopes to host immunity to overcome tumor-specific tolerance in the context of immune stimulation by activating and selectively expanding tumor-specific lymphocytes within the native effector cell repertoire while maintaining immune-regulatory protection against autoimmunity[144]. Vaccine-mediated stimulation of tumor-specific immunity provides a physiologic stimulus for T-cell activation, fostering a potentially more-sustained native immune response with greater durability for long-term antitumor surveillance. Alike engineered ACT, cancer vaccines may be designed to target TAAs or neoantigens, of which HCC exhibits many with high specificity[10]. Classical cancer vaccines rely on exogenous administration of antigens or antigen-pulsed dendritic cells (DCs). The only cancer therapy vaccine to be approved by the FDA to date is Sipuleucel-T, a DC-like anticancer vaccine for prostate cancer[145]. In HCC, vaccine constructs are mainly based on RNA, peptides, proteins, or DCs [31,140].

Early investigations in cancer patients and pre-clinical models have demonstrated the synergistic capacity of cancer vaccines in combination with anti-PD-1 and anti-CTLA-4 checkpoint blockade, with evidence also reported for HCC[146-148]. Vaccines can reverse immune tolerance and exhaustion seen in patients treated with ICIs by providing a stimulus to prime and expand tumor-specific T-cells that preserve their effector functions through the effect of ICIs[31]. Moreover, phase I/II studies have shown that vaccines are tolerable and can reduce recurrence rate[149] and prolong recurrence-free survival[150, 151] in patients treated with ablative therapy. However, trials with tumor lysate vaccines have failed to show promising results in terms of efficacy[31,151]. Today, combination of vaccines with ICIs are being investigated clinically in phase I/II trials in HCC (NCT04912765, NCT04248569, NCT04251117), as are combinations with ablative therapies (NCT03674073, NCT03942328), and other treatments.

Virotherapy: Cancer virotherapy represents the most common type of cancer gene therapy and involves the transfer of genetic material (transgenes) into cells to modify their gene-expression profiles *via* viral vectors[31]. Oncolytic viruses (OVs) are a type of cancer virotherapy incorporating modified viral agents that selectively replicate in cancerous cells resulting in tumor cell lysis[152]. By contrast to classical vaccines, OVs represent an in situ cancer vaccine[35]. The primary immune effect aimed with virotherapy is to reduce tumor burden and broaden TCR repertoire[31]. Yet, scientists have increasingly realized that most anti-cancer efficacy observed with OVs is attributable to enhanced immune response activation triggered by immunogenic cell death caused by the destruction of cancer cells and uptake of tumor antigens by antigen presenting cells – often enhanced by the arming of OVs with cytokine encoding genes, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) – rather than their oncolytic properties[153]. Additionally, OVs display short-lived efficacy and are not effective on repeated doses, due to brief vector replication and transgene expression as well as neutralizing antibody development after first vector administration, respectively. However, long-term vectors may broaden applications[31]. Hence, OVs are better candidates as an adjunct therapy to trigger adaptive antitumor responses which require maintenance and expansion with additional immunotherapies[154]. To date, three OVs have been approved for cancer therapy: RIGVIR for melanoma[155], Oncorine for head and neck cancer[156], and T-Vec for melanoma[157], though only the latter has been granted FDA approval.

In HCC, several OVs have been investigated clinically with some featuring promising results. JX-594 (Pexa-Vec), an oncolytic poxvirus carrying human GM-CSF genes, is the only OV to have successfully reached phase III investigation. Unfortunately, phase IIb/III trials showed that Pexa-Vec failed to improve treatment efficacy in patients previously treated with sorafenib[158], in combination with sorafenib[159], and in sorafenib-naïve patients[160]. Investigation of Pexa-Vec combination with nivolumab was also terminated early due to futility in other pivotal trials[161]. Still, phase I/II trials are ongoing to investigate different virotherapy agents as monotherapy (NCT00028496, NCT04246671), and in combination with pembrolizumab (NCT02509507, NCT02432963) for HCC.

CONCLUSION

The progress achieved within the landscape of immunotherapy for HCC is remarkable. In the last five years, ICIs have become a cornerstone systemic treatment approach in the routine clinical management of aHCC. The year 2020 saw ICIs become frontline treatments for aHCC in combination with bavacizumab, rendering sorafenib frontline only for patients who are ineligible for or contraindicated to receive immunotherapy or anti-angiogenics. In 2nd-line setting, several ICIs continue to be standard of care, with more agents emerging in the horizon. Still, much progress is yet to be made, especially concerning the lack of real-world data to support the generalizability and applicability of clinical trial

findings to a broader cohort of aHCC patients who are not subjectable to stringent clinical trial inclusion and exclusion criteria. The main obstacles to immunotherapy frequently encountered in real-world practice surround patient ineligibility for immunotherapy because of contraindications, comorbidities, or poor performance status; lack of response, efficacy, and safety data; and cost-effectiveness. Hence, the reality of immunotherapy treatment for HCC outside of trial setting is far from ideal. Further real-world data from high-quality large prospective cohort studies as well as evidence from institutional experiences of immunotherapy in patients with aHCC outside of clinical trials is mandated to aid evidence-based clinical decision-making for this cohort of individuals who indeed represent the vast majority of patients encountered. At the same time, ongoing trials investigating novel approaches to optimize systemic regimens and enhance ICI efficacy through combination with locoregional ablation, other systemic agents, and novel immune-based approaches are necessary to break new grounds. With multiple ICI agents undergoing investigation, more ICIs are likely to enter the treatment landscape for aHCC. The development of new models such the *cancer immune cycle* theory to better understand and reverse limiting steps in cancer immune evasion; the characterization and subgrouping of different tumor immune microenvironment phenotypes in aHCC; and novel means of employing machine learning algorithms to predict patient response to select targeted therapies further advance the field of precision medicine in HCC into a new era. In the years to come, ACT including CAR T cells and CIK cells are likely to become part of the treatment armamentarium against aHCC. We eagerly await to monitor the field as it advances.

FOOTNOTES

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MINIREVIEWS

Circulating tumor DNA for diagnosis, prognosis and treatment of gastrointestinal malignancies

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Abstract

Minimally invasive detection of circulating tumor DNA (ctDNA) in peripheral blood or other body fluids of patients with gastrointestinal malignancies *via* liquid biopsy has emerged as a promising biomarker. This is urgently needed, as conventional imaging and plasma protein-derived biomarkers lack sensitivity and specificity in prognosis, early detection of relapse or treatment monitoring. This review summarizes the potential role of liquid biopsy in diagnosis, prognosis and treatment monitoring of gastrointestinal malignancies, including upper gastrointestinal, liver, bile duct, pancreatic and colorectal cancer. CtDNA can now be part of the clinical routine as a promising, highly sensitive and specific biomarker with a broad range of applicability. Liquid-biopsy based postoperative relapse prediction could lead to improved survival by intensification of adjuvant treatment in patients identified to be at risk of early recurrence. Moreover, ctDNA allows monitoring of antineoplastic treatment success, with identification of potentially developed resistance or therapeutic targets during the course of treatment. It may also assist in early change of chemotherapy in metastatic gastrointestinal malignancies prior to imaging findings of relapse. Nevertheless, clinical utility is dependent on the tumor's entity and burden.

Key Words: Cell-free tumor DNA; Circulating tumor DNA; Gastrointestinal cancer; Liquid biopsy; Esophageal cancer; Gastric cancer; Liver cancer; Bile duct cancer; Pancreatic cancer; Colorectal cancer

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Core Tip: This review provides an update on the state-of-the-art circulating tumor DNA detection *via* liquid biopsy for diagnosis, prognosis and treatment in gastrointestinal malignancies and presents the strengths and limitations of this innovative method.

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INTRODUCTION

Historically, tissue biopsy or plasma protein-derived tumor markers have been the fundamental pillars of cancer diagnosis, selection of treatment, monitoring of treatment effect and estimation of prognosis [1]. As cancer is a dynamic and likely progressive disease, histological analysis of a single lesion (*i.e.*, primary tumor or metastasis) at a single time point is now being replaced by minimally invasive detection of cell-free deoxyribonucleic acid (cfDNA) *via* liquid biopsy to monitor the continual change of the disease process[1-3]. Furthermore, disconcordance and genetic differences within the primary tumor tissue over time (temporal heterogeneity) or between the primary tumor and its metastases (spatial heterogeneity) can be observed *via* next-generation sequencing (NGS)[2,4]. Thus, single conventional biopsies do not accurately reflect the cellular and genetic composition of malignancies[1]. In contrast, liquid biopsies include nucleic acids or cancer cells from the entire tumor burden of the patient and can easily be conducted serially[4].

Discovered in 1989 in patients with gastrointestinal (GI) malignancies, circulating tumor DNA (ctDNA) derives from apoptotic, necrotic, or circulating cancer cells, and constitutes a small subset (< 0.01%) of cfDNA in the plasma of peripheral blood or other body fluids[5]. cfDNA has become a promising tool for diagnosis, monitoring of antineoplastic treatment effect, and early detection of relapse, in addition to evaluating potential new drug targets[6-8]. ctDNA is thought to be actively released *via* microvesicles (exons) of double-stranded DNA. Passive release of DNA fragments into the circulation from apoptotic and necrotic cells has been demonstrated[3,9]. The amount of cfDNA is significantly higher in cancer patients than in healthy individuals, but serum levels can easily be biased by various factors; ctDNA is considered tumor-specific and more robust[1]. Nevertheless, both values are strongly influenced by preanalytical and analytical variables. The influence of differences in type of sample collection tubes, sample storage time, performing the assay with plasma or serum, use of short or long amplification assays, or the time of blood collection have been evaluated[10]. Plasma is considered superior to serum because of its robust DNA data with higher KRAS allele frequency. A greater absolute amount of DNA is yielded by serum samples, but is also more affected by contamination or lysis)[10]. Although the American Society of Clinical Oncology and the College of American Pathologists highly recommend plasma analysis for DNA detection, many investigators in the past used serum samples. Two reviews[10,11] published in 2018 claimed that 100% of gastrointestinal stromal tumor (commonly known as GIST) studies, 62% of gastric cancer studies, 29% of esophageal cancer studies, and 20% of colorectal cancer (CRC) studies used serum samples for ctDNA analysis.

Over the years, several amplification techniques, such as real-time quantitative polymerase chain reaction (referred to as qPCR)[12], digital droplet PCR (ddPCR)[13,14], beads, emulsion amplification, and magnetics (BEAMing)[15] or NGS[16] have been adopted for clinical use[4]. The most commonly employed are digital PCR (dPCR) or ddPCR techniques using water-in-oil emulsion droplets with dispersed individual DNA strands. These fluorescently labeled samples allow a binary identification system of target mutations (*i.e.* mutant *vs* wild-type alleles), leading to a very low limit of detection (LOD) ranging from 0.1%-0.001%[4]. One of the most used dPCR systems for ctDNA detection is the Bio-Rad QX-200 platform[14]. BEAMing provides a high analytical sensitivity of < 0.01% minor allele frequency (MAF) by combining emulsion PCR and flow cytometry with a focus on rare mutations in *a priori* known target mutations[4]. NGS, on the other hand, can cover a broad range of mutations in multiple cancer-associated genes but is less sensitive than dPCR (~ 1%)[4]. Safe-SeqS is one of the first and most commonly used NGS platforms (LOD 1%)[17], whereas CAPP-Seq/iDES is a newer NGS technique with LODs of 0.002%-0.00025%[18]. Depending on the entity under investigation, approaches have emerged for detection within samples with a known mutation target and those without a known mutation. In the following section we describe currently promising prospects of this new and easily harvested biomarker for diagnosis, early relapse detection, and treatment efficacy.

UPPER GI CANCER

Diagnosis

Upper GI (UGI) cancer subsumes esophageal cancer, cancer of the gastroesophageal junction, and the gastric cancer. Unfortunately, detection rates of UGI cancers are low in the early stages (approximately 20%) and reporting studies, thus, have low case numbers[19]. However, potential targets for molecular tracking are: HOXD10 (higher methylation rates in more advanced disease); ZIC1, RUNX3, and TP53 (53%); or receptor tyrosine kinases, including KRAS (15%), FGFR2, EGFR (17%), ERBB2, PIK3CA (13%), or HER2 (17%)[7,20,21]. NGS of metastatic UGI cancer in small case studies revealed detection rates of up to 87.5%[21]. Detection rates greatly depend on the MAF in the site of associated metastases, with only 23.3% in the lung, 19.2% in the liver, and only 2.5% in peritoneal metastases; the primary tumor burden is represented by tumor volume[20].

Prognosis

Relapse prediction following neoadjuvant treatment is a substantial issue in UGI cancer that affects almost all patients undergoing surgery. A study including more than 1600 patients reported postsurgical detection rates of up to about 32% and that MAF cutoff levels of > 0.25% (100% sensitivity) were associated with worse progression-free survival (PFS) (12.5 mo *vs* not reached, $P = 0.03$, $n = 22$)[20]. A significant survival disadvantage was observed in patients undergoing treatment with checkpoint inhibitors when detecting a MAF of > 3.5% prior to treatment initiation (8.8 mo *vs* 2.5 mo, $P = 0.04$, $n = 27$)[20]. If detectable, some mutations like PIK3CA (3.8 mo *vs* 13.6 mo, $P = 0.006$) or BRAF (5.6 mo *vs* 13.7 mo) indicate especially poor survival among stage IV patients[20]. On the other hand, targeted therapy, when detecting HER2 or EGFR mutation, can lead to significant survival benefits (21.1 mo *vs* 14.4 mo, $P = 0.001$)[20]. These findings need to be evaluated in larger prospective studies.

Treatment monitoring

Serial measurement of ctDNA in stage IV UGI cancer has found a significant survival benefit for patients with a > 50% decrease of the maximum MAF (13.7 mo *vs*. 8.6 mo, $P = 0.02$, $n = 35$) during the course of first-line therapy[21]. The Personalized Antibodies for Gastroesophageal Adenocarcinoma ("PANGEA") study revealed promising results in 68 patients undergoing ctDNA-guided individualized monoclonal antibody treatment compared with historical chemotherapy controls (1-year survival of 66%, median overall survival (OS) of 15.7 mo, $P = 0.0024$, median PFS of 8.2 mo, and first-line response rate of 74% *vs* about 50%)[22].

LIVER AND BILE DUCT CANCER

Diagnosis

CtDNA has been investigated in liver cancer patients for several years, and although it is still not in routine clinical use, liquid biopsy was shown to be superior to conventional plasma-derived biomarkers. For example, alpha-fetoprotein has a diagnostic sensitivity of 50% for hepatocellular carcinoma (HCC)[23]. Unfortunately, HCC has a broad range of potentially mutated genes. The most common are TP53 (c.747G>T), TERT (c.1-124C>T), and CTNNB1 (c.121A>G and c.133T>C)[24]. Generally, detection rates using liquid biopsy are expected to reach 56% in resectable HCC patients (ddPCR of 48 samples)[24]. A study published in 2006 reported sensitivity and specificity values of 69% and 93%, respectively, for discrimination of HCC and controls using cfDNA cutoff levels[25]. Subsequently, the presence of a combination of different methylated tumor suppressor genes, which rarely occur in the DNA of healthy tissue, had a reported sensitivity of 83.3% and specificity of 90.5% for detection of HCC[26]. Apart from detection of malignancies, liquid biopsy and stratification following detection of methylated peroxisome proliferator-activated receptor gamma (commonly known as PPARg) gene promoter has also shown promise for prediction of fibrosis grade in nonalcoholic fatty liver disease[27].

On the contrary, data on mutation detection *via* ctDNA in bile duct cancer is sparse, as cholangiocarcinoma is a rare disease. It has an estimated incidence 0.5-3.5/100.000), is often diagnosed at a metastasized stage, and the reported data is frequently pooled with liver or pancreatic cancer[28]. Overall, about 28% of patients with bile duct cancer show TP53 mutations, followed by 17% with ARID1A mutations and 16% with KRAS mutations[28]. However, bile duct cancer has very heterogenous mutation patterns. Using liquid biopsy in cases with a histologically verified mutation, Ettrich *et al*[28] reported a detection rate of 92% in intrahepatic cholangiocarcinoma (IHCC) and only a 55% detection rate in extrahepatic cholangiocarcinoma (referred to as EHCC).

Prognosis

Both the untargeted (cfDNA) and targeted detection of mutation, primarily of TP53 (32%), CTNNB1 (17%), and TERT (51%), has shown prognostic potential indicating poorer disease-free survival and OS in patients with HCC, regardless of tumor stage[23,24,29-31]. Moreover, vascular invasion, tumor mass,

and level of postoperative cfDNA have emerged as independent risk factors for recurrence in patients with resectable HCC[32].

Regarding cholangiocarcinoma, some studies reported poorer PFS when detecting mutations *via* liquid biopsy, especially in cases with ctDNA assay of TP53, KRAS, BAP1, or PBRM1 in settings of both curative and palliative intent, as compared with patients with nondetectable mutation[33-35]. Again, the data was obtained in IHCC patients; most studies could not detect a significant correlation regarding PFS in EHCC patients[28].

Treatment monitoring

Serial ctDNA measurement in advanced HCC has revealed progression of the disease before imaging or alpha-fetoprotein dynamics could indicate recurrence, but the studies included small case numbers[36]. As ctDNA MAF of both IHCC and EHCC correlate with tumor load, some authors estimate a potential for treatment efficacy detection in bile duct cancer, but that needs further evaluation, as serial measurement for treatment monitoring has not yet been performed[28,37]. In a January 2021 publication, Felden *et al*[31] reported prospective findings of ultra-deep sequencing and ddPCR in 121 patients that supported the treatment-monitoring potential of ctDNA as a biomarker response to antineoplastic treatment.

PANCREATIC CANCER

Diagnosis

While surgical resection can improve 5-year survival by 15%-25%, fewer than 20% of patients qualify for a primarily surgical approach[38]. In 2018, more than 50% of patients were diagnosed with distant metastases and had a 5-year survival rate of only about 3%[39]. The mean 5-year survival of all stages of pancreatic ductal adenocarcinoma (PDAC) stages is reported to be about 6%-8%, which is also due to the early systemic spread of the disease[39]. Thus, highly sensitive and reliable biomarkers are urgently needed for earlier diagnosis. Theoretically, PDAC, which accounts for 90% of all pancreatic cancers, could be an ideal entity for agnostically driven ctDNA determination as a screening biomarker because of the high rate of histologically detectable early KRAS mutations (> 90%)[40,41]. However, detection rates in histologically verified PDAC *via* liquid biopsy are significantly lower, controversially reported in literature, and very much depend on the stage of the disease (43%-54% in stages I–II; 67% in stage IV, and up to 95% if a mutation had already been detected in the tissue)[42-44]. Another study suggests much lower detection rates for early-stage PDAC[45]. Using ddPCR, Berger *et al*[46] reported in 2016 that mean cfDNA values (KRAS, GNAS) discriminated potentially premalignant cysts (*e.g.*, intraductal papillary mucinous neoplasms) and harmless pancreatic cysts. Nevertheless, the sensitivity and specificity were too low for applicability as a potential screening method. Thus, ctDNA offers little clinical application in diagnosis of localized pancreatic cancer, as it is inferior to the plasma-protein derived tumor marker CA 19-9, which has high sensitivity (70%-95%) and specificity (70%-90%). It also has a high vulnerability to coincident Lewis-negative blood group, acute cholangitis, obstructive jaundice, or chronic pancreatitis[47-51]. Therefore, the gold standard for diagnosis remains imaging combined with histological verification with endoscopic ultrasound and fine needle aspiration (commonly known as EUS-FNA)[38].

Prognosis

Although lacking usability in the initial diagnosis of PDAC, several studies demonstrated a significant correlation between both pre- and postoperative ctDNA positivity and OS [hazard ratio (HR): 2.093, P = 0.028] and PFS (HR: 4.543, P = 0.006) for both localized and metastatic PDAC (referred to as lPDAC and mPDAC, respectively)[52-55]. In 2010, Chen *et al*[56] reported a median OS of 3.9 mo *vs* 10.2 mo (P < 0.001) positivity in 91 patients with mPDAC, associated with mutKRAS ctDNA. In 2019, Lee *et al*[57] reported an OS of 5.8 mo *vs* 16.3 mo in lPDAC. The same group showed 100% of patients remaining ctDNA-positive after systemic neoadjuvant treatment following an early relapse, with a median PFS of 5 mo.

Treatment monitoring

Liquid biopsy allows earlier detection of relapse compared with plasma protein-derived tumor markers (CA 19-9), with lead times of 1 mo to 2 mo, and is more sensitive (83%) to changes in ctDNA levels[43,58]. This could indicate a potential opportunity for monitoring treatment during palliative chemotherapy using serial liquid biopsies, and ultimately making a change of the antineoplastic agent. Data on serial measurement in advanced PDAC mostly lacks large patient numbers, although promising results have raised the hope of early response to therapy and, ultimately, relapse identification[43]. Kruger and colleagues[43] were the first to report the potential of ctDNAs to indicate response as early as 14 d after treatment initiation, demonstrating major superiority to plasma-protein derived tumor markers, with a specificity of 100%. Nevertheless, the clinical survival benefit by eventual change of treatment in

patients with detected relapse using serial ctDNA measurements still needs to be explored. Targeted therapy could be another promising field of future research. Liquid biopsy has already found usage in PDAC patients suffering from BRCA1/2 mutations by providing PARP-inhibitors[59].

CRC

Diagnosis

CRC is the third leading newly diagnosed malignancy worldwide[60]. CtDNA is detectable in about 73% of stage II–III cases and 90% of patients with localized and metastatic CRC, positioning this entity as the ideal target for liquid biopsy[61,62]. Until now, liquid biopsy has not been included in the routine screening for CRC, but samples are easily assessable. Tests are becoming more cost effective and the presence of ctDNA in early-stage CRC (46% detection rate in stage I) was reported in 2015[63]. Acceptance of liquid biopsy in the general population appears to be high, based on a 2014 German study finding that patients not willing to undergo colonoscopy preferred blood tests over other noninvasive screening tools, like fecal occult blood tests[64].

Prognosis

Multivariate analyses conducted in several studies have confirmed that postoperative detection of ctDNA is an independent marker of recurrence, regardless of stage and location of the primary tumor. The 3-year PFS was 33% *vs* 87%[65,66]. In 2019, Tie *et al*[65] serially measured plasma ctDNA in 159 patients with locally advanced rectal carcinoma (T3/4 and/or N+) and treatment-naïve stage, post-chemoradiotherapy, of 4–10 wk after primary curative resection with adjuvant treatment. In an analysis that was blinded to ctDNA status, HRs and 3-year PFS significantly differed with positive liquid biopsy results. The 3-year PFS was 33% *vs* 87% after chemoradiotherapy (HR: 6.6, $P < 0.001$) and was 13.0 ($P < 0.001$) after resection, which allowed for stratification of patients with very high and very low risk of relapse[65]. Based on those findings, several ongoing prospective international studies are evaluating the potential additional clinical benefit from postoperative ctDNA positivity in CRC to identify patients at high risk of recurrence[7].

CtDNA-positive patients with stage II disease (*i.e.* with no clear recommendation for adjuvant treatment) might benefit from additional adjuvant chemotherapy. That is being tested in the DYNAMIC (ACTRN-12615000381583), COBRA (NCT04068103), and CIRCULATE AIO-KRK/PRODIGE 70 (NCT04089631/NCT04120701) trials. Whether stratifying stage III CRC patients by ctDNA results can guide decision making for intensification or de-escalation of adjuvant treatment or surveillance is being tested in the DYNAMIC-III (ACTRN-12617001566325) study[7,67-69]. Postoperative ctDNA detection has proven to be a strong indicator of distant recurrence, with a median lead time of 10 mo compared with conventional modalities such as computed tomography (commonly known as CT) and plasma-derived biomarkers like carcinoembryonic antigen (CEA)[70]. Regarding metastatic CRC, studies have demonstrated a significant survival benefit of wild-type carrier patients compared with patients with detectable ctDNA, dependent on the particular mutation[71].

Treatment monitoring

At diagnosis, approximately 80% of patients with CRC present without distant metastases and undergo primary curative resection. Over 50% of patients with stage II or III cancer also show rather unspecific abnormalities, such as elevated CEA (20%), CT abnormalities (40%), or both (13%) during the 5-year surveillance recommended by the American Society of Clinical Oncology (known as the ASCO)[72-74]. Nevertheless, until now there is no evidence of OS improvement resulting from 5-year surveillance, including clinical and endoscopic examinations, CEA measurement, and imaging[75,76]. Liquid biopsy could help to clarify uncertain findings. For example, ctDNA is positive in 85% of persons who experience imaging-verified relapse, whereas increased CEA levels are observed in only about 41% of radiologically verified recurrences[77]. Moreover, lead time of liquid biopsy compared with CEA is reported to be about 8 mo[76]. Thus, serial ctDNA measurement as a postoperative treatment monitoring method during surveillance could provide earlier detection of relapse.

The clinical benefit for OS has to be evaluated in future studies. Among other ongoing ctDNA studies investigating the benefit of adjuvant chemotherapy based on liquid biopsy findings, Danish investigators (IMPROVE-IT2; NCT04084249) have evaluated the surveillance improvement when implementing supplementary fludeoxyglucose-positron emission tomography/CT follow-up evaluation every 3 mo, based on ctDNA positivity (*i.e.* ddPCR every 4 wk) for 2 years after surgery for stage II–III CRC and early detection of relapse[78]. Most centers use NGS prior to the start of antineoplastic treatment for identification of potential therapeutic targets, providing in advance a mutational target for liquid biopsy. Interim analysis of our own ongoing study revealed detection rates of more than 92% in metastatic CRC with ddPCR of 28.5 mL plasma samples and known mutations found in tissue samples prior to analysis. That is in line with the 8% disconcordance rate in a BEAMing analysis of 236 patients reported in 2018[79].

Serial liquid biopsies allow response prediction prior to that obtained by conventional methods in metastatic-stage patients undergoing palliative chemotherapy[80]. Furthermore, studies have demonstrated the PFS and OS benefits of repeated mutational status determination for eventual rechallenge with cetuximab/irinotecan-based regimes in initially RAS/BRAF wild-type patients or patients with acquired resistance during the course of treatment[81,82].

CONCLUSION

CtDNA is ready to be integrated into routine clinical use in order to improve survival and relapse prediction in nonmetastatic GI cancers. It also allows for monitoring of antineoplastic treatment success for early detection of nonresponders, with potential early change of chemotherapy in metastatic GI malignancies prior to imaging findings of relapse. For some entities, especially CRC, rapid progress in liquid biopsy research could lead to fundamental changes in therapeutical strategies, accompanied by the desired survival improvement. The test is simple, cost effective, and easily assessable, although there are large differences in suitability, detection rate, progress of research (Table 1), tumor volume, and site of metastasis. Overall, lymph node metastases or peritoneal carcinosis lead to significantly lower amounts of detectable ctDNA compared with liver or lung metastases. Various techniques of target mutation detection have been established in clinical trials, and several potential preanalytical variables have to be taken into account when implementing these into routine clinical practice. Depending on the technologies in clinical use, the limits of detection range from about 1% (qPCR, Safe-SeqS) to 0.01% (ddPCR, BEAMing, CAPP-Seq/iDES)[4]. Nevertheless, mutation detection *via* liquid biopsy has several potential pitfalls and limitations. Firstly, standardization of sample drawing and the processing methods could help avoid common mistakes leading to very heterogenous sensitivity and specificity. Secondly, measured ctDNA levels are strongly affected by the period of time between blood draw and surgery or the initiation of chemotherapy. The ideal post-interventional interval for sample assessment needs to be further explored and eventually standardized, as ctDNA levels initially increase but continuously decline over the following weeks. The same applies for systemic antineoplastic treatment, as Maron *et al*[20] reported considerable differences in ctDNA MAF in untreated stage IV UGI patients (mean MAF: 11.6%) compared with patients receiving treatment up to 14 d prior to sample collection (mean MAF: 5%).

UGI cancer

Liquid biopsy could provide essential benefits for adjuvant and palliative treatment decision making, but low detection rates in nonmetastatic UGI cancers hinders this. Positive ctDNA after neoadjuvant treatment can identify patients with significantly increased risk of relapse (HR: 18.7), distant metastases (HR: 32.1), and cancer-associated death (HR: 23.1), but identifying how this issue should be addressed for significant survival benefit is a key question for further studies[7]. Moreover, liquid biopsy may become integrated into treatment response prediction, especially of immunotherapy, in advanced UGI cancers[83].

Liver cancer

Liquid biopsy offers significant prognostic potential in resectable HCC and was recently established as a promising biomarker for early response prediction of systemic therapy in advanced HCC; although, improvement regarding the LOD is necessary to implement these findings into clinical practice[31]. Ongoing studies are attempting to lower the LOD using multifocal screening panels, which could establish ctDNA as a valuable diagnostic and predictive biomarker for HCC patients, regardless of the disease stage[23].

Bile duct cancer

Until now, no prospective studies have investigated the benefits of liquid biopsy in bile duct cancer. Detection rates of mutations *via* liquid biopsy in histologically verified patients distinguishes between extra- and intrahepatic cholangiocarcinoma in favor of IHCC[28]. Nevertheless, screening for certain mutations, like IDH1 or FGFR, could help to establish personalized first-line palliative antineoplastic treatment, for example with ivosidenib (IDH1) or FGFR-kinase-inhibitors in the future[84,85].

Pancreatic cancer

For localized or locally advanced pancreatic cancer, ctDNA positivity prior to treatment is predictive of survival and relapse. This finding could assist decision making for additional perioperative or adjuvant antineoplastic treatment of high-risk patients. Negative ctDNA, on the other hand, holds no additional informative value in those with pancreatic cancer. Since 2014, pancreatic cancer has been known to release significantly lower amounts of detectable circulating tumor cells into the bloodstream compared with most other tumors, including colorectal, gastric, lung, breast, ovarian, prostate, bladder, or renal cancer[86]. However, some recent, small pilot studies have shown promising screening rates using specially designed detection methods. For example, hTERT promoter-regulated oncolytic herpes

Table 1 Detection rates and impact on outcome of circulating tumor DNA in gastrointestinal cancer

Entity	Detection rate	Common target	OS ctDNA −/ +	PFS ctDNA −/ +
mPDAC	67%-75%[43]	> 90% KRAS, but also TP53, SMAD4	8.4 *vs* 3.2[89]	5 *vs* 3.9[43]
lPDAC	21%-69%[42]		16.3 *vs* 5.8[57]	19 *vs* 8[57]
mCRC	> 90%[79]	KRAS, NRAS, BRAF, PIK3CA, NRAS, APC, TP53, EGFR, ERBB3/4	36.5 *vs* 17.1[90]	RAS 8.3 *vs* BRAF 4.5 *vs* wild-type 22.9[72]
lCRC	73%(43%-80%)[62,63]		-	87% *vs* 33%[65]
				3-yr PFS
mUGIC	87.5%[21]	TP53, HER2, MET, EGFR, KRAS	13.7 *vs* 8.6[20]	7.4 *vs* 4.9[83]
lUGIC	20%[19]		66.9 *vs* 37.7[10]	12.5 *vs* not reached[20]
HCC	56.3%[24]	TP53, CTNNB1, TERT	61% *vs* 24%[29]	47% *vs* 22%[29]
			3-yr OS	3-yr PFS
mIHCC	92%[28]	TP53, KRAS, ARID1A	16.4 *vs* 7.4[91]	8.2 *vs* 4.6[91]
mEHCC	55%[28]		NS[28]	NS[28]

−/+: ctDNA negative/positive; ctDNA: Circulating tumor DNA; HCC: Hepatocellular carcinoma; lCRC: Localized colorectal carcinoma; lPDAC: Localized pancreatic ductal adenocarcinoma; lUGIC: Localized upper gastrointestinal carcinoma; mCRC: Metastatic colorectal carcinoma; mEHCC: Metastatic extrahepatic cholangiocarcinoma; mIHCC: Metastatic intrahepatic cholangiocarcinoma; mPDAC: Metastatic pancreatic ductal adenocarcinoma; mUGIC: Metastatic upper gastrointestinal carcinoma; NS: Not significant; OS: Overall survival in months; PFS: Progressive-free survival in months.

simplex virus-1 targeting telomerase reverse transcriptase was positive in 88.2% of 17 patients with PDAC in all stages of disease. A parallel-flow microfluidic chip detected 91.7% of 12 mPDAC patients[4,87,88]. Methods like these need further study before they can be integrated into the clinical routine.

CRC

Significant progress has been made in ongoing trials of liquid biopsy in nonmetastatic CRC, especially on ctDNA-guided change in adjuvant therapeutic regimes, which may have a fundamental impact in future care. CRC is the ideal entity for liquid biopsy because of high rates of mutation detection and the total amount of cf/ctDNA in the plasma. This is in addition to the fact that tissue samples for Safe-SeqS/NGS are available for a sufficient proportion of patients to allow for guided mutation detection, thus resulting in very high specificity and sensitivity rates. Metastatic CRC offers even higher detection rates and could optimally benefit from the use of liquid biopsy in prognosis estimation and treatment evaluation in the future.

FOOTNOTES

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MINIREVIEWS

Current treatment landscape for oligometastatic non-small cell lung cancer

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Abstract

The management of patients with advanced non-small cell lung carcinoma (NSCLC) has undergone major changes in recent years. On the one hand, improved sensitivity of diagnostic tests, both radiological and endoscopic, has altered the way patients are staged. On the other hand, the arrival of new drugs with antitumoral activity, such as targeted therapies or immunotherapy, has changed the prognosis of patients, improving disease control and prolonging survival. Finally, the development of radiotherapy and surgical and interventional radiology techniques means that radical ablative treatments can be performed on metastases in any location in the body. All of these advances have impacted the treatment of patients with advanced lung cancer, especially in a subgroup of these patients in which all of these treatment modalities converge. This poses a challenge for physicians who must decide upon the best treatment strategy for each patient, without solid evidence for one optimal mode of treatment in this patient population. The aim of this article is to review, from a practical and multidisciplinary perspective, published evidence on the management of oligometastatic NSCLC patients. We evaluate the different alternatives for radical ablative treatments, the role of primary tumor resection or radiation, the impact of systemic treatments, and the therapeutic sequence. In short, the present document aims to provide clinicians with a practical guide for the treatment of oligometastatic patients in routine clinical practice.

Key Words: Oligometastatic; Non-small cell lung carcinoma; Non-small cell lung cancer; Oligometastasis

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Core Tip: The treatment of oligometastatic non-small cell lung cancer patients remains controversial. The lack of solid evidence for the best therapeutic strategy and the multiple options currently available for both systemic and local treatments make this particular population of patients a challenge for clinicians. Improvement of surgical and radiotherapy techniques and the appearance of different ablative methods, such as radiofrequency or cryoablation, have made it possible to radically treat metastases in any location. In addition, recent prospective studies suggest that combining these ablative therapies with systemic treatments improve patient outcomes. We discuss the current status of the management of oligometastatic patients.

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INTRODUCTION

Up to two-thirds of patients diagnosed with non-small cell lung cancer (NSCLC) present with advanced disease on diagnosis, or develop incurable metastases during the course of the disease[1]. Despite the heterogeneity of this group of patients, their treatment is largely systemic. In recent years, new systemic treatments have appeared, such as tyrosine kinase inhibitors (TKIs) with molecular targets or immunotherapy (ICI), which have significantly improved the efficacy of these systemic treatments, leading to prolonged survival in candidates for targeted therapies or immunotherapy. Moreover, the prognosis is very different for patients with a low metastatic volume. This is reflected in the 8th tumor-node-metastasis (TNM) classification, which distinguishes between patients with a single extra-thoracic metastasis, M1b stage IVA, and patients with multiple lesions in one or multiple organs, M1c stage IVB [2]. Patients with a low metastatic burden, also referred to as oligometastatic, can benefit from local treatment of the primary tumor and metastatic sites. The term oligometastatic, coined by Hellman and Wichselabum in 1995[3,4], refers to an intermediate situation between potentially curable local neoplastic disease and incurable widespread metastatic cancer. In the case of NSCLC, oligometastatic patients constitute 26% to 50% of patients with advanced disease, depending on whether the cutoff is taken at ≤ 3 or ≤ 5 metastatic locations[5,6]. Precisely, the main challenge for an optimal approach to oligometastatic disease has been the lack of consensus in its definition. Recently, the European Consensus defined oligometastatic state as a maximum of five metastases from up to three different sites[7], although this definition is not unanimously accepted by the scientific community, and some

prospective studies developed in this context define oligometastatic stage as a maximum of three metastases.

Initially, surgery was the only radical treatment that could be offered to these patients. Now, however, thanks to technological advancements, they can receive ablative irradiation doses by stereotactic body radiotherapy (SBRT) at cranial and extracranial levels, which is both safe and well-tolerated. When local treatment of metastases is combined with systemic treatment, 5-year survival rates between 8.3% and 86% can be achieved[8]. Three randomized studies in oligometastatic patients have shown that this radical local treatment of metastatic locations increases progression-free survival (PFS) and even benefits overall survival (OS)[9-11]. However, it is still unclear which patients can benefit from this strategy. Although there is a lack of consensus about the definition of oligometastases, for some patients with oligoprogression, local treatment of these sites can increase PFS without exhausting new lines of systemic chemotherapy (CT).

In this review, we propose to explore the most controversial aspects of patients with oligometastatic NSCLC, examining in greater depth aspects such as: the definition of this condition, the selection of patients, and the combination of systemic and local treatments.

THE DEFINITION OF OLIGOMETASTATIC DISEASE

Oligometastatic lung cancer refers to a group of patients with stage IV NSCLC, who present with limited metastatic disease in terms of the number of lesions and organs affected. The incidence of oligometastatic NSCLC has been estimated at between 27% and 55%, depending on the series published [12]. The most frequent oligometastatic location is the brain (36%), followed by the contralateral lung (34%), suprarenal gland (13%), bones (9%), and liver (2%)[13]. Oligometastatic disease, more accurately referred to as an oligometastatic state, can have a more indolent biology than widespread metastases, or at the least, microscopic disease that can be eradicated with systemic therapy. This limited metastatic phenotype could benefit from local aggressive therapy known as consolidation therapy. In fact, an ongoing study is examining different epigenetic markers such as microRNAs[14], to determine their ability to distinguish between the oligometastatic state and widespread metastases. This distinction together with the determination of different prognostic factors are crucial to select patients in whom radical treatment of the primary tumor and of the oligometastases could improve PFS and OS[9].

Currently, the concept of limited metastatic disease is not clearly defined and there is some discrepancy among authors. A European multidisciplinary group recently agreed to accept the definition of oligometastatic disease as the presence of up to five metastases in three different organs[7]. However, this is not universally accepted and additional studies are required to standardize the concept of oligometastases. Within the oligometastatic state, different patterns of presentation of the disease and its response to treatment can be clearly distinguished. The term synchronic or "*de novo*" oligometastatic disease refers to the initial simultaneous diagnosis of both the primary lung tumor and a limited number of metastases. This presentation pattern appears to have a worse prognosis than metachronic oligometastatic disease or oligorecurrence, in which the patient develops distance metastases after having received radical treatment with curative intent of the primary lung tumor, with an apparent local control of the disease[12,15]. In both patterns, the oligometastatic phenotype seems to reflect the biology of the underlying tumor rather than being related to any specific previous therapy. Another two patterns correspond to patients with initially widespread metastases who receive systemic treatment and achieve a partial response, consisting of the stable persistence of a small number of oligometastases (oligopersistent disease or "induced oligometastasis") with possible later progression (oligoprogression). These scenarios are more common among patients treated with targeted therapies who present acquired resistance to treatment.

ALTERNATIVES TO ABLATIVE TREATMENT OF METASTASES

The main local treatments in oligometastatic disease correspond to surgical resection, radiotherapy treatment, and ablative radiofrequency techniques[16]. Although there are no prospective studies that compare the efficacy of these treatments, the main characteristics and published evidence for each of these therapeutic alternatives are described below.

SURGERY

Traditionally, surgery has always been the elective approach in oligometastatic patients[17].Surgical indication depends on several factors relating to the metastases (size, number, and locations), and also on patient-related factors (age, performance status, comorbidities, and prognosis). Over the past decade, the rate of metastectomies among NSCLC patients has increased and these mainly correspond to

interventions on lung, brain, and adrenal gland metastases. Moreover, mortality has declined with the development of less invasive advanced surgical techniques[18]. Most evidence for the benefits of surgery can be found in studies on patients with brain metastases. Patchell *et al*[19] randomized 48 patients with a single brain metastasis (77% of whom were diagnosed with NSCLC) to whole brain radiotherapy (WBRT) or surgical resection of the metastasis followed by WBRT. The results demonstrated an increased local control and OS in the group treated surgically. Few studies on the surgical resection of extracranial metastases have been published and most of these are retrospective and highly heterogeneous regarding time of onset of the metastases and their location[20-22].

RADIOTHERAPY

Thanks to technological advances in recent years, large doses of radiation can be delivered with high precision to several sites. Brain metastases are treated with stereotactic radiosurgery and extracerebral lesions with SBRT, or stereotactic ablative radiotherapy (SABR). One of the advantages of these treatments is that they require fewer sessions, each of a short duration. They are also safe, produce minimum toxicity, and do not require long interruptions in systemic chemotherapy.

Most studies are retrospective, but some prospective randomized phase II studies focusing on the efficacy and safety of these techniques have produced promising results[8-10,23-28] (Table 1). Results are pending, over the next few years, for several ongoing phase 3 studies[29,30] (Table 2).

RADIOFREQUENCY ABLATION

The radiofrequency ablation (RFA) technique consists of applying high frequency microwaves by means of a catheter inserted inside the tumor to destroy the tissue with heat. RFA has been used for both primary lung tumors and pulmonary metastases. Simon *et al*[31] treated 153 patients with primary or medically-inoperable metastatic NSCLC with RFA. For stage I NSCLC, they reported OS at 1, 2, and 5 years of 78%, 57%, and 27%, respectively. Tumoral control was 83%, 64%, and 47% at 1, 2, and 5 years for tumors of 3 cm or less, and for tumors larger than 3 cm, was 45%, 25%, and 25%, respectively. The incidence of pneumothorax was 28.4% (52 of 183 sessions) and 9.8% (18 cases) required placement of a drain. More recently, Picchi *et al*[32] reported a retrospective series of 174 patients with lung cancer treated with 264 CT-guided ablation sessions. In patients with primary lung lesions, the OS rates were 66.73% at 1 year, 23.13% at 3 years, and 16.19% at 5 years. In patients affected by metastatic lung lesions, the OS rates were 85.11%, 48.86%, and 43.33%, respectively, at 1, 3, and 5 years[32]. Although evidence is scarce, these experiences support CT-guided RFA in patients with primary and metastatic lung cancer as an alternative therapy in non-surgical candidates.

CRYOABLATION

This technique destroys the tissues by extreme cold and freezing. Cryoablation is currently used routinely to treat lung cancers with specific clinical indications. Bronchoscopic cryoablation is an accepted, standard-of-care for the safe and effective treatment of obstructing endobronchial tumors in the central airways[33-36]. Cryoablation has also been used as a treatment option for unresectable primary and secondary peripheral lung tumors[37,38].

Recently, high rates of tumoral control and promising survival outcomes have been reported in a series of patients with metastatic lung cancer lesions treated with this technique[39], although more research is required to verify these findings.

THE ROLE OF SURGERY AND RADIOTHERAPY IN PRIMARY TUMORS

Local therapies in primary tumors should conform with the principles governing a good control of the pulmonary neoplastic disease and, in this context, the concept of oligometastatic disease has become a different entity. The most important prognostic factor is the stage of spread according to the TNM classification, but in recent years histological subtype, lymphovascular spread, and genetic and molecular alterations have gained in importance[40].

In the treatment of primary tumors per se, the type of patients is an important prognostic factor that can affect survival[41]. The lymph nodes should be examined thoroughly to rule out pathological mediastinal or hilar involvement. This is an important prognostic factor as it could indicate lymphatic and hematogenic spread, thus limiting the options of intrathoracic control and would also increase the risk of spread of the metastatic disease[12].

Table 1 Main studies on stereotactic body radiotherapy for the treatment of oligometastatic non-small cell lung carcinoma

Ref.	Year	Patients (*n*)	Site of oligo-metastasis	N	Dose (Gy/fraction)	Systemic therapy	Median follow-up (mo)	Median PFS (mo)	Median OS (mo)
Retrospective studies									
Inoue *et al*[27]	2010	41[1]	Brain, lung, adrenal	< 5	48/8 (adrenal)35-60/4-8 (lung)	NA	20	3-yr PFS 20%	24
Hasselle *et al*[28]	2012	25	Multiple	< 5	24-70/3-20	Various	21	4.2 (all); 12 (1 met)	23 (1 met)
De Rose *et al*[26]	2016	60	Lung	< 5	48-60/3-8	Chemo	28	32.2 (actuarial)	32.1 (actuarial)
Single arm prospective trials									
Salama *et al*[23]	2012	61[1]	Multiple	< 5	24-48/3	Chemo	20.9	2-yr PFS 22%	2-yr OS 56.7%
De Ruysscher *et al* [20]	2012	40	Multiple	< 5	54/3[2]	Chemo	27.7	12.1	13.5
Collen *et al*[29]	2014	26	Multiple	< 5	50/10	Chemo	16.4	11.2	23
Randomized phase II trials									
Gomez *et al*[25]	2016	49	Multiple	< 3	NR	Chemo	12.4	14.2 *vs* 4.4	41.2 *vs* 17
Iyengar *et al*[10]	2018	29	Multiple	< 5	21-37.5/1-5	Chemo	9.6	9.7 *vs* 3.5	Not reached *vs* 17
Palma *et al*[11]	2019	99	Multiple	< 5	35-60/3-8	Chemo	25	12 *vs* 6	41 *vs* 28

[1]Diverse primary histology including non-small cell lung carcinoma.
[2]Only 1patient received stereotactic body radiotherapy.
Chemo: Chemotherapy; N: Number of oligometastatic lesions per patient; NA: Not applicable; NR: Not reported; OS: Overall survival; PFS: Progression-free survival.

On the other hand, the type of local therapy chosen should guarantee complete local control. Surgery is the most frequent local treatment in published studies[41]. Moreover, for a therapeutic approach to oligometastatic disease, complete resection (R0) must be performed[42]. In the case of surgery, the patient's clinical condition must be good enough to ensure not only that the tumor can be resected, but that the patient can withstand an operation. In other words, that the patient's overall cardiologic and respiratory functional status are sufficient to permit surgical intervention.

The role of radiotherapy and its modalities depend upon the stage of the primary tumor. In the case of external curative radiotherapy (EBRT), this is defined by delivery of a biologically effective dose (BED) higher than or equal to $60Gy_{10}$. In the case of SBRT with intention-to-treat, a BED higher than or equal to 100 Gy_{10} is required[43]. In the initial stages, SBRT is indicated when surgical intervention is not possible, or when the patient refuses surgery[43]. EBRT is only used in non-operable patients, who do not fulfil criteria for SBRT. In stage III, if the lesion is potentially resectable, the combination of radiotherapy and chemotherapy plays a dominant role within multimodal treatments, either pre or post-operatively[44].

In this stage, if the tumor is unresectable, the elective treatment is radiotherapy delivered concurrently with chemotherapy. Sequential administration is possible if the size of the tumor makes it difficult to deliver sufficient radiation. Radiation therapy can also be delivered alone if chemotherapy is contraindicated[45].

SYSTEMIC TREATMENT IN THE OLIGOMETASTATIC PATIENT

In the management of oligometastatic NSCLC patients, local treatments of surgery or radiotherapy have been used to reduce tumoral burden and prolong OS and PFS. For years, the evidence supporting this strategy was mainly provided by retrospective studies in which encouraging results were observed in patients treated with local ablative therapies compared to those receiving systemic therapy alone[7]. The recent publication of some randomized prospective studies has provided valuable information to help

Table 2 Ongoing studies on stereotactic body radiotherapy in oligometastatic non-small cell lung carcinoma

Title	Patients	Study design	Estimated completion
Stereotactic Ablative Radiotherapy for Oligometastatic Non-Small Cell Lung Cancer. A Randomised Phase III Trial Institution: University College London Clinical Trials.gov identifier: NCT02417662	340	Phase 3 multicenter: chemotherapy alone or chemotherapy + radical radiotherapy (conventional RT and SABR) Primary histology: all NSCLC 1-3 oligometastatic lesions Primary outcome measure: OS	August 2022
Maintenance Systemic Therapy Versus Local Consolidative Therapy (LCT) Plus Maintenance Systemic Therapy for Limited Metastatic Non-Small Cell Lung Cancer (NSCLC): A Randomized Phase II/III Trial (NRG LU-002) Institution: NRG Oncology Clinical Trials.gov identifier: NCT03137771	400	Phase 2/3 multicenter: maintenance chemotherapy or SBRT + maintenance chemotherapy Primary histology: all NSCLC 1-3 oligometastatic lesions Primary outcome measure: PFS	August 2022
Randomized Phase III Trial of Local Consolidation Therapy after Nivolumab and Ipilimumab for Immunotherapy-naive Patients with Metastatic NSCLC (LONESTAR)-Strategic Alliance: BMS Institution: M.D. Anderson Cancer Center Clinical Trials.gov identifier: NCT03391869	360	Phase 3 multicenter; systemic treatment only with nivolumab and ipilimumab, or induction nivolumab and ipilimumab followed by local consolidative therapy with surgery and/or radiotherapy Primary histology: all NSCLC 1 oligometastatic lesions Primary outcome: OS	December 2022
A Randomised Trial of Conventional Care Versus Radioablation (Stereotactic Body Radiotherapy) for Extracranial Oligometastases Institution: Royal Marsden NHS Foundation Trust Clinical Trials.gov identifier: NCT02759783	245	Phase 2/3 multicenter: standard of care + SBRTPrimary histology: breast, prostate or NSCLC 1-3 oligometastatic lesions Primary outcome measure: PFS	October 2024
A Randomized Phase III Trial of Stereotactic Ablative Radiotherapy for the Comprehensive Treatment of 4-10 Oligometastatic Tumors (SABR-COMET 10) Institution: Lawson Health Research Institute Clinical Trials.gov identifier: NCT03721341	159	Phase 3 multicenter: stereotactic ablative radiotherapy, plus standard of care treatment; chemotherapy, immunotherapy, hormones, or observation given at the discretion of the treating oncologist Various histology including NSCLC 4-10 oligometastatic lesions Primary outcome: OS	January 2029
Randomized Phase II Trial of Osimertinib With or Without Local Consolidation Therapy (LCT) for Patients With EGFR-Mutant Metastatic NSCLC (NORTHSTAR) Institution: M.D. Anderson Cancer Center Clinical Trials.gov identifier: NCT03410043	143	Phase 2 multicenter: osimertinib followed by local consolidative therapy with surgery and/or radiotherapy or maintenance osimertinib alonePrimary histology: NSCLC > 1oligometastatic lesion Primary outcome: PFS	January 2023
A Multicenter Single Arm Phase II Trial Assessing the Efficacy of Immunotherapy, Chemotherapy and Stereotactic Radiotherapy to Metastases Followed by Definitive Surgery or Radiotherapy to the Primary Tumor, in Patients With Synchronous Oligometastatic Non-small Cell Lung Cancer Institution: European Thoracic Oncology Platform Clinical Trials.gov identifier: NCT03965468	47	Phase 2 multicenter: durvalumab, carboplatin/paclitaxel chemotherapy, followed by SBRT to all oligometastases. Restaging at 3 mo definitive local treatment with surgical resection of primary tumor or RT 60-66 Gy to the primary tumor if not disease progression 1-3 oligometastatic lesions Primary outcome: PFS	December 2023

OS: Overall survival; PFS: Progression-free survival; RT: Radiotherapy; SABR: Stereotactic ablation radiotherapy; SBRT: Stereotactic body radiotherapy.

treatment decisions in this setting.

The SABR-COMET study is a phase II prospective clinical trial in which 99 patients with different types of oligometastatic tumor (a maximum of 5 metastatic sites) were randomized to receive SBRT and standard systemic treatment, or systemic treatment alone[11]. Ablative treatment with SBRT significantly increased OS [41 mo *vs* 28 mo, hazard ratio (HR): 0.57, 95% confidence interval (CI): 0.30-1.10]. Only 18 patients in this cohort had a primary lung tumor, thus making it difficult to extrapolate

the results for application in this patient group. In the SBRT-treated group, a higher proportion of patients had breast and prostate cancer. The less aggressive history of these entities could also affect outcomes. However, a post-hoc analysis which excluded patients with breast and prostate cancer still found a significant benefit for patients receiving ablative radical treatment, with a survival rate at 5 years of 33% compared with 16% in patients receiving the standard treatment.

More recently, the findings of several phase II clinical trials in patients with lung cancer have been published. Iyengar *et al*[10] published the results of a phase II clinical trial in 29 patients with oligometastatic advanced NSCLC who had completed induction chemotherapy with disease response or stabilization. Patients were randomized to receive maintenance chemotherapy *vs* SBRT on all tumoral sites followed by maintenance chemotherapy[10]. A significant increase in PFS was observed in the patient group receiving the radical treatment (9.7 mo *vs* 3.5 mo; P = 0.01), with excellent local control of irradiated sites and no rise in toxicity. Similarly, Gomez *et al*[9] published the results of a phase II trial in which 49 patients with advanced lung cancer, with three or fewer metastases at diagnosis, had been treated with induction therapy and were randomized to receive local radical treatment and maintenance with standard systemic therapy *vs* systemic therapy exclusively. They found significant differences in both PFS (14.2 mo *vs* 4.4 mo; P = 0.022), and in OS (41.2 mo *vs* 17 mo; P = 0.017) in favor of the combined treatment[9].

More recently, the annual conference of the American Society of Clinical Oncology reported the results of the SINDAS study. This phase III randomized clinical trial explored the role of stereotactic radiotherapy combined with first or second generation tyrosine kinase inhibitors of epidermal growth factor receptor (EGFR) (tyrosine kinase inhibitor [TKI]-EGFR) *vs* TKI-EGFR alone in first-line treatment of patients with EGFR-mutant advanced oligometastatic lung adenocarcinoma, with five or fewer metastatic lesions[46]. A total of 133 patients were included (65 in the TKI arm) and 68 in the TKI-SBRT arm, finding a significant difference in favor of the experimental arm for both PFS [20.2 mo *vs* 12.5 mo, HR: 0.6188 (95%CI: 0.3949-0.9697); P < 0.001) and OS [25.5 mo *vs* 17.4 mo, HR: 0.6824 (95%CI: 0. 4654-1.001); P < 0.001), with no increase in toxicity[46].

CONCLUSION

Oligometastatic disease (OMD) is a unique condition characterized by a limited number of metastases and an indolent evolution. Because of the different prognosis of this condition, the TNM classification considers stage IV with a single metastatic site as a different category, called stage IV1b[2]. However, the World Health Organization classification is much more heterogeneous and includes patients with a greater number of metastases (up to 5 for some authors). Because of this difference, in an attempt to reach a consensus, the European Organization for Research and Treatment of Cancer established the most accepted definition to be the presence of five metastases and three affected organs after staging with computed tomography/positron emission tomography (CT/PET) and brain magnetic resonance imaging[14]. Noteworthy, the main points to consider in OMD are that all lesions (primary lesions and metastases) can be managed with an intention-to-treat approach and that the goal of treatment must be curative.

Growing interest in OMD has arisen from three main developments. First, an improvement in diagnostic techniques, mainly with the use of CT/PET in lung cancer staging, has resulted in an increasing number of patients being diagnosed with fewer metastases. The prognosis of this group is also better[21]. Moreover, technological advances in the field of radiotherapeutic oncology mean that high doses of radiation can be applied to specific sites. This non-surgical approach is preferred by patients with OMD as they can avoid the morbidity and mortality derived from surgical intervention. Over the past few years, the number of studies into the use of SBRT in OMD has been increasing. These have not only focused on brain metastases, but also on metastases of liver, lung, bone, and multiple organs, reporting local control rates of 70%-90% and a toxicity ≥ grade 3 lower than 10%[47]. The final aspect to consider, but not the least important, concerns the employment of immunotherapy in lung cancer treatment. Ionizing radiations can alter the tumor (beyond merely reducing the number of viable cells) and also its microenvironment, producing a specific immune response (antigenic tumoral death) that can trigger an immune response in non-irradiated sites (abscopal effect)[48]. This immunogenic effect is more pronounced with SBRT, in which high doses are delivered in few fractions[49], making this even more attractive as a treatment of OMD.

A question frequently posed in OMD is whether the better prognosis is due to the ablative treatment or the more indolent course of the disease. In a retrospective study of 90 patients with ≤ 3 metastases, after adjusting for factors that could potentially affect OS and PFS, it was found that patients who received local intensive treatment with CT + radiotherapy or surgery, or both, had better OS and PFS than those who received less intensive treatments, such as palliative CT alone[50]. However, randomized studies in both the general population[9,11] and in the population with EGFR mutations [46] have shown that the addition of local ablative treatment to systemic treatment is associated with increased PFS and OS.

One of the greatest remaining challenges is to distinguish between patients with OMD, characterized by a reduced number of metastases, and those with pre-widely metastatic disease, in other words, those diagnosed as having a small number of metastases but who develop multiple metastases in the following weeks or months. A search is currently underway for genetic profiles[51,52], either epigenetic modifications by overexpression or inhibition of microRNA[53,54] or methylations of genetic loci, that regulate the expression of the microRNA they encode[55]. These could possibly explain the limited rather than extensive spread of the disease in these patients. However, a greater knowledge of the immune system has revealed the importance of its interaction with the tumor for tumoral control or spread. Pitroda *et al*[56] *via* integrated transcriptional analysis, describe three molecular subtypes of liver metastases of colon cancer, all biologically different and each with a clinical course that is independent of known clinical risk factors. Canonical and stromal subtypes are characterized by a lack of, or a reduction in, T cell infiltration and the expression of non-immune inflammatory pathways, and are linked to a higher recurrence rate and a greater number of metastases. By contrast, the immune subtype, characterized by upregulation of the immune genes and a greater infiltration of T cells in the tumor, is associated with better survival, with relapse limited to between one and three metastases. These findings are in line with studies that show that the adaptive immune response plays a key role in controlling metastatic spread[57]. From these findings, it could be hypothesized that OMD would represent a point of equilibrium between tumoral growth and its inhibition by the immune system.

Over the next few years, further research into the immune and molecular profiles of OMD patients, combined with the application of radiotherapy with its immunogenic role, and treatment with new immunomodulator agents could be beneficial for these patients.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Molecular docking of DS-3032B, a mouse double minute 2 enzyme antagonist with potential for oncology treatment development

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Abstract

BACKGROUND

It is known that p53 suppression is an important marker of poor prognosis of cancers, especially in solid tumors of the breast, lung, stomach, and esophagus; liposarcomas, glioblastomas, and leukemias. Because p53 has mouse double minute 2 (MDM2) as its primary negative regulator, this molecular docking study seeks to answer the following hypotheses: Is the interaction between DS-3032B and MDM2 stable enough for this drug to be considered as a promising neoplastic inhibitor?

AIM

To analyze, *in silico*, the chemical bonds between the antagonist DS-3032B and its binding site in MDM2.

METHODS

For molecular docking simulations, the file containing structures of MDM2 (receptor) and the drug DS-3032B (ligand) were selected. The three-dimensional structure of MDM2 was obtained from Protein Data Bank, and the one for DS-3032B was obtained from PubChem database. The location and dimensions of the Grid box was determined using AutoDock Tools software. In this case, the dimensions of the Grid encompassed the entire receptor. The ligand DS-3032B interacts with the MDM2 receptor in a physiological environment with pH 7.4; thus, to simulate more reliably, its interaction was made with the calculation for the prediction of its protonation state using the MarvinSketch® software. Both ligands, with and without the protonation, were prepared for molecular docking

using the AutoDock Tools software. This software detects the torsion points of the drug and calculates the angle of the torsions. Molecular docking simulations were performed using the tools of the AutoDock platform connected to the Vina software. The analyses of the amino acid residues involved in the interactions between the receptor and the ligand as well as the twists of the ligand, atoms involved in the interactions, and type, strength, and length of the interactions were performed using the PyMol software (pymol.org/2) and Discovery Studio from BIOVIA®.

RESULTS

The global alignment indicated crystal structure 5SWK was more suitable for docking simulations by presenting the p53 binding site. The three-dimensional structure 5SWK for MDM2 was selected from Protein Data Bank and the three-dimensional structure of DS-3032B was selected from PubChem (Compound CID: 73297272; Milademetan). After molecular docking simulations, the most stable conformer was selected for both protonated and non-protonated DS-3032B. The interaction between MDM2 and DS-3032B occurs with high affinity; no significant difference was observed in the affinity energies between the MDM2/pronated DS-3032B (-9.9 kcal/mol) and MDM2/non-protonated DS-3032B conformers (-10.0 kcal/mol). Sixteen amino acid residues of MDM2 are involved in chemical bonds with the protonated DS-3032B; these 16 residues of MDM2 belong to the p53 biding site region and provide high affinity to interaction and stability to drug-protein complex.

CONCLUSION

Molecular docking indicated that DS-3032B antagonist binds to the same region of the p53 binding site in the MDM2 with high affinity and stability, and this suggests therapeutic efficiency.

Key Words: DS-3032B; Mouse double minute 2 antagonist; Molecular docking; Tumor suppressor p53

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Core Tip: The knowledge, at the molecular level, of the complexes formed by therapeutic drugs and their target in the body are relevant to understand the efficiency of the drug. These data can be provided, with high reliability, by bioinformatics tools, which saves time in relation to *in vitro* and *in vivo* analyses. The drug DS-3032B has been a potential candidate for oncogenic treatment in preclinical trials, but clinical studies are scarce. This work shows data on chemical interactions between this drug and its target, mouse double minute 2, that corroborate the preclinical data and demonstrate the stability of the therapeutic complex.

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INTRODUCTION

Cancer is a genetic disease whose evolution leads to numerous changes in DNA. According to a survey conducted by the International Agency for Research on Cancer in 2018, cancer was considered the second leading cause of death in the world, affecting 18.1 million people and causing the death of 9.6 million people around the world[1]. For Brazil, the estimate for the triennium 2020-2022 predicts that 625000 new cases of cancer will occur[2].

While proto-oncogenes are genes responsible for the positive regulation of cell proliferation, tumor suppressor genes are genes responsible for negative regulation; in other words, it inhibits cell multiplication. An example of this class is the p53 gene, which is found mutated in about half of human cancers[3]. p53 is a transcription factor activated by signs of stress, such as DNA damage, oncogenes activation, and nutritional deprivation[4]; it also has an essential function in DNA damage repair and antioxidant response regulation[5].

Therefore, overactivation of p53 is considered as an option for selective therapies against cancer, providing a targeting of neoplastic cells and sparing unaffected normal tissue[6]. One proposed treatment aims to inhibit tumor growth by activating the tumor suppressor protein p53 through inhibition of the mouse double minute 2 (MDM2)[7].

MDM2 is an E3 ubiquitin ligase enzyme that has a negative regulatory role of the tumor suppressor p53. This enzyme controls transcriptional activity and stability of p53. MDM2 expression is regulated in several tumors, resulting in loss of p53-dependent activities, such as apoptosis and cell cycle arrest[8].

p53 is targeted for degradation by the proteasome by MDM2. Through E3 ubiquitin ligase activity, MDM2 promotes ubiquitination of p53, leading to increased p53 degradation. In some human tumors, MDM2 has been shown to be abnormally upregulated leading to enhanced degradation and reduction of p53 activity[9].

The molecule DS-3032B (Milademetan or RAIN-32) is an MDM2 antagonist that prevents its interaction with p53. Clinical trials with DS-3032B have been conducted by the National Institute of Health (phase I) in patients with leukemia and lymphoma and showed clinical efficacy[10]. A phase I trial evaluated the safety, tolerability, efficacy, and pharmacokinetics of DS-3032B in Japanese patients with solid tumors who relapsed after or refractory to standard therapy, and dose-limiting toxicities, safety, tolerability, maximum tolerated dose, pharmacokinetics, and recommended dose for phase II clinical trial were determined[11].

However, the trials are empirical, as the mechanism and biochemistry of interaction of DS-3032B with MDM2 are not known. Although there is a lot of preclinical evidence of the action of MDM2 inhibitors as monotherapy or in combination, clinical experience with these agents is limited. Thus, information, at the molecular level, about the complex formed between the inhibitor and its target will help to clarify the nature of the interaction and its stability[9].

Such information is important to understand the functioning of the compound and even increase its efficiency through structural alterations. Since obtaining these data by crystallization and X-ray diffraction is laborious and time consuming, a plausible alternative has been facilitated by computational methods, such as molecular docking that has proven useful and reliable for predicting the possible interactions and affinity of ligands with macromolecules. *In silico* methods have been gaining increasing prominence since the experimental determination of complex three-dimensional structures is quite complex and costly[12]. Thus, the aim of this study was to analyze the interaction of the DS-3032B to its binding site in MDM2, the chemical bonds between drug and protein, and the affinity of the formed complex in order to clarify the stability of the interaction and thus help in elucidating the molecular mechanism of therapeutic action of the antagonist.

MATERIALS AND METHODS

Receptor preparation

For molecular docking simulations, the structures of MDM2 (receptor) and the drug DS-3032B (ligand) were selected. The three-dimensional structure of MDM2 was selected from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do) after a previous global alignment of all available primary sequences using CLUSTAL X 2.0 software. The 3D structure file was obtained in the extension ".pdb" (input file). The selected receptor's three-dimensional structure was prepared for molecular docking simulations using AutoDock Tools software; the water molecules were deleted, since they do not belong to the molecule and can interfere in the docking process and hydrogen atoms were also added. Then it was determined through the AutoDock Tools software the location and dimensions of the Grid (virtual box that delimits the region where the ligand will perform possible interactions with the receptor). In this case, the dimensions of the Grid encompassed the entire receptor. The Grid data and coordinates were used in molecular docking.

Ligand preparation

The three-dimensional structure of the drug DS-3032B was solved experimentally and deposited in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/); the file was obtained in the extension ".sdf" and converted to ".pdb" (input file). The ligand DS-3032B interacts with the MDM2 receptor in a physiological environment with pH 7.4; thus, to simulate more reliably, its interaction was made with the calculation for the prediction of its protonation state using the MarvinSketch 5.7® software from ChemAxon®. Both ligands, with and without the protonation, were prepared for molecular docking using the AutoDock Tools 1.5.6 software. This software detects the torsion points of the drug and calculates the angle of the torsions.

Molecular docking

Molecular docking procedures for a rigid protein and a flexible ligand were used. A grid of points in x, y, and z directions was built with a grid spacing of 1.0 Å using the AutoGrid component of the software. Molecular docking simulations were performed using the tools of the AutoDock platform (http://autodock.scripps.edu/) connected to the Vina 1.1.2 software (http://vina.scripps.edu/). The software used associates two components: A search algorithm and a score function. First, the algorithm was responsible for the search of possible combinations in the bonds, exploring the rotational, translational, and conformational degrees of freedom of the ligand as well as of the proteins. Then, the score function was used to choose the best binding modes. These functions were obtained according to the force fields

of molecular mechanics and empirical parameters from free energy calculations.

In silico analysis of drug-protein interactions

The analyses of the amino acid residues involved in the interactions between the receptor and the ligand, as well as the twists of the ligand, atoms involved in the interactions, and type, strength, and length of the interactions were performed using the PyMol 2.5 software (pymol.org/2) and Discovery Studio from BIOVIA®.

RESULTS

Receptor and ligand selected

During the search for the three-dimensional structure of the receptor, the alignment of primary sequences of three-dimensional structures available in the PDB corresponding to MDM2 was performed. The 5SWK, from *Homo sapiens*, was indicated as the best structure by having structure resolved by X-ray crystallography with high resolution (1.92 Å) and greater coverage of the site responsible for the antagonism of the protein. The antagonist binding site is located in chain A of MDM2, so chain A was isolated to perform molecular docking simulations. Figure 1 shows the primary sequence alignment between 5SWK chain A (153 residues) from PDB and whole MDM2 (466 residues). The consensus region presents the binding site for the MDM2 receptor antagonist. The three-dimensional structure of the antagonist DS-3032B was obtained from PubChem database with the CID (compound identification number) 73297272. MarvinSketch® software showed that at pH 7.4, 97.37% of DS-3021B was distributed in its protonated form.

Analysis of the drug-protein complex

A ranking of nine conformations presenting different affinity energies was obtained in molecular docking for each ligand (protonated and non-protonated), and the conformation with the lowest energy (Figure 2) was selected for subsequent analysis of drug-protein interactions. In addition, two root mean square deviation (RMSD) metric variants are also available: RMSD/L.b. (lower limit of RMSD) and RMSD/u.b. (upper limit RMSD), which differ by the way the atoms are matched in the distance calculation. While in RMSD/u.b. each atom is matched in one conformation to itself in the other conformation, ignoring any symmetry, in RMSD/L.b., each atom is matched in one conformation to the nearest atom of the same element type in the other conformation. No significant difference was observed between the affinity energies between both MDM2/protonated DS-3032B and MDM2/non-protonated DS-3032B conformers (Table 1); therefore, the protonated form, which prevails under physiological conditions, was better analyzed in this study. Sixteen amino acid residues of MDM2 are involved in chemical bonds with the protonated DS-3032B. Polar bond, hydrophobic interactions (pi-sigma and alkyl), and Van der Waals were observed (Table 2). These 16 residues of MDM2 chain A belong to the p53 biding site region. Four out of 16 interactions are more relevant, and they range from 2.18 to 3.96 Å; the shortest bond is a hydrogen bond between an oxygen atom of leucine residue 54 (LEU 54) of MDM2 and a nitrogen atom of one of the rings of the DS-3032B antagonist (Figure 3).

DISCUSSION

The MDM2, also called E3 ubiquitin ligase enzyme, is commonly overexpressed in various cancers[13], inactivating directly p53 by interacting with its transcriptional activation domain and inducing its degradation through ubiquitination[4]. DS-3032B is a compound derived from dispiropyrrolidine, also called milademetan. It impairs the binding of MDM2 to the transcriptional activation domain of p53[14].

Pharmacological inhibition of the p53-MDM2 interaction has been evaluated as a therapeutic approach to exert p53-mediated antitumor effects. Because MDM2 antagonists can produce nongenotoxic activation of wild-type p53, leading to anticancer activity, these agents are candidates to improve the therapeutic index of current chemotherapy regimens while minimizing the risk of resistance to single-agent MDM2 inhibition[15]. MDM2 inhibitors have demonstrated in preclinical and clinical studies their antineoplastic effects arising from p53 activation caused by negative regulation of MDM2 in solid and hematological tumors.

Identifying the interactions between drugs and their targets is critical in the discovery of new drugs. This helps to understand better the mechanism of the disease and to identify unexpected therapeutic activity or adverse side effects of the drugs. Therefore, the prediction of interaction between drugs and targets becomes important in the context of pharmacology and drug redefinition[16]. The precise and efficient identification of interactions between drugs and their targets in the body can reveal hidden functions of these drugs and target proteins as well as speed up the drug development process[17]. Drug development is a time-consuming process, the experimental identification of interactions between drugs and their targets is very costly, and modern technologies have mitigated this problem. The

Table 1 Affinity energy and RMSD values of both conformers mouse double minute 2/protonated DS-3032B and mouse double minute 2/non-protonated DS-3032B

Conformer	Affinity energy (kcal/mol)	Dist. From	
		RMSD l.b.	RMSD u.b.
MDM2/protonated DS-3032B	-10.0	0.000	0.000
MDM2/non-protonated DS-3032B	-9.9	0.000	0.000

MDM2: Mouse double minute 2; RMSD: root mean square deviation; RMSD l.b.: RMSD lower limit; RMSB u.b.: RMSD upper limit.

Table 2 Chemical interactions between mouse double minute 2 receptor and protonated DS-3032B antagonist

Residues of MDM2	Interaction	Bond size (Å)
Leu 54	Hydrogen bond	2.18
Leu 57	Alkyl	3.92
Ile 61	Alkyl	3.91
Tyr 67	Pi-Sigma	3.96
Ile 103	Van der Waals	-
Phe 86	Van der Waals	-
Phe 91	Van der Waals	-
Ile 99	Van der Waals	-
Tyr 100	Van der Waals	-
Val 75	Van der Waals	-
Val 93	Van der Waals	-
Gln 71	Van der Waals	-
Gln 72	Van der Waals	-
Gly 58	Van der Waals	-
His 73	Van der Waals	-
Met 62	Van der Waals	-

MDM2: Mouse double minute 2.

computational prediction of drug target interactions has been shown to be fundamental for the study of drugs, since it reduces the time and costs of the process[18].

In fact, through *in silico* approach, it was possible to observe that DS-3032B is able to connect to the p53 binding site of MDM2 chain A with a significant affinity. The interaction between the antagonist and MDM2 involves 16 amino acid residues by polar and nonpolar bonds throughout the entire structure of the drug. The arrangement of the connections contributes to the low bind energy value and consequently to the stability of the complex. Complex stability and high affinity indicate promising therapeutic potential for DS-3032B.

There are three clinical studies of MDM2 inhibitors in various cancers, including the MDM2 antagonist, DS-3032B, still in early phase (solid tumors and lymphoma: NCT01877382; myeloma: NCT02579824; leukemia: NCT02319369; acute myeloid leukemia: NCT03671564; acute myeloid leukemia, being associated with Quizartinib: NCT03552029; refractory leukemia, being associated with Cicarabine: NCT03634228)[19]. Clinical responses in these trials have been limited overall, but some patients have clearly achieved clinical benefit through monotherapy with MDM2 inhibitors[20]. Although monotherapy with MDM2 inhibitors has benefits, clinical responses are usually modest; association with other inhibitors has shown synergism and more efficient clinical responses[9]. However, the association of more medications can increase the presence of unwanted effects and even decrease patients' adherence to therapy. The action of drugs is related to their interaction with their therapeutic target; drug-protein affinity predicts complex stability, longer interaction time, and greater pharmacological effectiveness.

```
MDM2          ---MCNTNMSVPTDGAVTTSQIPASEQETL-----------------------VLFYL  32
SSWK_A|Chains GPHMCNTNMSVPTDGAVTTSQIPASEQETLVRPKPLLLKLLKSVGAQKDTYTMKEVLFYL  60
                 ***************************                       *****

MDM2          GQYIMTKRLYDEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTMIYRNLVVVNQQESSD  92
SSWK_A|Chains GQYIMTKRLYDEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTMIYRNLVVVNQQESSD 120
              ************************************************************

MDM2          SGTSVSENRCHLEGGSDQKDLVQELQEEKPSSSHLVSRPSTSSRRRAISETEENSDELSG 152
SSWK_A|Chains SGTSVSENRCHLEGGSDQKDLVQELQEEKPSSS---------------------------- 153
              ********************************

MDM2          ERQRKRHKSDSISLSFDESLALCVIREICCERSSSSESTGTPSNPDLDAGVSEHSGDWLD 212
SSWK_A|Chains ------------------------------------------------------------ 153

MDM2          QDSVSDQFSVEFEVESLDSEDYSLSEEGQELSDEDDEVYQVTVYQAGESDTDSFEEDPEI 272
SSWK_A|Chains ------------------------------------------------------------ 153

MDM2          SLADYWKCTSCNEMNPPLPSHCNRCWALRENWLPEDKGKDKGEISEKAKLENSTQAEEGF 332
SSWK_A|Chains ------------------------------------------------------------ 153

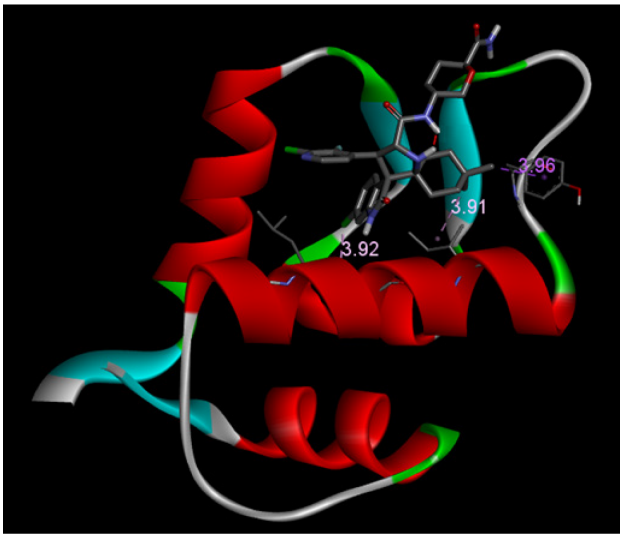
MDM2          DVPDCKKTIVNDSRESCVEENDDKITQASQSQESEDYFQPSTSSSIIYSCQEDVKEFERE 392
SSWK_A|Chains ------------------------------------------------------------ 153

MDM2          ETQDKEESVESSLPLNAIEPCVICQGRPKNGCIVHGKTGHLMACFTCAKKLKKRNKPCPV 452
SSWK_A|Chains ------------------------------------------------------------ 153

MDM2          CRQPIQMIVLTYFP 466
SSWK_A|Chains -------------- 153
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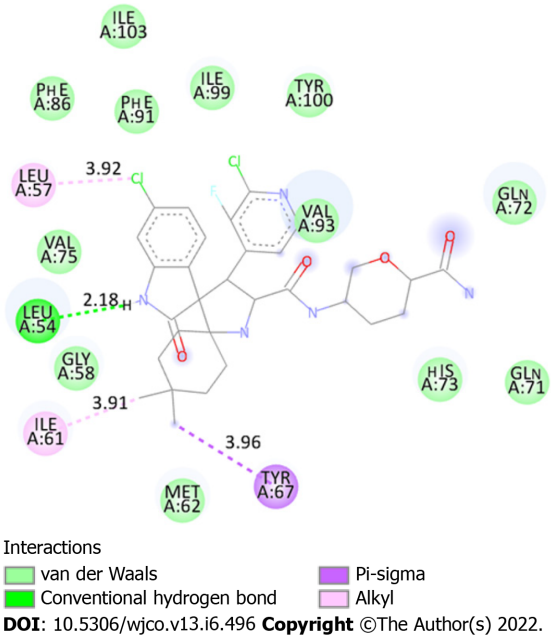
Figure 1 Global alignment between 5SWK chain A (153 residues) and whole mouse double minute 2 (466 residues). Consensus region is located at position 56 - 153 of 5SWK chain A. The color of the residues represents the chemical characteristic of their side chains. MDM2: Mouse double minute 2.



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Figure 2 Mouse double minute 2/protonated DS-3032B conformer. Mouse double minute 2 (MDM2) receptor is shown in ribbon and DS-3032B is shown in sticks. Only LEU 57, ILE 61, and TYR 67 bonds are shown. Bond size in Å.

Thus, knowledge about the molecular interactions between the test drug and its therapeutic target becomes interesting to predict the behavior of the drug in the body, predict its efficiency and stability, and can help predict how this drug can affect the physiology of the individual. Since *in silico* studies can provide this data satisfactorily and reliably and much faster compared to *in vitro* and *in vivo* studies, this methodology has been used to support scientific studies. The analyses performed in this study, with the DS-3032B, indicate that the complex formed between this drug and its target is quite stable, indicating high therapeutic efficiency. This efficiency, measured indirectly, through affinity energy may be responsible for the good preclinical results of ds-3032B, and it may be more effective as monotherapy



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Figure 3 Two-dimensional diagram demonstrating interactions between mouse double minute 2 chain A (5SWK) and protonated DS-3032B. Light green: Residues involved in Van der Waals interactions. Green: Residue involved in hydrogen bond. Lilac: Residue involved Pi-Sigma interaction. Pink: Residues involved in alkyl interactions. Numbers in spheres indicate the residue position. Bond size are shown (Å).

than current inhibitors.

Regarding the *in silico* analyses performed, the ligand position is selected based on calculations that are ranked according to docking score that represents the binding affinity between the ligand and the receptor and is expressed in kcal/mol[21]. Molecular docking is an established *in silico* structure-based method widely used in drug discovery. Docking enables the identification of novel compounds of therapeutic interest, predicting ligand-target interactions at a molecular level[22]. In this study it was obtained as a result of molecular docking, the ranking of the nine conformations with the highest affinities for the receptor, in parallel with two metric variants of RMSD: RMSD/L.b. and RMSD/u.b., which differ by the way the atoms are matched in the distance calculation. While in RMSD/u.b. each atom is matched in one conformation to itself in the other conformation, ignoring any symmetry, in RMSD/L.b. each atom is matched in one conformation to the nearest atom of the same element type in the other conformation. Whereas that for RMSD, the tolerance value respected is at most 2.0Å[23]. In addition, the affinity of the conformation presents the binding energy between the receptor and the ligand, being considered significant values less than -6.0 kcal/mol[13]. The compound that requires lower energy for the interaction to occur forms a more stable complex, in other words, has greater biological activity[24].

It is important to highlight that the DS-3032B, in addition to presenting satisfactory preclinical data regarding its anti-tumor potential, which are supported by computational findings, this drug is administered orally[11]. Thus, the DS-3032B becomes an attractive therapy compared to invasive and uncomfortable administrations for the patient.

CONCLUSION

In this study, higher biological activity means greater antagonism of MDM2 and consequent restoration of the tumor suppressor, p53. The confirmation was provided by the results obtained during molecular docking calculations, given that the conformer with the highest affinity showed -9.9 kcal/mol and therefore can be considered a promising candidate to inhibit the MDM2 protein. These dyspyrolidine-derived compounds may represent a starting point for the development of new drugs to treat cancers with overexpression of the MDM2 protein. The identified results reinforce that bioinformatics offers great direction in the search and validation of treatment targets, because it presents itself as a starting point for improving the knowledge involving drug- protein interactions. In addition, it also promotes cost and time reduction when compared to traditional research methods and directs the treatment so that the new drugs have their side effects minimized. However, *in silico* processes are complementary and do not rule out the need for *in vitro* and *in vivo* tests.

ARTICLE HIGHLIGHTS

Research background

Mouse double minute 2 (MDM2) is the main negative regulator of tumor suppressor p53; in this context, the effective inhibition of MDM2 is an alternative for cancer treatments.

Research motivation

DS-3032B is an MDM2 antagonist, and its activity is known only empirically, so bioinformatics analyses can point to molecular characteristics of complex interaction.

Research objectives

To analyze, *in silico*, the interactions between the antagonist DS-3032B and MDM2 and infer the antineoplastic potential of the drug.

Research methods

The analysis of chemical bonds, interaction of the drug-protein complex, and its stability were done by molecular docking.

Research results

Molecular docking simulations between MDM2 chain A (PDB: 5SWK) and DS-3032B (CID: 73297272) in its protonabed form indicated a complex with significant affinity energy, -10.0 kcal/mol. The results indicate a stable complex, maintained by hydrophilic and hydrophobic bonds involving 16 amino acid residues of MDM2.

Research conclusions

DS-3032B is able to bind to MDM2 with high affinity and stability, suggesting therapeutic efficiency.

Research perspectives

Analyze the DS-3032B/MDM2 complex using molecular dynamics and verify the possibility of structural changes of the drug to increase its efficiency.

FOOTNOTES

Author contributions: da Mota VHS, Freire de Melo F, and Teixeira KN contributed to study conceptualization, methodology, validation, and investigation and writing of the original draft; Freire de Melo F contributed to study conceptualization, methodology, and investigation, and manuscript review; de Brito BB and Silva FAFD contributed to study validation, visualization, and formal analysis and manuscript writing, review, and editing; Silva FAFD contributed to study validation, visualization, and formal analysis, manuscript writing, review, and editing, and supervision; Teixeira KN contributed to study conceptualization, methodology, and investigation, original draft writing, and supervision.

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ORIGINAL ARTICLE

Basic Study

Nicotinic receptors modulate antitumor therapy response in triple negative breast cancer cells

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Abstract

BACKGROUND

Triple negative breast cancer is more aggressive than other breast cancer subtypes and constitutes a public health problem worldwide since it has high morbidity and mortality due to the lack of defined therapeutic targets. Resistance to chemotherapy complicates the course of patients' treatment. Several authors have highlighted the participation of nicotinic acetylcholine receptors (nAChR) in the modulation of conventional chemotherapy treatment in cancers of the airways. However, in breast cancer, less is known about the effect of nAChR activation by nicotine on chemotherapy treatment in smoking patients.

AIM

To investigate the effect of nicotine on paclitaxel treatment and the signaling pathways involved in human breast MDA-MB-231 tumor cells.

METHODS

Cells were treated with paclitaxel alone or in combination with nicotine, administered for one or three 48-h cycles. The effect of the addition of nicotine (at a concentration similar to that found in passive smokers' blood) on the treatment with paclitaxel (at a therapeutic concentration) was determined using the 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The signaling mediators involved in this effect were determined using selective inhibitors. We also investigated nAChR expression, and ATP "binding cassette" G2 drug transporter (ABCG2) expression and its modulation by the different treatments with Western blot. The effect of the treatments on apoptosis induction was determined by flow cytometry using annexin-V and 7AAD markers.

RESULTS

Our results confirmed that treatment with paclitaxel reduced MDA-MB-231 cell viability in a concentration-dependent manner and that the presence of nicotine

reversed the cytotoxic effect induced by paclitaxel by involving the expression of functional α7 and α9 nAChRs in these cells. The action of nicotine on paclitaxel treatment was linked to modulation of the protein kinase C, mitogen-activated protein kinase, extracellular signal-regulated kinase, and NF-κB signaling pathways, and to an up-regulation of ABCG2 protein expression. We also detected that nicotine significantly reduced the increase in cell apoptosis induced by paclitaxel treatment. Moreover, the presence of nicotine reduced the efficacy of paclitaxel treatment administered in three cycles to MDA-MB-231 tumor cells.

CONCLUSION

Our findings point to nAChRs as responsible for the decrease in the chemotherapeutic effect of paclitaxel in triple negative tumors. Thus, nAChRs should be considered as targets in smoking patients.

Key Words: Breast cancer; Paclitaxel; Nicotinic acetylcholine receptors; Drug therapy; Signal transduction; Drug transporter

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Core Tip: Smokers with lung tumors are more likely to generate resistance to chemotherapy than non-smokers. However, little is known about the effect of nicotinic activation during the treatment of breast cancer, a cancer which arises close to the lung. In triple negative human breast cells, nicotine reduces the chemotherapeutic effect of paclitaxel through the participation of several kinases, as well as by modulating ATP "binding cassette" G2 drug transporter expression and inducing resistance to treatment. These results indicate that nicotinic acetylcholine receptors are a new possible target in antitumor therapy for this subtype of breast cancer.

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INTRODUCTION

Cigarette smoke contains many harmful components for human health[1]. Among them, nicotine (NIC), which has addictive properties[2], exerts its effects by activating nicotinic acetylcholine receptors (nAChRs)[3]. These receptors belong to the Cys-loop family of pentameric ionic channels activated by ligands[4]. nAChRs can be made up of 17 different subunits, whose assembly creates several homopentameric and heteropentameric channel subtypes in the cell membrane[5].

Originally, nAChRs were found in the nervous system. However, their expression has been described in several organs such as the lungs[6,7], kidney[8,9], intestine[10,11] and breast, where mainly the α7 and α9 subunits are expressed[12,13]. The activation of nAChRs can induce an increase in the levels of intracellular calcium[14], which has been related to tumorigenesis in the lungs[15], liver[16], pancreas [17] and brain[18]. This increase in intracellular calcium can in turn activate kinase signaling pathways [19,20], which regulate different parameters of tumor biology such as proliferation, migration and invasion[21-23].

Previous evidence has demonstrated that the activation of these receptors can decrease the effectiveness of different antitumor agents in various tumor types such as those from the oral and nasal cavity [14,24], pancreas[25], head and neck[26] and lungs[27-29]. In the lungs, many authors have described that NIC may exert its modulatory effect on the actions of chemotherapeutic agents through the activation of signaling pathways involving protein kinases[30-33]. However, little is known about the effect of nicotinic activation during the treatment of breast cancer, a cancer that arises close to the lungs.

Breast cancer is characterized by a high incidence that causes a high number of deaths in women worldwide[34]. According to their genetic profile, breast tumors are classified in different subtypes, a fact that allows doctors to choose the most effective antitumor therapy. Tumors can be categorized according to the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67 protein by immunohistochemistry. This allows defining four subtypes of breast tumors: luminal A, luminal B, HER2+ and normal-like or triple negative (TN)[35,36]. TN tumors lack the expression of ER, PR and HER2, but present high expression of Ki-67 protein and are usually very invasive and aggressive[37]. Since these tumors do not exhibit a defined therapeutic

target, there is no specific treatment, and they respond poorly to taxanes like paclitaxel (PX), usually administered as conventional chemotherapy to other breast cancer patients.

PX is an effective drug used not only in the treatment of breast cancer, but also in non-small cell lung cancer, prostate cancer, and head and neck cancer[38]. It is a cytostatic compound that causes hyper-stabilization of polymerized microtubules, inhibiting the mitotic spindle and arresting cells in G_2/M phases[39]. The persistence of cell arrest eventually produces cell death by apoptosis[40]. Thus, a low level of apoptosis could be an important factor in the development of resistance to treatment.

In this study, we evaluated the ability of NIC to interfere in the treatment of human TN breast cancer MDA-MB-231 cells with PX and the signaling pathway involved in this action.

MATERIALS AND METHODS

Cell culture

The human breast adenocarcinoma cell lines MDA-MB-231 (TN, CRM-HTB-26), MDA-MB-468 (TN, HTB-132) and MCF-7 (luminal A, HTB-22) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, United States) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen Inc., Carlsbad, CA, United States) supplemented with 100 mL/L heat inactivated fetal bovine serum (FBS) (Internegocios SA, Mercedes, Buenos Aires, Argentina), L-glutamine (0.3 mg/L) and gentamicin (80 mg/L). The cultures were maintained at 37 °C in humidified air with 50 mL/L CO_2 and the medium was replaced three times a week. Cells were detached with 250 mg/L trypsin in Ca^{2+}- and Mg^{2+}-free PBS containing 20 mg/L EDTA. Cell viability was determined by the Trypan blue exclusion test and the absence of mycoplasma was observed by Hoechst staining[41].

Cell viability assay

Cell viability after treatment was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining method (Life Technologies, Eugene, OR, United States). A suspension containing 4×10^3 cells/well was added to each well of a 96-well plate in culture medium supplemented with 50 mL/L FBS and cells were then left to attach overnight. When cells reached 60%-70% of confluence, they were deprived of FBS for 24 h to induce the synchronization of cultures. Then, cells were treated with PX (Bristol-Myers Squibb, Buenos Aires, Argentina) and/or NIC (which is a non-selective nAChR agonist) in medium supplemented with 20 mL/L FBS, for 48 h in triplicate. To inhibit the action of the nicotinic agonist, cells were previously treated with the antagonists mecamylamine (MM), methyllycaconitine (MLA) or luteolin (Lut) at 10^{-6} mol/L; these three are respectively nAChR non-selective, α7 nAChR selective and α9 nAChR selective antagonists. To determine the participation of kinases in the effects of PX, cells were previously treated with inhibitors of: protein kinase C (PKC) [staurosporine (Stau), 10^{-8} mol/L], mitogen-activated protein kinase kinase (MEK) [PD098059 (PD), 10^{-5} mol/L], Ras [S-trans, trans-farnesylthiosalicylic acid (FTS), 10^{-6} mol/L], extracellular signal-regulated kinases (ERK1/2) (U126, 10^{-5} mol/L), p38 mitogen-activated protein kinases (p38MAPK) [SB203580 (SB), 10^{-5} mol/L] or the mediator of the activation of the NF-κB pathway IκB kinase (IKKβ) [IMD354 (IMD), 5×10^{-8} mol/L].

To determine the modulation of the sensitivity of MDA-MB-231 cells to chemotherapy, cells were treated with PX in the absence or presence of NIC for three 48-h cycles with 24 h intercycles without treatment. Then, surviving cells were treated with a new cycle of PX for 48 h. After treatment, the medium was removed and 100 μL of MTT solution (500 mg/L medium free of phenol red and FBS) was added. Plates were incubated for 4 h at 37°C and the production of formazan was measured by analyzing the absorbance at 540 nm with an ELISA reader (BioTek, Winooski, VT, United States). Values are indicated as mean ± SD and expressed as the percentage of cell viability in comparison to cells without treatment considered 100%.

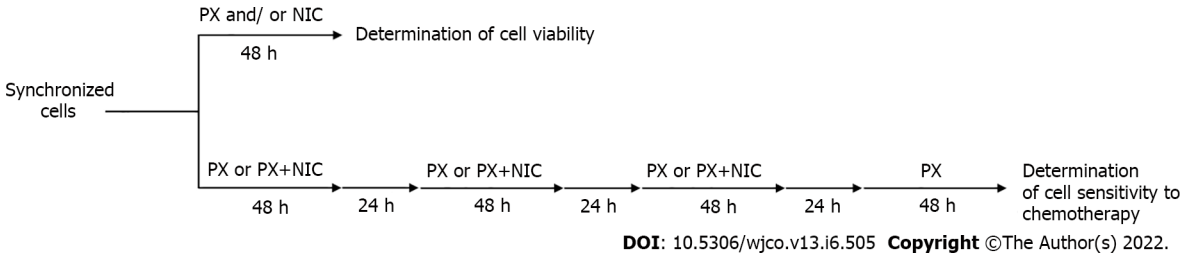
A diagram of the administration schedule for the determination of cell viability or cell sensitivity to chemotherapy is shown in Figure 1.

Calculation of the effective concentration 50

Dose–response data were transformed, changed to percentage and fitted to a sigmoidal curve, following a maximal effective concentration (Emax) model with at least six data points, using the GraphPad Prism 6 software. This allowed calculating the effective concentration 50 (EC50) and Emax values. Only data with a coefficient of variation lower than 20% were considered for the EC50 values.

Detection of nicotinic receptors by Western blot

MDA-MB-231 cell proteins were extracted by washing them with a buffer containing 6 g/L Tris-HCl, 3 g/L NaCl, 210 mg/L NaF, 480 mg/L $MgCl_2$, 300 mg/L EDTA, 380 mg/L EGTA, 870 mg/L phenylmethanesulfonyl fluoride, 10 mL/L Triton X-100 and 10 mg/L trypsin inhibitor, aprotinin and leupeptin, at pH 7.4. Samples were incubated on ice for 1 h and centrifuged at 800 G for 20 min at 4°C, afterwards the supernatants were collected and saved at -80°C. Protein concentrations were determined by the



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Figure 1 Diagram of the administration schedule for the determination of cell viability or cell sensitivity to chemotherapy. PX: Paclitaxel; NIC: Nicotine.

Bradford assay[42].

Samples (80 μg protein per lane) were separated by 10% SDS-PAGE minigel electrophoresis and then transferred to nitrocellulose membranes. They were then blocked with 50 g/L skim milk and incubated overnight with rat anti-human-α7 nAChRs monoclonal antibody or mouse anti-human-α9 nAChRs monoclonal antibody (Santa Cruz Biotechnology Inc., Dallas, TX, United States), both diluted 1:200. Then, strips were incubated with anti-rat or anti-mouse IgG coupled to horseradish peroxidase diluted 1:10000 in buffer containing 2.4 g/L Tris-HCl buffer, 9 g/L NaCl and 500 mg/L Tween 20 (TBS-T) at 37°C for 1 h. α7 nAChR and α9 nAChR bands were detected by chemiluminescence and quantified by densitometric analysis using Image J software (NIH). The results are expressed as optical density (O.D.) units relative to the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology Inc., Dallas, TX, United States), which was used as the loading control[43].

Trypan blue exclusion assay

Cell viability was also determined by the trypan blue dye exclusion test. Briefly, cells were treated with the corresponding drugs for 48 h in 48-well plates at a density of 10^4 cells/well. Cells were collected and centrifuged at 900 r/min for 10 min. Then, pellets were resuspended in DMEM and the trypan blue solution was added in a 1:1 ratio. The number of viable cells, identified as non-stained cells, was counted using a hemocytometer under an inverted microscope at 10X magnification and the percentage of these cells with respect to the total cell number was calculated.

Apoptosis determination by flow cytometry

For apoptosis determination, MDA-MB-231 cells were grown in six-well plates and treated for 48 h with PX, NIC or their combination, in the presence or absence of selective and non-selective nicotinic antagonists and kinase inhibitors. Then, cells were harvested and resuspended in binding buffer, and 2 μL of AnnexinV-FITC was added to each sample. Cells were then incubated for 15 min at room temperature in the dark. After that, 2 μL of 7AAD was added and the samples were immediately analyzed by the BD Accuri C6 Plus Flow Cytometer. Data were analyzed by the BD Accuri C6 Plus software.

ATP binding cassette transporter G2 detection by Western blot

For the detection of ATP "binding cassette" G2 drug transporter (ABCG2), cells (2×10^6) were treated for 48 h with the different drugs and samples were prepared as indicated to detect nicotinic receptors. Then, samples (80 μg protein per lane) were separated by 12% SDS-PAGE minigel electrophoresis, transferred to nitrocellulose membranes, blocked with 50 g/L skim milk and incubated overnight with a rabbit anti-human ABCG2 polyclonal antibody (Santa Cruz Biotechnology Inc., Dallas, TX, United States) diluted 1:200. Then, strips were incubated with horseradish peroxidase-linked anti-rabbit IgG, diluted 1:10000 in TBS-T at 37°C for 1 h. Bands were detected by chemiluminescence and then quantified by densitometric analysis using the Image J program (NIH) and expressed as O.D. units in comparison to the expression of GAPDH, which was used as the loading control[44].

Statistical analysis

Results are expressed as mean ± SD and statistical analysis was performed using the GraphPad Prism6 software. To determine differences between mean values, one-way ANOVA was performed with Tukey´s post-hoc analysis. $P < 0.05$ was accepted as statistically significant. The data and statistical analysis complied with the recommendations on experimental design and analysis in pharmacology [45].

RESULTS

Effect of NIC on MDA-MB-231 cell viability

First, we analyzed the effect of NIC administered for 48 h to MDA-MB-231 cells in culture. The addition of different concentrations of NIC increased cell viability from 10^{-10} mol/L, with an Emax of 189.3% ± 5.2% (Figure 2A). This effect was reduced by the pre-treatment of cells with different nicotinic antagonists: MM (non-selective) (Emax: 118.4% ± 3.36%), MLA (α7 nAChR selective) (Emax: 143.6% ± 1.38%), or Lut (α9 nAChR selective) (Emax: 135.6% ± 5.69%), all of them added at 10^{-6} mol/L (all P < 0.001 *vs* NIC treatment) (Figure 2A). The nicotinic antagonists alone did not modify cell viability (data not shown). In addition, expression of α7 and α9 nAChRs in MDA-MB-231 cells was detected by Western blot assay (Figure 2B).

Paclitaxel treatment of MDA-MB-231 cells in the presence of NIC

In the next set of experiments, we analyzed the action of PX on tumor cells in the presence of NIC. First, we confirmed that PX reduced MDA-MB-231 cell viability in a concentration-dependent manner and that the PX effect was significant at concentrations equal to or higher than 10^{-8} mol/L (EC50: 1.0 × 10^{-7} mol/L) (Figure 3). On the other hand, the presence of the first effective concentration of NIC (10^{-10} mol/L) shifted the concentration-response curve to the right, increasing the EC50 value by more than one order (EC50: 1.2 × 10^{-6} mol/L) (Figure 3).

After three cycles of PX (48 h each) in the presence of NIC, followed by 24 h without drugs, surviving cells were less sensitive to a subsequent PX cycle in comparison to the same treatment in the absence of NIC. The latter was evidenced by an increase in the EC50 value obtained from the concentration-response-curve (PX+NIC EC50: 3.6 × 10^{-6} mol/L; PX EC50: 4.8 × 10^{-7} mol/L) (Figure 4).

It has been documented that 10^{-10} mol/L can be considered a concentration of NIC similar to that present in the bloodstream of passive smoking patients[46]. To analyze the ability of NIC to interfere with the action of therapeutic concentrations of PX, we treated MDA-MB-231 cells with a combination of 10^{-10} mol/L NIC and 10^{-7} mol/L PX for 48 h. We determined that NIC reduced PX effectiveness by increasing tumor cell viability to 110.8% ± 1.9% in comparison to PX treatment in the absence of NIC (51.1% ± 3.9%, P < 0.01). This effect of NIC on PX action was partially reduced by the pre-treatment of cells with nicotinic antagonists (MM, MLA or Lut) added at 10^{-6} mol/L (P < 0.001 *vs* PX+NIC) (Figure 5A) in a manner similar to that of NIC treatment alone (NIC: 138.16 ± 6.08; NIC+MM: 116.52 ± 0.12; NIC+Lut: 126.16 ± 5.96; NIC+MLA: 119.69 ± 4.26; P < 0.001; P < 0.05 and P < 0.001 *vs* NIC, respectively). We confirmed that the PX effect was independent of nAChR activation since pre-treatment with nicotinic antagonists did not modify the effect of PX treatment alone (PX+MM: 52.12 ± 6.65; PX+Lut: 49.97 ± 5.88; PX+MLA: 54.6 ± 4.01). To confirm the action of drugs on cell viability, we next analyzed the ratio of living cells by the Trypan blue exclusion test after the different treatments. The results plotted in Figure 5B (control: 100 ± 3.3; NIC: 129.3 ± 1.1, P < 0.001; PX: 64.1 ± 4.3, P < 0.001; PX+NIC: 92.3 ± 2.5, P=ns; PX+NIC+MM: 56.3 ± 3.4, P < 0.001; PX+NIC+MLA: 66.7 ± 5.4, P < 0.001; PX+NIC+Lut: 52.3 ± 6.0, P < 0.001; all being % or significance with respect to the control) show values similar to those obtained by the MTT assay (Figure 5A).

To confirm that nicotinic agonists can modulate the action of PX in reducing viability in other breast cancer cells, we tested the effect of PX (10^{-7} mol/L) in the absence or presence of NIC (10^{-10} mol/L) on MDA-MB-468 and MCF-7 cell viability. We determined that the presence of NIC reduced the effect of PX in MDA-MB-468 and MCF-7 cells, and that these effects were prevented by pre-incubating cells with 10^{-6} mol/L of MM (Table 1).

Signal transduction pathways and mechanism involved in the effect of paclitaxel on NIC-treated tumor cells

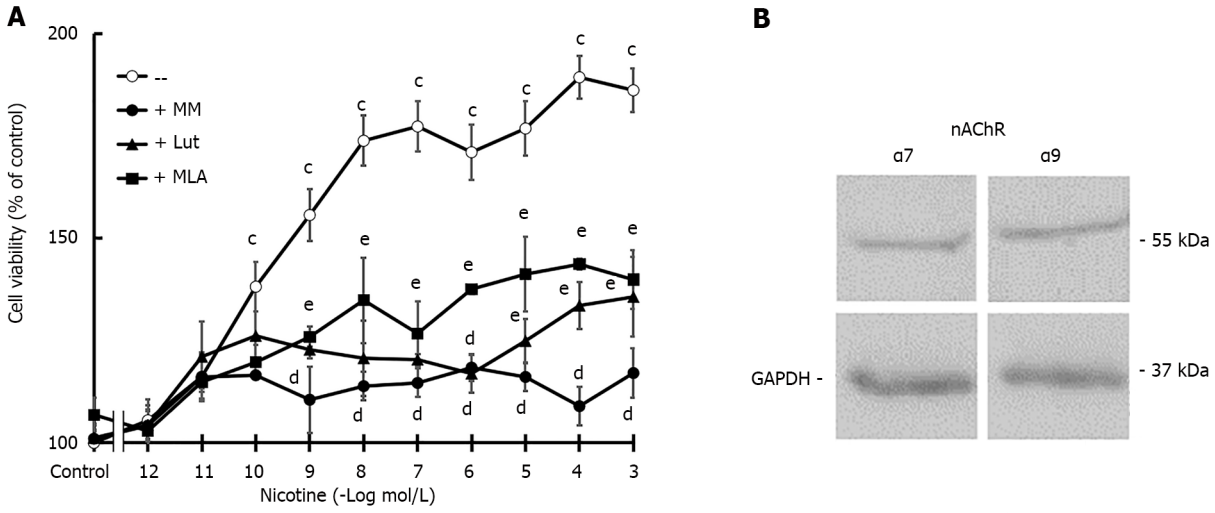
Previous reports have indicated that the activation of protein kinases is essential to modulate cell viability by triggering pro/anti-apoptotic gene transcription[47,48]. Additionally, the induction of the expression of these proteins could implicate activation of the NF-κB pathway. In the present study, we observed that the effect of PX on MDA-MB-231 cells is mediated, at least in part, by the Ras, p38MAPK (P < 0.01 *vs* PX) and NF-κB pathways (P < 0.05 *vs* PX) since the addition of their specific inhibitors FTS, SB or IMD respectively modified PX action (Figure 6A). On the other hand, the presence of NIC during PX treatment not only involved the previously mentioned molecules, but also the PKC (P < 0.05 *vs* PX), MEK (P < 0.01 *vs* PX) and ERK1/2 (P < 0.01 *vs* PX) pathways, as revealed by the action of Stau, PD and U126, respectively, which were added to the cultures (Figure 6B). We confirmed that the inhibitors alone did not modify cell viability (data not shown).

Apoptosis is a cell death mechanism that could improve antitumor actions of chemotherapy, and the activation of protein kinases is frequently associated with the activation of pro/anti-apoptotic signaling pathways. As expected, we found a reduction in cell viability due to the apoptosis induced by PX (PX: 16.7% ± 1.4%; control: 6.1% ± 0.7%, P < 0.001). The presence of NIC reduced the effect of PX. The percentage of apoptotic cells was 9.3 ± 0.6 (P < 0.05 *vs* control or P < 0.001 *vs* PX) (Figure 7). We also observed that the effect of NIC on PX-treated cells was totally reversed in the presence of nicotinic antagonists (P > 0.05 *vs* PX) (Figure 7).

Table 1 Effect of the combination of paclitaxel with nicotine on MDA-MB-468 and MCF-7 cell viability

Treatment	MDA-MB-468, cell viability (% of control)	MCF-7, cell viability (% of control)
PX	61.01 ± 3.79	65.36 ± 4.86
NIC	137.79 ± 3.69[c]	141.94 ± 4.07[c]
PX+NIC	79.15 ± 6.94[a]	117.99 ± 10.06[c]
PX+NIC+MM	62.37 ± 4.71	69.13 ± 7.22

[a]$P < 0.05$ *vs* control.
[c]$P < 0.001$ *vs* control.



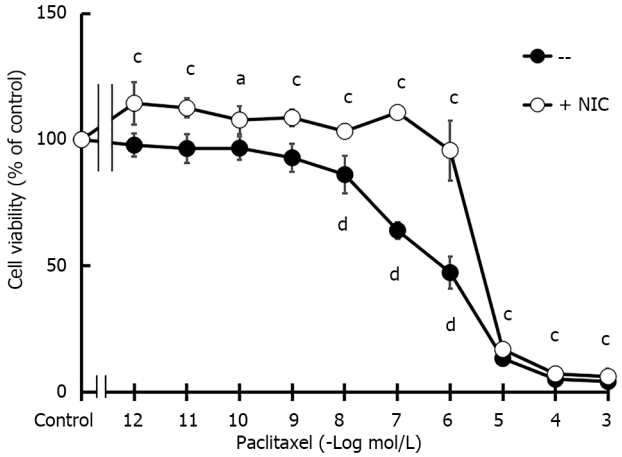
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Figure 2 MDA-MB-231 cell viability. A: Concentration-response curves of nicotine on cell viability in the absence or presence of nicotinic antagonists: mecamylamine [non-selective for nicotinic acetylcholine receptors (nAChRs)], methyllycaconitine (selective for α7 nAChRs), or luteolin (selective for α9 nAChRs) at a concentration of 10^{-6} mol/L. Values are the mean ± SD of five experiments performed in duplicate. [c]$P < 0.001$ *vs* control; [d]$P < 0.001$ *vs* nicotine; [e]$P < 0.001$ *vs* control or nicotine; B: Western blot assay to detect α7 and α9 nAChR expression. Molecular weights are indicated on the right. The expression of glyceraldehyde 3-phosphate dehydrogenase was used as the loading control. One representative experiment of three is shown. MM: Mecamylamine; MLA: Methyllycaconitine; Lut: Luteolin; nAChRs: Nicotinic acetylcholine receptors; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

An important aspect in cancer chemotherapy is the development of resistance. This phenomenon has been linked to several proteins, including ABCG2, which is a drug extrusion pump that decreases the effectiveness of antitumor drugs. Thus, we next analyzed ABCG2 expression by Western blot. As shown in Figure 8, MDA-MB-231 cells expressed this protein and the addition of PX caused a significant increase in its expression ($P < 0.05$). The presence of NIC during PX treatment potently increased ABCG2 levels in tumor cells ($P < 0.001$ *vs* PX). The pretreatment of cells with nicotinic antagonists reduced NIC action on ABCG2 expression ($P > 0.05$ *vs* PX+NIC) (Figure 8).

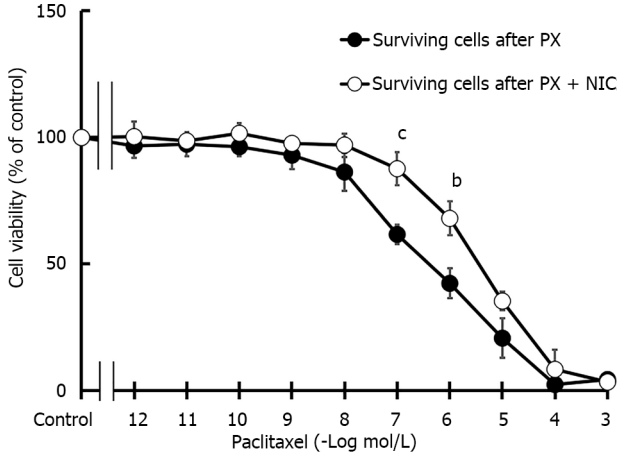
DISCUSSION

Our results reveal the mechanisms by which NIC decreases the cytotoxic effects of PX on human TN breast cancer MDA-MB-231 cells. Several authors have described the effect of nAChR activation on cell proliferation. Regarding the latter, NIC from tobacco smoke stimulates nAChRs expressed in the oral cavity[49], esophagus[50], stomach[51], intestine[52] and lungs[53-55]. NIC can also trigger malignant transformation in smoking patients and increases the risk of developing lung cancer[56,57]. Less is known about the effects of NIC on developing tumors or promoting malignant growth in other organs near the lung that also express nAChRs, such as is the case of the breast. Previous studies have shown that human breast tumors express the α7 and α9 nAChR subtypes[58,59]. In the present study, we confirmed that these receptors are functional in MDA-MB-231 tumor cells as treatment with NIC increased cell viability in a concentration-dependent manner. This effect was reversed by the pre-treatment of cells with selective nicotinic antagonists.



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Figure 3 MDA-MB-231 cell viability. Concentration-response curves of paclitaxel on cell viability in the absence or presence of nicotine (NIC) (10^{-10} mol/L). Values are the mean ± SD of six experiments performed in duplicate. $^{a}P < 0.05$; $^{c}P < 0.001$ *vs* Control; $^{d}P < 0.001$ *vs* +NIC. NIC: Nicotine.

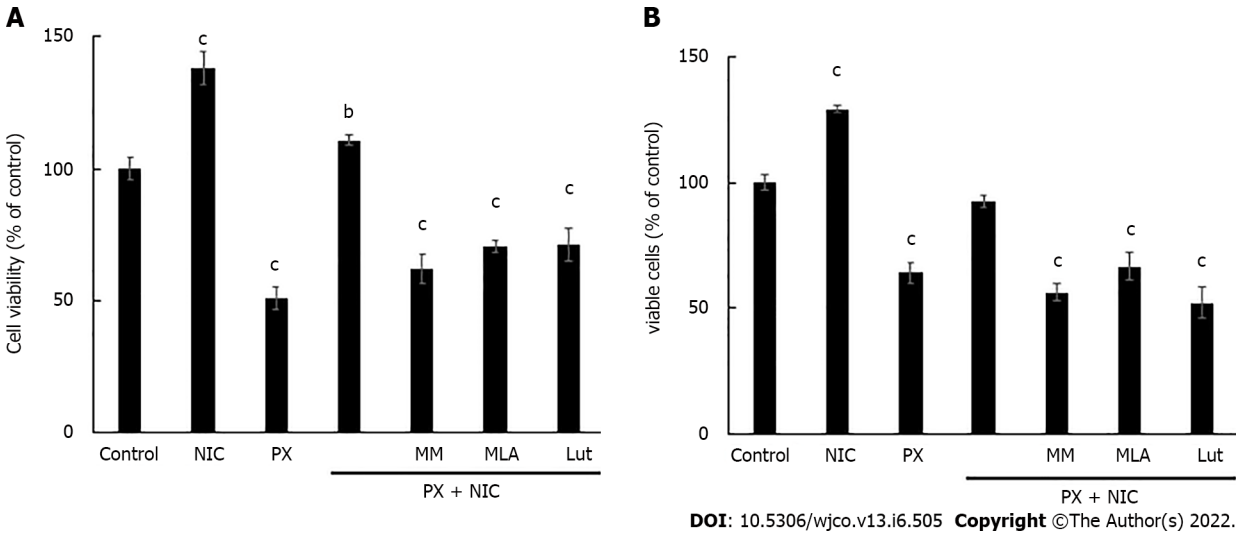


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Figure 4 Sensitivity of MDA-MB-231 cells to chemotherapy. Concentration-response curves of paclitaxel (PX) on the viability of surviving cells after three cycles of PX treatment (10^{-7} mol/L) in the absence or presence of nicotine (10^{-10} mol/L). Values are the mean ± SD of three experiments performed in duplicate. $^{b}P < 0.01$; $^{c}P < 0.001$ *vs* surviving cells after three cycles of PX treatment. PX: Paclitaxel; NIC: Nicotine.

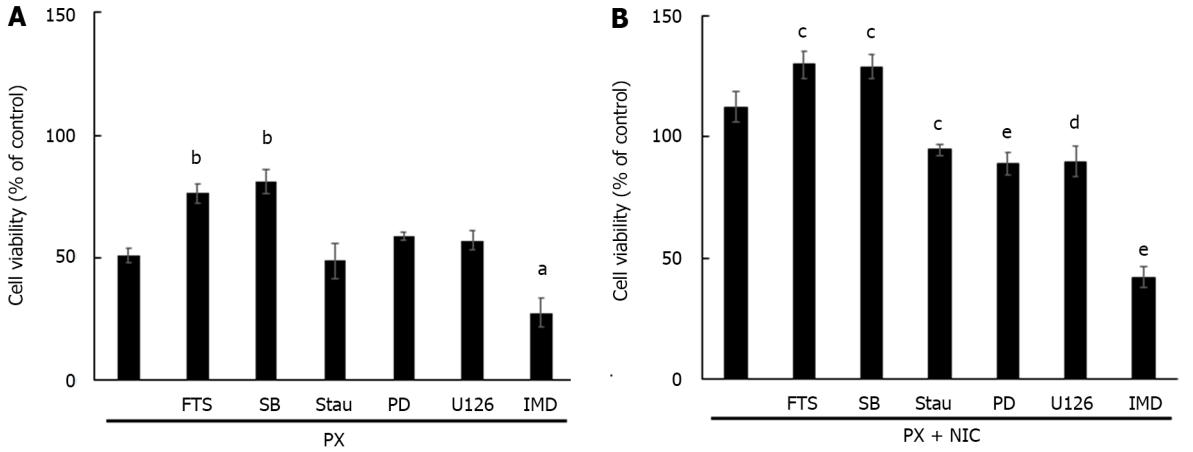
Previous reports have indicated that the presence of NIC (due to smoking) reduces the effectiveness of chemotherapy in lung cancer patients[60]. Less evidence is available about the effect of NIC on breast cancer patients during chemotherapy administration. PX is a first-choice drug in breast cancer treatment due to its antimitotic ability and its effect on inhibiting different tumor progression pathways[61]. Our results confirm that PX acts on MDA-MB-231 tumor cells by reducing their viability in a dose-dependent manner. The presence of NIC at a concentration similar to that present in the blood of passive smokers[62,63] reduced the potency of PX by more than one order of magnitude. It is also important to highlight that the administration of NIC is effective in reducing the PX effect in other TN tumor cells such as MDA-MB-468, and in luminal A MCF-7 tumor cells. In a human gastric cancer model, Tu *et al*[64] observed a reduction in the effect of PX on cell viability due to NIC through the activation of α7 nAChRs. Similarly, here we demonstrated that both the α7 and α9 nAChR subtypes are involved in this effect, a fact also evident when PX chemotherapy was administered in cycles to TN breast tumor cells.

Several authors have demonstrated that chemotherapeutic drugs control tumor tissue growth by reducing cell viability[28,65,66] and activating distinct signaling transduction pathways that involve PKC[67], MEK[68], ERK1/2[69], Ras[70], p38MAPK[71] and NF-κB[72]. In particular, PX exerts its effects *via* the activation of different kinase signaling pathways depending on the cell type analyzed[73-75]. In our model, PX reduced cell viability through activation of the Ras, p38MAPK and NF-κB pathways. These results are in line with those obtained by Lu *et al*[76], who described that treatment with PX decreases ovarian carcinoma cell viability by activating the p38MAPK pathway, as well as with those of Okano and Rustgi[77], who observed that the treatment of human esophageal squamous cancer



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Figure 5 Effect of nicotine on paclitaxel treatment. A: Viability determination of MDA-MB-231 cells treated with nicotine (10^{-10} mol/L) and paclitaxel (10^{-7} mol/L) alone or in combination, in the absence or presence of nicotinic antagonists: mecamylamine [non-selective for nicotinic acetylcholine receptors (nAChRs)], methyllycaconitine (selective for α7 nAChRs), or luteolin (selective for α9 nAChRs) at a concentration of 10^{-6} mol/L; B: Determination of percentage of living MDA-MB-231 cells treated with the same drug combinations as those shown in Figure 5A. Values are the mean ± SD of four experiments performed in duplicate. $^{b}P < 0.01$; $^{c}P < 0.001$ *vs* control, considered as 100%. MM: Mecamylamine; MLA: Methyllycaconitine; Lut: Luteolin; PX: Paclitaxel; NIC: Nicotine.



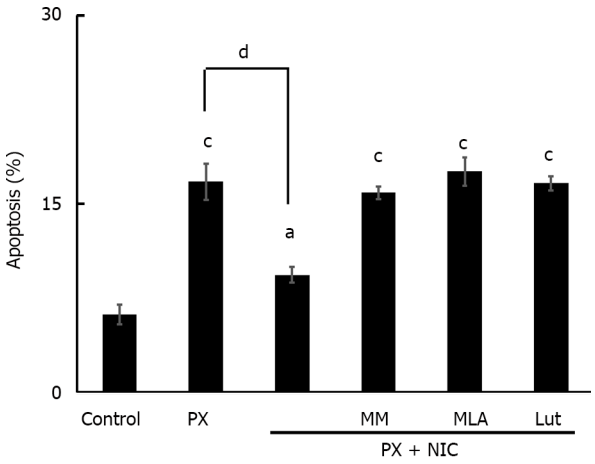
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Figure 6 Effect of paclitaxel and nicotine on MDA-MB-231 cell viability. A: Cells were treated with paclitaxel (PX) (10^{-7} mol/L) and the mediators were evaluated in the absence or presence of the kinase inhibitors for: PKC (staurosporine, 10^{-8} mol/L), MEK (PD098059 PD, 10^{-5} mol/L), Ras (S-trans, trans-farnesylthiosalicylic acid, 10^{-6} mol/L), ERK1/2 (U126, 10^{-5} mol/L), p38MAPK (SB203580, 10^{-5} mol/L) or IKKβ (IMD354, 5×10^{-8} mol/L); B: Cells were treated with the combination of PX and nicotine (NIC) (10^{-10} mol/L) as well as with the same inhibitors as those shown in Figure 6A. Values are the mean ± SD of four experiments performed in duplicate. $^{a}P < 0.05$; $^{b}P < 0.01$ *vs* PX. $^{c}P < 0.05$; $^{d}P < 0.01$; $^{e}P < 0.001$ *vs* PX+NIC. FTS: S-trans, trans-farnesylthiosalicylic acid; SB: SB203580; Stau: Staurosporine; PD: PD098059; PX: Paclitaxel; NIC: Nicotine.

cells with PX increases cell death through the activation of Ras.

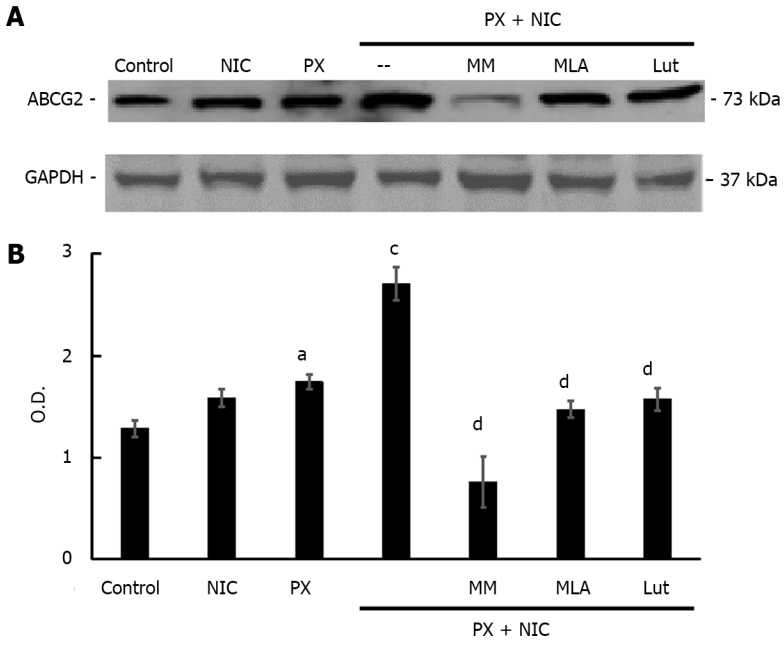
In the present study, when we analyzed signaling pathways involved in PX treatment in the presence of NIC, we determined that PKC, MEK and ERK1/2 also participate in the reduction of breast tumor cell viability. These results are similar to those of Chernyavsky *et al*[78], who described that, in human corneal epithelial cells, the stimulation of α7 nAChRs can activate the PKC-MEK-ERK1/2 signaling pathway through an increase in intracellular calcium, which induces an up-regulation of E cadherin expression related with corneal re-epithelization. In the same line of evidence, Wang *et al*[79] described that the activation of α7 nAChRs can mediate the proliferation of hepatocellular carcinoma through a TRAF6/NF-κB-dependent mechanism similar to that observed in the present study. Our results and those of others thus indicate the presence of antagonistic effects between PX and NIC on cell viability.

PX reduces breast cell viability, at least in part, through the induction of apoptosis[71]. We confirmed that this effect also occurs in our model as an increase in MDA-MB-231 cell apoptosis was observed after 48 h of treatment. The effect of PX on cellular apoptosis was attenuated by the activation of α7 and α9 nAChRs with NIC.



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Figure 7 Effect of nicotine on paclitaxel-induced apoptosis in MDA-MB-231 cells. Tumor cells were treated with paclitaxel (10^{-7} mol/L) in the absence or presence of the following nicotinic antagonists: mecamylamine [non-selective for nicotinic acetylcholine receptors (nAChRs)], methyllycaconitine (selective for α7 nAChRs), or luteolin (selective for α9 nAChRs) at a concentration of 10^{-6} mol/L. The percentage of apoptotic cells was determined by flow cytometry. Values are the mean ± SD of four experiments performed in duplicate. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$ *vs* control; $^{d}P < 0.001$. MM: Mecamylamine; MLA: Methyllycaconitine; Lut: Luteolin; PX: Paclitaxel; NIC: Nicotine.



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Figure 8 Effect of nicotine on paclitaxel-induced expression of ATP binding cassette transporter G2 protein in MDA-MB-231 cells. A: ATP binding cassette transporter G2 expression was determined by Western blot assays in cells treated with paclitaxel (10^{-7} mol/L), nicotine (10^{-10} mol/L) or both, in the absence or presence of the nicotinic antagonists mecamylamine [non-selective for nicotinic acetylcholine receptors (nAChRs)], methyllycaconitine (selective for α7 nAChRs) or luteolin (selective for α9 nAChRs) at a concentration of 10^{-6} mol/L. Molecular weights are indicated on the right; B: The densitometric analysis of the bands is expressed as optical density units relative to the expression of glyceraldehyde 3-phosphate dehydrogenase protein used as the loading control. One representative experiment of three is shown. Values are the mean ± SD of three experiments. $^{a}P < 0.05$; $^{c}P < 0.001$ *vs* Control; $^{d}P < 0.001$ *vs* PX+NIC. ABCG2: ATP "binding cassette" G2 drug transporter; O.D.: Optical density; MM: Mecamylamine; MLA: Methyllycaconitine; Lut: Luteolin; nAChRs: Nicotinic acetylcholine receptors; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; PX: Paclitaxel; NIC: Nicotine.

A frequent undesirable effect of antitumor therapy is innate or acquired resistance. In particular, PX can induce acquired resistance[80,81] by the transactivation of signaling pathways[82] or by triggering the overexpression of several proteins[83,84]. Considering the latter, the ABCG2 transporter plays an important role in the generation of resistance to PX treatment in breast adenocarcinomas[85]. In the present study, PX induced an increase in ABCG2 expression and this effect was potentiated by α7 and α 9 nAChR activation by NIC. The increase in ABCG2 expression should lead to a higher rate of PX extrusion, partly explaining the reduction in the cytostatic effect of PX in the presence of NIC, as well as

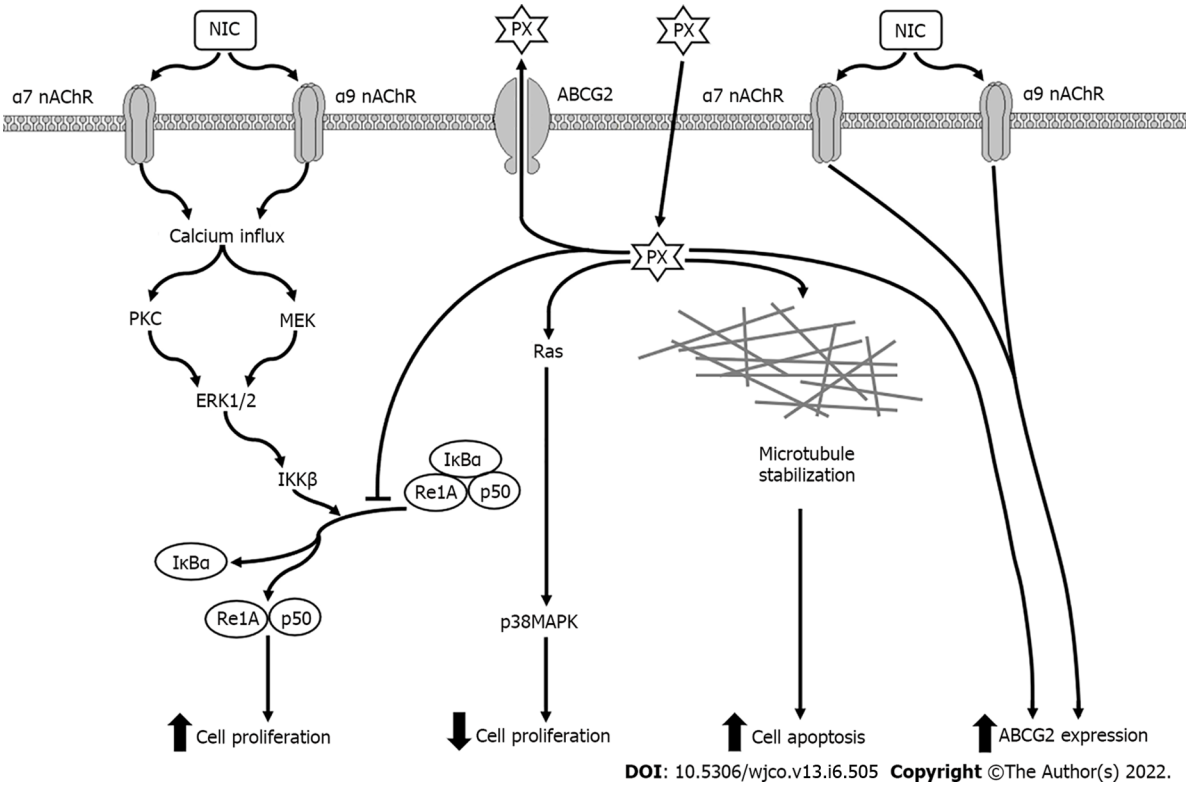


Figure 9 Possible signal transduction pathways in MDA-MB-231 cells activated by paclitaxel in the absence or presence of nicotine. NIC: Nicotine; PX: Paclitaxel; nAChR: Nicotinic acetylcholine receptors; ABCG2: ATP "binding cassette" G2 drug transporter; PKC: Protein kinase C; MEK: Mitogen-activated protein kinase kinase; ERK: Extracellular signal-regulated kinases; p38MAPK: p38 Mitogen-activated protein kinases; IKKβ: IκB kinase; IκBα: κB inhibitors.

the decrease in the sensitivity to PX when administered in cycles in the presence of NIC. Similar results have been obtained by Mukherjee *et al*[86] and Amawi *et al*[87], who described that PX treatment increases the activation and expression of ABCG2 in different breast cancer cell lines and that this factor could mediate the resistance to treatment[88]. Moreover, Nimmakayala *et al*[89] and An *et al*[90] observed that the exposure of pancreatic and lung tumor cells to cigarette smoke caused an increase in the expression of this pump.

A possible mechanism of action of PX treatment in the presence of NIC in a TN tumor-bearing smoking patient is proposed in Figure 9.

CONCLUSION

Our results demonstrate that NIC at a concentration similar to that present in passive smokers' plasma can negatively modulate the cytotoxic/apoptotic effect of PX in TN breast tumors. The reduction in the sensitivity to PX could be due to an increase in the expression of the ABCG2 transporter in malignant cells. Additionally, our findings demonstrated the participation of different kinases and the NF-κB pathway, which would modulate cell viability in this effect. This information could allow the development of better strategies to improve TN breast cancer therapy, such as blocking nAChRs together with PX during chemotherapy administration to passive smoking patients.

ARTICLE HIGHLIGHTS

Research background

Triple negative is the subtype of breast cancer with the worst prognosis, showing an increase in resistance to chemotherapy in smoking patients, who have high levels of nicotine in their blood. In lung cancer, it has been proposed that the activation of nicotinic acetylcholine receptors could be responsible for the modulation of several parameters of tumor biology and the loss of effectiveness of chemotherapeutic treatment, but it is not known what occurs in a nearby organ such as the breast.

Research motivation

Given that breast tumor-bearing patients have a low efficiency to antitumor therapy, knowledge of the signaling pathways involved in this phenomenon is important to generate new therapeutic targets that improve sensitivity to treatment.

Research objectives

This research aimed to determine the signaling pathways involved in the nicotinic modulation of the cytostatic effect of paclitaxel in human triple negative breast cancer cells.

Research methods

The modulatory effect of nicotine on paclitaxel treatment was assessed by the 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The trypan blue exclusion assay was used to evaluate viable cells in response to different treatments. Protein expression levels were evaluated by Western blot assays and apoptosis was determined using immunofluorescence assays with annexin V and 7AAD.

Research results

Nicotine decreased paclitaxel's inhibition of viability and apoptosis in MDA-MB-231 breast cancer cells. This modulation of viability is mediated by the activation of α7 and α9 nicotinic acetylcholine receptors and protein kinases PKC, Ras, MEK, ERK, p38MAPK and the NF-κB pathway. Cells surviving paclitaxel treatment in the presence of nicotine are less sensitive to another cycle with the chemotherapeutic agent probably due an increase in the protein expression of ATP binding cassette transporter G2.

Research conclusions

Nicotine modulates the cytotoxic/apoptotic effects of paclitaxel and knowledge of its signaling pathway mediators could allow the development of better strategies to improve triple negative breast cancer therapy, such as nicotinic acetylcholine receptors blockage together with paclitaxel during chemotherapy administration to smoking patients.

Research perspectives

Knowledge of the mediators that participate in the nicotinic modulation of paclitaxel's effect will allow the development of new antitumor strategies that could be applied not only to other subtypes of mammary tumors, but also to tumors in other organs of smoking patients.

FOOTNOTES

Author contributions: Español A performed cell assays, supervised the work, analyzed and interpreted the data, and wrote the manuscript; Sanchez Y and Salem A performed cell assays and contributed to the writing of the manuscript; Obregon J carried out lab work as part of her grade thesis, and helped to analyze and interpret the data; Sales ME supervised the work and edited the manuscript draft; all authors read and approved the final manuscript.

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Abstract

BACKGROUND

Although PNENs generally have a better prognosis than pancreatic cancers, some PNENs display malignant behavior including lymph node (LN) metastasis. Complete tumor resection can be the only potentially curative treatment for patients with resectable PNENs. However, the indications for LN dissection are still controversial. Over the last decade, minimally invasive surgery such as laparoscopic pancreatic surgery (LPS) has been increasingly performed for pancreatic tumors including PNENs.

AIM

To investigate the risk factors for LN metastasis in PNENs and to select appropriate patients for limited surgery by LPS.

METHODS

From April 2001 to December 2019, 92 patients underwent pancreatic resection for PNENs at Kumamoto University Hospital. Finally, 82 patients were enrolled in this study. Using perioperative factors, we examined the predictive factors for LN metastasis in PNENs.

RESULTS

Among the 82 patients, the percentage of LN metastasis according to the pathological findings was 12% (10/82 cases). The median tumor size was 12 mm (range: 5-90 mm). The median tumor size in the LN-positive group (37 mm) was significantly larger than that in the LN-negative group (12 mm) (P = 0.0001). Multivariate analyses revealed that larger tumor size (≥ 20 mm) was an inde-

pendent risk factor for LN metastasis (odds ratio 16.8, $P = 0.0062$). In patients with small tumors (≤ 10 mm), LN metastasis was not found.

CONCLUSION
Larger tumor size (≥ 20 mm) is an independent risk factor for LN metastasis in PNENs. In smaller PNENs (≤ 10 mm), we may be able to choose limited surgery without LN dissection.

Key Words: Lymph node metastasis; Pancreatic neuroendocrine neoplasms; Risk factor; Tumor size

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Core Tip: Among the 82 patients, the percentage with lymph node (LN) metastasis according to the pathological findings was 12% (10/82 cases). The median tumor size was 12 mm (range: 5-90 mm). The median tumor size in the LN-positive group (37 mm) was significantly larger than that in the LN-negative group (12 mm) ($P = 0.0001$). Multivariate analyses revealed that large tumor size (≥ 20 mm) was an independent risk factor for LN metastasis. In patients with small tumors (≤ 10 mm), LN metastasis was not found. In conclusion, large tumor size (≥ 20 mm) is an independent risk factor for LN metastasis in PNENs. In smaller PNENs (≤ 10 mm), we may be able to choose limited surgery without LN dissection.

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INTRODUCTION

Pancreatic neuroendocrine neoplasms (PNENs) are relatively rare and represent 1%-2% of all pancreatic neoplasms[1]. Although patients with PNENs generally have better prognosis than those with pancreatic cancers, some PNENs display malignant behavior including local invasion, lymph node (LN) metastasis, and distant metastasis[2]. The natural history of PNENs is not fully understood because of their relative rarity, and therefore, it is difficult to predict the malignant potential of PNENs precisely.

Complete tumor resection can be the only potentially curative treatment for patients with resectable PNENs. However, optimal surgical management procedures have not yet been established[3,4]. Especially, the indications for LN dissection are still controversial, especially in early PNENs. This is partly caused by the difficulty of predicting LN metastasis. Therefore, it is important to establish appropriate indications for LN dissection to treat PNENs.

Over the last decade, minimally invasive surgery such as laparoscopic pancreatic surgery (LPS) has been increasingly performed for pancreatic tumors including PNENs[5-7]. Non-comparative studies have shown that LPS for pancreatic tumors is safe and equivalently effective to open pancreatic surgery (OPS)[8-10]. In well-selected groups of patients with pancreatic lesions, LPS provides good peri and post operative outcomes, such as reduced intraoperative blood loss, and postoperative pain and length of postoperative day[8,10-13]. As a limited type of LPS, laparoscopic spleen-preserving distal pancreatectomy and excisional resection for PNENs has also been performed in selected cases[7,14,15]. However, the indications for limited surgery by LPS for patients with PNENs remain unclear.

The aims of this study are to investigate the risk factors for LN metastasis in PNENs and to select appropriate patients for limited LPS.

MATERIALS AND METHODS

Study cohort

From April 2001 to December 2019, 92 patients underwent pancreatic resection for PNENs at Kumamoto University Hospital. Of them, 10 patients (11%) were excluded from this analysis because of distant metastases and coexisting tumors other than PNENs. Finally, 82 patients were enrolled in this study. The patients were identified retrospectively from a prospectively maintained database, and additional data were obtained by reviewing each patient's medical records. Written informed consent was obtained from all patients before treatment, and this study was approved by the Institutional Review Board of Kumamoto University (number 1291).

Treatment strategy

Before treatment, all patients underwent routine diagnostic laboratory tests and imaging modalities including enhanced computed tomography (CT), magnetic resonance imaging (MRI), and endoscopic ultrasound (EUS). The final diagnoses were confirmed pathologically using resected specimens. Tumors were classified as functional PNENs according to the clinical signs and symptoms of hormonal excess and increased levels of corresponding serum peptides and hormones. Tumors were classified as non-functional if they were not associated with distinct clinical manifestations or hormonal alterations[16]. Surgical procedures were selected based on each tumor's location and extent and the patient's general condition. Pancreatic resection was considered the first-choice treatment for patients with PNENs.

Postoperative workup

After treatment, all of the patients underwent regular follow-up examinations including routine laboratory tests and imaging studies including EUS, CT, or MRI to detect any pancreatic recurrence or distant metastasis, as described previously[17]. When tumor recurrence was confirmed, various treatment modalities were selected, including repeat surgery, chemotherapy, or a combination of these methods, according to tumor location and patient condition.

Statistical analysis

Continuous variables were expressed as median (range). Continuous and categorical variables were compared using Mann-Whitney U and χ^2 tests, respectively. Survival analyses were performed using the Kaplan-Meier method, with comparisons using the log rank test. Overall survival (OS) was calculated from the date of surgery until death or last follow-up. Variables in which the *P* value for LN metastasis was < 0.05 in univariate analysis were subjected to subsequent multivariate analysis by stepwise backward elimination procedures. All statistical analyses were performed using JMP® version 13.1 (SAS institute, Cary, NC, United States). All *P* values were two-sided, and $P < 0.05$ was considered as statistically significant.

RESULTS

The 82 patients' demographic and clinical characteristics are summarized in Table 1. There were 41 male and 41 female patients, with a median age of 59 years (range, 18-81 years). Thirty five patients (43%) had symptoms at the first consultation. Preoperative contrast-enhanced CT showed that the majority of patients had tumors with hyper enhanced pattern (72 patients, 88%). Of the 31 patients (38%) who had functional PNENs, the most frequent type of functional PNEN was insulinoma (26 patients, 32%), followed by glucagonoma (2, 2.5%), gastrinoma (2, 2.5%), and VIPoma (1, 1%). There were 51 patients (62%) who had non-functional PNENs. Their 2017 WHO classifications were: G1, 70 (85%); G2, 9 (11%); and G3 or NEC, 3 (4%). Fourteen patients (17%) had multiple tumors, and the median tumor size was 12 mm (range, 5-90 mm). Among the 82 patients, 23 (28%) received pancreatoduodenectomy (PD), 38 (46%) received distal pancreatectomy, 2 (2.5%) received PD + DP, and 19 (23%) received enucleation or partial pancreatectomy.

Among the 82 patients, 10 (12%) were identified as having LN metastasis. The demographic and clinical characteristics of the 10 patients with LN metastasis were compared with those of the 72 patients without LN metastasis (Table 1). LN metastases of PNENs were positively associated with pathological grade: 6%, 44%, and 67% of cases with LN metastases were classified as G1, G2, and G3/NEC, respectively ($P = 0.0009$). In the LN metastasis-positive group, tumor size was significantly larger than that in the negative group (median, 12 *vs* 37, $P = 0.0001$). Univariate analysis showed that the following three factors were related to LN metastasis: tumor size ≥ 20 mm [Odds Ratio (OR) 31.5, $P < 0.0001$], WHO 2017 classification ≥ G2 (OR 20.1, $P = 0.0001$), and non-functional type of PNEN (OR 6.43, $P = 0.035$). Multivariate logistic regression analyses revealed that tumor size ≥ 20 mm was an independent risk factor for LN metastasis (OR 16.8, $P = 0.0062$) (Table 2).

Figure 1 shows the rate of LN metastasis according to tumor size. The rates of LN metastasis according to tumor size were as follows: 0% (0/29 cases, ≤ 10 mm group), 3% (1/31 cases, 11 mm-20 mm group), 25% (2/8 cases, 21-30 mm group), 50% (3/6 cases, 31-40 mm group), and 50% (4/8 cases, > 40 mm group) (Figure 1). The median length of follow-up after surgery was 51.8 months (range, 0.4-224.2). The cumulative OS rate after surgery for patients with no LN metastasis was significantly higher than that for those with LN metastasis ($P = 0.009$) (Figure 2).

DISCUSSION

PNENs are rare tumors[1]. The oncological history is not yet fully understood due to their often-lazy course, because it is not easy to find correct diagnosis and treatment. Furthermore, PNENs have wide variety biological behaviors, such as benign tumors and malignant status[18]. Because of the hetero-

Table 1 Comparisons of patients' characteristics according to the presence of lymph node metastasis of pancreatic neuroendocrine neoplasm

Variables	Total (*n* = 82)	N^- (*n* = 72)	N^+ (*n* = 10)	*P* value
Age, median (range)	59 (18-81)	58 (18-80)	63 (18-81)	0.65
Gender (male/female)	41/41	35/37	6/4	0.50
Tumor size, median, mm (range)	12 (5-90)	12 (5-90)	37 (12-75)	0.0001
Tumor number (single/multiple)	68/14	59/13	9/1	0.50
Tumor location (Ph/Pb/Pt/Ph and Pt)	32/23/25/2	23/22/25/2	9/1/0/0	0.15
Symptoms (yes/no)	35/47	31/41	4/6	0.85
CT Enhancement (hyper/hypo)	72/10	64/8	8/2	0.17
Type of PNEN, *n* (%)				NS
Insulinoma	26 (32)	26	0	
Gastrinoma	2 (2.5)	1	1	
Glucagonoma	2 (2.5)	2	0	
VIPoma	1 (1)	1	0	
Non functional	51 (62)	42	9	
WHO classification 2017, *n* (%)				0.0009
NET G1	70 (85)	66	4 (6%)	
NET G2	9 (11)	5	4 (44%)	
NET G3/NEC	3 (4)	1	2 (67%)	
Surgical procedure, *n* (%)				NS
Pancreatoduodenectomy (PD)	23 (28)	15	8	
Distal pancreatectomy (DP)	38 (46)	37	1	
PD + DP	2 (2.5)	2	0	
Enucleation/partial pancreatectomy	19 (23)	18	1	

N^-: Negative for lymph node metastasis; N^+: Positive for lymph node metastasis; NEN: Neuroendocrine neoplasms; WHO: World Health Organization; NET: Neuroendocrine tumor; PD: Pancreatoduodenectomy; DP: Distal pancreatectomy; CT: Computed tomography; PNEN: Pancreatic neuroendocrine neoplasm. NS: Not significant.

geneity of PNENs, it is very difficult both to construct the effective clinical treatment policy systems and to confirm the surgical method for cure.

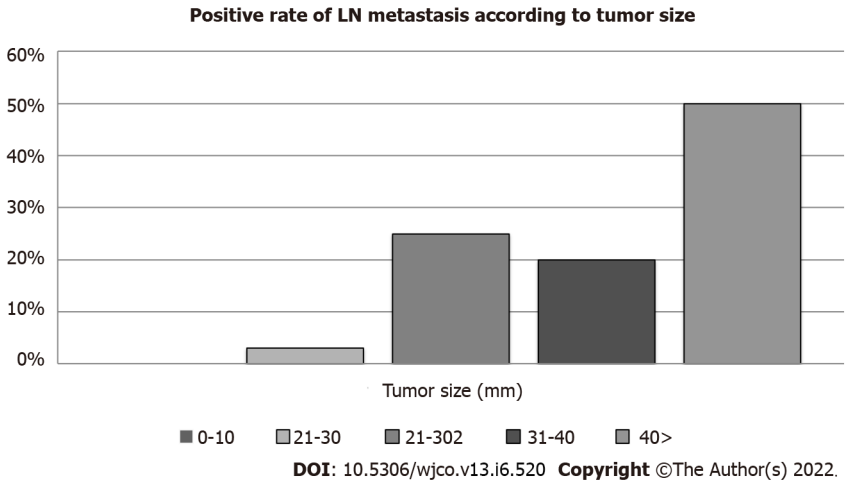
Some reports have associated LN metastasis with shorter OS[3,19-25], while others have found that LN status did not affect survival[26-29]. LN metastasis is positively correlated with pathological grade, with 15%-20%, 30%-40%, and > 50% of patients with LN metastasis classified as G1, G2, and G3, respectively[30]. In our study, we also reported that the LN metastasis-positive group of PNENs had poor OS after surgery. Further, we reported that LN metastases of PNENs are positively associated with pathological grade, with 6%, 44%, and 67% of patients with LN metastases classified as G1, G2, and G3/NEC, respectively (P = 0.0009; Table 1). Therefore, patients with PNENs and LN metastasis have poor prognosis and high malignant potential. However, previous reports have not clearly shown that to omit LN dissection may increase the possibility of recurrence. Some previous studies shows that local LN metastases of PNENs have oncologic effects[30,31]. A past study related with non-functional G1 PNENs who underwent surgery of pancreas reported that LN metastases of PNENs do not adversely affect oncological outcomes and do not require routine local lymphadenectomy[32]. Partelli *et al*[33] reported that a lot of insulinomas (well-differentiated) and non-functional PNENs located in the distal pancreas are very small, rarely associated with LN metastases, and there is no radiographic evidence of positive of LN metastases. Thus, the significance of LN metastasis in patients with PNENs is very complicated, and the indications for regional LN dissection are still controversial.

Previous studies have focused on the associations of LN metastasis or and/or prognosis with tumor size[17,21,34-39]. Although LN metastasis has been seen even in patients with tumors < 10 mm, LN metastasis occurs more often in patients with large tumors than in those with smaller ones. In our study, there were no cases of LN metastasis in patients with tumors ≤ 10 mm. LN metastases of PNENs were

Table 2 Factors related to lymph node metastasis of pancreatic neuroendocrine neoplasm

Factors	Univariate analysis		Multivariate analysis		
	Odds ratio	*P* value	Odds ratio	95%CI	*P* value
Age ≥ 60	0.54	0.40			
Gender (male)	0.63	0.50			
Symptoms (yes)	1.13	0.85			
CT Enhancement (hyper)	4	0.17			
Tumor number (multiple)	1.98	0.50			
Tumor size (≥ 20 mm)	31.5	< 0.0001	16.8	2.15-35.4	0.0062
WHO classification 2017 (≥ G2)	20.1	0.0001		NS	
Type of PNEN (non functional)	6.43	0.035		NS	

NEN: Neuroendocrine neoplasms; WHO: World Health Organization; CT: Computed tomography; PNEN: Pancreatic neuroendocrine neoplasm; NS: Not significant.

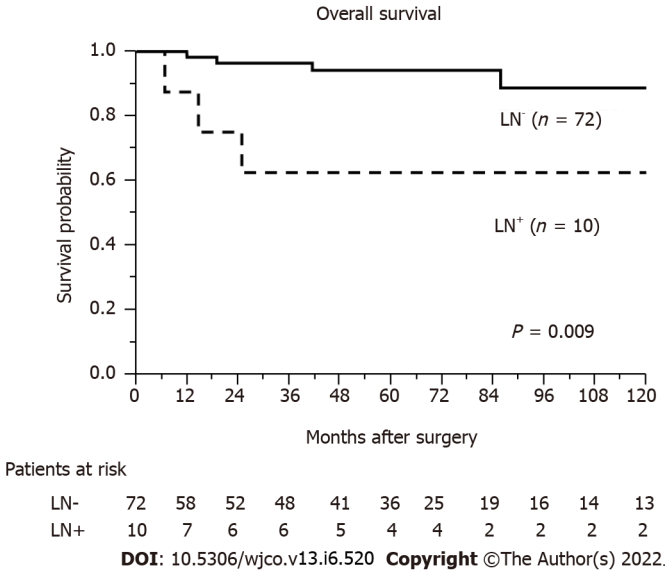


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Figure 1 Rate of lymph node metastasis according to tumor size. The rates of lymph node metastasis according to tumor size were as follows: 0% (0/29 cases, ≤ 10 mm group), 3% (1/31 cases, 11 mm-20 mm group), 25% (2/8 cases, 21-30 mm group), 50% (3/6 cases, 31-40 mm group), and 50% (4/8 cases, > 40 mm group). LN: Lymph node.

positively associated with tumor size, being present in 0%, 3%, 25%, and 50% of patients with primary tumors ≤ 10 mm, 11-20 mm, 21-30 mm, and > 30 mm, respectively. If we can predict the presence of LN metastasis according to tumor size, we can select appropriate patients for limited LPS.

Over the last decade, the use of laparoscopy in pancreatic surgery has increased significantly, and previously almost all open surgery can now be performed in a minimally invasive method. In general, these minimally invasive surgery should be limited to high-volume centers with extensive experience in pancreatic surgery with open surgery. Patients with small-sized PNENs in the body and tail of the pancreas are particularly well suited for minimally invasive surgery, and the laparoscopic procedures gives better result than open surgical method[40,41]. Laparoscopic distal pancreatectomy have the potential to be superior to the open surgical method in patients with benign tumors, resulting in less operative bleeding, shorter postoperative days, and equivalent rates of complications[13]. According to the review by 11 studies, which involve 906 PNENs patients, of whom 22% and 78% underwent LPS and OPS, respectively, it reported that overall complication rate of laparoscopic method was significantly lower (38% *vs* 46%, $P < 0.001$) and the postoperative days in hospital is shorter ($P < 0.001$) [40]. LPS is now considered to be a safe approach for PNENs and should be included in the patient's surgical equipment. Many surgeons have reported that the rates of overall complication in small or benign tumors were lower with LPS than OPS. Although in the cases of patients with malignant PNENs, we need advanced surgical skills, LPS was not associated with compromised oncologic resection and provided benefits including reduced postoperative pain, shorter hospital stay, and shorter postoperative recovery period. Thus, it is important to investigate the risk factors of LN metastases in PNENs and to select appropriate patients for limited LPS. Our results offer certain recommendations in this regard.



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Figure 2 Overall survival after surgery of 82 patients according to the presence of lymph node metastasis. The cumulative overall survival rate after surgery among patients who had no lymph node (LN) metastasis was significantly higher than that for those who had LN metastasis. LN: Lymph node.

However, this study had several limitations, including its retrospective design, the small number of subjects, and the lack of data on certain pathologic variables (especially the Ki-67 indices and mitotic rates) for all patients. The number of examined LNs was not sufficient, and data on the number of positive LNs were not available for all PNENs. Therefore, further research regarding advanced PNENs is required.

CONCLUSION

In conclusion, larger tumor size (≥ 20 mm) is an independent risk factor for LN metastasis in PNENs. In smaller PNENs (≤ 10 mm), we may be able to choose limited surgery without LN dissection.

ARTICLE HIGHLIGHTS

Research background

The indications for lymph node (LN) dissection are still controversial.

Research motivation

Over the last decade, minimally invasive surgery such as laparoscopic pancreatic surgery (LPS) has been increasingly performed for pancreatic tumors including pancreatic neuroendocrine neoplasms (PNENs).

Research objectives

The aim of this study was to investigate the risk factors for LN metastasis in PNENs and to select appropriate patients for limited surgery by LPS.

Research methods

From April 2001 to December 2019, 92 patients underwent pancreatic resection for PNENs at Kumamoto University Hospital. Finally, 82 patients were enrolled in this study. Using perioperative factors, we examined the predictive factors for LN metastasis in PNENs.

Research results

Among the 82 patients, the percentage of LN metastasis according to the pathological findings was 12% (10/82 cases). The median tumor size was 12 mm (range: 5-90 mm). The median tumor size in the LN-positive group (37 mm) was significantly larger than that in the LN-negative group (12 mm) (P = 0.0001). Multivariate analyses revealed that large tumor size (≥ 20 mm) was an independent risk factor for LN metastasis (odds ratio 16.8, P = 0.0062). In patients with small tumors (≤ 10 mm), LN metastasis

was not found.

Research conclusions

Large tumor size (≥ 20 mm) is an independent risk factor for LN metastasis in PNENs. In smaller PNENs (≤ 10 mm), we may be able to choose limited surgery without LN dissection.

Research perspectives

In smaller PNENs (≤ 10 mm), we may be able to choose limited surgery without LN dissection.

FOOTNOTES

Author contributions: Nakao Y, Hayashi H and Yamashita Y designed the research study; Nakao Y, Takashi O, Matsumura K, Uemura N, Kitamura F, Itoyama R, Yusa T, Taki K, Miyata T, Higashi T, Nakagawa S, Okabe H and Imai K performed the research; Yamashita Y and Baba H contributed new reagents and analytic tools; Nakao Y, Hayashi H and Takashi O analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

Institutional review board statement: Institutional Review Board of Kumamoto University (number 1291).

Informed consent statement: Consent was obtained from the patient and family according to Institutional Review Board protocols.

Conflict-of-interest statement: All the authors have no conflicts of interest in association with this study. No financial support was received for the work described in this manuscript.

Data sharing statement: No additional data are available.

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ORIGINAL ARTICLE

Prospective Study

Significance of serum carcinoembryonic antigen in metastatic breast cancer patients: A prospective study

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Abstract

BACKGROUND

Carcinoembryonic antigen (CEA) is an important serum tumour marker with a substantial role in diagnosis and monitoring of various solid tumours. About 36%-70% of breast cancers have elevated serum CEA. And the available studies show discrepancy in addressing the prognostic significance of CEA in advanced breast cancer.

AIM

To estimate the serum CEA level in our metastatic breast cancer patients and correlate it with response to treatment and clinical outcome.

METHODS

This was a prospective clinical study conducted on 50 metastatic breast cancer patients treated at breast clinic, with newly diagnosed metastatic breast cancer planned for palliative chemotherapy, targeted therapy, and hormonal treatment. We estimated the proportion of patients with elevated serum CEA level at baseline and after palliative treatment and also studied the association of serum CEA levels with known prognostic factors. The response to treatment was correlated with the serum CEA levels in the context of responders and non-responders.

RESULTS

The median pre-treatment and post-treatment CEA levels were 7.9 (1.8-40.7) ng/mL and 4.39 (1.4-12.15) ng/mL, respectively, in the whole study population (P = 0.032). No statistically significant difference was seen in baseline serum CEA between responders and non-responders. Even in the luminal group, pre-treatment serum CEA was not a predictor of response, but post-treatment CEA was a significant predictor of tumour progression. In patients with liver and lung metastases, post-treatment CEA level difference was not statistically significant in both responders and non-responders though the values were higher in non-

responders. Among those with bone metastases, 69.5% had elevated post-treatment serum CEA, and only 37.5% had elevated serum CEA in those with no bone metastases.

CONCLUSION
Elevated post-treatment serum CEA levels are associated with disease progression and poor response to therapy. Persistently elevated post-treatment serum CEA levels are significantly associated with bone metastases. Elevated serum CEA and hormonal status are significant predictors of treatment response.

Key Words: Carcinoembryonic antigen; Metastatic breast cancer; Serum tumour marker; Luminal and non-luminal metastatic breast cancer; Palliative chemotherapy

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Core Tip: In breast cancer patients, elevated serum carcinoembryonic antigen (CEA) levels are particularly noted in advanced disease. Our study suggested that serum CEA has potential clinical value in monitoring the treatment response of metastatic breast cancer patients, especially in those with bone metastasis.

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INTRODUCTION

Breast cancer, one of the leading causes of malignancy related morbidity and mortality among women, comprises of a spectrum of clinically and histologically heterogeneous group of diseases with distinct molecular portraits[1]. In spite of increasing awareness, advanced screening, and diagnostic methodologies, we still witness a significant proportion of patients who present with advanced stage disease. Deciding optimal treatment and monitoring strategies for patients with metastatic and recurrent disease remains a diagnostic challenge for physicians.

Carcinoembryonic antigen (CEA) is an important serum tumour marker with a substantial role in diagnosis and monitoring of colorectal cancer. Globally, cancer antigen 15-3 (CA15-3) and CEA are used serum tumor markers in breast cancer[2-4]. In breast cancer patients, elevated serum CEA levels are particularly noted in metastatic and recurrent disease. Studies have reported a varying incidence of serum CEA positivity ranging from 36%-70%[5]. Elevated levels are known to positively correlate with tumour burden, grade of tumour, and site of metastasis, and they also translate into poor overall survival (OS) and progression-free survival[6]. The clinical utility of serial tumour marker measurements is not indicated in asymptomatic women for surveillance after treatment of breast cancer [7-9]. The main applications are used in metastatic disease monitoring during treatment, especially CA15-3. Among serum tumour markers in breast cancer, CA15–3 and CEA have been the commonly used ones[10-13]. Hence, serum CEA estimation can be proposed as an auxiliary tool for response assessment, monitoring, and gaining prognostic information. In spite of these, due to discordant results, their clinical utility remains unclear[14-16]. There are very few studies addressing the prognostic significance of CEA and the available studies show discrepancy. Hence, we conducted this study to estimate the serum CEA level in our metastatic breast cancer (MBC) patients and correlate it with response to treatment and clinical outcome.

MATERIALS AND METHODS

This was a prospective experimental study conducted on 50 MBC patients treated at Breast Clinic, Department of Medical Oncology during the period December 2019 to November 2020. Patients with newly diagnosed MBC planned for palliative chemotherapy, targeted therapy, and hormonal treatment were included. Routine protocol for MBC work-up included biopsy from breast lump or metastatic lesion, histopathology and immunohistochemistry for oestrogen, progesterone, and Her2 receptors, computed tomography of the chest, abdomen, and pelvis, bone scan, and serum biochemistry. Patients with inflammatory breast cancer and active inflammatory conditions were excluded in this study due to the fact that they could cause elevation of serum CEA levels. Five milliliters of venous blood was drawn

from MBC patients who consented for study participation and serum was isolated after centrifugation at 3000 rpm for 10 min, transported into new disposable tubes, and stored at -20 ℃. In patients with hormone positive MBC with visceral crisis and triple negative breast cancer (TNBC) patients, sample for serum CEA levels was collected before initiation of first cycle of palliative chemotherapy and after completion of six cycles of chemotherapy. In patients with hormone positive MBC without visceral crisis, serum CEA sample was collected before initiation of endocrine agents and at 6 mo after initiation. In patients with HER2 positive MBC, blood sample was collected before initiation of first cycle of palliative chemotherapy plus trastuzumab and after completion of six cycles of chemotherapy plus trastuzumab.

Concentrations of the serum tumour marker CEA were measured with an automated sandwich ELISA test system using the manufacturer's recommended kits (ELISA 2010, Roche Company). CEA concentrations were recorded in nanogram *per* millilitre. CEA value more than 3.8 ng/mL was considered positive. Patient treatment and response evaluation were as *per* the institutional protocol. Treatment and follow-up details of the patients were noted from the medical case records. We estimated the proportion of patients with elevated serum CEA level in MBC and also studied the association of serum CEA levels with known prognostic factors. The radiological response was assessed using Response Evaluation Criteria in Solid tumours (RECIST1.1). The response to treatment were correlated with the serum CEA levels in the context of responders and non-responders.

Statistical analysis

Statistical calculations were performed using the SPSS for Windows, version 15.0 (SPSS, Inc., Chicago, United States). Categorical variables are expressed using frequencies and percentages. Continuous variables are presented in terms of the mean and standard deviation. Association between two categorical variables was analyzed using Chi square or fisher's exact test. Non-parametric tests were used for finding the statistical significance. Wilcoxon signed rank test was used for comparing pre- and post-treatment serum CEA in different categories. Comparison of serum CEA in different clinical categories was carried out using Mann-Whitney test and Kruskal-Wallis test.

The optimal cut-off values of the CEA were determined using receiver operator characteristic (ROC) curve. A P value < 0.05 was considered significant.

RESULTS

The median age of diagnosis was 57.5 (48.7-63.2) years. Median duration of symptoms was 4 (1.75-6.0) mo. About 24% (12/50) of the patients were premenopausal and 76% (38/50) were post-menopausal. The main comorbidities were diabetes mellitus (24%; 12/50), hypertension (28%; 14/50), and coronary heart disease (4%; 2/50). About 64% (32/50) of the patients had distant nodal metastases, 50% (25/50) had bone metastases, 72% (36/50) had lung metastases, 36% (18/50) had liver metastases, and 6% (3/50) had oligometastatic diseases. About 96% (48/50) had invasive ductal carcinoma (IDC) and 4% (2/50) had other histology. Approximately 72% (36/50) were hormone positive and 38% (19/50) were HER2 positive. Grade 2 IDC accounted for 24% (12/50) and grade 3 IDC accounted for 76% (38/50). Among the study population, luminal type was seen in 70% (35/50), HER2 positive type in 8% (4/50), and TNBC in 22% (11). The pre-chemotherapy CEA levels were more than 3.8 in 72% (36/50) of the patients. About 82% (41/50) were treated with chemotherapy and 18% (9/50) treated with hormonal agents. Anti-Her2 treatment was received by 16% (8/50) of the patients. The median number of cycles of chemotherapy was 6 (4-6). The main palliative chemotherapy agents were docetaxel (68%; 34/50), paclitaxel (4%; 2/50), capecitabine (2%; 1/50), doxorubicin plus cyclophosphamide (2%; 1/50), carboplatin (2%; 1/50), and paclitaxel plus carboplatin (4%; 2/50). About 6% (3/50) of the patients received palliative radiation to their painful bone metastases.

About 36% (18/50) of the patients progressed on treatment while 64% (32/50) had responded to palliative systemic treatment. Among responders (64%), 2% (1/50) had complete remission, 32% (16/50) had partial response, and 30% (15/50) had stable disease. About 36% (18/50) had progressive disease.

Serum CEA and its correlation with other variables

Serum CEA value more than 3.8 ng/mL was considered positive. Baseline serum CEA and its correlation with other variables in MBC are given in Table 1. None of the factors like menstrual status, grade of the tumour, number and sites of metastases, presence or absence of metastases, HER2 status, and TNBC status showed any statistical significance except luminal type (P = 0.016).

Serum CEA as a predictor of response to treatment

The median pre-treatment and post-treatment CEA levels were 7.9 (1.8-40.7) ng/mL and 4.39 (1.4-12.15) ng/mL, respectively, in the whole study population (P = 0.032). Serum CEA and response to treatment in responders and non-responders are given in Table 2. Among responders, median pre-treatment CEA was 8.87 (2-49.6) ng/mL and post-treatment CEA was 2.07 (1-8.7) ng/mL (P = 0.001). Among non-responders, median pre-treatment CEA was 5.4 (1.7-36.01) ng/mL and post-treatment CEA was 11

Table 1 Association of baseline serum carcinoembryonic antigen with other variables in study population

CEA level	Less than or equal to 3.8	More than 3.8	*P* value
Pre-menopausal	3	9	0.79
Post-menopausal	11	27	
Grade 2	3	9	0.79
Grade 3	11	27	
Luminal	6	30	0.016
Her2 Neu	1	2	
TNBC	7	4	
Luminal	6	30	0.012
Non luminal	8	6	
Bone metastases	6	19	0.682
No bone metastases	7	17	
Lung metastases	11	25	0.487
No lung metastases	2	11	
Liver metastases	3	15	0.392
No liver metastases	10	21	
Less than 5 metastases	1	2	0.78
More than 5	12	34	
PR/SD/CR	9	23	0.79
Progression	5	13	

CEA: Carcinoembryonic antigen; TNBC: Triple negative breast cancer; PR/SD/CR: Partial response/stable disease/complete response.

Table 2 Serum carcinoembryonic antigen and response to treatment in responders and non-responders

Serum CEA	Responders	Non-responders	*P* value
Median pre-treatment serum CEA	8.87 (2-49.6)	5.4 (1.7-36.01)	0.527
Median post-treatment serum CEA	2.07 (1-8.7)	11 (4.65-22.5)	0.002
P value	0.001	0.06	

CEA: Carcinoembryonic antigen.

(4.65-22.5) ng/mL (P = 0.06). Since there was no statistically significant difference between responders and non-responders in baseline serum CEA, it cannot be taken as a predictor of response but post-treatment increase in CEA was associated with non-response or progression.

Pre-treatment and post-treatment ROC curves for the whole study population and luminal type breast cancer are given in Figure 1. We tried to find optimal pre-treatment cut-off for serum CEA in luminal breast cancer using ROC curve. The cut-off can be taken as 29.7 ng/mL as a predictor of tumour progression, with a sensitivity of 50% and specificity of 64%, but that cut-off was not statistically significant. ROC curve analysis for finding the cut-off for post-treatment CEA was also done. Post-treatment CEA for predicting the progression was taken as 2.16 ng/mL, with a sensitivity of 94.1% and specificity of 54.8%. For hormone positive tumours, post-treatment cut-off can be taken as 9.46 ng/mL with a sensitivity of 88.9% and specificity of 75.9% (P = 0.02). With a cut-off of 9.41, we found statistical significance in the whole group of patients (P = 0.006).

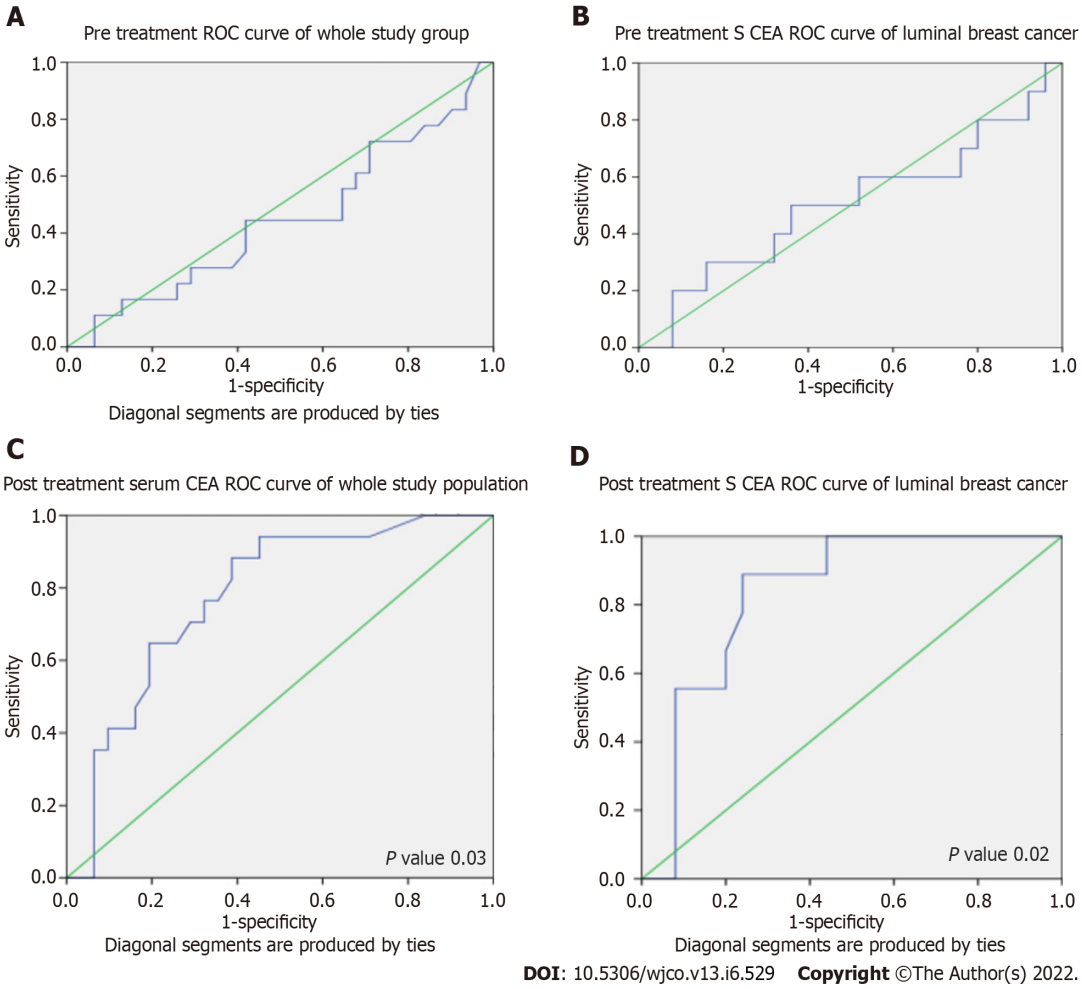
Serum CEA and luminal and non-luminal MBC

Table 3 shows serum CEA and response to treatment in responders and non-responders according to breast cancer type. Among responders, median pre-treatment CEA for luminal type was 14.7 (5.4-50.6) ng/mL and post-treatment CEA was 3.0 (1-10) ng/mL (P = 0. 001). Even in the luminal group, pre-

Table 3 Serum carcinoembryonic antigen and response to treatment in responders and non-responders according to breast cancer type

Classification		Responders			Non-responders		
		Median pre-CEA	Median post-CEA	*P* value	Median pre-CEA	Median post-CEA	*P* value
Hormonal classification	Luminal	14.7 (5.4-50.6)	3 (1-10)	0.001	22.39 (3.9-84.4)	21.00 (10.6-164.15)	0.26
	Non-luminal	1.85 (1-3.65)	1.25 (0.5-3)	0.046	4.15 (0.85-10.17)	5.65 (2.65-12.05)	0.161
Genomic classification	Luminal	14.7 (5.4-50.6)	3 (1-10)	0.001	22.39 (3.9-84.47)	20.67 (10.6-164.17)	0.260
	HER2	4 (1.2-4)	3.25 (0.5-3.25)	0.18	11.7	13	_
	TNBC	1.85 (0.74-2.4)	1.25 (0.67-1.88)	0.144	4 (0.5-5.6)	5.3 (2.2-9.2)	0.237

TNBC: Triple negative breast cancer; CEA: Carcinoembryonic antigen.



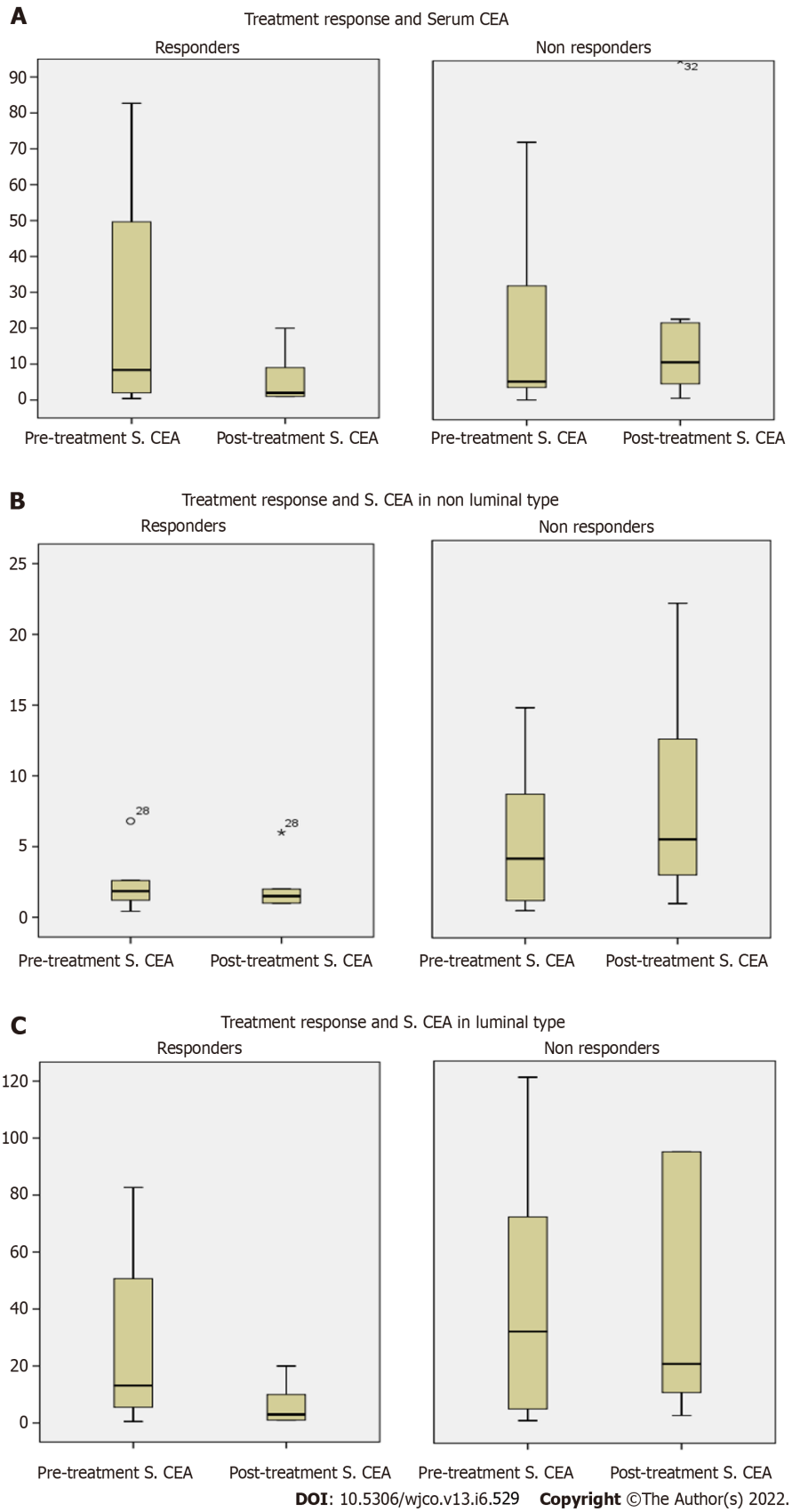
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Figure 1 Receiver operator characteristic curves. A: Pre-treatment receiver operator characteristic (ROC) curve for whole study population; B: Pre-treatment ROC curve for luminal type; C: Post-treatment ROC curve for whole study population; D: Post-treatment ROC curve for luminal type. ROC: Receiver operator characteristic; CEA: Carcinoembryonic antigen.

treatment serum CEA was not a predictor of response, but post-treatment CEA was a significant predictor of tumour progression (Figure 2).

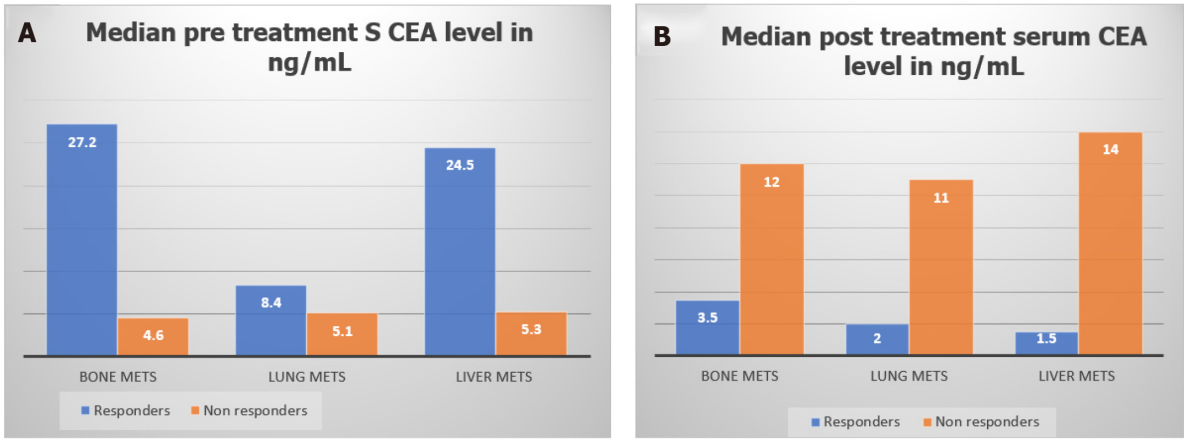
Association of serum CEA with various sites of MBC

Figure 3 shows median pre-treatment and post-treatment serum CEA levels in responders and non-responders according to site of metastasis. Among responders, median pre-treatment serum CEA levels of patients with bone metastases, lung metastases, and liver metastases were 27.2 ng/mL, 8.4 ng/mL, and 24.5 ng/mL respectively. Among non-responders, median post-treatment serum CEA levels of



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Figure 2 Treatment response. A: Association of treatment response with serum carcinoembryonic antigen (CEA) (pre-treatment and post-treatment) in whole study population; B: Association of treatment response with serum CEA in non-luminal type; C: Association of treatment response with serum CEA in luminal type. CEA: Carcinoembryonic antigen.



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Figure 3 Median pre-treatment and post-treatment serum carcinoembryonic antigen levels in responders and non-responders according to various sites of metastasis. A: Median pre-treatment serum carcinoembryonic antigen (CEA) level; B: Median post-treatment serum CEA level. CEA: Carcinoembryonic antigen.

patients with bone metastases, lung metastases, and liver metastases were 12 ng/mL, 11 ng/mL, and 14 ng/mL, respectively.

Table 4 shows serum CEA and response to treatment in bone, liver, and lung metastases**.** In patients with liver and lung metastases, post-treatment CEA level difference was not statistically significant in both responders and non-responders though the values were higher in non-responders.

In non-responders, comparing patients with or without bone metastases, the median post-treatment serum CEA of patients with bone metastases was 12 ng/dL whereas median post-treatment serum CEA in those without bone metastases was 10 ng/mL; post-treatment CEA level difference was statistically significant (P = 0.063). Among those with bone metastases, 69.5% had elevated post-treatment serum CEA, and only 37.5% had elevated serum CEA in those with no bone metastases (Figure 4).

DISCUSSION

The measurement of serum tumour marker levels could provide useful information for earlier detection of recurrence or accurate prediction of outcomes after recurrence in various cancers. They are more useful when patients have elevated level at baseline. The commonly studied tumour markers in breast cancer are CA15-3 and CEA. The significance of these markers remains unclear[17,18]. Even though the prognostic value of CA15-3 in breast cancer had been documented in some studies, serum CEA is less widely investigated as a prognostic factor than CA15-3 because of its poor sensitivity and specificity[18,19]. Elevated serum levels of CA15-3 and CEA preoperatively were significantly associated with tumour size, axillary node metastasis, and advanced stage[20-23]. A recent meta-analysis investigated the prognostic value of these two markers (serum CA15-3 and CEA) in 12993 breast cancer patients and indicated that elevated CA15-3 level significantly corresponded with poor disease-free survival and OS of breast cancer[23].

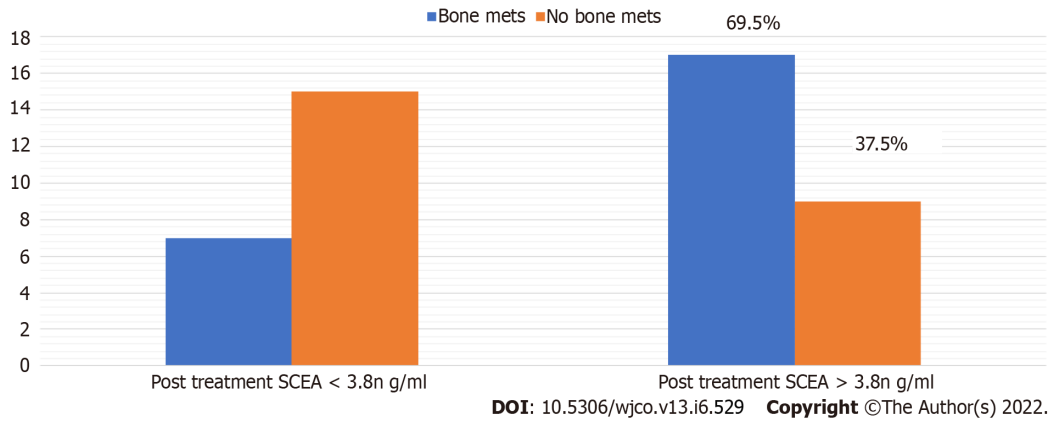
In our study, no clinically meaningful significance was seen in factors like menstrual status, grade of the tumour, number and sites of metastases, presence or absence of metastases, HER2 status, and TNBC status except luminal type. This finding was consistent with a study by Geng *et al*[23]. Elevated CEA levels were significantly associated with breast cancer molecular subtypes and luminal subtypes exhibited a higher percentage of elevated CEA levels compared to non-luminal subtypes. The reason for this differential expression of CEA is that the expression patterns of luminal, HER2 positive, and basal-like tumours are closely associated with their maturation and differentiation. Luminal subtypes have high expression of hormone receptor related genes, whereas HER2 positive or basal-like tumours have low expression of hormone receptor related genes, which explains the association between CEA elevation and luminal subtype. Our study showed that pre-treatment serum CEA cannot be taken as a predictor of response even in luminal subtype but post-treatment CEA was a significant predictor of tumour progression. Hence, we can conclude that monitoring CEA levels in luminal MBC at the end of treatment is a significant predictor of treatment response.

The correlation between tumour marker levels and various metastatic sites in MBC is poorly defined [24,25]. A study by Yerushalmi *et al*[26] identified that tumour marker elevation was documented in the majority of patients with MBC and luminal subtypes expressed more frequently compared with the non-luminal groups[26]. CEA elevation was not different between different sites of metastasis. Whereas in our study, in patients with liver and lung metastases, post-treatment CEA level difference was not

Table 4 Serum carcinoembryonic antigen and response to treatment in bone, liver, and lung metastases

Serum CEA (ng/mL)	Bone metastases	No bone metastases	*P* value
Median pre-treatment serum CEA	11.7 (2.9-48.4)	6.8 (2-32.3)	0.788
Median post-treatment serum CEA	9 (2-20)	2 (1-9)	0.063
	Liver metastases	**No liver metastases**	
Median pre-treatment serum CEA	11.7 (4.4-62.7)	6.8 (1.9-22.7)	0.244
Median post-treatment serum CEA	8 (1.2-19.75)	3 (1.25-11.5)	0.352
	Lung metastases	**No lung metastases**	
Median pre-treatment serum CEA	7.8 (1.9-31.3)	9.78 (5.15-66.64)	0.353
Median post-treatment serum CEA	3.5 (1-13.75)	5 (1.5-10)	0.93

CEA: Carcinoembryonic antigen.



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Figure 4 Association of post-treatment serum carcinoembryonic antigen with bone metastases. CEA: Carcinoembryonic antigen.

statistically significant in both responders and non-responders even though the values were higher in non-responders.

A study by Yazdani *et al*[27] showed that age, menopausal status, number of axillary lymph node metastases, tumor size, and ALP were identified as prognostic factors for bone metastasis in patients with breast cancer, whereas significantly persistent elevated post-treatment serum CEA levels were seen with bone metastases in our study[27]. Kosaka *et al*[28] proposed that in hormone receptor positive breast cancer, nodal metastasis and elevated serum CEA were associated with a poor prognosis and there was a significant rate of recurrence in those with high serum CEA levels compared with those with low levels of CEA[28]. Elevated serum levels of HER2, BCL2, CA15-3, and CEA in breast cancer patients are useful markers for predicting aggressive behaviour and relapse[29,30].

One major limitation of our study is the small sample size (50 patients) and it limits the predictive power of these markers and needs larger studies to confirm the findings.

CONCLUSION

Pretreatment serum CEA is elevated in luminal subtype. With treatment, responders have a significant fall in serum CEA level but it is clinically significant in luminal breast cancer type. Elevated post-treatment serum CEA levels are associated with disease progression and poor response to therapy. Persistently elevated post treatment serum CEA levels are associated with bone metastases. Elevated serum CEA and hormonal status are significant predictors of treatment response.

ARTICLE HIGHLIGHTS

Research background

In breast cancer patients, elevated serum carcinoembryonic antigen (CEA) levels are particularly noted in metastatic and recurrent disease and its significance in clinical practice is doubtful.

Research motivation

We aimed to estimate the serum CEA level in our metastatic breast cancer patients and correlate it with response to treatment and clinical outcome.

Research objectives

To evaluate the efficacy of serum CEA levels as a prognostic marker in metastatic breast cancer patients.

Research methods

This is a prospective clinical study of 50 patients with metastatic breast cancer treated at a breast clinic with newly diagnosed metastatic breast cancer planned for palliative chemotherapy, targeted therapy, and hormone therapy. We estimated the proportion of patients with elevated serum CEA levels at baseline and after palliative care, and investigated the association of serum CEA levels with known prognostic factors. Response to treatment was correlated with serum CEA levels in both responders and non-responders.

Research results

Pretreatment serum CEA was elevated in luminal subtype. With treatment, responders had a significant fall in serum CEA level but it was clinically significant in luminal breast cancer type. Metastatic breast cancer patients with bone metastases had significantly elevated post-treatment serum CEA levels after treatment.

Research conclusions

Based on our results, we suggest that serum CEA has potential clinical value in monitoring the treatment response of metastatic breast cancer patients, especially in patients with bone metastasis.

Research perspectives

Serum CEA as a tumour marker warrants further studies in metastatic breast cancer especially with bone metastases.

FOOTNOTES

Author contributions: Anoop TM designed the study, drafted the manuscript, and supervised the study and treatment; Joseph P R participated in the design and supervision of the study and treatment; Chacko S participated in the design of the study; Soman S was involved in data collection, analysis, and statistics; Mathew M participated in data collection; all authors read and approved the final manuscript.

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CASE REPORT

Epstein-Barr virus-associated smooth muscle tumors in immunocompromised patients: Six case reports

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Abstract

BACKGROUND

Epstein-Barr virus associated smooth muscle tumor (EBV-SMT) is a rare oncological entity. However, there is an increasing incidence of EBV-SMTs, as the frequency of organ transplantation and immunosuppression grows. EBV-SMT diagnosis relies on histopathology and immunochemical staining to distinguish it from post-transplant lymphoproliferative disorder (PTLD). There is no clear consensus on the treatment of EBV-SMTs. However, surgical resection, chemotherapy, radiation therapy, and immunosuppression reduction have been explored with varying degrees of success.

CASE SUMMARY

Our case series includes six cases of EBV-SMTs across different age groups, with different treatment modalities, adding to the limited existing literature on this rare tumor. The median latency time between immunosuppression and disease diagnosis is four years. EBV-SMTs present with variable degrees of aggressiveness and seem to have worse clinical outcomes in patients with tumor multiplicity and worse immunocompetency.

CONCLUSION
It is imperative to continue building on this knowledge and keeping EBV-SMTs on the differential in immunocompromised individuals.

Key Words: Epstein-Barr virus; Smooth muscle tumors; Human immunodeficiency virus; Epstein-Barr virus-associated smooth muscle tumors; Immunocompromised; Solid Organ Transplant; Orthotopic heart transplant; Orthotopic liver transplant; Living related kidney transplant; Post-transplant lymphoproliferative disorders; Case report

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Core Tip: Epstein-Barr virus associated smooth muscle tumor (EBV-SMT) is a rare oncological entity. Only a handful of case series have shed light on the presence of EBV-SMT in individuals, most of whom are immunocompromised. EBV-SMT should not be confused with post-transplant lymphoproliferative disorder. Histopathology should help guide the diagnosis.

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INTRODUCTION

Epstein-Barr virus associated smooth muscle tumor (EBV-SMT), first reported in 1970[1], is a rare oncological entity. Though EBV is present in 50%-89% of children and > 90% of adults worldwide, the virus often remains dormant until an individual becomes immunocompromised[2,3]. EBV is better known for other malignancies including nasopharyngeal carcinomas and lymphomas but in a minority of cases, it can trigger the proliferation of smooth muscle cells, resulting in mesenchymal tumors termed EBV-SMT.

There are three different types of EBV-SMTs identified to date: (1) Post-transplant associated smooth muscle tumors (PT-SMT); (2) Human immunodeficiency virus (HIV) associated smooth muscle tumors (HIV-SMT); and (3) Congenital immunodeficiency associated smooth muscle tumors (CI-SMT) such as in GATA2 and CARMIL2 deficiency[4]. EBV-SMTs can be encountered at any age, though it is more common in children[5,6]. EBV-SMTs can arise in any organ system, however, they are most common in the liver, followed by the lungs, central nervous system (CNS), adrenal glands, and gastrointestinal tract [7,8]. Unlike primary leiomyosarcomas where the histological grade is associated with disease severity, EBV-SMTs behave with variable severity, independent of their histological grade[9]. Clinical presentation is thus non-specific and depends on the location, size, and degree of organ involvement. EBV-SMTs can manifest either synchronously or metachronously in multiple organ systems and have a low propensity to metastasize[7,8].

PT-SMT can be confused with EBV associated post-transplant lymphoproliferative disorder (EBV-PTLD) as they are both caused by the same virus and occur in immunocompromised patients. Clinical presentation and radiographic findings cannot be used to tell the two entities apart. Instead, histopathology, immunohistochemistry, and EBV-encoded small RNA *in situ* hybridization (EBER ISH) are used to aid the diagnosis. Given its rarity, it is difficult to quantify the incidence of EBV-SMTs, however, it is estimated each subtype above may impact < 1%-5% of individuals. EBV-PTLD, on the other hand, is more prevalent than EBV-SMT and has an incidence ranging from 1%-20%[10] with mortality rates around 50%-90%[11,12].

Given how rare these tumors are, there is no standard treatment for EBV-SMTs which are instead treated on a case-by-case basis. Individuals with HIV-SMT are kept on appropriate antiretroviral treatment, and those with PT-SMT are treated with a reduction of immunosuppression. Surgical removal of tumors is considered when tumors impinge on the involved organ. Chemotherapy and radiation have also been utilized but without any obvious benefits on the disease course[6]. Allogeneic hematopoietic stem cell transplantation has also been used to treat CI-SMT with good outcomes[13,14].

Given their rarity, much of what we know about EBV-SMTs is through case reports and case series. However, as the frequency of organ transplantation grows with reliance on immunosuppression to prevent graft loss, it is pertinent to continue gathering information on EBV-SMTs. Many questions remain regarding incidence, prevalence, latency period, survival rates, and appropriate treatment

strategies, amongst other crucial facts. Here, we describe six cases of EBV-SMT at a quaternary academic referral center.

CASE PRESENTATION

Chief complaints

Case 1: Fever, thrush, diarrhea.

Case 2: Weight loss, headaches, and myalgias.

Case 3: Severe headaches and altered mental status.

Case 4: New-onset hematuria.

Case 5: Shortness of breath.

Case 6: Neck, back, and shoulder pain.

History of present illness

Case 1: A five-year-old female with a history of idiopathic dilated cardiomyopathy status post orthotopic heart transplant (OHT) at eight months old, on tacrolimus, and previous EBV viremia at age 22 months presented with her third infection-related hospitalization in the previous six months.

Case 2: A 20-year-old male with a history of Idiopathic dilated cardiomyopathy status post OHT at age 17 on tacrolimus and mycophenolate mofetil (MMF) presented to the hospital with weight loss, headaches, and myalgias.

Case 3: A 16-year-old female with a history of dilated cardiomyopathy status post OHT at 12-year-old, on MMF and tacrolimus presented to the hospital with severe headaches and altered mental status.

Case 4: A 61-year-old male with a pre-transplant negative cytomegalovirus (CMV) and EBV serology underwent orthotopic heart and liver transplant from a CMV and EBV positive donor (CMV Donor+/Recipient-, EBV Donor+/Recipient-) at the age of 58 years for cirrhosis secondary to Hereditary Familial Amyloidosis (Thr60A1a mutation) was admitted for new-onset hematuria and acute kidney injury.

Case 5: A 63-year-old male with a history of a living-related donor kidney transplant at age 55 for end-stage renal disease of unknown etiology was hospitalized for community-acquired pneumonia.

Case 6: A 45-year-old female with a long-standing history of HIV (CD 4 count unknown) on anti-retroviral therapy presented to the primary care clinic with neck, back, and shoulder pain with associated proximal and distal left upper extremity numbness and paresthesias.

History of past illness

Case 1, 2: Idiopathic dilated cardiomyopathy status post OHT.

Case 3: History of dilated cardiomyopathy status post OHT at 12-year-old.

Case 4: Status post Orthotopic heart and liver transplant from a CMV and EBV positive donor (CMV Donor+/Recipient-, EBV Donor+/Recipient-) for cirrhosis secondary to Hereditary Familial Amyloidosis (Thr60A1a mutation).

Case 5: History of a living-related donor kidney transplant for end-stage renal disease of unknown etiology.

Case 6: Long-standing history of HIV (CD 4 count unknown) on anti-retroviral therapy with dolutegravir, abacavir, and lamivudine

Personal and family history

Cases 1-6: No relevant family history.

Physical examination

Physical examination findings are not applicable.

Laboratory examinations

Laboratory findings are summarized in Table 1 for cases 1-6.

Table 1 Demographic characteristics and clinical summary of six Epstein-Barr virus associated smooth muscle tumor at a major quarternary care hospital

Cases	Sex	Type of transplant/IS	IS	Age at transplant (yr)	Age at diagnosis (yr)	EBV titer at diagnosis (copies per mL)	Tumor location	Histopathology findings	Treatment	Outcome (years post EBV-SMT)	Follow up
1	F	OHT	Tacrolimus	8 mo	6	286000	Colon	Smooth muscle spindle cell proliferation with strongly positive *in-situ* EBER hybridization and focal desmin+	Tacrolimus reduced	Alive (age 16)	None
2	M	OHT	Tacrolimus, MMF	17	20	161476	Thymus, lung, liver, mesenteric, and retroperitoneal lymph nodes, ascending colon, and proximal left femoral bone marrow	The spindled cells were positive with antibodies to smooth muscle antigen and desmin. Tumor cells were focally positive with antibodies to caldesmon. The spindle cells were negative with antibodies to S100 protein and cytokeratin AE1/3. *In situ* hybridization revealed that up to 80% of tumor cell nuclei were positive for the presence of EBER	Supportive	Death (age 20)	NA
3	F	OHT	Tacrolimus; Sirolimus	12	16	11848	Brain, lung, kidney	Hepatic parenchyma focally replaced by spindle cell proliferation. The spindle cells are arranged in a haphazard pattern and contain nuclei with minimal nuclear pleomorphism and abundant eosinophilic cytoplasm. (1) The tumor cells are strongly positive for h-caldesmon and smooth muscle actin; (2) Desmin+; (3) EBER positive by CISH; (4) CD20+, MUM-1+. CD3-, CD10-. EBER ISH+ (CNS); and (5) H-caldesmon+, smooth muscle actin+, desmin+ (focal). EBER ISH+ (liver)	Intrathecal rituximab, methotrexate, systemic chemotherapy, whole brain radiation, and T-cell therapy	Alive (age 22); course c/b seizures	Yearly brain and liver MRI
4	M	OHT/OLT	Tacrolimus	58	61	25458	Kidney, liver, lung	(1) Monotonous proliferative of relatively uniform spindle cells arranged in intersecting fascicles with pale eosinophilic cytoplasm and elongated, blunt-ended nuclei with dark vesicular chromatin.; (2) Mitotic figures are not readily identified and there is no significant cytologic atypia, pleomorphism, or necrosis; (3) The tumor cells are strongly immunoreactive for SMA and negative for CD117, DOG-1, desmin, S100, SOX10, CD34, and STAT6; and (4) EBER positive by CISH	L lateral segmentectomy w superficial wedge resection;Tacrolimus reduced, stopped, and eventually started given concern for ACR	Death (age 61)	NA
5	M	LRD kidney	Prednisone, MMF, Sirolimus	55	63	4765	Liver	(1) Fascicles of malignant appearing spindle cells diffusely positive for desmin and H-caldesmon; and (2) EBER positive by CISH	MMF. Sirolimus dosing decreased	Alive (age 73)	Repeat MRI with stable/decreased size hepatic lesions; no new lesions noticed
6	F	HIV	Abacavir-Lamivudine-	NA	45	NA	Thoracic spine	(1) Spindle cell neoplasm staining positive for smooth muscle actin; (2) Negative for desmin;	s/p T8-T10 Laminectomy; no change in HAART	Alive (age 55); total hip	None

	Dolutegravir for HIV	and (3) EBV positive by CISH	arthroplasty for bl avascular necrosis

IS: Immunosuppressant; EBV: Epstein-Barr virus; EBER: EBV-encoded small RNA; MRI: Magnetic resonance imaging; F: Female; M: Male; OHT: Orthotopic heart transplant; MMF: Mycophenolate mofetil; NA: Not available; OHT/OLT: Combined orthotopic heart and liver transplant; LRD: Living related donor; HIV: Human immunodeficiency virus.

Imaging examinations

Case 1: Unrevealing total body computed tomography (CT) scan. EGD and colonoscopy with 6-10 mm polypoid lesions with central ulceration in the sigmoid colon and rectum. Biopsy of lesions confirmed EBV-SMT (Figures 1 and 2).

Case 2: Neck magnetic resonance imaging (MRI) revealed a mass in the right Meckel's cave. Liver biopsy confirmed EBV-SMT (Figure 3). PET scan showed multiple fluorodeoxyglucose avid foci (Figure 4).

Case 3: MRI brain showed a left temporal ring-enhancing lesion (Figure 5). Whole body PET scan showed right hepatic mass (Figure 6).

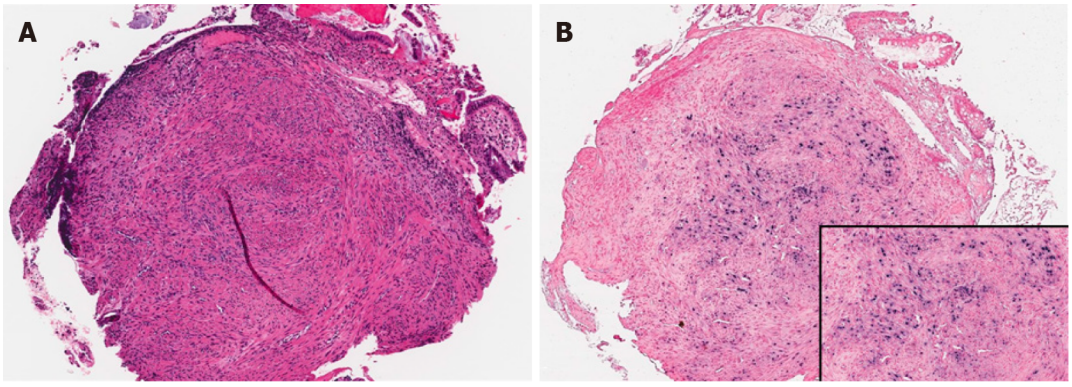
Case 4: CT scan of the liver revealed multiple indeterminate liver masses (Figure 7).

Case 5: MRI imaging of the abdomen showed over 20 cystic hepatic masses, with the largest measuring 12.5 cm x 9.2 cm, resolved on subsequent imaging (Figure 8).

Case 6: Cervical and thoracic spine MRI which showed a 1.9 cm x 1.2 cm x 2.4 cm intradural heterogeneous mass centered within the left T9-T10 neuroforamina, with abutment of the left lateral spinal cord resulting in rightward cord displacement as well as moderate canal stenosis (Figure 9).

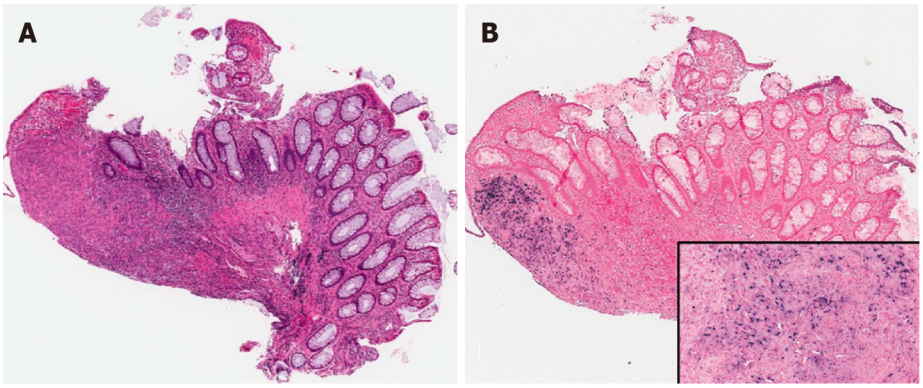
Case series details

Case 1: A five-year-old female with a history of idiopathic dilated cardiomyopathy status post orthotopic heart transplant (OHT) at eight months old, on tacrolimus, and previous EBV viremia at age 22 mo presented with her third infection-related hospitalization in the previous six months. Presenting symptoms included fever, thrush, diarrhea, and neutropenia with an absolute neutrophil count of 260 cells per μL. She was placed on broad-spectrum antibiotics and antifungals but continued to have diarrhea, failure to thrive, as well as new oral ulcers. Due to concern for PTLD, she underwent total body CT which was unrevealing. She also underwent esophagogastroduodenoscopy and colonoscopy. In addition to the presence of colonic candidiasis, colonoscopy revealed 6-10 mm polypoid lesions with central ulceration in the sigmoid colon and rectum with biopsies consistent with an EBV-driven smooth muscle tumor (Figures 1 and 2). The patient was EBV negative before transplant with repeat EBV titers high on admission (Table 1). She was treated for colonic candidiasis as well as a pseudallescheria boydii complex infection of her lungs with symptomatic improvement. She did not undergo any EBV-SMT-directed treatment or surveillance and remains well at age 16 years.



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Figure 1 Epstein-Barr virus associated smooth muscle tumor in the sigmoid colon in case 1. A: Proliferation of smooth muscle cells undermining and distorting the colonic mucosa (HE stain, 70 x); B: Epstein-Barr virus-encoded small RNA *in situ* hybridization is positive within the smooth muscle cell population (70 x, inset box 200 x).

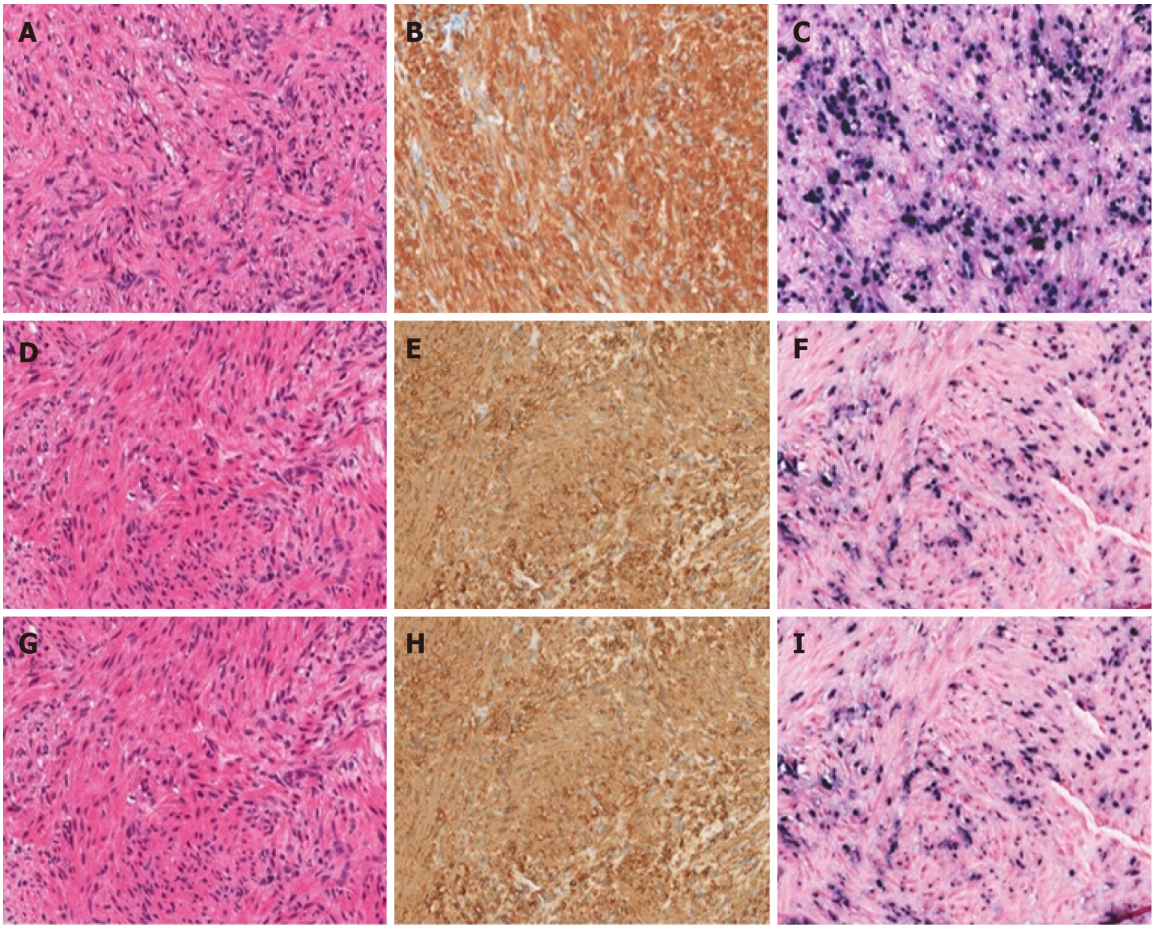


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Figure 2 Epstein-Barr virus associated smooth muscle tumor in the rectum in case 1. A: Proliferation of smooth muscle cells undermining the rectal mucosa (HE stain, 40 x); B: Epstein-Barr virus-encoded small RNA *in situ* hybridization is positive within the smooth muscle cell population (40 x, inset box 200 x).

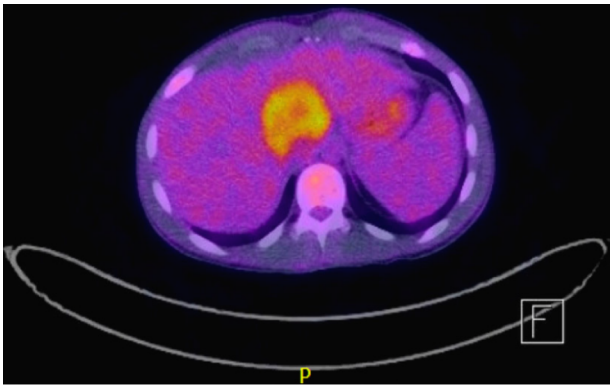
Case 2: A 20-year-old male with a history of idiopathic dilated cardiomyopathy status post OHT at age 17 on tacrolimus and mycophenolate mofetil (MMF), Crohn's disease, alopecia, juvenile idiopathic arthritis, and common variable immune deficiency (CVID) presented to the hospital with weight loss, headaches, and myalgias. The patient had abruptly stopped his immunosuppression agents a month before admission. He was treated with antibiotics for Group A streptococcus infection. The patient was previously EBV seronegative before transplant but had elevated titers on admission (Table 1). A whole-body positron emission tomography (PET) scan showed multiple fluorodeoxyglucose (FDG) avid foci suspicious for widespread PTLD in the thymus, lung, liver, mesenteric lymph nodes, retroperitoneal lymph nodes, ascending colon, and proximal left femoral bone marrow (Figure 4). A neck magnetic resonance image (MRI) revealed a mass in the right Meckel's cave. The patient's tacrolimus was deceased due to suspicion for PTLD. Biopsies taken during colonoscopy and bone marrow biopsies were negative for PTLD. A liver biopsy was performed and showed a proliferation of bland spindle cells with pale eosinophilic cytoplasm and ovoid nuclei with smooth contours and pale chromatin (Figure 3A). Immunohistochemical stain for smooth muscle actin showed strong, diffuse reactivity in the neoplastic cells (Figure 3B). EBER highlighted the presence of viral genetic material, confirming the diagnosis of PT-SMT (Figure 3C). During this admission, he became febrile prompting a chest X-ray which revealed left upper lobe haziness, concerning for pneumonia. He rapidly deteriorated, developing acute renal failure requiring dialysis and respiratory distress with encephalopathy requiring intubation. He then sustained a fatal cardiac arrest. An autopsy revealed severe acute cellular rejection, negative for antibody-mediated rejection, and confirmed multisite PT-SMT.

Case 3: A 16-year-old female with a history of dilated cardiomyopathy status post OHT at 12-year-old, on MMF and tacrolimus presented to the hospital with severe headaches and altered mental status. MRI brain showed a left temporal ring-enhancing lesion (Figure 5). The patient was previously EBV seronegative before transplant but had elevated titers on admission (Table 1). She underwent a brain



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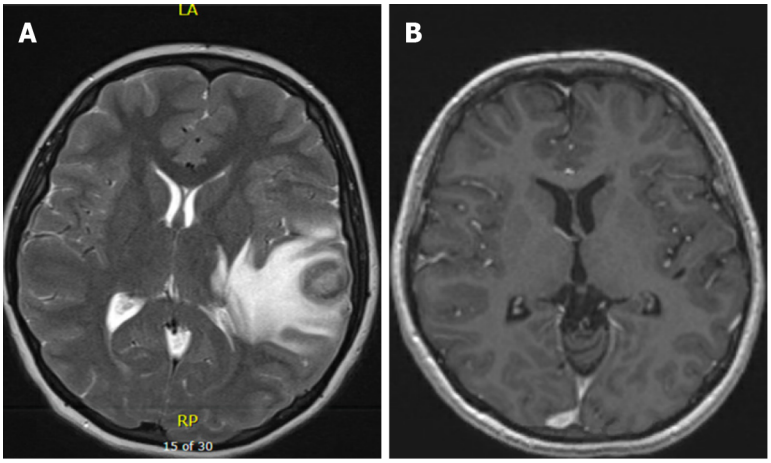
Figure 3 Epstein-Barr virus associated smooth muscle tumor liver biopsies for cases 2-4 (HE stain, 200 x). Hematoxylin and eosin-stained tissue sections showed fascicles of well-differentiated spindle cells with pale eosinophilic cytoplasm and blunt-ended, ovoid nuclei with smooth nuclear contours. A, D, G: No significant cytologic atypia or nuclear pleomorphism is appreciated; B, E, H: All three cases show strong, diffuse reactivity for smooth muscle actin, confirming smooth muscle differentiation, confirming smooth muscle tumor lineage; C, F, I: Chromogenic *in situ* hybridization studies for the Epstein-Barr virus-encoded small RNA confirm the presence of viral genetic material in all three cases.



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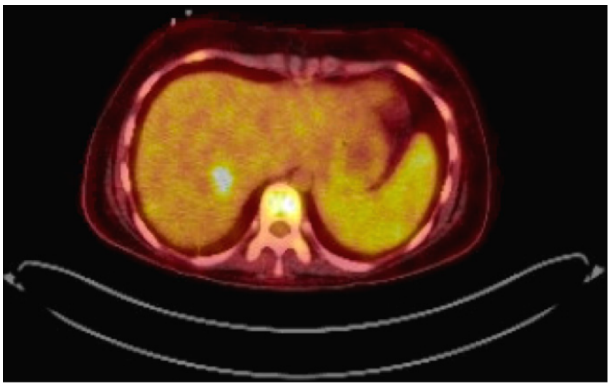
Figure 4 Positron emission tomography scan showing a hypermetabolic mass arising from the medial segment of the left liver lobe, measuring about 5.1 cm x 4.7 cm in the axial and anteroposterior dimension and 6.9 cm in the craniocaudal dimension in case 2.

biopsy which showed EBV-PTLD, with morphology consistent with diffuse large B-cell lymphoma. Her immunosuppression therapy was reduced. She underwent a CT abdomen which showed a 2 cm low-attenuating hypervascular lesion in the right hepatic dome and another 0.6 cm lesion in the right anterior inferior hepatic lobe, redemonstrated on a PET scan (Figure 6). Liver biopsy showed a proliferation of spindle cells with eosinophilic cytoplasm, mild to moderate nuclear pleomorphism, and focal



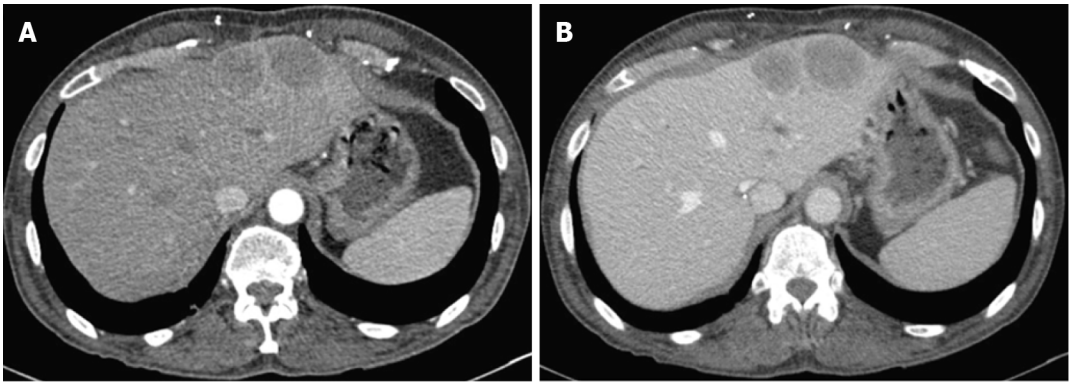
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Figure 5 Magnetic resonance imaging brain. A: T2 weighted turbo spin echo magnetic resonance images of the brain showing ring-enhancing lesion in the left temporal region in case 3; B: T1 FI3d magnetic resonance images of the brain showing resolution of ring-enhancing lesion six years post-treatment in case 3.



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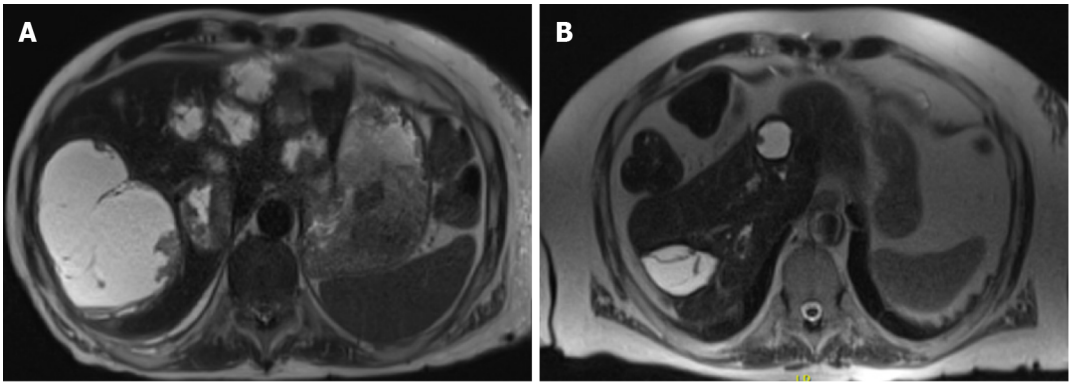
Figure 6 Positron emission tomography scan showing a hypermetabolic right hepatic mass in case 3.



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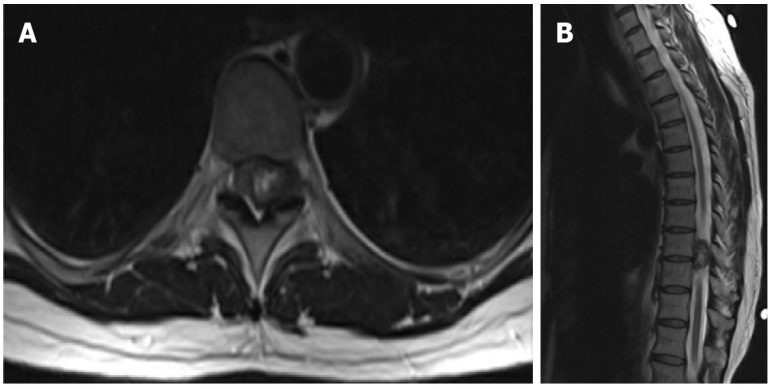
Figure 7 Computed tomography scan. A: The liver with intravenous contrast in arterial phase showing multiple hepatic lesions in case 4; B: The liver in venous phase showing multiple hepatic lesions in case 4.

tumor necrosis (Figure 3D). No severe nuclear pleomorphism or mitotic activity was identified. The neoplastic cells were strongly immunoreactive for smooth muscle actin, and showed diffuse nuclear reactivity for EBER, confirming the diagnosis of PT-SMT (Figure 3E and F). A subsequent CT scan of the abdomen and pelvis showed two nonspecific small low density lesions in the left kidney, three months after the initial diagnosis of PTLD. For her CNS-PTLD, the patient had a ventriculoperitoneal shunt



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Figure 8 Axial HASTE sequence abdominal magnetic resonance imaging. A: demonstrating numerous cystic appearing hepatic Epstein-Barr virus associated smooth muscle tumors, including the largest lesion in segment VII in case 5; B: Demonstrating stable/deceased hepatic lesions with no new lesions in case 5.



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Figure 9 Axial (A) and sagittal (B) cuts of a T2 weighted magnetic resonance imaging of the thoracic spine demonstrating an Epstein-Barr virus associated smooth muscle tumor centered in the left T9/T10 neuroforamina with a rightward displacement of the spinal cord in case 6.

placed and underwent six cycles of intrathecal rituximab with methotrexate, systemic chemotherapy, whole-brain radiation, and T-cell therapy with subsequent decrease and ultimate resolution in the CNS lesion on subsequent imaging (Figure 5). Her cerebrospinal fluid studies were negative for infection. She started having generalized tonic-clonic seizures in the post-treatment setting and was treated with anti-epileptics. She remains well at the age of 22 years, with yearly liver MRI showing stable hepatic lesions (Figure 6).

Case 4: A 61-year-old male with a pre-transplant negative CMV and EBV serology underwent orthotopic heart and liver transplant from a CMV and EBV positive donor (CMV Donor+/Recipient-, EBV Donor+/Recipient-) at the age of 58 years for cirrhosis secondary to Hereditary Familial Amyloidosis (Thr60A1a mutation). He was on tacrolimus for immunosuppression and post-transplant developed asymptomatic EBV and CMV viremia as well as stage 3 chronic kidney disease secondary to calcineurin inhibitor nephrotoxicity. He was admitted for new-onset hematuria and acute kidney injury. A kidney ultrasound noted a 2 cm hypoechoic lesion in the right inferior liver lobe. A CT scan of the liver revealed multiple indeterminate liver masses (Figure 7A and B). Biopsy of one lesion showed a monotonous proliferation of relatively uniform spindle cells arranged in intersecting fascicles with pale eosinophilic cytoplasm and elongated, blunt-ended nuclei with darkly staining vesicular chromatin (Figure 3G). There was again, no significant cytologic atypia, nuclear pleomorphism, or tumor necrosis. The tumor cells were strongly immunoreactive for smooth muscle actin and showed diffuse nuclear reactivity for EBER (Figure 3H and I). These morphologic and immunohistochemical findings were consistent with a diagnosis of PT-SMT. He subsequently underwent a left lateral liver segmentectomy with additional superficial hepatic wedge resections in segments 4A, 4B, and 8. Pathology showed positive margins. Immunosuppression was initially lowered and eventually discontinued. He was later found to have elevated liver enzymes on follow-up testing and a liver biopsy confirmed severe acute

cellular rejection (ACR). He was treated with steroids and restarted on sirolimus and tacrolimus with resolution of ACR as proven on subsequent biopsy and improvement of liver enzymes on laboratory testing. He also completed a course of valganciclovir for CMV viremia. However, he developed worsening neutropenia with bone marrow suppression, low-grade fevers, altered mental status requiring intubation, and anuria requiring dialysis. He was empirically started on broad-spectrum antibiotics. Infectious workup demonstrated streptococcus Gordonii bacteremia. Despite optimal treatment and supportive measures, his condition deteriorated rapidly and ended up having multi-organ failure, followed by a fatal cardiac arrest.

Case 5: A 63-year-old male with a history of a living-related donor kidney transplant at age 55 for end-stage renal disease of unknown etiology, was maintained on prednisone, MMF, and sirolimus. The patient was hospitalized for community-acquired pneumonia complicated by a parapneumonic effusion necessitating antibiotics and decortication. On CT and subsequent MRI imaging of the abdomen, he was incidentally found to have over 20 cystic hepatic masses, with the largest measuring 12.5 cm x 9.2 cm (Figure 8A). Fine needle aspiration confirmed PT-SMT. EBV DNA quantification was at 4,765 copies per milliliter with no prior pre-transplant levels. He had no evidence of distant or intra-cranial disease on imaging. MMF was stopped and sirolimus was decreased from 1.5 mg to 1 mg daily initially, and down to 0.5 mg one year later. He was also treated with a course of valganciclovir at the time of diagnosis. Annual hepatic MRIs demonstrated initial size reduction followed by stable disease without any new lesions, with the most recent imaging performed at age 72 (Figure 8B). He continues to do well with intact kidney function, despite decreasing his immunosuppression.

Case 6: A 45-year-old female with a long-standing history of HIV (CD 4 count unknown) on anti-retroviral therapy with dolutegravir, abacavir, and lamivudine presented to the primary care clinic with neck, back, and shoulder pain with associated proximal and distal left upper extremity numbness and paresthesia. With worsening symptoms after conservative therapy, she underwent cervical and thoracic spine MRI which showed a 1.9 cm x 1.2 cm x 2.4 cm intradural heterogeneous mass centered within the left T9-T10 neuroforamina, with abutment of the left lateral spinal cord resulting in rightward cord displacement as well as moderate canal stenosis (Figure 9). She underwent T8-T10 Laminectomy with mass excision, with pathology consistent with EBV-SMT. Subsequent PET revealed no FDG avid lesions. Serum EBV viral levels were not obtained and prior seronegative status was also unknown. She did not undergo any further treatment or surveillance and has not had any complications of her disease, now at age 55.

FINAL DIAGNOSIS

EBV-SMT in all six cases.

TREATMENT

Case 1

Immunosuppression reduction.

Case 2

Supportive treatment.

Case 3

Intrathecal rituximab, methotrexate, systemic chemotherapy, whole brain radiation, and T-cell therapy.

Case 4

Left lateral segmentectomy with superficial wedge resection; reduction in immunosuppression, and eventually started given concern for ACR.

Case 5

Reduction in immunosuppression.

Case 6

T8-T10 Laminectomy; no change in HAART regimen.

OUTCOME AND FOLLOW-UP

Cases 2 and 4 died. Cases 1, 3, 5, 6 alive and well with no recurrence in EBV-SMT.

DISCUSSION

Here we report six cases of EBV-SMT, two of which were in pediatric patients with ages ranging from 6-61. There was an equal number of males and females in the cohort. Five out of six cases were PT-SMT while the sixth patient had HIV-SMT. One patient had CVID in addition to being a transplant recipient. Two patients died at the time of diagnosis, though neither death was attributed directly to the SMTs. Three patients in the group had single organ involvement while the rest had multiple organs involved. Two patients with CNS involvement (one with EBV-PTLD and another with HIV-SMT) underwent surgical removal of the tumor without recurrence. Interestingly, one patient in this cohort had both biopsy-proven PTLD and EBV-SMT.

A systemic review by Chen *et al*[15] on EBV-SMT with CNS invasion found that HIV-SMTs have a predilection for the central nervous system. This was seen in our patient with HIV-SMT whose EBV-SMT was found in the thoracic spine. In contrast, patients with PT-SMT have a propensity for extra-CNS involvement, primarily lung and liver as confirmed in a study by Jonigk *et al*[7]. The pathophysiology is unclear but it has been proposed these organ systems are hypervascular and may attract the proliferation of smooth muscle tumors. This is also seen in other lymphoproliferative disorders such as PTLD and non-Hodgkin lymphomas in patients with Acquired Immunodeficiency Syndrome (AIDS). Interestingly, Chen *et al*[15] notice a concomitant lung and liver involvement in patients with PT-SMT. This was true for two of our patients who had both lung and liver SMTs; one patient with solitary lung involvement. This raises the question of whether a liver lesion increases the chance of getting a lung lesion and not vice versa. Regardless, concomitant lung and liver lesions should be kept in mind during workup.

The latency period between either HIV infection or immunosuppression initiation and the occurrence of EBV-SMT is variable. In PT-SMT patients, previous studies have found an average latency period of three years in children compared to four years in adults[7,15]. The latency period in our patients ranged from three to eight years. For HIV-SMT patients, it was more difficult to determine the timeline between HIV infection and diagnosis of AIDS. However, latency time could be as high as 8.5 years[16,17]. For our patient with HIV, we were unable to determine this latency period. In contrast, PTLD can develop at any point after transplant, up to 10 years later, whereby a majority of cases occur within the first year post transplantation[13,14].

Additionally, multiple cases of synchronous or metachronous EBV-SMTs were seen in our patients, consistent with prior publications[15,18,19]. Jonigk *et al*[7] showed that patients with multiple organs involvement had worse overall survival than those with single organ involvement, while individuals with intracranial disease had the worst outcomes. However, in the study by Chen *et al*[15], presence of CNS SMTs, tumor multiplicity, or pre-existing medical conditions did not impact the survival rate. In our case series, one-third of our patients died and had tumor multiplicity in addition to a multitude of chronic medical conditions. These patients did not die directly due to EBV-SMTs but died of the disease while battling other complications. Age differences did not impact survival, raising the question whether age has any role in prognosis. However, it does supplement the theory that the degree of immunocompetency may determine the degree of disease aggressiveness and subsequent survival rates.

EBV-SMTs have been generally thought of as slow-growing tumors with a 1-year overall survival rate of 50%-76% for patients with HIV-SMT and PT-SMT patients and 0% for CI-SMT[8,15]. 5-year survival rate is estimated at 60% for patients with CNS involvement[15]. Patients with HIV-SMTs have been known to have higher survival but with a shorter follow-up period. A separate analysis by Jonigk *et al*[7] suggested that PT-SMT and CI-SMT have better outcomes than HIV-SMT. Four out of six patients in our group continue to live with stable disease or no evidence of disease. It will be prudent to continue follow-up for all EBV-SMTs to help us study the disease course and prognosis over time.

Previous studies have found that pre-transplant EBV seronegativity is a risk factor for EBV-PTLD[20]. However, the role of this factor is not known for EBV-SMT, though it is postulated that pre-transplant seronegativity and post-transplant primary EBV infection could be considered a risk factor for PT-SMT[21]. In our study, EBV status was not known for HIV patient, however, the other transplant patients were seronegative at the time of transplant and became highly seropositive at the time of diagnosis (table 1). It would be interesting to see if any absolute levels of EBV titers have any bearing on the severity of disease course and outcomes.

There is no standardized treatment for EBV-SMT, given its rarity. In the study by Jonigk *et al*[7], patients who underwent surgical resection had similar outcomes to those who underwent reduced immunosuppression alone without any surgery, suggesting that either may be an appropriate strategy. Most patients with CNS involvement undergo surgical resection to alleviate parenchymal tumor compression[15]. No statistically significant difference was seen in outcomes between PT-SMT and HIV-SMT in these patients. Surgical resection to alleviate parenchymal tumor compression in individuals

with CNS involvement is a reasonable strategy.

CONCLUSION

EBV-SMTs are a rare oncological entity found in immunocompromised patients with either primary immunodeficiency as in Congenital Immunodeficiency or secondary immunodeficiency as seen in patients with HIV or post-transplant patients on long term immunosuppression. As the number of patients who undergo organ transplantation increases with time, the incidence of EBV-SMT may also increase. It is imperative to keep EBV-SMT on the differential in immunosuppressed individuals who develop tumors. Questions regarding the best treatment modality remain as patients are treated on a case-by-case basis.

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FOOTNOTES

Author contributions: Khan AA wrote the manuscript; Bassam BN and Esfeh JM conceptualized and proofread the paper; Yalamanchali A and Niang D contributed to the paper; Savage E, Fulmer CG, and Gosnell HL provided input on pathology including preparing slides; and All authors have read and approved the final manuscript.

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REVIEW

Nanomedicine approaches for treatment of hematologic and oncologic malignancies

Polyxeni Nteli, Danae Efremia Bajwa, Dimitrios Politakis, Charalampos Michalopoulos, Anastasia Kefala-Narin, Efstathios P Efstathopoulos, Maria Gazouli

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Abstract

Cancer is a leading cause of death worldwide. Nowadays, the therapies are inadequate and spur demand for improved technologies. Rapid growth in nanotechnology and novel nanomedicine products represents an opportunity to achieve sophisticated targeting strategies and multi-functionality. Nanomedicine is increasingly used to develop new cancer diagnosis and treatment methods since this technology can modulate the biodistribution and the target site accumulation of chemotherapeutic drugs, thereby reducing their toxicity. Cancer nanotechnology and cancer immunotherapy are two parallel themes that have emerged over the last few decades while searching for a cure for cancer. Immunotherapy is revolutionizing cancer treatment, as it can achieve unprecedented responses in advanced-stage patients, including complete cures and long-term survival. A deeper understanding of the human immune system allows the establishment of combination regimens in which immunotherapy is combined with other treatment modalities (as in the case of the nanodrug Ferumoxytol). Furthermore, the combination of gene therapy approaches with nanotechnology that aims to silence or express cancer-relevant genes *via* one-time treatment is gradually progressing from bench to bedside. The most common example includes lipid-based nanoparticles that target VEGF-A and KRAS pathways. This review focuses on nanoparticle-based platforms utilized in recent advances aiming to increase the efficacy of currently available cancer therapies. The insights provided and the evidence obtained in this paper indicate a bright future ahead for immuno-oncology applications of engineering nanomedicines.

Key Words: Nanomedicine; Cancer; Immunotherapy; Gene; Cell therapy

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Core Tip: Despite many years of fundamental and clinical examination and preliminaries of promising new treatments, cancer stays a significant reason for dreariness and mortality. Ongoing investigations propose that nanomedicine gives benefits over conventional treatments for cancer therapy. Immunotherapeutic strategies, such as cancer vaccines, immunomodulatory agents, immune checkpoint inhibitors, natural killer cells, peptides, nucleic acids, and chimeric antigen receptor T-cells, have augmented the development of this treatment either by stimulating cells or blocking the so-called immune checkpoint pathways. The efficacy of nanomedicine treatments and the examination of the advancement in the synergistic plan of immune-targeting combination therapies reviewed in this manuscript have been validated in clinical trials. The field of nanomedicine, therefore, generates new approaches regarding oncologic malignancies.

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INTRODUCTION

Cancer refers to a diverse group of more than 100 different diseases that exhibit a highly complex and multifactorial profile and together serve as one of the leading causes of death worldwide, accounting for nearly 10 million deaths in 2020 alone[1]. The global prevalence of cancer was estimated to rise from 17 million cases annually in 2018 to 27.5 million in 2040[2]. Although the etiology of each type is hugely varying and the clinical manifestations quite heterogeneous, aberrated cellular and tissue regulation is among the trademarks of cancer[3]. Fundamentally, each type results in the accumulation of genetic and epigenetic alterations that dysregulate the cell cycle and promote abnormal cell growth[4].

Collectively, these alterations impair cellular control mechanisms and the responsible regulatory signaling pathways and drive the transformation of normal cells into malignant cells. The malignant cells acquire new biological abilities, referred to as the hallmarks of cancer[5], a set of distinct features including sustained proliferation, evasion of growth suppression and cell death, altered response to metabolic and stress cues, vascularization, invasion and metastasis, and immune modulation. Various genes have been implicated in carcinogenesis, from activated oncogenes and anti-apoptotic genes to inactivated tumor suppressor genes[6,7]. Large-scale genomic analysis and functional studies have facilitated the identification of distinct mutations in different tumor types, allowing the development of valuable diagnostic biomarkers[8] and the stratification of patients towards more personalized therapeutic approaches[9].

The field of oncology focuses on the prevention, diagnosis, and treatment of cancer while implementing various strategies and tools for clinical application. Conventional cancer therapeutics, including chemotherapy, radiation therapy, and surgery, are widely accepted and used; however, they suffer from various drawbacks due to the lack of tumor-specific targeting, dosing, pharmacokinetic limitations, and severe complications[10]. Concurrently, a tremendous body of research work has been generated over the last decades to advance on the one hand, the understanding of the complicated events governing tumorigenesis[11] and, on the other hand, to develop early diagnostic and efficient therapeutic approaches[12]. Towards that goal, a promising branch of biomedicine, nanomedicine, aims to boost these current cancer management strategies. Nanomedicine can be defined as the use of nanomaterials (materials with at least one dimension ranging from 1-100 nm) for the prevention, diagnosis, and ultimately, treatment of diseases[13].

Nanomedicine, the application of nanotechnology in medical practice, aims to bridge the gap between different scientific principles such as physics, chemistry, pharmaceutics, and biology to harness nanotechnology's knowledge and tools with the goal of serving medicine. The unique phenomena that govern the nanoscale enable novel medical applications and are responsible for the exceptional properties that make nanomaterials excellent candidates for therapeutic applications[14]. Despite their minuscule size, nanoparticles hold great potential as drug delivery systems for cancer treatment, and tremendous research has taken place in the last decades to bring this technology from bench to bedside [15].

Nanoparticles as drug carriers have proven to be an effective tool in the fight against cancer[16]. The improved selectivity afforded by these nanocarriers resulted in a significant increase in the efficacy of the carried medicine, while side effects in the host were minimized. It is also feasible to include

targeting moieties specific for cell organelles, which boosts the efficacy of the transported medicines even more[17]. Nanoscale platforms come in various sizes, geometries, materials, and targeting moieties, allowing them to target organs, tissues, and individual cells[18]. Because of their distinct benefits, nanomedicines have emerged as a viable alternative to viral vectors, including low toxicity and immunogenicity, sustained and controlled release features, scale-up capacity, and low-cost manufacturing[17].

This review thus focuses on nanoparticle-based platforms utilized in recent advances aimed to increase the efficacy of currently available cancer therapies.

TYPES OF CANCER NANOMEDICINES

Immunomodulatory agents

Recently, some of the alternate approaches to treat cancer are based on immunomodulation which employs the host's own natural defense mechanisms to recognize and selectively eliminate the cancer cells by inducing the immune system[19]. Nanomaterial-mediated immunomodulation can be achieved either directly or indirectly[20]. To the first group belong nanomaterials that act as vaccine adjuvants, as several systems have been reported to improve antigenicity of conjugated weak antigens, while engineered nanosystems have also shown inherent antigenic properties[21]. Recent studies have highlighted the inherent tendency of liposomes to interact extensively with the immune system leading to several immunomodulatory effects, concerning tumor growth[22]. More specifically, circulating proteins are rapidly integrated to the surface of liposomes, forming a protein corona which can function as the interface for biological interactions and contributes to the formation of immune complexes and immunogenic epitope generation from self-antigens, ultimately resulting in the activation or suppression of immune responses[22]. Moreover, increasing evidence is emerging that indicates the functional ability of nanoparticles to polarize macrophages[23]. On the other hand, the multicomponent cargo capacity of delivering immunomodulatory agents in a targeted manner enables their function as delivery platforms that bolster the immune response.

Nanomaterials used in the combating of the immune evasion strategies of cancer operate in three different approaches that include the immunogenic targeting of cancer cells, the reshaping of the tumor's immune microenvironment, and the stimulation of the peripheral immune network[24].

When targeting cancer cells, nanomedicines typically aim to induce immunogenic cell death (ICD), thereby triggering an immunogenic cascade that leads to an antigen-specific immune response against a broad spectrum of solid tumors. It is now established that ablative cancer treatments, such as radiotherapy, photodynamic therapy, hyperthermia, and photothermal therapy, as well as certain chemotherapeutics can cause tumor cell death[11].

In the context of tumor immune microenvironment (TIME), nanomaterials can be used to modulate the immunosuppressive tumor microenvironment by targeting tumor-associated macrophages (TAMs), regulatory T cells (Treg cells), regulatory B cells, myeloid-derived suppressor cells (MDSCs), as well as cancer-associated fibroblasts. Several nanoparticle-based strategies that target TAMs for suppressing tumor progression include TAM depletion, inhibiting monocyte recruitment, and TAM reprogramming [25]. Recent studies showed that the utilization of dendrimer nanoparticles carrying the chemotherapeutic methotrexate that specifically recognize the folate receptor-2, which is overexpressed in TAMs, increases therapeutic efficacy by depleting TAMs[25]. Considering monocyte recruitment, it is reported that silver nanoparticles have an adjuvant effect inducing recruitment and activation of local macrophages[26]. As for the reprogramming of macrophages, there have been attempts for creating an albumin-derived nanoplatform that delivers both the disulfiram/copper complex and macrophage modulator regorafenib for reprogramming macrophage[27]. In the context of down-regulating Treg cells, a common strategy is the use of checkpoint blockade antibodies (anticytotoxic T lymphocyte-associated protein 4)[25]. A modulating strategy for abnormal MDSC differentiation has been introduced, using lipid-coated biodegradable hollow mesoporous silica nanoparticles[28] in order to induce differentiation of MDSCs to mature DCs, macrophages, and granulocytes.

Nanomedicines can furthermore be applied in cancer vaccination to target the peripheral immune system[29]. This application's grounds are based on the notions that intradermally or subcutaneously injected nanoparticles drain to LNs and that antigens bound to a nanoparticle are more efficiently processed by APCs. Instead of triggering APCs to present antigens to naive T cells, nanomedicines have also been designed to replace APCs by directly generating cytotoxic T cells[29].

During the last decade, nanoparticle-based immunotherapy formulations have passed from the preclinical stage in the clinical trials and several new treatments have been approved. Ferumoxytol is a nanoparticle formulation that contains iron oxide cores that are coated with carboxymethyl dextran. It enhances the production of reactive oxygen species by macrophages *via* the Fenton reaction, as M1 macrophages release hydrogen peroxides[30]. As an outcome, cancer cell cytotoxicity is enhanced while continued M1 polarization triggered by apoptotic cancer cells creates an autocrine feedback loop that maintains the production of tumor necrosis factor. Because ferumoxytol is FDA-approved, the drug is accessible for cancer patients through 'off-label' use[30]. The only cancer vaccines currently in routine

clinical use are the Sipuleucel-T and the Talimogene laherparepvec (T-VEC). The FDA-approved nanomedicine for the treatment of prostate cancer is sipuleucel-T which is a personalized vaccine encompassing patients' *ex vivo* processed dendritic cells that express a key tumor antigen, prostatic acid phosphatase (PAP)[31,32]. T-VEC is an engineered oncolytic herpes simplex virus type 1 in which the neurovirulence factor ICP34.5 is replaced by the coding sequence for GM-CSF and acts as a single agent in patients with skin and soft tissue metastases[32]. GM-CSF functions to recruit antigen presenting cells to the tumor microenvironment and promote cytotoxic T-cell responses to tumor associated antigens.

Immune checkpoint inhibitors

The last decade cancer immunotherapies have changed the perspective of cancer treatment (Table 1). The basic immunotherapy options approved are immune checkpoint inhibitors (ICIs).

ICIs have stirred up the field of tumour therapy and are now considered first-line therapies for various solid and liquid tumours. The approval of anticytotoxic T lymphocyte-associated protein 4 for advanced stage melanoma in 2011, opened up a new field of exploration that led to the 2018 Nobel Prize in Medicine to James P. Allison and Tasuku Honjo for inhibiting negative immune regulation in cancer [33].

Cancer immunotherapies are defined as therapies that directly or indirectly target any component of the immune system that is involved in the anti-cancer immune response, including the stimulation, enhancement, suppression, or desensitization of the immune system. These therapies are composed of monoclonal antibodies targeting the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-1 receptors and the PD-1 ligand PD-L1, which are involved in the regulation of T cells. As shown in Table 1, there is a plethora of ICIs approved for the treatment of various cancer.

As a general concept, T cell activation needs two signals, first theantigen recognition by the T cell receptor (TCR), and then the antigen presentation by major histocompatibility complex class II molecules on the surface of antigen-presenting cell that leads to signal modulation by CD80 or CD86 binding to the CD28 receptor[34].

CTLA-4 is found on the T cell surface competing with the CD28 receptor to bind CD80 or CD86, thereby blocking T cell activation. Furthermore, CTLA-4 inhibitors block CTLA-4-CD80 or CTLA-4-CD86 binding to facilitate T cell activation (Figure 1A). In Figure 1B, we see PD-1 as a surface receptor that is expressed by T cells and promotes apoptosis of antigen-specific T cells and reduces apoptosis of regulatory T cells through its interaction with its ligand, PD-L1, which is expressed by tumour cells and myeloid cells[35,36]. This interaction is useful in preventing autoimmunity in physiological conditions, but cancer cells exploit this process to escape from immune system activity, upregulating PD-L1 expression[37,38]. PD-1 and PD-L1 inhibitors disrupt the PD-1-PD-L1 interaction, facilitating T cell activation and survival (dashed lines).

The use of ICIs for cancer therapy is increasing; however, only a minority of patients treated with ICIs achieve a durable response. A portion of patients that receive ICIs do not respond to treatment, while others respond initially but ultimately acquire resistance. Primary and acquired resistance are the effect of constantly changing interactions among cancer cells and the immune system. Even in patients with melanoma, which has one of the highest rates of response to ICI, 60%-70% of patients do not experience an objective response to anti-PD-1 therapy[39,40]. Moreover, 20%-30% of patients demonstrate eventual tumour relapse and progression. A key challenge that has emerged with the progressive implementation of ICIs in clinical practice is their uncontrolled collateral effects on the immune system that can lead to so-called immune-related adverse events (irAEs).

ICIs have a different spectrum of toxicities[41] from standard chemotherapy or other biological agents, and most toxicities result from excessive immunity against normal organs. All the primary and secondary (acquired) resistance are a result of complex and constantly evolving interactions between cancer cells and the immune system. The most frequently noted irAEs involve inflammation of gastrointestinal, dermatologic, endocrine, or pulmonary organs. Several clinical trials for ICIs including adjuvant and neo-adjuvant therapies are still in progress.

The role of nanomedicine in ICIs is to ensure an increased therapeutic outcome by using specific nanocarriers. Several formulations are currently investigated in both pre-clinical and clinical studies [42]. Starting from the preclinical studies, at least 12 different nanocarriers are being investigated. These include gold nanostars that are being tested for the PD-L1 blockage, PLGA combined with anti-CTLA4, and incorporation of anti-PD-1 and anti-TIM-3 with liposomes[43-46]. All these preclinical studies are held in mouse models for bladder, breast (4T1 cells), and colon cancer.

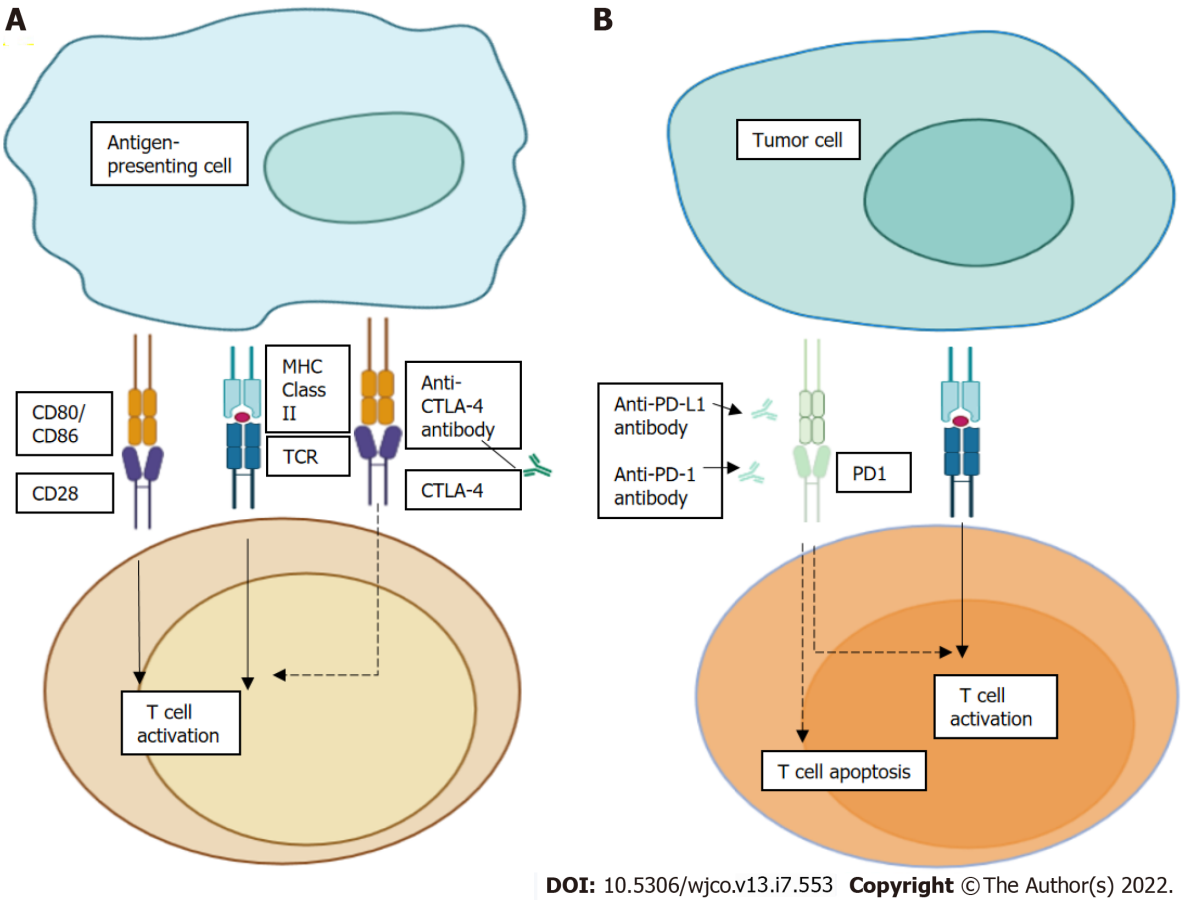
An important number of clinical trials are also being performed. These mainly include Nanoparticle Albumin Bound (Nab) formulations that combine ICIs with standard chemotherapeutics (as in the case of paclitaxel and carboplatin in Pembrolizumab, Atezolizumab, and Nivolumab formulations). Non-Nab strategies in nanoparticle-based immunotherapy include the radiosensitizer molecule NBTXR3[42].

Most of these studies will require a considerable amount of time to be completed and for the efficacy of these nanoformulation to be investigated. The poor lymphatic drainage of tumours (part of a phenomenon known as the EPR effect) could theoretically offer an advantage of nanoformulations over conventional ones although due to its complicated nature of EPR, this needs to be validated in these studies[47,48]. At the same time, further considerations are constantly being applied for future applications as the concept of smart nanoplatforms that will be triggered only upon an external stimulus[49-

Table 1 Approved immune checkpoint inhibitors according to cancer type

Anti-CTLA-4 antibodies
Ipilimumab; Colorectal cancer; Melanoma; Renal cell carcinoma
Anti-PD-1 antibodies
Nivolumab; Bladder cancer; Colorectal cancer; Head and neck cancer; Hepatocellular carcinoma; Hodgkin lymphoma; Melanoma; Non-small-cell lung cancer; Renal cell carcinoma; Cemiplimab; Cutaneous squamous cell carcinoma; Pembrolizumab; Bladder cancer; Cervical cancer; Gastro-oesophageal junction cancers; Head and neck cancer; Hepatocellular carcinoma; Hodgkin lymphoma; Merkel cell carcinoma; Metastatic solid tumours classified as microsatellite instability high or deficient mismatch repair; Non-small-cell lung cancer; Primary mediastinal large B cell lymphoma; Stomach cancer
Anti-PD-L1 antibodies
Atezolizumab; Bladder cancer; Breast cancer; Non-small-cell lung cancer; Avelumab; Bladder cancer; Merkel cell carcinoma; Durvalumab; Bladder cancer; Non-small-cell lung cancer

Anti-CTLA-4: Anticytotoxic T lymphocyte-associated protein 4; PD-1: Programmed cell death protein - 1.



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Figure 1 Mechanism of action of immune checkpoints and immune checkpoint inhibitors. A: Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors block CTLA-4-CD80 or CTLA-4-CD86 binding to facilitate T cell activation; B: We see PD-1 as a surface receptor that is expressed by T cells and promotes apoptosis of antigen-specific T cells and reduces apoptosis of regulatory T cells through its interaction with its ligand, PD-L1, which is expressed by tumour cells and myeloid cells. CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; MHC: Major histocompatibility complex; PD1: Programmed cell death protein 1.

51].

Triggers of natural killer cells

Cancer immunotherapy is considered to eliminate primary as well as metastatic tumors and it is shown to develop immunological memory. It is important to say that nanomedicine can deliver a vast number of immunological agents to the targeted site (*i.e.*, tumor)[52]. Nanomedicines have been explored thoroughly for tumor-targeted drug delivery and reducing the side effects of chemotherapeutic drugs. Tumor targeting is mainly mediated by passive targeting and/or active targeting and has been evaluated based on the average targeting efficiency and clinical impact (Table 2). There are three targeting strategies to boost cancer immunotherapy, including targeting and killing cancer cells to

Table 2 Clinical trials on natural killer cells in hematological and solid tumors

Condition	Interventions	Phase	Ref.	Status
Solid tumor	ROBO1 CAR-NK cells	I/II	NCT03940820	Recruiting
Ewing sarcoma; Neuroblastoma; Rhabdomyosarcoma; Osteosarcoma; CNS tumors	Allogeneic HCT; Donor NK cell infusion	II	NCT02100891	Active, not recruiting
Brain and CNS tumors; leukemia; lymphoma; chronic myeloproliferative disorders; lymphoproliferative disorder multiple myeloma and plasma cell neoplasm; myelodysplastic syndrome; myelodysplastic/ myeloproliferative neoplasm; unspecified adult solid tumor, protocol specific	Donor NK cell infusion	I/II	NCT00823524	Completed
Malignant solid tumors	NK Immunotherapy	II	NCT02853903	Completed
Malignant solid tumors	NK Immunotherapy	I/II	NCT02857920	Completed
Multiple myeloma	CIML NK cells plus KP1237 and low dose IL-2	I/II	NCT04634435	Recruiting
Hematological malignancy;	NK cell infusion	I	NCT01853358	Completed
leukemia; lymphoma; myeloma; Hodgkin's disease	NK-92 cells	I	NCT00990717	Completed
Acute lymphoblastic leukemia; chronic lymphoblastic leukemia; B-cell lymphoma	Fludarabine + Cyclophosphamide + CAR-NK-CD19 Cells	I	NCT04796688	Recruiting
Leukemia; lymphoma	NK cell infusion	I	NCT01287104	Completed

NK: Natural killer.

induce specific forms of ICD, TIME, and targeting the peripheral immune system[53].

Natural killer (NK) cells, part of the innate immune system, have been identified as the next-generation therapy for cancer. These cells are lymphocytes with antitumor and antiviral abilities that have several applications. NK cells have memory-like and memory responses after cytokine preactivation, viral infections, and hapten exposure, in addition to being classified as innate lymphoid cells [54]. They have various mechanisms for directly killing cancer cells and enhancing the immune system's ability to fight cancer. Over the last 40 years, NK cell immunotherapy has demonstrated encouraging effects in both preclinical and clinical studies. These cells have been used for years and have been approved by the FDA. The NK-92 cell line (CD56+/CD3-), isolated from a patient with lymphoma, has expected high cytotoxic movement and can be extended under acceptable assembling practice conditions in recombinant interleukin-2[55].

Many nanoparticles have been discovered to be immunotherapy carriers, delivering antitumor immunotherapeutics specifically to tumor cells. These nanoparticles could provide stability, increase solubility, and cause less toxicity to healthy cells. Nanoparticles have the potential to deliver immunotherapeutics directly to cancer sites, which can be explained by their increased duration in the bloodstream without altering the body's physiochemical properties. The lymphoid node secures the nanoparticles prior to their drug conveyance priority and the elimination of toxic waste products. When nanoparticle immunotherapy is used passively to target cancer, there is a significant reduction in cellular cytotoxicity and a favorable outcome. Thus, to achieve an effective outcome, the delivery system must be modified so that the immunotherapeutic carrier enters the intracellular space before accomplishing the immunotherapy. There are several types of nanoparticles, which are classified based on their size, morphology, and physical and chemical properties[56]. Nanotechnology, specifically nanoparticles as drug delivery systems (DDSs), eases targeted medicines and theragnostics. Most nanomedicines include a targeting element, but some do not, yet[57].

Magnetic nanoparticles, Fe_3O_4, were modified with meso-2,3-dimercaptosuccinic acid, as the affinity of the electron-rich carboxyl group was higher and the orbital in the Fe atom was empty. After obtaining CD56 antibody-modified Fe_3O_4 nanoparticles aided by a shorter co-culture period, NK-92 cell recruitment and infiltration into solid tumors were improved in the presence of a magnetic field. Biohybrid treatment with NK-CD56 nanoparticles effectively suppressed tumor growth and significantly prolonged the survival of cancer-bearing mice. Finally, by synergizing immune cells with a directional magnetic field that promotes infiltration into solid tumor tissue under magnetic resonance imaging control, antitumor efficiency is significantly improved. Magnetic nanoparticles and NK cells can be utilized for various biomedical applications, as they have proved to possess flexible characteristics to operate in biomedicine[58].

Gold nanoparticles (AuNPs) were coated with PEG and D-(β)-glucosamine, as glucose coating increases the cellular uptake of the nanoparticles. The K562 human erythroleukemia cell line (positive target) and the 888 human melanoma cell line (negative control) were co-cultured with AuNP-labeled NK-92 cells. The results indicate that AuNP-labeled NK-92 cells can specifically identify target cells and

keep their cytokine secretion and antitumor function. In addition, gold nanoparticles do not undermine the therapeutic effect of NK-92 cells *in vivo*. AuNPs could assist NK cells to achieve their aim, the regression of the tumor; therefore, this combinatorial therapy for cancer will reduce or even end the dosage of radiation[59].

CHIMERIC ANTIGEN RECEPTOR T-CELL BASED TREATMENTS

Worldwide, approximately 97% of active clinical trials are chimeric antigen receptor (CAR) T-cell-based therapies. Nanoparticles can engineer NK cells to produce CAR-NK therapy by targeting several ligands, such as antibodies to nanoparticles, and accomplish successful targeted delivery[60]. Axicabtagene ciloleucel (refractory diffuse large B-cell lymphoma) and tisagenlecleucel (B-cell precursor acute lymphoblastic leukemia) were two CAR T-cell therapies that cure blood cancer approved by the FDA [61]. Functional antitumor immune response has been shown by adoptive cell transfer studies, such as CAR T-cell therapy [62].

PEPTIDES

Peptides are a powerful tool in cancer diagnosis and treatment with many advantages and numerous ways to alter their function and use them in oncology. They present with excellent biocompatibility (degradation products are amino acids, which are a natural source of cells). They can be formulated and introduced with all kinds of modifications. By using the process of self-assembly, we can improve the stability of a peptide sequence and create the conditions for better targeting of the diseased organ. Their big advantage depends on their small size and better tissue/cell penetration[63,64]. To minimize the nonselective side effects of chemotherapy, a specific peptide sequence or motif can be used. Nanoparticles based on peptides, can be used to target cancer cells, to minimize systemic drug exposure and increase efficiency of the drug that is to be delivered[63].

Some examples of therapeutic peptides in clinical use nowadays are GnRH agonists for the treatment of prostate and breast cancer (*e.g.*, Buserelin and Nafarelin), GnRH antagonists for the treatment of prostate and breast cancer (*e.g.*, Cetrorelix and Abarelix), and somatostatin agonists for the treatment and diagnosis of GH-producing tumors (*e.g.*, Ocreotide and Lanreotide). In the future, many more peptides will take part in the treatment process against oncology, such as Chlorotoxin and its analogue TM601 (phases I, II, and III clinical trials for diagnosis of glioma), BT1718 (phases I and II for treatment of solid tumors), and P28 (phase I for treatment of various solid tumors)[64].

Drug conjugates is a modern method of using peptides as a tool for drug delivery. They are chemotherapeutic or cytotoxic agents linked to an antibody or a peptide *via* a linker. They provide enhanced function, higher circulation time, and lower off-target toxicity (to healthy tissues)[65]. An example is the conjugation of paclitaxel to a peptide (Angiopep-2) *via* an ester/amide bond. Angiopep-2 goes into the cell *via* transcytosis and crosses the blood-brain barrier, thus facilitating the uptake of the conjugate into the brain for the treatment of patients with solid tumors and brain metastases. The esterase enzyme, which is present in lysosomes, breaks down the ester bond, thus releasing paclitaxel in the brain. In this way, ANG 1005 overcomes the main disadvantage of paclitaxel and gains access into the blood-brain barrier. ANG1005 has been studied in several clinical trials (phase I and phase II) in patients with metastatic brain cancers and the results have shown that it works well against CNS tumors, improves symptoms, and increases survival[66].

Peptide self-assembly is a process in which peptides spontaneously or by a trigger form aggregates. In that form, the transport mechanism provides a higher efficiency of drug loading with better molecule stability and a simultaneous lower ratio of drug loss[67]. The method uses monomers of short amino acid sequences or repeated amino acid sequences that assemble together to form nanostructures. The nanostructure can be made by various building blocks such as dipeptides (the simplest form), surfactant-like peptides, and cyclic peptides[68]. The resultant nanostructure can take the form of nanofibers, nanotubes, micelles, and hydrogels[69-71].

Self-assembly of peptides is divided into spontaneous and trigger types. If the assembly happens in an aqueous solution, it is spontaneous. The peptide molecules that are dissolved in the aqueous solution form non-covalent interactions, such as hydrogen bonding bonds, van der Waals forces, electrostatic, and π-π stacking interactions[69]. If the process of assembly is driven by external factors and does not happen spontaneously, such as temperature, ion concentration, and pH changes, it is called trigger aggregation. The above-mentioned nanostructure can be used for drug delivery, drug stabilization, crossing the blood-brain barrier, neuronal or liver cell regeneration, fibroblast migration, *etc*[69].

NUCLEIC ACIDS

Albeit their central role in governing cell physiology, nucleic acids had not been considered as possible drug candidates until relatively recently when successful protein production was demonstrated upon *in vivo* administration[70]. Since then, a novel class of drugs, referred to as nucleic acid therapeutics, have emerged[71]. Conventional therapeutics generally exhibit a transient effect and exert their action *via* protein targeting. This action mode poses significant disadvantages as only a fraction of human proteins can be targeted by pharmaceutical compounds[72]. Combined with the limitations of conventional therapeutics in oncology discussed above, gene therapy offers a promising approach as a one-time treatment targeting the route of the disease - genetics - while contributing to long-standing therapeutic outcomes with high specificity[73]. Developments in nucleic acid design and chemical modifications have assisted in overcoming stability, toxicity, and immunogenicity issues[74-76], and by further harnessing the power of nanomaterials, nucleic acid therapeutics can be loaded into nanocarriers to formulate DDSs with enhanced pharmacokinetic properties[77].

Due to the arduous nature of the causation and phenotype of cancer, it is evident that nucleic acid-based therapeutics must implement a plethora of strategies *via* different modes of action to target relevant genes and their products in cancer cells or stimulate an immune response against them[78]. Table 3 summarizes the current status of nucleic acid nanomedicines available for cancer treatment. The first strategy implemented in oncology utilizes antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs) to target disease-relevant mRNAs and inhibit their translation. ASOs are synthetic oligonucleotides complementary to a gene of interest that bind on the pre-mRNA or mRNA of the target gene, hindering cellular post-transcriptional and translational machinery and eventually leading to altered splicing patterns or gene silencing, respectively[79,80]. Lipid nanoparticle (LNP)-based ASOs are under clinical evaluation to treat leukemia[78,81] and solid tumors[81] *via* targeting Grb2. Furthermore, targeting the anti-apoptotic gene Bcl-2 is also being examined as a possible target in patients with advanced lymphoid malignancies[82].

siRNAs are a class of double-stranded RNA molecules involved in the biological process of RNA interference that regulates gene expression[83]. By administering a siRNA complementary to its target mRNA, this natural process is harnessed to selectively silence genes *via* multiprotein complexes[84]. Activation of the oncogene *KRAS* is a hallmark of pancreatic ductal adenocarcinoma, the most common type of pancreatic cancer[85]. siG12D-LODER is a biodegradable polymer-based system loaded with siRNA against *KRAS*, which has completed phase I and is currently being tested in phase II in combination with chemotherapy to determine treatment efficacy[86-88]. Other delivery systems based on LNPs have also completed or are currently being tested in phase I trials against advanced solid cancers in various modalities targeting tumor proliferation or microenvironment[89-92].

Additional strategies based on small activating RNA molecules aim to upregulate the expression of physiologic master gene regulators[93] and are undergoing pre-clinical development for the treatment of hepatocellular carcinoma[94] and advanced solid tumors[95]. Moreover, since misregulated miRNA expression is another feature of cancer, miRNA mimics are also being developed to mimic endogenous miRNAs and restore physiological expression levels[96,97].

The rise of mRNA vaccines has led to a new era of cancer immunotherapy offering considerable benefits, including increased safety and efficacy with expeditious cost-effective manufacturing pipelines, aiming to elicit an immune response upon exposure to a tumor antigen[98,99]. Several LNP-based mRNA vaccines encoding known tumor-specific antigens are being investigated in early phase clinical trials in patients with HPV-driven squamous cell carcinoma, melanoma, ovarian, pancreatic, lung, and colorectal cancer[100-103]. Technological progress in next-generation sequencing has enormously facilitated the discovery of patient-specific neoantigens, novel epitopes arising from tumor-specific mutations that can be used as a template to generate personalized neoantigen vaccines[104]. Such vaccines are being assessed clinically for the treatment of melanoma and breast cancer[105,106].

CONCLUSION

The field of cancer nanomedicines is rapidly expanding and is expected to revolutionize available treatment options. Nanomaterial-mediated immunomodulation offers a dual aspect of immunomodulation therapies, as they can themselves act as immunomodulatory agents, or they can function as delivery platforms for targeted delivery of other immunomodulating agents[20]. Their unique and tunable properties can be utilized to target the cancer-immunity flow in multiple steps, offering advanced systems that pave a way to reshaping the landscape of clinical cancer treatment. ICIs have launched a new field far beyond CTLA-4 and PD-1. First of all, co-inhibitory signaling pathways, such as HVEM-BTLA and Galectin-9-TIM3, are being studied in cancer and other diseases[107]. Once we learn more about them, we may design rational combinational strategies to concurrently target two or more inhibitory pathways to gain better therapeutic efficacy. Moreover, good results are shown with the combination of immune checkpoint blockade with other immunotherapy regimens to eliminate primary cancer and metastases more effectively. One such strategy has been to combine anti-PD-1/PD-L1 or

Table 3 Summary of nanomedicines based on nucleic acids

Name	Category	Structure	Mode of action	Status
ASO	Inhibition of translation of cancer or angiogenesis associated proteins	Synthetic ssDNA or ssRNA oligos complementary to mRNA of interest	Rnase H mediated mRNA degradation	In clinical trials; LNP-based anti-Grb2 ASOs for leukemia[70] and solid tumors[71]; LNP-based anti-Bcl-2 ASOs for advanced lymphoid malignancies[72]
siRNA	Inhibition of translation of cancer or angiogenesis associated proteins	Synthetic dsRNA oligos complementary to mRNA of interest	Dicer induces cleavage of dsRNA and RNA-induced silencing complex mRNA degradation	In clinical trials; Polymeric anti-KRAS siRNAs for pancreatic ductal adenocarcinoma[78]; LNP based anti-PKN3 siRNAs in patients with advanced solid tumors[79]; LNP based anti-KSP and anti-VEGF-A siRNAs in patients with solid tumors[80,81]; LNP based anti-PLK1 siRNAs in patients with solid tumors[82]
saRNA	Forced exogenous gene expression	Synthetic dsRNA oligos complementary to mRNA of interest	Target gene promoters to induce transcriptional gene activation	In clinical trials; LNP based formulations for treatment of hepatocellular carcinoma[84] and advanced solid tumors[85]
miRNA mimics	Regulation of post-transcriptional mRNA expression	Chemically modified dsRNA molecules designed to mimic endogenous microRNAs	Translational repression and gene silencing	Currently only in basic research[87]
mRNA vaccines	Forced exogenous antigen expression	Synthetic mRNA	Induction of immune response against cancer cells	In clinical trials; LNP-based mRNA vaccines encoding known tumor-specific antigens are being investigated in early phase clinical trials in patients with HPV-driven squamous cell carcinoma[90], melanoma[90], ovarian[92], pancreatic, lung, and colorectal cancer[93]; Personalized vaccines based on patient specific neo-antigens are being assessed clinically for the treatment of melanoma[95] and breast cancer[96]

ASO: Antisense oligonucleotides; saRNA: Small activating RNA; siRNA: Small interfering RNAs; LNP: Lipid nanoparticle.

anti-CTL4 with oncolytic viruses[108,109]. Meanwhile, other types of cancer immunotherapies, including adoptive transfer of CAR T cells, TCR-modified T cells, and cancer vaccines using neo-antigens, have made significant progress in recent years and have shown promise in clinics[110-112]. Future NK cell products will be able to suppress inhibitory signals and tumor proliferation but enhance the activation of the immune system. Evidence of increased NK cell-mediated tumor cell killing has emerged in targeted therapies. To enhance that, nanomedicine approaches immunity with T-cell activation, specific antigen delivery, and the appropriate nanoparticle for the targeting[113]. Nanoparticles will tackle all the obstacles to delivery and engage multiple aspects of the immune system by producing therapeutics to target current and forthcoming diseases[114]. These are only a small portion of the application of nanoparticles with NK cells and their clinical activity because of the heterogeneity of human diseases[115]. These findings, combined with the ability of NK cells to detect immune responses, suggest that NK cells are the keys to the next-generation onco-immunotherapy. In the future days, peptides will play a significant role in the continuous research of cancer therapy and human well-being. Cell-penetrating peptides have the ability to deliver molecules such as drugs, oligonucleotides, and nanoparticles inside cells, without any size restriction[116].

Future research will set the basis for the ideal drug-delivery system, where peptides would reach their target site efficiently without any degradation before and the cargo would be rapidly released and act on the site. Also, the problem of non-selective cellular uptake will be eliminated and thus modern therapy tools for anti-cancer treatment will be created[117,118]. The nanoparticle-mediated delivery of guide RNAs and programmable nucleases such as Cas9 and Cas13 has expanded the portfolio of *in vivo* tissue-specific genome editing tools available for cancer research in pre-clinical models[119-122]. Alongside advancements in nucleic acid drugs, innovative nanoparticle delivery systems will vastly benefit the field by implementing novel delivery systems, such as nanoclews and surface modifications, allowing the manufacturing of sophisticated nanoparticles[123-127]. Despite the numerous nanotherapeutics being clinically scrutinized, cancer nanomedicines often fail to reach their primary endpoint, and the correlation of the drug behavior between animal models and patient cohorts is often inconsistent[128,129]. Therefore, *in silico* models should also be implemented to aid in understanding and predicting biological interactions[130]. Finally, multi-omics data, including but not limited to genomics, epigenomics, transcriptomics, and radiomics, can comprehensively be evaluated and reform the field of personalized nanomedicine by allowing the design of customizable medicines based on the patients' profile[131].

FOOTNOTES

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MINIREVIEWS

Simple approach for the histomolecular diagnosis of central nervous system gliomas based on 2021 World Health Organization Classification

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Abstract

The classification of central nervous system (CNS) glioma went through a sequence of developments, between 2006 and 2021, started with only histological approach then has been aided with a major emphasis on molecular signatures in the 4th and 5th editions of the World Health Organization (WHO). The recent reformation in the 5th edition of the WHO classification has focused more on the molecularly defined entities with better characterized natural histories as well as new tumor types and subtypes in the adult and pediatric populations. These new subclassified entities have been incorporated in the 5th edition after the continuous exploration of new genomic, epigenomic and transcriptomic discovery. Indeed,

the current guidelines of 2021 WHO classification of CNS tumors and European Association of Neuro-Oncology (EANO) exploited the molecular signatures in the diagnostic approach of CNS gliomas. Our current review presents a practical diagnostic approach for diffuse CNS gliomas and circumscribed astrocytomas using histomolecular criteria adopted by the recent WHO classification. We also describe the treatment strategies for these tumors based on EANO guidelines.

Key Words: Central Nervous System glioma; Classification; World Health Organization 2021; European Association of Neuro-Oncology guidelines

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Core Tip: Central nervous system (CNS) gliomas went through a sequence of development since 2006. The guidelines of 2021 World Health Organization (WHO) classification of CNS tumors and European Association of Neuro-Oncology (EANO) utilized molecular signatures in the diagnostic approach for CNS gliomas. We herein presents a practical diagnostic approach and the treatment strategies for diffuse CNS gliomas and circumscribed astrocytomas using histomolecular criteria based on the WHO classification.

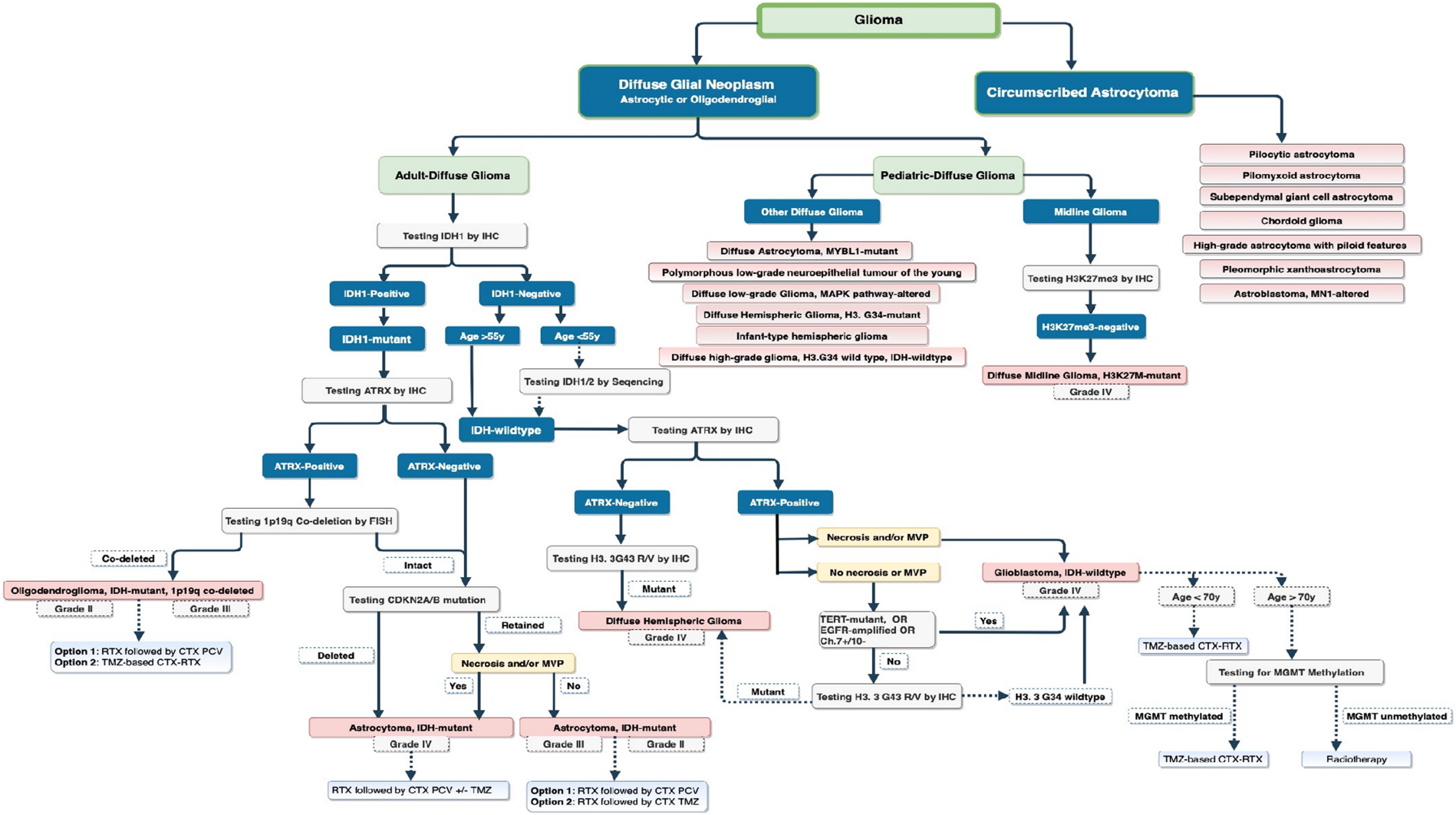
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INTRODUCTION

Brain tumors are defined as masses derived from various cells originating from the brain (primary tumors) or distally (secondary tumors, most commonly lung, breast, renal, prostate, and skin cancers) that have undergone metastatic spread[1]. The most prevalent primary intracranial tumors are gliomas, with 80% of the population, and World Health Organization (WHO) grade 4 astrocytoma (previously named glioblastoma) is present in nearly one-half of the patients with astrocytoma, with a 95% mortality rate in a 5-year follow-up period irrespective of age and gender[2,3,4]. Gliomas usually present with headache, nausea and vomiting, blurred vision, focal neurological deficit, alteration in sensation, and other manifestations of high intracranial pressure that warrant investigation by neuroimaging, preferably magnetic resonance imaging (MRI) of the brain[5]. In imaging, cystic change, multicentric enhancement, and hemorrhage are commonly observed[6]. Therefore, histopathological examination is considered the gold standard method for diagnosing and grading the tumors.

According to the WHO classification, central nervous system (CNS) gliomas can be classified based on their histological and molecular features[1]. The classification initially includes diffuse and non-diffuse gliomas, and it has undergone significant changes since its establishment to the 4th edition in 2016, aided by molecular signatures[2]. The recent 5th edition of the WHO classification has replaced the entity with type and variant with (subtype) group. In addition, the WHO classification has adopted Arabic numerical over the former Roman numerical grading system for grading brain gliomas for easier reading and to avoid confusion when interpreting pathology reports[7]. However, using the Roman numerical grading system is still considered acceptable. Some of the most important changes in the 5th edition involve the classification of gliomas, differentiating gliomas that occur primarily in adults from those that occur mainly in children[8]. Gliomas are traditionally subclassified into three major categories: diffuse gliomas, pediatric diffuse low- and high-grade gliomas, and circumscribed astrocytic gliomas (Figure 1). In addition, glioneuronal tumors, neuronal tumors, and ependymomas were also included in the classification as separate types[7,8].

Further propositions of the 2021 WHO classification of CNS tumors was adapted by the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy - Not Officially WHO (cIMPACT-NOW), published in 2020/2021. Moreover, they included various molecular genomic studies, including methylation profiling, isocitrate dehydrogenase (*IDH1-2*) codon mutation, and alpha-thalassemia-mental retardation X-chromosome (*ATRX*) mutation. This genomic profiling has affected such tumors' management and treatment modalities[9]. Moreover, three major subtypes of diffuse gliomas have been emphasized, based on molecular genomic signatures, including *IDH*-mutant oligodendroglioma with 1p/19q codeletion, *IDH*-mutant astrocytoma, and *IDH*-wild-type glioblastoma [8].



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Figure 1 A simple consensus approach to diagnose diffuse central nervous system gliomas using immunohistochemistry and molecular profiling, aided with the treatment strategy.

In this review, we presented a simple and practical diagnostic approach using histomolecular characteristics to define diffuse CNS gliomas accurately. We also associated the newly published 5th edition of the 2021 WHO classification with the current and updated European Association of Neuro-Oncology (EANO) treatment guidelines.

DISCUSSION

Before 2016, the classification of CNS tumors was established based on histological findings and immunohistochemical tests. Between 2016 and 2021, molecular biomarkers were incorporated into the diagnostic criteria to differentiate CNS tumors into clustered groups[7,9]. The current 5th edition of the 2021 WHO classification does not recommend a specific assessment method to identify molecular alterations unless a distinct tumor subtype is in the differential diagnosis. The cIMPACT-NOW has emphasized the confirmatory diagnosis of astrocytoma with the presence of *IDH* mutation or wild type, *ATRX* loss, *TP53* mutation, and lack of 1p/q19 codeletion[7,10]. In comparison, diffuse gliomas (classified as WHO grades 2 and 3) are either *IDH* wild type or *IDH1*$^{-}$ mutant[11,12]. As a key feature of oligodendroglioma, it, by definition, must harbor an *IDH* mutation and 1p/19q codeletion.

If *IDH1*^{R132H} is immunonegative in astrocytic or oligodendroglial tumors of WHO grades 2 and 3 or in patients aged less than 55 years, IDH1 (132 codon) and/or IDH2 (172 codon) Deoxyribonucleic acid (DNA) sequencing should be performed using the Sanger method or polymerase chain reaction (PCR) [13,14]. Otherwise, IDH1 immunonegative in patients aged greater than 55 years is likely to be IDH-wild type with an incidence rate of < 1% and acts like a high-grade glioma. Meanwhile, IDH1/2 DNA sequencing is preferable for detecting non-canonical mutations[15]. A non-canonical IDH mutation and a loss of ATRX mutation and O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation[7] have been associated with a family history of cancer and astrocytoma of the infratentorial region being identified at 80% of the time in multicentric astrocytoma[16].

ATRX mutation should also be tested in all gliomas, whether *IDH1/2* is a mutant or wild type. *ATRX* can be tested by immunohistochemistry (IHC). In oligodendroglioma, positive *ATRX*, negative *TP53*, and *TERT* mutations in *IDH*-mutant tumors are prevalent; nonetheless, 1p19q codeletion using fluorescence in situ hybridization (FISH) should be tested. However, false-positive results are expected to be less than 4% in most cases[17,18]. In both astrocytomas and oligodendrogliomas, the presence of homozygous deletion of *CDKN2A/B* at 9p21 is associated with poor prognosis in such groups, leading to decreased overall survival (OS)[11]. *CDKN2A/B* gene mutation can be tested through next-generation sequencing or the Sanger method if the tumor is *IDH*-mutant and 1p19q is not codeleted[19] (Figure 1). Loss of p16 expression (a marker of CDKNA/B) is associated with poor prognosis in *IDH*-mutant tumors and those with 1p/19q codeletion[20]. *CDKN2A/B* deletion is detected in both the wild-type and mutant *IDH*; however, it has been related to a reduction in OS in wild-type tumors[21-23].

Glioblastomas are routinely diagnosed based on histological findings of microvascular proliferation (MVP) and/or necrosis, and are molecularly defined as either *IDH*-mutant (10%) or *IDH*-wild-type (90%) tumors with significantly different biology and prognoses[8]. The terminology of *IDH*-mutant glioblastoma was omitted from the cIMPACT as they are biologically different from *IDH*-wild-type astrocytomas and were defined as *IDH*-mutant WHO grade 4 astrocytomas. Glioblastoma is referred to as *IDH*-wild-type glioblastoma or astrocytoma. There are no longer *IDH*-mutant glioblastomas. Another new terminology is an astrocytic glioma with *IDH* wild type, but with the presence of a histone 3 (H3) mutation, classified as a WHO grade 4 astrocytoma[9,24] (Figure 1). In glioblastoma, Telomerase reverse transcriptase (*TERT)* promoter mutations, Epidermal growth factor receptor (*EGFR)* mutations, and/or loss of chromosome 10 with gain of chromosome 7 are all prevalent[25]. If one of these features is detected, glioblastoma should be immediately diagnosed regardless of the presence of necrosis and/or MVP[26]. The presence of the H3F3A G34 histone mutation with chromosomal 1q gain distinguishes pediatric diffuse hemispheric glioma from adult diffuse hemispheric glioma. *EGFR*, *TERT*, *CDKNB2A/B*, and Ch10-Ch7+ are usually identified in adults; however, they should be tested for H3F3A gene mutation[27,28]. In comparison, the 2021 WHO classification has included *IDH* wild type and *H3F3A* in diffuse high-grade pediatric astrocytoma (most commonly from the pons in more than two-thirds of the cases, followed by the spinal cord and thalamus)[29].

H3-K27 is another commonly reported histone mutation in midline gliomas, ependymomas, and gangliogliomas[30-32]. Altered mitogen activated protein kinase (*MAPK)* pathway and *H3-K27*-mutant tumors are associated with diffuse low-grade midline gliomas and are associated with prolonged OS of more than 10 years[33]. The astroblastoma tumor is a new entity, *MN1*-altered, with better OS compared to C11Orf95-*RELA* and *BRAF*-positive astrocytic tumors[34]. Four other categories of diffuse astrocytomas were described in the 5th edition of the WHO classification, which included angiocentric gliomas, polymorphous low-grade neuroepithelial tumors of the young, and diffuse low-grade gliomas (*MAP* altered) (Figure 1). Infantile gliomas have a special genetic signature with fusion genes of *MET*, *ROS1*, *ALK*, or *NTRK1/2/3*[35,36].

Guidance to treatment modalities

A positive prognostic factors in diffuse gliomas include young patients, good Karnofsky performance status (KPS), total resection, and *MGMT* promoter methylation[37]. Surgical resection is considered the cornerstone of therapy, with a 5-year survival rate (80%) followed by watchful waiting in low-grade gliomas. However, total resection may result in neurological deficits; thus, awake craniotomy and imaging modalities, such as tractography, may be utilized on a case-by-case basis[38,39]. Postoperatively, MRI can detect residuals, and perfusion studies may detect progression[40]. Therefore, the care plan must be through a multidisciplinary approach with neurooncologists, neuropathologists, and neurosurgeons on board to discuss management modalities.

For low-grade gliomas, such as *IDH*-mutant and 1p/19q codeleted oligodendroglioma (WHO grade 2), careful, a watchful waiting strategy is an option, particularly for totally resected tumors or younger patients (< 40 years) with incomplete tumor resection. However, this would come at a cost: the patient's life, neurological impairments development, and a substantial increase in histological grading over time [41,42]. As a result, disease progression must be monitored with neuroimaging every 2–3 months. According to the National Comprehensive Cancer Network guidelines, patients should be followed up every 2 months with an MRI brain scan, then every 3 months if they have been off therapy for a year [40]. Progression may occur after 4–8 weeks, necessitating a brain MRI. Perfusion studies and spectroscopy can help to differentiate between progression and pseudoprogression, and, in doubt, a multidisciplinary team should be counseled.

Adjuvant radiotherapy

Post-surgical resection radiotherapy is the best therapeutic effect to prevent recurrence or delay the progression of diffuse gliomas (WHO grades 2-4)[43]. The role of radiotherapy is to maintain the control of tumor progression, but it leads to neurotoxicity if used in high doses. Thus, the most used doses are 50-60 Gy administered 3-5 times postoperatively[44]. Choi *et al*[45] found that patients with WHO grade 4 astrocytoma who received 50-60 Gy lived an average of 9 months longer than those who received 45 Gy for 3 months.

Radiotherapy is recommended for all WHO grade 2 gliomas with incomplete resection or for patients aged > 40 years and all WHO grade 3–4 gliomas. Early radiotherapy has been shown to prolong progression-free survival but not OS[46]. Whole-brain radiation therapy is usually not preferable in clinical practice because it is associated with cognitive effects[45]. A follow-up MRI 3-4 weeks after radiotherapy completion is typically performed to monitor disease progression[40]. The use of chemotherapy without radiation remains under investigation but might be an option if radiotherapy is not possible, for example, in patients with large tumors or elderly patients who might not be candidates for radiation.

Chemotherapy

Another adjunct treatment modality for diffuse gliomas is pharmacological treatment, mainly for patients with high-grade gliomas. The most used drug is the alkylating agent temozolomide (TMZ) for its immunomodulation and contribution to tumor-acquiring cell death. TMZ was first discovered in 1987 and has been widely applied as an effective first-line chemotherapeutic agent for treating patients with glioblastoma since the Food and Drug Administration has approved its efficacy in 2005[47,48]. Other drugs used as adjuvants in treating gliomas include nitrosourea, which includes lomustine, carmustine, nimustine, and fotemustine. However, this class of drugs may cause pronounced low platelet count when administered long-term. As a result, patients with oligodendroglioma frequently receive a procarbazine, vincristine, and lomustine (PCV) regimen, as dose-related side effects are more evident in other nitrosourea classes, such as gastrointestinal disorders, decreased cell count, and ototoxicity[48]. Anti-vascular endothelial growth factor antibody (bevacizumab) has also been used as an adjuvant treatment, but it's clear benefit is uncertain[49].

Recent update in glioma treatment

In the new 2021 WHO, cIMPACT-NOW, and EANO guidelines, therapeutic options are targeted at the genomic types of tumors[7,9]. As total surgical resection remains the standard treatment for all CNS gliomas, radiotherapy is still considered the first-line targeted therapy after surgical resection for all WHO grade 2 and 3 oligodendrogliomas or astrocytomas, as mentioned previously[50-52].

In patients with *IDH*-mutant oligodendroglioma (WHO grade 2 or 3) with 1p19q codeletion, aged > 40 years or with no totally resected tumor, and associated with comorbidities, residuals, or recurrence > 15 cm[3], radiotherapy followed by PCV chemotherapy regimen is recommended[51]. According to the two trials (EORTC 26951 and RTOG 9402), the combination of radiotherapy and PCV regimen showed a considerable benefit[52,53].

Radiotherapy followed by chemotherapy is recommended for all patients with *IDH*-mutant WHO grade 2, 3, or 4 astrocytomas, particularly in patients aged > 40 years, with incompletely resected tumors, and associated with neurological deficits[51]. TMZ is often preferred over PCV owing to its safety and ease of administration. However, radiotherapy followed by PCV constitutes the current standard of care for patients with *IDH*-mutant astrocytomas (WHO grade 2)[51]. The RTOG 9802 trial

reported a major prolongation of OS with the addition of PCV to radiotherapy, from 7 to 13 years in patients with WHO grade 2 gliomas who had undergone a subtotal resection or in those aged ≥ 40 years [54]. To prevent functional deficits, diffusion tensor imaging and functional MRI can be utilized[55]. In recurrence, repeat surgery with radiation and chemotherapy (TMZ and nitrosourea) can be considered equally efficient in treatment[9]. For *IDH*-mutant WHO grade 3 astrocytoma, the EORTC 26053 trial of radiotherapy alone, with concomitant or maintenance TMZ, showed a significant prolongation of OS in patients receiving radiotherapy followed by maintenance TMZ[56]. Therefore, TMZ chemotherapy is considered as the standard treatment for tumour progression after surgery and radiotherapy for most patients with *IDH*-mutant gliomas (WHO grade 2 or 3).

Glioblastoma (*IDH*-wild-type grade 4 astrocytoma) is best managed by gross total resection followed by radiotherapy[51]. In non-feasible or nearly total resection cases with age ≥ 70 years, radiotherapy (60 Gy in 30 fractions) or over fractionated radiotherapy (40 Gy in 15 fractions) is preferable to increase OS [9]. Higher survival rates are recorded in younger age groups < 65 years at diagnosis, with a median of up to 40 weeks. However, *TERT* mutation, gain of chromosome 7, and loss of chromosome 10 are associated with poor prognosis[57]. Neurocognitive outcomes can be affected by overfractionated radiotherapy. In patients with good KPS and aged < 70 years, a combination of radiotherapy and chemotherapy (TMZ) is the standard therapy[58]. Combined TMZ with lomustine in early diagnosis may increase OS, particularly in *MGMT*-methylated glioblastomas[59-61]. Hypofractionated radiotherapy is preferable for patients aged ≥ 70 years. The standard-of-care treatment for patients with recurrent glioblastoma has not yet been clarified; treatment is selected based on the prior therapy, patient's age, KPS score, MGMT promoter methylation status, and disease progression. Therefore, surgery and radiotherapy should be considered. Nitrosourea regimens, TMZ, with consideration of bevacizumab are options for pharmacotherapy but have an unconfirmed effect on OS. In patients who did not benefit from adjuvant radiotherapy or had an early symptomatic progression, a second surgery could be considered 6 months after the initial surgery aiming to increase OS[62].

There is a limitation of surgical management in *H3-K27M*-mutant diffuse midline glioma (WHO grade 4) because of its eloquent structures, including the pituitary, thalamus, midbrain, pons, and medulla, and it has a 5-year survival period of < 1%. Radiotherapy is often used but is associated with a poor prognosis. However, in hemispheric glioma, chemoradiotherapy drugs can be used because most of these tumors are *MGMT*-methylated[63].

CONCLUSION

In this review, we presented a simple diagnostic approach to differentiate diffuse CNS gliomas into molecularly defined subtypes using histomolecular features, based on the 5th edition of 2021 WHO classification of CNS tumors. This is to emphasize that molecular profiling is important in the diagnostic classification and grading of diffuse CNS gliomas. We also defined the role of different treatment modalities of surgery, radiotherapy, and pharmacotherapy in the treatment of these molecular defined gliomas. In fact, our review intends to serve as a simple reference for the diagnosis of CNS gliomas for healthcare providers.

FOOTNOTES

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ORIGINAL ARTICLE

Case Control Study

Edmonton Symptom Assessment Scale may reduce medical visits in patients undergoing chemotherapy for breast cancer

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Abstract

BACKGROUND

Adjuvant chemotherapy is recommended in high-risk breast cancer. However, no universally accepted guidelines exist on pre-chemotherapy assessment. In particular, the number and frequency of medical visits vary according to each institution's policy. We hypothesised that the Edmonton Symptom Assessment Scale (ESAS) may have a favourable impact on the pre-treatment assessment in candidates for adjuvant chemotherapy.

AIM

To investigate whether the ESAS can be used to safely reduce the number of medical visits in women with breast cancer undergoing adjuvant chemotherapy.

METHODS

In a retrospectively prospective matched-pair analysis, 100 patients who completed the ESAS questionnaire before administration of adjuvant chemotherapy (ESAS Group) were compared with 100 patients who underwent chemotherapy according to the traditional modality, without ESAS (no-ESAS Group). Patients of the ESAS Group received additional visits before treatment if their ESAS score was > 3. The primary endpoint was the total number of medical visits during the entire duration of the chemotherapy period. The secondary endpoints were the occurrence of severe complications (grade 3-4) and the number of unplanned visits during the chemotherapy period.

RESULTS

The study variables did not statistically differ between patients of the ESAS Group and no-ESAS Group (age $P = 0.880$; breast cancer stage $P = 0.56$; cancer histology $P = 0.415$; tumour size $P = 0.258$; lymph node status $P = 0.883$; immunohistochemical classification $P = 0.754$; type of surgery $P = 0.157$), except for premenopausal status ($P = 0.015$). The study variables did not statistically differ between patients of the ESAS Group and no-ESAS Group regarding age, cancer stage, histology, tumour size, lymph node status, immunohistochemical classification, and type of surgery. Unplanned visits during the entire duration of chemotherapy were 8 in the ESAS Group and 18 in the no-ESAS Group visits ($P = 0.035$). Grade 3-4 toxicity did not differ between the study groups ($P = 0.652$). Forty-eight patients of the ESAS Group received additional visits due to an ESAS score > 3. The mean number of medical visits was 4.38 ± 0.51 in the ESAS Group and 16.18 ± 1.82 in the no-ESAS group ($P < 0.001$). With multivariate analysis, women of the ESAS group were more likely to undergo additional visits for an ESAS score > 3 if they were aged 60 or older, received a mastectomy, or had tumour stage II/III.

CONCLUSION

The ESAS score may safely reduce the number of medical visits in candidates for adjuvant chemotherapy for early breast cancer. Our results suggest that the ESAS score may be used for selecting a group of breast cancer patients for whom it is safe to reduce the number of medical visits in the setting of adjuvant chemotherapy. This may translate into several advantages, such as a more rational utilization of human resources and a possible reduction of coronavirus pandemic infection risk in oncologic patients.

Key Words: Edmonton system assessment scale; Adjuvant chemotherapy; Breast cancer; Medical visits; Patient-reported outcomes

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Core Tip: Adjuvant chemotherapy is recommended in high-risk breast cancer. We hypothesized that the Edmonton Symptom Assessment Scale (ESAS) can be used to safely reduce the number of medical visits in women with breast cancer undergoing adjuvant chemotherapy. The main result of this case-matched analysis is that ESAS screening may safely reduce the frequency of medical visits in the setting of AC in patients with breast cancer. This finding may have some advantageous implications in oncological practice, especially in the current scenario, where an increase in coronavirus pandemic cases throughout the world has imposed measures for minimising the risk of infection among patients and health care providers.

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INTRODUCTION

The multidisciplinary treatment of breast cancer has permitted achieving high survival rates over the last 20 years[1-4]. According to current accepted worldwide guidelines, many patients with breast cancer receive recommendation for adjuvant chemotherapy (AC), which continues to be a cornerstone of treatment for high-risk patients. In fact, AC has been linked to a reduced risk of developing locoregional and systemic recurrences, as well as to increased overall survival in some subgroups of patients who have undergone surgery for breast cancer[5-7]. However, it is known that toxicity of chemotherapy regimens can expose patients to adverse effects, unplanned medical visits, or hospitalisation[4,7,8].

There are no globally standardised guidelines that regulate the pre-treatment assessment of candidates for AC. While it is established that administration of chemotherapy drugs should be done by oncology nurses under the supervision of a medical oncologist, some aspects of the treatment vary according to each institution's policy. In common practice, prior to every session of chemotherapy patients are evaluated during a medical visit. A pre-chemotherapy medical visit before every cycle of AC represents a time- and resource-demanding practice, especially in high-volume centres. The Edmonton Symptom Assessment Scale (ESAS) is a useful and simple tool for evaluating patients

undergoing therapy for cancer. The ESAS consists of a questionnaire developed to rate the intensity of nine common symptoms experienced by patients with cancer[9-11]. We hypothesised that the ESAS can be used to safely reduce the number of medical visits in women undergoing AC for breast cancer. Therefore, we conducted a prospective matched-pair analysis to evaluate the impact of the ESAS in this subgroup of patients.

MATERIALS AND METHODS

Study population

Patients receiving treatment for breast cancer were prospectively registered in an institutional board-registered database at the Breast Unit of the University Hospital of Sassari (Italy). According to the institutional policy, all patient cases were presented in a weekly multidisciplinary meeting, in which preoperative and postoperative management was discussed. After metastatic work up, each patient received neoadjuvant chemotherapy or upfront surgery (mastectomy or breast-conserving surgery [BCS] and sentinel node biopsy with or without axillary lymphadenectomy, according to the status of the sentinel node). Radiotherapy was given after BCS and in selected high-risk patients after mastectomy, in accordance with current guidelines. Adjuvant endocrine therapy was administered for 5 years to all women with oestrogen receptor-positive breast cancer after the completion of chemotherapy. Trastuzumab was recommended for women with HER2-positive tumours (immunohistochemistry 3+) for a total duration of 1 year. For the purpose of this study, we asked our database for patients who had undergone AC for Stages I-III breast cancer from January 2018 to November 2021. To be eligible for the present study, patients had to fulfil the following criteria: female gender, age ranging from 18 to 75 years old, diagnosis of unilateral or bilateral operable primary breast carcinoma without distant metastases, and sequential chemotherapy comprising epirubicin and cyclophosphamide followed by taxane. Exclusion criteria were neoadjuvant chemotherapy, metastatic disease, recurrent breast cancer, pregnancy, or lactation.

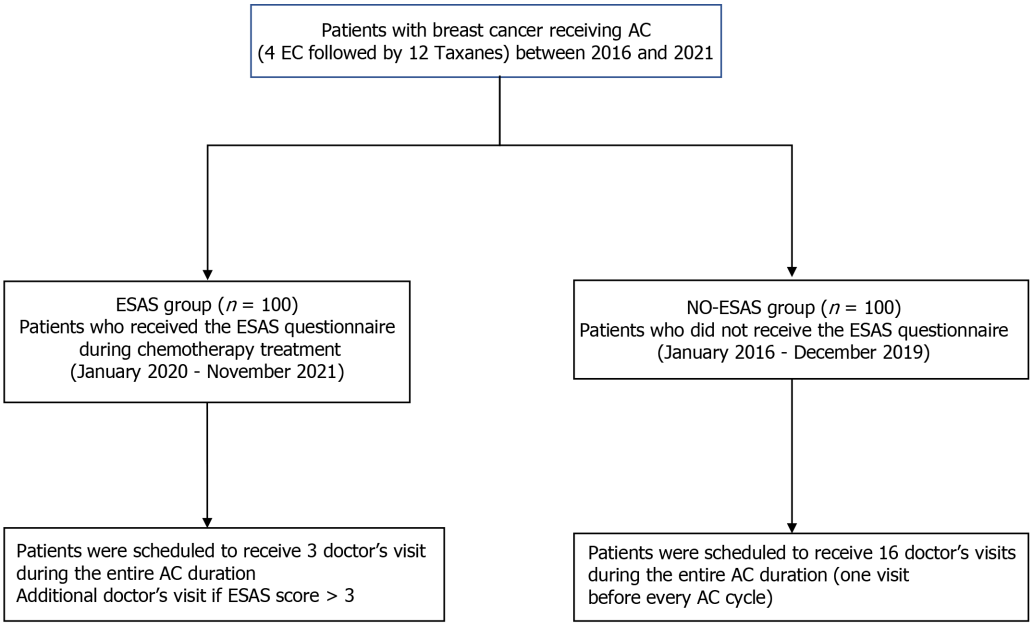
Study design

The study was approved by the Institutional Board of the AOU of Sassari. From January 2020, patients scheduled for AC were offered to participate in a programme where the ESAS was provided during the chemotherapy treatment period. All patients signed a written consent form before entering the ESAS programme. In a case-matched analysis, data from 100 patients taking the ESAS (the ESAS Group) in the period January 2020 to November 2021 were compared with data of 100 patients who underwent AC according to the traditional modality, without the ESAS (the no-ESAS Group) during the previous period (January 2016-December 2019). All patients of the study were scheduled to receive the following sequential regimen: Four cycles of epirubicin and cyclophosphamide followed by 12 cycles of paclitaxel (4EC-12T). Patients of the ESAS Group received the ESAS questionnaire translated into Italian before every cycle of AC; a medical visit was scheduled before the first cycle of epirubicin and cyclophosphamide, before the first cycle of taxane, and before the last cycle of taxane[12,13]. Therefore, each patient of the ESAS Group was scheduled to receive a total of three medical visits for the entire AC duration; an additional medical visit before each chemotherapy session was carried out according to the ESAS score (specifically in the all cases where the ESAS score was > 3). Patients of the no-ESAS Group received a medical visit before every cycle of AC. Therefore, each patient of the no-ESAS Group was scheduled to receive a total of 16 medical visits for the entire AC duration (Figure 1).

The matching variables included age and breast cancer stage. We decided to perform a case-matched analysis to obtain a more homogenous control group, and to minimise differences between groups due to the extent of disease. For all patients, the following data were extracted: Age, year of diagnosis, menopausal status, tumour size, histological type, axillary lymph nodes status, immunohistochemical classification, type of upfront surgery, and breast cancer stage. In the ESAS Group, patients who needed additional medical visits based on the ESAS score > 3, were identified. In both study groups (ESAS and no-ESAS) percentage of patients requiring unplanned medical visits (defined as visits for problems related to the surgical procedure or chemotherapy-related side effects), the number of unplanned medical visits, and grade 3-4 adverse effects during chemotherapy treatment, were calculated.

Study endpoints and statistical analysis

The primary endpoint was the total number of medical visits per patient during the entire duration of AC. The secondary endpoints were the occurrence of severe complications (grade 3-4) during the administration of AC and the number of unplanned visits during the cycles of chemotherapy. In addition, independent factors associated with the likelihood of receiving additional visits due to an ESAS score > 3 were analysed. Quantitative variables are presented as a mean; qualitative variables are presented as absolute numbers and percentages. Categorical variables were compared by the chi-square test or Fisher's exact test where appropriate. Continuous variables were assessed by Student's *t*-test or the Mann-Whitney U test. A P value < 0.05 was used as the threshold for statistical significance. In the ESAS Group, the likelihood of receiving additional visits on the basis of an ESAS score > 3 was analysed



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Figure 1 Study design. AC: Adjuvant chemotherapy; ESAS: Edmonton Symptom Assessment Scale.

with a multivariable logistic regression model. Each factor was dichotomised to a binary variable: Age (≤ 60 years *vs* > 60 years), type of surgery (BCS *vs* mastectomy), immunohistochemical classification (luminal *vs* non-luminal), and tumour stage (stage I *vs* stage II/III). Covariates were chosen on the basis of clinical significance. For each dichotomous variable, a reference category was chosen, generally the majority category, and compared with the other category. The odds ratio (OR) in each category *vs* the reference category was estimated. The goodness of fit of the model was assessed by the Hosmer-Lemeshow test, and $P > 0.05$ indicated a good fit. Statistical analyses were conducted by using SPSS Statistics 20 (IBM Corp., Armond, NY, United States).

RESULTS

Patient and tumour characteristics

Demographic and tumour characteristics are presented in Table 1. The mean age at diagnosis was 57.2 years. Tumour size was ≤ 2 cm in 48% of patients and > 2 cm in 52%. The most common histology was invasive ductal carcinoma (86%), followed by lobular invasive carcinoma (14%). Thirty-five per cent of patients were premenopausal. The majority of patients had tumours of stage II/III (60%). Fifty-three per cent of patients underwent BCS, while 47% underwent a mastectomy. Axillary lymph node status was positive in 37% of cases and negative in 63%. Regarding the immunohistochemical classification, the most frequent subtype was HER2-enriched (54%), followed by luminal B (23%), triple-negative (13%), and luminal A (10%) tumours. The study variables did not differ significantly between patients of the ESAS Group and the no-ESAS Group (mean age $P = 0.524$; age ≤ 60 years $P = 0.880$; breast cancer stage $P = 0.56$; cancer histology $P = 0.415$; tumour size $P = 0.258$; axillary lymph node status $P = 0.883$; immunohistochemical classification $P = 0.754$; type of surgery $P = 0.157$), except for premenopausal status, which was more frequent in the ESAS Group ($P = 0.015$). There were there 8 additional unplanned visits for 6 patients in the ESAS Group, and 18 additional visits for 12 patients in the no-ESAS Group ($P = 0.035$) Six patients of the ESAS Group and 12 of the no-ESAS Group needed one or more unplanned visit during the AC duration, for a total of 8 and 18 visits, respectively ($P = 0.057$). Grade 3-4 toxicity occurred in two and three patients of the ESAS Group and the no-ESAS Group, respectively ($P = 0.652$). Forty-eight patients of the ESAS Group received an additional visit due to an ESAS score > 3. Globally, the mean number of medical visits was 4.38 ± 0.51 in the ESAS Group and 16.18 ± 1.82 in the no-ESAS Group ($P < 0.001$) (Table 2).

Based on multivariate analysis, women of the ESAS Group were more likely to undergo additional visits before chemotherapy for an ESAS score > 3 if they were aged > 60 years, received a mastectomy, or had tumour stage II/III (Table 3). We did not find any association between additional visits and immunohistochemical tumour classification or lymph node status. Age > 60 years was the strongest predictor of receiving additional medical visits before chemotherapy (OR 4.93, 95% confidence interval 1.26-19.25).

Table 1 Clinicopathological characteristics of the study population

Characteristic	Group A (ESAS) (*n* = 100)	Group B (No-ESAS) (*n* = 100)	*P* value
Age (mean ± SD)	57.7 ± 11.5	56.6 ± 12.4	0.524
Age groups, *n* (%)			0.880
≤ 60 yr	64 (64)	62 (62)	
> 60 yr	36 (36)	38 (38)	
Premenopausal status	51 (51)	34 (34)	0.015
Breast cancer stage, *n* (%)			
I	44 (44)	36 (36)	0.506
II	28 (28)	31 (31)	
III	28 (28)	33 (33)	
Cancer histology, *n* (%)			
Ductal	84 (84)	88 (88)	0.415
Lobular	16 (16)	12 (12)	
Tumour size (mean ± SD) , *n* (%)			0.258
≤ 2 cm	52 (52)	44 (44)	
> 2 cm	48 (48)	56 (56)	
Lymph node status, *n* (%)			0.883
N0	66 (66)	59 (59)	
N+	34 (34)	41 (41)	
Himmunohistochemical classification, *n* (%)			0.754
Luminal A	12 (12)	8 (8)	
Luminal B	21 (21)	25 (25)	
HER2 positive	55 (55)	54 (54)	
TNBC	12(12)	13 (13)	
Type of surgery, *n* (%)			0.157
BCS	58 (58)	48 (48)	
Mastectomy	42 (42)	52 (52)	

ESAS: Edmonton Symptom Assessment Scale; BCS: Breast-conserving surgery.

DISCUSSION

Various chemotherapy regimens, which can be associated with either minor or major toxicity, are commonly used for AC in patients undergoing surgery for breast cancer[4,14]. However, no recognised guidelines exist regarding some aspects of this important part of the multidisciplinary treatment. The main result of this case-matched analysis is that ESAS screening may safely reduce the frequency of medical visits in the setting of AC in patients with breast cancer. This finding may have some advantageous implications in oncological practice, especially in the current scenario, where an increase in coronavirus pandemic 2019 (COVID-19) cases throughout the world has imposed measures for minimising the risk of infection among patients and health care providers.

Pre-chemotherapy assessment varies among oncology services. On a general basis, during the medical visit before chemotherapy, relevant information to manage any possible treatment side effect are collected, and a physical examination might be carried out. In the present study, we have used the ESAS score as a patient-reported outcomes tool. The ESAS is one of the first multidimensional assessment tools that has been used in clinical practice. The scale was created for the clinical assessment of the increase and modification of symptoms in patients with advanced cancers admitted to palliative care units[11,15,16]. The ESAS score has subsequently been validated in various studies and used as a tool for the detection of symptoms divided by clusters, favouring the implementation of interventions for symptom management[17]. In patients with breast cancer, correct symptom assessment and

Table 2 Outcomes of interest during the chemotherapy treatment in the study population

Variable	Group A (ESAS)	Group B (No ESAS)
N of doctor visit scheduled for each patient	3	16
Total No. of scheduled doctor visits	300	1600
N of patients requiring adjunctive visit on the bases of ESAS score > 3	48	-
N of adjunctive visits on the bases of ESAS score > 3	130	-
N of patients requiring unplanned doctor visits	6	12
N of unplanned doctor visit	8	18
Effective total No. of doctor visits[a]	438	1618
N of visits for each patient (mean ± SD)[b]	4.38 ± 0.51	16.18 ± 1.82
Adverse effects during chemotherapy treatment	2	3

[a]$P < 0.001$.
[b]$P < 0.001$. ESAS: Edmonton Symptom Assessment Scale.

Table 3 Multivariate logistic regression for factors associated with the need of additional medical visits before chemotherapy in patients the Edmonton Symptom Assessment Scale Group (Edmonton Symptom Assessment Scale score > 3)

Variable	Odds ratio	St. Error	Z-score	95%CI	*P* value
Age					
> 60 (*n* = 36)	4.93	0.695	1.596	1.26-19.25	0.022[a]
≤ 60 (*n* = 64)	Ref.				
Lymph node status					
Positive (*n* = 44)	0.50	0.662	0.691	0.13-1.83	0.297
Negative (*n* = 66)	Ref.				
Type of surgery					
Mastectomy (*n* = 42)	0.15	0.726	-1.895	0.03-0.62	0.009[b]
BCS (*n* = 58)	Ref.				
IHC classification					
Luminal (*n* = 33)	1.96	0.699	0.674	0.49-7.73	0.335
Non-Luminal (*n* = 67)	Ref.				
Tumour stage					
I (*n* = 44)	0.86	0.880	0.149	1.12-35.44	0.036[c]
II/III (*n* = 56)	Ref.				

[a]$P < 0.05$.
[b]$P < 0.05$.
[c]$P < 0.05$. BCS: Breast-conserving surgery.

management still represent a challenge for medical oncologists[18]. Specifically, in the early setting of the disease, the correct assessment and management of symptoms is essential to improve quality of life and patient adherence to treatments and, therefore, the effectiveness of adjuvant therapies.

Several studies have explored the role of the ESAS to predict patient-related outcomes in patients with breast cancer, especially in the setting of advanced disease[19]. In a recent review including nine articles, the authors reported that the ESAS score is a promising tool for predictive modelling of time to death in patients with breast cancer receiving palliative care[19]. However, few studies have investigated the role of the ESAS in the setting of breast cancer. In patients with non-metastatic breast cancer who received radiotherapy, the ESAS score has been used to identify significant symptoms linked to a worse overall quality of life[20].

In the series described herein, we found that the patients who completed the ESAS questionnaire received significantly fewer medical visits during chemotherapy period compared with patients of the control group. In the series described herein, we found that the use of the ESAS questionnaire allowed to identify patients who required additional medical visits before a chemotherapy cycle. To note, the reduction in the number of scheduled visits based on the ESAS score, did not affect the occurrence of complications from chemotherapy, and was associated to a reduced number of unplanned medical visits. In fact, patients of the ESAS Group were scheduled to receive only three visits; additional visits were deemed necessary only when the ESAS score was > 3. These findings are consistent with the experience of Barbera *et al*[14], who demonstrated that screening with the ESAS was associated with decreased emergency department visits by patients with breast cancer receiving AC. It has been suggested that screening of routine symptoms, using tailored patient-reported outcomes tools, could be useful for improving patient/physician communication, helping to monitor the treatment response and identifying unrecognised problems[20-23].

In this study, we hypothesised that the ESAS score in the setting of AC would be able to safely reduce the number of medical visits. We used the occurrence of grade 3-4 chemotherapy toxicity as a surrogate of safety; this measure did not differ between the two study groups. The need for medical visits in patients undergoing AC for breast cancer depends on many tumour- and patient-related factors[4,14]. In our experience, patients aged > 60 years had a fourfold increased risk of receiving additional visits based on the ESAS score, reflecting the importance of patient age regarding anticancer treatments. Of note, the number of unplanned medical visits due to acute toxicity experienced by patients was lower in the ESAS Group. In another study involving a cohort of 2541 patients with stage I-III breast cancer, women undergoing chemotherapy for breast cancer screened with the ESAS had a 43% lower rate of emergency department visits than those who were not screened with the ESAS[14].

Medical visits for pre-chemotherapy assessment represent a significant burden on the oncological care system. There are several potential advantages of reducing the number of medical visits in patients receiving AC. First, although we did not calculate the time spent on every visit, we can assume that the reduced number of medical visits does translate to a significant sparing of time in oncology departments; hence, oncologists and nurses may spend their time on other clinical activities. This may have important implications especially in high-volume oncology centres. Second, the ESAS score permits patients to take an active role in deciding the course of their AC treatment. Generally, patient-reported outcomes have been gaining importance for describing subjective symptoms and improving quality of life[4,23,24]. Studies have compared the description of toxicity and adverse effects by using patient-related-outcome tools in comparison with physician-reported findings. A possible underestimation of the incidence and the entity of symptoms reported by physicians has been evidenced[25,26]. Baratelli *et al* demonstrated, in a cohort of 211 patients receiving active anticancer treatment, that these tools produced high patient satisfaction and a significant quality-of-life improvement, compared with the traditional modality of a medical visit[23]. Third, in the current scenario, where contact restrictions are encouraged, use of the ESAS questionnaire may reduce the risk of COVID-19 infections among oncologic patients. In fact, the decrease in medical visits could reduce both personal contacts and the duration of stay in oncology units among patients with chemotherapy-induced immunosuppression. At the time of writing, the world is experiencing a new wave of the pandemic due to the delta and omicron variants of severe acute respiratory syndrome coronavirus 2.

Several studies have investigated the role of the ESAS score on quality-of-life perception, supportive care needs and symptom assessment in patients with cancer; however, to the best of our knowledge, this is the first study focussing on its impact on medical visits in the setting of AC. We recognise that this work has some limitations, the main one being the small sample size. Furthermore, we arbitrarily decided to set the ESAS score cut-off point for patients to receive additional medical visits for AC administration as 3. Regarding this matter, the optimal cut-off points for the symptoms and quality indicators of the ESAS remain ill defined[27,28].

CONCLUSION

In summary, our work provides evidence that the use of the ESAS score may safely reduce the number of medical visits in patients undergoing AC. Moreover, it implies that ESAS may help to identify patients who do not need to visit a doctor during each course of chemotherapy, as well as to identify a group of patients with a high risk of complications in whom a treatment adjustment is needed. This may result in several advantages for both patients and health care providers, especially in the current COVID-19 pandemic. Additional studies are needed to gain new insights into the role of patient-reported outcome strategies in the management of AC in the setting of breast cancer.

ARTICLE HIGHLIGHTS

Research background

Adjuvant chemotherapy (AC) represents a fundamental part of multidisciplinary treatment of women with high-risk breast cancer, since it has been associated to a reduced risk of developing cancer recurrence, as well as to an increased survival. However, no standardised guidelines that regulate the pre-treatment assessment of patients candidates for AC exist. In common practice, a pre-chemotherapy medical visit before every cycle of AC is scheduled, and this represents a time- and resource-demanding practice.

Research motivation

Accurate use of the Edmonton Symptom Assessment Scale (ESAS) may lead to identify patients who do not need to visit a doctor during each course of AC.

Research objectives

To evaluate the value of the ESAS in safely reduce the number of medical visits prior adjuvant chemotherapy.

Research methods

One-hundred breast cancer women candidates to AC were administered the ESAS score (ESAS Group), and were scheduled to receive a total of three medical visits for the entire AC duration. They were prospectively compared to a to a matched-pair group of 100 patients who received adjuvant chemotherapy without ESAS (no-ESAS Group) and were scheduled to receive 16 medical visits for the entire AC duration. Study endpoints were the number of medical visits, occurrence of severe complications, and the number of unplanned visits.

Research results

The mean number of medical visits was 4.38 ± 0.51 in the ESAS Group and 16.18 ± 1.82 in the no-ESAS group ($P < 0.001$). Unplanned visits during the entire duration of chemotherapy were 8 in the ESAS Group and 18 in the no-ESAS Group visits ($P = 0.035$). Grade 3-4 toxicity did not differ between the study groups ($P = 0.652$). Forty-eight patients of the ESAS Group received additional visits due to an ESAS score > 3. With multivariate analysis, women of the ESAS group were more likely to undergo additional visits for an ESAS score > 3 if they were aged 60 or older, received a mastectomy, or had tumour stage II/III.

Research conclusions

Our results suggest that the ESAS score may be used for selecting a group of breast cancer patients for whom it is safe to reduce the number of medical visits in the setting of AC. This may permit a more rational utilization of human resources and a possible reduction of coronavirus pandemic 2019 infection risk in oncologic patients.

Research perspectives

Additional studies are needed to gain new insights into the role of patient-reported outcome strategies in the management of AC in the setting of breast cancer.

FOOTNOTES

Author contributions: Sanna V and Fancellu A designed the study, supervised, wrote and edited the final version; Deiana G, Ninniri C and Alicicco MG provided original data, collected variables, and analysed data; Fedele *P* and Santoro AN provided technical support, figures, tables, and reviewed the manuscript; Pazzola A envisioned the study, and edited the final manuscript.

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ORIGINAL ARTICLE

Case Control Study

Awareness, knowledge, and attitudes towards sun protection among patients with melanoma and atypical mole syndrome

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Abstract

BACKGROUND

Patients with atypical mole syndrome (AMS) have a 3- to 20-fold higher risk of developing malignant melanoma (MM) than individuals without. The most modifiable risk factor for developing MM is the ongoing ultraviolet exposure.

AIM

To assess awareness, knowledge, and attitudes towards sun protection among patients with MM and AMS.

METHODS

From January 2020 till December 2021, a written survey was administered to patients with MM and AMS and a control group who attended a specialist mole clinic at the Dermatology Department of the University Hospital of Heraklion in Heraklion, Crete, Greece. Demographic data and photoprotective practices, knowledge, and perceived barriers were collected. Relevant statistical analyses were performed using SPSS IBM 25.

RESULTS

In total, 121 subjects consented and participated in the survey. Their mean age was 43.92 ± 12.55 years. There were 66 (54.4%) females and 55 (45.4%) males. Forty-seven (38.8%) patients had AMS, 26 (21.5%) had a past medical history of

MM, and 48 (39.7%) attended the clinic for a full skin checkup for their naevi without having AMS or MM. Although 104 (86%) participants reported using sunscreen with the majority of them (59/121 = 48.8%) wearing sunscreen with a sun protection factor of > 50, only 22 (18.2%) patients did so every day and only 20 (16.5%) all year round. Approximately 74.4% of patients recalled having received advice on how to protect their skin from sunlight, and 73% were interested in receiving education about sun protection. The most mentioned barriers in photoprotection were concerns over adequate vitamin D and lack of time.

CONCLUSION

Despite mentioning having received adequate education in photoprotection, adherence to photoprotection practices is suboptimal in patients with MM and AMS.

Key Words: Atypical mole syndrome; Dysplastic naevi; Malignant melanoma; Photoprotection; Skin cancer

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Core Tip: There are no previous studies assessing awareness, knowledge, and attitudes towards sun protection among patients with malignant melanoma (MM) and atypical mole syndrome (AMS). Our study highlights the importance to raise awareness regarding photoprotection in patients with MM and AMS to prevent skin cancer.

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INTRODUCTION

The term atypical mole syndrome (AMS) refers to people who have multiple naevi (> 100), including some naevi larger than 8 mm in diameter with atypical features[1,2]. Patients with AMS have a 3-20 times higher risk of developing malignant melanoma (MM) than individuals without[3-6]. The most modifiable risk factor for developing MM is ongoing ultraviolet (UV) exposure[7]. Eliminating UV exposure *via* photoprotective practices is an important strategy for reducing MM risk in patients with AMS[8-10].

Through the implementation of a written survey, our aim for this study was to assess awareness, knowledge, and attitudes toward sun protection among patients with MM, those with AMS, and a control group who attended a specialist mole clinic at the dermatology department of a tertiary hospital in Greece.

MATERIALS AND METHODS

From January 2020 through December 2021, we administered a written survey to patients who attended a specialist mole clinic at the Dermatology Department of the University Hospital of Heraklion in Heraklion, Crete, Greece. Having approached 140 patients, we obtained consent from 121 patients (a response rate of 121/140 = 86.42%). The participants completed the surveys in person, and we included all the data in our analysis.

The specialist mole clinic at the Dermatology Department of the University Hospital of Heraklion is a dedicated clinic for patients at high risk of developing skin cancer, such as those who have a past medical history (PMH) of MM, non-melanoma skin cancer, or AMS or who have received immunosuppression (*e.g.*, transplant patients). All these patients undergo annual or biannual full skin checkups and receive photoprotection counselling.

The study was approved by the Ethics Committee of the University Hospital and all participants gave consent for inclusion in the study.

Survey contents

The written survey that we administered included basic demographic data, Fitzpatrick skin phototypes, medical histories, comorbidities, and collected information regarding awareness and knowledge of

Table 1 Demographic and clinical characteristics of 121 patients who were included in our study

	Patients with a PMH of MM, *n* = 26/121 (21.5%)	Patients with AMS, *n* = 47/121 (38.8%)	Control group, *n* = 48/121 (39.7%)	All participants, *n* = 121	*P* value
Mean age (± SD)	45.65 (± 12.61)	43.21 (± 12.61)	43.67 (± 12.65)	43.92 (± 12.55)	0.88
Gender, *n* (%)					0.39
Male	10/26 (38.5)	25/47 (53.2)	20/48 (41.7)	55/121 (45.5)	
Female	16/26 (61.5)	22/47 (46.8)	28/48 (58.3)	66/121 (54.5)	
Employment Status, *n* (%)					0.40
Student	2/26 (7.7)	3/47 (6.4)	2/48 (4.2)	7/121 (5.8)	
Employed	16/26 (61.5)	36/47 (76.6)	33/48 (68.8)	85/121 (70.2)	
Unemployed	2/26 (7.7)	4/47 (8.5)	7/48 (14.6)	13/121 (10.7)	
Retired	5/26 (19.2)	3/47 (6.4)	3/48 (6.3)	11/121 (9.1)	
Housewife	1/26 (3.8)	1/47 (2.1)	3/48 (6.3)	5/121 (4.1)	
Educational level, *n* (%)					0.61
Elementary school	9/26 (34.6)	1/47 (2.1)	3/48 (6.3)	4/121 (3.3)	
High school	0/26 (0)	20/47 (42.6)	21/48 (43.8)	50/121 (41.3)	
Technical studies	6/26 (23.1)	8/47 (17)	5/48 (10.4)	19/121 (15.7)	
University level	11/26 (42.3)	18/47 (38.3)	19/48 (39.6)	48/121 (39.7)	
Fitzpatrick skin phototype, *n* (%)					0.81
Skin type I (Always burns, does not tan)	0/26 (0)	2/47 (4.3)	2/48 (4.2)	4/121 (3.3)	
Skin type II (Burns easily, tans poorly)	10/26 (38.5)	14/47 (29.8)	15/48 (31.3)	39/121 (32.2)	
Skin type III (Tans after initial burn)	14/26 (53.8)	24/47 (51.1)	22/48 (45.8)	60/121 (49.6)	
Skin type IV (Burns minimally, tans easily)	2/26 (7.7)	7/47 (14.9)	9/48 (18.8)	18/121 (14.9)	
BMI (± SD)	25.07 (± 4.06)	26.92 (± 5.12)	25.58 (± 5.20)	25.99 (± 4.96)	0.281
Eye colour, *n* (%)					0.466
Dark	1/26 (3.8)	2/47 (4.3)	5/48 (10.4)	8/121 (6.6)	
Brown	16/26 (61.5)	31/47 (66)	31/48 (64.4)	78/121 (64.5)	
Blue	3/26 (11.5)	9/47 (19.1)	5/48 (10.4)	17/121 (14)	

Green	6/26 (23.1)	5/47 (10.6)	7/48 (14.6)	18/121 (18)	
Natural hair color, *n* (%)					0.649
Red	0/26 (0)	1/47 (2.1)	0/48 (0)	1/121 (0.8)	
Blond	5/26 (19.2)	6/47 (12.8)	8/48 (16.7)	19/121 (15.7)	
Brown	16/26 (61.5)	34/47 (72.3)	28/48 (58.3)	78/121 (64.5)	
Black	5/26 (19.2)	6/47 (12.8)	12/48 (25)	23/121 (19)	
Number of naevi, *n* (%)					0.000
< 25 naevi	9/26 (34.6)	0/47 (0)	23/48 (47.9)	32/121 (26.4)	
25-50 naevi	8/26 (30.8)	2/47 (4.3)	12/48 (25)	22/121 (18.2)	
50-100 naevi	3/26 (11.5)	11/47 (23.4)	7/48 (14.6)	21/121 (17.4)	
100 naevi	6/26 (23.1)	34/47 (72.3)	6/48 (12.5)	46/121 (38)	
Smoking status, *n* (%)					
Current smoker	8/26 (30.8)	15/47 (31.9)	9/48 (18.8)	32/121 (26.4)	0.198
No smoker	15/26 (57.7)	25/47 (53.2)	28/48 (58.3)	68/121 (56.2)	
Ex-smoker	3/26 (11.5)	7/47 (14.9)	11/48 (22.9)	21/121 (17.4)	
Sunburn before the age of 18, *n* (%)					0.000
No	9/26 (34.6)	30/47 (63.8)	40/48 (83.3)	79/121 (65.3)	
Yes	17/26 (65.4)	17/47 (36.2)	8/48 (16.7)	42/121 (34.7)	
Leisure sun exposure, *n* (%)					0.393
No	17/26 (65.4)	31/47 (66)	38/48 (79.2)	86/121 (71.1)	
Yes	9/26 (34.6)	16/47 (34)	10/48 (20.8)	35/121 (28.9)	
Occupational sun exposure, *n* (%)					0.35
No	19/26 (73.1)	27/47 (57.4)	30/48 (62.5)	76/121 (62.8)	
Yes	7/26 (26.9)	20/47 (42.6)	18/48 (37.5)	45/121 (37.2)	
Significant time spent outdoors, *n* (%)					0.356
No	11/26 (42.3)	24/47 (51.1)	28/48 (58.3)	63/121 (52.1)	
Yes	15/26 (57.7)	23/47 (48.9)	20/48 (42.7)	58/121 (47.9)	
Mean weeks of vacation spent before the age of 10 (± SD)	7.35 ± 5.61	6.87 ± 5	7.42 ± 4.36	7.19 ± 4.86	0.7444

Mean weeks of vacation spent before from the age of 11 till 18 (± SD)	6.12 ± 4.27	6.38 ± 4.44	6.94 ± 4.20	6.55 ± 4.29	0.740
Mean weeks of vacation spent after the age of 18 (± SD)	3.92 ± 2.1	4.02 ± 2.77	4.98 ± 4.35	4.39 ± 3.40	0.806

AMS: Atypical mole syndrome; PMH: Past medical history; MM: Malignant melanoma; SD: Standard deviation; BMI: Body mass index.

photoprotection measures and current sun-protective practices. The participants were asked to report any difficulties that discouraged them from practicing photoprotective measures. We administered the survey to patients after they received counseling on photoprotection from the dermatology outpatient mole clinic.

Statistical analysis

Descriptive statistics, ANOVA, Kruskal-Wallis test, *t* tests, and Pearson correlation tests were performed using SPSS version 25.0.

RESULTS

Demographic data

Of the 140 patients that we approached who attended the specialist mole clinic at the Dermatology Department of the University Hospital of Heraklion in Heraklion, Crete, Greece from January 2020 until December 2021, 121 consented to and participated in the study, making our response rate be 121/140. Their mean age was 43.92 ± 12.55 years. There were 66 (54.4%) females and 55 (45.4%) males. Forty-seven (38.8%) patients had AMS, 26 (21.5%) had a PMH of MM, and 48 (39.7%) attended the clinic for a full skin checkup for their naevi without having AMS or MM. The main demographic and clinical characteristics of these 121 patients are summarized in Table 1. There were no statistical differences among the three groups of patients for the following demographics and clinical characteristics: Age; gender; employment status; educational level; Fitzpatrick skin phototype; body mass index; eye and natural hair color; smoking status; leisure and occupational sun exposure; significant time spent outdoors; and mean weeks of vacation spent before the age of 10, from the ages of 11 to 18, and after the age of 18. There was a significant statistical difference among the three groups regarding history of sunburn before the age of 18 ($P < 0.001$). As expected, patients with a PMH of MM more frequently had a history of sunburn before the age of 18 than the group with AMS and the control group.

Photoprotective practices

Although 104 (86%) participants reported using sunscreen, with most of them (59/121 = 48.8%) reporting wearing sunscreen with a sun protection factor (SPF) of > 50, only 22 (18.2%) patients did so every day and only 20 (16.5%) did so all year round. Of all participants, 89 (73.6%) reported wearing sunscreen only during the summer and 94 (77.7%) only in direct sunny weather. Fifty-two patients reported reapplying sunscreen while outdoors and only a minority (37/121 = 30.58%) reported

Table 2 Sun protection practices in patients with malignant melanoma, those with atypical mole syndrome, and controls

	Patients with a PMH of MM, *n* = 26	Patients with AMS, *n* = 47	Control group, *n* = 48	All participants, *n* = 121	*P* value
Do you use sunscreen? *n* (%)					
No	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121(14)	0.461
Yes	21/26 (80 .8)	40/47 (85.1)	43/48 (89.8)	104/121 (86)	
If yes, which SPF sunblock rating do you use? *n* (%)					
< 30	2/26 (7.7)	5/47 (10.6)	9/48 (18.8)	16/121 (13.2)	0.222
≥ 30	7/26 (26.9)	10/47 (21.3)	14/48 (29.2)	31/121 (25.6)	
≥ 50	12/26 (46.2)	25/47 (53.2)	20/48 (41.7)	57/121 (47.1)	
No sunscreen use	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121 (14)	
How frequently do you use sunscreen? *n* (%)					
Everyday	5/26 (19.2)	6/47 (12.8)	11/48 (22.9)	22/121 (18.2)	0.663
Most days	4/26 (15.4)	13/47 (27.7)	10/48 (20.8)	27/121 (22.3)	
Occasionally	11/26 (42.3)	18/47 (38.3)	18/48 (37.5)	47/121 (38.8)	
Rarely	1/26 (3.8)	3/47 (6.4)	4/48 (8.3)	8/121 (6.6)	
No sunscreen use	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121 (14)	
During which seasons do you apply sunscreen? *n* (%)					
Only during the summer	17/26 (65.4)	37/47 (78.7)	30/48 (62.5)	84/121 (69.4)	0.353
All year-round	4/26 (15.4)	3/47 (6.4)	13/48 (27.1)	20/121 (16.5)	
No sunscreen use	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121 (14)	
In which of the following weather conditions do you apply sunscreen? *n* (%)					
Only in direct sunny weather	17/26 (65.4)	38/47 (80.9)	34/48 (70.8)	89/121 (73.6)	0.606
Both sunny and cloudy weather	4/26 (15.4)	2/47 (4.3)	9/48 (18.8)	15/121 (12.4)	
No sunscreen use	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121 (14)	
While outdoors, do you reapply sunscreen? *n* (%)					
No	16/26 (61.5)	21/47 (44.7)	33/48 (68.8)	70/121 (57.9)	0.31
Yes	10/26 (38.5)	26/47 (55.3)	15/48 (31.3)	51/121 (42.1)	
Do you reapply sunscreen after swimming or perspiring heavily? *n* (%)					
No	14/26 (53.8)	20/47 (42.6)	28/48 (58.3)	62/121 (51.2)	0.139
Yes	12/26 (46.2)	27/47 (57.4)	20/48 (41.7)	59/121 (48.8)	
Wearing UV-protective sunglasses, *n* (%)					
Everyday	13/26 (50)	21/47 (44.7)	12/48 (25)	46/121 (38)	0.303
Most days	5/26 (19.2)	10/47 (21.3)	16/48 (33.3)	31/121 (25.6)	
Occasionally	2/26 (7.7)	7/47 (14.9)	13/48 (27.1)	22/121 (18.2)	
Rarely	1/26 (3.8)	4/47 (8.5)	4/48 (8.3)	9/121 (7.4)	
Never	5/26 (19.2)	5/47 (10.6)	3/48 (6.3)	13/121 (10.7)	
Wearing a broad-brimmed hat, *n* (%)					
Everyday	5/26 (19.2)	3/47 (6.4)	3/48 (6.3)	11/121 (9.1)	0.535
Most days	1/26 (3.8)	6/47 (12.8)	6/48 (12.5)	13/121 (10.7)	
Occasionally	6/26 (23.1)	14/47 (29.8)	13/48 (27.1)	33/121 (27.3)	
Rarely	7/26 (26.9)	13/47 (27.7)	6/48 (12.5)	26/121 (21.5)	

Never	7/26 (26.9)	11/47 (23.4)	20/48 (41.7)	38/121 (31.4)	
Wearing long-sleeved shirts or long plants made from tight fabric weave, *n* (%)					
Everyday	1/26 (3.8)	2/47 (4.3)	1/48 (2.1)	4/121 (3.3)	0.275
Most days	4/26 (15.4)	11/47 (23.4)	9/48 (18.8)	24/121 (19.8)	
Occasionally	7/26 (26.9)	13/47 (27.7)	16/48 (33.3)	36/121 (29.8)	
Rarely	5/26 (19.2)	11/47 (23.4)	12/48 (25)	28/121 (23.1)	
Never	9/26 (34.6)	10/47 (21.3)	10/48 (20.8)	29/121 (24)	
Avoiding the sun during hours of peak sunlight intensity (10:00 am to 16:00 pm), *n* (%)					
Everyday	8/26 (30.8)	9/47 (19.1)	5/48 (10.4)	22/121 (18.2)	0.492
Most days	10/26 (38.5)	20/47 (42.6)	26/48 (54.2)	56/121 (46.3)	
Occasionally	4/26 (15.4)	11/47 (23.4)	13/48 (27.1)	28/121 (23.1)	
Rarely	4/26 (15.4)	5/47 (10.6)	0/48 (0)	9/121 (7.4)	
Never	0/26 (0)	2/47 (4.3)	4/48 (8.3)	6/121 (5)	

PMH: Past medical history; MM: Malignant melanoma; AMS: Atypical mole syndrome; UV: Ultraviolet.

reapplying sunscreen after swimming or perspiring. Photoprotective practices are summarized in Table 2.

Forty-six (46/121 = 38%) patients reported daily use of UV sunglasses. There was a tendency of more frequent daily use of sunglasses in the MM and AMS groups in contrast to the control group, but this was not statistically significant (P = 0.303). Eleven (9.1%) and four (3.3%) patients reported daily use of broad-brimmed hats and long-sleeved shirts, respectively, with no significant difference among the three groups. Only a minority of patients (22/121 = 18.2%) avoided the sun daily during peak hours of sunlight intensity.

Photoprotection education and perceived barriers

Most of the patients, 90/121 (74.4%), had been given advice on how to protect their skin from sunlight, with 86/121 (71.1%) receiving that advice from their family doctor. Photoprotection education is summarized in Table 3.

One third of patients (45/121 = 37%) were given sun protection education from a health-care professional more than three times; half of them (63/121 = 52.1%) were educated from multimedia sources; and most of them (104/121 = 86%) were given written photoprotective advice.

Most of the patients (88/121 = 73%) were interested in receiving education. Eighty-eight patients (72.7%) were interested in receiving sun protection advice from a health-care worker and 74 (61.2%) were interested in receiving photoprotection advice from multimedia sources.

Half of the patients (63/121 = 52.1%) had encountered barriers that discouraged them from practicing sun protection. These barriers are summarized in Table 4. A quarter of them (27/121 = 22.3%) claimed that they did not have time to practice photoprotection measures. Concerns over adequate vitamin D levels and financial concerns were reported by 28.9% and 15.7%, respectively. Only a minority reported appearance concerns (4.1%), difficulty in obtaining materials (5.8%), or previous unpleasant experiences with and bad reactions to sunscreen (7% and 0.8%, respectively). There was no statistical difference among the three groups in our study.

DISCUSSION

To the best of our knowledge, we have here presented the first study of its kind describing demographic and clinical characteristics and assessing awareness, knowledge, attitudes, and barriers toward photoprotective practices among patients with MM and AMS and a control group. We conducted our study in the city of Heraklion, Crete, Greece, which has a very high UV index and a significantly homogeneous population. Limitations of our study include the small sample of patients and the single-center location.

Our evidence indicates that adapting effective photoprotective practices, such as the daily use of high SPF sunblock, wearing a broad-brimmed hat and a long-sleeved shirt, and avoiding sun exposure between the peak hours of 10:00 a.m. to 4:00 p.m. protect against the development of skin cancer[8-12]. Therefore, assessing photoprotective education and attitudes and providing sun protection education

Table 3 Sun protection education in patients with malignant melanoma, those with atypical mole syndrome, and controls

	Patients with a PMH of MM, *n* = 26	Patients with AMS, *n* = 47	Control group, *n* = 48	All participants, *n* = 121	*P* value
Have you ever been given advice on how to protect your skin from sunlight? *n* (%)					
No	6/26 (23.1)	9/47 (19.1)	16/48 (33.3)	31/121 (25.6)	0.59
Yes	20/26 (76.9)	38/47 (80.9)	32/48 (66.7)	90/121 (74.4)	
Have you ever received sun protection education from a family doctor? *n* (%)					
No	6/26 (23.1)	11/47 (23.4)	18/48 (37.5)	35/121 (28.9)	0.109
Yes	20/26 (76.9)	36/47 (76.6)	30/48 (62.5)	86/121 (71.1)	
On how many occasions have you received sun protection education from a healthcare professional? *n* (%)					
Never	5/26 (19.2)	10/47 (21.3)	14/48 (29.2)	29/121 (24)	0.316
Once	4/26 (15.4)	5/47 (10.6)	6/48 (12.5)	15/121 (12.5)	
Twice	3/26 (11.5)	6/47 (12.8)	8/48 (16.7)	17/121 (14)	
3 times	4/26 (15.4)	5/47 (10.6)	6/48 (12.5)	15/121 (12.5)	
3 times	10/26 (38.5)	21/47 (44.7)	14/48 (29.2)	45/121 (37)	
Have you ever received sun protection education from Media (*i.e.*, television, newspaper)? *n* (%)					
No	11/26 (42.3)	22/47 (46.8)	25/48 (52.1)	58/121 (47.9)	0.546
Yes	15/26 (57.57)	25/47 (53.2)	23/48 (47.9)	63/121 (52.1)	
Have you ever received written advice about sun protection? *n* (%)					
No	21/26 (80.8)	38/47 (80.9)	45/48 (93.8)	17/121 (14)	0.055
Yes	5/26 (19.2)	9/47 (19.1)	3/48 (6.3)	104/121 (86)	
Would you be interested in receiving education about sun protection? *n* (%)					
No	4/26 (15.4)	12/47 (25.5)	17/48 (35.4)	33/121 (27)	0.619
Yes	22/26 (84.6)	35/47 (74.5)	31/48 (64.6)	88/121 (73)	
Would you be interested in receiving photoprotection advice about sun protection from a healthcare worker? *n* (%)					
No	4/26 (15.4)	16/47 (34)	17/48 (35.4)	33/121 (27.3)	0.154
Yes	22/26 (84.6)	31/47 (66)	31/48 (64.6)	88/121 (72.7)	
Would you be interested in receiving photoprotection advice about sun protection from multimedia? *n* (%)					
No	11/26 (42.3)	16/41 (34)	20/48 (41.7)	47/121 (36.8)	0.693
Yes	15/26 (57.7)	31/41 (66)	28/48 (58.3)	74/121 (61.2)	

PMH: Past medical history; MM: Malignant melanoma; AMS: Atypical mole syndrome.

are both important and effective in preventing skin cancer, especially in areas with high UV indexes such as Crete, Greece.

Our survey highlighted that although most of the patients used sunscreen (104/121 = 86%), and half of them (57/121 = 47.1%) used sunscreen with an SPF of > 50, only a small proportion of them (22/121 = 18.2%) applied it daily, and the majority (84/121 = 69.4%) applied it only during the summer. Many participants reported never having worn a broad-brimmed hat (38/121 = 31.4%), a long-sleeved shirt, or long pants (29/121 = 24%) to protect themselves from sunlight. There was no statistical difference among the three groups regarding sun protection practices.

Most of the patients (90/121 = 74.4%) recalled having received advice on how to protect their skin from sunlight. This shows high recall of receiving photoprotective education (this number has varied

Table 4 Perceived barriers to implementation of photoprotection practices in patients with malignant melanoma, those with atypical mole syndrome, and controls

	Patients with a PMH of MM, *n* = 26	Patients with AMS, *n* = 47	Control group, *n* = 48	All participants, *n* = 121	*P* value
Have any of the following barriers discouraged you from practicing sun protection? *n* (%)					
No	7/26 (26.9%)	27/47 (57.4%)	24/48 (50%)	58/121 (47.9%)	0.656
Yes	19/26 (73.1%)	20/47 (42.6%)	24/48 (50%)	63/121 (52.1%)	
Skepticism ("I do not believe skin cancer is a serious health threat"), *n* (%)					
No	22/26 (84.6%)	43/47 (91.5%)	45/48 (93.8%)	110/121 (90.9%)	0568
Yes	4/26 (15.4%)	4/47 (8.5%)	3/48 (6.3%)	11/121 (9.1%)	
Hassle/lack of time, *n* (%)					
No	21/26 (80.8%)	35/47 (74.5%)	38/48 (79.2%)	94/121 (77.7%)	0.639
Yes	5/26 (19.2%)	12/47 (25.5%)	10/48 (20.8%)	27/121 (22.3%)	
Concerns over adequate Vitamin D, *n* (%)					
No	20/26 (76.9%)	34/47 (72.3%)	32/48 (66.7%)	86/121 (71.1%)	0.486
Yes	6/26 (23.1%)	13/47 (27.7%)	16/48 (33.3%)	35/121 (28.9%)	
Cost/financial concerns. *n* (%)					
No	25/26 (96.2%)	38/47 (80.9%)	39/48 (81.3%)	102/121 (84.3%)	0.810
Yes	1/26 (3.8%)	9/47 (19.1%)	9/48 (18.8%)	19/121 (15.7%)	
Appearance ("I do not like how using sun protection will make me look"), *n* (%)					
No	24/26 (92.3%)	44/47 (93.6%)	48/48 (100%)	116/121 (95.9%)	0.090
Yes	2/26 (7.7%)	3/47 (6.4%)	0/48 (0%)	5/121 (4.1%)	
Difficulty obtaining materials (sunscreen, sunglasses, hats, *etc*), *n* (%)					
No	25/26 (96.2%)	44/47 (93.6%)		114/121 (94.2%)	0.962
Yes	1/26 (3.8%)	3/47 (6.4%)		7/121 (5.8%)	
Sunscreen is uncomfortable or unpleasant, *n* (%)					
No	22/26 (84.6%)	43/48 (91.5%)	47/48 (97.9%)	112/121 (93%)	0.149
Yes	4/26 (15.4%)	4/48 (8.5%)	1/48 (2.1%)	9/121 (7%)	
Previous "bad" reaction to sunscreen (please specify), *n* (%)					
No	25/26 (96.2%)	47/47 (100%)	48/48 (100%)	120/121 (99.2%)	0.765
Yes	1/26 (3.8%)	0/47 (0%)	0/48 (0%)	1/121 (0.8%)	
None/no barriers have discouraged me, *n* (%)					
No	14/26 (53.8%)	21/121 (44.7%)	23/48 (47.9%)	58/121 (47.9%)	0.840
Yes	12/26 (46.2%)	26/121 (55.3%)	25/48 (52.1%	63/121 (52.1%)	

PMH: Past medical history; MM: Malignant melanoma; AMS: Atypical mole syndrome.

from 27.5% to 96% in previous papers). Our survey highlights that, despite recalling having received adequate photoprotection education, the implementation of sun protective practices in all the three groups remained suboptimal. Our study showed that adherence to photoprotective practices did not correlate with education level. Previous studies have documented that a lack of post-secondary education was correlated with a reduced adoption of sun protective behaviors[13-18].

Several barriers regarding photoprotection have been reported in the literature. In our cohort, the three most-cited barriers were "concerns over adequate vitamin D" (35/121 = 28.9%), "hassle/lack of

time" (27/121 = 22.3%), and "cost/financial concerns" (19/121 = 15.7%). Only the barrier "lack of time" was consistent with previous studies[19-23].

We also found that 72.7% of the subjects expressed interest in receiving photoprotection advice from a health-care worker and 61.2% from multimedia sources. This indicates that patients might prefer receiving verbal advice from a health-care professional, and that electronic devices might also play a crucial role in relevant education[24-27]. However, the use of multimedia methods in educating people on photoprotective practices may be inefficient for older patients.

Our study has both strengths and limitations. A dermatologist assessed all participants, and the questionnaire was not only self-reported but also the patient and the dermatologist completed the questionnaire together at the same time. The dermatologist, who examined the patient, gave more accurate data. Furthermore, the design of our study involves consecutive patients who were recruited during a specific timeline. Limitations include the small sample of patients and the single-center hospital-based nature of the study. We recruited patients and controls consecutively from a tertiary referral mole clinic who were dermatology department patients. These patients might be more motivated toward skin cancer prevention knowledge and photoprotection measures, which may limit the generalizability of our results.

CONCLUSION

Considerable efforts should be made to raise awareness regarding photoprotection practices with the aim to prevent skin cancer in patients with MM and AMS.

ARTICLE HIGHLIGHTS

Research background

Patients with atypical mole syndrome (AMS) have a 3- to 20-fold higher risk of developing malignant melanoma (MM) than individuals without.

Research motivation

The most modifiable risk factor for developing MM is the ongoing ultraviolet exposure.

Research objectives

To assess awareness, knowledge, and attitudes towards sun protection among patients with MM and AMS.

Research methods

A written survey was administered to patients with MM, those with AMS, and a control group who attended a specialist mole clinic in Heraklion in Greece.

Research results

In total 121 subjects participated in the study. Their mean age was 43.92 ± 12.55 years. There were 66 (54.4%) females and 55 (45.4%) males. Forty-seven (38.8%) patients had AMS, 26 (21.5%) had a past medical history (PMH) of MM, and 48 (39.7%) attended the clinic for a full skin checkup for their naevi without having AMS or MM. 104 (86%) participants reported using sunscreen. Approximately 74.4% of patients recalled having received advice on how to protect their skin from sunlight. The most mentioned barriers in photoprotection were concerns over adequate vitamin D and lack of time.

Research conclusions

Despite mentioning having received adequate education in photoprotection, adherence to photoprotection practices is suboptimal in patients with MM and AMS.

Research perspectives

Larger prospective studies could be performed comparing awareness, knowledge, and attitudes towards photoprotection among patients with MM and AMS before and after receiving education in photoprotection.

FOOTNOTES

Author contributions: Koumaki D, Papadakis M, and Krasagakis K contributed to designing the study; Koumaki D

contributed to collecting and analyzing the data, and writing the paper; Papadakis M contributed to analyzing the data; Kouloumvakou S contributed to collecting the data; Koumaki D, Papadakis M, and Krasagakis K contributed to revising and approving the paper.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Short term safety of coronavirus disease 2019 vaccines in patients with solid tumors receiving systemic therapy

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Abstract

BACKGROUND

There are currently three coronavirus disease 2019 (COVID-19) vaccines approved by the United States Food and Drug Administration to prevent coronavirus infection. However, robust data are unavailable on the adverse events of the vaccines in patients with solid tumor malignancies undergoing systemic therapies.

AIM

To evaluate the safety of COVID-19 vaccines in patients with solid tumors undergoing systemic therapies.

METHODS

The study included patients with solid tumors treated in an academic tertiary care center who received COVID-19 vaccination between January 1, 2021 and August 15, 2021, while undergoing systemic therapy. Electronic medical records were accessed to collect information on patient characteristics, systemic therapies, type of vaccine received, and adverse effects associated with the vaccine administration. Adverse events (AEs) were graded according to Common Terminology Criteria for Adverse Events, version 5.0.

RESULTS

The analysis included 210 patients; the median age was 70 years, and 51% of patients were female. The most common chemotherapy, immunotherapy, and targeted therapy administered were taxane-based regimens 14.2% (30/210), anti-programmed death 1 (PD-1) agents 22.8% (48/210), and antiangiogenic agents

7.1% (15/210), respectively. The most common cancers were gastrointestinal 43.8% (92/210), thoracic 30.4% (64/210), and genitourinary 17.6% (37/210). Patients received the following vaccines: 2 doses of BNT162b2 by Pfizer 52% (110/210), 2 doses of mRNA-1273 by Moderna 42% (89/210), and 1 dose of JNJ-78436735 by Johnson & Johnson 5% (11/210). At least 1 AE attributable to the vaccine was observed in 37 patients 17.6% (37/210). The total number of AEs attributable to vaccines was 62: Fifty-three grade 1 and nine grade 2. Most adverse events occurred after the second dose 59.7% (37/62). The most frequent grade 1 AEs included fatigue 17% (9/53), fever 15% (8/53), injection site reaction 13.2% (7/53), and chills 9.4% (5/53). The most frequent grade 2 AEs were fatigue 33.3% (3/9) and generalized weakness 22.2% (2/9). Therapy was delayed by 2 wk because of the AEs possibly related to vaccine administration in 3 patients 1.4% (3/210).

CONCLUSION

The present study demonstrates that the adverse events associated with COVID-19 vaccination are infrequent, mild, and rarely delay treatment in patients with solid tumors receiving systemic therapies.

Key Words: COVID-19; Adverse events; Solid tumor; Chemotherapy; Immunotherapy; Targeted therapy

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Core Tip: The current study evaluates the safety and spectrum of adverse events associated with coronavirus disease 2019 (COVID-19) vaccination in solid tumor patients receiving systemic therapy. While COVID-19 vaccination has been shown to be safe and effective in the healthy population, the data confirming the safety of COVID-19 vaccines in cancer patients are sparse. The lack of safety data in cancer patients has caused significant hesitancy to receive COVID-19 vaccination among the patient population with cancer. Our study showed that the administration of COVID-19 vaccines in solid tumor patients receiving systemic therapy is safe and should be encouraged.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has profoundly impacted and transformed healthcare systems across the globe. In addition to comprehensive modification in healthcare delivery, patients have encountered immeasurable emotional and socioeconomic hardships[1,2]. SARS-CoV-2 is a novel single-stranded, enveloped RNA virus that primarily spreads *via* the respiratory route and causes respiratory infection, including pneumonia with or without multiorgan failures[3]. While many patients remain asymptomatic, infection with the SARS-CoV-2 virus has been shown to cause a myriad of symptoms, including severe acute respiratory distress syndrome[1]. Analysis of comprehensive observational data has shown increased mortality, hospitalization, and intensive care admission in cancer patients who received anticancer therapy within 3 mo of infection[4,5]. A study from China reported a 3.5-fold increased risk of respiratory failure requiring mechanical ventilation in cancer patients infected with the SARS-CoV-2 virus[6]. The interplay between COVID-19 infection and cancer is complex, attributable to a wide variety of factors including immunosuppression, co-morbidities, aging, and the biology of the cancer itself[7].

The United States Food and Drug Administration approved three COVID-19 vaccines to prevent coronavirus infection. These include the BNT162b2 from Pfizer, mRNA-1273 from Moderna, and JNJ-78436735 vaccine from Johnson & Johnson. Patients with cancer should be considered a high-priority group for COVID-19 vaccination due to their higher risk of morbidity and death associated with COVID-19 disease[5,6,8-10]. However, the trials reporting efficacy and safety of COVID-19 vaccines were conducted in healthy volunteers, excluding the immunocompromised cancer patients on treatment [11-13]. Although several cancer societies recommend COVID-19 vaccination in patients with cancer, the data confirming the safety of vaccines are sparse[14,15]. This lack of rigorous scientific inquiry into vaccine safety has led to increased apprehension and hesitation to receive vaccination in the patient

population with cancer. As the incidence of cancer continues to rise, solid tumor malignancies continue to emerge among the most prevalent diagnoses. Frequently used therapeutic regimens include chemotherapy, immunotherapy, and targeted therapy. We conducted a study to assess the safety and determine the spectrum of adverse events (AEs) associated with COVID-19 vaccination in patients with solid tumors receiving systemic therapy.

MATERIALS AND METHODS

The aim of this study was to determine the real-world incidence and spectrum of AEs in patients with solid tumor malignancies receiving systemic therapy. This was a retrospective study of cancer patients who received COVID-19 vaccination between January 1, 2021 and August 15, 2021 at Froedtert and the Medical College of Wisconsin Cancer Center (Milwaukee, WI, United States of America). Inclusion criteria required that patients be at least 18 years of age at the time of inoculation and have a histologically confirmed solid tumor diagnosis for which they were receiving systemic therapy (chemotherapy, immunotherapy, or targeted therapy). Patients were excluded from the study if they had an active hematologic malignancy, were being treated with hormonal therapy, or had a benign tumor diagnosis that did not require anti-neoplastic treatment. Patients for this study were identified from the cancer center database using a tool available in the electronic medical record software (EPIC SlicerDicer tool). The initial screen identified 1480 cancer patients who received COVID-19 vaccines. Of these, 349 were omitted due to an active hematologic malignancy, and 183 patients were excluded due to diagnoses of benign solid tumors. An additional 401 patients who were receiving hormonal therapies (*i.e.*, leuprolide for prostate cancer or tamoxifen/anastrozole for breast cancer) were excluded. Finally, 337 patients were excluded who were not receiving active treatment for malignancies (*e.g.*, patients on surveillance following completion of their initial treatment) or active malignancies being treated with modalities other than chemotherapy, immunotherapy, or targeted therapies (*e.g.*, radiation therapy). After review, 210 patients were found to meet the study requirements (Figure 1). Electronic medical records for these patients were examined to collect information on patient characteristics, tumor characteristics, details of systemic therapy, type of vaccine received, and any AEs associated with the vaccine administration. Clinic and hospital notes were further analyzed to capture AEs occurring in a period between the first vaccination and day 30 after the second/final vaccination. In the case of the Johnson & Johnson vaccines, patient charts were reviewed for the 30-d period following the single dose of vaccination. AEs were graded in accordance with version 5.0 of the Common Terminology Criteria for Adverse Events [16]. The institutional review board of the Medical College of Wisconsin approved this study protocol.

RESULTS

Patient characteristic, systemic therapy, and vaccination types

Between January 1, 2021 and August 15, 2021, 210 patients were included in the study (Table 1). The median age of the cohort was 70 years (range, 23-91), 51% (108/210) of patients were female, and 87.1% (183/210) of the study population was Caucasian. Distribution of vaccine types included BNT162b2 from Pfizer 52.3% (110/210), mRNA-1273 from Moderna 42.3% (89/210), and JNJ-7843 vaccine from J&J 5% (11/210). All patients who received either the Pfizer or Moderna vaccine completed the 2-dose vaccination series. Gastrointestinal cancers were the most frequent diagnoses 43.8% (92/210), followed by thoracic cancers 30.4% (64/210) and genitourinary cancers 17.6% (37/210).

In the study cohort, 117 patients were receiving systemic chemotherapy at the time of vaccination. The median age of this cohort was 69 years, with a slight female predominance at 53% (62/117). Distribution of vaccine types were BNT162b2 from Pfizer 55.6% (65/117), mRNA-1273 from Moderna 40.1% (47/117), and JNJ-7843 vaccine from J&J 4.2% (5/117). The most common chemotherapeutic regimens included were taxane-based 25.6% (30/117) regimens followed by oxaliplatin-based regimens 22.2% (26/117).

Fifty-one patients were receiving immunotherapy at the time of vaccination. The median age of this cohort was 72 years, with a slight male predominance at 56.9% (29/51). Distribution of vaccine types were BNT162b2 from Pfizer 47% (24/51), mRNA-1273 from Moderna 45.1% (23/51), and JNJ-7843 vaccine from J&J 7.8% (4/51). The most common immunotherapeutic regimens consisted of programmed death 1 (PD-1) blocking agents 94% (48/51).

Forty-two patients were receiving targeted therapy at the time of vaccination. The median age of this cohort was 68 years, with a slight female predominance at 57% (24/42). Distribution of vaccine types were BNT162b2 from Pfizer 50% (21/42), mRNA-1273 from Moderna 45.2% (19/42), and JNJ-7843 vaccine from J&J 4.8% (2/42). The most common targeted therapy treatment administered was Osimertinib 14.2% (6/42).

Table 1 Characteristics of solid tumor patients receiving coronavirus disease 2019 vaccination

Patient characteristics	*n* = 210, %
Age at vaccination, median (range), yr	70 (23-91)
Sex	
Male	102 (49)
Female	108 (51)
Race	
Caucasian	183 (87)
African American	19 (9)
Other	8 (4)
Site of primary tumor	
Gastrointestinal	92 (44)
Thoracic	64 (30)
Genitourinary	37 (18)
Other	17 (8)
Type of systemic therapy	
Chemotherapy	117 (56)
Immunotherapy	51 (24)
Targeted therapy	42 (20)

Adverse events

The total number of AEs attributable to vaccination in the current cohort was 62 (Table 2). At least 1 unique AE was noted in 17.6% of patients (37/210). The number of patients who experienced any grade AEs was 20 in the chemotherapy group, 12 in the immunotherapy group, and 5 in the targeted therapy group. There were 33 AEs related to the Pfizer vaccine, 26 to the Moderna vaccine, and 3 to the J&J vaccine. In total, there were fifty-three grade 1 AEs 85.5% (53/62) and nine grade 2 AEs 14.5% (9/62). Following the first vaccination, there were twenty-one grade 1 and four grade 2 AEs. The most frequent grade 1 AEs were injection site reaction 23.8% (5/21), fatigue 23.8% (5/21), and fever 9.5% (2/21). The four grade 2 AEs noted included fatigue, nausea, chills, and maculopapular rash. Following the second vaccination, there were thirty-two grade 1 and five grade 2 AEs. The most frequent grade 1 AEs were fever 18.8% (6/32), fatigue 12.5% (4/32), chills 12.5% (4/32), and myalgia 12.5% (4/32). The five grade 2 AEs included 2 cases of fatigue, 2 cases of generalized muscle weakness, and 1 case of fever.

Cumulatively, the most frequent grade 1 AEs included fatigue 17% (9/53), fever 15% (8/53), injection site reaction 13.2% (7/53), and chills 9.4% (5/53). The most frequent grade 2 AEs were fatigue 33.3% (3/9) and generalized muscle weakness 22.2% (2/9). Of the grade 2 AEs, 6 were associated with the Pfizer vaccine and 3 with the Moderna vaccine. No grade 2 AEs were noted in the J&J vaccine population. In those who received the Pfizer or Moderna vaccine, the majority of AEs occurred after the second dose of vaccination 59.7% (37/62).

Treatment was delayed in 3 patients 1.4% (3/210) after the second dose of the Moderna vaccine by 2 wk because of AEs possibly related to vaccine administration. None of the patients had displayed any AEs after the first vaccination dose. Two of these 3 patients receiving immunotherapy developed generalized weakness that resolved within 2 wk without any specific treatment. The third patient developed malaise and fatigue, which also resolved spontaneously. No grade 3-5 AEs or anaphylaxis were noted in this patient cohort.

DISCUSSION

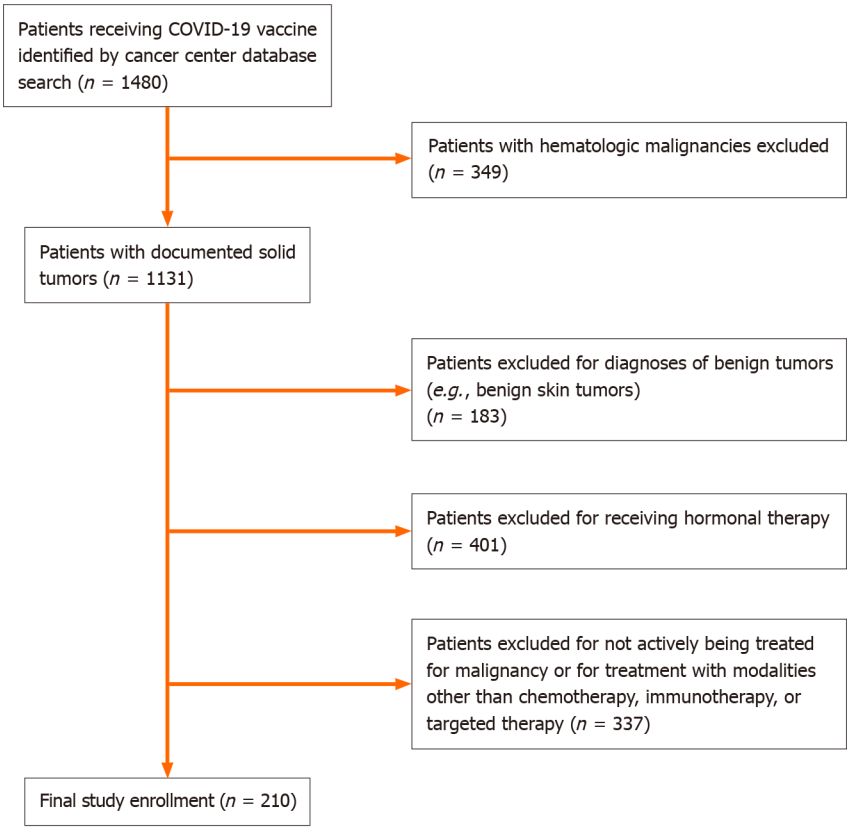
Data on the safety of COVID-19 vaccines in cancer patients undergoing systemic therapies are sparse. The current study aimed to address this unmet need by collecting data on COVID-19 vaccine-associated AEs in real-world cancer patients with solid tumors receiving various systemic therapies. The study revealed that COVID-19 vaccines cause infrequent and minor side effects in this patient population.

The pandemic caused by the novel coronavirus SARS-CoV-2 has significantly impacted cancer care delivery and cancer treatment globally. The COVID-19 pandemic has affected many aspects of cancer

Table 2 Adverse events (AEs) observed with coronavirus disease 2019 vaccination in patients with solid tumors receiving systemic therapies

	Chemotherapy	Immunotherapy	Targeted therapy
Patient number	117	51	42
Median age (yr)	69	72	68
Gender (Male/Female)	55/62	29/22	18/24
Type of vaccine administered(Moderna/Pfizer/J&J)	47/65/5	23/24/4	19/21/2
AEs (Grade 1 + 2), number (%)	37 (60)	18 (29)	7 (11)
Therapy delayed because of AEs, #	1	2	0

AEs: Adverse events.



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Figure 1 Consort diagram illustrating patient enrollment. COVID-19: Coronavirus disease 2019.

care, including delay in cancer diagnosis and treatment, the long-term ramifications of which are yet to be determined[15]. The rapid development of coronavirus vaccines has brought the hope of preventing infection and restoring normalcy. While the initial clinical trials with COVID-19 vaccines demonstrated a high safety profile of the vaccines in the healthy population[11-13], limited safety data have been reported in cancer patients. Consequently, significant hesitancy in adopting widespread vaccination has been observed among patients with active cancer[17-20]. In a cross-sectional, internet-based survey, hesitancy to receive COVID-19 vaccination was reported in 13.4% of patients with cancer[19]. In a study with breast cancer patients, 26% of patients were hesitant to receive vaccination due to their concerns regarding vaccine-related AEs[20]. As patients with cancer are at increased risk of COVID-19 infection-associated complications and mortality[8-10,21,22], data confirming the safety of COVID-19 vaccines in cancer patients are urgently needed. Our study provides important safety information on COVID-19 vaccines in cancer patients undergoing active cancer treatment.

Several studies have investigated the safety of COVID-19 vaccines (summarized in Table 3). Oosting and colleagues have reported a prospective, multicenter study from the Netherlands in which patients with solid tumors received the Moderna vaccine while undergoing treatment with chemotherapy,

Table 3 Adverse events associated with coronavirus disease 2019 vaccination in published studies

Ref.	Sample size (*n*)	Cancer type	Systemic therapy	Vaccines administered	Patients with Grade 3 or worse AE, %	Immune-related AEs	Comment
Oosting *et al* [23]	544	Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy	mRNA-1273 (Moderna)	10/544 (1.8%)	4% in both immunotherapy and chemoimmunotherapy group	Total 4 serious AEs were potentially related to the vaccination
Cavann *et al* [24]	257	Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy; Chemotherapy plus biological therapy; Biologic therapy	PfizerModerna	0/257 (0%)	NA	Approximately 1/3rd of patients reported mild local reactions (pain, erythema) at the injection site
Waissengrin *et al*[25]	134	Solid Tumors	Immune checkpoint inhibitor; Chemoimmunotherapy	BNT162b2 mRNA vaccine (Pfizer)	0/134 (0%)	Nonattributable to the vaccination	Fatigue (34%), headache (16%), muscle pain (34%)
Di Noia *et al* [26]	816	Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy; Targeted therapy	Pfizer	3.3% after the 1st dose, 1.4% after the second dose	NA	AE occurred in 359 (44%) and 301 (38.3%) patients after the first and second dose, respectively
Shmueli *et al* [27]	129	Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy; Biological Therapy; Hormonal Therapy; Radiotherapy	Pfizer	0/129 (0%)	NA	AE was reported by 39% of patients after the first dose and 58% of patients after the second dose- all mild to moderate in severity
Tamura *et al* [29]	120	Solid Tumor	Chemotherapy; Immunotherapy; Targeted Therapy; Chemoimmunotherapy	Pfizer Moderna	0/120 (0%); CTCAE was not used	NA	Study limited to patients receiving treatment for lung cancer only. No serious AEs or treatment delay was observed
Kian *et al*[28]	210	Solid & Non-Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy; Biological Therapy; Hormonal Therapy; Radiotherapy; Radio-hormonal; Chemo-biological	Pfizer	0.004% after 1st dose, 1.9% after the second dose	NA	AE occurred in 65 (31%) and 65 (31%) patients after the first and second dose, respectively. Injection site pain was the most common AE after both doses

AEs: Adverse events; NA: Not available.

immunotherapy, or chemoimmunotherapy[23]. In this study, the incidence of grade 3 or worse AEs were reported in 2% of patients treated with immunotherapy, 2% of patients treated with chemotherapy, and 1% of patients treated with chemoimmunotherapy. No vaccine-related death was reported. A similar study from Italy reported that patients with solid tumors undergoing active treatment also demonstrated a low incidence of significant AEs associated with COVID-19 vaccination [24]. In this study, none of the 257 evaluable patients experienced grade 3 or higher AEs. The most frequently reported AE was injection site pain and/or redness occurring in 31.5% and 33.4 % of patients after the first and second vaccinations. The most frequently reported AEs after the first dose were weakness (7%), headache (8%), and muscle pain (2.7%), and after the second dose were weakness (8.9%) and fever (5.8%). A study from Israel also reported a low incidence of AEs in patients with solid tumors receiving immunotherapy with checkpoint inhibitors, with injection site pain being the most frequently reported AE at 21% (28/134)[25]. Several other studies have demonstrated similar results[26-29]. The results of our study, in conjunction with the studies discussed above, indicate that COVID-19 vaccination is safe in solid tumor patients undergoing active treatment. The high mortality rate associated with COVID-19 disease (as high as 40% in certain patient populations)[30] and the safety data available far justify routine COVID-19 vaccination in patients with solid tumors undergoing active treatment. This recommendation is further supported by several oncology societies[14,15] and echoed by the American Society of Clinical Oncology endorsement (https://www.asco.org/covid-resources/vaccines-patients-cancer) which states: At this time, patients undergoing treatment may be offered vaccination against COVID-19 as long as any components of the vaccine are not contraindicated.

It is important to reiterate that COVID-19 vaccines in cancer patients treated with immunotherapy did not cause a higher incidence of immune-related AEs, a finding supported by several other studies [23,25]. While 2 patients in our study receiving immune checkpoint inhibitors experienced treatment delay secondary to vaccination-associated AEs, their symptoms resolved quickly with supportive care only. The remaining patients in our immunotherapy cohort demonstrated mild grade 1 AEs with rapid resolution of symptoms.

Although the current study provides valuable information on COVID-19 vaccine safety in a real-world setting, it has several limitations that include the inherent biases associated with a retrospective study design, modest sample size, and reliance on physician documentation for the data related to the AEs.

CONCLUSION

Our study demonstrates that the COVID-19 vaccines cause infrequent and mild AEs in patients with solid tumors receiving systemic therapies. The study results support routine COVID-19 vaccination in cancer patients receiving active treatment.

ARTICLE HIGHLIGHTS

Research background

In the wake of the coronavirus disease 2019 (COVID-19) pandemic, the United States Food and Drug Administration approved 3 vaccines to prevent coronavirus infection. The rapidity of vaccine approval and the limited scientific inquiry into vaccine-related adverse events notably expanded apprehension towards vaccination in patients with malignancies. Our study reports real-world data on the severity and spectrum of adverse events in solid tumor cancer patients receiving systemic therapy.

Research motivation

The motivation behind this project was to promote awareness regarding the short-term safety of COVID-19 vaccines in cancer patients with solid tumor malignancies. Our results help lessen the societal apprehension and hesitation surrounding the safety of COVID-19 vaccination.

Research objectives

The main objective of this study was to evaluate the short-term safety of COVID-19 vaccines in patients with solid tumors undergoing treatment with systemic therapies. Through rigorous analysis, we were able to document the incidence and spectrum of vaccine-related adverse events in our patient cohort. Our research forms the groundwork for future studies on long-term adverse events secondary to vaccination.

Research methods

Our study was a retrospective analysis of cancer patients who received COVID-19 vaccination between January 1, 2021 and August 15, 2021. Eligible patients were identified using the EPIC SlicerDicer tool in the Froedtert and the Medical College of Wisconsin Cancer Center database. Once identified, patients were further screened based on study inclusion/exclusion criteria. Electronic medical records for the final patients were examined to collect information on patient characteristics, tumor characteristics, details of systemic therapy, type of vaccine received, and any adverse events associated with the vaccine administration.

Research results

Analysis of our 210 patients revealed at least 1 adverse event attributable to vaccination in 17.6% of our study cohort. Of these adverse events, fifty-three were grade 1 and nine were grade 2. Our data further bolsters the sparse scientific literature regarding COVID-19 vaccination in patients with cancer.

Research conclusions

The present study demonstrates that the adverse events associated with COVID-19 vaccination are infrequent, mild, and rarely delay treatment in patients with solid tumors receiving systemic therapies. This knowledge further begs the question of whether or not patients receiving systemic therapies are mounting an appropriate response to immunogenic antigens. Further scientific inquiry exploring vaccine efficacy and adverse events in our patient cohort *vs* a healthy control group could elucidate the role of systemic therapy in vaccine-related adverse events.

Research perspectives

Future research will be focused on increasing study enrollment and exploring the long-term adverse events secondary to COVID-19 vaccination.

FOOTNOTES

Author contributions: Cox RE, Parish M, Oxencis C, McKenna E, Thapa B, and Chakrabarti S contributed equally to this work; All authors have read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Medical College of Wisconsin Institutional Review Board (Approval No. PRO00040038).

Informed consent statement: Per institutional review board approval, consent forms were not necessary since the project did not include direct contact with subjects.

Conflict-of-interest statement: Sakti Chakrabarti has received fees for serving as a speaker for Natera. Sakti Chakrabarti has received Honoraria from Haliodx and QED Therapeutics. Ronald Cox has no conflicts of interest. Marie Parish has no conflicts of interest. Carolyn Oxencis has no conflicts of interest. Bicky Thapa has no conflicts of interest. Edward McKenna has no conflicts of interest.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Decreased incidence of febrile neutropenia in Michigan following masking and social distancing orders for the COVID-19 pandemic: A population based cohort study

Michael Gerard Baracy Jr, Karen Hagglund, Sanjana Kulkarni, Fareeza Afzal, Katherine Arends, Robert T Morris, Leigh Ann Solomon, Muhammad Faisal Aslam, Logan Corey

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Abstract

BACKGROUND

It has been theorized that 75%-80% of febrile neutropenia (FN) is caused by endogenous pathogens, while up to 20% of cases are thought to be caused by a viral infection. It is unknown if precautions such as masking and social distancing reduce the risk of FN in susceptible populations.

AIM

To determine whether coronavirus disease 2019 (COVID-19) infection mitigation efforts, namely masking and social distancing, were associated with a reduction in the incidence of FN.

METHODS

This was a retrospective population based cohort study comparing the incidence of FN in the 13 mo prior to (Year 0) and 13 mo following (Year 1) the public health executive orders (PHEO) in Michigan. Data was queried for all emergency department (ED) visits from April 1, 2019 to March 31, 2021 from the National Syndromic Surveillance Program, a program which collects data that is voluntarily submitted by approximately 89% of Michigan EDs. The primary study outcome was the incidence of FN as a proportion of ED visits in the 13-mo before and 13-mo after COVID-19 mitigations efforts, namely masking and social distancing. We hypothesized that there would be a significant decrease in the incidence of FN in the period following the PHEO aimed at reducing the spread of the severe acute respiratory syndrome coronavirus 2 virus.

RESULTS

There was a total of 8979221 total ED visits captured during the study period. In Year 0 there were 5073081 recorded ED visits and 3906140 in Year 1. There was a significant reduction in the proportion of total ED visits with a diagnosis of FN, decreasing 13.3% across periods (0.15% *vs* 0.13%, $P = 0.036$). In patients with a hematologic malignancy a more impressive reduction in the incidence of FN was evident following PHEO (22% *vs* 17%, $P = 0.02$).

CONCLUSION

We found a significant association between social distancing and mask guidelines implemented on a large public scale with decreased rates of FN, particularly in those with a hematologic malignancy. These findings may be useful in the design of future research and recommendations regarding the prevention of FN.

Key Words: Febrile neutropenia; COVID-19; SARS-CoV-2; Malignancy; Hematology; Public health

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Core Tip: There has been a proven reduction in respiratory viral infections (*e.g.*, flu, common cold, *etc.*) with the implementation of social distancing and masking during coronavirus disease 2019 mitigation efforts. It has been theorized that up to 20% of febrile neutropenia is caused by viral infections. We found a significant reduction in the incidence of febrile neutropenia following the implementation of public health interventions, namely masking and social distancing, with the overall incidence of febrile neutropenia decreasing by approximately 13%. The largest reduction in febrile neutropenia was found for hematologic malignancies where the incidence of febrile neutropenia declined by 22%.

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INTRODUCTION

Febrile neutropenia (FN) is defined as neutropenia in the setting of a temperature greater than or equal to 100.4 degrees Fahrenheit and is one of the most common and costly complications associated with cancer treatment[1,2]. Granulocyte colony stimulating factor is the most effective prophylaxis against neutropenia and the progression to FN[3,4]. Yet, little is known about non-pharmacological strategies, such as masking, for the prevention of FN in at-risk populations.

Currently, FN prevention strategies include hand hygiene and the avoidance of sick contacts and crowds, however the impact of these efforts is uncertain[5]. Furthermore, the majority of FN with culture-proven bacteremia are thought to be the result of translocation of gut bacteria[6]. Although it has been theorized that 75%-80% of FN is caused by endogenous microorganisms, up to 20% of cases are thought to be caused by communicable pathogens, such as virions[7]. Accordingly, mitigating viral infection in at risks populations would theoretically reduce the incidence of FN. However, we are not aware of any population-based studies evaluating the impact of infection mitigation practices on the incidence of FN.

The public health crisis due to coronavirus disease 2019 (COVID-19) and its corresponding public health interventions provided a unique opportunity to evaluate the impact of social distancing and masking on the incidence of FN. In March of 2020, the state of Michigan issued an Executive Order that

urged residents to remain at home and socially distance, which was amended shortly thereafter to include a mask mandate[8]. The aim of our study was to evaluate the association of these mitigation efforts and the incidence of FN in patients presenting to emergency departments (EDs) in the state of Michigan.

MATERIALS AND METHODS

This is a retrospective cohort study comparing the incidence of FN in the 13 mo prior to (Year 0, March 1, 2019-March 31, 2020) and the 13 mo following (Year 1, April 1, 2020-April 31, 2021) the public health executive orders (PHEO) in Michigan. Approximately 89% of EDs across Michigan voluntarily submit encounter data (patient's chief complaint, associated diagnoses, age, sex, intake temperature, intake percent oxygen saturation, and blood pressure) to the National Syndromic Surveillance Program (NSSP). A query was made for all encounters with a diagnosis of Neutropenia (ICD-10-CM D70) from March 1, 2019-April 31, 2021. FN was defined as an intake temperature greater than or equal to 100.4 degrees Fahrenheit and an ICD-10-CM D70. The incidence of FN in Year 0 was compared to the incidence of FN in Year 1. To account for the impact of the COVID-19 pandemic on total ED visits, the incidence of each ICD code was analyzed as a proportion of ED visits for the corresponding year. Associated ICD-10-CM codes were grouped according to Clinically Relevant Groups (CRG) (Supplementary Table 1), modified from the Healthcare Cost and Utilization Project[9]. Analysis of the incidence of FN in each CRG was also performed. A visit containing multiple ICD-10-CM diagnostic codes was included in multiple CRGs, if applicable. This study was deemed exempt by the responsible institutional review board.

Statistical analysis

Descriptive statistics were generated to characterize the study cohorts. Continuous variables are described as the mean with standard deviation or median with range or interquartile range. Categorical variables are described as frequency distributions. Univariable analysis of factors associated with FN were assessed using Student's *t*-test, analysis of variance, and the chi-squared analysis. Multivariable analysis was done using logistic regression. Analyses were conducted using SPSS version 25.0 and a *P* value less than 0.05 was considered statistically significant.

RESULTS

From March 1, 2019 to April 31, 2021 there were 8979221 total ED visits in the state of Michigan with data submitted to the NSSP and at least one viable ICD-10 code. In Year 0, there were 5073081 recorded ED visits and 3906140 in Year 1, a decrease of 23% (Table 1). There was a total of 5717 encounters with a diagnosis consistent with neutropenia. There was a significant reduction in the proportion of total ED visits with a diagnosis of FN, decreasing 13.3% from Year 0 to Year 1 (0.15% *vs* 0.13%, $P = 0.036$).

In a sub-analysis of all patients with FN, in patients with a concomitant diagnosis of hematologic malignancy, FN was significantly lower in the period following PHEO (22% *vs* 17%, $P = 0.02$, Table 2). In Year 0 there was a 29.3% incidence of FN in neutropenic patients with a CRG of hematologic malignancy, *vs* a 21.2% incidence in neutropenic patients without a CRG of hematologic malignancy ($P < 0.0001$, Figure 1). This difference was not observed in Year 1 (23.8% *vs* 20.2%, $P = 0.12$). Hematologic malignancy was the only CRG diagnosis to have a relatively higher rate of FN in Year 0 compared to Year 1.

DISCUSSION

The public health response to COVID-19 in the state of Michigan provided a unique opportunity to analyze the impact of social distancing and masking on the incidence of FN. Masking and social distancing designed to prevent the spread of COVID-19 have resulted in the decline of other non-covid viral illnesses[10,11]. Our study is the first to document an association between this phenomenon and a decline in the incidence of FN, both overall and in patients with an ICD-10-CM diagnosis consistent with hematologic malignancy.

In the majority of cases, the underlying cause of FN is unknown, and therefore little is known about the efficacy of non-pharmacological efforts to prevent development of FN in neutropenic patients[12]. We found a significant association between the implementation of public health measures to prevent the spread of communicable diseases and the incidence of FN associated with hematologic malignancies. This is an important finding as patients with hematologic cancers are particularly vulnerable to FN and its associated morbidity and mortality[13]. Our findings suggest that a significant proportion of FN in patients with malignancy may have a viral etiology. Accordingly, health measures, such as masking,

Table 1 Baseline demographics

	Year 0, *n* (%)	Year 1, *n* (%)	*P* value
Total ED visits	5073081 (56.4)	3906140 (43.6)	-
Visits with neutropenia			
Male	1572 (48)	1189 (49)	0.59
Female	1704 (52)	1252 (51)	
Age (yr)	55.1 ± 23.6	54.7 ± 23.7	0.55
Visits with febrile neutropenia			
Male	2145 (48)	616 (50)	0.35
Female	2327 (52)	629 (50)	
Age (yr)	56.2 ± 22.6	50.3 ± 26.6	< 0.0001

Data reported at mean ± SD or *n* (%) unless otherwise specified. ED: Emergency department.

Table 2 Frequency of clinical relevant group diagnosis among patients with neutropenic and febrile neutropenia in Year 0 and Year 1

	Neutropenia			Febrile neutropenia		
	Year 0[1]	Year 1[2]	*P* value	Year 0[1]	Year 1[2]	*P* value
Diagnoses	*n* = 3276	*n* = 2441		*n* = 740	*n* = 505	
Common infections	1482 (45)	974 (40)	**< 0.0001**	403 (55)	255 (51)	0.17
Any malignancy	1237 (38)	833 (34)	**0.01**	276 (37)	182 (36)	0.65
Solid malignancies	722 (22)	511 (21)	0.32	120 (16)	101 (20)	0.09
Hematologic malignancies	564 (17)	357 (15)	**0.01**	165 (22)	85 (17)	**0.02**
Benign neoplasms	147 (5)	111 (5)	0.91	43 (6)	17 (3)	**0.05**
Non-malignant blood dyscrasias	1580 (48)	1213 (50)	0.27	315 (43)	227 (45)	0.41
Endocrine, nutritional, and metabolic disorders	1485 (45)	1119 (46)	0.70	268 (36)	200 (40)	0.23
Skin, musculoskeletal, psychiatric, and nervous system disorders	1224 (37)	870 (36)	0.18	211 (29)	134 (27)	0.44
Disorders of the cardiopulmonary system	1639 (50)	1125 (46)	**0.003**	324 (44)	210 (42)	0.44
Disorders of the gastrointestinal and genitourinary systems	1332 (41)	952 (39)	0.21	226 (31)	159 (32)	0.72
Coronavirus disease 2019	6 (0)	207 (9)	< 0.0001	0 (0)	45 (9)	-

[1]Includes the 13 mo prior to Michigan's state-wide public health executive orders (PHEO) and coronavirus disease 2019 (COVID-19) mitigation efforts; March 1, 2019 to March 31, 2020.
[2]Includes the 13 mo following Michigan's state-wide PHEO and COVID-19 mitigation efforts; April 1, 2020 to April 31, 2021.
Data reported at *n* (%) unless otherwise specified.

may reduce the risk of FN in vulnerable patients.

Strengths and limitation

The strengths of this study include a large number of encounters and associated accurate objective data points (ICD codes and temperature). Additionally, Michigan adopted the stay-at-home orders and mask mandates quickly and broadly, with one of the highest compliance rates of the country during Year 1 (Supplementary Figure 1)[14]. As a result, our results likely accurately reflect the effect of COVID-19 mitigation efforts on FN.

Our study has several limitations in addition to the inherent vulnerability to unmeasured biases found in retrospective studies: (1) There may be a small number of encounters of FN that are missed in this dataset; (2) Only the intake vital signs were available, and a temperature of 100.4°F was selected as the cutoff for diagnosing FN (rather than 101 F) in an effort to have a more inclusive cohort; (3) Each ED visit was treated as a separate encounter. Therefore, a patient with FN who presented to the ED on multiple occasions would be captured multiple times; (4) It is impossible to account for individual

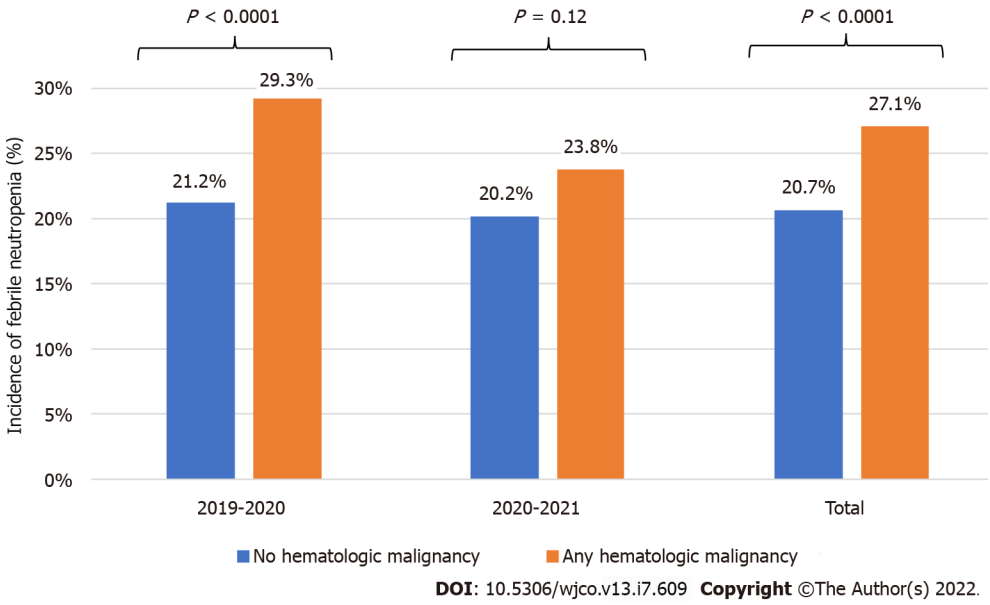


Figure 1 Incidence of febrile neutropenia in patients with an associated diagnosis of hematologic malignancy compared to those without a diagnosis of hematologic malignancy. Patients with a diagnosis of a hematologic malignancy were significantly more likely to be febrile than those without a diagnosis of hematologic malignancy in Year 0 (21.2% *vs* 29.3%, $P < 0.0001$). This difference was not seen after implementation of public health guidelines in Year 1 (20.2% *vs* 23.8%, $P = 0.12$).

patient hesitancy on presenting to ED[14]; (5) Charts review was not possible, so the effect of active myelosuppressive therapy could not be assessed; and (6) The number and identify of facilities contributing data changes over time, and the use of diagnosis codes could be inconsistent across and within facilities.

CONCLUSION

Our study found a significant association between the implementation of social distancing and mask guidelines and the incidence of FN in ED patients with neutropenia. This reduction was most pronounced in those with a hematologic malignancy. These findings may be useful in the design of clinical trials as well as informing future recommendations for the prevention of FN in vulnerable patients.

ARTICLE HIGHLIGHTS

Research background

It has been theorized that 75%-80% of febrile neutropenia (FN) is caused by endogenous pathogens, while up to 20% of cases are thought to be caused by a viral infection. It is unknown if precautions such as masking and social distancing reduce the risk of FN in susceptible populations.

Research motivation

There has been a proven reduction in respiratory viruses (*e.g.*, flu, common cold, *etc.*) with the implementation of social distancing and masking in the effort to prevent the spread of coronavirus disease 2019 (COVID-19). We sought to elucidate whether such public health measures would concomitantly reduce the incidence of FN in susceptible populations, namely those with malignancies.

Research objectives

To determine whether COVID-19 infection mitigation efforts, namely masking and social distancing, was associated with a reduction in the incidence of FN.

Research methods

This is a retrospective population based cohort study comparing the incidence of FN in the 13 mo prior to and 13 mo following the public health executive orders in Michigan. Data was queried for all emergency department visits from April 1, 2019 to March 31, 2021 from the National Syndromic

Surveillance Program.

Research results

There was a significant reduction in the proportion of total ED visits with a diagnosis of FN, decreasing 13.3% across periods (0.15% *vs* 0.13%, $P = 0.036$). In patients with a hematologic malignancy a more impressive reduction in the incidence of FN was evident following PHEO (22% *vs* 17%, $P = 0.02$).

Research conclusions

Masking and social distancing appear to decrease the risk of FN in susceptible populations, especially among patients with hematologic malignancies.

Research perspectives

Masking and social distancing appear to decrease the risk of FN in patients with malignancies, supporting the theory that a proportion of FN may be secondary to communicable infectious particles. Well-designed studies and clinical trials are needed to guide recommendations regarding masking and social distancing for the prevention of FN in vulnerable patients.

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FOOTNOTES

Author contributions: Corey L conceived of the idea; Baracy Jr MG and Corey L designed the protocol and wrote the manuscript; Baracy Jr MG and Corey L organized operative diagnoses, CPT codes, ICD-9 and ICD-10 codes and ensured the integrity of codification; Hagglund K verified the protocol was methodologically sound and analyzed the data; Arends K procured the data; Kulkarni S, Afzal F, Solomon LA, Morris RT and Aslam MF were integral in the design and execution of the project; all authors discussed the final results and contributed to the final manuscript.

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ORIGINAL ARTICLE

Retrospective Cohort Study

iCEMIGE: Integration of CEll-morphometrics, MIcrobiome, and GEne biomarker signatures for risk stratification in breast cancers

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Abstract

BACKGROUND

The development of precision medicine is essential for personalized treatment and improved clinical outcome, whereas biomarkers are critical for the success of precision therapies.

AIM

To investigate whether iCEMIGE (integration of CEll-morphometrics, MIcro - biome, and GEne biomarker signatures) improves risk stratification of breast cancer (BC) patients.

METHODS

We used our recently developed machine learning technique to identify cellular morphometric biomarkers (CMBs) from the whole histological slide images in The Cancer Genome Atlas (TCGA) breast cancer (TCGA-BRCA) cohort. Multivariate Cox regression was used to assess whether cell-morphometrics prognosis score (CMPS) and our previously reported 12-gene expression prognosis score (GEPS) and 15-microbe abundance prognosis score (MAPS) were independent prognostic factors. iCEMIGE was built upon the sparse representation learning technique. The iCEMIGE scoring model performance was measured by the area under the receiver operating characteristic curve compared to CMPS, GEPS, or MAPS alone.

Nomogram models were created to predict overall survival (OS) and progress-free survival (PFS) rates at 5- and 10-year in the TCGA-BRCA cohort.

RESULTS

We identified 39 CMBs that were used to create a CMPS system in BCs. CMPS, GEPS, and MAPS were found to be significantly independently associated with OS. We then established an iCEMIGE scoring system for risk stratification of BC patients. The iGEMIGE score has a significant prognostic value for OS and PFS independent of clinical factors (age, stage, and estrogen and progesterone receptor status) and PAM50-based molecular subtype. Importantly, the iCEMIGE score significantly increased the power to predict OS and PFS compared to CMPS, GEPS, or MAPS alone.

CONCLUSION

Our study demonstrates a novel and generic artificial intelligence framework for multimodal data integration toward improving prognosis risk stratification of BC patients, which can be extended to other types of cancer.

Key Words: Breast cancer; Gene signature; Microbiome signature; Cellular morphometrics signature; Multimodal data integration; Prognosis

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Core Tip: Cancer heterogeneity consistently results in a large variation in the prognosis of patients after a certain treatment. The discovery of biomarkers for predicting prognosis can significantly assist clinical oncologists in making treatment decisions for cancer patients. Our results revealed that iCEMIGE (integration of cell-morphometrics, microbiome, and gene biomarker signatures) significantly improves risk stratification of BC patients. The clinical utility of iCEMIGE needs to be further validated in retrospective and prospective cohort studies to determine whether the iCEMIGE score can provide sufficient predictive information to stratify patients by risk and guide treatment. If so, the iCEMIGE score could assist clinicians in decision-making about cancer treatment and enable more personalized cancer therapy.

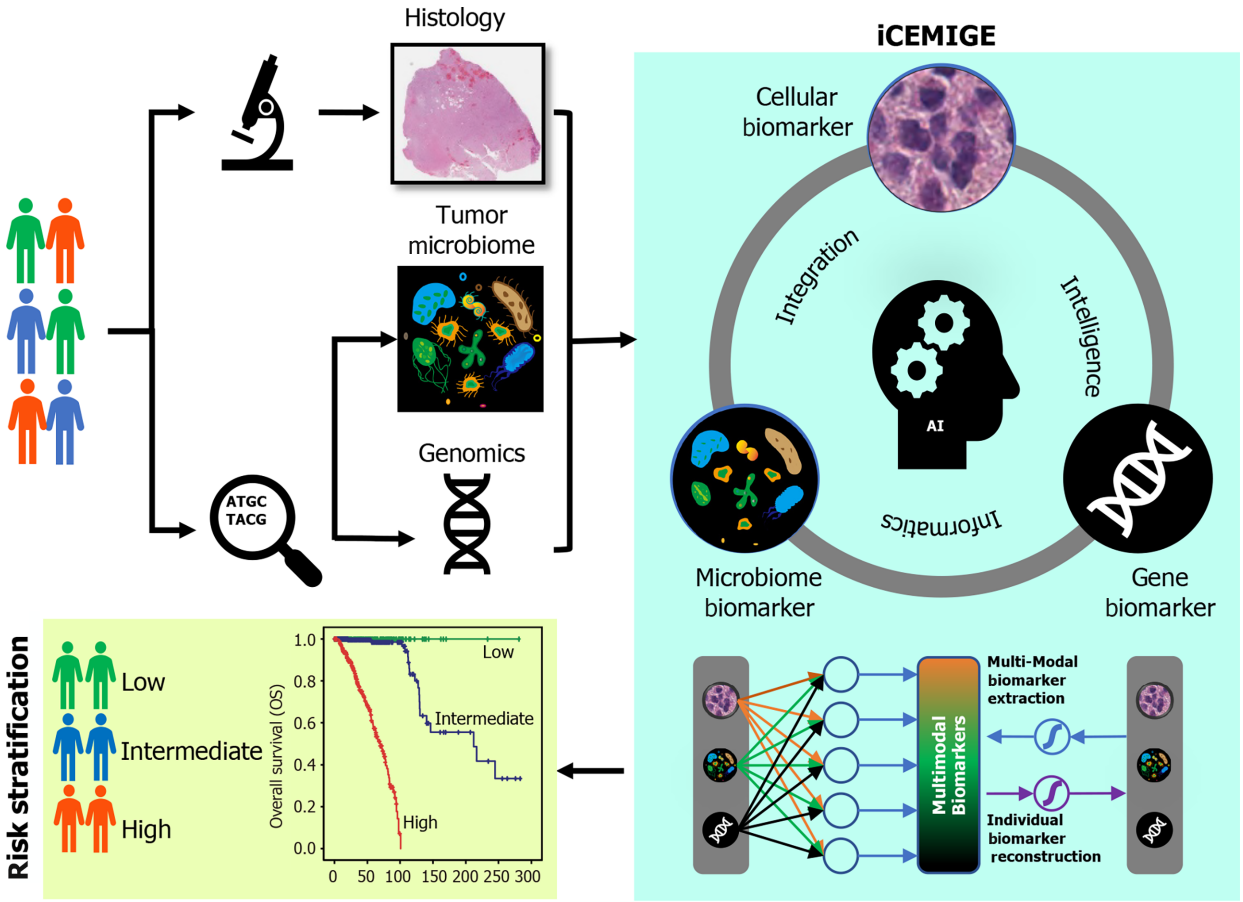
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INTRODUCTION

Cancer is a complex and heterogeneous disease that displays many morphological, genetic, and epigenetic features[1]. Cancer heterogeneity consistently results in a large variation in clinical outcomes of patients after a certain treatment[2], and therefore the development of precision medicine is essential for personalized treatment and improved clinical outcome[3-6]. The discovery of biomarkers for predicting prognosis, a critical step toward precision medicine, can significantly assist clinical oncologists in making treatment decisions for cancer patients[7-9].

Microscopic examination of the histology, which encompasses the morphological features of cancer cells, is the oldest and most basic way of cancer classification. A complete and accurate pathological cancer classification is still crucial to deciding on the best treatment plan for patients. Recently, we developed a framework powered by artificial intelligence (AI) technique for identifying cellular morphometric biomarkers (CMBs) and cellular morphometric subtypes (CMSs) from the whole slide images (WSI) of Hematoxylin and Eosin (H&E)-stained tissue histology[10,11]. We demonstrated that CMSs were significantly associated with specific molecular alterations, immune microenvironment, and prognosis in lower-grade gliomas[10].

With the rapid biotechnological development, such as next-generation sequencing, different aspects of genomic heterogeneity have been uncovered in cancers[12], which dramatically speed the discovery of molecular biomarkers for precision diagnosis and therapy. For example, several molecular biomarkers have been developed for clinical practice in breast cancer (BC)[13,14], including PAM50 (Prosigna, South San Francisco, United States), OncotypeDx (Exact Sciences Corp., Madison, United



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Figure 1 A schematic illustration for the study design. Using an advanced unsupervised representation learning neural network, iCEMIGE realizes efficient and effective multi-modal biomarker mining and extraction, ensuring the optimal integration of reconstructable individual biomarkers.

States), and MammaPrint (Agendia, Amsterdam, Netherlands).

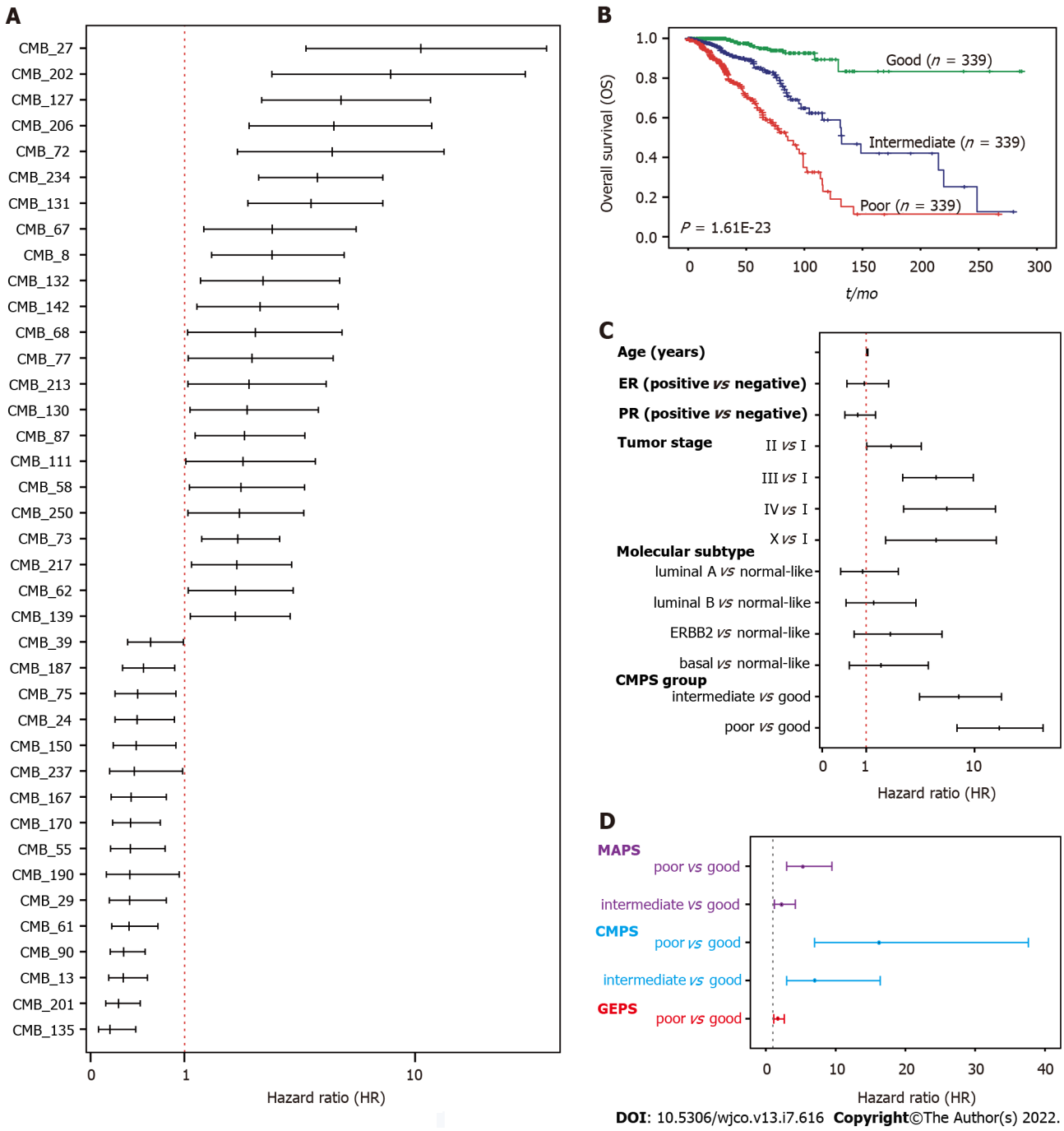
In addition to cancer genomic heterogeneity, a significant number of studies have revealed the diversity of the microbiome in cancer and the roles of the microbiome in cancer development and response to therapies[15-18]. We have recently developed a novel cancer microbiome signature for predicting the prognosis of BC patients[19]. Given the importance of tissue histology, genomics, and microbiome in cancer diagnosis and treatment, efficient and effective integration of these multimodal data is believed to open a new era for precision oncology[20].

In this study, we developed a strategy to integrate multimodal data (Figure 1) and investigated whether iCEMIGE (integration of cell-morphometrics, microbiome, and gene biomarker signatures) improves the risk stratification of BC patients. We first used our recently developed machine learning technique (CMS-ML) to identify the CMBs from the WSIs in The Cancer Genome Atlas (TCGA) breast cancer (TCGA-BRCA) cohort and established a cellular-morphometrics prognosis score (CMPS). We then demonstrated that CMPS, together with our previously reported 12-gene expression prognosis score (GEPS)[21] and the 15-microbe abundance prognosis score (MAPS)[19] were independent prognostic factors. Finally, we established the iCEMIGE scoring system and assessed its clinical value and prognosis predictive power compared to GEPS, MAPS, and CMPS alone.

MATERIALS AND METHODS

Study design and dataset

The TCGA-BRCA cohort was used in this study. The patient diagnostic tissue histology slides were downloaded from GDCportal (https://portal.gdc.cancer.gov/). TCGA-BRCA microbiome, transcriptome, and clinical data, including PAM50-based molecular subtypes, were downloaded from the cBioPortal (https://www.cbioportal.org/)[22,23]. No additional modifications were made to the downloaded data during our analyses.

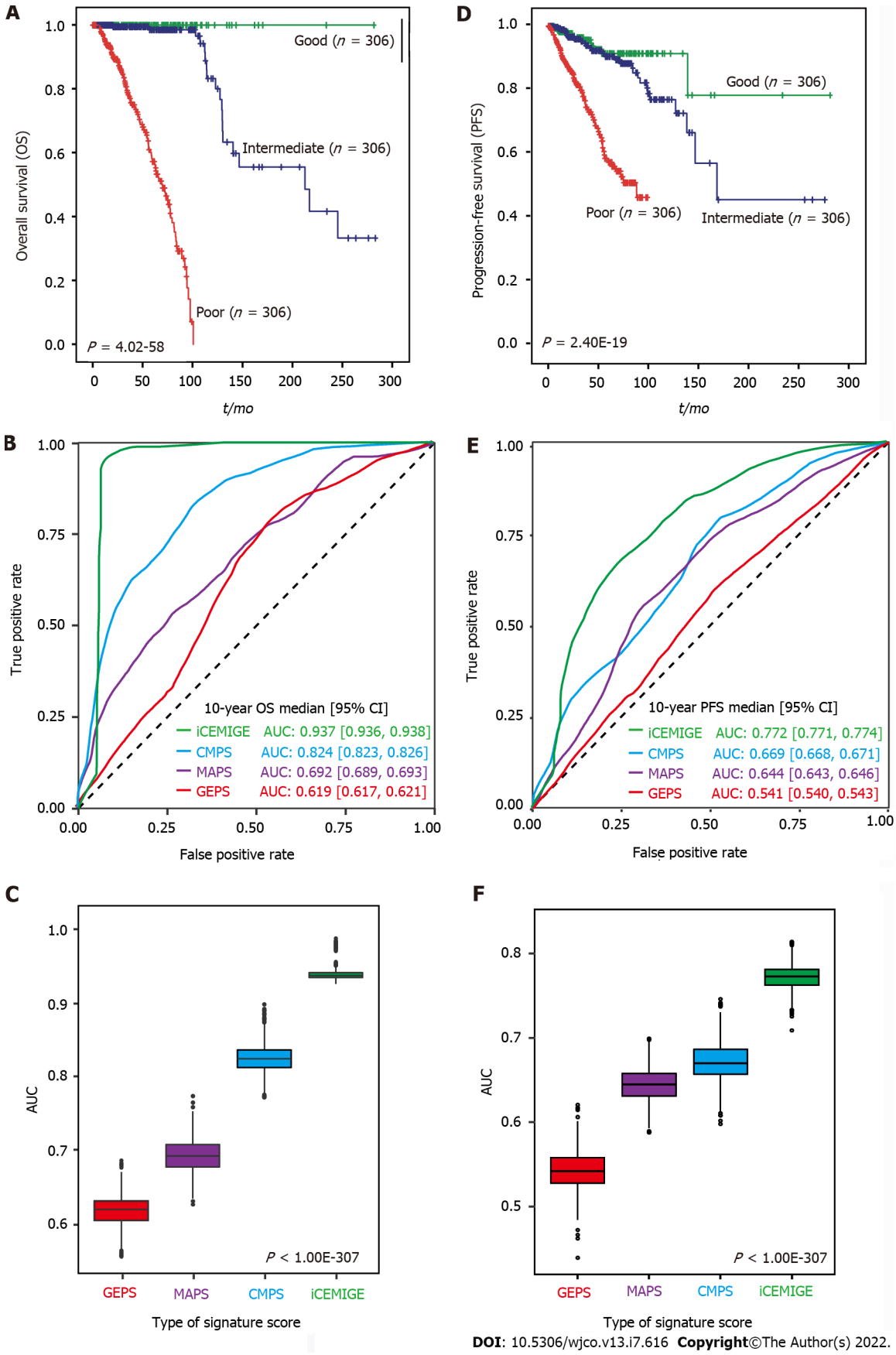


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Figure 2 Prognostic value of the cellular morphometric biomarker signature. A: Multivariate Cox regression analysis with the hazard ratio (HR) represented as a forest plot for cellular morphometric biomarkers; B: Kaplan-Meier curves on overall survival for breast cancer patients are presented with respect to the cellular morphometric prognosis score (CMPS) groups; C: Multivariate Cox regression analysis with hazard ratio (HR) represented as a forest for CMPS groups, clinical factors, and PAM50 subtypes; D: Multivariate Cox regression analysis with the HR represented as a forest plot for CMPS, MAPS, and GEPS.

Extraction of cellular morphometric characteristics and stratification of breast cancer patients

Following our previous work[10], we deployed an unsupervised feature learning pipeline, which was based on the stacked predictive sparse decomposition (SPSD)[24,25], for unsupervised discovery of underlying cellular morphometric characteristics from 15 cellular morphological features that were extracted from the diagnostic slides from the TCGA-BRCA cohort. 256 cellular morphometric biomarkers (CMB) were defined for cellular object representation. Specifically, we used a single network-layer with 256 dictionary elements (*i.e.*, CMBs) and a sparsity constraint of 30 at a fixed random sampling rate of 1000 cellular objects per WSIs from the TCGA-BRCA cohort. The pre-trained SPSD model reconstructed each cellular region (represented as a vector of 15 morphometric properties) as a sparse combination of pre-defined 256 CMBs and thereafter represents each patient as an aggregation of all delineated cellular objects belonging to the same patient.



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Figure 3 iCEMIGE significantly outperforms cellular morphometric prognosis score, 15-microbe abundance prognosis score, and cellular morphometric prognosis score in prognosis prediction in the Cancer Genome Atlas breast cancer cohort. A: Kaplan-Meier overall survival (OS) curves for breast cancer (BC) patients are presented according to iCEMIGE score groups; B: ROC curves for 10-year OS prediction across different signature scores. C: Area under the curve (AUC) of 10-year OS prediction across different signature scores; D: Kaplan-Meier progress-free survival (PFS) curves for BC patients are

presented according to iCEMIGE score groups; E: Receiver operating characteristic (ROC) curves for 10-year PFS prediction across different signature scores. F: AUC of 10-year PFS prediction across different signature scores. The Kaplan-Meier p-values were calculated by the log-rank test among the three groups. The *P* values for AUC were obtained from Kruskal-Wallis test.

The prognostic effect of high or low levels of each CMB on overall survival (OS) was assessed by Kaplan-Meier analysis (survminer package in R, Version 0.4.8) and log-rank test (survival package in R, Version 3.2-3), where the TCGA-BRCA cohort was divided into two groups (*i.e.*, CMB-high and CMB-low groups) based on each CMB (survminer package in R, Version 0.4.8). The set of CMBs as a prognostic signature were selected *via* a multivariate CoxPH regression model including these CMBs with a significant effect on OS.

Finally, we calculated the cellular morphometric prognosis score (CMPS) using the formula below, where the coefficients of the final CMBs as categorical variables were obtained from multivariate CoxPH regression analysis:

$$\mathrm{CMPS} = \sum_{i=1}^{N} \left(coefficient\ of\ CMB_Category^{i} \right) * \left(CMB_Category^{i} \right)$$

Where N is the number of final CMBs that were independently and significantly associated with OS, and $CMB_Category^{i}$ is the category of the i^{th} CMB (*i.e.*, CMB-high: 1; CMB-low: 0).

Mining of multi-modal iCEMIGE biomarker signature

We extended the unsupervised feature learning pipeline (SPSD)[24,25] to achieve efficient and effective mining of multi-modal biomarker signatures from prebuilt cellular-morphometrics, microbiome, and gene biomarkers. Given $X = [x_1,\ldots,x_N] \in R^{m\times N}$ as a set of patients (N) with a combination of biomarkers from different modalities (*i.e.*, cellular-morphometrics, microbiome, and gene biomarkers), the formulation of the iCEMIGE multi-modal biomarker mining model was defined as follows.

$$\boldsymbol{min}_{\mathbf{B,Z,W,G}} \ /\mathbf{X} - \mathbf{BZ}/_F^2 + /\mathbf{Z} - \mathbf{G}\sigma(\mathbf{WX})/_F^2 + \lambda_1 /\mathbf{Z}/_1$$

$$\boldsymbol{s.t.}\ /\boldsymbol{b_i}/_,^2 = \mathbf{1}, \forall \boldsymbol{i} = \mathbf{1}, \ldots, \boldsymbol{h}$$

Where $B = [b_1,\ldots,b_h] \in R^{m\times h}$ was a set of multi-modal biomarkers to be mined. Each multi-modal biomarker (b) was composed of *m* individual biomarker (*e.g.*, m = 66 in our study); $Z = [z_1,\ldots,z_N] \in R^{h\times N}$ was the sparse multi-modal biomarker expression matrix, where z_i was the sparse multi-modal biomarker expression profile of the original patient biomarkers (x_i), consisting of relative abundances of all (*h*) multi-modal biomarkers that contributed to the reconstruction of x_i; $W \in R^{h\times m}$ was the auto-encoder for efficient and effective extraction of sparse multi-modal biomarker expression matrix (*Z*) from original patient biomarker data (X); $G = diag\ (g_1,..,g_h) \in R^{h\times h}$ was a scaling matrix with *diag* being an operator aligning vector $[g_1,..,g_h]$, along the diagonal; $\sigma(\cdot)$ was an element-wise sigmoid function; λ_1 was the regularization constant to ensure the sparsity of *Z*, such that only a subset of multi-modal biomarkers was utilized during the reconstruction of original patient biomarker data.

The first constraint: $\| \mathbf{X} - \mathbf{BZ} \|_{\mathbf{F}}^{\mathbf{2}}$, penalized the reconstruction error of original patient biomarker data (X) with multi-modal biomarker (B) and the corresponding sparse multi-modal biomarker expression matrix (*Z*), which helped minimize the loss of individual biomarker information; the second constraint: $\| \mathbf{Z} - \mathbf{G}\sigma(\mathbf{WX}) \|_{\mathbf{F}}^{\mathbf{2}}$, penalized the approximation error of sparse multi-modal biomarker expression matrix (*Z*) with the auto-encoder, which helped improve the accuracy of multi-modal biomarker extraction for new patients; the third constraint: $\| Z \|_1$, penalized the sparsity of the multi-modal biomarker expression matrix, which helped ensure the utilization/activation of dominant multi-modal biomarkers during the learning process.

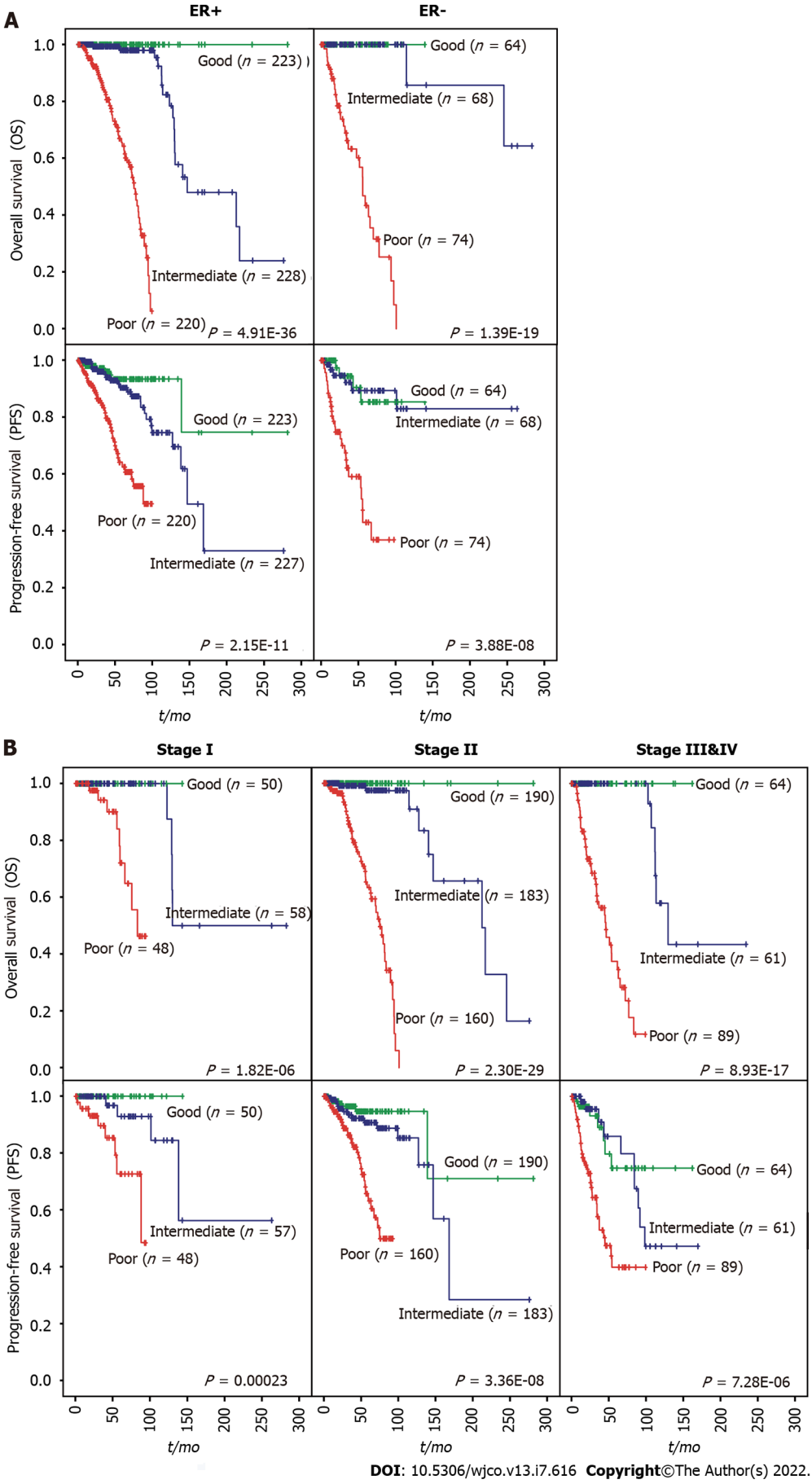
Construction of the iCEMIGE score

After multi-modal biomarker mining (*i.e.*, 256 multi-modal biomarkers mined in this study), a multivariate Cox regression was performed on 256 multi-modal biomarker signatures, defined as 256 covariates using the TCGA-BRCA dataset. The iCEMIGE score of each patient was calculated by the following formula:

$$iCEMIGE\ score = \sum_{i=1}^{256} (\text{ covariate } i\ coefficient) * (\text{ covariate } i\ expression\ level)$$

Nomogram, receiver operating characteristic and C-index

A nomogram model (rms package in R, Version 6.0-1) was constructed to predict 5- and 10-year OS probability of BC patients. The time-dependent receiver operating characteristic (ROC) curve (survival ROC package in R, Version 1.0.3) and concordance index (C-index) were used to evaluate the



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Figure 4 Prognostic value of iCEMIGE score on overall survival and progress-free survival according to ER status and tumor stage. A:

Kaplan-Meier curves on overall survival (OS) (top panel) and progress-free survival (PFS) (bottom panel) for ER+ and ER- breast cancer (BC) patients are presented according to iCEMIGE score groups; B: Kaplan-Meier curves on OS (top panel) and PFS (bottom panel) for Stage I, II, and III&IV BC patients are presented according to iCEMIGE score groups. The *P* values were obtained from the log-rank test among the three groups.

performance of the nomogram model, where the C-index was repeated with 1000 bootstrapping iterations and an 80% sampling rate per iteration. Mann-Whitney non-parametric test was used for the comparison across models.

Statistical analysis

The cohort of patients were divided into three groups (Poor: top third; Intermediate: middle third; and Good: bottom third) based on CMPS or iCEMIGE score. The independent prognostic impact of different scores (CMPS and iCEMIGE) was assessed by multivariate CoxPH regression including the clinical factors (age, stage, ER, and PR status) and PAM50-based molecular subtype. All statistical analyses were performed through either SPSS 24.0 (IBM, NY, United States) or R (version 4.0.2, https://www.r-project.org/). Graphic visualizations were generated by R (ggpubr package, Version 0.4.0; ggplot2 package, Version 3.3.3) or SPSS. The statistical significance was defined as $p<0.05$ (two-tails).

RESULTS

Identifying cellular morphometric biomarkers for prognosis of BC patients

Over 300 million cellular objects from 1085 diagnostic slides of 1017 TCGA-BRCA patients were recognized and delineated by an unsupervised feature learning pipeline based on SPSD[24]. Each cellular object was represented with 15 morphometric properties as described in our previous work[10].

Next, we optimized and trained our SPSD model based on pre-quantified cellular objects randomly selected from the TCGA-BRCA cohort to discover the underlying cellular morphometric biomarkers (CMBs). After training, the prebuilt SPSD model reconstructed each cellular object as a sparse combination of the pre-identified 256 cellular morphometric biomarkers, which led to the novel representation of every single cellular object as 256 sparse code (reconstruction coefficient); and thereafter, the corresponding 256-dimensional cellular morphometric context representation of each patient as an aggregation of all delineated cellular objects belonging to the same patient (Supplementary Table 1). The final patient-level cellular morphometric context representation consisted of 256 CMBs.

We next evaluated the association of 256 CMBs with OS in the TCGA-BRCA cohort. Survival analysis revealed that 148 of 256 CMBs had a significant prognostic impact ($p < 0.05$, Supplementary Table 2). Among these 148 CMBs, 39 CMBs demonstrated independent and significant association with OS by multivariate CoxPH regression analysis (Figure 2A; Supplementary Figure 1; Supplementary Table 3), which were defined as a 39-CMB signature.

Assessing prognostic value of the 39-CMB signature

To further evaluate the prognostic value of the 39-CMB signature, we constructed the cellular morphometric prognosis score (CMPS) (see Methods) and divided TCGA-BRCA cohort into three groups (Poor: top third; Intermediate: middle third; and Good: bottom third) based on CMPS (Supplementary Table 4). Patients with good scores had significantly longer OS than those with poor scores. The OS of patients with intermediate scores was between these two groups (P = 1.61E-23, Figure 2B). Moreover, CMPS provided additional prognostic value to clinical factors (age, ER, PR, and stage) and PAM50-based molecular subtypes (Figure 2C).

Establishing the iCEMIGE prognostic model

Omics analyses of cancers have further revealed their genomic heterogeneity. FDA has approved many genomic biomarkers for clinical use, such as PAM50. Based on the omics data, we have previously identified 12-gene[21] and 15-microbe signatures[19] for the prognosis of BC patients (Supplementary Table 3). We conducted a multivariate Cox regression analysis to address whether GMPS, MAPS, and GEPS are independent prognostic factors. Indeed, CMPS, MAPS, and GEPS were significantly and independently associated with OS (Figure 2D). We then integrated 39 CMBs, 15 microbes, and 12 genes in an unsupervised representation framework ("iCEMIGE") and mined 256 multi-modal biomarkers (Supplementary Table 3) with experimentally optimized parameters for C-index for OS (Supplementary Figure 3). The optimal iCEMIGE score was then constructed to assess a patient's risk for death and disease progression (Supplementary Table 4, details see Materials and Methods).

Evaluating the prognostic value of the iCEMIGE score

A total of 919 BC patients in the TCGA-BRCA cohort with full signature (iCEMIGE) data were included in this evaluation (Supplementary Table 5). 919 BC patients were stratified into different prognostic

groups (Poor: top third; Intermediate: middle third; and Good: bottom third) according to the iCEMIGE score. Patients within the poor prognosis group had significantly shorter OS compared to those within the intermediate and good prognosis groups (P = 4.02E-58, Figure 3A). Importantly, we showed that the iCEMIGE score was more effective in predicting OS of BC patients than CMPS, MAPS, and GEPS alone (Figure 3B and C; Supplementary Figure 2A and B). Moreover, we found that the iCEMIGE score was also significantly associated with PFS (P = 2.40E-19, Figure 3D) and had more effective in predicting PFS (Figure 3E and F; Supplementary Figure 2C and D).

We then evaluated whether the prognostic value of the iCEMIGE score was independent of ER status, stage, and molecular subtypes. As shown in Figure 4A, patients with poor iCEMIGE scores had significantly shorter OS and PFS compared to those with good iCEMIGE scores in both ER+ and ER- groups. Moreover, the iCEMIGE score was significantly associated with OS and PFS in all different stages (Figure 4B) and subtypes (Figure 5).

Finally, using multivariate Cox regression analyses (including pathological stage, age, PR status, ER status, molecular subtype, iCEMIGE), we demonstrated that iCEMIGE was an independent prognostic factor for both OS (Figure 6A) and PFS (Supplementary Figure 4A). These findings indicate that the iCEMIGE score has an independent prognostic value in BCs.

To further assess the clinical value of the iCEMIGE score, we established a nomogram model, a valuable clinical tool for prognosis prediction, where we integrated iCEMIGE with clinical factors (age, stage, ER, and PR), PAM50-based molecular subtypes to predict the 5- and 10-year OS probability of BC patient (Figure 6B). The iCEMIGE score significantly improved the predictive power of prognosis (Figure 6C). Similar results were found for PFS (Supplementary Figure 4B and C).

DISCUSSION

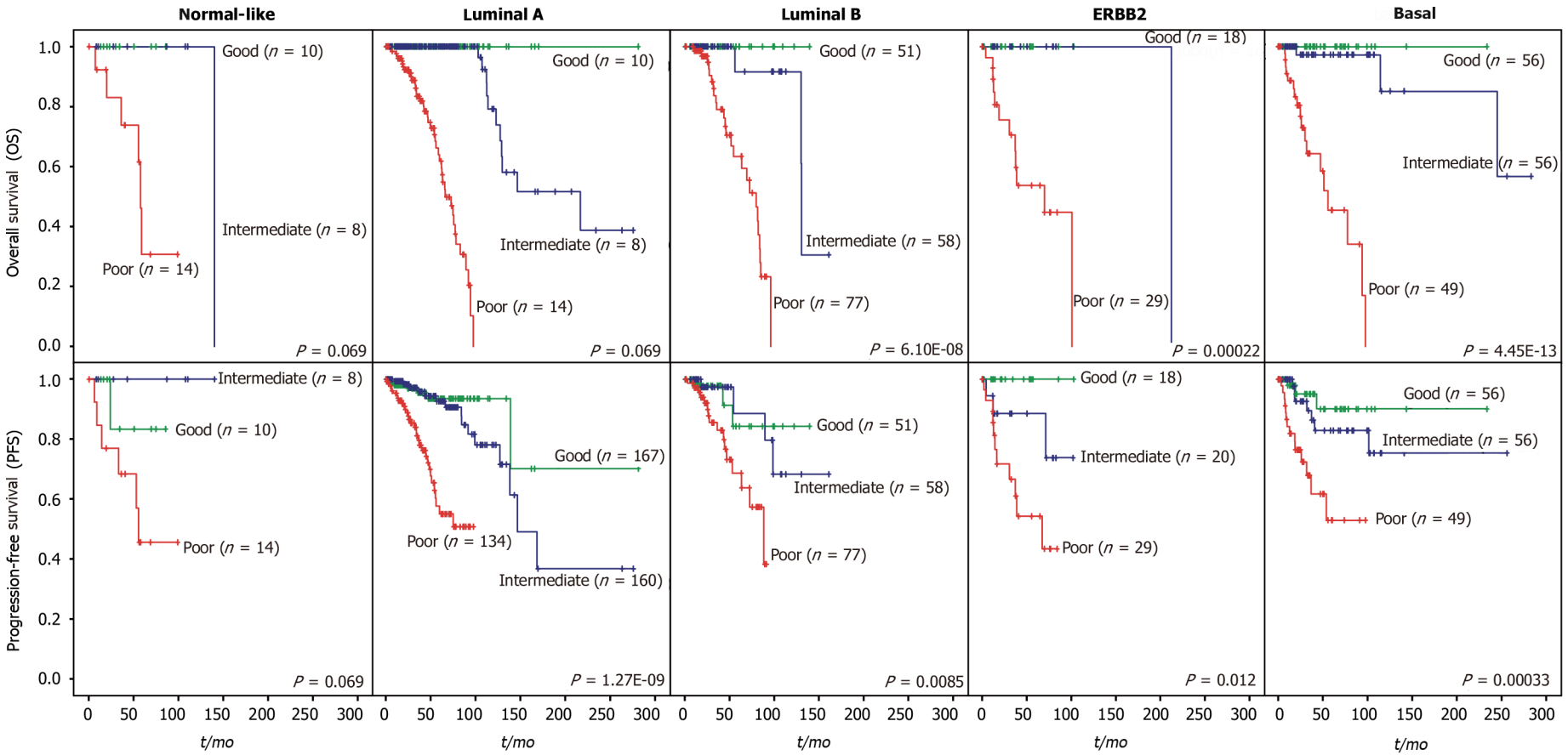
High BC heterogeneity brings up a significant challenge for predicting a patient's response to treatment or prognosis. In this study, we established a new strategy for tackling this challenge by integrating multimodal signatures and demonstrated that such approach significantly improved the power for prognostic prediction compared to the single modal biomarker. In addition, we showed that iCEMIGE is significantly superior in predicting OS and PFS compared to the PAM50-based molecular subtype in the TCGA-BRCA cohort, although additional validation is required, as stated later in the limitations of this study.

The majority of biomarker developments are limited to a single modal data[20]. In the past, we followed the same path to define the 12-gene expression prognosis score (GEPS)[21] and the 15-microbe abundance prognosis score (MAPS)[19] in BC. Here, we developed the 39-CMB prognosis score (CMPS) using an AI-driven CMB detection technique[10]. We found that CMPS, MAPS, and GEPS had an independent prognostic value. This suggests that different modal data provide unique clinical value for prognosis prediction and raises the possibility that integrating multimodal biomarkers can advance precision oncology by more accurately predicting the risk of treatment failure, relapse *etc.*

Integrating multimodal data to yield improved performance compared with each modality alone remains challenging. In this study, we presented a multi-step approach to integrate cellular morphometric, molecular, and microbiome landscapes into a multimodal prognostic system for BC. Firstly, we identified the biomarker signature and systematically assessed its prognostic value in each type of modal data. Secondly, we investigated whether these modal-specific biomarker signatures are independent prognostic factors. Thirdly, we established the final predictive model incorporating all modal biomarker signatures with significantly improved prognostic risk stratification compared with each modality alone. Finally, we systematically evaluated the clinical value of the final predictive model. Such a strategy can extend to other types of cancers.

Modern clinical instruments are generating massive amounts of multimodal data, including radiology, histology, and molecular data, where each of them provides unique value for cancer diagnosis and treatment. Therefore, the efficient and effective integration of multimodal data becomes critical and, however, remains challenging in terms of robustness, interpretability, and translational impact, even with the current advancesin artificial intelligence techniques[26-28]. Two major trends in multimodal integration in cancer research are modal-specific raw data integration (MDI)[29,30] and modal-specific representation integration (MRI)[31,32]. The MDI strategy handles each modality (*e.g.*, histology and genomics) using different neural network structures and then combines the corresponding output of each neural network branch in subsequent network layers to predict the health outcome. Trained in an end-to-end fashion (*i.e.*, black-box fashion), this strategy delivers a convenient and powerful utilization of information and interaction across modalities; however, in general, it lacks biomedical interpretability. In addition, such a strategy does not guarantee the learning of clinically significant and independent information per each modality, and thus the alternative deployment of an individual modality or a subset of modalities is nearly impossible.

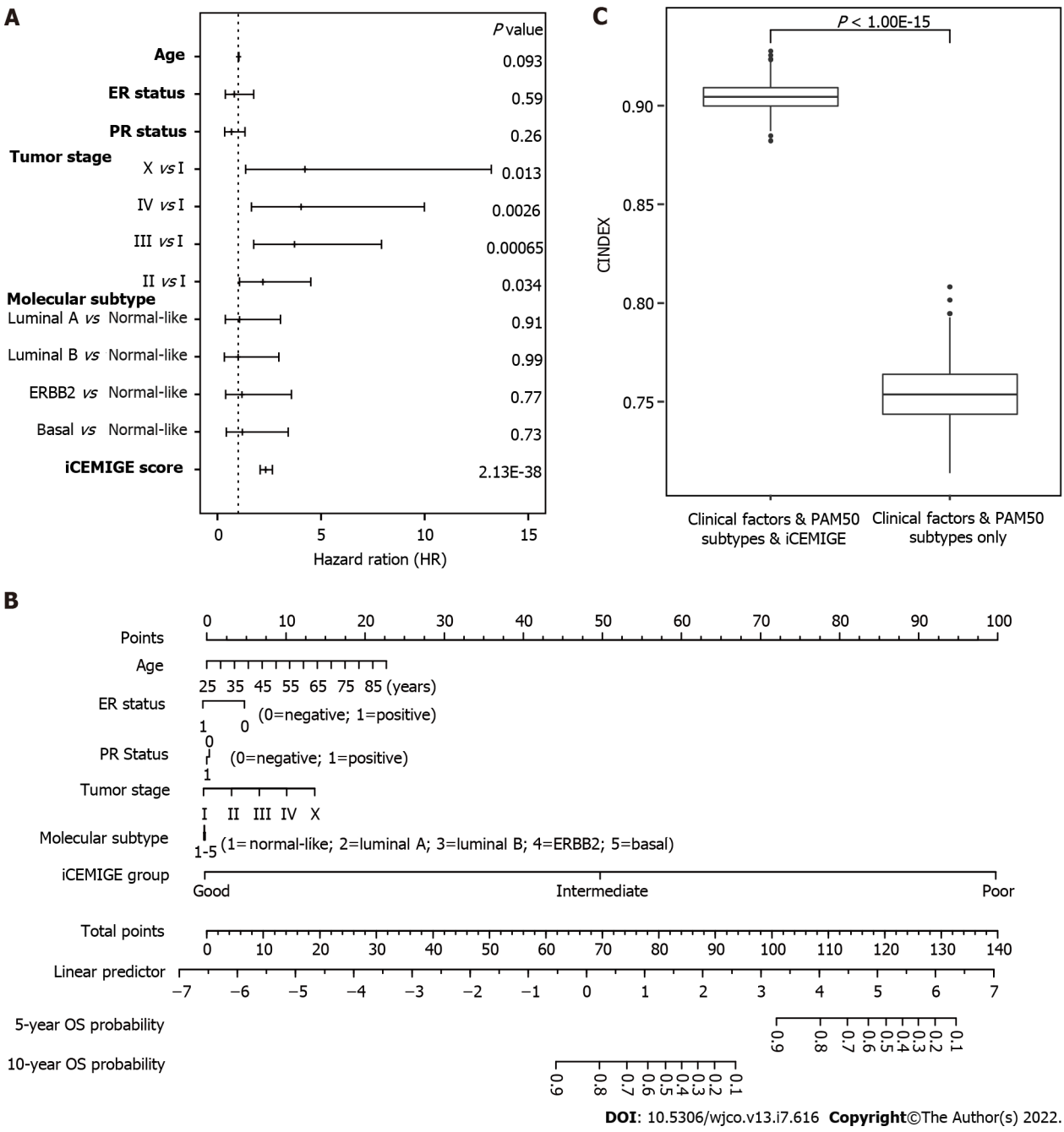
In contrast, the MRI provides a stepwise strategy, where the first step consists of outcome-driven representation mining per modality, and the second step integrates modal-specific representation towards the outcome. Obviously, MRI is more likely (without guarantee) to mine model-specific repres-



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Figure 5 Prognostic value of iCEMIGE scores on overall survival and progress-free survival within different molecular subtypes. Kaplan-Meier curves on overall survival (top panel) and progress-free survival (bottom panel) for breast cancer patients are presented with respect to the iCEMIGE score groups in different molecular subtypes. The *P* values were calculated by the log-rank test among the three groups.

entation with independent clinical value *via* a stepwise mechanism and consequently provides more flexibility in individual/subset modality deployment. This flexibility is important in clinical practice, especially when all modalities are not available. Extended from the MRI strategy, our work realizes the modal-specific knowledge integration (MKI) by enforcing the mining and utilization of biomedically interpretable, clinically significant and independent, and double-blindly validated knowledge (*i.e.*, cellular morphometric biomarkers, microbiome biomarkers, and genomic biomarkers) through an AI-powered systems biology workflow for maximized clinical implications and translation impact.



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Figure 6 iCEMIGE score provides significant and additional value for overall survival prediction. A: Multivariate Cox regression analysis of overall survival (OS) with hazard ratio represented as a forest for iCEMIGE score, clinical factors, and PAM50 subtypes; B: Nomogram for predicting OS was constructed based on integrating clinical factors and molecular subtype with iCEMIGE; C: C-index comparison for OS in different nomogram models with and without iCEMIGE. The *P* value was calculated by Mann-Whitney non-parametric test.

Our study established a new promising strategy for integrating multimodal data to enhance prognostic prediction. A significant limitation was that we did not have independent cohorts to validate our findings. In addition, due to the limited clinical information in the TCGA-BRCA cohort, we were unable to comprehensively explore the potential confounding clinical factors, including tumor size, different cancer treatments, *etc*. The clinical utility of iCEMIGE needs to be further validated in retrospective and prospective cohort studies to determine whether the iCEMIGE score can provide sufficient predictive information to stratify patients by risk and guide treatment. If so, the iCEMIGE score could assist clinicians in decision-making about cancer treatment and enable more personalized cancer therapy.

CONCLUSION

Our study demonstrates a novel and generic AI framework for multimodal data integration toward improving prognosis risk stratification of BC patients, which can be extended to other types of cancer.

ARTICLE HIGHLIGHTS

Research objectives

To develop a strategy to integrate multimodal data and to investigate whether iCEMIGE (integration of cell-morphometrics, microbiome, and gene biomarker signatures) improves the risk stratification of breast cancer patients.

Research motivation

Modern clinical instruments are generating massive amounts of multimodal data, including radiology, histology, and molecular data, where each of them provides unique value for cancer diagnosis and treatment. Efficient and effective integration of these multimodal data is believed to open a new era for precision oncology.

Research background

Cancer heterogeneity consistently results in a large variation in clinical outcomes of patients after treatment. The discovery of biomarkers for tailoring cancer treatments is a critical step toward personalized medicine.

Research perspectives

The iCEMIGE score could assist clinicians in decision-making about cancer treatment and enable more personalized cancer therapy.

Research conclusions

Our study indicates that multimodal integration (iCEMIGE) can more accurately predict the prognostic risk of breast cancer patients.

Research results

iCEMIGE is significantly superior in predicting overall and progression-free survival of breast cancer patients compared to single modal biomarker and the PAM50-based molecular subtype, which is one of FDA approved biomarkers and is currently used in clinical practice.

Research methods

The artificial intelligence pipeline powered is used to identify cellular morphometric biomarkers. Single modal biomarker signatures are integrated using the sparse representation learning technique to establish iCEMIGE. Clinical value of iCEMIGE is evaluated using different statistical methods.

FOOTNOTES

Author contributions: Perez-Losada J, Chang H, and Mao JH planned the project; Chang H, Mao XY, Perez-Losada JP, and Mao JH wrote the manuscript; Mao XY, Chang H, and Mao JH designed the algorithm, performed the bioinformatics analyses, and conducted statistical tests; Abad M, Rodríguez-González M, and Rodríguez CA provided pathological and clinical interpretation; All authors have read and edited the manuscript; Chang H and Mao JH are accountable for communications with requests for reagents and resources; Mao JH and Chang H contributed equally to these senior authors.

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Informed consent statement: The data used in this study are from the public databases. Therefore, the informed

consent is not applicable.

Conflict-of-interest statement: All the authors declare no conflicts of interest.

Data sharing statement: All data used in the study were downloaded from a publicly available source (GDCportal and cBioPortal).

STROBE statement: All the authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Clinical characteristics and outcomes in carbohydrate antigen 19-9 negative pancreatic cancer

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Abstract

BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of death from cancer worldwide. Tumor markers like carbohydrate antigen 19-9 (CA 19-9) have been proven valuable as a diagnostic tool and a predictor for tumor staging and response to therapy.

AIM

To delineate the phenotype of normal CA 19-9 PDAC according to clinical features, disease staging and prognosis as compared with high CA 19-9 PDAC cases.

METHODS

We performed a retrospective single-center analysis of all PDAC cases admitted in our Gastroenterology department over a period of 30 mo that were diagnosed by endoscopic ultrasound-guided tissue acquisition. Patients were divided into two groups according to CA 19-9 levels over a threshold of 37 U/mL. We performed a comparison between the two groups with regard to demographic and clinical

data, biomarkers, tumor staging and 6-mo survival.

RESULTS

Altogether 111 patients were recruited with 29 having documented normal CA 19-9 (< 37 U/mL). In the CA 19-9 negative group of patients, 20.68% had elevated levels of both CEA and CA 125, 13.79% for CA 125 only whilst 17.24% for CEA only. The two groups had similar demographic characteristics. Abdominal pain was more frequently reported in positive *vs* negative CA 19-9 PDAC cases (76.83% *vs* 55.17%), while smoking was slightly more prevalent in the latter group (28.04% *vs* 31.03%). Tumors over 2 cm were more frequently seen in the positive CA 19-9 group, reflecting a higher proportion of locally advanced and metastatic neoplasia (87.7% *vs* 79.3%). Six-month survival was higher for the negative CA 19-9 group (58.62% *vs* 47.56%).

CONCLUSION

Elevated CA 19-9 at diagnosis seems to be associated with a more pronounced symptomatology, high tumor burden and poor prognosis compared to negative CA 19-9 PDAC cases. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

Key Words: Pancreatic cancer; Carbohydrate antigen 19-9; Survival; Lewis; Outcome

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Core Tip: Given the large heterogeneity of pancreatic cancer, delineation of subgroups with different tumor biology is essential for personalized management. We outlined the phenotype of carbohydrate antigen 19-9 negative pancreatic cancer according to clinical features, disease staging and prognosis.

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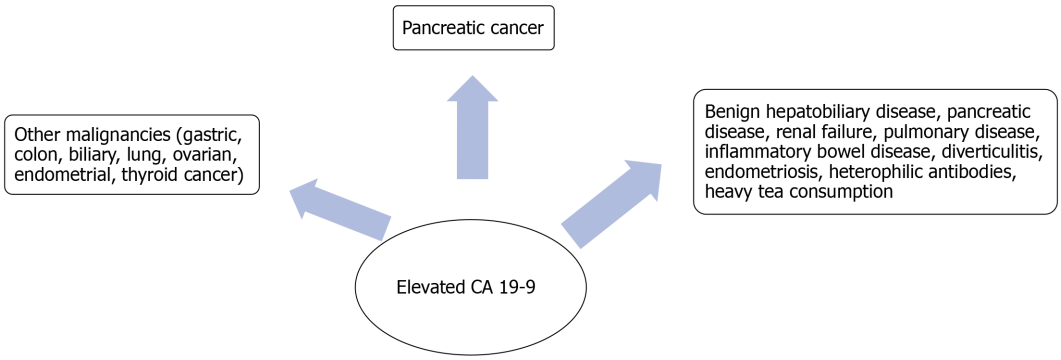
INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of death from cancer worldwide, mostly due to late-stage diagnosis and resistance to chemotherapy. According to Globocan statistics 2020, pancreatic cancer has an incidence rate of 4.9/100000 and mortality almost equal to its incidence of 4.5/100000[1]. In fact, while mortality rates from other types of cancer are decreasing, pancreatic cancer is the only malignancy with an unfavorable trend[2].

Because of its aggressive tumor biology, early diagnosis is very important in order to maximize outcomes. Several strategies have been considered for setting an early accurate diagnosis, from case-finding tools to surveillance of high-risk patients. Alongside the imaging evaluation, there is a great interest in the development of biomarkers for optimizing the management of pancreatic adenocarcinoma[3].

The most commonly used biomarker for PDAC is carbohydrate antigen 19-9 (CA 19-9), which is related to Lewis blood group antigens, and has been proven valuable as a diagnostic tool and in tumor staging, resectability and response to therapy[3]. CA 19-9, also called sialylated Lewis (a) antigen, is synthesized by pancreatic and biliary ductal cells and by other types of epithelium (stomach, colon, uterus, lung, salivary glands), which makes it a nonspecific biomarker for PDAC[4,5]. Elevated CA 19-9 has been reported in both benign and malignant pathology (Figure 1)[6,7]. Expression of CA 19-9 requires the presence of Lewis antigens A [Le(a+b-)] or B [Le(a-b+)], meaning that [Le(a-b-)] are theoretically non-producers of CA 19-9[8]. Lewis negative individuals ([Le(a-b-)]) lack the enzyme α1-3,4 fucosyltransferase, which is required for CA 19-9 biosynthesis. This dysfunction of the Lewis gene is associated with deficient protein fucosylation, which has been involved in cancer development[9].

As CA 19-9 secretion is dependent on the Lewis antigen expression, undetectable false negative results can occur in Lewis antigen-negative individuals, meaning [Le(a−b−)] non-expressors[10]. This could represent a cause of delayed diagnosis in these patients and a pitfall in screening strategies based on CA 19-9. While red cell phenotyping for Lewis antigen status would provide insight in such situations, this is not routinely performed in clinical practice. However, despite the relationship between CA 19-9 secretion and Lewis antigen status, not all Lewis negative individuals with PDAC are non-secretors of CA 19-9, which makes CA 19-9 retain its diagnostic utility at least partially even in this



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Figure 1 Causes of elevated carbohydrate antigen 19-9. CA 19-9: carbohydrate antigen 19-9.

patient category[11-13] (Figure 2).

Given the large heterogeneity of PDAC, delineation of subgroups with different tumor biology is considered of paramount importance for personalized management. Currently available literature is inconsistent regarding the clinical features and outcomes of patients with CA 19-9 or Lewis negative PDAC. Some authors have shown a better prognosis, while others have revealed worse outcomes compared to high CA 19-9 PDAC[14,15]. Our aim was to delineate the phenotype of CA 19-9 negative PDAC according to clinical features, disease staging and prognosis as compared with high CA 19-9 PDAC cases.

MATERIALS AND METHODS

Study design and patient population

We performed a retrospective analysis of patients admitted to our Gastroenterology department during a period of 30 mo, from January 2019 to July 2021, who were diagnosed with PDAC by endoscopic ultrasound guided tissue acquisition. Demographic, clinical, laboratory work-up and imaging data were collected from patients' medical records. Staging was carried out based on pancreatic-protocol computed tomography scan, according to the International Association of Pancreatology criteria for resectability-resectable, borderline resectable, locally advanced or metastatic disease[16]. Regarding tumor location, we grouped cases into lesions extended to head, uncinate and neck of the pancreas comprising one set and tumors of the body and tail representing another set. A 6-mo follow-up aimed at assessing survival was carried out either by reaching out to the general practitioner/oncologist or by contacting the patient/patient's family by phone. Patients with missing data according to items assessed in this research were excluded from analysis. Also, patients lost from follow-up were excluded as survival could not be determined.

For the purpose of this study, we divided patients into two groups according to CA 19-9 levels. A threshold was set at 37 U/mL, and patients were classified as CA 19-9 negative or normal (for values < 37 U/mL)-group A and CA 19-9 positive (≥ 37 U/mL)-group B. We then compared the two groups according to demographic and clinical data, biomarkers, tumor staging and 6-mo survival.

Statistical analysis

Data analysis was carried out using SPSS Statistics 25 software (Armonk, NY, United States). Continuous variables were reported as mean, and categorical variables were reported as count and percentage. Comparison among the two groups was done using χ^2 tests for categorical variable and a two-sample *t*-test for continuous variables at a significance of $\alpha = 0.05$.

RESULTS

Altogether 111 patients were analyzed for the purpose of this study; 29 had documented normal CA 19-9 (< 37 U/mL) and 82 were CA 19-9 positive (≥ 37 U/mL). Demographic data, tumor characteristics and outcomes among the two groups was summarized in Table 1.

With regard to sex distribution, a male predominance was seen in the study cohort (75/111, 67.5%), mostly owing to a higher male:female ratio in group B (2.4:1). Median age was similar between the two groups.

Table 1 Characteristics of study patients according to carbohydrate antigen 19-9 value

	Group A (*n* = 29)	Group B (*n* = 82)	*P* value
Patient demographics			
Age in yr, median	64	67	0.241
Male sex	58.62	70.73	0.333
At risk behaviors			
Smoking	31.03	28.04	0.946
Drinker	20.68	23.17	0.987
Clinical findings			
Abdominal pain	55.17	76.83	0.048
Jaundice	27.58	29.26	0.946
Weight loss	62.06	63.41	0.924
Diabetes mellitus	34.48	34.14	0.845
Tumor localization			
Head, neck and uncinate	62.06	57.31	0.820
Body and tail	37.93	42.68	
Tumor size in cm			
< 2	10.34	2.43	0.447
2-4	58.62	64.63	
> 4	31.03	32.92	
Staging			
Resectable	13.79	7.31	0.714
Borderline resectable	6.89	4.87	
Locally advanced	20.68	24.39	
Metastatic	58.62	63.31	
Outcome			
6-mo survival	58.62	47.56	0.308

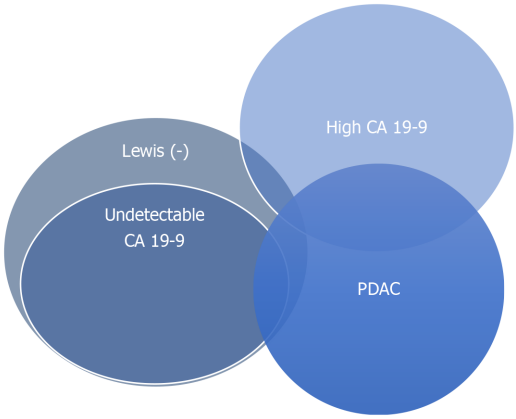
Data are %, unless otherwise indicated. Group A: Patients classified as CA 19-9 negative or normal (< 37 U/mL); Group B: Patients classified as CA 19-9 positive (≥ 37 U/mL).

Considering at risk behavior among the patient population, a higher proportion of smokers was seen in group A (31.03% *vs* 28.04%), while heavy alcohol consumption was seen slightly more frequently in group B (23.17% *vs* 20.68%). Concerning the symptoms, abdominal pain was more prevalent in patients from group B (76.83% *vs* 55.17%), while weight loss and jaundice were noted in similar proportions in both patient groups. Also, diabetes mellitus was seen in about one-third of patients in both groups (34.48% *vs* 34.14%).

The average value of CA 19-9 was 16904.85 for group B compared with 8.48 for group A. In this latter group of patients, 20.68% had elevated levels for both CEA and CA 125, 13.79%for CA 125 only and 17.24% for CEA only. For both groups analyzed, most tumors (62.06%-group A, 57.31%-group B) were located in the head or uncinate process, while the remaining 37.93% and 42.68%, respectively, developed in the body or tail region. Regarding tumor size, there were no significant differences among the two groups in tumors over 4 cm. A higher proportion of lesions under 2 cm was reported in group A (10.34% *vs* 2.43%), while tumors sized 2-4 cm were more frequently seen in group B (64.63% *vs* 58.62%).

Analysis of tumor staging revealed there were more resectable (13.79% *vs* 7.31%) or borderline resectable tumors (6.89% *vs* 4.87%) in group A, while locally advanced and metastatic tumors were predominant in group B (24.39% *vs* 20.68%, 63.41% *vs* 48.62%). Six-month survival was higher in group A (58.62%) compared to group B (47.56%).

We further performed a subgroup analysis according to sex, taking into account the male predominance of our study cohort. While there were more men with elevated CA 19-9 than women (77.33% *vs* 66.67%), the proportion of locally advanced or metastatic tumors was higher in subgroup B females than



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Figure 2 Interrelation between Lewis phenotype, carbohydrate antigen 19-9 and pancreatic ductal adenocarcinoma. CA 19-9: carbohydrate antigen 19-9; PDAC: Pancreatic ductal adenocarcinoma.

males (95.83% *vs* 84.48%). Regarding symptomatology, abdominal pain was more frequent in group B for both sexes, but the difference seen with group A was higher for males (72.41% *vs* 47.06%) than females (87.50% *vs* 66.67%) without being statistically significant. We also conducted an analysis according to an age threshold set at 65 years. While advanced tumors were seen more in subgroup A less than 65 years of age compared to over 65 (86.7% *vs* 71.4%), in group B 90.5% of elderly patients had locally advanced or metastatic neoplasia compared to 83.9% in those under 65 years. Six-month survival was similar in subgroups A and B according to the 65-year threshold (57.1% and 49.0% for patients under 65 years and 60.0% and 45.2%, respectively, for those 65 years or older).

DISCUSSION

CA 19-9 is the most widely used biomarker for PDAC, but its major drawbacks are represented by false positive results in benign inflammatory conditions and extra-pancreatic neoplasms and by false negative results in Lewis negative individuals, which comprise about 10% of the Caucasian population [5]. However, in several aspects CA 19-9 remains a valuable biomarker for PDAC management, from screening and diagnosis to treatment response, prognosis and recurrence (Figure 3)[9,17-21].

In our study, we enrolled PDAC patients and divided them into two groups: CA 19-9 positive (n = 82) and CA 19-9 negative (n = 29), according to a threshold of 37 U/mL. Six-month survival was better in the CA 19-9 negative patients (58.62% *vs* 47.56%), reflecting a lower proportion of locally advanced and metastatic disease in this group. This could be explained by triggering of imaging studies in patients with elevated CA 19-9, leading to an early stage diagnosis and thus a better prognosis, while in patients with negative CA 19-9 further investigations are often deferred due to lack of concern, leading to delayed diagnosis in advanced stages and poorer prognosis.

Some authors have proposed genotyping Lewis antigen along with CA 19-9 dosing in order to improve its diagnostic accuracy[22,23], but recent studies have shown that CA 19-9 retains its utility even in Lewis negative individuals[11]. CA 19-9 values over 37 U/mL were seen in 27.4% of Lewis negative patients, and areas under the receiver operating characteristic curve for the diagnostic accuracy of CA 19-9 were similar in Lewis negative PDAC patients compared to all PDAC patients (0.842 *vs* 0.898). This was also shown by Kwon *et al*[14], who also found that not all Lewis negative PDAC patients are non-secretors of CA 19-9. In this study, 172/375 (45.87%) of patients in the Le(a−b−) group had a serum CA 19-9 over 37 U/mL. The paradoxical elevation of CA 19-9 in Lewis negative individuals might be explained by partial secretion of the protein, which can be detected by enzymatic immunoassays or by cross-reactivity of the antibodies used for CA 19-9 dosing; treating the collected specimen with blocking agents has been proposed as a method to eliminate interference with heterophilic antibodies[5,13,24]. Therefore, PDAC prognosis is different if patients are stratified according to either CA 19-9 or to Lewis antigen.

A literature search of studies assessing PDAC outcomes according to CA 19-9 and Lewis antigen status has shown inconsistent results (Table 2)[11,14,25-40]. While low CA 19-9 PDAC has been associated with better prognosis, some have shown that Lewis negative PDAC harbors a more aggressive tumor biology and has a poorer outcome[15]. Discordant results might be due to different patient populations and different timeframes of studies, and not least to overlap of Lewis-negative with detectable CA 19-9 PDAC patients. Some authors have concluded that the usefulness of the 37 U/mL threshold for CA 19-9 is more appropriate for PDAC diagnosis than predicting prognosis. However, others have shown a strong correlation of CA 19-9 with tumor burden, survival and recurrence[41,42].

Table 2 Studies reporting on pancreatic ductal adenocarcinoma prognosis according to carbohydrate antigen 19-9 level or Lewis antigen status

Survival analysis according to CA 19-9 values			
Ref.	***n***	**CA 19-9 in U/mL**	**Survival**
			Overall median survival in mo
Berger *et al*[30], 2004	7	Undetectable	32
	21	≤ 37	35
	44	38-200	22
	57	> 200	16
Ferrone *et al*[33], 2006			Mean survival time in yr
	29	< 37	2.3
	82	≥ 37	1.6
Waraya *et al*[28], 2009			Disease-specific survival in mo
	23	≤ 37	30.6
	66	> 37	12.7
Hirakawa *et al*[29], 2011			Median survival in mo
	41	Normal	39.0
	84	Elevated	16.9
Hartwig *et al*[32], 2011			Median survival in mo
	232	< 37	28.0
	418	37-399	23.5
	239	≥ 400	14.5
Turrini *et al*[40], 2009			Median survival in mo
	50	< 37	22
	53	400-900 (n = 27), > 900 (n = 26)	15
Katz *et al*[34], 2010			Median survival in mo
	21	< 37	52.8
	78	> 37	21.2
Kondo *et al*[35], 2010			Preoperative 3-yr survival (%)
	32	< 37	57%
	77	> 37	30%
Hata *et al*[36], 2012			Preoperative median survival in mo
	51	< 37	16.2
	218	> 37	16.4
Bergquist *et al*[37], 2016			Median OS in mo
	3666	< 37	19.1
	7140	> 37	14
Jia *et al*[38], 2019			Median OS in mo
	13	< 35	21
	107	≥ 35	11
Mattiucci *et al*[25], 2019			Median OS in mo
	39	0-5.0	25
	167	5.1-37.0	38

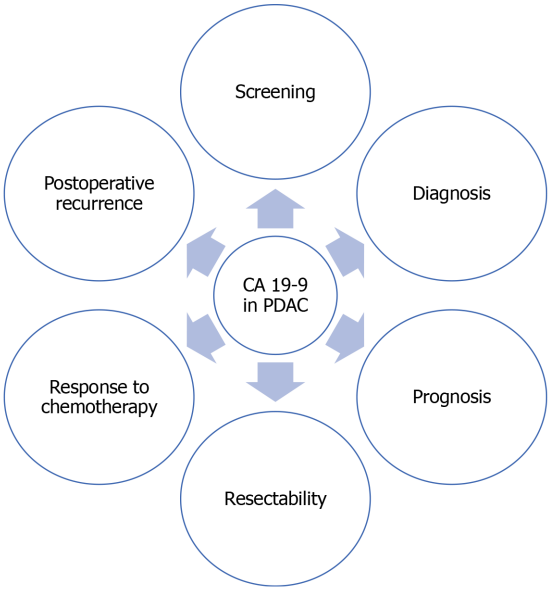
	139	37.1-100.0	32
	178	100.1-353.0	22
	177	> 353.1	20
Kondo *et al*[26], 2017			Median survival in mo
	65	< 37	52.0
	84	≥ 37	23.7
	88	< 50	52.0
	61	≥ 150	20.9
	101	< 300	46.7
	48	≥ 300	18.8
Dong *et al*[27], 2014			Median OS in mo
	18	< 37	21.6
	102	≥ 37	14.2
Kang *et al*[31], 2007			Disease free survival in mo
	18	< 50	22.20
	43	≥ 50	19.31
Kwon *et al*[14], 2020			Median survival in d
	408	< 37	644
	779	> 37	340
Survival analysis according to Lewis antigen status			
Luo *et al*[39], 2017			Median survival in mo
	682	137 CA 19-9 (-)	Stage I, II: 16.6 in Lewis (-), 17.6 in Lewis (+)
		47 Lewis (-)	Stage III, IV: 6.0 in Lewis (-), 7.8 in Lewis (+)
Luo *et al*[11], 2018			Median survival in mo
	1482	19.8% CA 19-9 (-)	8.0 in Lewis (-)
		8.4% Lewis (-)	10.0 in Lewis (+)
Kwon *et al*[14], 2020			Median survival
	1187	203 CA 19-9 (-)	356 d in Lewis (-)
		375 Lewis (-)	477 d in Lewis (+)

CA 19-9: Carbohydrate antigen 19-9; OS: Overall survival.

In order to better predict outcomes, some have proposed measuring other markers such as CA 242, CA 50, CEA, CA 125 or periostin complementary to CA 19-9 for PDAC[43-48]. Additional markers, such as CEMIP, apolipoprotein A-I and transferrin[49,50], were shown to be useful especially in PDAC with normal CA 19-9 levels. Lee *et al*[49] showed that CEMIP (also called KIAA1199) had a diagnostic yield of 86.1% in CA 19-9 negative PDAC, and the combination of CEMIP + CA 19-9 had a significantly improved area under the receiver operating characteristic curve over CA 19-9 alone (0.94 *vs* 0.89, $P < 0.0001$). In our study, 34.47% of CA 19-9 negative PDAC cases had elevated levels of CA 125, 37.92% for CEA and 20.68% for both. Concerning the patients with negative CA 19-9 and positive CA 125 and CEA, 83.33% had metastatic disease at the time of the diagnosis and only 50.00% survived at 6 mo.

Similar results were seen in the paper by Luo *et al*[39]. In Lewis negative patients, high values of CEA were seen in 63.8% of patients, and CA 125 was seen in 51.1%. They concluded that CEA and CA 125 should be routinely measured for PDAC. Considering the metastatic burden and survival among 853 pancreatic cancer patients, Liu *et al*[15] observed that Lewis negative PDAC constitutes an aggressive tumor subtype, with low secretion of CA 19-9 and high secretion of CA125. In line with Luo *et al*[39], others have highlighted the fact that CEA and CA 125, similar to CA 19-9, can also be used to monitor therapeutic response[51].

Interestingly, several papers have shown that CA 19-9 and the other biomarkers are upregulated early in the course of PDAC development-up to 2 years before clinical diagnosis and can be used to



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Figure 3 Usefulness of carbohydrate antigen 19-9 in pancreatic ductal adenocarcinoma management. CA 19-9: carbohydrate antigen 19-9; PDAC: Pancreatic ductal adenocarcinoma.

detect preclinical pancreatic cancer[52,53]. This could be useful for screening strategies of high-risk groups, keeping in mind that Lewis negative individuals might be missed by this approach. Moreover, clinicians should take note that CA 19-9 is also of limited value in the follow-up of Lewis negative patients, in order to avoid erroneous decisions in PDAC management.

The current study has several limitations. Patients recruited in this study were from a hospital-based setting, which had either an acute presentation (jaundice, pancreatitis) or were referred for diagnostic procedures. Also, we acknowledge the lack of Lewis antigen genotyping in our study population, which might have provided further insight into PDAC outcomes according to both CA 19-9 and Lewis antigen status. Another important limitation is the sample size, which makes it very difficult to obtain a statistically significant analysis.

CONCLUSION

In our study, patients with negative CA 19-9 had a better prognosis than those with values over 37 U/mL. Elevated CA 19-9 at diagnosis seems to be associated with a more pronounced symptomatology and higher tumor burden. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

ARTICLE HIGHLIGHTS

Research background

Carbohydrate antigen 19-9 (CA 19-9) is the most widely used biomarker for pancreatic ductal adenocarcinoma (PDAC), but its use is hindered by both false-positive and false-negative results.

Research motivation

There are inconsistent results regarding the outcome of CA 19-9 negative PDAC cases.

Research objectives

To delineate the phenotype of negative CA 19-9 PDAC according to clinical features, disease staging and outcome.

Research methods

Retrospective single-center analysis of PDAC cases over a period of 30 mo.

Research results

Among 111 recruited patients, 29 had normal CA 19-9. Patients with elevated CA 19-9 had higher tumor burden and more advanced staging. Six-month survival was higher for the negative CA 19-9 group (58.62% *vs* 47.56%).

Research conclusions

Negative CA 19-9 PDAC has a better prognosis than PDAC with high CA 19-9 values. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

Research perspectives

Negative CA 19-9 PDAC cases warrant in-depth analysis of tumor biology to assess if there is indeed a different phenotype of neoplasia.

FOOTNOTES

Author contributions: Balaban DV proposed the research idea; Balaban DV, Marin FS, Manucu G and Zoican A drafted the study design; Jinga M critically reviewed the manuscript; all authors were involved in patient recruitment, data analysis, literature review and drawing of figures and tables and contributed to the initial version of the manuscript.

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Data sharing statement: Data sharing is available upon request from the corresponding author.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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ORIGINAL ARTICLE

Retrospective Study

Necessity of neutrophil-to-lymphocyte ratio monitoring for hypothyroidism using nivolumab in patients with cancer

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Abstract

BACKGROUND

Low neutrophil-to-lymphocyte ratio (NLR) has been shown to be associated with a favorable therapeutic response to nivolumab. The activation of immunocompetent cells such as lymphocytes exhibits an antitumor effect; however, the development of excessive immune responses in autologous organs along with the breakdown of self-tolerance causes immune-related adverse events, including hypothyroidism. Therefore, the possibility that NLR is associated with immune response shows that NLR can be not only a predictive factor for good response to nivolumab but also a predictive factor for the development of hypothyroidism.

AIM

To evaluate whether continuous NLR monitoring during nivolumab treatment is useful for predicting the incidence and onset period of hypothyroidism.

METHODS

This retrospective study comprised patients who received nivolumab for treating all types of cancer at our hospital between January 2015 and December 2019. The NLRs of patients were measured before each administration, and the patients were followed up till the administration of 12 doses. NLR at treatment initiation was compared between patients with and without hypothyroidism. Patients who developed hypothyroidism were categorized into three groups: those with NLR < 3.5, 3.5 to < 5, and ≥ 5 according to their maximum NLR from treatment initiation to hypothyroidism development. Further, the onset periods of hypothyroidism were compared between the groups.

RESULTS

Overall, 104 patients were included in the analysis. Twenty-one patients developed hypothyroidism throughout the observation period. NLR at treatment initiation was significantly lower (2.54 ± 1.21 *vs* 4.58 ± 4.03; $P = 0.017$) in patients with hypothyroidism than in those without hypothyroidism, and patients with NLR < 5 had a significantly higher incidence of hypothyroidism than those with NLR ≥ 5 (26%: 20 of 78 patients *vs* 4%: 1 of 26 patients; $P = 0.022$). Additionally, treatment continuity in patients with hypothyroidism was significantly longer than in those without hypothyroidism (median not reached *vs* 7 times administration, $P = 0.010$). Patients with maximum NLR < 3.5 until the development of hypothyroidism had a significantly earlier onset of hypothyroidism than those with maximum NLR ≥ 5 (hazard ratio for low tertile [NLR < 3.5] *vs* high tertile [NLR ≥ 5]: 5.33, $P = 0.011$).

CONCLUSION

Low NLR at treatment initiation increases the incidence of treatment-induced hypothyroidism. Furthermore, its persistence may be a risk factor for the early onset of hypothyroidism.

Key Words: Nivolumab; Hypothyroidism; Immune checkpoint inhibitors; Immune-related adverse event; Neutrophil-to-lymphocyte ratio

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Core Tip: This study evaluated whether continuous monitoring of neutrophil-to-lymphocyte ratio (NLR) during nivolumab treatment is useful for predicting the incidence and onset period of hypothyroidism. Patients with hypothyroidism had a significantly lower NLR at treatment initiation, and hypothyroidism incidence was higher among those with NLR < 5. Patients with persistently low NLR (< 3.5) developed hypothyroidism earlier than those with an NLR of 3.5 to < 5 and ≥ 5. Low NLR at treatment initiation increases the incidence of treatment-induced hypothyroidism. Furthermore, its persistence may be a risk factor for the early onset of hypothyroidism.

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INTRODUCTION

The immune checkpoint inhibitor nivolumab restores and activates antigen-specific T cells that have become unresponsive to cancer cells by inhibiting the binding of programmed death-1 (PD-1) to PD-1 Ligands (PD-L1) and exerts antitumor effects[1]. Nivolumab has been successfully used to treat various types of cancer, including advanced melanoma, non-small-cell lung cancer, renal cell carcinoma, classical Hodgkin lymphoma, head and neck cancer, gastric cancer, and malignant pleural mesothelioma. Although nivolumab exerts a remarkable effect on cancer, it requires a certain period until the manifestation of treatment response[2-10]. Considering that other treatments may be required if nivolumab does not achieve a good treatment response, early identification of predictive factors for its efficacy is highly desired. Treatment with nivolumab is accompanied by immune-related adverse events (irAEs), such as hypothyroidism[11]. A recent study suggested that the development of irAEs was associated with treatment benefit[12-15]. The mechanism by which nivolumab elicits an antitumor and antithyroid immune response has not been fully elucidated. The activation of immunocompetent cells by nivolumab results in an antitumor effect. However, the development of excessive immune responses in autologous organs along with the breakdown of self-tolerance causes irAEs, such as hypothyroidism. Neutrophil-to-lymphocyte ratio (NLR) has gained attention as a predictive factor for the efficacy of nivolumab; particularly, low NLR at treatment initiation has been associated with a favorable therapeutic response[16-20]. Therefore, it is assumed that the association of NLR with an immune response shows that NLR is both a predictive factor for nivolumab efficacy and an indicator of the risk for hypothyroidism. In our previous study with patients who responded to six or more doses of nivolumab, we showed that patients with NLR < 5 at the 6th administration had a significantly higher incidence of hypothyroidism[21]. Although we showed the effect of low NLR on the incidence of hypothyroidism, NLR was evaluated only at a fixed observation point, i.e., at the 6th administration of nivolumab. In this study, we investigated whether continuous monitoring of NLRs during nivolumab

treatment is necessary to predict the frequency and onset period of hypothyroidism.

MATERIALS AND METHODS

Patients

This single-center retrospective study comprised patients who received nivolumab regardless of the type of cancer at the Jikei University Hospital between January 2015 and December 2019. The dosage of nivolumab was 3 mg/kg every 2 wk up to October 2018, and due to the revision in guidelines, the dosage of nivolumab was 240 mg/person every 2 wk thereafter. This study included patients who underwent thyroid-stimulating hormone (TSH) and free thyroxine (FT4) measurements at every or alternate administration of nivolumab to assess fluctuation in NLR. The exclusion criteria were as follows: patients with a history of hypothyroidism, thyroid cancer; those at treatment initiation; and those with TSH levels above the upper limit or FT4 Levels below the lower limit of the reference values. Patients who discontinued nivolumab after single administration were also excluded from the analysis because fluctuations in laboratory data could not be analyzed. The reference values of TSH and FT4 Levels were 0.34-4.04 μIU/mL and 0.88-1.67 ng/dL, respectively, based on the Japanese Committee for Clinical Laboratory Standards. In this study, hypothyroidism was defined as TSH levels exceeding the upper limit or FT4 Levels falling below the lower limit of the reference values twice in a row during the nivolumab observation period, with the follow-up period being up to the 12th administration.

NLR and nivolumab treatment continuity

NLR was calculated by dividing the absolute neutrophil and lymphocyte counts measured in peripheral blood samples at each administration. The follow-up period was up to the 12th administration, and each NLR from treatment initiation to the 12th administration was investigated. The decision to discontinue treatment was made by the clinician depending on the progression of disease or the development of severe irAEs. Fluctuations in NLRs were assessed for the following groups of patients: Those who discontinued treatment after administering nivolumab < 6 times, those who discontinued treatment after administering nivolumab 6-11 times, and those who administered nivolumab ≥ 12 times. In particular, we compared NLR fluctuation at treatment initiation and discontinuation among the patients who received nivolumab < 6 times and 6-11 times. Among the patients who received nivolumab ≥ 12 times, we compared NLR fluctuation at treatment initiation and the 12th administration.

Furthermore, we categorized the patients into three groups according to the tertiles of their mean NLR as follows: NLR < 3.5, NLR 3.5 to < 5, and NLR ≥ 5 during the observation points. This analysis compared the differences in treatment continuity between the NLR 3.5 to < 5 and NLR ≥ 5 groups relative to the NLR < 3.5 group.

NLR and hypothyroidism

Patients were classified into two groups according to the presence or absence of hypothyroidism, and the difference in treatment continuity between the two groups was evaluated.

Patients who developed hypothyroidism were categorized into three groups according to the tertiles of their maximum NLR from treatment initiation to development of hypothyroidism as follows: NLR < 3.5, NLR 3.5 to < 5, and NLR ≥ 5. The onset period of hypothyroidism was defined as the number of times nivolumab was administered until the onset. This analysis compared the differences in onset period of hypothyroidism between the NLR < 3.5 and NLR 3.5 to < 5 groups relative to the NLR ≥ 5 group.

Statistical analysis

The distribution of continuous variables was evaluated using the Shapiro-Wilk test. Based on the distribution of the data, continuous variables were statistically analyzed using the Student t test or Mann-Whitney's *U*-test. Categorical variables were statistically analyzed using Fisher's exact test. For comparing the NLR levels during nivolumab treatment or at discontinuation, we used the Wilcoxon signed-rank test for the following groups: patients who discontinued treatment after administering nivolumab < 6 times, those who discontinued treatment after administering nivolumab 6-11 times, and those who administered nivolumab ≥ 12 times. The differences in nivolumab treatment continuity and onset period of hypothyroidism were calculated using the Kaplan-Meier method and analyzed using the log-rank test and Cox proportional hazards analysis. All statistical data were analyzed using the BellCurve for Excel (Social Survey Research Information Co., Ltd. Tokyo, Japan). The significance level of the tests was set at 0.05.

RESULTS

Patients and NLR at treatment initiation

A total of 104 patients were included in the analysis. Nivolumab was administered primarily at 2-week intervals, but it was temporarily administered at 3-week intervals when the hospital was closed or requested by the patient. Table 1 summarizes the background characteristics of patients who received nivolumab and their types of cancers. Throughout the observation period, 21 of 104 (20%) patients developed hypothyroidism. NLR at treatment initiation in patients with hypothyroidism was significantly lower than that in patients without hypothyroidism (2.54 ± 1.21 *vs* 4.58 ± 4.03; $P = 0.017$). Patients with NLR < 5 had a significantly higher incidence of hypothyroidism than those with NLR ≥ 5 (26%: 20 of 78 patients *vs* 4%: 1 of 26 patients; $P = 0.022$).

Association between NLR and nivolumab treatment continuity

The median values of NLR at treatment initiation in patients who received nivolumab administration < 6, 6-11, and ≥ 12 times were 4.01, 3.03, and 2.64, respectively (Figure 1). A significant increase in NLR was observed at discontinuation in 40 patients who discontinued treatment after administering nivolumab < 6 times (median NLR, 4.01 *vs* 5.92, $P = 0.020$; Figure 1A). The reasons for the discontinuation of nivolumab in these patients were progression of disease in 34 patients and development of severe irAEs in six patients (pneumonitis: two patients, rashes: one patient, myocarditis: one patient, hypophysitis: one patient, and eosinophilia: one patient). A significant increase in NLR was observed at discontinuation in 32 patients who discontinued treatment after administering nivolumab 6-11 times (median NLR, 3.03 *vs* 3.50, $P = 0.038$; Figure 1B). The reasons for the discontinuation of nivolumab in these patients were progression of disease in 26 patients and severe irAEs in six patients (pneumonitis: three patients, rashes: two patients, and colitis: one patient). Finally, no significant differences in NLR were observed between the treatment initiation and the 12th administration in 32 patients who received nivolumab ≥ 12 times (median NLR, 2.64 *vs* 2.32, $P = 0.940$; Figure 1C).

When the population was categorized into three groups based on the tertiles of their mean NLR during the observation period as NLR < 3.5, 3.5 to < 5, and ≥ 5, we observed a significant difference in treatment continuity between the three groups, as shown in Figure 2. The median number of times that nivolumab was administered in each group with mean NLR < 3.5, 3.5 to < 5, and ≥ 5 was 11.5, 8, and 4, respectively. The groups with mean NLR < 3.5 and 3.5 to < 5 had significantly longer treatment continuity than the group with NLR ≥ 5 (hazard ratio [HR] for low tertile compared with high tertile: 0.23; 95% confidence interval [CI]: 0.13-0.41, $P < 0.001$; HR for middle tertile compared with high tertile: 0.32; 95%CI: 0.17-0.60; $P < 0.001$).

Association between NLR and hypothyroidism

Treatment continuity was significantly longer in patients who developed hypothyroidism than in patients without hypothyroidism (median not reached *vs* 7 times administration, $P = 0.010$; Figure 3).

No patients discontinued nivolumab due to hypothyroidism. In patients who developed hypothyroidism, the reasons for discontinuing nivolumab during the observation period were progression of disease in nine patients and severe irAEs in two patients (pneumonitis: one patient and rashes: one patient). In patients without hypothyroidism, the reasons for discontinuing nivolumab during the observation period were progression of disease in 51 patients and severe irAEs in ten patients (pneumonitis: four patients, rashes: two patients, myocarditis: one patient, colitis: one patient, eosinophilia: one patient, and hypophysitis: One patient).

When the population was categorized into three groups based on the tertiles of their maximum NLR from treatment initiation to development of hypothyroidism, we observed a significant difference in the onset period, as shown in Figure 4. The median onset periods of each group with maximum NLRs of < 3.5, 3.5 to < 5, and ≥ 5 were at 5th, 6th, and 9th administrations, respectively. The groups with maximum NLR < 3.5 had a significantly earlier onset of hypothyroidism than the group with NLR ≥ 5, whereas there was no significant difference in the onset periods of the groups with maximum NLRs of 3.5 to < 5 and ≥ 5 (HR for low tertile compared with highest tertile: 5.33; 95%CI: 1.47-19.33, $P = 0.011$; HR for middle tertile compared with highest tertile: 3.15; 95%CI: 0.83-11.89, $P = 0.091$).

DISCUSSION

This study evaluated treatment outcomes as the number of times of nivolumab administration. The median values of NLR at treatment initiation in patients who administered nivolumab < 6, 6-11, and ≥ 12 times were 4.01, 3.03, and 2.64, respectively. Previous studies have found that low NLR at treatment initiation is associated with favorable therapeutic outcomes[16-20]; the results of this study are similar to those previously reported. Because the cancer treatment response to nivolumab is assessed up to the 6th administration[2-10], patients who discontinue after administering nivolumab < 6 times are considered to show a lack of therapeutic effect, whereas those who discontinue after administering nivolumab 6-11 and ≥ 12 times are considered to show a therapeutic effect. Therefore, patients with high NLR at

Table 1 Characteristics of patients at treatment initiation

	All patients (*n* = 104)	Hypothyroidism		
		Yes (*n* = 21)	No (*n* = 83)	*P* value
Male/female	69/35	14/7	55/28	1.000
Median age (min-max) (years)	68.5 (32-91)	70.0 (45-91)	68.0 (32-88)	0.382
Body weight (kg)	52.7 ± 11.9	54.0 ± 9.6	52.4 ± 12.4	0.340
Cancer type				
Head and neck cancer	29	6	23	1.000
Non-small-cell lung cancer	29	6	23	1.000
Malignant melanoma	16	1	15	0.183
Renal cell cancer	15	4	11	0.497
Gastric cancer	15	4	11	0.497
Laboratory data				
TSH (μIU/mL)	2.08 ± 0.80	2.34 ± 0.78	2.02 ± 0.79	0.058
FT3 (pg/mL)	2.21 ± 0.50	2.12 ± 0.32	2.23 ± 0.54	0.584
FT4 (ng/dL)	1.18 ± 0.20	1.11 ± 0.20	1.20 ± 0.19	0.064
NLR	4.17 ± 3.73	2.54 ± 1.21	4.58 ± 4.03	0.017
NLR < 3.5	60	16	44	0.082
NLR ≥ 3.5	44	5	39	
NLR < 5	78	20	58	0.022
NLR ≥ 5	26	1	25	

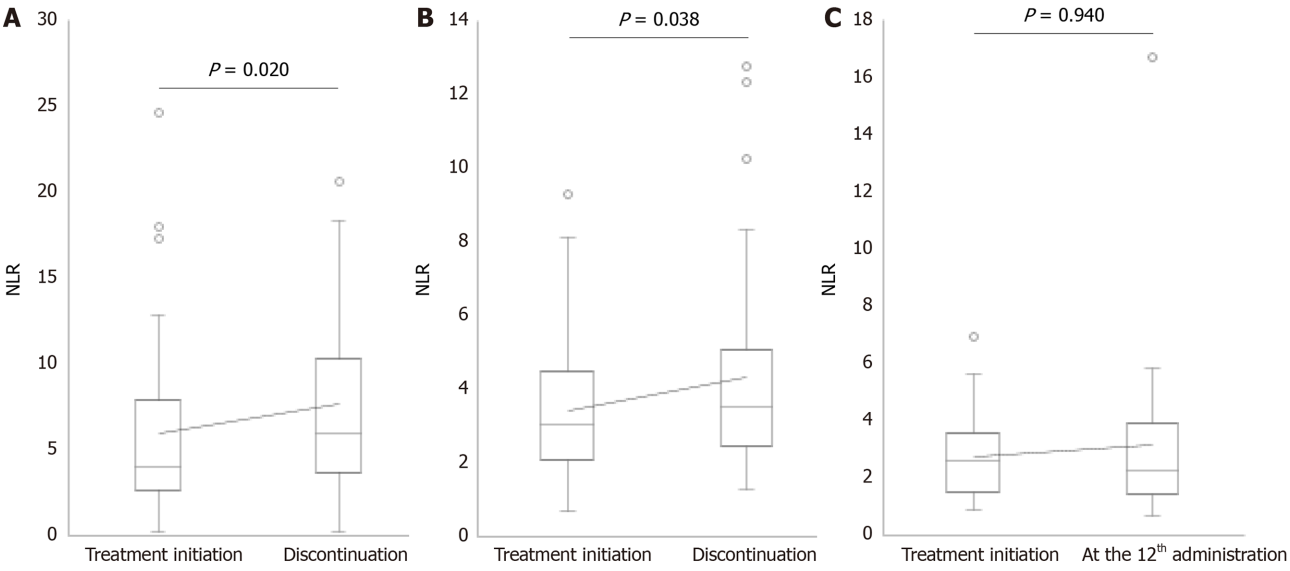
TSH: Thyroid-stimulating hormone; FT3: Free triiodothyronine; FT4: Free thyroxine; NLR: Neutrophil-to-lymphocyte ratio; min: Minimum; max: Maximum.

treatment initiation may not show a therapeutic effect until the 6th administration, increasing the possibility of discontinuation.

A previous study reported that low NLR at the 4th administration of nivolumab was associated with prolongation in overall survival and that responding patients showed a decline in their longitudinal NLR over time[22,23]. We found that patients with mean NLR < 3.5 and 3.5 to < 5 had significantly longer treatment continuity than those with mean NLR ≥ 5. Thus, we suggest that low NLR (mean NLR < 5) can be useful for predicting treatment continuity. Interestingly, a significant increase in NLR was observed at treatment discontinuation (Figure 1A-C). PD-1 expressed on activated T cells binds to PD-L1 expressed on cancer cells to transmit an inhibitory signal to T cells; however, nivolumab promotes the reactivation of the immune response by suppressing this inhibitory signal[1,24]. Thus, low NLR levels indicates that the antitumor effect of nivolumab sustains the lymphocyte-dominant immune state, whereas an increase in NLR indicates that the weakened immune activation affects the discontinuation of nivolumab.

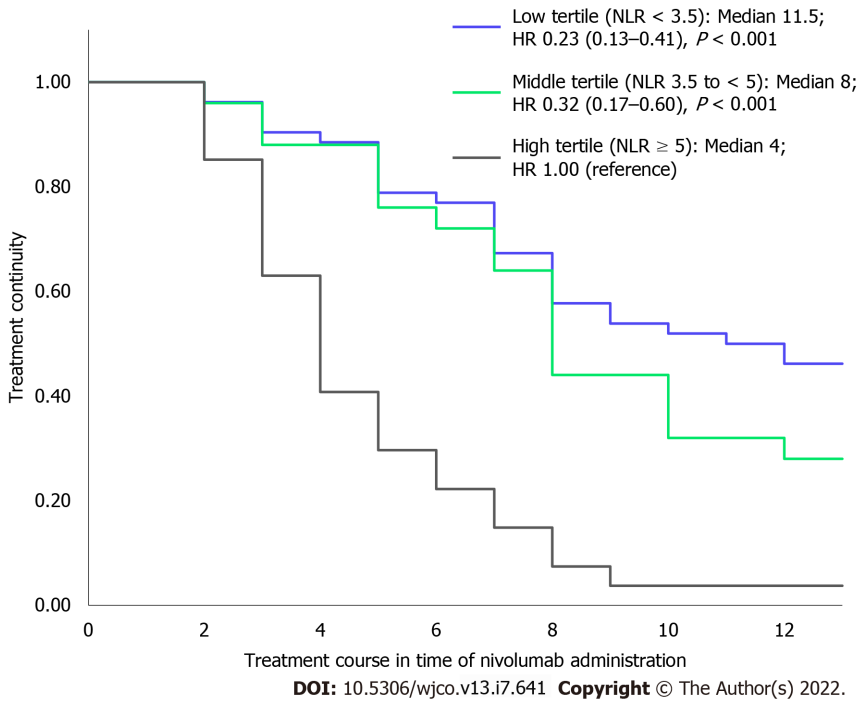
Patients who developed irAEs have shown favorable treatment response to nivolumab[12,13]. Furthermore, it has recently been reported that patients who developed hypothyroidism, one of the irAEs, during treatment also showed a favorable therapeutic response[14,15]. Our study showed that patients with hypothyroidism have a longer treatment continuity than those without hypothyroidism, supporting the results of the previous studies.

Although it has been mentioned above that monitoring NLR fluctuations during treatment is useful for predicting the therapeutic effect, whether NLR fluctuations can be used to predict the onset period of hypothyroidism is an interesting topic. However, Matsukane *et al*[25] showed that there was no significant change in NLR from the period of treatment initiation to development of hypothyroidism in patients who developed hypothyroidism after administering nivolumab. Thus, NLR fluctuations during treatment cannot predict the development of hypothyroidism. However, the present study revealed that patients who developed hypothyroidism showed significantly lower NLR at treatment initiation and patients with NLR < 5 showed a significantly higher incidence of hypothyroidism than those with NLR ≥ 5. We further investigated whether the persistence of low NLR affected the difference in the onset period of hypothyroidism. In particular, we investigated whether patients with NLR < 3.5 and NLR 3.5 to < 5 at treatment initiation had an earlier onset period than those with NLR ≥ 5. This study showed



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Figure 1 Neutrophil-to-lymphocyte ratio fluctuation in patients who discontinued treatment after administering nivolumab < 6 times, who discontinued treatment after administering nivolumab 6-11 times, and who administered nivolumab ≥ 12 times. A: A significant increase in neutrophil-to-lymphocyte ratio (NLR) was observed at the discontinuation (n = 40, median NLR = 4.01 *vs* 5.92, P = 0.020); B: A significant increase in NLR was observed at the discontinuation (n = 32, median NLR = 3.03 *vs* 3.50, P = 0.038); C: No significant difference in NLR was observed between treatment initiation and the 12th administration (n = 32, median NLR = 2.64 *vs* 2.32, P = 0.940). NLR: Neutrophil-to-lymphocyte ratio.

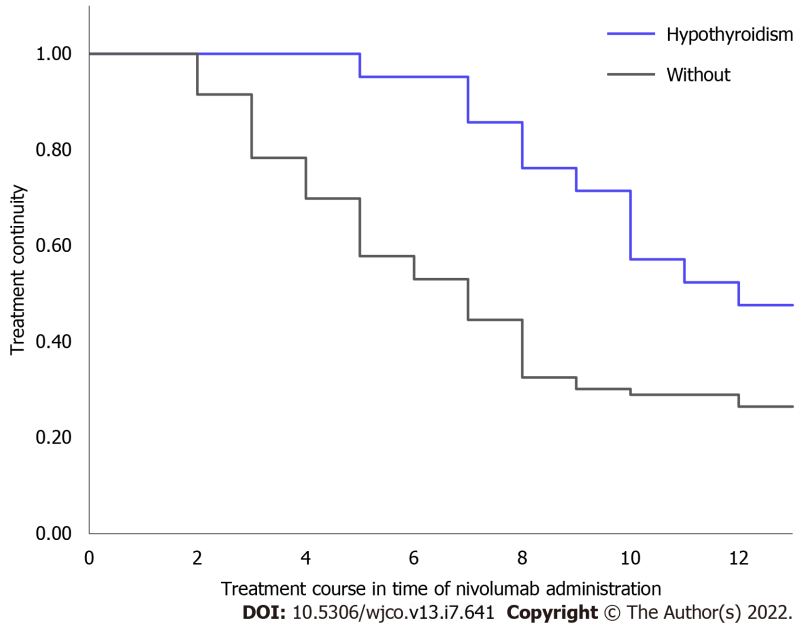


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Figure 2 Relationship between neutrophil-to-lymphocyte ratio and nivolumab treatment continuity. The median numbers of nivolumab administration in each group with mean neutrophil-to-lymphocyte ratio (NLR) < 3.5, 3.5 to < 5, and ≥ 5 were 11.5 (n = 52), 8 (n = 25), and 4 (n = 27), respectively. The groups with mean NLR < 3.5 and 3.5 to < 5 had significantly higher treatment continuity than those with mean NLR ≥ 5 (hazard ratio [HR] for low tertile compared with high tertile: 0.23; 95% confidence interval [CI]: 0.13-0.41, P < 0.001; HR for middle tertile compared with high tertile: 0.32; 95%CI: 0.17-0.60; P < 0.001).

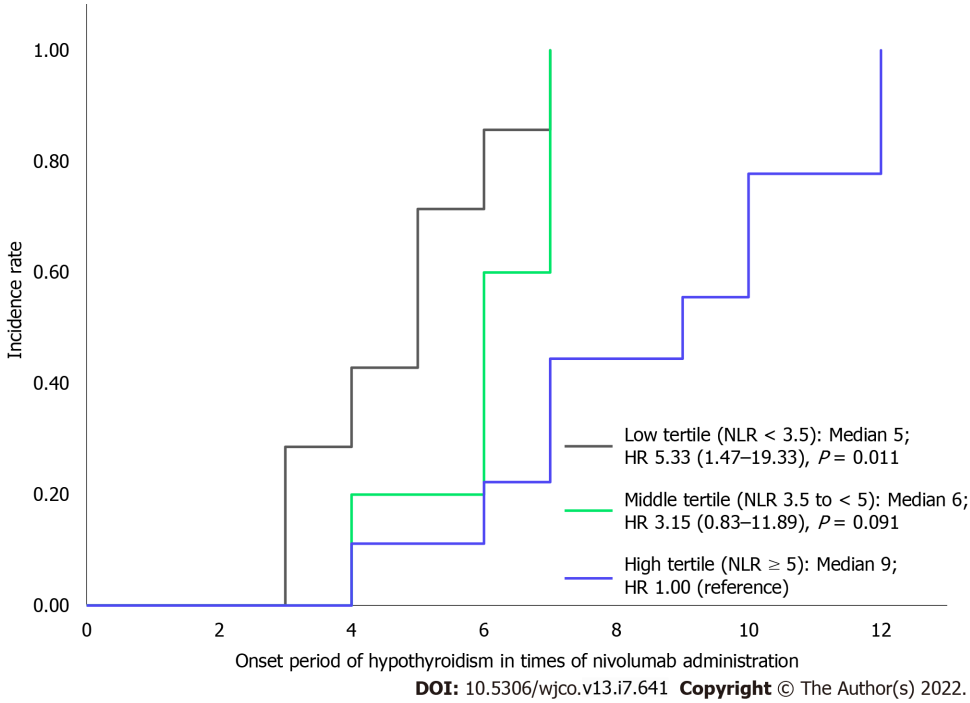
that patients with maximum NLR of < 3.5 until the development of hypothyroidism had a significantly earlier onset of hypothyroidism than those with NLR ≥ 5. Thus, persistently low NLR may be a risk factor for the early development of hypothyroidism. Monitoring the maximum NLR using a cutoff value of < 3.5 as a reference is clinically helpful in predicting the early onset of hypothyroidism.

This study has several limitations. First, this was a retrospective study conducted at a single institution, and the cancer types of patients were not specified. Additionally, there was a bias in cancer



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Figure 3 Nivolumab treatment continuity in patients who developed hypothyroidism. Treatment continuity in patients who developed hypothyroidism was significantly longer than in those who did not develop hypothyroidism (n = 104, median not reached *vs* 7 times administration, P = 0.010).



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Figure 4 Relationship between neutrophil-to-lymphocyte ratio and the onset period of hypothyroidism. The median onset periods of each group with maximum neutrophil-to-lymphocyte ratio (NLR) values of < 3.5, 3.5 to < 5, and ≥ 5 were at 5th (n = 7), 6th (n = 5), and 9th administration (n = 9), respectively. The groups with a maximum NLR of < 3.5 had a significantly earlier onset of hypothyroidism than the group with NLR ≥ 5, whereas there was no significant difference in the onset periods of the groups with maximum NLR values of 3.5-5 and ≥ 5 (HR for low tertile compared with highest tertile: 5.33; 95%CI: 1.47-19.33, P = 0.011; HR for middle tertile compared with highest tertile: 3.15; 95%CI: 0.83-11.89, P = 0.091).

types of the patient population. Second, due to the limited sample size of this study population, follow-up with larger populations is needed for verification. Third, the follow-up period was limited to the 12th dose of nivolumab. In fact, in some patients, hypothyroidism develops after 12 doses; hence, the incidence of hypothyroidism should be evaluated throughout the treatment period. Fourth, we analyzed the treatment continuity of nivolumab rather than its therapeutic response as a criterion of therapeutic effect. Further studies are needed on NLR fluctuations *via* treatment response.

The involvement of antithyroid peroxidase antibody or antithyroglobulin antibody has been shown as a factor related to the development of hypothyroidism[26]. However, these laboratory data are not measured regularly in daily clinical practice. Alternatively, as the neutrophil and lymphocyte counts are regularly measured, the possibility of using NLR as a predictive factor was considered to be useful for the evaluation of the treatment continuity of nivolumab and associated adverse effects.

CONCLUSION

Low NLR at treatment initiation increased the incidence of treatment-induced hypothyroidism. Low NLR levels were also associated with the treatment continuity of nivolumab. Thus, the persistence of low NLR may be a risk factor for the early development of hypothyroidism.

ARTICLE HIGHLIGHTS

Research background

The activation of immunocompetent cells by nivolumab exerts an antitumor effect. However, excessive immune responses developed in autologous organs along with the breakdown of self-tolerance causes immune-related adverse events (irAEs), such as hypothyroidism.

Research motivation

Low neutrophil-to-lymphocyte ratio (NLR) values have been shown to be associated with a favorable therapeutic response to nivolumab. The possibility that NLR is associated with immune response implies that NLR can be not only a predictive factor for good response to nivolumab but also a predictive factor for the development of hypothyroidism.

Research objectives

To evaluate whether continuous monitoring of NLRs during nivolumab treatment is useful for predicting the incidence and onset period of hypothyroidism.

Research methods

NLR of patients who received nivolumab treatment was measured before each administration. NLR at treatment initiation was compared between patients with and without hypothyroidism during the treatment period. Patients who developed hypothyroidism were categorized into three groups as those with NLR < 3.5, NLR 3.5 to < 5, and NLR ≥ 5 according to their maximum NLR from treatment initiation to hypothyroidism development, and the onset periods of hypothyroidism were compared.

Research results

Patients with hypothyroidism showed significantly lower NLR at treatment initiation, and the incidence of hypothyroidism was higher among those with NLR < 5. Patients with persistently low NLR (< 3.5) developed hypothyroidism earlier than those with NLR 3.5 to < 5 and NLR ≥ 5.

Research conclusions

Low NLR at treatment initiation increases the incidence of treatment-induced hypothyroidism. Moreover, its persistence may be a risk factor for the early onset of hypothyroidism.

Research perspectives

The follow-up period in this study was limited to the 12th dose of nivolumab. The incidence of hypothyroidism should be evaluated throughout the treatment period.

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FOOTNOTES

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for important intellectual content; Kawakubo T provided the final approval for this article.

Institutional review board statement: The study protocol was approved by the Ethics Committee of the Jikei University [No. 31-048 (9547)].

Informed consent statement: This study was a retrospective observational study conducted using the opt-out method. Informed consent for the study was not required because the analysis used anonymous clinical data obtained after each patient had agreed to treatment through written consent. For full disclosure, the details of the study were mentioned in the opt-out document in the Jikei University School of Medicine.

Conflict-of-interest statement: Kazuyoshi Kuwano received study support from Ono Pharmaceutical Co., Ltd., Astellas Pharma Inc., Kyorin Pharmaceutical Co., Ltd. and Nippon Boehringer Ingelheim Co., Ltd. These companies did not have a role in conducting this study. All authors have no financial relationships to disclose.

Data sharing statement: No additional data are available.

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ORIGINAL ARTICLE

Retrospective Study

Assessing radiation dose for postoperative radiotherapy in prostate cancer: Real world data

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Abstract

BACKGROUND

Approximately 30% of patients with localized prostate cancer (PCa) who undergo radical prostatectomy will develop biochemical recurrence. In these patients, the only potentially curative treatment is postoperative radiotherapy (PORT) with or without hormone therapy. However, the optimal radiotherapy dose is unknown due to the limited data available.

AIM

To determine whether the postoperative radiotherapy dose influences biochemical failure-free survival (BFFS) in patients with PCa.

METHODS

Retrospective analysis of patients who underwent radical prostatectomy for PCa followed by PORT-either adjuvant radiotherapy (ART) or salvage radiotherapy (SRT)-between April 2002 and July 2015. From 2002 to 2010, the prescribed radiation dose to the surgical bed was 66-70 Gy in fractions of 2 Gy; from 2010 until July 2015, the prescribed dose was 70-72 Gy. Patients were grouped into three categories according to the total dose administered: 66-68 Gy, 70 Gy, and 72 Gy. The primary endpoint was BFFS, defined as the post-radiotherapy prostate-specific antigen (PSA) nadir + 0.2 ng/mL. Secondary endpoints were overall survival (OS), cancer-specific survival (CSS), and metastasis-free survival (MFS; based on conventional imaging tests). Treatment-related genitourinary (GU) and gastrointestinal (GI) toxicity was evaluated according to Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer

criteria. Finally, we aimed to identify potential prognostic factors. BFFS, OS, CSS, and MFS were calculated with the Kaplan-Meier method and the log-rank test. Univariate and multivariate Cox regression models were performed to explore between-group differences in survival outcome measures.

RESULTS

A total of 301 consecutive patients were included. Of these, 93 (33.6%) received ART and 186 (66.4%) SRT; 22 patients were excluded due to residual macroscopic disease or local recurrence in the surgical bed. In this subgroup (n = 93), 43 patients (46.2%) were Gleason score (GS) ≤ 6, 44 (47.3%) GS 7, and 6 (6.5%) GS ≥ 8; clinical stage was cT1 in 51 (54.8%), cT2 in 35 (39.3%), and cT3 in one patient (1.1%); PSA was < 10 ng/mL in 58 (63%) patients, 10-20 ng/mL in 28 (30.6%), and ≥ 20 ng/mL in 6 (6.4%) patients. No differences were found in BFFS in this patient subset versus the entire cohort of patients (P = 0.66). At a median follow-up of 113 months (range, 4-233), 5- and 10-year BFFS rates were 78.8% and 73.7%, respectively, with OS rates of 93.3% and 81.4%. The 5-year BFFS rates in three groups were as follows: 69.6% (66-68 Gy), 80.5% (70 Gy) and 82.6% (72 Gy) (P = 0.12):the corresponding 10-year rates were 63.9%, 72.9%, and 82.6% (P = 0.12), respectively. No significant between-group differences were observed in MFS, CSS, or OS. On the univariate analysis, the following variables were significantly associated with BFFS: PSA at diagnosis; clinical stage (cT1 *vs* cT2); GS at diagnosis; treatment indication (ART *vs* SRT); pre-RT PSA levels; and RT dose 66 -68 Gy vs. 72 Gy (HR: 2.05; 95%CI: 1.02-4.02, P = 0.04). On the multivariate analysis, the following variables remained significant: biopsy GS (HR: 2.85; 95%CI: 1.83-4.43, P < 0.001); clinical stage (HR: 2.31; 95%CI: 1.47-4.43, P = 0.01); and treatment indication (HR: 4.11; 95%CI: 2.06-8.17, P < 0.001). Acute grade (G) 1 GU toxicity was observed in 11 (20.4%), 17 (19.8%), and 3 (8.3%) patients in each group (66-68 Gy, 70 Gy and 72 Gy), respectively (P = 0.295). Acute G2 toxicity was observed in 2 (3.7%), 4 (4.7%) and 2 (5.6%) patients, respectively (P = 0.949). Acute G1 GI toxicity was observed in 16 (29.6%), 23 (26.7%) and 2 (5.6%) patients in each group, respectively (P = 0.011). Acute G2 GI toxicity was observed in 2 (3.7%), 6 (6.9%) and 1 (2.8%) patients, respectively (P = 0.278). No cases of acute G3 GI toxicity were observed.

CONCLUSION

The findings of this retrospective study suggest that postoperative radiotherapy dose intensification in PCa is not superior to conventional radiotherapy treatment.

Key Words: Prostate cancer; Postoperative radiotherapy; Dose intensified; Radiation dose; Biochemical relapse free survival

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Core Tip: This retrospective study was performed to evaluate whether higher doses of postoperative radiotherapy influence biochemical failure-free survival rates in patients with prostate cancer. Our results show no significant differences in biochemical failure-free survival, cancer-specific survival, metastasis-free survival, or overall survival regardless of the radiotherapy dose (66-68 *vs* 70 *vs* 72 Gy). No differences in treatment-related toxicity were observed. These findings suggest that radiation dose intensification is not superior to conventional radiotherapy treatment.

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INTRODUCTION

In the year 2020, prostate cancer (PCa) was the 4th most common cancer worldwide, with an annual incidence of 1414259 cases, and the 8th leading cause of cancer mortality, with 375304 deaths[1]. Radical prostatectomy (RP) is one of the primary treatments for localized PCa, with good long-term results[2]. However, up to 30% of surgically-treated patients will develop biochemical recurrence, which is primarily observed in patients who present high-risk factors in the surgical specimen, positive surgical margins, Gleason score (GS) ≥ 8, extracapsular extension, and/or involvement of the seminal vesicles[3].

In this clinical context, the main international clinical guidelines recommend postoperative radiotherapy (PORT)[4]. There are two main treatment modalities for PORT, adjuvant radiotherapy (ART) or salvage radiotherapy (SRT). ART is defined as the prophylactic administration of RT after RP but before recurrence (when prostate-specific antigen [PSA] levels remain undetectable) in patients with a high risk of recurrence due to adverse pathologic features. By contrast, SRT involves the administration of RT to the prostate bed in patients with confirmed biochemically-recurrent PCa (without evidence of distant metastasis) after surgery[5].

Despite the recent publication of several studies[6-9], the optimal timing of PORT (*i.e.*, ART *vs* SRT) remains unclear in some patient subgroups. The optimal dose for both ART and SRT has not been established, nor is it clear whether dose escalation is appropriate in these patients. Although several studies suggest that dose intensification may be more effective than conventional doses in terms of biochemical control[10-15], other studies have found that dose intensification does not provide any benefits compared to conventional dosing and is also associated with greater toxicity[16].

In this context, the aim of the present retrospective study was to describe long-term clinical outcomes and treatment-related toxicity (acute and chronic) according to the PORT dose (66-68 Gy, 70 Gy, and 72 Gy) in patients treated at our hospital between 2002 to 2015.

MATERIALS AND METHODS

This was a retrospective analysis of patients with PCa who underwent radical prostatectomy followed by PORT (ART or SRT) at the Ramón y Cajal University Hospital in Spain between April 2002 and July 2015. From 2002 to 2010, the dose to the surgical bed was 66-70 Gy; in 2011, the dose was increased to 70-72 Gy. In all cases, the doses were delivered in fractions of 2 Gy, 5 d a week according to the protocol established in that centre at that time and the clinical criteria of the radiation oncology specialist.

Treatment planning was performed with the patient in the supine position, with a full bladder and empty rectum. Contouring of the surgical bed was performed in accordance with Radiation Therapy Oncology Group guidelines[17,18]. Until April 2006, three-dimensional (3D) conformal radiotherapy was used. Thereafter, patients were treated with intensity-modulated radiotherapy (IMRT).

Follow-up was performed by specialists from the Radiotherapy Oncology or Urology Departments at our hospital. The first follow-up visit, consisting of a clinical evaluation and PSA determination, was conducted three months after treatment completion. Subsequent visits were performed every 3-6 mo during the first five years and annually thereafter.

Acute toxicity was defined as any toxicity from the start of radiotherapy until six months after treatment finalisation. Treatment-related toxicity observed > six months after treatment completion was defined as chronic toxicity.

The primary aim of this study was to evaluate BFFS, defined as the PSA nadir + 0.2 ng/mL after completion of RT. Patients were classified into three groups according to the total radiotherapy dose administered to the surgical bed (66-68 Gy, 70 Gy, and 72 Gy). Secondary objectives were as follows: overall survival (OS), cancer-specific survival (CSS), and metastasis-free survival (MFS)-assessed by conventional imaging tests (computed tomography [CT] and bone scan); and genitourinary (GU) and gastrointestinal (GI) toxicity according to Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria[19]. Finally, we evaluated the following variables as potential prognostic factors: PSA level prior to the start of RT; clinical and pathological stage; GS; margin status; radiotherapy dose; hormonal therapy; perineural invasion and treatment indication (ART *vs* SRT).

Statistical analysis was performed with the SPSS statistical software, v.20 (IBM-SPSS Corp). BFFS, OS, CSS and MFS were calculated from the start of RT, using the Kaplan-Meier method and the log-rank test with a significance level of $P < 0.05$. Univariate and multivariate Cox regression models were performed to explore between-group differences in survival measures.

RESULTS

We evaluated 301 consecutively-treated patients. Of these, 93 (33.6%) received ART (≤ six months after surgery) due to unfavourable histological factors (involved or close margins or stage pT3b-T4). A total of 186 patients (66.4%) were treated with SRT after biochemical recurrence. Twenty-two patients were excluded due to residual macroscopic disease or local recurrence in the surgical bed. Lymph node dissection was performed simultaneously with radical prostatectomy in 135 patients (48.6%). The clinicopathologic characteristics of the patients by radiotherapy dose to the surgical bed are shown in Table 1.

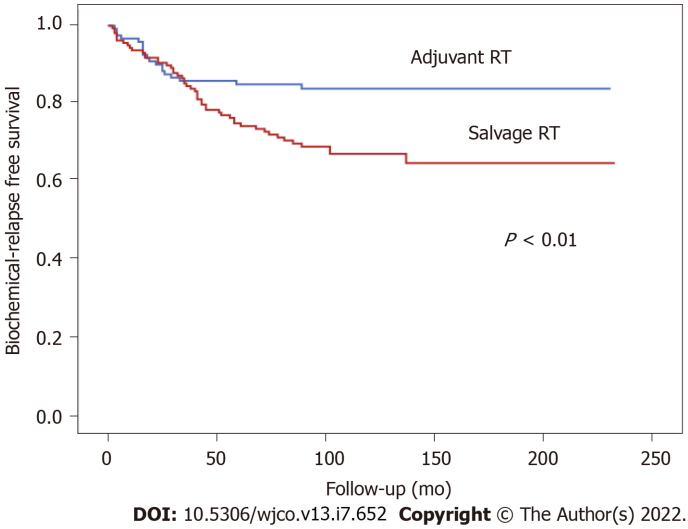
At a median follow-up of 113 mo (range, 4-233), 5- and 10-year survival rates, respectively, were as follows: BFFS: 78.8% and 73.7%; OS: 93.3% and 81.4%; CSS: 95.9% and 88.4%; and MFS: 96.8% and 91.8%. Local recurrence in the surgical bed was observed in four cases (1.5%), lymph node recurrence in 22 patients (8.3%), and distant metastases in 27 patients (10.1%).

Table 1 Clinicopathologic characteristics of the patients according to the radiotherapy dose to the surgical bed

	Total dose			*P* value
	66-68 Gy	70 Gy	72 Gy	
Variable	*n* (%)			
Patients, *n* (%)	59 (21.1)	131 (50)	89 (31.9)	
Median age, range	63.3 (50-74)	62.7 (48-75)	62.3 (41-75)	
PSA preRT				0.36
≤ 0.4 ng/mL	26 (44.1%)	59 (48.8%)	49 (55.7%)	
>0.4 ng/mL	33 (55.9%)	62 (51.2%)	39 (44.3%)	
cT stage				0.43
cT1	38 (67.9%)	65 (57%)	53 (66.2%)	
cT2	18 (32.1%)	46 (40.4%)	26 (32.5%)	
cT3	0 (0%)	3 (2.6%)	1 (1.2%)	
pT stage				0
pT1-T2	45 (80.4%)	94 (75.2%)	41 (47.1%)	
pT3-T4	11 (19.6%)	31 (24.8%)	46 (52.9%)	
pN stage				0.32
N0	25 (42.4%)	60 (46.9%)	42 (48.3%)	
N1	1 (1.7%)	5 (3.9%)	1 (1.1%)	
Nx	33 (55.9%)	63 (49.2%)	44 (50.6%)	
GS (biopsy)				0.06
≤ 6	31 (53.4%)	67 (52.8%)	32 (37.6%)	
7	25 (43.1%)	53 (41.7%)	42 (49.4%)	
≥ 8	2 (3.4%)	7 (5.5%)	11 (12.9%)	
GS (prostatectomy)				0.01
≤ 6	7 (17.9%)	28 (29.5%)	9 (12.2%)	
7	27 (69.2%)	49 (51.6%)	41 (55.4%)	
≥ 8	5 (12.8%)	18 (18.9%)	24 (32.4%)	
Margin status				0.81
Positive	33 (55.9%)	74 (56.9%)	54 (60.7%)	
Negative	26 (44.1%)	56 (43.1%)	35 (39.3%)	
Hormonotherapy				0
Yes	28 (48.3%)	50 (40.3%)	15 (18.1%)	
No	30 (51.7%)	74 (59.7%)	68 (81.9%)	
RT indication				0.68
Adjuvant RT	19 (32.2%)	41 (31.5%)	33 (37.1%)	
Salvage RT	40 (67.8%)	90 (68.5%)	56 (62.9%)	

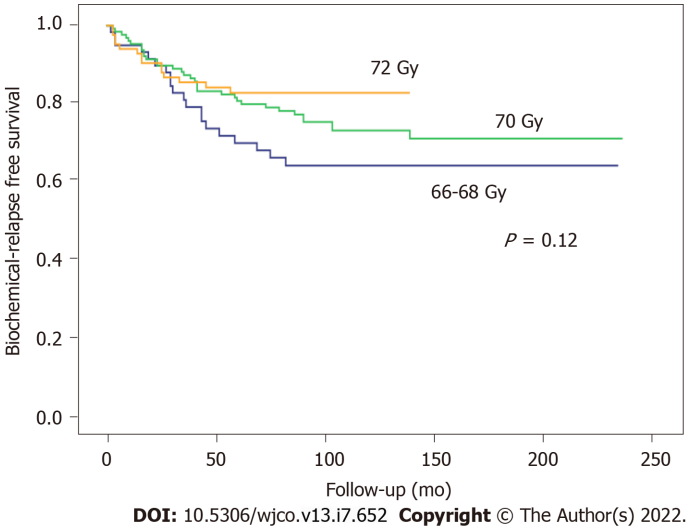
GS: Gleason score; preRT: Pre-radiotherapy; PSA: Prostate-specific antigen

At 5 and 10 years, BFFS was 89.1% and 89.1% in the ART group *vs* 73.3% and 65.5%, respectively, in the SRT group (Figure 1). By total dose, the median BFFS (Figure 2) was not reached in any of the subgroups; the 5- and 10-year BFFS rates in these three groups were 69.6%, 80.5% and 82.6% (P = 0.12) and 63.9%, 72.9% and 82.6% (P = 0.12), respectively; the 5- and 10-year CSS rates in these three groups were 100%, 98.4% and 98.8% and 89.3%, 96.4% and 97.3% (P = 0.067), respectively; the 5- and 10-year OS rates in these three groups were 93.1%, 94.5% and 91.5% and 76.6%, 81.3% and 88.9% (P = 0.519),



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Figure 1 Biochemical relapse free survival according to radiotherapy indication (adjuvant versus salvage radiotherapy). RT: Radiotherapy.



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Figure 2 Biochemical failure-free survival according to radiotherapy dose to the surgical bed.

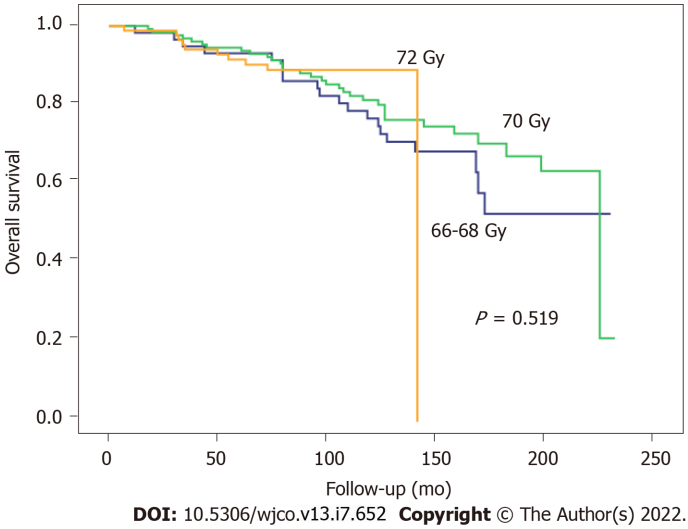
respectively, Figures 3 and 4.

On the univariate analysis, the following variables were significantly associated with BFFS: PSA at diagnosis (hazard ratio [HR]: 1.05; 95% confidence interval [CI]: 1.77-5.11, $P = 0.00$); clinical stage cT1 *vs* cT2 (HR: 3.01; 95%CI: 1.67-4.75, $P < 0.001$); GS at diagnosis 6 *vs* 7 (HR: 2.31; 95%CI: 1.31-4.08, $P = 0.004$) and 6 *vs* 8-9 (HR: 7.88; 95%CI: 3.76-16.52, $P < 0.001$); (ART *vs* SRT; HR: 3.40; 95%CI: 1.74-6.66, $P = 0.00$); PSA level prior to RT (HR: 1.25; 95%CI: 1.14-1.38, $P < 0.001$); and RT dose 66-68 Gy *vs* 72 Gy (HR: 2.05; 95%CI: 1.02-4.02, $P = 0.04$). None of the following variables were associated with BFFS: preoperative androgen blockade ($P = 0.66$), perineural invasion ($P = 0.15$), or involved margins ($P = 0.36$).

On the multivariate Cox regression analysis, the following variables remained significantly associated with BFFS: GS in the biopsy (HR: 2.85; 95%CI: 1.83-4.43, $P < 0.001$); clinical stage (HR: 2.31; 95%CI: 1.47-3.43, $P = 0.01$); and the indication for external beam radiation therapy (ART *vs* SRT), (HR: 4.11; 95%CI: 2.06-8.17, $P < 0.001$).

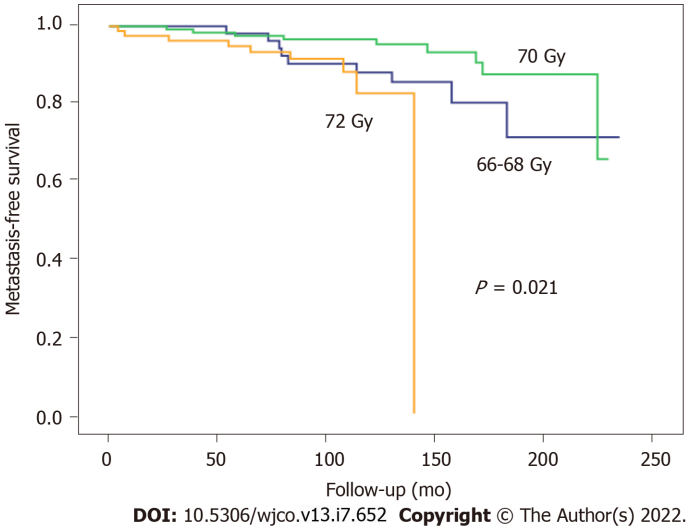
On the univariate analysis, the following variables were significantly associated with OS: Age (HR: 1.07; 95%CI: 1.02-1.12, $P = 0.003$); GS in the surgical specimen: GS 6 *vs* 8-9 (HR: 2.36; 95%CI: 1.01-5.52, $P = 0.048$); PSA prior to RT: ≤ 4 *vs* > 4 ng/mL (HR: 1.81; 95%CI: 1.07-3.06, $P = 0.027$); and distant metastases (HR: 2.49; 95%CI: 1.37-4.53, $P = 0.003$). On the multivariate analysis, only age (HR: 1.09; 95%CI: 1.03-1.13, $P = 0.002$) and distant metastases (HR: 2.82; 95%CI: 1.54-5.16, $P = 0.001$) remained significant.

Acute grade (G)1 GU toxicity was observed in 11 (20.4%), 17 (19.8%), and 3 (8.3%) patients in each group (66-68 Gy, 70 Gy, and 72 Gy), respectively, ($P = 0.295$). Acute G2 GU toxicity was observed in 2 (3.7%), 4 (4.7%) and 2 (5.6%) patients, respectively, ($P = 0.949$). Only one patient (in the 72 Gy group)



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Figure 3 Overall survival according to total radiotherapy dose to the surgical bed.



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Figure 4 Metastasis-free survival according to the total radiotherapy dose to surgical bed.

developed G3 toxicity (Table 2). Acute G1 GI toxicity was observed in 16 (29.6%), 23 (26.7%) and 2 (5.6%) patients, respectively, (P = 0.011). Acute G2 toxicity was observed in 2 (3.7%), 6 (6.9%) and 1 (2.8%) patient, (P = 0.278). No cases of acute G3 GI toxicity were observed in any of the groups (Table 2). Chronic GU toxicity was as follows: G1-2 in 3 patients (11.5%) and G3 in one (3.8%) patient in the 70 Gy group, and in 11 (13.4%) and 1 (1.2%) of those who received 72 Gy, respectively (P = 0.338). Chronic G1-G2 GI toxicity was observed in 2 (7.7%) of the patients who received 70 Gy and in one (2%) who received 72 Gy (P = 0.262), with no G3 chronic GI toxicity in any of the groups (Table 3).

DISCUSSION

In this retrospective study, higher total postoperative radiation doses to the surgical bed were not associated with better BFFS or OS outcomes, a finding that is consistent with data from randomised clinical trials[16].

In this series, the patients' clinical characteristics were indicative of an aggressive disease profile: 139 patients (48%) were stage pT3-T4, 47 (16.8%) had a GS ≥ 8, 161 (58.9%) had positive margins in the surgical specimen, and 124 (49%) had a pre-RT PSA > 0.4ng/mL. In addition, 93 patients (34.8%) received short-term androgen blockade prior to surgery, as this was standard clinical practice at some centres based on the available evidence at that time, even though preoperative androgen deprivation therapy is no longer prescribed in these cases[20]. Given the time period (2002-2015) of this study, none of the patients were prescribed concurrent hormonal therapy with postoperative radiotherapy, even

Table 2 Acute gastrointestinal and genitourinary toxicity (Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria) according to total radiotherapy dose to the surgical bed

	Total dose								
	66-68 Gy (*n* = 54)			70 Gy (*n* = 86)			72 Gy (*n* = 36)		
Grade (G)	GI	GII	GIII	GI	GII	GIII	GI	GII	GIII
Acute GU toxicity	11 (20.4%)	2 (3.7%)	0	17 (19.8%)	4 (4.7%)	0	3 (8.3%)	2 (5.6%)	1 (2.8%)
Acute GI toxicity	16 (29.6%)	2 (3.7%)	0	23 (26.7%)	6 (6.9%)	0	2 (5.6%)	1 (2.8%)	0

G: Grade; GI: Gastrointestinal; GU: Genitourinary.

Table 3 Chronic gastrointestinal and genitourinary toxicity (Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria) according to total radiotherapy dose to the surgical bed

	Total dose					
	66-68 Gy (*n* = 5)		70 Gy (*n* = 26)		72 Gy (*n* = 51)	
Grade (G)	GI-II	GIII	GI-II	GIII	GI-II	GIII
Chronic GU toxicity	0	0	3 (11.5%)	1 (3.8%)	11 (13.4%)	1 (1.2%)
Chronic GI toxicity	0	0	2 (7.7%)	0	1 (2%)	0

GI: Gastrointestinal; GU: Genitourinary.

though this approach is now common clinical practice-due to the proven clinical benefits-in well-selected patients who meet the clinical criteria[21,22].

The patients in this series did not undergo prophylactic nodal radiation due to the conflicting and controversial evidence in the literature[23-25]. Nevertheless, only 22 patients (8.3%) developed nodal recurrence; of these, 70% received SRT, 64% presented perineural invasion, and 45% were stage pT3-4. Given the low rate of nodal recurrence in this series, we were unable to identify any significant predictors.

A recent randomised trial compared ART to SRT (64 Gy) without hormonal therapy or prophylactic nodal irradiation[8]. The 5-year BFFS was 87%, which was higher than the 78.8% observed in our series, probably due to the less aggressive disease profile and the application of treatment volumes that differed from those recommended in the Radiation Therapy Oncology Group contouring guidelines[17,18].

In our series, 5- and 10-year BFFS was significantly better in the patients who received ART *vs* SRT, a finding that is consistent with previous reports[3,26-30]. Although numerous studies have sought to determine the optimal timing of EBRT after radical prostatectomy, this remains uncertain[3,27,31,32] Several recent phase III trials-RADICALS, Groupe d´ Etude des Tumeurs Uro-Genitales (GETUG)-AFU 17 and RAVES, and the ARTISTIC meta-analysis-have compared ART to SRT, demonstrating that early SRT is superior to ART[6-9]. However, because those trials included a limited number of patients with highly unfavourable clinicopathologic characteristics (involved margins, pathologic lymph nodes, stage pT3b and/or GS ≥8) more studies are needed to determine whether early SRT is indicated in all surgically-treated patients, or whether some patient subgroups might benefit from ART.

In the comparison of treatment outcomes according to the radiotherapy dose to the surgical bed, dose intensification did not improve the results, a finding that contrasts with several retrospective reports that reported better clinical outcomes in patients who received higher doses[10-15]. However, it is important to emphasize that we did not randomise patients and, moreover, there were important differences among the subgroups in terms of the clinicopathologic characteristics. In fact, this is a study limitation given that patients who received 72 Gy had more severe disease (stage pT3-T4, higher GS) and were less likely to receive hormonal therapy than patients included in the other two subgroups (70 Gy and 66-68 Gy). Both the European Association of Urology (EAU) and the GETUG have developed criteria to identify patients with a high risk of developing metastatic disease in this clinical scenario[21,33]. Nonetheless, in our multivariate analysis, most of these criteria failed to predict the effectiveness of PORT. Consequently, a more comprehensive analysis in a larger sample that stratifies patients according to their baseline clinical characteristics could help to better elucidate the true potential of dose intensification, thus allowing for more individualized treatment.

In terms of toxicity, previous studies have found that dose-escalated PORT is associated with a significant increase in both GU and GI toxicity[16]. However, various factors could influence this

association, including the technique (*i.e.*, 3D-CRT *vs* IMRT), irradiation or not of the uninvolved nodal areas, the contouring criteria for the treatment volumes, pretreatment urinary function, as well as several other factors described elsewhere[34]. We found no significant between-group differences in acute or chronic GI or GU toxicity, regardless of the radiotherapy dose, a finding that is consistent with the randomised trial conducted by Qi *et al*[35]. In that trial, the authors compared outcomes in patients (n = 144) randomised to receive either 66 or 72 Gy to the surgical bed. They found no significant between-group differences in acute and/or chronic GI or GU toxicity. In our study, the use of more advanced radiotherapy techniques (IMRT/rotational techniques) in approximately 50% of the patients may have contributed to the good treatment outcomes. However, these findings should be interpreted cautiously given the retrospective study design and small sample size. Given these limitations, we cannot draw any definitive conclusions. Consequently, larger, more comprehensive studies are needed.

CONCLUSION

The findings of this study suggest that dose-intensified postoperative radiotherapy in patients with PCa is not superior to conventional dosing. Consequently, there is a clear need for randomised clinical trials with well-selected patients to determine the optimal individualized radiotherapy dose scheme in patient subgroups with highly aggressive disease.

ARTICLE HIGHLIGHTS

Research background

Approximately 30% of patients with localized prostate cancer (PCa) who undergo radical prostatectomy will develop biochemical recurrence. In these patients, the only potentially curative treatment is postoperative radiotherapy (PORT) with or without hormone therapy. However, the optimal radiotherapy dose is unknown due to the limited data available.

Research motivation

Our article analyses the changing landscape of the management of prostate cancer patients who receive postoperative radiotherapy, shedding light on an area, optimal radiation dose, applicable to clinical practice, for which the current evidence base is constantly fluctuating with a growing need to optimize the treatment of these patients.

Research objectives

To determine whether the postoperative radiotherapy dose influences biochemical failure-free survival (BFFS) in patients with prostate cancer.

Research methods

Retrospective analysis of patients who underwent radical prostatectomy for PCa followed by PORT-either adjuvant radiotherapy or salvage radiotherapy-between April 2002 and July 2015. From 2002 to 2010, the prescribed radiation dose to the surgical bed was 66-70 Gy in fractions of 2 Gy; from 2010 until the present, the prescribed dose was 70-72 Gy. Patients were grouped into three categories according to the total dose administered: 66-68 Gy, 70 Gy, and 72 Gy. The primary endpoint was BFFS, defined as the post-radiotherapy prostate-specific antigen (PSA) nadir + 0.2 ng/mL. Secondary endpoints were overall survival (OS), cancer-specific survival (CSS), and metastasis-free survival (MFS; based on conventional imaging tests). Treatment-related genitourinary (GU) and gastrointestinal (GI) toxicity was evaluated according to Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria. Finally, we aimed to identify potential prognostic factors. BFFS, OS, CSS, and MFS were calculated with the Kaplan-Meier method and the log-rank test. Univariate and multivariate Cox regression models were performed to explore between-group differences in survival outcome measures.

Research results

301 consecutive patients were included. At a median follow-up of 113 mo (range, 4-233), 5-and 10-year BFFS rates were 78.8% and 73.7%, respectively, with OS rates of 93.3% and 81.4%. The 5-year BFFS rates in the three groups were as follows: 69.6% (66-68Gy), 80.5% (70Gy) and 82.6% (72Gy) (P = 0.12): at 10 years, the corresponding rates were 63.9%, 72.9% and 82.6% (P = 0.12), respectively. No significant between-group differences were observed in MFS, CSS, or OS. No significant differences were found in GU or GI toxicity between the 3 radiation-dose groups except acute grade 1 GI toxicity that was observed in 16 (29.6%), 23 (26.7%) and 2 (5.6%) patients in each group (66-68Gy, 70Gy and 72Gy), respectively (P = 0.011).

Research conclusions

Postoperative radiotherapy dose intensification in PCa is not superior to conventional radiotherapy treatment.

Research perspectives

A more comprehensive analysis of the radiation dose in prostate cancer patients who receive postoperative radiotherapy could help to better elucidate the true potential of dose intensification, thus allowing for more individualized treatment.

FOOTNOTES

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Institutional review board statement: The study was reviewed and approved by the Ethics Research Committee of Hospital Universitario Ramón y Cajal.

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FIELD OF VISION

Potential of mRNA vaccines to become versatile cancer vaccines

Shiu-Ying Tsao

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Abstract

For centuries, therapeutic cancer vaccines have been developed and tried clinically. Way back in the late 19th century, the Father of Immunotherapy, William Coley had discovered that bacterial toxins were effective for inoperable sarcomas. In the 1970s, the Bacillus Calmette-Guérin (BCG) vaccine was repurposed, *e.g.*, for advanced melanomas. Then, therapeutic cancer vaccines based on tumor-associated antigens (found on the surfaces of cancer cells) were tried clinically but apparently have not made a really significant clinical impact. For repurposed pathogen vaccines, only the BCG vaccine was approved in 1989 for local application to treat nonmuscle-invading bladder cancers. Although the mildly toxic vaccine adjuvants deliberately added to conventional pathogen vaccines are appropriate for seasonal applications, when repurposed for continual oncology usage, toxicity may be problematic. In 2010, even with the approval of sipuleucel-T as the very first cancer vaccine (dendritic cell) developed for designated prostate cancers, it has also not made a really significant clinical impact. Perhaps more "user friendly" cancer vaccines should be explored. As from approximately 30 years ago, the safety and effectiveness of mRNA vaccination for oncology had already been studied, the current coronavirus disease 2019 pandemic, though disastrous, has given such progressively advancing technology a kickstart. For oncology, other virtues of mRNA vaccines seem advantageous, *e.g.*, rapid and versatile development, convenient modular design, and entirely cell-free synthesis, are being progressively recognized. Moreover, mRNAs encoding various oncology antigens for vaccination may also be tested with the combination of relatively non-toxic modalities of oncology treatments, *e.g.*, metformin or metronomic (low-dose, prolonged administration) chemotherapy. Admittedly, robust clinical data obtained through good quality clinical trials are mandatory.

Key Words: Cancer vaccine; Cyclophosphamide; Metformin; Metronomic chemotherapy; mRNA vaccine; Myocarditis; Tumor microenvironment

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Core Tip: Although vaccines are effective for pathogen prevention and cancers, hitherto, oncology vaccines have not yet made a very significant clinical impact. Currently, as mRNA vaccines already have a proven safety profile, it is highly appropriate to further develop the decades-old mRNA technology for oncology. Compared to other approved cancer vaccines, oncology mRNA vaccines may be more versatile, pragmatic, affordable, and effective. To combat the notoriously resistant tumor microenvironment, the probable mutual enhancement effects with, *e.g.*, metronomic chemotherapy should be fully explored, especially as no significant added toxicity is anticipated. Clearly, undertaking much more research work (especially clinical) is mandatory.

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INTRODUCTION

The term "cancer vaccine" includes vaccines, pathogen, or otherwise that induces the innate and adaptive immunities for specific purposes; as such, it does not include items like oncolytic viruses. Although cancer immunotherapy is now well recognized to be a significant modality of treatment, interestingly, way back in the 1890s, an American orthopedic surgeon, William Coley had already documented an unexpected regression of a sarcoma when a surgical wound failed to close due to wound infections. Coley hypothesized that the tumor regression may be related to the patient's febrile erysipelas infection (caused by *Streptococcus pyogenes* bacteria). Eventually, he had developed the very first cancer vaccine containing toxins from killed *Streptococcus pyogenes* and *Serratia marcescens* bacteria [1]. However, with variable successes across other patients, Coley's approach unfortunately waned in popularity especially upon the advent of radiotherapy and the then very novel cancer chemotherapy. Nevertheless, he had most remarkably pioneered the concept of bacterial toxins inducing immunity that was also effective for eradicating cancer cells. For this, he was subsequently honored as the "Father of Immunotherapy". Rather unfortunately, cancer vaccines had not subsequently made very significant clinical impacts since then. Actually, specific cellular vaccines have been made to induce satisfactory immune responses against cancer cells, for instance, autologous cell-based cancer vaccines, *e.g.*, for hematological and other cancers[2,3]. However, these may be less versatile, too time consuming to process, and too costly to exert a significant impact on a good number of cancer patients. Apparently, as the very first cancer vaccine [dendritic cell (DC)] developed for specific prostate cancers (sipuleucel-T) has also not made a really significant clinical impact, it may be appropriate to explore other more "user friendly" cancer vaccines.

TUMOR-ASSOCIATED ANTIGENS

Remarkably, cancer immunotherapy in the form of cancer therapy vaccines (that followed William Coley's discoveries) waxed and waned probably because both radiotherapy and chemotherapy were developing steadily by then. Yet, by the 1970s, the Bacillus Calmette-Guérin (BCG) vaccine had actually been repurposed for cancer therapy and tried clinically, *e.g.*, for melanoma[4]. Another form of immunotherapy involved stimulating the cancer patient's own innate and adaptive immunities using the cancer's tumor-associated antigens (TAAs) for developing cancer vaccines. Basically, TAAs are related to antigen molecules present on tumor cell surfaces, *e.g.*, embryonic proteins and glycoprotein antigens. These have been exploited to develop TAA cancer vaccines[4]. However, even though most TAAs are being overexpressed on cancer cells, they are actually not specific enough as these antigens are also expressed in normal tissues[5]. Thus, as TAAs may arise, *e.g.*, as oncofetal antigens, a peripheral tolerance may have already developed to these antigens and would thus preclude a satisfactory immune response to TAAs. Admittedly, despite the encouraging evaluation of numerous vaccine strategies targeting various tumors, the efficacy of therapeutic cancer vaccines has not been clearly demonstrated through robust clinical trials[6]. Notably, most of the tumor antigens employed for cancer vaccines were non-mutated, overexpressed self-antigens, eliciting mostly T cells having low-affinity T cell receptors (TCRs) that were deemed the most appropriate to mediate an effective anti-tumor response. Taken together, TAA cancer vaccines have not yet made a significant clinical impact on cancer control[6,7].

REPURPOSING PATHOGEN VACCINES

Repurposing pathogen vaccines for oncology has also been proposed as a feasible modality of cancer immunotherapy. Actually, even in the 1970s, the BCG vaccine had already been tried clinically, *e.g.*, for melanoma[4]. It was felt that, despite some demonstrable effect due to BCG, it did not seem to influence significantly the course of the advanced melanoma. Subsequently, pre-clinical studies of some other pathogen vaccines seemed to be more encouraging. However, it was found that possibly, one of the best applications was intratumoral administrations of pathogen vaccines to turn "cold" tumors into "hot" ones, *i.e.*, having more abundant immune cells (see Section "Tumor Microenvironment"). Admittedly, although this may be very helpful, low-dose cyclophosphamide injected more conveniently through tie intravenous route, would also have a similar effect[8]. Currently, of all the pathogen vaccines, only BCG is approved in 1989 for local treatment of nonmuscle-invasive bladder cancers, even though the exact mechanism is still controversial[9,10].

Importantly, although the mildly toxic vaccine adjuvants deliberately added to conventional pathogen vaccines to boost the immune response was appropriate for seasonal application, continual oncology usage may be controversial[11]. Even though aluminum salts are the commonest vaccine adjuvants, in extreme cases, heavy metal poisoning may occur especially if very frequent administrations of these repurposed pathogen vaccines are given. Currently, the potential toxicity of aluminum is increasingly recognized[12]. Perhaps intratumoral administrations would be most appropriate, except for the fact that the mode of administration is technically more complicated[13,14]. Taken together, with repurposing of pathogen vaccines for oncology, the frequency of administration should be noted well. For instance, a study administering a weekly combination of several repurposed pathogen vaccines for lung cancer [NCT02333474] might have problems related to vaccine adjuvant toxicity.

MRNA VACCINES

Remarkably, during this coronavirus disease 2019 (COVID-19) pandemic, mRNA vaccinations have demonstrated their remarkable success and good safety profile. mRNA for incorporation into vaccines is synthesized *in vitro* to mimic the host mRNA in order to increase mRNA stability and translation efficiency[15]. Moreover, unlike conventional pathogen vaccines, mRNA vaccines are devoid of any cellular or animal components. Additionally, some mRNA vaccines do not require any adjuvants to boost their immune effectiveness[16]. As booster pathogen mRNA vaccines are often given at intervals of 5 mo or less for healthy subjects, when applied for cancer patients, repeated applications would most probably be feasible even at shorter intervals. This is especially so for those mRNA vaccines that have no added adjuvants. Of course, more robust data upon further clinical studies are required for confirmation.

DEVELOPMENT OF MRNA VACCINES

Currently, three major types of mRNA vaccines are available: (1) Conventional, non-amplifying mRNA molecules; (2) Base-modified, non-amplifying mRNA molecules incorporating chemically modified nucleotides; and (3) Self-amplifying mRNAs (saRNAs) that maintain the auto-replicative activity derived from an RNA virus vector. Thus, saRNAs would encode both the antigen and the viral replication machinery which enables intracellular RNA amplification and ample protein expression[17]. saRNAs may thus be advantageous as they maintain all the advantages of mRNA vaccines (rapid development, convenient modular design, and entirely cell-free synthesis), let alone a significantly lower dose of mRNA is now feasible, due to the self-replicating properties[17].

Admittedly, despite much work on TAA vaccines, there are still no very significant clinical impacts. On the other hand, mRNA vaccines may generate potent and protective immune responses of both cellular and humoral types. Basically, mRNA is an intermediate between the translation of protein-encoding DNA and protein production[16]. Notably, unlike pathogen vaccines, adjuvants to enhance vaccine immunity are no longer essential and so, repeated administration for oncology therapy would unlikely be problematic. Moreover, through billions of administrations of mRNA vaccines, the safety profile can be better confirmed. Lastly, it is also most unlikely to have any chance of incorporation into potential oncogenic sites within the genome[15].

REFINING MRNA VACCINES

Although pioneer mRNA vaccines (for oncology) were naked, *e.g.*, the version employed by a German group, subsequent work had appropriately enabled encapsulation in a lipid nanoparticle (LNP)[18,19]. This effectively limits detection by the innate immune system, enhances the cellular uptake of the

mRNA, and prolongs as well as enhances protein expressions. Moreover, the ionizable cationic lipid can also improve the release of mRNA from the endosome to the cytoplasm and markedly prolong protein expressions[16]. Importantly, encapsulation may also serve as a self-adjuvant purpose (see below).

For administration, they can be injected subcutaneously, intradermally, or directly into lymph nodes or tumors. Notably, the production of mRNA vaccines is potentially faster, more flexible, and less expensive and can even be used for precise and individualized therapies. During this pandemic, the rapid and safe vaccine production was clearly shown[16]. Vaccine adjuvants are usually not required as the LNP already induces an innate immune response – a self-adjuvant. With continual mRNA vaccine development, the structured 5′ as well as 3′ termini and the double-stranded RNA replication intermediates of saRNA vaccines would be recognized, leading to a type I interferon (IFN1) response (see below). Remarkably, this immune stimulation would serve as a self-adjuvant to increase vaccine immunogenicity[15].

Lastly, vaccine quality may be improved by nucleoside modification or complexed mRNAs, and further shaped or influenced by the choice of the delivery routes and formats, *e.g.*, LNP vaccines. It was also found that the introduction of noninflammatory modified nucleosides into the mRNA was advantageous as they induce potent T follicular helper and also germinal center B cell responses[20].

TWO MRNA VACCINES FOR COVID-19

By December 2020, two mRNA vaccines from BioNTech/Pfizer and Moderna were approved against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, representing the very first approval for any mRNA vaccines. For development, BioNTech/Pfizer had previously compared several RNA-based COVID-19 pandemic vaccine candidates in clinical studies in Germany and the US. Despite incomplete data publication of all technical details then, mRNA vaccines were known to be LNP-formulated and nucleoside-modified. Eventually, two most promising vaccine candidates were selected: BNT162b1 (encoding the SARS-CoV-2 receptor–binding domain and BNT162b2 (encoding a modified version of the SARS-CoV-2 full-length spike protein)[21]. As BNT162b2 was found to exhibit a good balance of efficacy and safety even at a low dose of 30 μg, it attained the international phase 2–3 clinical trials[22]. Approximately 44000 adults were subjected to two intramuscular injections of 30 μg of BNT162b2 (21 d apart) (NCT04368728). That regimen could confer 95% protection against SARS-CoV-2. Moreover, the titers of SARS-CoV-2–neutralizing antibodies either resembled or exceeded those found in patients who had recovered from COVID-19[16].

SAFETY ASPECTS OF MRNA FOR COVID-19

Before 2020, no mRNA vaccine had ever been approved. During this pandemic, with the authorization and approval of mRNA vaccines against SARS-CoV-2, the safety profile become better recognized. As mRNA vaccines are devoid of any cellular or animal components, they have since been shown to be generally safe and well-tolerated. Integration into the subjects' genome is deemed not possible. With storage at very low temperatures, microbial contaminations are also extremely unlikely. Serious adverse effects were few, although local pain and redness at the injection sites may occur. Rarely, systemic allergic reactions may also ensue. Besides systemic inflammatory reactions, a theoretical risk of inflammation and autoimmunity may occur, likely due to the induced IFN1 response[23]. In patients with systemic lupus erythematosus or other similar autoimmune diseases, anti-self RNA antibodies may develop and could worsen their autoimmunity[24]. Moreover, toxic side effects related to the delivery compounds or complexing agents and potentially, to inserted nucleotides may occur[16]. Although rare, a serious adverse event of myocarditis and/or pericarditis may occur especially in younger adults and adolescents, predominantly in males (12.6 cases/million doses of the second-dose mRNA vaccine) – a few days after the dose, chest pain may occur, with ECG changes, raised serum cardiac troponin levels, and myocarditis features on MRI. Although the mechanism is unclear, it mostly resolves spontaneously [25]. Obviously, many more long-term clinical studies on a wider population spectrum are mandatory.

EFFECT OF EXOGENEOUS MRNA ON IMMUNITY

Upon confirmation that exogenous mRNA is being processed as for any endogenous mRNA, for more efficient T cell activation, a costimulatory signal was found to be helpful for inducing a better immune response with consecutive cytokine production[26]. Naturally, DCs would express these costimulatory signals (such as B7 molecules) after sensing pathogen-associated molecular patterns (PAMPs) that indicate microbial infection or imminent danger. Pharmacologically, this can be achieved by exploiting toll-like receptor (TLR) ligands[27]. TLRs are related to the innate immune system's ability to detect PAMPs. Induction of IFN1 by viruses or other pathogens is indispensable for innate immune responses

and would thus confer anti-microbial activities[28]. Upon sensing PAMPs, an immediate innate inflammatory response (including IFN1 induction) is initiated. Exogeneous mRNAs are likewise sensed by TLRs and double-strand RNAs can induce a strong IFN1 response. Eventually, clonal expansion of antigen-specific B and T cells results in target cell elimination. Although this may be less complicated for infection prophylaxis, significant problems abound for effective control of advanced cancers. Conceptually, resistant cancers often have immunosuppressive tumor microenvironments (TMEs) through recruiting myeloid-derived suppressor cells (MDSCs), regulatory T cells, macrophages, *etc*., to the TME, let alone the production of immunosuppressive cytokines. Hence, very much more robust modalities of treatments than infection prophylaxis are called for (see Section "Tumor Microenvironment").

PIONEERING MRNA TECHNOLOGY FOR ONCOLOGY

Remarkably, even as early as 1995, the feasibility of using mRNA technology for oncology was noted [29], with mRNA transcripts encoding luciferase and human carcinoembryonic antigen (CEA) then. Subsequently, DCs were transfected with either mRNA encoding TAAs[4] or mRNA technology was employed for *in vivo* induction of T cell immunity[30]. Notably, DCs could utilize mRNA encoded TAAs for the induction of anti-cancer immunity[31]. Throughout the decades, more and more research work had validated the feasibility and possible efficacy of mRNA vaccination for immunotherapy against cancers.

Eventually, knowledge on mechanisms involved in innate and adaptive immune sensing ensued. Moreover, various novel approaches of mRNA delivery and complexing of the vaccine could be implemented. Undoubtedly, these may have paved the way to current successful clinical trials on COVID-19. Even for oncology mRNA vaccines, these may be generated using *ex vivo* loaded or electroporated DCs, usually with a known carrier. DCs are then isolated and subsequently transfected with mRNA encoding, *e.g*., TAA(s) before re-infusion into the patient. For instance, transfection by electroporation has been found to be safe for cancer patients[32]. DCs electroporated with mRNA encoding ovalbumin or tumor-derived mRNAs can actually generate robust tumor-specific immune responses in different murine melanoma models and even in patients undergoing vaccination trials (see Table 1).

EARLY CLINICAL TRIALS OF MRNA FOR ONCOLOGY

In 2002, oncology mRNA vaccines for enhancing immunity were reported to be useful for a patient with a carcinoembryonic antigen-expressing adenocarcinoma[33]. As mRNA was known to be basically a copy of the coding genomic information, it was thus found to be useful for the expression of therapeutic proteins[18]. In mice, naked mRNA coding for tumor antigens was administered by injecting intradermally. Most interestingly, it resulted in protein expression as an immune response. Subsequently, the same protocol was applied to 15 melanoma patients in the first phase I/II trial and found to be safe[19]. Notably, some patients even had an increase in antitumor humoral immune response. After the injection of the mRNA cancer vaccine, the encoded protein was translated and presented to the immune system – closely resembling the natural course of a viral infection and its consecutive induction of a protective immune response. Importantly, upon entering to the cytoplasm, the exogenous mRNA had been found to be processed as for any endogenous mRNA[16].

THERAPEUTIC MRNA VACCINES: INJECTION SITES

Although pathogen mRNA vaccines are nearly always given intramuscularly, there are various other different routes for therapeutic mRNA vaccines. These other injection sites may impact on the induced immune response. As the human skin has many antigen-presenting cells (APCs), especially interstitial DCs in the dermis[34], after intradermal injection, exogenous mRNAs are taken up and locally expressed by ample APCs there. Despite scanty immune cells in muscles, circulating immune cells would eventually reach the injection site to process and present the antigen locally. This is just like the expected actions caused by traditional pathogen vaccine adjuvants. It is where the usual local inflammatory reaction at injection sites promotes significant immune cell activities[16]. Even as technical details are beyond the scope of this article, upon local injection of mRNA vaccines, mRNAs will eventually be processed by APCs reaching there and antigen-specific CD8+ T cells are induced.

IMMUNOGENIC CELL DEATH

It is increasingly recognized that the cancer cell killing by chemotherapy (ChT) is not just by direct

Table 1 Selected national registered clinical trials on combination mRNA oncology vaccines[a]

mRNA vaccine	I.S.	Combo agent	Ph	Cancer	Oncol status	Yr	Country	Trial status	NCT number
mRNA-2752	i.t.	+ durva	1	Solid ca, lymph	R/R	2018	United States	Recruiging	03739931
BI 1361849	i.d.	+ durva +/- treme	1/2	NSCLC	Adv	2017	United States	Completed	03164772
mRNA-4157	i.m.	+/- pembro	1	Solid ca	Resected	2017	United States	Recruiting	3313778
mRNA-5671/V941	i.m.	+/- pembro	1	NSCLC/ CRC/ pancCA	Adv	2019	United States	Not yet recruiting	03948763
TriMix[b]	i.t.	Neoadj ChT +/- TriMix[b]	1	Breast	Early	2018	Belgium	Recruiting	03788083
W_ova1	i.v.	+ neoad + adj ChT	1	Ovarian ca	Early	2019	Nether- lands	Recruiting	04163094
W_pro1	i.v.	+/- cemip	1/2	mCRPC	Adv	2020	United States	Recruiting	04382898
Trivalent DCs[c]	i.d.	TMZ/RT +/- DCs	2/3	GBM	Post-op	2018	Norway	Recruiting	03548571
PSCT19[d]	i.v.	allo-SCT +/- PSCT19[d]	1/2	Hemat	Post- allo-SCT	2015	Nether- lands	Completed	02528682
WT1 DC	i.d.	adj TMZ +/- WT1 DC	1/2	GBM	Post-op	2016	Belgium	Recruiting	02649582
pp65 DC[e]	i.d.	adj TMZ +/- pp65 DC[e]	2	GBM	Post-op	2015	United States	Recruiting	02465268
pp65 DC[f]	i.d.	+/- varli	2	GBM	Post-op	2018	United States	Recruiting	03688178
RO7198457	i.v.	+/- pembro	2	Melanoma	Adv	2019	United States	Not yet recruiting	03815058
RO7198457	i.v.	+/- atezo	1	Solid tumors	Adv	2017	United States	Not yet recruiting	03289962

[a]For combinations with therapeutic mRNA vaccines, in principle, the best candidates are those without immune suppressive properties, *e.g.*, while maximum tolerated dose chemotherapy (ChT) may suppress immunity induced by mRNA vaccines, ironically, mChT could have the opposite effect of priming resistant tumors to be more responsive ones[8,63].
[b]CD40L, CD70, and constitutively active toll-like receptor 4.
[c]Dendritic cells (DCs) transfected with mRNA of neoantigens (survivin, hTERT) and autologous tumor stem cells.
[d]PSCT19: MiHA-loaded PD-L-silenced DC Vaccination.
[e]pp65-shLAMP mRNA (autologous) DCs with GM-CSF.
[f]Human CMV pp65-LAMP mRNA-pulsed autologous DCs. Adj: Adjuvant; Adv: Advanced; Allo-SCT: Allogeneic stem cell transplantation; Atezo: Atezolizumab; Ca: Cancer; Cemip: Cemiplimab; ChT: Chemotherapy; CRC: Colo-rectal cancer; Durva: Durvalumab; GBM: Glioblastoma multiforme; Hemat: Hematological malignancies; i.d.: Intradermal; I.S.: Injection site; i.t.: Intratumoral; Lympho: Lymphoma; mCRPC: Metastatic castration-resistant prostate cancer; MTD: Maximum tolerated dose; Neoadj: Neoadjuvant; NSCLC: Non-small-cell lung cancer; Oncol: Oncology; PancCA: Pancreatic cancer; Pembro: Pembrolizumab; Ph: Phase; Post-op: Post-operative; R/R: Relapsed/residual; RT: Radiotherapy; SCT: Stem cell transplant; TMZ: Temozolomide; Treme: Tremelimumab; Varli: Varlilumab.

cytotoxicity, but also by restoring immunity primed by the mechanism of immunogenic cell death (ICD). Intriguingly, dying cancer cells may be immunogenic provided that they emit a set of immunostimulatory signals inducing an activation of intracellular stress response pathways. As the phenomenon of ICD has already been described elsewhere, it is not repeated in this perspective article[35,36]. Briefly, ICD is characterized by cancer cell killing through cell-surface translocation of calreticulin (CRT), extracellular release of ATP and high mobility group box 1 (HMGB1), as well as stimulation of IFN1 responses. For ICD, emission of signals or "damage-associated molecular patterns" (DAMPs) is required. It is akin to a significant quantity of specific cancer cell death debris that may induce strong immune effects. Although ICD is a very attractive oncology phenomenon, maximum tolerated dose chemotherapy (MTD ChT) may have suppressed much of the immunity so induced, and metronomic chemotherapy (mChT) is preferred[37,38]. Moreover, certain mChT agents, *e.g.*, cyclophosphamide, also induce ICD itself (see Section "Combining mRNAs with Metronomic Chemotherapy"). Notably, the tumor infiltrating lymphocytes (TILs) are also modulated and would reactivate antitumor immunity within the notorious and immuno-suppressive TME.

TUMOR MICROENVIRONMENT

Most advanced cancers would deliberately produce a TME to disable and evade the body's immunity. The TME is now well recognized to be the main culprit for the vast majority of cancer resistance. With a serious lack of essential nutrients, *e.g.*, glucose, and oxygen, infiltrating immune cells are thus starved in this deliberately hostile environment[39]. Yet, cancer cells in the TME manage to survive readily through consuming minimal nutrients. Moreover, they also can manage by-products to their own advantage, *e.g.*, lactic acid which can reduce immune cell functions to the cancer cells' advantage. Taken together, the TME is most elusive and resilient and has various "plan Bs" and "plan Cs" to enable an almost intractable resistance to most conventional oncology treatments, especially immunotherapy, except perhaps, some immune checkpoint inhibitors (ICIs, see below). To tackle such TMEs, a multi-prong approach is most appropriate.

Notably, tumors having a robust TME may also be described as "cold" tumors, being unresponsive to most oncology treatments, whereas "hot" tumors are the exact opposite. Now, various innovative methods may be required to render such "cold" tumors into "hot" ones (having abundant immune cells). Notably, intratumoral mRNA vaccines might turn "cold" tumors into "hot" ones[40]; similarly, low-dose intravenous cyclophosphamide has also been found to act likewise[8]. This mechanism may be very useful for resistant tumors as there may be a much desired effect of "mutual enhancement" to tackle tumors which would go hand-in-hand with very evasive TMEs.

COMBINING MRNA VACCINES WITH IMMUNE CHECKPOINT INHIBITORS

In this era of cancer immunotherapy, ICIs have been widely applied for managing cancer patients. Although ICIs do not share similar toxicities with cancer ChTs, ICIs have their own disadvantages as has been discussed elsewhere[36]. Briefly, the response rates are too low and the adverse effects (mostly autoimmune related) may also be significant, so much so that patients with pre-existing autoimmune disorders are deprived of the benefits of ICIs. Realistically, the majority of cancer patients would not derive any benefit from ICIs. Moreover, the "one size fits all" dosage commonly approved for ICI prescriptions may be associated with higher adverse effect rates, especially in Asians who usually have smaller body builds than Caucasians. Although the combination with mRNA cancer vaccines might be beneficial [NCT03948763 (see Table 1)], *e.g.*, to raise the response rates, as both modalities of treatments are immune related, whether immune-related adverse effects might be even more common would require careful documentation, even as the higher cost of such combinations could be ignored.

COMBINING MRNAS WITH METRONOMIC CHEMOTHERAPY

Recently, the advantages of using mChT as one of the ways to patch up immunotherapy deficits have been detailed elsewhere[36]. Briefly, mChT agents not only act akin to targeted therapy agents but are also much less toxic than MTD ChTs so that they would not suppress immunity generated by combination cancer immunotherapy agents. Actually, as some ChT agents have the ICD phenomenon, immunity is enhanced (see Section "Immunogenic Cell Death"). Although MTD ChT has been designed to achieve maximum cancer cell killing, such very high dosages would likely suppress any immunity so generated, be it by mRNA vaccines or by the ICD phenomenon. Thus, as mChT usually does not suppress immunity, it is more appropriate for these combinatory purposes.

Intriguingly, mChT, *e.g.*, very short courses of intravenous low-dose cyclophosphamide, may ironically have a useful action of turning "cold" tumors "hot"[8]. For cyclophosphamide, the personal experience[36] and others[42] tally with such an action, even though the mechanism was entirely unknown decades ago. Importantly, the current evidence is on enhancing immunity mainly by modifying regulatory T cells (Tregs). mChT may even prime "cold" tumors into "hot" ones (see Section "Tumor Microenvironment"). Coincidentally, mRNA vaccines can also act likewise[40] so that there would now be a most desirable mutual enhancement effect. Such combinations are highly worth exploring further, especially as currently, mRNA vaccines may become a potential oncology breakthrough – thus, mChTs with ICD mechanisms[36] would work hand-in-hand with mRNA vaccines for the desired mutual enhancement effect. Although far too few clinical trials have been done on its combination, the remarkable safety profile of mChT is advantageous as no untoward toxicities are expected upon the combination.

COMBINING MRNA VACCINES WITH METFORMIN

Another similar agent deemed suitable for combination with mRNA vaccines is metformin. It has a similarly good safety profile as mChTs[43]. The details have already been reviewed elsewhere[41].

Briefly, despite its discovery around 100 years ago as an anti-diabetic, it is recently known as an agonist of the adenosine monophosphate-activated protein kinase (AMPK) that inhibits the mammalian target of rapamycin (mTOR), especially as mTOR is activated in cancer cells and would even convey drug resistance[44]. Metformin also has an ability of preferentially targeting cells that have abnormal or altered glycolysis, including cancer associated fibroblasts (CAFs). These cells may thus be rendered more susceptible than other cells to the action of cisplatin ChT[45]. This is valuable as CAFs play a vital role in the TME, currently deemed to be the worst culprit for cancer resistance.

Importantly, metformin can actually eradicate cancer stem cells, a pivotal aspect of cancer therapy, but conventional MTD ChT agents can hardly do so[46]. Moreover, MDSCs, being a main player of the TME[47], are also targeted by metformin[48,49]. For usually resistant cancers, *e.g.*, basal breast cancers, pre-clinical studies showed that a combination of metformin and a targeted therapy (erlotinib) could have encouraging results[50]. Thus, apart from observational and preclinical studies revealing metformin's activities on various cancers, it may now be worthwhile to undertake clinical trials (Figure 1). On the safety profile, despite being an anti-diabetic agent, hypoglycemia is hardly a significant problem, unlike most other anti-diabetics. Actually, over many decades, it has proven to be well tolerated and safe.

DISCUSSION

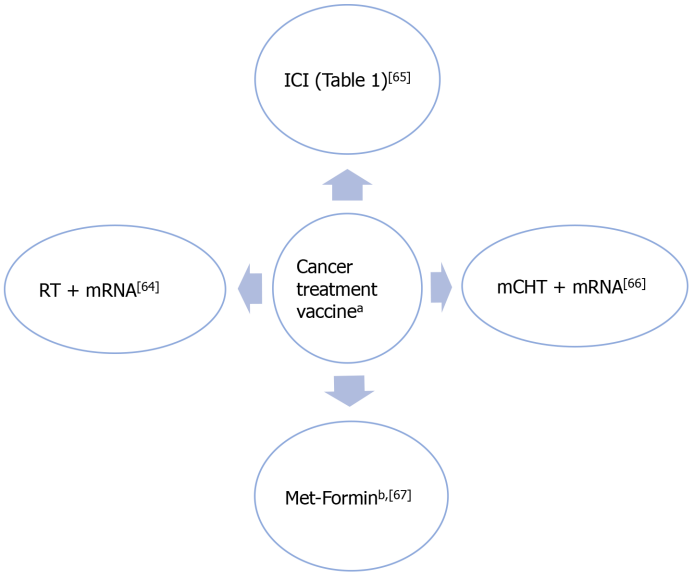
Although mRNA vaccines have already been tried clinically for oncology even two decades ago, the implementation for oncology has obviously been lagging behind. Actually, there has been significant technical advancements[51,52]. The current COVID-19 pandemic, though most disastrous, has ironically provided a good platform to highlight the safety profile of mRNA vaccines when the nucleoside-modified mRNA-LNP vaccines have a remarkable safety track record[53]. Actually, mRNA-LNPs can induce superior T follicular helper cell responses than that of an adjuvanted protein subunit vaccine even though the exact mechanism is still unclear. Moreover, although conventional pathogen vaccines usually require adjuvants to boost the much desired immunity, LNPs readily act as self-adjuvants[54]. Repeated oncology administrations would thus be facilitated, as there is hardly any issue of possible toxicity due to vaccine adjuvants typical of repurposed pathogen vaccines.

Importantly, mRNA vaccines represent a promising platform for the development of oncology vaccines as they can induce potent T cell responses and can also be readily modified[55]. Moreover, as mRNA vaccine design is highly flexible, it would enable the development of personalized neoantigen cancer vaccines, unless the cost becomes a significant concern, *e.g.*, during the current severe economic recession. As various aspects of novel developments, pivotal considerations, as well as current challenges for successful development of the self-amplifying RNA (saRNA) vaccines are already fully discussed elsewhere, suffice it to say that, even though the saRNA is very remarkable for enabling lower vaccine doses, the stability and manufacturing may still be challenging[17,56-59]. Encouragingly, it is now feasible to design very promptly a new saRNA vaccine for testing, as in the case of the Imperial College London[59]. The rapid and easy manufacture of saRNA vaccines could enable local productions so as to reduce logistical and cold-chain issues of current mRNA vaccines. Importantly, minimizing the required dose is highly desirable as it reduces side effects, *e.g.*, myocarditis and permits repeated usage for oncology practice.

Although testing of new modalities of oncology treatments often involve advanced cancers, the TME is actually very well known to be a major factor preventing successful testing of treatment options designed to cater for advanced cancers[60]. It would be more appropriate to test clinically these novel agents without the interference of the TME. For instance, for advanced melanomas, a recent randomized phase II clinical trial was on the efficacy of autologous DCs co-electroporated with mRNA coding for TriMix as well as mRNA encoding one of four TAAs linked to one HLA class II targeting signal (TriMixDC-MEL) (see Table 1)[61]. The randomization involved 41 patients (21 receiving TriMixDC-MEL; 20 had placebo). All patients had stage III/IV melanomas but no evidence of any residual disease (after resecting all macro-metastases). The vaccine was found to be tolerable and the 1-year disease free survival rate was 71% for the TriMixDC-MEL arm *vs* 35% of the placebo arm[61]. Admittedly, although not all melanoma metastases could likewise be resected, this trial would still demonstrate the vaccine's tolerability and probable effectiveness. This could not have been accomplished had the trial been performed on patients with significant TMEs.

CONCLUSION

The future development of mRNA vaccines for oncology is two pronged. On the one hand, as neoantigens of cancer cells are often dissimilar among individual patients, personalized vaccines are most appropriate, *e.g.*, the intranodal vaccine injection with free mRNA encoding 10 neoepitopes on 13 advanced melanoma patients could generate T cell immunity against multiple neoepitopes in all 13 patients[56,62]. Several personalized cancer vaccines using lipid nanoparticle–mRNA formulations have



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Figure 1 Selected combinations with cancer treatment vaccines: Immune checkpoint inhibitors, radiotherapy, metronomic chemotherapy, and metformin. [a]Especially mRNA cancer vaccines: cell-free, rapid production, versatile and inherent adjuvant properties outperforming pathogen vaccines repurposed for oncology. Even balancing innate and adaptive immunities is feasible with mRNA. [b]Metformin's long standing safety track record, ready availability and eminent affordability may enable an ideal combination with mRNA cancer vaccines. ICI: Immune checkpoint inhibitors; mChT: Metronomic chemotherapy; RT: Radiotherapy.

also entered clinical trials, *e.g.*, mRNA-4157 is being tried actively both as monotherapy and in combination with ICIs (see Table 1).

On the other hand, such most impressive personalized oncology treatments, though much more specific, probably effective, and now with reduced processing time than other personalized vaccines, may not be readily affordable for the vast majority of cancer patients especially at this very trying period of severe economic recession. Therefore, for priming tumors having highly evasive TMEs, combination chemotherapy, radiation, and vaccines may have better efficacy[63]. As there may even be a highly beneficial mutual enhancement effect of turning "cold" tumors into "hot" ones[8,40], it really pays to explore further by performing robust clinical trials to document if such combinations have the potential of being a more versatile approach.

FOOTNOTES

Author contributions: Tsao SY designed the plan of the article, performed the reference search, wrote the manuscript, and has read and approved the final manuscript.

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ORIGINAL ARTICLE

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Eight hub genes as potential biomarkers for breast cancer diagnosis and prognosis: A TCGA-based study

Nan Liu, Guo-Duo Zhang, Ping Bai, Li Su, Hao Tian, Miao He

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Abstract

BACKGROUND

Breast cancer (BC) is the most common malignant tumor in women.

AIM

To investigate BC-associated hub genes to obtain a better understanding of BC tumorigenesis.

METHODS

In total, 1203 BC samples were downloaded from The Cancer Genome Atlas database, which included 113 normal samples and 1090 tumor samples. The limma package of R software was used to analyze the differentially expressed genes (DEGs) in tumor tissues compared with normal tissues. The cluster Profiler package was used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of upregulated and downregulated genes. Univariate Cox regression was conducted to explore the DEGs with statistical significance. Protein-protein interaction (PPI) network analysis was employed to investigate the hub genes using the CytoHubba plug-in of Cytoscape software. Survival analyses of the hub genes were carried out using the Kaplan-Meier method. The expression level of these hub genes was validated in the Gene Expression Profiling Interactive Analysis database and Human Protein Atlas database.

RESULTS

A total of 1317 DEGs (fold change > 2; $P < 0.01$) were confirmed through bioinformatics analysis, which included 744 upregulated and 573 downregulated genes in BC samples. KEGG enrichment analysis indicated that the upregulated genes were mainly enriched in the cytokine-cytokine receptor interaction, cell

cycle, and the p53 signaling pathway ($P < 0.01$); and the downregulated genes were mainly enriched in the cytokine-cytokine receptor interaction, peroxisome proliferator-activated receptor signaling pathway, and AMP-activated protein kinase signaling pathway ($P < 0.01$).

CONCLUSION

In view of the results of PPI analysis, which were verified by survival and expression analyses, we conclude that *MAD2L1*, *PLK1*, *SAA1*, *CCNB1*, *SHCBP1*, *KIF4A*, *ANLN*, and *ERCC6L* may act as biomarkers for the diagnosis and prognosis in BC patients.

Key Words: Breast cancer; Bioinformatics; Hub gene; The Cancer Genome Atlas; Protein-protein interaction

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Core Tip: This study identified 1317 DEGs related to the occurrence and development of breast cancer (BC), 165 DEGs related to prognosis, and 8 hub genes (*MAD2L1*, *PLK1*, *SAA1*, *CCNB1*, *SHCBP1*, *KIF4A* , *ANLN*, and *ERCC6L*). Each of these eight hub genes has different expression levels in BC and is significantly related to prognosis. The results of this study indicate that studying these DEGs may help provide a full understanding of the molecular mechanisms underlying BC pathogenesis and progression. Moreover, these hub genes may serve as potential prognostic markers and therapeutic targets, which provide a reference for more in-depth and extensive prospective clinical research.

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INTRODUCTION

Breast cancer (BC) is the most common malignant tumor in women. In 2019, 268600 new BC patients and 41760 new BC deaths were reported, accounting for 30% of all new cancer cases and 15% of cancer-related deaths, respectively. The mortality of BC is second only to lung cancer[1]. In recent years, BC outcome has significantly improved and treatment strategies such as surgery, chemotherapy, radiotherapy, endocrine therapy, and targeted therapy have achieved fine clinical benefits[2], whereas patients with distant metastases are almost incurable[3]. In addition, even after resection of the primary tumor, 30% of early BC is prone to recurrence in distant organs[4]. In clinical practice, the treatment and prognosis of different molecular subtypes of BC are significantly different: estrogen receptor-positive (ER+) patients prefer endocrine therapy, human epidermal growth factor receptor 2-positive (HER2+) patients prefer targeted therapy, and poorly differentiated tumors are usually associated with a poor prognosis[5-7].

Recent studies have found that the occurrence and development of BC are related to many molecular markers. For example, the expression of cluster of differentiation 82 is significantly decreased in BC and is associated with disease progression and metastasis[8]. In addition, a study on triple-negative BC suggested that multiple long noncoding RNAs are associated with prognosis, including MAGI2-AS3, GGTA1P, NAP1L2, CRABP2, SYNPO2, MKI67, and COL4A6[9]. Advances in microarray and high-throughput sequencing technology provide strong support for the development of more reliable prognostic markers[10,11]. Genome wide expression profiling can reveal molecular changes in the process of tumorigenesis and development, and has proven to be an efficient method to identify key genes[12]. Therefore, it is particularly important to explore more sensitive and specific biomarkers to further understand the pathogenesis of BC and the choice of treatment strategies.

This public database-based study explored potential hub genes in the occurrence and development of BC through bioinformatics analysis of the gene expression profile and clinical characteristics of BC, in order to provide new biological targets and directions for the clinical diagnosis and treatment of BC.

MATERIALS AND METHODS

Data sources and processing

The Cancer Genome Atlas (TCGA) database is a cancer research project established by the. National Cancer Institute and National Human Genome Research Institute. It aims to understand the mechanism

of carcinogenesis and development of cancer cells and develop new diagnosis and treatment methods by collecting various types of cancer-related omics data. In this study, 1203 breast samples (fragments per kilobase million [FPKM] format) were downloaded from TCGA database (https://portal.gdc.cancer.gov/), including 1090 tumor samples and 113 normal samples. For a more accurate comparison of gene expression, FPKM data were converted to transcripts per million (TPM). At the same time, 1097 tumor samples containing clinical information were downloaded, and the data that did not match the expression samples were excluded. The remaining 1089 tumor samples were included in the univariate Cox regression analysis. Overall survival (OS) was taken as the endpoint event, and gene expression in TPM format was converted to $\log_2 (x + 1)$.

DEG acquisition

Limma package of R software (version 3.6.3) was employed for differential gene analysis[13], using the adjusted *P*-value (adj *P*-value) to avoid false-positive results. The inclusion criteria of DEGs were: | log2 fold change (FC) | > 2 and adjusted $P < 0.01$. The ggplot2 package of R software was used to generate a volcano plot to visualize these differential genes.

Functional enrichment analysis

DEGs were converted into gene ID through org.Hs.eg.db package of R software, and then Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was carried out by R software's clusterProfiler and enrichlot program package. ggplot2 program package was used to display the top 10 enrichment items, and adjusted $P < 0.05$ was considered statistically significant.

Univariate Cox regression analysis

The survival package of R software was used to carry out univariate Cox regression analysis on 1089 BC samples with survival information. The median value of expression was set as the cut-off point between the high expression and low expression groups, and differential genes related to prognosis were obtained for subsequent analysis. $P < 0.05$ was considered statistically significant.

Construction of PPI

The STRING database (https://string-db.org/) is a search tool for searching interacting genes, which aims to construct protein-protein interaction (PPI) networks of different genes based on known and predicted PPIs, and analyze the proteins that interact with each other[14]. Based on the online tool STRING, PPI of prognosis-related DEGs was constructed, and the confidence score was ≥ 0.4. Then the PPI network was visualized by Cytoscape software (version 3.7.2). In addition, using the CytoHubba plug-in of Cytoscape software to calculate the gene degree through the "degree" method, the top 10 genes were taken as the hub genes for subsequent analysis and verification.

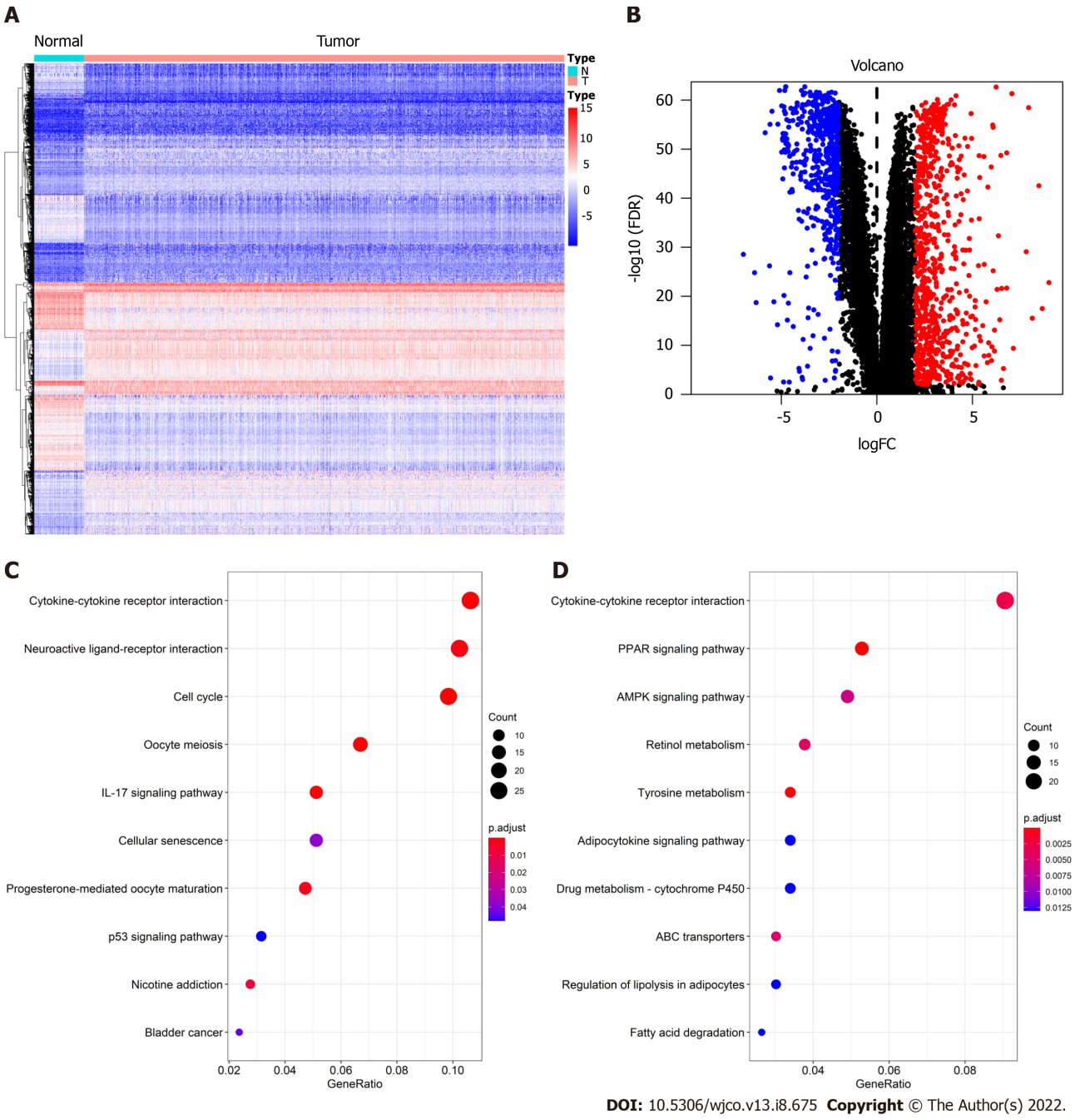
Survival analysis of hub genes

The Kaplan-Meier plotter (http://kmplot.com/analysis/) can use 18674 cancer samples to evaluate the impact of 54675 genes on survival[15]. These studies included recurrence-free survival and OS information of 5143 cases of BC, 1816 cases of ovarian cancer, 2437 cases of lung cancer, 1065 cases of gastric cancer, and 364 cases of liver cancer, which are mainly based on Gene Expression Omnibus, TCGA, and European Genome-phenome Archive databases. The role of the tool is to benefit patients in clinical decision making, health care policy, and resource allocation through meta-analysis of biomarker assessment[16]. In this study, we analyzed the OS rate of 10 hub genes in BC using the Kaplan-Meier plotter. According to the median expression of each hub gene in Kaplan-Meier plotter, the patients were divided into two groups to present the difference in survival probability between the high expression group and the low expression group. A total of 14 datasets were enrolled in our analysis according to the Kaplan-Meier web tool and detailed retrospective clinical information in http://kmplot.com/analysis/. $P < 0.05$ was considered statistically significant.

To further investigate the prognostic value of the hub genes selected above, we performed the log-rank test on these hub genes in molecular subtypes of BC based on TCGA cohort. Through the PAM50 algorithm, TCGA cohort was separated into five major subtypes: luminal A, luminal B, HER2 enriched, basal-like, and normal-like. This method was completed through utilizing the "genefu" R package according to detailed operation protocol.

Expression analysis of hub genes

The Gene Expression Profiling Interactive Analysis (GEPIA) database was employed to verify the mRNA expression levels of 10 hub genes in normal breast tissues and cancer tissues. GEPIA database contains data from 9736 tumor samples and 8587 normal samples, which were used to display the mRNA expression levels of each key gene in cancer and non-cancer tissues[17]. The protein expression levels of 10 hub genes in human normal tissues and BC tissues were analyzed using the human protein atlas database (HPA), which contains immunohistochemical expression data covering about 20 of the most common types of cancer[18].



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Figure 1 Screening and functional enrichment analysis of differentially expressed genes. A: Heat map of differentially expressed genes (DEGs); B: Volcano Plot of DEGs; C: Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of upregulated genes; D: KEGG enrichment analysis of downregulated genes.

RESULTS

Identification and functional analysis of DEGs

After DEG analysis of 113 normal breast samples and 1090 BC samples, we found that there were 1317 DEGs, of which 744 were upregulated and 573 were downregulated in BC. As shown in Figure 1A, red represents high expression and blue represents low expression. At the same time, the volcano plot was used to present the distribution of DEGs (Figure 1B), the red dots represent upregulated genes and the blue dots represent downregulated genes.

To further understand the biological function of these 1317 DEGs, the clusterProfiler and enrichplot packages of R software were used to perform KEGG enrichment analysis on these DEGs. The enrichment analysis results of upregulated genes and downregulated genes are shown in Figure 1C and D, respectively. The top 10 upregulated genes were the cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction, cell cycle, oocyte meiosis, interleukin 17 signaling pathway, cellular senescence, progesterone-mediated oocyte maturation, p53 signaling pathway, nicotine

Table 1 Summary of the top 10 hub genes according to their grade

Genes	Gene name	Grade
MAD2L1	MAD2 mitotic arrest deficient-like 1	24
PLK1	Polo-like kinase 1	22
SAA1	Serum amyloid A1	22
CCNB1	Cyclin B1	20
SHCBP1	SHC SH2-domain binding protein 1	18
KIF4A	Kinesin family member 4A	18
ANLN	Actin binding protein	16
ERCC6L	Excision repair cross-complementation group 6-like	16
CXCL2	Chemokine (C-X-C motif) ligand 2	16
WT1	Wilms tumor 1	14

addiction, and bladder cancer. The 10 ten downregulated genes were the cytokine-cytokine receptor interaction, peroxisome proliferator-activated receptor (PPAR) signaling pathway, AMP-activated protein kinase (AMPK) signaling pathway, retinol metabolism, tyrosine metabolism, adipocytokine signaling pathway, drug metabolism - cytochrome p450, ATP-binding cassette transporters, regulation of lipolysis in adipocytes, and fatty acid degradation.

Screening of hub genes

To screen the DEGs related to the prognosis of BC, we used the survival package of R software to perform univariate Cox regression analysis on 1317 DEGs, and found that the prognosis of 165 genes was statistically significant (Supplementary Table 1). As shown in Figure 2, further analysis of the PPI of these 165 genes revealed that there were a total of 164 nodes and 156 interactions (edges), and the confidence score adopted default value ≥ 0.4. The CytoHubba algorithm of Cytoscape software was used to calculate the degree score of each node. The top 10 genes were MAD2L1, PLK1, SAA1, CCNB1, SHCBP1, KIF4A, ANLN, ERCC6L, CXCL2, and WT1 (Figure 3). The upregulated genes were represented by red and round nodes, and the downregulated genes were represented by blue and diamond nodes. The node size represented the level, and most of the hub genes were upregulated DEGs. Gene annotation and grade scores are shown in Table 1.

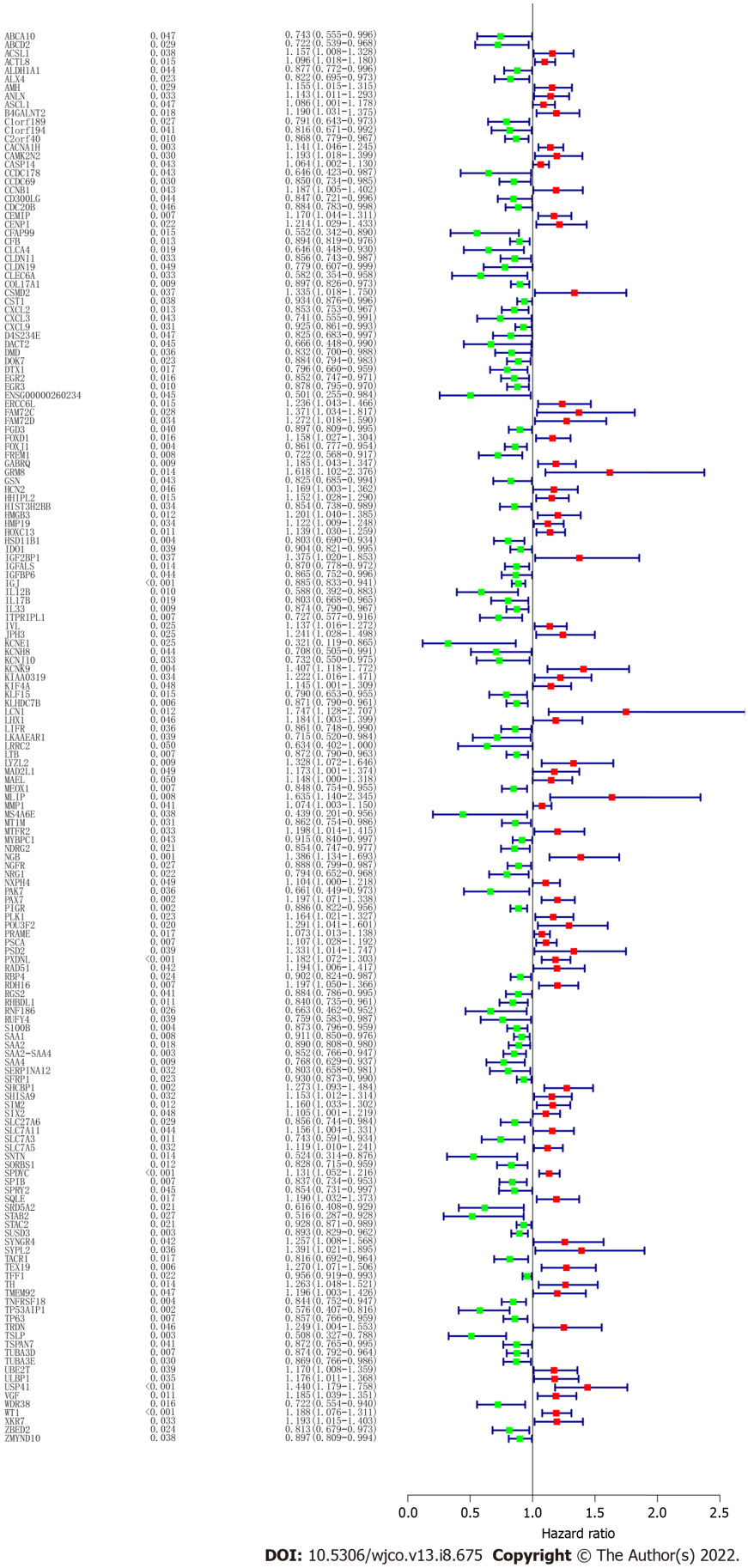
Survival analysis of hub genes

Kaplan-Meier plotter was used to explore the prognostic value of 10 hub genes in BC. The results showed that, except for *CXCL2* [hazard ratio (HR) 0.86 (0.69-1.07); $P = 0.170$] and *WT1* [HR 1.03 (0.83-1.28); $P = 0.760$], the highly expressed *MAD2L1* [HR 2.02 (1.62-2.51); $P = 1.8e-10$], *PLK1* [HR 1.42 (1.15-1.76); $P = 0.0012$], *CCNB1* [HR 1.42 (1.04-1.94); $P = 0.028$], *SHCBP1* [HR 1.76 (1.42-2.19); $P = 2.1$ e-07], *KIF4A* [HR 1.8 (1.44-2.23); $P = 8.8e-08$], *ANLN* [HR 1.48 (1.08-2.03); $P = 0.014$], and *ERCC6L* [HR 1.68 (1.35-2.09); $P = 2e-06$] were related to the poor OS rate of BC patients. By contrast, the high expression of *SAA1* [HR 0.71 (0.57-0.88); $P = 0.018$] was associated with a better OS rate for BC patients (Figure 4).

We also conducted the survival analysis of these 10 hub genes in TCGA molecular subtypes. As a result, TCGA cohort was successfully divided into five subtypes based PAM50 identifier: 563 of luminal A, 215 of luminal B, 82 of HER2-enriched, 189 of basal-like, and 39 of normal-like. Then survival analysis of these 10 genes was performed in each subtype group. The results indicated that *CXCL2* (HR = 0.45; $P < 0.05$) and *SAA1* (HR = 0.53; $P < 0.05$) were protective factors in the luminal A subtype (Figure 5). *ANLN* (HR = 2.12; $P < 0.05$), *ERCC6L* (HR = 3.04; $P < 0.05$), *KIF4A* (HR = 2.50; $P < 0.05$), *PLK1* (HR = 2.40; $P < 0.05$), and *SHCBP1* (HR = 2.42; $P < 0.05$) were hazard factors in luminal B subtype, whereas the *CXCL2* (HR = 0.45; $P < 0.05$) showed protective effects. Finally, *KIF4A* (HR = 4.31; $P < 0.05$) acted as a risk factor in *HER2*-enriched patients and *CXCL2* played a satisfactory role among basal-like patients (HR = 0.46; $P < 0.05$).

Expression analysis of hub genes

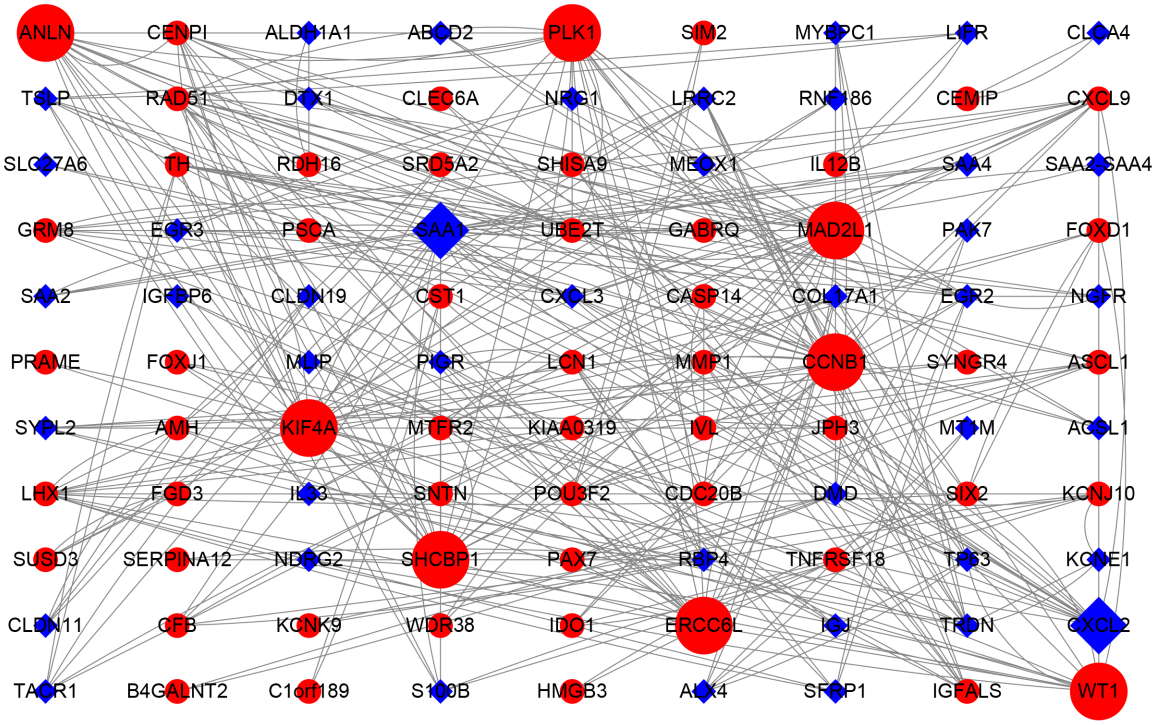
To verify the expression differences of key genes in BC, *GEPIA* was employed to analyze the mRNA expression levels of *MAD2L1*, *PLK1*, *SAA1*, *CCNB1*, *SHCBP1*, *KIF4A*, *ANLN*, *ERCC6L*, *CXCL2*, and *WT1* between BC and non-cancerous tissues (Figure 5). Compared with non-cancerous tissues, MAD2L1 (Figure 5A), PLK1 (Figure 5B), CCNB1 (Figure 5D), SHCBP1 (Figure 5E), KIF4A (Figure 5F), ANLN (Figure 5G), and ERCC6L (Figure 5H) in BC tissues were significantly upregulated ($P < 0.01$); SAA1 (Figure 5C) and CXCL2 (Figure 5I) were significantly downregulated in BC ($P < 0.01$); and WT1 (Figure 5J) tended to increase in BC tissues. After verifying the mRNA expression level of hub genes, we



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Figure 2 Protein-protein interaction network analysis of prognosis related differentially expressed genes. The upregulated genes are

represented by red and round nodes, whereas the downregulated genes are represented by blue and diamond nodes. The size of the node represents their grade.



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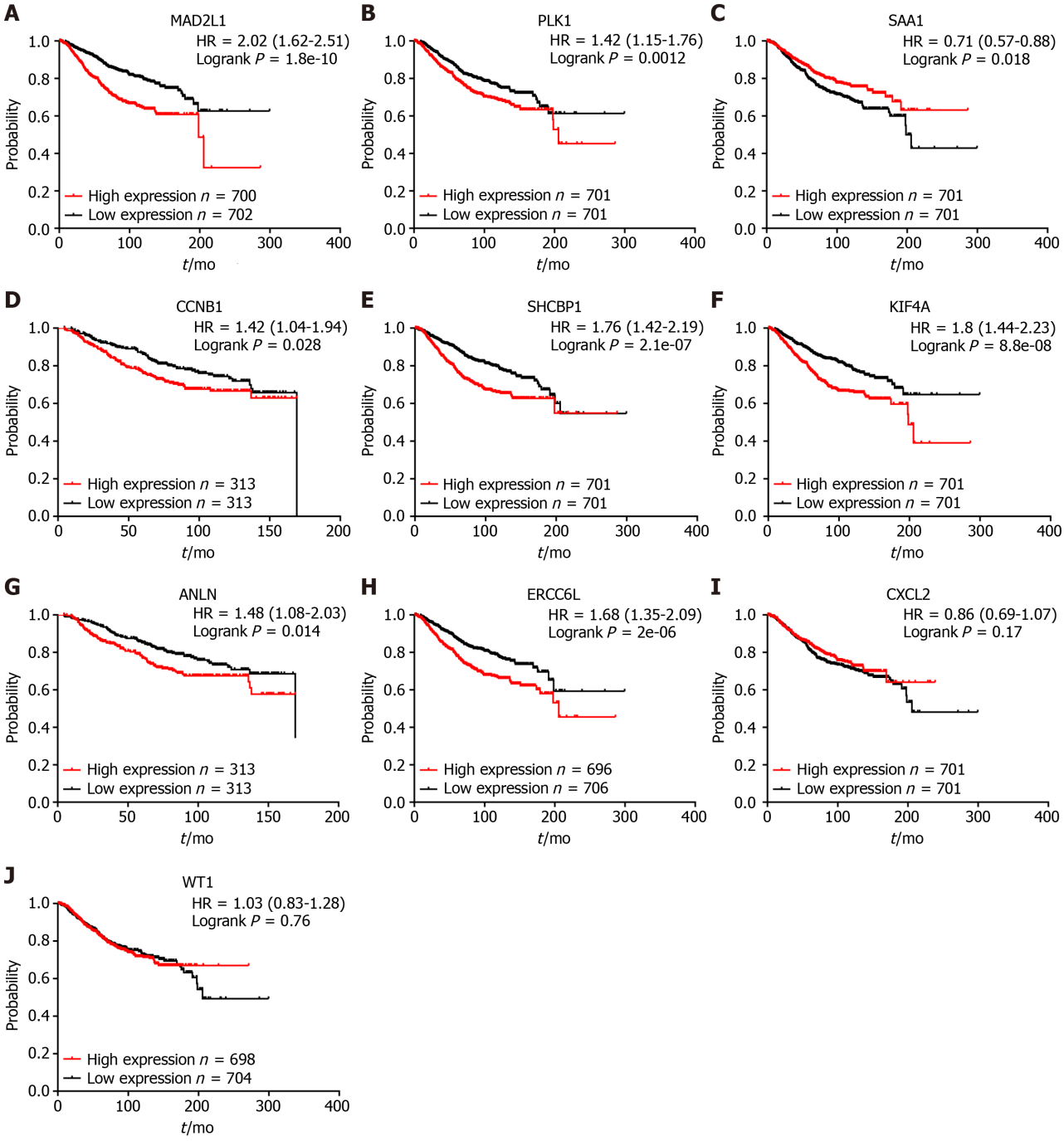
Figure 3 Survival analyses of the 10 hub genes were verified by Kaplan-Meier plotter.

used the HPA database to verify the protein expression level of these hub genes in BC. It is worth noting that MAD2L1 (Figure 6A), PLK1 (Figure 6B), CCNB1 (Figure 6C), SHCBP1 (Figure 6D), ANLN (Figure 6F), ERCC6L (Figure 6G), and WT1 (Figure 6H) were not expressed in normal breast tissues, but expressed in different levels in BC tissues. KIF4A (Figure 6E) was moderately expressed in normal breast tissues and highly expressed in BC tissues. In short, the expression of hub genes was consistent with the results of differential analyses at both the mRNA and protein levels.

DISCUSSION

In this study, we used bioinformatics analysis to screen and verify potential biomarkers associated with BC. After comparing the gene expression matrix of breast tissue retrieved from TCGA database, 744 upregulated DEGs and 573 downregulated DEGs were successfully identified. Combined with the survival data, 165 prognostic-related DEGs were analyzed. According to PPI network analysis, the top 10 node genes were ranked: *MAD2L1*, *PLK1*, *SAA1*, *CCNB1*, *SHCBP1*, *KIF4A*, *ANLN*, *ERCC6L*, *CXCL2*, and *WT1*. After subsequent survival analysis and expression analysis verification, the expression and prognosis of *MAD2L1*, *PLK1*, *SAA1*, *CCNB1*, *SHCBP1*, *KIF4A*, *ANLN*, and *ERCC6L* in BC were finally confirmed. These eight hub genes may play a vital role in the occurrence and development of BC.

Among the 1317 identified DEGs, significant gene expression dysregulation was observed in the cell cycle, PPAR signaling pathway, and AMPK signaling pathway. Cell cycle is a highly conserved process in human evolution and is essential for the normal growth of cells. Abnormal cell cycle is a hallmark of human cancer[19]. Recent studies have also identified several genes related to the cell cycle, including *CCNB1*, *ANLN*, *MAD2L1*, and *PLK1*. For example, CCNB1 may be a biomarker for the prognosis of ER+ BC patients and monitoring the efficacy of hormone therapy[20]. Recent studies have found that the occurrence and proliferation of gastric cancer cells induced by ISL1 is mediated by the expression and regulation of CCNB1, CCNB2, and C-MYC[21]. In addition, the high expression of ANLN in BC cell nuclei is significantly related to tumor tissue size, histopathological grade, high proliferation rate, and a worse prognosis[22]. *MAD2L1* is a mitotic spindle checkpoint gene. In patients with primary BC, compared with patients with ER+, PR+ and low-grade tumors, patients with ER-, PR- and high-grade tumors have higher expression of MAD2L1, and high expression of MAD2L1 is associated with a poor OS[23]. PLK1 is a key oncogene that can regulate the transition of cells in the G2-M phase, thus

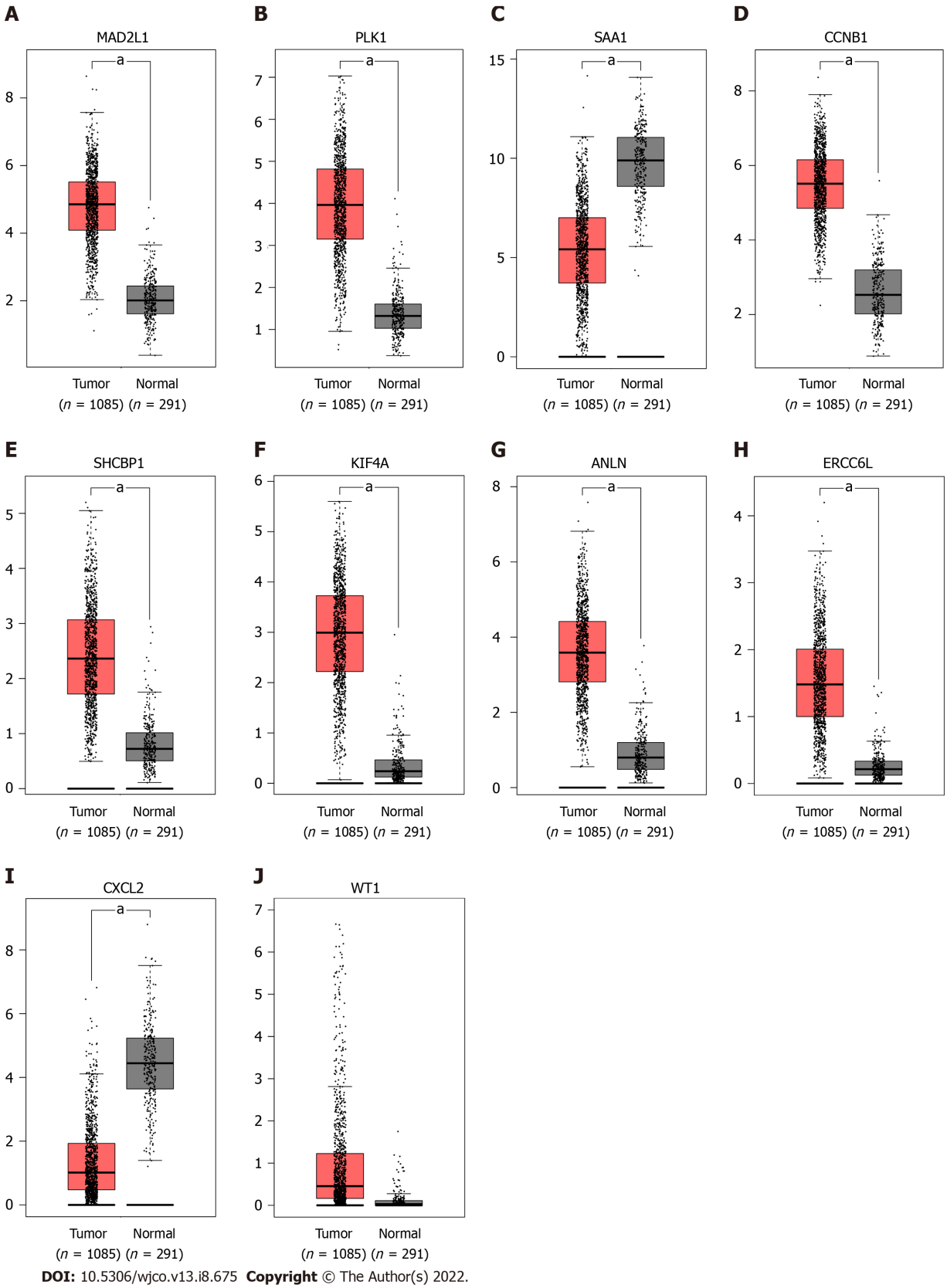


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Figure 4 Subtype survival analysis of these 10 hub genes in breast cancer patients among The Cancer Genome Atlas cohort. The results are presented by a heatmap and the detailed value on each cell represent the hazard ratio of survival plot.

promoting the growth and metastasis of tamoxifen resistant BC[24]. These studies are consistent with our current conclusion that *CCNB1*, *ANLN*, *MAD2L1*, and *PLK1*, as key genes, are overexpressed in BC tissues, and their overexpression is correlated with poor prognosis. Meanwhile, the PPAR signaling pathway may be an important predictor of BC response to neoadjuvant chemotherapy[25], and activation of the AMPK signaling pathway can inhibit the activity of the Wnt/β-catenin signaling pathway, thereby inhibiting the growth of BC cells[26]. These studies showed that the identified DEGs play a critical role in the occurrence and development of BC, and the hub genes among them may serve as prognostic markers and are worth further investigation.

With the exception of *CCNB1*, *ANLN*, *MAD2L1*, and *PLK1*, the gene combination model of CD74, MMP9, RPA3, and SHCBP1 in the tumor microenvironment (TME) can effectively predict the prognosis and disease risk of BC patients[27], while their potential mechanism remains unknown. In addition, the circKIF4A-miR-375-KIF4A axis can regulate the development of triple-negative BC through competing endogenous RNA, and circKIF4A can act as a prognostic biomarker and therapeutic target for triple-

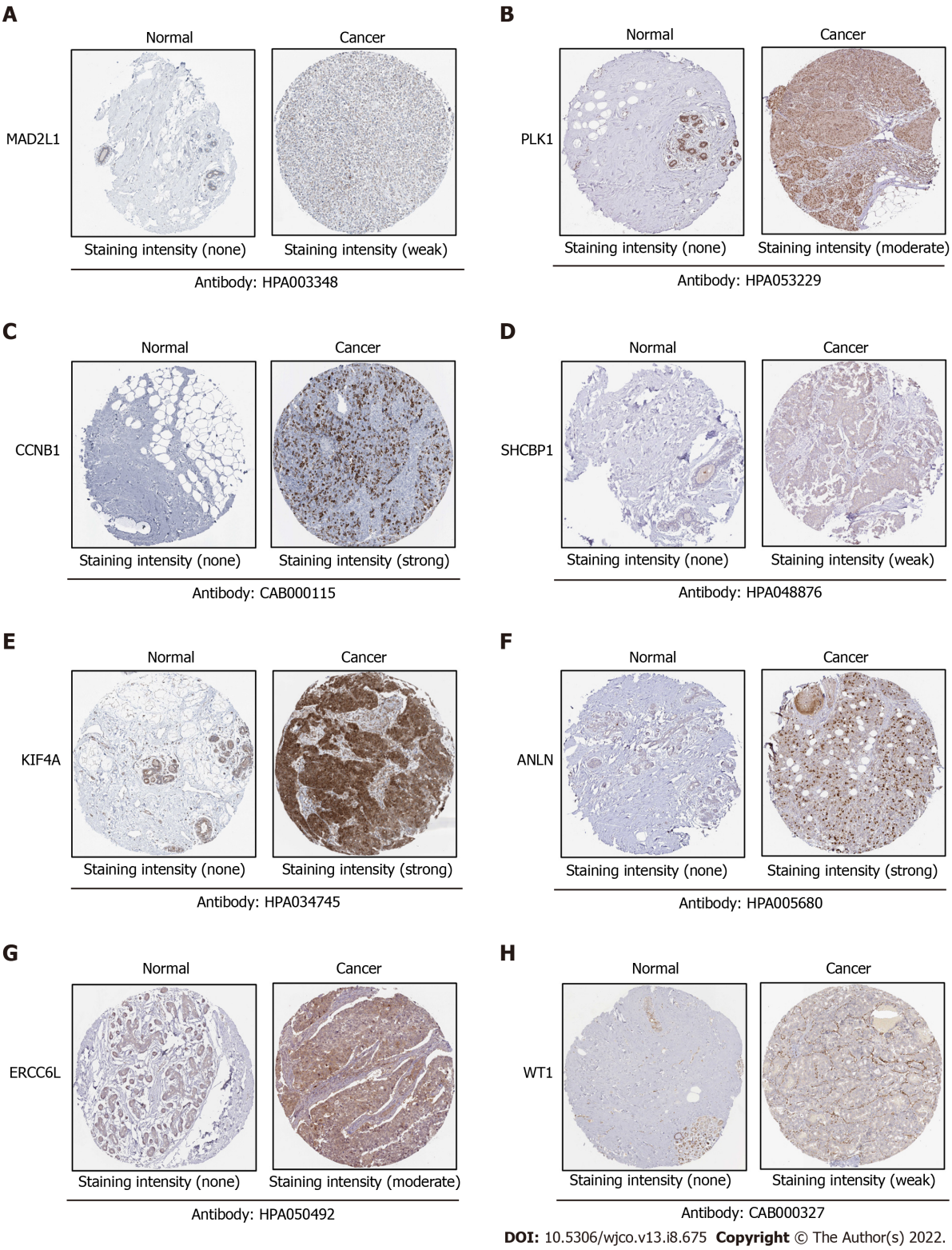


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Figure 5 mRNA expression of the 10 hub genes were verified by the Gene Expression Profiling Interactive Analysis database. $^{a}P < 0.05$.

negative BC[28].

SAA1 is a serum amyloid protein family member that is highly expressed in non-small cell lung cancer, and is associated with a poor prognosis and tyrosine kinase inhibitors[29]. SAA1 has low expression in hepatocellular carcinoma, and the high expression of SAA1 is associated with a better prognosis[30]. To date, SAA1 has not been reported in BC, and the specific role and function of this gene



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Figure 6 Protein expression of the eight hub genes were verified by human protein atlas database. The database lacks expression data on serum amyloid A1- and chemokine (C-X-C motif) ligand 2-related proteins.

in BC require further experimental exploration and clinical specimen verification. ERCC6L is a newly discovered DNA helicase. In the human BC cell line MDA-MB-231, exogenous interference with the expression of ERCC6L can inhibit the growth of BC cells[31]. However, its role and specific mechanism in clinical specimens are still unknown. The expression of ERCC6L is upregulated in clear cell renal cell carcinoma, and the highly expressed ERCC6L can promote the proliferation of clear cell renal cell carcinoma cells by regulating the mitogen-activated protein kinase signaling pathway[32]. In this study, we found that SAA1 and ERCC6L may be used as prognostic markers for BC, whereas there are few reports on these two genes, and further research is necessary.

In this study, we found that the differential expression of the eight hub genes are related to the occurrence and development of BC, and are significantly related to the OS rate, which indicate that these hub genes may be utilized as potential prognostic biomarkers and therapeutic targets for BC. This study had some limitations. First, due to the complexity of the dataset in the public database, it is difficult to consider some important confounding factors such as different ages, races, regions, and tumor stages when analyzing DEGs. Second, according to the results, seven key genes were upregulated in BC and one key gene was downregulated, but the mechanism of their differential expression is still unclear, and more studies are needed to confirm their biological basis. Finally, this study focused on the expression level and OS rate of the eight hub genes, and whether these key genes can be used as biomarkers and can improve the diagnostic accuracy and specificity of BC requires further research.

CONCLUSION

In conclusion, based on comprehensive bioinformatics analysis, this study identified 1317 DEGs related to the occurrence and development of BC, 165 DEGs related to prognosis, and 8 hub genes (*MAD2L1*, *PLK1*, *SAA1*, *CCNB1*, *SHCBP1*, *KIF4A*, *ANLN* and *ERCC6L*). Each of these eight hub genes has different expression levels in BC and is significantly related to prognosis. The results of this study indicate that studying these DEGs would help us have a deeper understanding of the molecular mechanisms of the pathogenesis and progression of BC. Moreover, these hub genes may serve as potential prognostic markers and therapeutic targets for BC, which provides a reference for more in-depth and extensive prospective clinical research.

ARTICLE HIGHLIGHTS

Research background

Breast cancer (BC) is the most common malignant tumor in women. In 2019, 268600 new BC patients and 41760 new BC deaths were reported, accounting for 30% of all new cancer cases and 15% of cancer-related deaths. Therefore, it is particularly important to explore more sensitive and specific biomarkers for further understanding the pathogenesis of BC and the choice of treatment strategies.

Research motivation

Exploring more valuable therapeutic targets would be helpful in treating with high efficacy.

Research objectives

This study aimed to identify novel biomarkers for BC.

Research methods

The limma package of R software and clusterProfiler package were used to analyze the differentially expressed genes (DEGs) in tumor tissues compared with the normal tissues, respectively. The protein-protein interaction network (PPI) analysis was used to investigate the hub-genes through cytohubba algorithm by the Cytoscape software. Survival analysis of the hub-genes were carried out through the Kaplan-Meier database. The expression level of these hub-genes was validated in the GEPIA database and the Human Protein Atlas database.

Research results

Upregulated genes mainly enriched in the cytokine-cytokine receptor interaction, cell cycle, and p53 signaling pathway ($P < 0.01$). The downregulated genes were mainly enriched in the cytokine-cytokine receptor interaction, peroxisome proliferator-activated receptor signaling pathway, and AMP-activated protein kinase signaling pathway ($P < 0.01$).

Research conclusions

MAD2L1, PLK1, SAA1, CCNB1, SHCBP1, KIF4A, ANLN, and *ERCC6L* may act as biomarkers for diagnosis and prognosis in BC patients.

Research perspectives

Proper validations must be made in future studies.

FOOTNOTES

Author contributions: Liu N performed the experiment and wrote the paper; Liu N, Zhang GD, and Bai P contributed to the bioinformatics analysis and figure preparation; Tian H and Su L modified the structure and language of the manuscript; He M and Tian H contributed to the conception and design of the study and the revisions of the manuscript; All authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Propensity-matched analysis of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma undergoing a liver transplant

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Abstract

BACKGROUND

Cholangiocarcinoma (CC) is a rare tumor that arises from the epithelium of the bile ducts. It is classified according to anatomic location as intrahepatic, perihilar, and distal. Intrahepatic CC (ICC) is rare in patients with cirrhosis due to causes other than primary sclerosing cholangitis. Mixed hepatocellular carcinoma-CC (HCC-CC) is a rare neoplasm that shows histologic findings of both HCC and ICC within the same tumor mass. Due to the difficulties in arriving at the correct diagnosis, patients eventually undergo liver transplantation (LT) with a presumptive

diagnosis of HCC on imaging when, in fact, they have ICC or HCC-CC.

AIM

To evaluate the outcomes of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma on pathological examination after liver transplant.

METHODS

Propensity score matching was used to analyze tumor recurrence (TR), overall mortality (OM), and recurrence-free survival (RFS) in LT recipients with pathologically confirmed ICC or HCC-CC matched 1:8 to those with HCC. Progression-free survival and overall mortality rates were computed with the Kaplan-Meier method using Cox regression for comparison.

RESULTS

Of 475 HCC LT recipients, 1.7% had the diagnosis of ICC and 1.5% of HCC-CC on pathological examination of the explant. LT recipients with ICC had higher TR (46% *vs* 11%; $P = 0.006$), higher OM (63% *vs* 23%; $P = 0.002$), and lower RFS (38% vs 89%; $P = 0.002$) than those with HCC when matched for pretransplant tumor characteristics, as well as higher TR (46% *vs* 23%; $P = 0.083$), higher OM (63% *vs* 35%; $P = 0.026$), and lower RFS (38% *vs* 59%; $P = 0.037$) when matched for posttransplant tumor characteristics. Two pairings were performed to compare the outcomes of LT recipients with HCC-CC *vs* HCC. There was no significant difference between the outcomes in either pairing.

CONCLUSION

Patients with ICC had worse outcomes than patients undergoing LT for HCC. The outcomes of patients with HCC-CC did not differ significantly from those of patients with HCC.

Key Words: Cholangiocarcinoma; Hepatocellular carcinoma; Liver; Prognosis; Recurrence; Survival analysis; Transplantation

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Core Tip: This retrospective cohort study analyzes the outcomes of patients undergoing liver transplantation (LT) with a presumptive diagnosis of hepatocellular carcinoma (HCC) in which explant analysis identified that they actually had intrahepatic cholangiocarcinoma (ICC) or mixed hepatocellular cholangiocarcinoma (HCC-CC). Propensity score matching was used to analyze tumor recurrence, overall mortality, and recurrence-free survival in LT recipients with pathologically confirmed ICC or HCC-CC matched 1:8 to those with HCC. Patients with ICC have worse outcomes than patients undergoing LT for HCC, even when matched for explant pathology. Outcomes did not differ significantly between patients with HCC-CC and patients with HCC.

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INTRODUCTION

Cholangiocarcinoma (CC) is a relatively rare, aggressive tumor that arises from the epithelium of the bile ducts. It is classified according to anatomical location as intrahepatic, perihilar, or distal[1]. CC is the most common tumor of the biliary tree, accounting for approximately 10%-25% of all hepatic malignancies[2]. It is the second most common hepatic malignancy[3].

Intrahepatic CC (ICC) represents 5%-10% of all CCs[1,4,5]. Although rare, its incidence is increasing in many countries[6-9]. In Brazil, ICC-related mortality in persons aged 45-64 years increased by 100% from 2002 to 2012, reaching 0.35 and 0.37 per 100000 person-years for men and women, respectively[9]. The increase is attributed, at least in part, to improved ICC classification, accurate diagnosis, and the negative impact of known risk factors, such as chronic hepatitis C virus (HCV) infection and obesity[10].

Mixed hepatocellular-cholangiocarcinoma (HCC-CC) is a rare neoplasm that histologically resembles both HCC and ICC within the same tumor mass[11]. It has an estimated incidence of 1%-4.7% among hepatic malignancies[12]. HCC-CC and ICC share the same risk factors[13]. The diagnosis of HCC-CC is typically made by pathology after resection or transplant, and a preoperative diagnosis is unlikely[14].

Although imaging findings suggestive of the diagnosis of HCC, ICC, or HCC-CC have been described[15-17], these tumors can be challenging to diagnose because of their rarity. In addition, HCC and ICC can coexist in separate nodules within the same liver or within the same tumor mass. Therefore, due to the difficulties in arriving at the correct diagnosis, patients eventually undergo a liver transplant (LT) with the presumptive imaging diagnosis of HCC when, in fact, they have ICC or HCC-CC[18,19].

The present study aimed to determine the prevalence of ICC or HCC-CC confirmed by explant pathology in patients who underwent LT with the presumptive diagnosis of HCC and to compare recurrence, recurrence-free survival, and overall mortality rates between these patients and LT recipients with HCC.

MATERIALS AND METHODS

Study design and population

This retrospective cohort study included patients aged ≥ 18 years with liver cirrhosis and imaging findings suggestive of HCC within the Milan criteria who underwent LT between June 1997 and July 2019 at a transplant referral center/teaching hospital in southern Brazil. Patients were followed up until April 2020 and divided into three groups according to the diagnosis on explant pathology: (1) Patients with HCC; (2) Patients with ICC; and (3) Patients with mixed HCC-CC. Well-established diagnostic criteria were followed, and immunohistochemical analysis was performed if necessary[12,20].

The following variables were analyzed: Age, sex, etiology of liver cirrhosis, Child-Pugh score, pretransplant tumor characteristics, including presence and type of neoadjuvant therapy, highest alpha-fetoprotein (AFP) level, and sum of nodule diameters on imaging; and posttransplant characteristics (explant), including number of nodules and sum of nodule diameters, cases within the Milan criteria or University of California San Francisco (UCSF) criteria, tumor grade/differentiation, presence of total necrosis, and microvascular invasion.

The outcomes analyzed were tumor recurrence, recurrence-free survival, and overall mortality.

Brazilian criterion for inclusion of patients with HCC in the transplant waiting list

In Brazil, patients with liver cirrhosis and imaging findings suggestive of HCC[21,22] can be placed on the LT waiting list upon detection of a lesion ≥ 2 cm and ≤ 5 cm or up to three lesions ≥ 2 cm and ≤ 3 cm.

Pretransplant locoregional therapy

Patients on the waiting list with an estimated waiting time for LT > 6 mo were treated with transarterial chemoembolization, radiofrequency ablation, or percutaneous ethanol injection.

Statistical analysis

The statistical methods of this study were reviewed by Mario B. Wagner, MD PhD DLSHTM, Full Professor of Epidemiology and Biostatistics, School of Medicine, Federal University of Rio Grande do Sul, Brazil.

Baseline patient characteristics were described using standard statistical methods. Continuous variables were compared using *t*-test or Mann-Whitney test when distributional assumptions were in doubt. Categorical variables were compared by the chi-square test or Fisher's exact test when needed. Propensity score matching (PSM) was used to assess whether tumor recurrence, overall mortality, and recurrence-free survival rates in patients with ICC or HCC-CC differed from those in patients with HCC. Additionally, hazard ratios (HRs) and their confidence intervals (CIs) were calculated. Progression-free survival rate and overall mortality rate were computed with the Kaplan-Meier method using Cox regression for comparison.

Propensity score matching

Patients with ICC and HCC-CC were matched to those with HCC using PSM based on the nearest neighbor algorithm according to a 1:8 ratio. Considering pretransplant and posttransplant variables, two matching sequences were run for patients with ICC and another two sequences for those with HCC-CC, which resulted in four matching datasets.

The variables considered for the pretransplant matching were highest AFP level, largest nodule diameter or the sum of the largest diameters in the case of multiple lesions, and year of LT. The posttransplant matching was based on variables collected during explant pathology which included tumor grade/differentiation, microvascular invasion, largest nodule diameter or the sum of the largest diameters in the case of multiple lesions, and year of LT.

Simple Cox regression was applied to the four datasets (pretransplant variable-matched sets ICC *vs* HCC and HCC-CC *vs* HCC, and posttransplant variable-matched sets ICC *vs* HCC and HCC-CC *vs* HCC) to obtain HRs and 95%CIs.

PSM groups were defined using R version 4.0 and the package MatchIT (software package MatchIT in R version 4.0.4; https://www.r-project.org/). Other analyses were conducted with IBM-SPSS version 25. *P* values < 0.05 were considered statistically significant.

Ethical aspects

The study followed the guidelines for the publication of observational studies[23]. The Institutional Review Board of Santa Casa de Misericórdia de Porto Alegre approved the study protocol (No. 4.250.889). Informed consent was waived due to the non-interventional design of the study and retrospective nature of data collection. All investigators signed a data use agreement to ensure the ethical and secure use of the data.

RESULTS

Over a period of 22 years, 475 patients with the presumptive diagnosis of HCC underwent LT at our center. According to a retrospective review of the LT database, 15 of these patients (3.1%) were found to have either ICC (n = 8) or HCC-CC (n = 7) detected in the pathological examination of the explant. The remaining 460 patients had the diagnosis of HCC confirmed by explant pathology (Figure 1). Most ICCs (6/8; 75.0%) were moderately or poorly differentiated and had the largest nodule diameter or the sum of the largest diameters < 5 cm. The patients with HCC-CC (7/7; 100%) were also moderately or poorly differentiated. In most HCC-CC cases (5/7; 71.4%), the largest nodule diameter or the sum of the largest diameters did not exceed 5 cm.

Comparison of ICC vs HCC transplant recipients, propensity score-matched for year of transplant and pretransplant and posttransplant tumor characteristics

Table 1 shows the comparison of patients with ICC (n = 8) matched 1:8 to those with HCC (n = 64) who underwent LT in the same year and had similar pretransplant tumor characteristics (median highest AFP level and cumulative radiologic tumor diameter). Demographic characteristics and mean age did not differ significantly between the two groups: most patients were men and the most common etiology of liver cirrhosis was HCV infection. The median highest AFP level of patients with ICC was higher than that of patients with HCC, although without statistical significance. Patients with ICC more commonly received bridging therapy for transplant (100% *vs* 67.2%; P = 0.036), but they were less responsive than patients with HCC (total necrosis: 12.5% *vs* 58.1%; P = 0.008). Also, according to explant pathology, patients with ICC had less differentiated tumors (grade 2 + 3: 75% *vs* 56.2%; P = 0.022) and higher rates of microvascular invasion (37.5% *vs* 9.4%; P = 0.056) (Table 1).

Figure 2 shows the risk of tumor recurrence, overall mortality, and recurrence-free survival. When comparing these risks between patients with ICC and HCC matched for pretransplant tumor characteristics, estimated by the simple Cox regression model, patients with ICC had a higher 3-year risk of recurrence (46% *vs* 11%; HR 7.14 [95%CI, 1.77-28.85]; P = 0.006) and overall mortality (63% *vs* 23%; HR 4.41 [95%CI, 1.72-11.32]; P = 0.002) and a lower recurrence-free survival rate (38% *vs* 77%; HR 4.42 [95%CI, 1.74-11.24]; P = 0.002).

Given the poorer outcomes of LT recipients with ICC and pretransplant tumor characteristics like those of LT recipients with HCC, we sought to assess whether these results would be explained by the potentially more aggressive nature of ICC. To this end, an additional PSM was performed by pairing patients with ICC and HCC with similar explant pathology (median cumulative tumor diameter, nuclear grade/differentiation, and microvascular invasion), but the groups did not differ significantly in these variables (Table 1). Compared with patients with HCC, those with ICC had a higher 3-year cumulative risk of tumor recurrence (46% *vs* 23%; HR 3.07 [95%CI, 0.86-10.94]; P = 0.083) and overall mortality (63% *vs* 35%; HR 2.78 [95%CI, 1.13-6.86]; P = 0.026) and a lower recurrence-free survival rate (38% *vs* 65%; HR 2.59 [95%CI, 1.06-6.31]; P = 0.037) (Figure 2).

Compared with HCC transplant recipients with similar pretransplant characteristics, patients with ICC had significantly higher 1- and 5-year overall mortality (62.5% and 81.2% vs 12.5% and 29.8%; P = 0.002) and lower 1- and 5-year RFS (37.5% and 18.8% *vs* 87.5% and 70.2%; P = -0.002). Compared with those with similar posttransplant characteristics (explant pathologic features), patients with ICC had significantly higher 1- and 5- year mortality (20.3% and 42.8% *vs* 12.5% and 29.8%; P = 0.002) and lower 1- and 5-year RFS (79.7% and 57.2% vs 87.5% and 70.2%; P = 0.002) (Figure 3).

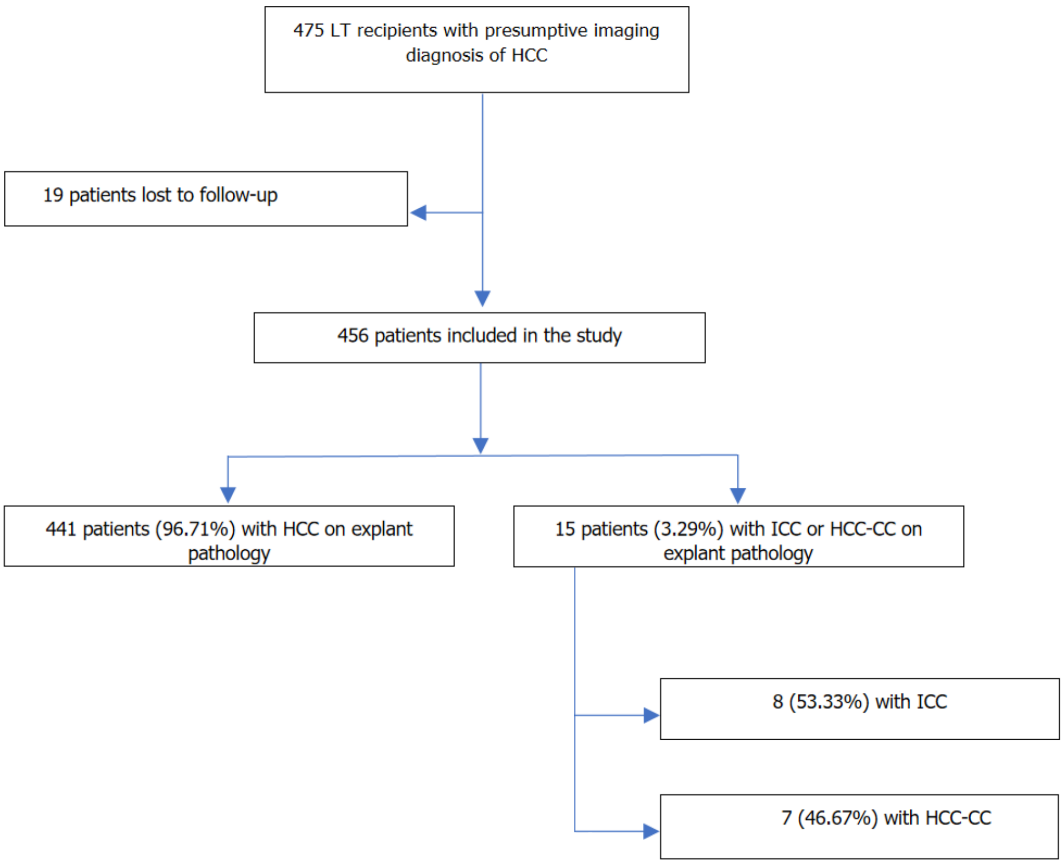
Comparison of HCC-CC vs HCC transplant recipients, propensity score-matched for year of transplant and pretransplant and posttransplant tumor characteristics

Two pairings were also performed, in a 1:8 ratio, between patients with HCC-CC (n = 7) and HCC (n = 56) who underwent LT in the same year. The first pairing considered similar pretransplant tumor

Table 1 Comparison of pretransplant tumor characteristics, locoregional therapy, and posttransplant tumor characteristics between patients with intrahepatic cholangiocarcinoma and hepatocellular carcinoma, matched 1:8 for pre-liver transplant factors and explant factors

Variable	Pre-LT factors			Explant factors	
	ICC (*n* = 8)	HCC (*n* = 64)	*P* value	HCC (*n* = 64)	*P* value
Recipient characteristics					
Age, mean ± SD	59.4 ± 7.6	61.5 ± 8.0	0.489	60.3 ± 8.6	0.774[2]
Male, *n* (%)	5 (62.5)	44 (68.8)	0.704	50 (78.1)	0.382[1]
Etiology of liver disease, *n* (%)			0.201		0.745[1]
HCV	6 (75.0)	50 (78.1)		45 (70.3)	
Alcohol	0	9 (14.1)		10 (15.6)	
HBV	0	1 (1.6)		3 (4.7)	
NAFLD	1 (12.5)	2 (3.1)		1 (1.6)	
Cryptogenic	1 (12.5)	1 (1.6)		2 (3.1)	
Other	0	1 (1.6)		3 (4.7)	
CTP class, *n* (%)			0.168		0.210[1]
A	7 (87.5)	43 (67.2)		40 (63.5)	
B	0	17 (26.6)		19 (30.2)	
C	1 (12.5)	4 (6.3)		4 (6.3)	
Maximum pretransplant AFP, ng/mL	28.5 (1.60-801.0)	10.8 (1.7-1133.0)	0.324	12.5 (1.3-6123.0)	0.620[3]
Radiographic tumor characteristics					
Cumulative tumor diameter, cm, *n* (%)			0.072		0.862[1]
< 2.1	1 (12.5)	21 (32.8)		9 (14.1)	
2.2-5.0	4 (50.0)	37 (57.8)		39 (60.9)	
> 5.1	3 (37.5)	6 (9.4)		16 (25.0)	
Neoadjuvant therapy, *n* (%)			0.036		0.016[1]
None	0	21 (32.8)		21 (32.8)	
TACE	8 (100.0)	32 (50.0)		29 (45.3)	
Other	0	11 (17.2)		14 (21.9)	
Pathologic tumor characteristics, *n* (%)					
Total necrosis among treated patients, *n*/total *n* (%)	1/8 (12.5)	25/43 (58.1)	0.008	7/43 (16.3)	0.741[1]
Within Milan criteria	3 (37.5)	52 (81.3)	0.015	35 (54.7)	0.463[1]
Within UCSF criteria	6 (75.0)	56 (87.5)	0.307	46 (71.9)	> 0.999[1]
Median cumulative nodule size			0.072		0.862[1]
< 2.1	1 (12.5)	21 (32.8)		9 (14.1)	
2.2-5.0	4 (50.0)	37 (57.8)		39 (60.9)	
> 5.1	3 (37.5)	6 (9.4)		16 (25.0)	
Tumor grade, *n*/total *n* (%)			0.225		0.214[1]
1	2 (25.0)	28 (43.8)		4 (6.3)	
2	4 (50.0)	31 (48.4)		40 (62.5)	
3	2 (25.0)	5 (7.8)		20 (31.3)	
Microvascular invasion	3 (37.5)	6 (9.4)	0.056	20 (31.3)	0.741[1]

[1]Fisher exact test.
[2]*t*-test.
[3]Mann-Whitney test. Data expressed as mean ± SD or median (interquartile range). CTP: Child-turcotte-pugh; HBV: Hepatitis B virus; HCV: Hepatitis C virus; LT: Liver transplant; NAFLD: Nonalcoholic fatty liver disease; AFP: Alpha-fetoprotein; TACE: Transarterial chemoembolization; UCSF: University of California San Francisco.



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Figure 1 Flow chart of eligible patients included in the analysis according to the diagnosis of hepatocellular carcinoma, intrahepatic cholangiocarcinoma, or mixed hepatocellular-cholangiocarcinoma on explant pathology (January 1998-July 2019, southern Brazil). LT: Liver transplant; HCC: Hepatocellular carcinoma; HCC-CC: Hepatocellular cholangiocarcinoma; ICC: Intrahepatic cholangiocarcinoma.

characteristics (imaging findings and highest AFP level), whereas the second pairing considered similar explant pathology. There was no statistically significant difference between the two groups (Table 2). Most patients were men, and HCV infection was the most common etiology of liver cirrhosis. Also, there was no statistically significant difference between recurrence, overall mortality, or recurrence-free survival rates in either pairing (by pretransplant or posttransplant tumor characteristics) (Figure 2).

Compared with HCC transplant recipients with similar pretransplant characteristics, patients with HCC-CC showed no significant differences in 1- and 5-year overall mortality (14.3% and 52.4% vs 14.3% and 45.9%; $P = 0.500$) and RFS (85.7% and 47.6% *vs* 85.7% and 54.1%; $P = 0.278$). Compared with those with similar posttransplant characteristics, patients with HCC-CC also showed no statistical differences in 1- and 5-year overall mortality (14.3% and 40.9% *vs* 14.3% and 45;9%; $P = 0.528$) and 1- and 5-year RFS (85.7% and 59.1% vs 85.7% and 54,1%; $P = 0.283$) (Figure 4).

DISCUSSION

The present study described the experience of a Brazilian LT center with the outcomes of LT recipients with ICC or HCC-CC who had a pretransplant radiological diagnosis of HCC. Over a 22-year period, the rate of incorrect diagnosis of ICC or HCC-CC and unintentional LT was 3.1%, similar to that identified in a single-center Spanish study analyzing a 10-year period[24].

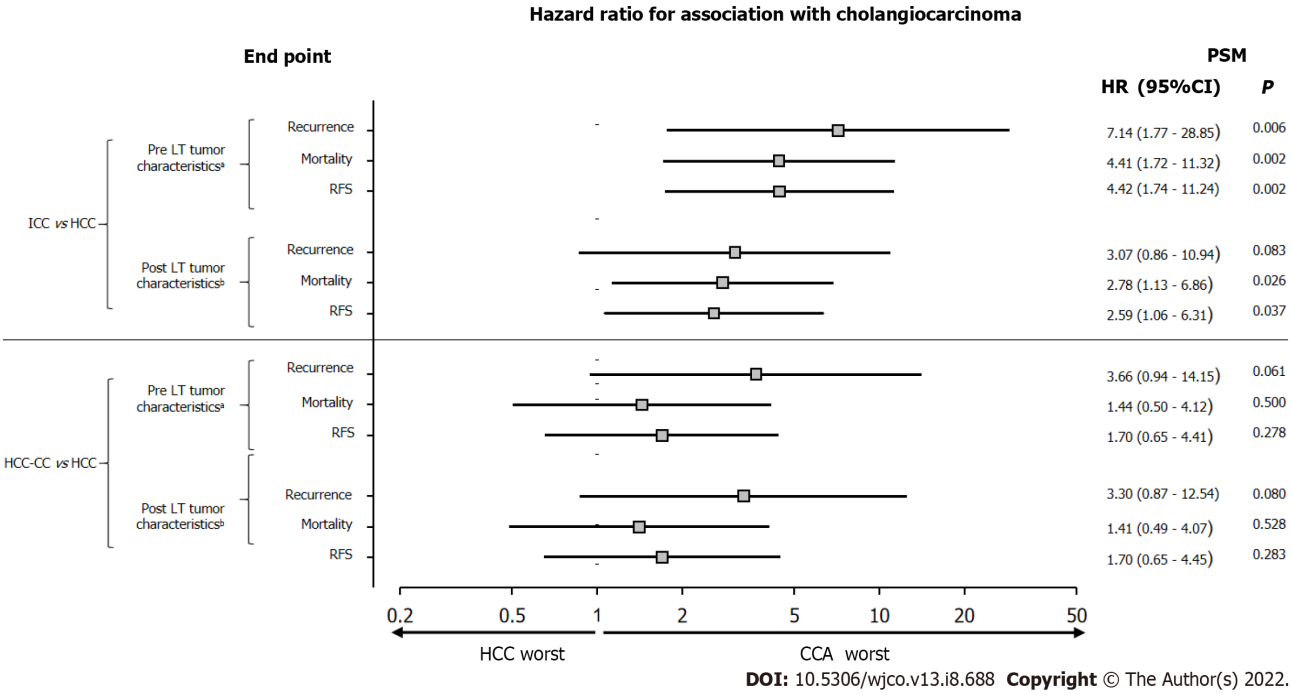
Table 2 Comparison of pretransplant tumor characteristics, locoregional therapy, and posttransplant tumor characteristics between patients with mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma, matched 1:8 for pre- liver transplant factors and explant factors

Variable	Pre-LT factors			Explant factors	
	HCC-CC (*n* = 7)	HCC (*n* = 56)	*P* value	HCC (*n* = 56)	*P* value
Recipient characteristics					
Age, mean ± SD	58.0 ± 6.9	60.3 ± 9.2	0.317	60.8 ± 7.1	0.289[2]
Male, *n* (%)	4 (57.1)	41 (73.2)	0.397	4 (57.1)	0.375[1]
Etiology of liver disease, *n* (%)			0.192		0.789[1]
HCV	6 (85.7)	42 (75.0)		41 (73.2)	
Alcohol	0	7 (12.5)		3 (5.4)	
HBV	0	5 (8.9)		5 (8.9)	
NAFLD	1 (14.3)	0		3 (5.4)	
Cryptogenic	0	2 (3.6)		4 (7.1)	
CTP class, *n* (%)			0.201		0.556[1]
A	3 (42.9)	21 (37.5)		29 (51.8)	
B	2 (28.6)	30 (53.6)		20 (35.7)	
C	2 (28.6)	5 (8.9)		7 (12.5)	
Maximum pretransplant AFP, ng/mL	35.3(4.3-357.0)	9.6(1.1-628.0)	0.150	16.5(1.1-6123.0)	0.668[3]
Radiographic tumor characteristics					
Cumulative tumor diameter, cm, *n* (%)			0.224		0.723[t]
< 2.1	1 (14.3)	25 (44.6)		11 (19.6)	
2.2-5.0	4 (57.1)	23 (41.1)		36 (64.3)	
5.1	2 (28.6)	8 (14.3)		9 (16.1)	
Neoadjuvant therapy, *n* (%)			0.085		0.081[1]
None	0	14 (25.0)		12 (21.4)	
TACE	3 (42.9)	22 (39.3)		28 (50.0)	
Other	4 (57.1)	20 (35.7)		16 (28.6)	
Pathologic tumor characteristics, *n* (%)					
Total necrosis among treated patients, *n*/total *n* (%)	3/7 (33.3)	20/42 (47.6)	0.8000	18/44 (40.1)	0.223[1]
Within Milan criteria	4 (57.1)	39 (69.6)	0.669	35 (62.5)	> 0.999[1]
Within UCSF criteria	6 (85.7)	49 (87.5)	> 0.999	45 (80.4)	> 0.999[1]
Median cumulative nodule size			0.224		0.723[1]
< 2.1	1 (14.3)	25 (44.6)		11 (19.6)	
2.2-5.0	4 (57.1)	23 (41.1)		36 (64.3)	
> 5.1	2 (28.6)	8 (14.3)		9 (16.1)	
Tumor grade, *n*/total *n* (%)			0.722		0.233[1]
1	0	1/36 (2.8)		0	
2	4/7 (57.1)	24/36 (66.7)		18/55 (32.7)	
3	3/7 (42.9)	11/36 (30.6)		37/55 (67.3)	
Microvascular invasion	0	9 (16.1)	0.580	0	0

[1]Fisher exact test.

[2]*t*-test.

[3]Mann-Whitney test. Data expressed as mean ± SD or median (interquartile range). CTP: Child-turcotte-pugh; HBV: Hepatitis B virus; HCV: Hepatitis C virus; LT: Liver transplant; NAFLD: Nonalcoholic fatty liver disease; AFP: Alpha-fetoprotein; TACE: Transarterial chemoembolization; UCSF: University of California San Francisco.

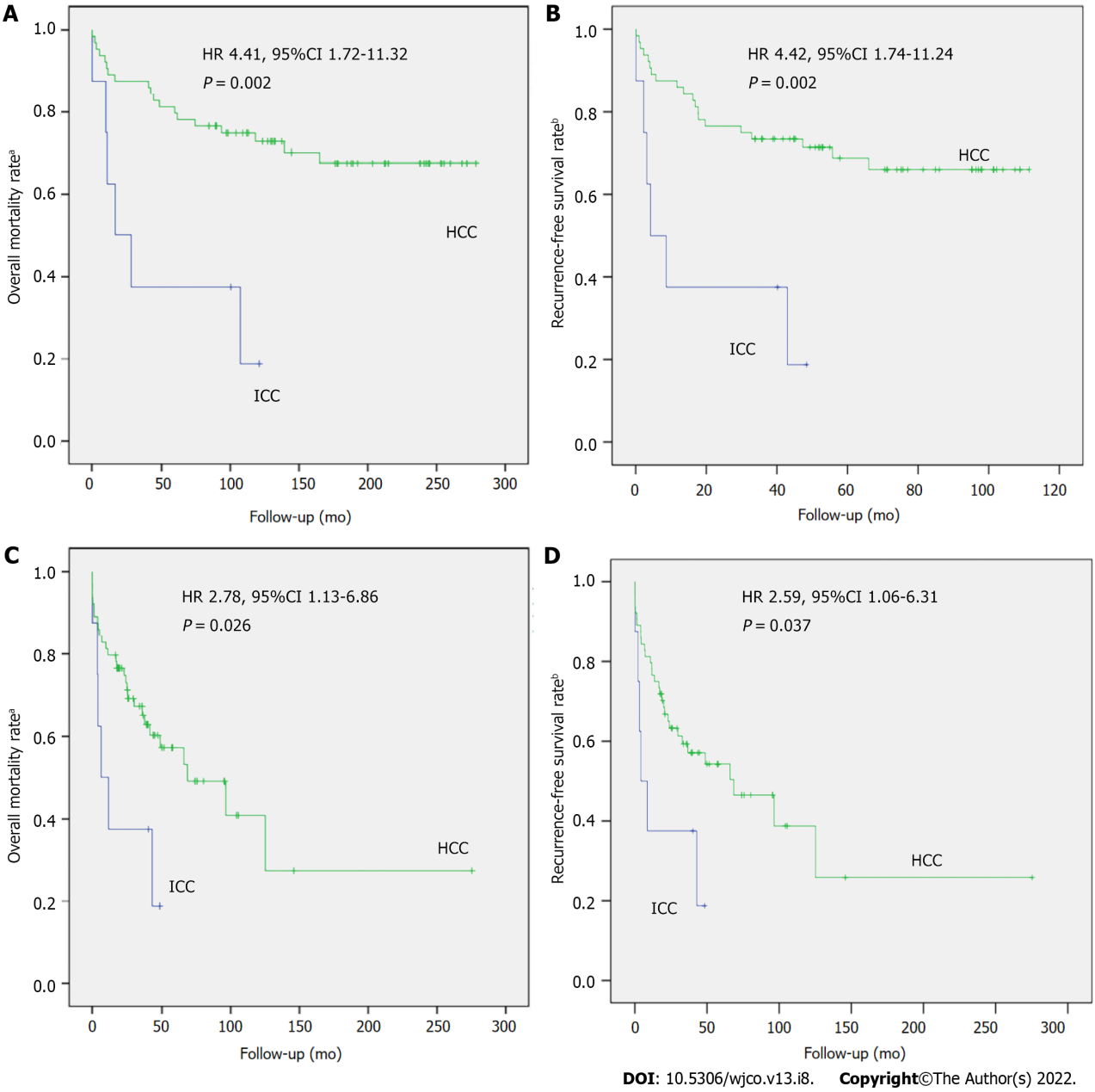


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Figure 2 Post-liver transplant 3-year risk of recurrence, overall mortality, and recurrence-free survival in patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma compared with patients with hepatocellular carcinoma, matched 1:8 for pretransplant tumor characteristics (Pre liver transplant characteristics) and pathologic tumor characteristics (Post liver transplant characteristics). [a]Pre liver transplant (LT) characteristics: serum alpha-fetoprotein and radiologic tumor diameter; [b]Post LT characteristics: Tumor diameter, grade/differentiation, microvascular invasion. PSM: Propensity score matching; LT: Liver transplant; HCC: Hepatocellular carcinoma; HCC-CC: Hepatocellular cholangiocarcinoma; ICC: Intrahepatic cholangiocarcinoma.

In order to assess outcomes of these entities (ICC or HCC-CC) after LT, we compared the outcomes of patients who had ICC or HCC-CC with the outcomes of patients transplanted for HCC. At first, we matched LT recipients with ICC and LT recipients with HCC for pretransplant tumor characteristics. Patients with ICC were more likely to have poorer tumor differentiation and higher microvascular invasion rates on explant pathology. To estimate the risk of recurrence, overall mortality, and recurrence-free survival in both groups, we used PSM followed by simple Cox regression. This comparative, propensity-matched analysis showed a higher risk of poorer outcomes after LT for ICC than HCC when patients were matched for pretransplant tumor characteristics. A previous study reported that worse tumor differentiation and presence of microvascular invasion are risk factors for recurrence in LT recipients with ICC[25]. Therefore, in order to assess the role of the potentially more aggressive nature of ICC, we matched patients with ICC and patients with HCC for explant pathology, which included nuclear differentiation, microvascular invasion, and cumulative tumor diameter, and repeated the same statistical analyses. Again, patients with ICC had worse outcomes (tumor recurrence, overall mortality, and recurrence-free survival) than those with HCC. That is, ICC was associated with worse outcomes even when high-risk factors for tumor recurrence were considered, indicating that ICC is an inherently more aggressive tumor whose risk factors for recurrence differ from those traditionally described for HCC. To our knowledge, this is the first time that posttransplant outcomes of patients with ICC and HCC have been comparatively evaluated by matching patients for explant pathology.

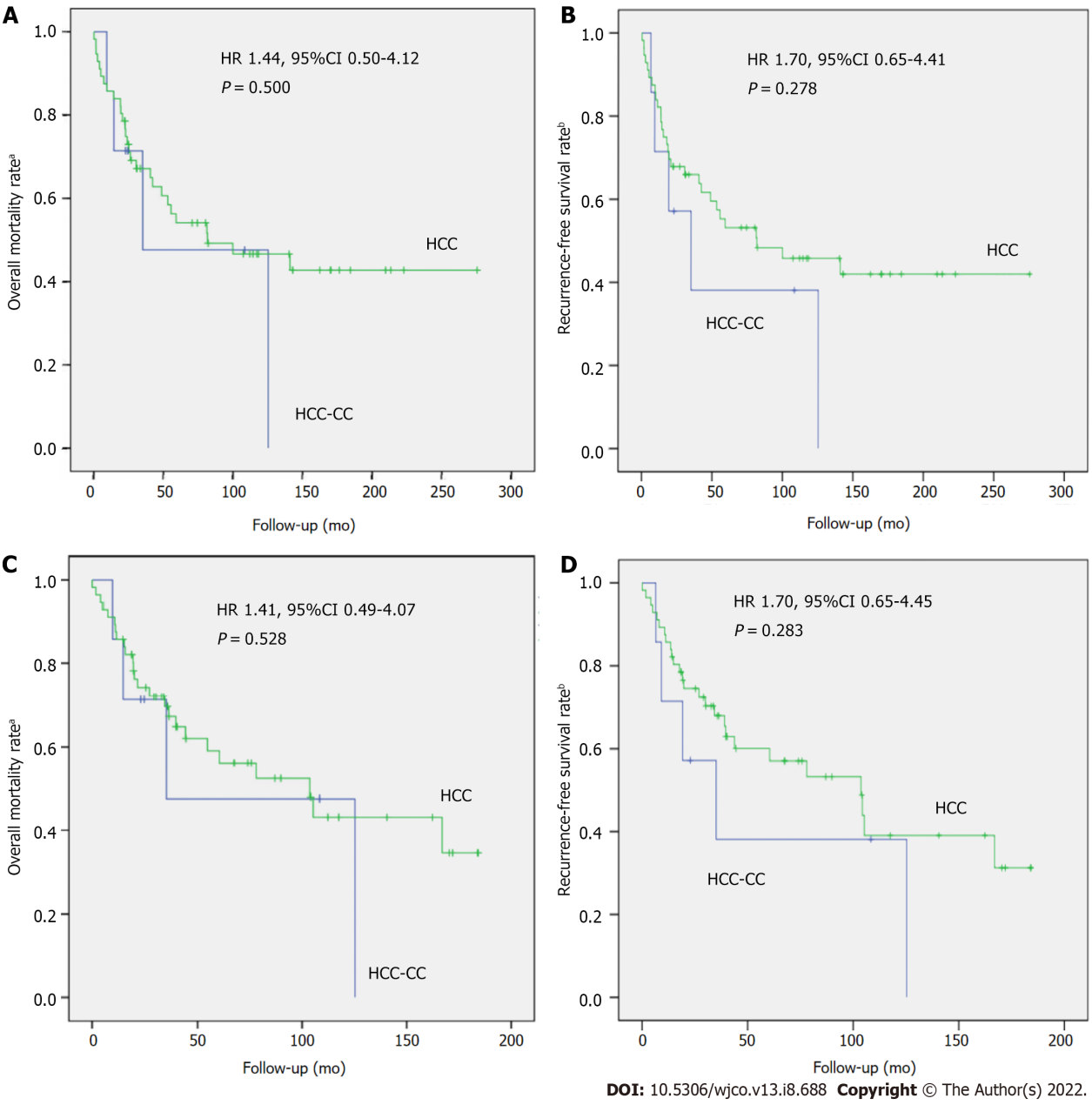
LT has been contraindicated in patients with ICC due to poor results[26-28]. The possibility of successfully transplanting patients with ICC began to change as it became clear that better patient selection was likely to impact posttransplant outcomes. Satisfactory results have been recently reported in LT of cirrhotic patients with grafts showing incidental ICC on explant pathology. Retrospective data from these patients demonstrated suitable 5-year overall and recurrence-free survival in patients with "very early" ICC (≤ 2 cm)[18,25,29]. A Japanese study found that patients with and without cirrhosis who underwent liver resection for ICC ≤ 2 cm reached a 100% 5-year survival rate. The authors identified 2 cm as a good cutoff point when selecting patients for hepatectomy[30]. Recently, French



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Figure 3 Kaplan-Meier curves representing. A: post-liver transplant overall survival; B: recurrence-free survival in patients with intrahepatic cholangiocarcinoma compared with patients with hepatocellular carcinoma, matched 1:8 for pretransplant tumor characteristics; C: post-liver transplant overall survival; D: recurrence-free survival in patients with intrahepatic cholangiocarcinoma compared with patients with hepatocellular carcinoma matched 1:8 for posttransplant tumor characteristics. CIs: Confidence intervals; HCC: hepatocellular carcinoma; HRs: Hazard ratios; ICC: intrahepatic cholangiocarcinoma.

researchers suggested that this ≤ 2 cm limit could be expanded by showing, in a retrospective multicenter study analyzing posttransplant outcomes of cirrhotic patients with incidental ICC detected on the pathological examination of the explant, that patients with clearly differentiated ICC up to 3 cm had similar survival to patients with tumors ≤ 2 cm. In this study, the only independent variable associated with tumor recurrence was its differentiation[31]. Prospective multicenter clinical trials are needed to confirm these results. The 2 cm cutoff point seems safe but limited because preoperative radiological diagnosis of these small tumors is challenging[15,16] and ICC features are still often underestimated during pre-LT diagnostic evaluation. Nevertheless, studies indirectly state that ICC is a more aggressive tumor by suggesting that LT should only be an option for patients with tumors ≤ 2 cm. This differs from the indication for LT in patients with HCC, who can undergo LT with tumors up to 5 cm in diameter, with acceptable recurrence rates[32]. It is important to note that, in our series, all patients with liver cirrhosis had ICCs > 2 cm. In order to expand the indication criteria for LT in patients with liver cirrhosis and unresectable ICC, the effectiveness of pretransplant neoadjuvant chemotherapy is being evaluated[33]. The International Liver Transplantation Society (ILTS) recommends resection as the treatment of choice for patients with ICC. When the procedure is contraindicated, LT may be considered when the tumor is ≤ 2 cm; if the tumor is > 2 cm, LT may be performed under strict clinical



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Figure 4 Kaplan-Meier curves representing. A: post-liver transplant overall survival; B: recurrence-free survival in patients with mixed hepatocellular-cholangiocarcinoma compared with patients with hepatocellular carcinoma (HCC), matched 1:8 for pretransplant tumor characteristics; C: post-liver transplant overall survival; D: recurrence-free survival in patients with mixed hepatocellular-cholangiocarcinoma compared with patients with hepatocellular carcinoma matched 1:8 for posttransplant tumor characteristics. HCC: hepatocellular carcinoma; HCC-CC: hepatocellular-cholangiocarcinoma; HRs: Hazard ratios; CIs: Confidence intervals.

protocols and only when the disease remains stable after neoadjuvant therapy[34].

We performed the same comparisons, using pretransplant and posttransplant tumor characteristics, for LT recipients with HCC-CC *vs* HCC, but no statistically significant differences were observed between the two groups. The statistical analyses (PSM and simple Cox regression) yielded similar risks for tumor recurrence, overall mortality, and recurrence-free survival when patients were matched for pretransplant or posttransplant tumor characteristics. As observed in ICC, patients with HCC-CC also had a worse prognosis than those with HCC, but the differences were smaller than those found for ICC *vs* HCC; consequently, in most outcomes, the differences did not reach statistical significance. This may suggest that LT recipients with ICC or HCC-CC have worse outcomes than those with HCC, but ICC appears to be more aggressive. Lunsford *et al*[35] analyzed posttransplant outcomes of 12 patients with HCC-CC *vs* 36 patients with HCC matched for the pretransplant and posttransplant variables reproduced in the present study. When patients were matched for explant pathology, those with HCC-CC had a slightly higher recurrence rate, without statistical significance, whereas recurrence-free survival and overall survival rates were equivalent to those of LT recipients with HCC[35]. Other authors also consider that a diagnosis of HCC-CC should not be an impediment to LT in well-selected cases[24,36,37]. However, for patients with HCC-CC, the ILTS expert panel believes that this tumor is

not an established indication for LT due to the limited worldwide experience, and prognostic factors need to be identified to improve patient selection and to obtain better results with the procedure[30].

Transplant oncology is a new concept encompassing multiple disciplines of transplantation medicine and oncology (transplant oncologists, hepatologists, gastroenterologists, transplant hepatobiliary surgeons, interventional radiologists, and immunologists) designed to push the envelope of the treatment and research of hepatobiliary cancers[38,39]. This field will certainly improve treatments and cure rates for patients with HCC, ICC, or HCC-CC, as well as other cancer types.

This study has limitations that need to be addressed. First, it is a retrospective study conducted at a single center with a limited number of cases. However, given the rarity of these tumors, most studies are retrospective and have also included a small number of patients, which makes it difficult to perform statistical analyses that can identify factors potentially associated with the outcomes[40]. Furthermore, because LT is a current contraindication for patients with ICC or HCC-CC, the diagnosis was made on explant. Finally, the study included patients receiving care over a long period of time. To minimize any bias that may have resulted from advances in research, management, and treatment during the study period, patients were also matched for year of transplant.

CONCLUSION

In this series, LT for ICC (all excepted one were larger than 2 cm) was associated with worse outcomes compared with LT for HCC, even when patients were matched for explant pathology. However, the outcomes after LT for mixed HCC-CC, despite being worse than those of LT recipients with HCC, did not reach statistical significance. Improvement in the detection of these rare tumors during pretransplant evaluation is essential for the eventual adoption of LT as an effective treatment for these patients.

ARTICLE HIGHLIGHTS

Research background

Cholangiocarcinoma (CC) is a rare tumor that arises from the epithelium of the bile ducts. It is classified according to anatomic location as intrahepatic, perihilar, or distal. Intrahepatic cholangiocarcinoma (ICC) is rare in patients with cirrhosis due to causes other than primary sclerosing cholangitis. Mixed hepatocellular-cholangiocarcinoma (HCC-CC) is a rare neoplasm with histologic findings of both hepatocellular carcinoma (HCC) and ICC within the same tumor mass.

Research motivation

Because of difficulties in reaching the correct diagnosis, patients eventually undergo liver transplantation (LT) with a presumptive diagnosis of HCC on imaging when, in fact, they have ICC or HCC-CC.

Research objectives

To determine the prevalence of ICC or HCC-CC confirmed by explant pathology in patients who underwent LT with the presumptive diagnosis of HCC and to compare tumor recurrence (TR), recurrence-free survival (RFS), and overall mortality (OM) rates between these patients and LT recipients with HCC.

Research methods

This retrospective cohort study included patients aged ≥ 18 years with liver cirrhosis and imaging findings suggestive of HCC within the Milan criteria who underwent LT between June 1997 and July 2019. Patients were divided into three groups according to the diagnosis on explant pathology: (1) Patients with HCC; (2) Patients with ICC; and (3) Patients with mixed HCC-CC. The analyzed outcomes were TR, RFS, and OM. Propensity score matching was used to assess whether TR, OM, and RFS rates in patients with ICC or HCC-CC differed from those in patients with HCC. Additionally, hazard ratios (HRs) and their confidence intervals were calculated. Progression-free survival and OM rates were computed with the Kaplan-Meier method using Cox regression for comparison.

Research results

Over a 22-year period, 475 patients with the presumptive diagnosis of HCC underwent LT, and 15 (3.1%) were found to have either ICC (n = 8) or HCC-CC (n = 7) detected in the pathological examination of the explant. LT recipients with ICC had higher TR (46% *vs* 11%; P = 0.006), higher OM (63% *vs* 23%; P = 0.002), and lower RFS (38% *vs* 89%; P = 0.002) than those with HCC when matched for pretransplant tumor characteristics, as well as higher TR (46% *vs* 23%; P = 0.083), higher OM (63% *vs*

35%; $P = 0.026$), and lower RFS (38% *vs* 59%; $P = 0.037$) when matched for posttransplant tumor characteristics. Two pairings were performed to compare the outcomes of LT recipients with HCC-CC *vs* HCC. There was no significant difference between the outcomes in either pairing.

Research conclusions

Patients with ICC had worse outcomes than patients with HCC undergoing LT. Preoperative diagnosis of HCC-CC should not prompt the exclusion of these patients from transplant options.

Research perspectives

This study reinforces the need for more accurate criteria: (1) To identify these rare tumors in pretransplant evaluation; and (2) To select patients who may benefit from LT.

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FOOTNOTES

Author contributions: All the authors solely contributed to this paper.

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Informed consent statement: Informed consent was waived due to the non-interventional design of the study and retrospective nature of data collection. All investigators signed a data use agreement to ensure the ethical and secure use of the data.

Conflict-of-interest statement: All authors have no conflicts of interest to disclose.

Data sharing statement: No additional data are available.

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ORIGINAL ARTICLE

Retrospective Study

Computer-aided clinical image analysis as a predictor of sentinel lymph node positivity in cutaneous melanoma

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Abstract

BACKGROUND

Delays in sentinel lymph node (SLN) biopsy may affect the positivity of non-SLNs. For these reasons, effort is being directed at obtaining reliable information regarding SLN positivity prior to surgical excision. However, the existing tools, *e.g.*, dermoscopy, do not recognize statistically significant predictive criteria for SLN positivity in melanomas.

AIM

To investigate the possible association of computer-assisted objectively obtained color, color texture, sharpness and geometry variables with SLN positivity.

METHODS

We retrospectively reviewed and analyzed the computerized medical records of all patients diagnosed with cutaneous melanoma in a tertiary hospital in Germany

during a 3-year period. The study included patients with histologically confirmed melanomas with Breslow > 0.75 mm who underwent lesion excision and SLN biopsy during the study period and who had clinical images shot with a digital camera and a handheld ruler aligned beside the lesion.

RESULTS

Ninety-nine patients with an equal number of lesions met the inclusion criteria and were included in the analysis. Overall mean (± standard deviation) age was 66 (15) years. The study group consisted of 20 patients with tumor-positive SLN (SLN+) biopsy, who were compared to 79 patients with tumor-negative SLN biopsy specimen (control group). The two groups differed significantly in terms of age (61 years *vs* 68 years) and histological subtype, with the SLN+ patients being younger and presenting more often with nodular or secondary nodular tumors ($P < 0.05$). The study group patients showed significantly higher eccentricity (*i.e.* distance between color and geometrical midpoint) as well as higher sharpness (*i.e.* these lesions were more discrete from the surrounding normal skin, $P < 0.05$). Regarding color variables, SLN+ patients demonstrated higher range in all four color intensities (gray, red, green, blue) and significantly higher skewness in three color intensities (gray, red, blue), $P < 0.05$. Color texture variables, *i.e.* lacunarity, were comparable in both groups.

CONCLUSION

SLN+ patients demonstrated significantly higher eccentricity, higher sharpness, higher range in all four color intensities (gray, red, green, blue) and significantly higher skewness in three color intensities (gray, red, blue). Further prospective studies are needed to better understand the effectiveness of clinical image processing in SLN+ melanoma patients.

Key Words: Melanoma; Skin cancer; Image processing; Sentinel lymph node; Presurgical

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Core Tip: Computer-aided image analysis can facilitate prediction of sentinel lymph-node positivity. Several color, sharpness and geometry parameters can predict positive lymph node occurrence, while color texture cannot determine sentinel lymph node positivity.

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INTRODUCTION

Cutaneous melanoma is a highly aggressive tumor that often spreads to local lymph nodes. Sentinel lymph node biopsy (SLNB) is commonly performed to identify nodal metastases because sentinel lymph node (SLN) status is a strong prognostic factor for survival in melanoma patients, especially in those without evidence of clinically positive lymph nodes[1]. In some subgroups, *e.g.*, in patients with thick melanomas (*i.e.* Breslow thickness > 4 mm) and patients with melanomas of the scalp, SLN status is considered the most important prognostic survival factor[2,3].

According to the existing guidelines, the decision for SLNB is based on the thickness of the primary tumor. SLNB is indicated for all primary tumors thicker than 1 mm and tumors thicker than 0.75 mm in the presence of ulceration or high mitotic rate (> 1 mm²). SLNB is of crucial importance in disease management because positive SLNB should be followed by lymph node dissection for regional disease control and staging purposes. Delays in SLNB may affect the positivity of non-SLNs[4]. For these reasons, an effort is being directed at obtaining reliable information regarding SLN positivity before surgical excision.

Dermoscopy is a non-invasive technique that facilitates early melanoma detection by revealing skin features invisible to the naked eye. However, dermoscopy does not recognize statistically significant predictive criteria for SLN positivity in melanomas because specific melanoma criteria strongly associated with a higher Breslow thickness, such as gray-blue areas or an atypical vascular pattern, do not seem to associate with SLN positivity[5].

Computer-aided clinical image analysis is also used to improve diagnostic accuracy for skin melanoma. We have shown that geometrical and color parameters objectively extracted by computer-aided clinical image processing may correlate with tumor thickness in patients with cutaneous melanoma[6]. However, to the best of our knowledge, there is no study investigating the possible association of computer-assisted objectively obtained color, color texture, sharpness and geometry variables with SLN positivity. The aim of this study was to investigate whether such an association exists.

MATERIALS AND METHODS

Patient recruitment

We retrospectively reviewed and analyzed the computerized medical records of all patients diagnosed with cutaneous melanoma in a tertiary hospital in Germany during a 3-year period. The study included patients with histologically confirmed melanomas with Breslow > 0.75 mm who underwent lesion excision and SLN biopsy during the study period and who had clinical images shot with a digital camera and a handheld ruler aligned beside the lesion. Patients with melanomas with Breslow < 0.75 mm and in situ melanomas as well as patients without digital images were excluded from the study. Patients referred to our center after primary excision to undergo SLN biopsy were also excluded from the study.

The study group consisted of patients with a positive SLN biopsy who were compared to patients with a negative SLN biopsy (control group). Clinical features studied included age, sex, tumor location and diagnosis date. Histopathologic features included tumor subtype [superficial spreading (de novo and nevus-associated) and nodular, including secondary nodular], Breslow thickness, Clark level, presence of ulceration, nevus pre-existence and SLN status (positive or negative). The study was approved by the institutional review board of the University of Witten-Herdecke.

Image collection, storage system and image database

All lesions were photographed at admission with the same commercial digital camera at a resolution of 1600 × 1200 pixels and with a handheld ruler aligned beside the lesion to allow for correct image scaling. All photos were obtained from the same educated nurse to minimize inconsistencies in methodology and were uploaded to a local server. The lesions were then excised under local anesthesia, and the diagnosis was histologically confirmed.

Image processing

The color images obtained underwent digital processing with an almost fully automated noncommercial software developed by one of the authors for study purposes. The software applies several kinds of algorithms to allow image segmentation and geometry, color and color texture analysis (Figure 1). The only manual involvement was the selection of the lesion border with the mouse cursor when the algorithm failed to do so (*i.e.* in very small lesions) (Figure 2). Such cases were independently analyzed twice by two of the authors (MP and GM) to avoid intraobserver errors (Figure 3). In cases of discrepancies, the mean scores were accepted and further analyzed. The 34 variables studied are classified as follows: (1) Geometrical variables (*i.e.* area, maximum diameter, perimeter, circularity, eccentricity and mean radius); (2) Color variables [*i.e.* range, standard deviation, coefficient of variation and skewness for all four color intensities (gray, red, green, blue)]; (3) Sharpness variables; and (4) Color texture variables (*i.e.* lacunarity)[7]. All variables are thoroughly described in Table 1.

Statistical analysis

Normal distribution was determined using histogram plots, box plots and the Shapiro-Wilk test. Continuous data are presented in mean-standard deviation form. Categorical variables were compared using the two-tailed Fisher's exact test and continuous variables using the two-tailed Student's *t* test. A *P* value of less than 0.05 was considered statistically significant. Univariate and multivariate analysis using logistic regression were employed to identify potential independent determinants of a positive SLN result. Data analyses were performed using SPSS 23.

RESULTS

Ninety-nine patients with an equal number of lesions met the inclusion criteria and were included in the analysis. Of these, 52 (52%) were males and the rest (48%) females. Histogram plots, box plots and the Shapiro-Wilk test demonstrated an almost normal distribution appearance for all continuous variables. The overall mean (standard deviation) age was 66 (15) years. The youngest patient was 14-years-old and the oldest 92-years-old. The study group consisted of 20 patients with tumor-positive SLN (SLN+) biopsy who were compared to 79 patients with tumor-negative SLN biopsy specimens (control group).

Table 1 Explanation of geometric, sharpness, color and color texture variables studied[7]

Classification	Parameter	Explanation
Geometry variables	Area	Lesion surface area, measured in cm^2
	Maximum diameter	The longest line that joins two points on the border of the lesion, measured in cm
	Perimeter	Total boundary length of the region of interest (*i.e.* lesion), measured in cm
	Circularity	Ratio of the perimeter of the lesion divided by the perimeter of a circle with the same midpoint and same area as the lesion
	Mean radius (Rm)	Mean value of the lesion's radii
	Standard deviation of Rm	Standard deviation of the mean radius
	Coefficient of variation of Rm	Expresses the standard deviation as a percentage of the mean
	Eccentricity	Distance between color and geometric midpoint within the lesion
	Eccentricity ratio	Distance between midpoint and color midpoint expressed as a fraction of the maximum diameter
Sharpness variables	SD of gray intensity	Intensity of gray on the border of the lesion
	Coefficient of variation of SD of gray intensity	SD of gray intensity in grayscale image. The higher the value, the more discrete the lesion is from the surrounding normal skin (Manousaki *et al*[7], 2016)
Color texture variables	Grayscale lacunarity of lesion (Lac gray)	It is estimated in grayscale image and assesses image texture heterogeneity or incomplete space filling within the lesion
Color variables	Range of gray, red, green, blue	Range of values of gray, red, green, blue intensity
	Mean gray, red, green, blue	Mean value of gray, red, green, blue intensity within the lesion
	SD of gray, red, green, blue	Standard deviation of gray, red, green, blue intensity within the lesion
	Coefficient of variation of gray, red, green, blue	Expresses the standard deviation of gray, red, green, blue intensity values as mean percentage
	Skewness from Gaussian curve (gray, red, green, blue)	Deviation of each color's histogram from the normal distribution curve

SD: Standard deviation.

The two groups differed significantly in terms of age (61 years *vs* 68 years) and histological subtype; the SLN+ patients were younger and presented more often with nodular or secondary nodular tumors ($P < 0.05$).

The study group patients also showed significantly higher eccentricity (*i.e.* the distance between color and geometrical midpoint) and higher sharpness (*i.e.* these lesions were more discrete from the surrounding normal skin, $P < 0.05$). Regarding color variables, SLN+ patients demonstrated a higher range in all four color intensities (gray, red, green, blue) and significantly higher skewness in three color intensities (gray, red, blue), $P < 0.05$. Color texture variables (*i.e.* lacunarity) were similar in both groups. Comparative data are summarized in Table 2.

Multivariate analysis of univariately significant variables ($P < 0.05$) revealed that younger age and higher eccentricity were independently associated with a higher probability of positive lymph node occurrence [for age: adjusted odds ratio (aOR) = 0.95, 95% confidence interval (CI): 0.91 to 0.99 and for eccentricity: aOR = 1.45, 95%CI: 1.12 to 1.89]. Nevus, nodular and secondary nodular histotypes were also significantly linked with higher odds of positive lymph node presence when compared to the superficial spreading histotype (for nevus: aOR = 14.19, 95%CI: 1.15 to 174.76, for nodular: aOR = 10.71, 95%CI: 1.48 to 77.48 and for secondary nodular: aOR = 18.21, 95%CI: 2.19 to 151.22). The proposed multivariate model can predict the presence of SLN+ with an accuracy of 85% and is summarized in Table 3.

DISCUSSION

Computer-aided image analysis is a noninvasive method and as such an established tool in the physicians' armamentarium to obtain reliable information regarding malignancy before surgical excision. SLN status is a strong prognostic factor for survival in melanoma patients (the tumor thickness threshold for SLNB being 1 mm) in the absence of risk factors. We herein investigated the possible association of computer-assisted objectively obtained color, texture and geometric variables with SLN

Table 2 Demographic, clinical and image processing characteristics of melanoma patients with positive and negative sentinel lymph node biopsy

Variable		Study group, SLNB+; *n* = 20	Control group, SLNB-; *n* = 79	*P* value
Demographics				
Sex				
Male		12 (60%)	40 (50%)	0.62
Female		8 (40%)	39 (50%)	
Age (yr)		61 (13)	68 (15)	0.05
Tumor thickness (mm)		2.6 (2.7)	2.2 (3.0)	0.64
Subtype	Superficial spread	3 (15%)	44 (56%)	0.04
	Nodular	10 (50%)	25 (32%)	
	Secondary nodular	7 (35%)	10 (12%)	
Geometric variables				
Area (cm^2)		3.4 (2.9)	2.8 (4.7)	0.49
MaxD (cm)		2.4 (1.2)	2 (1.1)	0.22
Perimeter (cm)		6.6 (3.0)	5.7 (3.3)	0.25
Circularity (ratio)		1.1 (0.1)	1.1 (0.1)	0.44
Rm (cm)		0.98 (0.5)	0.84 (0.5)	0.21
SDRm		0.14 (0.1)	0.12 (0.1)	0.48
CVRm		13 (7.1)	13 (5.5)	0.88
Delta (cm)		0.04 (0.03)	0.03 (0.03)	0.04
Delta ratio		1.8 (0.9)	1.6 (1.3)	0.42
Sharpness variables				
Sharpness		31 (9)	26 (8)	0.02
CV sharpness		22 (6)	18 (7)	0.02
Color texture variables				
Lac gray		2 (0.28)	1.97 (0.30)	0.65
Color variables				
Mean gray		104 (21)	113 (24)	0.12
SD gray		32 (6)	30 (7)	0.12
CV gray		8 (2)	10 (1)	0.07
Range gray		201 (21)	187 (32)	0.03
Skewness gray		0.52 (0.5)	0.23 (0.6)	0.03
Mean red		144 (26)	157 (32)	0.06
SD red		37 (9)	33 (11)	0.14
CV red		27 (10)	23 (11)	0.14
Range red		205 (23)	190 (37)	0.04
Skewness red		0.03 (0.43)	-0.04 (0.83)	0.003
Mean green		89 (23)	96 (23)	0.24
SD green		33 (7)	31 (6)	0.19
CV green		39 (12)	35 (13)	0.13
Range green		209 (24)	195 (33)	0.04

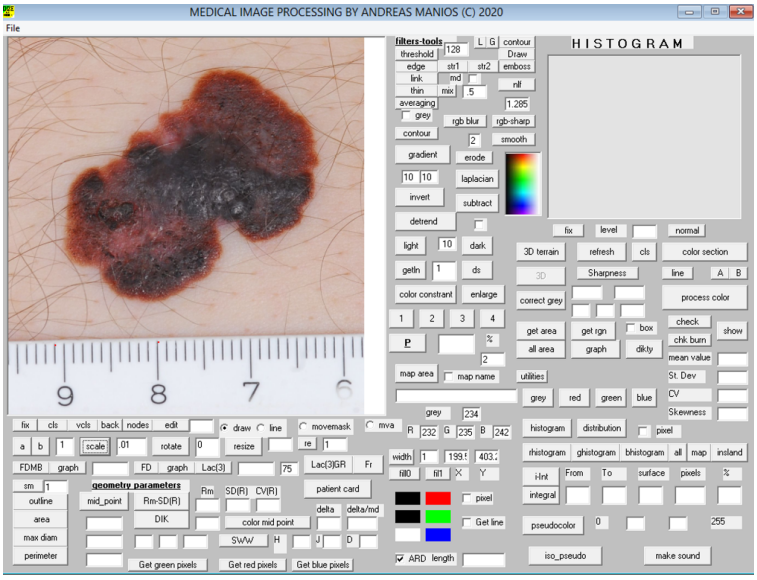
Skewness green	0.64 (0.46)	0.41 (0.60)	0.08
Mean blue	87 (22)	94 (25)	0.22
SD blue	33 (7)	31 (6)	0.24
CV blue	40 (10)	35 (13)	0.14
Range blue	218 (23)	201 (34)	0.02
Skewness blue	0.67 (0.47)	0.42 (0.56)	0.05

All continuous variables were normally distributed and therefore expressed in mean-deviation form. Categorical variables were expressed in terms of absolute (n) and relative (%) frequencies. Statistical significance: *P* value < 0.05. SD: Standard deviation; CV: Coefficient of variation; MaxD: Maximum diameter; SLNB+: Tumor-positive sentinel lymph node biopsy; SLNB-: Tumor-negative sentinel lymph node biopsy; Rm: Mean radius; SDRm: Standard deviation of the mean radius; CVRm: Coefficient variation of the mean radius; Lac: Lacunarity.

Table 3 Multivariate analysis of univariately significant predictors of a positive sentinel lymph node result

Variable	Coefficient (β)	Standard Error	Wald χ^2	*P* value	Odds ratio	95%CI
Age	-0.05	0.20	7.60	0.006	0.95	0.91 to 0.99
Subtype, nevus-associated	2.65	1.28	4.28	0.038	14.19	1.15 to 174.76
Subtype, nodular	2.37	1.01	5.51	0.019	10.71	1.48 to 77.48
Subtype, secondary nodular	2.90	1.08	7.22	0.007	18.21	2.19 to 151.22
Eccentricity	0.38	0.13	7.84	0.005	1.46	1.12 to 1.89

95%CI: The 95% confidence interval for the estimated odds ratios.



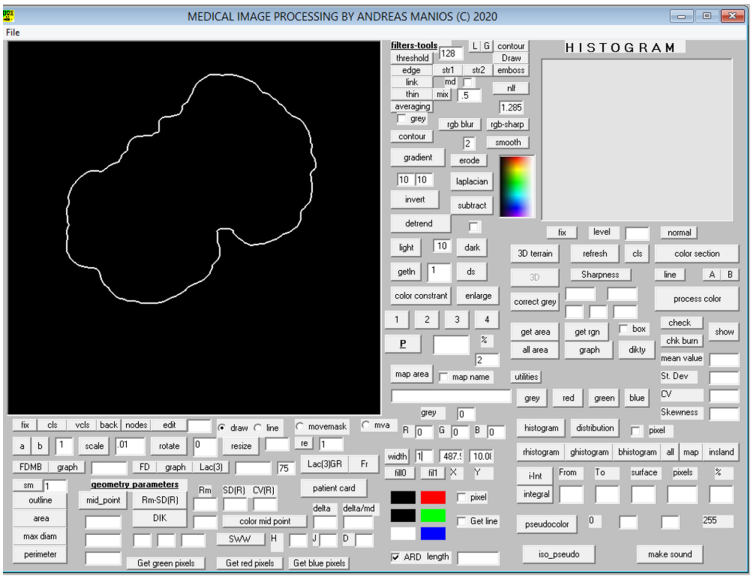
DOI: 10.5306/wjco.v13.i8.702 **Copyright** © The Author(s) 2022.

Figure 1 Scaling of the lesion.

positivity.

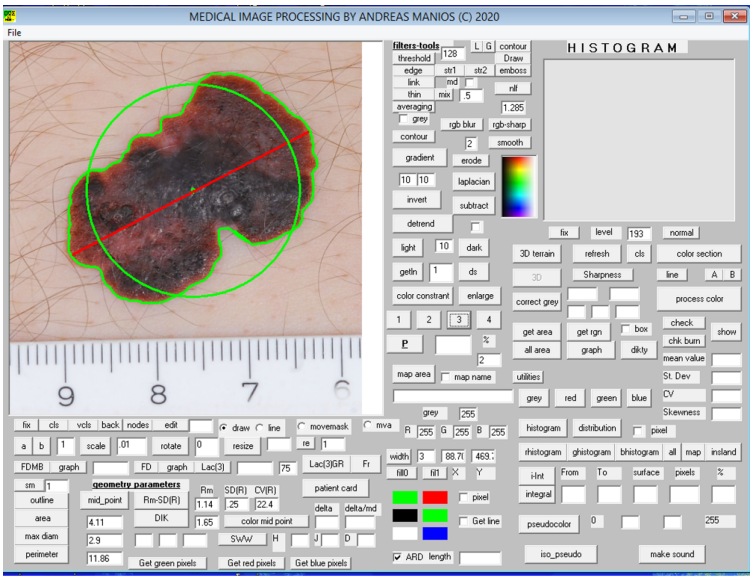
SLN+ patients have a higher range in all four color intensities (gray, red, green, blue) and significantly higher skewness in three color intensities (gray, red, blue). Blue and black pigmentation is associated with the presence of nodular melanoma, which in our study accounted for 50% of the SLN+ tumors[8]. Malignant epidermal structures, (*e.g.*, atypical pigment network, radial streaks and pseudopods) are rarely seen in SLN+ melanomas, while they are observed in one-fourth of SLN negative lesions[5].

Despite the significant differences in all color intensities, we found that lacunarity (a measure of the variation of the color intensity) cannot predict SLN status in melanoma patients although it is a proven



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Figure 2 Automatic recognition of the lesion's borders.



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Figure 3 Measurements.

promising parameter in the automated differentiation of melanoma from non-melanoma[9]. We also found the lesions of SLN+ patients have significantly higher eccentricity, which is an index of uneven lesion coloration. Eccentricity represents a special case of asymmetry, and as shown before eccentric lesions may be thicker[6]. Dermoscopically, only the presence of ulceration and blotch correlate with positive SLNB[10].

Regarding histological type, González-Álvarez *et al*[10] found nodular melanomas to be the most associated with SLN positivity, reporting an OR of 3.98. This is consistent with our findings, where half of the SLN+ tumors were nodular, and the OR was 14.4. Moreover, we found that secondary nodular tumors are much more often associated with SLN+, with the OR exceeding 25. This may reflect more aggressive tumor growth because angiolymphatic invasion is observed in the majority of nodular melanomas with SLN+[5]. According to our findings, a multivariate model consisting of age, histological type and eccentricity can predict the presence of SLN+ with an accuracy of up to 85%.

A recent study described a deep learning-based digital biomarker to predict SLN+ from digitized hematoxylin and eosin slides of primary melanoma tumors. Artificial neural networks predicted SLN status with an accuracy of 55%-62%[11]. This relatively low accuracy is attributed to morphological changes of the tumor cells or tumor architecture. Moreover, the histopathological workup may have

caused tumor cells to be missed in the lymph nodes. Neural networks failed to detect features other than thickness and age that predict SLN+. We found that higher values of eccentricity, sharpness, blue, gray, green range, red skewness and red mean could also predict positive lymph node occurrence.

Our study is limited regarding its retrospective nature and small sample size. First, it is a single-center study of German individuals, and therefore the results cannot be easily generalized. Second, only melanoma patients with available clinical images made before the diagnosis was established were included. Therefore, there is a high risk of bias due to no consecutive cases being included. Selection bias may have led to suspect cases being more frequently photographed. Moreover, we excluded all patients with melanomas with Breslow thickness < 0.75 mm. However, the specimens were analyzed by different pathologists during the study period, so the interpretation bias of tumor thickness may have led to eligible cases being excluded.

CONCLUSION

In conclusion, computer-aided image analysis can facilitate the prediction of SLN+. SLN+ patients demonstrated significantly higher eccentricity, higher sharpness and higher range in all four color intensities (gray, red, green, blue) as well as significantly higher skewness in three color intensities (gray, red, blue). Further prospective studies are needed to better understand the effectiveness of clinical image processing in SLN+ melanoma patients.

ARTICLE HIGHLIGHTS

Research background

Computer-aided clinical image analysis is used to improve diagnostic accuracy for skin melanoma.

Research motivation

To the best of our knowledge, there is no study investigating the possible association of computer-assisted objectively obtained color, color texture, sharpness and geometry variables with sentinel lymph node positivity (SLN+).

Research objectives

To investigate a possible association of computer-assisted objectively obtained color, color texture, sharpness and geometry variables with SLN+.

Research methods

The study included patients with histologically confirmed melanomas with Breslow > 0.75 mm who underwent lesion excision and SLN biopsy during the 3-year study period and who had clinical images shot with a digital camera and a handheld ruler aligned beside the lesion. All the color images obtained underwent digital processing with an almost fully automated noncommercial software developed by one of the authors for study purposes.

Research results

Ninety-nine patients with an equal number of lesions met the inclusion criteria and were included in the analysis. The study group consisted of 20 patients with SLN+ biopsy who were compared to 79 patients with tumor-negative SLN biopsy specimen (control group). The study group patients showed significantly higher eccentricity (*i.e.* distance between color and geometrical midpoint) as well as higher sharpness (*i.e.* these lesions were more discrete from the surrounding normal skin, $P < 0.05$). Regarding color variables, SLN+ patients demonstrated higher range in all four color intensities (gray, red, green, blue) and significantly higher skewness in three color intensities (gray, red, blue), $P < 0.05$. Color texture variables, *i.e.* lacunarity, were comparable in both groups.

Research conclusions

Computer-aided image analysis can facilitate the prediction of SLN+. SLN+ patients demonstrated significantly higher eccentricity, higher sharpness and higher range in all four color intensities (gray, red, green, blue) as well as significantly higher skewness in three color intensities (gray, red, blue).

Research perspectives

Further prospective studies are needed to better understand the effectiveness of clinical image processing in SLN+ melanoma patients.

FOOTNOTES

Author contributions: Papadakis M designed the research, performed the research, analyzed the data and wrote the paper; Paschos A participated in the data collection; Papazoglou A analyzed the data, Manios A contributed a new software used for the study; Zirngibl H contributed literature sources; Manios G analyzed the data; Koumaki D designed the research, performed the research, analyzed the data and wrote the paper; All authors reviewed and approved the final manuscript.

Institutional review board statement: The study was approved from the Ethics Committee of University Witten/Herdecke and was performed in accordance with institutional guidelines. Written informed consent was waived for retrospective study participation.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: All authors declare no conflicts of interest.

Data sharing statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Prospective Study

Impact of cytochrome P450 2D6 polymorphisms on decision-making and clinical outcomes in adjuvant hormonal therapy for breast cancer

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Abstract

BACKGROUND

There are concerns that tamoxifen is less effective in Asian women because of the high prevalence of impaired function cytochrome P450 2D6 (*CYP2D6*) polymorphisms.

AIM

To evaluate how knowledge of *CYP2D6* genotype impacted the choice of hormonal agent and how *CYP2D6* genotype and agent were associated with clinical outcomes.

METHODS

Eighty-two women were recruited. Seventy-eight completed *CYP2D6* genotyping and were categorized into poor, intermediate (IM) and extensive or ultra metabolizer phenotypes. Women with poor metabolizer and IM phenotypes were recommended aromatase inhibitors as the preferred agent.

RESULTS

More than 70% of the women had an IM phenotype, 32% an extensive or ultra metabolizer phenotype, and 0% had a poor metabolizer phenotype. Regardless of genotype, more women opted for aromatase inhibitors. Overall, 80% of women completed 5 years of hormonal therapy. Five women developed recurrence, 3 contralateral breast cancer, 5 died, and 1 was diagnosed with a second primary cancer. Five-year recurrence-free and overall survival were slightly better in women with the extensive or ultra metabolizer phenotype compared to those with the IM phenotype, though not statistically significant [P = 0.743, hazard ratio (HR): 1.441, 95% confidence interval (CI): 0.191 to 10.17 and P = 0.798, HR: 1.327, 95%CI: 0.172 to 9.915, respectively]. Women receiving aromatase inhibitors also appeared to have a better, but also nonsignificant, 5-year recurrence-free and overall survival (P = 0.253, HR: 0.368, 95%CI: 0.031 to 0.258 and P = 0.292, HR: 0.252, 95%CI: 0.005 to 4.951, respectively).

CONCLUSION

The IM phenotype was highly prevalent but was not associated with clinical outcome.

Key Words: Functional cytochrome P450 2D6 polymorphisms; Breast cancer; Hormonal therapy

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Core Tip: We studied the role of cytochrome P450 2D6 (*CYP2D6*) polymorphisms in guiding the selection of hormonal agents in women with hormone-responsive breast cancer. The CYP2D6 intermediate metabolizer phenotype was highly prevalent in our women, while the poor metabolizer phenotype was rare. We did not observe any significant association between the CYP2D6 phenotypes and recurrence-free or overall survival in our study, although it could be because most women opted for aromatase inhibitors regardless of CYP2D6 phenotype. There was a non-significant trend towards better survival associated with aromatase inhibitor use over tamoxifen.

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INTRODUCTION

In many centers, aromatase inhibitors (AIs) are now the first-line adjuvant hormonal therapy agents recommended for hormone-responsive breast cancer. While there are several reports of superior efficacy with AIs[1-3], some have questioned whether this is seen only in women with impaired tamoxifen metabolism[4,5]. Tamoxifen undergoes extensive first pass oxidative metabolism by the cytochrome P450 2D6 (CYP2D6) enzyme into the metabolically active derivative endoxifen (4-hydroxy-N-desmethyl-tamoxifen)[6,7]. The effect of impaired tamoxifen metabolism has particular significance among certain patient groups including Asians, where only 50% have functional CYP2D6 alleles. The reduced function allele *CYP2D6*10* is highly prevalent among Asians and results in a 60% reduction in CYP2D6 enzyme activity[8-14]. The lower levels of the active metabolite endoxifen could imply that a large number of Asian women may have sub-therapeutic levels of tamoxifen and consequently suboptimal risk reduction. In contrast to tamoxifen, AIs act by inhibiting the aromatase enzymatic conversion of androgens to estradiol and is not affected by CYP2D6 metabolism.

At the time when this study was initiated, cost and the treatment duration were significant factors contributing to patient cost of the hormonal agent. Previously, AIs cost almost 25 times more than tamoxifen, and AIs were recommended for 5 years, whereas tamoxifen began to be recommended for 10 years. These factors are less relevant today. The cost of AIs are now relatively similar to tamoxifen with generic preparations of AIs now available, and extended AI therapy is also more often recommended. In spite of this, tamoxifen remains an important hormonal agent, particularly in premenopausal women, where the use of an AI will require concomitant use of gonadotropin-releasing hormone inhibitors. This is seldom done unless the risk of recurrence risk is high. Tamoxifen also remains a valuable alternative in women who cannot tolerate the musculoskeletal side effects of AIs or who develop osteoporosis from accelerated bone loss.

In this study, we evaluated the frequency of *CYP2D6* polymorphisms and examined how the knowledge of the CYP2D6 phenotype impacted patient's choice of hormonal agent. We also evaluated

the association with clinical outcome through endpoints such as disease recurrence, mortality, contralateral breast cancer and 5-year recurrence-free and overall survival. We also evaluated the adverse effects reported by patients and the compliance to each agent, including the frequency of a switch to an alternative agent or premature discontinuation of hormonal therapy.

MATERIALS AND METHODS

Description of study cohort and CYP2D6 phenotype classification

The study was designed as a single-arm prospective study and recruited 82 women with breast cancer. The study was granted Ethics Committee approval (2011/00017). Women included into the study were: (1) Post-menopausal; (2) Histologically confirmed with invasive breast carcinoma, Stage I to III; (3) Proven to have estrogen receptor (ER) and/or progesterone receptor (PR)-positive tumors (tumors with cells with at least 1% of cells staining positive for ER or PR were considered positive); (4) Had completed curative breast cancer surgery; (5) Had been recommended adjuvant hormonal therapy by the multidisciplinary tumor board; and (6) Were capable of providing informed consent. Exclusion criteria were: (1) Ductal carcinoma in situ; (2) Microinvasive disease; (3) Metastatic disease at presentation (including those found with metastatic disease on staging scans done after surgery); (4) Prior personal history of breast cancer or other primary cancers; and (5) Specific contraindications to tamoxifen and AIs, such as previous deep venous thrombosis, pulmonary embolism, cerebrovascular accident and severe osteoporosis.

Blood was sampled from patients who satisfied both criteria for *CYP2D6* genotyping. The Qiagen DNA extraction kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from blood collected; DNA concentration, purity and integrity were verified for all samples. Three poor metabolizer (PM) alleles (*4, *5, *6) and three intermediate metabolizer (IM) alleles (*9, *10 and *41) were identified using the pyrosequencing method, as previously described[15]. These six alleles were selected based on the reported prevalence among women of Chinese and Malay ethnicity, who make up the majority of the study cohort[8-14]. Primers were designed using the pyrosequencing software (http://techsupport.pyrosequencing.com); primer sequences are listed in Table 1.

The PM phenotype was defined by being homozygous or compound heterozygous for two PM alleles. The IM phenotype was defined by being homozygous for two IM alleles or heterozygous for a PM allele and an IM allele. All other combinations are considered either extensive metabolizer (EM) or ultra metabolizer (UM) phenotypes. A PM or IM allele in combination with an EM allele results in an EM phenotype (tamoxifen metabolism being comparable). Patients were classified according to the following combinations: (1) PM: *4/*4, *4/*5, *4/*6*, *5/*5, *5/*6, *6/*6; (2) IM: *9/*9, *9/*10, *9/*41, *10/*10, *10/*41, *41/*41, *9/*4, *9/*5, *9/*6, *10/*4, *10/*5, *10/*6, *41/*4, *41/*5, *41/*6, heterozygotes for *4, *5, *6, *9, *10, *41; (3) EM/UM: all other combinations.

Hormonal therapy recommendations and outcomes based on CYP2D6 genotyping

Results of the *CYP2D6* genotyping were made known to the patient during the discussion for hormonal therapy. Patients identified to have PM or IM phenotypes were recommended anastrozole at a dose of 1 mg daily or letrozole at a dose of 2.5 mg daily, but they were still permitted to opt for tamoxifen. Those with an EM/UM phenotype were given a choice between an AI or tamoxifen (20 mg daily). All agents were recommended for a duration of 5 years, which was the standard practice at the time of the study. The benefit and potential treatment-related side effects of either agent was discussed with the patient, and the women themselves made the final decision regarding the choice of hormonal agent. The women were assessed every 6 mo with clinical history and physical examination during regular surveillance visits, and any side effects or disease progression were documented. Women receiving AIs also received calcium and vitamin D supplements, and bone mineral density was monitored every year. Women receiving tamoxifen were referred to the gynecology clinic for surveillance that included a yearly ultrasound of the pelvis to evaluate endometrial thickness specially; this was in addition to the routine 3-yearly PAP smear screenings. While this study was originally designed to follow up for the 5-year duration of hormonal treatment, we continued to collect data from the women who remained on extended therapy and on follow-up with the breast clinic in view of the increasing use of hormonal agents beyond 5 years as the study progressed. All 70 women included in the final analyses remained on follow-up until study completion. Median follow-up was 86 mo (30.57 to 99.50 mo), and median overall survival rate was 75.95 mo (30.57 to 94.10 mo).

Statistical analyses

The associations between CYP2D6 phenotype and the specific hormonal agent received and with standard clinicopathological parameters and outcomes were evaluated with univariate analyses (χ^2 test, Fisher's test, one-way analysis of variance, χ^2 test for trend for ordinal data) and were performed using GraphPad Prism version 6 (GraphPad Software, San Diego, CA, United States). Recurrence-free survival was defined as the time interval from surgery to the development of either locoregional or distant

Table 1 Primers for cytochrome P450 2D6 genotyping

	Position/change	Forward primer (5'→3')	Reverse primer (5'→3')
*4	1846G>A	AGA GGC GCT TCT CCG TGT CC	AAA TCC TGC TCT TCC GAG GC
*5	Gene deletion	CTC CAG CCT CCA CCA GTC CAG	CAG GCA TGA GCT AAG GCA CCC AGA C
*6	1707delT	CGC AAC TTG GGC CTG GGC AAG AAG TCG CTG GAC TAG	CTC GGG AGC TCG CCC TGC AGA GAC TC
*9	2613AGA>del	GGT CAG TGG TAA GGA CAG GCA GGC CC	CAC CCT TGC CCC CCA CCG TGG CAG CCA CTC TAA GCT
*10	100C>T	GAT GCA CCG GCG CCA ACG CTG GGC TGC ACG GTA C	CAA ACC TGC TTC CCC TTC TCA GCC
*41	2988G>A	CGT GAG CCC ATC TGG GAA A	CTG ACA CTC CTT CTT GCC TCC TA

recurrence. Overall survival was defined as the time interval from surgery to death, whether from breast cancer specific mortality or from any other causes. Contralateral cancer was defined as the occurrence of a metachronous cancer in the contralateral breast more than 6 mo following the diagnosis of the first cancer. Kaplan Meier survival curves were performed to compare survival rates, and the log rank test was used to compare between the two arms. A two-tailed test was used for all analyses, and a value of $P < 0.05$ was considered statistically significant.

RESULTS

Study cohort demographics

Over a 3-year period from 2011 to 2013, a total of 82 women were recruited into the study. Eleven women later opted to withdraw for personal reasons, and one woman was withdrawn after she developed metastatic disease while on adjuvant chemotherapy treatment (prior to the start of hormonal treatment). *CYP2D6* genotyping was completed in 78 women, and study endpoints were evaluated in the 70 women who remained in the study and completed at least 5 years of follow-up (apart from 3 women who died). Median patient age was 61 years (ranging from 47 years to 86 years), and the majority (70 of 82, 85.4%) were of Chinese ethnicity. More than half (62 of 82, 75.6%) of the women had at least one pre-existing co-morbidity; hypertension was the most common. All the women had undergone curative surgery, with 64.6% having had a mastectomy. Disease was staged as Stage I in 34 of 82 women (41.5%), Stage II in 27 (32.9%) and Stage III in 21 (25.6%); 40.2% of women had node-positive disease. Invasive ductal carcinoma not otherwise specified was the most common histological type (78%). Median invasive tumor size was 2 cm (ranging from 0.1 to 8.0 cm), and median tumor grade was 2.

All but 2 women had ER-positive tumors; they had ER-negative/PR-positive tumors. Median ER staining intensity was strong, and the median proportion of tumor cells staining positive for ER was 90.7%. Details of tumor ER expression is as follows: less than 10% of tumor cells stained positive in 4 of 80 women (4.9%), 11% to 49% of cells stained positive in 8 women (8.9%), 50% to 89% of cells stained positive in 17 women (24.3%) and more than 90% of cells stained positive in 41 women (58.7%). Tumors were positive for PR in 61 women (87.1%) and human epidermal growth factor receptor-2-positive in 31 (44.3%) women.

The majority of women (73.1%) were classified as having an IM phenotype, 25 women (32.1%) as having an EM/UM phenotype, and none were classified as having a PM phenotype (Table 2). The *10 allele was highly prevalent and was found in 52 (66.7%) women. Another 2 (2.5%) women were found with the *41 allele. The *4 and *5 PM alleles were found in 5 (6.4%) women, and in all instances occurred together with a *10 allele. Of the 8 women who withdrew from the study and who were not included in endpoint analyses, 5 were of the IM phenotype and 3 were of the EM/UM phenotype. Women with IM and EM/UM phenotypes shared similar characteristics (Table 3). Specifically, the IM phenotype did not correlate with tumor receptor status nor with the intensity of ER staining or proportion of tumor cells staining positive for ER.

Details of hormonal agent choice and treatment

Following a discussion with their attending clinician, more women opted for an AI over tamoxifen regardless of CYP2D6 phenotype. More than 80% of the women (43 of 52, 82.7%) with an IM phenotype opted for an AI, and 72.2% of women with an EM/UM phenotype (13 of 18) also opted for an AI. Only 14 women (9 with an IM phenotype and 5 with an EM/UM phenotype) opted for tamoxifen. There were no significant differences between the group who opted for an AI compared to those who chose

Table 2 Details of cytochrome P450 2D6 genotyping for the 78 women classified into two phenotype groups: Intermediate metabolizers and extensive metabolizers/ultra metabolizers

Phenotype	CYP2D6 genotype	Number of women, *n* = 78
IM	*10/*10	25
	*10/41	2
	*10/*4	2
	*10/*5	3
	*10/EM/UM	20
	*5/EM/UM	5
EM/UM	EM/UM	21

IM: Intermediate metabolizers; EM/UM: Extensive metabolizers/ultra metabolizers; CYP2D6: Cytochrome P450 2D6.

tamoxifen (Table 4). The difference in median ages between the two groups (P = 0.008) was not likely clinically significant. Similar numbers of women on AI and tamoxifen choose mastectomy, but more women opting for AI had received other modes of systemic treatment (chemotherapy with or without trastuzumab) (P = 0.045).

Overall, 56 of the 70 women (80%) completed 5 years of hormonal therapy; comprising 46 of the 56 women (82.1%) who opted for an AI and 10 of the 14 women (71.4%) who opted for tamoxifen. Nineteen women who started on an AI and three of those who started on tamoxifen continued with extended therapy after 5 years of treatment. Thirty of the 56 women (53.5%) completed 5 years of the initial AI agent they were started on. Side effects were reported in 19 women: severe myalgia and arthralgia in 8 women, skin rashes in 4 women and osteoporosis in 7 women. Two women with intolerable musculoskeletal side effects opted to discontinue hormonal therapy. Of the other 12 women with musculoskeletal side effects or rashes, 7 switched to another AI, and 3 switched to tamoxifen. Four of the seven women who developed osteoporosis switched to tamoxifen, while the remaining three remained on AIs but started on bisphosphonates. All the women who switched to another AI agent eventually completed 5 years of treatment, although 2 switched to a third AI agent and 3 switched to tamoxifen before completion. Of the 7 women who switched to tamoxifen, 6 completed 5 years of treatment with tamoxifen, while the remaining patient switched back to another 2 different AI agents before completing the 5 years of treatment. Of the 14 women who started on tamoxifen, 2 women (14.3%) switched to an AI after developing skin rashes; though one of them later discontinued the AI after developing musculoskeletal side effects. The hormonal agent was discontinued in 8 patients upon the development of new events. Another 3 women chose to discontinue hormonal therapy but had not reported any side effects.

Clinical outcomes

Over the follow-up period of 96 mo, 3 women developed contralateral breast cancer, 5 women developed recurrences, 5 women died, and 1 woman was diagnosed with a nasopharyngeal carcinoma. Contralateral breast cancer was diagnosed after a median interval of 48.8 mo (ranging from 47.83 to 59.27 mo). All 3 occurred in women of the IM phenotype, of which 2 women had received AI and 1 received tamoxifen. Recurrence was systemic in all 5 women and had occurred after a median interval of 24.93 mo (ranging from 23.07 to 48.10 mo). Four of these women later died, although one death was attributed to a non-breast cancer-related cause. The last mortality occurred in a woman who had remained disease-free up to the time of death from a non-breast cancer-related cause. Disease recurrence did not show a clear association with the hormonal agent received (P = 0.260) (Table 4). Recurrence developed in 3 women who had received an AI (2 of the IM phenotype and 1 of the EM/UM phenotype) and in 2 women who had received tamoxifen (both of the IM phenotype). One of the women developed both locoregional and systemic recurrence.

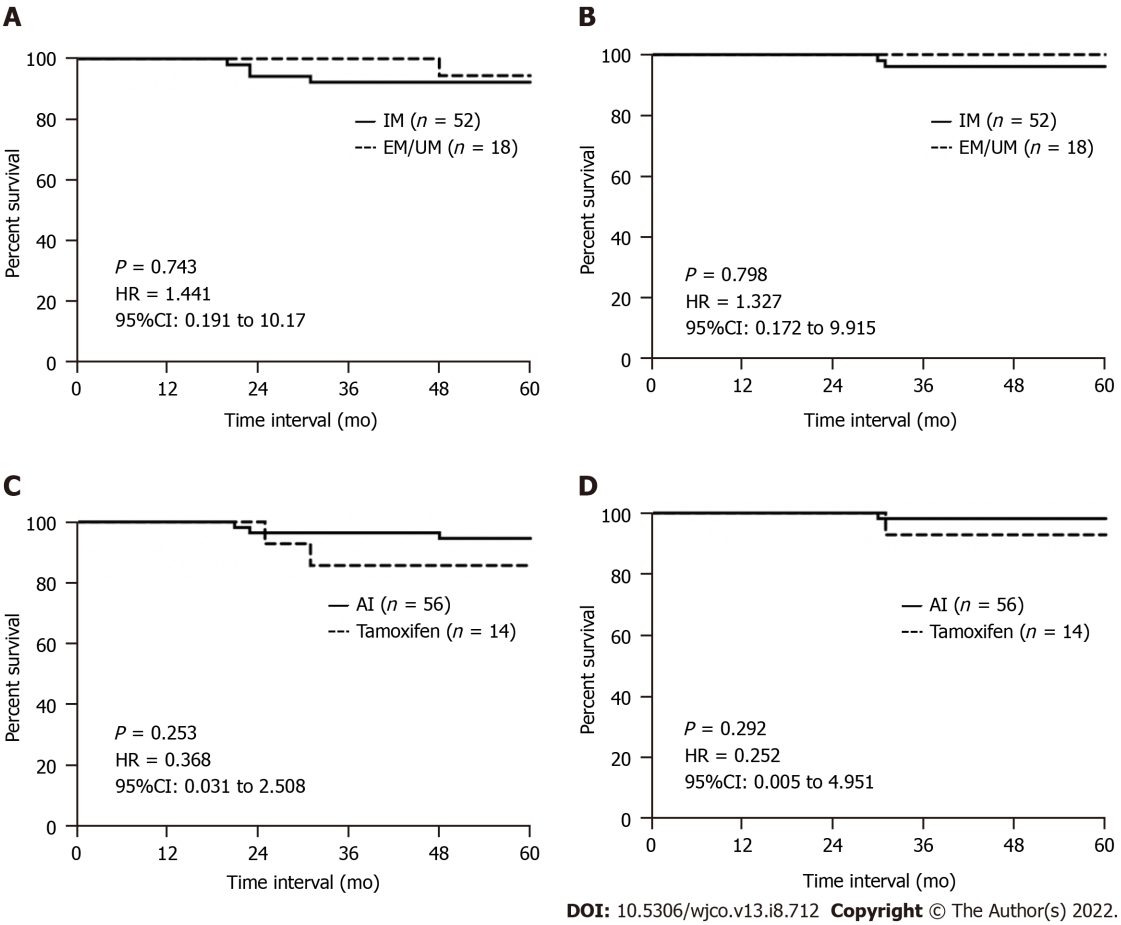
Five-year recurrence-free and overall survival appeared slightly better in women with an EM/UM phenotype compared to those with an IM phenotype but was not statistically significant [P = 0.743, hazard ratio (HR): 1.441, 95% confidence interval (CI): 0.191 to 10.17 and P = 0.798, HR: 1.327, 95%CI: 0.172 to 9.915, respectively) (Figure 1A and B). When stratified by the hormonal agent received, women who had received an AI appeared to have better 5-year recurrence-free and overall survival compared to those who had received tamoxifen, but again these were not statistically significant (P = 0.253, HR: 0.368, 95%CI: 0.031 to 0.258 and P = 0.292, HR: 0.252, 95%CI: 0.005 to 4.951, respectively) (Figure 1C and D).

Table 3 Univariate correlation analyses of clinicopathological parameters with cytochrome P450 2D6 phenotype classification

	IM phenotype, *n* = 52	EM/UM phenotype, *n* = 18	*P* value
Median age in yr	62.5 (51-86)	61 (50-84)	0.574
Disease stage			0.595
I	24	6	
II	17	8	
III	11	4	
Ethnicity			0.148
Chinese	47	13	
Malay	3	3	
Indian	1	2	
Others	1	0	
Comorbidities			0.728
Yes	43	14	
No	9	4	
Tumor histology			0.518
IDC	40	14	
ILC	7	1	
Others	5	3	
Tumor grade			0.247
1	16	3	
2	25	10	
3	11	5	
Median tumor size in mm	16.5 (1.2 to 70.0)	20.0 (3.0 to 45.0)	0.334
Lymphovascular invasion			0.527
Present	16	7	
Absent	36	11	
ER intensity			0.528
Low	2	0	
Moderate	11	2	
High	38	15	
Negative	1	1	
Proportion of tumor cells staining ER-positive			0.267
1% to 10%	4	0	
11% to 49%	6	1	
50% to 89%	14	3	
More than 90%	27	14	
PR intensity			0.631
Low	5	1	
Moderate	9	5	
High	31	11	
Negative	7	1	
Proportion of tumor cells staining PR-positive			0.785

1% to 10%	11	3	
11% to 49%	8	2	
50% to 89%	18	7	
More than 90%	11	6	
HER2 status			0.495
Positive	11	2	
Negative	41	16	
Clinical subtypes			0.692
ER+/HER2-	41	14	
ER+/HER2+	10	2	
ER-/HER2+	1	0	

ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor-2; IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; IM: Intermediate metabolizers; EM/UM: Extensive metabolizers/ultra metabolizers.



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Figure 1 Survival outcomes stratified by cytochrome P450 2D6 phenotype and by hormonal agent used. A: Kaplan-Meier curves showing 5-year recurrence-free survival stratified by cytochrome P450 2D6 phenotype (n = 70); B: Kaplan-Meier curves showing 5-year overall survival stratified by cytochrome P450 2D6 phenotype (n = 70); C: Kaplan-Meier curves showing 5-year recurrence-free survival stratified by hormonal agent received (n = 70); D: Kaplan-Meier curves showing 5-year overall survival stratified by hormonal agent received (n = 70). IM: Intermediate metabolizers; EM/UM: Extensive metabolizers/ultra metabolizers; AI: Aromatase inhibitors; HR: Hazard ratio; CI: Confidence interval.

DISCUSSION

AIs are now widely used as first-line hormonal agent in many clinical units. With the introduction of generic letrozole, AIs have become more affordable for our local women. The common practice here is to start women on AIs unless they have contraindications or develop intolerable side effects. Tamoxifen

Table 4 Univariate correlation analyses of clinicopathological parameters and clinical outcome with type of hormonal agent received

	Aromatase inhibitors, *n* = 56	Tamoxifen, *n* = 14	*P* value
CYP2D6 phenotype			0.339
IM	43	9	
EM/UM	13	5	
Median age in yr	62 (50 to 80)	63 (52 to 86)	0.008
Disease stage			0.795
I	23	7	
II	21	4	
III	12	3	
Ethnicity			0.126
Chinese	50	10	
Malay	3	2	
Indian	3	1	
Others	0	1	
Comorbidities			1.000
Yes	45	12	
No	11	2	
Tumor histology			0.918
IDC	45	11	
ILC	6	2	
Others	5	1	
Tumor grade			0.686
1	15	4	
2	27	8	
3	14	2	
Median tumor size in mm	16.5 (1.2 to 70.0)	19.0 (1.6 to 53.0)	0.747
Lymphovascular invasion			0.526
Yes	17	6	
No	39	8	
ER intensity			0.509
Low intensity	1	1	
Moderate intensity	10	4	
High intensity	44	9	
Negative	1	0	
Proportion of tumor cells staining ER-positive			0.386
1%-10%	3	1	
11% to 49%	4	3	
50% to 89%	15	2	
More than 90%	33	8	
PR status			1.000
Positive	49	13	
Negative	7	1	

PR intensity			0.161
Low intensity	3	3	
Moderate intensity	10	4	
High intensity	36	6	
Negative	7	1	
Proportion of tumor cells staining PR-positive			0.318
1%-10%	11	3	
11% to 49%	6	4	
50% to 89%	21	4	
More than 90%	15	2	
HER2 status			0.700
Positive	10	3	
Negative	46	10	
Tumor subtypes			0.754
ER+/HER2-	46	10	
ER+/HER2+	9	3	
ER-/HER2+	1	0	
Type of surgery			0.756
Mastectomy	35	10	
Wide local excision	21	4	
Treatments received			0.045
Systemic therapy[1] and hormonal therapy	12	2	
Systemic therapy[1], radiation and hormonal therapy	18	0	
Radiation and hormonal therapy	12	5	
Hormonal therapy alone	14	7	
Disease recurrence[2]			0.260
Yes	3	2	
No	53	12	
Mortality[2]			0.344
Yes	1	1	
No	55	13	
Contralateral breast cancer[2]			0.551
Yes	2	1	
No	54	13	

[1]Systemic therapy refers to chemotherapy with or without trastuzumab.
[2]Events occurring within 5 years.
ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor-2; IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; IM: Intermediate metabolizers; EM/UM: Extensive metabolizers/ultra metabolizers; CYP2D6: Cytochrome P450 2D6.

is now first-line only in premenopausal women or in those with contraindications to AIs. Otherwise, it is often the second-line agent that women are switched to should they develop intolerable side effects from tamoxifen. The move towards adopting AIs as first-line came following reports of superior efficacy and because AIs were previously recommended for 5 years, whereas tamoxifen was recommended for 10 years. However, it has also been suggested that AIs are superior to tamoxifen primarily in women with nonfunctional or reduced function *CYP2D6* polymorphisms[4,5], specifically in those with a PM or IM phenotype where impaired CYP2D6 enzyme metabolism results in lower serum concentrations of

active metabolite endoxifen responsible for tamoxifen efficacy[16,17]. Observations that serum endoxifen levels correlated with the frequency and severity of adverse effects raised the possibility that clinical outcomes could likewise be adversely affected by impaired CYP2D6 metabolism[18,19].

More than 70% of Caucasians have functional *CYP2D6* alleles, yet only 50% of Asians have functional *CYP2D6* alleles[8-10]. It has been variously reported that 40% to 70% of Asians carry reduced function alleles, particularly the *CYP2D6*10* allele[11-14]. The high prevalence of *CYP2D6*10* was confirmed in our study, where it was present in two-thirds of the women included. The *41 allele was present in 2 women. The *CYP2D6*10* allele results in an approximately 60% reduction in CYP2D6 enzyme activity, and Asians have been reported to metabolize tamoxifen and other CYP2D6-mediated drugs more slowly than Caucasians[20-23]. Nonfunctional alleles were uncommon, and all 5 women (6.4%) in our study with *4 and *5 PM alleles were heterozygotes. This is relatively similar to the prevalence reported in other studies[9-11,13,14,22,23]. We did not find any women with the *CYP2D6*9* allele, which we had included as it was observed in 3% of Malays in Malaysia[9].

Five women (7.1%) in our study developed recurrence during the study, including 4 women who were of the IM phenotype; 2 of whom received an AI. We did observe a trend towards better survival in women with the EM/UM phenotype. This was in agreement with the findings of a prospective study window-of-opportunity study conducted at our unit where women received up to 2 wk of tamoxifen prior to surgery. Women with at least one wild-type *CYP2D6* allele demonstrated a significantly greater Ki-67 response, suggesting that tamoxifen produced a greater inhibitory effect in those with functioning *CYP2D6* polymorphisms[24]. The lack of statistical significance was likely because of the small sample size and perhaps because there were no women with the PM phenotype in our study. Women with a PM phenotype were reportedly at a 7% higher risk of recurrence, which appeared to be incremental over time[5]. Like others, we did not observe *CYP2D6* polymorphisms to correlate with tumor size, grade or nodal status, which meant that survival differences were not likely a result of unfavorable tumor factors[25].

Mathematical modelling showed that survival outcomes in women without nonfunctional alleles were not different whether they received AIs or tamoxifen[5], and reduced function *CYP2D6* polymorphisms were only associated with clinical outcomes in those treated with tamoxifen[25,26]. We observed that women who received AIs had a slightly better but nonsignificant survival and that these women were more likely to have received systemic treatments (chemotherapy with or without trastuzumab), perhaps indicating a clinician bias towards AIs in those deemed to have more 'high-risk' disease. However, our study numbers are small and too few women received tamoxifen for a meaningful analysis. A larger cohort would be needed to stratify the effect of the hormonal agent used by disease stage and other systemic treatments. Overall, we observed little difference in contralateral breast cancer rates and disease recurrence between women with IM and EM/UM phenotypes, which was perhaps due to many women in our study, including 72% of those with an EM/UM phenotype, opting for an AI over tamoxifen.

Despite the differences in cost at the time of the study, many women opted for the more costly AI. This was so even in those with an EM/UM phenotype, in whom there were suggestions that tamoxifen efficacy was comparable to AI. However, only about half of the women in our study completed 5 years of the AI agent they were initially started on. In 60% of cases, the discontinuation was initiated by the women themselves because of intolerable myalgia, arthralgia and skin rashes [10 (53%) switched to another AI, and 5 had further problems with the second AI and eventually switched to tamoxifen or a third AI]. Four women who started on an AI were switched to tamoxifen by their clinician because of osteoporosis from accelerated bone loss. On the other hand, the majority of women who started on tamoxifen appeared to tolerate it well, and only 2 were switched to an AI after developing skin rashes. Overall, more women who started on tamoxifen completed 5 years of treatment compared to those who started on an AI. Excluding the 8 women who progressed on hormonal therapy, 90% of the women completed 5 years of hormonal therapy.

Like others, we did not find *CYP2D6* polymorphisms to correlate with any clinicopathological factors, implying that genotyping would be the only means of ascertaining the phenotype. The prevalence of the IM phenotype in our local women may mean that *CYP2D6* genotyping at the offset may be of little benefit since AIs are now the initial hormonal agent of choice. On the other hand, given that a significant number of women do develop AI-related side effects and in those whom a switch is being considered, *CYP2D6* genotyping could help clinicians decide whether to switch to another AI or to tamoxifen. Those with an EM/UM phenotype can be switched to tamoxifen since outcomes are probably comparable with those on AIs[5]. Furthermore, tamoxifen-related side effects appear to be less common. An unpublished review of women on follow-up at our unit did not find a higher incidence of endometrial cancer among those treated with tamoxifen. Those with an IM phenotype should consider switching to another AI agent, based on reports of impaired function variants being associated with higher risks of recurrence. This would have particular significance in the setting of ER-positive disease where late recurrences are more common and since the majority of women survive for many more years after breast cancer treatment.

CONCLUSION

The prevalence of the IM phenotype was high in our study, with more than two-thirds of the women having the *CYP2D6*10* allele. We did not observe the IM phenotype to be associated with any clinicopathological parameter and did not observe any correlation with clinical outcome. The hormonal agent used was not associated with a difference in outcome. Compliance was good, and most women completed 5 years of hormonal therapy, although more women who started on an AI required a switch to another hormonal agent because of side effects.

ARTICLE HIGHLIGHTS

Research background

There are concerns that tamoxifen is less effective in Asian women because of the high prevalence of impaired function cytochrome P450 2D6 (*CYP2D6*) polymorphisms.

Research motivation

Tamoxifen is still the first-line agent for premenopausal women and for those with intolerable AI-related side effects. It is therefore necessary to verify the effectiveness of tamoxifen in view of the high prevalence of reduced function *CYP2D6* polymorphisms in Asians.

Research objectives

We evaluated the frequency of *CYP2D6* polymorphisms and its association with clinical outcome. We also evaluated treatment-related side effects in order to better determine the risk:benefit ratio.

Research methods

We designed a single-arm prospective study to evaluate how knowledge of *CYP2D6* genotype impacted the choice of hormonal agent and how *CYP2D6* genotype and agent were associated with clinical outcomes.

Research results

More than 70% of the women in our study had an intermediate metabolizer phenotype. Regardless of genotype, more women opted for aromatase inhibitors. Women with the extensive or ultra metabolizer phenotype had slightly better but nonsignificant 5-year recurrence-free and overall survival compared to women with the intermediate metabolizer phenotype. Women on AIs appeared to have better but also nonsignificant 5-year recurrence-free and overall survival.

Research conclusions

The intermediate metabolizer phenotype was highly prevalent in our local women but was not associated with clinical outcome.

Research perspectives

Data on the effect of *CYP2D6* polymorphisms on tamoxifen efficacy remains conflicting. More studies in Asian women would help to clarify this association.

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FOOTNOTES

Author contributions: Tan EY designed and performed the research study, analyzed the data and wrote the manuscript; Bharwani L and Chia YH performed study procedures in the protocol; Soong RCT was responsible for *CYP2D6* genotype and verification of the phenotype; Lee SSY, Chen JJC and Chan PMY contributed to data collection, data analyses and review of the manuscript; All authors have read and approved the final manuscript.

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LETTER TO THE EDITOR

COVID-19 and oral cancer: Critical viewpoint

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Abstract

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has marked the beginning of a new pandemic named coronavirus disease 2019 (COVID-19). The World Health Organization has announced it as a health emergency that is of international concern. The disease has been reported to cause respiratory illness, pneumonia and even hinder the immunity of an individual. Individuals with disturbed immune responses have been found to be quite susceptible to this viral infection. Oral cancer patients are also at high risk in this pandemic situation and might encounter severe detrimental outcomes. Angiotensin receptors, documented in studies as the path of entry of this virus, are highly expressed in the epithelial cells of oral mucosa, making the group of individuals with oral cancers even more vulnerable. Extracellular matrix metalloproteinase inducer is another potential target for SARS-CoV-2. An exhaustion of angiotensin converting enzyme 2 cell receptors leads to protumoral effects, whereas a downregulation of extracellular matrix metalloproteinase inducer leads to antitumoral effects. Thus, it causes a variation of the biological behavior of the tumor. This article focusses on the molecular mechanisms, effects and pathophysiology of COVID-19 in oral squamous cell carcinoma patients. The different molecular changes in oral squamous cell carcinoma in the background of COVID-19 will modify various environmental factors for this pathology and have an effect on the carcinogenesis process. Understanding the behavior of the tumor will help plan advanced treatment strategies for oral squamous cell carcinoma patients in the background of COVID-19.

Key Words: COVID-19; SARS-CoV-2; Oral cancer; Head and neck carcinomas; Oral squamous cell carcinoma

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Core Tip: The outbreak of coronavirus disease 2019 (COVID-19) has evoked concern worldwide. The rapid spread of the disease during the first and the second waves caused severe respiratory illness. Individuals are facing a suppressed immune response. An impaired immune response has made patients with head and neck cancer highly susceptible to the viral infection. The two potential receptors of severe acute respiratory syndrome coronavirus 2, angiotensin receptors and extracellular matrix metalloproteinase inducer, have contrasting effects on cancer progression. Thus, the molecular mechanisms and the biological behavior of oral squamous cell carcinoma show varying effects in the background of COVID-19.

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TO THE EDITOR

The outbreak of coronavirus disease 2019 (COVID-19) has posed a major health impact, affecting populations all over the world with significant morbidity and mortality. With the introduction of the second wave in many countries, the doubling time of infectivity has reduced drastically. This also means that we should doubly prepare for all the consequences that we faced in the first wave. Individuals with disturbed immune responses have been found to be quite susceptible to this viral infection. Cancer patients have been considered to be at high risk in this pandemic situation because of immunosuppression[1]. Not only the underlying malignant condition but also co-morbidities, advanced age and poor host response have been held responsible for the vulnerability of cancer patients during the COVID-19 pandemic[2,3].

Studies have identified the angiotensin converting enzyme 2 (ACE2) cell receptors as the path of entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into a host cell[4]. ACE2 receptors are reportedly found to be highly expressed on the epithelial cells of oral mucosa making the group of individuals with oral cancers even more vulnerable. ACE2, a key enzyme of renin-angiotensin system, breaks down angiotensin II (Ang II) into Ang 1-7[5]. Ang II is a protumoral agent that plays a major role in carcinogenesis[6]. It helps in tumor cell proliferation and angiogenesis. It also facilitates the metastasis of cancer cells. Thus, Ang II aids in progression of the disease, while ACE2 and Ang 1-7 inhibit the progression. ACE2 maintains a balance of renin-angiotensin system[5]. However, these propositions might alter due to changes in the viral component, specially mutation in the spike protein.

The SARS-CoV-2 attaches to ACE2 cell receptors through the S-spikes on the virus surface. The SPIKE (S protein) expressed by the virus attaches to the extracellular part of ACE2 receptors, and the S protein breaks down into subunits S1 and S2[7]. The virus fuses with the cell membrane and gains entry into the cell *via* endocytosis. An exhaustion of ACE2 receptors takes place due to the viral infection. ACE2 receptors being highly expressed in tongue, gingiva and buccal epithelial cells, oral squamous cell carcinoma (OSCC) patients are at high risk during this pandemic[7]. The viral infection will cause a reduction in ACE2 concentration leading to an increase in Ang II concentration[5]. This could have a protumoral effect facilitating the progression of OSCC.

Besides ACE2 receptors, extracellular matrix metalloproteinase inducer (EMMPRIN), also known as BASIGIN/CD147 has been identified as another potential target for SARS-CoV-2[8]. EMMPRIN is a cell surface glycoprotein belonging to the immunoglobulin family. It helps in activation of molecules of several matrix metalloproteinases. Thus, it helps in proliferation of tumor cells and their invasion and migration[9]. EMMPRIN also promotes angiogenesis by stimulating vascular endothelial growth factors in the tumor microenvironment[10]. It is speculated that EMMPRIN expression is increased in oral carcinogenesis. The upregulation of EMMPRIN expression in OSCC patients might make them more susceptible to COVID-19 infection[8]. The virus attaches itself to EMMPRIN receptors through S receptors; thus, COVID-19 in OSCC patients will lead to downregulation of EMMPRIN receptors. This will inhibit the progression of the tumor due to scarcity of EMMPRIN receptors.

The COVID-19 infection in OSCC patients will reduce the availability of ACE2 receptors. This will lead to upregulation of Ang II concentration, thus promoting carcinogenesis. In such situations of nonavailability of ACE2 receptors, SARS-CoV-2 attaches to its next potential target, EMMPRIN receptors, to gain entry into the host cells[8]. This in turn causes downregulation of EMMPRIN receptors leading to antitumoral effects. The two potential receptors of SARS-CoV-2 have contrasting effects on OSCC progression.

COVID-19 infections in OSCC patients modulate the events of carcinogenesis and control the biological behavior of the tumor. Future molecular studies are required to have a better insight into the role of the two receptors in the pathophysiology of OSCC. Moreover, angiotensin converting enzyme

inhibitors and angiotensin receptor blockers, which are administered in cancer patients, have been thought to have varying effects on tumor progression. The use of these drugs in OSCC patients during this pandemic still remains doubtful and requires clinical studies.

The expression of ACE2 in various pathologies like oral cancer, oral submucus fibrosis and periodontitis modulate their disease process. The biological behavior of not only OSCC, but also other oral potentially malignant disorders, in the background of COVID-19 requires in-depth studies and research. This can only be achieved by representative clinical material, *i.e.* COVID-19 positive OSCC patients, appropriate disease model and their long-term follow-up. While keeping these interactions in mind, one should not forget the delay in cancer treatment worldwide. The COVID-19 pandemic has caused deviation of attention from many medical emergencies; cancer and OSCC is not an exception to it. Thus, it is mandatory to formulate guidelines for safe and effective delivery of therapeutics to cancer patient in this difficult time.

Impact of the pandemic on cancer management

Due to mandatory lockdowns during the pandemic, many healthcare specialty services were affected including cancer management. Many countries reported more than 50% reductions in the registration of new cancer patients[11]. These repercussions of the pandemic are mainly related to travel restrictions, conversion of hospitals to COVID-19 centers, fear in the mind of patients, human resource shortages, *etc.* To mitigate the reduction in the number of cases many cancer hospitals have started telecommunication and teleconsultations, but it is premature to comment on its effectiveness especially for head and neck cancer.

Due to compromised primary medical and dental services across the world, the early detection of oral cancer is at stake. Already, head and neck cancers are detected at advanced stages; further delay in the detection would lead to extremely poor prognoses. According to one study in the United States, there was a 25% reduction in newly diagnosed oral cancer cases[12]. Currently, COVID-19 is at declining stages in many countries, and this opportunity should be exploited to perform maximum screening for early detection.

FOOTNOTES

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MINIREVIEWS

Hyperprogression under treatment with immune-checkpoint inhibitors in patients with gastrointestinal cancer: A natural process of advanced tumor progression?

Mo-Xuan Wang, Shu-Yue Gao, Fan Yang, Run-Jia Fan, Qin-Na Yang, Tian-Lan Zhang, Nian-Song Qian, Guang-Hai Dai

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Abstract

Immunotherapy has shown great promise in treating various types of malignant tumors. However, some patients with gastrointestinal cancer have been known to experience rapid disease progression after treatment, a situation referred to as hyperprogressive disease (HPD). This minireview focuses on the definitions and potential mechanisms of HPD, natural disease progression in gastrointestinal malignancies, and tumor immunological microenvironment.

Key Words: Hyperprogressive; Immunotherapy; Natural process; Gastric cancer; Colorectal cancer

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Core Tip: There have been several literature reviews on the definition, incidence, predictors, potential biomarkers, and prognosis of hyperprogressive disease. However, this is a minireview of two conflicting concepts: HPD is a new form of immunological response *vs* a natural process of advanced tumor progression. It also takes a look at cellular and molecular mechanisms of the pathways of the tumor microenvironment and recent clinical trials exploring the risk factors and mechanisms of HPD in gastrointestinal cancer.

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INTRODUCTION

A minority group of patients with gastrointestinal cancer during the treatment of checkpoint inhibitors (ICIs) show paradoxical acceleration in tumor growth. Patients with hyperprogressive disease (HPD) show a shortened progression-free survival or overall survival as compared to patients with natural progressive disease (PD)[1]. According to a recent meta-analysis, the overall incidence of HPD was 13.4% (95% confidence interval [CI], 10.2%-16.6%), with a range of 5.9% to 43.1%[2]. However, this might be an underestimation and the true incidence could be higher, as a certain number of patients might not be diagnosed due to clinical disease progression. Colonic and gastric cancers are the fifth and sixth most common types of cancer, ranking second and fourth worldwide in terms of mortality, respectively[3]. The survival benefits of ICIs such as nivolumab and pembrolizumab in gastrointestinal cancer vary in clinical studies due to different molecular targets and cytotoxicity[4]. To make ICIs safer and more effective in treating gastrointestinal cancer, there is an immense need to explore the molecular mechanism of HPD. This minireview will discuss two contradicting viewpoints in this regard: Is the development of HPD unique to immunotherapy in gastrointestinal cancer patients or is it a natural process of progression of advanced cancers?

DEFINITION

The most widely used indexes for HPD diagnosis include tumor growth kinetics (TGK), time to treatment failure (TTF), response evaluation criteria in solid tumors (RECIST), and tumor growth rate (TGR) or their combinations. However, there has been no consensus on the medical diagnosis criteria for HPD so far.

Kato *et al*[5] suggested three criteria to define HPD in patients with non-small cell lung cancer (NSCLC): Progression increase (TGR) of at least two times, a tumor burden increase of 50%, and TTF < 2 mo. Kim *et al*[6] defined HPD as a progressive disease (PD) based on TGK or TGR, *i.e.*, an increase of more than two folds of TGR or TGK during the treatment time interval as compared to that of the reference times in sicker populations already diagnosed with PD by RECIST 1.1 at the first response assessment after PD-1/PD-L1 inhibitors. Ten Berge *et al*[7], Petrioli *et al*[8], and Refae *et al*[9] adopted the same definition for HPD by using RECIST 1.1 at first assessment and TGRPOST/TGRPRE ≥ 2.

In a retrospective analysis of 270 patients with pan-cancer, three criteria were used in defining HPD: (1) 40% increase in sum of target lesions (STL) *vs* baseline or/and; (2) 20% increase in STL *vs* baseline plus the appearance of new lesions in at least two different organs; and (3) Minimum increase in the measurable lesions of > 10 mm and PD by RECIST at first 8 wk after treatment initiation[10]. In the other two retrospective studies about advanced gastric cancer (AGC), HPD was similar for Aoki *et al*[2] and Lu *et al*[11], who defined it as TGKPOST/TGKPRE ≥ 2. There are a few retrospective AGC studies evaluating the incidence of HPD, which are summarized in Table 1.

In a recent meta-analysis, subgroup analysis of varied underlying malignancies suggested that the overall incidence of HPD was 19.4% (95%CI: 9.7%-29.1%) in patients with AGC[2]. An optimal definition of HPD should be comprehensive and contain few variables (early tumor burden increase, TGR, TGK, new lesions, TTF, and clinically associated criteria, *etc.*). There is a need to establish quantifiable criteria based on Eastern Cooperative Oncology Group (ECOG) performance status or Karnofsky Performance Scale score, a systematic measure of tumor growth acceleration, and alternative diagnostic criteria.

Table 1 Incidence and definition of hyperprogressive disease in advanced gastric cancer patients receiving immunotherapy

Study agent	Tumor	HPD definition	Number of patients	Incidence of HPD, %	Ref.
Nivolumab	AGC	(1) An increase of ≥ 50% in the sum of longest diameter (SLD) of target lesions at 8 wk post baseline; (2) An increase of ≥ 20% in the sum of longest diameter of target lesions at 8 wk post baseline; and (3) An increase of ≥ 100% in the sum of longest diameter of target lesions at 8 wk post baseline	243; 243; 243	5.4; 27.6; 1.2	Feng *et al* [75], 2018
PD-1 inhibitor monotherapy	AGC	$TGK_{POST}/TGK_{PRE} \geq 2$	9	55.6	Sugimoto *et al*[76], 2018
PD-1 inhibitor monotherapy	AGC	$TGR_{POST}/TGR_{PRE} \geq 2$	105	24.8	Sunakawa *et al*[77], 2019
PD-1 inhibitor monotherapy	AGC	$TGR_{POST}/TGR_{PRE} \geq 2$	218	17.4	Suzuki *et al* [78], 2020

HPD: Hyperprogressive disease; AGC: Advanced gastric cancer; TGK: Tumor growth kinetics; TGR: Tumor growth rate.

MAIN VIEWPOINTS

Natural process

First, HPD is not caused by immunotherapy alone. A *post hoc* analysis from the OAK study (a randomized phase 3 study to describe results of atezolizumab therapy in NSCLC) suggested that fast progression is a universal phenomenon that coexists with ICIs and chemotherapy. The proportion of patients encountering fast progression criteria was analogous between the docetaxel and atezolizumab cohorts (n = 41 [9.6%] *vs* n = 44 [10.4%], respectively)[12]. However, a retrospective study by Aoki *et al* found that HPD incidence was slightly higher with nivolumab (29.4%) than irinotecan (13.5%) (P = 0.0656)[13], suggesting that hyperprogression after baseline is not unique to PD-L1 blockade therapy in NSCLC and AGC. There are also unbalances in the arms that may affect the therapeutic response. For instance, the irinotecan group had fewer patients with recurrent disease in contrast with the ICI group (18 of 66 [28.8%] *vs* 19 of 34 [52.9%]; P = 0.028 < 0.05). A higher proportion of patients treated with ICIs had posterior line therapy (13 of 34 [38.2%]) compared with those in the chemotherapy (20 of 66 [30.3%]; P = 0.502)[13]. After immunotherapy, it is possible that therapeutic resistance in patients who do not respond to ICIs developed secondarily to past chemotherapy. The large real-world data regarding gastric tumor treated with nivolumab had showed an insignificant difference in median overall survival (2.40 *vs* 2.79 mo; P = 0.8)[14] in patients with PD with or without HPD.

HPD is not unique to immunotherapy as it is also present in chemotherapy, but the incidence is higher in the former. This phenomenon also applies to NSCLC under treatment of Sorafenib (a multi-target tyrosine kinase inhibitor) and in metastatic renal cell carcinoma[15,16]. According to published data, HPD incidence is correlated to the type of tumor[17]. The incidence of immunotherapy-related HPD in AGC cases ranges from a few percent to about 21% (13 of 62)[18], according to a recent study, while the incidence stands at 6% in colorectal cases[19].

There is increasing evidence demonstrating that the predictive factors for HPD include age > 65 years, metastasis burden (number of sites of metastatic disease), local and regional relapse (TGKR ≥ 2: 90% *vs* TGKR < 2: 37%; P = 0.008 < 0.01), but do not include distant or local recrudescence, liver metastases, a large tumor at baseline, and ECOG performance status of 1 or 2[12,18-23].

A recent study suggested that hyperprogression is usually associated with high risk genetic alternations (*i.e.*, MDM2/4, epidermal growth factor receptor [EGFR], DNMT3A, AKT1 E17K, KRAS, and FBXW7) [18,23,24], which correlate with a shorter time to TTF. For instance, MDM2/4 is an oncogenic gene which functions through inactivation of p53, a tumor suppressing transcription factor. Experiments have demonstrated that MDM2 mediates resistance to immunotherapy by reducing T cell activation in malignancies[18]. However, the relationship between MDM2/4 amplification and hyperprogression remains unclear, although some scholars hypothesize about the involvement of a genomic site on the MDM2 amplicon[25,26]. Another study reported that one of 36 patients with AGC under nivolumab treatment had MDM2 gene amplification[27]. There is also evidence showing that two of 47 patients with AGC had MDM2 gene amplification, where one patient developed HPD under nivolumab treatment[18]. Data from a few studies investigating MDM2 inhibitors suggested that the combination of MDM2 inhibitors and immunotherapy could be an alternative strategy for patients with MDM2 AMP tumor and hyperprogression.

The EGFR signaling cascade is a key regulator in cancer development, survival, differentiation, and cell proliferation. It belongs to the ERBB family of tyrosine kinase receptors[28]. During nivolumab administration as anti-PD1 treatment in patients with AGC, three patients with ERBB2 mutation or amplification showed HPD (P = 0.48 or 1)[18]. Despite that all patients with FBXW7 mutation or KRAS amplification developed HPD in this study, the association between these genetic alterations and

hyperprogression needs to be further explored. There is evidence supporting the presence of EGFR mutated tumors (EGFR E746-A750 del and T790M mutation or EGFR exon 20 insertion mutation and MYC amplification) in patients with non-gastrointestinal (non-GI) cancer such as NSCLC who showed a less satisfactory response to ICIs and rapid progression[29]. According to a case report, the subtype of EGFR Kinase Domain Duplication, somatic alteration EGFR exon 2-28 duplication is present in a patient with esophageal squamous cell carcinoma who developed hyperprogression under camrelizumab treatment, existed[30]. A retrospective study on pan-cancer reported that the mutated type of KRAS mutation was associated with HPD in colorectal cancer (23.5% in non-HPD *vs* 80.0% in HPD; $P = 0.039 < 0.05$)[31].

A case report presented that a 64-year-old man with stage IIIA colon tumor remained disease-free for 10 years during the treatment with adjuvant chemotherapy. After recurrence in the liver, lymph nodes, and ureters, the patient was treated with FOLFIRI and bevacizumab, followed by cetuximab and irinotecan. In 2016, he was started on compassionate use of pembrolizumab for 9 mo until his CEA progressively increased and PET-CT imaging displayed progression in the liver and ureters. Atezolizumab was given for his urothelial tumor; however, the CEA rapidly increased 3 mo later. After discontinuing pembrolizumab and atezolizumab and following treatment with nivolumab and ipilimumab combination for four cycles, his CEA decreased to a stable level, and PET-CT imaging revealed a lower uptake in his original cancer as well as other metastases[32]. If hyperprogression is strongly correlated with immunotherapy, immunotherapy should be terminated after the occurrence of disease progression, although in this case, the patient was treated effectively with sequential PD-1/PD-L1 blockades as well as dual checkpoint inhibitors with good control of tumor burden. In the non-GI tumors, a patient with metastatic breast tumor developed HPD during the treatment of pembrolizumab, then the patient switched to the chemotherapy plus the PD-L1 inhibitor atezolizumab[33]. The patient maintained a partial response to rechallenge with atezolizumab for more than 8 mo. Repeated exposure to different ICIs after failure of initial ICI treatment existed in the other types of tumors, such as NSCLC [34-36].

These phenomena may indicate that PD-L1 blockade relieves B7.1 sequestration *in cis* through PD-L1 in dendritic cells[37], which leads to a B7.1/CD28 reaction to increase T cell priming, and rechallenging with other PD-L1/PD-1 inhibitors might synchronously revive immune response in the tumor microenvironment (TME)[38,39]. Further research is needed to explore what patient population are most likely to benefit from successive ICIs and the basic molecular and cellular mechanisms of different ICIs by analyzing gene expression and genetic mutations, and molecular dynamics simulations of the cancer microenvironment.

Most importantly, large-scale randomized controlled trials are urgently warranted to clarify the correlation among predictive factors for HPD, the molecular mechanisms of hyperprogression, and the natural progression of advanced malignant neoplasms in GI tumors. Prospective observational studies are also essential to compare treatment courses after each treatment.

This minireview has several limitations. Most of the referenced studies were not randomized controlled trials and instead were mostly retrospective. The incidence of HPD is lower in GI tumors than in lung cancers. The future perspective on HPD in GI tumor patients should be focused on the predictive biomarkers of response to immunotherapy, immuno-oncology mechanism, and the murine model of HPD.

Clear effect of immunotherapy

Tumor infiltrating lymphocytes in patients with HPD are rich in regulatory T (Treg) cells, a subset of $CD4^+$ T cells with immunosuppressive function. They highly express PD-1 or CTLA-4 and thus can be targeted by ICIs[40,41]. PD-1 blockade or deficiency in T cells enhances T cell receptor and CD28 signaling, which leads to the activation of Treg and conventional T cells. The former suppresses and the latter promotes antitumor immunity[42,43]. Anti-PD-1 antibody in Treg cells highly augments their proliferation and inhibition of antitumor immunity[44] in AGC patients[45,46]. Up-regulation of the EGFR pathway suppresses immune responses by activating Tregs after using ICIs[23]. Moreover, high Treg ratio is associated with poorer survival in patients with colorectal carcinoma and gastric cancer[47, 48].

INF-γ hypothesis

When utilizing ICIs, the $CD8^+$ T cells release INF-γ and up-regulate PD-L1 expression in tumor cells to make NLRP3 induce immunosuppressive myeloid-derived suppressor cells into the TME, which results in suppression of P53 and tumor growth[49].

Indoleamine 2,3-dioxygenase, an immunosuppressive enzyme, contributes to immune tolerance, inhibition of inflammation, and autoimmunity[50]. Up-regulation of indoleamine 2,3-dioxygenase inhibitors (IDOI) promotes the release of the immunosuppressive cytokines IL-10, angiopoietin 2, and INF-γ into the TME. It enhances the infiltration and proliferation of effector T cells and hyperactivates the JNK pathway, resulting in P53 suppression and activation-induced cell death (AICD), which leads to T-cell depletion[50-53]. IFN-γ also induces overexpression of interferon regulatory factor 8 by activating JAK-STAT signaling, which might stimulate mouse double minute 2 homolog (MDM2) expression[54-56]. Mechanistically, MDM2 negatively regulates T-cell activation through degradation of the

transcription factor NFATc2[57] or inhibits P53 activity by its direct interaction[58-60], suggesting a potential role of MDM2 in immune evasion.

CD38 hypothesis

CD38, a multifunctional ectoenzyme, modulates adenosine receptor signaling in the TME, leading to the inhibition of T-cell proliferation and function[49]. Adenosine in the TME has two dominating aspects: It increases the number of T-regulatory cells and the polarization of M2 macrophages; active adenosine A2A receptor in tumor cells induces treatment resistance and under-regulation of P53[61,62]. CD38 leads to the expression of AICD and FasL on T-cells[63] and angiopoietin 2 that promotes angiogenesis and triggers more invasive M2 macrophages expressing PD-L1. CD38 also makes tumor cells express HIF-1α to release insulin-like growth factor and vascular endothelial growth factor[64], which recruit Treg cells or promote tumor growth by initiating paracrine or autocrine signaling.

Other mechanisms

ICIs may stimulate tumor-infiltrating dendritic cells to secrete IL-10. It impedes antigen presentation and co-stimulation, which inhibits antigen-specific T cell responses. Alternative immune checkpoints increasing T cell depletion, such as LAG-3, T2M-3, and CTLA-4, might result in HPD[65]. TH1 and TH17 recruit neutrophil populations, causing inflammation that contributes to proliferation and survival of malignant cells, angiogenesis, metastasis, and subversion of adaptive immunity[66]. Group 3 innate lymphoid cells produce IL-22 to promote tumor growth through STAT3 activation[67]. Fc receptor promotes functional reprogramming by ICIs to make related immune cells, such as tumor-associated macrophages or M2-like $CD163^{+}CD33^{+}PD\text{-}L1^{+}$ epithelioid macrophages, more aggressively cause HPD [68,69]. CD74-MIF was found absent in HPD, thus we speculated that it potentially impairs proliferation of effector T cells, resulting in HPD[54]. Radiotherapy can lead to changes in the TME by inhibiting TGF expression[70]. Studies have confirmed that TGF-derived epithelial-mesenchymal transition increases mesenchymal cells, leads to tissue fibrosis, and restricts T cell movement and anti-tumor responses[71-73]. By limiting the infiltration of inflammatory/immune cells, it suppresses $CD8^{+}$ T cells and NK cell-mediated anti-tumor response[74]. HPD is associated with flared expansion of FoxP3 T-regulatory cells in gastric cancer patients[25].

CONCLUSION

The scientific community does not have a consensus on HPD definition, and different criteria are used for different cancer types. Whether ICIs are used or not, what appears to be HPD could be the natural progression of advanced cancer associated with MDM2/4 or EGFR signaling. The INF-γ and CD38 hypotheses have been studied in depth in the development of HPD. In the setting of immunotherapy, a large number of immunosuppressive and inflammatory factors affect the TME, resulting in decreased P53 expression or inducing oncogenic signaling, which are all potential mechanisms of HPD.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Whipple's pancreaticoduodenectomy at a resource-poor, low-volume center in Trinidad and Tobago

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Abstract

BACKGROUND

Many authorities advocate for Whipple's procedures to be performed in high-volume centers, but many patients in poor developing nations cannot access these centers. We sought to determine whether clinical outcomes were acceptable when Whipple's procedures were performed in a low-volume, resource-poor setting in the West Indies.

AIM

To study outcomes of Whipple's procedures in a pancreatic unit in the West Indies over an eight-year period from June 1, 2013 to June 30, 2021.

METHODS

This was a retrospective study of all patients undergoing Whipple's procedures in a pancreatic unit in the West Indies over an eight-year period from June 1, 2013 to June 30, 2021.

RESULTS

This center performed an average of 11.25 procedures per annum. There were 72 patients in the final study population at a mean age of 60.2 years, with 52.7% having American Society of Anesthesiologists scores ≥ III and 54.1% with Eastern Cooperative Oncology Group scores ≥ 2. Open Whipple's procedures were performed in 70 patients and laparoscopic assisted procedures in 2. Portal vein resection/reconstruction was performed in 19 (26.4%) patients. In patients undergoing open procedures there was 367 ± 54.1 min mean operating time, 1394 ± 656.8 mL mean blood loss, 5.24 ± 7.22 d mean intensive care unit stay and 15.1 ± 9.53 d hospitalization. Six (8.3%) patients experienced minor morbidity, 10 (14%) major morbidity and there were 4 (5.5%) deaths.

CONCLUSION
This paper adds to the growing body of evidence that volume alone should not be used as a marker of quality for patients requiring Whipple's procedures. Low volume centers in resource poor nations can achieve good short-term outcomes. This is largely due to the process of continuous, adaptive learning by the entire hospital.

Key Words: Pancreas; Surgery; Pancreatectomy; Whipple's; Pancreaticoduodenectomy

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Core Tip: Although conventional recommendations suggest that Whipple's procedures should only be performed in high-volume centers, this is not practical in many nations. This paper adds to the growing body of evidence that volume alone should not be used as a marker of quality for patients requiring Whipple's procedures. Low volume centers in resource poor nations can achieve good short-term outcomes. This is largely due to the process of continuous, adaptive learning by the entire hospital.

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INTRODUCTION

Whipple's procedure is a major operation designed to treat malignant peri-ampullary lesions[1,2]. Many Whipple's procedures tend to be concentrated in high-volume hospitals, usually found in high income nations[3,4,5].

Pancreatic surgeons in the West Indies work in limited-resource environments and perform small numbers of resections annually[5]. There are no centers that qualify as high-volume centers in this setting. Traditional teaching suggests that this scenario is not ideal. In this paper, we examine outcomes of Whipple's procedures at a low volume/resource environment in a West Indian nation.

MATERIALS AND METHODS

The country of Trinidad and Tobago is comprised of two small islands with a cumulative population of 1.35 million persons. A pancreatic surgery unit was established in the main referral hospital in 2013. This unit was led by a single fellowship-trained pancreatic surgeon, one dedicated senior resident and two junior residents. We received permission from the institutional review board to collect and examine data from all consecutive patients who underwent Whipple's procedures in this setting over an eight-year period from June 1, 2013 to June 30, 2021.

We identified patients by reviewing the hospital records and operating room log books. The hospital records for all patients who underwent Whipple's operations were retrieved for detailed review. The data extracted included diagnoses, performance scores, estimated operative blood loss, duration of operation (from incision to closure), therapeutic outcomes, post-operative morbidity and mortality. Complications were classified according to the modified Clavien-Dindo system[6]. Pancreatic leak was categorized according to the International Study Group on Pancreatic Fistula criteria. Cardiopulmonary complications included myocardial infarction, arrhythmia, congestive heart failure, pneumonia, pulmonary embolus, and respiratory failure. Statistical analyses were performed using SPSS ver 16.0.

RESULTS

There were 90 patients with operable peri-ampullary neoplasms who had Whipple's procedures attempted (mean annual case volume of 11.25). The detailed paper-based hospital record could not be retrieved in 14 cases. A search of the intensive care unit (ICU) and hospital registers indicated that these 14 patients were discharged from hospital alive, but they were excluded from the final analysis since their clinical details were not available. We also excluded 4 patients who were deemed irresectable at the time of operation and had palliative bypasses. The final study population included 72 patients who underwent Whipple's procedures.

There were 32 men and 40 women at a median age of 61 years (range 46-77; mean 60.2; SD ± 9.28). There were 62 (86%) patients with > 1 comorbid condition, 38 (52.7%) with American Society of Anesthesiologists (ASA) scores > II and 39 (54.1%) with performance scores > 1 (Tables 1 and 2). Pancreatic ductal adenocarcinoma was the commonest pathology, as outlined in Figure 1.

The operation was anticipated to be technically difficult in 26 (36.1%) persons due to: vein involvement requiring resection and reconstruction (19 patients), prior open surgery for abdominal sepsis (5 patients) and planned laparoscopic approach (2 patients).

Operative details

Four patients had palliative bypasses as they were deemed irresectable at the time of operation due to: invasion of common hepatic artery (1 patient), metastatic disease (2 patients) and portal vein encasement (1 patient). Data on these patients were excluded from further analysis.

In 70 cases, the operation was planned *via* the open approach using a modified Makuuchi incision, aided by an Omnitract® retractor (Integra Life Sciences, Princeton, NJ). This was our preferred incision as it afforded us good access to the pancreatico-duodenal complex in the retro-peritoneum.

Two patients underwent laparoscopic-assisted Whipple's procedures. In these cases, kocherization of the duodenum, dissection of the pancreatic neck tunnel, dissection of the gallbladder and structures in the hepatoduodenal ligament, transection of the stomach and full mobilization of the duodenum were completed laparoscopically. A 7 cm midline incision was used to create a pancreatico-gastrostomy, hepatico-jejunostomy and for specimen removal. Both of these patients had ampullary lesions and none required vein resection or reconstruction.

Clinical outcomes

The median operating time for open Whipple's procedures was 350 min (range 260-485; SD ± 54.1; mean 367). The median blood loss was 1.2 L (range 0.6-4.0; SD ± 0.7; mean 1.4) and 2 packed red cell units was the median transfusion rate (range 0-5; SD ± 1.4; mean 1.88).

Nineteen (26.4%) patients underwent planned vein resections and reconstruction. Reconstruction was performed with primary anastomoses in 13 cases, vein patches in 4 cases and interposition grafts in 2 cases.

In the patients with technically difficult operations, the duration of operation was 374 ± 57.34 minutes (mean ± standard deviation), estimated blood loss was 1494 ± 815 mL (mean ± standard deviation) and 2 ± 1.6 packed red cell units (mean ± standard deviation) were transfused per patient.

We insisted on a policy of mandatory admission to intensive care (ICU) after Whipple's resection since institutional limitations prevented the expected level of supportive care to be delivered in other areas. Our patients stayed in the ICU for 3 ± 7.22 d (mean ± standard deviation), with 29 (40.3%) needing extended stay > 72 h for ventilator and/or inotropic support. The median hospital stay for all patients was 12 ± 9.6 d (mean ± standard deviation).

Morbidity/mortality analysis

There were no complications in 56 patients within this series. Patients without complications remained in ICU for 3.5 ± 1.5 (mean ± standard deviation) d and remained hospitalized for 14 ± 7.9 (mean ± standard deviation) d. Patients who experienced a complication remained in ICU for 9.8 ± 11.3 (mean ± standard deviation) d and remained hospitalized for 15.3 ± 8.4 (mean ± standard deviation) d.

There were 16 (22.2%) patients with overall morbidity - 6 (8.3%) minor and 10 (14.0%) major (Table 3). There were 4 (5.5%) in-patient deaths: (1) A man at 53 years of age with pancreatic head adenocarcinoma who developed massive bleeding from a pseudoaneurysm that could not be controlled at re-operation; (2) A 59-year-old man who did not receive pre-operative stenting and had frank pus in the biliary system at operation. He developed bacteremia and septic shock; (3) A 48-year-old man who developed a leak from the jejuno-jejunal anastomosis, leading to intra-abdominal sepsis and multiple organ failure; and (4) A 70-year-old man with no prior cardiac history who succumbed to a massive myocardial infarction on day 5 post-operation.

In an attempt to analyze the outcomes chronologically, we tabulated the case volume by year (Table 4). The case volume remained relatively stable with time, although there was a notable reduction in the case volume for the last period (June 2020 to June 2020) due to effects of the coronavirus disease 2019 pandemic. We also analyzed complications and mortality chronologically as outlined in Figure 2. Although minor morbidity reduced over time, there was no statistically significant change in outcomes over time.

We also analyzed clinical outcomes according to patient risk as stratified by their performance scores and ASA scores (Table 5). Although there were trends toward greater morbidity and mortality in high-risk patients, none of these parameters attained statistical significance.

Table 1 American society of anesthesiologists scores for patients undergoing Whipple's procedures in a low volume caribbean centre

Score	American Society of Anesthesiologists Descriptor	No (%)
I	Completely healthy	10 (13.9)
II	Mild systemic disease	24 (33.3)
III	Severe systemic disease that is not incapacitating	30 (41.7)
IV	Incapacitating disease that is a threat to life	8 (11.1)
V	Moribund and not expected to survive > 24 h	0

Table 2 Performance scores for patients undergoing Whipple's procedures in a low volume caribbean centre

Grade	Eastern Cooperative Oncology Group performance status	No (%)
0	Fully active, able to carry out all activities without restriction	13 (18.1)
1	Restricted in physically strenuous activity, but ambulatory and able to carry out light work	20 (27.8)
2	Ambulatory and capable of self care, but unable to carry out work activities. Up and about > 50% of waking hours	34 (47.2)
3	Capable of limited self care and confined to bed or chair for more than 50% of waking hours	4 (5.6)
4	Completely disabled and cannot carry on self care. Confined to bed or chair	1 (1.4)
5	Dead	0

Table 3 Complications after Whipple's procedures in a low volume caribbean centre

Morbidity	Description	No	%
Overall	Number of patients with any complication	16	22.2
Minor	Clavien-Dindo I or II	6	8.3
	Pneumonia	2	
	Deep vein thrombosis	1	
	Delayed gastric emptying	1	
	Gastrointestinal bleeding	2	
Major	Clavien-Dindo III or IV	10	13.9
	Anastomotic dehiscence	1	
	Massive upper gastrointestinal bleeding	1	
	Myocardial infarction	3	
	Pseudoaneurysm	2	
	Biliary sepsis as a source of septicemia	2	
	Post-operative pancreatic fistula/intra-abdominal collection	1	
Mortality	30-d mortality: All causes: (1) Massive bleeding from a pseudoaneurysm; (2) Generalized sepsis secondary to cholangitis; (3) Jejuno-jejunal anastomotic leak leading to multiple organ failure; and (4) Myocardial infarction	4	5.6

DISCUSSION

Although it is a high-risk operation, Whipple's procedure is the only existing treatment with the potential to cure peri-ampullary malignancies[7,8]. Early series in the late 1960s reported 60% post-operative morbidity and mortality rates approaching 25%[9-11], but with better surgical equipment and supportive care, the safety profile has improved. Modern reports document 30-d mortality rates between 4%-6%[7,8,12-14].

Much of the recent progress in surgical treatment has been aimed at minimizing peri-operative morbidity with a multidisciplinary approach to care[1,2], advanced cross-sectional imaging[1,15], specialized surgical equipment[5], appropriate support services[16,17], full-time intensive care, interventional radiology and gastroenterology services[1,14-17] and quaternary surgical training. In our

Table 4 Chronologic analysis of clinical outcomes after Whipple's procedures in a low volume caribbean centre

Year	Case volume	Minor morbidity	Major morbidity	Mortality
2013-2014	9	0	1	1
2014-2015	10	1	2	0
2015-2016	11	1	3	0
2016-2017	8	1	1	1
2017-2018	10	1	2	1
2018-2019	8	0	0	0
2019-2020	11	1	1	1
2020-2021	5	1	0	0
Total	72	6 (8.3%)	10 (13.9)	4 (5.6%)

Table 5 Clinical outcomes stratified according to patient risk

American Society of Anesthesiologists scores			
Parameter	ASA I-II (34)	ASA III-IV (38)	*P*
Overall morbidity	7 (20.6%)	9 (23.7%)	0.7843
Overall mortality	2 (5.9%)	2 (5.3%)	1.000
Eastern Cooperative Oncology Group performance status			
Parameter	ECOG 0-2 (67)	ECOG 3-4 (5)	*P*
Overall morbidity	14 (20.9%)	2 (40%)	0.307
Overall mortality	3 (4.5%)	1 (20%)	0.255

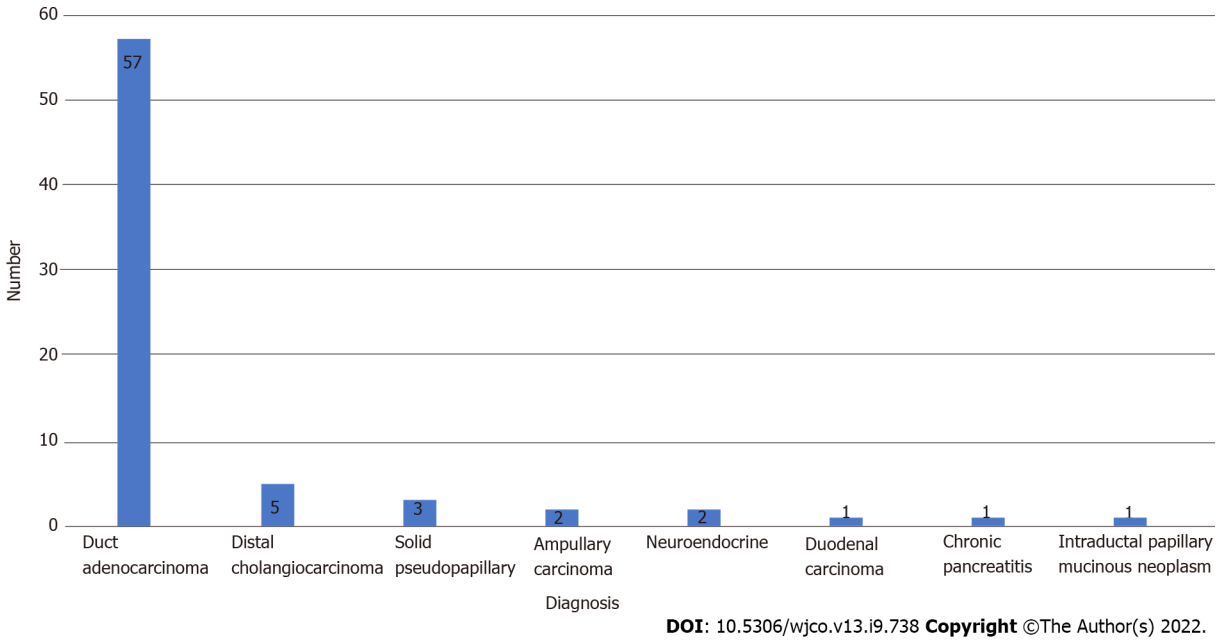
ASA: American Society of Anesthesiologists; ECOG: Eastern Cooperative Oncology Group.

center, we have been able to achieve many of these goals.

Another change was the centralization concept, that was popularized in the early 21st century[3,4,11-13]. Published data showed reductions in morbidity[1,2,18], cost[19], mortality[1,2,18,10,20-22] and hospitalization[1,18] in high-volume hospitals. However, the high-volume definition remained elusive. Some have designated centers performing as few as 3 Whipple's procedures per annum as high-volume centers[12,14,19], while others reserve this designation for facilities performing ≥ 30 per annum[18,23,24]. Most researchers quote numbers ≥ 18 Whipple's procedures per annum as high volume[2,7,11,18,22-26]. Using this definition, our facility did not qualify as high-volume. Although our center has documented the largest volume of Whipple's procedures per annum in the region[5], we still only performed an average 11.25 cases per annum.

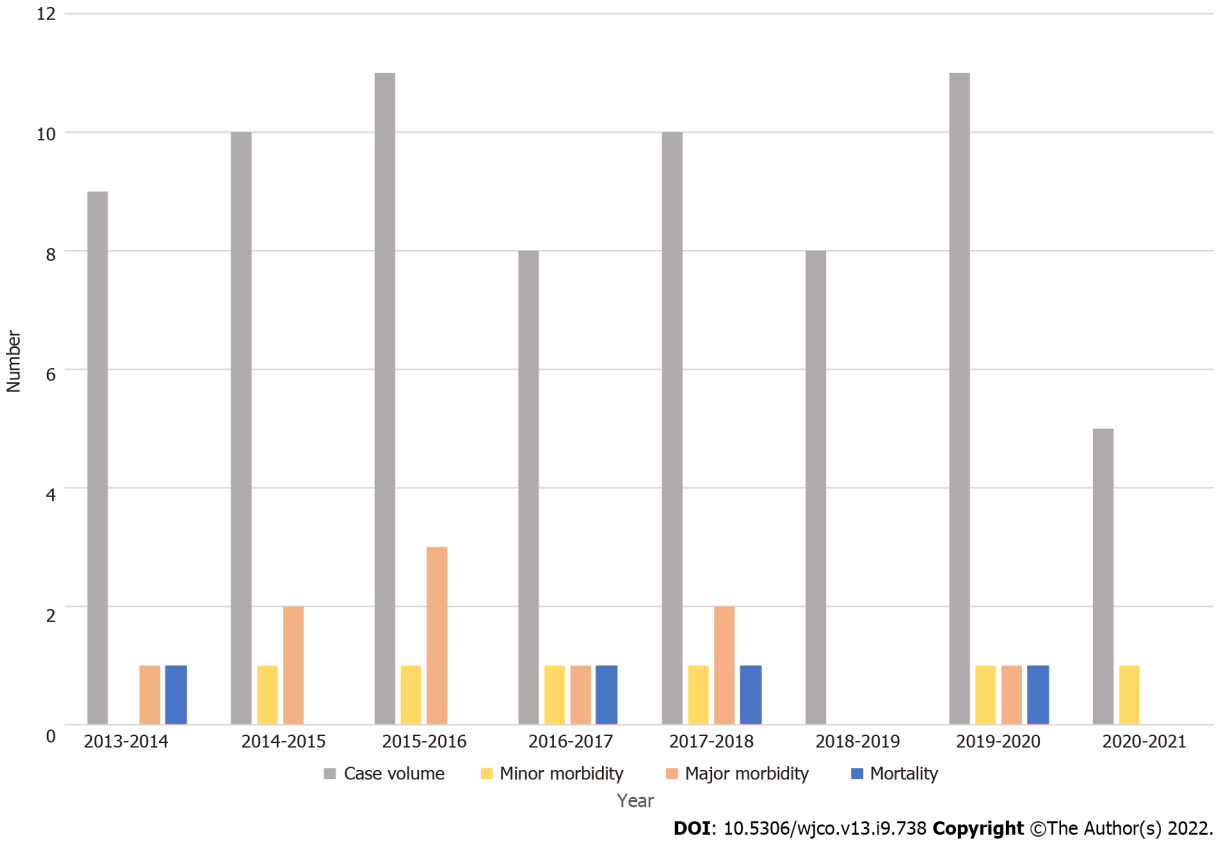
Centralization remains controversial. Even in developed countries, most Whipple's procedures are still performed in low-volume centers[7,20,27]. In Texas, Riall *et al*[27] reported that ≥ 25% of Whipple's procedures were performed in hospitals doing < 5 cases per annum and 35% were done in hospitals performing < 10 cases per annum. Similarly, McPhee *et al*[22] reported that 61% of Whipple's procedures across the United States in the year 2007 were done outside of high-volume centers. Furthermore, it has been documented that centralization contributes to health care inequity, with significantly fewer females[27], non-caucasians[18,27-31], persons from low socio-economic brackets [32], persons from low-income zip codes and persons without private health insurers[18,27-30] being able to access care in these centers. We do not believe that the traditional centralization concept is practical for the West Indies due to travel restrictions, low health insurance rates, financial limitations and absent social support pathways.

Despite the fact that these surgeons performed small numbers of operations in a setting with scarce blood products, limited operating time and restricted intensive care support, short-term outcomes were still reasonable. The 30-d mortality in high-volume centers ranged widely, but most high-volume centers maintained 30-d mortality rates between 4%-6%[2-4,7,11-14,18,22,26,27]. At 5.5%, our 30-d mortality compared favorably. Similarly, our major morbidity rates compared favorably with high-volume centers reporting figures that ranged from 16%[33] to 26%[1]. Several authors have advocated documentation of procedure-related complications that include pancreatic fistula, delayed gastric emptying, intra-abdominal sepsis and intra-abdominal haemorrhage[34]. In our series the incidence of



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Figure 1 Histologic diagnoses of patients undergoing Whipple's procedure.



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Figure 2 Chronologic analysis of clinical outcomes.

these procedure-specific complications was acceptable (1.4%, 1.4%, 2.8% and 2.8% of cases respectively).

It has been demonstrated in the medical literature that procedure-related complications are similar between low- and high-volume hospitals, but there is a significant difference in medical complications such as aspirations, pneumonia, pulmonary failure, renal failure and septicemia[33,34]. This reinforces the thinking that, while surgical expertise is necessary, it alone is not sufficient to guarantee good post-operative outcomes[2,33,34]. Medical complications occurred in 8.3% of our cases, suggesting that there may still be room for us to optimize support care/medical services.

Schmidt *et al*[2] introduced the concept of the "experienced surgeon" as being distinct from a "high-volume surgeon." They defined the "experienced surgeon" as one who performed ≥ 50 Whipple's procedures in their career, regardless of the interval[2]. They also made the point that experienced surgeons may not be high volume surgeons (which was time dependent) and demonstrated that experienced surgeons with low annual volumes had equivalent outcomes to high-volume surgeons[2]. The pancreatic surgeon in this setting was experienced, having performed ≥ 100 Whipple's procedures. We believe that this contributed to the outcomes reported in this paper, and gives support to Schmidt's concept of the experienced surgeon.

In their paper, Schmidt *et al*[2] counted the number of procedures in which a vein resection was performed as a surrogate marker of technical complexity and surgeon experience. In our series, 26.4% of patients had portal vein resection and reconstruction. It should be noted, however, that while these were experienced surgeons, they would have accrued much of their experience in high volume centers in developed countries during fellowship training. These facilities operate under different circumstances. Upon repatriation to the West Indies, these surgeons would have to adapt to challenging, new working environments. These surgeons adapted their practices to the new environment, focusing on peri-operative management and inter-disciplinary cooperation that evolved with time and were hospital-specific. This interaction and continuous institutional learning are difficult to measure and would evolve specific to each surgeon's health care environment. Several authors have alluded to the concept of continuous, adaptive learning by the institution[1,2,7,18,35-37]. This is not limited to the surgeons alone, but includes pre-operative evaluation, multidisciplinary team interaction, intra-operative anaesthesia care, surgeon training, post-operative care pathways, post-procedure nursing care, ICU care, availability of emergency medical doctors and experienced subspeciality supportive care [1,2,7,18,35-37].

We agree that Whipple's procedure is a complex operation that depends heavily on surgeon experience. At the same time, we believe that there is more to experience than technical facility. For example, the experienced surgeon would know how to resect and reconstruct the portal vein when required to achieve negative margins[2], when not to operate on patients[2], to recognize aberrant anatomy[2], how to get out of trouble when complications occur intra-operatively[7]. These can only be learned with experience and proper mentorship[2].

Recently, there has been focus on learning curves as a part of the concept of surgeon volume and surgeon experience. Tseng *et al*[38] suggested that after 60 Whipple's procedures, surgeons improved on peri-operative outcomes such as blood loss, operation time, hospital stay and margin status. However, the most senior author in our paper performed over 300 Whipple's and felt that he was still improving well beyond 200 cases, although the steepest part of the curve was the first 50. Similarly, the first author who performed all Whipple's procedures in this series felt that he continued to improve during this series. It seems reasonable to conclude that the learning curve lies somewhere between 50 and 70 cases.

We believe that multiple factors contributed to the outcomes in our setting: (1) Population-based data [5]; (2) Training of unit staff; (3) Developing an intimate knowledge of the hospital; (4) Fostering teamwork; (5) Diligent administration of care; and (6) Regular audits. We also advocate two experienced surgeons operating to maximize experience. Also, if one surgeon is more experienced it speeds up the learning curve for the second surgeon. The key is overall team experience because, in addition to reducing intraoperative complications, effectively managing post-operative complications is important.

LIMITATIONS

It is tempting to think that the outcomes reported here may be biased due to case selection. However, we do not believe that this was the case in our setting because this was a government funded hospital and we were required to provide care for all patients who presented to this unit. In addition, many of our patients were physiologically challenging, with 52.7% having ASA scores ≥ III and 54.1% with ECOG scores ≥ 2.

The retrospective study design did limit our ability to collect detailed clinical information, such as accurate blood loss, adherence to post-care pathways and, as previously noted, we were unable to locate paper-based records for 14 patients who underwent Whipple's procedures.

CONCLUSION

This paper adds to the growing body of evidence that volume alone should not be used as a marker of quality for patients requiring Whipple's procedures. Low volume centers in resource poor nations can achieve good short-term outcomes. This is largely due to the process of continuous, adaptive learning by the entire hospital and includes: Population-based data, good teamwork, effective staff training, regular audit and due diligence in care administration.

ARTICLE HIGHLIGHTS

Research background

Whipple's operations are high-risk operations that should be done in high-volume centers for optimal outcomes. This is supported by data from several high-volume hospitals.

Research motivation

High-volume centers are usually in developed nations. There are no high-volume centers in the West Indies. In this setting, pancreatic surgeons have to perform Whipple's operations in resource-poor, low-volume settings. This scenario is not ideal, but it is the reality on the ground.

Research objectives

We sought to document the clinical outcomes when Whipple's operations were performed in resource-poor, low-volume centers in the West Indies. If the outcomes are poor, this would be impetus not to perform these operations in this setting or to develop service centralization with high-volume centers.

Research methods

A retrospective audit of all Whipple's operation performed at a referral center over an eight-year period was performed. Data collected from hospital records included: diagnoses, performance scores, estimated operative blood loss, duration of operation, therapeutic outcomes, post-operative morbidity and mortality. Statistical analyses were performed using SPSS version 16.0.

Research results

This facility performed 11.25 Whipples procedures per annum. There were 72 patients in the final study population at a mean age of 60.2 years. Open Whipple's procedures were performed in 70 patients and laparoscopic assisted procedures in 2. Portal vein resection/reconstruction was performed in 19 (26.4%) patients. In patients undergoing open procedures there was 367 ± 54.1 min mean operating time, 1394 ± 656.8 mL mean blood loss, 5.24 ± 7.22 d mean intensive care unit stay and 15.1 ± 9.53 d hospitalization. Six (8.3%) patients experienced minor morbidity, 10 (14%) major morbidity and there were 4 (5.5%) deaths.

Research conclusions

Low volume centers in resource poor nations can achieve good short-term outcomes once they pay attention to continuous, adaptive learning. Volume alone should not be used as a marker of quality for patients requiring Whipple's procedures.

Research perspectives

The direction of future research is to identify specific hospital-based pathways and/or team-focused processes that improve clinical outcomes in low-volume facilities.

FOOTNOTES

Author contributions: Cawich SO, Naraynsingh V, Thomas D and Pearce NW designed and coordinated the study; Pearce NW, Thomas D and Naraynsingh V acquired and analyzed data; Cawich SO, Naraynsingh V, Thomas D and Pearce NW interpreted the data; Cawich SO, Naraynsingh V, Thomas D and Pearce NW wrote the manuscript; all authors approved the final version of the article.

Institutional review board statement: The study was reviewed and approved by the University of the West Indies Institutional Review Board (CREC-SA.1623/06/2022).

Informed consent statement: This was a retrospective audit of written hospital records and so informed consent was waived by the institutional review board.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Data sharing statement: All data are available from the corresponding author upon reasonable request at tt.liver.surgery@gmail.com.

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ORIGINAL ARTICLE

Observational Study

Factors predicting upstaging from clinical N0 to pN2a/N3a in breast cancer patients

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Abstract

BACKGROUND

With sentinel node metastasis in breast cancer (BC) patients, axillary lymph node (ALN) dissection is often omitted from cases with breast-conserving surgery. Omission of lymph node dissection reduces the invasiveness of surgery to the patient, but it also obscures the number of metastases to non-sentinel nodes. The possibility of finding ≥ 4 lymph nodes (pN2a/pN3a) preoperatively is important given the ramifications for postoperative treatment.

AIM

To search for clinicopathological factors that predicts upstaging from N0 to pN2a/pN3a.

METHODS

Patients who were sentinel lymph node (SLN)-positive and underwent ALN

dissection between September 2007 and August 2018 were selected by retrospective chart review. All patients had BC diagnosed preoperatively as N0 with axillary evaluation by fluorodeoxyglucose (FDG) positron emission tomography/computed tomography and ultrasound (US) examination. When suspicious FDG accumulation was found in ALN, the presence of metastasis was reevaluated by second US. We examined predictors of upstaging from N0 to pN2a/pN3a.

RESULTS

Among 135 patients, we identified 1-3 ALNs (pN1) in 113 patients and ³4 ALNs (pN2a/pN3a) in 22 patients. Multivariate analysis identified the total number of SLN metastasis, the maximal diameter of metastasis in the SLN (SLNDmax), and FDG accumulation of ALN as predictors of upstaging to pN2a/pN3a.

CONCLUSION

We identified factors involved in upstaging from N0 to pN2a/pN3a. The SLNDmax and number of SLN metastasis are predictors of ≥ 4 ALNs (pN2a/pN3a) and predictors of metastasis to non-sentinel nodes, which have been reported in the past. Attention should be given to axillary accumulations of FDG, even when faint.

Key Words: Breast cancer; Axillary lymph node metastasis; Positron emission tomography/computed tomography; Sentinel lymph node; Predictive factors of lymphnode metastasis; Standardized uptake value max; Diameter of sentinel lyphonode metastasis

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Core Tip: This is the first report to include the results of preoperative positron emission tomography/computed tomography (PET/CT) and to examine results related to the upstaging of pN2a/pN3a (more than 4 axillary lymph node metastases) in breast cancer (BC) patients. Specifically, 135 patients who were sentinel lymph node (SLN)-positive and underwent ALN dissection were selected by retrospective chart review, all of whom had BC diagnosed preoperatively as N0 with axillary evaluation by fluorodeoxyglucose (FDG) PET/CT and ultrasound. Our results suggest that the size and number of SLN metastases were still important factors. And, attention should be given to axillary accumulations of FDG, even when faint.

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INTRODUCTION

Sentinel lymph node (SLN) biopsy is usually performed in N0 cases. If SLN biopsy yields a positive result, an axillary lymph node (ALN) dissection is performed. However, since the publication of findings from the American College of Surgeons Oncology Group (ACOSOG) Z-0011 trial, many cancer centers have been omitting ALN dissection from breast-conserving surgeries[1]. The problem with omitting axillary dissection is that the number of non-SLN metastases cannot be ascertained, which may lead to over or under-treatment with adjuvant chemotherapy and radiation therapy. For example, patients with pN2a [4-9 ALN metastases (ALNMs)] or pN3a (³10ALNMs) need to be irradiated to not only the residual breast, but also the supraclavicular region after breast-conserving surgery. However, without knowing the number of metastases, no accurate decision on the need for irradiation can be made. Post-mastectomy radiation (PMRT) is also required for pN2a/N3a patients. Some reports have noted that PMRT after reconstruction impairs conformity[2-4]. Further analyses have shown that direct implants and autologous tissue reconstruction have fewer complications from PMRT[5,6]. The ability to predict the necessity of postoperative irradiation before surgery would affect surgical planning, including reconstructive surgery. A variety of factors and nomograms have been reported to allow preoperative prediction of the presence or absence of ALNMs[7-9], but few reports have examined factors predicting the presence of ³4 ALNMs. Preoperative fluorodeoxyglucose (FDG) positron emission tomography (PET)/computed tomography (CT) is reportedly excellent for predicting metastasis preoperatively and may have influenced the prediction of ALNMs in the present study. Although many

papers have described predictors of non-SLN metastasis, few have rigorously assessed ALNM preoperatively using PET/CT. In this study, all patients were evaluated preoperatively by PET/CT, and cases with false-negative results on other imaging modalities were excluded. This is the first report to include the results of preoperative PET/CT and to examine results related to the upstaging of pN2a/pN3a.

MATERIALS AND METHODS

Patients

A retrospective chart review was conducted for patients who were SLN-positive and underwent ALND between September 2007 and August 2018. All patients had breast cancer (BC) diagnosed preoperatively as N0 with axillary evaluation by PET/CT and ultrasound (US) examination. This study was conducted with approval from the institutional review board and with the informed consent of each patient. Axillary dissection was performed in all patients with SLN metastasis > 2 mm in diameter. In the case of total mastectomy, axillary dissection was performed for metastases > 0.2 mm in diameter. A flow chart of the eligible/included patients is shown in Figure 1.

About ultrasound examination

A EUB-7500 scanner with a EUP-L54MA 9.75-MHz linear probe (Hitachi Medical Systems, Tokyo, Japan) or Aplio XG scanner with a PLT-805AT 8.0-MHz linear probe (Toshiba Medical Systems, Tochigi, Japan) was used for US examinations. If ultrasound or PET/CT findings were suspicious for metastatic lymph nodes, cytology was performed.

Protocol for FDG-PET/CT

All patients were intravenously administered ^{18}F-FDG (3.7 MBq/kg; 0.1 mCi/kg) after a minimum 4-h fasting period. Next, whole-body images were routinely obtained using a PET/CT system (Aquiduo; Toshiba Medical Systems, Tokyo, Japan). In addition, CT was performed using the following parameters: pitch factor, 0.938; gantry rotation time, 0.5 s; table time, 30 mm/s; auto-exposure control (SD20), 120 kVp; and slice thickness, 2.0 mm. Notably, contrast media were not used for CT examinations. Approximately 60 min after ^{18}F-FDG administration, whole-body PET was performed using the following parameters: emission time per bed, 2 min; bed positions, 7-8; slice thickness, 3.375 mm; and matrix, 128 × 128.

Data analysis of FDG-PET/CT

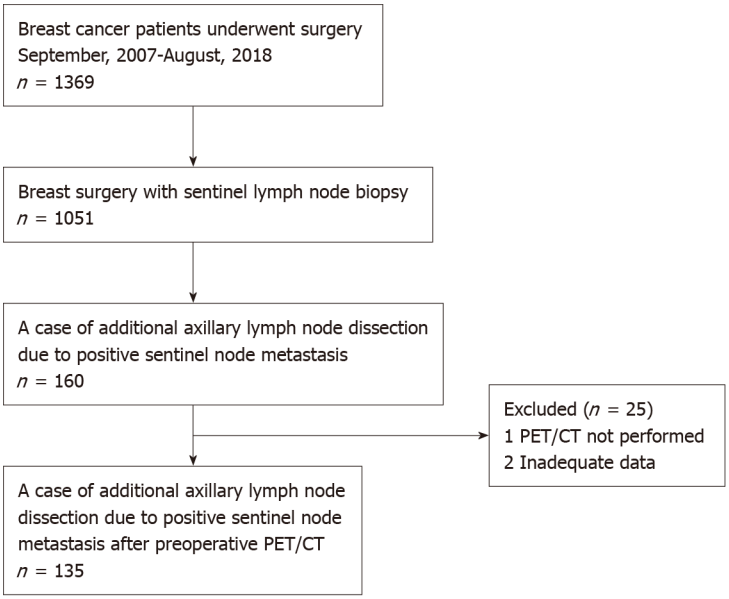
In the present study, ^{18}F-FDG-PET/CT findings at each examination were assessed using a consensus reading by two breast radiologists (T.F. with 14 years of experience in breast imaging; M.M. with 10 years of experience in breast imaging). We performed visual analysis of primary lesions and ALNs without defining a cut-off value. Of note, lesions with an ^{18}F-FDG uptake value higher than that of the background tissue were defined as FDG-positive.

Immunohistochemical examination

All specimens were analyzed by pathologists from our institution, and specimens were considered estrogen receptor (ER) positive on immunohistochemistry (IHC) for staining rates higher than 10%. For human epidermal growth factor receptor 2 (HER2) values, and IHC result of 3+ was defined as BC with strong, complete membrane staining observed in at least 10% of tumor cells. For HER2 overexpression of 2+, gene amplification with fluorescence in situ hybridization was performed in this study.

Statistical analysis

Differences in proportions of categorical data were tested using Fisher's exact probability test. Unless otherwise indicated, significant differences among mean values of numerical data were analyzed using Mann-Whitney test. Relationships between the size of SLN metastases and the number of ALNMs were measured using Spearman rank correlation analysis, which can have a magnitude ranging from 0 to 1, with 0 denoting no correlation at all and 1 denoting complete correlation. Predictors of upstaging to pN2a/pN3a were determined by univariate and multivariate logistic regression analyses. Values of $P < 0.05$ were regarded as statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interfaces for R (The R Foundation for Statistical Computing, Vienna, Austria)[10]. More precisely, EZR is a modified version of R Commander designed to add statistical functions frequently used in biostatistics.



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Figure 1 A flowchart of eligible patients. PET/CT: Positron emission tomography/computed tomography.

RESULTS

Demographic and clinical characteristics of study subjects

We retrospectively analyzed 135 BC patients who were SLN-positive and had undergone ALN dissection. FDG-PET/CT was performed in all cases preoperatively. The detailed clinicopathological characteristics of subjects are summarized in Table 1. Among these, 113 patients showed 1-3 ALNMs (pN1) and 22 patients had ³4 ALNMs (pN2a/pN3a). FDG accumulation in ALNs was found on PET/CT in 19 cases (14.1%). The mean standardized uptake value (SUV) max was 1.48 (range, 0.8-2.0). Preoperative PET/CT showed accumulation in the ALNs, and second-look US was performed in cases where metastasis could not be ruled out. The mean number of excised SLNs was 1.6 (range, 1-5) and the mean number of SLN metastasis was 1.2 (range, 1-3). The median maximal diameter of metastasis in the SLN (SLNDmax) was 3 mm. The correlation between the SLNDmax and the number of ALNMs is shown in Figure 2. A strong correlation was identified between SLNDmax and the number of ALNMs ($P < 0.001$). We measured the cut-off value for the SLNDmax from the receiver operating characteristic (ROC) curve and the cut-off value for upstaging from N0 to pN2a/pN3a was set at 5 mm (area under the curve: 0.873; 95%CI: 0.808–0931) (Figure 3). Table 2 shows a comparison between pN1 and pN2a/pN3a.

Univariate logistic regression analysis of risk factors associated with upstaging

To search for risk factors for upstaging to pN2a/N3a, univariate and multivariate analyses were performed for associations with clinicopathological factors in two groups of pN1 and pN2a/N3a cases. Invasive diameter at the primary site, number of SLN metastasis, FDG accumulation in ALNs, and SLNDmax were associated with upstaging to pN2a/pN3a, but age, ER status, HER 2 status, tumor grade, and SUVmax at the primary site were not (Table 3).

Multivariate logistic regression analysis of risk factors associated with upstaging

Multivariate logistic regression analysis of clinicopathologic factors was used to examine risk factors for upstaging to pN2a/pN3a. The number of SLN metastasis, SLNDmax, and FDG accumulation in ALNs were associated with upstaging (Table 3).

DISCUSSION

The ACOSOG Z-0011 trial concluded that ALN dissection is not always necessary for women undergoing breast-conserving surgery with 1-2 positive SLNs[1]. However, the overall number of ALNMs represents crucial clinical information. Although avoidance of ALN dissection reduces the degree of surgical invasiveness of a procedure, the number of ALNMs cannot be ascertained. If more than 4 metastatic lymph nodes are present, radiation to the breast or chest wall as well as to the supraclavicular area is necessary[11]. Thus, not knowing the number of ALNMs may lead to over-or under-

Table 1 Background characteristics of all patients

Background characteristics (*n* = 135)	
Age (yr)	56 (35-84)
SUVmax of primary tumor	3.05 (0.8-11.9)
pN1	113
pN2a or pN3a	22
The invasion diameter of the primary lesion	
≤ 20 mm	67
< 50 mm	55
≥ 50 mm	11
NA	2
Maximal diameter of metastasis in the sentinel lymph nodes (mm)	3 (0.1-25)
The number of SLN metastasis	
1	112
2	19
3	4
ER	
Positive	118
Negative	14
Unknown	3
HER2	
Positive	8
Negative	125
Unknown	2
Nuclear grade of biopsy specimen	
1, 2	103
3	26
Unknown	6
FDG accumulation of axillary lymph nodes	
Yes	19
No	116

SUV: Standardized uptake value; NA: Not applicable; SLN: Sentinel lymph node; ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; FDG: Fluorodeoxyglucose.

treatment with radiation therapy postoperatively. The presence or absence of postoperative radiation may also affect the choice of reconstructive technique. Radiation during the insertion of an expander is associated with a greater risk of complications, while radiation after implant placement or to autologous tissue is reported to have fewer complications[2,3]. Pre- and postoperative prediction of the number of ALNMs affects not only the optimal extent of axillary dissection, but also the choice of radiotherapy and, indirectly, reconstruction methods. In the present study, we searched for predictors of upstaging from clinical N0 to pN2a/pN3a using factors identified pre- or intraoperatively, including FDG-PET/CT. This imaging modality is generally considered useful in searching for ALNMs[12]. However, few reports have specified whether preoperative PET/CT was performed when examining factors predicting non-SLN metastasis. As a result of examining various factors for upstaging, we extracted SLNDmax, mild accumulation of FDG in the axilla, and the number of SLN metastasis. The SLNDmax and the number of SLN metastasis have been reported in the past as predictors of metastasis to non-SLNs[13-16]. This is the first study to show that these factors are also important in upstaging from N0 to pN2a/pN3a. Various cut-offs for the SLNDmax have been reported as a predictor of non-SLN

Table 2 Comparison between pN1 and pN2a/pN3a

Total (*n* = 135)	pN1	pN2a/pN3a	*P* value
	n = 113	*n* = 22	
Age	57.96 ± 12.14	59.64 ± 12.73	0.56
The invasion diameter of the primary lesion (mm)	22.77 ± 14.76	39.86 ± 21.73	< 0.001
Maximal diameter of metastasis in the SLNs	3.46 ± 2.80	9.19 ± 5.66	< 0.001
The number of SLN metastasis			0.014
1	98	14	
2 and more	15	8	
ER			0.47
Positive	100	18	
Negative	11	3	
Unknown	2	1	
HER2			1
Positive	7	1	
Negative	104	21	
Unknown	2	0	
Nuclear grade of biopsy specimen			0.77
1, 2	87	16	
3	21	5	
Unknown	4	1	
SUVmax of the primary tumor	3.80 ± 2.50	4.13 ± 2.77	0.61
FDG accumulation of axillary lymph nodes			< 0.001
No	104	11	
Yes	8	11	
Unknown	1	0	

ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; SLN: Sentinel lymph node; SUV: Standardized uptake value; FDG: Fluorodeoxyglucose.

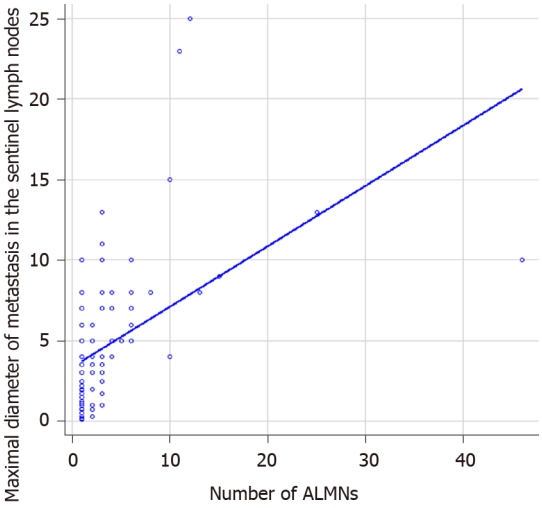
metastasis, and we used ROC curves in our search. As a result, the predictor for upstaging from N0 to pN2a/pN3a was set at 5 mm. If the number of SLN metastasis is large (that is, two or more) or the diameter of metastasis is large (more than 5 mm), or if FDG accumulation in the axilla is mild even if no metastases have been confirmed, irradiation to the chest wall and supraclavicular region may need to be considered even in cases where axillary dissection has been omitted. PET/CT is often performed preoperatively for different cancers. On the other hand, findings from this modality are reportedly less significant for low-stage BC, given the low likelihood of distant metastasis. Some reports have suggested that SUV at the primary site may offer a useful predictor of metastasis to non-SLNs[17]. Ueda *et al*[18] evaluated ALNMs using PET/CT and reported low sensitivity but high specificity. In the present study, we found PET/CT to be useful in predicting multiple ALNMs, but the positive predictive value was not particularly high (57.9%). Many questions remain unanswered, such as the optimal cut-off value for integrated SUV, the influence of the histological type of the primary tumor, and the suitable timing of biopsy.

Further accumulation and analysis of cases are needed. Institutions that aggressively omit axillary dissection should be aware of the dangers of remaining ignorant of the number of ALNMs when multiple risk factors are identified pre- or intraoperatively.

Table 3 Uni- and multivariate logistic regression analysis of risk factors associated with upstaging pN2a/pN3a

Factors	Univariate logistic regression analysis to predictive factors of pN2a/pN3a			Multivariate logistic regression analysis to predictive factors of pN2a/pN3a		
	Odds ratio	95%CI	*P* value	Odds ratio	95%CI	*P* value
Age (yr) (< 50 *vs* ≥ 50)	0.863	0.333-2.24	0.76			
ER (positive *vs* negative)	0.66	0.167-2.60	0.55			
HER2 (positive *vs* negative)	0.833	0.954-7.28	0.87			
Invasive diameter of the primary site (≤ 20 mm *vs* > 20 mm)	4.3	1.48-12.50	0.007	3.53	0.963-13.0	0.057
Nuclear grade (1, 2 *vs* 3)	1.29	0.426-3.393	0.65			
Total positive SLNs (1 *vs* 2 and more)	3.73	1.34-10.40	0.012	3.92	1.01-15.3	0.048
Maximal diameter of metastasis in the SLNs (< 5 mm *vs* ≥ 5 mm)	21.2	4.68-96.3	< 0.001	15.6	3.08-79.2	< 0.001
SUVmax of primary tumor (< 3.1 *vs* ≥ 3.1)	0.96	0.351-2.62	0.94			
FDG accumulation of axillary lymph nodes (yes *vs* no)	13	4.32-39.2	< 0.001	4.84	1.29-18.2	0.02

ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; SLN: Sentinel lymph node; SUV: Standardized uptake value; FDG: Fluorodeoxyglucose.

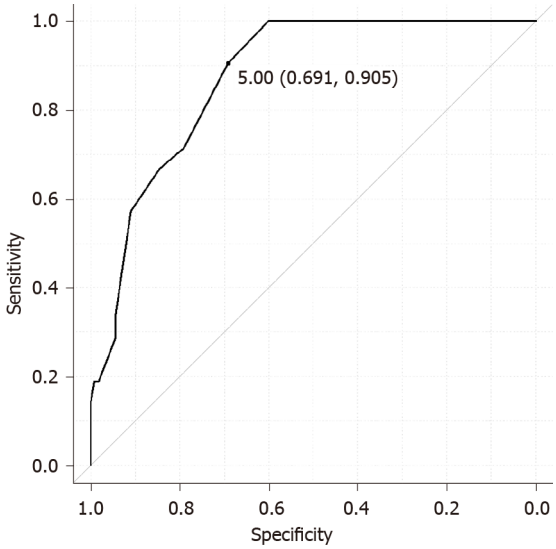


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Figure 2 Correlation between the maximal diameter of metastasis in the sentinel lymph nodes and number of axillary lymph node metastases. A strong correlation is evident between maximal diameter of metastasis in the sentinel lymph nodes and the number of axillary lymph node metastases (*P* < 0.001). ALMNs: Axillary lymph node metastases.

CONCLUSION

The present study investigated factors predictive of upstaging from clinical N0 to pN2a/pN3a. Factors such as the number of metastases and SLNDmax, which have previously been reported as predictors of metastasis to non-SLNs, were also useful in predicting upstaging to pN2a/pN3a and emphasized the utility of FDG-PET/CT. The only factor that predicts preoperatively, but not intraoperatively, is the result of FDG-PET/CT.



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Figure 3 Cut-off value for the maximal diameter of metastasis in the sentinel lymph node from the receiver operating characteristic curve and cut-off for upstaging from N0 to pN2a/pN3a set to 5 mm (area under the curve: 0.873, 95%CI: 0.808-0931).

ARTICLE HIGHLIGHTS

Research background

With sentinel node metastasis in breast cancer (BC) patients, axillary lymph node (ALN) dissection is often omitted from cases with breast-conserving surgery. Omission of lymph node dissection reduces the invasiveness of surgery to the patient, but it also obscures the number of metastases to non-sentinel nodes.

Research motivation

The possibility of finding ≥ 4 lymph nodes (pN2a/pN3a) preoperatively is important given the ramifications for postoperative treatment.

Research objectives

The purpose of this study is to search for clinicopathological factors that predict upstaging from N0 to pN2a/N3a.

Research methods

Patients who were SLN-positive and underwent ALN dissection between September 2007 and August 2018 were selected by retrospective chart review. All patients had BC diagnosed preoperatively as N0 with axillary evaluation by fluorodeoxyglucose (FDG) positron emission tomography/computed tomography and ultrasound (US) examination. When suspicious FDG accumulation was found in ALN, the presence of metastasis was reevaluated by second US. We examined predictors of upstaging from N0 to pN2a/pN3a.

Research results

Among 135 patients, we identified 1-3 ALNs (pN1) in 113 patients and ³4 ALNs (pN2a/pN3a) in 22 patients. Multivariate analysis identified the total number of SLN metastasis, the maximal diameter of metastasis in the SLN (SLNDmax), and FDG accumulation of ALN as predictors of upstaging to pN2a/pN3a.

Research conclusions

We identified factors involved in upstaging from N0 to pN2a/pN3a. The SLNDmax and number of SLN metastasis are predictors of ≥ 4 ALNs (pN2a/pN3a) and predictors of metastasis to non-sentinel nodes, which have been reported in the past. Attention should be given to axillary accumulations of FDG, even when faint.

Research perspectives

It is somewhat possible to predict upstaging to pN2a/pN3a by searching for clinicopathological factors

FOOTNOTES

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LETTER TO THE EDITOR

Neoadjuvant immunotherapy in non-small-cell lung cancer: Times are changing—and fast

Carlos Aguado, Unai Jiménez Maestre, Xabier Mielgo-Rubio

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Abstract

Recent data from a phase 3 trial have shown that the addition of immunotherapy to neoadjuvant chemotherapy improves event-free survival in patients with non-small-cell lung cancer (NSCLC). This is the first positive phase 3 trial in this setting, although several phase 3 trials are currently investigating the efficacy of neoadjuvant and adjuvant immunotherapy in resectable NSCLC.

Key Words: Neoadjuvant; Immunotherapy; NSCLC; Perioperative; Checkmate-816; nivolumab; Chemo-immunotherapy

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Core Tip: Recent data from a phase 3 trial show that the addition of immunotherapy to neoadjuvant chemotherapy in patients with non-small-cell lung cancer (NSCLC) improves pathologic complete response and event-free survival. This is the first positive phase 3 trial in this setting, although several other phase 3 studies are currently investigating the efficacy of neoadjuvant and adjuvant immunotherapy in resectable NSCLC. We describe the results of the CheckMate-816 phase 3 trial, which found that neoadjuvant chemoimmunotherapy was superior to chemotherapy alone. We also briefly review the main phase 3 studies currently underway to evaluate the role of immunotherapy in the perioperative setting of NSCLC.

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TO THE EDITOR

The management of localized non-small-cell lung cancer (NSCLC) is set to undergo an important change in the first few months of this year (2022) due to the recent publication of the second primary endpoint—event-free survival (EFS)—from the Checkmate-816 trial. The data show that the combination of chemotherapy + nivolumab yielded a mean disease-free survival of 31.6 m in the experimental arm *vs* 20.8 m [hazard ratio (HR): 0.63] in the control arm (chemotherapy alone), with a 2 year-EFS rate of 64% *vs* 45%, respectively[1]. These results, in addition to previously reported results showing an improvement in pathological complete response (pCR) of 24% *vs* 2%, confirm the combination of three cycles of chemotherapy + neoadjuvant nivolumab as the new standard of care in resectable NSCLC[2].

This is the first time that pCR has been validated as a surrogate marker for survival in a randomized trial. In the experimental arm, the median EFS was 26.6 m in patients without pCR and not reached in those with pCR (HR: 0.13). Although the results in terms of overall survival are still immature, a trend towards better survival was observed in the experimental arm, in which 12% more patients were alive at 2 years (HR: 0.57).

This new change in clinical practice comes with several questions that need be resolved in the next few years, including the following: The role of adjuvant therapy; the selection of the most suitable candidates; comparison with adjuvant chemoimmunotherapy; the optimal approach in stage I-II disease; standardization of pathological response assessment; changes in resectability criteria; and changes in the preoperative algorithm.

The perioperative management of NSCLC will undoubtedly undergo a major transformation in the coming years due to the arrival of targeted therapy in this clinical setting, mainly the incorporation of pre- or post-operative immunotherapy[3]. The CheckMate 816 study was the first phase 3 trial to report positive results for the addition of immunotherapy to neoadjuvant chemotherapy[1]. However, other ongoing phase 3 trials evaluating other PD-1 axis inhibitors are expected to report results soon, such as the Impower-030 trial (atezolizumab)[4], KeyNote-671 trial (pembrolizumab)[5], and the Aegean trial (durvalumab)[6] (Table 1). Likewise, atezolizumab has already obtained FDA approval for use in the adjuvant setting in patients with resected PD-L1 positive stage II-IIIA NSCLC[7], and positive results have also been reported from an interim analysis of the KeyNote-091 trial, showing the benefits of pembrolizumab in resected stage IB-IIIA NSCLC[8]. Nivolumab and durvalumab are also being evaluated in the adjuvant setting in several other phase 3 trials (ANVIL, NADIM-Adjuvant, Mermaid-1)[9-11] (Table 2). As a result, the panorama for the treatment of early-stage NSCLC is becoming increasingly interesting, and the data suggest that it will be crucial to personalize treatment to offer the best treatment scheme for each individual patient.

These new options bring hope of a cure to a greater number of patients, but also new challenges for the multidisciplinary team and other professionals involved in the treatment of these patients. Once again, coordinated multidisciplinary work will be essential, especially among medical oncology, thoracic surgery, and radiation oncology.

Table 1 Main phase 3 trials evaluating neoadjuvant chemoimmunotherapy in non-small-cell lung cancer

Neoadjuvant NSCLC				
Study	**IO agent**	**Strategy**	**Objective**	**Status**
CheckMate-816[1]	Nivolumab (anti-PD1)	ChT + IO	EFS and pCR	FDA approved
Impower-030[4]	Atezolizumab (anti-PD-L1)	ChT + IO	PFS and OS	Completed. Results pending
KeyNote-671[5]	Pembrolizumab (anti-PD1)	ChT + IO	EFS and OS	Active, not recruiting
Aegean[6]	Durvalumab (anti-PD-L1)	ChT+ IO	pCR and EFS	Recruiting

IO: Immunotherapy; ChT: Chemotherapy; EFS: Event-free survival; pCR: Pathologic complete response; PFS: Progression-free survival; OS: Overall survival; FDA, Food and Drug Administration; NSCLC: Non-small-cell lung cancer.

Table 2 Main phase 3 trials evaluating adjuvant immunotherapy in non-small-cell lung cancer

Adjuvant NSCLC				
Study	**IO agent**	**Strategy**	**Objective**	**Status**
Impower-010[7]	Atezolizumab (anti-PD-L1)	IO mono	OS in selected PD-L1 population	FDA approved in II-IIIA NSCLC PD-L1+
KeyNote-091 (PEARLS)[8]	Pembrolizumab (anti-PD-L1)	IO mono	DFS	Interim analysis: positive in IB-IIIA NSCLC all corners
ANVIL[9]	Nivolumab (anti-PD1)	IO mono	OS and DFS	Active, not recruiting
NADIM-Adjuvant[10]	Nivolumab (anti-PD-1)	ChT + IO	DFS	Recruiting
Mermaid-1[11]	Durvalumab (anti-PD-L1)	ChT + IO	DFS in MRD+	Recruiting

IO: immunotherapy; mono: monotherapy; OS: overall survival; NSCLC: non-small-cell lung cancer; DFS: disease-free survival; ChT: chemotherapy; MRD: minimal residual disease; FDA, Food and Drug Administration; NSCLC: Non-small-cell lung cancer.

FOOTNOTES

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REVIEW

Systems biology and OMIC data integration to understand gastrointestinal cancers

Iasmin Moreira Costa Bispo, Henry Paul Granger, Palloma Porto Almeida, Patricia Belini Nishiyama, Leandro Martins de Freitas

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Abstract

Gastrointestinal (GI) cancers are a set of diverse diseases affecting many parts/organs. The five most frequent GI cancer types are esophageal, gastric cancer (GC), liver cancer, pancreatic cancer, and colorectal cancer (CRC); together, they give rise to 5 million new cases and cause the death of 3.5 million people annually. We provide information about molecular changes crucial to tumorigenesis and the behavior and prognosis. During the formation of cancer cells, the genomic changes are microsatellite instability with multiple chromosomal arrangements in GC and CRC. The genomically stable subtype is observed in GC and pancreatic cancer. Besides these genomic subtypes, CRC has epigenetic modification (hypermethylation) associated with a poor prognosis. The pathway information highlights the functions shared by GI cancers such as apoptosis; focal adhesion; and the p21-activated kinase, phosphoinositide 3-kinase/Akt, transforming growth factor beta, and Toll-like receptor signaling pathways. These pathways show survival, cell proliferation, and cell motility. In addition, the immune response and inflammation are also essential elements in the shared functions. We also retrieved information on protein-protein interaction from the STRING database, and found that proteins Akt1, catenin beta 1 (CTNNB1), E1A binding protein P300, tumor protein p53 (TP53), and TP53 binding protein 1 (TP53BP1) are central nodes in the network. The protein expression of these genes is associated with overall survival in some GI cancers. The low TP53BP1 expression in CRC, high EP300 expression in esophageal cancer, and increased expression of Akt1/TP53 or low CTNNB1 expression in GC are associated with a poor prognosis. The Kaplan Meier plotter database also confirmed the association between expression of the five central genes and GC survival rates. In conclusion,

GI cancers are very diverse at the molecular level. However, the shared mutations and protein pathways might be used to understand better and reveal diagnostic/prognostic or drug targets.

Key Words: Gastrointestinal cancers; Genome; Cellular pathways; Protein-protein interaction; Prognosis; OMIC data

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Core Tip: We highlight the genomic mutations and cellular pathways in gastrointestinal (GI) cancers. These are responsible for the cell's behavior, allowing unlimited cell replication and invasion of other tissues. Using the STRING database, we found that Akt1, catenin beta 1, E1A binding protein p300, tumor protein p53 (TP53), and TP53 binding protein 1 are central nodes in the GI cancer protein network. Their expression is associated with poor survival in some GI cancers, which was confirmed by the Kaplan Meier plotter database. This information points to crucial and shared aspects of the most frequent GI cancers.

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INTRODUCTION

In 2020, the number of cancer cases in the digestive system was 5 million and 3.5 million deaths worldwide[1,2]; the physiologic system with the highest number of cases and among the highest percentage of deaths[3] (Table 1). The cancer types in this system can be classified as organ origin and cell type. The most frequent are esophageal cancer (EC), gastric cancer (GC), liver cancer, pancreatic cancer, and colorectal cancer (CRC)[2,3]. GC, liver cancer, and CRC are among the most common causes of cancer deaths annually[2]. Gastrointestinal (GI) cancers also have specific molecular changes in genetic/genome, epigenetics, gene expression, and cellular pathways contributing to tumor behavior. This information might be helpful in diagnosis, prognosis, and new drug development.

EC

EC has two subtypes: esophageal squamous cell cancer (ESCC) and esophageal adenocarcinoma (EAC) [4]. The incidence of ESCC increases globally and predominantly in Eastern Asia and Eastern/Southern Africa[4-7]. However, the ESCC decreases while EAC increases in the United States and a few European countries[5]. The ESSC and EAC incidence differences are geographically observed in sex and ethnic patterns[4,5].

There is also a well-established genetic factor associated with sex, and although it is still not well understood, it is known that the ratio between men to women is 2.5-4.4:1[4,6]. Studies indicate a protective effect of female sex hormones, including a lower risk of cancer for women previously breastfed. Nevertheless, environmental factors also influence this prevalence as, for example, men tend to abuse alcohol and tobacco, which are primary risk factors for the manifestation of EC[4,8].

The risk factors for ESCC are smoking, a low vegetables/fruit diet, and alcohol consumption[9], whereas for EAC, the risk factors are obesity and gastroesophageal reflux disease[9,10]. When alcohol and tobacco are used together, there is an increased risk. This combination is believed to be responsible for 70%-90% of cases, mainly because they cause chronic irritation and inflammation of the esophageal mucosa. In the case of obesity, the greater the abdominal circumference, the greater the intra-abdominal pressure increases the probability of developing gastroesophageal reflux[4,6,11-14].

Early diagnosis is fundamental to improving prognosis. However, dysplasia usually is asymptomatic [4,11,12,15] and manifests at an average age of 67 years, when there is a high incidence of metastasis, mainly in lymph nodes, liver, lungs, and bones[11,12]. These features make the EC an aggressive malignancy with a 15%-23% 5-year survival rate[9,10].

GC

GC has the fourth highest incidence and mortality worldwide[1,2]. The primary risk factors for GC are genetics, diet (high amount of salt and low consumption of fruits and vegetables), *Helicobacter pylori* or Epstein–Barr virus infection, smoking, alcohol intake, and sedentary life[16-19]. The principal risk factor for GC is *H. pylori* infection, accounting for 80% of the cases. Although the incidence of *H. pylori* infection is decreasing, GC deaths are still high. While the primary risk factor is *H. pylori* infection, many

Table 1 Gastrointestinal cancer data

Tissue	Incidence[1]	Mortality[1]	ASR incidence[1]	ASR mortality[1]	5-yr survival rate
Colorectum	1931590	935173	2.3	0.94	60%-70.9%[1]
Esophagus	604100	544076	0.78	0.68	14.6%-23.2%[1]
Liver	905677	830180	1.1	1.0	18%[3]
Pancreas	495773	466003	0.55	0.51	7.9%-14.3%[1]
Gastric	1089103	768793	1.3	0.90	20.8%-32.8%[1]

Age-standardized rate in 100000 people. ASR: Age-standardized rate.

genes are associated with GC[16,18,20], and some genetic variations that can interact with *H. pylori* increase the GC risk[21,22]. The incidence of GC is higher in males (1.32-2.2) and in Eastern/Central Asia and Latin America[16,18].

Obesity can induce inflammation of the stomach lining through tumor necrosis factor (TNF), interleukin 6 (IL-6), and C-C motif chemokine ligand 2. By contrast, a diet rich in fruits and vegetables has proven to be an ally in cancer prevention because it contains numerous antioxidants that prevent metabolic damage, especially vitamin C[18].

A relevant factor in the decline of GC has been the successful prevention and treatment of infections by *H. pylori*[18]. According to the International Agency for Research on Cancer, this is a carcinogen from group 1, meaning there is sufficient evidence of human carcinogenicity[23,24]. *H. pylori* infection affects more than half of the world's population, and its eradication may considerably decrease the chances of stomach cancer. However, it would increase the chances of esophageal adenocarcinoma. However, it is unknown how this esophageal protection mechanism occurs[18,24,25].

Hepatocellular carcinoma

There are about 1 million new cases of liver cancer each year, with hepatocellular carcinoma (HCC) responsible for most patients (90%) and the second most common cause of cancer death worldwide[26,27].

HCC presents a poor prognosis due to a late diagnosis. Multiple different tumors may occur in a single patient, leading to intra-tumor and intra-patient heterogeneity, which makes it difficult to establish a treatment line for HCC[27,28]. This heterogeneity can be caused by environmental factors and genomic and biological changes caused by the tumor lesion[27].

Cirrhosis and non-alcoholic fatty liver disease are risk factors associated with alcohol abuse and obesity that can lead to the onset of HCC. Genetic factors such as diabetes, exposure to carcinogens (aflatoxins), and biological factors, especially hepatitis virus infection, can be highlighted[28].

The HCC development is a multistep process. It starts as a chronic liver disease that leads to inflammation, fibrosis, or aberrant hepatocyte regeneration. This set of conditions can progress to cirrhosis and later malignancy. The causes of this inflammation can be hepatitis B virus/hepatitis C virus infection, fatty liver disease, excessive alcohol intake, and aflatoxin consumption[26,29]. The outcome of this inflammation can be influenced by epigenetics and the immunological response in the tumor microenvironment to create a preneoplastic lesion until producing cells with highly proliferative, invasive, and survival skills[26].

The geographic regions most affected by HCC are Southeast Asia and sub-Saharan Africa, where there is endemic infection by the hepatitis virus and high exposure to aflatoxin, which are responsible for 70%-90% of cases in these places[28]. Currently, there is no line of therapy based on biomarkers suitable for HCC, although some candidate genes already exist[30].

Pancreatic cancer

Pancreatic cancer, characterized by pancreatic ductal adenocarcinoma (PDAC), is the seventh leading cause of cancer-related deaths worldwide[31]. Its incidence is higher in Europe, followed by North America and Oceania, mainly in people over 70-years-old. Incidence and mortality increase with aging and are more common in men than women[32].

It is highly fatal because it presents aggressive growth and a lack of symptoms in the disease's initial stage. As the tumor progresses, a picture of nonspecific symptoms begins, including jaundice, weight loss, abdominal pain, and fatigue[32]. About 80% of diagnoses are made in the advanced clinical stages, leading to a low 5-year prognosis of survival after surgery[33]. Surgical resection is the single strategy capable of curing pancreatic cancer. Besides, using chemotherapy concomitantly improves survival rates [34].

The main risk factors for the onset of pancreatic adenocarcinoma are smoking, alcohol, obesity, *H. pylori*, and type 2 diabetes[34]. Other factors, such as fat infiltration into the pancreas, have been associated with developing intraepithelial neoplasms. Pancreatic cancer can also arise from genetic

factors that can cause familial syndromes, such as Peutz-Jeghers syndrome[31]. A history of pancreatic cancer in first-degree relatives leads to a 2- to 3-fold increase in incidence risk due to inherited genetic predispositions[35].

CRC

CRC is the second most deadly cancer worldwide (1.3 million) and is the third leading cause of cancer-related deaths (540000) annually[2]. CRC is responsible for about 10% of cancer-related deaths worldwide, and in the last 45 years, there has been an increase in this mortality rate[36]. Its incidence is higher in developed countries such as Australia and New Zealand, followed by countries in Europe, East Asia, and North America. The frequency increases as individuals age, usually appearing in people over 50 years[37].

The tumor can originate in both the colon and the rectum. However, usually fuse because they have similar clinical and biological characteristics, with adenocarcinoma as the primary cell type of the tumor [37]. Many factors are associated with this increase in the diagnosis/mortality rate, such as an increase in life expectancy, poor dietary habits, and risk factors: smoking, red meat consumption, sedentary lifestyle, obesity, alcohol intake, and genetics[36,38-40]. These factors change the genetic/molecular in colon epithelial cells deactivating suppressor tumor genes and activating oncogenes to create aggressive and malignant behavior[40].

In the early stages, the disease has no clinical manifestation. The patient may be asymptomatic for years, but as the disease progresses, it advances to a more severe condition, with symptoms such as changes in intestinal motility, hidden or evident colorectal bleeding, cramps, loss of weight, weakness, and fatigue are manifesting[37].

GENOME DATA IN GI CANCERS

EC

There are several generalized genomic changes when esophageal carcinoma cells are analyzed. The most evident is a somatic mutation in tumor protein p53 (TP53) that appears in about 83% of cells. The p53 protein is a tumor suppressor and one of the most important transcription factors for regulating proliferation, apoptosis, autophagy, and cell cycle. However, this gene has a high mutation percentage in cancer cases, reaching 75% in tumor cells[12,41].

There are also changes in genes that control cell cycle and differentiation, including cyclin-dependent kinase inhibitor 2A (CDKN2A), nuclear factor erythroid derived 2-like 2, checkpoint kinase 1/2, and Notch1/3. Others may appear overexpressed such as cyclin D1 (CCND1) and CDK4/6[12,42-44]. The B cell translocation gene 3 protein can regulate the cell cycle's progression; its low expression is related to the appearance of esophageal adenocarcinoma, and its expression level is directly correlated with lymph node metastasis[12,45].

The presence of mutations in the growth factors in cancer cells is well documented in the literature. Overexpression of epidermal growth factor receptor (EGFR) in carcinoma cells is associated with lymph node metastasis, and its expression level also influences the patient's clinical stage. Another growth factor correlated with esophageal carcinoma is vascular endothelial growth factor C (VEGFC), encoded by the Fms related receptor tyrosine kinase 1 gene, and its levels in the tissues correlate with tumor stages and metastasis state[12,41].

Using next-generation sequencing, frequent mutations in carcinoma cells have been observed in the lysine methyltransferase 2D (KMT2D), SET domain containing 2 histone lysine methyltransferase, Notch1, retinoblastoma 1, CDKN2A, BRCA1-associated protein-1, forkhead box O3, and MutS homolog 6 (MSH6) genes compared to adenocarcinoma. It was also observed that some copy number variations in fibroblast growth factor 3 (FGF3), FGF4, FGF19, and CCND1 are more expressed in carcinoma compared to adenocarcinoma[46].

GC

Besides the infectious causes, the genetic data have helped to classify the GC into three additional subtypes: microsatellite instability (21.7%), genome stability (19.6%), and chromosome instability tumors (49.1%)[47].

Although infection is environmental, GC caused by infection is associated with genetic modifications such as phosphoinositide 3-kinase catalytic subunit (PIK3CA) mutations or gene amplification of Janus kinase (JAK), programmed death-ligand 1/2, or ERBB2. The infectious pathogen can also induce epigenetic modifications in this type of GC as DNA methylation in the phosphatase and tensin homolog (PTEN) gene promoter[48] and tumor-suppressor gene adenomatous polyposis coli (APC)[49]. Microsatellite instability is more associated with many truncating or missense mutations. The genes with the highest number of mutations in microsatellite instability GC are EGFR, ERBB3, KRAS/NRAS, and PIK3CA[50].

Genomically stable tumors present many mutations, especially genes well associated with cancer. The gene Ras homolog family member A works as signal transduction inducing cell proliferation, actin cytoskeleton structure, and cell movement associated with metastasis[51,52]. The genes claudin 18 and Rho-GTPase-activating proteins are frequently translocated in genomically stable GC tumors. The gene cadherin 1 (CDH1) encodes a cell-cell adhesion protein, which is also currently mutated in this type of cancer[53]. Furthermore, CDH1 has a role in cell proliferation, invasive behavior, and migration[54-56]. In the CDH1 gene, autosomal dominant mutations increase stomach cancer risk, especially when one of its copies is lost, generating a scenario of diffuse hereditary GC[18].

The chromosomal alterations involve gene amplification of EGFR, ERBB2/3, KRAS/NRAS, and RASA1; gene deletion of PTEN. These genetic modifications probably would result in gene activation or deactivation, which would result in tumor cell phenotypes. EGFR, ERBB2/3, JAK2, FGFR2, MET, KRAS/NRAS, and PIK3CA are predicted to be active, while RASA1, PTEN, and PIK3R1 would be inactive.

HCC

Numerous genetic changes in HCC cells, including mutations, changes in the number of copies, and chromosomal rearrangements, lead to a very complex genomic picture. Its complexity is further aggravated when etiological factors that precede the tumor development for years are considered[57].

Some genes play a fundamental role in cancer development, which is why they appear more frequently as TP53, MYC, WNT, and CTNNB1. Also highlighted are genes related to the cell cycle, such as CCND1 and CDKN2A[57].

A study integrating RNA sequencing, DNA sequencing, T cell receptor sequencing, and single nucleotide polymorphism array was carried out to investigate the space-time interactions between cancer and immune cells. A difference in the interaction of the adaptive immune system was detected in different regions of the same tumor. The TP53 and CTNNB1 genes expressed clonal mutations. High-level amplifications have been reported for CCND1, FGF19, and VEGFA. Mutations related to environmental risk factors such as smoking and alcohol were found in telomerase reverse transcriptase (TERT), CTNNB1, TP53, axin 1, and AT-rich interactive domain-containing protein 1A (ARID1A). There were also mutations without an apparent etiological factor in TERT, KMT2B, CCNA2, and CCNE1[58].

HCC results from of a multistep process involving genetic, epigenetic, and transcriptomic interactions. Among these interactions, epigenetics is among the most affected, leading to profound gene expression changes that can facilitate tumor formation The most common form of epigenetic silencing of tumor suppressor genes is hypermethylation of DNA. This epigenetic change usually occurs in CpG islands of gene-promoting regions such as deleted in liver cancer 1, tissue factor pathway inhibitor 2, CDKN2A, and PTEN[30].

Pancreatic cancers

The etiology of PDAC is mainly related to genetic predisposition, environmental factors such as smoking, obesity, and poor nutritional diet. These factors lead to chromosomal instability, affecting cell cycle pathways, chromatin remodeling, WNT, MYC, NOTCH signaling, and DNA damage repairs[35,59]. Among the mutated genes, the one that appears most frequently is KRAS[60]. It is also possible to highlight mutations in MLH1, MSH2, PMS2, and MSH6 responsible for Lynch Syndrome and mutations in the germ lines of PALB26, 11, 12, and ATM7, 12, 13[35].

Pancreatic cancer genome analyses showed a homogenous profile with somatic mutations in a few genes shared KRAS, TP53, CDKN2A, and SMAD4. However, other less frequent genes are also involved including mitogen-activated protein kinase kinase 4 (MAP2K4), lysine demethylase 6A, ring finger protein 43, ARID1A, transforming growth factor beta receptor 2 (TGFβR2), GNAS, Ras responsive element binding protein 1, and Polybromo 1[61-63]. These mutations can vary, and it is observed that non-silent mutations, gene amplification (> 8 copies, deletions, and structural variants)[63]. The set of genes that appear often mutated in pancreatic cancer plays a role in oncogenes, DNA damage repair, and chromatin modification[61,64]. The pancreatic cancer genome has chromosomal rearrangements classified into four subtypes: stable, locally rearranged, scattered, and unstable[61]. The mutation event more frequent is non-silent single nucleotide variants and copy number change (loss)[61]. The pancreatic cancer stable subtype was found in 20% of samples and had very few structural rearrangements (< 50 structural rearrangements) and more chromosomal mutations (aneuploidy). The locally rearranged subtype was found in 30% of samples with a high number of structural rearrangements (> 200) in a few chromosomes (three or fewer chromosomes), and there is more gene amplification. The scattered subtype is the most frequent (36% of samples) and has 50-200 structural rearrangements. Besides, the mutation type gene amplification is more frequent than in the other subtypes. The unstable subtype is less frequent (14% of the samples) and has the highest number of structural rearrangements (> 200 structural rearrangements), such as intrachromosomal, translocations, inversion, deletions, and duplication. Besides the frequent mutation described in pancreatic cancer, the unstable subtype is also associated with BReast CAncer gene 1 (BRCA) pathway mutations (BRCA1, BRCA2, and PALB2)[61].

CRC

Most CRC cases are sporadic (70%), and only 30% are inherited[38]. The genes most affected are DNA mismatch-repair genes, APC, or mutY DNA glycosylase[39,40]. The DNA mismatch-repair proteins malfunctioning creates the condition of genetic mutation accumulation and tumor cells rising.

The CRC has three genetic subtypes based on their genomic alterations. The genomic alterations are chromosome or microsatellite instability or epigenetic changes of CpG islands (CpG island methylator phenotype - CIMP)[65,66]. Chromosomal instability is the most frequent in CRC, present in 71%-85%[65, 66]. The genetic differences also lead to overall survival differences in CRC. The CIMP subtype is associated with poor prognosis, followed by chromosome instability, and microsatellite instability showed the best survival[66-68]. The CIMP's poor prognosis indicates the importance of CpG methylation dysregulation in CRC tumorigenesis. The methylation dysregulation might affect the proto-oncogenes and tumor-suppressor genes. The worst prognosis in the CIMP subtype indicates that a different approach is necessary to deal with molecular modifications. Epigenetic modifications can also be therapeutic targets to improve the treatment.

The genetic/genomic diversity in GI cancers shows the importance of molecular characterization to improve the treatment and prognosis.

PATHWAYS

The cellular pathways show the main activities and functions present in a cell when proteins work together. The cancer pathways are responsible for the cell's behavior, allowing unlimited cell replication, survival, and tissue invasion. The pathways also are responsible for the molecular changes driving tumorigenesis. Understanding how a set of proteins work together to develop a cancer cell might point to the target proteins to block these processes.

The pathways most present among the GI cancers discussed here are apoptosis, focal adhesion, and p21-activated kinase (PAK), PI3K/Akt, TGF-β, and Toll-like receptor (TLR) signaling pathways (Table 2) [69-93].

Apoptosis plays a role in maintaining the balance in cell division and death during development and life. The unbalance of apoptosis leads to survival and uncontrolled division in tumorigenesis[94]. The apoptosis pathway is triggered by irreparable DNA damage, and it has many proteins that can fail and be blocked to inhibit cell death. The intrinsic process is mediated by mitochondria releasing cytochrome C after BH3 proteins activate B-cell lymphoma 2 (Bcl-2)-associated X protein and Bcl-2 homologous antagonist/killer. The cytochrome C and apoptotic protease activating factor 1, and caspase-9 create the apoptosome to continue the apoptosis process. The extrinsic process has death receptor ligands (cluster of differentiation 95 ligand [CD95L], TNF-related apoptosis-inducing ligand, and TNFα), death receptors, and associated proteins (Fas-associated death domain and TNF receptor 1-associated death domain protein) that transduce the death signal until caspase-8. Both intrinsic and extrinsic processes act on caspase-3/6/7 to induce the apoptosis cascade. Cell death by apoptosis results in a non-inflammatory process, which attracts research to the development of therapies that use apoptosis to treat cancer[95-97].

The PAK1 signaling pathway has six members divided into two groups and induces proliferation, survival, and motility[98]. PAK1 participates in cancer tumorigenesis after being highly expressed. The crosstalk of PAK1 with the MAPK/extracellular signal-regulated kinase (ERK) and PI3K/Akt pathways induces proliferation and survival, respectively[99]. PAK1 also connects with the Wnt signaling pathway through CTNNB1 and continues to stimulate growth and metastasis[98]. PAK1 expression protects the cell from apoptosis after interaction with Raf, which inactivates Bcl-2 family members (BCL2 associated agonist of cell death [BAD]) in mitochondria[98,100].

TLRs are part of the family of pattern knowledge receptors and operate on innate immunity, participating in the body's first line of defense against invasion of microbial pathogens, tissue damage, and cancer. Its signaling pathway controls immune cell activation, maturation, and immune functions, especially the secretion of cytokines, influencing the tumor's metabolism, proliferation, and spread [101]. They are expressed by several immune cells such as macrophages, dendritic cells, B lymphocytes, natural killer cells, non-immune cells such as epithelial cells, and cancer-associated fibroblasts[102]. When expressed in the tumor, TLRs can release cytokines and chemokines into the tumor environment to recruit other immune cells to release more proinflammatory cytokines, pro-angiogenic factors, and growth factors[101].

The TGF-β signaling pathways are pleiotropic, regulating multiple functions such as cell growth, differentiation, apoptosis, angiogenesis, motility, invasion, and immune response. Modifications in this pathway might play an essential role in developing tumors and metastasis. These modifications can affect not only the tumor cells but also the environment. At this level, the TGF-β generates an environment conducive to tumor growth and metastasis at all carcinogenesis stages. TGF-β has a contradictory behavior at the cellular level, acting as a suppressor and a tumor promoter[103,104]. Initially, the TGF-β pathway promotes cell cycle arrest and apoptosis. It promotes cancer cell motility, invasion, tumor progression, and metastasis in advanced stages. Thus, the accumulation of mutations is

Table 2 Pathways enriched in transcriptional analyses in esophageal, gastric, liver, pancreas, and colorectal cancers

Pathway	CRC	EC	GC	HCC	PDAC	Ref.
Focal adhesion	X	X	X	X	X	[69-76]
Apoptosis	X	X	X	X	X	[71,72,77-79]
PAK pathway	X	X		X	X	[80-83]
PI3K/Akt signaling pathway	X	X	X			[71,84-86]
TGF-beta pathway		X	X	X	X	[75,76,87-90]
Toll-like receptor signaling pathway		X	X	X		[88,91-93]

CRC: Colorectal cancer; EC: Esophageal cancer; GC: Gastric cancer; HCC: Hepatocellular carcinoma; PAK: p21-activated kinase; PDAC: Pancreatic ductal adenocarcinoma; PI3K: Phosphoinositide 3-kinase; TGF: Transforming growth factor beta.

responsible for guiding the evolution from a suppressor pathway to a tumor promoter[105].

The HCC RNA sequencing study identified four subtypes of HCC using 212 samples. The pathway analyses using the expression data reveal the enriched pathways metabolism RNA processes such as RNA processing, binding, and splicing. Although all the samples are from HCC, this result indicates different gene expression, cell activity, and behaviors. These enriched processes are not shared by the four HCC groups funded. However, at least three groups shared translation, ribosome, metabolism of proteins, and cytoplasm ribosomal proteins[106]. The microarray analysis using 25 HCC samples identified thousands of differentially expressed genes, and the pathways of cell cycle response, DNA damage response, cell survival, and apoptosis were identified. In addition, it was also linked to pathway terms and poor prognosis clinical parameters. These results also agree with RNA sequencing study point transcriptional regulation, RNA processing, and cell cycle regulation. The single-cell RNA sequencing analysis indicates 119 genes associated with HCC. The pathways analysis using Gene Ontology showed an acute inflammatory response, oxidative stress, and humoral response. Simultaneously, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways indicate IL-17 and TNF signaling pathways, infectious disease, and rheumatoid arthritis. These samples present more immunological functions[107]. According to the OncoVar database, the KEGG pathways associated with HCC are mainly cancer pathways, viral infection, cell longevity (growth and death), antineoplastic drug resistance, and transduction signaling pathways (Wnt and Hippo signaling pathways)[108]. The molecular pathways in HCC are not entirely understood, and these results showed a notable variation of response in the differentially expressed genes working together to express a function.

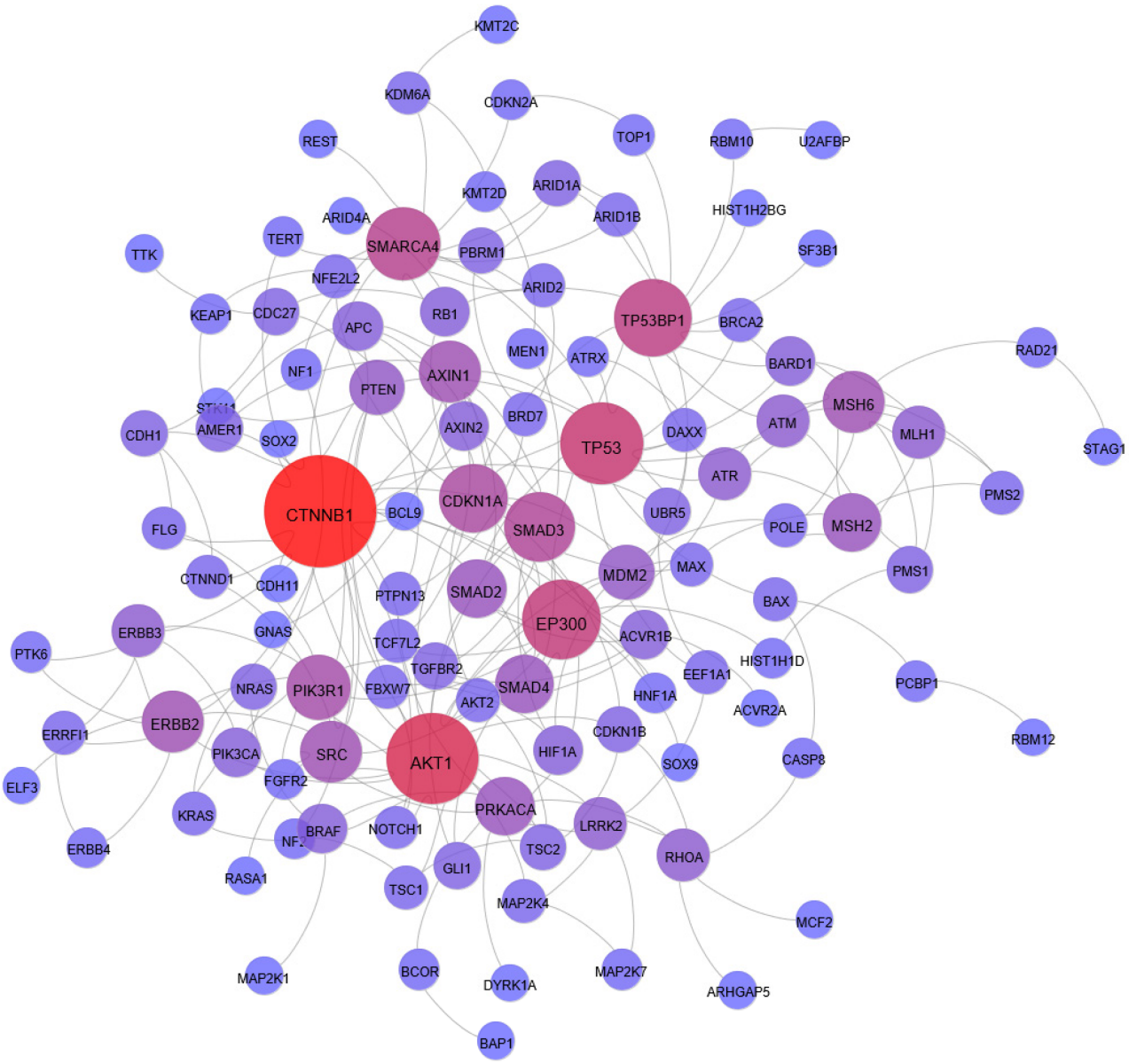
Analysis combining CRC and endometrial cancer microarray samples identified 139 genes upregulated in both studies. These genes operate in the cellular functions of cell proliferation, Wnt signaling pathway, fatty acid beta-oxidation, transcription, exocytosis, dopaminergic neuron differentiation, and platelet degranulation. The KEGG pathways enriched were tight junctions, rheumatoid arthritis, renal cell carcinoma, and cancer pathways signaling. The rheumatoid arthritis pathway was enriched in more than one study with the genes (ATP6V0D1, ATP6V1D, CD28, CTLA4, CTSK, FOS, IL-18, and JUN)[109]. Other microarray meta-analysis studies using CRC samples point to also the KEGG pathways related to the cell cycle, pathways in cancer, and the Wnt signaling pathway. These pathways are linked; as a result, they share proliferation and block apoptosis[65]. Together, these processes induce the normal cell to convert to a tumor cell.

THE PROTEIN-PROTEIN INTERACTION IN CANCER

The number of GI cancer projects in different OMIC levels found many genes working in tumorigenesis. The GI cancers discussed here sum 178 different genes with associated mutations. The number of genes with mutations associated with GI cancers ranges from 41 to 89 genes in HCC and GC.

Each of these cancers has variation and can be classified into subtypes according to cell origin, chromosomal structural rearrangements, gene expression, and cell behaviors. However, there are 46 genes shared by at least two types of cancers. These genes should be investigated to understand better how they assist in the cell transformations to tumors, biomarkers of tumor cells, and potential drug or therapy targets. The genes present in all five types of cancers are activin A receptor type 2A, APC, ARID1A, and CTNNB1.

We used information from STRING database to check the protein-protein interaction (PPI) from these 178 genes. We used the experimental information only to build this PPI network. The PPI investigation allows for building a network with 111 genes connected (Figure 1)[110]. The number of nodes in the PPI network indicates that these genes work together in GI cancer tumorigenesis.



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Figure 1 Protein-protein interaction of genes with mutations associated with gastrointestinal cancers. The nodes represent the genes, and the edges represent the protein interactions. The network was built using information from experimental data only from the STRING database[110]. The node size represents the number of protein interactions (degree), indicating the node's centrality.

We analyzed the GI cancer network to identify in this PPI most connected protein (high degree) as central nodes. The proteins CTNNB1, Akt1, TP53, EP300, and TP53-binding protein 1 (53BP1) are the central nodes with the highest degree.

The CTNNB1 gene encodes a beta-catenin protein expressed in the adherens junctions[53]. The beta-catenin is a cytoplasm protein that works in the adhesion between cells. The beta-catenin binds the actin in the cytoskeleton and the E-cadherin protein in the cell membrane, connecting neighboring cells[111]. The beta-catenin is also a mediator in the Wnt signaling pathway. When activated, the Wnt signaling pathway induces the accumulation of beta-catenin in the nucleus, activating target genes' transcription [53]. The WNT protein binds the receptor in the membrane and induces beta-catenin to accumulate, promoting cell survival and proliferation[65]. The mutations in CTNNB1 gene are frequently found in HCC (13%)[112,113], CRC (6%)[114], and it is mutated in 4% of GC[47].

The Akt1 is a central protein in cell transduction signaling, which, when induced by PI3K, induces process cell proliferation, survival, and angiogenesis. The activation of the mammalian target of rapamycin (mTOR) complex by ATK is investigated as a drug target to treat PDAC[115-117]. The Epstein-Barr virus and *H. pylori* induce inflammation and the expression of Akt in GC. The outcome is cell proliferation and telomerase activation[118,119]. The investigation of blockage of Akt in GC resulted in suppression of growth and metastasis[120]. The investigation of critical proteins in HCC PPI identified several functions crucial in tumorigenesis, cell proliferation, anti-apoptosis, and metastasis.

The PPI network showed Akt1 as a potential drug target[104]. These results indicate Akt1 central position in tumorigenesis and a potential drug target.

The 53BP1 protein has a role in DNA damage response and cycle arrest, triggering the expression of p53; the malfunctioning of this protein might lead to the development of genomic instability and molecular diseases. The lack of function of 53BP1 is associated with poor prognosis, angiogenesis, and metastasis[121]. The decreased expression of 53BP1 in CRC induces radiotolerance and chemoresistance. Moreover, CRC cells with lower expression of 53BP1 have a higher proliferating rate, decreased apoptosis, and poor prognosis[122-124]. The 53BP1 also interacts with p53, as indicated in CRC and EC, when the reduction of 53BP1 induces the downregulation of p53[122,123,125]. The 53BP1 is expressed as soon as DNA damage treatment occurs in human pancreatic cells[126]. The 53BP1 might also influence tumor outcome in pancreatic cancer, as shown when the variation of 53BP1 expression changes the association of carbohydrate 19-9, a well-known pancreatic cancer marker, and overall survival[100].

The p300 protein (encoded by the *EP300* gene) is a histone acetyltransferase that participates in chromatin remodeling and interacts with basal transcriptional machinery to improve DNA binding, affecting gene transcription in normal and cancer cells[127]. The EP300 mutations are common in CRC and GC by frameshift in microsatellite regions[128]. The mutation in EP300 is frequent in EC (10%), and it correlates with a poor prognosis, associated with cell proliferation, migration, and invasion (metastasis)[129,130]. The role of p300 in remodeling the chromatin makes it appropriate to investigate epigenetic therapies, and the use of natural nutrients as potential prevention and treatment has already been discussed with GC[131].

ESSENTIAL GENES AND KAPLAN-MEIER SURVIVAL ANALYSIS

All GI cancers discussed here have a low 5-year survival rate, except CRC (Table 1). The esophagus, liver, and pancreas have the lowest 5-year survival rate. The late diagnosis, metastasis, and aggressive behavior are associated with a low 5-year survival rate. Many studies describe the poor prognosis as associated with gene expression[97,122,129,132-135].

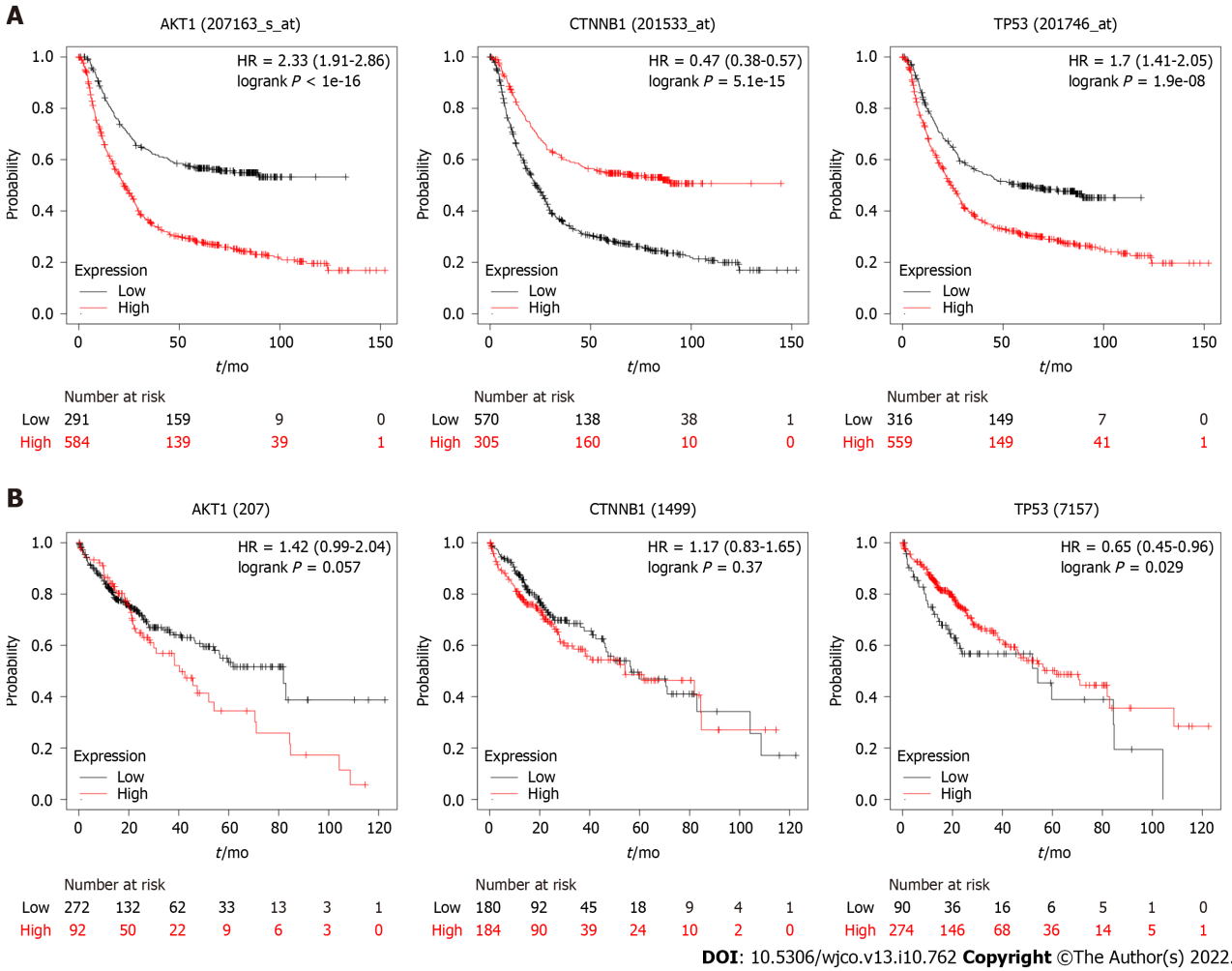
The expression levels are crucial information that might work as a prognostic factor in GI cancers. The association between TP53BP1 expression and overall survival analyses in CRC indicate a connection with low expression and low survival in the I-IIA stage, T3-T4, and N0[122]. Again, this protein has an essential role in CRC, not only to a high degree but also as a prognostic marker. The EP300 gene has high expression associated with poor survival in ESCC[129]. The long non-coding RNAs (lncRNAs) have a critical role in cancer development, and the high expression of ANRIL and homeobox A11-antisense RNA (HOXA11-AS) lncRNA is associated with poor survival in GC[132,133]. The overexpression of lncRNA ANRIL is significantly associated with GC progression and can serve as an independent predictor of patient survival[136]. The high expression of ANRIL combined with polycomb repressive complex 2 significantly silences microRNA 99a (miR-99a) and miR-449a at the transcriptional level, which increases the expression of mTOR, CDK6, and E2 transcription factor 1[132]. The HOXA11-AS gene reduces the expression of suppressor tumor genes Krüppel-like Factor 2 (KLF2) and protease serine 8 at the transcriptional level[133]. KLF2 downregulation is associated with migration, invasion, and poor survival[137,138]. KLF2 inhibits growth and migration and induces pancreatic cancer cells to senescence.

ESCC has poor survival when low esophageal cancer-related gene 4 expression occurs compared to the high-expression group[139]. EAC has worse overall survival when IL11 expression increases. Poor survival is also observed in a low expression of neuronal pentraxin 1, inositol 1,4,5-trisphosphate receptor type 1, and platelet derived growth factor D[140].

PDAC analyses show that high expression of the centromere protein F, sciellin, serpin family B member 5, solute carrier family 2 member 1 (SLC2A1), SLC6A14, transmembrane channel like 7, and transmembrane serine protease 4 is associated with a lower probability of survival compared to the same genes in low expression[141].

We investigated the gene expression and overall survival of the central genes present in the PPI network (Figure 1). We used information from the Kaplan Meier plotter (https://kmplot.com)[142] to investigate the potential prognosis of the central genes. Three of the five genes investigated have gene expression associated with survival (Akt1, TP53, and CTNNB1) (Figure 2).

The high expression of Akt1 and TP53 in GC is associated with a poor prognosis. In contrast, low CTNNB1 expression is correlated with reduced survival. The expression values and survival curves for TP53 (mRNA) in the Kaplan Meier plotter agree with tumor protein p53 expression in GC[143,144]. The TP53 expression is low and has a short half-life in normal cells, whereas in tumor cells, this gene has high expression and a long half-file[145]. The higher expression of TP53 is indicative of the worst prognosis. Akt1 expression was not indicative of prognosis[146]. However, they found that EGFR and Akt1 expression are mutually exclusive and associated with poor survival. This result might be due to the two proteins acting in the same pathway. The phosphorylated Akt1 and CTNNB1 high expression are associated with poor survival[147,148].



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Figure 2 Prognostic value of Akt1, catenin beta 1, tumor protein p53 for gastric cancer (A) and hepatocellular carcinoma (B) in Kaplan Meier plotter (https://kmplot.com)[142]. Kaplan-Meier survival curves for patients of gastric cancer and hepatocellular carcinoma with high and low indicated gene expression. CTNNB1: Catenin beta 1; TP53: Tumor protein p53.

There is no significant difference between Akt1 or CTNNB1 high and low expression groups in liver cancer. Regarding the TP53 gene, the differences in expression are not significant in the initial stage of carcinoma. However, this high expression predicts a poor prognosis and a higher mortality rate than a low expression. The results are not according to the TP53 gene expression for HCC, where TP53 high expression is present in poor prognosis groups[149].

However, the prognosis markers based on expression have limitations, and the result must be taken together with other markers.

CONCLUSION

The OMIC information about GI cancer is very complex, and each organ/region has subtypes and particularities. We presented information about and brought to light the most common genomic changes among these cancers. The pathways shared by these molecular diseases also point to the standard functions and the crosstalk of these pathways and the PAK1 pathway centrality, connecting to MAPK/ERK, PI3K/Akt, apoptosis, and Wnt signaling pathways. The PPI network pointed to five central genes, and the literature corroborates the crucial role in GI cancer with expression and poor prognosis association. This information might help in the target choice of drug and therapy research.

FOOTNOTES

Author contributions: Bispo IMC, Granger HP, and de Freitas LM wrote the paper; Almeida PP collected the data and contributed to the analyses; Nishiyama PB and de Freitas LM revised the manuscript.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Somatic mutations in FAT cadherin family members constitute an underrecognized subtype of colorectal adenocarcinoma with unique clinicopathologic features

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Abstract

BACKGROUND

The FAT cadherin family members (FAT1, FAT2, FAT3 and FAT4) are conserved tumor suppressors that are recurrently mutated in several types of human cancers, including colorectal carcinoma (CRC).

AIM

To characterize the clinicopathologic features of CRC patients with somatic mutations in FAT cadherin family members.

METHODS

We analyzed 526 CRC cases from The Cancer Genome Atlas PanCancer Atlas dataset. CRC samples were subclassified into 2 groups based on the presence or absence of somatic mutations in *FAT1*, *FAT2*, *FAT3* and *FAT4*. Individual clinicopathological data were collected after digital slide review. Statistical analysis was performed using *t* tests and chi-square tests.

RESULTS

This CRC study cohort had frequent mutations in the *FAT1* (10.5%), *FAT2* (11.2%), *FAT3* (15.4%) and *FAT4* (23.4%) genes. Two hundred CRC patients (38.0%) harbored somatic mutations in one or more of the *FAT* family genes and were grouped into the FAT mutated CRC subtype. The FAT-mutated CRC subtype was more commonly located on the right side of the colon (51.0%) than in the rest of

the cohort (30.1%, $P < 0.001$). It showed favorable clinicopathologic features, including a lower rate of positive lymph nodes (pN1-2: 33.5% *vs* 46.4%, $P = 0.005$), a lower rate of metastasis to another site or organ (pM1: 7.5% *vs* 16.3%, $P = 0.006$), and a trend toward an early tumor stage (pT1-2: 25.0% *vs* 18.7%, $P = 0.093$). FAT somatic mutations were significantly enriched in microsatellite instability CRC (28.0% *vs* 2.1%, $P < 0.001$). However, FAT somatic mutations in microsatellite stable CRC demonstrated similar clinicopathologic behaviors, as well as a trend of a better disease-free survival rate (hazard ratio = 0.539; 95% confidence interval: 0.301-0.967; log-rank $P = 0.073$).

CONCLUSION

FAT cadherin family genes are frequently mutated in CRC, and their mutation profile defines a subtype of CRC with favorable clinicopathologic characteristics.

Key Words: *FAT* cadherin family genes; Colorectal adenocarcinoma; Clinicopathologic features; Prognosis; The Cancer Genome Atlas

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Core Tip: Colorectal carcinoma (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide. In this study, we aimed to characterize the clinicopathologic features of CRC patients with somatic mutations in FAT cadherin family members. CRC cases have frequent mutations in *FAT* family genes. The FAT-mutated CRC subtype is more commonly located on the right side of the colon and shows favorable clinicopathologic features, including a lower rate of positive lymph nodes and a lower rate of metastasis to another site or organ, suggesting that the *FAT* somatic mutation is a potentially independent prognostic factor in CRC.

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INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide, with more than 1.9 million new cases and 935000 deaths in 2020[1]. Except for a few CRC cases (5%-10%) with inherited gene mutations, most CRC cases occur sporadically and exhibit chromosomal instability that leads to changes in chromosome numbers and structure, featuring aneuploidy, loss of heterozygosity, subkaryotypic amplification, and chromosomal rearrangement. Along with karyotypic abnormalities, mutations in specific tumor suppressor genes and oncogenes, such as the adenomatous polyposis (*APC*) gene, tumor protein p53 (*TP53*) and KRAS proto-oncogene GTPase, also contribute to CRC tumorigenesis. Notably, mutation of the *APC* gene, which leads to the activation of Wnt/β-catenin signaling, is an essential and early event in the development of CRC[2,3].

Despite the well-defined genetic and epigenetic alterations in CRC initiation and progression, recent studies have shown that the Hippo pathway may interact with Wnt/β-catenin signaling and play a crucial role in controlling intestinal stem cell proliferation and CRC development[4]. The Hippo pathway is an emerging tumor suppressor pathway. As a proposed upstream component of the Hippo pathway, the atypical cadherin FAT acts as a receptor to activate the Hippo pathway[5], and its mutation appears to be a recurrent event in human cancers in association with dysregulation of the Hippo pathway[6].

The human *FAT* cadherin gene family comprises the *FAT1*, *FAT2*, *FAT3* and *FAT4* genes[7-10]. The encoded proteins FAT1-4 are human homologs of Drosophila FAT, of which FAT1 and FAT4 have been reported to be involved in the regulation of planar cell polarity[11] and tumor suppression[12,13]. FAT1 also promotes actin-mediated cell migration[14,15] and plays a role in epithelial mesenchymal transition [16]. Somatic mutations of *FAT* family genes have been detected in different human cancers, including squamous cell carcinoma of the head and neck (*FAT1*, *FAT2* and *FAT4*)[17-20], breast cancer (*FAT1*)[21], melanomas (*FAT4*)[22], leukemia (*FAT1*)[23,24], hepatocellular cancer (*FAT1*, *FAT4*)[25,26], esophageal squamous cell carcinoma (*FAT1*)[27-29], pancreatic cancer (*FAT1*, *FAT3* and *FAT4*)[30,31], and gastric cancer (*FAT4*)[32,33]. Alterations in FAT family genes are associated with tumorigenesis and prognosis. For instance, upregulation of the *FAT1* gene is associated with poor prognosis and early relapse in acute

lymphoblastic leukemia patients[24] and invasive progression of ductal carcinoma *in situ*[21], while loss of *FAT4* is associated with invasiveness in gastric cancer[34]. Until now, the role of FAT family genes in CRC tumorigenesis has not been well studied. In this study, we characterized the clinicopathologic features of *FAT* family gene mutations in CRC patients.

MATERIALS AND METHODS

Study design

In total, 526 CRC cases were selected from The Cancer Genome Atlas (TCGA) PanCancer Atlas dataset. cBioPortal (https://www.cbioportal.org/) was used to download whole-exome somatic mutation data and clinical information. There are certain sample inclusion criteria for the TCGA PanCancer Atlas on colorectal adenocarcinoma. The biospecimens were collected from newly diagnosed colorectal adenocarcinoma patients undergoing surgical resection, regardless of histologic grade or tumor stage. The patients had not received prior chemoradiation therapy. The histological sections contained an average of 60% tumor cells with less than 20% necrosis[35].

In the TCGA PanCancer Atlas dataset, the somatic mutation profiles of *FAT1*, *FAT2*, *FAT3* and *FAT4* were analyzed for each tumor. Furthermore, the CRC cases were categorized into two groups based on their mutational status on FAT family genes: The cases with mutant *FAT1-4* and the cases with wild-type *FAT1-4*. Standard demographic and clinicopathological data were retrieved for each patient, including age, sex, tumor location, pT stage, pN stage, pM stage, differentiation grade, tumor type, lymphovascular invasion, month of disease-free survival (DFS) and overall survival (OS).

Statistical analyses

Demographic and clinicopathological details were stratified according to *FAT1-4* mutation. Quantitative and qualitative variables were expressed as the means ± SD and the frequencies. Comparisons between the groups were analyzed with *t* tests and chi-square tests. DFS and OS were analyzed using the Kaplan-Meier method, and the log-rank test was used to assess differences. The figure was prepared using GraphPad Prism 9 software (GraphPad Software, San Diego, California, United States). *P* values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

The study included 526 patients with CRC from TCGA PanCancer Atlas Dataset. The mean age of the patients was 65.8 years (SD 13.0 years; range: 31-90 years). Based on the available clinicodemographic information, two hundred fifty-two patients were female, and two hundred seventy-two patients were male. Of them, 254 (48.3%) patients had left-sided colon cancer, and 197 (37.5%) patients had right-sided colon cancer. The majority (72.4%) of the CRCs were moderately differentiated adenocarcinomas. The detailed demographics, histopathologic stage and features are summarized in Table 1.

Somatic mutations of FAT family genes in CRC

Among the 526 CRC cases, 200 (38.0%) patients harbored one or more somatic mutations of the FAT cadherin family genes, including mutations in the *FAT1* (10.5%), *FAT2* (11.2%), *FAT3* (15.4%), and *FAT4* (23.4%) genes. The somatic mutation types of the *FAT* family genes include missense mutation, nonsense mutation, splicing mutation, frameshift deletion, frameshift insertion and in-frame deletion, with missense mutation being the most common somatic mutation type (Table 2). Interestingly, these somatic mutations were significantly enriched in the extracellular cadherin domain (*FAT*1, 49.0%; *FAT*2, 63.4%; *FAT*3, 40.1%; *FAT*4, 57.8%) (Table 2).

Based on the presence or absence of somatic mutations in *FAT1-4* genes, these cases were subclassified into 2 groups in our study. The clinicopathologic features of these 2 subtypes are summarized in Table 3. In the FAT-mutated CRC subtype, the median patient age was 66.5 years (range: 33-90 years), and 102 (51.0%) patients were male. Compared with the rest of the cohort, the FAT-mutated CRC subtype was more commonly located on the right side of the colon (51.0% *vs* 30.1%, $P < 0.001$) and more commonly associated with favorable histopathologic features, including lower pathological nodal stage (pN0: 66.5% *vs* 52.8%, $P = 0.005$), lower rate of metastasis to another site or organ (pM1: 7.5% *vs* 16.3%, $P = 0.006$), and a trend of lower pathological tumor stage (pT1-2: 25.0% *vs* 18.7%, $P = 0.093$).

FAT somatic mutations are enriched in microsatellite-instable CRC

Human *FAT* family genes encode large atypical cadherin proteins with a large number of cadherin repeats. Given the overlapping features found in the FAT-mutated CRC subtype and microsatellite-instable (MSI) CRC (right sided with favorable clinicopathological features), we further explored the association between *FAT* mutations and MSI. Interestingly, FAT somatic mutations were significantly

Table 1 Clinicodemographics and histologic features in 526 patients with colorectal adenocarcinoma (PanCancer Atlas)

Feature	Level	Number	MSS number
Age (yr), mean ± SD		65.8 ± 13.0	65.4 ± 12.7
Gender	Female	252 (47.9%)	218 (47.1%)
	Male	272 (51.7%)	243 (52.5%)
	Unknown	2 (0.4%)	2 (0.4%)
Histopathologic differentiation	Well	19 (3.6%)	18 (3.9%)
	Moderate	381 (72.4%)	351 (75.8%)
	Poor	114 (21.7%)	83 (17.9%)
	Unknown	12 (2.3%)	11 (2.4%)
Tumor location	Left	254 (48.3%)	248 (53.6%)
	Right	197 (37.5%)	149 (32.2%)
	Left and right	3 (0.6%)	3 (6.5%)
	Unknown	72 (13.7%)	63 (13.6%)
Tumor staging (pT)	T1	18 (3.4%)	17 (3.7%)
	T2	94 (17.9%)	83 (17.9%)
	T3	355 (67.5%)	310 (67.0%)
	T4	57 (10.8%)	52 (11.2%)
	TX	2 (0.4%)	2 (0.4%)
Nodal staging (pN)	N0	305 (58.0%)	255 (55.1%)
	N1	128 (24.3%)	120 (25.9%)
	N2	90 (17.1%)	85 (18.4%)
	NX	3 (0.6%)	3 (6.5%)
Metastasis (pM)	M0	388 (73.8%)	338 (73.0%)
	M1	68 (12.9%)	66 (14.3%)
	MX	70 (13.3%)	59 (12.7%)
Lymphovascular invasion	Present	178 (33.8%)	157 (33.9%)
	Absent	230 (43.7%)	202 (43.6%)
	Unknown	118 (22.4%)	104 (22.5%)
Ethnicity	Caucasian	273 (51.9%)	236 (51.0%)
	African-American	60 (11.4%)	51 (11.0%)
	Asian	12 (2.3%)	11 (2.4%)
	Unknown	181 (34.4%)	165 (35.6%)
Subtype	CIN	328 (62.4%)	
	MSI	63 (12.0%)	
	GS	58 (11.0%)	
	POLE	10 (1.9%)	
	Unknown	57 (10.8%)	
Total		526	463

CIN: Chromosomal instability; MSI: Microsatellite instability; GS: Genomically stable; POLE: Polymerase epsilon mutation; MSS: Microsatellite stable.

enriched in MSI CRC (28.0% *vs* 2.1%, $P < 0.001$) (Table 3).

Table 2 Genetic mutation types and numbers in FAT family genes in colorectal adenocarcinoma (PanCancer Atlas)

Gene	Missense mutation	Nonsense mutation	Splicing mutation	Frame shift deletion	Frame shift insertion	Inflame deletion	Total mutation	Mutation in Cadherin domains
FAT1	85	5	2	3	2	1	98	48 (49.0%)
FAT2	90	2	3	5	1	0	101	64 (63.4%)
FAT3	124	6	0	5	2	0	137	55 (40.1%)
FAT4	198	19	0	10	4	0	230	133 (57.8%)

To control for confounding in the analysis, we focused on cases of microsatellite-stable (MSS) CRC. As shown in Table 1, the MSS CRC cases showed similar clinicodemographic and histologic features as the entire cohort. We also categorized the MSS CRC cases into 2 groups based on the mutation status of *FAT* family genes. Similar to the entire cohort we described earlier, the FAT-mutated MSS CRC subtype was also more commonly located on the right side of the colon (39.6% *vs* 28.8%, $P = 0.038$) and more commonly associated with favorable histopathologic features, such as a lower rate of metastasis to another site or organ (pM1: 9.0% *vs* 16.6%, $P = 0.038$). It also showed a trend of lower pathological tumor stage (pT1-2: 26.4% *vs* 19.1%, $P = 0.083$) and lower pathological nodal stage (pN0: 60.4% *vs* 52.7%, $P = 0.079$) (Table 3). Therefore, even though it is enriched in MSI CRC, the FAT somatic mutation is a potentially independent prognostic factor in CRC.

The median DFS for CRC patients was 26.0 mo (0.5-148.0 mo), and the OS was 21.0 mo (0-148.0 mo). Consistent with the favorable pathologic features, the FAT-mutated MSS CRC subgroup showed a trend toward a better DFS rate [hazard ratio (HR) = 0.539; 95% confidence interval (CI): 0.301-0.967; log-rank $P = 0.073$]. However, FAT mutation status did not show a significant impact on the OS rate (HR = 1.198; 95%CI: 0.770-1.864; log-rank $P = 0.440$) (Figure 1).

DISCUSSION

To our knowledge, this is the first study to assess the impact of somatic mutations in *FAT* family genes on clinicopathologic features, with an emphasis on prognosis in CRC patients. Our study shows that somatic mutations in *FAT* family genes are associated with favorable clinicopathologic features, including a lower rate of lymph node and distal metastasis. It also showed a trend toward a lower tumor stage with a relatively favorable DFS.

In addition to the *APC-β-catenin* pathway, which represents the most prominent signaling pathway in CRC, components of the Hippo pathway have been reported to be involved in CRC tumorigenesis[36-40] and have been proposed as prognostic factors in CRC[41-44]. As an upstream organizer and activator of the Hippo pathway[6], *FAT* family genes have emerged as an important mechanism that orchestrates epithelial development as well as human cancer initiation and progression. The *FAT* family genes (*FAT1-4*) encode atypical cadherins that contain multiple extracellular cadherin repeats, laminin G motifs and EGF-like motifs[45]. Among these, *FAT*1 and *FAT*4 are relatively well studied. Loss of *FAT*4 expression has been reported in some primary breast cancers and breast cancer cell lines[46]. Low *FAT*4 expression was also observed in gastric cancers and was associated with a poor prognosis, including high pathologic T stage, an increase in perineural invasion, high lymph node metastasis and reduced DFS[47]. Similarly, a study reported recurrent *FAT1* mutations in multiple human cancers, including glioblastoma, CRC, and head and neck cancer, and *FAT1* mutations affected patient survival by promoting Wnt signaling and tumorigenesis[48]. Our study demonstrates that somatic mutations in FAT family genes are frequent recurrent events in CRC and that FAT mutations are associated with favorable clinicopathologic features. These somatic mutations are highly enriched in the extracellular cadherin domains (Table 2). FAT proteins are large single transmembrane receptors characterized by 32-34 extracellular cadherin repeats. These cadherin repeats contain highly conserved binding sites for proteins, such as beta-catenin and p120-catenin, which are important for the FAT protein to execute its role in migration, polarity and cell adhesion by linking it to the actin cytoskeleton.

Our study also revealed the significant enrichment of FAT-mutated CRC (28.0%) in the MSI subgroup. However, the clinicopathologic characteristics in FAT-mutated MSS CRC are quite compatible with the entire FAT-mutated CRC cohort in our study, suggesting that MSI only partially contributes to its pathologic features and clinical outcomes. Interestingly, FAT-mutated MSS CRC cases showed a trend of favorable DFS but not OS. The underlying mechanisms of this discrepancy are currently unclear. Notably, DFS does not always correlate with OS in CRC, such as in the case of liver-only metastatic CRC[49].

Similar to the findings in our study, Wang *et al*[33] reported a superior prognosis in gastric adenocarcinoma with *FAT* family gene mutations. In their study, *FAT* gene mutations were significantly associated with better progression-free survival and OS, which was likely attributed to the significantly

Table 3 Association of clinicopathologic features with FAT somatic mutations in colorectal adenocarcinoma (PanCancer Atlas)

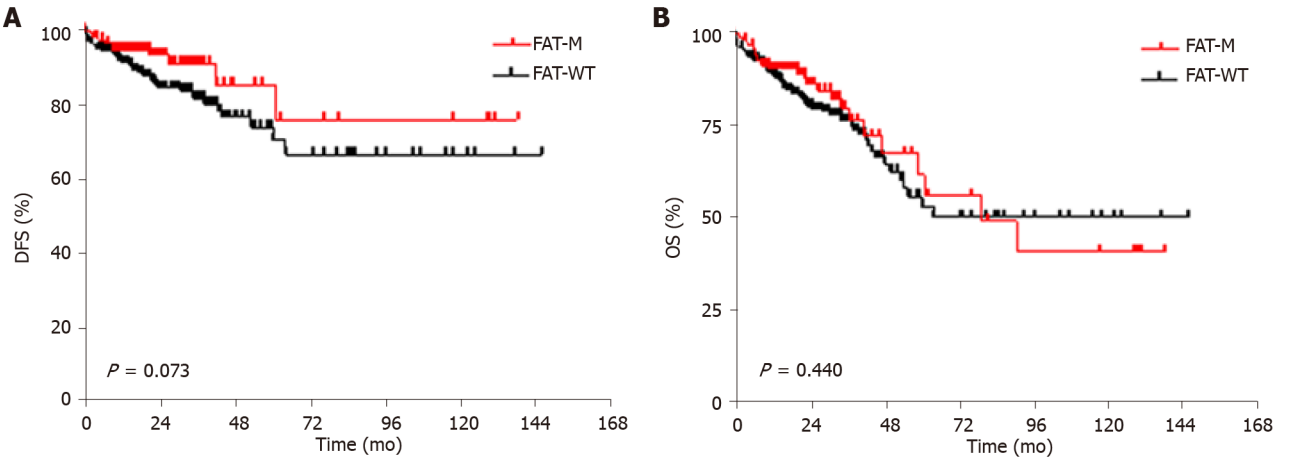
Clinicopathologic features	Mutated FAT genes	Wildtype FAT genes	*P* value	Mutated FAT genes (MSS)	Wildtype FAT genes (MSS)	*P* value
Mean age (mean ± SD)	66.5 ± 12.9	65.3 ± 13.0	0.912	65.6 ± 12.1	65.3 ± 12.9	
Sex			0.689			0.825
Female	98 (49.0%)	154 (47.2%)		67 (46.5%)	151 (47.3%)	
Male	102 (51.0%)	170 (52.1%)		77 (53.5%)	166 (52.0%)	
Location			< 0.001[a]			0.038[a]
Left side	65 (32.5%)	181 (55.5%)		70 (48.6%)	178 (55.8%)	
Right side	102 (51.0)	98 (30.1%)		57 (39.6%)	92 (28.8%)	
pT stage			0.093			0.083
pT1-2	50 (25.0%)	61 (18.7%)		38 (26.4%)	61 (19.1%)	
pT3-4	150 (75.0%)	263 (80.7%)		106 (73.6%)	256 (80.3%)	
pN stage			0.005[a]			0.079
pN0	133 (66.5%)	172 (52.8%)		87 (60.4%)	168 (52.7%)	
pN1	44 (22.0%)	84 (25.8%)		39 (27.1%)	81 (25.4%)	
pN2	23 (11.5%)	67 (20.6%)		18 (12.5%)	67 (21.0%)	
pM stage			0.006[a]			0.038[a]
pM0	153 (76.5%)	235 (72.1%)		110 (76.4%)	228 (71.5%)	
pM1	15 (7.5%)	53 (16.3%)		13 (9.0%)	53 (16.6%)	
Differentiation grade			0.332			0.172
G1-2	145 (72.5%)	255 (78.2%)		117 (81.3%)	252 (79.0%)	
G3	47 (23.5%)	67 (20.6%)		20 (13.9%)	63 (19.7%)	
Subtype			< 0.001[a]			
CIN	92 (46.0%)	236 (72.4%)				
MSI	56 (28.0%)	7 (2.1%)				
GS	25 (12.5%)	33 (10.1%)				
Lymphovascular invasion			0.313			0.516
Positive	61 (30.5%)	117 (35.9%)		44 (30.6%)	113 (35.4%)	
Negative	90 (45.0%)	140 (42.9%)		63 (43.8%)	139 (43.6%)	
Total	200 (38.0%)	326 (62.0%)		144 (31.1%)	319 (68.9%)	

[a]$P < 0.05$.

CIN: Chromosomal instability; MSI: Microsatellite instability; GS: Genomically stable; MSS: Microsatellite stable.

higher tumor mutational burden and an inflamed tumor microenvironment[33]. Whether the tumor microenvironment plays a similar role in CRC still awaits further investigation.

Our study has several limitations. First, our findings were obtained from a bioinformatics study on somatic mutation profiles through the TCGA PanCancer Atlas dataset. The protein expression levels of individual FAT family members were not systemically examined in the study, and the underlying molecular mechanisms related to the prognostic role of the FAT family in colorectal cancer need further experimental validation. Second, all the patients in the study were untreated, with no therapy response data and a short follow-up. Therefore, the evaluation of advanced-stage CRC is relatively limited. Third, we tried to address the impact of MSI status, a confounding factor, by analyzing the MSS samples. However, there are still additional potential confounding factors, such as histopathological subtypes, *TP53* mutation status, and intratumoral spatial and temporal heterogeneity. The ability of our study to address these potential confounding factors is hampered by intrinsic limitations of the TCGA database, the landmark cancer program heavily focused on cancer genomics datasets. A randomized, large-scale clinical cohort is necessary to validate our conclusion and to establish somatic mutations in *FAT* family



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Figure 1 Kaplan-Meier curves of disease-free survival and overall survival in microsatellite-stable colorectal adenocarcinoma patients without and with FAT family gene mutations. A: Disease-free survival; B: Overall survival. FAT-M: FAT mutated; FAT-WT: Wild-type FAT; DFS: Disease-free survival; OS: Overall survival.

genes as independent prognostic factors for CRC in future studies.

CONCLUSION

In summary, our study shows that somatic mutations in *FAT* family genes are recurrent genetic events detected in approximately 38% of CRC cases and therefore represent an underrecognized subtype of CRC. The FAT-mutated CRC subtype shows unique clinicopathologic features, including a right-side location, a lower rate of positive lymph nodes, a lower rate of metastasis to another site or organ, and a trend toward favorable DFS. Our study suggests that somatic mutations in *FAT* family genes are potential prognostic biomarkers for CRC.

ARTICLE HIGHLIGHTS

Research background

The human *FAT* cadherin gene family comprises the *FAT*1, *FAT*2, *FAT*3 and *FAT*4 genes. Somatic mutations of *FAT* family genes have been detected in different human cancers.

Research motivation

Until now, the role of *FAT* family genes in colorectal carcinoma (CRC) tumorigenesis has not been well studied. In this study, we characterized the clinicopathologic features of *FAT* family gene mutations in CRC patients.

Research objectives

In total, 526 CRC cases were selected from The Cancer Genome Atlas PanCancer Atlas dataset.

Research methods

CRC cases were categorized into two groups based on their mutational status on *FAT* family genes: The cases with mutant *FAT1-4* and the cases with wild-type *FAT1-4*. Standard demographic and clinicopathological data were retrieved for each patient, including age, sex, tumor location, pT stage, pN stage, pM stage, differentiation grade, tumor type, lymphovascular invasion, month of disease-free survival and overall survival.

Research results

The FAT-mutated CRC subtype is more commonly located on the right side of the colon and shows favorable clinicopathologic features, including a lower rate of positive lymph nodes and a lower rate of metastasis to another site or organ.

Research conclusions

FAT cadherin family genes are frequently mutated in CRC, and their mutation profile defines a subtype of CRC with favorable clinicopathologic characteristics.

Research perspectives

FAT somatic mutation is a potentially independent prognostic factor in CRC.

FOOTNOTES

Author contributions: Wang LL, Zheng W, Liu XL and Yin F collected and analyzed the data, made the tables and figures, and wrote and finalized the manuscript; and all authors have approved the final manuscript.

Institutional review board statement: This study is solely based on the publicly available TCGA PanCancer Atlas database. The Institutional Review Board Approval is not applicable.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Outcomes after natural orifice extraction *vs* conventional specimen extraction surgery for colorectal cancer: A propensity score-matched analysis

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Abstract

BACKGROUND

Natural orifice specimen extraction (NOSE) *via* the anus or vagina replaces conventional transabdominal specimen retrieval *via* the transabdominal route through a limited mid-line laparotomy or Pfannenstiel incision. Reducing the number of laparoscopic ports further decreases operative abdominal wall trauma. These techniques reduce the surgical wound size as well as the risk of incision-related morbidity.

AIM

To compare short-term outcomes following 3-port NOSE surgery with a matched cohort of conventional non-NOSE colorectal cancer surgery.

METHODS

Patients who underwent elective 3-port laparoscopic colorectal NOSE surgery between February to October 2021 were identified. Selection criteria for NOSE surgery was adapted from the 2019 International Consensus on Natural Orifice Specimen Extraction Surgery for colorectal cancer. Patients with clinical T4 or N2 tumors on staging computed tomography were also excluded. The propensity score-matched cohort was identified amongst patients who underwent conventional laparoscopic colorectal surgery from January 2019 to December 2020. Matching was performed in the ratio of 1:4 based on age, gender, type of resec-

tion, and p - tumor node metastasis staging.

RESULTS

Over the eight-month study duration, 14 consecutive cases (nine female, five male) of elective 3-port laparoscopic surgery with NOSE were performed for colorectal cancer. Median age and body mass index were 70 (range 43-82) years and 24.1 (range 20.0-31.7) kg/m^2 respectively. Six patients underwent transanal NOSE and eight had transvaginal NOSE. Median operative time, intraoperative blood loss and postoperative length of stay were 208 (range 165-365) min, 30 (range 10-150) mL and 3 (range 2-6) d respectively. Two (14%) suffered minor postoperative compilations not attributable to the NOSE procedure. Median follow-up duration was 12 (range 8-15) mo. No instances of mortality, local or distant disease recurrence were recorded in this cohort. Compared to the conventional surgery cohort of 56 patients, the 3-port NOSE cohort had significantly quicker mean return of bowel function (2.6 *vs* 1.2 d, $P < 0.001$), reduced postoperative pain and patient-controlled analgesia use, and decreased length of hospital stay (6.4 *vs* 3.4 d, $P < 0.001$). There were no statistical differences in surgical duration and perioperative complication rates between the NOSE and non-NOSE cohorts.

CONCLUSION

3-port laparoscopic colorectal surgery with NOSE is a feasible technique, augmenting the minimally invasive nature of surgery and producing good outcomes. Appropriate patient selection and expertise in conventional laparoscopy are required.

Key Words: 3-port laparoscopy; Colorectal surgery; Natural orifice specimen extraction; Transanal; Transvaginal; Minimally invasive surgery

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Core Tip: This paper demonstrates the benefit of reduced port laparoscopic colorectal surgery with natural orifice specimen extraction compared to conventional laparoscopic colorectal surgery. This technique represents a natural progression towards scarless surgery - the holy grail of minimally invasive surgery.

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INTRODUCTION

Minimal access abdominopelvic surgery has come a long way since the advent of laparoscopic colorectal surgery in the early 90 s. New technologies and platforms have been introduced, including robotic and transanal minimally invasive surgery. The primary objective remains the same - complete tumor extirpation along with the draining lymphatic tissue. Without deviating from the principles of surgical oncology, increasing experience and expertise of laparoscopic surgeons has encouraged continued surgical innovation, resulting in improved operative technique and patient outcomes.

Natural orifice specimen extraction (NOSE) is a logical progression in the evolution of minimally invasive colorectal surgery. Removal of the surgical specimen *via* a natural bodily orifice such as the vagina or anus replaces the need for conventional specimen extraction (CSE) *via* the transabdominal route through a limited mid-line laparotomy or Pfannenstiel incision. This greatly reduces the surgical wound size as well as the risk of incision-related morbidity.

The first use of NOSE in colorectal surgery was reported in 1993 by Franklin *et al*[1], who described laparoscopic colectomy with transanal specimen retrieval. There has been continued interest in this technique almost three decades later. Three meta-analyses comparing laparoscopic colorectal resection with NOSE *vs* CSE have been published in the last two years[2-4]. These studies consistently demonstrate the benefits of NOSE, in terms of overall complications, incision-related complications, intraoperative blood loss, postoperative pain, return of gastrointestinal function and length of hospital stay. However, NOSE required a longer operating time than CSE. No significant differences were observed for cancer-specific outcomes, including local and distant recurrences, 3- and 5-year disease-free survival and overall survival[2-4].

Furthermore, patients who underwent NOSE colectomy were found to have better perception of body image and cosmetic appearance compared to CSE at a median follow-up of approximately 3-years after surgery[5]. Quality of life and gastrointestinal function following NOSE were also found to be superior to a propensity score-matched cohort of CSE at 3-mo post-surgery[6]. We recently demonstrated the feasibility of NOSE following combined colectomy and liver resection[7].

Conventional laparoscopic colorectal surgery is performed using 4 or 5 ports: 1 camera port, 2 operator ports and 1 or 2 assistant ports. Reducing the number and size of the ports further decreases the operative trauma to the abdominal wall. 3-port colorectal surgery with 1 camera port and 2 operator working ports has previously been demonstrated to be feasible[8-10]. A recent study showed equivalent long-term oncologic outcomes with 3-port right hemicolectomy compared to the conventional 5-port technique; the former was also associated with significantly less operative blood loss[11].

Logically, the minimally invasive nature of surgery is augmented utilizing 3-port surgery in addition to NOSE, enhancing the overall benefit to the patient. In this study we aimed to compare the short-term outcomes following 3-port NOSE surgery with a matched cohort of conventional laparoscopic non-NOSE surgery across a variety of colorectal cancer resection types. We also discuss the in-depth technical approach to NOSE surgery.

MATERIALS AND METHODS

From 1 February to 1 October 2021, all cases of elective 3-port laparoscopic colorectal surgery with NOSE for colorectal cancer were included in the study. Selection criteria for NOSE surgery was adapted from the 2019 International Consensus on Natural Orifice Specimen Extraction Surgery for colorectal cancer[12]. Colectomy for benign diagnoses were excluded from the analysis. Patients with clinical T4 or N2 tumors on staging computed tomography were also excluded. Final decision to proceed with the NOSE procedure was only made following laparoscopic assessment.

The propensity score-matched cohort was identified amongst anonymized subjects who underwent elective laparoscopic colorectal surgery with CSE for colorectal cancer from January 2019 to December 2020. Matching was performed in the ratio of 1:4 based on age, gender, type of resection, and p - tumor node metastasis staging. Statistical analysis was performed using R statistical software (version 4.1.2). Continuous variables were compared with the Mann-Whitney U test and independent t-test, while dichotomous variables with compared using chi-squared test.

Ethics approval for the study was granted by the SingHealth centralized institutional review board (reference number 2022/2114), conforming to the provisions of the Declaration of Helsinki. All patients who underwent NOSE surgery provided written informed consent for participation in the study.

Surgical technique

The 3-port laparoscopic NOSE technique involves 3 phases: (1) Standard laparoscopic bowel mobilization and oncologic resection; (2) Natural orifice specimen extraction; and (3) Intestinal reconstruction. We utilized the port placements and operative set-up as shown in Figure 1.

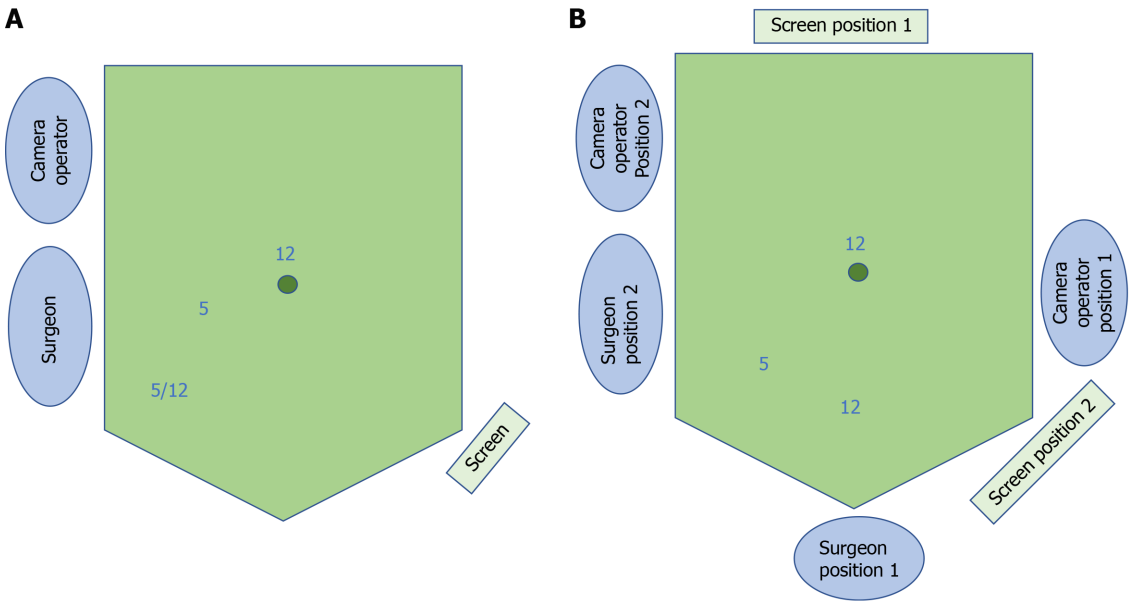
NOSE procedure: For left-sided resections, transanal NOSE was the only possible natural orifice extraction method in males, and preferable over transvaginal NOSE in females to avoid an additional vaginal incision. The transvaginal route *via* a posterior vaginotomy was chosen to allow retrieval of larger specimens due to the increased elasticity of the vagina[11]. For both transanal and transvaginal NOSE, the specimen was delivered through an extra small Alexis® dual-ring wound protector with the inner ring inserted fully into intraperitoneal space and the outer ring opened against the perineum to shorten the length of the channel (Figure 2). Reducing the length of the channel for extraction is of particular importance for sigmoid cancer surgery where the full length of the rectum is preserved.

For right-sided resections, only females were selected for the NOSE procedure. All specimens were thus extracted transvaginally. We recently reported our technique for 3-port laparoscopic D3 right hemicolectomy with transvaginal NOSE[13]. Transanal NOSE has been successfully performed and described following right-sided colonic surgery, in both male and female patients[14,15]. However, this approach requires an additional rectal incision and was avoided in our cohort, due to the added risk of luminal content spillage.

Care was taken to ensure surgical specimens were delivered complete (Figure 2) and did not tear or rupture during the extraction process. Following transvaginally delivery, the posterior vaginotomy was closed continuously with a barbed suture (Figure 2).

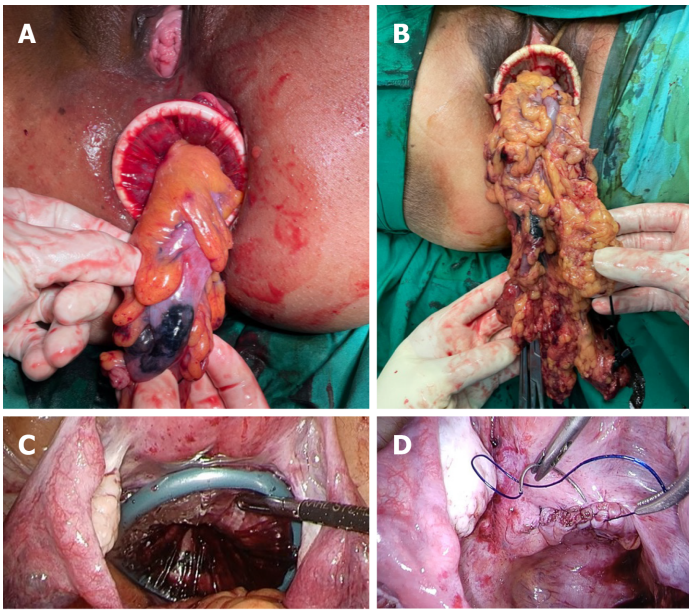
Intestinal reconstruction: Restoration of intestinal continuity following left-sided NOSE surgery requires management of the proximal and distal bowel ends prior to anastomosis, which was performed with a circular stapler.

Two methods were used to secure the anvil to the proximal bowel. The first involved transanal or transvaginal colonic pull-through to allow extracorporeal anvil application (Figure 3). This required complete mobilization of splenic flexure for length. In our cohort, medial-to-lateral splenic flexure takedown did not require additional port placement. The second technique involved securing the anvil



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Figure 1 Schematic of 3-port natural orifice specimen extraction surgery port positioning and operative set-up. A: For left-sided colorectal resection; the right iliac fossa port can be 5 or 12 mm depending on whether a linear stapler is used; B: For right-sided or subtotal/total colectomy, comprising position 1 for the initial phase of surgery and position 2 for the natural orifice specimen extraction procedure.

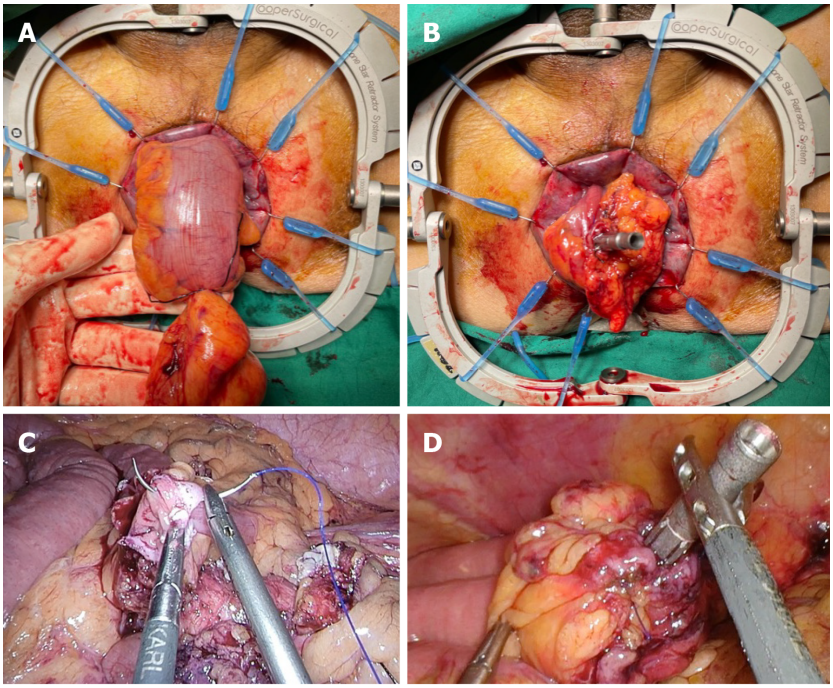


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Figure 2 Operative images of the natural orifice specimen extraction procedure. A: Transanal extraction; B: Transvaginal extraction; C: Intraperitoneal application of the dual-ring wound protector with the uterus hitched to the anterior abdominal wall; D: Closure of the posterior vaginotomy.

to cut end of the proximal bowel using an intracorporeal purse-string suture (Figure 3). This approach required less colonic mobilization but had a theoretical risk of luminal content spillage in a poorly bowel-prepped patient.

Rectal stump closure was performed using several techniques. The first was conventional distal transection with a linear stapler, where transvaginal NOSE was planned. A linear stapler was also used to seal the open rectal stump following transanal NOSE (Figure 4). Otherwise, a purse-string suture could be applied to the rectal stump and anchored to the spike of circular stapler. For high anastomoses, the purse-string could be applied laparoscopically. For low rectal anastomoses, transanal application of the purse-string was preferred, with the aid of a transanal minimally invasive surgery access device (Figure 4).



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Figure 3 Methods of securing the circular stapler anvil to the proximal bowel. A and B: Bowel pull-through and extracorporeal anvil application; C and D: Securing the anvil with an intracorporeal purse-string suture.

The hypothetical advantage of rectal purse-string closure is the creation of a double purse-string single-stapled anastomosis (Figure 4). This method eliminates the "dog-ears" of the anastomosis, with theoretical points of weakness at the corners of the linear staple line and the cross-stapled junctions between the linear and circular staple lines[16]. Furthermore, the double purse-string anastomosis enabled the use of a smaller 5 mm port instead of 12 mm, as a linear stapler was not required (Figure 1).

Ileocolic anastomoses following right-sided resections were performed in an antiperistaltic side-to-side fashion, with the linear stapler introduced *via* the 12mm suprapubic port (Figure 1). This was previously demonstrated in a video correspondence[13].

RESULTS

Over the eight-month study duration, 14 consecutive cases (nine female, five male) of elective 3-port laparoscopic colorectal surgery with NOSE were performed by a single surgeon. Patient and surgical characteristics of these are shown in Table 1. Six patients underwent transanal NOSE and eight had transvaginal NOSE. Median age and body mass index (BMI) were 70 (range 43-82) years and 24.1 (range 20.0-31.7) kg/m^2 respectively. All patients with left-sided resections underwent pre-operative bowel preparation with 2 L polyethylene glycol. No bowel preparation was administered for right-sided resections.

Operative data and postoperative outcomes are given in Table 2. Median operative time, intraoperative blood loss and postoperative length of stay were 208 (range 155-365) min, 30 (range 10-150) mL and 3 (range 2-6) d respectively. All patients recovered gastrointestinal function within the first two postoperative d, defined as passage of flatus and non-mucoid stool. All surgical margins were clear (R0) and all had more than 12 harvested lymph nodes.

Overall complication rate was 14% (n = 2), although both were minor without requiring return to the operating theatre. One patient had low-volume chylous ascites (Clavien-Dindo grade I) and the other had high ileostomy output requiring antimotility agents (Clavien-Dindo grade II); the latter was re-admitted to hospital on postoperative day 18 for dehydration. Neither complication was attributable to the natural orifice extraction or reconstruction technique. Postoperative abdominal appearance following 3-port NOSE surgery is shown in Figure 5. Median follow-up duration was 12 (range 8-15) mo. No instances of mortality, local or distant disease recurrence were recorded.

Propensity score matching identified 56 patients from an anonymized, prospectively maintained, retrospective database, who underwent conventional laparoscopic colorectal surgery at our unit from 2019 to 2020. Comparisons of characteristics and perioperative outcomes between the NOSE and non-NOSE cohorts are shown in Table 3 and Table 4 respectively. Due to inconsistent documentation in the non-NOSE group, operative blood loss was not compared between the cohorts.

Table 1 Patient and surgical characteristics for patients who underwent 3-port laparoscopic colorectal surgery with natural orifice specimen extraction

Patient	Age	ASA	Sex	BMI (kg/m^2)	Surgery	Indication
1	80	3	F	29.1	Anterior resection, transvaginal NOSE	Sigmoid cancer pT3N1M0
2	59	1	F	20.0	Left hemicolectomy, transvaginal NOSE	Splenic flexure cancer pT3N1M0
3	82	3	F	22.4	Anterior resection, transvaginal NOSE	Sigmoid cancer pT3N0M0
4	43	2	F	31.7	Anterior resection, transanal NOSE	Sigmoid cancer pT1N0M0
5	78	3	F	21.6	Right hemicolectomy (D3), transvaginal NOSE	Transverse colon cancer pT3N1M0
6	63	2	F	28.0	Right hemicolectomy (D3), transvaginal NOSE	Hepatic flexure cancer pT1N0M0
7	77	2	M	20.3	Anterior resection with DI, transanal NOSE	Mid rectal cancer pT3N1M0
8	50	2	F	28.0	Anterior resection, transvaginal NOSE	Sigmoid cancer pT3N0M0
9	77	2	M	24.3	Anterior resection with DI, transanal NOSE	Mid rectal cancer pT3N1M0
10	79	3	M	24.3	Anterior resection, transanal NOSE	Upper rectal cancer pT3N1M0
11	73	3	M	22.4	Anterior resection, transanal NOSE	Sigmoid cancer pT4N2M1
12	58	2	F	23.5	Anterior resection, transvaginal NOSE	Sigmoid cancer pT2N0M0
13	67	2	M	27.6	Anterior resection, transanal NOSE	Sigmoid cancer pT2N1M0
14	58	2	F	23.8	Left hemicolectomy, transvaginal NOSE	Splenic flexure cancer pT1N0M0

ASA: American Society of Anesthesiologists score; BMI: Body mass index; M: Male; F: Female; NOSE: Natural orifice specimen extraction; DI: Defunctioning ileostomy.

Table 2 Intraoperative data and postoperative outcomes for patients who underwent 3-port laparoscopic colorectal surgery with natural orifice specimen extraction

Patient	Operative time (min)	Blood loss (mL)	Time to first flatus/ BO (d[a])	Postoperative LOS (d[a])	Postoperative complications
1	235	30	1/1	4	Nil
2	170	20	1/2	3	Nil
3	210	30	2/2	3	Nil
4	200	20	1/1	3	Nil
5	260	100	2/1	3	Nil
6	255	50	2/2	5	Chylous ascites
7	265	80	1/1	3	Nil
8	175	10	1/1	3	Nil
9	300	150	1/1	6	High stoma output
10	365	100	1/1	3	Nil
11	205	20	1/2	3	Nil
12	155	30	1/2	2	Nil
13	205	10	2/2	3	Nil
14	180	50	1/2	3	Nil

[a]Postoperative days. BO: Bowel opening; LOS: Length of stay.

There were no statistical differences in surgical duration and perioperative complication rates between the NOSE and non-NOSE cohorts. The 3-port NOSE group had significantly quicker return of bowel function, reduced postoperative pain and analgesia use, with a mean use of zero mg of patient-controlled morphine on the second postoperative day. Notably, the average length of hospital stay was

Table 3 Comparison of characteristics between laparoscopic 3-port colorectal natural orifice specimen extraction surgery with a propensity score-matched cohort of conventional surgery

Characteristic	NOSE, *n* = 14 Frequency (%)	Non-NOSE, *n* = 56 Frequency (%)	*P* value
Age, mean ± SD (yr)	67.4 ± 12.4	73.1 ± 10.2	0.182
Gender			
Male	5 (35.7)	21 (37.5)	0.765
Female	9 (64.3)	35 (62.5)	
BMI, mean ± SD (kg/m^2)	24.8 ± 3.5	24.0 ± 4.1	0.526
ASA score			
1	1 (7.1)	1 (1.8)	0.265
2	8 (57.1)	43 (76.8)	
3	5 (35.7)	12 (21.4)	
Tumor location			
Caecum to transverse colon	2 (14.3)	18 (32.1)	0.219
Splenic flexure to sigmoid	9 (64.3)	22 (39.3)	
Rectum	3 (21.4)	16 (28.6)	
Surgery			
Anterior resection	7 (50.0)	23 (41.1)	0.576
Low anterior resection	3 (21.4)	13 (23.2)	
Left hemicolectomy	2 (14.3)	3 (5.4)	
Right hemicolectomy	2 (14.3)	17 (30.4)	
Defunctioning stoma creation			
Yes	3 (21.4)	9 (16.1)	0.634
No	11 (78.6)	47 (83.9)	
AJCC pT stage			
T1	4 (28.6)	7 (12.5)	0.527
T2	2 (14.3)	10 (17.9)	
T3	7 (50)	33 (58.9)	
T4	1 (7.1)	6 (10.7)	
AJCC pN stage			
N0	6 (42.9)	33 (58.9)	0.236
N1	7 (50.0)	15 (26.8)	
N2	1 (7.1)	8 (14.3)	
M stage			
M0	13 (92.9)	55 (98.2)	0.282
M1	1 (7.1)	1 (1.8)	
Stage			
I	4 (28.6)	13 (23.2)	0.162
II	1 (7.1)	20 (35.7)	
III	8 (57.1)	22 (39.3)	
IV	1 (7.1)	1 (1.8)	

NOSE: Natural orifice specimen extraction; ASA: American Society of Anesthesiologists score; BMI: Body mass index; SD: Standard deviation, AJCC:

American Joint Committee on Cancer Staging 8th Edition.

Table 4 Comparison of perioperative outcomes between laparoscopic 3-port colorectal natural orifice specimen extraction surgery with a propensity score-matched cohort of conventional surgery

Outcome	NOSE *n* = 14 Frequency (%)	Non-NOSE *n* = 56 Frequency (%)	*P* value
Duration of surgery, mean ± SD (min)	227 ± 55	261 ± 96	0.463
Intraoperative complications			
Yes	0 (0)	2 (3.6)	0.473
No	14 (100)	54 (96.4)	
30-day postoperative complications			
Yes	2 (14.3)	13 (23.2)	0.466
No	12 (85.7)	43 (76.8)	
Clavien-Dindo classification			0.202
1	1 (7.1)	5 (8.9)	
2	1 (7.1)	7 (12.5)	
3	0	0	
4	0	1 (1.8)	
5	0	0	
Time to first bowel movement, mean ± SD (d)	1.2 ± 0.4	2.6 ± 2.0	<0.001
Length of hospitalization stay, mean ± SD (d)	3.4 ± 1.0	6.4 ± 5.3	<0.001
POD 1 highest pain score[a], mean ± SD	1.5 ± 1.9	3.0 ± 2.0	0.012
POD 2 highest pain score[a], mean ± SD	0.7 ± 1.5	1.8 ± 2.2	0.066
POD 1 PCA total morphine use, mean ± SD (mg)	1.4 ± 3.3	6.7 ± 8.1	0.002
POD 2 PCA total morphine use, mean ± SD (mg)	0	2.5 ± 5.1	0.005

[a]Measured using visual analog scale. NOSE: Natural orifice specimen extraction; SD: Standard deviation; POD: Postoperative day; PCA: Patient-controlled analgesia.

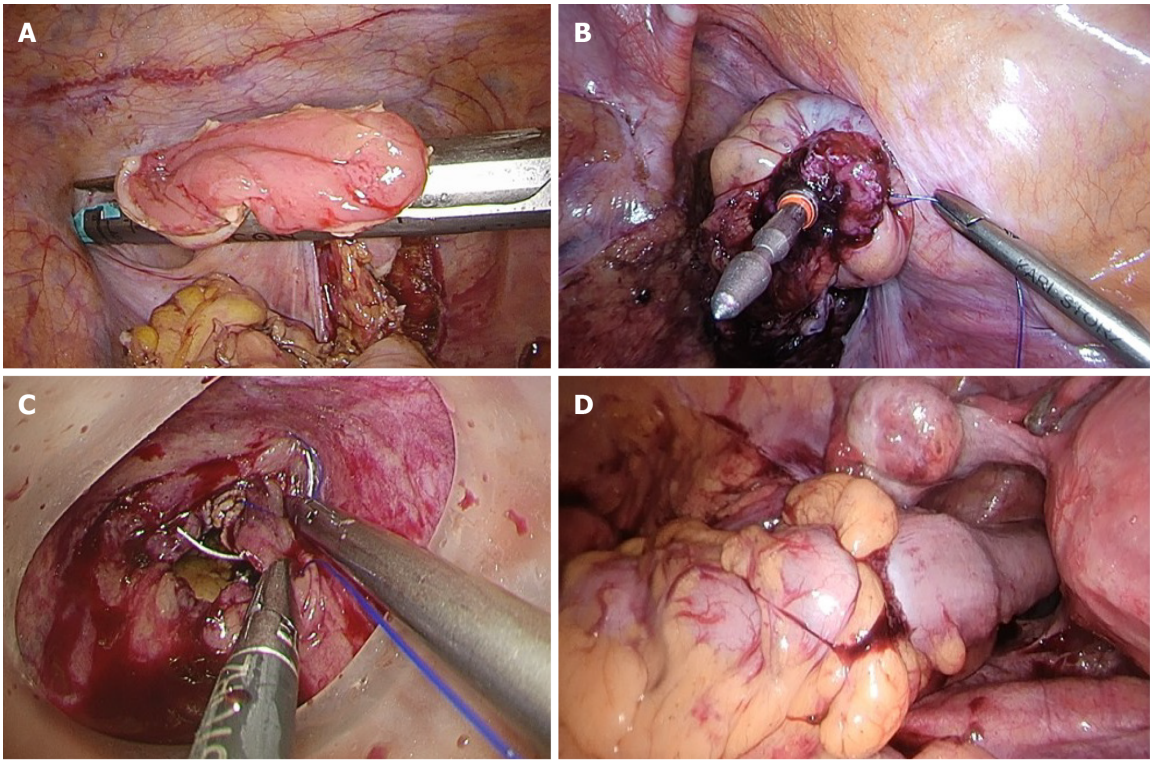
almost twice as long in the non-NOSE group compared to the NOSE group.

DISCUSSION

As recommended by the international NOSE surgery consensus, the maximum tumor dimension for transanal and transvaginal NOSE are 3 cm and 5 cm respectively[12]. While tumor size can be estimated on preoperative imaging, the decision to proceed with the NOSE procedure can often only be established intraoperatively, due radiological limitations on assessment of peritumoral desmoplastic reaction and mesocolic or mesorectal bulkiness, which may add considerably to the overall specimen diameter.

Moreover, while absolute diameter is an important consideration, the relative size of the specimen to the width of the pelvic outlet as well as the laxity of the chosen bodily orifice may be more crucial in determining the success or failure of the procedure. As illustrated by a recent series of NOSE following sigmoidectomy for volvulus, surgery for benign colorectal disease without a physical mass is ideal for NOSE[17].

BMI limits of 30 kg/m^2 and 35 kg/m^2 were suggested for transanal and transvaginal NOSE respectively[12]. Obese patients often possess a bulkier mesocolon or mesorectum which increases the difficulty of extraction. Nonetheless, the benefits of reduced incision may be more apparent in a patient with a thicker abdominal wall, who is at an increased risk of wound complications including infection and herniation. We previously demonstrated a successful transvaginal NOSE technique in a patient with BMI of 37 kg/m^2[18]. A large retrospective Australian study also demonstrated the feasibility of NOSE in obese patients[19].



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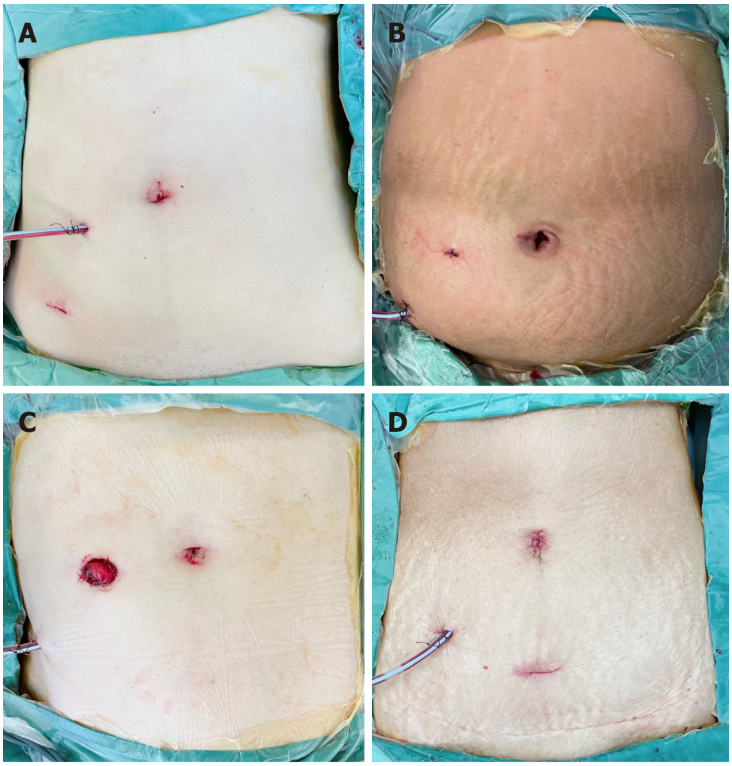
Figure 4 The hypothetical advantage of rectal purse-string closure is the creation of a double purse-string single-stapled anastomosis. A and B: Methods of rectal stump closure linear-stapled closure, intracorporeal purse-string suture onto the fully extended spike of the circular stapler; C: Transanal purse-string suture with a transanal access device; D: A double purse-string single-stapled anastomosis.

Unlike prior reports, NOSE did not significantly add to operative time in our experience, even with the removal of assistant ports[2-4]. Following our findings, routine postoperative patient-controlled opioid anaesthesia (PCA), a feature of our unit's enhanced recovery program, was discontinued for NOSE patients in view of minimal use[20]. Furthermore, postoperative ileus was virtually eliminated in the studied cohort. This may be explained by the relative lack of extracorporeal bowel exposure, as well as quicker patient mobilization. There were also no infective complications recorded, despite known concerns regarding contamination during transanal specimen extraction[21].

While the reduction of several laparoscopic ports may ostensibly offer only minor improvement over traditional 4- or 5-port surgery, reduced-port colorectal surgery represents another incremental step towards the holy grail of scarless surgery. In the modern era of minimally invasive surgery, an accumulation of several small gains may be required to make meaningful clinical differences to patient outcomes. In our opinion, the reduced-port technique is synergistic with natural orifice specimen extraction techniques to further minimize abdominal wall trauma. Another advantage of reduced-port surgery is the removal of dependence on a surgical assistant, particularly in the setting of limited manpower resources.

Single incision laparoscopic surgery (SILS), and 2-port laparoscopic surgery (using a SILS multi-channel umbilical port and one separate working port), have been demonstrated in colorectal surgery [22-24]. While these techniques reduce the number of ports even further, a larger umbilical incision is generally required for insertion of a multi-channel access device, which offsets the decrease in overall number of ports. Considerable operative challenges can also be anticipated with a SILS access device, including clashing of the laparoscopic instruments with the endoscope, and operator discomfort due to awkward surgical posture. In our experience, the 3-port technique provides the optimal balance between minimizing abdominal trauma and allowing operator as well as cameraman comfort by enabling adequate optical and working port triangulation.

A technical learning curve exists for 3-port NOSE surgery, and the 3-port technique and natural orifice extraction each present with a separate set of challenges. The issue of lack of tissue traction by an assistant can be overcome *via* positional changes of the operating table. The uterus should be hitched to the anterior abdominal wall for all female patients (Figure 2), facilitating pelvic visualization during rectal mobilization or the NOSE procedure. Additional assistant ports should be used if difficulties are encountered. In event of a problematic natural orifice extraction, transabdominal specimen extraction can be performed instead of NOSE with minimal added detriment to the patient. Operators should be proficient in conventional laparoscopic colorectal surgery before attempting the 3-port NOSE technique.



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Figure 5 Postoperative abdominal incisions and appearance. A: Anterior resection with transvaginal natural orifice specimen extraction (NOSE); B: Anterior resection with transanal NOSE; C: Low anterior resection and defunctioning ileostomy with transanal NOSE; D: D3 right hemicolectomy with transvaginal NOSE.

Our study is limited by the small sample size in the NOSE cohort. Furthermore, the benefits shown in the 3-port NOSE group may have been largely contributed by the reduced abdominal incision, consistent with the findings from previous studies, rather than the reduced number of ports used[2-4]. Nonetheless, the feasibility and clinical applicability of the 3-port NOSE technique is still demonstrated across a range of colorectal resection types, with considerable improvements in short-term outcomes compared to conventional laparoscopy.

CONCLUSION

3-port laparoscopic colorectal surgery with NOSE is a feasible and safe technique, and together augment the minimally invasive nature of surgery producing excellent cosmesis and good outcomes. Appropriate patient selection and expertise in conventional laparoscopy are required. Larger studies are necessary to draw conclusive results.

ARTICLE HIGHLIGHTS

Research background

Natural orifice specimen extraction (NOSE) *via* the anus or vagina replaces conventional transabdominal specimen retrieval *via* the transabdominal route through a limited mid-line laparotomy or Pfannenstiel incision. Reducing the number of laparoscopic ports further decreases operative abdominal wall trauma. These techniques reduce the surgical wound size as well as the risk of incision-related morbidity.

Research motivation

To our knowledge, the technique of 3-port colorectal cancer surgery with NOSE has never been evaluated or described in-depth.

Research objectives
To compare short-term outcomes following 3-port NOSE surgery with a matched cohort of conventional non-NOSE colorectal cancer surgery.

Research methods
This was a retrospective cohort study of patients who underwent elective 3-port laparoscopic colorectal NOSE surgery between February to October 2021. The propensity score-matched cohort was identified amongst patients who underwent conventional laparoscopic colorectal surgery from January 2019 to December 2020. Matching was performed in the ratio of 1:4 based on age, gender, type of resection, and p - tumor node metastasis staging.

Research results
Our results showede no statistical differences in surgical duration and perioperative complication rates between the NOSE and non-NOSE cohorts. As hypothesized, the 3-port NOSE cohort had significantly quicker mean return of bowel function (2.6 *vs* 1.2 d, $P < 0.001$), reduced postoperative pain and patient-controlled analgesia use, and decreased length of hospital stay (6.4 *vs* 3.4 d, $P < 0.001$), compared to the conventional surgery cohort.

Research conclusions
3-port laparoscopic colorectal surgery with NOSE is a feasible technique, augmenting the minimally invasive nature of surgery and producing good outcomes.

Research perspectives
Studies with larger patient numbers are necessary to draw definitive conclusions. A defined criteria should be evaluated for more objective selection of patients who are considered for colorectal NOSE surgery.

FOOTNOTES

Author contributions: Seow-En I conceived the research and study design; Seow-En I, Chen LR, Li YX, and Tan EKW contributed to data acquisition and analysis; Zhao Y performed the statistical analysis; all authors were involved in interpretation of data and writing of the manuscript, as well as editing and revision of the article, read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the SingHealth Institutional Review Board.

Informed consent statement: All natural orifice specimen extraction study participants provided informed written consent prior to study enrolment.

Conflict-of-interest statement: All authors declare no conflict-of-interest.

Data sharing statement: No additional data are available.

STROBE statement: All authors have read the STROBE Statement - checklist of items, and the manuscript was prepared and revised according to the STROBE Statement - checklist of items.

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ORIGINAL ARTICLE

Retrospective Study

Oncology and reproductive outcomes over 16 years of malignant ovarian germ cell tumors treated by fertility sparing surgery

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Abstract

BACKGROUND

Malignant ovarian germ cell tumors (MOGCT) are rare and frequently occur in women of young and reproductive age and the oncologic and reproductive outcomes after fertility-sparing surgery (FSS) for this disease are still limited.

AIM

To evaluate the oncology and reproductive outcomes of MOGCT patients who underwent FSS.

METHODS

All MOGCT patients who underwent FSS defined as the operation with a preserved uterus and at least one side of the ovary at our institute between January 2005 and December 2020 were retrospectively reviewed.

RESULTS

Sixty-two patients were recruited for this study. The median age was 22 years old and over 77% were nulliparous. The three most common histology findings were immature teratoma (32.2%), dysgerminoma (24.2%), and yolk sac tumor (24.2%). The distribution of stage was as follows; Stage I, 74.8%; stage II, 9.7%; stage III, 11.3%; and stage IV, 4.8%. Forty-three (67.7%) patients received adjuvant chemotherapy. With a median follow-up time of 96.3 mo, the 10-year progression-free survival and overall survival were 82.4% and 91%, respectively. For reproductive outcomes, of 43 patients who received adjuvant chemotherapy, 18 (41.9%) had normal menstruation, and 17 (39.5%) resumed menstruation with a median time of 4 mo. Of about 14 patients who desired to conceive, four were pregnant and delivered good outcomes. Only one case was aborted. Therefore, the successful pregnancy rate was 28.6%

CONCLUSION

The oncology and reproductive outcomes of MOGCT treated by FSS are excellent.

Many patients show a long survival time with normal menstruation. However, the obstetric outcome is not quite satisfactory.

Key Words: Malignant ovarian germ cell tumor; Fertility-sparing surgery; Oncology outcome; Reproductive outcome; Pregnancy rate; Survival rate

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Core Tip: The oncology and reproductive outcomes of malignant ovarian germ cell tumors treated by fertility-sparing surgery were satisfying. Even though most patients developed normal menstruation, nearly 1/3 were successfully pregnant and delivered.

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INTRODUCTION

Malignant ovarian germ cell tumors (MOGCT) account for about 5% of all ovarian cancer cases and approximately 70% develop in young women[1]. With the introduction of chemotherapy consisting of bleomycin, etoposide, and cisplatin (BEP) for MOGCT treatment after surgery, the outcome of this malignancy is excellent even in the advanced stage[2]. The cure rate of MOGCT in the early stage and the advanced stage was 100% and 75%, respectively[3]. Therefore, in patients who were of young and reproductive age, the role of fertility-sparing surgery (FSS) defined as cytoreductive surgery with preservation of the contralateral adnexa and uterus is the standard treatment for these patients[4]. We previously reported a 10-year overall survival (OS) rate as high as 86.2% but did not focus on the patients who underwent FSS[5]. Therefore, with the limited data on oncology and reproductive outcomes of FSS especially in Southeast Asia, this study was conducted to identify these outcomes of MOGCT patients who were treated by FSS.

MATERIALS AND METHODS

Study population

After the protocol was approved by the local ethics committees, the medical records of the MOGCT patients who underwent FSS defined as surgical cytoreduction with preservation of the uterus and unilateral adnexa at Chiang Mai University Hospital from January 2005 through December 2020 were reviewed. The patients who developed other histologic types arising in germ cell tumors were excluded. The basic clinical data, histology, staging, type of surgery, chemotherapy regimen, and outcomes were identified. All pathology specimens were examined by gynecologic pathologists in our institute. The decision of treatment depended on the preference of the physicians.

Oncology outcome

After complete treatment, the surveillance schedule was set every 3 mo in the first year, every 4 mo in the second year, every 6 mo in the third to fifth year, and annually thereafter. At that time, all of the patients were examined for a blood test for MOGCT and were examined by gynecologic oncologists. Pelvic ultrasonography was done at each visit for unmarried patients. Other imaging modalities such as CT were utilized when clinically indicated or with a rising of tumor markers.

Progression-free survival (PFS) was defined as the time between the month of the primary surgery and the month of tumor progression or recurrence detection or last contact, whereas OS was defined as the similar starting time of PFS to the month of patient death or last contact. The death data was also sought from the Thai Civil registration system *via* the National identification card number. Both PFS and OS were estimated by the Kaplan-Meier method using the SPSS for Windows program (Version 22; IBM Corporation, Armonk, New York, United States). Descriptive data of all studied patients are presented as the mean with range and discrete data are reported as numbers and percentages.

Reproductive outcome

The reproductive outcome after FSS was identified by collecting the data on the menstrual status during and after treatment, the number of pregnancies and childbirth before and after treatment, the present marital status, the childbearing desire, the method of pregnancy, gestational age at delivery, birth weight of the baby, and obstetrical complications from the medical records and direct contact with the patients by phone for more information.

RESULTS

Among 98 MOGCT, 62 patients underwent FSS in the studied period. The clinical data are presented in Table 1. The median age of these patients was 22 with a range of 4-34 years old. Over 77% of them were nulliparous and the three most common presentations were pelvic mass, abdominal pain, and abdominal distension. Approximately 54.8% of the tumors were located on the right side and 41.9% on the left side.

The details of FSS are as follows: Unilateral salpingo-oophorectomy (SO) in 49 cases, unilateral ovarian cystectomy in four, and unilateral SO with contralateral ovarian cystectomy in the rest. A frozen section was done in 26 cases. About staging procedures, peritoneal cytology was done in 39 cases with ten cases revealing positive malignancy cells, while omentectomy was done in 44 cases and lymphadenectomy was performed in 30, with four cases each having positive results. Half of the studied patients underwent an appendectomy. Regarding the surgical outcomes, 75.8% had complete resections.

The three leading histology types were immature teratoma (32.3%), dysgerminoma (24.2%), and yolk sac tumor (24.2%). The majority of the patients were in stage I (74.2%) and about 4.8% were in stage IV. Nearly 70% of the patients were given adjuvant chemotherapy. All except one was BEP regimen. Only one case was given etoposide + methotrexate + actinomycin D + cyclophosphamide + vincristine (EMACO). This case was diagnosed with stage IV choriocarcinoma. About one-third of the patients received four to six cycles of chemotherapy. Concerning the long-term side effects of chemotherapy, numbness occurred in three cases, lung fibrosis occurred in two, and hearing problems in two. Five patients died; two died from neutropenic sepsis and the rest from the progression of the disease.

Oncology outcome

With a median follow-up time of 96.3 mo, the 10-year PFS and OS were 82.4% and 91% as shown in Figures 1 and 2, respectively. However, 62.9% did not continue regular follow-ups.

Four cases developed progression after primary FSS. The details of these patients are summarized in Table 2. One of them lived while the others died from the disease progression during treatment. The surviving case was a 17-year-old with stage IC1 grade 2 immature teratoma. The primary surgery was right SO and appendectomy with pelvic recurrence developing 1 mo after the operation. She underwent tumor debulking and received six cycles of the BEP regimen. She is still alive without disease with an overall survival of 109 mo. The other three cases had stage IV disease. The histology was yolk sac tumor in two cases with choriocarcinoma in the remainder. All of them underwent FSS and received multiple chemotherapy regimens with unfavorable outcomes and died of disease after primary surgery at 16, 28, and 30 mo. One case developed lung fibrosis after being administered two cycles of the BEP regimen.

Regarding four patients who underwent only a cystectomy, the pathology was immature teratoma in two cases (stage IA grade 2 and stage IC grade 1), papillary thyroid cancer arising from mature teratoma (1), and carcinoid tumor (1). Only one case of stage IA grade 2 immature teratoma received four cycles of BEP regimen while the other received only an operation. All of them are still alive at present with an overall survival of 44-173 mo.

Reproductive outcome

Of 62 patients, 43 received adjuvant chemotherapy with BEP in 41 cases and EMACO in the rest. The menstrual history of these patients is summarized in Table 3. Forty-two percent of the patients had menstruation while receiving chemotherapy, while 39.5% resumed menstruation after complete treatment with a median resumption time of 4 mo. One case was five years old at the treatment time with menarche at age 12 (seven years later).

Eight patients were without menstruation after chemotherapy. The one case without menarche at presentation was 12 years old. She was diagnosed with stage I mixed MOGCT and received six cycles of BEP regimen after undergoing right SO at 5 years of age. She was followed regularly with no evidence of recurrence. The remaining seven patients developed premature menopause. One case was diagnosed as having a stage IIA endodermal sinus tumor at 29 years old. She received six cycles of BEP regimen after undergoing right SO and omentectomy on January 1, 2017. One year after that, she developed a left ovarian tumor measuring 10 cm × 15 cm and received a hysterectomy with left SO. The final pathology revealed grade 1 endometrioid CA. The patient was given six cycles of carboplatin with a disease-free survival of 61 mo and received estradiol valerate 2 mg as hormonal therapy. The other two patients underwent FSS and received three and six cycles of BEP, respectively. Both cases did not resume menstruation after completing treatment. One case received hormonal therapy. However, both cases

Table 1 Clinical data (*n* = 62)

	***n* (%)**
Median age (range; yr)	22.0 (4-34)
Parity before surgery	
0	48 (77.4)
1	8 (12.9)
2	6 (9.7)
Presentation	
Pelvic mass	24 (38.7)
Abdominal pain	18 (29.0)
Abdominal distension	15 (24.2)
Others[1]	5 (8.1)
Tumor side	
Right	34 (54.8)
Left	26 (41.9)
Bilateral	2 (3.2)
Detail of FSS	
Unilateral SO	49 (79.0)
Unilateral cystectomy	4 (6.5)
Unilateral SO & cystectomy	9 (14.5)
Frozen section	26 (41.9)
Cytology	
Not done	23 (37.1)
Negative	29 (46.8)
Positive	10 (16.1)
Omentectomy	
Not done	18 (29.0)
Negative	40 (64.5)
positive	4 (6.5)
Lymphadenectomy	
Not done	32 (51.6)
Negative	26 (41.9)
Positive	4 (6.5)
Appendectomy	32 (51.6)
Surgical outcome	
No residual	47 (75.8)
Optimal	5 (8.1)
Suboptimal (residual tumor > 1 cm)	10 (16.1)
Histology	
Dysgerminoma	17 (27.4)
Immature teratoma	20 (32.3)
Yolk sac tumor	15 (24.2)
Mixed type	5 (8.1)

Others[2]	5 (8.1)
Stage	
I	46 (74.2)
II	6 (9.7)
III	7 (11.3)
IV	3 (4.8)
Adjuvant chemotherapy	
None	19 (30.6)
BEP	42 (67.7)
EMACO	1 (1.6)
Cycles of chemotherapy	
1-3	6
4-6	33
> 6	4
Long-term side effect	
None	46 (74.2)
Numbness	3 (4.8)
Lung fibrosis	2 (3.2)
High-frequency hearing loss	1 (1.6)
Tinnitus	1 (1.6)
Progression of disease	4 (9.5)
Death	5 (8.1)
Alive	55 (88.7)
Missing data	2 (3.2)

[1]Others: Amenorrhea (2), anti-NMDAR (N-methyl D-aspartate receptors) encephalitis (1), incidental finding during cesarean section (1), and hyperthyroidism (1).
[2]Others: Struma ovarii (1), carcinoid (1), chorioCA (1), steroid cell tumor (1), and papillary thyroid CA arising in teratoma (1).
Death: SN 43, 52, 74, 108, and 110. SO: Salpingo-oophorectomy; BEP: Bleomycin etoposide cisplatin; EMACO: Etoposide methotrexate actinomycin D cyclophosphamide vincristine.

were followed for only 1 year after FSS. Four cases died, two from neutropenic sepsis, and two from disease progression after multiple chemotherapy regimens. The details of these patients are summarized in Table 4.

Regarding 19 patients who underwent only FSS without adjuvant chemotherapy, one was lost to follow-up since surgery while the remaining 18 had no problem with menstruation. One case was diagnosed with stage I immature teratoma and received left SO with omentectomy and appendectomy at 4 years old. At 15 years old, her menarche occurred.

For pregnancy outcomes, the data was available in 30 patients and revealed that 14 cases attempted to become pregnant and four of them (28.6%) succeeded in delivering a term baby after 1 year for two cases and 6 years for one case. One patient was known to give one term birth due to unavailable contact details. Three cases underwent unilateral SO and the rest received a unilateral ovarian cystectomy. The histology of these four cases was grade 1 carcinoid tumor neuroendocrine tumor (1), dysgerminoma (2), and grade 1 immature teratoma (1). Moreover, one case developed a spontaneous abortion 2 years after treatment and was never pregnant again. She was diagnosed with a steroid cell tumor. None of the patients who attempted to conceive actively tried to become pregnant by going to an infertility clinic. The details of these patients are shown in Table 5.

Table 2 Progression cases (*n* = 4)

SN	Year	Age	Stage	Histology	Chemotherapy	Cycle	Site	Primary surgery	Note
50	2013	17	IC1	Immature teratoma grade 2	None	-	Right	Right SO and appendectomy	PFS 3 mos →pelvic recurrence → debulking tumor and BEP × 6 cycles → alive without disease DFS 103 mos, overall survival 109 mo
52	2013	15	IV	Yolk sac tumor	BEP	8	Right	Right SO and left cystectomy and omentectomy	Progression after BEP × 8: Liver & lung metastasis → TIP x 2 → PT × 1 → ifosfamide × 1 → progression → death (overall survival 16 mo)
74	2010	18	IV	Choriocarcinoma	EMACO	6	Left	Left SO and omentectomy and appendectomy and PAN sampling	EMACO × 6 → progression (PFS 7 mos) → cisplatin and ifosfamide × 5 → paclitaxel × 1 → Act D and 5 FU × 1 → VAC × 1 → TAH and right SO (19/4/2011) → EMA/EP × 9 → TP/TE × 1 → BEP × 2 → palliative treatment → death 5/7/2012 overall survival 28 mo
110	2005	16	IV	Yolk sac tumor	BEP × 2 → EP × 11	12	Right	Right SO and appendectomy	Progression after EP × 11 → ifosfamide × 1 → EMA × 1 → single paclitaxel × 1 → palliative RT 25/1/2006 → VAC × 1 → Death 4/7/2007,OS 30 mo (lung fibrosis after BEP × 2)

PFS: Progression-free survival; DFS: Disease-free survival; SO: Salpingo-oophorectomy; PAN: Para-aortic lymph node; BEP: Bleomycin etoposide cisplatin; EMACO: Etoposide methotrexate actinomycin D cyclophosphamide vincristine; TIP: Paclitaxel ifosfamide cisplatin; PT: Paclitaxel carboplatin; Act D; actinomycin d; EMA/EP: Etoposide methotrexate actinomycin D etoposide cisplatin; 5FU: Fluorouracil; VAC: Vincristine dactinomycin and cyclophosphamide; TP/TE: Paclitaxel cisplatin/paclitaxel etoposide; TAH: Total abdominal hysterectomy; EP: Etoposide cisplatin, palliative; RT: Radiotherapy.

Table 3 Menstrual data of studied patients who received chemotherapy (*n* = 43)

	n (%)
No menstruation after treatment	8 (18.6)
No menarche[1]	1
Premature menopause	7
Resume menstruation[2]	17 (39.5)
1 mo	2
2 mo	2
3 mo	2
4 mo	3
5 mo	1
6 mo	6
Median 4 mo (1-6)	
Menstruation during and post-treatment	18 (41.9)

[1]SN 4, no menarche at age 12 years old.
[2]SN 64, menarche at age 12.

DISCUSSION

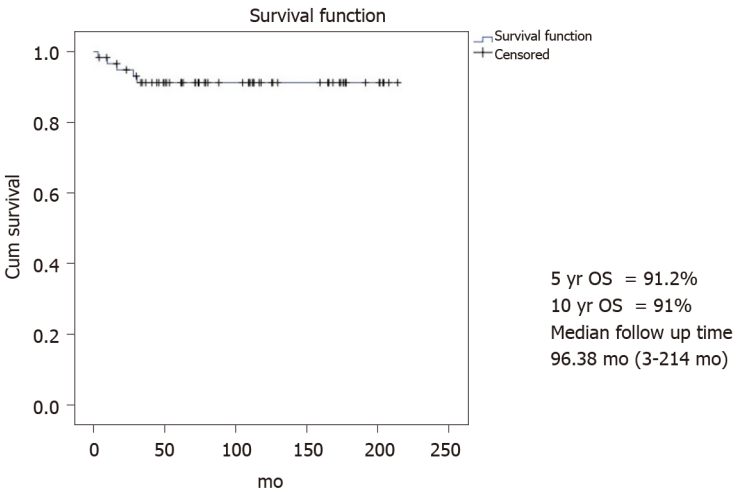
Oncology outcome

The outcome of 62 MOGOT patients who were treated by FSS in the present study was excellent with the 10-year PFS and OS being 82.4% and 91%, respectively. These results were close to the previous reports. Zamani *et al*[6] studied 79 MOGCT over 15 years and showed the 10-year OS as 94.4%. This study recruited only stages I-III while our study recruited all stages including three progressed cases of

Table 4 Details of premature menopausal patients (*n* = 7)

SN	Year	Age	Stage	Histology	Chemotherapy	Cycle	Site	Primary surgery	Note
25	2017	29	IIA	Yolk sac tumor	BEP	6	Right	Right SO and omentectomy January 6, 2017	16/1/18 abdominal pain and pelvic mass size 10 cm × 15 cm, solid and cystic, movable AFP 2.2 → TAH and left SO 19/1/18 → endometrioid CA IA → carboplatin × 6 → complete response → DFS 61 mo, HRT
43	2014	16	III	Dysgerminoma	BEP	4	Bilateral	Left SO and omentectomy	Partial response during BEP, overall survival of 3 mo, death from sepsis (neutropenia)
52	2013	15	IV	Yolk sac tumor	BEP	8	Right	Right salpingo-oophorectomy with left ovarian cystectomy	PFS 15 mo → TIP × 2 cycles → PT × 1 → ifosfamide × 1 cycle → death OS 16 mo
87	2008	28	I	Dysgerminoma	BEP	3	Left	Left SO	HRT icycloprogynova lost to follow up since 2009, unknown status
108	2005	15	III	Yolk sac tumor	BEP	6	Right	Right SO	Febrile neutropenia → sepsis → death 2005 OS 9 mo
110	2005	16	IV	Yolk sac tumor	BEP × 2 → EP × 11	12	Right		Progression after EP × 11 → ifosfamide × 1 → EMA × 1 → single paclitaxel × 1 → palliative RT January 25, 2006 → VAC × 1 → Death July 4, 2007, OS 30 mo (lung fibrosis after BEP × 2)
114	2005	23	II	Dysgerminoma	BEP	6	Right	Right SO	Alive, loss after 12 mo since start treatment, no HRT

BEP: Bleomycin etoposide cisplatin; SO: Salpingo-oophorectomy; TAH: Total abdominal hysterectomy; AFP: Alpha-fetoprotein; DFS: Disease free survival; HRT: Hormonal replacement therapy; PFS: Progression free survival; TIP: Paclitaxel ifosfamide cisplatin; PT: Paclitaxel carboplatin; OS: Overall survival; EP: Etoposide cisplatin; EMA: Etoposide methotrexate + actinomycin D; palliative RT: Radiotherapy.



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Figure 1 Overall Survival. 5-year overall survival (OS) = 91.2%, 10-year OS = 91%, and median follow-up time = 96.38 mo (3-214 mo). OS: Overall survival.

stage IV. Another study from Korea[1] studied 171 MOGCT patients who underwent FSS for 23 years (1992-2015). They reported the 5-year PFS and OS as 86% and 97%, respectively. About 14.6% developed recurrent disease and the death rate of disease was 2.9%. This recurrence rate was higher than our study which showed the progression of the disease at only 1.6%. However, due to over 2/3 of our patients without regular follow-up, the actual number of recurrence patients might be missed. However, the death rate of this disease in our study was 4.8%, which is near the Korean report. In addition, Beiner *et al* [7] reviewed eight retrospective studies comparing FSS with the conventional operation for MOGCT patients and found that both types of surgery were not significant for recurrence.

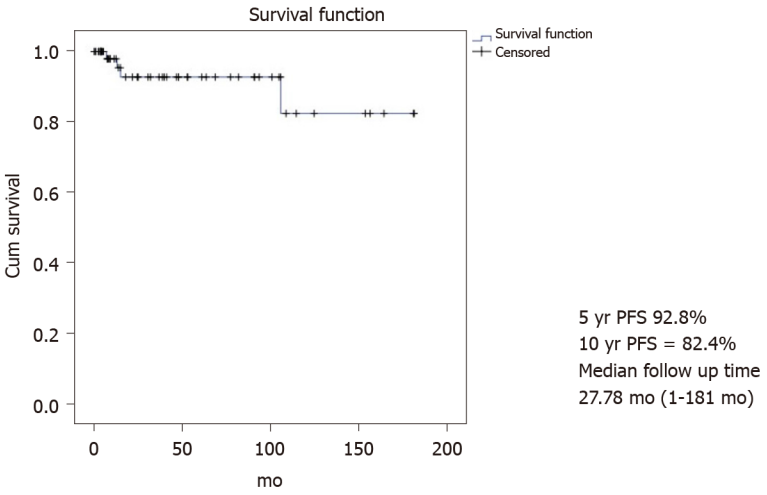
Regarding ovarian cystectomy in MOGCT, although this operation was not the standard of FSS, Tamauchi *et al*[8] showed an excellent outcome in eight patients who were diagnosed with early-stage

Table 5 Pregnancy outcomes

SN	Year	Age Dx	Age Preg	Stage	Histology	Site	Chemotherapy	Cycle	Parity	Primary surgery	Pregnancy outcome
19	2018	30	31	IA	Carcinoid tumor neuroendocrine tumor grade 1 arising in mature cystic teratoma	Left	None	-	1001	Laparoscopic left ovarian cystectomy June 1, 2018	1 Term pregnancy, GA 39 wk, NL August 1, 2019, BW 3030 gm
53	2013	21	27	IC	Dysgerminoma	Right	BEP	4	-	Right SO and omentectomy and appendectomy June 15, 2013	1 Term pregnancy, C/S GA 38 wk April 23, 2019, BW 2780 gm, ompholocele 9 cm × 8 cm, and atrial septal defect → surgical correction
84	2008	19	21	IA	Steroid cell tumor	Right	None	-	-	Right SO and omentectomy and PNS and PAS December 18, 2008	1 spontaneous abortion 31/1/2010
92	2005	34	35	IA	Immature teratoma grade[1]	Left	None	-	1001	Left SO	1 Term pregnancy, GA 38 wk, NL 10/12/2006, BW 2700 gm
94	2007	24	26	IA	Dysgerminoma	Left	None	-	1001	Left SO and omentectomy October 9, 2007	1 Term pregnancy with no available data of birth date, GA, and BW

[1]Parity before primary surgery.

NB: 14 of 30 patients who could be contacted for this information revealed an attempt to conceive. GA: Gestational age; BW: Birth weight; BEP: Bleomycin etoposide cisplatin; SO: Salpingo-oophorectomy; C/S: Cesarean section; PNS: Pelvic lymph node sampling; PAS: Para-aortic lymph node sampling.



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Figure 2 Progression free survival. 5-year progression free survival (PFS) = 92.8%, 10-year PFS = 82.4%, and median follow-up time = 27.78 mo (1-181 mo). PFS: Progression-free survival.

immature teratoma treated by ovarian cystectomy. Five patients received chemotherapy. With a median follow-up time of 4.7 years, all patients were still free of disease. The authors suggested that cystectomy followed by adjuvant chemotherapy showed impressive outcomes for early-stage MOGCT, especially in immature teratoma. For our study, four cases underwent ovarian cystectomy with one case of stage IA grade 2 immature teratoma and received adjuvant chemotherapy. All of them were still alive at a duration time of 44-173 mo after surgery.

Reproductive outcome

The 70.8% of patients who had no menstruation during treatment by FSS and chemotherapy in this study resumed menstruation with a median time of 4 mo. The true premature ovarian failure from chemotherapy occurred in only two (3.2%) cases. Both underwent unilateral SO with three and six cycles of the BEP regimen. Turkmen *et al*[9] used the Tokai Ovarian Tumor Study Group database on ovarian cancer patients and selected 110 MOGCT patients who received FSS with a median follow-up

period of 10.4 years for the study. In this Japanese report, 63.9% of the patients received the BEP regimen and about 30.6% received the cisplatin + vincristine + bleomycin regimen. They revealed premature menopause which was close to our study of 2.9%.

Regarding the obstetric outcome, our study reported that the rate of term pregnancy was 28.6%. This result was different from that of a Japanese study[9]. The authors revealed that 45 patients attempted to become pregnant with 40 patients succeeding in deliveries with total pregnancies as term deliveries in 54 (83.1%) cases, preterm delivery in two (3.2%), and abortion in 12 (18.5%). Seven cases received fertility treatment. A publication from Iran reported that 19 of 26 (73%) MOGCT patients who underwent FSS were successful in delivery without infertility treatment[6]. In addition, Mikuš *et al*[10] reported that the pregnancy rate in 20 German patients with MOGCT who desired to become pregnant of their series was 50%. The pregnancy rate from previous studies was higher than that of our study, which showed the successful pregnancy rate was only 28.6%. The difference might be from the current trend of Thai culture to have fewer children, the missing data from the patients unable to be contacted, and those non-actively who tried to conceive in our patients.

The strength of our study was the real-world series of patients with MOGCT treated by FSS in a single institute to show the oncology and reproductive outcomes. However, with the limitation of the retrospective study, about 2/3 of the patients were not followed for a long time. Therefore, some data were missed.

CONCLUSION

In conclusion, the oncology and reproductive outcomes of MOGCT treated by FSS were good. Many patients showed a long survival time with normal menstruation. However, the obstetric outcome in patients who attempted to conceive was not quite as high.

ARTICLE HIGHLIGHTS

Research background

Malignant ovarian germ cell tumors (MOGCT) are rare and frequently occur in women of young and reproductive age. Fertility-sparing surgery (FSS) is the main treatment for these patients. However, oncologic and reproductive outcomes after FSS for this disease are still limited.

Research motivation

Due to the limited data on oncology and reproductive outcomes of FSS especially in Southeast Asia, this study was conducted to identify these outcomes of MOGCT patients who were treated by FSS.

Research objectives

To evaluate the oncology and reproductive outcomes of MOGCT who underwent FSS.

Research methods

All MOGCT patients who underwent FSS defined as the operation with a preserved uterus and at least one side of the ovary at our institute between January 2005 and December 2020 were retrospectively reviewed.

Research results

Sixty-two patients were reviewed in this study. The median age was 22 years old and over 77% were nulliparous. The three most common histology findings were immature teratoma (32.2%), dysgerminoma (24.2%), and yolk sac tumor (24.2%). The distribution of stage was as follows: Stage I, 74.8%; stage II, 9.7%; stage III, 11.3%; stage IV, 4.8%. About 2/3 of the patients received adjuvant chemotherapy. With a median follow-up time of 96.3 mo, the 10-year progression-free survival and overall survival were 82.4% and 91%, respectively. For reproductive outcomes, of 43 patients who received adjuvant chemotherapy, 18 (41.9%) had normal menstruation and 17 (39.5%) resumed menstruation with a median time of 4 mo. Of about 14 patients who desired to conceive, four were pregnant and delivered good outcomes. Only one case was aborted. Therefore, the successful pregnancy rate was 28.6%.

Research conclusions

The oncology and reproductive outcomes of MOGCT treated by FSS were excellent. Many patients showed a long survival time with normal menstruation. However, the obstetric outcome was not quite high.

Research perspectives

The strength of our study was the real-world series of patients with MOGCT treated by FSS in a single institute to show the oncology and reproductive outcomes. However, with the limitation of the retrospective study, about 2/3 of the patients were not followed for a long time. Therefore, some data were missed. A good plan follow-up is needed in the future.

FOOTNOTES

Author contributions: Rungoutok M and Suprasert P contributed equally to this work; Suprasert P designed the research study; Rungoutok M and Suprasert P performed the research and contributed analytic tools; Suprasert P analyzed the data and wrote the manuscript; all authors have read and approved the final manuscript.

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Informed consent statement: Patients were not required to give informed consent for the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at psuprase@gmail.com. Participants consent was not obtained but the presented data are anonymized and risk of identification is low.

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ORIGINAL ARTICLE

Clinical Trials Study

Clinical relevance of the use of Dentoxol® for oral mucositis induced by radiotherapy: A phase II clinical trial

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Abstract

BACKGROUND

Severe oral mucositis associated with cancer therapy is a frequent complication that may affect a patient's systemic condition, resulting in interruption and/or prolongation of cancer therapy. Dentoxol® is a medical solution in the form of a mouthwash that has been shown to result in statistically significant improvement in the prevention of severe oral mucositis. However, knowing the measures of the clinical significance of this therapy is important for accurate decision-making.

AIM

To describe the clinical impact of Dentoxol® use in severe oral mucositis.

METHODS
Clinical significance was measured using the results obtained in a randomized controlled clinical trial previously conducted by the same group of researchers. The measures of clinical significance evaluated were the absolute risk or incidence, relative risk, absolute risk reduction, relative risk reduction, number needed to treat, and odds ratio.

RESULTS
The data obtained show that the impact of Dentoxol® on reducing the severity of oral mucositis has important clinical relevance.

CONCLUSION
The results of this study justify the incorporation of Dentoxol® mouth rinse into clinical protocols as a complement to cancer therapy to prevent and/or treat oral mucositis secondary to radiotherapy.

Key Words: Clinical trial; Dentoxol; Oral mucositis; Prevention; Radiotherapy; Treatment

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Core Tip: Oral mucositis associated with cancer therapy is a frequent complication. Dentoxol® is a medical solution that has been shown to prevent severe oral mucositis. The clinical significance of Dentoxol® was measured using the results obtained in a randomized controlled clinical trial previously conducted by the same group of researchers. The data obtained show that the clinical impact of Dentoxol® on oral mucositis justifies its incorporation into clinical protocols as a complement to cancer therapy to prevent and/or treat oral mucositis.

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INTRODUCTION

Oral mucositis is a complication that arises from cancer treatment (chemotherapy and/or radiotherapy) and manifests as erythematous and ulcerative lesions of the oral mucosa. These lesions cause considerable pain and functional impairment that can compromise nutritional status and prevent adequate oral hygiene in patients, increasing the risk of local infection and systemic spread. Additionally, in some cases, it can limit the dose or continuity of cancer therapy[1-3].

The available scientific evidence indicates that between 94%-96% of patients treated with head and neck radiotherapy develop some degree of oral mucositis, while 66% present with severe oral mucositis [4,5].

The pain caused by lesions often compromises a patient's ability to eat, frequently leading to the need for *via* nasogastric or gastrostomy tubes, which can impact the general condition of the patient due to weight loss 5%[6] as well as the overall cost of therapy by requiring hospitalization. Approximately 16% of patients with head and neck radiotherapy require hospitalization due to oral mucositis. In addition, 11% of patients who received radiotherapy for head and neck cancer had unplanned interruptions in radiotherapy due to severe oral mucositis[7].

The pathogenesis of oral mucositis is complex and involves different pathways. One of the events involved in the development of mucositis is the inflammatory response of tissues to cancer therapy[8,9]. Within these tissues, the participation of proinflammatory cytokines such as TNF- and IL-1 plays key roles in both the onset of tissue damage and acceleration of the process[10-13]. Likewise, these cytokines induce the expression of cyclooxygenase-2, which is responsible for the production of proinflammatory prostanoids such as prostaglandin E2 and prostacyclin I2 and for tissue injury and pain at the inflammation site[14-16].

Additionally, ulcers caused by oral mucositis can be colonized by bacteria from the patient's own oral flora. This secondary colonization may aggravate the clinical picture of mucositis through the release of bacterial products (lipopolysaccharides) capable of generating greater tissue damage and inhibiting the

healing process[13]. Sobue *et al*[17] evaluated the growth of and inflammatory responses against *Candida albicans, Candida glabrata,* and 2 streptococcal species of the mitis group (*S. oralis and S. mitis*), which are frequently associated with oral mucositis, in an organotypic model to represent chemotherapy-induced mucositis. Although a nonsignificant increase in growth was observed for the species studied, the authors reported an exacerbated proinflammatory response to *C. albicans, C. glabrata*, and *S. oralis*[17]. Recently, a positive correlation was found between ≥ grade 2 oral mucositis and the presence of *Bacteroidales G2, Capnocytophaga, Eikenella, Mycoplasma*, *Sneathia,* and the periodontopathogens *Porphyromonas* and *Tannerella*. Additionally, a large amount of *Fusobacterium, Haemophilus, Tannerella, Porphyromonas*, and *Eikenella* on buccal mucosa influenced oral mucositis susceptibility[18]. Bacteriome disturbance has been shown to have a strong and independent association with oral mucositis severity through decreases in commensal organisms such as those belonging to the *Streptococcus, Actinomyces, Gemella, Granulicatella,* and *Veillonella* genera and increases in gram-negative bacteria such as *Fusobacterium nucleatum* and *Prevotella oris*[19].

The complex nature of oral mucositis requires a comprehensive preventive and therapeutic approach that can address the different pathways involved to achieve a successful outcome[20]. Managing only inflammation or overinfection is not sufficient for efficient and adequate control.

In this context, Dentoxol®, an aqueous solution used as a mouthwash, whose main mode of action is mechanical sloughing of the superficial epithelial cell layer of the oral mucosa, thus stimulating local regeneration of the epithelium, was developed. The interaction of its components (purified water, xylitol, sodium bicarbonate, eugenol, camphor, parachlorophenol, and peppermint essence) in specific concentrations sloughs and eliminates cells damaged by radio/chemotherapy as well as particles and detritus present in the oral cavity, such as bacteria and organic debris. The clinical effect observed is a result of the interaction of its components acting on the different aspects of the physiopathogenesis of oral mucositis (antioxidant, bacteriostatic, bactericidal, anti-inflammatory, and moisturizing properties and mucosal regenerative stimulation). As a result, Dentoxol® can prevent oral mucositis by physically moisturizing and lubricating the oral mucosa to provide flexibility and resistance. Accordingly, it affects several pathways that influence the severity of oral mucositis[21].

Recently, a randomized controlled clinical trial conducted by this research team evaluated the effect of Dentoxol® mouthwash on the prevalence of severe oral mucositis and found statistically significant results regarding the prevention and reduction in the severity of oral mucositis[21]. Many clinical studies present their results based on statistical significance. However, clinical measures of significance are essential for evaluating the relevance and usefulness of a therapy in daily clinical practice[22].

Considering the high incidence of oral mucositis in patients undergoing head and neck cancer therapy as well as the relevant impact of this pathology on patient morbidity and quality of life, in addition to the associated economic costs, the clinical significance of an agent that can successfully treat oral mucositis needs to be analyzed. The aim of the present study is to objectively and clearly present the clinical impact of Dentoxol® on affected tissues based on statistical results obtained in a previously conducted clinical trial, with the aim of providing a clearer picture of the impact that clinicians responsible for managing this pathology should expect in their daily work when using this preventive and therapeutic tool to manage and control oral mucositis.

MATERIALS AND METHODS

Definition

Severe oral mucositis: Grade 3 or 4 mucositis based on the scale described by the World Health Organization (Table 1).

A descriptive study was conducted on the clinical significance of Dentoxol® in treating oral mucositis based on results obtained in a randomized controlled clinical trial with a parallel arm design (1:1) evaluating the effect of Dentoxol® mouthwash (test group) versus a placebo mouthwash (control group) on the incidence of severe oral mucositis associated with cancer therapy. The full methodology of the clinical trial was previously published by Lalla *et al*[21].

A total of 108 patients older than 18 years (Dentoxol® group = 55 and control group = 53) participated in the study.

Once the statistical results of the clinical trial were obtained, clinical significance measures such as the absolute risk (AR), relative risk (RR), absolute risk reduction (ARR), relative risk reduction, number necessary to treat (NNT), and odds ratio were calculated using a contingency table (Table 2).

RESULTS

Patient selection

A total of 108 patients were considered for the analysis of the outcomes of the randomized controlled clinical trial evaluating the use of Dentoxol®.

Table 1 Absolute frequencies and percentages of patients with and without severe oral mucositis by follow-up week

Week	Dentoxol® (*n* = 55)				Control (*n* = 53)				
	No severe oral mucositis		Severe oral mucositis		No severe oral mucositis		Severe oral mucositis		*P* value
	n	%	*n*	%	*n*	%	*n*	%	
1	49	94.2	3	5.8	49	98.0	1	2.0	0.327
2	49	100	0	0.0	42	95.5	2	4.5	0.131
3	47	95.9	2	4.1	30	76.9	9	23.1	0.007[a]
4	42	91.3	4	8.7	29	70.7	12	29.3	0.013[a]
5	33	73.3	12	26.7	19	52.8	17	47.2	0.055
6	29	64.4	16	35.6	15	46.9	17	53.1	0.125
7	22	57.9	16	42.1	11	44.0	14	56.0	0.280
8	11	57.7	6	35.3	4	44.4	5	55.6	0.320

[a]Statistically significant.

Table 2 Odds ratio were calculated using a contingency table

	Severe oral mucositis	No severe oral mucositis	Total
Dentoxol®	a	b	a + b
Control	c	d	c + d
Total	a + c	b + d	a + b + c + d

a: Number of patients who presented severe oral mucositis and received Dentoxol® treatment; b: Number of patients who did not present severe oral mucositis and received Dentoxol® treatment; a + b: Total number of patients in the Dentoxol® group; c: Number of patients who presented severe oral mucositis and received control treatment; d: Number of patients who did not present severe oral mucositis and received control treatment; c + d: Total number of patients in the control group.

Oral mucositis severity

Table 1 shows the number and percentage of patients who presented with severe oral mucositis in each treatment group. The Dentoxol® and control groups showed a progressive increase in the frequency of severe oral mucositis, with a peak at seven weeks.

Compared with the control group, the Dentoxol® group presented a lower number of patients with severe oral mucositis every week except for the first week, with a statistically significant difference observed at weeks 3 and 4 of the follow-up (see Table 1).

Clinical relevance

Table 2 shows the measures of clinical significance. The ARs of severe oral mucositis in the Dentoxol® group were 0.04 and 0.09 or 4% and 9% for weeks 3 and 4, respectively, versus 0.23 and 0.29 or 23% and 29%, respectively, in the control group. Additionally, from week 2 onward, the relative risk of severe oral mucositis in the Dentoxol® group was less than 1, indicating that Dentoxol® use acted as a protective factor.

Dentoxol® use was positively associated with a reduction in severe oral mucositis from week 2 onward, showing ARR values greater than 0. The values at weeks 3 and 4, ARR = 0.19 or 19% and 0.21 or 21%, respectively, indicate that if 100 patients were treated with Dentoxol®, 19 and 21, respectively, fewer cases of severe mucositis would occur compared to the control group. Likewise, during weeks 3 and 4, when statistically significant differences between the groups were noted, 5 patients (NNT) would need to be treated with Dentoxol® to prevent 1 additional case of severe oral mucositis (Table 3).

DISCUSSION

Measures of clinical significance allow making well-founded decisions when evaluating a therapy and can be applied in daily clinical practice and especially in recommendations for massive clinical protocols because through these measures, expected results with a real impact on the population can be obtained.

Table 3 Measures of clinical significance for the effect of Dentoxol® on severe oral mucositis

Week	AR		RR (95%CI)	ARR	RRR	NNT	OR (95%CI)
	Dentoxol (%)	Control (%)	Dentoxol®	Dentoxol® (%)	Dentoxol® (%)	Dentoxol®	Dentoxol®
1	5.77	2	2.88 (0.31-26.82)	-4	-188	-27	3 (0.30-29.85)
2	0	4.55	-	5	100	22	-
3	4.08	23.08	0.18 (0.04-0.77)	19	82.31	5	0.14 (0.03-0.70)
4	8.7	29.27	0.3 (0.1-0.85)	21	70.29	5	0.23 (0.07-0.78)
5	26.67	47.22	0.56 (0.31-1.02)	21	43.53	5	0.41 (0.16-1.03)
6	35.56	53.13	0.67 (0.4-1.12)	18	33.07	6	0.49 (0.29-1.23)
7	42.11	56	0.75 (0.45-1.25)	14	24.81	7	0.57 (0.21-1.58)
8	35.29	55.56	0.64 (0.27-1.52)	20	36.47	5	0.44 (0.08-2.27)

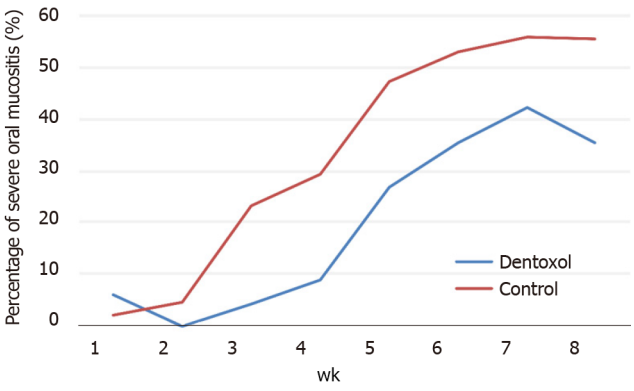
ARR: Absolute risk reduction; RR: Relative risk; RRR: Relative risk reduction; NNT: Number necessary to treat; OR: Odds ratio.

In the present study, the group that used Dentoxol® showed a lower incidence (AR) of severe oral mucositis than the control group (Figure 1) from the 2nd week of evaluation to the 4th week, representing the greatest difference between the groups (Table 2). The results shown in Table 2 demonstrate the strong potential of Dentoxol® to lower treatment complications. Treating 5 patients with Dentoxol® will prevent 1 additional case of severe oral mucositis that may need percutaneous endoscopic gastrostomy tubes, liquid diet supplements, pain medications, *etc*. Moreover, the results in Table 1 show that Dentoxol® delayed the onset of severe mucositis such that even patients with a severe grade who received it had the complication for a shorter period, with benefits on cost and quality of life.

Another important conclusion of the clinical trial was that the most beneficial effects of therapy with Dentoxol® are observed in patients who follow the instructions to rinse 4 times a day (Figure 1). Therefore, following the recommended instructions specifying that more frequent rinses yield better clinical results is important. Furthermore, the rinsing time should be longer than 1 min to allow the product to exert its effects on the oral mucosa. This is not a minor point because cancer patients very often have nausea and vomiting, which should be controlled to allow rinses at the appropriate frequency and time. A clinically recognized effective strategy is to begin with rinses days or ideally weeks before starting cancer therapy to prepare the mucosa for toxic effects and their consequences and thus more effectively prevent the onset of mucositis. Therefore, continuing to study different clinical protocols based on the experience of clinicians regarding this aspect through well-designed controlled clinical trials is essential.

With respect to the latter, the literature contains multiple studies evaluating different products and protocols to reduce the onset and severity of oral mucositis[23-25]. Properly designed studies allow their results to be comparable in terms of clinical effectiveness for correct decision-making. In this sense, 1 multicenter clinical trial evaluated the ability of Caphosol®, an electrolyte solution with concentrated calcium phosphate, to reduce oral mucositis in patients who received radiotherapy for head and neck cancer[26]. When observing the percentages of severe oral mucositis, 29.3% and 42.4% of grade 3 mucositis occurred during the 3rd and 4th weeks, respectively, and 8.6% and 18.6% of grade 4 mucositis occurred during the same weeks. If we compare these results with those obtained in the Dentoxol® trial, at the 3rd and 4th weeks, only 4.1% and 8.7% of patients who rinsed with Dentoxol® had severe oral mucositis, respectively (grades 3 and 4, respectively). Another more recent clinical trial showed no reduction in the incidence or duration of severe oral mucositis with Caphosol® use in patients with head and neck cancer versus the control group (64.1% *vs* 65.4%)[24]. Given the results obtained in these studies, the benefit of Caphosol® is not clear. On the other hand, Dentoxol® showed a statistically significant clinical benefit for patients undergoing radiotherapy for head and neck cancer.

Systematic reviews are also useful for comparing the different applications and clinical effectiveness of multiple therapeutic alternatives. Accordingly, a 2017 Cochrane Library review evaluated the effect of cytokines and growth factors in the prevention of oral mucositis[25]. The main agent evaluated was keratinocyte growth factor (KGF). The results indicated that KGF decreased the risk of severe oral mucositis in patients undergoing head and neck cancer therapy, with an RR = 0.79 and a 95% confidence interval (CI) = 0.69-0.90 (obtained from 3 studies), and that 7 patients (95%CI = 5-15) would need to be treated to prevent 1 case of severe mucositis[25]. If we compare those findings with the results for Dentoxol®, the latter agent had a lower RR from the 3rd week of follow-up, with RR values = 0.18 to 0.75, and between 5 and 7 patients (depending on the week of follow-up) would be required to prevent an additional case of severe oral mucositis. Although these results may seem similar, notably, KGF is a drug with important limitations: it is not indicated for solid tumors because it may enhance their growth; the cost is much higher; and it must be administered by IV infusion. Other products used for



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Figure 1 Line graph for the absolute risk (%) by treatment group.

similar clinical conditions could be considered for comparative evaluations[27].

To better present the results of the Dentoxol® study and to facilitate comparisons with other results, we must note that the placebo used in the clinical trial from which the analysis of the present study was performed was not a totally inactive agent. Due to ethical reasons, the control group could not be deprived of minimum protection; therefore, the placebo used was a mouthwash composed of an aqueous solution of sodium bicarbonate and xylitol, thus reducing the actual difference between the Dentoxol® group and the control group. Therefore, the benefit provided by rinses with Dentoxol® is even greater in reality.

In conclusion, more well-designed controlled clinical trials are needed to increase scientific evidence and test different clinical protocols and therapeutic strategies to offer patients effective solutions based on scientific evidence. To facilitate comparisons with other interventions, the type of cancer presented by the patients, the type of therapy (chemo- and/or radiotherapy), the frequency, dose, starting point, and duration of therapy for oral mucositis, *etc.*, should be considered. Additionally, the timing of evaluation considering the pathogenesis of mucositis is also an important factor.

CONCLUSION

In this study, the safety and clinical efficacy of Dentoxol® were demonstrated for the prevention and treatment of severe oral mucositis, an unwanted pathology that is a complication of treatments for much more serious diseases, including cancer. However, this complication can impact the costs and continuity of cancer treatment and, above all, the quality of life of patients. In this study, the effects of Dentoxol® were clinically evident and detectable in a small number of treated patients; therefore, the inclusion of Dentoxol® in clinical protocols is highly recommended for the management and control of the side effects of cancer treatments, which is as important as the other components of the therapeutic arsenal for cancer.

ARTICLE HIGHLIGHTS

Research background

Oral mucositis is a complication that arises from cancer treatment (chemotherapy and/or radiotherapy) and manifests as erythematous and ulcerative lesions of the oral mucosa. The pathogenesis of oral mucositis is complex and involves different pathways. One of the events involved in the development of mucositis is the inflammatory response of tissues to cancer therapy The complex nature of oral mucositis requires a comprehensive preventive and therapeutic approach that can address the different pathways involved to achieve a successful outcome. Managing only inflammation or overinfection is not sufficient for efficient and adequate control. In this context, Dentoxol®, an aqueous solution used as a mouthwash, whose main mode of action is mechanical sloughing of the superficial epithelial cell layer of the oral mucosa, thus stimulating local regeneration of the epithelium, was developed. Recently, a randomized controlled clinical trial conducted by this research team evaluated the effect of Dentoxol® mouthwash on the prevalence of severe oral mucositis and found statistically significant results regarding the prevention and reduction in the severity of oral mucositis.

Research motivation

Many clinical studies present their results based on statistical significance. However, clinical measures of significance are essential for evaluating the relevance and usefulness of a therapy in daily clinical practice.

Research objectives

The aim of the present study is to objectively and clearly present the clinical impact of Dentoxol® on affected tissues based on statistical results obtained in a previously conducted clinical trial, with the aim of providing a clearer picture of the impact that clinicians responsible for managing this pathology should expect in their daily work when using this preventive and therapeutic tool to manage and control oral mucositis.

Research methods

Once the statistical results of the clinical trial were obtained, clinical significance measures such as the absolute risk (AR), relative risk (RR), absolute risk reduction (ARR), relative risk reduction, number necessary to treat (NNT), and odds ratio were calculated using a contingency table.

Research results

The ARs of severe oral mucositis in the Dentoxol® group were 0.04 and 0.09 or 4% and 9% for weeks 3 and 4, respectively, versus 0.23 and 0.29 or 23% and 29%, respectively, in the control group. Additionally, from week 2 onward, the relative risk of severe oral mucositis in the Dentoxol® group was less than 1, indicating that Dentoxol® use acted as a protective factor. Dentoxol® use was positively associated with a reduction in severe oral mucositis from week 2 onward, showing ARR values greater than 0. The values at weeks 3 and 4, ARR = 0.19 or 19% and 0.21 or 21%, respectively, indicate that if 100 patients were treated with Dentoxol®, 19 and 21, respectively, fewer cases of severe mucositis would occur compared to the control group. Likewise, during weeks 3 and 4, when statistically significant differences between the groups were noted, 5 patients (NNT) would need to be treated with Dentoxol® to prevent 1 additional case of severe oral mucositis.

Research conclusions

In this study, the effects of Dentoxol® were clinically evident and detectable in a small number of treated patients; therefore, the inclusion of Dentoxol® in clinical protocols is highly recommended for the management and control of the side effects of cancer treatments, which is as important as the other components of the therapeutic arsenal for cancer.

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FOOTNOTES

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Clinical trial registration statement: ClinicalTrials.gov Identifier, No. NCT02885376.

Informed consent statement: All the participants have read and understood the provided information and have had the opportunity to ask questions. All authors understand that their participation is voluntary and that they were free to withdraw at any time, without giving a reason and without cost. All authors understand that they will be given a copy of this consent statement.

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SYSTEMATIC REVIEWS

Neutrophil-to-lymphocyte ratio as a prognostic factor for survival in patients with colorectal liver metastases: A systematic review

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Abstract

BACKGROUND

The inflammatory response to tumor has been proven to be closely related to the prognosis of colorectal cancer. Neutrophil to lymphocyte ratio (NLR) is a widely available inflammatory biomarker that may have prognostic value for patients with colorectal liver metastasis (CRLM).

AIM

To assess the role of NLR as a prognostic factor of survival and tumor recurrence in patients with CRLM.

METHODS

A systematic literature search of PubMed, Cochrane Library and clinicaltrials.gov was conducted by two independent researchers in order to minimize potential errors and bias. Conflicts were discussed and settled between three researchers. Studies including patients undergoing different types of medical interventions for the treatment of CRLM and evaluating the correlation between pretreatment NLR and disease-free survival (DFS) and overall survival (OS) were included in the review. Nineteen studies, involving 3283 patients matched our inclusion criteria.

RESULTS

In the studies included, NLR was measured before the intervention and the NLR thresholds ranged between 1.9 and 7.26. Most studies used 5 as the cut-off value.

Liver metastases were treated with hepatectomy with or without chemotherapy regimens in 13 studies and with radiofrequency ablation, radioembolization, chemoembolization or solely with chemotherapy in 6 studies. High NLR was associated with decreased OS and DFS after liver resection or other medical intervention. Moreover, high NLR was associated with poor chemosensitivity. On the contrary, CRLM patients with low pretreatment NLR demonstrated improved OS and DFS. NLR could potentially be used as a predictive factor of survival and tumor recurrence in patients with CRLM treated with interventions of any modality, including surgery, chemotherapy and ablative techniques.

CONCLUSION
NLR is an inflammatory biomarker that demonstrates considerable prognostic value. Elevated pretreatment NLR is associated with poor OS and DFS in patients with CRLM who are submitted to different treatments.

Key Words: Neutrophil-to-lymphocyte ratio; Colorectal liver metastasis; Prognosis; Survival

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Core Tip: Colorectal cancer is the third most common cancer globally and liver is the most common site of metastasis. Even though surgery and chemotherapy are the main treatment options, prognostic markers are also essential for the progress and future management of the disease. Neutrophil-to-lymphocyte ratio (NLR) is a promising biomarker that has been recently proposed as an indicator for the survival and recurrence of various malignancies. In our review we assess the role of NLR in the overall survival of patients with colorectal liver metastases.

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INTRODUCTION

According to GLOBOCAN 2020 Data, colorectal cancer (CRC) is the third most frequent cancer in both men and women with an estimated 1931500 new cases and 935173 deaths worldwide in 2020. The liver is the most common site of metastasis in patients with CRC as almost 50% of these patients will develop liver metastases (LM) during the course of their disease of whom 15%-25% have LM at initial diagnosis. The remaining 18%-25% will have metachronous LM in the next 5 years[1,2]. The management of patients with colorectal liver metastases (CRLM) consists of different treatment options such as tumor resection, radiofrequency ablation (RFA), which can destroy the tumor by the use of high-frequency electromagnetic current and can be applied in unresectable CRLM, or microwave ablation. Other treatment options include systemic therapy, such as Irinotecan-loaded drug-eluting beads and radioembolization (RE), that administer high doses of chemotherapy and radiation, respectively, and chemotherapy. The intra-arterial techniques aim specifically at the tumor's vasculature, thus minimizing systemic toxicity, and may be an option in patients not eligible for surgery or ablation[3]. Different treatment methods are selected depending on the patient's clinical and radiological data[4]. Advancements in treatment for patients with CRLM have resulted in improved 5-year survival rates as high as 46%; however, survival remains low in patients where all sites of disease are not surgically resectable[5]. The low 5-year overall survival (OS) and the fact that recurrences occur in more than half of CRLM patients, highlights the need for more prognostic factors that could be easily applied to predict OS as well as disease-free survival (DFS)[6].

Many studies have examined the prognostic role of neutrophil to lymphocyte ratio (NLR) in CRLM patients. NLR is a widely available, low-cost prognostic index that is calculated by dividing the number of neutrophils by the number of lymphocytes and reflects the inflammatory response of the patient against the tumor, which is correlated with tumor development and poor outcomes[7,8]. Neutrophils play a role in cancer development and metastases, while lymphocytes mediate an immune response against the malignancy, consequently an elevated NLR value could indicate a protumorigenic status.

In this systematic review we investigated the association between NLR and the prognosis of CRLM in patients treated with interventions of any modality including surgery, chemotherapy and ablative techniques[9,10]. High NLR was associated with poor survival in CRLM patients in the systematic

review and meta-analysis by Tang *et al*[11], which included 8 studies and in the systematic review by Haram *et al*[12] which also included 8 studies. Our systematic review includes 19 studies thus making the analysis results more robust. It consists of 12 studies including 2442 patients treated surgically, 6 studies including 641 patients treated with RFA or RE or solely chemotherapy and 1 study (Kishi *et al*[15]) including 200 patients treated surgically and 90 different patients treated with RFA. We studied the different impact of pretreatment NLR as a prognostic factor depending on the medical intervention and we present the analysis results in two categories. The first category included 2642 patients who were treated surgically and the second category included 731 patients who were treated with ablative techniques or solely chemotherapy. All the studies included demonstrated that CRLM patients with low pretreatment NLR had better survival and DFS in comparison to high pretreatment NLR patients regardless of the medical intervention received.

MATERIALS AND METHODS

Data extraction and risk of bias

A systematic literature search of PubMed and the Cochrane Library was performed using the following search terms: "Neutrophil to Lymphocyte Ratio and liver metastas* and survival", "NLR and liver metastas* and survival", "NLR and liver metastasis and prognostic factor", "NLR and liver metastas*" and "NLR". The same search strategy was used for the trial registry "ClinicalTrials.gov" in order to minimize publication bias by identifying unpublished studies.

The titles of the articles were screened and relevant abstracts were assessed for eligibility. After excluding duplicates, eligible articles were further evaluated and then the references of those studies were also checked. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart is shown in Figure 1.

In order to minimize possible errors and bias, two independent researchers blindly reviewed the literature and extracted data using the method of completely independent data extraction. After that, any potential differences were cleared up through discussion between them and a third reviewer. The following data were extracted from each study: (1) Patients' clinicopathological characteristics; (2) The treatment modalities used to treat CRLM; (3) The median survival, 3-year and 5-year OS, 3-year and 5-year DFS; and (4) The univariate and multivariate analysis outcomes.

Inclusion and exclusion criteria

In order to be included in the analysis, the studies must meet all of the following criteria: (1) Include patients older than 18 years of age diagnosed with CRLM; (2) Define NLR as the absolute number of neutrophils divided by the absolute number of lymphocytes in the peripheral blood; (3) Clearly stated pretreatment NLR values and NLR thresholds; and (4) Analyzing the correlation between pretreatment NLR value and OS outcome and/or DFS. The following exclusion criteria were applied: (1) Not specifically reported colorectal metastasis to the liver; (2) Patients with liver metastases originating from outside the colorectum; (3) Pre-clinical studies; and (4) Studies published in a language other than English.

Definitions

NLR was defined as the absolute number of neutrophils divided by the absolute number of lymphocytes in the peripheral blood. OS was defined as the time between treatment (hepatectomy, RFA, RE, chemotherapy) and death or last follow-up. DFS was defined as the time between the treatment and the first detection of disease recurrence, including local tumor recurrence, intrahepatic recurrence and extrahepatic metastases. Progression-free survival (PFS) was defined as the duration between primary tumor resection and disease progression.

RESULTS

NLR is a predictor of survival after hepatectomy with neoadjuvant or adjuvant chemotherapy. Eleven studies assessed the prognostic significance of NLR for patients undergoing hepatectomy for CRLM after neoadjuvant chemotherapy. Details on patient demographics and the different NLR thresholds are shown in Tables 1 and 2. Five studies that included 902 patients in total, used 5 as the cut-off value for the NLR. Elevated NLR was significantly associated with worse OS[13-17]. Peng *et al*[18] used 4.63 as the NLR threshold in 59 patients who received neoadjuvant chemotherapy yielded the same results. Elevated NLR was also significantly correlated with poor OS when the threshold was 1.9, 2.3, 2.4, 2.5, 2.6 or 7.26[19-23].

Ninety-eight patients in 3 studies received only adjuvant chemotherapy after metastasectomy. Elevated NLR was associated with significantly worse DFS[19,24,25]. The OS was also significantly shorter with elevated preoperative NLR in two of the studies[19,24]. However, the NLR cut-off value

Table 1 Patient demographics, neutrophil to lymphocyte ratio cut-off value, follow-up time and time of sample acquisition for patients after hepatectomy

Ref.	Number of patients	Sex	Mean age (years)	NLR threshold	Follow up (mo), mean (range)	Sample acquisition
Erstad *et al*[13]	151	84 M, 67 F	58	5	41.3 ± 36.7 (2-186)	Within 6 mo prior to surgery and prior to chemotherapy
Halazun *et al*[14]	440	289 M, 151 F	64 (32-88)	5	24 (11-97)	1 d prior to surgery
Kishi *et al*[15]	290	Resection group 132 M, 68 F; non resection group 61 M, 29 F	Resection, 57; non resection, 56	5	28 (2-102)	Resection group: Before chemotherapy and before resection
Neal *et al*[16]	302	192 M, 110 F	64.8 (26-85)	5	29.5 (4-96)	Prior to surgery
Hand *et al*[17]	322	205 M, 117 F	252 p < 70 yr; 70 p > 70 yr	5	41	On admission; the night prior to or on the morning of surgery
Peng *et al*[18]	150	97 M, 53 F	58 (20-82)	4.63	36 (2-126)	Within 7 d prior to surgery
Kim *et al*[19]	83	62 M, 21 F	59.5	1.94	NS	Within 1 wk prior to surgery
Mao *et al*[20]	183	123 M, 60 F	67 p > 60 yr	2.3	36.3	Within 10 d before chemotherapy and surgery
Neofytou *et al*[21]	140	88 M, 52 F	78% < 70 yr	2.4	33 (1-103)	Within 10 d prior to surgery
Giakoustidis *et al*[22]	169	104 M, 65 F	135 p < 70 yr, 34 p > 40 yr	2.5	34.6	10 d prior to surgery - after preoperative chemotherapy
Dupré *et al*[23]	343	236 M, 107 F	65.8 ± 10.9	2.5, 2.6 and 7.26[1]	49	Within 1 wk prior to surgery
Hamada *et al*[24]	29	20 M, 9 F	63 ± 11.6 (41-83)	4.1	51 (2-97)	NS
Zeman *et al*[25]	130	70 M, 60 F	60 (33-82)	5	39.3	NS

[1]Cut-off values that reached statistical significance.

M: Male; F: Female; p: Patients; NS: Not stated.

was different in each cohort (4.1, 1.94 and 5)[19,24,25]. Further information on the OS and DFS, the tumor characteristics as well as the results of univariate and multivariate analyses for the studies mentioned above are shown in Tables 3 and 4.

Non-surgical methods (RFA, RE, only chemotherapy)

Five studies included 494 patients who underwent RFA or RE or intraarterial therapy and they investigated the correlation between NLR and OS or DFS.

Chang *et al*[26] and Zhang *et al*[27] included 190 patients with CRLM without concomitant metastases outside of the liver. Patients were treated with RFA and both studies showed that preoperative high NLR (> 2.5) was associated with worse OS and DFS. Weiner *et al*[28] and Tohme *et al*[29] enrolled 235 patients, 100 of whom had extrahepatic metastases and an unspecified number of patients had unresected primary CRC both of which affect NLR and its correlation to OS. All of the patients underwent RE and high NLR was significantly associated with reduced OS. The fifth study investigated the correlation between NLR and OS in CRLM patients with unresectable CRC who were treated with Irinotecan drug-eluting beads against a control group and high NLR was significantly associated with reduced OS[30].

Two studies included 145 patients with unresectable or potentially resectable liver-only metastases from CRC and all of them were treated with primary tumor resection followed by chemotherapy. Both studies revealed that high NLR was significantly associated with worse survival and that prolonged survival was anticipated when NLR was normalized after chemotherapy. Wu *et al*[31] demonstrated that the normalization of high NLR was significantly associated with better PFS[15,31]. More details of patient demographics, medical treatments provided to the patients and survival outcomes are shown in Tables 5 and 6.

Table 2 Survival and disease characteristics

Ref.	Median Survival	5-year OS	5-year DFS	Extrahepatic Disease	Primary Tumor	Chemotherapy
Erstad *et al* [13]	3.1 yr, NLR > 5; 6.3 yr NLR < 5	28.7%, NLR > 5; 59.6%, NLR < 5	NS	No	Previous resection of rectum or colon	Neoadjuvant
Halazun *et al* [14]	NS	22%, NLR > 5; 43%, NLR < 5	12%, NLR > 5; 42%, NLR < 5	No disseminated or unresectable EHD	Previously resected	Neoadjuvant
Kishi *et al*[15]	34 mo, NLR > 5; 45 mo, NLR < 5	26%, NLR > 5; 36%, NLR < 5	NS	No	Previously resected	Neoadjuvant, *n* = 200; Without resection, *n* = 90
Neal *et al*[16]	27.8 mo, NLR > 5; 39.8 mo, NLR < 5	18.5% NLR > 5; 30.6% NLR < 5	22.3%, NLR > 5; 35.2%, NLR < 5[2]	NS	Rectum *n* = 149, Colon *n* = 153	Adjuvant, *n* = 126
Hand *et al*[17]	59 mo	Chemotherapy group, 50.8%; No chemotherapy group, 42.5%	NS	NS	No	Neoadjuvant, *n* = 202
Peng *et al*[18]	NS	18.8%, NLR > 4.63; 46.7%, NLR < 4.63	NS	No	58% colon, 42% rectum	Neoadjuvant, *n* = 59
Kim *et al*[19]	NS	NS	NS	No	NS	Neoadjuvant, *n* = 24
Mao *et al*[20]	31.1 mo NLR > 2.3 43.1 mo NLR < 2.3	NS	NS	No	Colon *n* = 104	Neoadjuvant, *n* = 183
Neofytou *et al* [21]	55 mo, NLR > 2.4; Not reached, NLR < 2.4	42%, NLR > 2.4; 69%, NLR < 2.4	Total 27%. 14%, NLR > 2.4; 40%, NLR < 2.4	No	Resection prior to hepatectomy in 81%	Neoadjuvant
Giakoustidis *et al*[22]	75 mo	51%, NLR > 2.5; 74% NLR < 2.5	NS	No	Synchronous resection, *n* = 26; 'liver first', *n* = 7	Neoadjuvant, *n* = 169
Dupré *et al* [23]	50.3, NLR < 2.5; 38.4, NLR > 2.5; 44.8, NLR < 7.26; 25.4, NLR > 7.26	NS	11.6, NLR < 2.5; 9.7, NLR > 2.5; 10.3, NLR < 7.26; 6.3, NLR > 7.26[1]	Resectable EHD in 36 patients	Synchronous, *n* = 169; Right colon, *n* = 73; Left colon, *n* = 126; Rectum, *n* = 142; Multiple, *n* = 2	Neoadjuvant, *n* = 198
Hamada *et al* [24]	NS	NS	NS	Yes, *n* = 5	NS	Adjuvant, *n* = 15
Zeman *et al* [25]	Resection group, 56 mo	46.6%, resection group; 9.5%, RFA group	30.5%, resection group, 21 mo median	No	Rectum *n* = 60, colon *n* = 70	Adjuvant, *n* = 25

[1]PFS: Progression-Free Survival.
[2]CSS: Cancer-Specific Survival.
OS: Overall survival; DFS: Disease-free survival; NLR: Neutrophil to lymphocyte ratio; NS: Not stated; EHD: Extra-hepatic disease.

DISCUSSION

Many studies have shown the significance of elevated NLR as a prognostic marker in various cancers and the role of NLR in predicting survival remains unanimous across diverse studies that included different cancer types, disease stages and sites[32]. In the studies we analyzed, the NLR cut-off values were determined either by using receiver operating characteristic (ROC) curve analysis that assigned patients in high and low NLR groups or arbitrarily by the authors based on previous studies or the decision-making for the threshold was not mentioned. There is currently no perfect cut-off value that could be used for all CRLM patients as the NLR is greatly affected by chemotherapy regimens, other inflammatory conditions and the tumor burden of each patient. The most frequently used cut-off values in CRLM patients are 2.5 and 5 but further research is needed in order to establish the ideal NLR threshold.

Association between NLR - inflammation - cancer

NLR is an inexpensive and easily calculated marker by dividing the total count of neutrophils by the total count of lymphocytes in peripheral blood as measured in a complete blood count (CBC) examination[11,12]. NLR is also immediately available as CBC is part of the routine examinations in patients with cancer.

Table 3 Univariate and multivariate analysis results after hepatectomy

Ref.	Univariate analysis	Multivariate analysis
Erstad *et al*[13]	NLR > 5 was significantly associated with reduced OS (P = 0.001)	NLR > 5 associated with reduced OS (P = 0.032)
Halazun *et al*[14]	NLR > 5 was associated with reduced OS (P < 0.0001); NLR > 5 was the sole positive predictor of recurrence (P < 0.0001)	NLR > 5 was associated with reduced OS (P < 0.0001)
Kishi *et al*[15]	NLR > 5 predicted worse survival (P = 0.011)	NLR > 5 predicted worse survival using variables available before surgery (P = 0.016) and after surgery (P = 0.048)
Neal *et al*[16]	NLR > 5 was significantly associated with worse OS (P = 0.001) and CSS (P < 0.001) following metastasectomy	NLR > 3 was associated with shorter survival (P < 0.001); NLR > 3 was associated with adverse outcomes regarding CSS (P < 0.001)
Hand *et al*[17]	Following index hepatectomy, patients with NLR > 5 had a median survival of 55 mo *vs* 70 mo when NLR < 5 (P = 0.027); Following neoadjuvant chemotherapy, no association between NLR and survival was demonstrated (P = 0.93); NLR > 5 had no impact on prognosis following repeat hepatectomy	There is an independent association between elevated preoperative neutrophil count and shortened overall survival (P = 0.001); no such association was found for NLR
Peng *et al*[18]	Patients with NLR > 4.63 were more likely to present multiple hepatic metastases than those with low NLR (68.8% *vs* 40.3%, P = 0.030); 5-year RFS and OS for high NLR were significantly inferior to those for low NLR (RFS: 12.5% *vs* 38.5%, P = 0.015; OS: 18.8% *vs* 46.7%, P = 0.004)	NLR was not identified as an independent inflammatory factor for better RFS
Kim *et al*[19]	NLR > 1.94 was a prognostic factor for poor OS (P = 0.035) and DFS; High NLR was associated with recurrence (P = 0.031)	NLR > 1.94 was an independent prognostic factor for OS (P = 0.01) and prognostic factor for poor DFS; High NLR was associated with recurrence (P = 0.006)
Mao *et al*[20]	Pre- and post-chemotherapy NLR > 2.3 was associated with decreased OS (P = 0.012)	NLR > 2.3 was a significant predictor both for worse OS (P < 0.001) and for RFS (P = 0.017)
Neofytou *et al*[21]	NLR > 2.4 was associated with decreased DFS (P = 0.033) and OS (P = 0.007)	No significant correlation was found between NLR and OS/DFS
Giakoustidis *et al*[22]	NLR > 2.5 was associated with decreased OS (P = 0.009) and decreased DFS (P = 0.09)	High NLR remained a significant prognostic factor for poor OS (P = 0.012)
Dupré *et al*[23]	NLR of 2.5 was an independent prognostic factor for OS and PFS	High NLR was significantly associated with decreased OS (P < 0.002)
Hamada *et al*[24]	NLR > 4.1 was significantly correlated with better CSS (P = 0.026)	Only univariate analysis was performed
Zeman *et al*[25]	NLR > 5 was significantly associated with DFS (P = 0.044); OS was significantly affected by the preoperative number of leukocytes (P = 0.0014) and neutrophils (P = 0.0036) but not by the NLR.	NLR > 5 was significantly associated with DFS (P = 0.03); Leukocyte number was significantly associated with OS (P = 0.0014); no effect of NLR was demonstrated on OS

NLR: Neutrophil to lymphocyte ratio; OS: Overall survival; CSS: Cancer-specific survival; RFS: Recurrence-free survival; DFS: Disease-free survival.

The association between high NLR and worse prognosis in CRLM patients is complicated and is being rigorously studied. Inflammation plays a significant role in tumor initiation, promotion and progression through pro-inflammatory cytokines, growth factors, chemokines and pro-angiogenic factors. Neutrophils promote tumorigenesis through several mechanisms. They induce DNA damage and mutations by producing toxic substances such as reactive nitrogen species, they promote neoangiogenesis and tumor progression by releasing matrix metalloproteinase-9 (MMP-9) followed by the release of vascular endothelial growth factor. Moreover, neutrophils release a granule protein, called Arg-1, which restricts CD3-cell mediated T cell activation, thus establishing an immunosuppressive microenvironment that contributes to cancer growth. Therefore, a high neutrophil count could indicate worse prognosis in patients with cancer[33,34]. On the contrary, a low lymphocyte count is associated with poorer tumor infiltration and insufficient cell immunity and therefore with worse prognosis in patients with cancer[32]. Consequently, high NLR as a result of increased neutrophils and/or decreased lymphocytes could be an indicator of poor host against tumor response and poor prognosis.

It is plausible that NLR could have an impact in clinical practice as it represents a readily-available biomarker which could potentially assist in the decision-making with prognostic significance. In the studies included in our literature review, patients were treated with different interventions such as surgery with or without adjuvant or neo-adjuvant chemotherapy and other patients were treated with RFA or RE or solely chemotherapy. High NLR was associated with worse OS and DFS in all of the studies.

Table 4 Patient demographics, NLR cut-off value, follow up and time of sample acquisition for patients treated with radio-frequency ablation, radioembolization or solely chemotherapy

Ref.	Number of patients and procedure	Sex	Mean age (yr)	NLR threshold	Follow up (mo)	Sample acquisition
Tohme *et al*[29]	104 RE	69 M, 35 F	60.8 ± 12.2, NLR > 5, 66.4 ± 12.2, NLR < 5	5	100 patients died during follow up	Same day before RE
Chang *et al* [26]	98 RFA	56 M, 42 F	62 (28-92)	2.5	35.2 ± 21.89	1 d before RFA - 1 mo after RFA
Zhang *et al* [27]	92 RFA	51 M, 41 F	59 (43-78)	5	27.1 ± 9.8 (range 5-62)	Preoperatively as part of the routine workup. 1-3 d before RFA
Weiner *et al*[28]	131 RE	84 M, 47 F	59	5	117 deaths during follow up	NS
Kishi *et al* [15]	90 chemotherapy	61 M, 29 F	56	5	28 (2-102)	
Wu *et al* [31]	55 chemotherapy	35 M, 20 F	59	4	Complete clinical records (no exact mention)	Preoperatively and before 2nd cycle of chemotherapy
Philips *et al*[30]	71	-	-	5	-	-

RE: Radioembolization; RFA: Radiofrequency ablation; M: Male; F: Female; NLR: Neutrophil to lymphocyte ratio; NS: Not stated.

Table 5 Survival and disease characteristics of patients that were treated with radio-frequency ablation, radioembolization or solely chemotherapy

Ref.	Median Survival	5-year OS	5-year DFS	Extrahepatic disease	Primary tumor	Chemotherapy
Tohme *et al*[29]	5.6 m high NLR 10.6 m low NLR	-	-	40 (less than 10% of tumor burden)	Some patients had undergone CRC resection (number not mentioned)	All patients
Chang *et al*[26]	-	-	(Preoperative NLR) 11.1% high NLR 22.6% low NLR (NLR increase after RFA 8.6%) (No postoperative NLR increase 22.2%)	No	All patients had undergone CRC resection	No mention
Zhang *et al*[27]	-	18,4% high NLR 41.7% low NLR	9.5% high NLR 26.7% low NLR	No	All patients had undergone CRC resection	If necessary after primary tumor resection (number not mentioned)
Weiner *et al*[28]	7.9 m high NLR 13 low NLR	-	-	59	79% had undergone primary CRC resection	All patients
Kishi *et al*[15]	11 m high NLR 21 m low NLR	0% high NLR 14% low NLR	-	No	All patients had undergone CRC resection	All patients
Wu *et al* [31]	24 m high NLR 56 m low NLR	-	-	No	All patients had undergone CRC resection	All patients
Philips *et al*[30]	14.7 m high NLR 31.9 m low NLR	-	-	Liver dominant disease	Not mentioned	All patients

RFA: Radio-frequency ablation; RE: Radioembolization; OS: Overall survival; DFS: Disease-free survival; NLR: Neutrophil to lymphocyte ratio; CRC: Colorectal cancer.

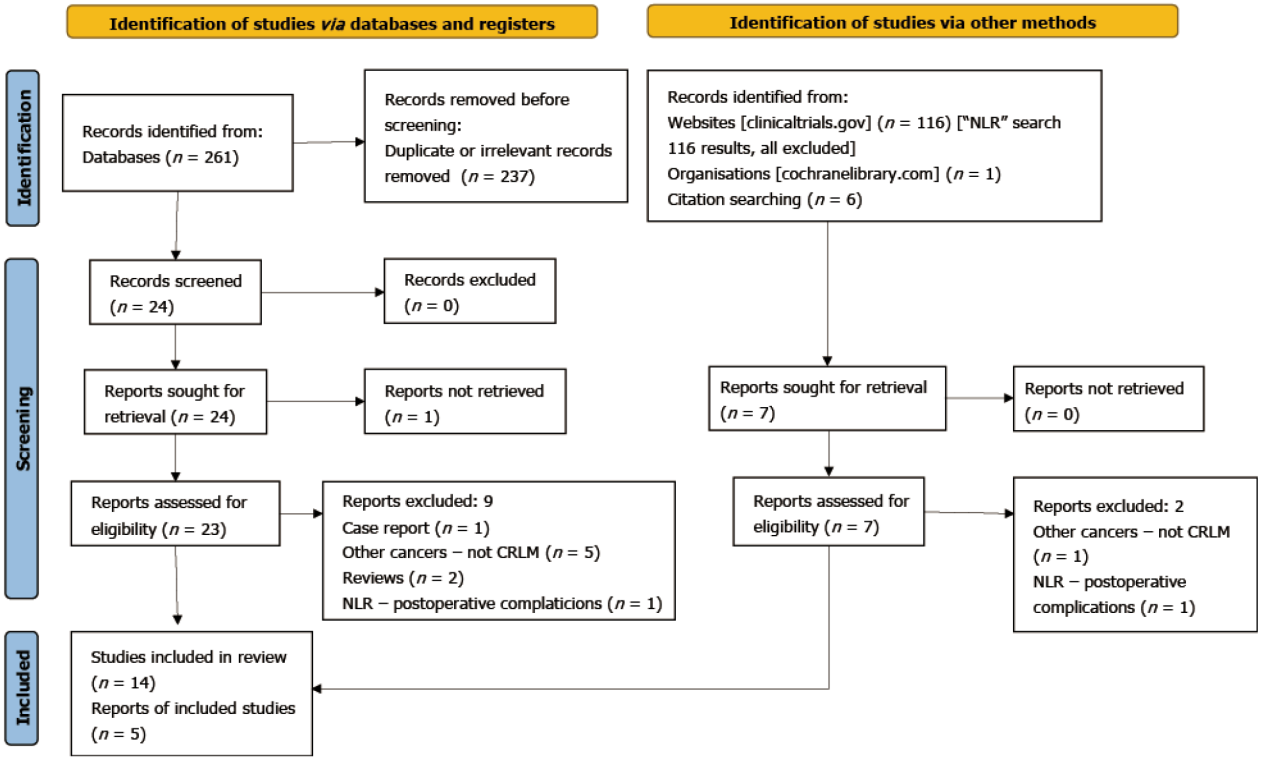
Chemotherapy may affect NLR - Surgical candidates

Patients with CRLM have a poor prognosis if not treated appropriately. The current surgical approach when applicable is to resect the primary tumor followed by liver metastases resection 2-3 mo later with or without chemotherapy, but in certain cases there can be synchronous resection of the primary colon cancer and the hepatic metastases or the 'liver first approach'[35]. The role of systemic chemotherapy before or after a surgical procedure is well-established both for resectable and non-resectable disease, as

Table 6 Univariate and multivariate analysis outcomes for patients treated with radio-frequency ablation, radioembolization or solely chemotherapy

Ref.	Univariate analysis	Multivariate analysis
Tohme *et al*[29]	High NLR associated with decreased OS $P < 0.001$	High NLR associated with decreased OS (HR = 1.927, 95%CI: 1.202-3.089, $P = 0.006$)
Chang *et al*[26]	Preoperative high NLR and postoperative increase in NLR were associated with decreased DFS ($P = 0.044$ and $P = 0.022$, respectively)	Only postoperative NLR increase was associated with decreased DFS ($P = 0.029$)
Zhang *et al*[27]	High NLR associated with decreased OS ($P = 0.007$) and DFS ($P = 0.007$)	High NLR associated with decreased OS ($P = 0.039$, HR = 3.59, 95%CI: 1.54-9.67) and DFS ($P = 0.022$, HR = 3.19, 95%CI: 1.87-8.24).
Weiner *et al*[28]	High NLR associated with decreased OS (HR = 2.18, 95%CI: 1.45-3.28, $P = 0.0002$)	High NLR associated with decreased OS (HR = 2.22, 95%CI: 1.46-3.38, $P = 0.0002$)
Kishi *et al* [15]	High NLR associated with decreased OS (HR = 3.1, 95%CI: 1.7-5.9, $P < 0.001$)	High NLR associated with decreased OS (HR = 2.9, 95%CI: 1.5-5.5, $P = 0.001$).
Wu *et al* [31]	High NLR (HR = 3.182, 95%CI: 1.277-7.933, $P = 0.013$) associated with decreased OS and DFS (HR = 2.284, 95%CI: 1.156-4.498, $P = 0.017$). Patients with normalization of NLR had better DFS than those with high NLR that did not decrease ($P = 0.002$).	No association between NLR and survival
Philips *et al*[30]	High NLR associated with decreased OS $P = 0.067$ (statistically significant)	No association between NLR and survival

CI: Confidence interval; RFA: Radio-frequency ablation; RE: Radioembolization; OS: Overall survival; DFS: Disease-free survival; NLR: Neutrophil to lymphocyte ratio.



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Figure 1 The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart.

it can improve OS[36].

This systematic review points out the role of the NLR as a prognostic factor for the OS and DFS of patients with CRLM. Patients with low preoperative NLR value had better outcomes with longer OS. Similar results were presented by another systematic review which concluded that preoperative NLR calculation could contribute to the identification of patients who could benefit from adjuvant therapies [12]. Measurement of the NLR is inexpensive and easily applied with its value possibly being able to add to the management strategy for patients.

It would be of interest if we could clarify whether different types of chemotherapy affect the NLR or vice versa. In two of the studies included, some patients received adjuvant and others neoadjuvant chemotherapy. The results showed that an elevated NLR is indeed significantly associated with worse survival, but the patients who received neoadjuvant chemotherapy were not separated from the adjuvant group[14,16]. However, Kishi *et al*[15] showed that preoperative chemotherapy normalized the NLR in 68% of patients with elevated pretreatment NLR, who eventually had similar survival to those with normal pretreatment NLR. Of note, Hand *et al*[17] showed that OS was significantly shorter for chemotherapy-naive patients with elevated NLR, but not for those who received neoadjuvant chemotherapy. For the latter, the OS resembled that of the patients with normal NLR. Hand *et al*[17] did not measure the NLR after chemotherapy, but their results are consistent with the fact that neoadjuvant chemotherapy could normalize NLR. Finally, the role of chemotherapy was also investigated in the study by Mao *et al*[20]. They separated patients into four groups depending on pretreatment and presurgical NLR values. Simultaneous pretreatment and presurgical NLR > 2.3 was significantly associated with worse OS, and the authors hypothesized that high NLR may also indicate poor chemosensitivity[20]. Wu *et al*[31] included patients with synchronous CRLM who were treated with chemotherapy after primary tumor resection. They showed that patients with normalization of the NLR after one cycle of chemotherapy had better PFS than patients in whom the NLR remained high after chemotherapy and CRC resection. Consequently, the reduction of NLR could imply better chemosensitivity.

Non-surgical candidates

To this day, hepatectomy is the "gold standard" treatment in patients with CRLM offering the longest OS, but as a matter of fact only 25% of those patients are eligible for surgery mainly because their clinical condition does not allow them to be surgical candidates or sometimes they refuse surgical treatment[37].

In two studies where patients were treated with RE, the median OS ranged between 5.6 to 7.9 mo in the high NLR group and between 10.6 to 13 mo in the low NLR group. Zhang *et al*[27] and Chang *et al*[26] included 190 patients with liver-only CRC metastases. They showed that high NLR patients had worse 5-year DFS ranging between 9.5 to 11% whereas low NLR patients had better 5-year DFS ranging between 22.6 to 26.7%. Zhang *et al*[27] also showed that high NLR is associated with worse 5-year OS (18.4%) after RFA in comparison to 41.7% in low NLR patients.

It is plausible that the studies investigating the correlation between NLR and OS or DFS in patients with unresectable tumors will demonstrate worse OS or/and DFS compared to studies in surgically treated patients, since as mentioned before non-surgical candidates present a worse clinical condition in general which affects their course of disease.

Different NLR thresholds

Even though increased preoperative NLR is correlated with shorter OS and DFS in general, the NLR cut-off values varied between individual studies. The most commonly used threshold was 5. However, the NLR threshold ranged from 1.94 to 7.26[19,23]. That wide distribution could be attributed to the NLR depending on many pro- and anti-inflammatory parameters and the extent of the body's inflammatory response to cancer, in other words the cancer's biology being unpredictable[7]. In a study where eight different cut-off values were used (2.2, 2.5, 2.6, 3, 3.5, 4, 5 and 7.26), elevated NLR was consistently associated with decreased OS, even though the results were not statistically significant for every cut-off value[23]. The optimal threshold is based on molecular data analyzed by computer applications, such as Cutoff Finder[38]. The cut-off value is calculated with various models, such as ROC curve analysis or Kaplan-Meier curves and proportional hazards regression[39].

Impact on clinical practice

NLR is an easily calculated tool with a possible prognostic significance. High NLR could inform the clinicians about the worse OS and DFS that would be expected. Since worse DFS would be expected, patients with high NLR could be submitted to earlier and more frequent diagnostic imaging examinations, in order to diagnose disease recurrence. Moreover, better prognosis would be anticipated in patients with low NLR since they present better OS and DFS.

Moreover, some patients are initially diagnosed with unresectable or potentially resectable CRLM. Many studies have shown that inoperable CRLM can be down-staged to resectable CRLM after chemotherapy, but this happens in less than 35% of patients with inoperable CRLM[40]. Therefore, more than 65% of patients with unresectable CRLM will not benefit from chemotherapy and it would be important to identify biomarkers that could identify chemosensitive patients. Later, those patients would be submitted to secondary CRLM curative resection. Mao *et al*[20] and Wu *et al*[31] demonstrated in their studies that the normalization of NLR after chemotherapy is correlated to chemosensitivity. Consequently, NLR could be used as an assisting tool in stratifying patients as chemosensitive or chemoresistant. Chemoresistant patients would possibly benefit more from interventions such as RFA or RE rather than chemotherapy. More studies are needed to assess whether NLR can be used as an indicator of chemosensitivity or if NLR could be combined with other biomarkers to increase accuracy.

Our results clearly demonstrate that elevated NLR is associated with adverse OS and DFS. These results are significant and they are the same across heterogeneous studies that included different populations, age groups, cancer stages, chemotherapy regimens and medical interventions, which shows that NLR could be an important prognostic factor that can be used in CRLM patients. High pretreatment NLR is associated with worse outcomes independently of the treatment received by the patients.

Prospective studies are needed in order to examine whether NLR could be used as part of an algorithm for the treatment of CRLM. It could also be potentially used in combination with other biomarkers or parameters such as CEA, CA19-9, primary tumor location and primary tumor TNM stage, which have been used in other studies in order to create a novel scoring system that would improve the predictive accuracy of recurrence and survival[19,41].

Limitations

One limitation of our study is that the cut-off values were different among the studies. That prevents the utilization of NLR as a tool for the management of patients in clinical practice. The timing of blood sampling was also not consistent among the studies. Regarding neoadjuvant chemotherapy, even though it appears to improve outcomes, there is a need for larger studies that distinguish different chemotherapy types and regimens to reach a certain conclusion. Finally, the heterogeneity of patient demographics and clinicopathological characteristics (*e.g.*, primary tumor location and treatment, size or extent of the metastases) prevented the conduction of a meta-analysis.

It is obvious that more research is needed in order to enhance the role of NLR as an inexpensive, independent, crucial prognostic marker. More prospective randomized trials should be designed and executed as all the articles that were available to us were retrospective except one. In upcoming studies the authors should clearly state the clinicopathological details of every patient, the dates of blood sampling, the primary tumor and liver metastasis characteristics and how they were treated. Ideally, all patients should have their primary colorectal tumor resected and not have extrahepatic metastasis as these raise the tumor burden of patients with CRLM and therefore affect NLR. Moreover, all of these patients should be treated with similar chemotherapy sessions and with interventions by surgeons with similar levels of experience and training.

CONCLUSION

Neutrophil to lymphocyte ratio calculation could potentially be an assisting tool in identifying patients with CRLM who have a higher probability of poor prognosis after treatment, so that the periprocedural management could be adjusted to benefit the patient. Overall, high pretreatment NLR was significantly associated with worse OS and DFS. Larger studies could help identify a standard, widely accepted cut-off value and therefore make the NLR's prognostic significance applicable in clinical practice.

ARTICLE HIGHLIGHTS

Research background

Patients with CRLM can be treated surgically or non-surgically, but regardless of the medical intervention they have low overall survival and disease-free survival.

Research motivation

It is important to develop prognostic biomarkers that could predict survival, tumor recurrence and response to treatment in order for patients to benefit most from medical interventions and receive personalized treatment.

Research objectives

To identify all possible articles related to our topic and examine the use of NLR as a prognostic factor in CRLM patients in clinical practice. We aimed to demonstrate that NLR is a possible significant biomarker that could assist in the management of CRLM patients by predicting survival, tumor recurrence or response to treatment.

Research methods

We performed an extensive search of PubMed, the Cochrane Library and also searched for unpublished articles in "clinicaltrials.gov". We used combinations of the words "Neutrophil to Lymphocyte ratio", "NLR", "survival", "prognostic factor", "metastasis", "metastases", "liver metastasis", "liver metastases". The results were screened by two independent researchers and any potential differences were resolved between them and a third researcher through discussion. The aim was to identify studies

that investigated the correlation between NLR and survival or tumor recurrence in CRLM patients.

Research results

We included 19 studies that included CRLM patients who were treated with different medical approaches, surgically or non-surgically. All the studies demonstrated that high NLR was associated with poor survival, disease-free survival and response to chemotherapy.

Research conclusions

The NLR could potentially be used as a predictor of survival, tumor recurrence and chemosensitivity in CRLM patients.

Research perspectives

Prospective, well-structured studies are needed in order to examine the role of the neutrophil to lymphocyte ratio (NLR) as a prognostic factor and establish it as part of the decision-making tools of clinicians in the management of colorectal liver metastasis (CRLM) patients.

FOOTNOTES

Author contributions: Papakonstantinou M and Fiflis S contributed equally to this work and wrote most of the manuscript; Papakonstantinou M, Fiflis S and Giakoustidis A designed the research study, performed the research and analyzed the data; Christodoulidis G offered guidance and assisted as a corresponding author; Giglio M offered guidance and performed manuscript revisions; Louri E and Mavromatidis S assisted in writing part of the introduction and performed manuscript revisions; Giakoustidis D and Papadopoulos VN assisted in writing part of the discussion and performed manuscript revisions; Giakoustidis A perceived the idea and assisted as a supervising author; all authors have read and approved the final manuscript.

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SCIENTOMETRICS

Current global research landscape on COVID-19 and cancer: Bibliometric and visualization analysis

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Abstract

BACKGROUND

Cancer is a severe public health issue that seriously jeopardizes global health. In individuals with coronavirus disease 2019 (COVID-19), cancer is considered an independent risk factor for severe illness and increased mortality.

AIM

To identify research hotspots and prospects, we used bibliometrics to examine the global production of COVID-19 literature published in the field of oncology.

METHODS
Data on publication output were identified based on the Scopus database between January 1, 2020, and June 21, 2022. This study used VOSviewer to analyze collaboration networks among countries and assess the terms most often used in the titles and abstracts of retrieved publications to determine research hotspots linked to cancer and COVID-19. The *Impact Index Per Article* for the top 10 high-cited papers collected from Reference Citation Analysis (RCA) are presented.

RESULTS
A total of 7015 publications were retrieved from the database. The United States published the greatest number of articles (2025; 28.87%), followed by Italy (964; 13.74%), the United Kingdom (839; 11.96%), and China (538; 7.67%). The *University of Texas MD Anderson Cancer Center* (n = 205, 2.92%) ranked first, followed by the *Memorial Sloan-Kettering Cancer Center* (n = 176, 2.51%). The *European Journal of Cancer* (n = 106, 1.51%) ranked first, followed by the *Frontiers in Oncology* (n = 104, 1.48%), *Cancers* (n = 102, 1.45%), and *Pediatric Blood and Cancer* (n = 95; 1.35%). The hot topics were stratified into "cancer care management during the COVID-19 pandemic"; and "COVID-19 vaccines in cancer patients".

CONCLUSION
This is the first bibliometric analysis to determine the present state and upcoming hot themes related to cancer and COVID-19 and vice versa using VOSviewer during the early stages of the pandemic. The emergence of hot themes related to cancer and COVID-19 may aid researchers in identifying new research areas in this field.

Key Words: Bibliometric; Scopus; COVID-19; Cancer; Coronavirus disease; VOSviewer; Reference Citation Analysis

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Core Tip: Many systematic reviews and meta-analyses found that the number of papers investigating the impact of coronavirus disease 2019 (COVID-19) on cancer in various countries increased during the pandemic. The existing literature on COVID-19, focusing on cancer research, has not been provided by any bibliometric analysis. The hot topics were stratified into "cancer care management during the COVID-19 pandemic"; and "COVID-19 vaccines in cancer patients". Cancer and COVID-19 have emerged as hot topics, which may help researchers uncover new research opportunities in this area.

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INTRODUCTION

The first confirmed case of coronavirus disease 2019 (COVID-19) was recorded in Wuhan, China, on December 31, 2019[1]. Since that time, COVID-19 has been spreading rapidly throughout the world. Although some individuals diagnosed with COVID-19 have no symptoms, patients who become symptomatic exhibit a wide range of severity, ranging from mild respiratory symptoms to critical lung disease, sepsis, multiple organ failure, or even death[2,3]. As of June 22, 2022, a total of 538321874 cases of COVID-19 have been confirmed worldwide, including 6320599 deaths[4]. According to the Sustainable Development Goals (SDGs) Report 2020, COVID-19 halts the progress of SDG 3, which seeks to guarantee well-being and a healthy life for everyone. During the crisis, health services for cancer screening have been disrupted or ignored in many places[5].

Cancer patients represent a district group in the population with a weakened immune system due to anticancer treatments and disease activity[6,7]. In pandemics like COVID-19, cancer patients may be deprived of receiving appropriate health care as many health institutes announced shortages of their resources, along with the inadequate information available in the literature to manage them properly [8]. Therefore, health care practitioners have to decide whether to initiate or defer anticancer treatments, considering the risks and benefits of such action. Notably, patients with active cancer are highly susceptible to COVID-19. They are suspected of having serious consequences, such as admission to the

intensive care unit, a requirement for mechanical ventilation, or death[9]. These unfavorable outcomes could sometimes be related to types of cancer, particularly hematologic malignancies and lung cancer [9].

Some studies reported a death rate of 28% among COVID-19 patients with cancer, which was far higher than the rate in the general population[10,11]. It was also found that certain demographics and disease-related factors, including male gender, smoking, old age, having ≥ two medical conditions, cancer status, and performance situation, were strongly associated with the mortality rate among COVID-19 cancer patients[12,13]. However, receiving antitumor therapy within four weeks of diagnosis with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was not associated with the death rate[10].

According to several systematic reviews and meta-analyses, the number of publications analyzing the impact of COVID-19 on cancer in various nations increased during the pandemic[14-18]. Although various bibliometric studies have been undertaken to evaluate COVID-19 research worldwide[19-23], limited studies have been identified that have presented the current literature on COVID-19, focusing on cancer research. The bibliometric methodology was utilized to measure and categorize research output, allowing for mapping the subject area based on the most involved authors, institutions, nations, citations, journals, and hot topics[24-28]. Therefore, this study sought to comprehensively analyze the current status of publications on COVID-19 in the oncology field through visual and bibliometric analysis. This study intends to be a valuable resource and guide for oncologists, clinicians, virologists, and epidemiologists conducting research on the emerging human coronavirus in the field of cancer in order to generate novel ideas for effective control measures and to outline COVID-19 vaccine guidance for cancer patients as soon as possible.

MATERIALS AND METHODS

Data source

The present study, which includes the analysis, was performed in June 2022. The authors utilized the Scopus database to find relevant publications as: (1) It is available to the author through the "Research4 Life" library; (2) it is the largest database available, and it has a greater number of indexed journals than other databases (*e.g.*, PubMed or Web of Science) and is completely inclusive of all journals in Medline [29-31]; and (3) it indexes journals in the disciplines of health, social sciences, life sciences, and physical sciences[32,33]. In addition, Scopus has previously been used to analyze and visualize research publications on various health-related topics[34-38].

Search strategies

In order to obtain all publications pertaining to COVID-19 and cancer published between January 1, 2020, and June 21, 2022, we employed the 'Advanced search' feature of the Scopus online database. The retrieval and export of data took place within one day to avoid the risk of bias induced by ongoing database changes (June 21, 2022). The following strategy was used to retrieve data for this study (Figure 1):

Step 1: The phrases associated with COVID-19 were entered into the Scopus engine to accomplish the study's objectives. They were drawn from previous bibliometric researches on COVID-19[20,21,39-41]. All selected "terms" were included in the "Article Title/Abstract/Keywords" section.

Step 2: The documents identified in step 1 were then limited to those having the phrases "cancer and related terms" in their titles. Cancer-related terms were taken from PubMed's Medical Subject Headings (MeSH), and from a previous systematic and meta-analysis on COVID-19 in the oncology field[14-17,42] and placed into the Scopus engine. Some documents (*i.e.*, erratum, and retracted) were excluded (Figure 1).

Bibliometric analysis

The following bibliometric indicators were compiled using an Excel spreadsheet: total number of publications, type of publication, prolific countries, prolific institutions, prolific journals, and top-cited publications. Reference Citation Analysis (RCA) data were used to calculate the *Impact Index Per Article* for the top ten most cited papers. Baishideng Publishing Group Inc. owns the RCA, an open transdisciplinary citation analysis database (Pleasanton, CA 94566, United States)[43].

Visualization analysis

The network visualization maps were created using the VOSviewer (version 1.6.16) software program [44,45]. VOSviewer was used in our study as it is well-known as a software tool for visualizing quantitative data. VOSviewer is widely used for mapping, networking, and visualization to emphasize international collaboration and create a co-occurrence matrix to identify research hotspots based on published evidence. A node represents a certain element, such as a country or term. Stronger

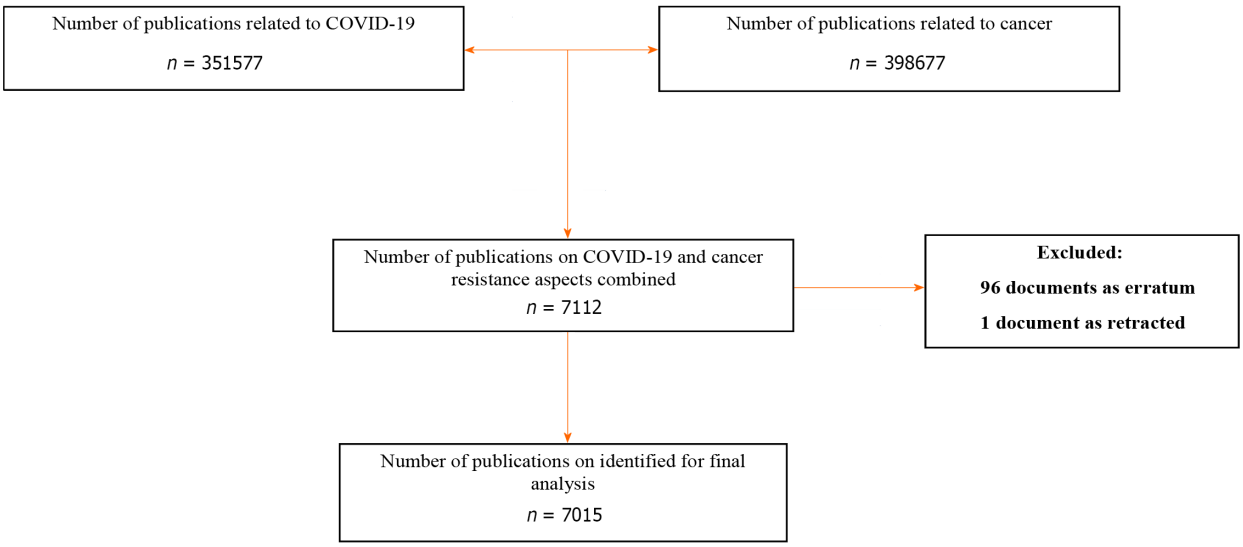


Figure 1 Flowchart for including and excluding literature studies.

cooperation is shown by wider links between nodes, whereas a bigger node size suggests a large number of publications[44,45]. The study themes in the collected literature were determined by mapping the most common terms in titles/abstracts. Using VOSviewer, it is possible to create an overlay visualization in which the most recently used author terms are shown in yellow. Terms overlay visualization was based on the occurrences and average publication per year scores.

RESULTS

Volume and types of publications

At the time of data collection (June 21, 2022), Scopus has published 351577 documents on COVID-19 throughout all research fields. During the study period (January 1, 2020, to June 21, 2022), Scopus identified 7015 papers on cancer and COVID-19 which were categorized into ten types. Among them, "Article" accounted for 57.59% of the total publications (4040 articles) and was the most frequent type, followed by letters to the editor (n = 1061; 15.12%), and reviews (n = 936; 13.34%). The remaining publication types were 978 documents (13.94%).

Contributions of countries to global publications

We ranked ten high-output countries according to the number of publications (Table 1). The United States published the greatest number of articles (2025; 28.87%), followed by Italy (964; 13.74%), the United Kingdom (839; 11.96%), and China (538; 7.67%). Figure 2 depicts a network map of the major participating countries' international research collaborations on cancer and COVID-19-related literature.

Active institutions/organizations

Table 2 shows the top ten active institutions. The *University of Texas MD Anderson Cancer Center* (n = 205, 2.92%) ranked first, followed by the *Memorial Sloan-Kettering Cancer Center* (n = 176, 2.51%) and the *Harvard Medical School* (n = 155; 2.21%). The majority of active institutions were from the United States (n = 4), followed by Italy (n = 3), Canada (n = 1), France (n = 1), and India (n = 1).

Active journals

For cancer and COVID-19-related literature, Table 3 shows the top ten active journals. The *European Journal of Cancer* (n = 106, 1.51%) ranked first, followed by the *Frontiers in Oncology* (n = 104, 1.48%), *Cancers* (n = 102, 1.45%), and *Pediatric Blood and Cancer* (n = 95; 1.35%).

Top cited publications

Table 4 lists the top ten most cited works in the field of COVID-19 and cancer, ranked by total citations. The citations in the top ten ranged from 2498 to 340[9-12,46-51]. Among the top 10 papers by total citation frequency, Liang *et al*[46], published in *The Lancet Oncology* in 2020, had the greatest overall citation frequency (number of citations = 2498). The *impact index per article* of the ten most cited articles ranged from 118.5 to 1017.0 (Table 4).

Table 1 Publication contributions of the top 10 productive countries

Ranking	Country	Number of documents	%
1st	United States	2025	28.87
2nd	Italy	964	13.74
3rd	United Kingdom	839	11.96
4th	China	538	7.67
5th	India	489	6.97
6th	France	403	5.74
7th	Canada	363	5.17
8th	Germany	349	4.98
9th	Spain	327	4.66
10th	Australia	237	3.38

Table 2 Top ten active institutions/organizations on research related to coronavirus disease 2019 and cancer

Ranking	Institution	Country	*n*	%
1st	University of Texas MD Anderson Cancer Center	USA	205	2.92
2nd	Memorial Sloan-Kettering Cancer Center	USA	176	2.51
3rd	Harvard Medical School	USA	155	2.21
4th	Dana-Farber Cancer Institute	USA	152	2.17
5th	University of Toronto	Canada	132	1.88
5th	Università degli Studi di Milano	Italy	127	1.81
7th	Istituto Europeo di Oncologia	Italy	121	1.72
8th	INSERM	France	120	1.71
9th	Fondazione IRCCS Istituto Nazionale dei Tumori, Milan	Italy	119	1.70
10th	Tata Memorial Hospital	India	116	1.65

Research themes in cancer and COVID-19-related literature

Mapping the most frequent appearing terms in the title/abstract fields of publications in cancer and COVID-19 with a minimum occurrence of 100 resulted in 253 terms being distributed into two clusters corresponding to the two primary study topics (Figure 3). The clusters are "cancer care management during the COVID-19 pandemic" (cluster 1, red), and "COVID-19 vaccines in cancer patients" (cluster 2, green); (Figure 3). The guideline, emergency, procedure, safety, process, recommendation, guidance, approach, and care are the most often used terms in cluster 2. The most often used terms in cluster 2 are vaccine, vaccination, immunotherapy, and development.

The evolution of color from dark blue to yellow represents the variation of the hot topic over time. As shown in Figure 4, researchers focused on topics related to COVID-19 vaccines in cancer patients during the last year and have become the hot research topics, attracting increasing attention.

DISCUSSION

This is the first bibliometric study in the field of cancer to assess and visualize COVID-19 research. We reviewed a total of 7015 publications from the Scopus database, and we present a detailed analysis of worldwide contributions and hotspots in COVID-19 and cancer research during the early stages of the pandemic. According to our study, the growing number of publications in cancer and COVID-19-related literature indicates that this topic is receiving considerable attention. During the COVID-19 pandemic, the popularity of sustainable development research has increased. The number of publications indicates that as the pandemic expanded internationally, more countries were impacted, which has led to an increase in researchers paying attention to the pandemic's influence on sustainable development[52].

Table 3 Top ten active journals on research related to coronavirus disease 2019 and cancer

Ranking	Journal	*n*	%	IF
1st	*European Journal of Cancer*	106	1.51	9.162
2nd	*Frontiers in Oncology*	104	1.48	6.244
3rd	*Cancers*	102	1.45	6.639
4th	*Pediatric Blood and Cancer*	95	1.35	3.167
5th	*Lancet Oncology*	90	1.28	41.316
6th	*Supportive Care in Cancer*	86	1.23	3.603
7th	*Cancer*	81	1.15	6.860
8th	*JAMA Oncology*	75	1.07	31.777
9th	*JCO Oncology Practice*	73	1.04	NA
10th	*Advances in Radiation Oncology*	60	0.86	NA

IF: Impact factor; NA: Not available.

Table 4 List of the top 10 cited articles for coronavirus disease 2019 studies related to cancer between January 1, 2020, and June 21, 2022

Ranking	Ref.	Title	Year	Source title	Cited by	Impact Index Per Article[1]
1st	Liang *et al* [46]	"Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China"	2020	The Lancet Oncology	2498	1017.0
2nd	Zhang *et al* [47]	"Clinical characteristics of COVID-19-infected cancer patients: a retrospective case study in three hospitals within Wuhan, China"	2020	Annals of Oncology	859	296.0
3rd	Tian *et al*[49]	"Pulmonary Pathology of Early-Phase 2019 Novel Coronavirus (COVID-19) Pneumonia in Two Patients With Lung Cancer"	2020	Journal of Thoracic Oncology	835	315.5
4th	Kuderer *et al* [12]	"Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study"	2020	The Lancet	825	292.5
5th	Dai *et al*[9]	"Patients with cancer appear more vulnerable to SARS-CoV-2: A multicenter study during the COVID-19 outbreak	2020	Cancer Discovery	774	118.5
6th	Yu *et al*[50]	SARS-CoV-2" "Transmission in Patients with Cancer at a Tertiary Care Hospital in Wuhan, China"	2020	JAMA Oncology	659	236.0
7th	Maringe *et al* [48]	"The impact of the COVID-19 pandemic on cancer deaths due to delays in diagnosis in England, UK: a national, population-based, modelling study"	2020	The Lancet Oncology	579	189.0
8th	Lee *et al*[10]	"COVID-19 mortality in patients with cancer on chemotherapy or other anticancer treatments: A prospective cohort study"	2020	The Lancet	574	210.5
9th	Mehta *et al* [11]	"Case fatality rate of cancer patients with COVID-19 in a New York Hospital system"	2020	Cancer Discovery	426	158.5
10th	Feldmann *et al*[51]	"Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed"	2020	The Lancet	340	140.0

[1]The Impact Index Per Article is presented based on Reference Citation Analysis.

One of the key hot issues in the current study was "Cancer care management during the COVID-19 pandemic". According to several studies, the probability of developing COVID-19 in cancer patients is considered twofold higher than in the normal population. Therefore, oncologists should employ appropriate therapeutic methods in the event of a pandemic, weighing the risks of mortality from COVID-19 against the risks and benefits of continuing anticancer therapy[53-55]. Additionally, managing patients efficiently during pandemics or big crises should be a key component of the cancer care continuum. Common immunosuppressive treatments are likely to make cancer patients more vulnerable to COVID-19-related severe outcomes. Although recent studies of immunocompromised people suggest that outcomes may be less severe, several malignancy studies show a link between



Figure 2 International collaboration in cancer and coronavirus disease 2019-related literature is visualized as a network map among the most active countries. This graphical collaboration map was created after a minimum of 50 publications were placed in each country. Of 143 countries working in this field, 33 met this threshold. The node size denotes the number of publications for that country.

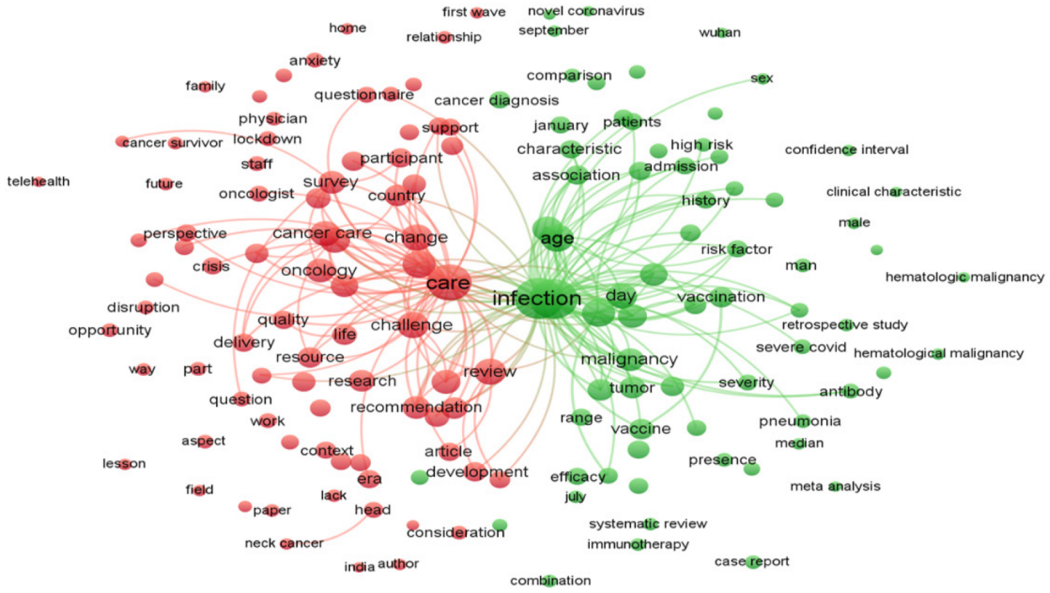


Figure 3 Map of terms in the title/abstract fields of papers relating to cancer and coronavirus disease 2019 as a network visualization. This graphical map of terms was created by placing the minimum number of term occurrences at least 100 times. Out of 75191 terms in this field, 253 terms met this criterion, grouped into three clusters and colored differently. The node size denotes the number of articles that contain that term.

increased fatality rates[12,56]. These risks are likely to differ depending on the type of cancer treatment and type of cancer[46,50,57]. According to the findings from a large systematic review and meta-analysis[58], it was shown that cancer is a comorbidity in between 1% and 2% of COVID-19 patients who are hospitalized in China, and in 5% to 7% of patients in Western nations. Based on these findings, it appears that the subjects clinically appear the same as normal individuals, and early research has shown that patients with cancer and COVID-19 have a greater in-hospital mortality risk.

Thus, this also minimizes harm in the event of a future pandemic, but it also empowers the gains generated by the current pandemic to improve overall health care delivery for all cancer patients and, by leveraging the efforts of many organizations across the cancer care stakeholders, helps all patients receive the highest-quality care while simultaneously fostering cooperation on a global scale[59,60].

Another hot subject is the COVID-19 vaccine in cancer patients. Since the early stages of the pandemic, patients with cancer have been designated as a high-risk group for COVID-19[61,62]. Therefore, the safety and effectiveness of COVID-19 vaccination in immunosuppressed persons must be better understood urgently, as excluding them and other susceptible groups from continuing trials of COVID-19 vaccines would result in inaccurate prognostic health models, which will impact subsequent

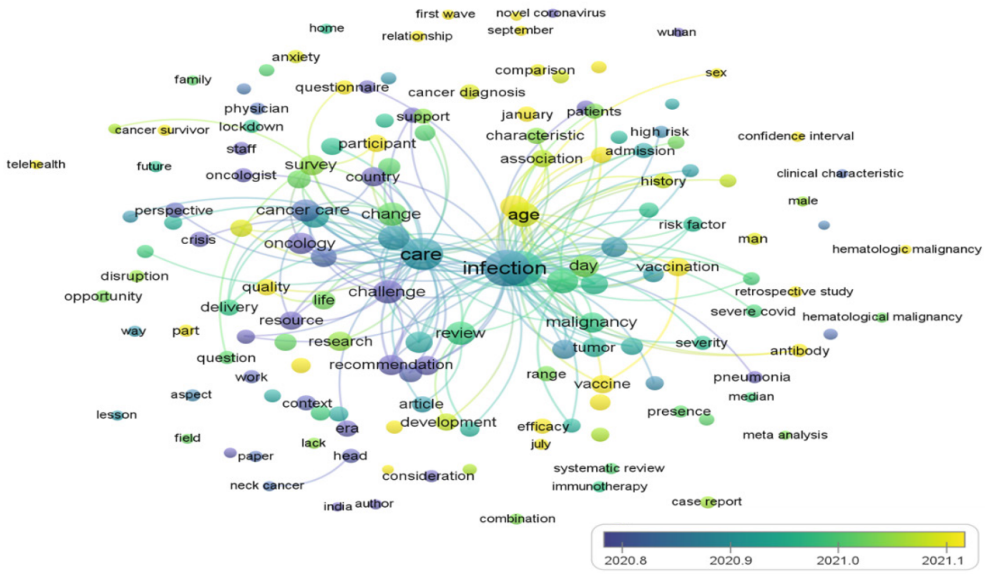


Figure 4 Overlay visualization of terms co-occurrence cluster analysis. The color of the nodes, which denotes the average publication year, changes from dark blue to yellow, representing the average publication year of the keyword from 2000 to 2022.

pandemic waves[63,64]. Given the significant risk of morbidity and death from COVID-19 in cancer patients, current information on the safety and efficacy of the approved COVID-19 vaccinations in these patients is limited. However, the benefits likely outweigh the risks of vaccine-related adverse effects[65].

Published documents that are often cited have a large academic influence. Table 4 lists the ten cancer and COVID-19-related documents with the highest citation frequency. The most frequently cited paper on the subject is "Cancer patients with SARS-CoV-2 infection: a nationwide analysis in China," published in *The Lancet Oncology* and cited 2498 times. This prospective observational study found that cancer patients were more likely to develop SARS-Cov-2 infection, require mechanical ventilation, and have an increased mortality risk[46]. It also showed that the clinical conditions of cancer patients got worse more rapidly than that of the other populations[46]. The paper by Zhang *et al*[47], which was published in *Annals of Oncology*, was the second most cited article. This study aimed to describe the clinical characteristics of COVID-19 patients who had cancer. The results revealed that more than 80% of patients had a dry cough, low lymphocyte count, high body temperature, low protein levels, and high value of inflammatory markers (C-reactive protein). In addition, patients who received anticancer therapy during the last two weeks were more likely to have serious consequences.

The third highest cited paper, published in *Journal of Thoracic Oncology*[49], analyzed two lung cancer tissue specimens of patients with COVID-19 and showed multinucleated giant cells, exudate-containing proteins, and central reactive hyperplasia of pneumocytes, along with infiltrated patches of inflammatory cells. The paper by Kuderer *et al*[12], which was published in the *Lancet*, was the fourth most cited article. This cohort analysis of 928 cancer patients diagnosed with COVID-19 noted that male gender, smoking, old age, having ≥ two medical conditions, use of chloroquine and azithromycin, cancer status, and performance situation were the determinants of death during one month. However, the types of malignancy or antitumor treatments used did not predict the death rate.

The paper by Dai *et al*[9], which was published in *Cancer Discovery*, was the fifth most cited article. The study was carried out to compare COVID-19 cancer patients *vs* non-cancer patients and their susceptibility to COVID-19. The risk of serious outcomes, including admission to the intensive care unit, developing serious symptoms, invasive ventilation, or death, was higher in cancer patients than in non-cancer cases. Hematologic malignancies, lung cancer, and metastatic tumors were the most frequent types of cancer to have such events.

The paper by Yu *et al*[50], which was published in *JAMA Oncology*, was the sixth most cited article. According to this study, which was conducted in one center in China, the risk of contracting COVID-19 among oncology patients was found to be 0.79%. In addition, the subgroup analysis revealed a greater rate of SARS-CoV-2 infection in non-small cell lung cancer patients over 60 years old compared to those under 60 years.

The seventh most cited article was by Maringe *et al*[48] and published in the *Lancet Oncology*. According to this study, the COVID-19 pandemic in the UK is predicted to significantly increase the number of preventable cancer deaths in England. The COVID-19 pandemic is predicted to impact cancer patients significantly, and urgent policy initiatives are needed to address the backlog in regular diagnostic services. The paper by Lee *et al*[10], which was published in the *Lancet*, was the eighth-most cited article. The outcomes of this study revealed a high mortality rate among COVID-19 patients with

active malignancy (28%). The mortality rate was significantly associated with old age, male gender, and other diseases. However, receiving anticancer treatment within four weeks of being diagnosed with SARS-CoV-2 infection was not related to the mortality rate.

The paper by Mehta *et al*[11], which was published in *Cancer Discovery*, was the ninth most cited article. This study reported a mortality rate of 28% (61/218) among COVID-19 cancer patients, which were distributed as 20 deaths of blood cancer (37%) and 41 of solid cancer (25%). The predictors of mortality were advanced age, presence of other medical conditions, a high level of inflammatory markers, and admission to the intensive care unit.

The tenth most cited article was by Feldmann *et al*[51] and published in the *Lancet*. This study revealed that tumor necrosis factor (TNF) is considered one of the main targeted therapies for certain inflammatory diseases, such as rheumatoid arthritis. Importantly, COVID-19 involves an inflammatory process with a role for TNF, indicating a possible benefit of using anti-TNF agents in COVID-19 patients. Moreover, no adverse outcome was found in COVID-19 patients who used anti-TNF therapy. Thus, there is an urgent need for clinical trials of anti-TNF treatment targeting COVID-19 patients.

Strengths and limitations

Publications in cancer and COVID-19-related literature were assessed and analyzed comprehensively and objectively using the largest abstract and citation database containing peer-reviewed research. Although this is the first bibliometric investigation of COVID-19 in the field of oncology, there are certain limitations: (1) The search was conducted on June 21, 2022, and included all documents from January 1, 2020, up to June 21, 2022, but the Scopus database would have been open for new documents from 2022, so this part was omitted; (2) Only publications containing the terms related to cancer in the title were retrieved; and (3) As the search was limited to Scopus indexed journals, a few publications not included in the Scopus database were missed. Other bibliometric studies have also noted some limitations[35,66,67].

CONCLUSION

In conclusion, this is the first bibliometric analysis to determine the present state and upcoming hot themes related to cancer and COVID-19 and vice versa using VOSviewer during the early stages of the pandemic. The top five most productive countries reporting high research on cancer and COVID-19-related literature are the United States, Italy, the United Kingdom, China, and India. In terms of publications in this discipline, the *University of Texas MD Anderson Cancer Center* and *the Memorial Sloan-Kettering Cancer Center* are the most prolific institutions. The results of the present bibliometric analysis revealed that most hot research topics have evaluated "cancer care management during the COVID-19 pandemic", and "COVID-19 vaccines in cancer patients". The emergence of hot themes related to cancer and COVID-19 may aid researchers in identifying new research areas in this field.

ARTICLE HIGHLIGHTS

Research background

In comparison to the general population, cancer patients with coronavirus disease 2019 (COVID-19) have a mortality rate that is two times higher.

Research motivation

Despite the fact that numerous bibliometric studies have been carried out to assess COVID-19 research across the globe, there are few studies that have focused on COVID-19 literature and cancer research.

Research objectives

Through visual and bibliometric analysis, this study aimed to thoroughly examine the current state of publications on COVID-19 in the field of cancer.

Research methods

The Scopus database was searched to identify publishing output data. To identify research hotspots related to cancer and COVID-19, this study used VOSviewer to analyze international collaboration networks and evaluate the terms most frequently used in the titles and abstracts of the articles retrieved. The *Impact Index Per Article* is shown for the top 10 highly cited publications gathered *via* Reference Citation Analysis (RCA).

Research results

The results of the present bibliometric analysis revealed that most hot research topics have evaluated

"cancer care management during the COVID-19 pandemic", and "COVID-19 vaccines in cancer patients".

Research conclusions

Based on a current review of hot topics and research patterns, the findings of this study may help researchers uncover new research areas in the field of cancer and COVID-19.

Research perspectives

For oncologists, clinicians and virologists, this study aims to be a valuable resource and guide for research on emerging COVID-19 in the field of cancer to generate novel ideas for effective control measures and to outline COVID-19 vaccine guidance for cancer patients in the most timely manner.

FOOTNOTES

Author contributions: Zyoud SH designed the study, collected the data, analyzed the data, made major contributions to the manuscript's existing literature search and interpretation, and drafted the manuscript; Koni A, Al-Jabi S, Amer R, Shakhshir M, Al subu R, Salameh H, Odeh R, Musleh S, Abushamma F, and Abu Taha A were involved in interpretation of the data, and made revisions to the initial draft; all authors provided a critical review and approved the final manuscript before submission.

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CASE REPORT

Ascending colon cancer and situs inversus totalis – altered surgeon position for successful laparoscopic hemicolectomy: A case report

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Abstract

BACKGROUND

Situs inversus totalis (SIT) is a rare congenital condition in which the structure of the abdominal and thoracic cavities is the mirror image of normal. This anatomic reversal makes laparoscopic surgery difficult when treating colorectal cancer.

CASE SUMMARY

We describe the successful laparoscopic hemicolectomy of a 68-year-old Chinese woman with SIT and ascending colon cancer. Based on preoperative imaging and careful consideration of the patient's anatomy, the position of the surgeon was modified such that the surgeon stood between her legs, while the surgical assistant and endoscopist stood to the surgeon's left. Trocar position was also adjusted appropriately. The surgery lasted 178 min, during which the patient lost 50 mL of blood. Pathology analysis of the resected tumor confirmed an adenocarcinoma in clinical stage pT3N0M0, without lymph node involvement. The patient experienced no postoperative complications and was discharged 10 d after surgery.

CONCLUSION

This case illustrates that careful positioning of the surgeon can facilitate laparoscopic surgery of SIT patients.

Key Words: Colon cancer; Situs inversus totalis; Laparoscopic surgery; Case report

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Core Tip: Situs inversus totalis (SIT) is a rare congenital anomaly in which the organs in the chest and abdomen are located in a mirror image reversal of their normal positions. We present a rare case of SIT accompanied by colon cancer. After careful consideration of the patient's anatomy, we modified the position of the surgeon to enable successful laparoscopic hemicolectomy. This case highlights that careful positioning of the surgeon can make laparoscopic surgery feasible and safe for SIT patients.

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INTRODUCTION

Situs inversus totalis (SIT) is a rare congenital anomaly in which organs in the chest and abdomen are positioned in the mirror image of normal. Incidence in the general population ranges from 1 per 8000 to 1 per 25000[1], and SIT patients with colon cancer are even rarer. Surgery in SIT patients, particularly laparoscopic procedures, are considered more difficult because of the anatomical abnormality[2,3].

Here, we report the case of a 68-year-old patient with SIT and ascending colon cancer who successfully underwent laparoscopic hemicolectomy with radical lymph node dissection. The success of the procedure was due to careful consideration of the patient's anatomy and optimization of the surgical team's position.

CASE PRESENTATION

Chief complaints

A 68-year-old Chinese woman visited our hospital in December 2020 due to gradual enlargement of a mass in the left lower abdomen.

History of present illness

The patient had experienced intermittent bloody stool for nearly 1 year.

History of past illness

The patient did not have any history of past illnesses.

Personal and family history

The patient had no remarkable personal or family history.

Physical examination

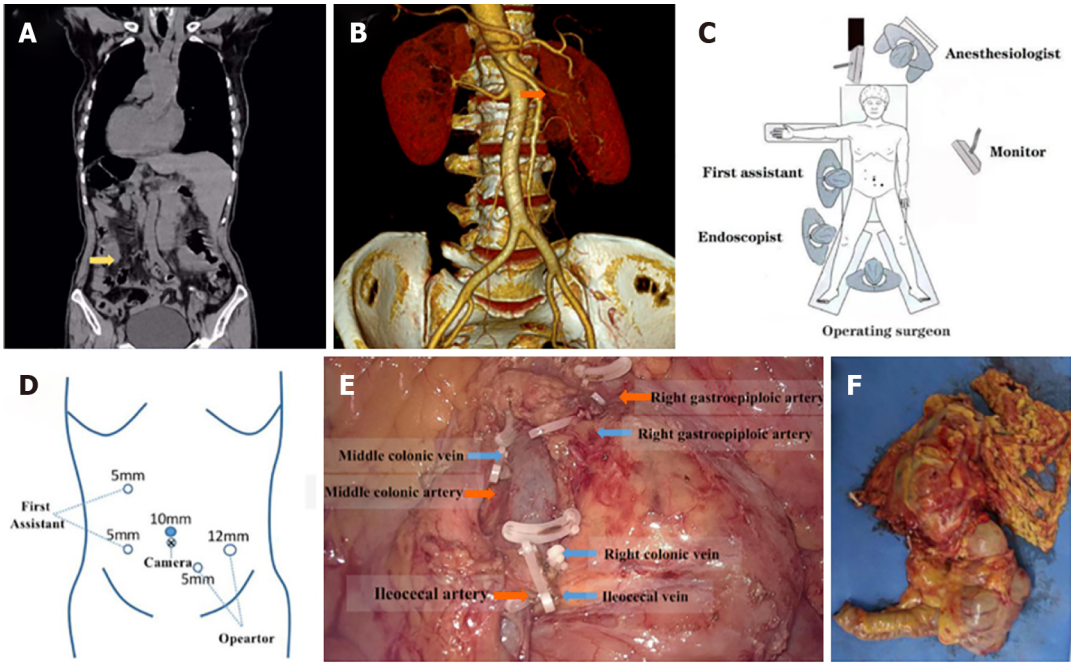
The patient was 142 cm tall and weighed 35 kg, corresponding to a body mass index of 17.4 kg/m^2. Physical examination revealed a mass measuring 4 cm × 5 cm in the left lower abdomen.

Laboratory examinations

Laboratory tests indicated no anemia, normal electrolytes, and no dysfunction of the liver or kidneys. The level of carcinoembryonic antigen in serum was slightly elevated (8.37 ng/mL; normal, < 2.5 ng/mL), while levels of carbohydrate antigen (CA) 12-5 and CA 19-9 were normal.

Imaging examinations

Chest and abdominal computed tomography (CT) revealed that the structure of the thoracic cavity and all abdominal organs were inverted from the normal position, leading to a diagnosis of SIT (Figure 1A). A mass in the ascending colon was confirmed (Figure 1A), and no evidence of distant metastasis was found. CT angiography showed that the superior mesenteric artery was located on the left side (Figure 1B). Colonoscopy revealed a mass in the ascending colon that occupied the complete diameter of the lumen, which together with intestinal stenosis prevented the passage of the colonoscope.



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Figure 1 Images of computed tomography and the surgery. A: Computed tomography (CT) showed complete transposition of the thoracic and abdominal viscera. The ascending colon tumor is marked with a orange arrow; B: Three-dimensional CT angiography of the superior mesenteric artery on the left side; C: Schematic of the surgical procedure; D: Positioning of the trocars; E: Exposure of blood vessels during operation; F: The excised tumor mass.

FINAL DIAGNOSIS

The patient was diagnosed with colon cancer and SIT.

TREATMENT

Laparoscopic hemicolectomy with radical lymphadenectomy was performed under general anesthesia. The patient was placed in a modified lithotomy position, with her head down and legs apart. The surgeon stood between her legs, and the first assistant and endoscopist stood on the surgeon's left, which is the opposite of the usual position for surgery (Figure 1C). The trocar placement was adjusted in order to facilitate surgical procedures (Figure 1D). The ileocolic vessels were carefully dissected, then the colon was dissected and reconstructed uneventfully (Figure 1E).

Pathology of resected tumor tissue revealed it to be moderately differentiated adenocarcinoma in stage pT3N0M0 involving invasion of the serosa (Figure 1F). All 22 resected regional lymph nodes were negative.

OUTCOME AND FOLLOW-UP

The entire surgery lasted 178 min, during which total blood loss was 50 mL. After surgery, the patient received six courses of chemotherapy with oxaliplatin and capecitabine, which proceeded uneventfully. At 12-mo follow-up, the patient reported being in good condition, and no symptoms or recurrence were noted.

DISCUSSION

SIT may arise from inherited or spontaneous genetic mutations that affect embryonic development[4]. Organ function is normal in most patients with SIT, and there are no obvious clinical symptoms, so most are diagnosed with the condition on the basis of X-ray imaging, ultrasonography, magnetic resonance imaging, or CT as in the present case. SIT can lead to misdiagnosis of colon cancer because the cancer manifests as the mirror opposite of the typical manifestations of obstruction, constipation, and diarrhea in the case of left colon cancer, or of anemia, weight loss, and fatigue in the case of right colon cancer.

Gastroscopy, colonoscopy, and CT are recommended to avoid misdiagnosis of cancer patients with SIT. As reported for other SIT patients[5], colonoscopy was successful in our patient, who was in the right decubitus position.

The success of treatment in the present case was due to the clinical team's experience and a clear understanding of the patient's anatomy, leading the team to adjust their normal positions for surgery. The team also remained flexible during the procedure in order to adapt to last-minute discoveries of vascular anomalies. Both CT angiography and CT colonography are useful for investigating anatomy and planning laparoscopic procedures[3,6,7]. Laparoscopic surgery, which is increasingly applied to a broad range of patients[8], can be a good option for SIT patients, following appropriate planning based on careful imaging[6,9-12]. While laparoscopic procedures on SIT patients can be more challenging for right-handed surgeons than for left-handed ones[13], adjusting the surgeon's position can help compensate for this[6].

Several adjustments to the laparoscopic procedures were made to compensate for our patient's SIT. The position of the surgical team was reversed from normal, and the trocar positions were correspondingly different, similar to those used to treat colorectal cancer on the left side. The surgeon in our case was right-handed, so he ligated the ileocolic vessels and mobilized the ascending colon using the right hand through a 12-mm trocar in the right lower quadrant. In this way, the surgeon compensated for the normal requirement to use the left hand during laparoscopic right hemicolectomy. In fact, the surgeon and his associates were able to complete the procedures smoothly despite the limited operating space due to the patient's small stature.

Our case report highlights that with careful preoperative imaging and planning, the surgical team can adjust their positions around the patient and the placement of trocars accordingly, allowing a safe and effective procedure. In this way, SIT patients with cancer can benefit from the minimal invasiveness of laparoscopic surgery like patients with normal anatomy.

CONCLUSION

Our case describes the successful laparoscopic hemicolectomy and radical lymphadenectomy of a SIT patient with ascending colon cancer. It highlights the importance of careful imaging assessment and preoperative planning, with the corresponding optimization of the surgical team's positioning around the patient. Laparoscopic surgery of SIT patients can be challenging but it remains a safe and effective minimally invasive option if appropriate steps are taken.

FOOTNOTES

Author contributions: Hu J, Wu K, and Li Q treated the patient, reviewed the literature, and contributed to manuscript drafting; All authors approved the final version.

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CASE REPORT

Mucinous adenocarcinoma arising from a tailgut cyst: A case report

Petra Malliou, Antonia Syrnioti, Triantafyllia Koletsa, Eleni Karlafti, Anestis Karakatsanis, Georgia Raptou, Stylianos Apostolidis, Antonios Michalopoulos, Daniel Paramythiotis

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Abstract

BACKGROUND

Retrorectal hamartomas or tailgut cysts (TCs) are rare. In most cases, they are asymptomatic and benign; however, rarely, they undergo malignant transformation, mainly in the form of adenocarcinoma.

CASE SUMMARY

A 55-year-old woman presented to our hospital with lower back pain. On magnetic resonance imaging, a large pelvic mass was found, which was located on the right of the ischiorectal fossa, extending to the minor pelvis. The patient underwent extensive surgical resection of the lesion through the right buttock. Histological examination confirmed the diagnosis of a retrorectal mucinous adenocarcinoma originating from a TC. Surgical resection of the tumour was complete, and the patient recovered without complications. The pilonidal sinus was then excised. One year later, semi-annual positron emission tomography-computed tomography and magnetic resonance imaging scans did not reveal any evidence of local recurrence or metastatic disease.

CONCLUSION

Preoperative recognition, histological diagnosis, and treatment of TCs pose significant challenges. In addition, the possibility of developing invasive mucinous adenocarcinoma, although rare, should be considered.

Key Words: Retrorectal tumour; Mucinous adenocarcinoma; Tailgut cyst; Mucosal tumour; Pilonidal cyst; Case report

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Core Tip: Retrorectal hamartomas or tailgut cysts are extremely rare. In certain cases, they undergo malignant transformation, predominantly in the form of adenocarcinomas. Mucinous adenocarcinomas are rare forms of carcinoma arising from tailgut cysts, with only 18 cases reported in the literature from 1988 to 2021. Furthermore, to our knowledge, coexistence of a pilonidal tract and mucinous adenocarcinoma is extremely rare; this being the second reported case in the literature. We present the case of a 55-year-old woman with a large pelvic mass on the right of the ischiorectal fossa and a pilonidal cyst. Surgical resection of the tumour and cyst was completed and the patient recovered well.

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INTRODUCTION

Retrorectal hamartomas or tailgut cysts (TCs) are very rare, with an incidence rate of approximately 1/40000[1]. TCs are believed to be embryologically derived from a remnant of the posterior intestine[2]. Alternative terminologies such as 'cyst of postanal intestine', 'retrorectal cystic hamartoma', 'tailgut vestiges', 'myoepithelial hamartoma of the rectum', and 'rectal cyst' have been used in the literature to describe these lesions[3]. These tumours are thin-walled, multi-layered structures lined by various glandular or transitional epithelia[4].

TCs occur more often in middle-aged women, whereas they are rare in children[5]. Forty percent of TCs occurring in children and new-borns are teratomas. Moreover, 10% of teratomas coexist with developmental disorders of the midline such as encephalocele[2,5]. In this age group, tumours could be benign, whereas malignant tumours are more common in older children[5]. Most TCs in adults are benign; however, malignant transformation has been reported in the literature, particularly in symptomatic cases[6].

TCs are mostly asymptomatic prior to clinical recognition. Symptoms are often associated either with a growing tumour mass and may include lower abdominal pain, rectal tenesmus and constipation or with infectious complications, even including fistulas[5].

Considering the rarity of this developmental anomaly, we present an interesting case of invasive mucinous adenocarcinoma originating from a TC associated with a pilonidal cyst that was managed in the Emergency Surgical Department of University Hospital.

CASE PRESENTATION

Chief complaints

A 55-year-old woman presented to the Emergency Surgical Department of the University Hospital with lower back pain.

History of present illness

The patient complained of pain in the previous 6 mo.

History of past illness

The patient had a history of ductal breast cancer, which was diagnosed 10 years ago and treated with lobectomy and adjuvant therapy. She also underwent hip arthroplasty 1.5 years ago and was under no medication and in good physical condition with good nutrition, according to her age.

Personal and family history

No pathological conditions were found.

Physical examination

The arterial blood pressure was 130/85 mmHg, temperature was 36.7 °C, and oxygen saturation level was 98%. Physical examination revealed a large, palpable gluteal mass.

Laboratory examinations

On her admission to our department, the routine laboratory test and carcinoembryonic antigen (CEA) results were within normal limits.

Imaging examinations

Magnetic resonance imaging (MRI) of the pelvis (Figure 1) revealed a pelvic mass that was located to the right of the rectus fossa, in contact with the uterus and rectum, which seemed to apply pressure on the adjacent structures and possibly on the sciatic nerve, and extended to the minor pelvis. The dimensions of the mass were 11 cm × 10 cm and 6 cm × 16.2 cm, and neural derivation was initially suspected.

Chest X-ray and computed tomography (CT) scans showed no abnormal findings. The abdominal CT scan revealed a large multifaceted formation located on the right side of the rectum, between the urinary bladder and coccyx and up to the fatty tissue of the buttocks, with enriched diaphragms. The appendix and ovaries were normal.

FINAL DIAGNOSIS

The CT findings raised the suspicion of a TC or cystic teratoma (Figure 1B and D).

TREATMENT

The patient underwent extensive surgical resection of the lesion through the right buttock (Figure 2).

An incision approximately 20 cm long was made, and sharp dissection was performed to carry the incision down directly into the midline until the presacral fascia was found. The medial gluteal fibres were then divided bilaterally to expose the attached mass which pushed the rectum and uterus away without infiltrating these structures. During dissection, it was crucial to avoid injury to the rectal wall, vagina, sciatic nerve, and urethra. This was facilitated by the use of rectoscopy during surgery, along with preoperative bowel preparation. A Foley catheter was used as a guide for the urethra. The lesion was resected, and the gluteal muscles were returned to the midline. The remaining layers of the incision were reapproximated and closed. Simultaneously, a pilonidal sinus was found and removed.

Preoperative planning concerned proper positioning of the patient. Lithotomy positioning was preferred because of the direct approach to the mass, rectum, and vagina and the potential need for a combined transabdominal incision.

Concerns were also raised about the contingent need for other specialists such as gynaecologists and urologists if the lesion was found to infiltrate the vagina or urinary tract. On that ground, these specialists stood by during the surgery.

The recuperation of the patient was uneventful, and she was discharged from the hospital on the seventh post-operative day because of delayed bowel movement.

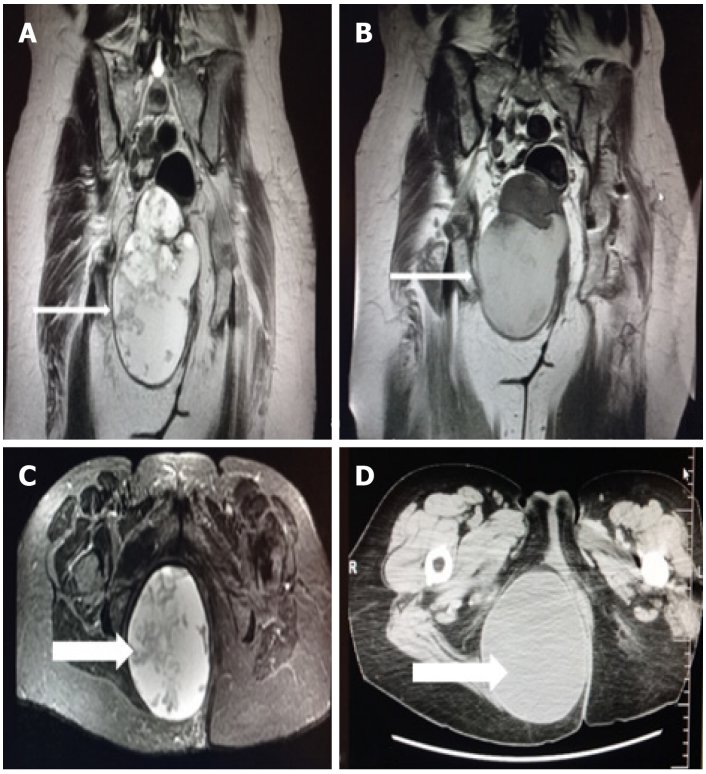
Wound care was performed as usual, and the skin sutures were removed 2 wk later, without any complications.

Both the mass and the pilonidal sinus were sent separately for histopathological examination. Upon grossing, a large mass was found to be cystic and filled with mucohaemorrhagic material. In a peripheral location, two smaller cystic spaces were identified, which were also filled with mucus and an amorphous material. Microscopic examination confirmed the presence of a cystic mass comprising thick fibrous bands that divided it into three cystic spaces, the largest of which corresponded to mucinous adenocarcinoma (Figure 3A). The neoplastic cells were medium to large in size, with roundish or irregular hyperchromatic atypical nuclei surrounded by an eosinophilic or pale cytoplasm (Figure 3B). Few "signet ring" cells were also observed. Tumour cells were arranged in glandular or cribriform structures, trabeculae, variably sized solid groups, and within large "lakes" of mucin. Rarely, isolated neoplastic cells floating in the mucin were identified. A large number of mitoses was observed. Regions of tumour necrosis and calcification were also observed. On immunohistochemical evaluation, the neoplastic cells exhibited the following immunophenotypes: CK20+ (Figure 3C), CDX2+, CK7+, GATA3-, ER-, PR-, and calretinin-.

Most current and similar published cases reported positivity, even partially, of TC or adenocarcinoma arising on the cyst to CK7 antibody.

Embryologically, the rectum is the last part of the tailgut, and both normal rectal mucosa and rectal adenocarcinomas present CK7 positivity in almost one-fifth of cases[7].

HER2 immunostaining showed faint, segmental, and membranous positivity in a small number of tumour cells (HER2 1+). The two other cystic spaces were lined with keratinising squamous or pseudostratified ciliated columnar or metaplastic squamous epithelia (Figure 3E). The mass was circumscribed with bundles of connective tissue at the periphery, and the surgical margins were tumour-free. Based on these findings, the diagnosis of an invasive mucinous adenocarcinoma, possibly on the grounds of the presence of a posterior rectal cyst sinus (TC), was established.



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Figure 1 Magnetic resonance imaging. A and C: Coronal and axial planes of the mass with smooth borders, lobed on the upper side with a beak sign. Cystic and solid elements, septa, and haemorrhagic and protein elements. It absorbs paramagnetic substance; B and D: Computed tomography scan - Coronal and axial planes of the mass. Differential diagnosis of tail gut cyst or cystic teratoma (arrows).

Gross examination of the sacrococcygeal pilonidal cyst revealed an elliptical skin-excision specimen. On the skin's surface, a hole measuring 0.1 cm in the greatest diameter was identified, which upon parallel sectioning was found to be continuous with a sinus tract that terminated in a brownish grey-coloured area. Microscopic examination revealed that the sinus tract was lined mainly by stratified squamous epithelium and partially by granulation tissue. Hair shafts were also focally identified around the sinus tract (Figure 3F). The latter extended to the deep tissue resection margin. No communication between the sinus tract and TC was found, albeit multiple sections.

OUTCOME AND FOLLOW-UP

The recuperation of the patient was uneventful, and she was discharged from the hospital on seventh post-operative day. According to the histopathology report, the oncology council recommended 22 sessions of radiotherapy while the patient completed the treatment. After 1 year, follow-up of the patient with semi-annual positron emission tomography-CT and MRI, did not show any evidence of local or metastatic recurrent disease.

DISCUSSION

Herein, we present an interesting case of mucinous adenocarcinoma arising on a TC. Mucinous adenocarcinoma is a rare type of carcinoma occurring on TCs, with only 18 cases reported in the English literature from 1988 to 2021. Furthermore, to our knowledge, coexistence of a pilonidal tract is extremely rare, this being the second reported case in the literature. A connection between the pilonidal sinus and TC was not established using imaging, intraoperatively, or on pathological examination.

Primary retrorectal tumours include congenital (55%-65% of all tumours in this region), neurogenic (10%-12%), osteogenic (5%-11%), inflammatory (5%), and other tumour types (5%-11%). According to their embryonic origin, cysts are classified into epidermal, dermal, neural, teratoma, enteric, rectal duplication, mucous-secreting, enterogenous, simplex, gland anal, rectal, hamartoma, and TCs[5,8]. TCs are found in the presacral space and are typically thin-walled cysts that may be single or multiloculated, branched, and may contain green opalescent colloid fluid[2].



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Figure 2 Patient underwent extensive surgical resection of the lesion through the right buttock. A: Preoperative view of the mass (arrow); B and C: Extensive surgical resection of the lesion through the right buttock; D: Removed mass.

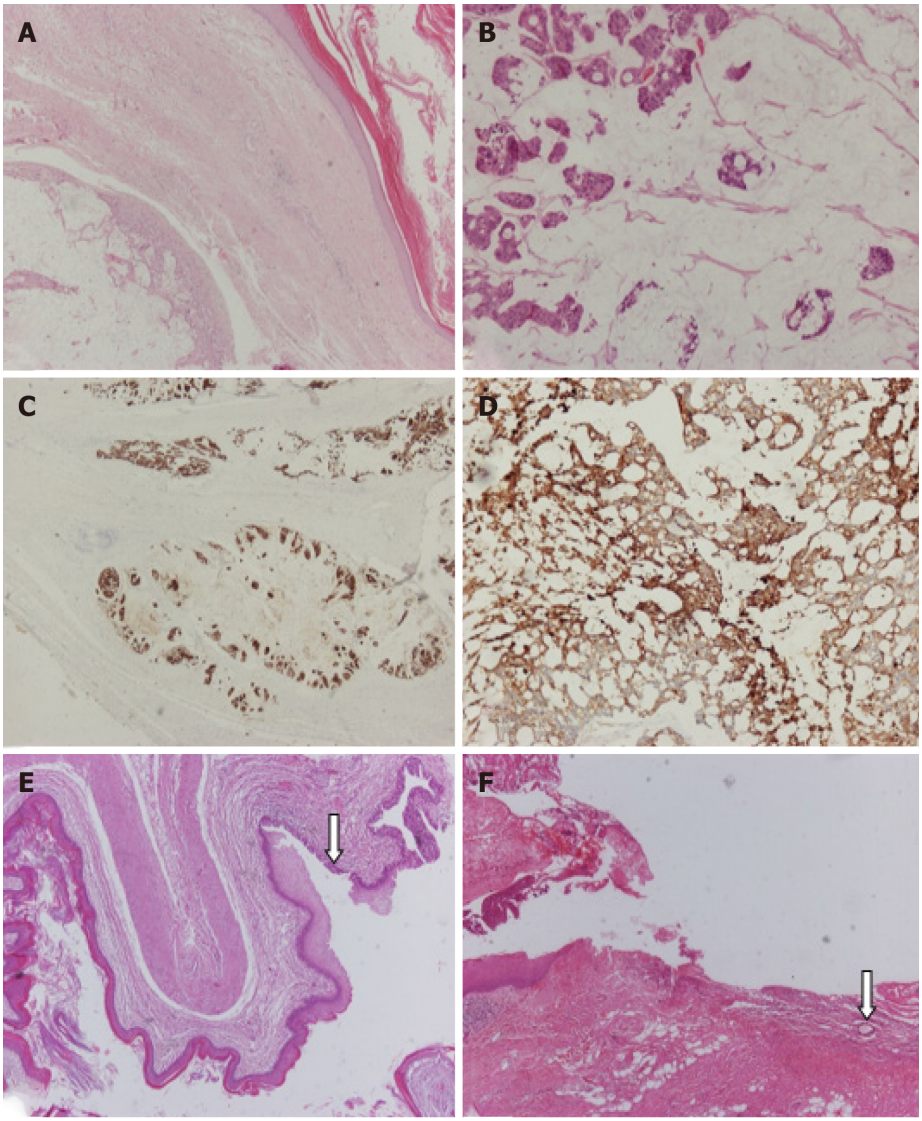
In 1885, Middeldorpf *et al*[9] reported the first case of a cystic mass in the retrorectal space in a 1-year-old girl, which was most likely a rectal duplication cyst. Hjermstad and Helwig reported the largest case series of TCs, which included 53 patients with an age range of 4 days to 73 years and average age of 36 years[10]. Based on the current literature, TCs may be asymptomatic or present with non-specific symptoms owing to the large size of the pelvic mass[2,5,8]. They can also lead to several complications including a neurogenic bladder, haemorrhage, faecal incontinence, faecal fistula, intestinal obstruction, infections, or malignant transformation as observed in the present case[11,12].

The diagnosis of TCs may be delayed because of the absence of typical symptoms[6]. Often, TCs are discovered incidentally through imaging tests during the investigation of other entities[5]. A CT scan typically shows a well-defined homogeneous retrorectal mass of water to soft-tissue density)[12]. A more solid appearance could also be described because of the keratinous or inflammatory debris within a cyst[3,5]. Higher-resolution scans may identify most TCs as multiloculated cysts[3]. On T1-weighted images, MRI scans reveal a hypointense lesion, whereas lesions are homogeneously hyperintense on T2-weighted images. However, MRI is not the gold standard for discriminating benign and malignant lesions[3].

A definitive diagnosis is established by histopathological examination[14]. TCs are congenital lesions that develop from the residual posterior remnant of the intestine, which retains its structure and architecture regarding the mature ectodermal, endodermal, and mesodermal tissue elements. The lining epithelia may vary, including squamous, ciliated columnar, pseudostratified, columnar, transitional, goblet columnar, and cuboidal epithelia[8,14]. Additionally, it is characterised by the presence of a smooth muscle layer and connective tissue, which may be disarrayed and do not encompass the nerve plexus or differentiated neuronal cells[14]. The immunophenotype of the mucinous adenocarcinoma in this case was that described in similar previously reported cases and is characterised by CK7, CK20, and CDX-2 positivity[15].

Most TCs are benign; nevertheless, rare cases of malignancies have been reported, including the present case[6]. Apart from adenocarcinomas, neuroendocrine, endometrioid, adenosquamous, and squamous cell carcinomas and sarcomas have also been described[13]. Although the option of needle biopsy seems attractive, it is not broadly recommended because of the possibility of false-negative results and the risk of tumour seeding[16].

Once a presacral tumour is diagnosed, the treatment of choice is extensive surgical removal due to the possibility of malignant transformation. The surgical approach depends on tumour location. Complete excision could be achieved with a posterior approach for tumours extending below the sacral spinal nerve 4 (S4), which is effective at a rate of 95%. For tumours that extend above S4, the abdominal or



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Figure 3 Microscopic examination confirmed the presence of a cystic mass that comprised thick fibrous bands that divided it into three cystic spaces, the largest of which corresponded to mucinous adenocarcinoma. A: Fibrous tissue separates two cystic spaces, one benign lined by keratinized squamous cell epithelium and the other corresponding to mucinous adenocarcinoma; B-D: Higher magnification of mucinous adenocarcinoma that is immunohistochemically positive (IHC-positive) for antibodies CK20 and CK7; E and F: A smaller cystic space with fibromuscular wall lined by keratinized squamous epithelium and partially by pseudostratified ciliated columnar epithelium (arrow) is observed. Section of the pilonidal cyst (arrow: Hair shaft). [A: Hematoxylin-eosin staining (HE), magnification × 40; B: HE × 100; C: IHC × 20; D: IHC × 100; E and F: HE × 40].

abdominal-perineal approach is suggested[16]. When TC is malignant, many studies suggest that treatment should include adjuvant radiation therapy alone or in combination with chemotherapy[6,17-19]. Martins *et al*[20] suggest both radiation therapy and chemotherapy. Liang *et al*[6] argue that the mainstream treatment method for TCs with adenocarcinoma is surgical resection followed by chemotherapy. Baverez *et al*[21] suggest that neoadjuvant chemoradiotherapy, similar to locally advanced rectal adenocarcinoma, decreases the risk of post-operative recurrence. Supplemental treatment can be administered as it is believed to contribute to the prevention of tumour recurrence. However, there is no clear evidence that it would improve the prognosis as there is no general consensus on treatment standards for TC-associated adenocarcinoma owing to its very low incidence rate[22]. Factors that determine prognosis include the stage during diagnosis, tumour histology and grade, and completeness of resection[3]. Compared with neuroendocrine tumours, adenocarcinomas arising from TCs have a poorer prognosis and carry a risk of local recurrence and metastasis[8]. Follow-up of the patient is also recommended, including monitoring for signs of recurrence with periodic positron emission tomography-CT and MRI scans in addition to serum CEA levels that serve as an indicator of the tumour's response to treatment and development of recurrence[19]. According to Chhabra *et al*[3], once a TC malignancy has been diagnosed and is associated with an elevated CEA level, CEA levels may be used as a simple measure to assess the tumour's response to treatment or development of recurrence. In our case, the patient did not have elevated CEA levels; therefore, this

measure was not monitored after surgery. Di Nuzzo *et al*[23] reported the use of combined MRI and endoscopy for post-operative follow-up.

CONCLUSION

TCs are rare clinical and pathological entities. The novelties of this case include the presence of a mucinous adenocarcinoma arising from a TC and that it is the second reported case of an association between TC and pilonidal cyst. Generally, TCs constitute both diagnostic and treatment challenges. Imaging tests may be helpful; however, a definitive diagnosis is usually established after complete surgical excision and histopathological examination. Guidelines for appropriate therapeutic management are required for TC-associated adenocarcinomas, although timely and extensive surgical resection along with adjuvant radiation therapy with or without chemotherapy have been used with good outcomes.

FOOTNOTES

Author contributions: Malliou P, Apostolidis S, Michalopoulos A, and Paramythiotis D were the patient's surgeons, and reviewed the literature and contributed to manuscript drafting; Karlafti E reviewed the literature and contributed to manuscript drafting; Syrnioti A, Koletsa, and Raptou G performed the microscopic examination and contributed to manuscript drafting; all authors were responsible for the manuscript's revision for important intellectual content and issued final approval for this version to be submitted.

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LETTER TO THE EDITOR

Diagnostic biopsy of cutaneous melanoma, sentinel lymph node biopsy and indications for lymphadenectomy

Efstathios T Pavlidis, Theodoros E Pavlidis

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Abstract

The incidence of cutaneous melanoma appears to be increasing worldwide and this is attributed to solar radiation exposure. Early diagnosis is a challenging task. Any clinically suspected lesion must be assessed by complete diagnostic excision biopsy (margins 1-2 mm); however, there are other biopsy techniques that are less commonly used. Melanomas are characterized by Breslow thickness as thin (< 1 mm), intermediate (1-4 mm) and thick (> 4 mm). This thickness determines their biological behavior, therapy, prognosis and survival. If the biopsy is positive, a wide local excision (margins 1-2 cm) is finally performed. However, metastasis to regional lymph nodes is the most accurate prognostic determinant. Therefore, sentinel lymph node biopsy (SLNB) for diagnosed melanoma plays a pivotal role in the management strategy. Complete lymph node clearance has undoubted advantages and is recommended in all cases of positive SLN biopsy. A PET-CT (positron emission tomography-computed tomography) scan is necessary for staging and follow-up after treatment. Novel targeted therapies and immunotherapies have shown improved outcomes in advanced cases.

Key Words: Surgical oncology; Malignant melanoma; Skin cancer; Cutaneous melanoma; Sentinel lymph node biopsy; Complete lymph node dissection

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Core Tip: The value of excision biopsy for the initial diagnosis of melanoma in every suspected cutaneous lesion is important. In positive cases, the roles of sentinel node biopsy and subsequent complete lymph node dissection, along with adequate margin excision of the primary lesion site are evaluated to improve the prognosis. Novel biological agents and molecular factors will open new horizons for future management policy.

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TO THE EDITOR

We read with great interest the recent paper by Koumaki *et al*[1] and we would like to congratulate the authors for their excellent trial on melanoma and atypical mole syndrome, which impressed us. This study is meticulous and arduous work that describes, for the first time, many details about the demographic and clinical characteristics of 121 patients. We absolutely agree with the authors that photoprotection education is required to prevent skin cancer development. Taking this opportunity, this paper presents some thoughts and observations from a surgical point of view on the latest developments in biopsy for the diagnosis of suspected primary lesions and the role of sentinel lymph node biopsy and the subsequent prophylactic or therapeutic lymphadenectomy.

The incidence of cutaneous melanoma has steadily increased over the past years. It has been estimated that this increase in the United States has reached up to 3% per year. However, most cases with early-stage disease (I and II) usually have a favorable prognosis[2]. The eighth edition of the American Joint Committee on Cancer (AJCC) staging system is the most widely used standard for the staging and classification of melanoma[2-4]. Cutaneous lesions with macroscopic features that raise the suspicion of melanoma can be used as an alternative for changes in color, outline, bleeding, rapid increase in size, nodular growth and ulceration.

Biopsy and histological examination will initially confirm the diagnosis and determine the stage of the disease, the extent of surgical resection and the management of the sentinel lymph node (SLN). The types of biopsy might be excisional, incisional, shave biopsy (superficial or deep scallop) or punch biopsies[5]. The most preferred excisional biopsy is reliable for defining the T stage in TNM staging. It resects the lesion beyond its margins to an extent of 1-3 mm according to NCCN (National Comprehensive Cancer Network) guidelines or 1-2 mm according to AJCC guidelines. This limit is crucial, given that avoiding lymphatic destruction ensures feasible detection of sentinel lymph nodes[6-8]. The other types of biopsy can potentially lead to misdiagnosis and inaccurate staging. The incisional biopsy removes a small part of the lesion for cosmetic reasons. It is indicated for large lesions of more than 2 cm in diameter that are mainly located on the face.

In a positive biopsy of the initial evaluation of the suspected skin lesion, sentinel lymph node biopsy (SLNB) follows. This is because the involvement of regional lymph nodes is considered an important prognostic factor for survival. SLNB is indicated by the current data and 15% to 20% of patients have regional node metastasis[9]. In addition, the presence or absence of nodal micrometastases is the most important prognostic factor in early-stage melanoma, particularly in intermediate thickness melanoma [10]. Thus, SLNB is considered the standard of care and has high diagnostic value. It is a minimally invasive procedure with a low complication rate[9,11]. The detection of sentinel lymph nodes is performed either 24 h preoperatively by Tc-99 administration and the use of a gamma probe or intraoperatively by methylene blue administration. Moreover, their combination can be used. A positive SLNB results in a complete lymph node dissection (CLND). This process provides adequate regional disease control and has an indication for adjuvant chemotherapy[11]. A negative SLNB has a minimal likelihood of metastasis. The final CLND biopsy ensures accurate staging and prognosis. Furthermore, CT (computed tomography) and PET (positron emission tomography) scans contribute to staging by defining the M (distant metastasis)[12]. However, the prognosis is influenced by disease progression [13].

The incidence of nodal metastases clearly depends on the thickness of the primary melanoma. Lesions more than 1 mm in thickness are more likely to have metastases in the sentinel node, and lesions between 1 mm and 2 mm only have metastases in the sentinel node. However, lesions more than 2 mm in thickness have metastases in additional lymph nodes and distant metastases[9]. According to the excision biopsy, when the depth of invasion (Breslow thickness) is less than 1 mm, or from others, less than 0.75 mm, then the positive SLNB will be less than 5%. An exception to this rule is the mitotic index (≥ 1 mitoses/mm^2), especially in cases with a Breslow thickness between 0.75 mm and 0.99 mm. The rate of false-negative SLNB reached 1.5% to 4.1%[11].

In the case of early-stage (pT1b, pT2a) melanoma with sentinel node micrometastases, when the deposits are less than 0.3 mm in maximum diameter, no adjuvant treatment will be necessary. Otherwise, when they are equal to or more than 0.3 mm, adjuvant systemic therapy could be beneficial [14].

The final differential diagnosis between melanoma and dysplastic nevus is made by histopathology. A molecular assay would be of value for early-stage lesions, but thus far, there is no such test[15].

PET-CT has the greatest diagnostic accuracy both for staging and follow-up. However, for the latter, the currently used immunotherapy can create various organ side effects; thus, radiologists should be aware of this[12].

The dataset of dermoscopic images is a useful tool for the early detection of skin cancer[16]. Ultrasound-guided fine needle aspiration cytology (FNAC) and core needle biopsy (CNB) can be used for the detection of subcutaneous or lymph node metastases[17]. Melanomas can be diagnosed in early stages (50%). They are more commonly located on the extremities in women and on the back in men. On the lower limbs, they can be more invasive and are without sex differences[18].

Current recommendations indicate complete excision biopsy to avoid residual disease in the complementary resection after partial excision biopsy. However, this treatment does not influence survival[19]. A recent large, retrospective study found that SLNB was more likely to be indicated for a Breslow depth >1 mm or mitotic rate ≥ 1/mm^2. It was less likely to be indicated in patients of older age (> 75-years-old) and those without an extremity location[20].

The prognostic value of complete lymph node dissection (CLND) after positive SNDB *vs* observation and therapeutic lymph node dissection (TLND) has been evaluated[21], despite the initial aspect of a nonsignificant difference between them[22]. A large, retrospective study from Italy including 2086 patients after CLND for lymph node involvement found improved survival. The 3-year survival was 79%, the 5-year survival was 70%, and the 10-year survival was 54%[23]. The preliminary results indicated that the clinicopathologic information (thickness, mitoses, age, and Breslow thickness 2 mm) and gene expression profiling (CP-GEP) were independent predictive factors for lymphatic metastases [24]. Similarly, 31-gene expression profiling (i31-GEP-SLNB) has become commercially available[25]. A vitamin D level < 9.25 ng/mL is another negative independent prognostic factor for survival. It is associated with ulceration formation in melanoma[26].

A stage-based follow-up scheme has recently been proposed by the European consensus for melanoma[27].

Tilmanocept, a CD206 receptor-targeted novel radiotracer, has recently been introduced for lymphoscintigraphy to assess nodal mapping[28].

Adequate margin excision (1-2 cm, depending on the invasion depth) has been the standard therapy, despite the de-escalation of its extent, together with SLNB[10,29]. Targeted therapy and immunotherapy have further improved the prognosis[30].

In conclusion, SLNB is indicated for melanoma stage IB (T1b ≤ 1 mm, ulceration, and mitoses >1 mm^2) and stage II. In positive cases, CLND is required instead of TLND. SLNB offers staging accuracy and has indications for adjuvant therapy. Thus, it can improve prognosis and survival. New diagnostic modalities and immunotherapies will contribute further to improved outcomes.

FOOTNOTES

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REVIEW

Influence of *Helicobacter pylori* oncoprotein CagA in gastric cancer: A critical-reflective analysis

Fabrício Freire de Melo, Hanna Santos Marques, Samuel Luca Rocha Pinheiro, Fabian Fellipe Bueno Lemos, Marcel Silva Luz, Kádima Nayara Teixeira, Cláudio Lima Souza, Márcio Vasconcelos Oliveira

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Abstract

Gastric cancer is the fifth most common malignancy and third leading cancer-related cause of death worldwide. *Helicobacter pylori* is a Gram-negative bacterium that inhabits the gastric environment of 60.3% of the world's population and represents the main risk factor for the onset of gastric neoplasms. CagA is the most important virulence factor in *H. pylori*, and is a translocated oncoprotein that induces morphofunctional modifications in gastric epithelial cells and a chronic inflammatory response that increases the risk of developing precancerous lesions. Upon translocation and tyrosine phosphorylation, CagA moves to the cell membrane and acts as a pathological scaffold protein that simultaneously interacts with multiple intracellular signaling pathways, thereby disrupting cell proliferation, differentiation and apoptosis. All these alterations in cell biology increase the risk of damaged cells acquiring pro-oncogenic genetic changes. In this sense, once gastric cancer sets in, its perpetuation is independent of the presence of the oncoprotein, characterizing a "hit-and-run" carcinogenic mechanism. Therefore, this review aims to describe *H. pylori*- and CagA-related oncogenic mechanisms, to update readers and discuss the novelties and perspectives in this field.

Key Words: *Helicobacter pylori*; Virulence factors; CagA; Gastric cancer; EPIYA motifs; Hit-and-run carcinogenesis

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Core tip: CagA is a translocated effector protein that induces morphofunctional modifications in gastric epithelial cells and persistent chronic gastric inflammation. Upon translocation, the bacterial oncoprotein acts as a promiscuous scaffold or hub protein, which is capable of disrupting multiple host signaling pathways, thereby inducing precancerous cellular alterations. This review aims to describe *Helicobacter pylori*- and CagA-related oncogenic mechanisms, as well as to discuss the novelties and perspectives in this field.

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INTRODUCTION

The multiple virulence mechanisms of *Helicobacter pylori* confer an ability to colonize the hostile gastric environment[1]. This pathogen infects > 50% of the global population and is a major health concern due to the serious repercussions related to its colonization[2]. Among the diseases predisposed by *H. pylori* infection, gastric adenocarcinoma is the fifth most common malignancy and third leading cancer-related cause of death worldwide[3]. Of note, the close relationship between *H. pylori* and gastric cancer has led the World Health Organization International Agency for Research on Cancer Working Group to consider the bacterium as a class 1 carcinogen based on epidemiological evidence and biological plausibility[4].

Various virulence factors contribute to successful *H. pylori* colonization and pathogenicity[5]. Among these factors is *cagA*, a well-known gene that encodes an oncogenic protein that seems to be a determining agent in *H. pylori*-related gastric carcinogenesis[6]. CagA seropositivity, regardless of *H. pylori* status, is associated with increased gastric cancer risk. The *cagA* gene is located in the pathogenicity island *cag* (*cag*PAI), a nucleic acid sequence that encodes the type IV secretion system (T4SS), which is a bacterial apparatus that delivers the CagA protein and peptidoglycans into gastric epithelial cells. Inside host cells, that virulence factor suffers phosphorylation at a Glu-Pro-Ile-Tyr-Ala (EPIYA) motif, a variable C-terminal region and, subsequently, promotes the activation of the SH2-containing protein tyrosine phosphatase (SHP2)[7,8]. SHP2, in turn, triggers various mechanisms that lead to important cell changes, including alterations in cellular morphology through the disturbing of cell polarity, which leads to a "hummingbird" phenotype, as well as carcinogenesis-related changes in cytoskeleton[9].

Despite the extensive number of studies on the relationship between CagA and *H. pylori* infection, much still has to be done in order to better understand the role of this oncoprotein in gastric carcinogenesis. Recent investigations have explored multiple host–pathogen interactions in this setting and found other CagA-triggered pathways that probably influence cancer development[10]. Moreover, the action of small RNAs in CagA post-transcriptional regulation has been investigated, showing a broad field to be explored[11]. This review aims to describe *H. pylori*- and CagA-related oncogenic mechanisms, and to discuss the novelties and perspectives in this field.

PATHOGENESIS OF *H. PYLORI* GASTRIC INFECTION

The capacity to resist severe stomach acid conditions is a notable aspect of *H. pylori*. To provide successful colonization, the pathogen uses various mechanisms, such as enhanced motility, adherence to gastric epithelial cells, enzymatic machinery, and virulence factors[12]. Besides that, the host immune system also plays an essential role during the infection, mainly by a Th1 response against the bacteria [13].

The bacterial flagella are crucial for reaching the protective mucus layer at the exterior of the gastric mucosa. After entering the stomach, *H. pylori* uses its flagella for swimming in gastric content, allowing the pathogen to arrive at the mucus layer[14]. Some studies have shown that the ferric uptake regulator performs an important role in bacterial colonization, positively regulating the flagellar motility switch in *H. pylori* strain J99[15]. Another factor described as a motility modulator is HP0231, a Dsb-like protein. It cooperates in redox homeostasis and is fundamental for gastric establishment[16].

H. pylori also depends on chemotaxis for its colonization. The essential pathogen chemoreceptors are T1pA, B, C, D, a CheA kinase, a CheY responsive regulator, and numerous coupling proteins, playing a pivotal role in bacteria pathogenesis[17]. The aggregation of the coupling proteins CheW and CheV1 culminates in the formation of the CheA chemotaxis complex, activating CheA kinase and optimizing the chemotaxis function[18].

An ideal balance between nickel absorption and incorporation is indispensable for *H. pylori* colonization, since nickel is an essential metal for bacterial survival and infection[12,19]. This metal is a cofactor for two significant enzymes: urease and hydrogenase. Both of them have a role in gastric infection, contributing, respectively, to bacterial colonization and metabolism signaling cascade to produce energy[20,21].

Adherence and outer membrane gastric cell receptors are also relevant in bacterial pathogenesis. The blood group antigen binding adhesin (BabA) is the best-studied molecule of *H. pylori*[22]. This protein sequence affects acid sensitivity and plays a critical role in bacterial acid adaptation during infection [12]. Pathogens with high BabA expression levels have increased virulence, leading to duodenal cancer and gastric adenocarcinoma[23]. Another adhesin has been described: HopQ. This molecule binds to cell adhesion molecules related to the carcinoembryonic antigen (carcinoembryonic antigen-related cell adhesion molecules, CEACAMs) 1, 3, 5 and 6, promoting cell signaling guided by this interaction, allowing the translocation of the oncoprotein CagA, the most important *H. pylori* virulence factor, and rising proinflammatory mediators in the infected cells[24,25].

Additionally, besides CagA, a wide range of virulence factors like vacuolating cytotoxin A (VacA), DupA and OipA have been reported as determinant molecules for *H. pylori* pathogenicity[26]. VacA, whose gene is found in most bacterial strains, promotes the formation of acidic vacuoles in gastric epithelial cells and modulate the immune response, leading to an immune tolerance and enduring *H. pylori* infection, due to its role in the activity of T cells and antigen-presenting cells [27,28]. These VacA functions can lead to gastritis and duodenal ulcer and gastric cancer development. The bacterial protein DupA provides acid resistance to the pathogen, and seems to cooperate with the production of interleukin (IL)-8, enhancing its levels in the gastric mucosa[29]. The outer membrane protein OipA contributes to the adhesion and activation of IL-8 production, increasing inflammation. OipA is a significant virulence factor on the infection outcome, resulting in increased development of gastric cancer and peptic ulcers[30].

H. pylori infection produces complex host immune responses, through diverse immune mechanisms [31]. During the first contact with the bacteria, a wide range of antigens like lipoteichoic acid and other lipoproteins bind to stomach cell receptors, known as Toll-like receptors (TLRs)[32]. After this interaction, NF-B and c-Jun N-terminal kinase activation takes place, among the proinflammatory cytokine release as signaling pathways[33]. Neutrophils and mononuclear cells infiltrate the gastric surface, producing nitric oxide and reactive oxygen species (ROS), and recruiting $CD4^+$ and $CD8^+$ T cells [34]. Finally, a Th1-polarized response occurs, with enhanced levels of IFN-γ, IL-1β, Il-6, IL-7, IL-8, IL-10 and IL-18[35,36].

A correlation has been shown between Th17 cells, a proinflammatory subset of $CD4^+$ T cells, and their affiliated cytokines (IL-17A, IL-17F, IL-21, IL-22 and IL-26) in persistent *H. pylori*-mediated gastric inflammation and the subsequent gastric cancer development[36,37]. We have demonstrated in a previous study that an IL-17 T-cell response predominates in *H. pylori*-associated gastritis in adults, whereas, in children, there is predominance of a T regulatory (Treg) cell response. IL-17A is known to play an important role in the recruitment and activation of polymorphonuclear cells that are vital for *H. pylori* clearance. Treg and Th17 cells are mutually controlled. Therefore, these findings could explain the higher susceptibility of children to the infection and bacterial persistence[38].

IL-27 expression differs between *H. pylori*-related diseases. Accordingly, we have shown that there is a high expression of IL-27 in the gastric mucosa and serum of *H. pylori*-positive duodenal ulcer patients. In contrast, IL-27 is absent in the gastric mucosa and serum of patients with gastric cancer. Consistent with the immunosuppressive role of IL-27 in Th17 cells and IL-6 expression, we observed that expression of Th17-cell-associated cytokines was lower in the patients with duodenal ulcer, which secreted a large concentration of proinflammatory Th1 representative cytokines. We demonstrated that there is high levels of IL-1β, IL-6, IL-17A, IL-23, and transforming growth factor-β, involved in IL-17A expression, on the gastric mucosa and in the serum of gastric cancer patients. Therefore, IL-27 may be involved in the development of different patterns of *H. pylori*-induced gastritis and its progression. Taking into account that IL-27 has evident antitumor activity, our results point to a possible therapeutic use of IL-27 as an anticancer agent[39].

Recently, the role of the interaction between *H. pylori* infection and the gastrointestinal microbiome in gastric carcinogenesis has also been investigated. In this regard, researchers explored the diversity and composition of the gastric microbiome at different stages of disease, including normal gastric mucosa, chronic gastritis, atrophic gastritis, intestinal metaplasia, and gastric cancer[40]. A recent meta-analysis indicated that *H. pylori*-positive gastric samples exhibit reduced microbial diversity, altered microbial community, composition, and bacterial interactions. An increased abundance of opportunistic pathogens (*e.g.*, *Veillonella* and *Parvimonas*) was observed concomitantly with a decrease in putative probiotics (*e.g.*, *Bifidobacterium*) throughout the stages of disease progression[41]. Another study has suggested that successful eradication of *H. pylori* could reverse the tendency towards gastric microbiota

dysbiosis and show beneficial effects on the gastric microbiota[42]. The next step in this field could be to conduct a prospective, multicenter, crosscultural study to validate these results and explore the mechanisms underlying the *H. pylori*–gastric microbiota interaction and its role in gastric cancer development[43].

CAGA — *H. PYLORI* TRANSLOCATED ONCOPROTEIN

CagA is a translocated effector protein that induces morphofunctional modifications in gastric epithelial cells and inflammatory responses, whose balance allows successful colonization of the acidic stomach environment[44,45]. The CagA gene is located in the *cag*PAI; a 40-kb DNA fragment that contains about 31 genes and confers virulence to some strains of *H. pylori*. Some genes of the island encode proteins that form a T4SS, which is responsible for the translocation of CagA into the cytoplasm of gastric epithelial cells through interaction of bacterial and host cell components[46-48]. The oncoprotein has a molecular weight of 128–145 kDa and its tertiary structure is characterized by a structured N-terminal region, split into Domains I–III and an unstructured C-terminal tail[49].

Injection of CagA depends on the recognition of its N-terminal and C-terminal portions by T4SS, which can occur simultaneously or not[50]. However, several other mechanisms are also required for its inoculation. We highlight the binding between T4SS CagL and CagY to human β1-integrins[51,52], along with the CEACAMs and *H. pylori* outer membrane protein Q (HopQ) interaction, that allow the translocation of CagA into the host cells[53]. A recent study noted that the instability of the relationship between *H. pylori* HopQ and mouse CEACAMs may explain why the pathogenesis of infection in the stomach of rodents infected with CagA positive *H. pylori* strains occurs differently from infection by the same strain in humans. The authors also demonstrated that the presence of functional T4SS is associated with a time-dependent reduction in human CEACAM1 levels after CagA-positive *H. pylori* infection [54]. However, it is still unclear whether the instability of the HopQ–CEACAMs relationship favors the host or the bacterium, so more studies are needed to investigate the possibility of these interactions being beneficial in favor of the patient infected with *H. pylori*. BabA is apparently also capable of increasing T4SS activity[55].

CagA is known to possess multiple phosphorylation segments in its tertiary structure. The phosphorylation sites are denominated EPIYA sequences; regions consisting of five amino acids (Glu-Pro-Ile-Tyr-Ala) located in the C-terminal portion of the oncoprotein. Four different EPIYA segments have been described according to the different amino acid sequences surrounding each motif, designated EPIYA-A (32 amino acids), B (40 amino acids), C (34 amino acids) and D (42 amino acids). EPIYA-A and EPIYA-B motifs have been identified in almost all CagA-positive *H. pylori* strains, followed by one, two, or three EPIYA-C sequences in western strains, or an EPIYA-D segment in East Asian strains[56,57]. Figure 1A summarizes the main structural domain differences between East Asian and western CagA.

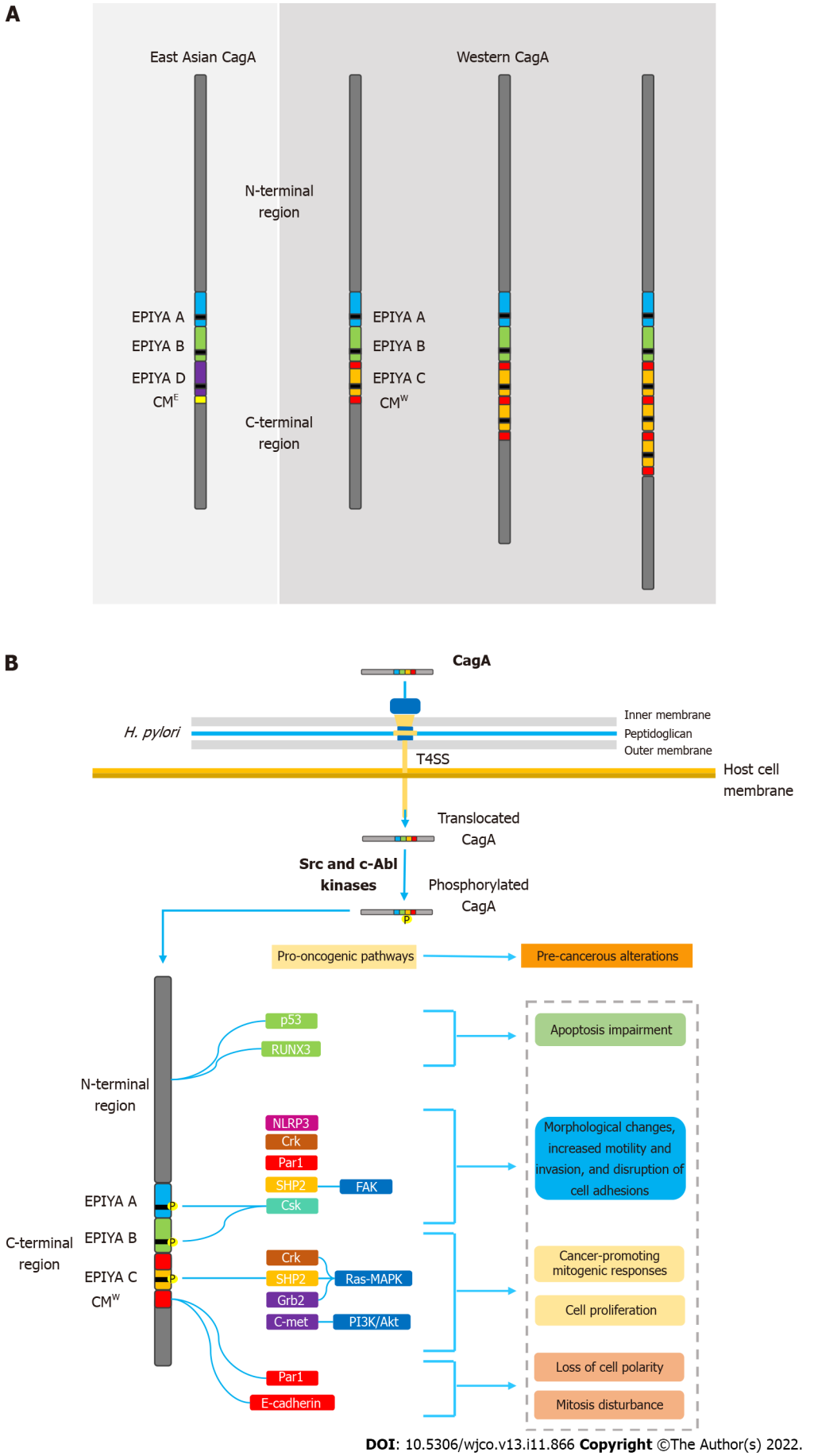
Once inside the gastric epithelial cells, the EPIYA segments are selectively tyrosine-phosphorylated by different kinases of the Src family (s-Src, Fyn, Lyn and Yes) or by Abl kinase of the host cells[58-61]. After the phosphorylation process, CagA moves to the cell membrane and acts as a promiscuous scaffold protein that simultaneously disturbs multiple signaling pathways, involved in the regulation of a large range of cellular processes, including proliferation, differentiation and apoptosis[62].

In this sense, a better understanding of the molecular structure of CagA and the interactions promoted by CagA-positive *H. pylori* strains to prevail in the host organism may be essential in identifying variations in prognosis, disease severity, and mechanisms that may be beneficial to the host, according to infections by different *H. pylori* strains.

ROLE OF CagA IN GASTRIC CARCINOGENESIS

The translocated effector protein CagA was found to be associated with gastric cancer development even before the initial elucidation of its pathogenic mechanisms[63,64]. Initially, the correlation between infection with CagA-positive *H. pylori* strains and carcinogenesis *in vivo* was established by experiments in Mongolian gerbil models[65-67]. More recently, transgenic expression of CagA in mice and zebrafish has also been shown to be associated with the development of gastric and hematopoietic neoplasms[68, 69]. Thereby, the oncogenic potential of the bacterial protein has become increasingly evident and, at the same time, different studies have sought to clarify its underlying mechanisms. Nowadays, CagA is considered a pathological analog of a scaffold or hub protein capable of disrupting multiple host signaling pathways and promoting a pro-oncogenic microenvironment[70].

Upon translocation, CagA localizes to the inner surface of the plasmatic membrane, where it undergoes tyrosine phosphorylation by multiple members of the Src family kinases and c-Abl kinases[8, 10,59,61]. As mentioned above, four different EPIYA segments were described according to the different amino acid sequences surrounding each motif, designated EPIYA-A–EPIYA-D[56,57]. Strains containing EPIYA-D or at least two EPIYA-C motifs are known to be associated with an increased risk of



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Figure 1 *Helicobacter pylori* oncoprotein: molecular structure and CagA-mediated carcinogenesis underlying mechanisms. A: Structural

domain differences between East Asian and western CagA. CagA tertiary structure is characterized by a structured N-terminal region and an unstructured C-terminal tail. The oncoprotein contains repetitive sequences in its C-terminal polymorphic region, known as the EPIYA motifs and CM motif. EPIYA-A and EPIYA-B motifs were identified in almost all CagA-positive *H. pylori* strains, followed by one, two, or three EPIYA-C sequences in western strains, or an EPIYA-D segment in East Asian strains. The CM motif, although highly conserved, possesses a 5-amino-acid difference between East Asian and western strains, hence distinguishing East Asian and Western CagA; B: Molecular mechanisms of CagA-mediated carcinogenesis. Upon translocation, CagA EPIYA motifs are tyrosine-phosphorylated by Src family or c-Abl kinases of the host cell. After phosphorylation, CagA localizes to the inner leaflet of the plasmatic membrane and acts as a promiscuous scaffold or hub protein that simultaneously disturbs multiple host signaling pathways, involved in regulation of a large range of cellular processes, including proliferation, differentiation and apoptosis. Ultimately, the disharmonic interaction between CagA and host proteins leads to pro-oncogenic cellular alterations. CM: CagA-multimerization; CM^{W}: western CagA.

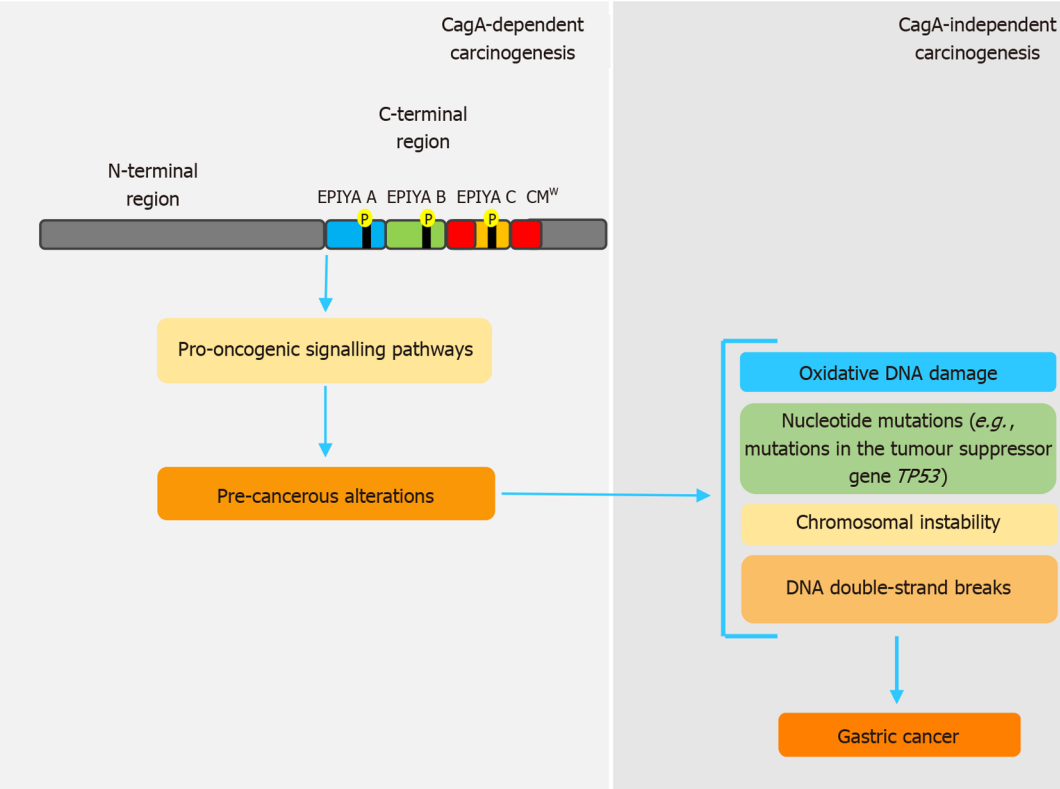
developing precancerous lesions and gastric cancer[71-75]. Queiroz *et al*[75] demonstrated in a Brazilian population that first-degree relatives of patients with gastric cancer were significantly and independently more frequently colonized by *H. pylori* strains with higher numbers of CagA EPIYA-C segments, which may be associated with an enhanced risk of developing this neoplasm[76].

Following tyrosine phosphorylation, EPIYA-C and D motifs acquire the ability to interact with SHP2 and induce pathological intracellular signaling[9]. Abnormal SHP2 activity leads to aberrant activation of the Ras–MAPK pathway, which has been associated with accelerated cell cycle progression and enhanced cell proliferation. CagA-activated SHP2 is also able to interact with proteins such as focal adhesion kinase, thence leading to cell elongation, increased motility, and cytoskeleton rearrangements [77,78]. All these pathological alterations in cell biology increase the risk of damaged cells acquiring precancerous genetic changes[79]. It is worth mentioning that CagA possessing EPIYA-D or a higher number of EPIYA-C segments binds more robustly to SHP2, which may explain their association with higher risk of gastric cancer development[8]. It has also been described that EPIYA-A and B motifs are able to interact with the SH2 domains of the tyrosine-protein kinase Csk, thereby inducing damage to actin binding proteins such as cortactin and vinculin. Consequently, rearrangements in the cytoskeleton reduce cell adhesion to the extracellular matrix and increase cell motility[80]. A recent study observed that *H. pylori*, through the T4SS encoded by *cag*PAI, interferes with the activity of cortectin binding partners, and stimulates overexpression of this molecule and promotes alterations in the actin cytoskeleton that may favor cell adhesion, motility and invasion of tumor cells contributing to the development of gastric cancer[81].

Recent studies have described that SHIP2, an SH2-containing phosphatidylinositol 5′-phosphatase, is a previously undiscovered CagA binding protein. Similar to SHP2, the SHIP2 protein is able to bind to EPIYA-C and D motifs in a tyrosine phosphorylation-dependent manner. In contrast, SHIP2 binds more robustly to EPIYA-C than to EPIYA-D sequences, thereby inducing changes in membrane phosphatidylinositol composition. This process enhances the subsequent delivery of CagA to the host cell, which binds to and dysregulates SHP2[82].

Other mechanisms have also been related to the aberrant induction of morphological changes, increased motility and cell proliferation[83]. In a tyrosine-phosphorylation-dependent manner, the interaction of CagA with the adapter molecule Crk is known to drive abnormal cell proliferation *via* MAPK signaling. Furthermore, the activation of Crk signaling pathways lead to the loss of cell–cell adhesion, hence inducing cell scattering/hummingbird phenotype and cell–cell dissociation[84]. It is important to mention that the EPIYA motif that corresponds to the binding site Csk has not been identified yet. In contrast, activation of the growth factor receptor-bound protein 2 (Grb2) protein and the *C-Met* oncogene in a tyrosine-phosphorylation-independent manner can also lead to similar pathological effects, highlighting the multiplicity of pro-oncogenic mechanisms of CagA[85,86]. Grb2 is an important regulator of Ras–MAPK pathway activation, capable of deregulating cell proliferation. Activation of C-Met-mediated PI3K/Akt signaling is associated with cancer-promoting mitogenic and inflammatory response[87-91].

The ability of CagA to disrupt the epithelial barrier has also been widely linked to its carcinogenic potential. In addition to the EPIYA sequences, the oncoprotein contains another repetitive sequence in its C-terminal polymorphic region, known as the CagA-multimerization (CM) motif[90]. With its number varying between different strains, the CM motif is composed of a 16-amino-acid sequence located downstream of the last EPIYA sequence[89]. This sequence, although highly conserved, possesses a 5-amino-acid difference between East Asian and western strains, hence distinguishing East Asian and western CagA (CM^{W})[91]. Thus, higher number of EPIYA-C motifs also increases the number of CM motifs in the CM^{W}. The CM motif is identified as the main mediator of the interaction between CagA and the partitioning-defective 1 (PAR1)/microtubule affinity-regulating kinase, which plays a pivotal role in establishing epithelial polarity. As a result, there is loss of cell polarity, as well as induction of morphological alterations also associated with the hummingbird phenotype[92]. CagA-mediated PAR1 inhibition also disrupts mitosis, causing increased cell division and impaired segregation of sister chromatids, thus leading to chromosomal instability (CI)[93]. Currently, CI is widely recognized for its multifactorial role in carcinogenesis and its microenvironment[94].



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Figure 2 Simplified model of CagA-mediated hit-and-run carcinogenesis. The pro-oncogenic properties of CagA are already well established. However, genetic and epigenetic alterations caused by this oncoprotein provide a favorable environment for carcinogenesis, independently of its presence, in a hit-and-run carcinogenesis mechanism. In this sense, through interaction with various host proteins, CagA leads to chromosomal instability, double-strand breaks and repeated nucleotide mutations, which are correlated to gastric cancer development. CM^W: western CagA.

From a different perspective, there is also a dangerous association between CM motifs and E-cadherin, a key protein in establishing cell polarity and maintaining epithelial integrity and differentiation[95-97]. It has been described that the CagA–E-cadherin interaction downregulates the β-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. Therefore, it was inferred that the oncoprotein plays an important role in the development of intestinal metaplasia, a precancerous transdifferentiation of gastric epithelial cells from which intestinal-type gastric adenocarcinoma emerges [98].

Also in this sense, a recent study observed that CagA seems to be able to induce gastric carcinogenesis by stimulating the migration of cancer cells through the activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome. The authors reported that CagA also participates in the generation of intracellular ROS and that ROS inhibition has the potential to disrupt the NLRP3 pathway and pyroptosis. With these findings, the authors concluded that NLRP3 plays a key role in the action of CagA on gastric cells and that silencing NLRP3 can limit the effects of migration and invasion of cancer cells caused by CagA[99].

It is worth emphasizing the ability of CagA to activate a variety of antiapoptotic pathways, upon interaction of its N-terminal portion with various tumor suppressor factors. For example, it is well described that CagA is able to impair the antiapoptotic activity of the tumor suppressor factor p53. CagA protein induces degradation of p53 protein in both ASPP2- and p14ARF-dependent manners[100, 101]. Furthermore, it is known that the interaction of the oncoprotein with Runt-related transcription factor 3 (RUNX3) is able to induce the ubiquitination and degradation of RUNX3 that blocks its antitumoral activity[102]. Figure 1B summarizes the main molecular mechanisms of CagA-mediated carcinogenesis.

Another important potential of CagA associated with gastric cancer pathogenesis is the ability to drive epithelial to mesenchymal transition (EMT); a phenomenon extensively related to carcinogenesis [103,104]. EMT is the result of a complex molecular program that allows cancer cells to suppress their epithelial characteristics, transforming themselves into mesenchymal epithelial cells. This change allows the cells to acquire motility, invasiveness, greater resistance to apoptosis, and the ability to migrate from the primary site[104,105]. In this regard, it has been shown that EMT gene expression is upregulated in gastric epithelial cells infected with *H. pylori* CagA-positive strains[106]. Nevertheless, multiple pathogenic mechanisms have been described, including reduction of glycogen synthase kinase-3 activity and triggering of the YAP oncogenic pathway, for example. However, the multiplicity of processes

involved in EMT and its role in gastric cancer development still requires further explanation[107,108].

Recent studies have not been limited to pathogen–host interactions in *H. pylori* infection, but have also investigated the mechanisms regulating bacterial virulence factors, such as CagA, and their relation to carcinogenesis. Eisenbart *et al*[11] identified a conserved, abundant nickel-regulated sRNA and named it NikS (nickel-regulated sRNA), whose expression is transcriptionally modulated based on the size of a variable thymine stretch in its promoter region. NikS, in dependence on nickel availability, directly represses several virulence factors of *H. pylori*, including the oncoprotein, CagA, and its effects on host cell internalization and epithelial barrier disruption. In this sense, multiple clinical repercussions of post-transcriptional modulation of CagA by NikS can be hypothesized, including decreased activation of procarcinogenic signaling. Kinoshita-Daitoku *et al*[109] described that NikS expression is lower in clinical isolates from gastric cancer patients than in isolates from noncancer patients, while the expression of virulence factors targeted by NikS, including CagA, is increased in isolates from gastric cancer patients. This field needs to be better explored, especially regarding the correlation of NikS with the multiple virulence factors of *H. pylori* and its potential clinical repercussions.

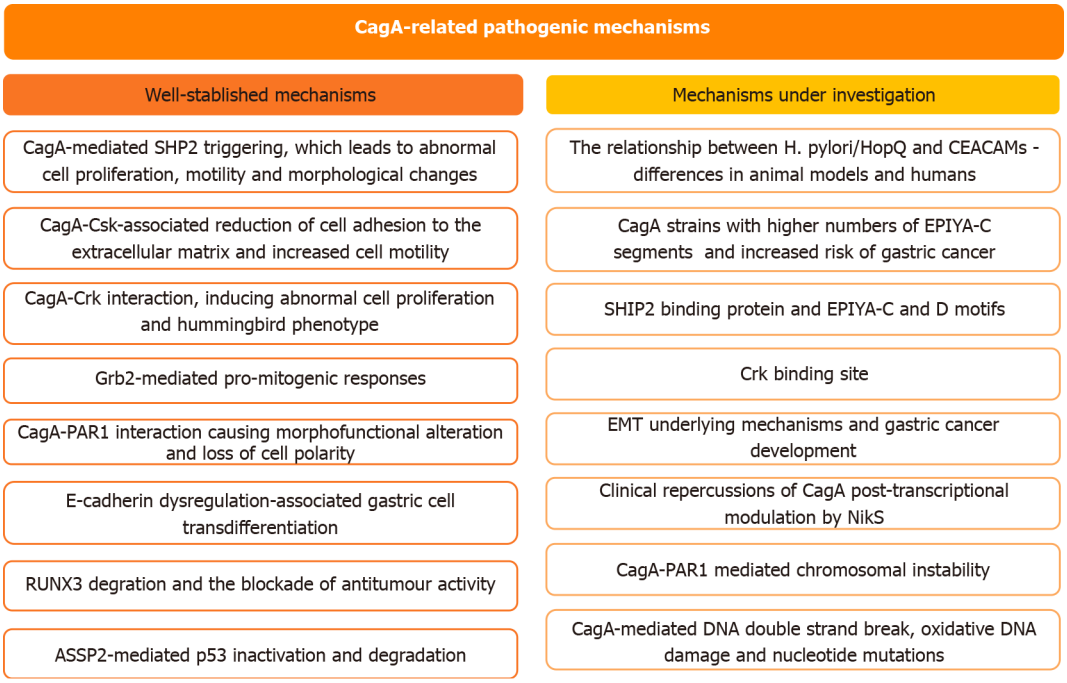
CAGA-MEDIATED HIT-AND-RUN CARCINOGENESIS

Even though CagA is notably a pro-oncogenic virulence factor, once gastric cancer sets in, its perpetuation is independent of the presence of this oncoprotein. In this sense, genetic and epigenetic changes caused by CagA seem to be responsible for this process, in a mechanism called "hit-and-run" carcinogenesis[58,92], firstly proposed by Skinner[110] for virus-induced cancers. Apparently, these changes are intrinsically related to disorders in the expression of activation-induced cytidine deaminase (AID), a crucial enzyme in the processes of somatic hypermutation and class-switch recombination of immunoglobulin genes in B cells[111,112]. Matsumoto *et al*[111] state that *cagA*-positive *H. pylori* induces disordered expression of AID *via* NF-B, which consequently leads to various nucleotide mutations, such as in the tumor suppressor gene *TP53*. *cagA*-positive *H. pylori* strains are also related to oxidative DNA damage, because they are able to promote increased levels of H_2O_2 and downregulation of heme oxygenase-1[89,113]. CagA also inhibits PAR1 kinase, leading to microtubule-based spindle dysfunction and consequent CI[93].

Some studies have shown that *H. pylori* can also promote DNA double-strand breaks (DSBs) in host cells[114,115], but whether or not this feature is CagA-related remains uncertain. However, a recent study showed that inhibition of PAR1b kinase by CagA hindered *BRCA1* gene phosphorylation, which leads to a BRCAness picture that, in turn, induces DSBs[116]. All these genetic and epigenetic changes support the hit-and-run mechanism, in which, regardless of the presence of CagA, the established pro-oncogenic environment maintains the acquired phenotype. Figure 2 summarizes the main features regarding CagA-mediated hit and run carcinogenesis mechanism.

CONCLUSION

In this study, we address the role of CagA in the development of *H. pylori*-mediated gastric carcinogenesis and discuss the perspectives in this field of study (Figure 3). CagA undergoes important translocation and tyrosine phosphorylation before disrupting several cell signaling pathways and promoting protein dysfunction. However, there is still much to be clarified regarding the steps involved in the role of CagA in gastric carcinogenesis. Recent discoveries such as the identification of the SHIP2 binding protein capable of binding to the EPIYA-C and D motifs and potentiating the delivery of CagA to infected cells, or discovery of the instability between the *H. pylori* HopQ and CEACAM relationship, are significant findings that can generate advances in the area, and therefore, need to be investigated in more depth. Exploration of the little-known field of regulatory RNAs seems promising for a broader understanding of the control mechanisms involved in *H. pylori* infection and CagA levels and has the potential to identify factors with important clinical repercussions for conditions such as gastric cancer. Finally, the ability of *H. pylori* to evolve as a pathogenic bacterium and as a carcinogen is undeniable, therefore, it is essential to clarify what is still unclear about the subject and periodically monitor the behavior of the bacterium in the infection, the molecular processes and elements such as CagA to advance the diagnosis and treatment of the disease.



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Figure 3 Status of the current understanding regarding CagA-related pathogenic mechanisms. EMT: Epithelial to mesenchymal transition; CEACAMs: Carcinoembryonic antigen-related cell adhesion molecules; Grb2: Growth factor receptor-bound protein 2; ASPP2: Apoptosis-stimulating protein of p53 2; SHP2: Domain-containing protein tyrosine phosphatase 2; SHIP2: Src homology 2 domain-containing inositol 5'-phosphatase 2; RUNX3: Runt-related transcription factor 3; PAR1: Partitioning-defective 1; NikS: Nickel-regulated sRNA.

FOOTNOTES

Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version; all authors agree to be accountable for all aspects of the work in ensuring that questions that are related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ORIGINAL ARTICLE

Basic Study

Folate receptor-targeted near-infrared photodynamic therapy for folate receptor-overexpressing tumors

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Abstract

BACKGROUND

Photodynamic therapy (PDT) is a minimally invasive form of cancer therapy, and the development of a novel photosensitizer (PS) with optimal properties is important for enhancing PDT efficacy. Folate receptor (FR) membrane protein is frequently overexpressed in 40% of human cancer and a good candidate for tumor-specific targeting. Specific active targeting of PS to FR can be achieved by conjugation with the folate moiety. A folate-linked, near-infrared (NIR)-sensitive probe, folate-Si-rhodamine-1 (FolateSiR-1), was previously developed and is expected to be applicable to NIR-PDT.

AIM

To investigate the therapeutic efficacy of NIR-PDT induced by FolateSiR-1, a FR-targeted PS, in preclinical cancer models.

METHODS

FolateSiR-1 was developed by conjugating a folate moiety to the Si-rhodamine derivative through a negatively charged tripeptide linker. FR expression in the designated cell lines was examined by western blotting (WB). The selective binding of FolateSiR-1 to FR was confirmed in FR overexpressing KB cells (FR+) and tumors by fluorescence microscopy and *in vivo* fluorescence imaging. Low FR expressing OVCAR-3 and A4 cell lines were used as negative controls (FR-). The NIR light (635 ± 3 nm)-induced phototoxic effect of FolateSiR-1 was evaluated by cell viability imaging assays. The time-dependent distribution of FolateSiR-1 and its specific accumulation in KB tumors was determined using *in vivo* longitudinal fluorescence imaging. The PDT effect of FolateSiR-1 was evaluated in KB tumor-

bearing mice divided into four experimental groups: (1) FolateSiR-1 (100 μmol/L) alone; (2) FolateSiR-1 (100 μmol/L) followed by NIR irradiation (50 J/cm^2); (3) NIR irradiation (50 J/cm^2) alone; and (4) no treatment. Tumor volume measurement and immunohistochemical (IHC) and histological examinations of the tumors were performed to analyze the effect of PDT.

RESULTS

High FR expression was observed in the KB cells by WB, but not in the OVCAR-3 and A4 cells. Substantial FR-specific binding of FolateSiR-1 was observed by *in vitro* and *in vivo* fluorescence imaging. Cell viability imaging assays showed that NIR-PDT induced cell death in KB cells. *In vivo* longitudinal fluorescence imaging showed rapid peak accumulation of FolateSiR-1 in the KB tumors 2 h after injection. *In vivo* PDT conducted at this time point caused tumor growth delay. The relative tumor volumes in the PDT group were significantly reduced compared to those in the other groups [5.81 ± 1.74 (NIR-PDT) *vs* 12.24 ± 2.48 (Folate-SiR-1), *vs* 11.84 ± 3.67 (IR), *vs* 12.98 ± 2.78 (Untreated), at Day 16, $P < 0.05$]. IHC analysis revealed reduced proliferation marker Ki-67-positive cells in the PDT treated tumors, and hematoxylin-eosin staining revealed features of necrotic- and apoptotic cell death.

CONCLUSION

FolateSiR-1 has potential for use in PDT, and FR-targeted NIR-PDT may open a new effective strategy for the treatment of FR-overexpressing tumors.

Key Words: Photodynamic therapy; Near-infrared; Photosensitizer; Folate receptor; Fluorescence; Cancer

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Core Tip: Photodynamic therapy (PDT) is a minimally invasive cancer therapy and photosensitizers (PS) with optimal properties are crucial for enhancing the efficacy of PDT. We evaluated the therapeutic efficacy of a folate receptor (FR)-targeted, near-infrared (NIR)-sensitive PS (FolateSiR-1). FolateSiR-1 showed FR specificity *in vitro* and *in vivo* and functions as a tumor-damaging PS by inducing necrosis, apoptosis, and cell growth inhibition upon activation with NIR light. Our findings suggest that FolateSiR-1 is effective in FR-targeted NIR-PDT with low side effects and has potential for presenting new and effective treatment options for FR-overexpressing tumors.

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INTRODUCTION

Cancer incidence and mortality is continually growing worldwide. GLOBOCAN 2020 estimated that there were 19.3 million new cancer cases and almost 10 million cancer deaths in 2020 and the global cancer burden is expected to reach 28.4 million cases in 2040[1]. Molecular-targeted approaches with various therapeutic modalities are expected to provide effective cancer diagnosis and treatment.

Photodynamic therapy (PDT) is one of the emerging options to combat various cancer types along with other conventional therapies such as surgery, chemotherapy, radiation therapy, and immunotherapy[2]. It is approved for the clinical treatment of several tumor types and certain non-malignant diseases as a minimally invasive therapy[3]. Although the precise mechanisms of tumor cell death by photodamage are not yet fully understood, they involve necrosis, apoptosis, tumor blood vessel damage, and nonspecific activation of the immune response against tumor cells[4]. Cell death through PDT generally occurs through a combination of these mechanisms, and no single pathway leads to cell death[5]. Photodynamic therapy involves the administration of a light-sensitive photosensitizer (PS) followed by the irradiation of targeted tumors with light corresponding to the specific absorbance wavelength of the PS[3]. Light activated PS accumulated in the cancer tissue leads to the local generation of reactive oxygen species (ROS), such as highly reactive singlet oxygen and/or radicals, which kill tumor cells through necrosis and cause tumor regression[6,7]. However, off-target toxicities and PS metabolic problems were encountered in clinical trials, and therefore, only a few PSs have been approved for anti-cancer therapies[8]. The tumor selectivity of the PS improves tumor localization in PDT, enhances tumor destruction, and reduces the side effects due to off-target localization[9].

Moreover, effective targeting reduces the required PS dose which additionally limits side effects. Tumor-localizing properties are generally dependent on the passive enhanced permeability and retention effect[10]. More effective active targeting is expected and achieved by diverse approaches such as the conjugation of receptor ligands, antibodies, carrier proteins, carbohydrates, and loading into targeted nanoparticles[11-14].

The first-generation of PSs were hematoporphyrin derivatives, and the first PS to be clinically used for cancer therapy was porfimer sodium (Photofrin®)[4]. Its poor tissue selectivity, low light absorption, and weak tissue penetration of emission demanded the development of second-generation PSs, porphyrinoids, and non-porphyrin compounds[15]. Moreover, third-generation PSs were developed by conjugating carrier biomolecules, such as receptor ligands or antibodies, to the second-generation PSs to selectively target tumors[4]. Nevertheless, the number of new clinically approved PSs remains low, and researchers are developing new improved PSs.

Folate receptor (FR) membrane protein is frequently overexpressed in 40% of human cancer types, including ovary, breast, head and neck, endometrium, lung, bladder, pancreatic, colon, and kidney cancer, whereas its expression is restricted to normal tissues[16]. It binds to extracellular folate with very high affinity and can physically deliver folate into the cell through endocytosis[17]. Therefore, FR is a good candidate for tumor-specific PS targeting[9,18]. Specific active targeting of PS to FR present on the cancer cell surface can be achieved by conjugation with the folate moiety[19]. Several studies have shown that some PSs conjugated with folic acid *via* appropriate linkers increase tumor uptake and enhance the efficacy of PDT. For instance, conjugation of folic acid to *meta*-tetra(hydroxyphenyl)chlorin (*m*-THPC)-like PS[20] and chlorin-based PS pheophorbide-a[21] has been reported. In addition, various materials such as polymers, nanomaterials, polysaccharides and cyclodextrins have been explored to overcome the shortcoming of PSs *via* tissue-specific delivery[22]. For example, folate-conjugated micelles containing temoporfin[23] and polydopamine nanoparticles conjugated with folic acid[24] showed potential for therapeutic applications. Kim *et al*[22] showed that ROS-sensitive and FR-targeted nanophotosensitizer conjugates are promising candidates for PDT of cervical cancer. Kato *et al*[25,26] reported that folate-porphyrin-lipid nanoparticles induced the selective destruction of lung cancer and malignant pleural mesothelioma in a preclinical model based on FR targeting. Baydoun *et al*[27] proved the efficacy of a new PS (pyropheophorbide a-polyethylene glycol-folic acid) on human ovarian cancer cells. Quilbe *et al*[28] developed a new PS targeting FR, protected by patent (WO2019 016397-A1) and evaluated its PDT efficacy on a mouse model of pancreatic cancer. These latest state-of-the-art studies encourage the use of folate-mediated PSs in tumor-targeted photodynamic therapy. In an alternative approach called photoimmunotherapy (PIT), PSs conjugated with monoclonal antibodies that have high affinity to tumor specific antigens instead of using receptor ligands have also investigated[29,30].

PS targeting was developed or improved by conjugating fluorophores to tumor-seeking molecules [31]. We previously developed a folate-linked near-infrared (NIR)-sensitive probe, folate-Si-rhodamine-1 (FolateSiR-1, Abs_{max}/Em_{max} = 652/674 nm, and fluorescence quantum yield of 7.6%) using folate glutamate as a tumor-seeking agent, a negatively charged peptide linker, and rhodamine derivative, 2,5-diCOOH SiR650, as a photoactive component (Figure 1A)[32]. Folate receptor α contains a folate-binding pocket[33]. The folate pteroate moiety is buried inside the receptor, whereas its glutamate moiety is solvent-exposed and protrudes from the pocket, which allows its conjugation to the fluorophore without adversely affecting FRα binding[33]. We chose to conjugate the linker to the folate glutamate moiety, as this allowed high affinity- and highly selective binding of FolateSiR-1 probe to FR [34]. One of the main drawbacks of currently approved PDTs is the insufficient penetration of light during treatment. This may be overcome by using NIR absorbing PSs[32] and appropriate NIR light sources because red and infrared light penetrate tissue more deeply than other visible light[3]. The photophysical properties of FolateSiR-1 and the wavelength of our laser system (635 ± 3 nm) used in this study resolved this issue. Fluorophores emitting NIR light in the phototherapeutic window (650-900 nm) are most suitable due to high tissue penetration and low autofluorescence, which results in a low background signal[35]. FolateSiR-1 exhibited very low background fluorescence and showed a high ratio of tumor-to-background fluorescent intensity[32].

In this study, we evaluated the *in vitro* and *in vivo* tumor specific targeting- and photodynamic activity of FolateSiR-1 using high-FR-expressing-KB cells (FR+) and low-FR-expressing-OVCAR-3 cells (Low FR). We revealed the potential of FolateSiR-1 as a candidate for use in the PDT of cancer by assessing its phototoxic cell death and antitumor effects. In addition, the cell death mode induced by FolateSiR-1-based PDT was investigated.

MATERIALS AND METHODS

Cell lines and culture

The human oral epidermoid carcinoma cell line, KB (a Hela subline), and human epithelial ovarian cancer cell line, OVCAR-3, were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, United States). The KB cells were cultured in Eagles's minimum essential medium (EMEM) (Wako Pure Chemical Corp., Osaka, Japan) supplemented with 10% fetal bovine serum (FBS)

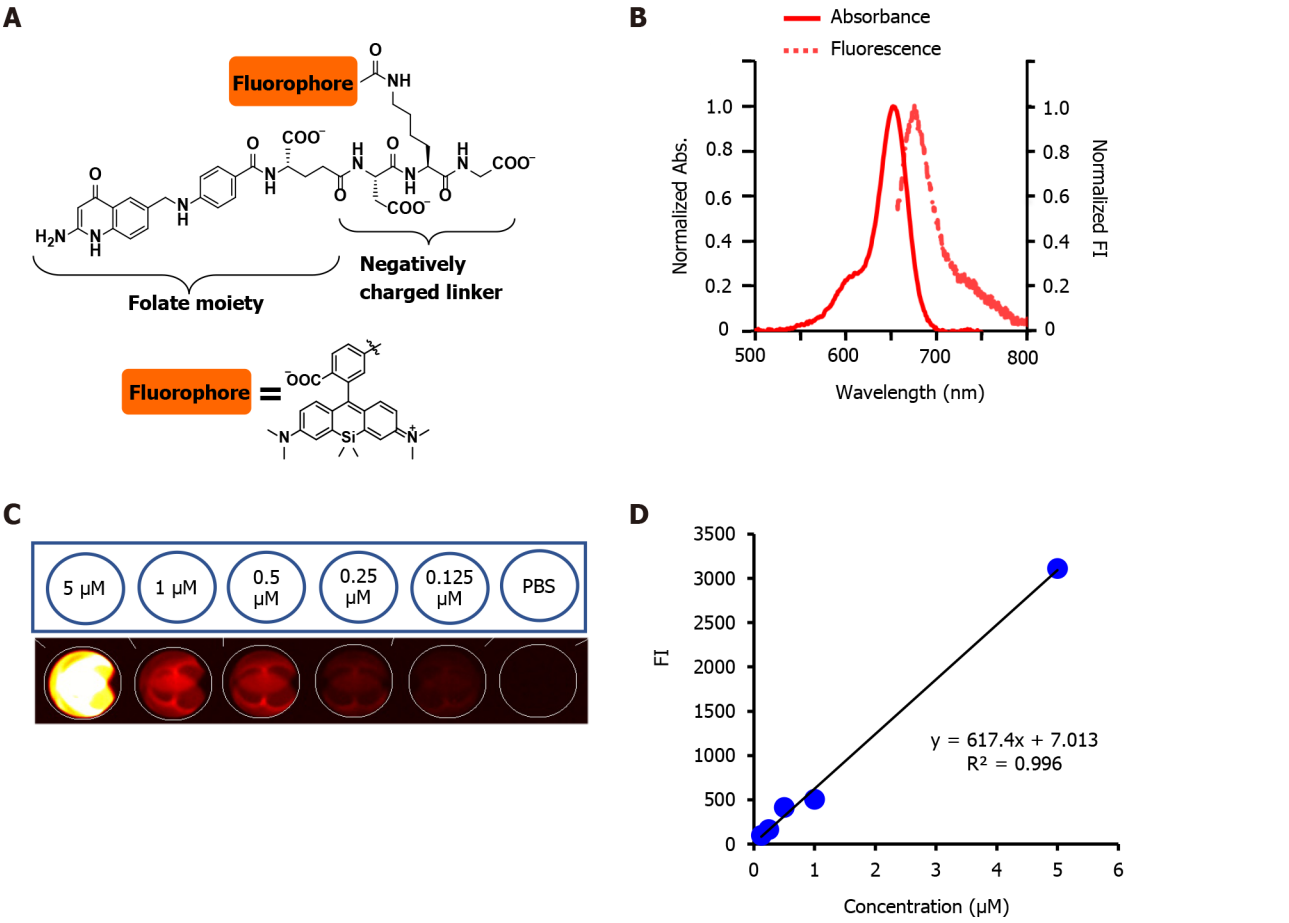


Figure 1 Folate-Si-rhodamine-1 characteristcs. A: Molecular design and chemical structure of folate-Si-rhodamine-1 (FolateSiR-1); B: Absorption and emission spectra of 1 μmol/L FolateSiR-1 in 100 mmol/L sodium phosphate buffer (pH 7.4) containing 0.1% DMSO: maximum absorption = 652 nm; maximum emission = 674 nm; C: Fluorescence images of FolateSiR-1 at a concentration of 5, 1, 0.5, 0.25, and 0.125 μmol/L in a 96-well plate captured using the VISQUE fluorescence imaging system; D: FolateSiR-1 concentration *vs* fluorescence signal intensity. Abs: Absorbance; FI: Fluorescence signal intensity; μM: μmol/L.

(Gibco, Life Technologies Japan Ltd., Tokyo, Japan), 100 U/mL penicillin-G sodium, and 100 μg/mL streptomycin sulfate (Wako) at 37 ˚C in a humidified incubator containing 5% CO_2. The A4 cell line is an immortalized mouse fibroblast cell line established from mouse 3T3 cells and transfected with human epidermal growth factor receptor 2-expression vector. The OVCAR-3 cells and A4 cells were similarly cultured in RPMI 1640 medium (Wako) supplemented with 10% FBS (Gibco), 100 U/mL penicillin-G sodium, and 100 μg/mL streptomycin sulfate (Wako).

FolateSiR-1 synthesis

FolateSiR-1 was designed and synthesized using folate glutamate as a tumor-seeking agent, a negatively charged peptide linker, and a rhodamine derivative, 2,5-diCOOH SiR650, as a photoactive component according to a previously described method[32]. Fluorescence spectroscopic analyses of FolateSiR-1 was conducted with a Hitachi F7000 spectrophotometer (Tokyo, Japan) using 2.5 nm excitation and emission slit widths and 700 V photomultiplier voltage. Different FolateSiR-1 concentrations (5, 1, 0.5, 0.25, 0.125 μmol/L) were prepared in 100 μL in a Nunc™ flat-bottom microplate (96 well, Black) (Thermo Fisher Scientific, Roskilde, Denmark). The fluorescence signal intensity (Fl) was determined using a VISQUE InVivo Smart fluorescence imaging and analysis system (Vieworks Co., Ltd, Gyeonggi, Korea) equipped with HyperRed light filter set (Ex/Em = 630-680/690-740 nm).

Animal and tumor model

Ethics Committee-approved animal studies were conducted in accordance with the institutional guidelines of the Animal Care and Use of Committee of National Institutes for Quantum and Radiological Science and Technology. The animal protocol was designed to minimize pain or discomfort to the animals. Four-week-old male BALB/cAJcl-nu/nu mice were obtained from CLEA (Shizuoka, Japan), kept in an air-conditioned facility (23 °C) with an artificial 12 h light-dark cycle, 50% humidity, and provided autofluorescence-free food and water *ad libitum* for one week prior to experimentation. Development of tumor models involved subcutaneous inoculation of early passage cells (5×10^6 KB cells/site, 1×10^7 OVCAR-3 cells/site, or 5×10^6 A4 cells/site, all in 100 μL medium) into both femoral

regions of nude mice. Mice were anesthetized with isoflurane during the experimental procedure. All animals were euthanized by cervical dislocation under anesthesia (inhalation of isoflurane) for tumor tissue collection.

Western blot analysis

Folate receptor expression in designated cells was examined by western blotting (WB). First, whole-cell lysate was prepared using radioimmunoprecipitation assay buffer (Wako) containing protease inhibitor cocktail (P8340; Sigma-Aldrich, St. Louis, MO, United States). Total protein concentration was determined using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, United States). Next, 50 μg of cell lysate protein samples were separated using 4%-20% polyacrylamide gel (ATTO Corporation, Tokyo, Japan) and transferred onto an Immobilon-P membrane (Millipore, Billerica, MA, United States). Mouse monoclonal antibody anti-FR of human origin (E-11) (Santa Cruz Biotechnology, Santa Cruz, CA, United States) (1:200 dilution) and goat polyclonal anti-human actin antibody (Santa Cruz Biotechnology) (1:500 dilution) were used as primary antibodies. Horseradish peroxidase (HRP)-linked anti-mouse IgG antibody (GE Healthcare, Little Chalfont, United Kingdom) (1:1000 dilution) and HRP-linked anti-goat IgG antibody (Santa Cruz Biotechnology) (1:1000 dilution) were used as secondary antibodies. The Enhanced Chemiluminescence Plus detection system (GE Healthcare) was used to visualize the immunoreactive bands.

Fluorescence microscopy

KB cells (1×10^5 cells/well) were seeded onto an EZVIEW glass bottom culture plate (24 well, black; IWAKI, Shizuoka, Japan) in complete EMEM medium (1 mL) and incubated for 24 h at 37 °C. The media was removed and each well was washed with PBS, subsequently, fresh medium was added with or without FolateSiR-1 (2.5 μmol/L in 0.5% DMSO) and incubated overnight at 37 ˚C. The medium was discarded, cells were washed with phenol red free EMEM α medium (Wako) two times, and each well was refilled with the same medium. The OVCAR-3 cells (5×10^5 cells/well) and A4 cells (1×10^5 cells/well) were prepared using RPMI 1640, and the cells were observed using a Keyence BZ-X700 fluorescence microscope (Keyence Japan Co, Ltd, Osaka, Japan) with an Ex/Em 590-650/665-732 nm filter for FolateSiR-1. The same exposure time was consistently used. Phase-contrast images were also captured.

Cell viability imaging

KB cells (1.5×10^5 cells/well) were seeded onto a EZVIEW glass bottom culture plate (24 well, black; IWAKI) in complete EMEM medium overnight at 37 °C. Spent medium was replaced with fresh medium with or without FolateSiR-1 (2.5 μmol/L in 0.5% DMSO) and the KB cells were incubated overnight. Next, the medium was discarded, cells were washed with phenol red free medium (Wako), and subsequently irradiated with NIR light from an infrared diode laser system (Laser Create Co., Tokyo, Japan) at 635 ± 3 nm and a power density of 10 J/cm^2 (0.1 W/cm^2 for 100 s). The distance between the sample and light source was set to 3 cm, and the light power was adjusted to 0.1 W/cm^2. The irradiation dose was measured using a Starlite thermal laser power sensor and optical power meter (OPHIR Japan, Saitama, Japan). After irradiation, each well was refilled with the same medium and incubated at 37 °C. Two hours later, two color staining assays were conducted using the ReadyProbes™ cell viability imaging kit (Thermo Fisher Scientific K.K, Tokyo, Japan) according to manufacturer's instructions. Nuclei of the viable cells and dead cells were stained with NucBlue® Live reagent and NucGreen® Dead reagent, respectively. Cell images were acquired with a Keyence BZ-X700 microscope (Keyence Japan Co, Ltd) with filter sets for NucBlue® (Ex/Em = 360/460 nm), NucGreen® (Ex/Em = 475/509 nm), and FolateSiR-1 (red, Ex/Em = 590-650/665-732 nm). Image acquisitions were conducted using similar exposure times.

Mice NIR fluorescence imaging

Intravenous injection of FolateSiR-1 (100 μmol/L in 100 μL saline and 10% DMSO) into the tail vein of tumor-bearing mice was followed by anesthetization and acquisition of fluorescent and white light images at the dorsal position. Pre-injection- and post-injection time points (5 min, 10 min, 15 min, 20 min, 30 min, 45 min, and 1 h, 2 h, 3 h, 4 h, 4.5 h, 5 h, 6 h, 24 h, and 48 h) were designated for longitudinal imaging. *In vivo* NIR fluorescence imaging was conducted using the VISQUE imaging system (Vieworks Co., Ltd,) equipped with HyperRed light filter (Ex/Em = 630-680/690-740 nm) using constant imaging parameters (exposure time: 100 ms, binning: 1 × 1, light intensity: high, mode: low gain). Tumor FI was acquired by subtracting nearby background FI using Clevue software (Vieworks). Two mice with OVCAR-3 and KB tumors in their left and right femoral regions, respectively, were imaged to confirm FR-specific binding. Similarly to the other control samples, KB tumor-bearing mice that did or did not receive the FolateSiR-1 injection were imaged together, and the A4 (low FR) tumor-bearing mouse was imaged after PS injection.

In vivo tumor PDT

Subcutaneous tumor lengths peaked at approximately 6-10 mm nine days after xenografting. These

mice were randomly assigned to one of the four groups (n = 5 in each group), and treatment was conducted using the designated schedule. FolateSiR-1 (100 μmol/L) was initially administered intravenously. After 2 h, the tumor was exposed to NIR light from a Diode Laser system (Laser Create Co.) at 635 ± 3 nm and 50 J/cm^2 (0.25 W/cm^2 for 200 s). The distance between the tumor and NIR light source was set to 3 cm. Whole body of the mouse, except for the tumor area, was shielded from the light using aluminum foil. Group 1 received PS injection, group 2 received PS injection followed by light irradiation at 2 h post-injection, group 3 was only irradiated, and group 4 received no treatment. Tumors sizes of all mice (n = 8 for each) were measured twice a week over 16 d using calipers, and volumes were calculated according to the formula: volume (mm^3) = [length (mm)] × [width (mm)]^2 × 0.5. The relative tumor volume was calculated based on the ratio of the volume on the indicated day and the starting volume prior to the treatment. Mice were weighed twice a week, while the skin condition of the light exposed area, and general conditions of the mice were monitored daily.

Immunohistochemical- and histological analysis

Twenty-four h after irradiation, a mouse from each group was sacrificed. The tumors were excised, fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5 μm-slices. Serial tissue sections were rehydrated and subjected to antigen retrieval for immunohistochemical (IHC) analysis. The sections were then stained for Ki-67 cell proliferation marker[36] using anti-human Ki-67 polyclonal antibody (Dako Denmark, Glostrup, Denmark) (1:200 dilution) as previously described[37]. Mouse monoclonal anti-FR antibody of human origin (E-11) (Santa Cruz Biotechnology) (1:200 dilution) was used to investigate tumor FR expression. Serial sections were stained with hematoxylin and eosin (H&E) dye to examine histopathological changes. Slides were examined with an Olympus BX43 microscope (Tokyo, Japan).

Statistical analysis

The quantitative results are presented as the mean ± SD. Relative tumor volume differences between groups were determined using ANOVA (two-factor with replication; Excel, Microsoft, Redmond, WA, United States), followed by Tukey's test. P values < 0.05 were considered statistically significant.

RESULTS

FolateSiR-1 conjugate characteristics

FolateSiR-1 probe (molecular weight 1232.78, Figure 1A) was developed from a Si-rhodamine derivative containing a carboxy group at the benzene moiety coupled to a folate ligand moiety through a negatively charged tripeptide[32]. FolateSiR-1 is expected to possess high affinity to FR with a tumor-seeking character of folate glutamate[38]. Rhodamine derivative 2,5-diCOOH SiR650 serves as a photoactive component. Maximum absorption and emission wavelengths of 1 μmol/L FolateSiR-1 in 100 mmol/L sodium phosphate buffer at pH 7.4 were 652 nm and 674 nm, respectively (Figure 1B)[32]. FolateSiR-1 fluorescence emission was most clearly visualized with HyperRed light filter (Ex/Em = 630-680/690-740 nm) using the VISQUE imaging system (Figure 1C). The quantitative evaluation of FI revealed that the linear correlation coefficient between fluorescence and FolateSiR-1 concentration was 0.996 (Figure 1D).

Folate receptor expression and FolateSiR-1 accumulation in cultured cells

High FR expression was observed in the KB cells by WB, but not in the OVCAR-3 and A4 cells (Figure 2A). Likewise, FolateSiR-1 accumulated in the KB cells at the cell membrane and their intracellular localization indicated substantial specific binding of FolateSiR-1 to FR and its internalization using fluorescence microscopy. However, this was not seen in almost any of the OVCAR-3 and A4 cells (Figure 2B). All three cell lines incubated in medium without FolateSiR-1 showed no fluorescence activity (data not shown).

Folate receptor-specific binding of FolateSiR-1 in in vivo tumors

Using the VISQUE imaging system 2 h after injecting FolateSiR-1 into tumor-bearing mice, the KB tumors inoculated in the right femoral regions showed high FI, while the OVCAR-3 tumors inoculated in the left femoral regions showed no FI (Figure 2C). Next, the KB tumor of the mouse that was administered FolateSiR-1 exhibited FI, but the negative control tumor of the mouse with no FolateSiR-1 injection did not (Figure 2D). Moreover, a mouse bearing A4 (low FR) tumor showed no FI (Figure 2E). After imaging, IHC examination of tumor tissue sections showed high FR expression in the KB tumor (Figure 2F). These results suggest that there was specific binding of FolateSiR-1 to FR in the tumors.

Phototoxic cell death induced by FolateSiR-1 mediated PDT

Immediate cell death induced by NIR-PDT was detected based on green colored cells by fluorescence microscopy, resulting from the staining with the NucGreen® Dead reagent, whereas viable cell nuclei

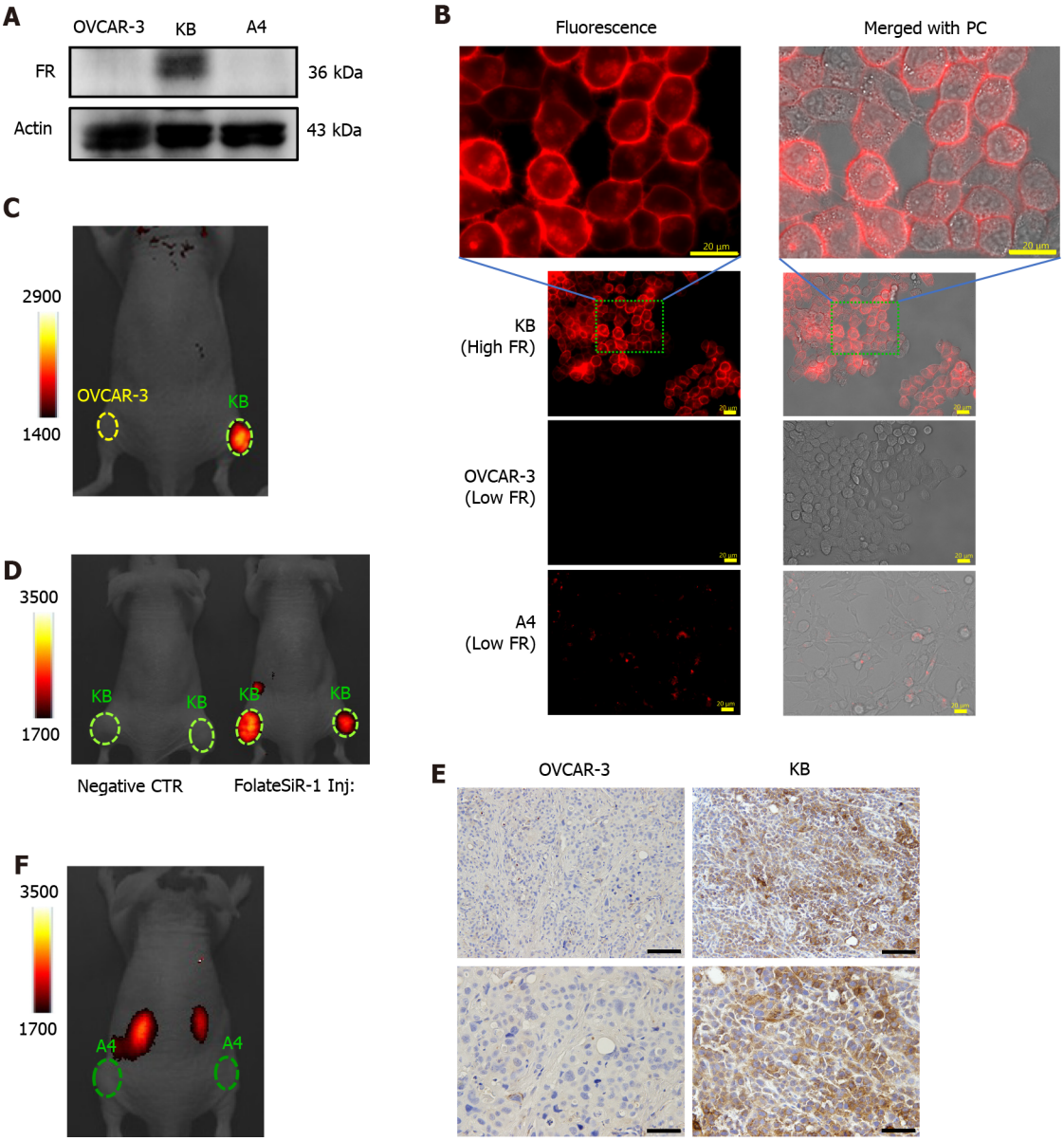


Figure 2 Folate receptor expression in designated cell lines and folate receptor-specific binding of folate-Si-rhodamine-1 in tumor *in vivo*. A: Folate receptor (FR) expression in cell lines OVCAR-3, KB, and A4 examined by western blotting; B: Cell lines were incubated with or without folate-Si-rhodamine-1 (FolateSiR-1) for 24 h and examined under a fluorescent microscope. FolateSiR-1 fluorescence was detected using the appropriate filters (Ex/Em 590-650/665-732 nm). The uppermost images are magnifications of the indicated portion of the lower image showing KB cells (scale bar = 20 μm, 40 × magnification, and 120 × magnification for the upper most image); C: Near-infrared (NIR) fluorescence image of a representative mouse bearing subcutaneous KB (right) and OVCAR-3 (left) tumors at 2 h after receiving the FolateSiR-1 injection: KB tumor (FR+) showed high fluorescence signal intensity (FI), and OVCAR-3 tumor (low FR) did not; D: NIR fluorescence image of mice bearing KB tumors: the right mouse received the FolateSiR-1 injection showed FI, while the left mouse did not (negative control tumor) show no FI; E: NIR fluorescence image of a mouse bearing A4 (low FR) tumor showed no FI; F: Immunohistochemical analysis of tumor tissue sections for FR expression: KB tumor (right column), OVCAR-3 tumor (left column). Scale bar = 100 μm, 20 × magnification for upper panel, and scale bar = 50 μm, 40 × magnification, for lower panel. FR: Folate receptor; Ex: Excitation; Em: Emission; FI: Fluorescence signal intensity; FolateSiR-1: Folate-Si-rhodamine-1; PC: Phase-contrast; NIR: Near-infrared; CTR: Control.

were stained blue with NucBlue® Live reagent (Figure 3). No noticeable phototoxic cell death was observed with FolateSiR-1 treatment alone, light treatment alone, or no treatment (Figure 3).

In vivo FolateSiR-1 biodistribution in tumorbearing mice

Time-dependent distribution of FolateSiR-1 and its specific accumulation in the tumors was determined based on serial images of a representative mouse following FolateSiR-1 injection (Figure 4A). The quantified FI in the tumor rapidly increased, peaking approximately 2 h after injection, and almost disappearing over 24 h (Figure 4B). Moreover, in another experiment, decreased tumor FI was observed in the right tumor that received PDT after 30 min compared with that of the non-irradiated left tumor (Figure 5A). Although there was a time-dependent decrease of FI due to normal washout of the PS from the tumor, the degree of FI reduction in the tumor treated with PDT was greater than in the untreated

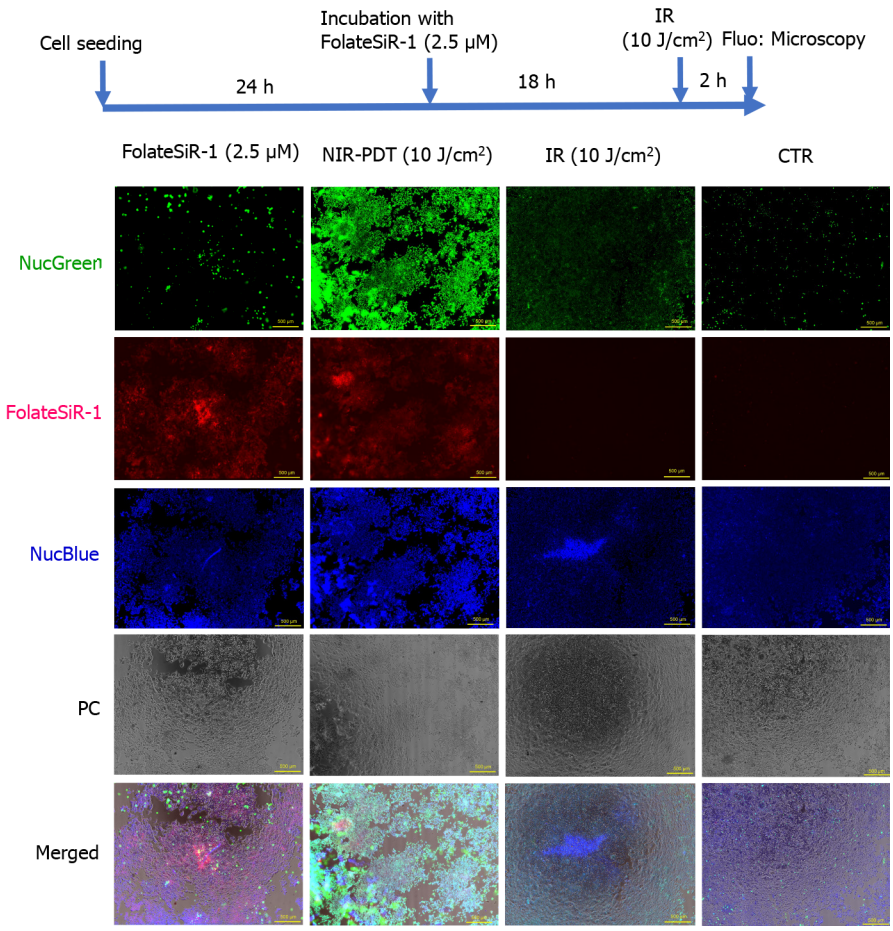


Figure 3 *In vitro* phototoxicity of folate-Si-rhodamine-1 after laser irradiation. The cells were stained using the reagents from the Cell viability imaging kit at 2 h after near-infrared photodynamic therapy (NIR-PDT). The nuclei of dead cells were stained with NucGreen® Dead reagent (green), while the viable cell nuclei were stained with NucBlue® Live reagent (blue). Rapid cell death-induced by PDT (10 J/cm^2) and abundant dead cells were clearly noted. (scale bar = 500 μm, 10 × magnification). NIR-PDT: Near-infrared photodynamic therapy; IR: Laser irradiation; CTR: Control; NucGreen: NucGreen® Dead reagent; NucBlue: NucBlue® Live reagent; PC: Phase-contrast; μM: μmol/L.

tumor. Therefore, the percentage decrease of tumor FI was higher in the PDT-induced tumors (Figure 5B). Mice were euthanized shortly after *in vivo* imaging, with tumors and other organs removed and analyzed by the VISQUE imaging system. *Ex vivo* NIR images showed higher FI in tumor tissues compared with major organs, and higher FI in the right tumor compared with that of the left tumor. Meanwhile, FI from organs including the liver and kidney was barely visible (Figure 5C). *Ex vivo* results showed similar trends in data as that in *in vivo* imaging data.

In vivo PDT efficacy of FolateSiR-1 on tumor size

The tumor-bearing mice were treated according to the scheme shown in Figure 6. Statistically significant tumor growth inhibition was observed after NIR-PDT treatment *vs* FolateSiR-1 treatment alone, NIR irradiation alone, and no treatment ($P < 0.05$, Figure 7A). No significant differences were observed amongst other groups. There was no significant body weight loss, skin damage, or abnormal general conditions in these mice (Figure 7B). Relatively decreased sizes of PDT-treated tumors were observed *ex vivo* 16 d after treatment compared with that in the other groups (Figure 7C).

Evaluation of tumor tissue response to PDT by IHC and histopathology

Proliferative factor Ki67 nuclear staining in the control tumor cells was observed by IHC analysis. In contrast, the tumor tissues treated with PDT showed weak staining and markedly reduced numbers of Ki-67-positive cells, suggesting that PDT inhibited tumor growth (Figure 8). Furthermore, PDT treated tumors showed rapid extensive damage with necrotic- and apoptotic cell death at the irradiated tumor site as indicated by H&E staining. PDT treated tumor cells showed some features of necrosis and apoptosis such as nuclear fading due to DNA degradation (karyolysis), anuclear necrotic cells with a glossier homogenous appearance, nuclear chromatin condensation (pyknosis), and nuclear fragmentation (karyorrhexis). No conspicuous damage was found in the tumors of the other control groups receiving FolateSiR-1 alone, irradiation alone, or no treatment (Figure 9).

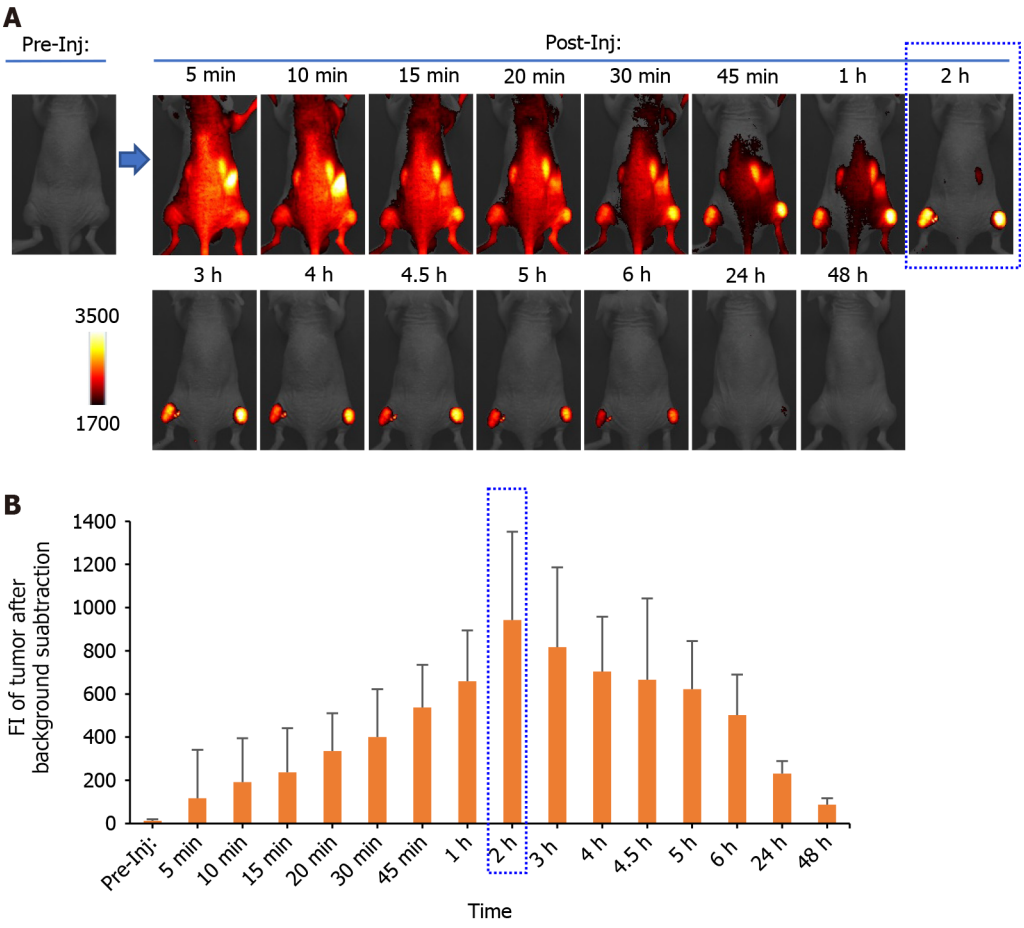


Figure 4 *In vivo* longitudinal near-infrared fluorescence imaging of folate-Si-rhodamine-1 in a KB tumor-bearing representative mouse. A: Imaging was conducted before and after folate-Si-rhodamine-1 (FolateSiR-1) injection. The indicated time points after injection are at 5 min, 10 min, 15 min, 20 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 4.5 h, 5 h, 6 h, 24 h, and 48 h. Time-dependent FolateSiR-1 distribution and its specific tumor accumulation is shown in serial images; B: Quantitative fluorescent signal intensity analysis of the tumors. Data are presented as the mean ± SD (n = 4). NIR: Near-infrared; FI: Fluorescent signal intensity; Pre-Inj: Pre-injection; Post-Inj: Post-injection.

DISCUSSION

FolateSiR-1 significantly accumulated in the FR-expressing KB tumors, but not in the FR-negative tumors and normal organs. This PS was activated by NIR light to evoke cytotoxic effects on cells and tumors, resulting in PDT induced necrosis and apoptosis. Our findings suggest that FolateSiR-1 has potential for application in the PDT of FR-expressing tumors.

High FR expression was observed in the KB cells, but not in the OVCAR-3 and A4 cells (Figure 2A). Furthermore, fluorescence microscopy revealed that KB cells incubated with FolateSiR-1 showed strong FI which contrasted with that of the other two cell lines (Figure 2B). These results encouraged us to use KB cells as a representative FR-expressing xenograft tumor in a mouse model. Stronger FI was observed in KB tumors, compared to OVCAR-3 tumors, A4 tumors, and other sites throughout the body, which was attributed to the presence of FolateSiR-1 (Figures 2C-E). Furthermore, strongly positive FR staining was observed in KB tumor cells, whereas it was almost entirely absent in OVCAR-3 cells based on IHC (Figure 2F). These findings and *ex vivo* images of normal tissues (Figure 5C) indicate that FolateSiR-1 binds FR with high specificity in FR-overexpressing cells and tumors.

In this study, FolateSiR-1 accumulation peaked at 2 h after injection in KB tumors, followed by its disappearance over the next 24 h based on quantification of serial fluorescence images. The rapid peak accumulation of FolateSiR-1 in tumors may be a more convenient clinical setting of PDT than the number of days required for PIT using an antibody as a PS carrier. Furthermore, rapid tumor contrast enhancement within a few hours after PS injection could be advantageous for real-time cancer detection in the intraoperative setting. Moreover, irradiation delivered at a shorter interval when PS is still present in the blood vessels results in a superior tumor response which causes marked vascular damage[39]. *In vivo* time-dependent images (Figure 4) and *ex vivo* images of tissues (Figure 5C) showed that FolateSiR-1 was cleared relatively quickly from normal tissues, including the main excretory organs (liver and kidney), indicating that the phototoxic side effects were minimized.

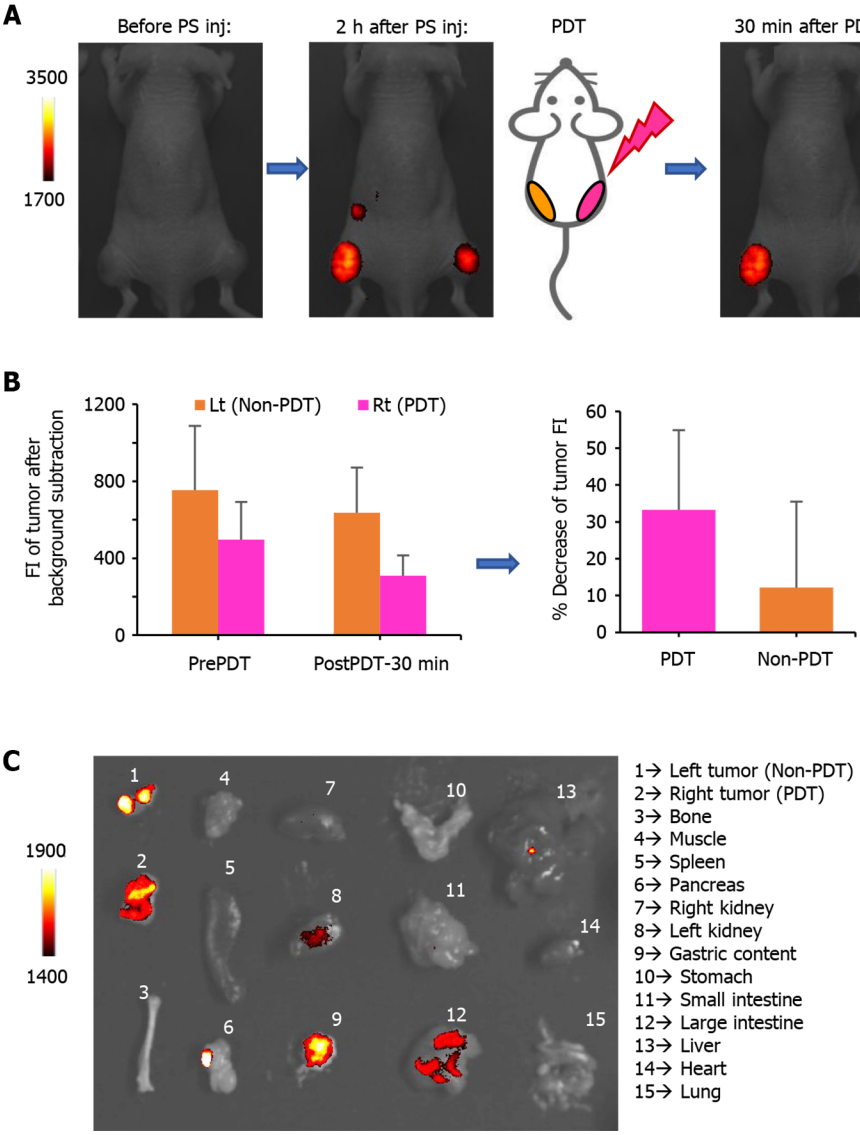


Figure 5 *In vivo* and *ex vivo* images before and after photodynamic therapy. A: Near-infrared fluorescence images of representative tumor bearing mice: before photosensitizer (PS) (FolateSiR-1) injection (left), 2 h after PS injection (center), and 30 min after photodynamic therapy (PDT) (right). The right tumor received PDT while the left tumor did not. Fluorescent signal intensity (FI) of the right tumor decreased immediately after PDT, and the extent of FI reduction is larger than that of the left tumor; B: Bar graph showing the FI of the tumor before- and 30 min after PDT. Data are presented as the mean ± SD (n = 4); C: *Ex vivo* fluorescence images captured 30 min after PDT. NIR: Near-infrared; Lt: Left; Rt: Right; PS inj: photosensitizer injection; PDT: Photodynamic therapy; Non-PDT: Without PDT; FI: Fluorescent signal intensity.

KB cells showed extensive bright fluorescence signals, whereas there was no obvious PS binding or uptake in the negative control cells indicating that FolateSiR-1 was selectively localized in KB cells (Figure 2B). Fluorescent signals were mainly observed at the plasma membrane of KB cells and in some cytoplasmic component sites. The cellular response to photodamage primarily depends on PS localization and PDT dose[40-42]. Photosensitizer localization and activation inside tumor tissues generates ROS which can directly kill malignant tumor cells[4]. Mitochondria-localized PS induces apoptotic cell death within a certain threshold of oxidative stress[6,43]. Necrosis is more often observed if the cell membrane is the site of action for the PS[44]. Mild oxidative damage by PS localized in the plasma membrane causes apoptosis, but severe damage leads to the loss of plasma membrane integrity and causes necrotic cell death[6,41,45]. Meanwhile, PSs targeting the endoplasmic reticulum, Golgi membranes, and lysosomes mediates necrosis[43]. Moreover, high PDT doses (dependent on the amount of PS and the irradiation dose) inactivate essential enzymes and other components of the apoptotic cascade resulting in increased cellular damage leading to necrosis rather than apoptosis[40, 46]. The merged fluorescence- and phase-contrast images showed strong FolateSiR-1 fluorescence intensity in the KB cell membrane and the cytoplasm, but not in the nucleus (Figure 2B). This finding suggests that FolateSiR-1 promotes necrosis and is a suitable probe for PDT. The probe alone did not penetrate the nuclear membrane and therefore did not cause genetic damage.

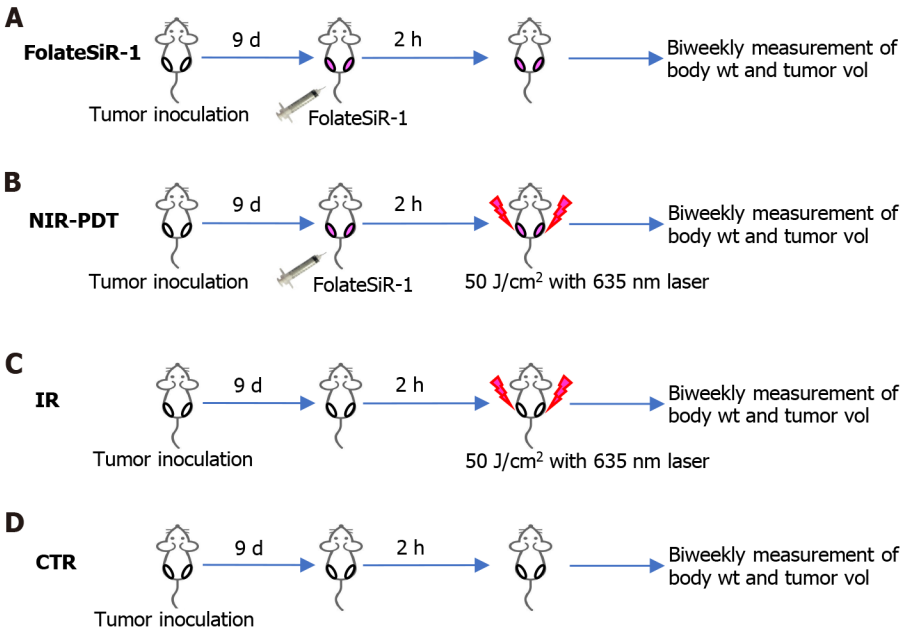


Figure 6 Experimental treatment scheme. Tumor bearing mice were assigned to four groups: A: folate-Si-rhodamine-1 injection alone; B: Near-infrared (NIR) photodynamic therapy (50 J/cm^2); C: NIR irradiation alone (50 J/cm^2); and D: no treatment. FolateSiR-1: Folate-Si-rhodamine-1; NIR-PDT: Near-infrared photodynamic therapy; IR: Laser irradiation; CTR: Control; wt: Weight; vol: Volume.

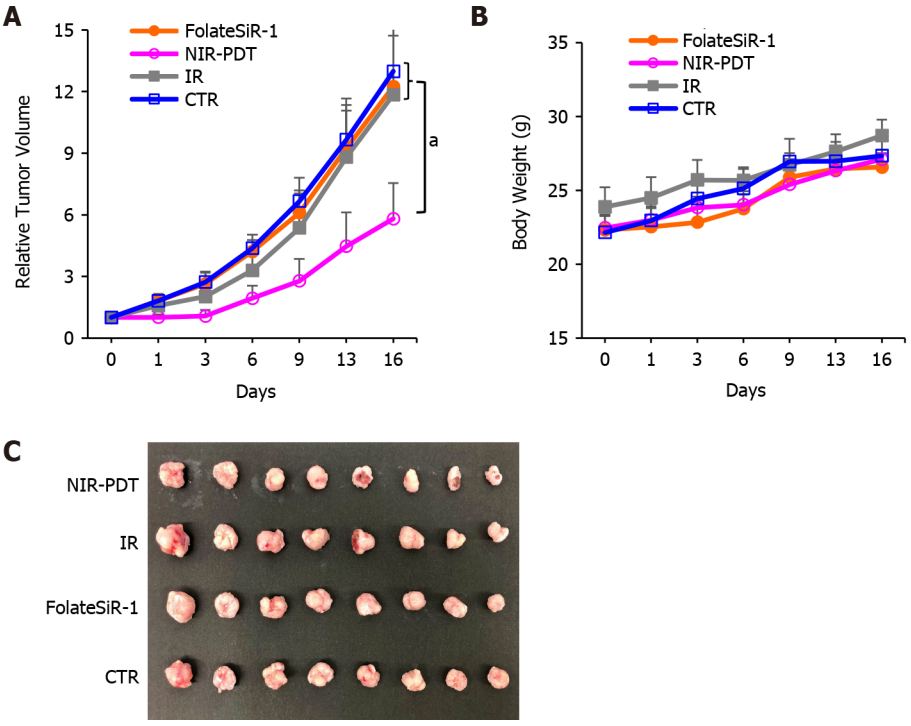


Figure 7 Phototherapeutic effects of photodynamic therapy on KB xenografts and body weight in mice. A: Tumor volume change is expressed as the relative tumor volume (volume on the indicated day/starting volume prior to the treatment. Tumors-treated by near-infrared photodynamic therapy (NIR-PDT) (50 J/cm^2), irradiation alone (50 J/cm^2), folate-Si-rhodamine-1 alone, and no treatment are shown in pink (○), gray (■), orange (●), and blue (□), respectively. Tumors-treated by NIR-PDT show significantly delayed growth compared to those of other groups. Values represent the mean ± SD (n = 8), $^aP < 0.05$ NIR-PDT *vs* other groups; B: Average body weight did not differ significantly among the four groups of mice; C: Images of *ex vivo* tumors excised 16 d after treatment. FolateSiR-1: Folate-Si-rhodamine-1; NIR-PDT: Near-infrared photodynamic therapy; IR: Laser irradiation; CTR: Control.

Our NIR-PDT regimen delivered a single dose of NIR light (50 J/cm^2) to KB tumors after intravenous administration of FolateSiR-1 (100 μg). The growth rate of tumors receiving PDT was significantly suppressed even with this single irradiation compared with the control groups (Figure 7). Complete tumor remission was not achieved with a single irradiation, however, partial remission for three days and delayed relapse was noted after PDT. Therefore, NIR-PDT using FolateSiR-1 is expected to be as

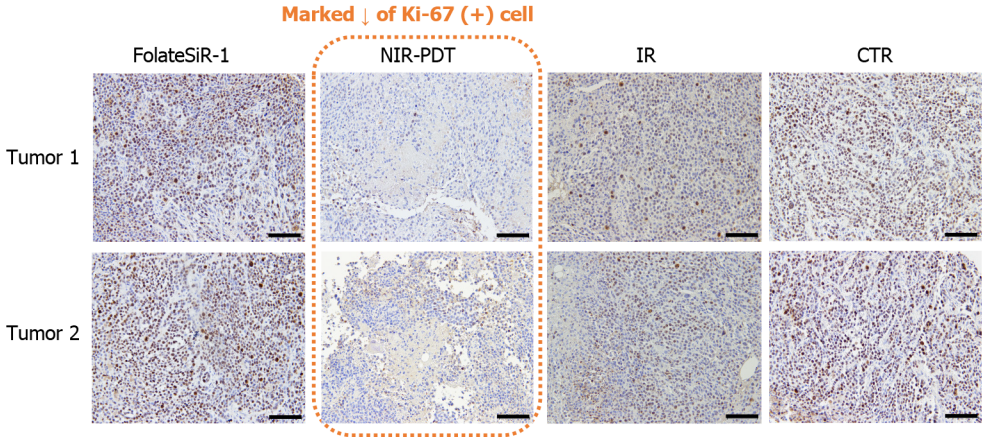


Figure 8 Tumor immunohistochemical staining of Ki-67 at 24 h after near-infrared photodynamic therapy. The weak staining and marked reduction of Ki-67-positive cells were detected in tumors treated with near-infrared photodynamic therapy (scale bar = 100 μm, 20 × magnification). FolateSiR-1: Folate-Si-rhodamine-1; NIR-PDT: Near-infrared photodynamic therapy; IR: Laser irradiation; CTR: Control.

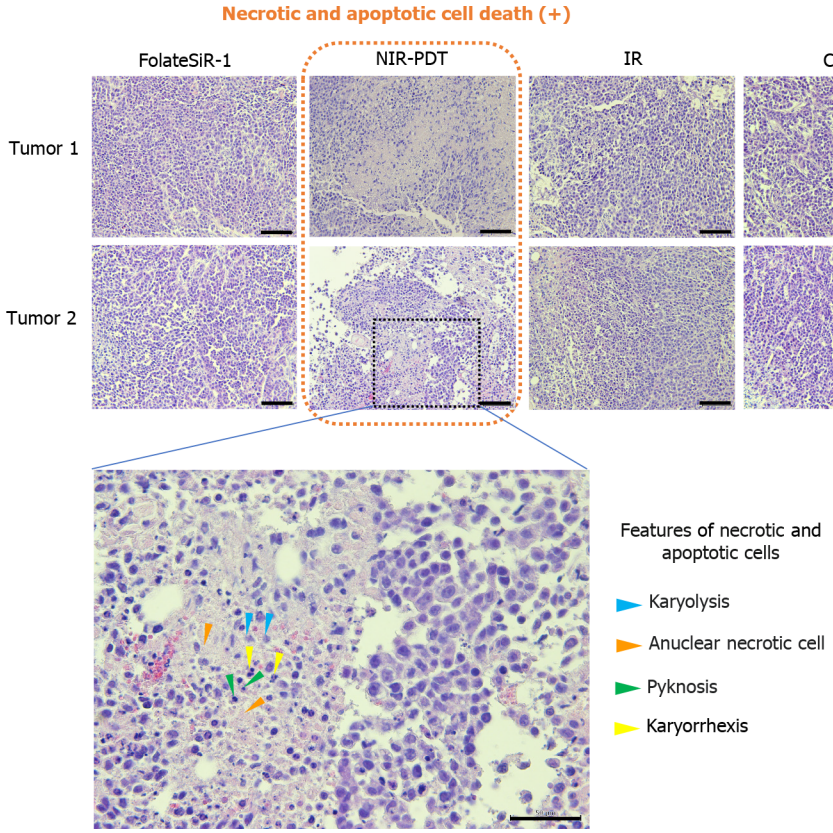


Figure 9 Histological examination of the destructive effects of near-infrared photodynamic therapy in tumor tissues 24 h after near-infrared photodynamic therapy. Tumors are stained with hematoxylin and eosin. The left lowermost image is a magnification of the indicated portion of the upper image. Some features of necrotic and apoptotic cells are shown with arrowheads: nuclear fading due to DNA loss, karyolysis (blue), anuclear necrotic cells with a glossier homogenous appearance (orange), nuclear chromatin condensation, pyknosis (green), nuclear fragmentation, and karyorrhexis (yellow). (Scale bar = 100 μm, 20 × magnification for upper two rows, and scale bar = 50 μm, 40 × magnification for lower row). FolateSiR-1: folate-Si-rhodamine-1; NIR-PDT: Near-infrared photodynamic therapy; IR: laser irradiation; CTR: control.

effective as other currently tested PDTs and PITs, because more precise treatment regimens can be easily designed by adjusting PS and irradiation doses, and repeating the treatment to improve the therapeutic efficacy and potentially achieving complete pathological remission. An extended study aiming to optimize the treatment will be performed in the future.

Photobleaching is the light-mediated destruction of PS. Linear correlation between protoporphyrin IX photobleaching and necrosis was used as a predictive tool of the PDT response in the rat ovarian cancer model[47]. Our limited mice experiments showed a tendency for FI reduction in tumors immediately

after PDT (Figure 5A and B), and this was probably due to some PS photobleaching and necrosis of cancer cells. An extended study is required to confirm this association.

Rapid phototoxic cell death-induced by NIR-PDT (10 J/cm^2) and abundant dead cells stained green with NucGreen® Dead reagent were clearly observed in viability imaging assays (Figure 3). In contrast, neither FolateSiR-1 alone nor irradiation alone induced noticeable cell death, suggesting that FolateSiR-1 is an effective PS with high phototoxicity and low dark toxicity.

Nuclear staining of the proliferative factor Ki67 in tumor cells using IHC indicating that these cells were actively proliferating at 24 h post-PDT. Meanwhile, tumors treated with PDT (50 J/cm^2) showed weak staining and a marked decrease in Ki-67 positive cells, suggesting that FolateSiR-1 mediated PDT inhibited cell proliferation in tumors (Figure 8). Therefore, some cells stop proliferating, but do not immediately undergo necrosis or apoptosis. In addition, tumors irradiated with targeted PDT exhibited rapid and extensive damage with a phenotype of necrosis and apoptotic cell death at the irradiated site as indicated by H&E staining. Some cells displayed necrotic cell death features such as nuclear fading (karyolysis) and anuclear necrotic cells, while other cells showed apoptotic cell death features such as chromatin condensation of the nucleus (pyknosis) and nuclear fragmentation (karyorrhexis). No distinct damage was found in the tumors of the other control groups (Figure 9). These results were consistent with that of tumor growth and revealed the presumptive mechanism of the effects of PDT.

The main mechanism of tumor destruction by PDT is the direct damage to cells[45], however, there are other indirect mechanisms such as damage to tumor blood vessels and nonspecific activation of the immune response against tumor cells[42,48]. As these mechanisms were not addressed in this study, additional research on this topic is required. Furthermore, only one FR-overexpressing cell line-derived subcutaneous tumor was used. The nature of the xenografted-tumor microenvironment does not reflect all of the characteristics of human cancer tissue. Not all tumors express high FR levels in a clinical setting. These drawbacks may limit the wide use of FR-targeted NIR-PDT. Nevertheless, further research may elucidate these limitations. The fascinating features of FolateSiR-1, including selectivity for tumor cells, membranous and subcellular localization, and cytotoxicity in combination with NIR irradiation, prompted the development of an alternative PS for NIR-PDT. The results of this study indicate that FR-targeted NIR-PDT using this PS may have potential for application in the treatment of FR-overexpressing tumors. Moreover, specific binding of FolateSiR-1 to FRs and emission of near-infrared light may be useful for decision making in various settings including patient screening for treatment selection, theranostic applications such as image-guided cancer detection and PDT, or in combination with other treatments.

CONCLUSION

FolateSiR-1 displayed FR-specificity *in vitro* and *in vivo* and functioned as a potential PS when irradiated with NIR light, causing cellular tumor damage by provoking the cumulative effect of necrosis, apoptosis, and cell proliferation-inhibition. These findings suggest that FolateSiR-1 may be effectively utilized in PDT with low side effects, and FR-targeted NIR-PDT can potentially provide new effective strategies for the treatment of FR-overexpressing tumors.

ARTICLE HIGHLIGHTS

Research background

Photodynamic therapy (PDT) is one of the emerging options to combat cancer and it requires photosensitizer (PS) and corresponding light irradiation. The tumor selectivity of the photosensitizer improves tumor localization in PDT, enhances tumor destruction, and reduces side effects due to off-target localization. Folate receptor (FR) membrane protein is frequently overexpressed in human cancer and specific active targeting of PS to FR can be achieved by conjugation with the folate moiety.

Research motivation

We previously developed a folate-linked, near-infrared (NIR)-sensitive probe folate-Si-rhodamine-1 (FolateSiR-1). The feasibility of NIR-PDT using FolateSiR-1 and appropriate light irradiation had not been determined and reqired elucidation.

Research objectives

The aim of this study was to evaluate the photodynamic therapeutic efficacy of FolateSiR-1 in a preclinical cancer model and determine the cell death mode induced by FolateSiR-1-based PDT.

Research methods

FolateSiR-1 was synthesized by conjugating a folate moiety to the Si-rhodamine derivative through a

negatively charged tripeptide linker. Utilizing FR-overexpressing cell line KB and low FR-expressing cell lines OVCAR-3 and A4, selective binding of FolateSiR-1 to FR was evaluated by fluorescence microscopy. Cell viability imaging assays was exploited to assess the phototoxic effect of FolateSiR-1. *In vivo* longitudinal fluorescence imaging was conducted to examine the time-dependent biodistribution of FolateSiR-1 and its specific accumulation in KB tumors. To evaluate PDT efficacy of FolateSiR-1, KB tumor-bearing mice were divided into four groups: (1) FolateSiR-1 alone; (2) FolateSiR-1 followed by NIR irradiation; (3) NIR irradiation alone; and (4) no treatment. Tumor volume measurement, as well as immunohistochemical (IHC) and histological examinations of tumors were performed to determine the effect of PDT.

Research results

FR-specific binding of FolateSiR-1 was observed by fluorescence microscopy and *in vivo* fluorescence imaging. Cell viability imaging assays indicated that NIR-PDT induced cell death. *In vivo* longitudinal fluorescence imaging showed rapid peak accumulation of FolateSiR-1 in KB tumors 2 h after injection. The tumor volumes in the PDT group were significantly reduced compared to the other groups ($P < 0.05$). IHC analysis revealed reduced numbers of proliferation marker Ki-67-positive cells in PDT treated tumors, and hematoxylin-eosin staining revealed features of necrotic- and apoptotic cell death.

Research conclusions

FolateSiR-1 may be effectively utilized in PDT with low side effects, and the FR-targeted NIR-PDT can potentially reveal new strategies for the treatment of FR-overexpressing tumors.

Research perspectives

The fascinating features of FolateSiR-1, including specificity to FR, cytotoxicity in combination with NIR irradiation and relatively fast clearance implying low toxicity, prompted the development of an alternative PS for NIR-PDT. The therapeutic effect was significant after a single dose of irradiation and may be optimized to achieve patient-specific clinical effects. Moreover, fluorescence emission from FolateSiR-1 may be used for real time cancer detection and patient screening for treatment selection. Further research may elucidate these additional details of these processes.

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FOOTNOTES

Author contributions: Aung W designed the study, conducted most of the experiments, analyzed the data, and wrote the manuscript; Hanaoka K, designed, synthesized, and provided the folate-Si-rhodamine-1; Tsuji AB and Higashi T coordinated the research and contributed to manuscript preparation; all authors critically reviewed and approved the final version of the article.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Is it possible to adopt the same oncological approach in urgent surgery for colon cancer?

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Abstract

BACKGROUND

Locoregional complications may occur in up to 30% of patients with colon cancer. As they are frequent events in the natural history of this disease, there should be a concern in offering an oncologically adequate surgical treatment to these patients.

AIM

To compare the oncological radicality of surgery for colon cancer between urgent and elective cases.

METHODS

One-hundred and eighty-nine consecutive patients with non-metastatic colon adenocarcinoma were studied over two years in a single institution, who underwent surgical resection as the first therapeutic approach, with 123 elective and 66 urgent cases. The assessment of oncological radicality was performed by analyzing the extension of the longitudinal margins of resection, the number of resected lymph nodes, and the percentage of surgeries with 12 or more resected lymph nodes. Other clinicopathological variables were compared between the two groups in terms of sex, age, tumor location, type of urgency, surgical access, staging, compromised lymph nodes rate, differentiation grade, angiolymphatic and perineural invasion, and early mortality.

RESULTS

There was no difference between the elective and urgency group concerning the longitudinal margin of resection (average of 6.1 in elective *vs* 7.3 cm in urgency, $P = 0.144$), number of resected lymph nodes (average of 17.7 in elective *vs* 16.6 in urgency, $P = 0.355$) and percentage of surgeries with 12 or more resected lymph

nodes (75.6% in elective *vs* 77.3% in urgency, $P = 0.798$). It was observed that the percentage of patients aged 80 and over was higher in the urgency group (13.0% in elective *vs* 25.8% in urgency, $P = 0.028$), and the early mortality was 4.9% in elective *vs* 15.2% in urgency ($P = 0.016$, OR: 3.48, 95%CI: 1.21–10.06). Tumor location ($P = 0.004$), surgery performed ($P = 0.016$) and surgical access ($P < 0.001$) were also different between the two groups. There was no difference in other clinicopathological variables studied.

CONCLUSION
Oncological radicality of colon cancer surgery may be achieved in both emergency and elective procedures.

Key Words: Colorectal cancer; Intestinal obstruction; Intestinal perforation; Surgical oncology; Lymph node excision

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Core Tip: The oncological radicality was compared between patients undergoing elective and urgent surgery for colon cancer. A total of 189 patients with nonmetastatic colorectal cancer who underwent surgical resection as the first therapeutic approach were included over two years in a single institution. The analysis of the oncological principles of the surgery, including the longitudinal margins of resection and the number of resected lymph nodes, revealed no statistical difference between elective and urgent surgeries. Therefore, the oncological principles of colorectal surgery should be followed in urgency as well as in elective cases.

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INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer worldwide, representing 1 148 515 new cases a year [1]. Although many signs of progress in early detection and systemic treatment have been achieved, surgical resection remains the only curative-intent treatment for localized colon cancer[2]. Therefore, the basic principles of surgery should be oncologically adequate[3]. Considering the inherent difficulty of urgent cases, mostly presenting with obstruction or bleeding, and surgical morbidity, the achievement of good oncological outcomes seems to be challenging. Thus, this study aimed to compare oncological radicality and surgical outcomes between patients who underwent colectomy for colon cancer in urgent or elective procedures.

MATERIALS AND METHODS

One hundred and eighty-nine consecutive patients with non-metastatic colon adenocarcinoma who underwent surgery with curative intent as the first therapeutic approach were selected, with or without colostomy, operated using urgent (66) or elective (123) procedures, from May 2016 to April 2018. All cases were operated at the General and Oncological Surgery Service of Hospital do Servidor Público Estadual de São Paulo (HSPE/SP), Brazil. The project was approved by the Universidade Federal de São Paulo Ethics Committee (CEP/UNIFESP: 0498/2019; approval decision: 3460953). The selected patients were divided into two groups: Urgency and Elective. Those admitted to the Emergency Room with a locoregional complication of colon cancer, whether obstruction or perforation, requiring a prompt surgical approach, were classified as "Urgency". In this study, no patient required urgent surgery for incoercible bleeding. Conversely, those who, despite the admission to the hospital *via* the Emergency Room, had their initial emergency controlled, making it possible to perform complete staging, preoperative examinations and assessments, and colon preparation according to the institution's routine, were classified as Electives, along with the cases scheduled on an outpatient basis. This study excluded patients with rectal cancer, metastatic disease, diagnosed before or during surgery, those who underwent other therapeutic interventions before surgical resection (colonic prosthesis, derivative surgery, neoadjuvant), histological types other than adenocarcinoma, as well as patients with

insufficient medical record data. Clinicopathological variables were selected to compare the "elective" and "urgency" groups. Histopathological analysis was performed by the Pathology Service of Hospital do Servidor Público Estadual, and no slide review was necessary to carry out in this study. All variables were collected by the chief researcher, retrospectively, through reviewing electronic medical records. The clinicopathological variables evaluated were longitudinal margin (cm), number of resected lymph nodes, percentage of surgeries with 12 or more resected lymph nodes, sex, age (years), tumor location, surgery performed, type of urgency, access route used, staging according to the AJCC UICC 8th edition (2017), rate of compromised lymph nodes, degree of differentiation, angiolymphatic and perineural invasion, and early mortality (up to 30 days). The oncological radicality for colectomies was assessed by the minimal longitudinal margin of resection of 5 cm and the harvesting of at least 12 lymph nodes (representing proximal ligature of colic vessels). Inferential analysis was performed using the R program version 3.5.2 R Core Team (2016). Pearson's Chi-square test or Fisher's exact test was applied when comparing groups for categorical variables. For numerical variables, the *t* test or Mann-Whitney test was applied in independent samples, and the Shapiro-Wilk test was used to determine the normality of numerical variables. In all conclusions obtained through inferential analyzes, an alpha significance level of 5% ($P < 0.05$) was used.

RESULTS

Of the 189 patients, 66 (34.9%) were in the urgency group and 123 in the elective group (65.1%). There was no difference between the two groups in terms of distribution by sex ($P = 0.632$). Higher mean age was observed in the urgency group (71.8 years) *vs* 68.1 years in the elective group, $P = 0.031$), with 25.8% of patients aged 80 years or older in the urgency group, against 130% in the elective group ($P = 0.028$). Regarding the type of urgency that led to surgery in the emergency room, 47 (71.2%) were due to obstruction and 19 (28.8%) to perforation, with no patient being operated on for bleeding. These general characteristics are summarized in Table 1. Urgency group had a higher early mortality (up to 30 d) than the elective group (15.2% *vs* 4.9%, $P = 0.016$, OR: 3.48, 95%CI: 1.21-10.06) and there was no difference in the interval for starting systemic chemotherapy when indicated (average of 75.2 *vs* 71.8 d, $P = 0.535$). There was a difference between the two groups directly related to the location of the tumor (Table 2). In both groups, there was a predominance of location in the sigmoid, followed by the ascending colon. The surgical access also differed between the two groups, with a higher frequency of surgeries performed by laparoscopy in the elective group (43.1%) *vs* 0% in urgency group ($P < 0.001$).

Pathological characteristics are summarized in Table 3. It was observed that there was no statistical difference between the groups concerning the T and N classification, staging, degree of differentiation, and presence of angiolymphatic and perineural invasion. It was noted that, in both groups, more than 80% of patients had advanced stages (II or III). The rate of compromised lymph nodes was also found to be similar between the two groups (8.1% *vs* 7.9%, $P = 0.785$). Regarding the variables referring to the oncological principles for colon cancer surgery, there was also no statistically significant difference in the urgency group when compared to the elective group (Table 4). The stratified analysis by the location of the tumor is summarized in Table 5. Tumors located in the cecum, ascending colon, and transversus (3 cases) were considered to be in the right colon; and those located at the splenic, descending colon, sigmoid, and transversus (2 cases) as in the left colon. Four cases of transverse tumors (2 in the elective group and 2 in the urgency group) were excluded from this stratification as they underwent transversectomy, and it was not able to assign them to the right or left colon. There was a difference in the longitudinal margin in the analysis of the left colon (4.8 in the elective *vs* 7.6 cm in urgency, $P = 0.003$), with all other variables being similar between the groups. Early mortality was analyzed (up to 30 d) in patients who underwent emergency surgery. It was observed that the mean age was significantly higher in patients who died (84.0 *vs* 69.6 years, $P < 0.001$, 95%CI: 7.2-21.6). Of the 10 patients who died, 8 (80.0%) were 80 years old or older, in contrast to the 56 patients who survived, in which only 9 (16.1%) were in this age group ($P < 0.001$). There was no statistically significant difference regarding early mortality between the two groups, as shown in Table 6.

DISCUSSION

One of the criteria for achieving oncological radicality involves the extension of the longitudinal margin of the colon, which must be 5 cm to 7 cm[4,5]. Regarding the radial margin, block resection of adjacent structures should be performed in case of direct invasion, given their tumoral involvement by contiguity[2,6]. Another oncological preconized principle is the complete resection of the main vascular pedicles with the corresponding lymphadenectomy[7]. The number of resected lymph nodes directly influences the prognosis of the patient with colon cancer[8,9], considering that at least 12 lymph nodes must be resected and evaluated for lymphadenectomy to be oncologically adequate[3]. A situation inherent to colon cancer is the presence of possible locoregional complications that lead to the need for urgent surgery[10], which can occur in up to 30% of cases[11]. Intestinal obstruction is the most common

Table 1 General characteristics of the patients

	Elective	Urgency	*P* value
Total	123 (65.1%)	66 (34.9%)	
Sex			0.632[a]
Male	51 (41.5%)	25 (37.9%)	
Female	72 (58.5%	41 (62.1%)	
Age (yr)			
mean ± SD	68.1 ± 11.1	71.8 ± 11.5	0.031[b]
< 80	107 (87.0%)	49 (74.2%)	0.028[a]
≥ 80	16 (13.0%)	17 (25.8%)	
Type of urgency			
Obstruction		47 (71.2%)	
Perforation		19 (28.8%)	

[a]Qui-square of Pearson test.
[b]*t* test for independent samples.

Table 2 Clinical and surgical characteristics

	Elective	Urgency	*P* value	OR and 95%CI
Location			0.004[c]	
Cecum/Ascendent	44 (35.8%)	18 (27.3%)		
Transverse	4 (3.3%)	5 (7.6%)		
Splenic Angle	0	4 (6.1%)		
Descendent	6 (4.9%)	9 (13.6%)		
Sigmoid	69 (56.1%)	30 (45.5%)		
Surgery1			0.016[c]	
Right colectomy	45 (36.6%)	20 (30.3%)		
Transversectomy	2 (1.6%)	2 (3.0%)		
Left colectomy	6 (4.9%)	11 (16.7%)		
Retosigmoidectomy	66 (53.7%)	27 (40.9%)		
Total colectomy	4 (3.3%)	6 (9.1%)		
Surgical access			< 0.001[a]	
Open	70 (56.9%)	66 (100%)		
Videolaparoscopy	53 (43.1%)	0		
Early mortality	6 (4.9%)	10 (15.2%)	0.016[a]	OR: 3.48, 95%CI: 1.21-10.06

[1]With or without enterostomy.
[a]Qui-square of Pearson's test.
[b]*t* test for independent samples.
[c]Exact Fisher's test.
[d]Mean and standard deviation.

locoregional complication, followed by intestinal perforation[12]. Incoercible bleeding is a less frequent cause of urgent indication for colon cancer because, in most cases, bleeding stops or reduces, either spontaneously or through endoscopic or hemodynamic therapies, allowing the elective surgery to be performed[13,14]. In the face of an emergency, whether perforation or obstruction, surgical resection should be proposed as the first therapeutic approach, provided that patients are in clinical conditions

Table 3 Pathological characteristics of the tumors

	Elective	Urgency	*P* value
T			0.278[c]
Tis	2 (1.6%)	0	
T1	6 (4.9%)	0	
T2	16 (13.0%)	6 (9.1%)	
T3	88 (71.5%)	54 (81.8%)	
T4	11 (8.9%)	6 (9.1%)	
N			0.943[a]
N0	76 (61.8%)	42 (63.6%)	
N1	32 (26.0%)	17 (25.8%)	
N2	15 (12.2%)	7 (10.6%)	
Staging			0.199[c]
0	2 (1.6%)	0	
I	20 (16.3%)	5 (7.6%)	
II	54 (43.9%)	37 (56.1%)	
III	47 (38.2%)	24 (36.4%)	
Differentiation grade			0.938[c]
Well	7 (6.0%)	3 (5.0%)	
Moderate	101 (87.1%)	52 (86.7%)	
Poor	8 (6.9%)	5 (8.3%)	
Compromised lymph nodes rated	8.1% ± 16.7	7.9% ± 17.1	0.785[c]
Angiolymphatic invasion	44 (36.4%)	21 (31.8%)	0.533[a]
Perineural invasion	19 (16.4%)	7 (11.1%)	0.339[a]
ALI + PNI	16 (13.0%)	5 (7.6%)	0.257[a]

[a]Qui-square of Pearson's test.
[b]*t* test for independent samples.
[c]Exact Fisher's test.
[d]Mean and SD.
[e]Mann Whitney test.
ALI: Angiolymphatic invasion; PNI: Perineural invasion.

for this purpose[15,16].

In emergency surgeries, however, it is observed that the oncological principles described above cannot always be contemplated, considering that locoregional complications can lead to abdominal sepsis, and patients may be complicated with pre-existing underlying diseases[17]. Thus, the surgeon must choose a less aggressive procedure to save the patient's life, avoiding any complications associated with more extensive surgeries[18]. In contrast, it is known that, despite the urgency, many patients are still able to undergo surgery with all the necessary oncological radical approaches.[19] As it is a frequent situation in the natural history of colon cancer, it is essential to be concerned regarding the oncological principles also in urgent surgeries. Teixeira *et al*[19] and Enciu *et al*[20] showed that it was possible to follow the oncological principles for colon cancer surgery even in emergency cases. In both studies there was no control group and, therefore, did not allow inferential analyzes to be carried out related to elective cases. Weixler *et al*[21] studied clinical and pathological data of patients with colorectal cancer who underwent emergency surgery, and included elective patients as a control group. In their study, , 747 patients were selected over 24 years, with 663 (88.8%) elective and 84 (11.2%) urgent cases. The percentage of patients who underwent emergency surgery was lower than that reported in other studies (about 30%), and the period of capturing patients was longer than most studies in this field[12,19,20]. The study showed that there was a statistically significant difference in relation to the percentage of surgeries with 12 or more resected lymph nodes ($P = 0.016$) and the presence of compromised margins ($P = 0.014$), showing a difference in the pattern of elective and urgent surgery. Despite these differences,

Table 4 Variables referring to oncological principles for colon cancer surgery

	Elective	Urgency	*P* value
Longitudinal margin (cm)			0.144[a]
mean ± SD	6.1 ± 4.7	7.3 ± 5.6	
Median	5.0	6.5	
Q1 e Q3	3.0 e 8.0	3.1 e 10.0	
Number of resected lymph nodes			0.355[a]
mean ± SD	17.7 ± 8.7	16.6 ± 8.3	
Median	16.0	14.0	
Q1 e Q3	12.0 e 22.0	12.0 e 20.0	
≥ 12 resected lymph nodes	93 (75.6%)	51 (77.3%)	0.798[b]

[a]Mann Whitney test.
[b]Qui-square of Pearson's test.
Q1: Quartil 1; Q3: Quartil 3.

Table 5 Variables referring to oncological principles for colon cancer surgery according to tumor location

	Right			Left		
	Elective	Urgency	*P* value	Elective	Urgency	*P* value
Total	45 (69.2%)	20 (30.8%)		76 (63.3%)	44 (36.7%)	
Longitudinal margin (cm)[1]	7.0 (5.0 - 10.0)	6.5 (2.8 - 10.3)	0.270[a]	4.0 (2.0 - 5.9)	6.5 (4.0 - 9.3)	0.002[a]
Number of resected lymph nodes[1]	19.0 (13.0 - 23.0)	19.5 (13.0 - 26.3)	0.446[a]	15.0 (11.0 - 19.3)	13.0 (11.0 - 17.3)	0.223[a]
≥12 resected lymph nodes	35 (77.8%)	19 (95.0%)	0.151[b]	56 (73.7%)	31 (70.5%)	0.832[c]

[1]p25 - p75.
[a]Mann Whitney test.
[b]Exact Fisher's test.
[c]Qui-square of Pearson's test.

the study demonstrated that the overall and disease-free survivals were not affected by emergency surgery[21].

Data available in the literature, therefore, are not able to show whether the emergency surgery for colon cancer is being performed with the same technical standard as the elective ones. The strength of the present study is based on the collected data from emergency and elective patients, in the same period of over two years in a single institution, which allowed the homogenization of the group of surgeons and pathologists. Elective patients constituted the ideal control group for the analysis of oncological radicality in the emergency surgery, which is the object of this investigation. In the hospital where the study was carried out, the same service is offered for elective and emergency oncological surgeries. Thus, in both situations, surgeons are duly qualified for coloncancer surgeries, ensuring the technical standard approach. It is known that the surgeon's experience and the volume of surgery at the institution have an impact on the short- and long-term prognosis of patients with colon cancer[22]. Although some patients underwent therapeutic interventions before surgical resection, such as colonic prosthesis or derivative surgery, they represented a small number of patients and there was a difficulty in allocating them between the elective and urgency groups. Thus, they were excluded from this study population. In the two main variables of the study, longitudinal margin and the number of resected lymph nodes, there was no statistically significant difference between the two groups. Thus, it was observed that, even in an emergency, it is feasible to perform an oncologically adequate surgery. Also, the percentage of surgeries with 12 or more resected lymph nodes was also similar between the groups, showing the same technical pattern of oncological radicality in urgency and elective approaches. The percentage values observed in this study are compatible with previous literature data[19,20]. The analysis of demographic characteristics revealed a statistically significant difference related to age. It was observed that urgency patients were older than elective ones. This data can be explained by the fact that elderly patients receive fewer screening tests for colon cancer, which increases the chance of presenting with symptomatic or complicated lesions. In addition, this hypothesis confirmation is not

Table 6 Analysis of early mortality (up to 30 d) after urgent surgery for colon cancer

	Early mortality	Survivals	*P* value	OR and 95%CI
Total	10 (15.2%)	56 (84.8%)		
Sex			0.076[a]	
Male	1 (10.0%)	24 (42.9%)		
Female	9 (90.0%)	32 (57.1%)		
Age (yr)				
mean and SD	84.0 ± 8.7	69.6 ± 10.7	< 0.001[b]	95%CI: 7.2-21.6
< 80	2 (20.0%)	47 (83.9%)	< 0.001[a]	
≥ 80	8 (80.0%)	9 (16.1%)		OR: 20.89, 95%CI: 3.79-115.00
Type of urgency			0.156[a]	
Obstruction	5 (50.0%)	41 (73.2%)		
Perforation	5 (50.0%)	15 (26.8%)		
Location			0.245[a]	
Cecum/Ascendent	2 (20.0%)	16 (28.6%)		
Transverse	2 (20.0%)	3 (5.4%)		
Splenic Angle	1 (10.0%)	3 (5.4%)		
Descendent	0	9 (16.1%)		
Sigmoid	5 (50.0%)	25 (44.6%)		
Surgery			0.059[a]	
Right colectomy	2 (20.0%)	18 (32.1%)		
Transversectomy	2 (20.0%)	0		
Left colectomy	1 (10.0%)	10 (17.9%)		
Retosigmoidectomy	5 (50.0%)	22 (39.3%)		
Total colectomy	0	6 (10.7%)		
T			0.407[a]	
T2	1 (10.0%)	5 (8.9%)		
T3	7 (70.0%)	47 (83.9%)		
T4	2 (20.0%)	4 (7.4%)		
N			0.100[a]	
N0	9 (90.0%)	33 (58.9%)		
N1	0	17 (30.4%)		
N2	1 (10.0%)	6 (10.7%)		
Staging			0.118[a]	
I	1 (10.0%)	4 (7.1%)		
II	8 (80.0%)	29 (51.8%)		
III	1 (10.0%)	23 (41.1%)		
Differentiation grade			> 0.999[a]	
Well	0	3 (5.4%)		
Moderate	9 (90.0%)	43 (76.8%)		
Poor	1 (10.0%)	4 (7.1%)		
Compromised lymph nodes rate	5.0% ± 15.8	8.4% ± 17.4	0.147[c]	
Angiolymphatic invasion	2 (20.0%)	19 (33.9%)	0.483[a]	

Perineural invasion	1 (10.0%)	6 (10.7%)	> 0.999[a]
ALI + PNI	1 (10.0%)	4 (7.1%)	0.573[a]
Margin (cm)1	5.3 (1.6-8.5)	7.0 (3.9-10.0)	0.400[c]
Number of resected lymph nodes1	12.5 (11.3-17.5)	14.0 (12.0-20.3)	0.306[c]
≥ 12 resected lymph nodes	7 (70.0%)	44 (78.6%)	0.683[a]

[1]p25-p75.
[a]Exact Fisher's test.
[b]*t* test for independent samples.
[c]Mann Whitney test.
Q1: Quartil 1, Q3: Quartil 3.

within the scope of this study. As for the type of urgency, it was observed that the prevalence of perforation (28.8%) was slightly higher than that reported in most of the literature, which is around 20% [11,12], yet similar distributions have also been demonstrated[23].

The early mortality rate was found about three times higher in urgency than in elective (15.2% *vs* 4.9%) cases. Morris *et al*[24] conducted an extensive population study in England, involving 160 920 individuals undergoing surgical resection for colorectal cancer, and also found a difference in early mortality with a similar proportion (14.9% *vs* 5.8%). Other studies have reported early emergency mortality rates ranging from 8.3%–34.0%, that is, it can be said that the mortality observed in this study is within the expected range according to previous literature data[19-22]. In the present study, when analyzing the clinicopathological characteristics between emergency patients who died and those who survived, a statistically significant difference was observed in relation to age. The percentage of the elderly was highest among those who died, of whom 80% were aged 80 years or over. Different from reports in the literature[25], there was no difference in pathological characteristics between those who died and survived, revealing those factors intrinsic to the patient would be more important than tumor staging for the outcome of early mortality. There was a statistically significant difference related to the resection approach employed, and it was observed that 43.1% of the elective surgeries were performed by laparoscopy, while all urgent surgeries were opened. The laparoscopic approaches in the urgency group were less expected based on their indissociable indications for urgency procedures (70.2% of bowel obstruction, and 28.8% of bowel perforation), and in older patients, possibly with more comorbidities. Nevertheless, the results suggest that even for patients in these unfavorable scenarios, patients of the urgency group obtained similar oncological outcomes concerning margin and node status to patients who underwent elective procedures. Laparoscopy was offered in the elective group, as much as possible, based on the current evidence in the literature that supports the oncological safety of minimally invasive colorectal surgery[26-28]. Thus, all patients regardless of their surgical approaches were used, in order not to exclude a certain group of patients or surgeons based on their practice. Ghazi *et al*[25] demonstrated the presence of more advanced tumors in the emergency room, with a higher rate of more advanced staging, greater angiolymphatic and perineural invasion, and a higher rate of compromised lymph nodes. However, our study did not reveal any significant difference between the elective and urgency groups in terms of staging, degree of differentiation, angiolymphatic invasion, perineural invasion and compromised lymph node rate. It is noteworthy that the present study revealed a low rate of early stages even in the elective group, which may be one of the reasons for not having observed this difference. Regarding the location of the tumor, some studies show a worse prognosis in the right colon compared to the left colon[29]. Furthermore, the extent of lymphadenectomy for colon cancer is still an object of study in the literature[30], most of which refer to the right colon, where there is a greater difficulty in standardizing the lymphadenectomy[9]. As there was a statistically significant difference between groups in terms of the location of the tumor in this study, this could bias the results regarding the oncological principles of surgery. However, in the analysis stratified by location, it was observed that, on the right, there was no difference between groups in relation to longitudinal margins, number of lymph nodes resected, or percentage of surgeries with 12 or more lymph nodes resected. On the left, lymphadenectomy was also similar between the groups, but there was a difference concerning the longitudinal margins, being lower in the elective group. The assessment of possible causes for this difference is out of the scope of this study.

Like any retrospective study, this study has limitations regarding the impact of inferential analyzes. In terms of long-term survival analyses, the study's limitations are associated with the immeasurable biases as seen in all retrospective studies, particularly those addressing oncologic outcomes. Selection bias based on several nonobjective criteria could have contributed to some of the differences between the two study groups. Because detailed data on systemic treatment, radiotherapy, or their toxicity were not reasonably available to analyze, they were not addressed in this study. However, for the investigation of oncological approach in urgency compared to elective surgeries, this is a useful and applicable model. The allocation to the elective or emergency group takes into account the patient's

clinical presentation, but not possible *via* any type of randomization. In 30-d mortality, our sample presented a small number of deceased patients to perform an adequate multivariate analysis. Thus, only univariate analysis was presented, and confounding bias cannot be excluded. In summary, we do believe that this study provides subsidies to recommend the oncologically adequate surgery to be performed even in an emergency for most patients. However, it also suggests that a more specific assessment of patients aged over 80 years is appropriate, especially due to the observed mortality. It should be noted that this conduct should be reserved for surgeons with experience in oncology surgery for colon cancer, as well as institutions with a high volume of this disease, as occurred in this study.

CONCLUSION

It is possible to achieve the oncological radicality of colon cancer surgery in both emergency and elective procedures.

ARTICLE HIGHLIGHTS

Research background

Locoregional complications of colon cancer may occur in up to 30% of patients. Many of these patients will need a surgical resection in an urgent scenario. Because of the patient's clinical deterioration, the oncological principles of surgery may be jeopardized.

Research motivation

We intended to determine whether the same oncological principles and surgical outcomes can be achieved in both urgent and elective colon cancer surgery.

Research objectives

This study aims to compare the oncological radicality of urgent surgery for colon cancer in comparison to elective cases.

Research methods

A total of 189 consecutive patients with colon cancer who underwent surgical resection as the first therapeutic approach were selected over two years in a single institution. The institution where the study was performed has a high volume of colorectal cancer patients (over 100 cases per year) and there are experienced surgeons in both elective and urgent situations. Patients were assigned to two groups: elective (123) and urgency (66). Clinicopathological variables were analyzed and compared retrospectively, including the longitudinal margin of resection and the number of harvested lymph nodes, between the two groups.

Research results

There was no significant difference between the two groups concerning the longitudinal margins of resection and the number of resected lymph nodes. A higher percentage of patients aged 80 and over was observed in the urgency group (25.8% *vs*. 13.0% in elective group, $P = 0.028$). Early mortality was higher in the urgency group (15.2% *vs* 4.9%), as expected according to previous studies.

Research conclusions

The oncological principles of colon cancer surgery can be adopted in urgency as well as in elective cases.

Research perspectives

Further studies are necessary to elucidate which patients should undergo classical oncological resection in urgency, especially in patients aged 80 and over, due to the higher early mortality in urgent approaches for this population. Intermediate interventions in urgent cases, such as derivative surgery or colonic prosthesis, require further studies as an alternative approach in high-risk patients.

FOOTNOTES

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Epidemiologic risk factors for patients admitted with chronic pancreatitis and pancreatic ductal adenocarcinoma in the United States

Daniel Lew, Fatima Kamal, Khiem Phan, Karamvir Randhawa, Sam Cornwell, Ayrton I Bangolo, Simcha Weissman, Stephen J Pandol

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Abstract

BACKGROUND

Epidemiological studies of chronic pancreatitis (CP) and its association with pancreatic ductal adenocarcinoma (PDAC) are limited. Understanding demographic and ethno-racial factors may help identify patients at the highest risk for CP and PDAC.

AIM

To evaluate the ethno-racial risk factors for CP and its association with PDAC. The secondary aim was to evaluate hospitalization outcomes in patients admitted with CP and PDAC.

METHODS

This retrospective cohort study used the 2016 and 2017 National Inpatient Sample databases. Patients included in the study had ICD-10 codes for CP and PDAC. The ethnic, socioeconomic, and racial backgrounds of patients with CP and PDAC were analyzed.

RESULTS

Hospital admissions for CP was 29 *per* 100000, and 2890 (0.78%) had PDAC. Blacks [adjusted odds ratio (aOR) 1.13], men (aOR 1.35), age 40 to 59 (aOR 2.60), and being overweight (aOR 1.34) were significantly associated with CP (all with $P < 0.01$). In patients with CP, Whites (aOR 1.23), higher income, older age (aOR 1.05), and being overweight (aOR 2.40) were all significantly associated with

PDAC (all with $P < 0.01$). Men (aOR 1.81) and Asians (aOR 15.19) had significantly increased mortality ($P < 0.05$). Hispanics had significantly increased hospital length of stay (aOR 5.24) ($P < 0.05$).

CONCLUSION

Based on this large, nationwide analysis, black men between 40-59 years old and overweight are at significantly increased risk for admission with CP. White men older than 40 years old and overweight with higher income were found to have significant associations with CP and PDAC. This discrepancy may reflect underlying differences in healthcare access and utilization among different socioeconomic and ethno-racial groups.

Key Words: Chronic pancreatitis; Pancreatic cancer; Ethno-racial; Risk factors; Hospitalization outcomes; Adult

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Core Tip: What is known chronic pancreatitis (CP) and pancreatic ductal adenocarcinoma (PDAC) rates are rising. Pancreatitis admissions costed 133 million dollars, and accounted for the 3rd leading cause of hospital admissions. There is lack of data identifying those at highest risk for admissions with CP and PDAC. What we found Black men between 40-59 years old and overweight are at significantly increased risk for admission with CP. White men with higher income were found to have significantly increased risk for admissions with CP and PDAC. Asians/Pacific Islanders had the highest risk for mortality from CP and PDAC.

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INTRODUCTION

The overall incidence of chronic pancreatitis (CP) is increasing worldwide, and accounts for significant healthcare utilization and costs. In the United States, CP hospital admissions costed 133 million dollars, and pancreatitis accounted for the 3rd leading cause of hospital admissions among gastrointestinal diseases in 2015[1]. The most common reason for CP-related admission is abdominal pain, but other complications can develop including pancreatic ductal adenocarcinoma (PDAC). PDAC is currently the fourth leading cause of cancer-related deaths in the United States and is projected to become the second leading cause by 2030[2]. CP is the major risk factor for developing PDAC[3-5].

There have been few epidemiological studies available for CP[6-14], and fewer studies for CP and PDAC[3,15-18]. Ethnic and socioeconomic factors including insurance status, median income, type and hospital factors are lacking.

Herein, we utilize hospital discharges from a large nationwide database to examine the demographic, ethno-racial, socioeconomic, and hospital factors associated with hospitalizations for CP and its association with PDAC. We also sought to determine the association between ethnicity/race on hospitalization outcomes in patients admitted with CP and PDAC.

MATERIALS AND METHODS

Data source

This retrospective cohort study utilized the 2016 and 2017 National Inpatient Sample (NIS) databases. The NIS is a database of inpatient stays derived from billing data based upon discharge abstracts. As such, it contains de-identified clinical and nonclinical elements at both the patient and hospital level. The NIS 2016 database contains data from 7.1 million hospital stays in 4575 hospitals in 47 states, while the 2017 database contains data from 7.1 million hospital stays in 4584 hospitals in 48 states. Using the combination of the NIS 2016 and 2017 databases allowed for inclusion of a greater total number of cases/patients.

Study population

Patients included in the study carried a primary diagnosis of CP based on ICD-10 codes (K86.0, K86.1, K90.3). The demographics and ethnic/racial profiles of those with CP were compared to the general population and examined for any associations. Subsequently, all patients with CP were then evaluated for PDAC based on ICD-10 diagnostic codes C25.0-C25.9. The demographic and ethnic/racial profiles of those with only CP were compared to the ethnic/racial profiles of those who also had PDAC and were examined for any associations. Finally, we analyzed the associations between ethnicity/race and hospitalization outcomes in patients admitted for PDAC.

Inclusion and exclusion criteria are shown in Figure 1. Institutional Review Board approval was not required for this study as it was performed using de-identified and nationally available data.

Census data

The estimated national population during 2016-2017 was obtained from the United States Census Bureau (www.census.gov).

Study variables

Patient demographics included age, gender, race, median household income, primary expected payer, hospital bed size, hospital teaching status, hospital region, and urban location. Burden of comorbidities was assessed using the Charlson comorbidity index.

Study outcomes

The primary outcomes were the ethno-racial factors associated with CP hospital admissions as compared to the general population and the ethno-racial factors associated between CP and PDAC. Secondary outcomes were the associations between ethnicity/race and hospitalization outcomes (mortality, hospital length of stay (LOS), expenses), in patients admitted with CP and PDAC.

Statistical analysis

Statistical analyses were performed using STATA, version 16.0 (StataCorp., College Station, Texas, United States). Weighting of patient-level observations was implemented. Univariate analysis was initially performed to calculate unadjusted odds ratio and determine confounders significantly associated with the outcomes. Multivariate regression analysis was used to adjust for potential confounders. Multivariate regression model was then built by including all confounders that were found to be significant by univariate analysis, to calculate an adjusted odds ratio (aOR). Logistic regression was used for binary outcomes and linear regression was used for continuous outcomes. Proportions were compared using Fisher's exact test, and continuous variables were compared using Student's *t*-test. All *P*-values were two-sided, with 0.05 as the threshold for statistical significance. Calculated rates of hospital admissions *per* 100000 population was performed using the United States Census Bureau national population estimates.

RESULTS

CP cohort

In our sample (NIS 2016-2017) of 14.2 million admissions, 371275 (2.6%) adult patients were found to have a diagnosis of CP. The mean age was 57.72 years, and most patients were white (64.82%) men (55.76%). Medicare was the primary payer insurance (40.2%). The majority of admissions were in teaching hospitals (69.54%). Additional patient and hospital characteristics are presented in Table 1. Overall hospitalization rate for 2016 and 2017 was 29 *per* 100000 (95%CI: 57.9-58.2).

On multivariate regressions analysis, men (aOR 1.35), Blacks (aOR 1.13), age between 40 and 59 years old (aOR 2.60), and body mass index (BMI) between 25 and 29.9 (aOR 1.34) were at higher risk of developing CP as compared to the general population ($P < 0.01$). On the contrary, women (aOR 0.65), Hispanics (aOR 0.63) or Asian/Pacific Islanders (aOR 0.50) and those above 80 years old (aOR 0.46) had a significantly decreased likelihood of having CP as compared to the general population (all with $P < 0.01$) (Table 2).

PDAC and CP cohort

Of the 371275 adult patients admitted with a primary diagnosis of CP, 2890 (0.78%) also had PDAC. Patients with PDAC and CP were significantly older ($P < 0.05$), White ($P < 0.01$), had Medicare as the primary insurance ($P < 0.01$), were from a higher income population ($P < 0.01$), were admitted to teaching hospitals ($P < 0.01$), and had a higher burden of comorbidities ($P < 0.01$) (Table 3). Overall hospitalization rate for 2016 and 2017 was 0.45 *per* 100000 (95%CI: 0.44-0.47).

On multivariate regressions analysis, older age (greater than 40 years old) (aOR 1.05), and BMI between 25 and 29.9 (aOR 2.40) had a higher risk of developing PDAC in patients with CP (all with $P < 0.01$). On the contrary, women (aOR 0.77), Blacks (aOR 0.77) and Hispanics (aOR 0.66) were associated

Table 1 Demographics, ethno-racial, socioeconomic, and hospital factors for patients hospitalized with chronic pancreatitis

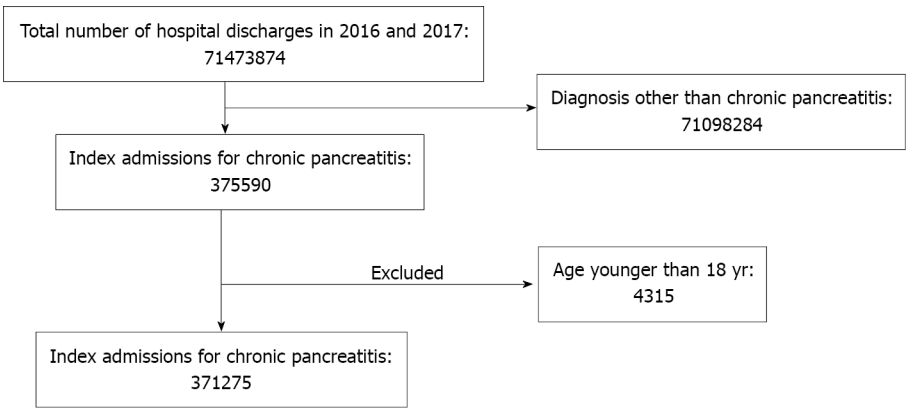
Variable	*n* = 371275
Race	
White	64.82%
Black	22.35%
Hispanic	8.4%
Asian or Pacific Islander	1.31%
Native American	0.87%
Other	2.25%
Gender	
Women	44.24%
Men	55.76%
Age	
Mean age, years	52.72
Age 18-39 yr	21.63%
Age 40-59 yr	49.84%
Age 60-79 yr	24.26%
Age > 80 yr	4.28%
Insurance provider	
Medicare	40.2%
Medicaid	30.28%
Private	22.53%
Uninsured	6.99%
Charlson comorbidity index	
0	24.09%
1	25.97%
2	17.37%
3 or more	32.58%
Median income in patient zip code	
$1–$38999	36.17%
$39000–$47999	26.11%
$48000–$62999	21.78%
$63000	15.94%
Hospital region	
Northwest	17.54%
Midwest	24.63%
South	40.23%
West	17.6%
Hospital location	
Rural	8.29%
Urban	91.71%
Hospital size	
Small	19%

Medium	28.34%
Large	52.65%
Type of hospital	
Teaching	69.54%
Non-teaching	30.46%

Table 2 Multivariate regressions analysis for demographic, socioeconomic, and hospital factors associated with hospitalizations secondary to chronic pancreatitis

Classification		*P* value
Age	aOR 0.98	< 0.01
Age 18-39 yr	Reference	
Age 40-59 yr	aOR 2.60	< 0.01
Age 60-79 yr	aOR 0.96	0.37
Age > 80 yr	aOR 0.46	< 0.01
BMI: 18-24.9	Reference	
BMI: 25-29.9	aOR 1.34	< 0.01
BMI: 30-39.9	aOR 0.62	< 0.01
Women	aOR 0.65	< 0.01
Whites	Reference	
Blacks	1.13	< 0.01
Hispanics	0.63	< 0.01
Asians/Pacific Islanders	0.50	< 0.01
Native Americans	1.05	0.31
Other	0.67	< 0.01

aOR: Adjusted odds ratio; BMI: Body mass index.



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Figure 1 Flowchart for patient inclusion.

with a significantly decreased likelihood of PDAC in patients with CP (all with $P < 0.01$) (Table 4).

Hospitalization outcomes in PDAC and CP

Men (aOR 1.81) and Asians/Pacific Islanders (aOR 15.19) had the highest mortality among patients with PDAC and CP (all with $P < 0.05$). Hispanics had the highest hospital LOS (aOR 5.24), charges (adjusted

Table 3 Demographics, ethno-racial, socioeconomic, and hospital factors for patients hospitalized with chronic pancreatitis and pancreatic ductal adenocarcinoma

Variable	CP without PDAC (*n* = 368385)	CP with PDAC (*n* = 2890)	*P* value
Race			< 0.01
Whites	64.75%	74.22%	
Blacks	22.4%	16.76%	
Hispanics	8.43%	4.97%	
Asians or Pacific Islanders	1.31%	1.29%	
Native Americans	0.87%	0.37%	
Other	2.25%	2.39%	
Gender			
Women	44.27%	40.31%	0.07
Men	55.73%	59.69%	
Age			
Mean age, years	52.61	66.32	< 0.05
Age 18-39 yr	21.78%	19%	
Age 40-59 yr	50.02%	26.99%	
Age 60-79 yr	23.97%	60.03%	
Age > 80 yr	4.23%	11.07%	
Insurance provider			< 0.01
Medicare	40.04%	60.64%	
Medicaid	30.44%	10.73%	
Private	22.5%	26.48%	
Uninsured	7.03%	2.15%	
Charlson comorbidity index			< 0.01
0	24.28%	0	
1	26.17%	0	
2	17.35%	19.38%	
3 or more	32.2%	80.62%	
Median income in patient zip code			< 0.01
$1–$38999	36.24%	28.22%	
$39000–$47999	26.1%	26.81%	
$48000–$62999	21.78%	21.34%	
$63000	15.88%	23.63%	
Hospital region			0.68
Northwest	17.53%	19.38%	
Midwest	24.64%	23.53%	
South	40.25%	38.24%	
West	17.59%	18.86%	
Hospital location			< 0.01
Rural	8.34%	1.56%	
Urban	91.66%	98.44%	
Hospital size			< 0.01

Small	19.08%	8.65%	
Medium	28.43%	16.78%	
Large	52.48%	74.57%	
Type of hospital			
Teaching	69.41%	86.16%	< 0.01
Non-teaching	30.59%	13.84%	

CP: Chronic pancreatitis; PDAC: Pancreatic ductal adenocarcinoma.

Table 4 Multivariate regressions analysis for demographic, socioeconomic, and hospital factors associated with hospitalizations in patients with chronic pancreatitis and pancreatic ductal adenocarcinoma

Classification		*P* value
Age	aOR 1.05	< 0.01
Age 18-39 yr	Reference	
Age 40-59 yr	aOR 6.79	< 0.01
Age 60-79 yr	aOR 24.17	< 0.01
Age > 80 yr	aOR 21.02	< 0.01
BMI: 18-24.9	Reference	
BMI: 25-29.9	aOR 2.40	< 0.01
Female	aOR 0.77	< 0.01
White	Reference	
Black	aOR 0.77	< 0.05
Hispanic	aOR 0.66	< 0.05
Asian or Pacific Islander	aOR 0.69	0.36
Native American	aOR 0.68	0.60
Other	aOR 0.97	0.94

aOR: Adjusted odds ratio; BMI: Body mass index.

coefficient $87285), and costs (adjusted coefficient $15212) amongst patients with PDAC and CP (all with $P < 0.05$). Gender was not found to be a significant contributing factor for hospital expenses.

DISCUSSION

In this nationwide cohort study, we found black men who were overweight had a significantly increased likelihood of being admitted with CP. These findings are consistent with previous studies, most likely due to alcoholic CP, though our current study is not able to definitively elucidate the cause of CP[14]. Our hospitalization rates for CP is much higher compared to the study by Yang *et al*[13], which also used the NIS database from 1988-2004 and found an incident rate of 7.0-8.1 *per* 100000 population[13]. Similarly, population-based studies from Olmsted County, MN, United States found an incident rate of 4.05 *per* 100000 population from 1997-2006 and Allegheny County, PA, United States found incident rate of 7.75 *per* 100000 population[11,12]. Our findings of an increased incidence rate may reflect recent trends in increased hospitalizations with pancreatitis being the 3rd most common reason for hospitalization in 2015 among gastrointestinal diseases[1]. This is likely due in part to increased use of cross-sectional imaging for the evaluation of abdominal pain in adults[19]. Another possible explanation is that our findings may be more of a reflection of prevalence rate rather than incidence rate. In 2014, Lévy *et al*[20] summarized 11 studies and found a prevalence rate ranging up to 41.76 *per* 100000 population[20]. Additionally, patients with CP are reported to have good overall survival despite complications with reported 10-year survival rate of 70%[17]. Taken together, our increased hospitalization rate is likely due to increased diagnosis of CP and longevity of patients with CP.

Other novel demographic findings from our study include the findings that patients with CP-related admissions had predominantly Medicare insurance and were from lower median income families. Additionally, we found patients were more likely to be admitted to teaching hospitals in large urban centers, predominantly in the Southern part of the United States. A potential reason for this finding may correlate with the South having the highest prevalence of obesity at 30% and higher. Furthermore, non-Hispanic Blacks were found to have the highest prevalence of obesity[21]. Our hope is that future studies can use our findings to further evaluate the exact association of CP-related admissions in this high-risk population in an effort to improve patient outcomes and decrease healthcare expenditures. While our study is not able to elucidate the reason for admission, it is presumed that abdominal pain is most likely the cause given extensive findings from previous studies[14,22]. Given that it is well established that abdominal pain is chronic, persistent, and can lead to significant impairment in quality of life including high rates of unemployment as well as recurrent hospitalizations and healthcare expenditure, future research in this area is needed[23,24]. The findings from our study can be used in future studies to target those patients at higher risk for CP-related hospitalizations to improve patient outcomes and limit healthcare expenditures.

A potential complication of CP is the development of PDAC. In our study, we found that 0.78% (approximately 0.39%/year) of patients with CP also carried a diagnosis of PDAC and that these patients were predominantly White men who were overweight and of older age. Our findings are similar to the 2014 Danish nationwide study by Bang *et al*[3], which found a 4.26% rate over a 15-year period (approximately 0.28%/year) of PDAC in CP patients occurring predominantly in older men[3]. While there is moderate amount of literature for the ethno-racial factors for CP and PDAC separately, there is limited data regarding ethno-racial factors specifically in patients with CP and PDAC. Bracci *et al*[25] in 2009 found in a multicenter study that majority of patients with PDAC were White men who were older, however, they did not provide demographic data specifically for PDAC in CP patients[25]. In a meta-analysis published in 2010 containing 22 studies, Raimondi *et al*[15] found a 13 fold increased risk for PDAC in CP patients but did not evaluate the ethno-racial factors[15]. A more recent meta-analysis published in 2017 by Kirkegård *et al*[4] also found an increased risk for PDAC in CP patients but again did not evaluate the ethno-racial factors[4]. To our knowledge, our study is the first to evaluate ethno-racial factors in patients with CP and PDAC. A major caveat to our findings is that we cannot definitively evaluate PDAC development as a progression of CP. Thus, there could be cases where PDAC occurred first and CP developed afterwards. However, current literature suggests the overwhelming likelihood of this occurring is extremely low and may actually be misclassifications given the similarities between the two diseases[4]. An interesting finding from our study was that we found that Blacks had a higher risk for having CP, but this did not translate into having a higher association with CP and PDAC. A potential explanation could be due to discrepancies in healthcare utilization given that our data showed patients with CP and PDAC were significantly more likely to have higher median incomes, lower rates of being uninsured, and high rates of being admitted to large urban teaching hospitals when compared to CP patients alone. Racial disparities have been shown for PDAC in blacks. Khawja *et al*[26] conducted an evidence-based review of PDAC in blacks and found significantly worse outcomes compared to whites. A 2019 review also showed blacks had worse outcomes, but also lower referral rates to see oncology or a surgeon and lower rates of surgical resection and adjuvant chemoradiation[26,27]. Potential reasons for this discrepancy include suboptimal patient communication, greater mistrust of the medical field, and unmeasured differences in morbidity/functional status. Future studies should further evaluate the accuracy of this finding and to evaluate the exact cause of this discrepancy if one exists. Another interesting finding from our study is that we found Asians/Pacific Islanders had an aOR 15.19. This is in contrast to recent data from the American Cancer Society, which showed Blacks had the highest risk of mortality at 15.0/100000 compared to 8.1/100000 with Asians/Pacific Islanders[2]. A possible explanation is that our study evaluated patients with PDAC and CP rather than all PDAC patients, though it is beyond the scope of this study as to why Asians/Pacific Islanders had the highest mortality. Finally, we found that Hispanics had the highest LOS and hospital expenses. Future studies should further evaluate these findings in an effort to identify reasons for increased mortality among Asians/Pacific Islanders and increased LOS and hospital expenses among Hispanics, respectively, to improve patient outcomes and decreased healthcare expenditures.

Limitations of this study include it being limited solely to inpatient encounters. Therefore, our study may not represent a complete epidemiological study given its lack of outpatient encounters. As mentioned previously, there are limitations associated with using the NIS database. We are not able to identify individual patients and review each patients' charts, thus we are not able to identify the reason for CP-related admissions though current literature suggest most likely related to abdominal pain. Additionally, we are not able to determine if patients with CP progressed to PDAC or if PDAC occurred before CP, but again current literature suggests majority of patients develop PDAC after CP. Incidence of PDAC in CP is related to etiology of CP. Risk is significantly higher in hereditary pancreatitis than with alcohol related pancreatitis. As the etiology is not captured in the database it might introduce bias. Duration of CP could not be found, as longer history associated with increased risk. Findings may be applicable only to the population studied and cannot be generalized.

CONCLUSION

In conclusion, based on this large, nationwide analysis, there is an increased trend in hospitalization rates for CP, predominantly among black men between 40-59 years old who were overweight. In patients with CP, white men older than 40 years old who were overweight with higher income had a higher risk of PDAC. The observed disparity may be a consequence of the difference in healthcare access among different ethnic groups.

ARTICLE HIGHLIGHTS

Research background

Chronic pancreatitis (CP) and pancreatic ductal adenocarcinoma (PDAC) rates are rising. Pancreatitis admissions costed 133 million dollars, and accounted for the 3rd leading cause of hospital admissions. There is lack of data identifying those at highest risk for admissions with CP and PDAC.

Research motivation

The main motivation of this study was to establish racial risk factors and their associations with PDAC.

Research objectives

This study had the objective to examine the demographic, ethno-racial, socioeconomic, and hospital factors associated with hospitalizations for CP and its association with PDAC.

Research methods

This retrospective study used the 2016 and 2017 National Inpatient Sample databases. ICD-10 codes compatible with CP and PDAC were used in the study. The ethnic, socioeconomic, and racial backgrounds of patients with CP and PDAC were analyzed.

Research results

Hospital admissions for CP was 29 *per* 100000, and 2890 (0.78%) had PDAC. Blacks [adjusted odds ratio (aOR) 1.13], men (aOR 1.35), age 40 to 59 (aOR 2.60), and being overweight (aOR 1.34) were significantly associated with CP (all with $P < 0.01$). In patients with CP, Whites (aOR 1.23), higher income, older age (aOR 1.05), and being overweight (aOR 2.40) were all significantly associated with PDAC (all with $P < 0.01$). Men (aOR 1.81) and Asians (aOR 15.19) had significantly increased mortality ($P < 0.05$). Hispanics had significantly increased hospital length of stay (aOR 5.24) ($P < 0.05$).

Research conclusions

There is an increased trend in hospitalization rates for CP, predominantly among black men between 40-59 years old who were overweight. Wealthy white men above the age of 40 had a higher PDAC diagnosis.

Research perspectives

Black men between 40-59 years old and overweight are at significantly increased risk for admission with CP. White men with higher income were found to have significantly increased risk for admissions with CP and PDAC. Asians/Pacific Islanders had the highest risk for mortality from CP and PDAC.

FOOTNOTES

Author contributions: Lew D, Kamal F, Phan K, Randhawa K, Cornwell S, and Weissman S assisted with data acquisition, analyses, and manuscript preparation; Lew D drafted and critically revised the manuscript; Lew D, Weissman S, Bangolo AI, and Pandol SJ provided input regarding methodology; Pandol SJ critically revised the manuscript and provided direct supervision and guidance; Weissman S is the article guarantor; All authors agree to the final version of this manuscript.

Institutional review board statement: This retrospective cohort study utilized the 2016 and 2017 National Inpatient Sample (NIS) databases. The NIS is a database of inpatient stays derived from billing data based upon discharge abstracts. As such, it contains de-identified clinical and nonclinical elements at both the patient and hospital level. Making the need for an Institutional review board approval dispensable.

Informed consent statement: The National Inpatient Database was a public-use dataset, of which the informed consent was waived.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: This retrospective cohort study utilized the 2016 and 2017 National Inpatient Sample (NIS) databases. The NIS is a database of inpatient stays derived from billing data based upon discharge abstracts. As such, it contains de-identified clinical and nonclinical elements at both the patient and hospital level. The dataset is publicly available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Efficacy of texture analysis of pre-operative magnetic resonance imaging in predicting microvascular invasion in hepatocellular carcinoma

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Abstract

BACKGROUND

Presence of microvascular invasion (MVI) indicates poorer prognosis post-curative resection of hepatocellular carcinoma (HCC), with an increased chance of tumour recurrence. By present standards, MVI can only be diagnosed post-operatively on histopathology. Texture analysis potentially allows identification of patients who are considered 'high risk' through analysis of pre-operative magnetic resonance imaging (MRI) studies. This will allow for better patient selection, improved individualised therapy (such as extended surgical margins or adjuvant therapy) and pre-operative prognostication.

AIM

This study aims to evaluate the accuracy of texture analysis on pre-operative MRI in predicting MVI in HCC.

METHODS

Retrospective review of patients with new cases of HCC who underwent hepatectomy between 2007 and 2015 was performed. Exclusion criteria: No pre-operative MRI, significant movement artefacts, loss-to-follow-up, ruptured HCCs, previous hepatectomy and adjuvant therapy. Fifty patients were divided into MVI

(n = 15) and non-MVI (n = 35) groups based on tumour histology. Selected images of the tumour on post-contrast-enhanced T1-weighted MRI were analysed. Both qualitative (performed by radiologists) and quantitative data (performed by software) were obtained. Radiomics texture parameters were extracted based on the largest cross-sectional area of each tumor and analysed using MaZda software. Five separate methods were performed. Methods 1, 2 and 3 exclusively made use of features derived from arterial, portovenous and equilibrium phases respectively. Methods 4 and 5 made use of the comparatively significant features to attain optimal performance.

RESULTS

Method 5 achieved the highest accuracy of 87.8% with sensitivity of 73% and specificity of 94%.

CONCLUSION

Texture analysis of tumours on pre-operative MRI can predict presence of MVI in HCC with accuracies of up to 87.8% and can potentially impact clinical management.

Key Words: Carcinoma; Hepatocellular; Magnetic resonance imaging; Liver neoplasms; Retrospective studies; Margins of excision

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Core Tip: This study demonstrates the utility of texture analysis on pre-operative magnetic resonance imaging to potentially impact clinical management in patients with surgically resectable hepatocellular carcinoma.

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INTRODUCTION

Macrovascular invasion and microvascular invasion (MVI) are independent prognostic factors of hepatocellular carcinoma (HCC) recurrence after curative partial hepatectomy or liver transplantation[1-3]. Previous studies have shown MVI to shorten disease-free survival and overall survival post-liver transplantation and liver resection[4]. While presence of macrovascular invasion can often be determined definitively on pre-operative cross-sectional imaging, microvascular invasion in HCC is typically only diagnosed post-operatively on histopathology. Moreover, the lack of consensus definition and grading of MVI, coupled with inter/intra-observer variability, has resulted in great heterogeneity in evaluation of this histopathological feature[5]. Previous studies have shown that the incidence of MVI ranges between 15% and 57.1%[4]. Pre-operative diagnosis of microvascular invasion to identify patients who are considered 'high risk' will allow for better patient selection, improved individualised therapy (such as extended surgical margins or adjuvant therapy) and pre-operative prognostication.

Texture analysis (TA) is a branch of computer vision that analyses and objectifies imaging characteristics that may be imperceptible to the human eye. It can be applied to any cross-sectional imaging and has been proven to reflect underlying heterogeneity. This technique may provide quantitative data and insight into tumor biology and thus has the potential to be used to diagnose and prognosticate disease [6-9]. Several groups have attempted to identify MVI pre-operatively using clinical data and imaging scoring systems albeit with variable and sometimes conflicting results[10-12], limiting its translation into clinical practice. Moreover, the detection of MVI by using pre-operative biopsy has proven to be unreliable as it did not correlate well with post-operative pathology[13]. Recent studies have sought to use TA to predict MVI on MRI and have identified certain imaging and textural features (such as tumour entropy) that may be associated with bad tumour behaviour[14,15]. While TA continues to grow as an emerging technology, before it can be considered for widespread clinical implementation, we sought to validate and replicate those findings. In this study, we aim to evaluate the accuracy of texture analysis on pre-operative contrast-enhanced MRI to predict microvascular invasion in HCC.

MATERIALS AND METHODS

Study cohort

Institutional review board was obtained and the requirement to obtain written consent was waived. All patients who underwent hepatectomy between January 1, 2007 and December 31, 2015 were considered for study inclusion. Clinical and pathologic parameters were retrospectively reviewed on electronic medical records. Inclusion criteria included histologically proven HCC with pre-operative MRI (done with 1.5T), tumour size ≥ 1 cm, treatment-naïve HCC. Exclusion criteria included loss to follow-up, collision tumours, ruptured HCCs, previous hepatectomy and interval adjuvant therapy, such as transarterial chemoembolization or radiofrequency ablation. For texture analysis evaluation, additional imaging-specific exclusion criteria included 3 T MRI, outdated MRI protocols, and images degraded by motion artefacts.

A total of 129 hepatectomies were performed on 129 HCC patients within the study period. 79 patients were excluded from the study for the following reasons: absence of pre-operative MRI (n = 37), interval adjuvant therapy or prior hepatectomy (n = 18), loss to follow-up (n = 12), 3 T MRI (n = 6), ruptured HCC (n = 2), old MRI protocol (n = 2), collision tumour (n = 1), movement artefact (n = 1). A representative flowchart is shown in Figure 1.

The primary outcome measure was the presence of MVI based on histopathological findings. Clinical factors that were potentially associated with MVI were analysed: age, gender, aetiology, hepatitis B surface antigen (HBsAg), serum alpha-fetoprotein, albumin, bilirubin, alanine amino-transferase, aspartate amino-transferase, alkaline phosphatase, ɣ-glutamyltranspeptidase (GGT) and Child-Pugh score.

MRI technique

Magnetic resonance (MR) imaging studies were performed using 1.5 T scanners (Signa HDxt, GE Medical Systems, Milwaukee, WI, United States; Ingenia, Philips, Amsterdam, The Netherlands). The liver imaging protocol included the following sequences: Breath-hold gradient-echo T1-weighted [4.2 ms repetition time (TR)/2 ms echo time (TE), 40 cm field of view, 12° flip angle, 5 mm section thickness], free-breathing spin-echo T2-weighted (10000 ms TR/82.8 ms TE, 40 cm field of view, 90° flip angle, 6 mm section thickness), diffusion-weighted (6000 ms TR / 66.1 ms TE, 40 cm field of view, 90° flip angle, 5 mm section thickness, b = 1000 s/mm^2) and T1-weighted contrast-enhanced sequences performed in the arterial (20-second scanning delay), portal venous (70-second scanning delay), and equilibrium phases (180-second scanning delay). Of note, one of the cases had no useable equilibrium phase image. Gadobenate dimeglumine (n = 36; MultiHance, BRACCO Altana Pharma, Constance, Switzerland), gadoterate meglumine (n = 8; Dotarem, Guerbet Roissy, France) and gadoxetic acid (n = 6; Primovist, Bayer Schering Pharma AG, Berlin, Germany) were used as contrast material. All MR examinations utilised 10 cc of the respective contrast agents.

Qualitative analysis

The pre-operative MR images were first independently evaluated on the picture archiving communication system by two radiologists with 13 and five years of experience (Tan CH and Low HM). The reviewers were aware that the patients had HCC but were blinded to the diagnosis of MVI by pathologic examination. The images were evaluated for tumour size (mm), tumour multiplicity, T1 pre-contrast signal intensity, post-contrast enhancement pattern in all phases, T2 hyperintensity, restricted diffusion, visibility of vessels in post-contrast sequences, peri-tumoral features, presence of hypodense halo, definition of border between tumour and liver, tumour margin smoothness and LI-RADS score (see Table 1). The reviewers reviewed the images in consensus and assessed the imaging features subjectively, with a binary "Yes/No" output.

Image segmentation and texture analysis

T1W post-contrast sequences in the arterial, portal venous (PV) and equilibrium phases were exported as DICOM (digital imaging and communications in medicine) files. A polygonal region of interest (ROI) was manually drawn on the largest cross-sectional area of the tumour. The segmented images were checked visually by a researcher and vetted by a third radiologist. Discordant findings were verified by one of the two senior radiologists (Tan CH). A typical segmentation result is shown in Figure 2.

Texture analysis was performed using MaZda software (MaZda, Technical University of Lodz)[16]. The MaZda software automatically extracts and analyses 290 texture parameters including area, histogram-based, gradient based, co-occurrence matrix based, autoregressive model, run-length matrix-based and wavelet analysis.

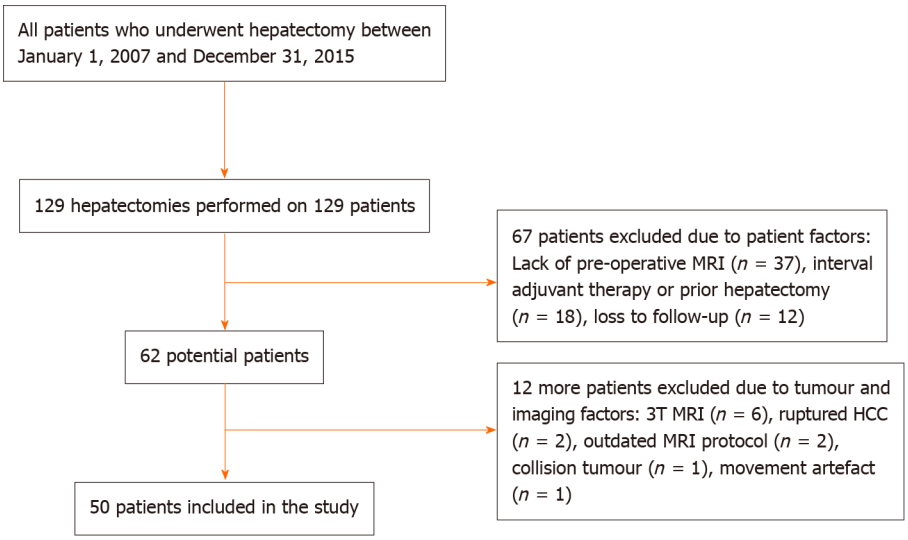
Five distinct methods were evaluated in an attempt to optimise the accuracy of the binary classification (presence or absence of MVI). Support vector machine classifiers developed for each study were tested using either bootstrapping method (1000 runs of five randomly selected test samples at a time, thus obtaining the average accuracy) or leave-one-out method.

Table 1 Tumor features on magnetic resonance imaging

	Total	MVI group	Non-MVI group	*P* value
Tumors, *n* (%)	50 (100)	15 (30)	35 (70)	
Size (mm), mean (SD)		56.4 (34.6)	41.3 (29.1)	0.107
Multiplicity, *n* (%)				0.087
Single	43 (86)	15 (100)	28 (80)	
Multifocal	7 (14)	0	7 (20)	
T1 (unenhanced), *n* (%)				0.346
Hypointense	46 (92)	13 (87)	33 (94)	
Isointense	2 (4)	1 (7)	1 (3)	
Hyperintense	2 (4)	1 (7)	1 (3)	
T1 (arterial phase), *n* (%)				0.663
Hypointense	6 (12)	1 (7)	5 (14)	
Isointense	6 (12)	1 (7)	5 (14)	
Hyperintense	38 (76)	13 (87)	25 (71)	
T1 (portal venous phase), *n* (%)				0.231
Hypointense	42 (84)	14 (93)	28 (80)	
Isointense	6 (12)	0 (0)	6 (17)	
Hyperintense	2 (4)	1 (7)	1 (3)	
T1 (equilibrium phase), *n* (%)				0.654
Hypointense	45 (90)	14 (93)	31 (89)	
Isointense	4 (8)	1 (7)	3 (9)	
Hyperintense	0	0 (0)	0 (0)	
T1 (HPB phase), *n* (%)				0.664
Hypointense	33 (66)	9 (60)	24 (69)	
Isointense	1 (2)	0	1 (3)	
Not performed	16 (32)	6 (40)	10 (28)	
T2 hyperintensity, *n* (%)				0.451
Present	42 (84)	12 (80)	30 (86)	
Absent	8 (16)	3 (20)	5 (14)	
Restricted diffusion, *n* (%)				0.745
Present	44 (88)	13 (87)	31 (89)	
Absent	5 (10)	2 (13)	3 (9)	
Not performed	1 (2)	0 (0)	1 (3)	
Visible vessels (arterial phase), *n* (%)				0.001
Present	11 (22)	8 (53)	3 (9)	
Absent	39 (78)	7 (47)	32 (91)	
Visible vessels (portal venous phase), *n* (%)				0.043
Present	8 (16)	5 (33)	3 (9)	
Absent	42 (84)	10 (67)	32 (91)	
Peritumoral enhancement, *n* (%)				0.248
Present	15 (30)	6 (40)	9 (26)	
Absent	35 (70)	9 (60)	26 (74)	

Hypodense halo, *n* (%)				0.524
Present	5 (10)	1 (7)	4 (11)	
Absent	45 (90)	14 (93)	31 (89)	
Border between tumour and liver, *n* (%)				0.428
Sharp	34 (68)	11 (73)	23 (66)	
Ill-defined	16 (32)	4 (27)	12 (34)	
Tumor margins, *n* (%)				0.477
Smooth	28 (56)	9 (60)	19 (54)	
Non-smooth	22 (44)	6 (40)	16 (46)	
LI-RADS, *n* (%)				0.05
LR-4	10 (20)	0 (0)	10 (29)	
LR-5	27 (54)	10 (67)	17 (49)	
LR-M	13 (26)	5 (33)	8 (22)	

MVI: Microvascular invasion.



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Figure 1 Flow chart of patient selection based on the inclusion and exclusion criteria. HCC: Hepatocellular carcinoma; MRI: Magnetic resonance imaging.

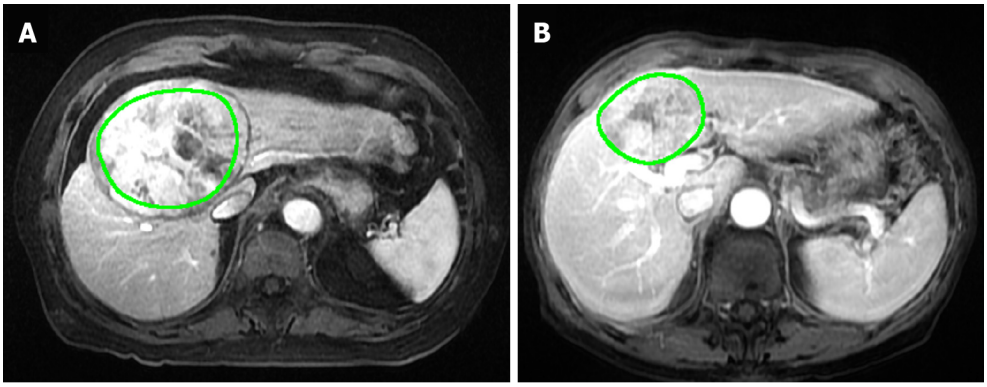
Methods 1, 2 and 3 exclusively used features from the arterial, PV, and equilibrium phase respectively. To optimise performance, 14 of the most significant features from methods 1-3 were manually selected and put through mutual information feature selection method for methods 4 and 5. Method 4 employed three features selected purely through mutual information, while study 5 incorporated recursive pruning and subsequent manual feature selection after mutual information. Method 5 eventually employed three features and was then tested with leave-one-out classification method to attain optimal performance.

Statistical analysis

Statistical analysis was performed using SPSS 20.0. The Mann-Whitney U-test or independent *t*-test were used for continuous variables, while the Fisher exact test was used for categorical variables.

RESULTS

Fifty patients (43 males, 7 females, and mean age 67 years, range 53-81 years) were included in the present study. All were new cases of histology-proven HCC in treatment-naive patients. The aetiologies



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Figure 2 Segmentation of two different biopsy-proven hepatocellular carcinoma across the largest cross-sectional area of the tumor in the T1-arterial phase and T1-portovenous phase. A: T1-arterial phase; B: T1-portovenous phase.

include hepatitis B (n = 28%, 56%), hepatitis C (n = 3%, 6%), hepatitis B and C (n = 1%, 2%), *de novo* (n = 9%, 18%), cryptogenic (n = 5%, 10%), alcoholic cirrhosis (n = 3%, 6%), and non-alcoholic steatotic hepatitis (n = 1%, 2%).

MVI was present in 30% (n = 15) of the resected specimens. Univariate analysis between MVI group and non-MVI group is shown in Table 2. Univariate analysis was also performed for MR imaging features collected for qualitative analysis (Table 1). We found a statistically significant difference of the pre-operative serum ɣ-glutamyltranspeptidase (GGT; $P \leq 0.01$) level between the two groups. We also noted a statistically significant difference in the rates of the following imaging features between the two groups: Visible intra-tumoral vessels in arterial phase (P = 0.01) and in PV phase (P = 0.043).

Of the five methods, Method 5 achieved the highest accuracy of 87.8%. Method 5 also achieved a sensitivity of 73.3%, specificity of 94.1% with positive and negative predictive value of 85% and 89% respectively. Methods 1 through 4 achieved accuracies between 70.0% to 85.5%. The details and results of the 5 methods are summarized in Table 3. The three TA parameters in method 5 were Arterial S(4,0) Correlat, Arterial S(4,-4) InvDfMom and PV (3,0)SumAverg. Incidentally, these textural features are found in relatively close proximity along the horizontal and diagonal position of the image (at a distance of 3 and 4 pixels), which could imply particular areas of the tumours contribute more to accuracy than others.

DISCUSSION

The results of our study show that texture analysis of pre-operative MRI can predict the presence of MVI pre-operatively with an accuracy of up to 87.8%. Although the exact relationship between the selected texture parameters and the image appearance is not easily explainable, this study shows that the grey value variation in the horizontal and the diagonal direction show usable differences between the two groups. The study by Ahn *et al*[14] also showed that application of TA increased diagnostic performance [area under the curve (AUC) improved from 0.7 to 0.83]. Unlike ours, the same study also found sphericity and discrete compactness to be the texture analysis variables that are significantly associated with MVI[14]. Wilson *et al*[15] achieved an AUC of 0.83 with their final texture analysis model and found tumour entropy to be significantly associated with MVI. Other studies have also identified entropy on post-contrast CT images to be an independent predictor of disease-free survival and overall survival[17,18].

TA is a post-processing technique for quantification of tissue heterogeneity. The method is based on analysis of the grey value distribution and relationship of pixels within any given ROI. The potential applications of texture analysis and radiomics in general are expanding, especially in solid tumour imaging[19-22]. In its present state, this technique is able to achieve good results without the need to obtain additional sequences or interventions. TA can also potentially be incorporated into future predictive models that incorporate clinicoradiological risk factors and radiomic features; early work has shown some promise in predicting MVI[21].

Qualitative MRI features of HCC may be useful in predicting MVI. Kim *et al*[23] found irregular circumferential peritumoral enhancement to be the only significant variable in MVI-present HCC, while Chandarana *et al*[24] demonstrated tumor multifocality takes precedence over all other imaging features. The presence of the aforementioned imaging features leads to high specificity for diagnosis of MVI, but with low sensitivity[25,26]. Furthermore, interobserver agreement tends to be only fair to moderate[27].

Table 2 Patient demographics

Variable	Total	MVI		*P* value
		MVI group = 15	Non-MVI group = 35	
Age (yr), mean (SD)	67.0 (7.0)	68.3 (8.8)	66.4 (6.1)	0.491
Gender				0.348
Male, *n* (%)	43 (86)	12 (80)	31 (89)	
Female, *n* (%)	7 (14)	3 (20)	4 (11)	
Aetiology				0.377
Alcohol, *n* (%)	3 (6)	0 (0)	3 (8)	
Hepatitis B, *n* (%)	28 (56)	7 (46)	21 (60)	
Hepatitis C, *n* (%)	3 (6)	1 (7)	2 (6)	
Hepatitis B and C, *n* (%)	1 (2)	1 (7)	0 (0)	
NAon-alcoholic steatohepatitis, *n* (%)	1 (2)	1 (7)	0 (0)	
Cryptogenic cirrhosis, *n* (%)	5 (10)	2 (13)	3 (8)	
De novo, *n* (%)	9 (18)	3 (20)	6 (17)	
HbSAg				0.676
Positive, *n* (%)	27 (54)	7 (46)	20 (57)	
Negative, *n* (%)	22 (44)	8 (54)	14 (40)	
Unknown, *n* (%)	1 (2)	0 (0)	1 (3)	
Pre-operative serology				
Alpha-fetoprotein (UG/L), mean (SD)	248.0 (893.2)	777.7 (1571.4)	32.9 (105.6)	0.065
Albumin (g/L), mean (SD)	36.3 (5.8)	35.4 (5.6)	36.7 (5.9)	0.374
Bilirubin (μmol/L), mean (SD)	19.5 (12.1)	18.6 (7.1)	19.9 (13.8)	0.797

MVI: Microvascular invasion.

Table 3 Features and results of methods 1 to 5

	Method 1	Method 2	Method 3	Method 4	Method 5
Feature set used	Arterial phase	Portovenous phase	Equilibrium phase	Selected features from all phases	Selected features put through recursive pruning
Classification accuracy (%)	81.4	84.7	70	85.5	87.8

Most recently, Hong *et al*[28] conducted a systematic review and meta-analysis on MRI features for predicting MVI and found 7 MRI features to be significantly associated with MVI: Larger tumour size (> 5 cm), rim arterial enhancement, arterial peritumoral enhancement, peritumoral hypointensity on hepatobiliary phase, non-smooth tumour margin, multifocality and hypointensity on T1W imaging.

Our study evaluated most of the aforementioned features, but found that only intra-tumoral vessel visibility on arterial and PV phase was significantly associated with MVI. Interestingly, vessel visibility in post-contrast sequences has not been extensively studied and may be a subject of future study. Further analysis of these two statistically significant features show sensitivity of 53.3% and 33.3% for vessel visibility on arterial and PV phase respectively. Both features demonstrate specificity of 91.4%. Using these as a measure of the qualitative analysis by radiologists, our model outperforms both features, achieving sensitivity of 73.3% and specificity of 94.1%.

The imaging features described in other papers, such as peritumoral arterial phase hyperenhancement, were not found to be significant for diagnosis of MVI; this may be due to small sample size in our current study. Of note, none of the parameters selected for Methods 4 and 5 included features from the equilibrium phase. This is consistent with the observations in current literature, where arterial phase and hepatobiliary phase findings predominate[28].

Current diagnosis of MVI relies on histopathologic examination that is done post-operatively. The presence of MVI indicates more aggressive tumour behaviour and poorer prognosis[1,2]. Furthermore, our study cohort comprises a high proportion of Hepatitis B aetiology (n = 29%; 58%) which has been shown to be associated with development of vascular invasion[29]. The ability to diagnose pre-operative MVI can significantly alter the clinical management for patients in several ways. Firstly, the informed consent process could include equivalent non-invasive options (*e.g.* Chemoembolisation and/or ablation techniques) which have been shown to produce similar oncologic outcomes, but with the added benefit of lower morbidity[30]. Secondly, the presence of MVI may prompt the use of more aggressive treatment options such as extended surgical margins or additional bridging treatment while awaiting liver transplantation[31-34]. Additionally, adjuvant therapy after surgical resection have also been shown to improve survival in patients with MVI[35,36]. Lastly, these patients could be followed up more closely given the susceptibility for early recurrence.

This study is not without its limitations. Firstly, this is a retrospective single-institution study with a small sample size, limiting our ability to compare it against the diagnostic performance of visual analysis by radiologists. The low sample size may also account for the relative paucity of statistically significant differences among the two study groups in terms of both clinical and radiological characteristics. Despite this, TA was able to achieve relatively high accuracy rates while identifying features that were significantly associated with MVI. Secondly, this study included MR examinations that were done with two types of hepatobiliary contrast agents (gadoterate meglumine, gadoxetate and gadobenate dimeglumine). Utilising a single (hepatocyte-specific) contrast agent would have helped to reduce heterogeneity of the MR images and allowed further analysis of the hepatobiliary phase. Due to small sample size, sub-group analysis of scans performed with each type of contrast agent could not be performed. However, standard imaging parameters were performed, in particular fixed timing of contrast enhanced phases, which reduced technical variability. Lastly, TA was only done across the largest cross-sectional area of the tumour. While analysing the entire volume of the tumour and the peritumoral tissues, would have been ideal, enough information was extracted from just a two-dimensional representation of the HCC to yield relatively accurate results.

CONCLUSION

In conclusion, texture analysis of HCC performed on pre-operative MR images can accurately predict the presence of MVI with an accuracy of up to 87.8%. It has potential to be incorporated into clinical routine as a reliable tool for making pre-operative treatment decisions. Larger studies should be performed to validate the texture parameters and its value over qualitative visual analysis.

ARTICLE HIGHLIGHTS

Research background

Presence of microvascular invasion (MVI) indicates poorer prognosis post-curative resection of hepatocellular carcinoma (HCC), with an increased chance of tumour recurrence. By present standards, MVI can only be diagnosed post-operatively on histopathology.

Research motivation

Texture analysis potentially allows identification of patients who are considered 'high risk' through analysis of pre-operative magnetic resonance imaging (MRI) studies. These findings may or may not be readily apparent to the human eye, thus the need for an analytic software. This will in turn allow for better patient selection, improved individualised therapy (such as extended surgical margins or adjuvant therapy) and pre-operative prognostication.

Research objectives

To evaluate the accuracy of texture analysis on pre-operative MRI in predicting MVI in HCC.

Research methods

We recruited patients who underwent hepatectomy. Both qualitative (performed by radiologists) and quantitative data (performed by software) were obtained. Radiomics texture parameters were extracted based on the largest cross-sectional area of each tumor and analysed using MaZda software. Final histology of the tumour was used as ground truth.

Research results

Texture analysis of tumours on pre-operative MRI can predict presence of MVI in HCC with accuracies of up to 87.8%.

Research conclusions

Texture analysis of HCC performed on pre-operative MR images can accurately predict the presence of MVI with an accuracy of up to 87.8%. It has potential to be incorporated into clinical routine as a reliable tool for making pre-operative treatment decisions. Larger studies should be performed to validate the texture parameters and its value over qualitative visual analysis.

Research perspectives

This study demonstrates the utility of texture analysis on pre-operative MRI to potentially impact clinical management in patients with surgically resectable hepatocellular carcinoma.

FOOTNOTES

Author contributions: Sim JZT contributed to drafting of manuscript, critical revision; Hui TCH contributed to acquisition of data, analysis and interpretation of data; Chuah TK contributed to acquisition of data, analysis and interpretation of data; Low HM, Shelat VG, and Tan CH contributed to study conception and design, critical revision.

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SYSTEMATIC REVIEWS

Gut microbiota diversity and composition in predicting immunotherapy response and immunotherapy-related colitis in melanoma patients: A systematic review

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Abstract

BACKGROUND

Gut microbiome (GM) composition and diversity have recently been studied as a biomarker of response to immune checkpoint blockade therapy (ICB) and of ICB-related colitis.

AIM

To conduct a systematic review on the role of GM composition and diversity in predicting response and colitis in patients with melanoma treated with ICB.

METHODS

The review protocol was registered in PROSPERO: CRD42021228018. From a total of 300 studies, nine studies met inclusion criteria. Two studies were phase I clinical trials, while the remainder were prospective observational studies. All but one study has moderate risk of bias. In addition, we conducted a relevant search by Reference Citation Analysis (*RCA*) (https://www.referencecitationanalysis.com).

RESULTS

Fecal samples enriched in Firmicutes phylum were associated with good response to ICB, whereas the Bacteroidales family was associated with poor response to ICB. Samples with greater GM diversity were associated with more favorable response to ICB [hazard ratio (HR) = 3.57, 95% confidence interval = 1.02-12.52, $P < 0.05$]. Fecal samples with a higher abundance in Firmicutes were more susceptible to ICB-related colitis ($P < 0.01$) whereas samples enriched in *Bacteroidetes* were more resistant to ICB-related colitis ($P < 0.05$). Overall, there was limited concordance in the organisms in the GM identified to be associated with response to ICB, and studies evaluating GM diversity showed conflicting results.

CONCLUSION

This highlights the need for further prospective studies to confirm whether the GM could be used as a biomarker and potential intervention to modulate ICB response in melanoma patients.

Key Words: Melanoma; Gut microbiome; Microbiota; Immunotherapy; Biomarker; Immune checkpoint blockade therapy

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Core Tip: Since the introduction of immune checkpoint inhibitors as part of standard of care for melanoma patients, there has been a growing interest in identifying biomarkers of response and immune related adverse events. Amongst these biomarkers, the composition of the gut microbiome has been one of the most intriguing discoveries. Our aim was to ascertain the current published evidence on the gut microbiome diversity and composition as a biomarker of response to immunotherapy. We demonstrated high variability in the results and limited concordance on the organisms identified. We highlight the conflicting aspects of these reports as well as their few commonalities.

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INTRODUCTION

Melanoma is the most lethal form of skin cancer accounting for 73% of skin-cancer related mortality and over 50000 deaths worldwide annually[1,2]. Survival for metastatic melanoma has significantly improved since the introduction of immunotherapy and targeted therapy with a 5-year survival rate of up to 50%[3-5]. Currently, the standard first-line therapy for metastatic melanoma include BRAF-targeted therapies and immune checkpoint blockade (ICB) consisting either anti-programmed death (PD)-1 monotherapy or combination of anti-PD-1 as well as anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) therapy[6]. Despite the considerable benefit of ICB, 40%-60% of melanoma patients do not experience objective responses to the therapy[7-9]. Thus, tremendous efforts are now focused on identifying novel biomarkers which could accurately predict the subset of patients who would benefit from ICB[10-14]. These biomarkers include tumor mutational burden, cytokines, circulating tumor DNA, human leukocyte antigen, gut microbiota (GM) diversity and composition, among many others [15].

The GM is a community of 100 trillion microorganisms of more than 1000 species mainly bacteria but also, archaea, viruses and fungi which colonize the human intestines[16]. The relationship which exists between GM and the host is a mutualistic relationship where one benefits the other[16]. In return for the nutrients derived from the host, the GM performs numerous critical functions such as fermentation of dietary fiber into short-chain fatty acids; synthesis of vitamins; protection against pathologic gut microbes; and induction and regulation of the immune system[17,18]. The gut microbial balance is pivotal in the optimal functioning of all of these roles and thus any discrepancy in this delicate

equilibrium could produce a state of dysbiosis which has been associated with many pathologies including cancer[19]. In the context of cancer, preclinical studies have demonstrated that some GM subpopulations have pro-tumorigenic effects, whereas others have tumor-suppressive effects[20-22]. Additionally, the GM has also been shown to modulate response to chemotherapy and immunotherapy [23-25]. This could be linked to the role of GM in metabolizing anti-cancer compounds and regulating the host's immune response[16]. Thus, GM has been studied intensely as a potential biomarker of response to ICB[12,26-31]. This is particularly relevant for melanoma, where ICB has become standard of care given its demonstrated pronounced effectiveness.

Studies investigating GM composition and/or diversity in patients with melanoma have identified distinct GM composition in responders to ICB compared to non-responders, offering hope of a novel biomarker for predicting response to ICB[12,26-32]. Additionally, studies exploring whether certain GM composition and diversity could be predictive of ICB-related colitis - one of the major factors of ICB treatment cessation and thus failure to derive full benefit of ICB - have also been conducted[27,33]. This systematic review will be the first to compile the existing data regarding the role of GM composition and diversity in predicting response to ICB and ICB-related colitis specifically in patients with melanoma. Notably previous reviews have combined multiple cancers.

MATERIALS AND METHODS

Literature search strategies

This review was conducted following the preferred reporting Items for systematic reviews and meta-analyses guidelines[34]. The review protocol was submitted to the international prospective register of systemic reviews (PROSPERO Registration number: CRD42021228018).

In this comprehensive literature search, original studies exploring the variation in GM community in fecal samples of melanoma patients who responded and did not respond to immunotherapy, experienced colitis and did not experience colitis were identified. Medline and Embase were searched for eligible papers published prior to December 2021 using the following search terms: (fecal OR gut) AND (microbiota OR microbiome) AND (melanoma) AND (immunotherapy OR checkpoint OR nivolumab OR ipilimumab OR pembrolizumab). OpenGrey and the Grey Literature Report were also searched for eligible unpublished papers and grey literature. The following keywords and its synonyms will be used for our search strategy: "fecal microbiota", "melanoma", "immunotherapy".

Duplicate and irrelevant publication types such as symposium agendas were removed from the initial search results. Titles and abstracts of relevant publications were screened independently by Oliver O and Simadibrata DM based on inclusion and exclusion criteria stated below. Subsequently, reference lists within each relevant publication were examined for further pertinent studies. The full texts of these publications were then reviewed.

Inclusion criteria

Inclusion criteria for the systematic review included randomized controlled trials (RCTs), original cohort, case-control studies published in a peer-reviewed journal exploring GM diversity and composition in fecal samples from melanoma patients treated with ICB which can be anti-PD-1 and/or anti-PD-L1 and/or anti-CTLA-4. Studies included should assess treatment outcome and/or ICB-related colitis incidence following treatment with ICB. Treatment outcomes should be determined by RECIST criteria and/or progression free survival (PFS) and/or overall survival (OS) and ICB-related colitis confirmed by colonoscopy.

Only studies which utilized fecal samples obtained from human subjects receiving ICB were included. Studies which assessed treatment response to immunotherapy in animal models were excluded. Two reviewers (Oliver O, Liu YY) independently screened and read the full text of the included articles for eligibility.

Data extraction

Two investigators (Oliver O, Liu YY) independently reviewed the eligible studies and extracted data from each study. Extracted variables included title, first author, year of publication, number of participants, type of immunotherapy received, GM analysis method, and study outcomes (GM composition and diversity in responders/non-responders and ICB-related colitis/non-ICB-related colitis patients). Extracted GM composition data included a list of the GM at the level of phyla, class, order, family, genus and species, whereas extracted GM diversity extracted included alpha diversity or the Shannon index. Any discrepancies found by the investigators on data extraction were resolved by consensus. In addition, we conducted a relevant search by Reference Citation Analysis (*RCA*) (https://www.referencecitationanalysis.com).

Quality assessment

Non-randomized studies, including cohort studies, case-control studies and single-arm clinical trial that

were included in this systematic review were independently evaluated by Oliver O and Simadibrata DM for any risk of bias using the Risk of Bias in Non-randomized Studies of interventions (ROBINS-I) assessment tool, a tool which assesses seven items: confounding, selection, intervention classification, deviation from intervention, missing data, measurement of outcome and selection of reported result. Each item was assessed according to the ROBINS-I guideline, where each bias domain can be classified as either low, moderate, serious or critical risk of bias, or no information mentioned.

RESULTS

Study selection and risk of bias assessment

The initial search from Medline, Embase, OpenGrey and Grey Literature retrieved 300 studies. After deduplication, the studies were screened by reviewing their abstracts and 10 articles selected for full assessment (Figure 1). One study by Vétizou *et al*[35] was excluded because while the fecal samples were obtained from patients treated with anti-CTLA-4, treatment response to ICB was assessed in an *in-vivo* mice model of melanoma following fecal transplantation rather than humans.

From the nine included studies, two studies were phase I clinical trials, while the remainder were prospective observational studies[26,28]. Unfortunately, no RCTs were available to date. According to the ROBINS-I assessment tool, all but one study was shown to have moderate risk of bias (Table 1). The study by Matson *et al*[29] had a serious risk of bias as there was a lack of clarity regarding the definition of intervention used.

GM composition and diversity in predicting immunotherapy response

Eight studies assessed the role of GM composition and/or diversity and response to ICB in melanoma patients (Table 2). Seven studies compared the GM between responders and non-responders to ICB, and two studies analyzed the GM in patients undergoing fecal microbiota transplant (FMT).

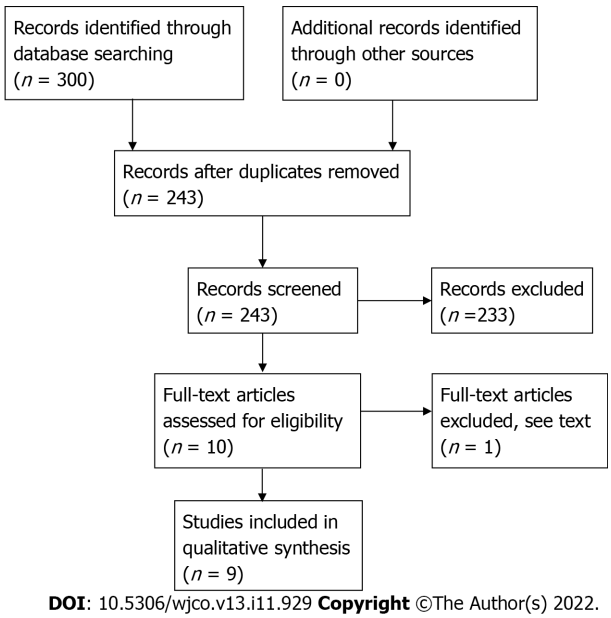
The study by Chaput *et al*[27] assessing fecal GM composition of 26 metastatic melanoma patients prior to and post commencing anti-CTLA-4 therapy revealed that GM composition varied according to response. Patients showing long term response to therapy (nine out of 26 patients) were found with fecal samples with significantly higher *Faecalibacterium* percentages ($P = 0.0092$) while patients with poor clinical benefit had higher proportions of *Bacteroides* ($P = 0.034$). When patients were grouped based on their microbiota composition, those with high prevalence of *Faecalibacterium* and other *Firmicutes* had a longer PFS ($P = 0.0039$) and to a lesser extent longer OS ($P = 0.051$) relative to patients whose fecal samples were abundant with *Bacteroides*. Additionally, these patient groups were noted to derive long-term clinical benefit compared to the latter (67% *vs* 0%; $P = 0.0017$)[28].

In an analysis of stool samples from 42 metastatic melanoma patients prior to treatment with anti-PD-1 ($n = 38$) and anti-CTLA-4 ($n = 4$) therapy, Matson *et al*[29] showed a significant difference in GM composition between responders (16 patients) and non-responders (26 patients) ($P < 0.01$). In responders, eight microbial species namely, *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum* were found to be more abundant in responders than in non-responders[29]. In non-responders, two microbial species, specifically, *Ruminococcus obeum* and *Roseburia intestinalis* were more abundant[29]. To further assess the applicability of GM composition as a biomarker of response to ICB, they explored the correlation between the ratio of total numbers of potentially "beneficial" and "nonbeneficial" operational taxonomic units (OTUs), and change in tumor size, as assessed by the RECIST[29]. Patients with an OTU ratio of greater than 1.5 demonstrated clinical response to ICB[29].

In another study by Gopalakrishnan *et al*[12], fecal samples of 43 metastatic melanoma patients prior to treatment with anti-PD-1 therapy were analyzed. In responders (30 patients), analysis of fecal samples revealed abundance of GM from *Ruminococcaceae* family of the *Clostridiales* order, whereas in non-responders (13 patients), abundance of GM from the *Bacteroidales* order was noted[12]. Further analyses demonstrated that *Faecalibacterium* genus was notably enriched in fecal samples from responders and *Bacteroides thetaiotaomicron*, *Escherichia coli*, and *Anaerotruncus colihominis* were enriched in non-responders[12]. In addition, to investigate durability of response, patients were stratified based on their fecal composition of *Faecalibacterium* genus and *Bacteroidales* order and correlated to their PFS [12]. Results demonstrated that patients with *Faecalibacterium*-enriched fecal samples have longer PFS than those with low abundance ($P = 0.03$) and patients with *Bacteroidales*-enriched fecal samples have shorter PFS than those with low abundance ($P = 0.05$). Beyond specific microbial taxa, GM diversity, as assessed by Simpson's reciprocal index, was higher in responders compared to non-responders ($P < 0.01$)[12]. Moreover, high GM diversity was significantly associated with anti-PD-1 therapy response, when compared to patient groups of intermediate diversity [hazard ratio (HR) = 3.60, 95% confidence interval (CI): 1.02-12.74, $P < 0.05$) and low diversity (HR = 3.57, 95%CI: 1.02-12.52, $P < 0.05$). Other important predictors of therapy response include abundance of *Faecalibacterium* (HR = 2.92, 95%CI: 1.08-7.89) and *Bacteroidales* (HR = 0.39, 95%CI: 0.15-1.03) in the fecal microbiome[12].

Table 1 Risk of bias assessment with Risk of Bias In Non-randomised Studies - of Interventions

Ref.	Confounding	Selection	Intervention classification	Deviation from intervention	Missing data	Measurement of outcome	Selection of reported result	Overall
Dubin *et al*[33], 2016	Moderate	Low	Low	Low	Low	Low	Low	Moderate
Chaput *et al*[27], 2017	Moderate	Low	Low	Moderate	Low	Low	Moderate	Moderate
Gopalakrishnan *et al*[12], 2018	Moderate	Low	No information	Low	Low	Low	Low	Moderate
Matson *et al*[29], 2018	Moderate	No information	Serious	No information	No information	Low	Moderate	Serious
Peters *et al*[30], 2019	Moderate	Low	No information	No information	Low	Low	Low	Moderate
Baruch *et al*[26], 2020	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate
Wind *et al*[31], 2020	Moderate	No information	No information	No information	No information	Low	Low	Moderate
Davar *et al*[28], 2021	Moderate	Moderate	Low	Low	No information	Low	Low	Moderate
Andrews *et al*[36], 2021	Moderate	Low	Low	Low	Low	Low	Low	Moderate



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Figure 1 Prisma flow diagram of study selection.

Peters *et al*[30] examined the correlation between GM taxa and PFS in pre-treatment fecal samples of 27 metastatic melanoma patients receiving anti-PD-L1 and/or anti-CTLA-4. GM which was associated with shorter PFS included genera *Bacteroides* and *Bilophila*, and species *Bacteroides ovatus*, *Blautia producta*, and *Ruminococcus gnavus*, whereas those which correlated with longer PFS included genera *Faecalibacterium* and *Parabacteroides* and species *Faecalibacterium prausnitzii*[12]. With regards to GM richness the authors compared the β-diversity or between-sample microbiome diversity relative to survival. Multivariate analysis adjusting for age, sex, BMI, stage, number of sites of metastases, and antibiotic use in the last 6 mo revealed that higher GM richness was correlated with longer PFS (number of 16S sub - OTUs: HR [95%CI] = 0.97 [0.95, 1.00], P = 0.02; number of shotgun subspecies: HR [95%CI] = 0.89 [0.79, 0.99], P = 0.03)[30]. Furthermore, analysis of the 16S but not shotgun dataset showed that higher diversity of GM, as assessed by the Shannon index, was associated with longer PFS (P = 0.02)[12].

Table 2 Characteristics of included studies exploring link between gut microbiome composition and diversity and response to immune-checkpoint blockade therapy in metastatic melanoma patients treated with immune-checkpoint blockade therapy

Ref.	Year	Therapy	Method	Sample size/ time point	Dominant microbes	Microbial diversity
Chaput *et al*[27], 2017	2017	Anti-CTLA-4	16S rRNA gene sequencing of fecal samples	26 before tx	Responders: *Faecalibacterium* and *Firmicutes*	N/A
Matson *et al*[29], 2018	2018	Anti-PD-1 or anti-CTLA-4	16S rRNA gene and shotgun metagenome sequencing of fecal samples; qPCR on selected bacteria	42 before tx	Responders: *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* Non-responders: *Ruminococcus obeum* and *Roseburia intestinalis*	N/A
Gopalakrishnan *et al*[12], 2018	2018	Anti-PD-1	16S rRNA gene and shotgun metagenome sequencing of fecal samples	43 before tx	Responders: *Clostridiales*, in particular *Faecalibacterium* Non-responders: *Bacteroidales*, in particular *Bacteroides thetaiotaomicron*; as well as *Escherichia coli*, and *Anaerotruncus colihominis*	Higher alpha diversity in patients with longer PFS
Peters *et al*[30], 2019	2019	Anti-PD-1 or anti-CTLA-4	16S rRNA gene and shotgun metagenome sequencing of fecal samples	27 before tx	Responders: *Faecalibacterium, Parabacteroides*, and *Faecalibacterium prausnitzii* Non-responders: *Bacteroides* and *Biophilia*	Higher microbial community richness and diversity was associated with longer PFS
Wind *et al*[31], 2020	2020	Anti-PD-1 or anti-CTLA-4	Shotgun metagenome sequencing of fecal samples	25 before tx	Responders: *Ruminococcus gnavus, Streptococcus parasanguinis*, and *Bacteroides massiliensis*. Non-responders: *Bifidobacterium longum* and *Peptostreptococcaceae*	No significant difference in alpha-diversity between responder and non responders
Baruch *et al*[26], 2020	2020	Anti-PD-1 refractory	16S rRNA gene and shotgun metagenome sequencing of fecal samples	10 anti-PD-1 refractory patients	FMT donors (responders): *Lachnospiraceae, Veillonellaceae*, and *Ruminococcaceae* Post FMT Responders: *Enterococcaceae, Enterococcus*, and *Streptococcus australis* Non-responders: *Veillonella atypica*	No significant difference in GM composition prior to FMT, but significant difference post-FMT between responders and non-responders Lower microbial richness in the donor of responding recipients
Davar *et al*[28]. 2021	2021	Anti-PD-1 refractory	Shotgun metagenomic sequencing of fecal samples	15 anti-PD-1 refractory patients, before FMT	Responders: Firmicutes (*Lachnospiraceae* and *Ruminococcaceae* families) and *Actinobacteria* (*Bifidobacteriaceae* and *Coriobacteriaceae* families)	Higher GM diversity of donors who were complete responders compared to donors who were partial responders No significant difference in GM diversity between donors and recipients prior to FMT
Andrews *et al* [36], 2021	2021	Combined ICB - Anti-PD-1 and anti-CTLA-4	16S rRNA gene and shotgun metagenome sequencing of fecal samples	38	Responders: *Bacteroides stercoris*, *Parabacteroides distasonis*, *Fournierella massiliensis*. Non-responders: *Klebsiella aerogenes* and *Lactobacillus rogosae*	No significant difference in GM diversity between responders and non-responders

Anti-CTLA-4: Anti-cytotoxic T lymphocyte-associated antigen-4; N/A: Not applicable; Anti-PD-1: Anti-programmed death-1; qPCR: Quantitative real-time polymerase chain reaction; FMT: Fecal microbiota transplant; GM: Gut microbiome; ICB: Immune checkpoint blockade therapy.

Similarly, Wind *et al*[31] analyzed fecal samples from 25 metastatic melanoma patients - 12 responders, 13 non-responders - prior to start of treatment with anti-PD-1 or anti-CTLA-4. Analysis revealed that the fecal samples of responders were mainly enriched in *Ruminococcus gnavus*, *Escherichia coli*, *Eubacterium biforme*, *Phascolarctobacterium succinatutens* and *Streptococcus salivarius*, whereas samples from non-responders were abundant in *Bifidobacterium longum*, *Prevotella copri*, *Coprococus* sp, *Eggerthella unclassified* and *Eubacterium ramulus*[31]. When correlated with survival, fecal samples of participants enriched in *Bacteroides massiliensis* and *Streptococcus parasanguinis* were associated with longer PFS (HR: 3.79, 95%CI: 1.06-13.52 P = 0.04) and OS (HR: 5.05, 95%CI: 1.33-19.21, P = 0.017) respectively, whereas those who were carriers of *Peptostreptococcaceae* were associated with shorter PFS (HR: 0.18, 95%CI: 0.05-0.62, P = 0.007) and OS (HR: 0.12, 95%CI: 0.01-0.96, P = 0.046)[31]. In terms of GM diversity, as assessed by Shannon index, no significant difference between responders and non-responders was noted[31].

The study by Andrews *et al*[36] analyzed gut microbiome samples from a subset of 77 metastatic melanoma patients - 27 responders, 11 non-responders - who underwent combined ICB. There was no significant association in *Firmicutes* phyla and *Clostridiales* order and response to ICB (P = 0.39 and P = 0.38, respectively) and no significant difference in alpha diversity between responders and non-responders to ICB[36]. Fecal samples from responders were mainly enriched with *Bacteroides stercoris*,

Parabacteroides distasonis and *Fournierella massiliensis* (P = 0.03, P = 0.04 and P = 0.008, respectively) while fecal samples from non-responders were abundant in *Klebsiella aerogenes and Lactobacillus rogosae* (P = 0.04 and P = 0.02, respectively)[36].

In a first clinical trial of its kind (phase 1), Baruch *et al*[26] demonstrated that FMT from anti-PD-1 treated metastatic melanoma patients who were complete responders (2 donors), triggered response to anti-PD-1 therapy in metastatic melanoma patients who were refractory to at least one line of anti-PD-1 therapy. Out of 10 patients included in the trial, 3 patients demonstrated objective responses with 1 achieving complete response and 2 patients achieving partial response[26]. Notably, the PFS milestone of 6 mo was reached in all responders[26]. Upon analysis of pre-treatment fecal samples of donors, donor of the responding recipients had a lower microbial richness than the other donor of the non-responding patients[26]. There was no significant difference on the GM composition prior to FMT of recipients who responded compared to those who did not respond[26]. Metagenome sequencing found that recipients post FMT have higher proportions of *Veillonellaceae* family and a lower relative abundance of *Bifidobacterium bifidum*. Donors were found with high amounts of *Lachnospiraceae, Veillonellaceae*, and *Ruminococcaceae*. Comparison of a small subset of non-responders with responders, found statistically significant higher abundance of *Enterococcaceae, Enterococcus*, and *Streptococcus australis*, and a lower relative abundance of *Veillonella atypica*. However clear deductions on specific GM taxa cannot be made, as there were non-responders and pre-treatment fecal samples with similar dynamics. it is crucial to note that this trial was primarily designed to assess safety of FMT and not statistically powered to assess efficacy[26].

In a separate trial, Davar *et al*[28] showed that fecal microbial transplant (FMT) from metastatic melanoma patients (7 donors) who had complete (4 donors) or partial response (3 donors) to anti-PD-1 therapy helped overcome resistance in anti-PD-1 treatment-refractory metastatic melanoma patients (15 patients). Following FMT and anti-PD-1 therapy, 6 out of 15 patients achieved clinical benefit, with 3 patients achieving objective responses and 3 patients experiencing stable disease lasting more than 12 mo[28]. Analysis of stools after FMT revealed that samples from responders were abundant in the phyla, *Firmicutes* (*Lachnospiraceae* and *Ruminococcaceae*) and *Actinobacteria* (*Bifidobacteriaceae* and *Coriobacteriaceae*) and had decreased proportions in phylum *Bacteroidetes*[28]. In terms of GM diversity assessed with inverse Simpson index, GM diversity of donors who were complete responders were more diverse than donors who were partial responders. There was no significant difference in GM diversity between donors and recipients prior to FMT[28].

Gut microbiota composition and diversity in predicting ICB-related colitis

To date only three studies have reported on the correlation between pre-treatment GM composition and/or diversity and ICB-related colitis (Table 3).

Firstly, a prospective study by Dubin *et al*[33] explored the link between GM composition, and subsequent colitis development in 34 metastatic melanoma patients treated with ipilimumab, showed that the *Bacteroidetes* phylum was more abundant (P = < 0.05) in fecal samples of the 24 patients who did not develop ipilimumab-induced colitis compared to those who did. Further analysis revealed that within the *Bacteroidetes* phylum, the population of *Bacteroidaceae, Rikenellaceae* and *Barnesiellaceae* was significantly more abundant in the former than the latter (P < 0.01, P < 0.05 and P < 0.05 respectively)[33]. However, there was no significant difference in microbial richness and diversity, as assessed by Shannon and inverse Simpson indices, between those who developed ipilimumab-induced colitis relative to those who did not[33].

In a similar study by Chaput *et al*[27], analysis of fecal samples of metastatic melanoma patients receiving ipilimumab demonstrated high proportions of *Firmicutes* in patients who developed ipilimumab-induced colitis (P = 0.009). In contrast, fecal samples of those that did not develop colitis were mainly enriched with *Bacteroidetes* (P = 0.011)[27]. Accordingly, patients with the former GM composition also tend to have a shorter colitis-free cumulative incidence compared with patients with the latter composition[27]. Several OTUs known to be predictive to colitis such as *F. prausnitzii* L2-6, *butyrate producing bacterium* L2-21 and *G. formicilis* ATCC 27749 were associated with longer OS[27].

Finally, Andrews *et al*[36], analyzed gut microbiome samples in metastatic melanoma patients undergoing combined ICB and their link to ileitis and colitis events. No significant difference in alpha diversity was observed between those that did and did not develop colitis[36]. Fecal samples of patients developing colitis were enriched in *Bacteroides intestinalis* and *Intestinibacter bartlettii* (P = 0.009 and P = 0.009, respectively) while those that did not were abundant in *Anaerotignum lactatifermentans* and *Dorea formicigenerans* (P = 0.016 and P = 0.06, respectively)[36]. For both *B. intestinalis* and *D. formicigenerans*, associations with their risk of colitis were still maintained after adjustment using a logistic regression model [OR = 4.54 (95%CI = 1.06-24.7) and OR = 0.35 (95%CI = 0.082-1.35), respectively][36].

DISCUSSION

Our review of current reports assessing the GM composition relative to response to ICB, indicated high variability in the results and limited concordance on the organisms identified (Figure 2). Amongst the

Table 3 Characteristics studies exploring link between gut microbiome composition and diversity and immune-checkpoint blockade therapy-related colitis in metastatic melanoma patients treated with immune-checkpoint blockade therapy

Ref.	Year	Therapy	Method	Sample size/ timepoint	Dominant microbes	Microbial diversity
Dubin *et al* [33], 2015	2015	Anti-CTLA-4 immuno-therapy	16S rRNA gene and shotgun metagenome sequencing of fecal samples	34	Colitis-resistant: *Bacteroidetes* (*Bacteroidaceae, Rikenel-laceae* and *Barnesiellaceae*)	No significant difference in microbial richness and diversity
Chaput *et al* [27], 2017	2017	Anti-CTLA-4 immuno-therapy	16S rRNA gene sequencing of fecal samples	26	Colitis-resistant: *Bacteroidetes*; Colitis-prone: *Firmicutes*	Decreased bacterial diversity was associated with colitis
Andrews *et al*[36], 2021		Combined ICB - Anti-PD-1 and anti-CTLA-4	16S rRNA gene and shotgun metagenome sequencing of fecal samples	38	Colitis resistant: *Firmicutes* ; Colitis prone: *Bacteri-odetes*	No significant difference in alpha diversity

Anti-CTLA-4: Anti-cytotoxic T lymphocyte-associated antigen-4; N/A: Not applicable; Anti-PD-1: Anti-programmed death-1; qPCR: Quantitative real-time polymerase chain reaction; ICB: Immune checkpoint blockade therapy.

few commonalities, we found that fecal samples enriched in organisms from the *Firmicutes* phylum (*Lachnospiraceae* and *Ruminococcaceae* family) especially the *Faecalibacterium* genus were associated with ICB responders in 4 of 9 studies[12,27,28,31], while *Bacteroidetes* phylum was found in higher proportions in non-responders in 2 of the studies[12,30]. However, other than these two findings, there was no clear correlation between specific GM composition and response to ICB.

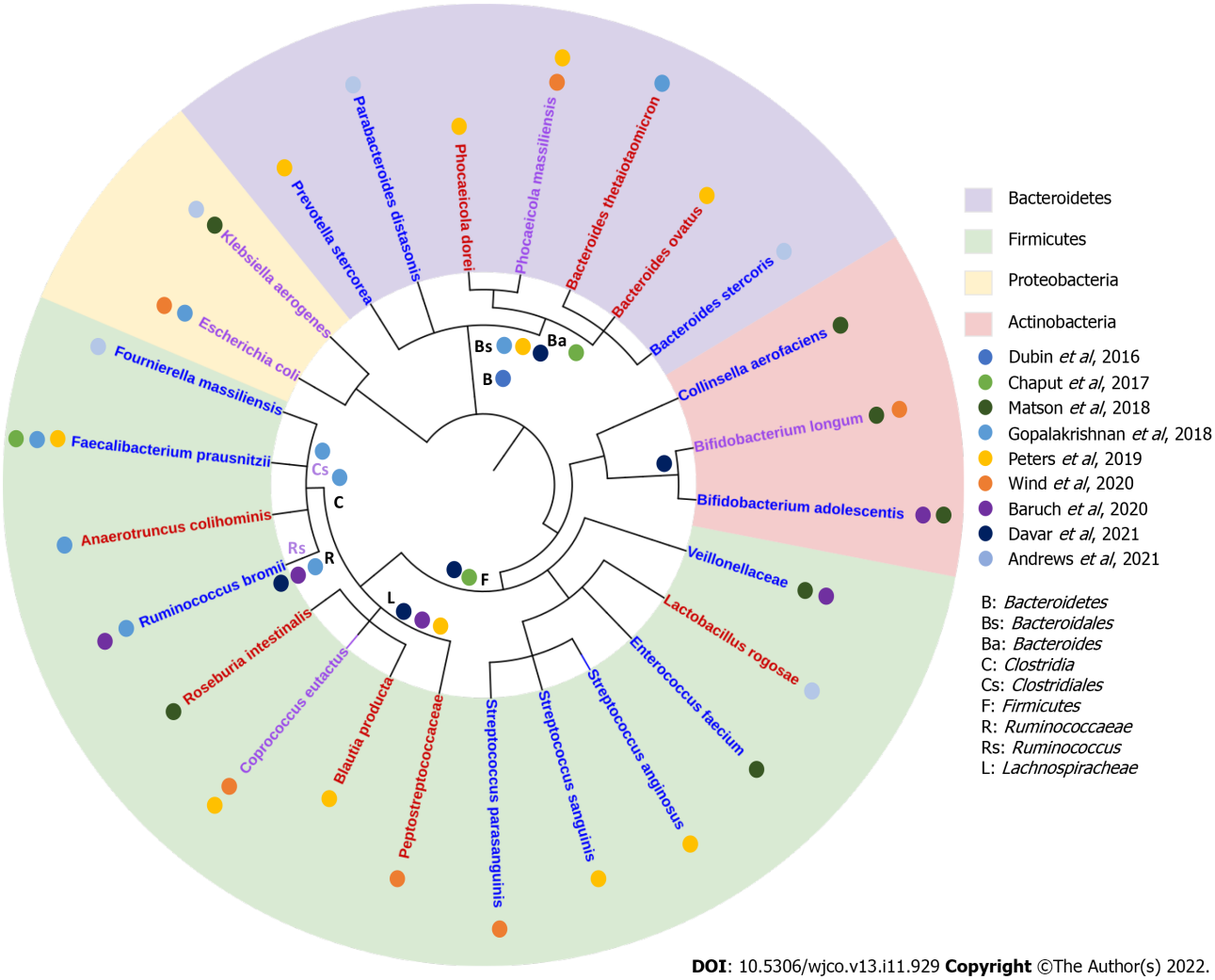
In fact, our analysis mainly identified inconsistencies in the GM composition reported to be associated with response to ICB. For instance, *Bifidobacterium longum* was found to be abundant in responders in the study by Wind *et al*[31], but found to be enriched in non-responders in the study by Matson *et al*[29]. Some species from the *Firmicutes* family were found in both responders and non-responders such as *Roseburia intestinalis*[29]. Similarly, species from the *Bacteroidales* order were found in both responders and non-responders, such as *Bacteroides massiliensis*[31]. The overlap in GM composition in responders and non-responders may suggest that the functional capacity of the GM may be more important than individual GM family/order/species in determining response to ICB[30].

In contrast to individual species or taxas, GM diversity have been heralded to a marker of good health[37]. Here four studies - Gopalakrishnan *et al*[12], Peters *et al*[30], Wind *et al*[31] and, Andrews *et al* [36] - assessed its potential to predict ICB responsiveness. The two first studies demonstrated that higher GM diversity in the responder group compared to non-responder arm[12,30]. However, the other two studies, Wind *et al*[31] and Andrews *et al*[36], found no differences in GM diversity between both groups. Nevertheless, in other cancer types such as renal cell carcinoma and non-small cell lung cancer, greater GM diversity has also been associated with improved responses to anti-PD-1 therapy[37-39].

Study results showing associations with GM diversity were consistent with previous studies which showed that greater GM diversity is prevalent in healthy state across multiple diseases, plausibly suggesting that a greater GM diversity produces the optimal immune environment needed for normal physiological functioning[40-42]. One major reason is the promotion of a favorable immune phenotype, as evidenced by the positive correlation between Shannon diversity index and several CD8+ T cell and NK cell signatures, required to produce a robust anti-tumoral response[38].

Previous studies have demonstrated that GM from *Firmicutes* family and *Bacteroidales* order play a significant role in mediating the response to immunotherapy in melanoma patients[12,27,29]. For instance, abundance of *Firmicutes* was associated with increased frequencies of CD4+, CD8+ T cells, CD 45+ myeloid and lymphoid tumor-infiltrating cells and preserved cytokine response to anti-PD-1 therapy[12]. Additionally, abundance of *Firmicutes* was linked with decreased frequency of intestinal and systemic regulatory T cells (Tregs) and $B7^+$ T cells, cells responsible for limiting immune response robustness[27]. This resulted in increased antigen presentation and effector T cell function in both the periphery and tumor microenvironment[12,27,29]. However, other GM such as *Bacteroidales* were unfavorable in terms of anti-tumoral response in that its abundance was associated with higher frequencies of Tregs and myeloid-derived suppressor cells and a blunted cytokine response[12]. These findings combined demonstrated that certain GM play a crucial role in mediating systemic and antitumor immune responses which have clear implications on efficacy on ICB therapy in metastatic melanoma patients.

Notably, GM has also been shown to potentially serve as not just a predictor of ICB therapy response, but also for boosting response to ICB therapy. FMT on anti-PD-1 treatment-refractory metastatic melanoma patients produced a complete response to anti-PD-1 therapy in one-third (9 out of 25 patients) of the otherwise therapy refractory patients[26,28].



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Figure 2 Phylogenetic tree showing family and species of gut microbiome abundant in responders and non-responders to immune-checkpoint blockade therapy in all included studies. Gut microbiome species highlighted in red: Abundant in non-responders to immune-checkpoint blockade therapy; blue: Abundant in responders to immune-checkpoint blockade therapy; purple: Abundant in both responders and non-responders.

Another aspect of the GM analyzed here, was its association with ICB-related colitis. The three studies included in this review demonstrated that GM which was abundant in ICB-related colitis-prone patients was enriched in responders to ICB (*Firmicutes*) while GM which was abundant in ICB-related colitis-resistant patients was enriched in non-responders to ICB (*Bacteroidetes*). This is consistent with the understanding that a more effective anti-tumoral response will produce greater off-target effects. The *Bacteroidetes* phyla has been linked with low-grade systemic inflammation, which could explain the observation that *Bacteroidetes* phyla was abundant in ICB-related colitis-resistant patients[27]. In line with this observation is the finding that level of *Bacteroidetes* is lower in inflammatory bowel disease - an autoimmune condition which produces chronic inflammation of the digestive tract - patients relative to healthy patients[43]. Conversely, *Firmicutes* phyla, especially *F. prausnitzii* has been associated with induction of Tregs which express high levels of CTLA-4, fueling speculation that it may cause sequestration of Tregs within the intestine[44]. Since Tregs express high levels of CTLA-4, their actions are inhibited, thereby limiting self-tolerance and promoting the development of colitis. These findings reiterate that GM has an immunomodulatory role, giving them the potential to be utilized as biomarkers of ICB-related colitis, in addition to response to ICB.

Our systematic review has several strengths. Firstly, unlike previous reviews which combined studies in various cancer types, this review focused solely on the effect of GM composition and diversity only in patients with melanoma. Secondly, we conducted a comprehensive search for RCTs and observational studies, performed a risk-of-bas assessment and studied clinically important outcomes - clinical response and ICB-related colitis - an adverse event reported in up to 25% of patients treated with ICB [45]. Thirdly, we only included studies which assessed response to immunotherapy in humans, not animals. However, several limitations exist in our systematic review. Studies which we included used distinct approaches when segregating patients into the responder and non-responder groups, using different response criteria to evaluate treatment response in patients. Additionally, there were

differences in methods of stool collection and analysis of GM composition and diversity. For example, Chaput *et al*[46] collected multiple stool samples every 3 wk of ICB, while other studies such as Dubin *et al*[33] and Matson *et al*[29] collected stool samples only prior to initiation of ICB. Furthermore, only 4 studies considered confounding factors such as variation in diet and antibiotic use[27,29-31]. Therefore, inter-study comparison of the GM composition and diversity in responders *vs* non-responders and those who experienced colitis *vs* non-colitis should be addressed with caution. Furthermore, included studies only enrolled a small number of patients, which could explain inconsistent results between studies.

CONCLUSION

In conclusion, GM composition and diversity holds some potential as a biomarker of response and toxicity to ICB in melanoma. Larger prospective studies with standardized experimental protocol ought to be conducted to elucidate whether distinct GM signatures are required for robust response to different ICB regimens. Additionally, more studies correlating metagenomic and metatranscriptomic data of GM to outcomes of melanoma patients on immunotherapy ought to be performed as the functional capacity may be more important rather than individual GM family/order/species. In addition, we eagerly await the outcome of multiple large-scale RCTs involving FMT in the context of ICB-refractory melanoma such as NCT04577729 and NCT04988841 (PICASSO) (ClinicalTrails.gov).We foresee that together with other promising biomarkers, GM composition and diversity will be integrated into a multiparameter model to accurately predict which subset of melanoma patients are likely to respond to ICB[10,11,47].

ARTICLE HIGHLIGHTS

Research background

Survival for metastatic melanoma has significantly improved since the introduction of immune checkpoint blockade (ICB) therapy. However, despite their considerable efficacy, 40%-60% of melanoma patients do not experience objective responses to the therapy. Additionally, some patients experience ICB-related colitis as a consequence of ICB therapy, preventing them from deriving the full benefit of ICB therapy. Recent studies have demonstrated that the gut microbiome (GM) may affect tumor immunity by regulating the host immune system and tumor micro-environment, thus suggesting that GM may affect response to ICB therapy and susceptibility of ICB-related colitis.

Research motivation

The GM has shown great potential as a biomarker of response to ICB therapy in melanoma patients. Previous studies investigating GM composition and/or diversity in patients with melanoma have identified distinct GM composition and diversity in responders to ICB compared to non-responders, as well as those more susceptible to ICB-related colitis than those who are not.

Research objectives

To be the first to compile the existing data regarding the role of GM composition and diversity in predicting response to ICB and ICB-related colitis specifically in patients with melanoma.

Research methods

Comprehensive literature search was done in various platforms using the following search terms: (fecal OR gut) AND (microbiota OR microbiome) AND (melanoma) AND (immunotherapy OR checkpoint OR nivolumab OR ipilimumab OR pembrolizumab). From a total of 300 studies, nine studies met inclusion criteria. Two studies were phase I clinical trials, while the remainder were prospective observational studies. All but one study has moderate risk of bias. Data from these studies including but not limited to, number of participants, type of immunotherapy received, GM analysis method, and GM composition and diversity were collected and interpreted.

Research results

Fecal samples enriched in *Firmicutes* phylum were associated with good response to ICB therapy, however they were associated with increased susceptibility to ICB-related colitis. Fecal samples enriched in *Bacteroidales* family were associated with poor response to ICB. Samples with greater GM diversity were associated with more favorable response to ICB. Fecal samples enriched in *Bacteroidetes* were associated with decreased incidence of ICB-related colitis. Overall, there was limited concordance in the organisms in the GM identified to be associated with response to ICB, and studies evaluating GM diversity showed conflicting results.

Research conclusions

GM composition and diversity holds some potential as a biomarker of response and toxicity to ICB in melanoma. Further prospective studies, including several RCTs that are underway, are needed to confirm whether the GM could be used as a biomarker and potential intervention to modulate ICB response in melanoma patients.

Research perspectives

With other promising biomarkers, GM composition and diversity holds potential to be integrated into a multiparameter model to accurately predict which subset of melanoma patients are likely to respond to ICB.

FOOTNOTES

Author contributions: Oey O, Simadibrata DM, Gray E and Khattak MA contributed to the study conception and design; Oey O and Liu Y performed data extraction; Oey O and Simadibrata DM performed risk of bias assessment; Oey O, Liu Y, Sunjaya AF, Simadibrata DM, Khattak MA and Gray E performed data analysis; Oey O written the first draft of the manuscript; all authors commented on previous versions of the manuscript, read and approved the final manuscript.

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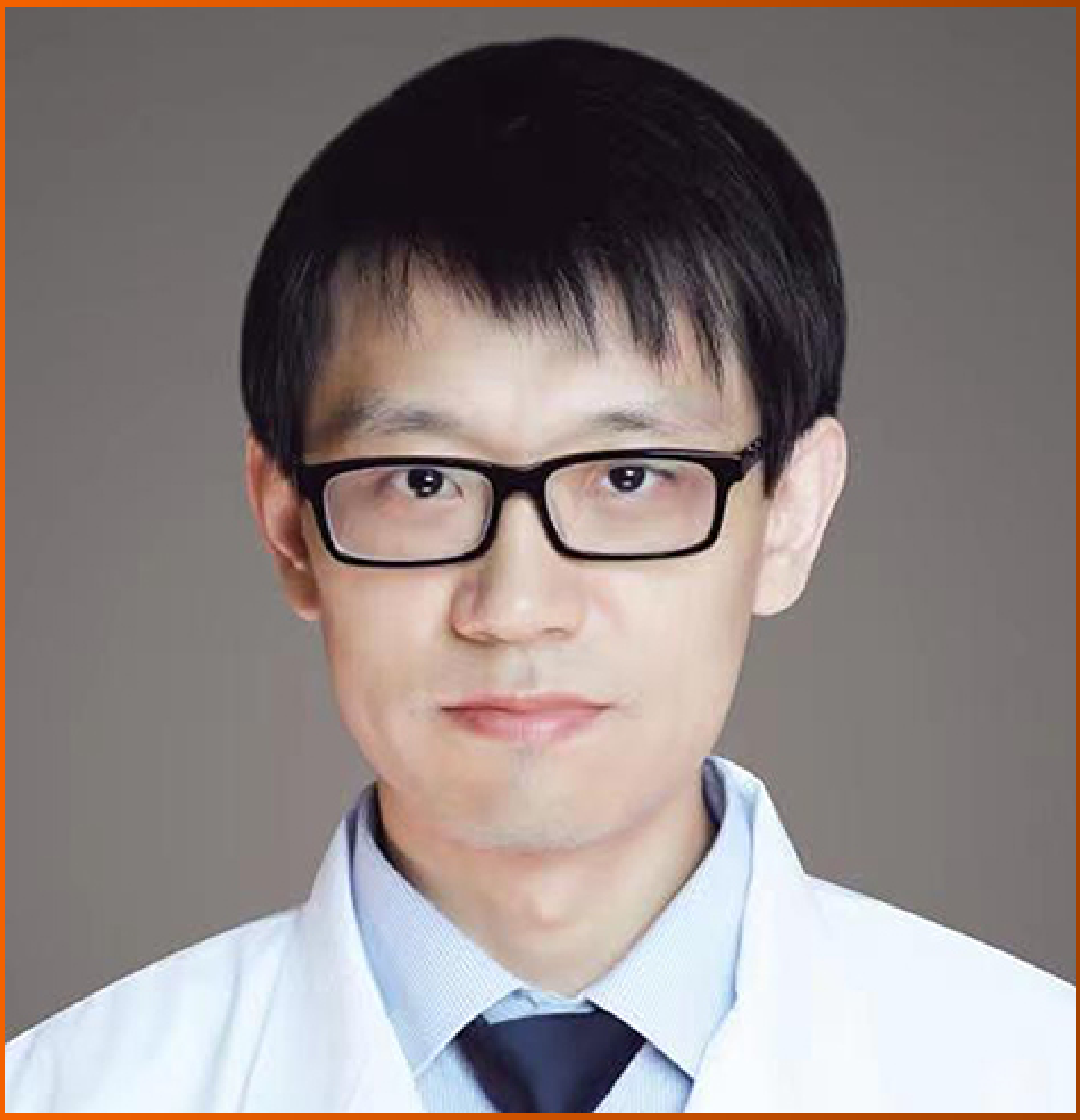


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Oncologic safety of colonic stenting as a bridge to surgery in left-sided malignant colonic obstruction: Current evidence and prospects

Sukit Pattarajierapan, Nattapanee Sukphol, Karuna Junmitsakul, Supakij Khomvilai

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Abstract

Approximately 7%-29% of patients with colorectal cancer present with colonic obstruction. The concept of self-expandable metal stent (SEMS) insertion as a bridge to surgery (BTS) is appealing. However, concerns on colonic stenting possibly impairing oncologic outcomes have been raised. This study aimed to review current evidence on the short- and long-term oncologic outcomes of SEMS insertion as BTS for left-sided malignant colonic obstruction. For short-term outcomes, colonic stenting facilitates a laparoscopic approach, increases the likelihood of primary anastomosis without a stoma, and may decrease postoperative morbidity. However, SEMS-related perforation also increases local recurrence and impairs overall survival. Moreover, colonic stenting may cause negative oncologic outcomes even without perforation. SEMS can induce shear forces on the tumor, leading to increased circulating cancer cells and aggressive pathological characteristics, including perineural and lymphovascular invasion. The conflicting evidence has led to discordant guidelines. Well-designed collaborative studies that integrate both oncologic outcomes and data on basic research (*e.g.*, alteration of circulating tumors) are needed to clarify the actual benefit of colonic stenting as BTS.

Key Words: Bridge to surgery; Colon cancer; Colorectal surgery; Emergency treatment; Intestinal obstruction; Self-expandable metal stent

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Core Tip: Although the concept of self-expandable metal stent (SEMS) insertion as a bridge to surgery in patients with left-sided malignant colonic obstruction is promising, there remain concerns of adverse oncologic outcomes. Nowadays, three possible mechanisms of tumor dissemination from SEMS have been proposed: (1) SEMS-related perforation; (2) increased circulating tumor cells; and (3) aggressive pathological features after SEMS placement. However, among these, only SEMS-related perforation clearly influences adverse oncologic outcomes. The other two mechanisms lack consistent clinical evidence for their association with decreased survival. Therefore, further collaborating studies are needed to validate the clinical impact of these hypotheses.

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INTRODUCTION

Colorectal cancer (CRC) is the fourth most common malignant disease worldwide, with more than 1.9 million new cases recorded in 2020[1]. Patients with CRC are presented with bowel obstruction for 7%-29%[2]. The outcomes of emergency surgery (ES) for patients with obstructed CRC are worse than those of elective surgery for patients without obstruction. Patients with obstructed CRC also have a higher mortality rate than those without obstruction (17% *vs* 6%, respectively)[3]. The causes of the high morbidity and mortality of ES are advanced-stage cancer, malnutrition, electrolyte abnormalities, colonic mucosa injury from distention, and fecal loading of the obstructed colon[4]. The self-expandable metal stent (SEMS) insertion as a bridge to surgery (BTS) concept, which converts an emergency condition to an elective one, is fascinating. Colonic decompression by SEMS gives time to stabilize medical conditions that distinctly benefit high-risk patients.

The benefits of SEMS as BTS for right-sided malignant colonic obstruction (RMCO, defined as an obstructed tumor located between the cecum and distal transverse colon) are limited. Currently, right colectomy with primary anastomosis is the recommended treatment for RMCO[5]. Ileocolic anastomosis is associated with the lowest incidence of leaks, ranging from 1% to 3%, and can be performed in cases with obstructive situation[6,7]. Therefore, the World Society of ES (WSES) guideline does not recommend SEMS as BTS for RMCO except in high-risk patients[5]. In contrast, SEMS insertion as BTS in left-sided malignant colonic obstruction (LMCO, defined as an obstructed tumor located between the splenic flexure and rectosigmoid junction) is very beneficial. In addition to the feasibility of laparoscopic resection, SEMS insertion allows the feasibility of elective single-stage colonic resection with a lower risk of permanent stoma creation[8].

However, the long-term oncologic outcomes are a matter of concern. SEMS induces shear force to the tumor and may lead to cancer cell dissemination into the peritoneal cavity, lymphatic fluid, and bloodstream[9,10]. A few studies suggested that SEMS insertion was associated with worse oncologic outcomes than ES, especially in patients with SEMS-related perforation[11,12]. Nevertheless, recent studies with low complication rates reported good oncologic outcomes with SEMS placement as BTS[13-19]. As a result, current guidelines are dynamic and discordant because of the conflicting evidence[20-24].

As such, this study aimed to perform a comprehensive review of the current evidence on the short- and long-term oncologic outcomes of SEMS insertion as BTS for LMCO.

TREATMENT OPTIONS FOR LMCO

ES

Emergent procedures for LMCO include various procedures such as Hartmann's procedure, segmental colectomy with/without on-table lavage, and subtotal/total colectomy. These procedures result in high morbidity and mortality because of the limited time to stabilize the patient's condition before surgery [4]. Among these, Hartmann's procedure remains one of the most common emergency procedures for the left colon because of the short operative time and avoidance of anastomotic leakage[25]. However, the rate of reversal of Hartmann's procedure is less than 50% because this operation is associated with high morbidity and possible mortality[26,27]. As a result, most of them turn to permanent stomas that severely affect the patient's quality of life. Laparoscopic approach for ES of LMCO has limited application because of technical difficulties during surgery. The WSES guideline does not recommend

its use except in selected cases in specialist centers[5].

Colonic stenting as BTS

Dohmoto *et al*[28] was the first to report the idea of using plastic tubes as colonic stenting for palliation of obstructed rectal cancer in 1991. One year later, Spinelli *et al*[29] started using SEMS insertion as a palliative modality with good results. After the success of palliative SEMS placement, the BTS concept was introduced by Tejero *et al*[30] in 1994. They described 3 phases of SEMS as BTS: (1) Relieving obstruction by SEMS; (2) recovering the patient's condition and mechanically preparing the colon; and (3) definitive elective surgery. It has been 30 years since the introduction of colonic stenting. Palliative SEMS placement is established as a preferred option in incurable malignant colonic obstruction because it confers superior quality of life by avoiding stoma and is associated with shorter time to initiation of chemotherapy than palliative surgery[21]. In contrast, the role of SEMS as BTS is still controversial, with concerns of adverse oncologic outcomes after SEMS insertion limiting its application as BTS.

Stent materials and technical considerations

There are different types of SEMS materials including stainless steel, elgiloy, and nitinol[31]. Endoscopists should be aware of the characteristics of each stent. Stainless steel stents are relatively stiff and interfere with magnetic resonance imaging (MRI) examination. Meanwhile, elgiloy stents have better elasticity and flexibility and do not interfere with MRI assessment. Nitinol stents are made of nickel-titanium and have poorer fluoroscopic visualization compared with elgiloy stents. Therefore, radiopaque markers, such as gold or silver markers, are added to both ends of these stents. Nitinol stents have superior flexibility and better memory to hold the original shape than stainless steel and elgiloy stents; consequently, nitinol stents are popular worldwide.

SEMS is classified as covered or uncovered. Covered SEMS has a silicone membrane on bare wires, preventing tumor ingrowth. For obstructed CRC, uncovered SEMS is recommended for both curative and palliative settings[21]. A recent meta-analysis, including one randomized controlled trial (RCT) and nine observational studies, compared covered and uncovered SEMS in curative and palliative settings. The study found that uncovered SEMS was associated with fewer complications (relative risk [RR]: 0.57; 95%CI: 0.44-0.74; $P < 0.001$), tumor ingrowth (RR: 0.29; 95%CI: 0.09-0.93; $P = 0.040$), and SEMS migration (RR: 0.29; 95%CI: 0.17-0.48; $P < 0.001$)[32]. Meanwhile, there was limited evidence regarding the optimal SEMS diameter[21]. Previous studies showed no association between SEMS diameter and success or perforation rate[33,34]. However, a few studies suggested an association between SEMS diameter < 24 mm and adverse events, especially migration[35-37]. Regarding SEMS length, it is recommended that the SEMS should be long enough to extend at least 1.5-2 cm on each side of the lesion, and the degree of SEMS shortening after deployment must be considered[21].

Colonic stenting can be performed endoscopically (through-the-scope technique) or fluoroscopically (over-the-wire technique). Several studies showed comparable technical success rate between endoscopic and fluoroscopic methods, but a combined endoscopic-fluoroscopic method showed the highest success rate[38-41]. Therefore, the European Society of Gastrointestinal Endoscopy (ESGE) guideline recommends that colonic stenting should be performed with the combined use of endoscopy and fluoroscopy[21]. For the combined technique, a soft-tipped hydrophilic guidewire is passed through the strictured lumen. Contrast injection helps to delineate the stenosis and to confirm guidewire placement under fluoroscopy. The SEMS is then passed over the guidewire and deployed under endoscopic visualization and fluoroscopic guidance[42] (Figure 1). Stricture dilation should not be performed either before or after colonic stenting as it increases the risk of perforation[21,43]. The recommended interval to curative resection after BTS stenting is approximately 2 wk[21].

Benefit of colonic stenting as BTS for LMCO

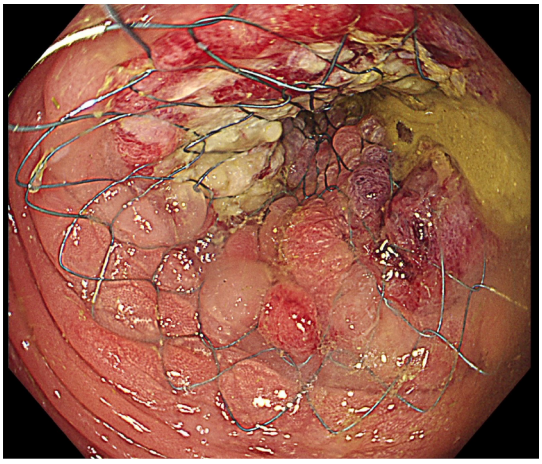
Owing to the high morbidity (45%-50%) and mortality (15%-20%) of ES for obstructed CRC, the BTS concept of avoiding an emergent situation is appealing[44]. After relieving the obstruction by SEMS placement, the clinicians can have time to stabilize the patients, improve their nutrition, correct electrolyte imbalance, and mechanically prepare the colon before definite resection. In addition, it is crucial that surgeons gain the ability to perform laparoscopic resection after BTS stenting (Figure 2). Compared with open resection, laparoscopic resection is associated with lower postoperative pain, earlier recovery of bowel function, and shorter hospital stay[45].

Nine RCTs have investigated the short-term outcomes of SEMS placement as BTS in comparison with those of ES for LMCO (Table 1). Notably, perforation after SEMS insertion and the success rate influenced postoperative outcomes. Four RCTs without perforation showed that SEMS insertion as BTS had a lower morbidity rate than ES[46-49]. Meanwhile, 1 RCT with a low stent success rate (70%) and 3 RCTs with 6.6%-12% perforation rate showed no difference in morbidity between SEMS insertion as BTS and ES[15,50-52]. Additionally, RCTs with low perforation rate also showed a significantly lower rate of postoperative stoma[15,46,53]. Stoma is well recognized to adversely affect quality of life. We conducted meta-analyses that included these nine RCTs. Of these, seven, nine, and seven studies reported the stoma rates, postoperative morbidity, and mortality rates, respectively. In the SEMS group, the stoma rate (RR: 0.68; 95%CI: 0.55-0.85, $I^2 = 19\%$) and postoperative morbidity (RR: 0.67; 95%CI: 0.48-0.94, $I^2 =$

Table 1 Short-term outcomes in randomized controlled trials of colonic stenting as a bridge to surgery

Ref.	Year	*n*	Perforation rate (%)	Stoma rate (%)	Morbidity (%)	Mortality (%)
Cheung *et al*[46]	2009	48	0	SEMS, 0; ES, 25, (P = 0.03)[a]	SEMS, 8; ES, 70; (P = N/A)	N/A
Van Hooft *et al*[51]	2011	98	12	SEMS, 57; ES, 66; (P = 0.35)	SEMS, 53; ES, 45; (P = 0.43)	SEMS, 19; ES, 17; (P = 0.84)
Pirlet *et al*[52]	2011	60	6.6	SEMS, 43; ES, 56; (P = 0.3)	SEMS, 50; ES, 56; (P = 1)	SEMS, 10; ES, 3; (P = N/A)
Alcántara *et al*[47]	2011	28	0	N/A	SEMS, 13; ES, 54; (P = 0.042)[a]	SEMS, 0; ES, 8; (P = 0.46)
Ho *et al*[50]	2012	39	0	SEMS, 10; ES, 31; (P = 0.12)	SEMS, 35; ES, 58; (P = 0.15)	SEMS, 0; ES, 16; (P = 0.1)
Ghazal *et al*[48]	2013	60	0	N/A	SEMS, 13; ES, 50; (P = 0.012)[a]	SEMS, 0; ES, 0
Arezzo *et al*[15]	2017	115	8.9	SEMS, 22; ES, 39; (P = 0.031)[a]	SEMS, 52; ES, 58; (P = 0.529)	SEMS, 7; ES, 5; (P = 0.943)
Elwan *et al*[49]	2020	60[1]	0	SEMS, 20; ES, 27; (P = N/A)	SEMS, 23; ES, 40; (P = 0.029)[a]	N/A
CReST trial[53]	2022	217[2]	3.3[3]	SEMS, 43; ES, 67; (P < 0.001)[a]	SEMS, 34; ES, 35; (P = 0.930)	SEMS, 4; ES, 6; (P = 0.480)

[a]P<0.05
[1]In this study, 85% of patients have left-sided malignant colonic obstruction, and 15% have right-sided malignant colonic obstruction.
[2]There are 217 potentially curative patients from 245 patients.
[3]The rate is reported in all patients (93% patients with potentially curable disease and 7%, palliative disease).
ES: Emergency surgery; SEMS: Self-expandable metal stent; N/A: Not available.



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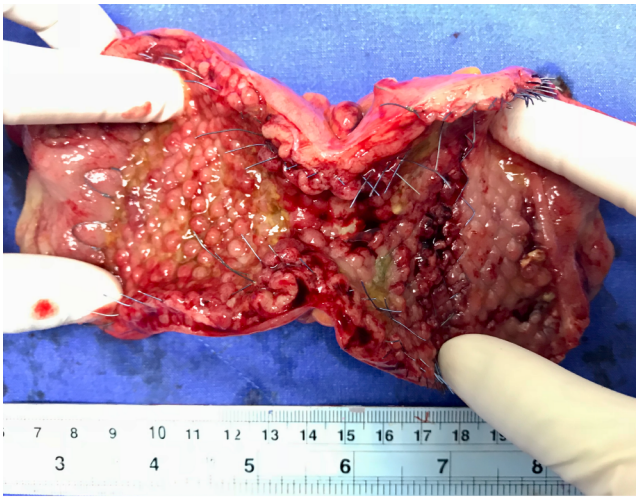
Figure 1 Endoscopic image of deployed stent.

65%) were significantly lower than those in the ES group (Figures 3 and 4). There were no differences in mortality rates between the SEMS and ES groups (RR: 0.95; 95%CI: 0.53-1.70, I^2 = 0%) (Figure 5).

In 2021, Cirocchi *et al*[54] conducted a systematic review and meta-analysis of RCTs and found that compared with ES, SEMS placement as BTS had a higher rate of successful primary anastomosis (RR: 1.26; 95%CI: 1.01-1.57), lower stoma rate (RR: 0.62; 95%CI: 0.45-0.85), and lower postoperative complication (RR: 0.61; 95%CI: 0.45-0.85). The mortality rate was comparable between the two modalities. To conclude, SEMS placement as BTS clearly has short-term benefits of higher primary anastomosis, lower stoma rate, and lower morbidity than ES. In this context, low perforation and high stenting success rates are needed to benefit from SEMS.

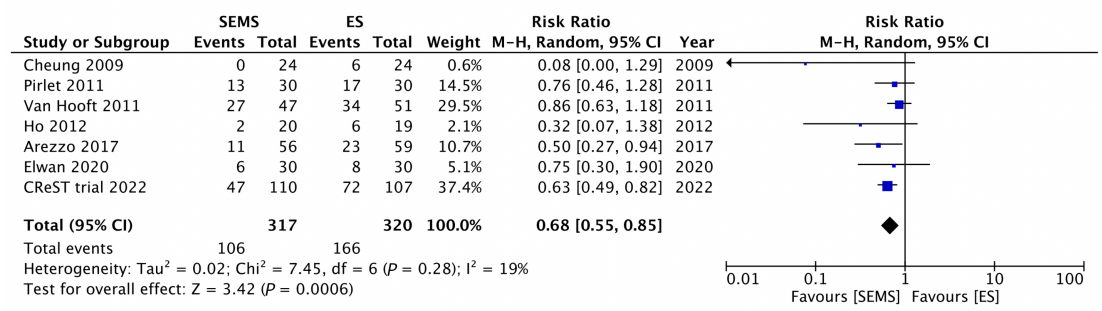
ONCOLOGIC OUTCOMES AFTER COLONIC STENTING IN CURABLE DISEASE

Despite the impressive short-term results of SEMS placement as BTS, its application has been debated due to concerns about adverse long-term oncologic outcomes. Theoretically, shear forces created by SEMS might lead to cancer cell dissemination through the following three possible mechanisms (Figure 6): (1) SEMS-related perforation; (2) increased circulating tumor cells; and (3) aggressive pathological features after SEMS placement.



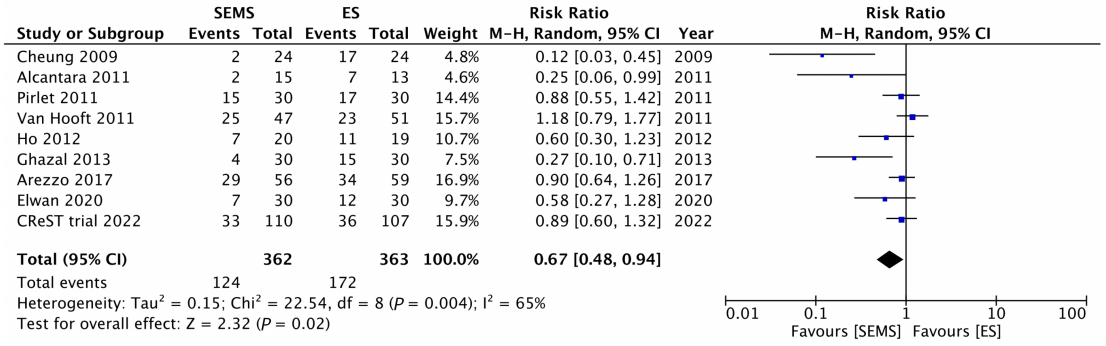
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Figure 2 Surgical specimen after laparoscopic colectomy following colonic stenting as a bridge to surgery.



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Figure 3 Forest plot showing the stoma rate. SEMS: Self-expandable metal stent; ES: Emergency surgery.

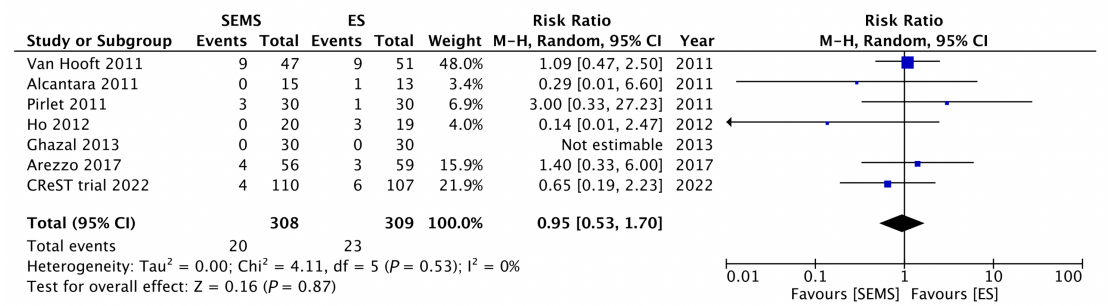


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Figure 4 Forest plot showing the postoperative morbidity rate. SEMS: Self-expandable metal stent; ES: Emergency surgery.

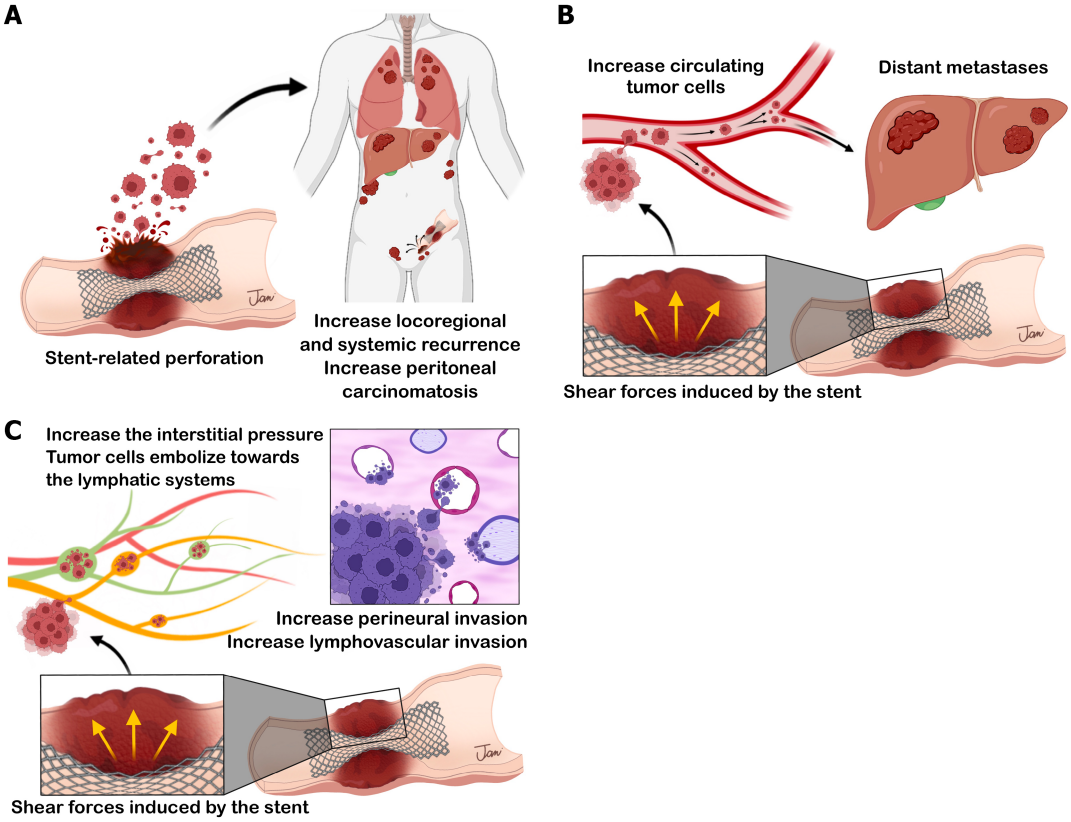
SEMS-related perforation

In obstructed CRC, manipulation of the ulcerated and necrotic tissue through SEMS may cause tumor perforation, which is the most feared complication of SEMS insertion. Perforation causes tumor dissemination into the peritoneal cavity and increases locoregional recurrence and peritoneal carcinomatosis, which affects long-term outcomes[55]. Perforation can be classified into clinical and silent perforation. Interestingly, some studies showed that silent perforation in SEMS as BTS can occur in up to 6%-27% of patients[43,51,52,56]. Further, this rate may still be underestimated because silent perforation can be diagnosed only from pathological assessment of the surgical specimen. Given that there have been sparse reports of silent perforation in the literature, its impact on oncologic outcomes is difficult to



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Figure 5 Forest plot showing the overall mortality rate. SEMS: Self-expandable metal stent; ES: Emergency surgery.



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Figure 6 Three possible mechanisms of tumor dissemination after self-expandable metal stent placement. A: self-expandable metal stent (SEMS)-related perforation; B: Increased circulating tumor cells; C: Aggressive pathological features after SEMS placement.

verify; however, it should not be disregarded.

In the Dutch Stent-In 2 trial, Sloothaak *et al*[57] found that 83% of patients with SEMS-related perforation have recurrence. Moreover, Gorissen *et al*[11] suggested that local recurrence was higher in patients who underwent SEMS placement as BTS than in those who underwent ES (32% *vs* 8%, $P = 0.04$). In this study, all patients with perforation had recurrence. Sabbagh *et al*[58] found that SEMS-related perforation was an independent risk factor for poor overall survival. Sensitivity analyses revealed that 3-year overall survival was better in studies with < 8% SEMS-related perforation rate than in those with ≥ 8%[59]. Balciscueta *et al*[55] recently conducted a systematic review and meta-analysis of 13 studies (1 RCT, 4 prospective studies, 8 retrospective studies) with long-term oncologic outcomes. The overall rate of SEMS-related perforation was 8.9%. The locoregional recurrence rate was higher in patients with perforation than in those without perforation (26.6% *vs* 12.5%; OR: 2.41; 95%CI: 1.33-4.34; $P = 0.04$), while the systemic recurrence rate was comparable.

In summary, SEMS-related perforation influences the occurrence of adverse oncologic outcomes; therefore, an endoscopist's experience and expertise are crucial with respect to deciding between SEMS placement as BTS or ES. The ESGE guideline recommends a shared decision-making discussion with the

patient that should include the availability of stenting expertise and the risk of perforation in the endoscopy unit[21].

Increased circulating tumor cells

SEMS placement could impair oncologic outcomes despite the absence of perforation. SEMS exerts shear forces on the tumor and makes tumor cells disseminate throughout the body[60]. Maruthachalam *et al*[9] found a more significant rise in cytokeratin 20 messenger RNA expression in peripheral circulation after SEMS placement than after conventional colonoscopy. The presence of messenger RNA coding for epithelial markers indicates the presence of tumor cells or shed debris in the circulation. Furthermore, Yamashita *et al*[10] found that SEMS placement induces tumor cell dissemination into the peripheral circulation. Using circulating cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) as indicators, Takahashi *et al*[61] recently found that SEMS insertion may cause massive cellular and tumor damage. The patients who underwent SEMS placement had higher postoperative plasma levels of both cfDNA and ctDNA than did those who underwent transanal tube decompression. On the contrary, Ishibashi *et al*[62] found that the increase of circulating tumor cells after SEMS insertion may be temporary, as in most cases, the number of circulating tumor cells decreased 4 d after SEMS placement. Although evidence of tumor cell dissemination after SEMS placement exists, there is inadequate clinical evidence of its negative effects on survival and prognosis.

Aggressive pathological features after SEMS placement

SEMS insertion leads to a sudden increase in interstitial pressure inside the tumor mass, possibly causing detachment of cells and tumor embolization towards the lymphatic systems and resulting in lymphatic invasion[63]. Hayashi *et al*[64] noted that the tumor pressure is important, not only for the number of tumor cells shed, but also for the size of emboli shedding into lymphatics around the tumor. Several studies revealed that SEMS insertion might promote perineural invasion found in surgical specimens, although these studies failed to translate higher perineural invasion into poorer oncologic outcomes[18,65-67]. Meanwhile, various studies found that SEMS had no significant effect on the incidence of perineural invasion compared with ES[8,68-70]. Conflicting findings with respect to other adverse pathological features such as lymphovascular and vascular invasion have also been reported [13,18,19,65,67,70-72]; therefore, cumulative data are needed. Balciscueta *et al*[73] recently conducted a meta-analysis of 1273 patients from 10 retrospective cohort studies and found higher perineural invasion (OR: 1.98; 95%CI: 1.22-3.21; P = 0.006) and lymphatic invasion (OR: 1.45; 95%CI: 1.10-1.90; P = 0.008) after SEMS insertion than after ES. Therefore, the use of SEMS as BTS should be carefully considered due to an increase in adverse pathological characteristics, although the long-term adverse oncological effects have not been demonstrated.

Oncologic outcomes of colonic stenting as BTS from RCTs

Six RCTs have reported long-term oncologic outcomes after SEMS placement as BTS compared with those of ES (Table 2). For the studies without SEMS-related perforation, the recurrence and survival outcomes are not significantly different between the two modalities[47,48,74]. In contrast, the Dutch Stent-In 2 trial, which had a high perforation rate of 23%, reported poorer disease-free survival in patients who underwent SEMS insertion as BTS than in those underwent ES[57]. This study underlined the strong association between SEM-related perforation and adverse oncologic outcomes. The long-term follow-up outcomes of the ESCO and CReST trials have been recently published. The ESCO trial reported comparable oncologic outcomes between the two modalities, with an 8.9% rate of SEMS-related perforation rate[75]. Similarly, the CReST trial found a comparable 3-year recurrence rate and overall survival between SEMS as BTS and ES, with a low SEMS-related perforation rate of 3.3%[53]. The latest systematic review and meta-analysis of five RCTs by Cirocchi *et al*[54] revealed comparable recurrence rates and oncologic outcomes between SEMS placement as BTS and ES. We conducted a meta-analysis of these six RCTs that reported the recurrence rate. There was no significant difference in recurrence rates between the SEMS and ES groups (RR: 1.45; 95%CI: 0.96-2.17, I^2 = 45%) (Figure 7).

These clinical studies show that among the three proposed mechanisms of cancer cell dissemination by SEMS, only SEMS-related perforation clearly influences adverse oncologic outcomes. Sensitivity analyses showed that a < 8% perforation rate is the oncologically safe cut-off point for SEMS insertion [59]. Therefore, endoscopy units that aim to perform SEMS as BTS should audit and improve their SEMS-related perforation rate to be lower than 8%. Two other mechanisms, including increased circulating tumor cells and aggressive pathological features, failed to produce consistent clinical evidence in decreased overall and disease-free survival in the SEMS group. Therefore, further studies are needed to validate the clinical impact of these hypotheses.

Table 2 Long-term outcomes in randomized controlled trials of colonic stenting as a bridge to surgery

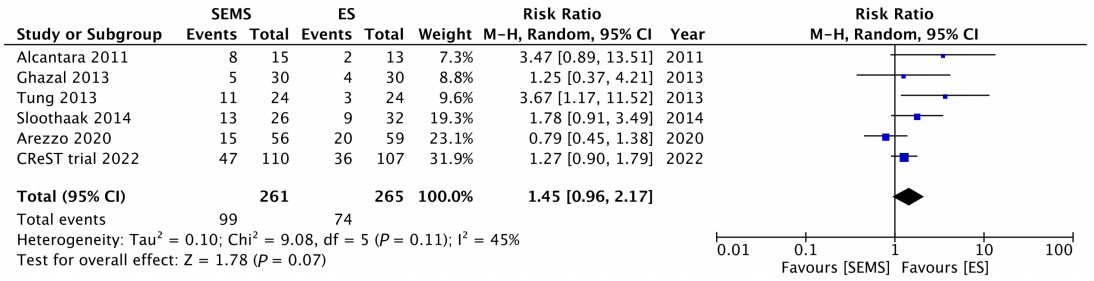
Ref.	Year	*n*	Perforation rate (%)	Median F/U time (mo)	Recurrence (%)	Overall survival (OS, %)	Disease-free survival (DFS, %)
Alcántara *et al*[47]	2011	28	0	38	SEMS, 53; ES, 15; (*P* = 0.055)	5-yr OS: SEMS, 60; ES, 68; (*P* = 0.843)	Disease-free period (mo): SEMS, 25; ES, 27; (*P* = 0.096)
Tung *et al*[74]	2013	48	0	32	SEMS, 46; ES, 13; (*P* = 0.400)	5-yr OS: SEMS, 48; ES, 27; (*P* = 0.076)	5-yr DFS: SEMS, 52; ES, 48; (*P* = 0.630)
Ghazal *et al* [48]	2013	60	0	18	SEMS, 17; ES, 13; (*P* = 0.228)	N/A	N/A
Sloothaak *et al*[57]	2014	58	23	43	SEMS, 50; ES, 28; (*P* = N/A)	4-yr OS: SEMS, 58; ES, 67; (*P* = 0.478)	4-yr DFS: SEMS, 30; ES, 49; (*P* =0.007)[a]
Arezzo *et al* [75]	2020	115	8.9	37	SEMS, 28; ES, 36; (*P* = N/A)	3-yr OS: SEMS, 63; ES, 68; (*P* = 0.822)	3-yr DFS: SEMS, 50; ES, 56; (*P* = 0.972)
CReST trial [53]	2022	217[1]	3.3[2]	N/A	3-yr recurrence: SEMS, 43; ES, 34; (*P* = 0.340)	3-yr OS: SEMS, 46; ES, 37; (*P* = 0.560)	N/A

[a]*P*<0.05

[1]There are 217 potentially curative patients from 245 patients.

[2]The rate is reported in all patients (93% patients with potentially curable disease and 7%, palliative disease).

ES: Emergency surgery; SEMS: Self-expandable metal stent; N/A: Not available.



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Figure 7 Forest plot showing the overall recurrence rate. SEMS: Self-expandable metal stent; ES: Emergency surgery.

CHEMOTHERAPY IN PATIENTS WITH COLONIC STENTS

Neoadjuvant chemotherapy in locally advanced colon cancer

The mainstay treatment of the potentially curable colon cancer is complete oncologic resection. However, one of the challenges is the risk of local and distant recurrence, which is estimated at 20%-30% in locally advanced colon cancer (defined as: T3 tumors with ≥ 5 mm invasion beyond the muscularis propria; T4; or extensive regional lymph node involvement without distant metastases)[76]. Recently, there was increasing evidence to support the use of neoadjuvant chemotherapy in locally advanced colon cancer[77-81]. The theoretical advantages include the early treatment of micrometastases, increased likelihood of clear resection (R0) margin, and ability to evaluate the chemosensitivity of the tumor[76]. Gosavi *et al*[76] conducted a meta-analysis of two RCTs and reported that neoadjuvant chemotherapy increased the likelihood of R0 resection in locally advanced colon cancer (RR 0.47; 95%CI: 0.47-0.96) without an increase in complications (anastomotic leak, wound infection, or re-operation). However, the safety of neoadjuvant chemotherapy after SEMS placement in obstructive colon cancer is also a concern.

There were a few studies using neoadjuvant chemotherapy after SEMS placement in obstructive colon cancer. The FOxTROT trial showed a significant decrease in R1 resection rate in patients who received neoadjuvant chemotherapy for locally advanced colon cancer. However, only a few patients in this trial underwent SEMS placement as BTS; therefore, a conclusion about SEMS safety could not be drawn[79]. Recently, Han *et al*[82] conducted a comparative study investigating the safety of neoadjuvant chemotherapy after SEMS placement. They found that the adverse events of preoperative chemotherapy were well-tolerated, and the neoadjuvant chemotherapy did not increase SEMS-related complications (*P* = 0.13). Moreover, this study revealed that patients who received neoadjuvant chemotherapy had better overall survival than those who received postoperative chemotherapy (mean overall survival, 53 *vs* 47 mo, respectively, *P* = 0.02). However, well-designed RCTs with larger sample

size and long-term follow-up are needed to confirm the safety and potential survival benefit of neoadjuvant chemotherapy after SEMS placement.

Chemotherapy in patients with incurable stage IV colon cancer

Patients with incurable stage IV colon cancer benefit from SEMS placement by avoiding palliative surgery and early initiation of chemotherapy. However, there is a concern that chemotherapy during SEM placement might induce complications. For palliative SEMS placement, many studies (including patients with and without chemotherapy) reported perforation rates of 7%-13%[83-88]. Therefore, the decision to perform SEM insertion in patients with incurable stage IV colon cancer must consider the risks of long-term SEMS-related complications weighted against SEMS benefits[89].

The administration of antiangiogenic agents (*e.g.*, bevacizumab) in patients who underwent SEMS placement was found to increase the risk of SEMS-related perforation. A retrospective study reported 3-fold higher perforation rate in patients who received bevacizumab after SEMS placement than in those who did not receive bevacizumab[90]. In a large retrospective study of 1008 patients who received bevacizumab for incurable colon cancer, Bong *et al*[91] found that SEMS placement is a significant risk factor for complications requiring surgery in patients who received bevacizumab (HR 5.69, 95%CI 2.37-13.64, $P < 0.001$). In contrast, a retrospective study reported no significant difference in perforation rate in patients who received chemotherapy with and without bevacizumab (7.3% *vs* 7.0%, respectively, $P = 0.925$)[92]. The updated 2020 ESGE guideline recommends chemotherapy as a safe treatment in patients who have undergone palliative SEMS insertion. However, SEMS placement should not be performed while patients are receiving antiangiogenic therapy[21].

FUTURE DIRECTION

Although many RCTs and prospective and retrospective studies have investigated the role of SEMS placement as BTS in comparison with that of ES in LMCO, current evidence is still conflicting, and the international guidelines are also dynamic and discordant[20,21]. In 2014, the ESGE guideline did not recommend using SEMS insertion as BTS based on previous studies with low success and high perforation rates[20]. Nevertheless, many comparative studies and one RCT published thereafter[13-19, 93] reported impressive short- and long-term oncologic outcomes. As such, the updated ESGE guideline released in 2020 considers SEMS placement as BTS a valid treatment option in patients with LMCO. The guideline emphasized that the medical team has to discuss the risks and benefits of SEMS with patients and SEMS insertion should be performed or directly supervised by a competent endoscopist[21].

The proficiency of the endoscopist is crucial when SEMS as BTS is considered. Previous RCTs with low perforation rate showed appreciable short-term outcomes of SEMS, including lower stoma rate, higher primary anastomosis, lower morbidity, and comparable oncologic outcomes to ES[15,46-49,74, 75]. Moreover, current evidence clearly demonstrates the association between SEMS-related perforation and negative oncologic outcomes. Therefore, SEMS placement as BTS is a valid option for competent endoscopists.

Impaired oncological outcomes after SEMS placement that result from increased circulating tumor and adverse pathological characteristics remain a concern. However, current evidence could not demonstrate adverse long-term oncological effects. Further well-designed collaborative studies are needed to investigate the association among the alteration of circulating tumors, adverse pathological characteristics, and oncologic outcomes.

CONCLUSION

Colonic obstruction is a common presentation of CRC that needs emergency intervention. SEMS placement as BTS, converting an emergency situation to an elective one, improves short-term outcomes in LMCO, including higher primary anastomosis, lower stoma rate, and lower postoperative morbidity, compared with ES. However, there remain concerns on adverse oncologic outcomes from shear forces induced by SEMS. There are three possible mechanisms of tumor dissemination from SEMS: (1) SEMS-related perforation; (2) increased circulating tumor cells; and (3) aggressive pathological features after SEMS placement. However, among these, only SEM-related perforation clearly influences adverse oncologic outcomes. Consistent clinical evidence supporting the association of the other two mechanisms with decreased overall and disease-free survival is lacking. Therefore, further well-designed collaborative studies are needed to validate the clinical impact of these mechanisms. Current guidelines consider SEMS placement as BTS a valid treatment option in patients with LMCO, but it should be performed by competent endoscopists.

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FOOTNOTES

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MINIREVIEWS

Colonic stent for bridge to surgery for acute left-sided malignant colonic obstruction: A review of the literature after 2020

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Abstract

It has been found that 8%-29% of colorectal cancers are obstructive. The use of "stent as bridge to surgery" is one of the most debated topics in obstructive left-sided colorectal cancer management. The endoscopic placement of a self-expanding metallic stent as bridge to surgery (BTS) could turn an emergency surgery to an elective one, increasing the number of primary anastomoses instead of stoma and facilitating the laparoscopic approach instead of an open one. However, in recent years the possible risk of perforations and microperforations facilitating cancer spread related to the use of self-expanding metallic stent for BTS has been highlighted. Therefore, despite the useful short-term outcomes related to BTS, the recent literature has focused on long-term outcomes investigating the disease-free survival, the recurrence rate and the overall survival. Due to discordant data, international guidelines are still conflicting, and the debate is still open. There is not agreement about using self-expanding metallic stent for BTS as the gold standard.

Key Words: Colorectal cancer obstruction; Anastomosis; Laparoscopy; Recurrence rate; Overall survival; Guidelines

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Core Tip: The most recent articles (published after 2020) about self-expanding metallic stent as bridge to surgery in left-sided colorectal cancer obstruction were collected. Both the short-term and long-term outcomes were analyzed, focusing on the role of stent-related microperforations in worsening disease-free and overall survival rates. Despite the growing number of studies published in recent years, the use of self-expanding metallic stent as bridge to surgery is not considered the gold standard due to conflicting reports. Updating meta-analyses, randomized studies and reviews will help determine new international guidelines and a shared treatment flow-chart.

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INTRODUCTION

It is known that 8%-29% of colorectal cancers (CRC) are initially characterized by obstruction[1]. In the management of left-sided colonic obstruction there are two different options: emergency surgery (ES) and stent placement as bridge to surgery (BTS). Historically, an ES was first considered for distal malignant obstruction[2]. In this context, three different surgical options could be considered[3]: (1) Three-stage management, in which the first intervention is a proximal stoma formation, followed by colonic resection and stoma reversal; (2) Two-stage management (Hartmann's intervention); and (3) One-stage management that consists of resection and primary anastomosis. About 20 years ago the SEMS placement was first used to decompress neoplastic stenosis[4].

ES is often conducted with an open approach because a distended bowel may hamper laparoscopy [5]. In addition to that, ES frequently concludes with stoma formation that negatively impacts patient quality of life[6]. This is why the interest in BTS has become increasingly important. In fact, authors have primarily focused on short-term outcomes of using SEMS for BTS, such as anastomosis and stoma rate, laparoscopic and open approach and postoperative hospital stay. On the other hand, the recent BTS literature is focused on long-term outcomes, such as disease-free survival (DFS), overall survival (OS) and progression-free survival. Due to the length of time since the start of using SEMS for BTS, a multitude of data have been collected (Figure 1).

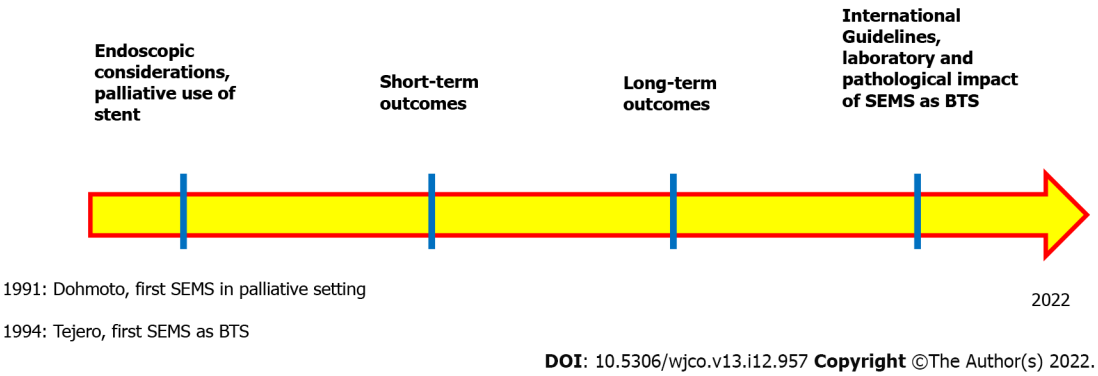
However, despite a growing number of articles about CRC obstruction, the use of SEMS for BTS is still debated. No uniform international guidelines have been published yet due to the hypothesized role of microperforations worsening the long-term outcomes of patients. The purpose of this review was to collect the latest (since 2020) research on SEMS for BTS use. In one of the more updated systematic reviews and meta-analyses[7], only two studies published after 2020 were included (Arezzo *et al*[8] multicentric study and Allievi *et al*[9] single center study). Both the international guidelines and the short-term/long-term outcomes focusing on the new data published after 2020 have been analyzed (the "update" part of each chapter).

GUIDELINES

In left-sided colonic obstructions, colonic stenting in a palliative setting is commonly accepted. However, the best treatment in a curative setting is still debated. There is still not agreement whether SEMS placement for BTS or upfront ES is better.

Some of the most important American guidelines recommend stent insertion as the first choice to solve colonic obstruction[10,11], while another considers both possibilities to be equally valid[12]. In general, the attitude of European guidelines is more moderate. No guidelines consider the insertion of a stent as the only option[13-15]. Both approaches are also considered in the World Society of Emergency Surgery[16]. Webster *et al*[17] analyzed high-quality international recommendations published between 2010 and 2019 and found that only two studies considered the use of a stent as the gold standard.

In this context, another discussed point is the "time to elective surgery," which is the time between stent insertion and elective surgery[18,19]. Theoretically a delayed interval between SEMS placement and definitive surgery could allow better recovery and improve nutritional status, but it could be burdened by a high rate of local tumor infiltration and fibrosis[20]. Not all guidelines indicate the ideal number of days between stent positioning and surgery. However, in the American Society for Gastrointestinal Endoscopy 6 d before elective surgery is considered the best interval. After the 6th day the risk of perforation is increased[10]. In the Eastern Association for the Surgery of Trauma an interval of 7 d is considered[21].



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Figure 1 Self-expandable metallic stent as bridge to surgery timeline. Topic of interest of self-expandable metallic stent (SEMS) used as bridge to surgery from the initial use to current use. BTS: Bridge to surgery.

Update

Recently, the European Society of Gastrointestinal Endoscopy (ESGE) guidelines-Update 2020 has been published[22]. They strongly recommend with high quality of evidence that stenting for BTS as an option in patients with potentially curable left-sided obstruction, and it must represent a shared decision-making process. This main recommendation is different from the 2014 ESGE guidelines, which stated that the stent placement could be considered as an alternative only for patients with American Society of Anesthesiologists ≥ III and/or age > 70 years[23]. However, in the 2014 and 2020 editions, the most important recommendation to use SEMS for BTS should be reserved for patients without signs of perforation (always strong recommendation)[22]. In the recent ESGE guidelines a time interval of 2 wk until surgery is considered (weak recommendation, low quality evidence)[22].

ENDOSCOPIC CONSIDERATIONS

For patients suspected of having a neoplastic left-sided colonic obstruction, an urgent colonoscopy is usually performed[24]. It may be useful to identify other colonic lesions and to stage cancer with more accuracy[25].

The SEMS placement remains a challenging procedure. The technical success is defined as the endoscopic correct stent placement, while the clinical success is the resolution of the obstruction[26]. SEMS can be covered and uncovered. The uncovered SEMS can be divided into through-the-scope and non-through-the-scope[27]. The through-the-scope SEMS is inserted through a guidewire. The diameter of the SEMS is about 18-22 mm[24]. In the majority of studies[24,25] the WallFlex enteral colonic stent and the Niti-S enteral colonic stent were used.

SEMS placement for BTS has been extensively studied for left-sided colonic obstruction, while limited data have been collected for the right-sided obstruction[28].

Although severe endoscopic adverse events complicate < 5% of procedures[10], early and late complications can sometimes occur[29]. Some early complications (within 30 d) are migration, perforation and bleeding, while some late complications (after 30 d) are a late obstruction, migration and perforation. The covered SEMS have a higher migration rate and lower obstruction rate, while uncovered SEMS have a lower migration rate[27]. Only a few studies investigated the predictors of technical failures, but it seems that a stenosis > 8 cm may be associated with a higher rate of technical failure[30].

Update

A central part of the updated ESGE guideline is dedicated to endoscopic technical considerations. Colonic stenting should be performed directly or supervised by a medical figure both with colonoscopy and fluoroscopic expertise[22].

In 2021, a multicenter prospective cohort study affirmed that the WallFlex stent was the most used globally[31].

Some authors tried to compare the feasibility and safety of SEMS for BTS based on the grade of colonic neoplastic obstruction, classified 0 to 2 by the Colorectal Obstruction Scoring System. No differences were found in safety and short-term outcomes in patients classified as 0, 1 or 2[26].

An effort to standardize the SEMS placement has been made. It has likely contributed to excellent short-term outcomes, technical success and low perforation rate[31].

According to the most recent literature, colonic stenting should be performed by endoscopists who demonstrate a good expertise as it represent a challenging procedure, In some articles a minimum number of procedures is indicated[32]. If stenting expertise is not available, decompressing stoma as a

bridge to elective surgery should be considered[22].

It was shown that delaying surgery can lead to a significantly higher recurrence rate[33].

LABORATORY AND ANATOMOPATHOLOGICAL CONSIDERATIONS

In the last 2-3 years there has been a growing interest about the possible association between SEMS for BTS and worse long-term oncological outcomes, such as perineural invasion (PNI) and vascular and lymphatic invasion[34]. The presence of PNI seems to decrease long-term survival[34]. The PNI negatively impacts recurrence and survival in CRC. In some studies, there was no difference in perineural invasion between the ES group and the SEMS for BTS group[35]. The tumor stage and vascular invasion were found to be independent risk factors for PNI in patients with obstructing colonic stenting[36]. According to Wang *et al*[37], PNI may be associated with obstruction but not with stent insertion.

In other articles, laboratory elements, such as circulating cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA), have been identified. cfDNA, which indicates cellular damage, is derived from apoptotic or necrotic cells. ctDNA indicates tumor-derived DNA possibly from apoptotic or necrotic cells, and it could contain gene mutations. During the endoscopic procedure of colonic stenting the manipulation of the tumor could increase plasma ctDNA and cfDNA[38]. Stent-induced neoplastic manipulation may lead malignant cells to local and distant invasion, worsening long-term outcomes [38].

Broholm *et al*[39] performed a gene expression analysis. The Nano String Counter Pan Cancer Immune Oncology Panel 360 gene expression was used. They observed that SEMS for BTS induced changes in gene expression in the neoplastic microenvironment, related to progression in CRC and may induce a more aggressive phenotype. These changes seemed to be caused by mechanical pressure of the cancer and the following inflammation of tissue. Six genes promoting angiogenesis were significantly upregulated. Tumor-promoting inflammation gene expression, such as *IL-6*, were involved. The use of anti-inflammatory drugs after stenting has been proposed[39].

SHORT-TERM OUTCOMES

In almost all studies postoperative complications are analyzed. Postoperative outcomes such as 30-d or 60-d mortality, anastomosis rate and laparoscopic rate are often considered.

The most important advantages of using a SEMS for BTS is transforming an urgent surgery to an elective one and maintaining bowel continuity by avoiding stoma creation[40,14]. In fact, a primary anastomosis can be created more safely in an elective setting than in an urgent or emergent setting. Wang *et al*[41] revealed in their meta-analysis that the use of colonic stenting could not increase the risk of anastomotic leakage incidence compared with emergency surgery.

Using a stent for BTS approach could be different as well. Donlon *et al*[42] reported a 78% rate of the laparoscopic approach. However, in the same year Boland *et al*[43] reported only three studies (41%) in which the laparoscopic approach was successfully completed after the stent insertion.

According to De Ceglie *et al*[44], postsurgical complications like infections were less frequent in patients undergoing urgent surgery. In the same article, the hospitalization rate was similar in the ES and BTS groups. On the other hand, Consolo *et al*[45] observed a different result, demonstrating a reduced hospital stay in the BTS group.

Arezzo *et al*[46] found in their high-quality meta-analysis published in 2017 a significantly lower rate of temporary and definitive stomas (33.9% *vs* 51.4%, $P < 0.001$ and 22.2% *vs* 35.2%, $P = 0.003$), while no difference in the 60-d mortality was observed.

Only a few studies analyzed the cost-effectiveness between using SEMS for BTS and ES[10]. Allievi *et al*[47] and Neo *et al*[48] concluded that more data about cost-effectiveness are needed.

Update

The updated review of Hiyoshi *et al*[49] demonstrated that the use of SEMS was associated with low hospital mortality, a higher rate of primary anastomosis and decreased stoma rate. For these reasons after colonic stenting for BTS, patients often have a better quality of life compared with immediate resection[50]. Better short-term outcomes of the BTS group were confirmed by Spannenburg *et al*[51]. Higher primary anastomosis, lower 30-d mortality rate, lower overall complications rate and shorter hospital stay were reported.

In June 2022, a study that compared BTS to diverting stoma was published[52]. Seven studies were included, and 1358 patient were recruited (646 in the first group and 712 in the second group). A lower Clavien-Dindo I/II complication rate was highlighted in the BTS group (8.68% *vs* 16.85%, $P = 0.004$), while the III-IV grade were similar (7.69% *vs* 8.79%, $P = 0.37$). There were no differences in short-term mortality, 3-year OS and permanent stoma rate.

LONG-TERM OUTCOMES

Even though SEMS for BTS short-term outcomes have been quite established, the long-term outcomes still remain uncertain. In the last few years, the literature on using SEMS for BTS focused on oncological outcomes. The DFS is the time between surgery and discovery of new cancer signs, while the OS is the time between surgery to death. In the early 2000s, Kim *et al*[53] suspected that SEMS insertion could negatively impact oncological outcomes.

Some authors tried to explain the mechanism. Some authors hypothesized that SEMS manipulation could cause a microperforation that may lead to peritoneal carcinomatosis. Other authors hypothesized that tumor compression was the cause of hematogenous diffusion[54]. Maruthachalam *et al*[55] found an increased expression of cytokeratin 20 mRNA (marker of tumor cells) in patients after stent insertion.

Amelung *et al*[56] found no significant differences in recurrence rate and 3-year and 5-year OS. Rodrigues-Pinto *et al*[57] also found no differences in tumor recurrence, recurrence-free survival and OS between the ES and BTS groups. The same result was also obtained by Matsuda *et al*[6] and Gibor *et al*[58].

These results were strongly supported by Arezzo *et al*[46] and Amelung *et al*[56]. The first was a multicentric prospective randomized trial conducted by the European Association for Endoscopic Surgery. The second was a meta-analysis in which no differences between the two groups in terms of 3-year and 5-year DFS and in 3-year and 5-year OS were confirmed.

However, a few studies showed differences in long-term oncological outcomes. In 2019 Foo *et al*[54] presented a higher distant recurrence rate in the BTS group (25.3% *vs* 15.0%, $P = 0.046$) and overall recurrence rate in the BTS group (37.0% *vs* 25.9%, $P = 0.049$). A 5-year follow-up is usually described in all studies, whereas Verstockt *et al*[59] presented a 1-year, 2-year, 3-year, 5-year and 10-year OS for all patients regardless of stage.

In recent literature, the cost-effectiveness has also been considered[60]. The American Society for Gastrointestinal Endoscopy guidelines considered the use of stent for BTS more cost-effectiveness than ES[10]. However, little data about this topic are available[47].

Update

According to Cirocchi *et al*[7], the overall recurrence and 3-year OS rates are similar for both the ES and BTS groups. In another recent study, 3-year and 5-year DFS and OS were not different despite a higher number of lymph nodes harvested in the BTS group than the ES group[51].

In the ESCO trial[8], neither OS nor DFS differed in the BTS and ES groups in a 36-mo follow-up study. However, as it has been reported in this paper, one randomized trial showed an increased rate of malignant recurrence[61]. The contradictory data need a well conducted prospective, randomized trial. The inclusion criteria used by ESCO were colonic cancer between the splenic flexure and 15 cm from the anal margin and diagnosed by computed tomography. Similar long-term oncological outcomes were observed. A significant time to progression was observed in the descending colon, possibly because it was the easiest endoscopic procedure (compared to flexure and sigmoid). No differences in terms of time to progression, DFS and OS were observed considering age, sex, body mass index and American Society of Anesthesiologists score[8].

In February 2022, a multicentric study[62] including 564 patients was published. The results showed the "non-inferiority" of BTS *vs* ES in terms of OS ($P = 0.012$). However, in another a recent study[63] considering only stage II and III patients, a higher frequency of distant metastatic recurrence was shown in BTS group than in the surgery alone group (30.4% *vs* 13.3%, $P = 0.035$).

In 2022, Yamada *et al*[64] tried to explain how tumor manipulation may worsen the prognoses in CRC patients after SEMS insertion. Seven days after stent insertion the cfDNA, ctDNA and serum lactate dehydrogenase levels were significantly higher. This indicated that SEMS injures cancer and spreads damage-associated molecular patterns released by necrotic cells that induce sterile inflammation. SEMS placement seems to induce unfavorable gene circulation, which results in an microenvironment associated with cancer progression. According to this last study, angiogenesis is also induced by cancer manipulation *via* miR-9 downregulation.

According to some authors, using a standardized and reproducible SEMS insertion method is essential for reducing the perforation rate[31].

Veld *et al*[65] compared SEMS for BTS with decompressing stoma bridge to resection instead of ES. The authors concluded that the two techniques have similar intermediate-term oncological outcomes[65].

A comprehensive literature review compared SEMS for BTS long-term outcomes with decompressing stoma for BTS and ES. The authors found that colonic stent and decompressing stoma may lead to better 5-year OS and DFS than ES. The decompressing stoma may have a better 5-year OS than the BTS strategy. According to these data, Tan *et al*[66] recommended decompressing stoma as the best choice for left-sided colonic obstruction.

CONCLUSION

In 2022, the BTS strategy is considered a safe strategy, and many studies have demonstrated better short-term outcomes than ES. By using a stent for BTS, it is possible to obtain an increased rate of primary anastomosis *vs* stoma rate, a laparoscopic approach *vs* open approach and a shorter postoperative stay.

The endoscopic stent placement is not a simple procedure, and it requires specific skills[57]. The most recent ESGE guidelines recommend that colonic stenting should be performed by an operator with competence both in colonoscopy and fluoroscopic technique[22]. The stent insertion could be followed by early (< 30 d) or late complications (> 30 d), such as migration, perforation, bleeding and obstruction. However severe adverse events only occur in < 5% of procedures[10]. Stents could be covered or uncovered, with covered stents having a lower obstruction rate[27].

The use of a stent for BTS changes an ES into an elective surgery. The role of BTS has been analyzed for both short-term and long-term outcomes. The short-term outcomes are represented by higher laparoscopic approaches *vs* open surgery[42,43], higher rate of anastomosis *vs* stoma rate[41,46,67], 30-d post-surgery complications and hospital stay[44]. The long-term outcomes include the DFS, the progression-free survival and OS.

Almost all articles about SEMS for BTS from the late 1990s to 2010 focused on the short-term outcomes. After stent placement in an elective setting, surgery can be completed with a laparoscopic approach. In addition, a higher rate of primary anastomosis and lower rate of temporary or definitive stoma rates improve patient quality of life[46].

Currently, more focus is being place on long-term outcomes than short-term outcomes. Initially, gastroenterologists and surgeons were enthusiastic due to the excellent short-term results from the use of SEMS for BTS. However, conflicting data about the worsening of OS and DFS due to BTS curbed the initial enthusiasm[68]. Although there are many hypotheses, the exact biological mechanism has not been described. The use of a stent for BTS seems to be burdened by a higher rate of perforations and microperforations resulting in cancer spread[64]. Most articles only have 1-year to 3-year or 5-year follow-up. Some rare cases have longer periods[59].

Because of all these contrasting data, international guidelines do not agree about using a stent for BTS as the gold standard. The American guidelines suggest the use of a stent for BTS as the gold standard [10], while the European guidelines suggest the surgical resection and the use of SEMS for BTS as possible treatments in patients with left-sided obstructing colonic cancer[22].

Some of the most important topics for further studies are the national and international agreement on therapeutic algorithm of treatment for patients with left-sided obstructive colonic cancer and a more detailed cost-benefit analysis. Furthermore, considering literature after the 2020, it could be interesting to prepare a specific study to understand the impact of the COVID-19 pandemic impact on the use of SEMS for BTS.

FOOTNOTES

Author contributions: Binetti M analyzed the data and wrote the manuscript; Lauro A contributed to designing the research; Tonini V analyzed and corrected the manuscript; all authors contributed to this work; All authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Machine learning-assisted ensemble analysis for the prediction of urinary tract infection in elderly patients with ovarian cancer after cytoreductive surgery

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Abstract

BACKGROUND

Urinary tract infection (UTI) is a common type of postoperative infection following cytoreductive surgery for ovarian cancer, which severely impacts the prognosis and quality of life of patients.

AIM

To develop a machine learning assistant model for the prevention and control of nosocomial infection.

METHODS

A total of 674 elderly patients with ovarian cancer who were treated at the Department of Gynaecology at Jingzhou Central Hospital between January 31, 2016 and January 31, 2022 and met the inclusion criteria of the study were selected as the research subjects. A retrospective analysis of the postoperative UTI and related factors was performed by reviewing the medical records. Five machine learning-assisted models were developed using two-step estimation methods from the candidate predictive variables. The robustness and clinical applicability of each model were assessed using the receiver operating characteristic curve, decision curve analysis and clinical impact curve.

RESULTS

A total of 12 candidate variables were eventually included in the UTI prediction model. Models constructed using the random forest classifier, support vector machine, extreme gradient boosting, and artificial neural network and decision

tree had areas under the receiver operating characteristic curve ranging from 0.776 to 0.925. The random forest classifier model, which incorporated factors such as age, body mass index, catheter, catheter intubation times, blood loss, diabetes and hypoproteinaemia, had the highest predictive accuracy.

CONCLUSION

These findings demonstrate that the machine learning-based prediction model developed using the random forest classifier can be used to identify elderly patients with ovarian cancer who may have postoperative UTI. This can help with treatment decisions and enhance clinical outcomes.

Key Words: Cytoreductive surgery; Machine learning; Ovarian cancer; Risk factors; Urinary tract infection

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Core Tip: Using a machine learning-based algorithm, we developed a feasible and robust method to identify factors that are significant for predicting urinary tract infections. The random forest classifier was especially robust and can improve the prediction and early detection of urinary tract infections in patients with ovarian cancer. In addition, the five most crucial factors were age, body mass index, catheter, catheter intubation times, blood loss, diabetes and hypoproteinaemia. Clinicians may find it extremely helpful to assess the individualised risk of urinary tract infections in clinical practice by incorporating the presentation of simple clinical data.

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INTRODUCTION

Ovarian cancer is a gynaecological malignant tumour with the highest degree of malignancy and mortality[1]. Approximately 70%–80% of patients have advanced to the middle and late stage at the initial diagnosis owing to the asymptomatic nature of ovarian cancer in the early stage and lack of sensitive screening methods. In addition, 80% of patients with ovarian cancer experience a relapse within 1-2 years after surgery[2,3]. According to the statistics of the International Union of Obstetrics and Gynaecology, patients with ovarian cancer have a 5-year overall survival rate of < 40%, and a 5-year clinical stage IV survival rate of < 5%[4,5]. Currently, the first-line treatment for ovarian cancer is carboplatin combined with paclitaxel platinum chemotherapy following surgery, with a clinical remission rate of 60%–80%[6-8].

Advanced ovarian cancer is surgically treated with tumour cytoreductive surgery, which is the most effective surgical procedure[8]. Tumour cytoreductive surgery with a satisfactory tumour reduction ratio can prolong the survival time of patients and improve their overall survival rate. However, the scope of surgical resection includes not only ovaries, uterus and omentum but also pelvic and abdominal metastases and affected lymph nodes with a diameter of > 2 cm[9]. The operation is challenging, the injury obtained from the procedure is significant, and there are numerous complications since the procedure often involves the intestinal tract, the urinary tract and pelvic vessels. Additionally, some patients must undergo 2-3 courses of neoadjuvant chemotherapy prior to the surgery to have sufficient operation conditions[10]. The postoperative rehabilitation process and the quality of life of patients will suffer significantly from the high incidence of postoperative complications.

Urinary tract infection (UTI) is a common type of postoperative infection following tumour cell reduction surgery for ovarian cancer. It is related to the surgical procedure and the unique physiological structure of the female urinary tract[11,12]. Elderly patients with ovarian cancer have a higher incidence of postoperative UTI owing to their weak immune system, poor organ reserve capacity and a high proportion of basic diseases[13]. The evaluation of related factors is crucial for the prevention and management of nosocomial infection. However, there is no specific study on the related factors of UTI in elderly patients with ovarian cancer who underwent cytoreductive surgery at home and abroad.

Nowadays, predictive models based on advanced algorithms have been gradually applied to the medical field, which also enables many diseases to be detected and diagnosed early[14,15]. Among them, the machine learning (ML) algorithm relies on repeated iterative operations to accurately output the results, so it can improve the accuracy and robustness of prediction. Given the superior ability of the

ML-based algorithm to improve the accuracy of muscular invasion prediction, we applied the ML-assisted decision-support model to assess the risk of UTI using clinical parameters and direct clinical decision-making prior to treatment decisions.

MATERIALS AND METHODS

Patient selection

As the research subjects, 674 elderly patients with ovarian cancer who received treatment at the Department of Gynaecology at Jingzhou Central Hospital between January 31, 2016 and January 31, 2022 and met the inclusion criteria were selected. A retrospective analysis of the postoperative UTI and related factors was performed using medical records. The inclusion criteria for patients were as follows: (1) All patients met the diagnostic criteria in the clinical practice guidelines for ovarian cancer developed by the National Comprehensive Cancer Network and were diagnosed by imaging examination and postoperative pathology[16]; (2) All the patients were older than 60-years-old. The clinical stages were stage III and above, and the pelvic and abdominal masses were fixed; and (3) All the patients were scheduled for cytoreductive surgery. The clinical data were complete, and the postoperative hospital stay exceeded 5 d. The exclusion criteria for patients were as follows: (1) Patients undergoing secondary cytoreductive surgery for recurrent ovarian cancer; (2) Patients with liver and kidney insufficiency, cardiovascular and cerebrovascular accidents, blood diseases, autoimmune diseases or immunodeficiency diseases and other malignant tumours; (3) Patients diagnosed with acute and chronic infection prior to surgery; and (4) Patients who had long-term usage of immunosuppressants or glucocorticoids. The guidelines of the Helsinki Declaration (2013 revision) were followed by the study protocol. It was approved by the Institutional Review Committee of Jingzhou Central Hospital (JZ-2022014). Owing to its traceability, patient information was managed with the utmost confidentiality, and informed consent was waived. The workflow for patient selection and model construction is summarized in Figure 1.

Diagnosis of postoperative urinary tract infection

The diagnostic criteria were as follows: The patient had urinary tract irritation symptoms such as frequent micturition, urgency and pain following the surgery. By microscopic examination of the urine sediment, the average number of leukocytes per high-power visual field was ≥ 5, and the urine pathogen was present. Based on a diagnosis of UTI, patients were divided into an infection group and a non-infection group.

Data collection and quality assessment

The following data were collected from all patients: age, body mass index (BMI), catheter retention time, catheter intubation times, operation time, intraoperative blood loss, length of hospital stay, diabetes, hypertension, prophylactic use of antibiotics and postoperative hypoproteinaemia. In most cases, the median was applied to variables with missing values. A variable was excluded from variable screening for the final model if ≥ 10% of its values were missing.

Development and validation of ML-based models

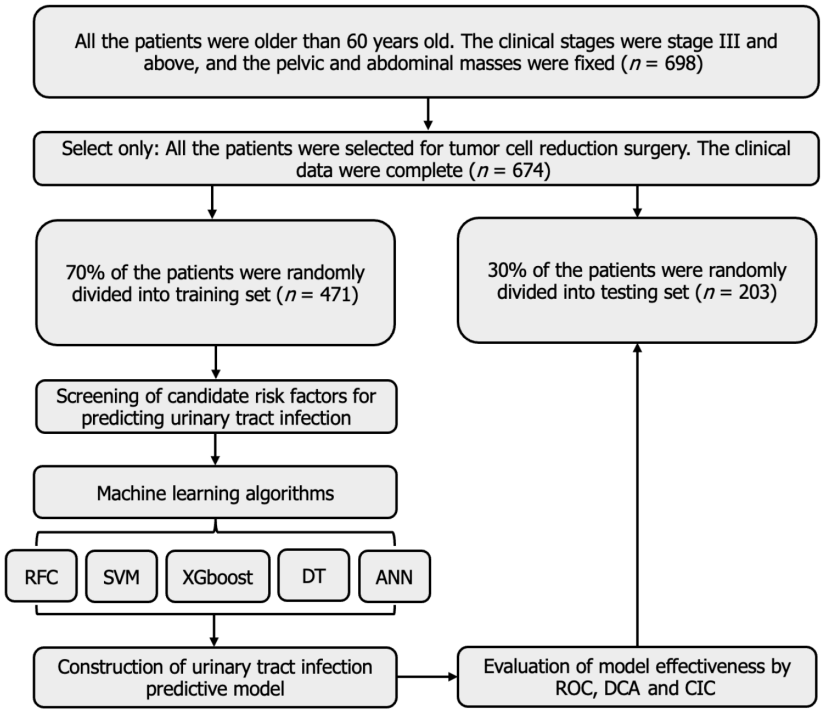
The data were randomly divided into a training set (70%) and a verification set (30%) to verify the prediction model. The inclusion principle of variables reported in previous studies was followed to screen variables. The principle of 'OOB error' was employed to screen the model variables (*i.e.* characteristic variables)[17], as follows: Gini (D) = 1-∑_(i = 1)^m P_i^2. If the Gini index was small, the probability of selecting mixed samples in the set was low, that is, the higher the purity of the set was and vice versa. However, the Gini index approaches zero if every sample in the set was of the same class. Based on the above algorithm principles, we have included five commonly used machine algorithm prediction models in this study, namely random forest classifier (RFC), support vector machine, extreme gradient boosting, artificial neural network (ANN) and decision tree (DT). Among them, RFC and DT are based on the algorithm principle of "branching and pruning," while ANN is based on "hidden layer" iteration. Support vector machine and extreme gradient boosting are also based on their iterative algorithm principle.

Prediction efficiency evaluation of ML-based models

The optimal subset variables for the modelling were obtained based on the intersection of variable sets. The receiver operating characteristic curve was used to evaluate the prediction accuracy of the model in the training and validation set. The discrimination ability of each model was quantified by the area under the receiver operating characteristic curve, decision curve analysis and clinical impact curve.

Statistical analysis

For descriptive analysis, median (interquartile range) and frequencies (%) were assessed for continuous



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Figure 1 Flow chart of the patient selection and data process. ANN: Artificial neural network; CIC: Clinical impact curve; DCA: Decision curve analysis; DT: Decision tree; RFC: Random forest classifier; ROC: Receiver operating characteristic curve; SVM: Support vector machine; XGboost: Extreme gradient boosting.

and categorical variables, respectively. Bonferroni corrected probability values were used to compare the qualitative data[18]. Wilcoxon rank-sum test or χ^2 test was used to compare the differences between diverse groups. The best subset of randomly selected explanatory variables or features was used to further divide each node during the selecting process, and the class prediction values generated by each tree were collected. Finally, the candidate variables of the prediction model, namely the Gini index, were determined according to the weight. All analysis was performed using the Python programming language (version 3.9.2, Python Software Foundation, https://www.python.org/) and R project for statistical computing (version 4.0.4, http://www.r-project.org/). All P values were two-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of the study population

The comprehensive clinical features and baseline data of 674 elderly patients with ovarian cancer are presented in Table 1. Using the caret package, patients were randomly divided into a training set (70%, $n = 471$) and a validation set (30%, $n = 203$) for internal validation of the model. As presented in Table 1 and Supplementary Table 1, 96 patients had postoperative UTI, with an infection rate of 14.24%. The clinical symptoms and signs of the patients were primarily urinary tract irritation, fever, poor urination or urinary retention, renal percussion pain and urethral mouth itching. In addition, there were significant differences in catheter retention time, catheter intubation times, intraoperative bleeding, length of hospital stay, the proportion of patients with diabetes and the incidence of postoperative hypoproteinaemia ($P < 0.05$) between the infection group and the non-infection group.

Selection of candidate variables

Feature selection is the aspect of ML that concentrates on selecting candidate variables[19]. The iterative analysis screened the candidate covariates of each algorithm. We executed 13 variables *via* Pearson correlation analysis. The correlation matrix revealed that UTIs significantly correlated with image factors and some clinical variables (Figure 2A). In addition, every significant candidate variable, such as age, BMI, catheter, catheter intubation times, blood loss, diabetes and hypoproteinaemia, contributed to the ML-based model (Figure 2B). These seven were the top predictors, which were consistent with the findings of the correlation analysis.

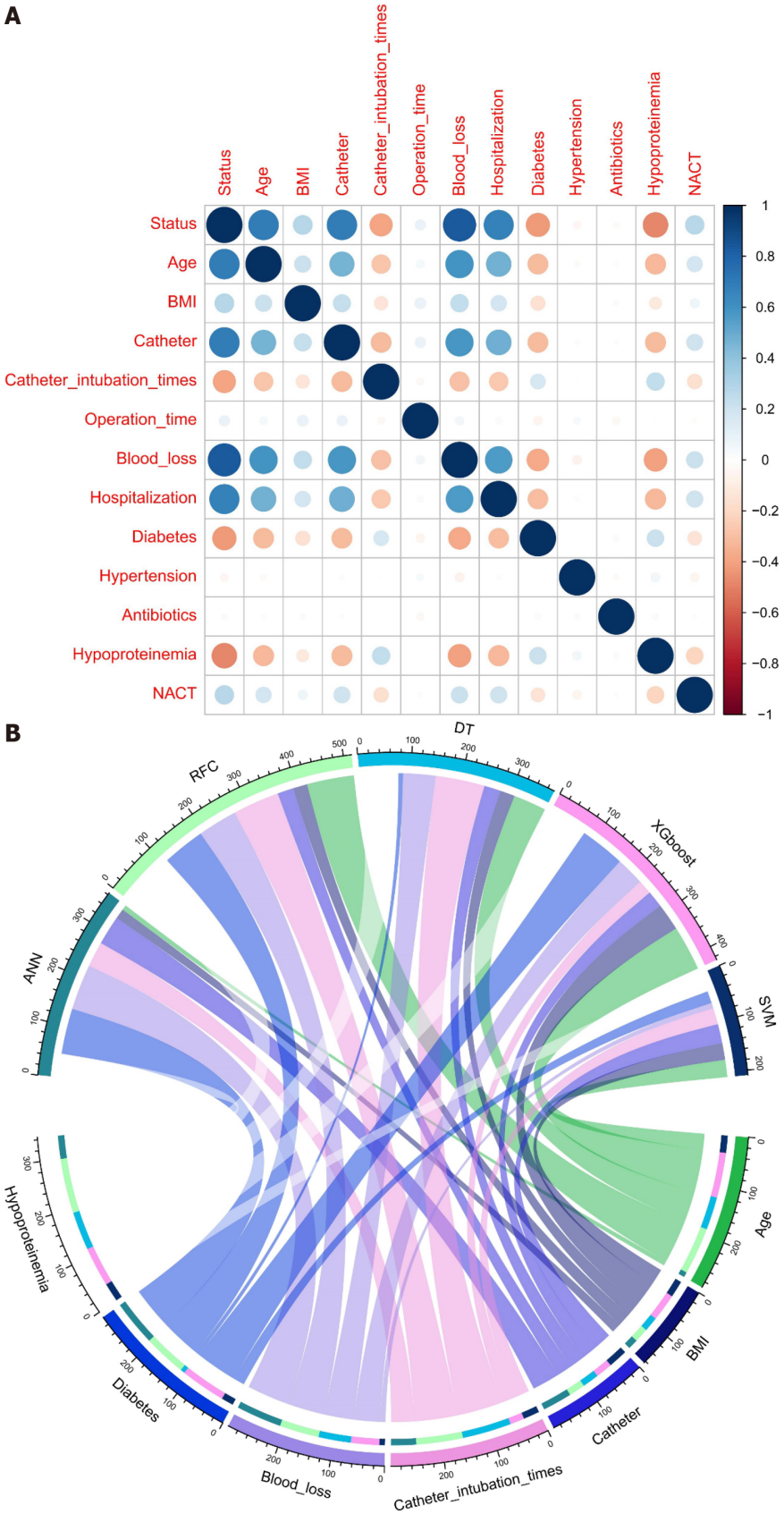
Table 1 Baseline demographic and clinicopathological characteristics of patients, *n* (%)

Variables	Training set				Testing set			
	Overall, *n* = 471	Yes, *n* = 70	No, *n* = 401	*P* value	Overall, *n* = 203	Yes, *n* = 26	No, *n* = 177	*P* value
Age [median (IQR)], yr	64.00 (63.00, 66.00)	69.00 (67.00, 71.00)	64.00 (62.00, 65.00)	< 0.001	64.00 (62.00, 65.50)	68.50 (65.25, 70.75)	63.00 (62.00, 65.00)	< 0.001
BMI [median (IQR)], kg/m^2	23.00 (22.00, 24.00)	24.00 (23.00, 25.00)	23.00 (22.00, 24.00)	< 0.001	23.00 (22.00, 24.00)	25.00 (23.00, 26.00)	23.00 (22.00, 24.00)	< 0.001
Catheter [median (IQR)], d	8.00 (7.00, 10.00)	13.00 (10.00, 14.00)	8.00 (7.00, 9.00)	< 0.001	8.00 (7.00, 9.00)	13.00 (11.00, 13.00)	8.00 (6.00, 9.00)	< 0.001
Catheter intubation times								
≥ 3	148 (31.4)	49 (70.0)	99 (24.7)	< 0.001	59 (29.1)	23 (88.5)	36 (20.3)	< 0.001
< 3	323 (68.6)	21 (30.0)	302 (75.3)		144 (70.9)	3 (11.5)	141 (79.7)	
Operation time [median (IQR)], h	3.60 (2.90, 4.40)	3.80 (3.10, 4.50)	3.60 (2.80, 4.30)	0.061	3.70 (2.90, 4.60)	3.85 (2.93, 4.60)	3.70 (2.90, 4.50)	0.373
Blood loss [median (IQR)], mL	476.00 (434.50, 515.50)	627.00 (592.75, 658.75)	465.00 (429.00, 499.00)	< 0.001	470.00 (432.00, 504.00)	646.00 (616.25, 670.00)	461.00 (426.00, 494.00)	< 0.001
Hospitalization [median (IQR)], d	11.00 (9.00, 13.00)	15.00 (14.00, 17.00)	10.00 (8.00, 12.00)	< 0.001	10.00 (8.00, 12.00)	16.00 (15.00, 17.00)	10.00 (8.00, 12.00)	< 0.001
Diabetes								
Yes	162 (34.4)	57 (81.4)	105 (26.2)	< 0.001	59 (29.1)	22 (84.6)	37 (20.9)	< 0.001
No	309 (65.6)	13 (18.6)	296 (73.8)		144 (70.9)	4 (15.4)	140 (79.1)	
Hypertension								
Yes	283 (60.1)	45 (64.3)	238 (59.4)	0.519	132 (65.0)	19 (73.1)	113 (63.8)	0.483
No	188 (39.9)	25 (35.7)	163 (40.6)		71 (35.0)	7 (26.9)	64 (36.2)	
Antibiotics								
Yes	295 (62.6)	47 (67.1)	248 (61.8)	0.477	128 (63.1)	16 (61.5)	112 (63.3)	1
No	176 (37.4)	23 (32.9)	153 (38.2)		75 (36.9)	10 (38.5)	65 (36.7)	
Hypoproteinaemia								
Yes	122 (25.9)	53 (75.7)	69 (17.2)	< 0.001	54 (26.6)	22 (84.6)	32 (18.1)	< 0.001
No	349 (74.1)	17 (24.3)	332 (82.8)		149 (73.4)	4 (15.4)	145 (81.9)	
NACT								
Yes	293 (62.2)	18 (25.7)	275 (68.6)	< 0.001	133 (65.5)	12 (46.2)	121 (68.4)	0.045
No	178 (37.8)	52 (74.3)	126 (31.4)		70 (34.5)	14 (53.8)	56 (31.6)	

BMI: Body mass index; IQR: Interquartile range; NACT: Neoadjuvant chemotherapy.

Construction of ML-based UTI predictive model

Positive or negative training results for each patient were entered for training data, and the final judgment result was the output, as indicated in the following formula: Gini(D)= 1-∑_(*i* = 1)^*m* *P_i*^2. The RFC algorithm represents a computational method for effectively navigating the free parameter space to obtain a robust model (Figure 3A). The variable Gini index in the RFC model is presented in Supplementary Table 2. The top seven candidate variables were age, BMI, catheter, catheter intubation times, blood loss, diabetes and hypoproteinaemia, which were consistent with the predicted results. In addition, data mining through the DT model, as demonstrated by impurity analysis: Gini (p) = ∑_(*K* = 1)^*K* [*Pk*(1-*Pk*)], was advantageous. At the branch of DT, age and catheter functioned as the irreplaceable weight in addition to clinical factor indicators (Figure 3B). In contrast, the RFC model outperformed the ANN model, which outperformed other models, in terms of prediction efficiency (Figure 4 and Supplementary Table 3).

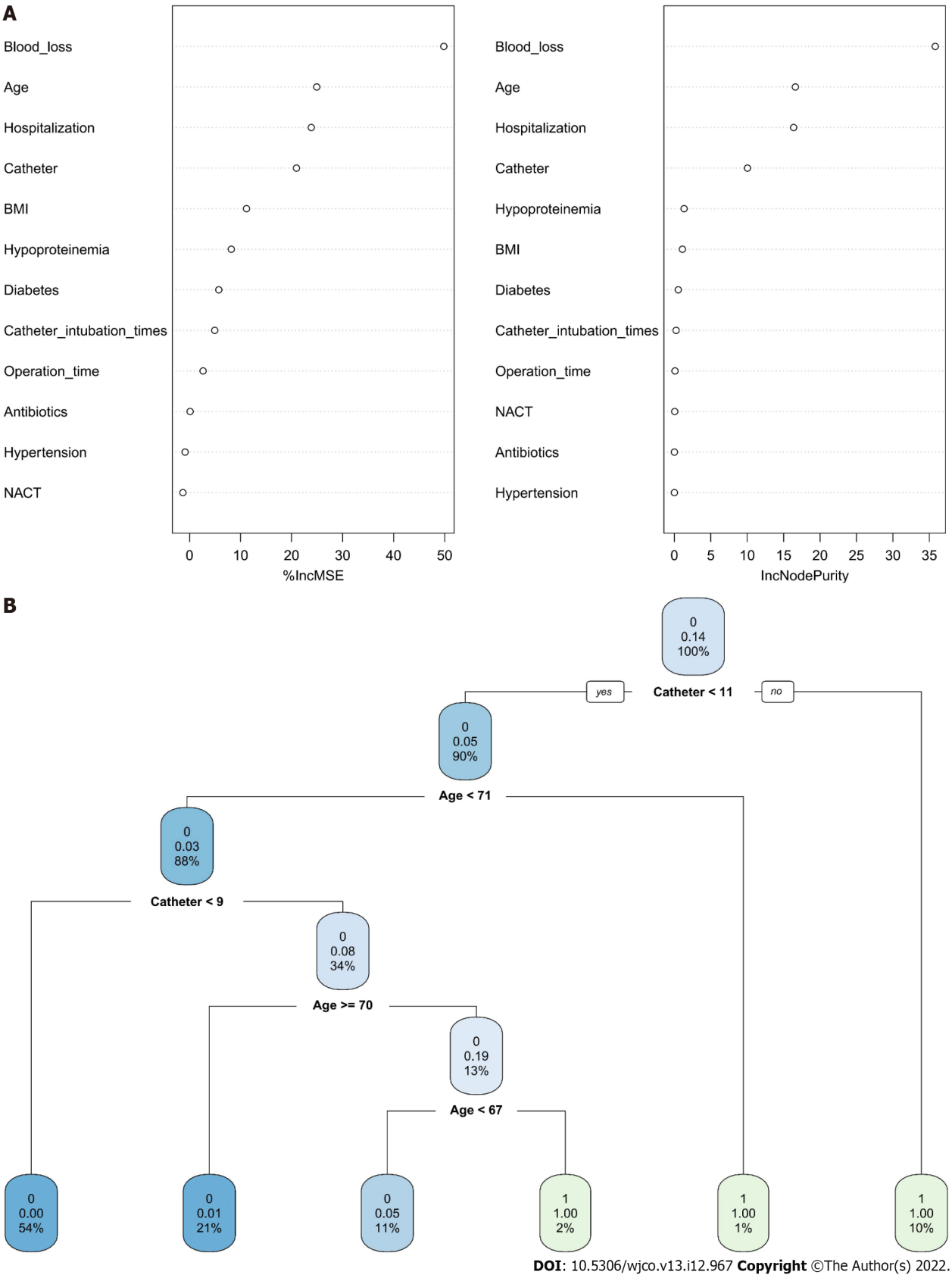


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Figure 2 Variable screening and weight allocation. A: Variable screening; B: weight allocation. ANN: Artificial neural network; BMI: Body mass index; DT: Decision tree; NACT: Neoadjuvant chemotherapy; RFC: Random forest classifier; SVM: Support vector machine; XGboost: Extreme gradient boosting.

Comparison across ML-based models

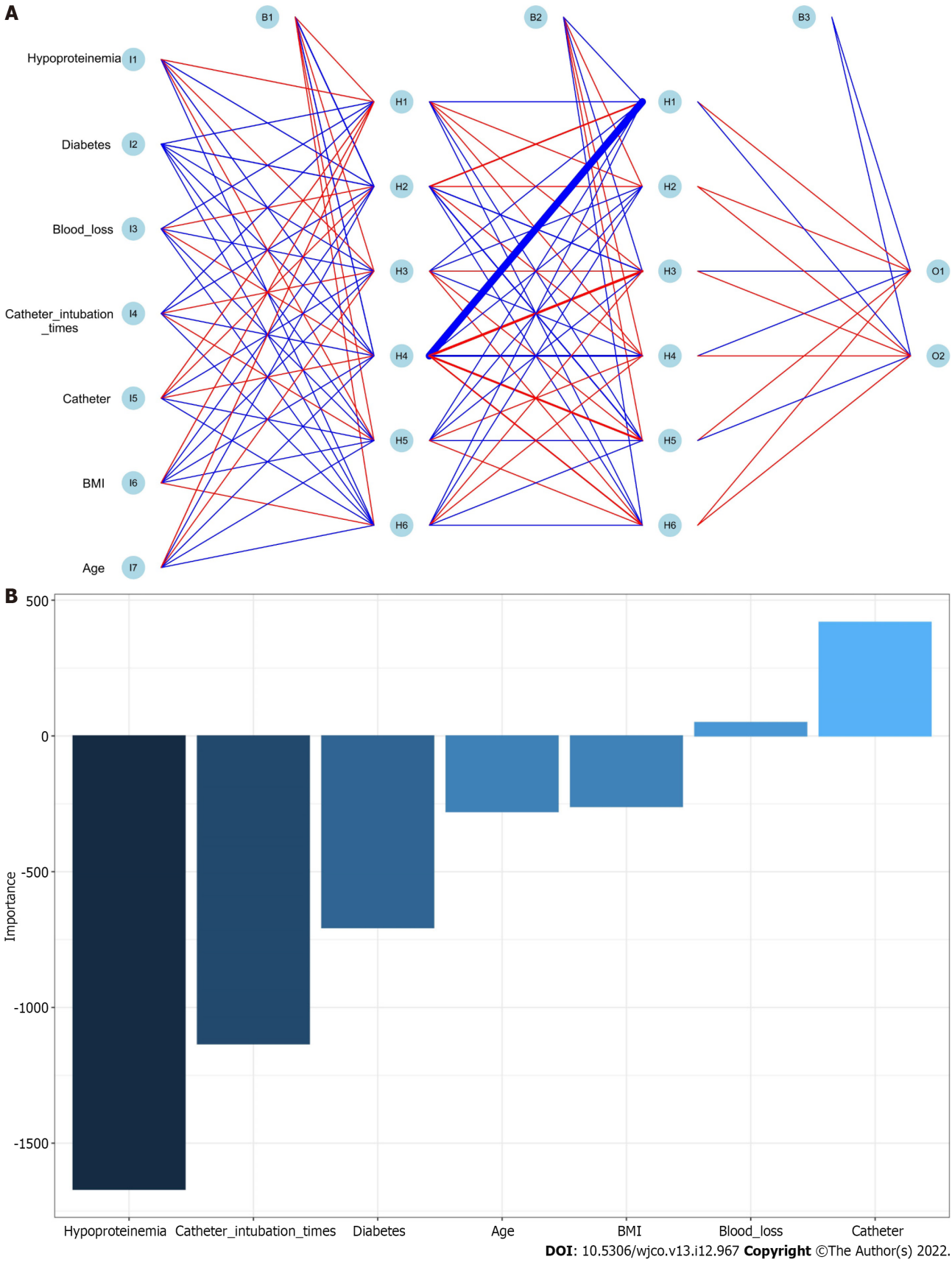
We used five supervised learning models for UTI assessment to investigate whether ML-based models



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Figure 3 Predictive model visualization based on machine learning-based algorithm. A: The random forest classifier algorithm represents a computational method for effectively navigating the free parameter space to obtain a robust model; B: At the branch of decision tree, age and catheter functioned as the irreplaceable weight in addition to clinical factor indicators. BMI: Body mass index; NACT: Neoadjuvant chemotherapy.

can improve prediction performance. The RFC model demonstrated a strong prediction performance in the training and validation cohorts based on decision curve analysis (Figure 5). In addition, the area under the curve of the RFC models peaked when the seven variables were added, followed by those of



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Figure 4 Predictive model visualization based on artificial neural network algorithm. BMI: Body mass index.

ANN, DT, support vector machine and extreme gradient boosting (Table 2 and Supplementary Table 4). Undoubtedly, RFC outperformed the generalised linear model in terms of prediction accuracy. Thus, both RFC and DT (ML-assisted decision-support) models were used to guide UTI prediction using the iterative algorithm analysis of supervised learning.

Table 2 Receiver operating characteristic curve analyses for predicting urinary tract infection in each machine learning-based model

Model	Training set			Testing set		
	AUC mean	AUC 95%CI	Variables[1]	AUC mean	AUC 95%CI	Variables[1]
RFC	0.925	0.868-0.982	7	0.918	0.861-0.975	7
SVM	0.787	0.730-0.844	7	0.779	0.722-0.836	7
DT	0.776	0.719-0.833	7	0.769	0.712-0.826	7
ANN	0.879	0.822-0.936	6	0.854	0.797-0.911	6
XGboost	0.797	0.740-0.854	7	0.788	0.731-0.845	7

[1]Variables included in the model. ANN: Artificial neutral network; AUC: Area under the curve; CI: Confidence interval; DT: Decision tree; RFC: Random forest classifier; SVM: Support vector machine; XGboost: Extreme gradient boosting.

Internal validation of the optimal predictive model

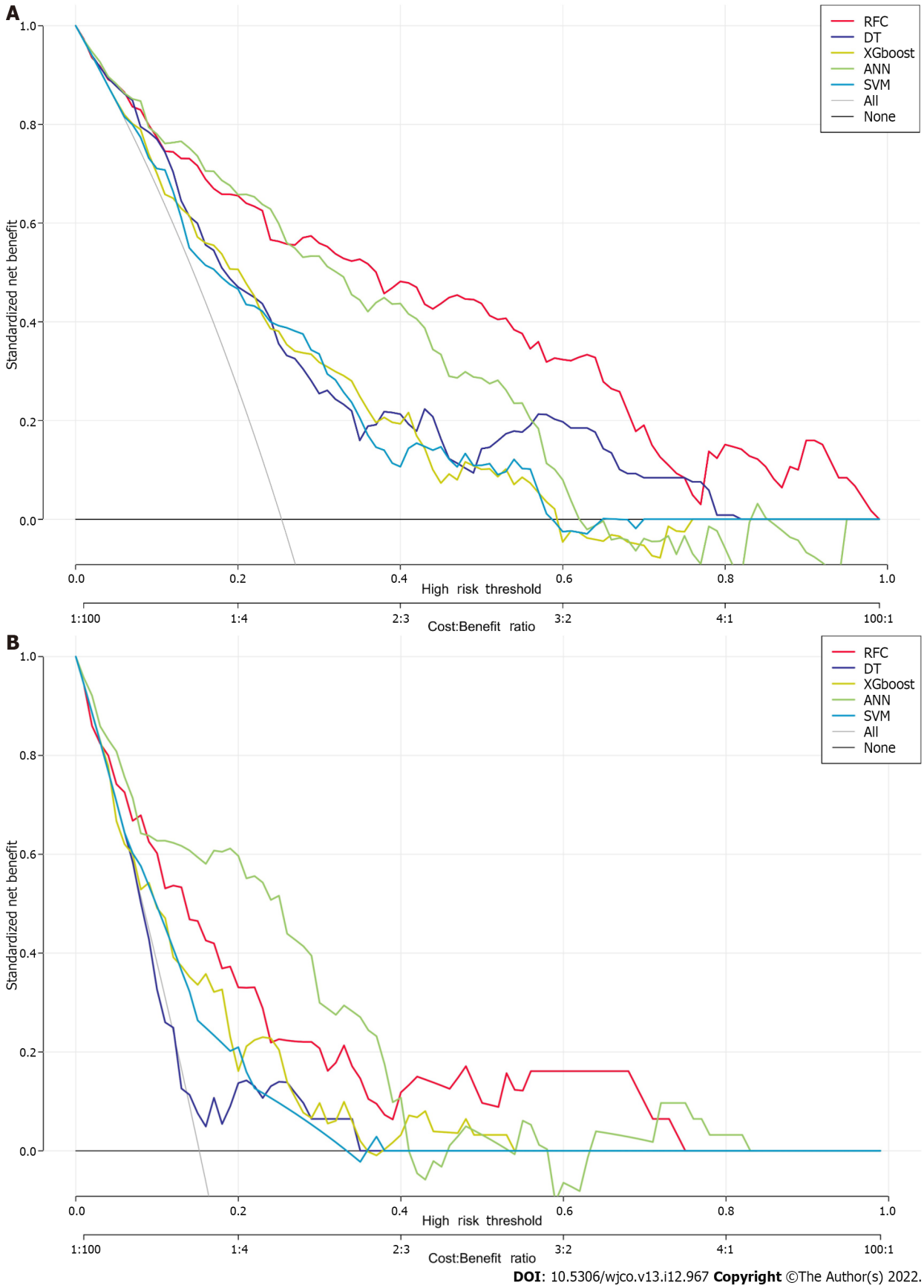
We also used the clinical impact curve to assess the accuracy to further validate the RFC model's ability to predict outcomes. The clinical impact curve revealed that UTI stratification was achieved in the training cohorts (Supplementary Figure 1). These were consistent with the results of validation cohorts, indicating that RFC performed best in terms of discrimination, calibration and overall performance, in particular the candidate systemic inflammation markers that were highly relevant to UTIs.

DISCUSSION

This study's findings indicated that the factors influencing the risk of UTI in elderly patients with ovarian cancer after tumour cell reduction include not only the patients' basic diseases but also their indwelling catheter and postoperative nutritional level. There has been a lack of specialised research on UTIs after tumour cell reduction in recent years, and some researchers have examined the operation or resection of patients with ovarian cancer. These findings demonstrated that the most common clinical manifestations of patients with UTI are urinary tract irritation, urinary retention, urethral mouth itching and urine turbidity, which is consistent with the results of a previous study[20]. However, the incidence of UTIs reported in these studies ranges from < 10% to > 40%. The infection rate reported in this study was 14.24%, which is considered moderate; this may be due to the exclusion of patients with severe basic diseases such as liver and kidney dysfunction[21,22]. In addition, concerning infection-related factors, these reports have drawn similar conclusions as this study, albeit they also stated that age, intubation times, length of hospital stay, paraaortic lymph node dissection and intestinal resection, haemoglobin and other factors can all affect the risk of infection.

In general, cytoreductive surgery is a relatively traumatic procedure for advanced ovarian cancer, and the scope of the operation is likely to involve the urinary system, causing a significant increase in the risk of postoperative UTI. Simultaneously, the risk factors for postoperative UTI in elderly patients differ from those in young and middle-aged patients. Therefore, the research objects with high heterogeneity are selected for analysis, and the demonstrability and repeatability of the results are insufficient. Furthermore, a thematic analysis for the patient population with specific surgical procedures and similar conditions and susceptibility factors should be performed. Considering this situation, this study included elderly patients with ovarian cancer who had undergone cytoreductive surgery as the research. Our findings indicated that actively controlling catheter-related UTIs and correcting postoperative malnutrition were important links to preventing and controlling UTIs in the elderly after ovarian cancer cell reduction.

Through clinical observation, researchers have discovered that postoperative UTI caused by an indwelling catheter is one of the most common postoperative infections in clinics in recent years. The operation, catheter selection, bladder flushing and patient factors are the main causes of infection, and catheter placement time, difficulty in catheter intubation, multiple intubations, previous catheter retention history, long anaesthesia time, history of diabetes, age, consciousness disorder improper bladder flushing, gastrointestinal decompression, enema, long replacement time of urine collection bag and other factors are related to postoperative catheter-related UTI[23]. Numerous studies have demonstrated that the time of catheter placement is an independent risk factor for postoperative UTI. Thus, to effectively prevent catheter-related UTI, it is necessary to strictly control the indications of a long-term indwelling catheter, reduce urethral injury, improve the skills of operators, prevent retrograde infection, improve the tightness of the catheter system and ensure the patency of the catheter system in clinical work.



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Figure 5 Prediction performance of candidate models based on machine learning-based algorithm. ANN: Artificial neural network; DT: Decision tree; RFC: Random forest classifier; SVM: Support vector machine; XGboost: Extreme gradient boosting.

One of the most common complications after major surgery is hypoproteinaemia. Its causes are complex, and it is closely linked to surgical trauma. Operation post-stress is related to infection and other factors[24,25]. Simultaneously, plasma albumin level in severe patients is correlated with the expression of serum inflammatory factors and peripheral blood T cell subsets. Hypoproteinaemia can improve the degree of inflammatory stress, cause immune dysfunction, significantly increase the risk of bacterial and fungal infection and have a serious adverse impact on the disease outcome[26,27]. In patients undergoing surgery for a malignant tumour or organ function decompensation, postoperative hypoproteinaemia can increase the incidence and mortality of complications, such as postoperative infection, and seriously impact the surgical efficacy. The incidence of postoperative complications significantly decreases as plasma albumin levels rise. Therefore, early postoperative nutritional support is an important link in the treatment and prevention of infection in elderly patients with ovarian cancer. Early intravenous nutrition support should be strengthened for patients who cannot use early enteral nutrition to correct the negative nitrogen balance caused by surgical stress and maintain normal nutrition levels in particular.

It is noteworthy that, owing to the limitations of clinical medical records, the risk factors associated with cytoreductive surgery for ovarian cancer in the elderly examined in this study are not comprehensive, which is a flaw in this study. In addition to the relevant factors analysed in this study, ascites volume, operation scope and other factors will also affect it. Laparoscopic cytoreductive surgery for advanced ovarian cancer is becoming more prevalent as laparoscopic technology advances, which reduces the risk of surgical trauma and postoperative infection to some extent. These factors must be researched and analysed further.

CONCLUSION

In conclusion, using an ML-based algorithm, we created a feasible and robust method for identifying factors important for predicting UTIs. The RFC in particular, which can improve the prediction and early detection of UTIs in patients with ovarian cancer, was robust. In addition, age, BMI, catheter, catheter intubation times, blood loss, diabetes and hypoproteinaemia were five crucial factors. In clinical practice, incorporating the presentation of simple clinical data may be helpful for clinicians to identify the individualised risk of UTI.

ARTICLE HIGHLIGHTS

Research background

Nowadays, predictive models based on advanced algorithms have been gradually applied to the medical field, which also enables many diseases to be detected and diagnosed early. Among them, the machine learning (ML) algorithm relies on repeated iterative operations to accurately output the results. Therefore, it can improve the accuracy and robustness of prediction.

Research motivation

Given the superior ability of the ML-based algorithm to improve the accuracy of muscular invasion prediction, we applied the ML-assisted decision-support model to assess the risk of urinary tract infection (UTI) using clinical parameters and direct clinical decision-making prior to treatment decisions.

Research objectives

We developed an ML assistant model for the prevention and control of nosocomial infection.

Research methods

A total of 674 elderly patients with ovarian cancer treated between January 31, 2016 and January 31, 2022 and met the inclusion criteria of the study were selected as the research subjects. A retrospective analysis of the postoperative UTI and related factors was performed by reviewing the medical records. Five ML-assisted models were developed using two-step estimation methods from the candidate predictive variables. The robustness and clinical applicability of each model were assessed using the receiver operating characteristic curve, decision curve analysis and clinical impact curve.

Research results

A total of 12 candidate variables were eventually included in the UTI prediction model. Models constructed using the random forest classifier (RFC), support vector machine, extreme gradient boosting, artificial neural network and decision tree had areas under the receiver operating characteristic curve ranging from 0.776 to 0.925. The RFC model, which incorporated factors such as age, body

mass index, catheter, catheter intubation times, blood loss, diabetes and hypoproteinaemia, had the highest predictive accuracy.

Research conclusions

These findings demonstrated that the ML-based prediction model developed using the RFC can be used to identify elderly patients with ovarian cancer who may have postoperative UTI. This can help with treatment decisions and enhance clinical outcomes.

Research perspectives

Using an ML-based algorithm, we developed a feasible and robust method to identify factors that are significant for predicting UTIs. The RFC, which can improve the prediction and early detection of UTIs in patients with ovarian cancer, was particularly robust. In addition, the five most crucial factors were age, body mass index, catheter, catheter intubation times, blood loss, diabetes and hypoproteinaemia. Clinicians may find it extremely helpful to assess the individualised risk of UTI in clinical practice by incorporating the presentation of simple clinical data.

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FOOTNOTES

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LETTER TO THE EDITOR

Circulating tumour DNA in gastrointestinal cancer in clinical practice: Just a dream or maybe not?

Andrea Pretta, Eleonora Lai, Clelia Donisi, Dario Spanu, Pina Ziranu, Valeria Pusceddu, Marco Puzzoni, Elena Massa, Mario Scartozzi

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Abstract

The evaluation of circulating tumor DNA (ctDNA) is increasingly integrated into the management of diagnosis and treatment of gastrointestinal cancer as it represents an innovative and minimally invasive biomarker that could allow us to reach clinical needs not met yet in randomized clinical trials. Recent research provided an interesting overview of the role of circulating tumor DNA in gastric, biliary, liver, pancreatic, and colorectal cancer. Data regarding upper gastrointestinal tumors are currently not practice changing. Tumor detection rates are low in the early stages, while in advanced stages ctDNA is useful for molecular tracking evaluation. Most of the evidence comes from colorectal cancer studies, where ctDNA was evaluated both in the early and advanced stages with the post-surgery minimal residual disease assessment and the response assessment, respectively. ctDNA qualifies as a promising tool in the era of precision medicine, with potential applications in the entire management of gastrointestinal cancer patients. Further evidence is needed to establish which setting may be influenced greatly by liquid biopsy in clinical practice.

Key Words: Circulating tumor DNA; Gastrointestinal cancer; Liquid biopsy; Esophageal cancer; Gastric cancer; Liver cancer; Bile duct cancer; Pancreatic cancer; Colorectal cancer

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Core Tip: Circulating tumor DNA is a promising tool in the era of precision medicine, with several potential applications in the entire management of gastrointestinal malignancies. Further evidence is needed to assess in which setting liquid biopsy might have a greater impact in clinical practice.

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TO THE EDITOR

We read with great interest the minireview by Kirchweger *et al*[1], entitled "Circulating tumor DNA (ctDNA) for diagnosis, prognosis and treatment of gastrointestinal malignancies". This paper provides a comprehensive overview on available literature data regarding the potential role of ctDNA in the management of gastric, biliary tract, liver, pancreatic and colorectal cancer (CRC). The authors discuss the application of ctDNA detection from diagnosis to prognosis and treatment monitoring of each disease analysed, by critically presenting to the readers the advantages and limitations of this tool.

We strongly agree with authors that ctDNA represents an innovative, minimally invasive biomarker that might allow us to reach unmet clinical needs in clinical practice for gastrointestinal cancer patients if further validated in randomised clinical trials. Indeed, considering the dynamic nature of tumor biology and the genetic heterogeneity of diseases such as CRC, the serial assessments of biomarkers of interest through liquid biopsy might reflect the continuous changes of tumour itself and be useful to clinicians[2].

Notably, not a large amount of data is available about the application of ctDNA for diagnosis, as well as about its role in gastric and liver cancer. We greatly appreciate the authors' effort to analyse these particular aspects and cancer types which are not the main field of research for this topic.

Indeed, main evidences regard prognosis and treatment monitoring, both in early stages (detection of minimal residual disease) and in advanced stages. Moreover, the majority of evidences derive from CRC studies.

Recently, Bregni *et al*[3] showed that baseline ctDNA was an independent prognostic factor for disease free survival (HR 3.35, 95%CI: 1.15-9.77, P = 0.03) in stage III CRC patients treated with neoadjuvant conventional 5-fluorouracil, oxaliplatin and folinic acid (FOLFOX) followed by surgery +/- adjuvant FOLFOX in the PePiTA trial[4]. These findings derive from a small sample size (80 patients) but represent a starting point needing to be confirmed in larger trials focusing on early-stage CRC.

Surely, as highlighted by the authors, ctDNA has been extensively studied for tailoring treatment with anti-EGFR in further lines for RAS wild type metastatic CRC[5]. Our group recently explored the liquid biopsy-driven cetuximab rechallenge in a *RAS* and *BRAF* wild type selected population[6]. This strategy was confirmed to be effective and despite the small sample size, clinical outcome was consistent with the findings of phase II studies. Moreover, we observed that in addition to the molecular selection through ctDNA analysis for *RAS-BRAF*, long anti-*EGFR* free interval was a prospective selection criterion for this therapeutic option. Thus, the combination of ctDNA analysis plus clinical elements might be a winning strategy overcoming the limitations of a single tool.

As for pancreatic cancer, the identification of prognostic and predictive biomarkers is an urgent medical need. Unfortunately, despite extensive research no robust validated factors to guide treatment choice are available, except for *BRCA* status, and no effective agents have drastically improved the management of this disease, including immunotherapy[7-10]. For this reason, we strongly agree with the authors that ctDNA detection appears as an appealing instrument to guide therapeutic choices across different treatment lines, in order to improve clinical outcomes pancreatic cancer patients. Indeed, liquid biopsy has shown to be more sensitive than carbohydrate antigen199 levels in predicting prognosis and treatment response[11].

Moreover, ctDNA evaluation has been shown to be more sensitive than current gold standard radiological methods (computed tomography) in the evaluation of the tumor burden at staging (for any micro dissemination or lymph node involvement) and for relapses detection[12-13].

At the present time, data regard small groups of patients and require validation in larger trials.

In conclusion, ctDNA qualifies as a promising main actor in the precision medicine era, with potential applications in the whole management of gastrointestinal cancer patients. Further larger and prospective studies are needed to assess the real impact of liquid biopsy in clinical practice, but for now, potential benefits are likely to overcome its limitations.

FOOTNOTES

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