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MINIREVIEWS

Neoadjuvant treatment in non-small cell lung cancer: New perspectives with the incorporation of immunotherapy

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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

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

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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

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

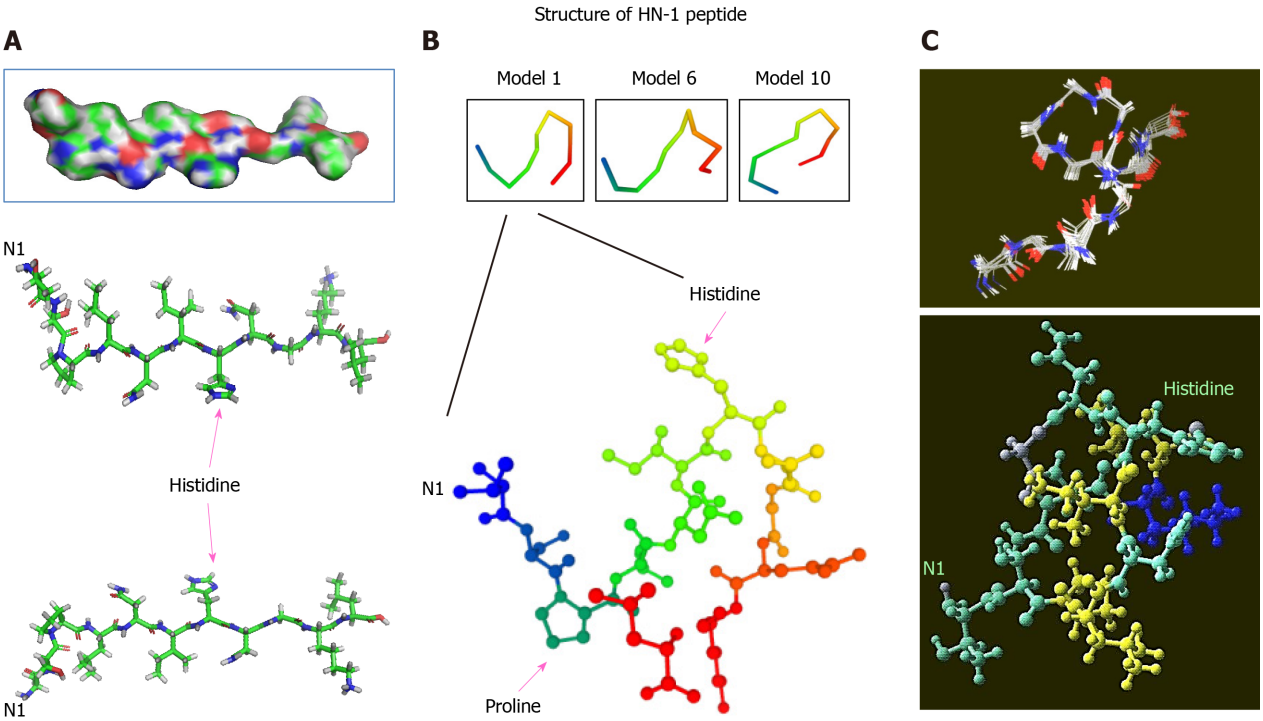

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Figure 1 Three-dimensional structure of HN-1 peptide. A: A 3D model of HN-1 peptide generated using PyMol molecular graphics system, version 1.2r3pre, Schrödinger, LLC. All graphics depict an identical configuration with the bottom two panels in the 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

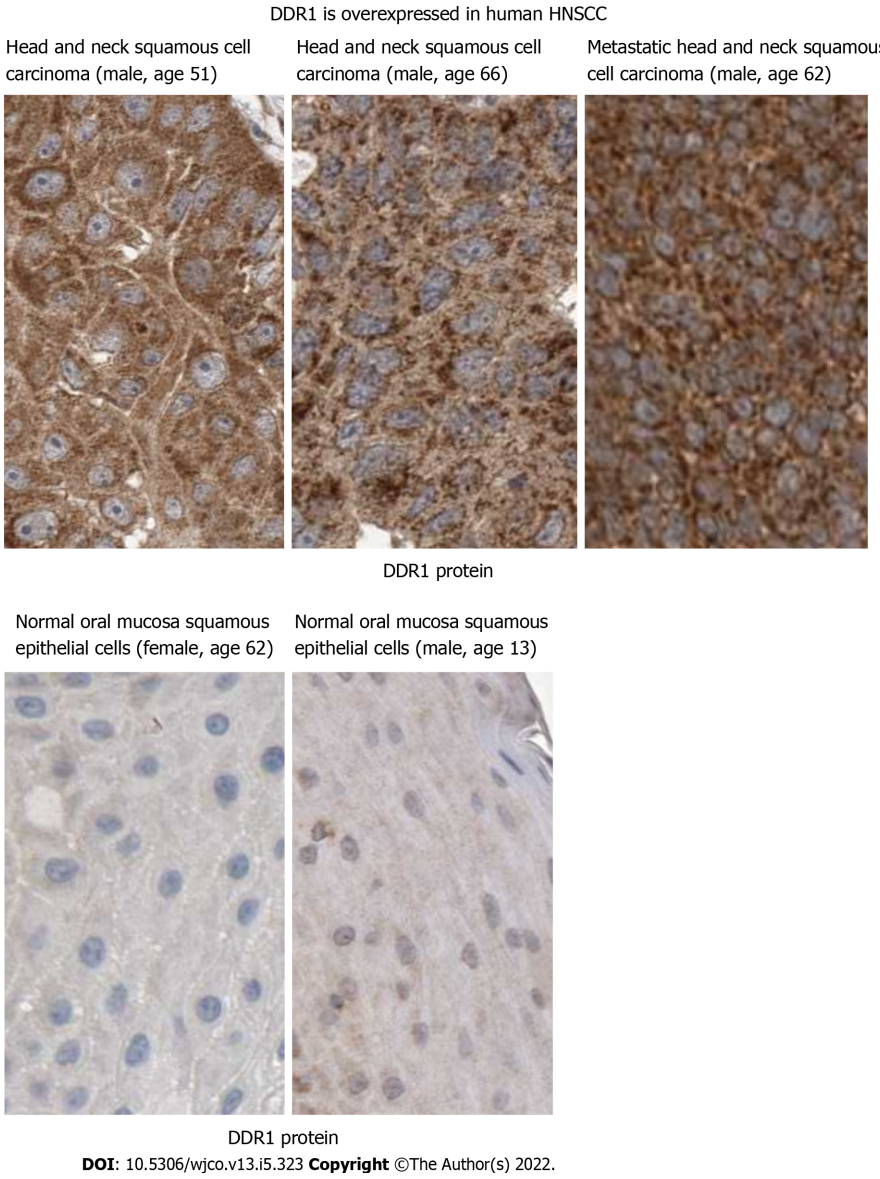

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Figure 2 Discoidin domain receptor 1 may mediate HN-1 endocytosis. Discoidin domain receptor 1 (DDR1) protein is upregulated in human head and neck squamous cell carcinoma. Immunohistochemical analysis of DDR1 was conducted by comparing tumor *vs* 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-

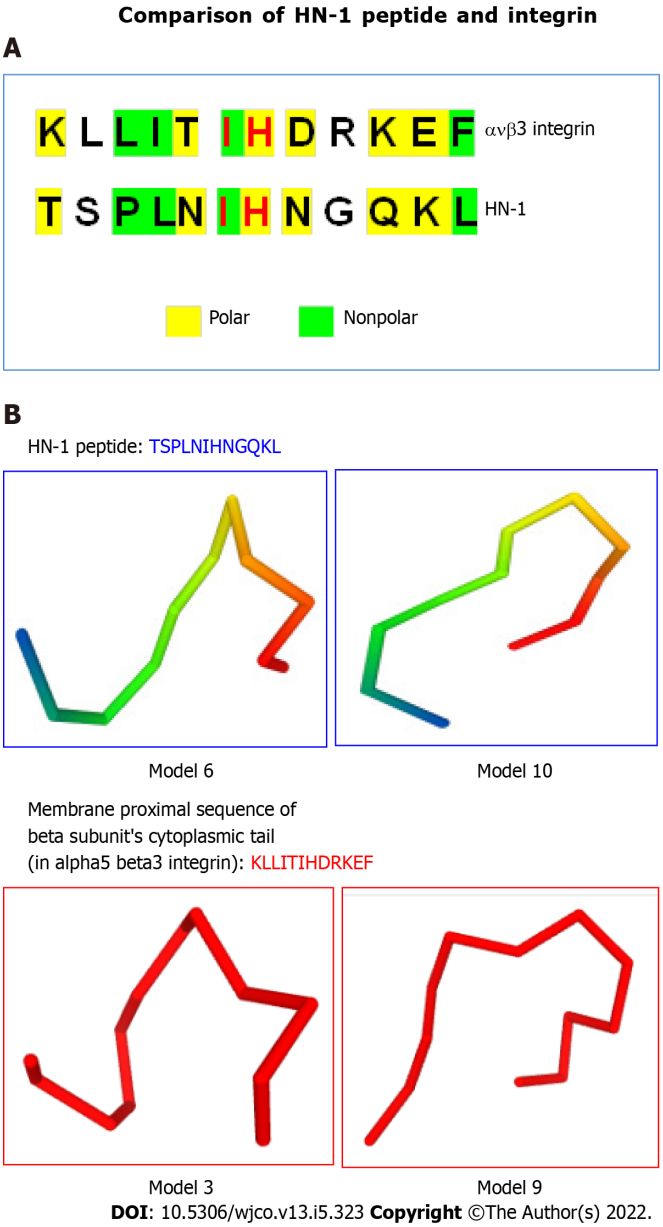

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Figure 3 HN-1 peptide exhibits similarity to integrin peptide. A: The similarities between the HN-1 sequence (TSPLNIHNGQKL) and a stretch of amino acids in alpha5 beta3 integrin (KLLITIHDRKEF) are highlighted. As HN-1 peptide lacks the recognition 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

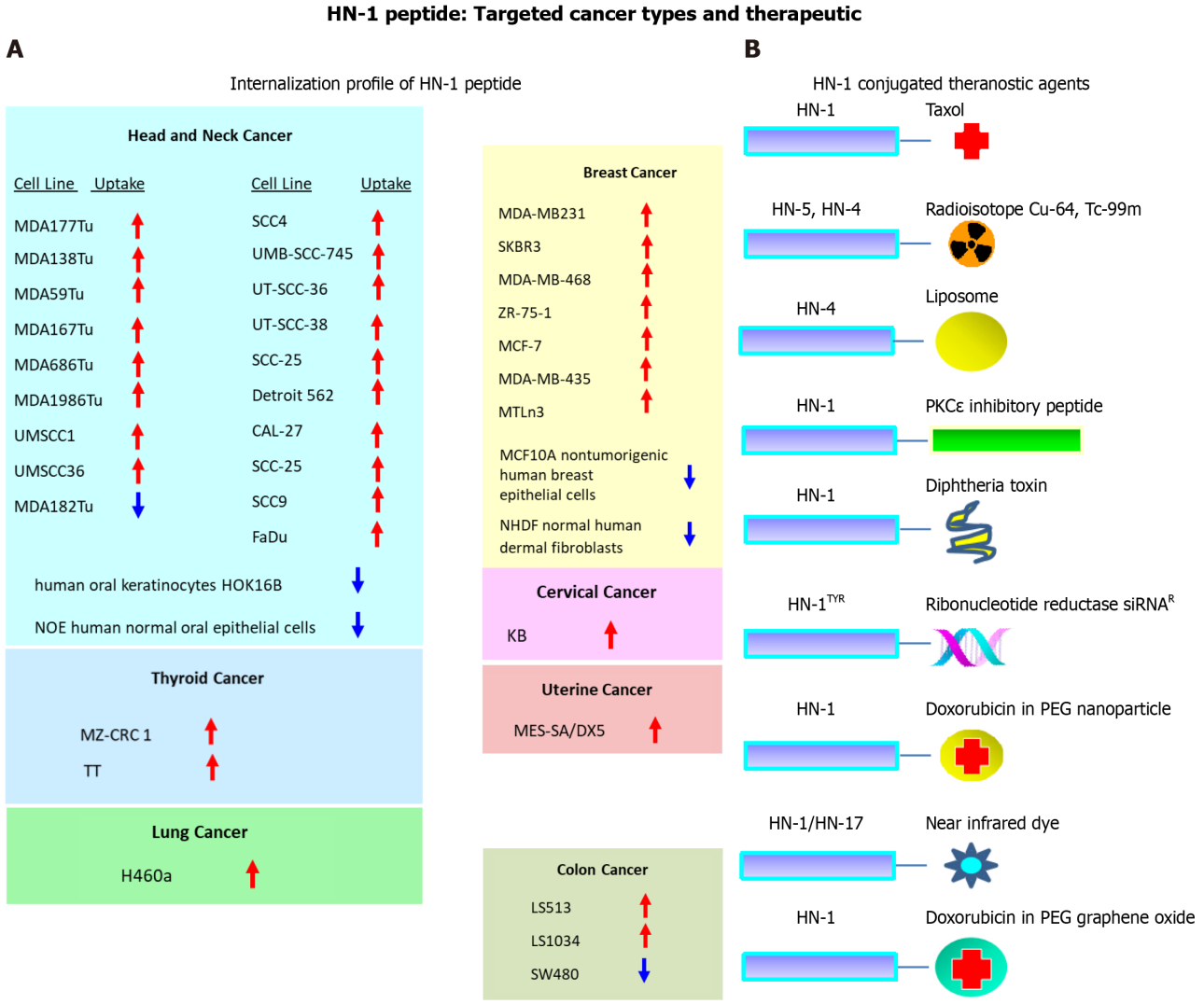

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Figure 4 The repertoire of targeted cancers and therapeutic application of HN-1 peptide. A: HN-1 uptake profile. The internalization of HN-1 peptide by various human cancer cells *vs* the corresponding normal human cells was compared. HN-1 uptake: 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.

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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

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

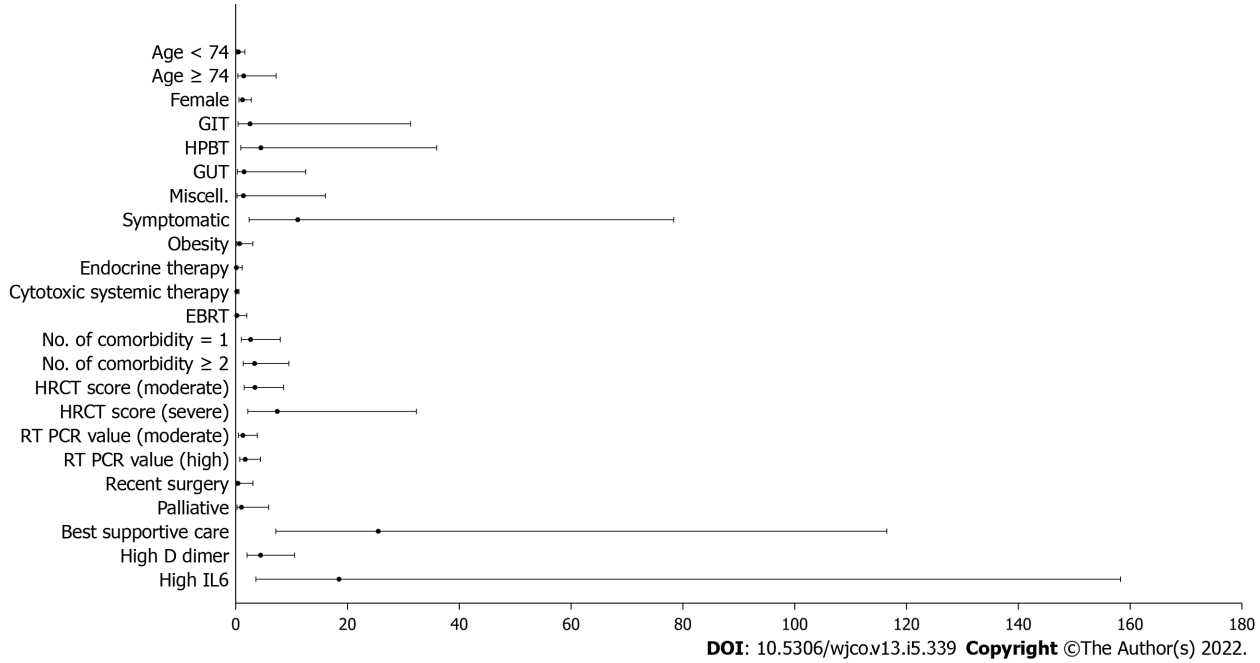

Figure 1 Forest plot for different parameters (demographic, clinical and laboratory) and 30-d mortality outcomes. GIT: Gastrointestinal tract; HPB: Hepatopancreatobiliary; GUT: Genitourinary tract; HPBT: Hepato-pancreato-biliary tract; EBRT: External beam radiotherapy.

(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

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.

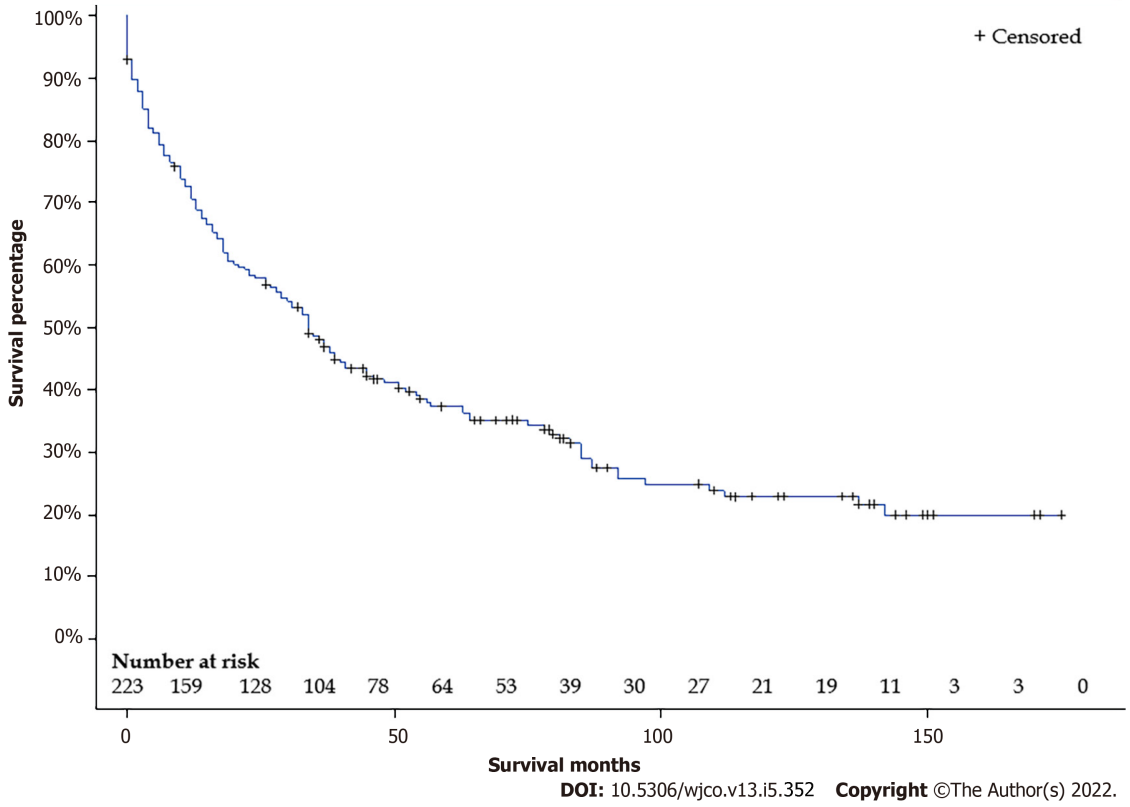

Figure 1 Overall Kaplan-Meier survival curve. Median survival for all patients is 34 (95%CI: 27-41) mo.

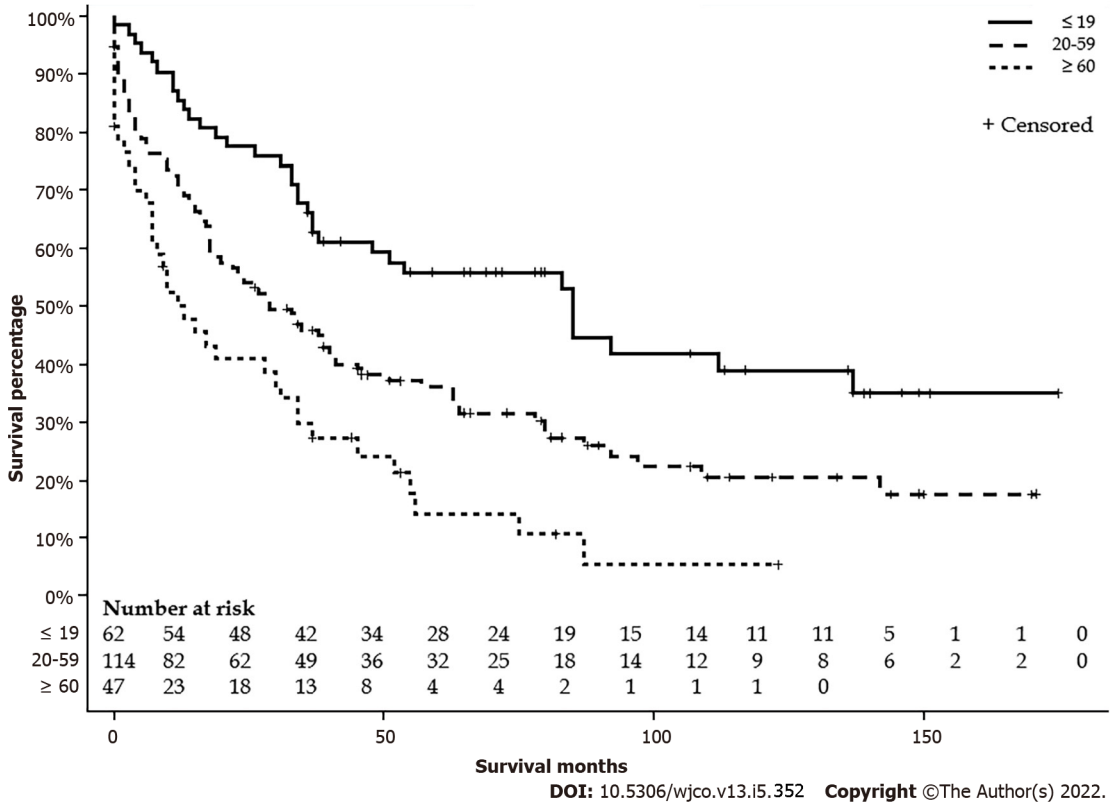

Figure 2 Kaplan-Meier survival curve by age groups. Median survival for patients ≤ 19-years-old is 85 (95%CI: 37-137) mo, patients between 20 and 59-years-old are 29 (18-41) mo, and patients ≥ 60-years-old is 12 (95%CI: 7-31) mo ($P < 0.001$).

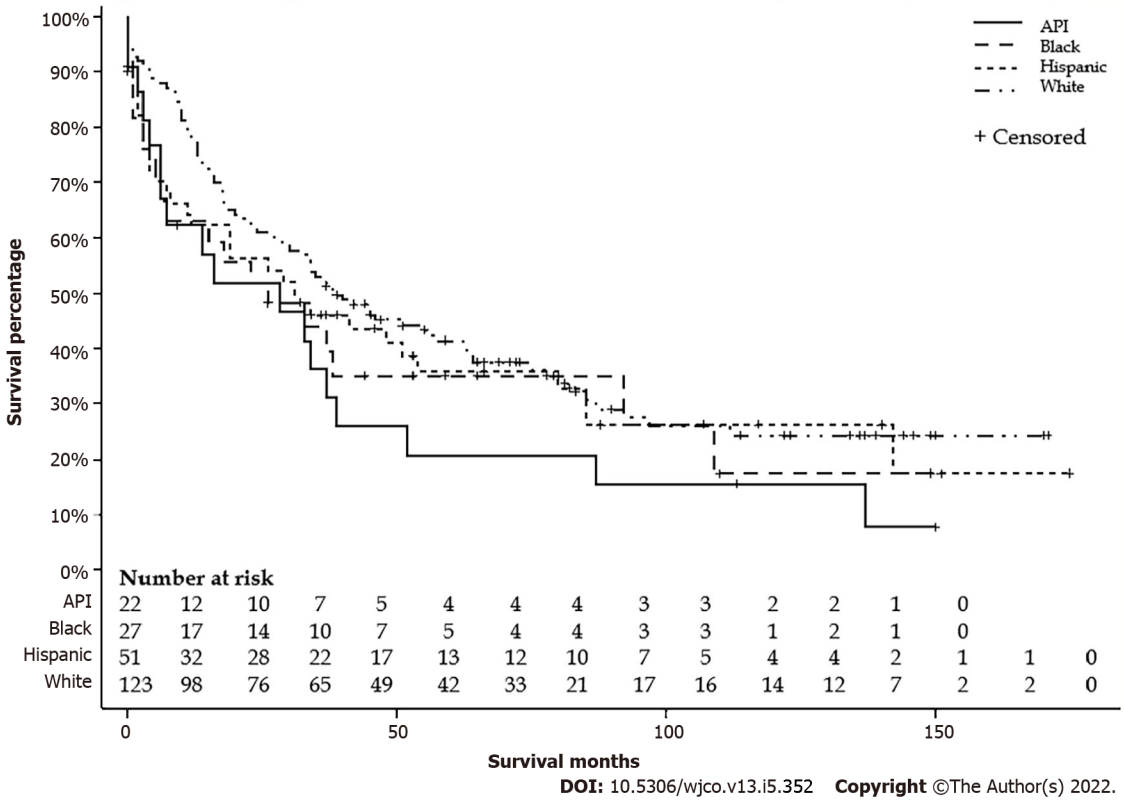

Figure 3 Kaplan-Meier survival curve by race (all race). Median survival for White 39 (95%CI: 29-63) mo, Black 26 (95%CI: 5-92) mo, Asian and Pacific Islander is 28 (95%CI: 6-39) mo, and Hispanic 31 (95%CI: 11-54) mo (P = 0.28).

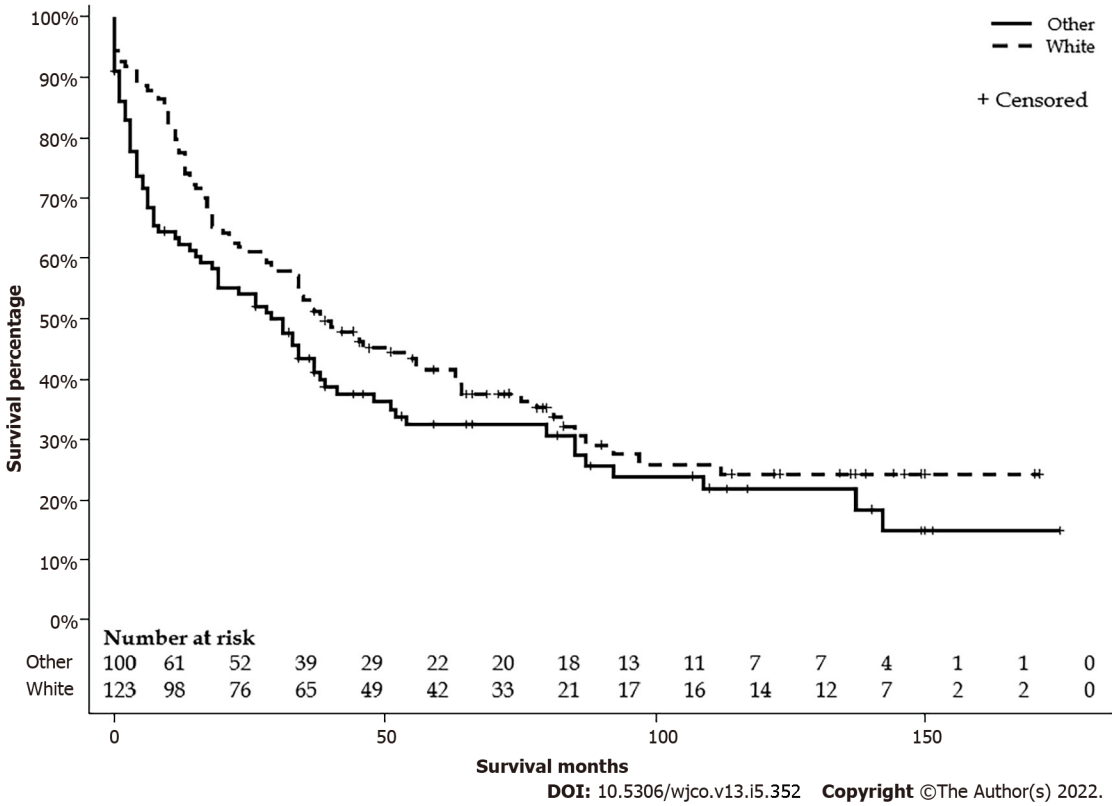

Figure 4 Kaplan-Meier survival curve by race (White *vs* non-White). Median survival for White 39 (95%CI: 29-63) mo, and Non-White is 29 (95%CI: 15-38) mo (P = 0.11).

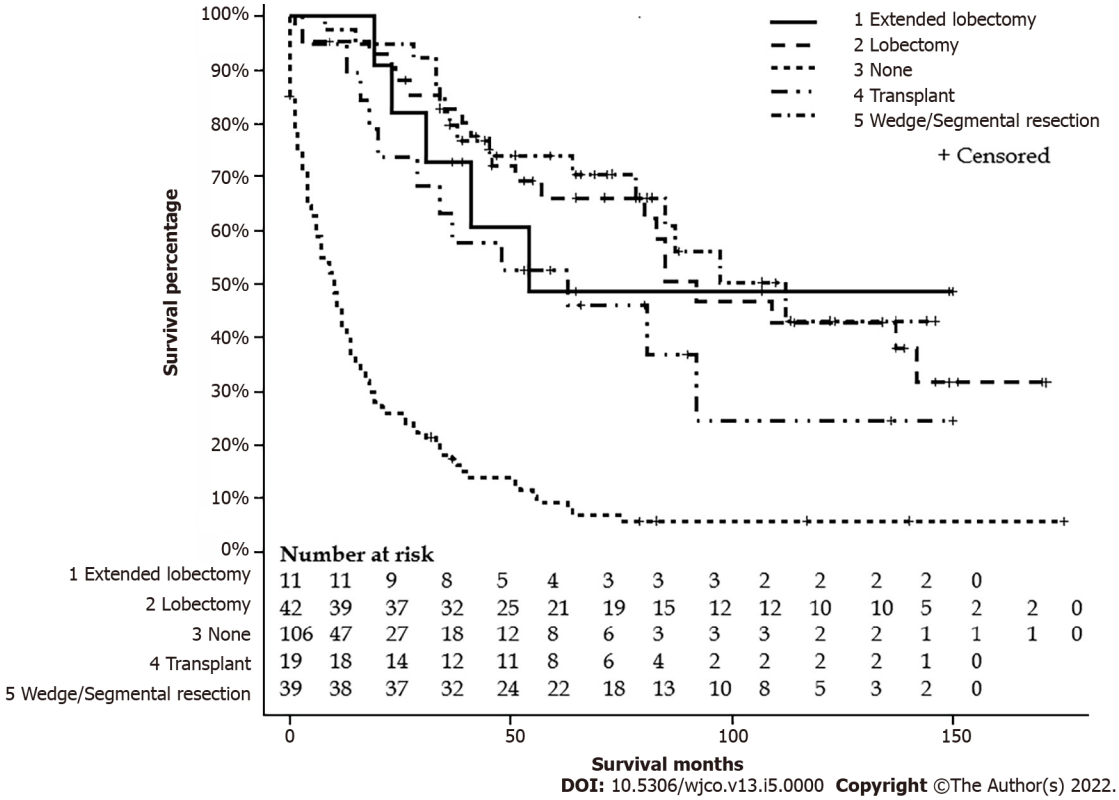

Figure 5 Kaplan-Meier survival curve by surgery types. Median survival for a wedge or segmental resection was 112 (95%CI: 78-NA) mo, lobectomy was 92 (95%CI: 57-NA) mo, extended lobectomy was 54 (95%CI: 23-NA) mo, none had 10 (95%CI: 6-13) mo, and the transplant was 63 (95%CI: 20-NA) mo ($P < 0.001$).

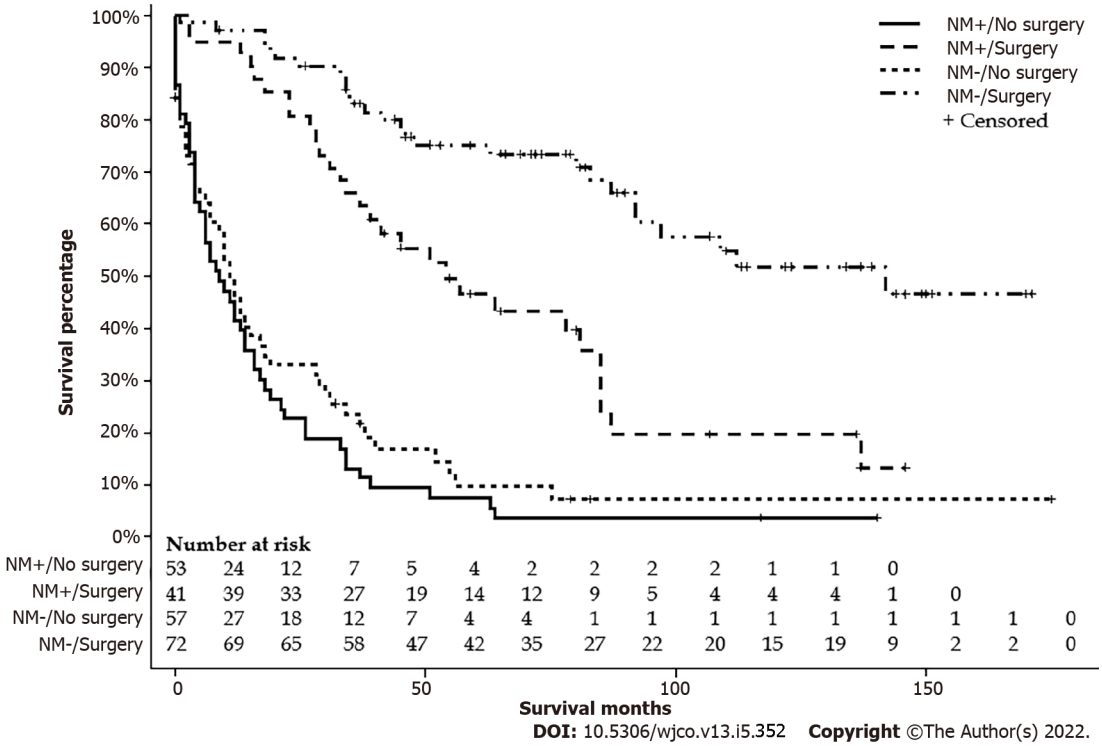

Figure 6 Kaplan-Meier survival curve by surgical status and metastasis status. Median survival for NM+/no surgery was 9 (95%CI: 4-14) mo, NM+/surgery was 54 (95%CI: 34-85) mo, NM-/no surgery was 11 (95%CI: 6-17) mo, and NM-/surgery was 142 (95%CI: 92-NA) mo ($P < 0.001$).

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.

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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

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

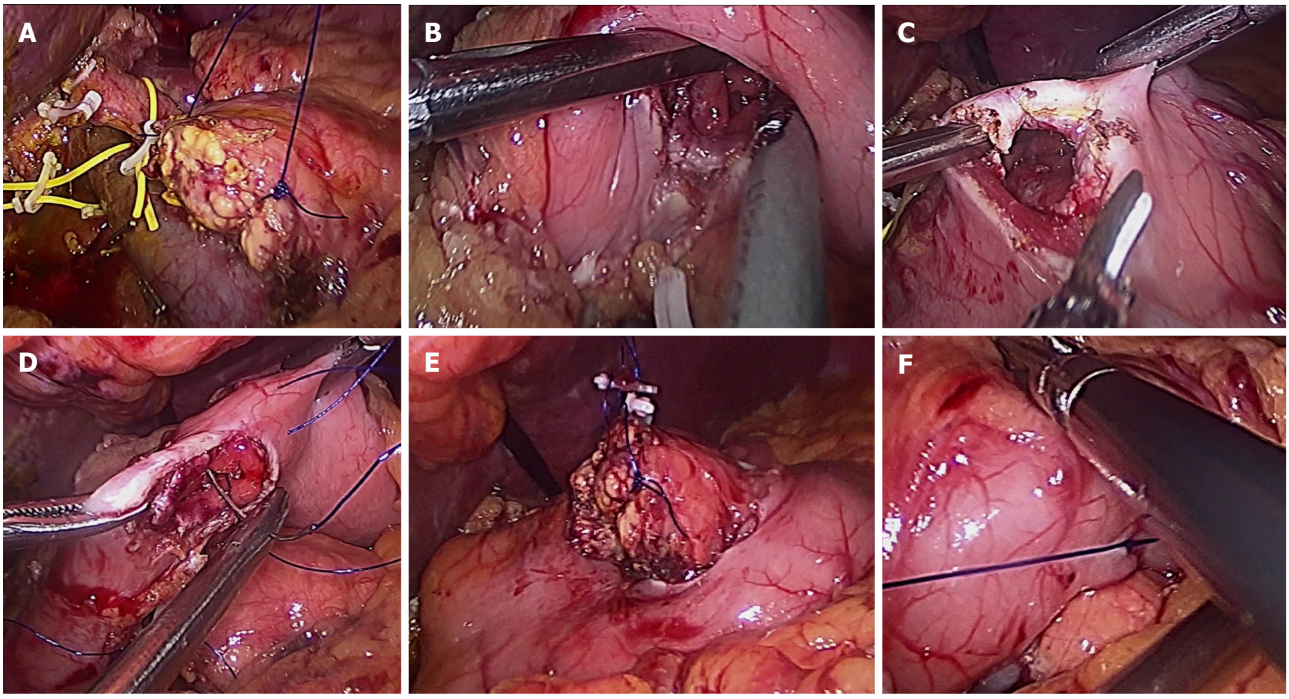

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Figure 1 Steps of modified binding pancreaticogastrostomy. A: Two full-thickness stay sutures are taken at the corners of the pancreatic cut surface; B: A posterior gastrotomy is made at a site where the pancreas can be invaginated without undue tension; 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).

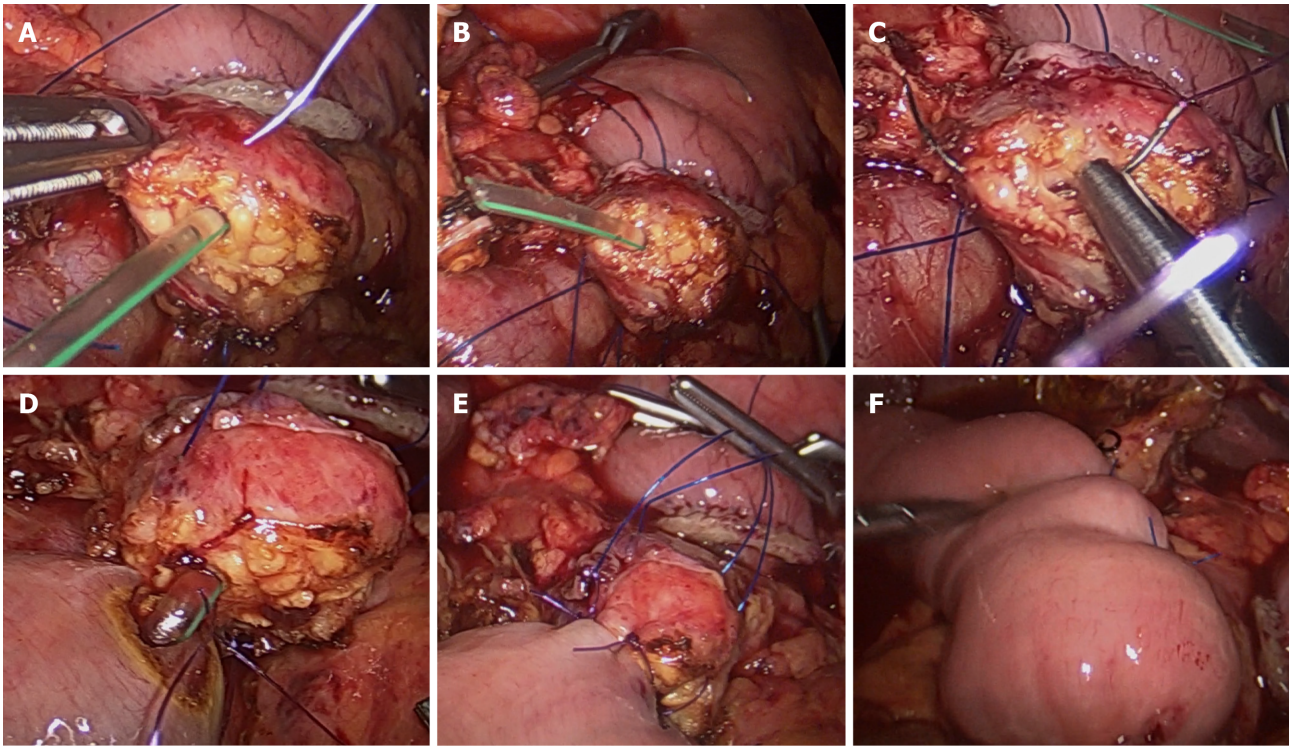

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Figure 2 Steps of modified Blumgart pancreaticojejunostomy. A: The 26 mm ½ circle round body needle of 3-0 polypropylene suture is straightened to facilitate the placement of transpancreatic suture; B: One transpancreatic U suture is taken on either 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).

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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

Masami Ikeda, Masashi Yoshida, Norio Mitsumori, Tsuyoshi Etoh, Chikashi Shibata, Masanori Terashima, Junya Fujita, Kazuaki Tanabe, Nobuhiro Takiguchi, Atsushi Oshio, Koji Nakada

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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

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.

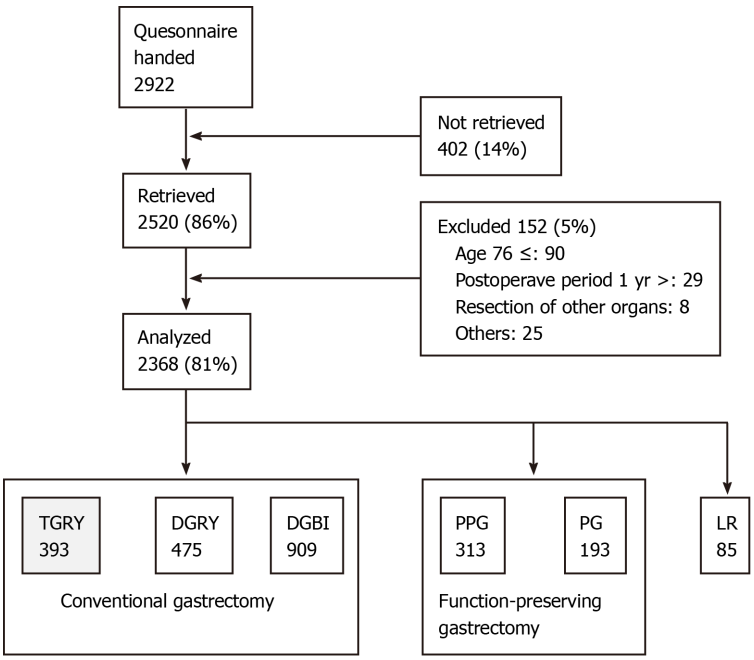

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Figure 1 Outline of the study. TGRY: Total gastrectomy with Roux-en-Y reconstruction; DGRY: Distal gastrectomy with Roux-en-Y reconstruction; DGBI: Distal gastrectomy with Billroth I reconstruction; PPG: Pylorus-preserving gastrectomy; PG: Proximal 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.

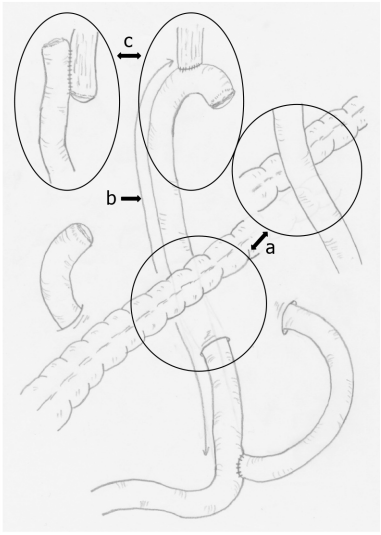

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Figure 2 Schema of Roux-en-Y reconstruction after total gastrectomy. a: Route of the Roux limb (antecolic or retrocolic); b: Length of the Roux limb defined as the distance from the esophago-jejunostomy to the jejunojejunostomy [shorter (≤ 39 cm), average (40 cm) or longer (≥ 41 cm)]; c: Anastomotic procedure for esophagojejunostomy (reconstruction using a circular or linear stapler).

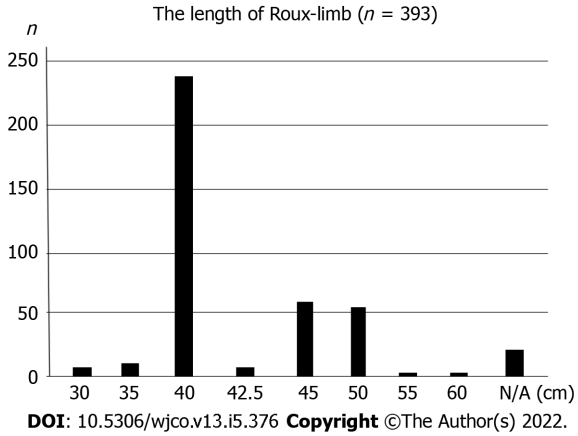

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Figure 3 The distribution of the length of Roux-limb after total gastrectomy. N/A: Not answered group indicated.

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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Informed consent statement: All study participants provided informed written consent prior to study enrollment.

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SYSTEMATIC REVIEWS

Immune checkpoint inhibitors in head and neck squamous cell carcinoma: A systematic review of phase-3 clinical trials

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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

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].

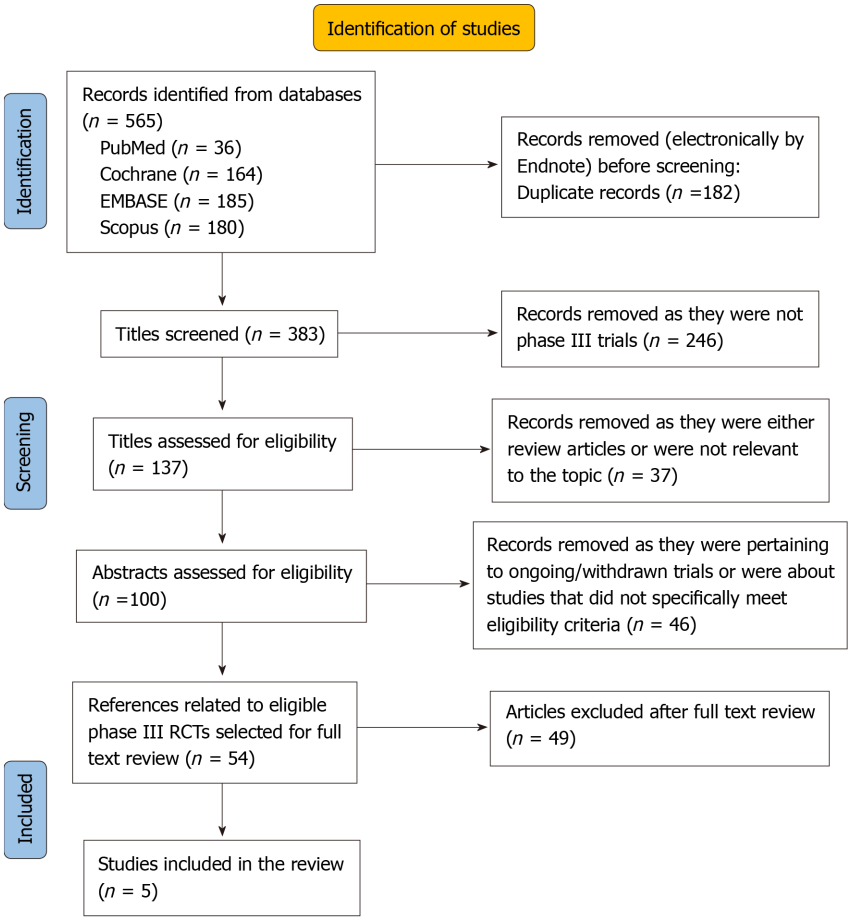

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Figure 1 Article selection flow diagram. RCTs: Randomized controlled trials.

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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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

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

Andrea Pretta, Dario Spanu, Stefano Mariani, Nicole Liscia, Pina Ziranu, Valeria Pusceddu, Marco Puzzoni, Elena Massa, Mario Scartozzi, Eleonora Lai

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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

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

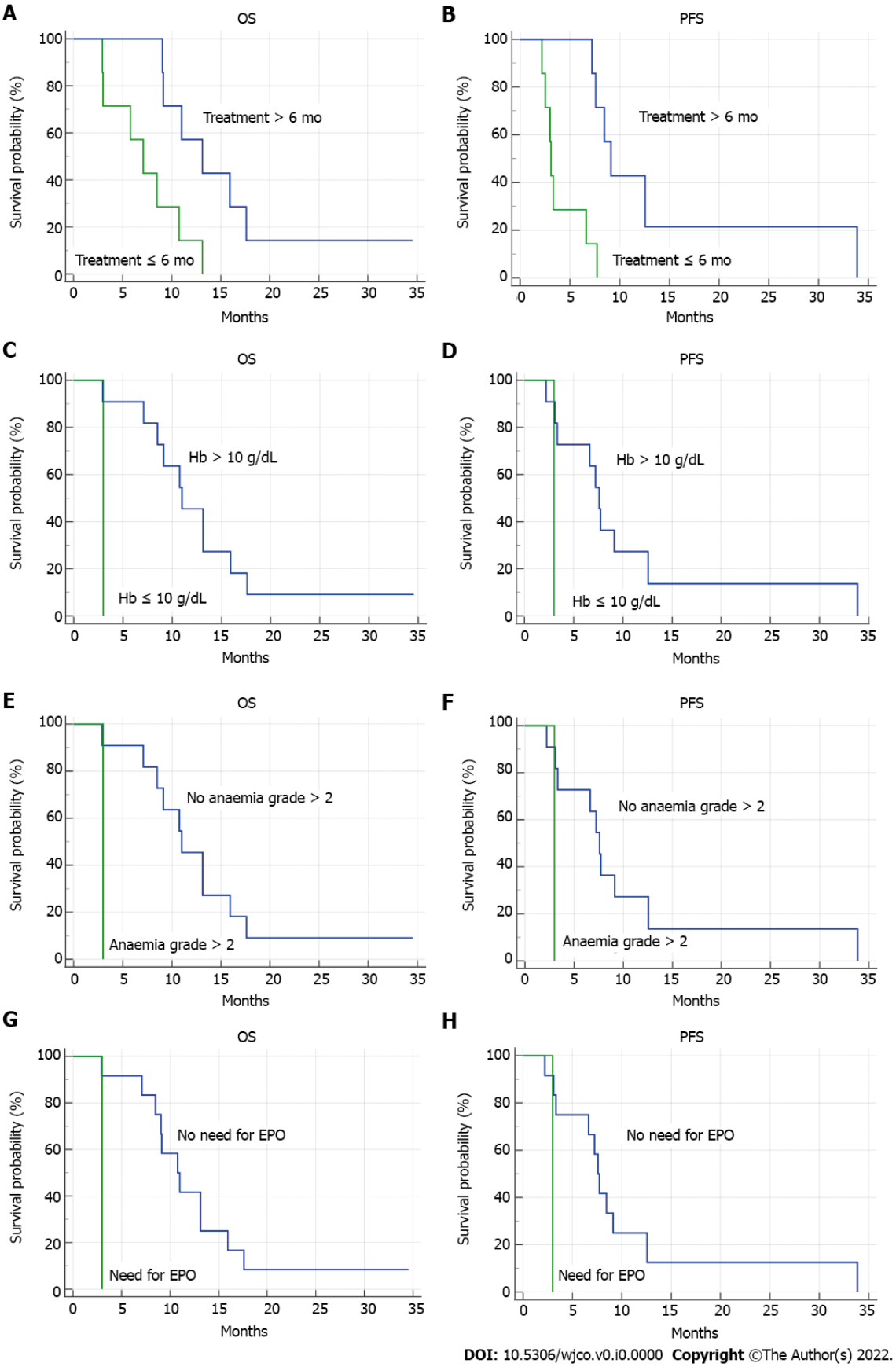

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Figure 1 Kaplan-Meier overall survival and progression free survival curves according to clinical variables. A: Overall survival (OS) Kaplan-Meier curve according to nal-IRI treatment duration; B: Progression free survival (PFS) Kaplan-Meier curve 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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