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EVIDENCE REVIEW

Postoperative radiotherapy in resected non-small cell lung cancer: The never-ending story

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Abstract

This manuscript collects in a joint and orderly manner the existing evidence at the present time about postoperative treatment with radiotherapy in non-small cell lung cancer. It also systematically reviews the current evidence, the international recommendations in the most relevant guidelines, the most controversial aspects in clinical and pathological staging, the specific technical aspects of radiotherapy treatment, and also collects all the potential risk factors that have been postulated as significant in the prognosis of these patients, evaluating the possibility of segmenting a particularly sensitive subpopulation with a high risk of relapse on which an adjuvant treatment with radiotherapy could have an impact on their

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clinical evolution. Finally, currently active trials that aspire to provide more evidence on this topic are reviewed.

Key Words: Non-small lung cancer; Radiotherapy; Postoperative; Lung cancer

Core Tip: The approach to the postoperative treatment of non-small cell lung cancer (NSCLC) is one of the pending subjects of the specialty of Radiation Oncology. Despite the enormous anticipation that the Lung-Art trial had produced, its results leave issues unresolved. In this article, we attempt to systematically recapitulate the currently existing evidence for the radiotherapeutic management of this pathology, in order to identify those patients who could potentially benefit more from postoperative treatment in NSCLC.

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INTRODUCTION

Historical evolution of postoperative radiotherapy

One of the great historical controversies in the field of thoracic oncology is the use of Postoperative radiotherapy (PORT) in patients with non-small cell lung cancer (NSCLC). The rationale for this therapeutic strategy is the high risk of locoregional recurrence (LRR) after radical surgery, especially in patients with pN2 disease, who account for up to 30% of patients. The development of LRR in patients with NSCLC has important clinical implications and is associated with worse survival outcomes[1]. Several different pathological variables have been associated with a higher risk of developing LRR, including tumour size > 3 cm, lymphovascular invasion, visceral pleural invasion, and involvement of multiple lymph nodes[2].

The role of PORT in NSCLC remains controversial, mainly because studies carried out over the last few decades have reported conflicting safety and efficacy results. Although multiple retrospective and prospective studies have been performed, we still lack high-quality evidence to confirm or definitively rule out PORT in these patients. A meta-analysis published in 1998 found that PORT was associated with lower overall survival (OS) rates in patients with stage I-II disease, with 2-year OS rates of 43% in the non-PORT group *vs* 30% in the patients that received PORT, although there was no clear evidence that PORT negatively influenced outcomes in patients with stage III pN2 disease[3]. In older studies, the poor outcomes of PORT could be due to the high levels of morbidity and mortality associated with obsolete radiotherapy techniques or inappropriate doses, fractionations, and/or irradiation volumes. In fact, a more recent meta-analysis demonstrated that PORT improves OS outcomes when modern technology (linear accelerators *vs* cobalt therapy units) is used to deliver the radiation dose[4].

Despite the contradictory findings described above, several studies have reported a clear benefit for PORT in patients with involved lymph nodes (pN2) in terms of improved local control and even OS[5-7]. Among those studies with positive findings, the most important is the study carried out by Mikell *et al*[7], who evaluated 2115 patients with pN2 NSCLC based on data retrieved from the National Cancer Database (NCBD). In that study, PORT was associated with a significant increase in OS (42 mo *vs* 38 mo, $P = 0.048$) in patients treated according to the therapeutic standards of the modern era [three-dimensional conformal radiotherapy (3D-CRT), adjuvant chemotherapy (ChT), *etc.*][7].

The long-awaited preliminary results of the Lung ART trial (NCT00410683)[8], which included patients with NSCLC who underwent complete resection with adjuvant ChT, were recently presented at the ESMO 2020 meeting. Lung ART is a

multi-institutional randomized phase III trial which included stage III N2 NSCLC cases comparing mediastinal PORT (54 Gy/27-30 fractions) to no PORT in very selected patients: PS 0-2, complete resection with optimal nodal exploration and proven N2 disease. The main endpoint was disease-free survival (DFS). Between August 2007 and July 2018, 501 patients were randomized after surgery or after ChT: 252 patients allocated to PORT, and 249 to no PORT. With a median FU of 4.8 years DFS HR was 0.85 (95%CI [0.67-1.07]); median DFS was 30.5 mo with PORT and 22.8 without PORT; 3-year DFS was 47.1% with PORT *vs* 43.8% without PORT (*P* = ns), and finally, 3-year OS was 66.5% with PORT *vs* 68.5% without PORT (*P* = ns). Early and late Gr 3-5 cardio-pulmonary toxicity was respectively 7% and 20% in PORT arm *vs* 3.2% and 7.7% in control arm. Nonetheless, PORT significantly decreased LRR in the mediastinum (46.1% *vs* 25% with and without PORT, respectively), a finding that suggests that PORT could offer a clinical benefit in a well-selected subgroup of patients.

However, these preliminary results raised further doubts about the role of PORT in NSCLC. The findings of this landmark trial are extremely important and may come to redefine the role of radiotherapy in NSCLC.

According to these data, PORT should not be routinely recommended to all resected stage III N2 NSCLC patients. The decision to prescribe o not PORT must be individualised according to the patient's specific characteristics. In general, PORT should be indicated only in highly selected patients with good performance status (PS 0-1), significant mediastinal lymph node involvement (pN2, extracapsular extension), and/or residual disease (R1-R2) after surgery. In addition, PORT must be only performed in cases with a favourable dose distribution that fulfils the dose restriction criteria for the organs of risk (OARs), especially cardiopulmonary restrictions.

CURRENT EVIDENCE AND RECOMMENDATIONS FOR PORT

The role of PORT in the treatment of NSCLC remains controversial. Although this therapeutic strategy has been evaluated in numerous retrospective and prospective studies, robust evidence to definitively support the value of PORT is still lacking, as can be seen in the lack of consensus among the clinical guidelines published by the main international scientific societies[9-13].

Currently, the most widely accepted indication for PORT, with the most evidence, is for the treatment of residual disease (including extracapsular extension) after radical surgery. Most international guidelines recommend PORT in patients with involved surgical margins (R1-R2) at the surgical bed due to the high risk of recurrence in this region, with a recommended dose ranging from 54-60 Gy (1.8-2 Gy/fraction)[14].

By contrast, in patients with stage pN2 disease, the current evidence suggests that the treatment decision should be assessed on a case-by-case basis by a multidisciplinary team to determine if the patient would be likely to benefit from PORT. The treatment decision should consider several key clinical characteristics, including the number of mediastinal nodal stations involved (≥ 1), the patient's general physical condition (PS 0-1), and cardiopulmonary function. Table 1 summarizes the recommendations proposed by the main international guidelines.

MANAGEMENT OF CASES WITH INVOLVED SURGICAL MARGINS

The rate of incomplete resections (microscopic or macroscopic; R1-R2) after radical surgery for lung cancer ranges from 1%-17%[15]. In these cases, the aim of PORT is to reduce the risk of local recurrence and improve OS. Although various clinical guidelines recommend salvage surgery in patients with positive surgical margins, this approach is not supported by robust data. Ghiribelli *et al*[16] evaluated OS in a series of patients with incomplete resections (R1), finding that survival was not correlated with the type of infiltration, nodal involvement, or histological type. As a result, in patients with microscopic residual tumours, the authors recommended salvage surgery only in patients with early stage (I-II) disease; by contrast, the recommended treatment in stage III pN2 disease is adjuvant radiotherapy.

A study published in 2012 evaluated the efficacy and toxicity of PORT according to histological subtype in patients (*n* = 41) with incompletely resected NSCLC[17]. Of the 41 patients, 23 had microscopic (R1) and 18 macroscopic (R2) residual disease. The histologic distribution was as follows: squamous cell carcinoma (SCC) (*n* = 23), adenocarcinoma (14), and other histologies (4). The predominant progression pattern

Table 1 Recommendations for postoperative radiotherapy according to the main international guidelines

Guidelines	Clinical scenario	Recommendation for PORT
NCCN[9]	Stage pN0-1	Not recommended
	Stage pN2, negative surgical margins (R0)	Sequential
	Microscopic or macroscopic surgical margins (R1-R2)	Concomitant (selected cases) or sequential
ASTRO[10]	Stage pN2	Sequential
	Microscopic or macroscopic surgical margins (R1-R2)	Concomitant (selected cases) or sequential
	ESTRO-ASTRO[11]	
	Multiple nodal stations involved	Sequential
	Extracapsular nodal extension	Sequential
ESMO[12]	Early stage (I-II) disease (R0)	Not recommended
	Positive margins or chest wall involvement (R1-R2)	Sequential
	Stage pN2	Only in selected cases
ASCO[13]	Early stage (I-II) disease (R0)	Not recommended
	Stage pN2	Only in selected cases

NCCN: National Comprehensive Cancer Network; ASTRO: American Sociedad of Radiation Oncology; ESTRO: European Society for Radiotherapy & Oncology; ESMO: European Society for Medical Oncology; ASCO: American Society of Clinical Oncology; PORT: Postoperative radiotherapy.

was distant disease, observed in 13% of patients with SCC and 64% of those with adenocarcinoma ($P < 0.01$). Survival rates at 5-years were as follows: OS, 56%; local control (LC), 63%; DFS, 37%; and metastasis-free survival (MFS), 49%. On the multivariate analysis, the only significant predictors of better survival (DFS and MFS) were SCC histology, stage N0-1, and R1 surgical margins. The authors concluded that, in patients with R1 margins, PORT provides good LC without severe toxicity, but systemic therapy should always be considered due to the high risk of distant metastasis.

Hancock *et al*[18] evaluated 3102 surgically treated NSCLC patients included in the NCDB registry. Of these, 1688 had microscopically positive margins (R1). The authors compared patients according to margin status (R1 *vs* R0), with significantly lower 5-year OS rates in the R1 group for all stages: stage I, 37% *vs* 62% (P <0.0001); stage II, 29% *vs* 41% ($P < 0.0001$); and stage III, 19% *vs* 33% ($P < 0.0001$). Administration of adjuvant ChT with PORT in the R1 group was associated with better OS than surgery alone, regardless of stage (stage I, 44% *vs* 35%, $P = 0.05$; stage II, 33% *vs* 21%, $P = 0.0013$; stage III, 30% *vs* 12%, $P < 0.0001$).

In a study published in 2015, Wang *et al*[19] evaluated 3395 patients with incompletely resected stage II-III NSCLC to determine the influence of PORT on survival outcomes, finding that PORT was associated with significantly better 5-year OS (32.4% *vs* 23.7%). Radiation doses between 50-70 Gy improved survival rates in the PORT group *vs* the non-PORT group. However, when higher doses (> 70 Gy) were administered, there were no between-group differences in OS. The authors of that study concluded that PORT improves OS in patients with incompletely resected stage II-III NSCLC and should therefore be considered as an adjuvant treatment. They also suggested that the radiation dose in patients with macroscopic residual disease (R2) should be the same as those used for radical radiotherapy (60-66 Gy).

MEDIASTINAL STAGING

Preoperative mediastinal staging

The appropriate management of NSCLC depends on accurate mediastinal staging. Contrast-enhanced chest computed tomography (CT) is currently the diagnostic test of choice for preoperative mediastinal staging. On CT imaging, nodes with a short-axis diameter ≥ 1 cm are considered pathological[20]. In recent years, 18F-fluorodeoxy-glucose (FDG) positron-emission tomography (PET)-CT has transformed lung cancer staging due to its greater sensitivity. However, PET-CT has some limitations in cases

with small nodes (< 1 cm) and in certain histologies in which FDG uptake is limited. PET-CT also has a high false positive rate (20%-25%) in the presence of intercurrent infections and inflammatory processes. Consequently, histopathologic confirmation of mediastinal node involvement is usually required, especially when the therapeutic approach depends directly on the results of this assessment[21-23]. Histological confirmation can be omitted in certain patients with small (≤ 3 cm) peripheral tumours without radiological evidence of suspected mediastinal involvement.

Mediastinal nodes can be obtained endoscopically through endobronchial ultrasound (EBUS) and/or endoscopic ultrasound (EUS) guided puncture, or surgically, through mediastinoscopy or video-assisted thoracoscopy (VATS). Endobronchial ultrasound (EBUS/EUS) is usually the first step in evaluating suspected mediastinal node involvement[24,25]. These minimally invasive endoscopic techniques are usually preferred to surgical approaches due to their good sensitivity and specificity profile and relatively low risk of morbidity. If the sample is negative, not assessable, or insufficient (despite radiological suspicion), staging should be completed with invasive techniques, which have a higher negative predictive value (NPV). For many years, conventional mediastinoscopy was the main surgical staging technique, despite the technical limitations of this procedure for the study of the posterior and inferior mediastinum, in which either extended cervical mediastinoscopy or VATS is necessary[26].

Mediastinal restaging after neoadjuvant therapy

Mediastinal restaging after neoadjuvant therapy (ChT or ChT+RT) is controversial. Some patients with stage IIIA, low volume N2 disease are classified as potentially resectable and may benefit from neoadjuvant therapy, which could increase the likelihood of achieving a complete response (CR) in the mediastinum, thus permitting surgical resection of the tumour[27]. In this clinical scenario, however, the value of CT for mediastinal restaging is questionable since CT-based assessment, although highly predictive of pathologic CR, tends to underestimate the true CR rate.

PET-CT is an excellent tool to assess the response of both the primary tumour and metastatic lesions, but it is less reliable in evaluating mediastinal involvement due to high rates of false negative and false positives (20% and 25%, respectively)[28,29]. Therefore, histopathologic confirmation is necessary in cases with radiological response if surgical resection is being considered.

EBUS/EUS restaging after neoadjuvant therapy has a low sensitivity and a low NPV. If the test is negative, the surgical technique should be escalated to reduce the false negative rate[30]. Restaging *via* mediastinoscopy has a high sensitivity (> 60%), specificity (≈ 100%), positive predictive value (PPV; 100%) and NPV (> 73%); however, this procedure is not routinely performed due to its technical complexity in this clinical context. Rather, the recommended strategy is initial confirmation of stage N2 disease by EBUS or EUS-guided transbronchial aspiration during the initial workup, thus reserving mediastinoscopy for restaging[31].

SELECTION OF CANDIDATES FOR PORT

Numerous studies have explored a wide range of prognostic factors potentially associated with an increased risk of LRR in order to identify high-risk patients suitable for adjuvant radiotherapy. In patients with NSCLC, the histological type is not currently considered a prognostic factor for adjuvant treatment due to the poor quality of the available data and contradictory findings in the literature. While some studies have found that SCC histology is associated with worse OS rates than adenocarcinoma [32,33], findings from other studies point in the opposite direction[34].

The findings of a recent meta-analysis involving 25780 patients from 13 studies (most retrospective) underscored the prognostic value of multiple mediastinal node involvement. That study showed that, in patients with pN2 disease with ≥ one positive node and/or multiple N2 station involvement, PORT significantly improved both DFS (HR 0.57, 95% confidence interval [CI], 0.38–0.85) and OS (HR 0.85, 95%CI, 0.79–0.92) [35].

The lymph node ratio (LNR)—defined as the number of involved nodes divided by the total removed or examined—has also been significantly associated with survival outcomes. A recent study evaluated 11341 patients with NSCLC and postoperative nodal involvement included the SEER (Surveillance, Epidemiology, and End Results Database) registry. The authors established three risk categories according to the LNR (LNR1 ≤ 0.28, LNR2 < 0.81, and LNR3 > 0.81), finding that LNR3 was an independent

prognostic factor for cancer-specific survival (CSS) (HR 2.54; 95%CI, 2.30–2.80; $P < 0.001$)[36].

Other parameters, such as the positive and negative lymph node counts (PLN and NLN, respectively), have been developed to quantify the tumour load in mediastinal nodes. Zhou *et al*[37] reviewed data from 39959 surgically-treated cases of NSCLC, demonstrating a significant association between mediastinal tumour burden and OS (PLN > 5; HR 2.0128, 95%CI: 1.6996–2.3836; NLN > 5; HR 0.7493, 95%CI: 0.7211–0.7785; LNR > 0.30; HR 1.7949, 95%CI: 1.5329–2.1016); and with CSS (PLN > 5; HR 2.2147, 95%CI: 1.8095–2.7106; NLN > 5; HR 0.7214, 95%CI: 0.6869–0.7575; LNR > 0.30; HR 1.9627, 95%CI: 1.6219–2.3752). In this same line of research, another study evaluated 5168 patients with stage IIIA-N2 NSCLC, finding that patients with PLN > 5 who underwent PORT had significantly better OS outcomes (HR 0.637, 95%CI: 0.518–0.784), a benefit that persisted even when compared to adjuvant ChT alone (HR 0.726, 95%CI: 0.564–0.934)[38].

The studies that have generated the most interest are those that have sought to stratify risk groups according to multiple clinical, pathologic, and molecular parameters. In this regard, the study by Deng and colleagues[39] is worth highlighting. Those authors evaluated numerous characteristics — age, sex, surgical technique, histological type, degree of differentiation, tumour size, number of nodes evaluated (LNR index) — in a large sample ($n = 2329$) of patients included in the SEER database. Based on that analysis, the authors proposed a prognostic scoring model that classified patients into two risk categories (high and low), which was a significant predictor of survival outcomes (OS and CSS)[40].

Jiang *et al*[40] recently developed a model that incorporated several molecular biomarkers, together with other well-known clinical variables, to predict clinical outcomes in patients with stage IIIA pN2 NSCLC. In that study, the following variables were significantly associated with the risk of LRR: epidermal growth factor receptor (EGFR) status: wild-type *vs* native (HR 3.666, 95%CI: 1.724-7.797); lymphocyte to monocyte ratio (LMR) < 4.69 (HR 2.364, 95%CI: 1.221-4.574); surgical procedure (VATS *vs* thoracotomy) (HR 0.348, 95%CI: 0.175 -0.693); and pN2 LNR ≥ 38.9% (HR 3.597, 95%CI: 1.832-7.062). The authors then used those data to develop a predictive model (Table 2) based on the four independent risk factors to determine the individual risk of LRR in each patient. This score, in turn, could be used to recommend or not adjuvant radiotherapy[41].

TECHNICAL RECOMMENDATIONS FOR THE TREATMENT OF PORT

Simulation

The generally accepted recommendations provided by clinical guidelines for the management of NSCLC should be followed for positioning, immobilization, and treatment simulation. Systems designed to improve immobilization and control respiratory motion (4D-CT) should be used, preferably with image-guided radiotherapy (IGRT), to obtain smaller treatment volumes and more precise radiotherapy to achieve a better dosimetric distribution.

In general, CT imaging (slice thickness, 2-3 mm) should be performed with intravenous contrast to improve contouring of the nodal areas[42,43]. The use of 5FDG-PET-CT for postoperative simulation is not recommended due to the lack of robust data; moreover, interpretation of these images in the immediate postoperative period can be challenging due to the inflammation, which can lead to false positives. Image interpretation after ChT is also difficult and it is easy to underestimate the residual disease (false negatives)[44].

Target volumes

The most important data for target volume definition were described in the Lung-ART clinical trial and based on contouring performed by 17 experienced thoracic radiation oncologists in two representative cases[45]. The clinical target volume (CTV) should include the bronchial stump, ipsilateral hilum, adjacent mediastinal pleura, and involved nodes (according to the pathology report). The involved nodal station and those immediately superior and inferior to that region should also be contoured, being careful to avoid oversizing the CTV. To generate the PTV (planning target volume), a margin of at least 0.5 cm in the mediolateral and dorsoventral directions (1 cm in the craniocaudal direction) should be applied to the CTV to minimize uncertainties related to tumour motion and patient positioning[46].

Table 2 Proposed predictive model for locoregional recurrence in stage IIIA N2 non-small cell lung cancer[41]

Risk model for LRR in stage pIIIA-N2 NSCLC		
Factor	**Category**	**Score**
EGFR status	Wild- type	4
LMR	LMR < 4.69	2
Type of surgery	Thoracotomy	3
LNR	LNR ≥ 38.9	4
Risk group	**Score**	**3-yr LRFS**
Low risk	0-2	71.4%
Medium risk	3-5	57.3%
High risk	6-13	13.6%

LRFS: Locoregional recurrence-free survival; LRR: Locoregional recurrence; NSCLC: Non-small cell lung cancer; LNR: Lymph node ratio; LMR: Lymphocyte-to-monocyte ratio.

The definition of critical organs (OARs)[47] and dose restrictions are the same as in NSCLC, although with more restrictive lung criteria. In post-lobectomy patients, Boonyawan *et al*[47], proposed limiting the lung volume that receives 10 and 20 Gy (V10 and V20) to < 30% and < 20%, respectively[48]. In patients older than age 65, the lung V5 should be reduced to ≤ 36%[49]; if IMRT is performed, the recommended V5 is < 64.9%, with mean lung dose (MLD) < 10.8 Gy[50]. In patients undergoing pneumonectomy, to ensure safety, these limitations should be even more restrictive, as follows: V5 < 30%, V20 < 13%, and MLD < 7.5 Gy[51]. If 3D-CRT is used, the V20 should be < 10%[52].

Dose and fractionation

In completed-resected (R0) surgeries, the recommended dose is 50-54 Gy using a conventional fractionation scheme (1.8-2 Gy/d)[53]. However, in high risk patients with R1 or R2 margins, the total dose may be increased up to 54-60 Gy, or even up to radical doses of 60-66 Gy if there is evidence of macroscopic residue in the surgical bed or mediastinal region.

The use of hypofractionated regimens is not advised due to the risk of increased toxicity. Currently, accelerated fractionation radiotherapy schemes (2 Gy/d, 7 d/wk) are being explored (NCT02189967)[54].

In terms of treatment sequencing, PORT should be administered after completing ChT if the surgical resection is complete (R0); however, in patients with postoperative R1-R2 margins, there is some controversy surrounding the use of concomitant or sequential RT and ChT. As a result, the treatment sequence should be individualized based on the expected tolerance[55,56].

Although several radiotherapy techniques — 3D-CRT, intensity modulated radiation therapy (IMRT), volumetric modulated arc therapy (VMAT), and tomotherapy — all provide optimal dosimetric results in the postoperative context [57], data from prospective studies support the routine use of the IMRT in NSCLC due to lower cardiac doses and a lower risk of severe pneumonitis.

FUTURE LINES OF RESEARCH IN PORT

At present, there is broad consensus among radiation oncologists that the current level of evidence is insufficient to recommend PORT for all patients with stage III pN2 NSCLC, which is mainly attributable to the heterogeneous characteristics of patients with pN2 disease and treatment-related cardiopulmonary toxicity, which remains high despite efforts to reduce it.

In terms of the lack of homogeneity, it is evident that TNM staging in patients with pN2 NSCLC does not provide sufficient information to indicate or not adjuvant therapy. Consequently, it is essential to explore and evaluate new clinical, pathological, and molecular factors to better differentiate between different risk subpopulations, which would then allow us to tailor the treatment indication based on

Table 3 Registered active studies related to postoperative radiotherapy

NCT	Title	Study type
NCT02977169	To Evaluate the Role of Postoperative Radiotherapy in Patients With IIIA(N2) Non-Small Cell Lung Cancer	Interventional
NCT02974426	To Evaluate the Optimal Timing of Postoperative Radiotherapy in Patients With IIIA(N2) Non-Small Cell Lung Cancer	Interventional
NCT04073745	Single Fraction Stereotactic Body Radiation Therapy After Surgery in Treating Patients with Non-small Cell Lung Cancer	Interventional
NCT03006575	Study of Split-course Chemoradiotherapy for Postoperative Locoregional Recurrence of Non-small Cell Lung Cancer	Interventional
NCT02555592	Strategy of Surgical Resection with Adjuvant Therapy for IIIA NSCLC and N2 Disease Only in Subaortic or Paraaortic Level	Observational
NCT02189967	Postoperative Radiotherapy of Non-small Cell Lung Cancer: Accelerated *vs* Conventional Fractionation	Interventional
NCT00880971	Postoperative Radiotherapy for Patients with IIIA (N2) Non-small Cell Lung Cancer	Interventional
NCT01112631	Prospective Study of Quality of Life in Non-small Cell Lung Cancer (NSCLC) Patients Treated With/Without Postoperative Radiotherapy	Observational

the patient's unique characteristics.

It is important to note that most of the prognostic factors identified to date have been derived from data obtained in large retrospective series or epidemiological records. Clearly, due to the important methodological limitations of those studies, it is difficult to extrapolate the findings of those studies into routine clinical practice without stronger supporting data. In this regard, new studies with more robust methodological designs are needed to obtain a higher level of evidence. Table 3 lists the main trials currently underway to evaluate PORT in NSCLC.

The studies performed to date have consistently found an association between PORT and a higher risk of cardiopulmonary morbidity and mortality, a finding that undermines the clinical benefits of this treatment. However, some studies have shown that IMRT is superior to 3D-CRT in NSCLC in terms of dosimetry and survival outcomes[58]. Heavy particle therapy seems to show certain dosimetric advantages *vs* IMRT in terms of protection of OARs, and could significantly reduce cardiopulmonary toxicity, although prospective studies confirming this clinical benefit are not yet available[59].

For all the reasons described above, it is evident that only advanced radiotherapy techniques, such as VMAT or IMRT, which allow for better dose conformity, should be used for the treatment of NSCLC. In addition, these techniques should be used in all future clinical trials of PORT to better determine the true value of PORT in patients with NSCLC.

CONCLUSION

In patients with stage pN2 disease, current evidence suggests that the treatment decision should be evaluated on a case-by-case basis by a multidisciplinary team to determine whether the patient is likely to benefit from PORT. The treatment decision should consider several key clinical features, such as the volume of nodal mediastinal tumor burden, physical condition (performance status) and individual cardiopulmonary risk, but another technological issues, like availability to modern functional imaging devices or high dosimetric conformation radiotherapy (IGRT or VMAT), may be critical for a correct indication.

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REVIEW

Relationship between Th17 immune response and cancer

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Abstract

Cancer is the second leading cause of death worldwide and epidemiological projections predict growing cancer mortality rates in the next decades. Cancer has a close relationship with the immune system and, although Th17 cells are known to play roles in the immune response against microorganisms and in autoimmunity, studies have emphasized their roles in cancer pathogenesis. The Th17 immune response profile is involved in several types of cancer including urogenital, respiratory, gastrointestinal, and skin cancers. This type of immune response exerts pro and antitumor functions through several mechanisms, depending on the context of each tumor, including the protumor angiogenesis and exhaustion of T cells and the antitumor recruitment of T cells and neutrophils to the tumor microenvironment. Among other factors, the paradoxical behavior of Th17 cells in this setting has been attributed to its plasticity potential, which makes possible their conversion into other types of T cells such as Th17/Treg and Th17/Th1 cells. Interleukin (IL)-17 stands out among Th17-related cytokines since it modulates pathways and interacts with other cell profiles in the tumor microenvironment, which allow Th17 cells to prevail in tumors. Moreover, the IL-17 is able to mediate pro and antitumor processes that influence the development and progression of various cancers, being associated with variable clinical outcomes. The understanding of the relationship between the Th17 immune response and cancer as well as the singularities of carcinogenic processes in each type of tumor

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is crucial for the identification of new therapeutic targets.

Key Words: Cancer; Immune response; Th17 cells; Interleukin-17; Tumor microenvironment; Pathophysiology

Core Tip: Cancer is still an important cause of death worldwide. Its development and progression are intimately related to the host immune response. In that context, the Th17 profile plays crucial roles in the pathogenesis of several cancers, promoting antitumor and protumor mechanisms. This study reviews the interactions occurring between Th17 responses and cancer.

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INTRODUCTION

Cancer represents the second leading cause of death worldwide and has been responsible for 8.97 million deaths from 2000 to 2016 according to the World Health Organization (WHO). The five most lethal malignancy types are lung, liver, gastric, breast, and colon cancers. Four out of the five aforementioned cancers are among the 20 main causes of death in the world and epidemiological studies predict a tendency of increasing mortality rates associated with these diseases in the next 40 years[1]. The knowledge on the relationship between cancer and the immune system was limited for a long time due to the tumor capacity of evading immune response through various mechanisms[2]. However, current evidence has emphasized that $CD4^+$ T cells are intimately associated with processes that unleash cancer as well as with the responses against the malignant cells through the detection of foreign antigens. These cells interact with each other and with other immune system components by releasing cytokines that are able to potentialize or suppress mechanisms in the tumor microenvironment, playing protumor and antitumor roles in that context[3].

Th17 cells is a type of T cell and its main related cytokine is the interleukin (IL)-17, which plays roles in the expression of other cytokines, including IL-6, IL-21, IL-22, interferon (IFN)-γ, and IL-10[4]. The Th17 cells are associated mainly with the immune response against bacteria and fungi, and also perform remarkable contributions in the promotion of inflammation and autoimmunity[5-7]. Moreover, the Th17 profile plays controversial roles in the tumor immunity and can be associated both to unfavorable and favorable outcomes[8]. The duality of Th17 cells in the setting of tumor immunity is potentialized by their plasticity, since they are able to switch to a Th1 phenotype, performing antitumor activities, and they are also capable of expressing a Treg phenotype, which can exert pro and antitumor activities, depending on the context of the immune response[9,10]. The Th17 cells have a peculiar relationship with the tumor microenvironment and use various molecular processes such as the induction and recruitment of further Th17 cells to maintain tumor infiltration[11-14]. Moreover, these cells play important roles in the growing and development of cancerous cells[15]. In that context, the mechanisms performed by Th17 cells that favor or impair tumor progression are complex and depend mainly on the type of cancer. These cells induce some mechanisms with a high carcinogenic potential such as angiogenesis, whereas they can also promote the recruitment of immune system cells to the tumor microenvironment and the activation of effector $CD8^+$ T cells, promoting antitumor activities [16].

The understanding of the mechanisms developed by Th17 cells to modulate the tumor microenvironment and to interact with other cells is a way to identify potential therapeutic targets for various types of cancer that are associated with cellular activities related to that immune profile. Those cells interact in interesting manners

with various interleukins and with cancer stem cells (CSCs)[17]. In addition, the relationship between Th17 cells and CSCs can be considered as bidirectional, with mutual modulation mechanisms[18-20]. This paper aims to review the role played by the Th17 response in various types of cancer, describing its presence in the tumor microenvironment and comparing the repercussions related to this immune profile in cancers, and to approach potential therapeutic targets associated with the immune system mechanisms related to Th17 cells.

CHARACTERIZATION OF THE TH17 RESPONSE

$CD4^+$ T cells can play proinflammatory and regulating roles in the immune response. The classic differentiation of $CD4^+$ T cells into two sets of cells with patterns of cytokine secretion and distinct functions, known as Th1 cells and Th2 cells, has changed with the discovery of a new set of cells known as Th17 cells[21]. Th17 cells express the RORγt (Retinoic-acid-receptor-related orphan nuclear receptor gamma), which is a molecular determinant for its polarization through IL-17A expression[22,23]. In rodents, these cells seem to have the same precursor as $Foxp3^+$ Treg cells, since naive $CD4^+$ T cells stimulated only by transforming growth factor (TGF)-β, convert into Treg cells. On the other hand, TGF-β and IL-6 together induce the emergence of Th17 cells. A study using splenocytes from mice found that an environment containing TGF-β predisposed the emergence of ex-Th17 Foxp3 cells, and simultaneous TGF-β and IL-6 stimuli led to enhanced production of IL-17$Foxp3^{++Neg}$ cells. In addition, that study showed that TGF-β, IL-6, and IL-23 together induced an increase in IL-17A release by Th17 cells[24]. IL-23, in its turn, plays the role of maintaining and expanding these cells. In humans, a relationship between Th17 cells and Th1 cells is evident. Naive $CD4^+$ T cells in the presence of IL-23 and IL-1β positively regulate RORγt, T-bet, IL-23R, and IL-12R. When the two aforementioned cytokines are expressed, IL-17 is produced alone or in combination with INF-γ. TGF-β inhibits the development of both Th1 cells and Th2 cells, and is not essential for the development or inhibition of Th17 cells; therefore, it indirectly favors the expansion of the latter[25,26]. The main function of Th17 cells is to contribute to the immune response against extracellular pathogens during infectious processes, but they are also suggested to play an important role in the pathogenesis of autoimmune and inflammatory diseases, as well as in acute graft-versus-host disease[5-7]. Th17 cells activate neutrophils, stimulate the emergence of CXCL chemokines and MUC5AC, the production of MUC5B mucins by bronchial epithelial cells, the expression of beta defensin-2 and CCL20 by lung epithelial cells, and contribute to the migration and activation of macrophages[7].

The IL-17 family is made up of six different cytokines: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F. IL-17 cytokines have proinflammatory properties, are expressed in various parts of the body, and signals by interacting with their transmembrane receptors. Five receptors from the IL-17 family have been identified: IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE. In addition to Th17 Lymphocytes, $CD8^+$ T cells, macrophages, and γδ T cells produce IL-17. These cells also express IL-23R and secrete IL-21 and IL-22. Among all IL-17 cytokines, IL-17F has the highest degree of conservation with the main cytokine in the family, IL-17A[27]. The IL-17 induces neutrophil recruitment and production of proinflammatory mediators, such as IL-1, IL-8, metalloproteinases 1 and 13, and prostaglandin E2[28,29].

Th17 cells can also produce IL-22. This cytokine is a member of the IL-10 family, and the IL-22R and IL-10R2 receptors have been identified as heterodimeric receptors mediating IL-22 signaling. Whereas IL-10R is ubiquitously expressed, IL-22R is restricted to cells harbored in tissues[30]. IL-22 targets epithelial and non-hematopoietic stromal cells and can promote cell proliferation, playing a role in tissue regeneration. In addition, it regulates host defenses on barrier surfaces. However, IL-22 has also been associated with the development of several diseases involving inflammatory mechanisms[31].

The inflammatory effects produced by IL-17 include stimulus for secretion of IL-6 by human fibroblasts and increased expression of the intercellular adhesion molecule-1[32]. In addition, Th17 cells that co-produce IL-21 regulate B cell responses, induce differentiation of plasma cells, and lead to the formation of antibodies[33]. In that context, the role of Th17 cells as key promoters of inflammation in various pathophysiological contexts, including cancer, has been investigated[8]. Of note, Th17 cells have already been identified in various types of human tumors[8], such as melanoma, ovarian cancer, colorectal cancer, and lung cancer[34,35].

Despite the advances in the knowledge about the roles of Th17 cells in complex biological contexts, many interactions involving these cells remain unknown mainly due to their plasticity[35]. The differentiation of T cells is no longer considered as linear and irreversible, since evidence has shown that populations of differentiated $CD4^+$ T cells can alter the spectrum of cytokines produced and, thus, the outcomes they promote[36]. The plasticity of Th17 cells stands out among the other T cells due to its high level of complexity, involving cytokine-dependent and -independent events, and due to the variability of functional phenotypes that can be adopted by the cells [37]. Th17 cells can play roles that are often heterogeneous, depending on the environmental conditions they are found in. Phenotypes similar to Th1 cells are expressed by Th17 cells in inflammatory environments, with a remarkable production of IFN-γ, contributing as an immunological support against fungal and extracellular bacterial infections, but also for intestinal inflammation observed in colitis, when the activity of these cells is not properly controlled[38-40]. The transfer of antigen-specific Th17 cells to a host has shown that Th17 cells can assume the phenotype of a Th2 cell in infections by *Nippostrongilus brasiliensis*[41]. This Th17/Th2 conversion has also been identified in the peripheral blood of patients with asthma, whose Th17/Th2 cells secreted cytokines from both profiles (Il-17, IL-22, IL-4, and IL-5), which evidences the pathogenic potential of these cells in the induction of intense inflammatory infiltrates [42]. In addition to their pro-inflammatory role, Th17 cells are also able to adopt a phenotype similar to that of Treg cells. In fact, both the conversion of Treg cells into Th17 cells and the conversion of Th17 cells into Treg cells are described by previous studies, possibly because both cell types share differentiation characteristics, given the participation of TGF-β in inducing the differentiation of both Treg and Th17 cells[43]. Although TGF-β, IL-6, and IL-23 alone tend to promote IL-17A release by Th17 cells, the presence of Prostalglandins E2 (PGE_2) makes the stimuli with these cytokines result in the conversion of Th17 cells into regulatory IL-$17A^{neg}$Foxp3 cells, which demonstrates the importance of the PGE_2 in the transdifferentiation of Th17 cells along with TGF-β[24]. Although it presents a certain stability, the aforementioned process has been shown to be bidirectional and, in the presence of Th17-polarizing cytokines, Th17-derived Treg cells can reassume their original Th17 cells phenotype (Figure 1) [9]. Moreover, in response to IL-27, Th17 cells acquire a phenotype similar to that observed in TR1 cells *via* activation of the Blimp-1 factor, which results in the secretion of cytokines such as IL-10[44].

RELATIONSHIP BETWEEN TH17 CELLS AND CANCER

Although advances have been achieved in the study of Th17 responses, the knowledge regarding its roles in the immune system is still limited. Moreover, the tumor microenvironment has been broadly studied because it is directly associated with cancer development and progression. The understanding on how the immune system cells behave in the aforementioned microenvironment and how this environment is influenced by those cells make possible a broader comprehension about cancer and the therapeutic possibilities against this disease[45]. The participation of Th17 cells in the different types of cancer is paradoxical, since they can play antitumor and protumor roles[8]. It is believed that the Th17 cells prevail in the tumor microenvironment through some mechanisms, including: (1) The induction of T cells involving the TGF-β 1 and IL-6. Signal transducer and activator of transcription 3 (STAT3)[11]; (2) Recruitment of Th17 cells dependent on various chemokines including CCL20, CCL17, CCL22, MIF, RANTES, and MCP1[12-15]; (3) Conversion from other cell types[15]; and (4) Th17 cell polarization through cytokines such as IL-1β and IL-13 that are produced in the tumor environment by specific myeloid cells[46]. In addition, the tumor microenvironment has antigens and metabolites that are able to suppress $CD4^+$ T cells, which start producing co-inhibitory and less effective molecules[47]. However, the Th17 cells from the tumor microenvironment seem to have an enhanced resistance to dysfunctionality because they present less exhaustion markers than other T cells as well as more CCR7, Lef1, and TCF7 markers[48,49].

An important aspect regarding Th17 cells in cancer settings is the aforementioned plasticity potential through their transformation into Treg cells, which occurs with TCR engagement leading to the expression of Foxp3 and subsequent imunossupressive roles by those cells. Of note, $IL17^+Foxp3^+$ T cells have been associated with the emergence of CSCs and with the inhibition of tumor-specific T $CD8^+$ cells in colorectal cancer. A study showed that $IL17^+Foxp3^+$ T cells induce the expression of markers such as CD133, CD44s, CD166, EpCAM, and ALDH1 in bone marrow-derived mononuclear

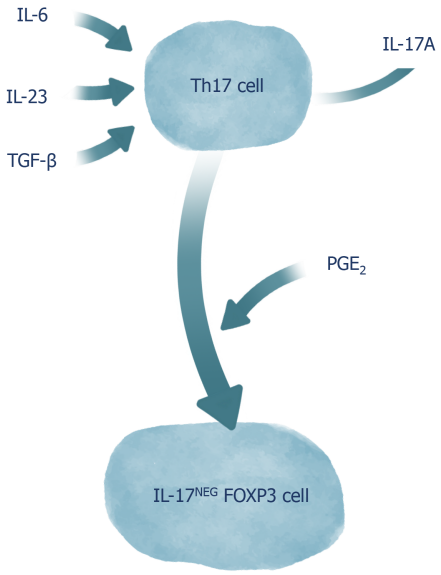

Figure 1 Interleukin-6, interleukin-23 e transforming growth factor-β stimuli for interleukin-17A production and the role of Prostalglandins E2 in Th17 cells transdifferentiation into interleukin-17^{NEG} FOXP3 cell. TGF: Transforming growth factor; IL: Interleukin; PGE_2: Prostalglandins E2.

cells and promoted their conversion into cancer-initiating cells[50,51].

Conversely, the production of IL-6 in mice with melanoma led to the conversion of Treg cells into Th17 cells, which resulted in the promotion of the activation of $CD8^+$ T cells and reduction in tumor growth[52]. A recent study on CpG (ODNs)/CpG 1826 oligodeoxynucleotides demonstrated the potential of these cells in the inhibition of Treg cells and in the stimulation of Th17 cells, extending survival among mice with leukemia[53]. Another study observed that the Treg/Th17 cell ratio was higher among patients with oral squamous cell carcinoma (OSCC) than in controls, suggesting that these cells are involved in the progression of OSCC and have the potential to be used as a prognostic indicator[54]. Considering the importance of the Th17/Treg axis in cancer-related immune response and inflammation, a study assessed the metabolic features involved in that setting. The researchers found that Th17 cells are more dependent on the synthesis of fatty acids than Treg cells, which primarily perform the oxidation of fatty acids to keep their energetic homeostasis. This metabolic differences can make the manipulation of the Th17/Treg axis possible for new therapeutic alternatives against neoplasms[55].

Interestingly, the plasticity of Th17 cells has also been observed with their conversion into Th1 Lymphocytes, exerting antitumor effects[10,56]. It is not well known if that transformation occurs inside the tumor microenvironment or if Th17/Th1 cells are recruited to the tumor microenvironment[57,58]. Muranski *et al*[48] reported that the Th17 cells polarization leads to the production of Th1 cells-related molecules such as INF-γ and T-bet, which are associated with remarkable antitumor activities[48].

The mechanisms taking place in tumor-related Th17 responses are various and depend on the type of cancer. A study observed that the transference of Th17 cells implied in the recruitment of $CD4^+$ T cells, $CD8^+$ T cells, and DCs for the tumor microenvironment. Additionally, when in contact with tumor antigens, Th17 cells acquired dendritic cell MCHI-peptide complexes and, through MHCI-TCR interaction and IL-2 release, there was an activation of $CD8^+$ T cells, reinforcing the role of Th17 cells in tumor immunity[16]. Studies have verified that IL-17 may indirectly potentialize the functions of cytotoxic T lymphocytes by stimulating the expression of IL-6 and IL-12, leading to antitumor effects[59]. The Il-17 acts in the recruitment and expansion of neutrophils that are essential to the destruction of tumor cells as well as contributes to the expression of various proinflammatory molecules including IL-1β, IL-6, tumor necrosis factor (TNF)-α, PGE_2, CXCL1, CXCL5, CXCL8, and GMCSF[60-65]. On the other hand, Wang *et al*[54] demonstrated that Th17 cells play protumor roles since IL-17 induces the expression of IL-6, which is responsible for activating oncogenic signal transducers and STAT3. The STAT3 aids in the promotion of tumor growth through the regulation of pro-angiogenic genes[66]. Differently from the IL-17 dualism, the IL-22 has shown to be a protumor interleukin that, through STAT3, is involved in the development of tumor cells[67-69].

The Th17 cytokines, especially IL-17, play important roles in the promotion of tumor angiogenesis[70]. Studies have described that the IL-17 induces the expression of vascular endothelial growth factor (VEGF), and that cytokine seems to be associated with a higher tumor vascular density[71]. In addition, the Il-17 induced the expression of angionenic chemokines including CXCL-1, CXCL-5, CXCL-6, and CXCL-8, leading to an increased angiogenic potential among immunocompromised rats with non-small cell lung cancer (NSCLC)[72]. Another study described that IL-17 is involved in the activation of angiogenic genes through the IL-6/STAT3 pathway[73]. The IL-22 has also been positively correlated with angiogenesis. A study suggested that it is involved in the proliferation, survival, and migration of endothelial cells. Moreover, that investigation described that IL-22 leads to vascular growth in animals[74]. Finally, a systematic review highlighted that, although there is a paradox in the behavior of the Th17 response in the different types of cancer, the expression of Th17 cells is often associated with better prognosis whereas the IL-17 is related to cancer progression[75].

Studies have described the IL-27 potential as a Th17 response inhibitor. This process occurs through the downregulation of RORγt[17]. The IL-27 is involved in the suppression of protumor cytokines such as IL-23 and IL-17[76]. A recent study described the existence of an inverse relationship between IL-27 and IL-17 as well as between IL-27 and IL-6. In patients with gastric cancer, studies have observed high concentrations of Th17 cells-related cytokines including IL-1β, IL-6, IL-17A, IL-23, and TGF-β and it is believed that this phenomenon occurs due to the low or null IL-27 Levels, since IL-27 inhibits RORγt and IL-6. This relationship has been observed in other cancers as well[77]. Additionally, another study observed that the IL-27 performed an antitumor activity through the IL-17 inhibition *via* RORγt among patients with small-cell lung cancer[78]. Of note, these findings suggest a therapeutic potential of the IL-27 through the inhibition of protumor Th17 cells-related mechanisms.

Interestingly, the cancerous cells inside individual tumors frequently exist in various phenotypic states. CSCs are a subpopulation of cells present in several types of cancer and that have self-renewal ability and tumorigenicity when transplanted to an animal host. Evidence on CSCs have aimed at developing a promising approach for the improvement of antitumor therapies. The CSCs are closely related to Th17 cells-related cytokines and other components in the tumor microenvironment, and they play crucial roles in the tumor progression and metastasis[79,80]. These cells can promote the differentiation of $CD4^+$ T cells into Th17 cells through the release of soluble mediators and cell-to-cell contact[18,19]. Moreover, a study observed that the interaction between stem cells and $CD4^+$ T cells can lead to the transformation of remaining Th17 cells through the activity of STAT3[81]. On the other hand, the IL-17 has shown to participate in the maintenance of CSCs through the action of the IL-17A receptor and the capacity of activating these cells in their quiescent state, configuring a more aggressive protumor behavior for the CSCs[20]. The recurrence of tumors after a primary treatment is still very frequent, and studies have supported that a successfull cancer therapy might be related to the elimination of CSCs[82]. In that context, the understanding of the relationship between these cells and the Th17 response in the various types of tumor can lead to new therapeutic possibilities[18].

ASSOCIATION BETWEEN TH17 RESPONSE AND DIFFERENT TYPES OF CANCER

Urogenital system cancers

Ovarian cancer: Ovarian cancer ranks fifth in cancer deaths among women[83]. This cancer usually has a late and advanced diagnosis causing resistance to treatment[84]. Studies have emphasized the involvement of the immune system in the process of development and progression of ovarian cancer, including the interactions of immune cells in the ovarian tumor microenvironment[85,86]. The expression of IL-23 is increased and positively correlated with IL-17 in immunohistochemical analyses of ovarian cancers[87]. Studies showed that IL-17 and IL-6 induce STAT3 phosphorylation, leading to the production of programmed death-ligand 1 (PD-L1), which is related to the inhibition of antitumor T cells[88-90]. Additionally, T lymphocytes and antigen presenting cells can stimulate the secretion of proinflammatory interleukins such as IL-1, IL-6, and IL-23, providing favorable means for the differentiation and expansion of Th17 cells[91]. In addition, ovarian tumor cell lines secrete IL-6, IL-8, IP-10, MCP-1, and VEGF in large amounts[91]. The IL-17 stimulates tumor progression due to proangiogenic effects and increased percentage of $CD4^+$/IL-17 T lymphocytes

among cells that infiltrate ovarian cancer[92,84]. The chemokine CXCL12 (SDF-1α) participates in the induction of angiogenesis verified in several types of tumors along with VEGF[93]. Curiel *et al*[94] demonstrated that ovarian cancer patients had increased levels of proangiogenic cytokines (IL-8 and TNF-α) that stimulate the development of new tumor blood vessels[94]. This abnormal vasculature allows the tumor to spread more effectively, facilitates the escape of immune surveillance, and impairs the action of antineoplastic agents, limiting the beneficial effects of these drugs [84,85]. Notably, IL-17A and gdT cells recruit small peripheral peritoneal macrophages that selectively secrete IL-17A receptors that trigger tumor growth and angiogenesis [95].

Another study reported that there is an inverse relationship between Treg and Th17 cells observed in ovarian cancer and this difference leads to an antitumor response, which significantly influences survival, and the presence of these polyfunctional Th17 cells is statistically related to more favorable clinical outcomes[96]. In contrast, the significant presence of regulatory T cells is associated with worse survival[97]. Studies suggest that the responses of T cells directed to ovarian cancer may be related to the expansion of Th17 cells and may neutralize the suppression of these cells through the activity of Treg cells[97,98]. Moreover, the decrease in Th17 Lymphocytes and the increase in Treg cells was related to an increased level of TGF-β and this, in turn, is associated with metastatic processes resulting in poorer clinical prognosis[99,100]. Additionally, in vivo studies observed that a high Treg cells/Th17 cells ratio seems to predispose tumor progression because this ratio has shown to be significantly higher in epithelial ovarian cancer and peritoneal metastasis than in benign ovarian tumors and benign peritoneum[101]. It is believed that the presence of tumor-associated macrophages may induce this imbalance[102]. M2 macrophages have been shown to modulate the tumor microenvironment and to promote a relative deviation to the Treg immune profile through the release of exosomes carrying miRNAs that are often overexpressed in some types of cancer, such as ovarian cancer[103,104].

Th17 cells in ovary cancer secrete IL-21, which, along with TGF-β, can interfere in the differentiation of T cells into Th17 Lymphocytes and stimulate the transformation of Th17 cells into Treg cells. In addition, there is a regulation in the distribution of Th17 and Treg cells *via* CCR6 chemokine receptors to direct Th17 cells to specific sites [105,106]. In this sense, some therapeutic methods have been developed aiming at increasing the patients' survival as well as at reducing the recurrence rates. Among these methods, stand out immunotherapy approaches based on therapeutic vaccines that stimulate the expansion of specific T cells in patients with ovarian cancer, using the alpha folate receptor (Fra) as the vaccine target antigen, since this receptor is overexpressed in patients with high-grade serous ovarian cancer[97,107]. The vaccine induces stimulation of Fra-specific INF-γ^+ and IL-17^+ T cells[97]. Furthermore, a study showed that the Th17 profile and IFN-γ induce the production of CXCL9 and CXCL10 that promote the migration of effector cells to ovarian tumors[98,108]. The use of vaccines that stimulate the expression of Th17 cells specifically in an ovarian tumor is associated with a reduction in recurrence rates, as well as in the improvement of survival rates[97,98].

Prostate cancer: Prostate cancer is the most prevalent type of cancer among the male population[109]. Chronic inflammation has been identified as a factor that is associated with the pathogenesis of various types of cancer, including prostate cancer[109,110]. In that context, IL-17 plays an important role in the inflammatory process related to the development and progression of that cancer[111]. Studies have shown that blocking IL-17 in mice inhibits the development of prostate cancer[112]. The imbalance in the proportion of $CD4^+$ and IL-17^+ cells and $CD4^+$ $Foxp3^+$ T cells, responsible for regulating Treg cells in the tumor microenvironment, may lead to worsening of the inflammatory process and promote carcinogenesis[113,114]. Treg cells are involved in the suppression of antitumor immune responses[115]. Blocking PD-1 can promote antitumor activity by balancing Th1/Th2 responses and stimulating Th17 cells, as well as inhibiting Treg cells and stimulating the Th17 response[116]. In that context, the use of vaccines that inhibit the action of programmed death 1 (PD-1)/PD-L1, which make up an immunological checkpoint, has demonstrated promising results[117,118]. In prostatic tumors, IL-17 has been shown to attract M1 and M2 macrophages. M1 macrophages inhibit tumor growth, whereas the M2 cells promote tumor growth[119-121].

Respiratory tract cancers

NSCLC and small cell lung cancer: Chronic inflammation is a crucial factor in the pathogenesis of different types of cancer, including lung cancer, which has proved to

be a major public health problem affecting 1.8 million patients each year[122]. Lung cancer is the leading cause of cancer-related deaths in recent decades and can be divided into two types: Small cell lung cancer and NSCLC[123]. In addition to Th17 cells and the association with tumor survival, chemokines and their receptors related to T cell migration were examined in NSCLC cases. The high expression of CCR6 was associated with shorter disease-free survival[124]. Likewise, CCL20, a chemokine known to interact with CCR6, was elevated in the tumor compared to tumor-free lung tissue. Thus, these results suggest that CCL20/CCR6 may facilitate the infiltration of Th17 cells in the NSCLC and promote tumor progression[125]. A regulatory role for Th17 cells in the tumor microenvironment of NSCLC was found to modulate the differentiation and activation of various subsets of local T cells[126]. IL-17A is associated with the concentration of VEGF in patients with NSCLC, suggesting that IL-17A may promote angiogenesis in that tumor[127]. In addition, patients with high levels of IL-17A demonstrated shorter survival compared to those with low expression of the cytokine[128]. Studies suggest that IL-17A/Th17 cells may play a pro-tumorigenic role, as an increased number of Th17 cells are found in lung cancer[129]. Th17 cells can be generated under oncogene activation or inhibition of tumor suppressors in human and murine models[130,131]. Oncogenic NSCLC models have shown a predominant pro-tumorigenic role of IL-17A[130,61], while downregulation of IL-17A in a tumor suppressor NSCLC model has been associated with antitumor activities[131].

Studies also demonstrated that IL-17A deficiency or blockade leads to the suppression of lung metastasis in experimental tumor models. This suggests that the key cytokine IL-17 produced by lung $CD4^+$ Th17 cells plays an important role in cancer regulation[132]. In addition, anti-IL-17A treatment in pulmonary adenocarcinoma modifies cytokine responses by lung $CD4^+$ T cells and induces production of TNF and IFN-γ by Th1 cells at the tumor site, leading to improved antitumor immune responses and suppression of tumor growth[133]. Other studies assessed the role of IL-6, a cytokine produced by Th17 cells, in a murine model of lung adenocarcinoma and human tumors, showing that IL-6 inhibits regulatory T cells and induces Th17 cells. In vivo treatment with anti-IL-17A antibodies reduced the production of IL-6 in the airways[132]. Thus, anti-IL-17A-mediated regulatory T responses can induce increased anti-tumor immune responses[134]. The main transcription factors of Foxp3 regulatory T cells, as well as the main transcription factors of Th17 in human cells, were increased in lung tumor tissues, resulting in a parallel local expansion of Th17 and Treg responses. These findings suggest a potential direct relationship between both T cell lines in lung cancer[135]. T-cell immunoglobulin-3 (TIM-3) expressed in Th1, Th17, and CD8 T cells, but not in Th2 cells, has already been described as a critical component of cell-mediated immunity against cancer[136]. Recent studies have supported an important role in the exhaustion of TIM-3 T cells in lung cancer[137]. TIM-3 as well as PD-1, another T cell exhaustion marker, are co-expressed in TCD8 in mice with lung tumors, exhibiting depleted phenotype as defined by the failure to proliferate and produce IL-2, TNF, and IFN-γ[137]. Blocking the TIM-3 and PD-1 pathways is more effective in controlling tumor growth than targeting either pathway alone, suggesting that these two pathways work synergistically in establishing T-cell exhaustion[138].

Gastrointestinal tract cancers

Gastric cancer: Gastric cancer is the fifth most common and the third most lethal malignancy around the world. The development of this cancer is mainly linked to factors such as the chronic inflammation induced by *Helicobacter pylori* infection and age[139]. It is well established that the immune response of the host infected by *Helicobacter pylori* leads to the activation of the IL-23 pathway, which induces the differentiation of $CD4^+$ naive T cells into Th17 cells. This immunological pathway is called the IL-23/IL-17 axis[140]. The IL-17 acts in the endothelium, monocytes, and gastric epithelial cells producing TNF-α, IL-1, IL-6, and IL-8 that stimulate the recruitment of neutrophils to the inflammatory site[140,141]. In an interesting study, a significant increase in the levels of IL-17 was observed in the serum of patients with gastric cancer. In addition, this increase in IL-17 expression was associated with a high density of microvessels, which assist in the development of the tumor[142]. An important study also found high levels of plasma IL-17 in a patient with gastric cancer and, interestingly, the results showed an increase in the expression of IL-17 and RORγt in the cancer tissue. In addition, a 26-fold increase in IL-17 expression was observed among patients who had metastasized[143].

Moreover, a study found high levels of IL-6, TGF-β1, FoxP3, and IL-17 expression in gastric cancer patients, highlighting the importance of the Th17/Treg axis in this neoplasm. IL17 and IL-6 have been associated with tumor progression, and Foxp3 and TGF-β1 were mainly expressed in patients with advanced gastric cancer. These findings suggest that these molecules play a role in the tumor immune response evasion and cancer progression[144].

The induction of Th17 cell expression is very important in the pathophysiology of gastric cancer. Moreover, it is well described that IL-1, IL-6, IL-21, IL-23, and TGF-β induce the differentiation of T naïve cells into Th17 cells[145]. In this sense, Su *et al* [143] also observed increased levels of TGF-β and IL-21 in gastric cancer tissues[143], positively regulating Th17 cells and, consequently, IL-17 levels. Some studies suggest that treatment with anti-IL-17A monoclonal antibodies such as Secukinumab and Ixekizumab may be beneficial in gastric cancer therapy[146]. An experimental study with rats, which had tumor growth stimulated by injection of gastric cancer cells of the YTN16 type, showed an expressive regression of the tumor with a complete elimination of the cancer in 8 of 10 mice using a combination of anti-IL-17A and anti-PD-1 monoclonal antibodies[147]. Despite being experimental results, they point to new paths regarding the treatment of gastric cancer with immunotherapy.

Pancreatic cancer: Pancreatic cancer is the 14th most common cancer and the 7th that kills the most in the world[148]. Obesity, type 2 diabetes, and smoking are the main risk factors for the development of this cancer[149]. Th17 cells are important in tumor-associated inflammation, stimulating migration, invasion, and induction of angiogenic factors[150]. A recent study observed that IL-17 stimulates an important mediator of pancreatitis (REG3-β) in pancreatic cells and can activate the gp130-JAK2-STAT3-dependent signaling pathway, which results in a greater acinar-ductal metaplasia and in the development of lesions of early pancreatic intraepithelial neoplasia[151]. An interesting study pointed to a similarity between the IL-17A-IL-17RA pathway and the IL-17B-IL-17RB pathway in tumor malignancy. It was observed that IL17-B increased the expression of IL-8, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and activating protein-1 (AP-1), which aid the tumor invasion in the pancreatic tissue and recruit neutrophils, lymphocytes, and endothelial cells[152]. Although advances have been achieved, the roles played by Th17 cells in the development of pancreatic cancer are not well understood. Previous investigations in mice suggest how this pathway works. However, the mechanism in humans needs further studies. Some studies have observed increased levels of Th17 cells in patients with pancreatic ductal adenocarcinoma, supporting theories about the role of this profile in the immune response involved in this cancer. He *et al*[153] reported that the frequency of these cells was higher in pancreatic tumor tissues when compared to other tissues of the organ (P = 0.031). Serum levels of IL-17 and IL-23 were significantly increased in pancreatic cancer patients when compared to healthy patients (P = 0.02)[153]. IL-17 blockade demonstrated an inhibition in neutrophil recruitment and increased activation of CD8$^+$ T cells in the environment close to the tumor. In addition, a synergy was observed between the inhibition of IL-17 and PD-1 in the therapy against that cancer[154]. Interestingly, a study that aimed at understanding the Th17/Treg axis and its implications in pancreatic cancer found that the balance between these immune response profiles was altered in the peripheral blood of patients with this malignancy, with an important deviation to the Treg immune profile. Furthermore, it was observed that this relationship becomes even more accentuated as the disease progresses, supporting the hypothesis that Treg cells might impair the antitumor immune response and contribute to the tumorigenesis in pancreatic cancer[155]. Despite these results, studies for the clinical use of these agents are still scarce.

Colorectal cancer: Colorectal cancer is the third most common cancer and the fourth with the highest mortality in the world[156]. The main risk factors for the development of this cancer are age, genetics, obesity, type 2 diabetes, and inflammatory bowel disease[157]. Possibly, IL-17A acts to increase IL-6 and VEGF, which are important in carcinogenesis and pro-angiogenic, respectively[158]. Other studies indicate that Th17 cells stimulate immunosuppressive factors such as TGF-β, CXCR3, chemokine receptor CC 6 (CCR6), and IL-6. In addition, they decrease the anti-tumor activities of CD8$^+$ T cells[159]. An interesting Chinese study observed the positive regulation of IL-17 in the progression of the adenoma-carcinoma sequence, with the levels of this cytokine being higher in cancer patients[160]. Although the pathogenesis of colorectal cancer has different pathways, in fact, IL-17 has an important role in the immune response and in the development of this cancer. The possible treatments that have been studied in this

setting involve blocking IL-23, IL17, IL-17R, and RORγt nuclear receptor antagonists, which can inhibit the differentiation of Th17 cells[159]. However, clinical trials with these agents have not been developed and further studies are needed to understand the effectiveness of these drugs in patients at different stages of this disease.

Liver cancer: Liver cancer is the fifth and ninth most common cancer in men and women, respectively, and has a high mortality rate around the world[161]. Infection with hepatitis B and C viruses, alcoholic liver disease, and, possibly, non-alcoholic fatty liver disease are the main risk factors for the development of hepatocellular carcinoma[162]. A recent Chinese study found that IL-17 has a direct effect over hepatocellular carcinoma with the induction of IL-6/JAK2/STAT3 by activating the AKT pathway. This pathway positively regulated IL-8, matrix metalloproteinases 2 (MMP2), and VEGF, and neutrophil recruitment, neoangiogenesis, and tumor growth were observed *in vivo*[163]. Another study also indicated the influence of the CCL20/CCR6/Th17 cells pathway in promoting vascular invasion and metastasis [164]. A study identified that the high intramural expression of IL-17 and IL-17E were predictors of a worse survival prognosis (P = 0.016; P < 0.001)[165]. It has been well described that IL-17 is important in the development and prognosis of hepatocellular carcinoma. However, there are not many studies on immunological therapy, and the concomitant use of IL-17 inhibitors with conventional treatments, may be a promising alternative to be explored by new studies.

Skin cancers

Non-melanoma skin cancer: According to the WHO, skin cancer has increased over the past 20 years. The global estimate is that there will be 2 to 3 million cases of non-melanoma cancer and 132000 melanoma cancers annually[166]. Previous animal model studies using the chemical inductors dimethylbenzanthracene (DMBA) and 12-O-tetradecanoylforbol-13-acetate (TPA) that promote the development of inflammation-associated skin cancer, have demonstrated that IL-17R-deficient mice are resistant to DMBA/TPA and that the depletion of this cytokine increases the immune control performed by $CD8^+$ T cells and inhibits the promotion of inflammation in the skin tumor. It has also been found that TPA-induced inflammation increases the susceptibility of tumor growth and the development of tumor-specific IL-17-producing T cells and that IL-17 blockade can inhibit the progression of existing skin tumors stimulated by chemical reagents and cancel the induced inflammation that contributes to tumor growth[167]. In another study using the same experimental model, in addition to looking at the importance of IL-17-produced by $CD4^+$ T cells in skin tumorigenesis, the researchers revealed an important regulatory role in the IL-17-STAT3 pathway in tumor development, verifying that IL-17 induces the oncogenic activity of STAT3 and promotes the proliferation of epidermal cells and hyperplasia. On the other hand the decrease in IL-17 reduced the activation of STAT3 and the unleashing of its protumor mechanisms[168]. In a research with samples of patients diagnosed with basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin, the role of the cytokines IL-17 and IL-22 in the progression of both types of cancer was evaluated. The results showed that the tumor microenvironment in both carcinomas is enriched with $IL\text{-}22^+$ and $IL\text{-}17^+$ T cells. The IL-17, alone or along with TNF-α, was able to induce the production of IL-6 and IL-8, important for tumor progression in the analysis of SCC CAL27 cells. Another important finding is that IL-17 positively regulated NF-κB signaling, while IL-22 activated the STAT3 pathway and the anti-apoptotic AKT protein in both cell lines. Corroborating the *in vitro* findings, experiments with mice that received CAL27 also demonstrated that IL-17 and IL-22 increased the size of the tumor[169]. Another study with human BCC biopsies characterized by a moderate-to-severe inflammatory infiltrate evaluated the expression of the cytokines IFN-γ, IL-23, IL-17, and IL-22 and their expression during treatment with the drug imiquimod (IMQ) and with photodynamic therapy (PDT). The results showed high expression of all cytokines in cancer, and they were related to the severity of the inflammatory infiltrate. It was also possible to observe a correlation between IFN-γ and IL-17 expression, and both cytokines were expressed by $CD4^+$ and $CD8^+$ T cells. In addition, there was an increase in all cytokines in response to IMQ/PDT treatment[170].

Melanoma skin cancer: Melanoma is the 19th most frequent neoplasm in the world with an incidence rate of 3.3 *per* 100 thousand people[171]. Malignant melanoma is the type of skin cancer with the worst prognosis, having a high probability of spreading metastases when in advanced stage[172]. IL-17 promotes tumor growth by facilitating angiogenesis and the exit of tumor cells from their primary focus[172,173]. The expression of IL-17A represents an important target in the study of the escape of

tumor cells from the immune system[171]. A research conducted in 2010 points to IL-17 as a useful biomarker for early diagnosis of melanoma in mucosae[174]. It is believed that the unregulated tissue inflammatory process may contribute to tumor expansion and metastasization[175-177]. A study analyzed the effect of IL-17 on the growth of melanoma, and wild type mice and IL-17-/-were inoculated with a B16 melanoma cell lineage. The results showed that melanoma growth was significantly inhibited in IL-17-/- mice compared with wild type mice. In this same study, the Hmgb1 (High-mobility group box 1) molecule and its receptor RAGE, which is associated with inflammation and cellular injury, were also analyzed. The growth of the B16 cell lineage was inhibited and the expression of IL-23 and IL-17 was significantly reduced in RAGE-/-mice, indicating that the Hmgb1-RAGE route contributes to the IL-17 expression dependent on the production of IL-23, and promotes tumor growth[176]. A previous study demonstrates that the production of IL-17 by B16 melanoma strains induces the production of IL-6 that activates the nuclear transcription factor STAT3, which acts activating the transcription of several genes related to cell proliferation and to an increase in the expression of VEGF, MMP9, and prostaglandins E1 and E2. Moreover, STAT3 increases the expression of anti-apoptotic genes[178]. Thus, it can be concluded that IL-17 promotes the growth of melanoma B16, while the blocking of IL-17 inhibits the growth of the tumor. On the other hand, Th17 cells might play a protumor role when they are converted into a hybrid phenotype expressing markers that characterize both this cell subtype and Treg cells. This conversion also occurs *via* the secretion of TGF-β and retinoic acid in the presence of suppressor cells derived from tumor-infiltrating myeloid cells. These findings reinforce that Th17 cells might play conflicting effects on melanoma, also contributing for its pathogenesis[179]. These studies support the involvement of Th17 cells and interleukins produced by this population of T lymphocytes, highlighting the cytokine IL-17, in the development and progression of skin cancer. In addition, there is also the possibility of new therapeutic approaches targeting this immune response profile. Finally, further studies are needed to better understand the relationship between Th17 responses and skin cancers.

Other types of cancer

Cervical cancer: Cervical cancer is the second leading cause of cancer death in young women worldwide. Virtually all cervical cancers begin with infection with high-risk human papillomavirus (HPV)[180]. Most HPV infections are eliminated naturally as a result of humoral and cellular immune responses[181]. However, the persistence of this infection induces an inflammatory response, which seems to contribute to tumor growth and disease progression, instead of inducing an effective immune response [182]. This response is partially induced by tumor cells that regulate the immune response negatively through the expression of human leukocyte antigen, which produces immunosuppressive cytokines, such as IL-10 and TGF-β, and attracts regulatory T cells[183].

A large number of Th17 cells was a factor that improved prognosis and survival in this type of cancer, suggesting that Th17 cells play important antitumor responses in cervical cancer settings[183]. Evidence emphasizes that the infiltrate of Th17 cells in the cervical tumor had an activated phenotype with increased expression of CCR6, the receptor for the CCL20 chemokine. It corroborates the hypothesis that the Th17/Treg axis imbalance may be involved in the promotion and progression of cervical cancer [184]. A recent study suggested that the imbalance between Th1/Th2 and Th17/Treg cells was related to the stage of cervical cancer, tumor size, metastasis, and vasoinvasion. The findings demonstrated that the peripheral immune cell levels reflect the patient's condition[185]. Although some questions can already be answered, further studies on the involvement of the Th17 response to cervical cancer are still needed.

Lymphoma: Every year, approximately 500000 people are diagnosed with non-Hodgkin's lymphoma and 80000 with Hodgkin's lymphoma, the most common cancers among the 90 lymphoma subtypes[186]. In a study conducted in China, a significantly decreased frequency of Th17 cells was observed in the peripheral blood of patients with non-Hodgkin's B-cell lymphoma compared to healthy individuals, along with an increase in Th1 cells[187]. This activity may be associated with the patient's response to treatment and the different stages of the disease[188]. Another study revealed that the number of Th17 cells in lymphomas is influenced by the amount of mast cells and granulocytes. The IL-6 expression by these cells contributes to the establishment of a pro-inflammatory environment for Th17 cells, favoring CXCL-13 production and its interaction with CXCR3 and CXCR5 receptors expressed in mast

cells[189]. It has been shown that drugs that block the IL-23/IL-17 axis, which are already available for the treatment of certain autoimmune diseases, can increase the therapeutic impact against classic Hodgkin's lymphoma[190,191]. Moreover, a recent study demonstrated that the prognostic implication of Th17 cells depends on the type of treatment employed, since the Th17 signature did not represent a negative prognosis in the treatment with the medication Lenalidomide in non-follicular lymphoma[192,193].

Breast cancer: Breast cancer is one of the main causes of death among women[194]. This type of cancer is a heterogeneous disease with different patterns of tumor infiltrating lymphocytes, depending on the molecular subtype and other factors of the tumor microenvironment that are important for prognosis and predictive for treatment [195]. Studies point to a relevant infiltrate, characterized mainly by $Foxp3^+$ cells and high levels of IL-6, in addition to revealing a high infiltration of IL-17-producing cells and a low amount of $CD8^+$ cells, suggesting that Th17 cells participate in an effective immune response to eliminate the tumor in patients with breast cancer[196,197]. IL-6 is expressed in breast cancer patients, and its levels are positively associated with the number of Th17 cells. In the breast cancer microenvironment, IL-6 enhances the differentiation and expansion of Th17 cells[198]. Another study showed that Th17 cells positively regulate the production of CXCL1 during the progression of breast cancer. CXCL1, which is produced by breast cancer cells, might promote the growth and development of cancer[199]. In a recent study carried out in China, the involvement of high salt intake was evidenced as a factor that accelerated the growth of breast cancer in addition to increasing Th17 cells circulation in mice. It was also demonstrated in an *in vitro* study that the elevation of Th17 cells was reversed with the application of 1.25 Vitamin D3, inhibiting the differentiation of these cells ($P < 0.001$)[200]. Although some studies point to the important protective role of Th17 cells, further investigation is needed in that context[201].

Bone-related cancers: Studies evaluating the relationship between Th17/Treg axis and bone marrow cancer concluded that deregulations in this axis leading to immune tolerance or impaired immune response might contribute to bone tumorigenesis. In that context, a study evaluating peripheral blood mononuclear cells and bone-marrow mononuclear cells from patients with multiple myeloma and healthy controls observed an enhanced expression of Th17 cell-related cytokines in the affected individuals. Moreover, that study demonstrated that the IL-17 has the potential to promote the growth of myeloma cells and colony formation through the activation of IL-17 receptors as well as to inhibit the Th1 immune system profile along with the IL-22[202]. Otherwise, studies have observed an increased number of Treg cells in patients with acute myelogenous leukemia (AML) compared to controls, which suggests that the Treg immune profile might contribute for an improper immune response against the malignancy and to a consequent progression of the AML[203, 204]. Furthermore, a study investigating the role of IL-22 produced by Th17 and Th22 cells in osteosarcoma demonstrated that its levels were enhanced in osteosarcoma cells and that it stimulates the proliferation and invasion of tumor cells *via* STAT3 signaling [205]. Finally, the aforementioned studies suggest that the understanding of the role of the Th17/Treg axis is essential for the study of bone-related cancers and should be explored as a potential therapeutic target in the treatment of those malignancies. Interestingly, vitamin D3 can act therapeutically over Th17 cells, reducing IL-17A and IFN-γ levels in rheumatoid arthritis. Therefore, vitamin D3 might could be used for the development of a therapeutic approach against bone-related cancers through the modulation of the Th17 immune profile (Figure 2)[206].

CONCLUSION

The Th17 response is intimately linked to the development of cancers and, in the last few years, the knowledge on the role of Th17 cells in the tumor microenvironment have significantly increased. However, much still has to be done in order to achieve a broader understanding on this issue. The IL-17 stands out among the Th17 cells-related inflammatory cytokines, being involved mainly in processes that promote tumorigenesis. In addition, the plasticity of Th17 cells, which allows a broader dynamics of the Th17/Treg axis in different tumor activities, and the Th17/Th1 axis, which is associated with antitumor mechanisms, are important issues to be taken into account in the immune-oncology field. The modulation of these immune system

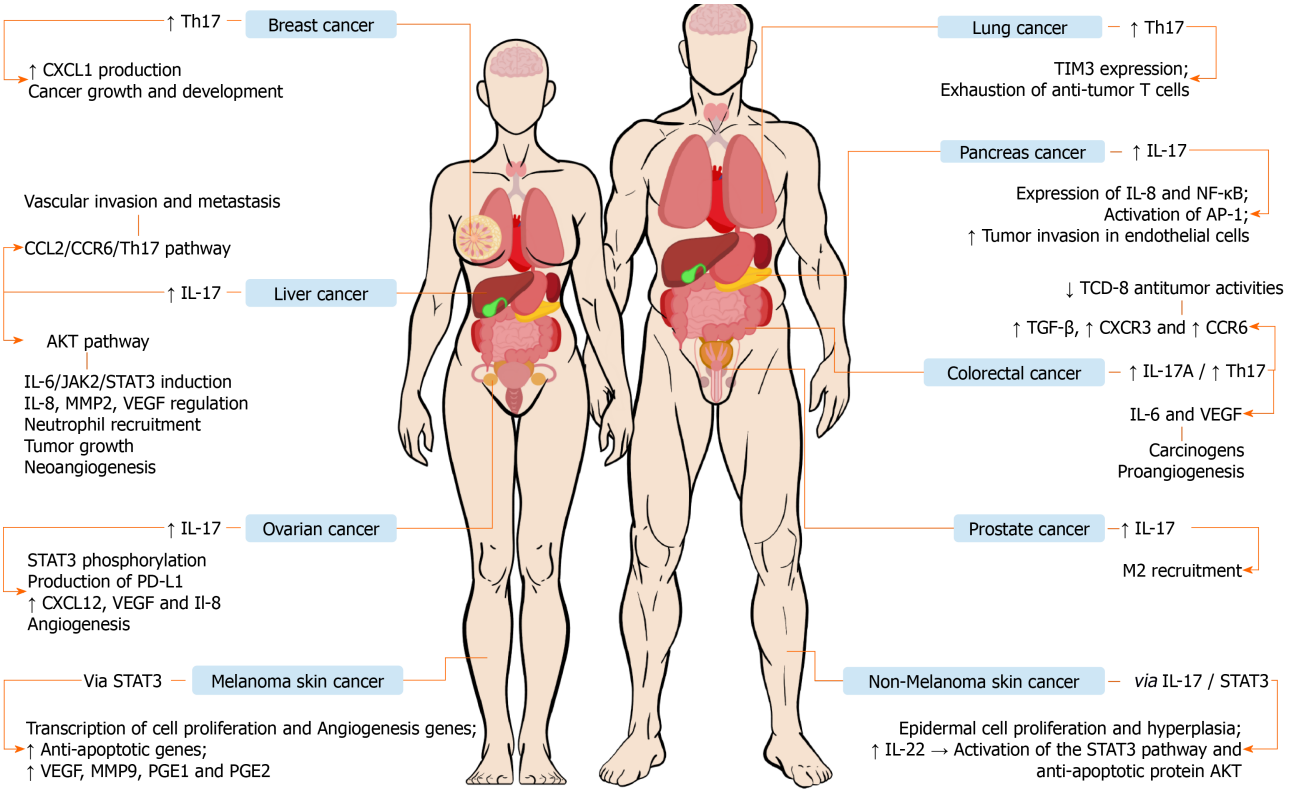

Figure 2 Main protumor mechanisms related to the Th17 immune response profile. STAT3: Signal transducer and activator of transcription 3; MMP2: Matrix metalloproteinases 2; VEGF: Vascular endothelial growth factor; PD-L1: Programmed death-ligand 1; PGE1: Prostaglandins E1; PGE_2: Prostaglandins E2; TIM-3: T-cell immunoglobulin-3; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; AP-1: Activating protein-1.

interplays might be a potential alternative for the development of new therapeutic interventions for various malignancies. Moreover, the role of the IL-27 should be further studied in various types of cancer since an important antitumor effect has been associated with this interleukin in some malignancies. Because IL-17 has shown to be so important in the pathogenesis of several malignant tumors, anti-IL-17 monoclonal antibodies are promising drugs that should be evaluated in numerous neoplasms. Finally, it has to be emphasized that there are several similarities and differences between Th17 responses in various cancers, being it highly dependent on the tumor context. The comprehension of the Th17 immune response in cancer is important not only to predict prognosis, but also to identify new therapeutic possibilities.

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MINIREVIEWS

Human epidermal growth factor receptor 2 targeted therapy in endometrial cancer: Clinical and pathological perspectives

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Abstract

Endometrial cancer is the most common gynecological cancer in developed countries, and its incidence has increased. The majority of patients with endometrial cancer have an early disease and favorable prognosis; however, a significant proportion of endometrial cancer, which mainly comprises high-grade or type II endometrial cancer such as serous, clear cell, and carcinosarcoma, shows advanced/recurrent disease and dismal prognosis. Novel therapeutic development is required for patients with aggressive endometrial cancers. Recent genomic and immunohistochemical analyses revealed human epidermal growth factor receptor 2 (HER2) overexpression/gene amplification in 20%-40% of patients with type II endometrial cancer. Historically, HER2 targeted therapy has been developed for various major cancers, including breast and gastric cancer. Notably, recent advances in HER2 targeted therapy for patients with type II endometrial cancer are also expected to change. Simultaneously, an optimized HER2 test for endometrial cancer as companion diagnostics should be established. In this review, we summarize the recent findings on endometrial cancer, current treatment, optimized HER2 testing, key clinical trials on HER2 targeted therapy, and future directions in aggressive endometrial cancer, including serous carcinoma and carcinosarcoma.

Key Words: Endometrial cancer; Serous carcinoma; Carcinosarcoma; Human epidermal growth factor receptor 2; Chemotherapy; Antibody-drug conjugates

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Core Tip: Endometrial cancer is the most common gynecological cancer in developed countries, and its incidence has increased. A significant proportion of endometrial cancer, which mainly comprises high-grade or type II endometrial cancer, including serous carcinoma and carcinosarcoma, shows a dismal prognosis. Recent molecular analyses revealed human epidermal growth factor receptor 2 (HER2) overexpression/gene amplification in 20%-40% of patients with type II endometrial cancer. Notably, HER2 targeted therapy for type II endometrial cancer has been dramatically developed. We review the recent findings on endometrial cancer, current treatment, optimized HER2 testing, key clinical trials on HER2 targeted therapy, and future directions in these aggressive endometrial cancers.

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INTRODUCTION

Endometrial cancer is the most common gynecologic malignancy, with 65000 cases diagnosed and 12000 deaths in the United States in 2020[1]. In Japan, approximately 16000 cases are diagnosed, 2500 deaths occur annually, and the number of patients is increasing[2,3]. The prognosis is relatively good, with a 5-year survival rate of 80%, and many cases are diagnosed at the localized stage[4]. However, a significant proportion of patients with endometrial cancer still have an advanced or recurrent disease and have a dismal prognosis.

Endometrial cancers can be classified into two types. Type I shows endometrioid morphology, which accounts for 80% of endometrial cancers, and type II shows non-endometrioid morphology, such as serous carcinoma, clear cell carcinoma, and carcinosarcoma, which account for the remaining 20%. Each type of carcinoma is known to have a different background and clinical course. Type I is reportedly caused by unopposed estrogen, and obesity is a trigger for carcinogenesis; it is diagnosed at a young age, has low grade and early stage, and is usually curable by surgery. In contrast to type I, type II is more common in elderly patients, occurs on a background atrophic endometrium, is diagnosed at an advanced stage, and is resistant to chemotherapy, resulting in a poor prognosis[5].

An analysis of The Cancer Genome Atlas revealed different molecular genetic pathways that are present in these two types. The results show that endometrial cancers can be divided into four clusters: *POLE* ultramutated (cluster 1), microsatellite instability hypermutated (cluster 2), copy-number low, endometrioid (cluster 3), and copy-number high; serous-like (cluster 4). Uterine serous carcinoma (USC), classified as Type II, accounts for the majority of cluster 4 cases[6]. Differences in common genomic alterations by cluster were characterized by *PTEN, PIK3R1, FBXW7*, and *KRAS* alterations in cluster 1, *KRAS* alterations in cluster 2, *CTNNB1* alterations in cluster 3, *TP53, FBXW7, PPP2R1A* alterations, *ERBB2*, and *CCND1* amplification in cluster 4[6].

Surgical treatment is appropriate for early-stage endometrial cancer, and chemotherapy and radiation therapy should be considered according to the risk of recurrence after resection. Women with low-risk endometrial cancer undergoing surgical treatment alone and no adjuvant treatment are indicated. For women with high-risk endometrial cancers undergoing surgery, adjuvant chemotherapy with or without radiation should be offered[7,8]. Although there is no consensus on adjuvant treatment among intermediate-risk cancers, some clinicians may offer adjuvant chemotherapy and/or radiation[5]. For advanced or recurrent uterine cancer, carboplatin, paclitaxel, adriamycin, and cisplatin are often chosen as chemotherapy regimens based on the GOG 122L[9], GOG 209L[10], and JGOG 2043 trials[11]. Furthermore, low-grade endometrial cancer with positive estrogen receptor and progesterone receptor can be expected to benefit from hormone therapy[12].

Molecular targeted agents for patients with endometrial cancer have been developed, similar to other solid tumors. In recent years, the efficacy of pembrolizumab has been demonstrated in solid tumors of microsatellite instability-high (MSI-H)/mismatch repair deficient (dMMR)[13,14]. MSI-H/dMMR is found in 20%-30% of endometrial cancers[6,15]. The combination of pembrolizumab and lenvatinib has been shown to be effective in patients with microsatellite stable/mismatch repair proficient, regardless of histologic subtype, with 40% of participants having type II, serous/clear cell carcinoma, and type I[16]. Notably, human epidermal growth factor receptor 2 (HER2) targeted therapy for patients with endometrial cancer has demonstrated practice-changing efficacy in the recent decade. In this review, we aim to overview recent advances in HER2 targeted therapeutics and HER2 testing for endometrial cancer.

HER2 AMPLIFICATION AND OVEREXPRESSION IN ENDOMETRIAL CANCER

HER2 is a cell surface receptor named after human epidermal growth factor receptor (EGFR)-related 2 in the 1980s. It is encoded by the *ERBB2* gene on chromosome 17. It is known to belong to the EGFR family that includes other receptors: EGFR, HER3, and HER4. When *HER2* is amplified, HER2 is overexpressed and forms active dimers that induce the downstream RAS/RAF/MAPK and PI3K/AKT pathways without ligand stimulation. This, in turn, activates the downstream pathway, contributing to oncogenesis[17,18].

HER2 amplification/overexpression is known to occur in a variety of solid tumors, including about 20% in breast cancer[17], 10%-20% in gastric cancer, 2%-10% in colorectal cancer, 5%-20% in biliary tract cancer, 10% in bladder cancer, and 2%-5% in lung cancer[19,20]. In all endometrial cancers, HER2 overexpression and amplification have been reported 18%-80% and 4%-69%[19]. HER2 positivity differs according to histopathological subtype. Although HER2 overexpression and amplification are rarely seen in low-grade endometrioid adenocarcinoma, USC has the highest prevalence of HER2 positivity. The rates of HER2 overexpression and amplification by histologic subtype are shown in Table 1.

HER2-targeted drugs are being used and developed for various malignancies. Currently, trastuzumab, pertuzumab, lapatinib, neratinib, tucatinib[21], trastuzumab emtansine, and trastuzumab deruxtecan[22] have shown clinical activity in HER2-positive breast cancer[19]. For HER2-positive gastric cancer, trastuzumab[23] and trastuzumab deruxtecan[24] have also shown clinical activity. In other carcinomas, HER2-targeted drug therapies are being developed for tumors with HER2 overexpression, amplification, or mutations[25-28]. In endometrial cancer, treatment options are very limited, especially for type II uterine carcinoma, which is resistant to cytotoxic chemotherapy. Therefore, new therapeutic targets and effective new drugs are required.

HER2 overexpression/amplification is currently assessed using immunostaining and fluorescent *in situ* hybridization (FISH)/dual-color *in situ* hybridization. However, there is variability in positive/negative results depending on the type of cancer. In this article, we summarize the methods used to assess HER2 in type II endometrial cancer and the related development of HER2-targeted drugs.

HER2 TESTING IN ENDOMETRIAL CANCER; PATHOLOGICAL PERSPECTIVE

A significant proportion of endometrial cancers show HER2 overexpression or gene amplification[19,29-31]. The percentage of HER2 positive cases varies by histological type, with serous carcinomas having the highest prevalence of HER2 positivity[29-31]. Standardization of HER2 testing accompanied with evidence-based treatment has not been performed for all endometrial cancers; thus, HER2 testing methods are currently optimized for each histological type, specifically in serous carcinoma and carcinosarcoma.

HER2 testing as companion diagnostics has been best established for breast and gastric cancers, and ASCO/CAP guidelines have been provided[32,33]. The HER2 assessment algorithm is based on the detection of protein overexpression by immunohistochemistry (IHC) and gene amplification by ISH to determine HER2-positive/-

Table 1 Human epidermal growth factor receptor 2 overexpression and gene amplification in endometrial cancer

Histological type	HER2 overexpression	*HER2* gene amplification	Ref.
Overall	4%-69%	18%-80%	[19,45,55]
Endometrioid (low grade)	rare	rare	[29,45]
Endometrioid (high grade)	8%-31%	15%-29%	[29,45]
Serous carcinoma	20%-60%	13%-29%	[29,42,43,45]
Clear cell carcinoma	33%-70%	22%-50%	[29,55]
Carcinosarcoma	0-25%	13%-20%	[38,48-51]

negative cases[32,33]. Although HER2 overexpression has been reported in other types of carcinomas, in most cases, these two HER2 testing methods are used[19,34] or modified (*e.g.*, in colorectal cancer[35,36]).

In endometrial cancer, HER2 testing has become more clinically relevant in serous carcinoma[37] and carcinosarcoma[38,39], with the accumulating knowledge of HER2 testing accompanied by ongoing therapeutic developments.

HER2 TESTING IN SEROUS CARCINOMA

Serous carcinoma accounts for up to 10% of endometrial cancers; however, it is aggressive and responsible for almost 40% of endometrial cancer deaths[40]. Histopathologically, serous carcinoma is usually composed of tumor cells with marked nuclear pleomorphism and presents a complex papillary pattern of typically small, bud-like papillae, irregular slit-like glands, an endometrioid-like glandular pattern, solid nests and sheets, and microcysts[41]. HER2 is overexpressed in 20%-60% of serous carcinomas[29,42-45]; however, less frequent HER2 gene amplification has been reported[43,44]. Notably, the staining pattern of HER2 in serous carcinoma cells has been well studied; approximately 75% of HER2 expressing carcinoma cells reportedly show a basolateral/Lateral membranous staining pattern, similar to that of gastric adenocarcinoma[37]. HER2 assessment of serous carcinoma has been performed using various methods, mainly based on the breast cancer criteria[42-44]. However, an optimal HER2 testing method specific for endometrial serous carcinoma has not been established in the clinical trial setting.

Recently, a randomized phase 2 clinical trial of carboplatin/paclitaxel *vs* carboplatin/ paclitaxel/trastuzumab in advanced or recurrent HER2 positive serous carcinoma demonstrated the survival benefit of adding trastuzumab[46]. Both prolonged progression-free survival (PFS) and overall survival (OS) were observed in the trastuzumab arm. For patient enrollment in this clinical trial, HER2 status was determined based on the modified 2007 ASCO/CAP breast criteria (Figure 1)[37]. Serous carcinoma showing intense complete or lateral/basolateral membranous HER2 staining in more than 30% of tumor cells were classified as score 3+, and score 2+ was assigned when intense complete or lateral/basolateral membrane staining was seen in ≤ 30%, or weak to moderate staining in ≥ 10% of tumor cells. FISH was performed only in tumors with an IHC score of 2+, and a HER2/CEP17 ratio of ≥ 2.0 was considered as amplified. Although this HER2 assessment criterion was justified in the context of the treatment effect of trastuzumab-containing regimens, it is still uncertain whether this HER2 testing algorithm should be used in patients receiving HER2 targeted drugs with a different mode of action from that of trastuzumab. For example, HER2-antibody drug conjugate (ADC) has been shown to be clinically effective in patients with low-level HER2 expressing breast cancer who have been assessed as HER2-negative according to the ASCO/CAP breast cancer criteria established for the prediction of response to trastuzumab[47]. The HER2 testing algorithm was optimized to maximize patient benefit and safety for each HER2 targeted therapy.

HER2 TESTING IN UTERINE CARCINOSARCOMA

Uterine carcinosarcoma (UCS) is a rare and high-grade subtype of endometrial cancer (WHO2020) and is characterized by the presence of both carcinomatous and

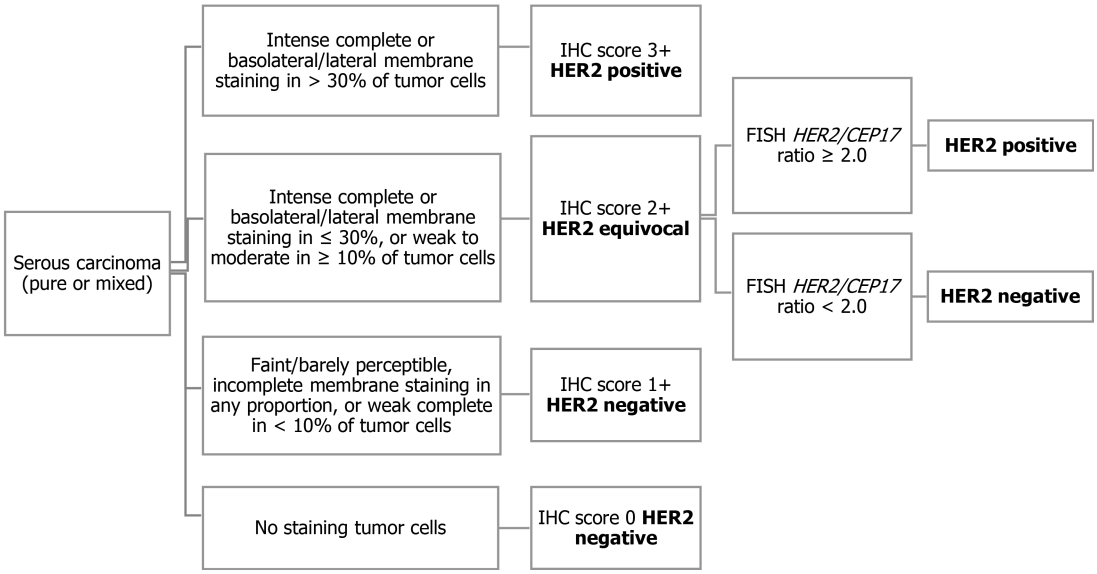

Figure 1 Human epidermal growth factor receptor 2 testing for serous carcinoma used in the Phase 2 clinical trial[37,46]. IHC: Immunohistochemistry; FISH: Fluorescent in situ hybridization.

sarcomatous components that are usually intimately admixed. The carcinomatous component is usually a high-grade endometrioid, serous, clear, or nonspecific carcinoma. HER2 is a promising therapeutic target for UCS. *HER2* gene amplification has been reported in 13%-20% of UCS cases[48,49]. Additionally, HER2 overexpression (IHC score 3+) reportedly ranges from 0 to 25%[38,49-51].

An evidence-based HER2 testing protocol for UCS has not been fully established; thus, several researchers have adopted HER2 testing protocols and assessment methods based on ASCO/CAP recommendations for HER2 testing for breast cancer and gastroesophageal cancer.

Recently, we proposed several requirements for HER2 testing in UCSs[39]. Our evaluation method has been used in the ongoing phase 2 clinical trial of HER2 ADC in patients with recurrent/metastatic UCS (STATICE trial)[52]. In this study, we identified that most UCS showed lateral/basolateral staining patterns (Figure 2), similar to endometrial serous carcinoma and gastric adenocarcinoma[39]. Based on our previous observations, we concluded that a HER2 testing protocol for UCS should contain the following requirements[39]: (1) Established pre-analytical factors of HER2 IHC[32,33] should be carefully controlled; (2) One representative section containing carcinoma components should be submitted from the hysterectomy specimen; (3) HER2 IHC should be performed using an IVD kit or a laboratory-developed test with appropriate quality control[32,33]; (4) Positive lateral/basolateral membranous staining patterns should be considered as positive, according to the 2016 ASCO/CAP gastric cancer criteria[32]; and (5) The proportion of HER2 expressing tumor cells should be determined as an approximate number of HER2-positive tumor cells divided by that of total tumor cells (both carcinoma and sarcomatous elements). Unfortunately, we did not provide supporting data for the best scoring system based on the patient clinical outcomes. However, we would report the correlation between treatment efficacy and HER2 score in patients with UCS in 1-2 years.

FUTURE DIRECTION OF HER2 TESTING IN ENDOMETRIAL CANCER

Most quality assurance of HER2 testing should be performed in accordance with well-established methods for breast and gastric cancer. In addition, we should consider specific issues for HER2 testing in endometrial cancers, including correlation with specific treatment response, intratumoral heterogeneity of HER2 status, discordant HER2 status between the primary site and metastasis, improvement of interobserver reproducibility of HER2 assessment, and use of liquid biopsy in the future.

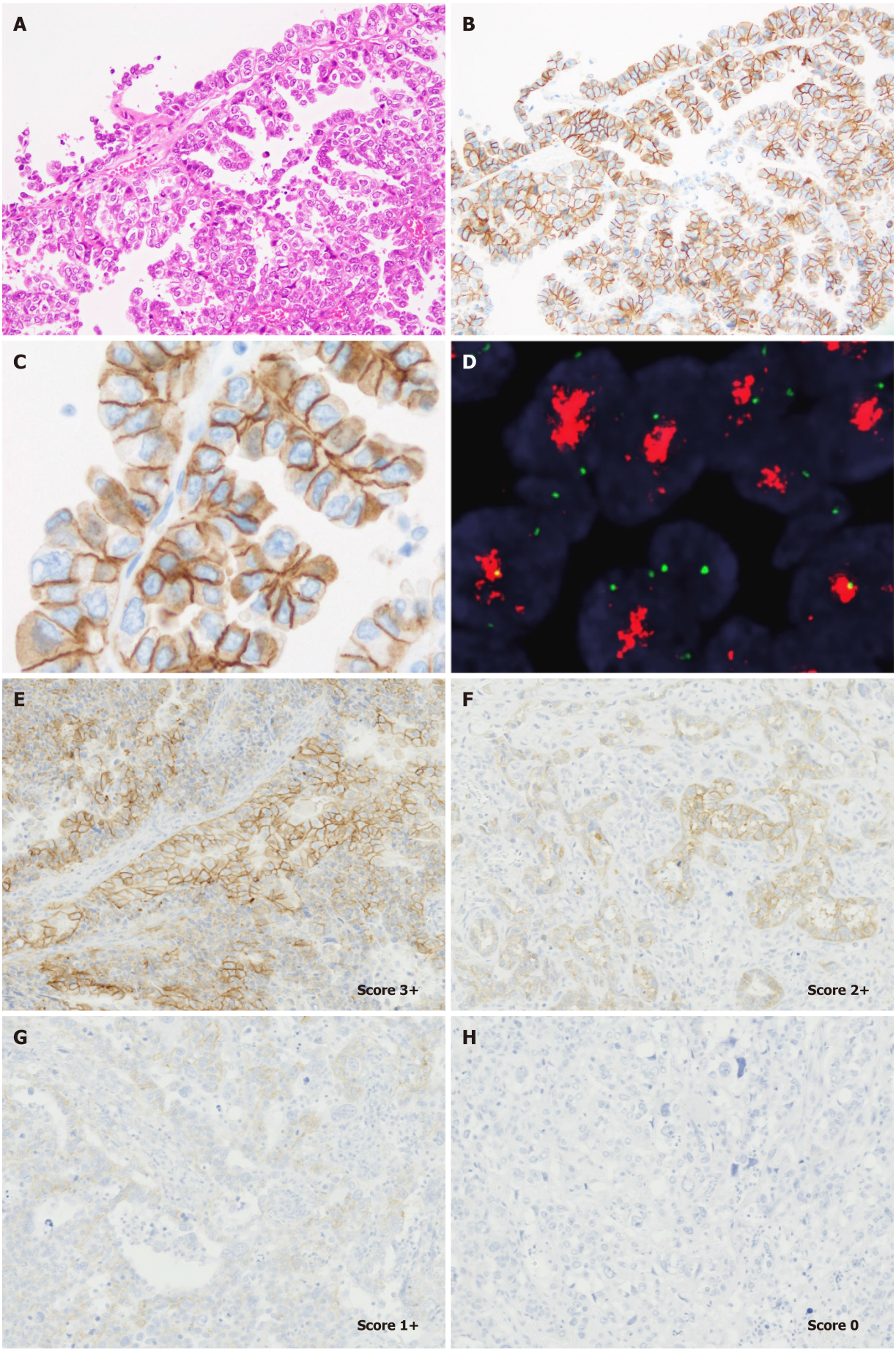

Figure 2 Representative staining patterns of human epidermal growth factor receptor 2 and fluorescent in situ hybridization results in

uterine carcinosarcoma. Serous-like carcinoma component (A) shows diffuse strong membranous staining of Human epidermal growth factor receptor 2 (HER2) (B). Most tumor cells show lateral or basolateral membranous staining patterns (C). *HER2* amplification observed in these tumor cells using fluorescent in situ hybridization (D). Representative images of HER2 score according to ASCO/CAP gastric cancer criteria. Score 3+ (E), score 2+ (F), score 1+ (G), and score 0 (H) (× 200). A (H&E, × 100); B, E-G, and H (HER2, × 100); C (HER2, × 400). FISH: Fluorescent in situ hybridization; H&E: Hematoxylin and eosin.

CURRENT THERAPIES AND FUTURE DIRECTIONS FOR HER2 TARGETED THERAPIES FOR UTERINE SEROUS CARCINOMA AND UTERINE CARCINOSARCOMA

Trastuzumab is the most popular HER2-targeted drug. Several case reports have shown that trastuzumab had clinical activity in patients with endometrial cancer[53, 54]. Fleming *et al*[55] investigated the efficacy of trastuzumab as a single agent in HER2-positive endometrial carcinoma in a GOG study (Table 2). However, the overall response rate was 0%, and no significant clinical activity was observed. A possible issue was that many endometrioid carcinomas with low HER2 positivity were screened, and the study had early termination with poor accrual. Lapatinib is a dual inhibitor of EGFR and HER2 used in HER2-positive breast cancer. The GOG 229D[56] trial examined the efficacy of lapatinib in uterine cancer (n = 2 for HER2-positive) in a phase II trial. The response rate was 3%, the median PFS was 1.82 mo, and clinical activity could not be demonstrated. This lack of clinical activity could be explained by the unselected patient population. Therefore, further studies are warranted to highlight type II endometrial carcinoma patients with high HER2-positivity.

Fader *et al*[46] conducted a randomized phase II trial to evaluate the effect of trastuzumab on carboplatin and paclitaxel, the standard of care for HER2-positive USC. The median PFS [12.6 mo *vs* 8.0 mo; hazard ratio (HR) = 0.44, 90% confidence interval (CI): 0.26-0.76, P = 0.005] was significantly different between the two groups. Updated analysis (median PFS 12.9 mo *vs* 8.0 mo; HR = 0.46, 90%CI: 0.28-0.76; P = 0.005, median OS 29.6 mo *vs* 24.4 mo; HR = 0.58, 90%CI: 0.34-0.99; P = 0.46) showed clinically significant benefit[57]. The addition of trastuzumab to carboplatin and paclitaxel chemotherapy represents a new standard treatment for USC. In the NCCN guidelines, carboplatin, paclitaxel, and trastuzumab combination therapy are recommended in Category 2A for advanced or recurrent USC[58].

Several basket trials in HER2-positive solid tumors, including endometrial carcinoma, have been reported. MyPathway is a phase II, multiple basket study with patients with advanced refractory solid tumors harboring molecular alterations such as *HER2, BRAF, EGFR*, and the Hedgehog pathway[25]. Patients with *HER2* alterations were treated with pertuzumab plus trastuzumab. Eight patients with HER2-positive endometrial carcinoma received trastuzumab plus pertuzumab. No responses were observed. The efficacy of trastuzumab emtansine has also been evaluated in a phase II basket trial, including HER2-positive endometrial carcinoma[59]. This trial demonstrated that 18 patients with uterine cancer (subtype unknown) were included, and two patients had a complete response and two had a partial response (PR), suggesting high efficacy.

Currently, several clinical trials of HER2-targeted drugs are ongoing. The DESTINY-PanTumor02 trial is a basket trial to evaluate the efficacy of trastuzumab deruxtecan in HER2-positive tumors, including endometrial carcinoma (NCT04482309). A phase I trial evaluating combination therapy with trastuzumab deruxtecan and olaparib in HER2-positive USC is also ongoing (NCT04585958). In HER2-positive UCS, the STATICE trial is a phase II trial to evaluate the efficacy of trastuzumab deruxtecan (UMIN00002956, NCCH1615)[52].

Novel therapeutic agents targeting HER2 have emerged. Trastuzumab duocarmazine (known as SYD985) is a novel HER2-targeted ADC that combines trastuzumab with duocarmazine, a DNA alkylating agent, as a payload. Trastuzumab duocarmazine has shown preclinical anti-tumor activity in USC[60]. Banerji *et al*[61] demonstrated phase 1 dose-escalation and dose-expansion study in breast, gastric, urothelial, and endometrial carcinomas that express HER2. Thirteen patients with endometrial cancer were included in the dose-expansion cohort. Five patients (39%) had PR, and the median PFS was 4.3 mo[61]. Treatment-related serious adverse events were reported in 11% of patients, and the frequency of cardiac toxicity did not increase compared with previous anti-HER2 drugs. Most patients had ocular adverse events, such as conjunctivitis, dry eye, and lacrimation. Trastuzumab duocarmazine has a manageable safety toxicity profile[61]. Further investigation of trastuzumab duocarmazine is ongoing in phase III trials for HER2-positive breast cancer (TULIP study,

Table 2 Human epidermal growth factor receptor 2-targeted therapies for uterine serous carcinoma and carcinosarcoma

Trial	Phase	Participants	Treatment	Efficacy/indentifer
GOG181B[55]	II	HER2-positive EC (n = 33, endometrioid: 13, serous: 11, clear: 3, others: 6)	Trastuzumab	ORR 0%, mPFS 1.84 mo, mOS 7.8 mo
GOG229D[56]	II	Persistent or recurrent EC (n = 31, endometrioid: 16, serous: 7, clear: 3, others: 5, HER2-positive: n = 2)	Lapatinib	mPFS 1.82 mo, mOS 7.33 mo
Fader *et al*[46,57]	II	HER2-positive USC (n = 61)	carboplatin+paclitaxel+trastuzumab *vs* carboplatin+paclitaxel	mPFS 12.9 mo *vs* 8.0 mo, mOS 29.6 *vs* 24.4 mo
MyPathway[25]	Phase IIa Multiple basket study	Solid tumor(HER2-positive EC, n = 7)	Trastuzumab+pertuzumab	ORR 0%(EC)
Li *et al*[59]	II	HER2 amplified cancer (endometrial cancer: n = 18)	Trastuzumab emtansine	Endometrial cancers: CR 2, PR 2
DESTINY-PanTumor02[77]	II	HER2 expressing tumor (urothelial, biliary tract, cervical, endometrial, ovarian, pancreatic, rare tumors)	Trastumab deruxtecan	NCT04482309
Veneris[78]	I	HER2-positive USC	Trastuzumab deruxtecan+olaparib	NCT04585958
Banerji *et al*[61]	I	Dose-expansion cohort: HER2-positive breast, gastric, urothelial, endometrial cancer	SYD-985 (Trastuzumab duocarmazine)	Endometrial cancer: ORR 39%, mPFS 4.3 mo
Koper[79]	I	HER2-positive solid tumor	SYD-985 (Trastuzumab duocarmazine)+Niraparib	NCT04235101
Hendriks[80]	II	HER2-positive endometrial cancer	SYD-985 (Trastuzumab duocarmazine)	NCT04205630
STATICE[52]	II	HER2-positive UCS	Trastuzumab deruxtecan	UMIN00002956 (NCCH1615)
Makker[81]	II	HER2-positive endometrial cancer, UCS	ZW25	NCT04513665
Ramos[82]	II	HER2-positive solid tumors	Tucatinib and trastuzumab	NCT04579380

EC: Endometrial cancer; USC: Uterine serous carcinoma; UCS: Uterine carcinosarcoma; CR: Complete response; mOS: Median overall survival; mPFS: Median progression-free survival; ORR: Objective response rate; PR: Partial response.

NCT03262935), phase II trials for HER2-positive endometrial cancer (NCT04205630), and phase I trial for HER2-positive solid tumor combination with niraparib (NCT04235101).

There are important considerations for future studies. Anti-HER2 therapy has been successful in HER2-positive breast cancer, but most of these drugs have not been successful in non-breast HER2-positive solid tumors. One explanation for these differences is the pattern of HER2 expression and heterogeneity within the tumor. Since the HER2 expression pattern of USC and UCS is similar to that of gastric cancer, but not breast cancer, it is possible that the same HER2-targeted therapy may have similar effects.

The first consideration may be to focus on combination therapies and ADC drugs rather than HER2 blockade alone in endometrial cancer. The second consideration is to overcome the resistance to HER2 targeted agents. Mechanisms of resistance to HER2 targeted therapy have been studied and can be classified into four groups: intratumoral heterogeneity, alterations in the binding site, activation of downstream signals, and overexpression of other HER2 family members[62]. The strategy against intratumoral heterogeneity could be a bystander effect of clinical activity against not only targeted cells but also surrounding cells. A novel HER2 ADC drug, trastuzumab deruxtecan, and trastuzumab duocarmazine showed a bystander effect *in vitro* and *in vivo*, and showed anti-tumor activity in clinical trials with low HER2 positivity[47,60,63-65]. Alteration in the binding site of HER2 and extracellular domain shedding could be overcome using irreversible inhibitors. In preclinical studies, afatinib and neratinib are irreversible pan-HER inhibitors that show anti-tumor activity in USC and gynecologic carcinosarcoma[66-68]. Tucatinib is an HER2 and HER3 kinase inhibitor, combination with trastuzumab are investigated in baskets study of Solid tumor with HER2 alterations, including uterine neoplasms. In downstream pathway alterations, the gain of function in PI3K is well known as resistance to HER2. Combined HER2 and PIK3CA dual inhibition using neratinib and taselisib were effective in cell lines and

xenograft models of USC[69,70]. Of overexpression of other HER2 family members, HER3 overexpression plays an important role. The formation of HER2-HER3 heterodimers is most related to resistance to anti-HER2 therapy. Pertuzumab is an HER2 antibody that binds to a different epitope from that of trastuzumab and inhibits dimerization. In breast cancer, the combination of trastuzumab and pertuzumab improved clinical outcomes[71]. Pertuzumab and trastuzumab showed anti-tumor activity in USC cell lines[72]. Although these drug combinations showed no clinical benefit in the Mypathway trial with HER2 amplification in endometrial cancer[25], further investigation is warranted. Several HER2 bispecific antibodies have been developed that simultaneously bind to two distinct HER2 epitopes, the same domain as trastuzumab and pertuzumab[73]. Researchers demonstrated a phase 1 basket trial to evaluate ZW25, one of HER2 bispecific antibodies, in HER2 positive solid tumors, including endometrial cancer. Of 17 evaluable patients, seven patients (41%) had an objective response, and the median PFS was 6.2 mo[74]. ZW25 is investigated in phase 2 clinical trial of HER2 overexpressed advanced endometrial cancer and carcinosarcomas.

In addition, there is rapidly growing HER2-directed immunotherapy in patients with HER2-positive solid tumors[19,75]. Several drugs with a different target, such as bispecific antibodies, immune-stimulating conjugates, vaccines, and adoptive T-cell therapies, are under investigation[76]. The bispecific HER2/CD3 antibodies BTRC4017A, GBR-1302 and M802 induce cytotoxic effect by interaction with HER2 on tumor cell and CD3 on cytotoxic T cell. NJH395 are immune-stimulating antibody conjugates which HER2 antibody links to payload as toll-like receptor 7 (TLR7) and TLR8. Stimulating TLR activated natural killer cells and antigen-presenting cells and facilitate invasion of CTLs to tumor tissues. PRS-343 increases tumor lymphocyte invasion *via* targeting HER2 and CD137 (4-1BB). CD137 is known as a co-stimulating factor of T cell activation[73]. We expect a further investigation of these drugs in patients with endometrial cancer.

CONCLUSION

In this review, we provided an overview of HER2-overexpression/amplification in endometrial cancer, pathological evaluation methods, and the current status of HER2-targeted therapies. With the advent of precision medicine, the development of therapies targeting biomarkers has become increasingly advanced. In the development of anti-HER2 inhibitors and ADC drugs targeting HER2, it may be important to develop not only a single drug but also combination therapies. Since there are limited therapeutic agents for endometrial cancer, especially for type II, the development of HER2-targeted therapy is urgently needed.

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MINIREVIEWS

Role of lenalidomide in the treatment of peripheral T-cell non-Hodgkin lymphomas

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Abstract

T-cell lymphomas (TCLs) represent a group of lymphoid neoplasms characterized by an aggressive clinical course, even after an anthracycline-containing regimen. Novel agents for patients with relapsed/refractory TCL are urgently needed. Lenalidomide is an oral drug with immunomodulatory, antiangiogenic and direct antineoplastic effects. These peculiar mechanisms of action make TCL an attractive target for lenalidomide. We have identified five clinical trials in which lenalidomide monotherapy was investigated to treat TCL, including cutaneous TCL (CTCL) and adult T-cell lymphoma/leukemia (ATLL). In the ATLL-002 study, the overall response rate (ORR) was 42% and median progression-free survival (PFS) and overall survival were 3.8 mo and 20.3 mo, respectively. In a phase II trial for CTCL, ORR was 28% and median PFS and overall survival were 8 mo and 43 mo, respectively. For nodal peripheral TCL, ORR was between 10% and 43% in three clinical trials, with a median PFS of about 4 mo, even if some patients had a durable response. Overall toxicity is manageable and grade 3-4 events are mainly hematological and reversible. Combination strategies did not improve PFS. In conclusion, lenalidomide could represent a suitable treatment option for relapsed/refractory TCL, especially for neoplasms with a T-follicular helper origin, such as angioimmunoblastic TCL.

Key Words: T-cell lymphomas; Lenalidomide; Therapy; Survival; Safety; T follicular helper

Core Tip: T cell lymphoma (TCL) are rare. Lenalidomide is an oral drug with an immunomodulatory, antiangiogenic and direct antineoplastic effect. These peculiar mechanisms of action makes TCL an attractive target for lenalidomide. We have

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identified 5 clinical trials in which lenalidomide monotherapy was investigated to treat TCL, including cutaneous TCL and adult T-cell lymphoma/leukemia. Overall response rate was between 10 % and 43%, with prolonged response in a significant proportion of cases and manageable toxicity. Lenalidomide could represent a suitable treatment option for R/R TCL, especially for neoplasms with a T-follicular helper origin, such as angioimmunoblastic TCL.

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INTRODUCTION

Peripheral T-cell lymphomas (TCLs), including primary nodal, extranodal, leukemic and cutaneous TCL (CTCL), are a heterogeneous group of lymphoid neoplasms characterized by aggressive clinical course and poor prognosis, representing about 10%-20% of non-Hodgkin lymphoma cases[1-3]. Due to its rarity, the treatment approach has historically been similar to that used for aggressive B-cell neoplasms, such as diffuse large B-cell lymphoma (DLBCL)[3,4]. However, except for anaplastic lymphoma kinase (ALK)-positive anaplastic lymphoma (ALCL), treatment efficacy is limited compared to DLBCL, disease relapse is frequent even after high-dose therapy and autologous stem-cell transplantation, and long-term responses are seldom observed[1,5-8]. Allogeneic stem-cell transplantation often remains the only curative option, but many elderly and/or relapsed/refractory (R/R) patients are not eligible, and treatment morbidity and mortality are not negligible[9]. Novel agents approved by the Food and Drug Administration and/or the European Medicines Agency for R/R TCL, as shown in Table 1, include pralatrexate, romidepsin, belinostat, brentuximab vedotin (only for ALCL and CD30-positive CTCL) and mogamulizumab [only for mycosis fungoides and Sézary syndrome (SS)][10-14]. Additional new drugs with different mechanisms of action are listed in the National Comprehensive Cancer Network guidelines, including bortezomib, alemtuzumab, crizotinib, cyclosporine, nivolumab and lenalidomide[15]. Interestingly, several drugs work very well on a few TCL subtypes, while their efficacy is limited for the others; this allows for speculation that disease biology could influence the therapeutic response[16].

Lenalidomide is an immunomodulatory drug initially approved as a treatment for patients with R/R multiple myeloma (MM)[17]. It was successfully used to treat myelodysplastic syndromes, chronic lymphocytic leukemia and R/R B-cell non-Hodgkin lymphoma, including DLBCL, mantle-cell lymphoma and follicular lymphoma[18-22]. Due to its peculiar mechanisms of action, TCL could represent an attractive target for lenalidomide; however, available data are limited[4,14]. According to this background, we would like to briefly summarize the pharmacological properties and to review the clinical efficacy and safety of lenalidomide monotherapy in previously treated TCL.

SEARCH CRITERIA FOR LITERATURE REVIEW

We performed a computerized search in MEDLINE to find full-text publications, in English, published to 2020, which focused on lenalidomide and TCL. We included nodal and extranodal peripheral TCL (PTCL), adult T-cell leukemia-lymphoma (ATLL) and CTCL. The key terms were "T-cell lymphoma OR TCL OR T-cell non-Hodgkin lymphoma OR mycosis fungoides (MF) OR Sézary syndrome (SS) OR cutaneous TCL OR peripheral TCL OR adult T-cell leukemia-lymphoma AND lenalidomide OR treatment OR immunotherapy OR combined modalities". We included prospective clinical trials, retrospective studies, letters to the editor and case reports. For each study, we extracted the following data, when available: number of patients, study design, patient population, treatment regimen, overall response rate (ORR), complete response (CR) rate, time to response (referred to as TTR), toxicity, and survival end-points, such as OS, PFS and duration of response (DOR). OS represents

Table 1 Clinical results of approved novel agents as monotherapy for relapsed/refractory T-cell lymphomas

Ref.	Patients, *n*	Study design	Treatment	Mechanism of action	Histology, (number of patients)	ORR	CR rate	Median PFS	Median DOR	Median OS
O'Connor *et al*[10], 2011	111	Multicenter phase II	Pralatrexate	Antifolate	Total evaluable (109)	29%	11%	3.5 mo	10.1 mo	14.5 mo
					PTCL-NOS (59)					
					ALCL (17)	32%	NA			
					AITL (13)	35%	NA			
					MF (12)	8%	NA			
					Other (8)	25%	NA			
						38%	NA			
Coiffier *et al*[11], 2014	130	Multicenter phase II	Romidepsin	Histone deacetylase inhibitor	Total evaluable -130	25%	15%	4 mo	28 mo	11.3 mo
					PTCL-NOS (69)	29%	14%			
					ALCL ALK- (21)	24%	19%			
					AITL (27)	30%	19%			
					Other (13)	/	/			
O'Connor *et al*[12], 2015	129	Multicenter phase II	Belinostat	Histone deacetylase inhibitor	Total evaluable -120	25.80%	10.80%	1.6 mo	13.6 mo	7.9 mo
					PTCL-NOS (77)	23%	NA			
					ALCL ALK- (13)	15%	NA			
					ALCL ALK+ (2)	/	NA			
					AITL (22)	46%	NA			
					Other (6)	16.60%	NA			
Pro *et al* [13], 2017	58	Multicenter phase II	Brentuximab vedotin	Monoclonal antibody anti-CD30	ALCL	86%	57%	20 mo	25.6 mo	NR (estimated 5-yr OS 60%)
Kim *et al* [14], 2018	186	Multicenter, randomized, phase III	Mogamulizumab	Monoclonal antibody anti C-C chemokine receptor 4	MF or Sézary syndrome	28%	2.70%	7.7 mo	14.1 mo	NR

AITL: Angioimmunoblastic T-cell lymphoma; ALCL: Anaplastic T-cell lymphoma; ALK: Anaplastic lymphoma kinase; CR: Complete response; DOR: Duration of response; MF: Mycosis fungoides; NA: Not available; NR: Not reached; ORR: Overall response rate; OS: Overall survival; PFS: Progression-free survival; PTCL-NOS: Peripheral T-cell lymphomas: not otherwise specified.

the most relevant outcome; PFS could be more accurate but the PFS definition has not been the same in different studies over the years. We included abstracts extracted from the last meetings of the European Hematology Association, American Society of Hematology, and International Conference on Malignant Lymphoma.

MECHANISMS OF ACTION AND PHARMACOLOGICAL CHARACTERISTICS OF LENALIDOMIDE

Lenalidomide is administered orally and rapidly adsorbed, without marked accumulation, even after multiple courses[23]. The drug is mainly excreted unchanged by renal elimination, thus a dose reduction is recommended in patients with reduced creatinine clearance due to renal impairment. Lenalidomide is characterized by an acceptable safety profile, and clinically relevant interactions with other drugs are unlikely[23].

The multiple mechanisms of action, as shown in Figure 1, include an immunomodulatory, antiangiogenic and direct antineoplastic effect[24]. Lenalidomide may be able to restore the function of the T-cell immune synapse and to suppress regulatory T-cells (*i.e.*, Tregs)[25,26]. Moreover, it could activate CD8-positive T-cells and favor a shift of T-helper (*i.e.*, T_H) response towards a T_H1 *vs* a T_{H2} subtype[27,28]. Moreover, it could improve natural killer (NK)-mediated immune function and NK cell activation, leading to the induction of a primarily NK-mediated tumor cells apoptosis[28,29]. Moreover, lenalidomide could inhibit the production of cytokines with a pro-inflammatory activity, such as interleukin (IL)-1, IL-6, IL-12 and tumor necrosis factor-α, while the production of anti-inflammatory molecules, such as IL-10, is increased[30].

The antiangiogenic effect is achieved by blocking the migration and adhesion of endothelial cells and by inhibiting the formation of microvessels (reduced microvessels density)[28]. In mantle-cell lymphoma mouse models, the drug could cause a depletion of immune cells associated with lymphomagenesis, such as monocytes and macrophages[31]. Moreover, a direct inhibitory effect of lenalidomide on the vascular endothelial growth factor production was demonstrated and was associated with an increased SPARC expression[32,33].

The antiproliferative effect is carried out by directly inducing G1 growth arrest of the cell cycle and apoptosis[34]. A relevant discovery was represented by the identification of cereblon, a component of a cullin-RING E3 ubiquitin ligase enzyme complex, which includes the deoxyribonucleic acid damage binding protein 1, cullin 4 and the cullin regulator 1[35,36]. After the direct binding of lenalidomide, cereblon leads to the ubiquitination and subsequent degradation of the substrate proteins Aiolos (IKZF3) and Ikaros (IKZF1), which are lymphoid transcription factors, with consequent cytotoxic and immunomodulatory effects[37,38]. In DLBCL, especially nongerminal center B-cell-like DLBCL, a direct, cereblon-dependent capability of lenalidomide to kill neoplastic cells was observed, through deregulation of interferon regulatory factor 4[39]. Remarkably, the antiproliferative effect can occur in a p53-independent manner[40].

LENALIDOMIDE IN THE TREATMENT OF TCLS

ATLL

ATLL represents an uncommon neoplasm linked to the human T-lymphotropic virus type 1 infection and characterized by an aggressive course with poor prognosis[41]. Although rare in Western countries, it represents a common TCL in Japan, where human T-lymphotropic virus type 1 is endemic[41]. ATLL is subdivided into four subtypes: smoldering; chronic (often with an indolent course); lymphoma; and acute (with a very aggressive behavior)[2,41]. Anthracyclines-containing regimens, such as cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP), showed limited efficacy, and most patients are refractory or relapse after an initial response[42]. R/R ATLL, due to the rarity of disease, has been frequently excluded from clinical trials investigating novel agents[42]. Ogura *et al*[43] investigated lenalidomide in relapsed ATLL or PTCL in a phase I study, in which 14 patients were enrolled. Maximum tolerated dose was 25 mg daily given continuously; out of 9 ATLL cases, 3/9 achieved a partial response (PR), leading to the design of a phase II study (ATLL-002), in which 26 relapsed or recurrent Japanese ATLL cases were enrolled[43,44]. The patients had received a median of two previous regimens (range: 1-4). Interestingly, 2 patients received lenalidomide for more than 1 year. As represented in Table 2, ORR was 42% (11/26 cases), with 4 CR and 1 unconfirmed CR; out of 11 responders, 2 were previously treated with mogamulizumab[44]. Median TTR, median PFS and OS were 1.9 mo, 3.8 mo and 20.3 mo, respectively, with manageable toxicity. According to disease subtype, ORR was 33% for acute ATLL and 57% for lymphoma ATLL[44]. Interestingly, in another Japanese experience, 2/4 relapsed ATLL cases after allo-stem-cell

Table 2 Clinical efficacy of lenalidomide single-agent in T-cell lymphomas

Ref.	Patient, *n*	Study design	Treatment	Histology (number of patients)	ORR	CR rate	Median PFS	Median DOR	Median OS
Ishida *et al*[44], 2016	26	Multicenter phase II	25 mg continuously until progression or unacceptable toxicity	ATLL (26)	42%	19%	3.8 mo	NR	20.3 mo
Querfeld *et al* [52], 2014	32	Multicenter phase II	25 mg for 21 d of a 28-d cycle; initial dose was reduced to 10 mg, with the possibility of increasing by 5 mg every cycle, until a maximum of 25 mg, until progression or up to 2 yr for SD or PR, while patients in CR received two additional cycles	Total evaluable (29)	28%	/	8 mo	10 mo	43 mo
				Mycosis fungoides (19)	36.8%				
				Sézary syndrome (13)	15.4%				
Zinzani *et al* [56], 2011	10	Phase II, bi-centric	25 mg for 21 d of a 28-d cycle for 4 cycles as induction phase; After the 4th cycle, patients who achieved at least a SD continued for other eight cycles as maintenance	PTCL-NOS (10)	30%	30%	NA	13 mo	NA
Morschhauser *et al*[51], 2013	54	Multicenter phase II	25 mg on d 1-21 of a 28-d cycle, until progression or unacceptable toxicity, for a maximum of 2 yr	Total evaluable (54)	22%	11%	2.5 mo (4.6 mo in AITL)	3.6 mo	NA
				AITL (26)	31%	15%			
				PTCL-NOS (20)	20%	NA			
				CTCL (3)	NA	NA			
				ALCL (3)	NA	NA			
				Cutaneous ALCL (1)	NA	NA			
				Extranodal NK/T-cell, nasal type (1)	NA	NA			
Toumishey *et al* [57], 2015	39	Multicenter phase II	25 mg daily on d 1-21 of a 28-d cycle, until progression or unacceptable toxicity	Total evaluable (39)	26%	7.7%	4 mo	13 mo	12 mo
				ALCL (10)	10%	/			
				AITL (9)	33%	11.1%			
				PTCL-NOS (14)	43%	14.3%			
				Other (6)	/	/			

AITL: Angioimmunoblastic T-cell lymphoma; ALCL: Anaplastic T-cell lymphoma; ATLL: Adult T-cell lymphoma/leukemia; CTCL: Cutaneous T-cell lymphoma; CR: Complete response; DOR: Duration of response; NA: Not available; NK: Natural killer; NR: Not reached; OS: Overall survival; PR: Partial response; ORR: Overall response rate; PFS: Progression-free survival; PTCL-NOS: Peripheral T-cell lymphomas: Not otherwise specified; SD: Stable disease.

transplantation achieved a CR, and 1 of them showed a prolonged remission[45].

Even if these findings have not been confirmed outside Japan, lenalidomide could represent a promising option for R/R ATLL, as single-agent or in the context of combination strategies[46].

CTCLS

CTCLs are a heterogeneous group of TCL, and cell of origin is represented by skin-homing, mature, CD4-positive T_H lymphocytes[47]. MF and SS are the most common subtypes and frequently relapse after systemic therapy; R/R MF/SS are not curable and characterized by dismal prognosis[48]. Moreover, treatment toxicity is elevated after cytotoxic therapy, thus biological agents are needed to improve therapeutic efficacy with limited toxicity[47,48]. As there is a prevalent T_H2 response with a reduction of CD8-positive T-cells in MF/SS, especially in advanced-stage disease, the immunomodulatory characteristics of lenalidomide represent a strong rationale for its use in CTCL patients[49,50].

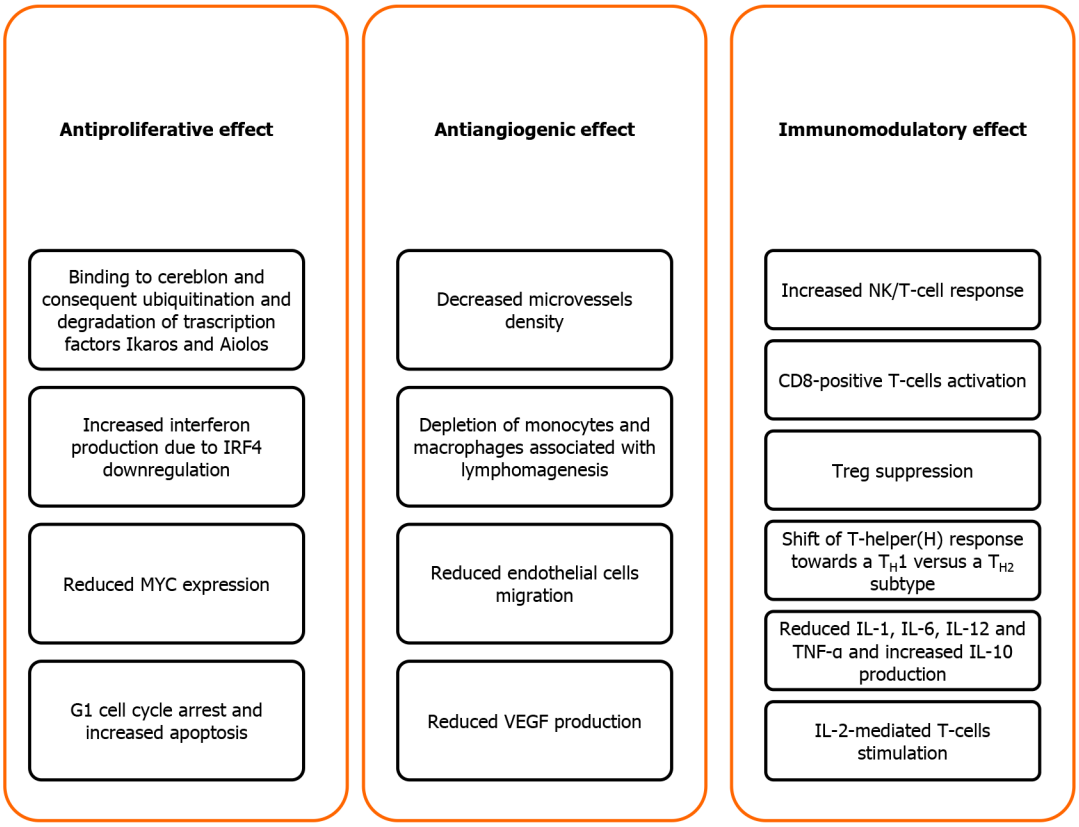

Figure 1 Lenalidomide mechanisms of action. IL: Interleukin; VEGF: Vascular endothelial growth factor.

Three MF patients were included in the phase II, single-arm EXPECT trial, in which lenalidomide dose was 25 mg once daily on days 1-21 of a 28-d cycle, given until progressive disease (PD) or unacceptable toxicity, for a maximum of 2 years. Unfortunately, treatment response for MF patients was not reported[51]. As illustrated in Table 2, Querfeld *et al*[52] enrolled in a phase II trial 32 patients with stage IB to IVB MF (18 cases), erythrodemic MF (3 cases) and SS (11 cases), who failed at least one prior therapy (skin-directed or systemic therapy). Lenalidomide was administered at a daily dose of 25 mg for 21 d of a 28-d cycle to the first 19 patients; the protocol was amended due to fatigue and tumor-flare reaction (referred to as TFR), and initial dose was reduced to 10 mg, with the possibility of daily dose increasing by 5 mg every cycle, based on treatment tolerance, up to a maximum of 25 mg[52]. Treatment was administered until PD or up to 2 years for patients who achieved a stable disease (SD) or PR, while patients in CR received two additional cycles before discontinuation. All patients received a median of six previous therapies (range: 1-14). In an intention-to-treat analysis, ORR was 28% (9/32 cases), all were PRs, with a median DOR of 10 mo [52]. The median TTR was 4 mo and 2 mo for an initial dose of 10 mg and 25 mg, respectively. Treatment response was achieved in a significant proportion of patients with blood or lymph node involvement and/or erythrodermic disease. Median PFS and OS were 8 mo and 43 mo, respectively; the main cause of death was PD (12/18 patients)[52]. Interestingly, immunophenotypic analysis showed lenalidomide effect could be associated with a reduction in circulating CD25-positive and CD4-positive T-cell count[52].

Nodal TCLS

The category of nodal PTCL includes angioimmunoblastic (AITL), anaplastic (ALCL) and PTCL not otherwise specified (NOS). PTCL-NOS represents the most common subtype, followed by AITL and ALCL (further divided in ALK-positive and ALK-negative ALCL)[1,2].

ALCL is characterized by a strong CD30 expression and large-cell anaplastic morphology; most ALK-positive cases occur in young adults and have a good prognosis, while ALK-negative cases are mainly reported in older population and display an aggressive behavior, with frequent disease relapse and/or chemorefractoriness[53]. AITL has peculiar pathophysiological features, such as the presence of an increased vascular proliferation and a reactive microenvironment (eosinophils, plasma

cells, epithelioid cells) in the lymph node, together with neoplastic T-cells[54]. Skin rash, B symptoms, hemolytic anemia and polyclonal hypergammaglobulinemia are frequently reported. Moreover, a deregulated T-cell response has been observed, with immune dysfunction[54]. A new category of neoplasms with derivation from CD4-positive T follicular helper (TFH) cells was defined in the last World Health Organization classification, which includes AITL, follicular TCL, and nodal lymphoma with a TFH phenotype[2]. PTCL-NOS is a heterogeneous subgroup of TCL cases, which do not fulfill the characteristics of more specific categories[1]. Due to this background, nodal PTCL, especially those with a TFH cell derivation, could represent an attractive target for lenalidomide.

To our knowledge, the first published study focused on lenalidomide for PTCL therapy is the interim report of a phase II trial by Dueck *et al*[55]. Both newly diagnosed (with contraindications to chemotherapy) and R/R TCL other than CTCL were enrolled from September 2006 to November 2008. The primary endpoint was ORR; patients received lenalidomide 25 mg daily on days 1-21 of a 28-d cycle, until PD or unacceptable side effects. Out of 24 patients, 20 were R/R; the diagnosis was PTCL-NOS (10 cases), AITL (7 cases), ALCL (5 cases), enteropathic-type and hepatosplenic γδ TCL (1 case each). The median number of previous regimens was 1 (range: 0-4), and 5/24 cases were refractory to last previous line. Out of 23 evaluable patients, ORR was 30% (all achieved a PR), and median PFS and OS were 96 d and 241 d, respectively (for patients with at least a SD, median PFS was 168 d)[55]. Among different histologies, ORR was 40% 29% and 33% for ALCL, AITL and PTCL-NOS, respectively. Interestingly, 2 PR patients were refractory to their previous regimen, and the development of skin rash was associated with treatment response[55].

All final reports of clinical trials focused on lenalidomide treatment for nodal TCL are illustrated in Table 2. Between November 2008 and June 2009, Zinzani *et al*[56] enrolled 10 R/R PTCL-NOS patients with stage II-IV disease in a prospective, single-arm, phase II trial. Lenalidomide initial dose was 25 mg for 21 d of a 28-d cycle for four cycles as induction phase. At disease restaging after the 4th cycle, patients who achieved at least a SD continued lenalidomide administration for another eight cycles with the same schedule as maintenance phase. The results after induction phase, with an ORR of 30% (3/10 cases, all CRs) and 1 SD, were encouraging, considering that most of patients were heavily pretreated (median number of 4 Lines, range of 2-7)[56]. After maintenance phase, CR was confirmed for all 3 patients, with 2/3 cases relapsed after 3 and 5 mo, respectively, while the remaining case maintained a durable CR at last follow-up after 11 mo. The patient with SD received all 12 cycles but developed PD 1 mo upon completion of the last treatment administration[56].

On 2013, the results of the above mentioned phase II EXPECT trial were published. Even if the primary end-point was met, the study was terminated early after 54 patients were enrolled, because the efficacy was considered unsatisfactory when compared to the results obtained with other investigated compounds[51]. Patients were heavily pretreated (median number of 3 prior therapies, range of 1-11), histological subtypes included AITL (26/54 cases), PTCL-NOS (20/54 cases), ALCL (3/54 cases) and 5 cases with extranodal TCL (3 MF, 1 cutaneous ALCL and 1 TCL nasal-type). The median drug exposure time was 42 d, while the median treatment duration was 176 d for patients achieving at least a PR[51]. ORR was 22% and CR rate was 11%; among the different histologies, ORR was 20% and 31% for PTCL-NOS and AITL, respectively[51]. The median DOR was 3.6 mo (not reached for patients in CR) and the response rate was slightly higher for younger *vs* older cases (27% and 18%, respectively). Median PFS for the entire cohort was 2.5 mo, while it was 4.6 mo and 1.9 mo for AITL and non-AITL cases, respectively; OS data were not shown[51].

On 2015, the final report of the above mentioned interim analysis was published by Toumishey *et al*[57]. Out of 39 enrolled patients (8 treatment-naïve cases), diagnosis was PTCL-NOS (14 cases), ALCL (10 cases), AITL (9 cases), lymphoblastic TCL, enteropathic-type and hepatosplenic γδ TCL (2 cases each). The median number of previous regimens was 1 (range: 0-5) and 11/39 cases were refractory to last previous line. ORR was 26% (10/39 cases), 3 patients achieved a CR. Among the different histologies, ORR was 10%, 33% and 43% for ALCL, AITL and PTCL-NOS, respectively [57]. The median PFS, DOR and OS were 4 mo, 13 mo and 12 mo, respectively. Interestingly, a trend between skin rash and treatment response was reported. When we analyzed newly diagnosed and R/R patients separately, ORR, median OS, PFS and DOR were 50% and 24%, 22 mo and 12 mo, 2 mo and 4 mo, 21 mo and 5 mo, respectively[57].

Due to the rarity of disease, we would like to mention several case reports about lenalidomide efficacy in R/R AITL. The peculiar pathophysiology of AITL makes the disease a suitable target for an immunomodulatory drug such as lenalidomide[58].

Our group administered lenalidomide to a patient with an unsatisfactory response despite three lines of chemotherapy. The patient received 12 treatment cycles (25 mg for four cycles and 15 mg for eight cycles) and maintained a durable CR after a follow-up of 30 mo[59]. In another report, a patient refractory to two previous lines received lenalidomide 15 mg continuously and achieved a CR, which was maintained after 2 years of follow-up[60]. Broccoli *et al*[61] administered reduced doses of lenalidomide (10 mg) to a refractory AITL patient with persistent disease and thrombocytopenia after autologous stem-cell transplantation. The patient achieved a long-lasting CR and continued therapy at an escalated dose of 15 mg for a total of 11 cycles. Finally, an 87-year-old woman with AITL and concurrent MM was refractory to multiple lines of chemotherapy and achieved a PR after four cycles of lenalidomide, with a dose escalation until 20 mg[62]. Interestingly, both AITL and MM improved after lenalidomide administration.

SAFETY

Lenalidomide is generally administered as an outpatient regimen and is well tolerated. As shown in Table 3, commonly observed side effects include neutropenia, thrombocytopenia, infections, skin rash and gastrointestinal disorders.

In the phase II study for R/R ATLL, the most common hematologic adverse event (AE) was thrombocytopenia (77%), while neutropenia and anemia occurred in 73% and 54% of total cases, respectively[44]. The incidence of grade 3-4 thrombocytopenia and neutropenia was 23% and 65%, respectively. The most frequent nonhematologic AEs were hypoalbuminemia (35%), constipation, hyponatremia and hypocalcemia (all with an incidence of 31%)[44]. Serious AEs were reported in 9 cases (35%), while AEs leading to treatment discontinuation were observed in 6 patients, including neutropenia and thrombocytopenia (2 cases each), toxic skin eruption (1 case), skin rash and hepatic failure (both in the same patient). Interestingly, no second primary malignancies (SPMs) were observed[44].

In the phase II trial focused on CTCL, the most common AEs were fatigue, lower leg edema and anemia, with an incidence of 59%, 47% and 41%, respectively[52]. Mild-to moderate constipation or diarrhea was observed in 11 cases (35%), while peripheral neuropathy was uncommon (19%). Grade 3-4 toxicity included fatigue (22%), infections (9%) and leukopenia (3%). Nine cases experienced a TFR, while no SPMs were reported[52].

In the Italian study, the incidence of grade 3-4 neutropenia, thrombocytopenia and asthenia was 25%, 15% and 10%, respectively[56]. In the EXPECT trial, grade 3-4 thrombocytopenia and neutropenia occurred in 20% and 15% of total cases, respectively[51]. Grade 3-4 infections and febrile neutropenia were observed in 15% and 4% of total cases, respectively; these events did not lead to treatment discontinuation. Grade 3-4 gastrointestinal disorders and TFR were experienced by 17% and 4% of total cases, respectively; only 1 patient had a treatment discontinuation due to TFR[51]. The most common AEs leading to treatment interruption or dose reduction were neutropenia and thrombocytopenia (11% of total cases each). Serious AE were reported in 54% of patients, the most frequently observed were hematological events and infections (19% of total cases each). SPMs were experienced by 3 patients, in 1 case the neoplasm was considered as therapy-related[51].

Finally, in the phase II trial by Toumishey *et al*[57], the most common AEs were pain (mainly considered as lymphoma-related rather than therapy-related), fatigue, gastrointestinal and hematological events[57]. The incidence of grade 3-4 anemia, neutropenia, febrile neutropenia, dyspnea, muscle weakness and dehydration was 11%, 16%, 8%, 13%, and 10%, respectively. Neutropenia was the main cause for dose reduction, while no deaths were considered as lenalidomide-related and no SPMs were reported[57].

NEW PERSPECTIVES

Lenalidomide monotherapy can produce a durable CR with manageable toxicity in R/R TCL, with an overall efficacy comparable to other investigated novel agents. New perspectives could be represented by combination strategies with lenalidomide in association with conventional chemotherapy and/or other novel agents, with the aim to have a place for earlier lenalidomide administration, even in a front-line regimen, especially for TCL with a TFH cell origin, such as AITL[56,57].

Table 3 Toxicity profile of lenalidomide in clinical trials for T-cell lymphomas

Toxicity/Adverse event	Ishida *et al*[44]	Querfeld *et al*[52]	Morschhauser *et al*[51]	Toumishey *et al*[57]
Hematological toxicity				
Anemia total	54%	41%	NR	26%
Anemia grade 3-4	19.2%	/	4%	11%
Leukopenia total	50%	22%	NR	NR
Leukopenia grade 3-4	38.5%	3%	4%	NR
Neutropenia total	73%	NR	NR	18%
Neutropenia grade 3-4	65.4%	NR	15%	16%
Thrombocytopenia total	77%	NR	NR	26%
Thrombocytopenia grade 3-4	23.1%	NR	20%	5%
Hypoalbuminemia	35%	28%	NR	NR
Grade 3-4	/	/	NR	NR
Constipation	31%	34%[1]	17%	44%
Grade 3-4	/	/	NR	3%
Nausea	23.1%	13%	NR	28%
Grade 3-4	3.8%	/	NR	/
Vomiting	23.1%	NR	NR	10%
Grade 3-4	/	NR	NR	/
Skin rash	23.1%	25%	NR	38%
Grade 3-4	7.6%	/	9%	11%
Fatigue	15.4%	59%	NR	56%
Grade 3-4	3.8%	22%	NR	11%
Diarrhea	NR	NR	NR	31%
Grade 3-4	NR	NR	NR	8%
Pain	NR	34%	NR	64%
Grade 3-4	NR	/	NR	21%
Infection	19.2%	34%	NR	26%
Grade 3-4	10.4%	9%	15%	5%
Neuropathy	NR	19%	NR	NR
Grade 3-4	NR	/	NR	NR
Lower leg edema	NR	47%	NR	28%
Grade 3-4	NR	/	NR	3%
Anorexia	NR	16%	NR	28%
Grade 3-4	NR	/	NR	5%
Respiratory disorders	10.4%	NR	NR	26%
Grade 3-4	7.6%	NR	13%	13%
Pulmonary embolism	NR	NR	NR	10%
Grade 3-4	NR	NR	NR	8%
Tumor flare reaction	NR	28%	14%	NR
Grade 3-4	NR	NR	4%	NR

[1]Considered together with diarrhea; Gastrointestinal disorders. NR: Not reported.

The genomic TCL landscape is being elucidated and a significant proportion of ATLL cases have shown alterations in interferon regulatory factor 4 and RHOA, which are involved in the mechanism of action of lenalidomide[63]. Due to the different mechanism of action, a combination regimen with mogamulizumab appears very promising[44-46]. The NK cells function is enhanced with lenalidomide, which could allow mogamulizumab to work better through an improvement of the antibody-dependent cell-mediated cytotoxicity[46].

The REVAIL study investigated lenalidomide in association with CHOP in newly diagnosed AITL patients. At the last International Conference on Malignant Lymphoma meeting, an ancillary study was presented in which bone marrow involvement (BMI), but no blood involvement, showed an association with reduced survival. Median PFS and OS for patients with or without BMI were 9 mo and 36 mo and 17 mo and 54 mo, respectively. The prognostic index for PTCL, including BMI, had the best power to divide the entire cohort between high-risk and low-risk cases, with a 2-year OS of 38% *vs* 79%. Moreover, BMI was associated with the presence of IDH-2 mutations[64].

An integrative analysis of this trial has been recently published, lenalidomide was given in association with CHOP every 21 d for eight total cycles[65]. CHOP was administered on day 1, and lenalidomide was added at a daily dose of 25 mg for 14 d every 21 d. Out of 78 patients included in the efficacy analysis, ORR was 56%, with a CR rate of 41%. The median dose intensity for lenalidomide was 81%, and 55% of total cases completed the study; the most common reasons for early treatment discontinuation were PD and toxicity[65]. After a median follow-up of 45 mo, 2-year PFS and OS were 42.1% and 59.2%, respectively; the presence of DNMT3A mutation appeared related with shorter PFS. Unfortunately, the primary end-point to improve a positron emission tomography-based CR rate from 45% to 60% was not reached, and the authors suggest the lack of benefit from adding lenalidomide to CHOP as AITL first-line therapy[65]. In 2 refractory AITL cases, lenalidomide was investigated in association with bortezomib and dexamethasone, achieving a CR and a PR with a manageable safety profile[66]. The rationale could be represented by a potential synergism between the immunomodulatory and antiangiogenic action of lenalidomide and the proteasome inhibitor activity of bortezomib.

In another phase I/II study, a combination of lenalidomide, vorinostat and dexamethasone was explored. Out of 8 enrolled R/R nodal TCL patients, 2 experienced a dose-limiting toxicity with a daily dose of 10 mg; thus, the maximum tolerated dose of lenalidomide was 5 mg/d[67]. ORR was 25% (1 CR and 1 PR), with a median PFS and OS of 2.2 and 6.7 mo, respectively. Due to these disappointing results, the authors did not find any additional benefit for this combination regimen compared to lenalidomide alone and discouraged further investigations[67].

The histone deacetylase inhibitor romidepsin showed a potential synergism with lenalidomide and could enhance tumor cell death in a TCL preclinical model[68]. This combination demonstrated a synergistic effect in the Hut-78 human TCL cell line and an additive effect in the Karpas-299 human TCL cell line; it was mainly related to the activation of a caspase-dependent pro-apoptotic pathway[68]. A phase Ib/IIa study, in which both PTCL and MM will be enrolled, is currently ongoing (NCT01755975).

Another promising strategy is represented by the use of lenalidomide as maintenance after debulking therapy, as previously published for R/R DLBCL cases[69]. Lenalidomide maintenance *vs* observation for advanced CTCL was investigated in a phase III, randomized trial at a daily dose of 25 mg for 21 d every 28 d[70]. Unfortunately, the trial was terminated early, following withdrawal of funding; out of 21 patients, 9/21 and 12/21 cases had been randomized to lenalidomide and observation. Median PFS was 5.3 mo and 2 mo, respectively, further suggesting a potential benefit for maintenance therapy, even if the reduced sample size did not permit a statistical comparison[70].

CONCLUSION

R/R TCL cases are characterized by poor prognosis and current guidelines showed a lack of satisfactory treatment options. Lenalidomide has been successfully used in several hematologic malignancies, such as mantle-cell lymphoma, MM, DLBCL and myelodysplatic syndromes. The multiple mechanisms of action, with immunomodulatory, antiangiogenic and direct antineoplastic properties, represent a strong rationale to investigate the drug, alone or in association, for the treatment of R/R TCL patients. Lenalidomide demonstrated a promising efficacy with manageable toxicity in the

treatment of ATLL, CTCL and nodal TCL, at least comparable to licensed drugs such as mogamulizumab, pralatrexate or romidepsin, even in heavily pretreated patients. Identification of the TFH cell as the cell of origin of several TCLs, including AITL, could explain the high efficacy of lenalidomide in the treatment of R/R AITL cases. To our knowledge, combination strategies did not show an additional benefit compared to monotherapy, even if further investigations are warranted.

In conclusion, lenalidomide as a single-agent prolongs PFS in R/R TCL with an acceptable toxicity and could represent a suitable treatment option for this patient population, especially for neoplasm cases with a TFH origin.

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MINIREVIEWS

Current update on imaging for pancreatic neuroendocrine neoplasms

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Abstract

Pancreatic neuroendocrine neoplasms (panNEN) are a heterogeneous group of tumors with differing pathological, genetic, and clinical features. Based on clinical findings, they may be categorized into functioning and nonfunctioning tumors. Adoption of the 2017 World Health Organization classification system, particularly its differentiation between grade 3, well-differentiated pancreatic neuroendocrine tumors (panNET) and grade 3, poorly-differentiated pancreatic neuroendocrine carcinomas (panNEC) has emphasized the role imaging plays in characterizing these lesions. Endoscopic ultrasound can help obtain biopsy specimen and assess tumor margins and local spread. Enhancement patterns on computed tomography (CT) and magnetic resonance imaging (MRI) may be used to classify panNEN. Contrast enhanced MRI and diffusion-weighted imaging have been reported to be useful for characterization of panNEN and quantifying metastatic burden. Current and emerging radiotracers have broadened the utility of functional imaging in evaluating panNEN. Fluorine-18 fluorodeoxyglucose positron emission tomography (PET)/CT and somatostatin receptor imaging such as Gallium-68 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid–octreotate PET/CT may be useful for improved identification of panNEN in comparison to anatomic modalities. These new techniques can also play a direct role in optimizing the selection of treatment for individuals and predicting tumor response based on somatostatin receptor expression. In addition, emerging methods of radiomics such as texture analysis may be a potential tool for staging and outcome prediction in panNEN, however further investigation is required before clinical implementation.

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Core Tip: Imaging plays a critical role in the diagnosis and management of pancreatic neuroendocrine neoplasms. Enhancement patterns and diffusion-weighted imaging aid the detection and classification of these lesions. Contrast-enhanced magnetic resonance imaging is useful for the evaluation of hepatic metastases. Dual-tracer positron emission tomography/computed tomography with Gallium-68 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid–octreotate and Fluorine-18 fluorodeoxyglucose may be particularly useful for distinguishing grade 3 pancreatic neuroendocrine tumor from pancreatic neuroendocrine carcinoma. Furthermore, these advanced imaging techniques can help in the staging and detection of distant metastases. Evaluation of somatostatin receptor expression and metabolic activity with functional imaging can help select optimal treatment.

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INTRODUCTION

Pancreatic neuroendocrine neoplasms (panNEN) represent a rare, diverse group of neoplasms[1]. These tumors account for less than 2% of pancreatic cancers and only 7% of all neuroendocrine tumors. These entities can manifest at any age but are most often diagnosed in individuals between 40 and 65 years old. The majority of panNEN are sporadic[2]. Up to 10% are associated with hereditary disorders including Von Hippel-Lindau disease, neurofibromatosis type 1, tuberous sclerosis complex, and multiple endocrine neoplasia type 1 (MEN1) syndrome, which increase a patient's predilection for neoplasms. PanNEN can be categorized into functioning and nonfunctioning neoplasms based on clinical findings. Recent discoveries on the mechanisms behind panNEN pathogenesis and molecular cytogenetics have resulted in significant changes regarding their classification, diagnosis, and treatment. In particular, new distinctions in classification between well-differentiated pancreatic neuroendocrine tumors (panNET) and poorly-differentiated pancreatic neuroendocrine carcinomas (panNEC) has emphasized the need for more advanced imaging techniques to guide diagnosis and follow-up[1]. In this review, we will discuss the most current classifications of panNEN based on pathology, genetic, and clinical features. In addition, we will review the use of anatomic imaging modalities like ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) for initial detection and management, along with molecular imaging techniques that have proven useful for identifying occult tumors and further characterization. The potential use of CT, MRI, and positron emission tomography (PET)/CT texture analysis to grade tumors and predict clinical outcome will also be briefly highlighted.

PATHOLOGY

PanNEN demonstrate two histopathological classifications: panNET and panNEC. PanNET account for more than 90% of panNEN and are characterized as well-differentiated neoplasms that manifest with little to moderate atypia. On gross examination, they appear well-circumscribed by a thin capsule. Cystic changes and hemorrhage may be identified. PanNEC can manifest as a small cell or large cell variant. The large cell variation comprises 60% of panNEC and exhibits expansile growth. Small cell panNEC exhibit more infiltrative growth. Necrosis and vascular invasion are com-

monly observed[3].

The 2010 World Health Organization (WHO) classification system for panNEN based categorization on a neoplasm's Ki-67 proliferation index and mitotic index. In this system, when both indices are greater than 20, the tumor is classified as panNEC. Subsequently, many large studies showed the existence of well-differentiated panNET presenting high mitotic and Ki-67 indices. Thus, the 2017 WHO classification system (Table 1) accounts for both the level of proliferation and differentiation of neoplasms, distinguishing a well-differentiated grade 3 panNET from a poorly-differentiated grade 3 panNEC[1,4]. Additional changes include the renaming of mixed adenoneuroendocrine carcinomas to mixed neuroendocrine-nonneuroendocrine neoplasms (MiNEN), in order to reflect their capacity to manifest not only as high-grade, malignant neoplasms, but also as low-grade, benign tumors. MiNEN are composed of both neuroendocrine and non-neuroendocrine components and have relatively non-specific features, tending to mimic panNEC[1].

Although WHO classification relies on pathological features to distinguish grading, single location biopsy may not be an accurate representation of all tumor burden due to the variance within and between lesions. In addition, grade transformation can occur following biopsy. Thus, imaging evaluation and follow-up often play an important role in dictating ongoing and future management, regardless of initial grading.

MOLECULAR CYTOGENETICS

Research focusing on the study of panNEN pathogenesis has significantly broadened the knowledge behind genetic mutations which may influence these lesions and their prognosis. The most common genetic alterations seen in panNET include mutations of the tumor suppressor gene *MEN1*, and chromatin-remodeling genes *ATRX* and *DAXX* [3]. *MEN1* encodes the protein menin, which is involved in histone methylation and cell cycle inhibition. *MEN1* mutations are seen in 31% to 44% of grade 3 panNET, resulting in the disruption of tumor suppression[5]. The majority of these mutations are sporadic, but some may be inherited and seen in association with MEN1 syndrome, Von Hippel Lindau syndrome, neurofibromatosis type 1 and tuberous sclerosis. *ATRX* and *DAXX* mutations are strongly associated with high grade tumors and poor outcomes. A mutation in one of the two genes is observed in more than 45% of well-differentiated neoplasms, and result in an alternative lengthening of telomeres phenotype which correlates with aggressive behavior. *DAXX* abnormalities are also associated with low expression of *TP53*, a tumor suppressor gene that is involved in apoptosis, cell proliferation, and DNA repair. Other molecular abnormalities that may be observed in panNET are mutations in *TSC1* and *TSC2*, *PTEN*, *PIK3CA*, and *DEPDC5*, which all play a role in the mammalian target rapamycin (mTOR) pathway. These mutations occur in approximately 15% of tumors[1,3].

The molecular abnormalities driving panNET do not usually occur in panNEC. Instead, these neoplasms commonly feature mutations in *TP53* and *Rb1*. *KRAS* and *SMAD4* mutations can also occur, but these are less frequent[1].

CLINICAL FEATURES

PanNEN have a wide range of clinical findings, depending on the subtype. The clinical presentation of functioning panNET is influenced by their characteristic hypersecretion of various hormones. Insulinomas account for 60% of functioning panNET and are composed of insulin-producing β cells[3]. They typically manifest with Whipple's Triad (*i.e.* fasting hypoglycemia, symptoms of hypoglycemia, and relief of symptoms following administration of IV glucose)[2]. About 10% of cases will present multiple insulinomas, usually in association with MEN1 syndrome. Gastrinomas represent the second most common functioning panNET. They usually arise in the gastrinoma triangle, a region enclosed by the pancreatic head and neck, the second and third part of the duodenum, and the cystic and common bile duct[1]. Overproduction of gastrin leads to the onset of Zollinger-Ellison syndrome, resulting in peptic ulcer disease, secretory diarrhea, or gastroesophageal reflux disease[3]. Glucagonoma is characterized by its hypersecretion of glucagon. Common manifestations include necrolytic migratory erythema, diabetes mellitus, deep vein thrombosis, and depression[3,6,7]. Other functioning panNET are somatostatinomas, vasoactive intestinal peptide-secreting tumors, and adrenocorticotropic hormone-secreting tumors, which comprise

Table 1 Comparison of 2010 and 2017 World Health Organization classification system for pancreatic neuroendocrine tumors

WHO 2010 Classification system	WHO 2017 Classification system	Ki-67 index (%)	Mitotic index[1]
Well-differentiated PanNET G1	Well-differentiated PanNET G1	< 3	< 2
Well-differentiated PanNET G2	Well-differentiated PanNET G2	3-20	2-20
	Well-differentiated PanNET G3	> 20	> 20
Poorly-differentiated PanNEC G3 (*i.e.* small cell carcinoma, large cell carcinoma)	Poorly-differentiated PanNEC G3 (*i.e.* small cell carcinoma, large cell carcinoma)	> 20	> 20
MiNEN	MANEC		

[1]Per 10 high-power fields. WHO: World Health Organization; PanNEN: Pancreatic neuroendocrine neoplasms; PanNET: Pancreatic neuroendocrine tumors; PanNEC: Pancreatic neuroendocrine carcinomas; MiNEN: mixed neuroendocrine-nonneuroendocrine neoplasms; MANEC: Mixed adenoneuroendocrine carcinomas.

less than 20% of cases[8].

Nonfunctioning panNET are usually asymptomatic until advanced stages, resulting in later presentation and diagnosis. These tumors can secrete polypeptides; however, such secretions do not lead to any associated clinical findings. When symptoms do appear, they are often a result of tumor burden and its mass effect. Up to 50% of nonfunctioning panNET present distant metastases, particularly in the liver, although other locations include the lungs, bone, peritoneum, adrenal glands, brain, and spleen [3]. Similarly, metastatic disease is a common clinical feature of panNEC. A retrospective study reported 88% of panNEC in their cohort demonstrated metastases upon diagnosis[9].

IMAGING FEATURES

Imaging plays a critical role in diagnosing and evaluating panNEN. Conventional modalities like US, CT, and MRI are often used in the initial detection of panNEN. Techniques using PET/CT and novel radiotracers have proven to be extremely useful in the identification and classification of these tumors.

US

On sonography, panNEN usually appear as a well-defined, solid, heterogeneous hypoechoic mass (Figure 1). Some lesions may present with cystic regions[8,10]. Hepatic metastases from panNEN are often hyperechoic in comparison to surrounding liver parenchyma, however they can also manifest as hypoechoic and targetoid lesions. Doppler US reveals increased vascularity. Endoscopic US (EUS) is the preferred modality for detecting small, occult panNEN that are difficult to see with noninvasive techniques[1]. EUS has been reported to have 80% to 90% sensitivity towards panNET, including those that remain undetected on CT and transabdominal US[11-14]. EUS sensitivity towards small insulinomas and duodenal gastrinomas is particularly useful, as these lesions can often be overlooked by other modalities. Following microbubble contrast, panNET show early, intense enhancement on EUS, differentiating these tumors from panNEC or pancreatic ductal adenocarcinoma (PDAC) which are generally hypovascular. Homogeneous enhancement typically indicates a lower Ki-67 index[1]. Other benefits of EUS include its capacity for tissue acquisition using fine needle aspiration or core biopsy; EUS-guided biopsies agree with surgical Ki-67 evaluation in up to 84% of cases[15-18]. Intraoperative US also plays a useful role in some cases by allowing for accurate localization of neoplasms in relation to adjacent structure, thus reducing the risk of postoperative fistulas[1].

CT

CT is commonly used for initial assessment of suspected panNET. Given its high spatial resolution, CT provides excellent diagnostic information with regards to the detection and characterization of the primary tumor and allows assessment of local vascular spread and distant metastatic spread. Typical CT protocol involves multiphasic imaging with pre-contrast acquisition and arterial, pancreatic, and venous phase acquisition following contrast[19]. Pre-contrast images may be useful in cases where there is hemorrhage. Following contrast administration panNEN are generally

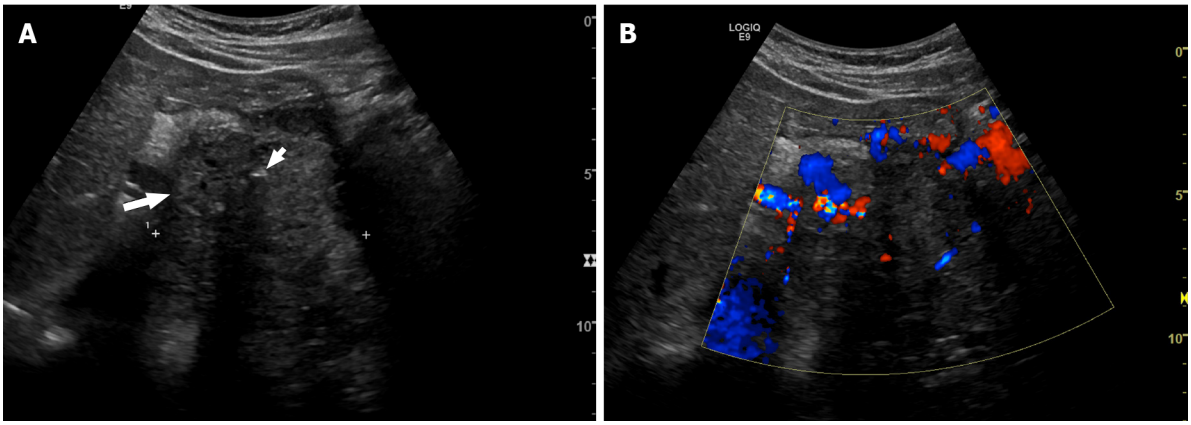

Figure 1 Forty-year-old man with pancreatic neuroendocrine neoplasm. A: Axial ultrasound shows a large solid heterogeneous mass (long arrow). Internal calcification (small arrow) is seen, causing posterior acoustic shadowing; B: Doppler ultrasound shows increased vascularity within the pancreatic tumor.

hyperenhancing (Figure 2) in comparison to surrounding pancreatic tissue on arterial phase and remain mildly hyperattenuating on venous and delayed phases. However, more subtle discrimination of enhancement patterns may allow further classification. Intense, homogeneous enhancement is typical of lower grade panNEN. Grade 1 and 2 neoplasms often appear as small, well-circumscribed lesions, best depicted on arterial phase. These tumors may contain cystic regions in up to 15%-20% of cases[20,21], and are more common in cases associated with MEN1. Pancreatic ductal dilation is more commonly seen in high-grade neoplasms and mixed tumors than well-differentiated panNEN; however, ductal dilation in low-grade tumors may be seen with secretion of serotonin. Grade 3 tumors are characterized as large, ill-defined masses that manifest with mild to low enhancement on arterial phase. They are typically hypointense on portal venous phase imaging. Heterogeneous attenuation due to necrosis and cystic change and the presence of lymphadenopathy or metastatic disease is common.

CT radiomics may be useful for distinguishing the grade of panNEN based on tumor heterogeneity and spatial variation when imaging findings are ambiguous. Texture analysis interprets the distribution of pixel values and position within an image to provide objective, quantitative evaluation of tissue heterogeneity. Guo *et al* [22] found texture parameters such as mean grey-level intensity, entropy, and uniformity demonstrated adequate sensitivity (73%-91%) and specificity (85%-100%) when differentiating grade 1 and 2 panNET from grade 3 panNEC, suggesting texture analysis may be useful for staging panNEN. Mean grey-level intensity showed up to a 100% sensitivity and 91% specificity for distinguishing grade 1 and grade 2 panNET. Canellas and colleagues reported significant differences between low-grade (grade 1) and high-grade (grade 2 and 3) panNEN in texture parameters including skewness, mean of positive pixels, and entropy. However, the only parameter that was an independent predictor of tumor grade was entropy. In addition, further investigation and standardization of postprocessing techniques is required before texture analysis can be applied in a clinical setting[23].

In conjunction with clinical findings, CT can also aid distinguishing functioning from nonfunctioning panNET. Functioning panNET tend to be smaller and more homogenous lesions. Gastrinomas may present ring-like enhancement. Nonfunctioning panNET are usually larger, heterogeneously enhancing masses, and are more likely to exhibit local or vascular spread. Necrosis, cystic changes, and calcifications may be observed[1,8]. Larger nonfunctioning panNET are more likely to exhibit aggressive behavior and often present with metastatic disease.

Hepatic metastases demonstrate intense enhancement on arterial phase imaging and only mildly enhance during the portal venous phase. Similar to gastrinoma, ring-like enhancement may also be seen and can be useful for differentiating panNEN-related metastatic disease from other hepatic lesions[1,11].

MRI

MRI provides improved detection of panNEN and hepatic metastases over abdominal sonography and CT given its superior contrast resolution (Figure 3). MRI enhancement patterns on arterial, venous, and delayed sequences are similar to those seen on CT. Fat-suppressed and diffusion-weighted imaging are particularly useful for identifying small, occult lesions and recognizing associated edema[11]. On MRI,

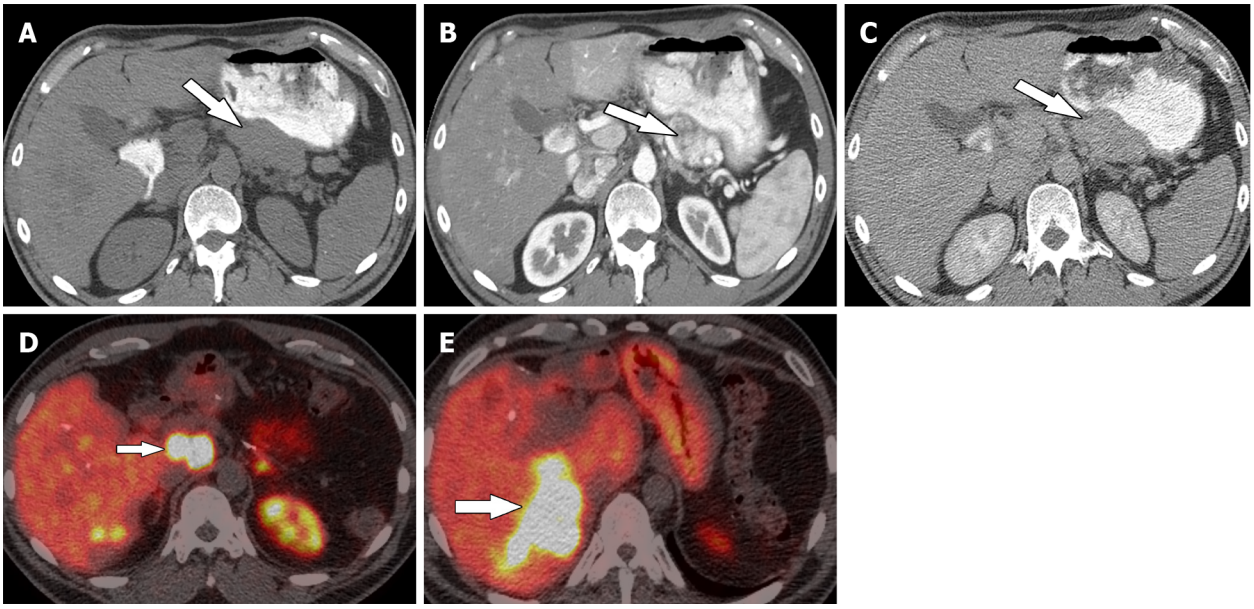

Figure 2 Thirty-eight-year-old woman with pancreatic neuroendocrine neoplasm. A: Axial precontrast computed tomography; B and C: Contrast-enhanced computed tomography in the arterial phase (B) and delayed phase (C) demonstrate pancreatic neuroendocrine neoplasm (arrow). Patient underwent surgical resection; D and E: Follow-up Gallium-68 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid–octreotate positron emission tomography/computed tomography shows metastatic adenopathy (short arrow) and liver metastases (long arrow).

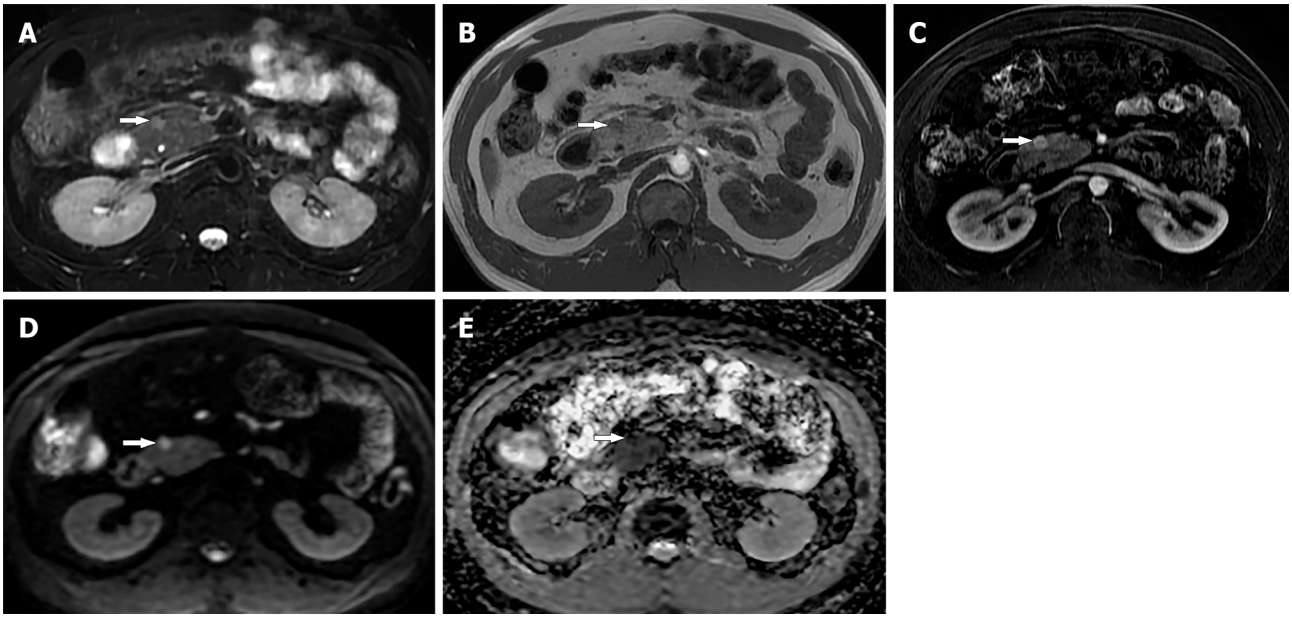

Figure 3 Thirty-five-year-old male with small pancreatic neuroendocrine neoplasm. A: Axial magnetic resonance T2 weighted image; B: T1 weighted image show a small 1 cm mass (arrow) in the head of pancreas; C: Arterial phase image shows avid enhancement in the tumor; D: Diffusion-weighted image; E: Apparent diffusion coefficient map show restricted diffusion within the tumor (arrow). Biopsy confirmed diagnosis of pancreatic neuroendocrine neoplasm.

panNEN typically manifest as hypointense on T1-weighted imaging and isointense on portal venous and delayed phases. Low-grade panNEN tend to exhibit high T2 signal while high-grade neoplasms typically exhibit low to intermediate hyperintensity on T2-weighted imaging[1].

Differentiating between panNEC and grade 3 panNET is challenging on imaging alone (Table 2). PanNEC usually share similar enhancement patterns to grade 3 panNET. Imaging features such as hypoenhancement or rim-like enhancement on arterial phase, persistent enhancement on portal venous phase, and hyperenhancement on delayed phase imaging may favor a diagnosis of panNEC over panNET. On diffusion-weighted imaging, panNEC also demonstrate high signal intensity and low apparent diffusion coefficient (ADC) in comparison to grade 3 panNET[1];

Table 2 Imaging features of grade 3 pancreatic neuroendocrine tumors *vs* grade 3 pancreatic neuroendocrine carcinomas

Grade 3 PanNET	Grade 3 PanNEC
Smaller, more defined lesions	Larger, ill-defined lesions
Absence of ductal dilation or metastatic disease	Ductal dilation or metastatic disease
Low to moderate homogeneous enhancement on arterial phase imaging	Heterogeneous or rim-like enhancement on arterial phase imaging
Hypointense on delayed phase imaging	Atypical persistence of enhancement on delayed phase imaging
Higher ADC values	Signal hyperintensity on diffusion-weighted MRI and lower ADC value
Low uptake on ^{18}F-FDG PET/CT	High uptake on ^{18}F-FDG PET/CT
Moderate uptake on ^{68}Ga-DOTATATE PET/CT	Low uptake on ^{68}Ga-DOTATATE PET/CT

MRI: Magnetic resonance imaging; ADC: Apparent diffusion coefficient; PanNET: Pancreatic neuroendocrine tumors; PanNEC: Pancreatic neuroendocrine carcinomas; ADC: Apparent diffusion coefficient; ^{18}F-FDG: Fluorine-18 fluorodeoxyglucose; PET/CT: positron emission tomography/computed tomography; ^{68}Ga-DOTATATE: Gallium-68 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid–octreotate.

however exact ADC cutoffs vary between studies and are not typically used in clinical practice to differentiate between panNEC and panNET[24-26]. The presence of ductal dilation and metastatic disease may indicate panNEC rather than panNET[1].

MRI is very helpful towards assessing the spread of panNEN to the liver[27]. Hepatic metastases are usually heterogeneously hyperintense on T2-weighted imaging, though atypical presentations include low to moderate T2 intensity. PanNEN hepatic metastases are typically hyperintense on the arterial phase of MRI. A peripheral ring of enhancement with gradual internal enhancement may also occur[1,11,28]. The apparent size of metastases can also vary depending on the dynamic contrast phase on which the dimension is measured. For estimation of tumor load, measurements on the hepatobiliary phase of gadoxetate MRI may be more accurate[29,30]. Histogram analysis of ADC maps could be useful for further indicating the aggressiveness and spread of panNEN. ADC entropy and kurotsis were reported to increase with tumor grade and vascular invasion. These parameters may also be useful for distinguishing panNEN with lymph node or distant metastasis, as both increase with the presence of metastases[31].

Functional imaging

The majority of panNEN express somatostatin receptors, allowing for excellent detection and characterization of these lesions using somatostatin analogs (SSA) coupled with radionuclide tracers. These techniques represent the forefront of panNEN imaging and can help to select patients for peptide receptor radionuclide therapy (PRRT)[1].

Somatostatin receptor scintigraphy (SRS) with Indium 111 (^{111}In)-pentetreotide can identify primary or metastatic disease throughout the body with 77% sensitivity and provides functional information on tumor somatostatin receptor expression[1,8]. However, SRS is limited due to its nonspecific uptake in other organs and inflammatory tissues. In addition, its poor spatial resolution and comparatively low affinity for somatostatin receptors has led to the adoption of substantially superior PET/CT techniques[32].

Gallium-68 (^{68}Ga) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)–octreotate, more commonly called ^{68}Ga-DOTATATE, has demonstrated consistently high specificity (81%-100%) and sensitivity (90%-100%) as a PET agent for panNET[33,34] (Figure 4). ^{68}Ga-DOTATATE PET/CT is particularly useful for distinguishing low-grade, well-differentiated panNEN, which show greater ^{68}Ga-DOTATATE uptake than high-grade panNEN. Grade 3 panNET exhibit moderate uptake, while panNEC exhibit relatively poor uptake[1]. Physiological uptake in the pancreatic uncinate process is observed in up to one-third of individuals. The European Association of Nuclear Medicine (EANM) recommends disregarding uptake in the pancreatic uncinate process unless corresponding imaging findings are seen [35]. Other ^{68}Ga-DOTA-peptides include DOTATOC and DOTANOC, which are reported to have similar diagnostic yields as to ^{68}Ga-DOTATATE.

The decrease of somatostatin receptors seen in higher grade, less differentiated neoplasms is accompanied by an increase in metabolic activity, making Fluorine-18 fluorodeoxyglucose (^{18}F-FDG) PET an ideal technique for identifying these lesions. Grade 3 tumors have a reported median maximum standardized uptake value of 11.7

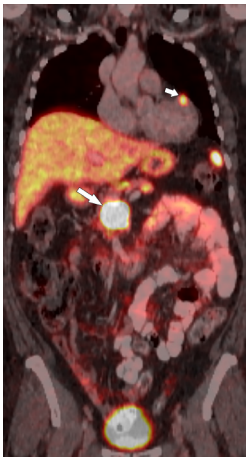

Figure 4 Sixty-two-year-old female with metastatic pancreatic neuroendocrine neoplasm. Coronal fused Gallium-68 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid–octreotate (DOTATATE) positron emission tomography/computed tomography shows a large soft tissue mass in the pancreatic head with intensely avid DOTATATE uptake. Note the subtle metastatic lesion in the pericardium (short arrow) along the left atrium.

for ^{18}F-FDG, *vs* 4.4 for ^{68}Ga-DOTATATE[1]. Conversely, tumors with a Ki-67 index lower than 10% showed minimal ^{18}F-FDG uptake, but high ^{68}Ga-DOTATATE uptake [36]. Dual-tracer PET/CT with ^{68}Ga-DOTATATE and ^{18}F-FDG may be useful for distinguishing grade 3 panNET from panNEC, as higher uptake of ^{68}Ga-DOTATATE indicates grade 3 panNET, while higher uptake of ^{18}F-FDG indicates panNEC[1,35]. The use of SSA-PET/CT combined with texture analysis may also be a useful indicator of prognosis. A multi-center retrospective study demonstrated higher entropy could predict greater overall survival[37].

A minority of insulinomas (< 10%) are negative on all conventional modalities due to their small size[35]. In such instances, SSA-PET/CT is a poor alternative, with a reported 25% sensitivity and specificity[38-43]. ^{18}F-dihydroxyphenylalanine (^{18}F-DOPA) PET/CT may aid localization of insulinomas, offering high sensitivity in cases of hyperinsulinemic hypoglycemia. However, this technique frequently results in positive findings for non-neuroendocrine pancreatic lesions and is only indicated for detecting non-pancreatic NENs by 2017 EANM guidelines[44]. Carbidopa premedication may increase ^{18}F-DOPA specificity towards insulinomas by inhibiting physiologic uptake. Multiple retrospective studies with small cohorts using ^{18}F-DOPA and carbidopa premedication have demonstrated insulinoma detection rates of 70-85% [45-47]. However, further investigation into the role of ^{18}F-DOPA PET/CT in panNEN is required.

Glucagon-like peptide receptor (GLP-1R) PET/CT may also prove useful for detecting insulinomas. The majority of benign insulinoma express GLP-1R, resulting in a sensitivity on GLP-1R -based PET/CT of more than 95%[47,48]. However, uptake in the pancreatic tail can be mistaken for physiological renal accumulation of radionuclides; uptake by duodenal Brunner gland may be mistaken for an insulinoma in the pancreatico-duodenal groove. In addition, malignant insulinomas express GLP-1R considerably less than their benign counterparts [35,49].

DIFFERENTIAL DIAGNOSIS

The differential diagnosis for panNEN includes other hypervascular pancreatic lesions. Pancreatic metastases from renal cell carcinoma, melanoma, and sarcoma may often appear as hypervascular masses resembling panNEN. In particular, renal cell carcinoma may present with late onset metastasis in pancreas, even 5 to 10 years following treatment of the primary tumor, causing diagnostic dilemma. A history of previous primary malignancies should alert to the possibility of pancreatic metastases over panNEN. Serous cystadenomas represent another possible mimic of panNEN, particularly the rare subset of cases which may appear solid on CT. T2-weighted usually reveals presence of multiple septated cysts in serous cystadenomas which may occasionally not be apparent on CT. Lack of uptake on ^{68}Ga-DOTATATE PET/CT is also useful for separating serous cystadenomas from panNEN. Intrapancreatic accessory splenules in the pancreatic tail may be another potential pitfall causing

diagnostic confusion, especially if only a single-phase CT is available. However, on MRI this diagnosis is generally straightforward. Splenules will have the same appearance as normal spleen on all MR sequences including T1-weighted, T2-weighted, diffusion-weighted and postcontrast sequences. In cases of diagnostic difficulty, uptake on technetium 99m (^{99m}Tc)-labeled heat-damaged red blood cell-tagged or ^{99m}Tc-labeled sulfur colloid scans may help. Cystic panNEN may be mistaken for other cystic pancreatic entities such as mucinous cystic neoplasms, in which case EUS-guided fine needle aspiration might be necessary to confirm diagnosis [1].

Distinguishing the typical well-differentiated panNET from PDAC is usually straightforward, as panNET typically are hypervascular, well-defined and do not typically cause ductal obstruction. Nevertheless, the imaging appearance of panNEC often overlap with PDAC given their shared hypovascularity and ill-defined borders. These similar radiologic findings may result in misdiagnosis of up to 57% of panNEC as PDAC[31]. Decreased portal phase enhancement and a lower enhancement ratio between arterial and portal phase may raise suspicion for PDAC over nonhypervascular panNEN[50-52]. Features that are more common in panNEC include tumoral calcification and vascular invasion[1,31]. CT texture analysis may be useful as panNEC typically demonstrate more intratumoral homogeneity than PDAC. Consequently, panNEC demonstrate higher uniformity and lower entropy than PDAC at portal phase imaging[50]. Texture analysis based on ADC values may also improve diagnostic capabilities; ADC histogram analysis of diffusion-weighted imaging revealed PDAC demonstrate higher kurtosis and skewness on ADC_{400} and ADC_{800} than panNEN, overall. PanNEN exhibited significantly lower entropy regardless of *b* value [31]. However, definitive discrimination between panNEC and PDAC using imaging alone is difficult, and histological diagnosis is usually warranted.

MANAGEMENT

The management of panNEN varies with their classification and the degree of local and metastatic spread. Localized, asymptomatic panNET less than 2 cm in size are usually treated conservatively with active surveillance[53]. However, larger or symptomatic panNEN require more comprehensive treatment such as symptom-directed therapy, SSA therapy, molecularly-targeted and conventional chemotherapy, or peptide receptor radionuclide therapy. Liver-specific therapy may be used to treat hepatic metastases[54,55].

Surgical resection and debulking

Surgical resection is currently used for nonfunctioning tumors larger than 2 cm, and functioning panNET of any size. Accurate tumor localization is critical for operative success. ^{68}Ga-DOTATATE PET/CT is the preferred imaging study for evaluating the spread of noninsulinoma panNET[54]. Selective arterial calcium stimulation with hepatic venous sampling is occasionally used to localize insulinomas that are difficult to assess on anatomic imaging. The emergence of GLP-1R PET/CT represents a superior alternative to this technique[54,56,57]. Simple enucleation may be sufficient for smaller, low-grade tumors that are at least 2-3 mm away from the main pancreatic duct. MR cholangiopancreatography and EUS are useful for estimating this distance [54]. The majority of functioning panNET require more extensive resection and lymphadenectomy[1]. A total pancreatectomy may be considered for multifocal disease. Noncurative surgical debulking may be pursued in cases of unresectable, metastatic panNEN to palliate symptoms and extend survival[1]. However, advanced disease is typically managed with non-surgical treatment strategies (Figure 5).

SAA therapy

SSA such as octreotide or lanreotide is often used in the management of advanced, progressive tumors. In addition to their antisecretory benefits these drugs have cytostatic effects on the tumor, as proven by the multicenter, phase III CLARINET and PROMID trials which demonstrated an increase in estimated progression-free survival [58,59]. However, this effect appears to be diminished in tumors that do not show adequate uptake on somatostatin imaging techniques. Koch *et al*[60] reported a 2.9-fold increased probability of achieving stable disease following SSA therapy in neuroendocrine tumors with high uptake on ^{68}Ga-DOTATATE PET, in comparison to tumors with low uptake. Thus, a multidisciplinary panel of experts convened by the Society for Medicine and Molecular Imaging (SNMMI) suggested the potential utility of ^{68}Ga-DOTATATE PET/CT in selecting patients with nonfunctioning panNET for soma-

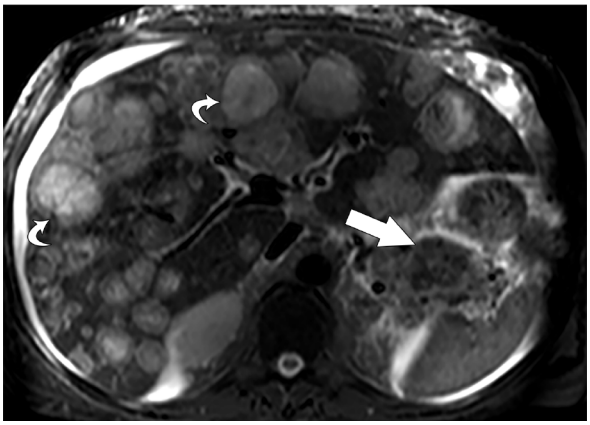

Figure 5 Thirty-nine-year-old male with metastatic pancreatic neuroendocrine neoplasm. Axial T2 weighted image shows innumerable bilobar metastases (curved arrows). Note the heterogeneous primary pancreatic neuroendocrine tumor (straight arrow). Patient was treated with capecitabine and temozolomide.

tostatin analog therapy. However, the SNMMI expert panel agreed that in the case of symptomatic manifestations, SSA therapy is indicated regardless of imaging findings [61].

Molecularly targeted chemotherapy

Molecularly targeted chemotherapy using agents such as everolimus, an mTOR inhibitor, and sunitinib, a tyrosine kinase inhibitor, have been reported to improve progression-free survival of individuals with grade 3 panNET and metastatic disease [1]. Early studies on emerging agents including multi-targeted kinase inhibitors and a combination of temsirolimus and bevacizumab, also show positive results[55,62].

Conventional chemotherapy

Although SSA and molecular therapies have shown significant benefits in patients with panNEN, conventional chemotherapy or PRRT is preferred for highly symptomatic patients and those with rapidly growing metastases. A streptozocin-based regimen or a combination of temozolomide and capecitabine is the optimal approach for panNET[55]. Platinum-based chemotherapies such as cisplatin with etoposide or irinotecan are the regimen of choice for panNEC, with reported response rates of 60% [1,63].

Peptide receptor radionuclide therapy

Peptide receptor radionuclide therapy (PRRT) uses SSA to deliver radionuclides such as yttrium-90 (^{90}Y) and lutetium-177 (^{177}Lu). These agents deliver beta radiation or high energy electrons, causing localized cellular necrosis at the site of accumulation, and have been associated with promising outcomes in grade 1 and 2 panNET. One phase II, single-center clinical trial demonstrated an increase in median survival by 26 mo in neuroendocrine tumor patients treated with PRRT[64-68]. However, PRRT may be less useful in panNEC due to their lower somatostatin receptor expression[1]. In addition, panNET with lower expression of somatostatin receptors may be susceptible to a similar decrease in response rate. Multiple studies propose the use of the "NETPET" scoring system developed by Chan and colleagues, and similar PET/CT-dependent classification, to select patients for PRRT[69-72]. In the NETPET system, tumors are graded from P1 to P5 based on their avidity on ^{68}Ga-DOTATATE and ^{18}F-FDG PET, with a score of 1 indicating positive results on ^{68}Ga-DOTATATE but not ^{18}F-FDG PET, and a score of 5 indicating positive results on ^{18}F-FDG PET but not on ^{68}Ga-DOTATATE PET. However, further investigation into the correlation between PRRT outcome and NETPET scores must be done to establish if such imaging-based classification systems have a role in clinical settings. Other methods for predicting PRRT response include the measurement of skewness and kurtosis based off ^{68}Ga-DOTATATE imaging; Önner *et al*[73] reported significantly higher skewness and kurtosis in tumors which did not response to treatment that those that did. Nevertheless, the diagnostic ability of the two metrics to indicate poor PRRT response remained moderate to low.

Liver-specific therapy

In the presence of hepatic metastases, liver-directed therapies including partial hepatectomy, ablation, or arterial chemo- and radioembolization may be useful. Resection is usually contraindicated in the presence of multifocal extrahepatic metastases, high-grade and poorly-differentiated carcinoma, liver disfunction, or diffuse bilobar involvement[55]. Previously, resection was only recommended if more than 90% of disease could be removed but more recent literature supports lowering this threshold to 70%[74-76]. Ablation is often reserved for the treatment of small metastases that do not qualify for surgical resection or may be done in addition to resection in the presence of multifocal disease. Arterial embolization, radioembolization, and chemoembolization can be used to diminish the secretory effects of functioning panNET. Liver transplantation is only considered in patients with significant hepatic tumor burden, without the presence of extrahepatic metastases, and is not routinely undertaken in metastatic panNET[1,55]. ^{68}Ga-DOTATATE PET/CT may be useful for determining suitability of patients for transplantation, as this technique allows for a whole-body acquisition in order to assess potential extrahepatic metastatic disease[35].

Symptom-directed therapy

Symptom-directed therapy plays an important role in the management of functioning panNETs. Treatment varies with each functioning panNET; common interventions include the use of diazoxide to suppress insulin secretion in insulinomas, and proton pump inhibitors to suppress hypersecretion by gastrinomas. Long-acting SSA may also be useful for controlling the secretory effects of these tumors, particularly vasoactive intestinal peptide-secreting tumors and glucagonomas[55].

CONCLUSION

Better understanding of the genetic and biological features of panNEN has led to significant changes in the diagnosis and management of these tumors. Imaging is crucial for diagnosing and staging of panNEN. CT and MR play a vital role in differentiating these tumors from other benign and malignant lesions of the pancreas. Recent studies indicate enhancement pattern of panNEN on cross sectional imaging and texture analysis may also be helpful in classifying these tumors or indicating prognosis. Diagnosis of panNEN is typically confirmed with EUS guided biopsy. Functional imaging techniques including SRS and PET/CT are very helpful in the management of panNEN. ^{68}Ga-DOTATATE and GLP-1R-based PET/CT may improve detection of occult lesions and their characterization. These techniques also have the potential to guide management, as information on somatostatin receptor expression and metabolic activity are useful for determining optimal treatment.

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MINIREVIEWS

Recent advances and new insights in the management of early-stage epidermal growth factor receptor-mutated non-small-cell lung cancer

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Abstract

Patients with early-stage non-small-cell lung cancer (NSCLC) are candidates for curative surgery; however, despite multiple advances in lung cancer management, recurrence rates remain high. Adjuvant chemotherapy has been demonstrated to significantly prolong overall survival (OS), but this benefit is modest

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and there is an urgent need for effective new therapies to provide a cure for more patients. The high efficacy of tyrosine kinase inhibitors (TKIs) against epidermal growth factor receptor-mutated (EGFR) in patients with advanced EGFR-mutated NSCLC has led to the evaluation of these agents in early stages of the disease. Multiple clinical trials have evaluated the safety and efficacy of EGFR TKIs as an adjuvant treatment, in patients with resected EGFR-mutated NSCLC, and shown that they significantly prolong disease-free survival (DFS), but this benefit does not translate to OS. Recently, an interim analysis of the ADAURA trial demonstrated that, surprisingly, osimertinib improved DFS. This led to the study being stopped early, leaving many unanswered questions about its potential effect on OS and its incorporation as a standard adjuvant treatment in this patient subgroup. These targeted agents are also being evaluated in locally-advanced disease, with promising results, although prospective studies with larger sample sizes are needed to confirm these results. In this article, we review the most relevant studies on the role of EGFR TKIs in the management of early-stage EGFR-mutated NSCLC.

Key Words: Non-small-cell lung cancer; Early stage; Epidermal growth factor receptor-mutated; Epidermal growth factor receptor-mutated-tyrosine kinase inhibitor; Adjuvant; Neoadjuvant

Core Tip: Epidermal growth factor receptor-mutated (EGFR) tyrosine kinase inhibitors (TKIs) have changed the natural history of advanced EGFR-mutated non-small-cell lung cancer (NSCLC). Multiple clinical trials conducted in the adjuvant setting have shown that EGFR TKIs prolong disease-free survival (DFS) but not overall survival (OS). Osimertinib demonstrated a surprising improvement in DFS in an interim analysis of the ADAURA study, which led to the study being stopped early, and left many unanswered questions about its potential effect on OS. Locally-advanced disease is also an attractive situation for assessment of the efficacy of these agents, with encouraging results so far. We discuss the recent advances in the management of early-stage EGFR-mutated NSCLC.

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INTRODUCTION

Non-small-cell lung cancer (NSCLC) represents 85% of all lung cancers, and more than 50% of patients with NSCLC are diagnosed in advanced stages[1,2]. Only 25%-30% are diagnosed in early stages, making them candidates for curative surgical treatment[3-6]; however, more than 50% of these patients go on to have recurrence and die from the disease[3,6-9]. NSCLC has a high metastatic potential, even in early stages, and the aim of adjuvant treatment is to eradicate residual micrometastases[10]. Platinum-based adjuvant chemotherapy has been shown to prolong overall survival (OS), but with an absolute improvement in 5-year OS of only 4%[11,12]. Therefore, there is a need for new effective and minimally-toxic treatments to increase the cure rate.

Treatment with EGFR tyrosine kinase inhibitors (TKIs) in patients with metastatic EGFR-mutated NSCLC has been demonstrated to increase survival more than chemotherapy, changing the natural history of the disease in this subgroup of patients [13-17]. This has raised the question of whether a molecularly-targeted adjuvant treatment with EGFR TKIs could improve the modest benefit afforded by chemotherapy in patients with completely-resected EGFR-mutated NSCLC. Multiple EGFR

TKIs have been assessed in this setting and have shown a significant benefit in disease-free survival (DFS) but not OS[9,18-20].

Unresectable and potentially-resectable locally-advanced disease are also attractive settings for evaluating the role of these agents. Multiple phase II clinical trials have shown encouraging results[9,21-23], although many questions remain to be answered.

In this review, we discuss the most relevant studies evaluating the role of EGFR TKIs in resectable, potentially-resectable, and unresectable locally-advanced NSCLC with EGFR-activating mutation.

EGFR TKIS AS TREATMENT FOR RESECTABLE DISEASE

EGFR mutations are the most common oncogenic drivers in NSCLC, occurring in 10-15% of Caucasian patients[24-26] and approximately 30% of patients in Latin America [27], while in the Asian population the prevalence of EGFR mutations is significantly higher, around 50%[28,29].

The presence of EGFR-activating mutations in patients with NSCLC confers high sensitivity to treatment with EGFR TKIs. In phase III clinical trials, multiple EGFR TKIs have shown dramatic, long-lasting responses that have translated to longer survival[13-17,30-36], never before seen in patients with advanced NSCLC treated with chemotherapy, positioning these targeted agents as the standard treatment in patients with advanced EGFR-mutated NSCLC.

The prevalence of EGFR mutations in NSCLC, the results observed in advanced disease, and the clinical need for new treatments to help cure more patients in early stages have led to the evaluation of EGFR TKIs as adjuvant therapy (Table 1).

Initially, studies were carried out in a population that was not selected for the presence of EGFR mutations. Based on the rationale that high EGFR expression in NSCLC confers aggressiveness and poor response to chemotherapy, Goss *et al* [37] conducted the phase III trial BR19, which compared gefitinib for 2 yr *vs* placebo in 503 patients with resected stage IB-IIIA NSCLC, and found no differences in DFS or OS between the two arms. There were only 15 patients with EGFR mutations, and no benefit was observed for gefitinib in this small subgroup, either in DFS [hazard ratio (HR): 1.84, 95% confidence interval (CI): 0.44-7.73; $P = 0.395$], or OS (HR 3.16, 95%CI: 0.61-16.45; $P = 0.15$)[37]. Similarly, the phase III trial RADIANT evaluated erlotinib for 2 yr *vs* placebo, after completion of standard adjuvant treatment, in 973 patients with resected stage IB-IIIA NSCLC with EGFR expression/amplification. There were no significant differences in DFS or OS between the two arms. However, when the data from the 161 patients with EGFR-activating mutations were analysed, DFS was better with erlotinib (46.4 *vs* 28.5 mo; HR: 0.61, 95%CI: 0.384-0.981; $P = 0.039$), but this did not reach statistical significance due to the hierarchical analysis established in the study design. The 2-year DFS was 75% and 54% for erlotinib and placebo, respectively[38]. Although there was a marked difference between the two study arms in patients with EGFR mutation, it should be noted that certain imbalances in the patient characteristics may have influenced these results (more patients with stage IB in the erlotinib arm; in the placebo arm, more patients were in stage IIIA and 44% of patients did not receive previous adjuvant chemotherapy).

Following the discovery that the presence of EGFR mutation favours response to EGFR TKIs, multiple trials have been performed to evaluate these agents as adjuvant treatment in patients with EGFR mutations. SELECT was a phase II single-arm trial that included 100 patients with resected stage IA-IIIA EGFR-mutated NSCLC, who, after completing standard adjuvant treatment, received erlotinib for 2 years. The 2-year DFS was 88%, which was significantly higher than the 76% observed in historic controls ($P = 0.0047$). The 5-year DFS and OS were 56% and 86%, respectively. It is important to mention that of the 40 patients who had disease recurrence, this occurred during treatment in only 4; in the other 36 it occurred after stopping erlotinib[39]. In the phase III trial ADJUVANT/CTONG1104, 222 patients with resected stage II-IIIA EGFR-mutated NSCLC were randomly assigned to receive gefitinib for 2 yr or cisplatin plus vinorelbine for 4 cycles. With a median follow-up of 36.5 mo, the median DFS was significantly longer with gefitinib than with cisplatin plus vinorelbine (28.7 *vs* 18 mo; HR: 0.60, 95%CI: 0.42-0.87; $P = 0.0054$)[40]. However, with longer follow-up, no statistically significant difference was observed between the two arms for 3-year DFS (39.6% *vs* 32.5%; $P = 0.316$), 5-year DFS (22.6% *vs* 23.2%; $P = 0.928$), or OS (75.5 *vs* 62.8 mo; HR: 0.92, 95%CI: 0.62-1.36; $P = 0.674$)[41]. Likewise, Tada *et al* conducted the phase III IMPACT study, which included 234 patients with resected stage II-III EGFR-mutated NSCLC randomized to receive gefitinib for 2 yr or cisplatin plus vinorelbine

Table 1 Clinical trials of adjuvant epidermal growth factor receptor-mutated tyrosine kinase inhibitors in epidermal growth factor receptor-mutated non-small-cell lung cancer

Clinical trial	Type of trial	Sample size	Primary outcome	Stage	Treatment	Previous adjuvant chemotherapy	TKI Duration	DFS	OS
BR19, Goss *et al*[37]	Phase III	503 (15 with EGFR mutation)	OS	IB-IIIA	Gefitinib *vs* placebo	Yes (17% in gefitinib arm and 17% in placebo arm)	2 yr	HR 1.84; $P = 0.395$[1]	HR: 3.16; $P = 0.15$[1]
RADIANT, Kelly *et al*[38]	Phase III	973 (161 with EGFR mutation)	DFS (ITT population)	IB-IIIA	Erlotinib *vs* placebo	Yes (45.1% in erlotinib arm and 55.9% in placebo arm)[1]	2 yr	46.4 *vs* 28.5 mo; HR: 0.61; $P = 0.039$[1]	Median OS NR in both arms; HR: 1.09; $P < 0.001$[1]
SELECT, Pennell *et al*[39]	Phase II	100	2-yr DFS	IA-IIIA	Erlotinib	Yes (not reported)	2 yr	Mean DFS NR; 2-yr DFS 88%; 5-yr DFS 56%	Median OS NR, 5-yr OS 86%
ADJUVANT/CTONG1104, Zhong *et al*[40,41]	Phase III	222	DFS	II-IIIA	Gefitinib *vs* cisplatin-vinorelbine	No	2 yr	28.7 *vs* 18 mo; HR: 0.60; $P = 0.0054$ 3-yr DFS 39.6% *vs* 32.5%; $P = 0.316$ 5-yr DFS 22.6% *vs* 23.2%; $P = 0.928$	75.5 *vs* 62.8 mo; HR: 0.92; $P = 0.674$
IMPACT, Tada *et al*[42]	Phase III	234	DFS	II-III	Gefitinib *vs* cisplatin-vinorelbine	No	2 yr	36 *vs* 25.2 mo; HR: 0.92; $P = 0.63$	Median OS NR in both arms; HR: 1.03; $P = 0.89$
EVAN, Yue *et al*[43]	Phase II	102	2-yr DFS	IIIA	Erlotinib *vs* cisplatin-vinorelbine	No	2 yr	42.4 *vs* 21 mo; HR: 0.268; $P < 0.0001$ 2-yr DFS 81.4% *vs* 44.6%; $P = 0.0054$ 3-yr DFS 54.2% *vs* 19.8%; $P = 0.0460$	Median OS NR in both arms; HR: 0.165; $P = 0.0013$
Neal *et al*[44]	Phase II	46	2-yr DFS	IA-IIIA	Afatinib 3 mo *vs* 2 yr	Yes (52% in 3-mo arm and 45% in 2-yr arm)	3 mo *vs* 2 yr	42.8 *vs* 58.6 mo 2-yr DFS 81% *vs* 70%; $P = 0.55$	Median OS NR in both arms
ADAURA, Wu *et al*[45]	Phase III	682	DFS in stages II-IIIA	IB-IIIA	Osimertinib *vs* placebo	Yes (60% in both arms)	3 yr	Stages II-IIIA: NR *vs* 19.6 mo; HR: 0.17; $P < 0.001$ 2-yr DFS 90% *vs* 44%ITT: NR *vs* 27.5 mo; HR: 0.20; $P < 0.001$ 2-yr DFS 89% *vs* 52%	Median OS NR in both arms (immature OS data)

[1]Results in EGFR-mutated population. EGFR: Epidermal growth factor receptor; DFS: Disease-free survival; HR: Hazard ratio; ITT: Intention to treat; NR: Not reached; OS: Overall survival; TKI: Tyrosine kinase inhibitor.

for 4 cycles. The results were recently reported, with no differences observed in DFS (36 *vs* 25.2 mo; HR: 0.92, 95%CI: 0.67-1.28; $P = 0.63$) or OS (median not reached in either arm; HR: 1.03, 95%CI: 0.65-1.65; $P = 0.89$) between the two arms[42]. The ADJUVANT/CTONG1104 and IMPACT trials, with similar designs, showed an initial separation of the DFS curves, which overlap around 48 mo, suggesting that adjuvant treatment with EGFR TKIs only delays relapse.

The lack of results demonstrating a benefit in OS and the heterogeneous populations (stages IA-IIIA) included in the various clinical trials prompted the phase II

randomised trial EVAN, which evaluated erlotinib for 2 yr *vs* cisplatin plus vinorelbine for 4 cycles, as an adjuvant treatment, in a specific population of 102 patients with resected stage IIIA EGFR-mutated NSCLC who had received no previous treatment[43]. The median DFS was significantly longer with erlotinib than with chemotherapy (42.4 *vs* 21 mo; HR: 0.268, 95%CI: 0.136-0.531; $P < 0.0001$). Both 2-year DFS (81.4% *vs* 44.6%; $P = 0.0054$), and 3-year DFS (54.2% *vs* 19.8%; $P = 0.0460$) were significantly higher with erlotinib. However, this study had several limitations, including the small sample size and the high percentage of patients (35%) in the chemo-therapy arm who did not meet the per protocol population criteria (8 patients who did not receive chemotherapy and 11 major protocol deviations), which could have influenced the difference in DFS between the two study arms. Furthermore, in this study, PET scan was not performed as part of screening; this, in addition to the patients with stage IIIA having a high probability of micrometastatic disease[10], means that a percentage of patients, rather than an adjuvant treatment, could have been receiving treatment for advanced disease—a situation in which it is already known that EGFR TKIs are superior to chemotherapy. Afatinib, the second-generation EGFR TKI, which was the first to demonstrate a benefit in OS in patients with advanced EGFR-mutated NSCLC[13], was also assessed as an adjuvant, in a phase II clinical trial comparing afatinib for 2 yr *vs* afatinib for 3 mo, in 46 patients with resected stage IA-IIIA EGFR-mutated NSCLC, who had previously received standard adjuvant treatment. The 2-year DFS was numerically higher with 2 yr of afatinib than with 3 mo (81% *vs* 70%; $P = 0.55$), but this difference did not reach statistical significance, although it must be recognised that certain limitations of the study such as the small sample size and low percentage of patients who completed treatment in the 2-year group (41%) could have influenced the lack of statistical significance[44].

Osimertinib, a third-generation EGFR TKI, was evaluated as first-line treatment for EGFR-mutated NSCLC in the phase III trial FLAURA, showing longer survival and greater central nervous system (CNS) efficacy than erlotinib or gefitinib[15,16]. The high efficacy demonstrated by this agent in advanced disease and the lack of robust results supporting the use of EGFR TKIs as adjuvant treatment led Wu *et al* to conduct the phase III trial ADAURA. This included 682 patients with resected stage IB-IIIA EGFR-mutated NSCLC, who, after completing standard adjuvant chemotherapy, were randomly assigned to receive osimertinib or placebo for 3 yr. The primary outcome of the study was DFS in patients in stages II-IIIA. After an interim analysis that was not planned as part of the protocol, the independent monitoring committee recommended unblinding of the study, due to evidence of a clear benefit in favour of osimertinib. In patients with stage II-IIIA disease, osimertinib markedly improved DFS (not reached *vs* 19.6 mo; HR: 0.17, 99.06%CI: 0.11-0.26; $P < 0.001$) in comparison with placebo, the 2-year DFS being 90% and 44%, respectively. These results were consistent in the total population (median not reached *vs* 27.5 mo; 2-year DFS 89% *vs* 52%; HR: 0.20, 99.12%CI: 0.14-0.30; $P < 0.001$), and a reduction was also observed in risk of CNS recurrence or death (HR 0.18, 95%CI: 0.10-0.33)[45].

While the benefit observed with osimertinib in terms of DFS was striking, many questions were raised regarding whether these results, with a median follow-up of 22 mo in an adjuvant trial, were sufficient to position osimertinib as a standard treatment in this situation. Recently, Zhong *et al*[41] published the updated data from the ADJUVANT/CTONG1104 trial, confirming a benefit in DFS, but which ultimately did not translate to an OS benefit. In addition, multiple meta-analyses have analysed the role of EGFR TKIs as adjuvant treatment in patients with NSCLC with an EGFR-activating mutation, showing a benefit in DFS but not OS[46-48].

Although, overall, the trials with first- and second-generation TKIs showed a benefit in DFS, the high number of recurrences after stopping adjuvant treatment with EGFR TKIs in the different studies was striking. In the ADJUVANT/CTONG1104 trial, the difference in DFS observed between the two arms was smaller with a longer follow-up [41], while in the SELECT trial, 90% of recurrences occurred after stopping erlotinib [39]. These findings suggest that adjuvant treatment with EGFR TKIs delays recurrence but does not prevent it, and therefore does not appear to be able to change the natural history of the disease by curing more patients.

Although multiple studies have evaluated the role of EGFR TKIs as adjuvant therapy, the question of whether previous adjuvant chemotherapy is necessary remains unanswered. An indirect comparison of the DFS results from RADIANT, SELECT, and the phase II afatinib trial with those from the ADJUVANT/CTONG1104 trial suggests that giving an EGFR TKI after standard adjuvant treatment provides a greater benefit in DFS than giving an EGFR TKI as a sole adjuvant treatment[18]. In the ADAURA trial, 60% of patients in the osimertinib arm received adjuvant chemotherapy, which could have led to a greater benefit in the experimental arm, and the

lack of adjuvant chemotherapy in 40% of the control arm patients could have led to a more marked difference between the two arms.

It should be borne in mind that EGFR TKIs given for a prolonged period cause toxicity[38,39,44,45], which can negatively affect quality of life in patients who are considered disease-free. If we were to treat all patients with resected EGFR-mutated NSCLC with adjuvant EGFR TKIs, we would be over-treating a group of patients that may already be cured, meaning we would not be adding any benefit and only worsening their quality of life.

Another point under discussion is the response to EGFR TKIs in patients with recurrence after receiving adjuvant therapy. In the SELECT study, only one patient with recurrence during erlotinib treatment was found to have a T790M mutation, and 65% of patients with recurrence were retreated with erlotinib, reaching a median treatment duration of 13 mo[39]. Likewise, in the ADJUVANT/CTONG1104 trial, 36.8% of patients with recurrence in the gefitinib arm were treated with EGFR TKIs, achieving a response rate of 46.4%[41]. While the results available so far suggest that adjuvant treatment with EGFR TKIs does not appear to affect sensitivity to these agents in patients with recurrence, there is still insufficient evidence and we cannot draw definitive conclusions regarding the potential development of resistance to these agents.

Targeted treatments and immunotherapy have been shown to significantly prolong OS in advanced NSCLC; however, high prices make access difficult, so many patients cannot benefit from these agents. Osimertinib is a very expensive drug and the ADAURA study proposed a prolonged treatment, so it is reasonable to demand a strong benefit in OS that justifies its use, especially as there would be a group of patients receiving adjuvant osimertinib who may already be cured and would therefore be overtreated at the expense of toxicity and a very high economic cost[19,49,50].

Finally, a significant improvement in DFS that does not translate to a significant improvement in OS has been a constant finding in adjuvant studies of EGFR TKIs, which raises the issue of whether DFS is a suitable primary outcome in adjuvant studies[19]. Although, in the past, DFS was considered a surrogate for OS in NSCLC [51], this is only applicable for chemotherapy[50], and nowadays, in the era of targeted therapies, with more treatment options available, there is a greater probability that OS will be affected by subsequent treatments, as has been seen in multiple studies with EGFR TKIs in patients with advanced disease[52]. One possible explanation for the lack of OS benefit in adjuvant studies is that, in patients in the control arm, treatment with EGFR TKIs at the time of recurrence could have attenuated a potential benefit in OS, if present[20]. This makes us question whether we really should treat all these patients with adjuvant EGFR TKIs, if treating only patients with recurrence would achieve the same results. We must await the OS results from the ADAURA trial, but it is likely that these will be affected by the early termination of the study[19], and that we will never know if this dramatic benefit in DFS translates to a higher patient cure rate. Currently, the phase III trial ALCHEMIST (A081105) is underway, which compares erlotinib for 2 yr *vs* placebo, in patients with completely-resected stage IB-IIIA EGFR-mutated NSCLC, after standard adjuvant treatment. The primary outcome of this study is OS[53], and it could give us more information on the role of EGFR TKIs in this setting.

EGFR TKIS AS TREATMENT FOR POTENTIALLY-RESECTABLE LOCALLY-ADVANCED DISEASE

Locally-advanced NSCLC is associated with a poor prognosis[54]. Although such patients are treated with curative intent, the 5-year OS rates are low. In this situation, pathological complete response (pCR) after a preoperative treatment has been correlated with OS[55]. However, neoadjuvant chemotherapy achieves pCR rates that range from 0-16%[56]. Currently, ongoing research aims to translate the benefits from targeted therapy and immunotherapy to potentially-resectable disease.

Neodjuvant immunotherapy, with or without chemotherapy, is being assessed in multiple clinical trials, with promising results[57,58]. Provencio *et al* carried out the phase II clinical trial NADIM, in which a surprising pCR of 63% was reported with chemotherapy plus nivolumab[59].

EGFR TKIs are also being assessed for use as neoadjuvant treatment in NSCLC (Table 2). Zhong *et al*[41] carried out a small phase II trial in which they assessed the feasibility of giving neoadjuvant treatment guided by EGFR status, in 24 patients with stage IIIA NSCLC. Patients with mutated EGFR received erlotinib for 42 d, while

Table 2 Clinical trials of neoadjuvant epidermal growth factor receptor tyrosine kinase inhibitors in epidermal growth factor receptor-mutated non-small-cell lung cancer

Clinical trial	Study type	Sample size	Primary outcome	Stage	Treatment	TKI duration	RR	R0 resectability rate	PR	OS
Zhong *et al*[60]	Phase II	24 (12 with EGFR mutation)	RR	IIIA	Erlotinib (patients with EGFR mutation) *vs* carboplatin-gemcitabine (patients with native EGFR)	42 d	58.3% *vs* 25%; *P* = 0.18	50% *vs* 71.4; *P* = 0.59	16.7% *vs* 25%; *P* = 0.64	14.5 *vs* 28.1 mo; *P* = 0.201
Xiong *et al*[61]	Phase II	25	Resectability rate	IIIA	Erlotinib	56 d	42.1%	68.4%	50%	51.6 mo
Xiong *et al*[62]	Phase II	31 (15 with EGFR mutation)	Resectability rate	IIIA	Erlotinib *vs* cisplatin-based chemotherapy	4-7 wk	67% *vs* 19%	80% *vs* 50%	67% *vs* 38%	51 *vs* 20.9 mo
EMERGING-CTONG 1103, Zhong *et al*[63], Wu *et al*[64]	Phase II	72	RR	IIIA	Erlotinib *vs* cisplatin-gemcitabine	42 d (12 mo after surgery)	54.1% *vs* 34.3%; *P* = 0.092	73% *vs* 63%	MPR: 9.7% *vs* 0%	42.2 *vs* 36.9 mo; HR: 0.83; *P* = 0.513

EGFR: Epidermal growth factor receptor; HR: Hazard ratio; MPR: Major pathological response; OS: Overall survival; PR: Pathological response; RR: Response rate; TKI: Tyrosine kinase inhibitor.

patients with native EGFR received carboplatin plus gemcitabine for 3 cycles. Although the response rate (RR) was numerically higher with erlotinib (58.3% *vs* 25%; *P* = 0.18), there was no increase in the N2 pCR (16.7% *vs* 25%; *P* = 0.64) or in OS (14.5 *vs* 28.1 mo; *P* = 0.201)[60]. A different phase II single-arm trial included 25 patients with stage IIIA EGFR-mutated NSCLC treated with neoadjuvant erlotinib for 56 d, observing a RR of 42.1%, with a resectability rate of 68.4%. On pathology, 50% partial responses were reported, but no complete response[61]. Similarly, Xiong *et al* conducted a phase II clinical trial in patients with stage IIIA NSCLC, in which they compared neoadjuvant treatment with erlotinib for 4-7 wk (15 patients with EGFR mutation) and cisplatin-based doublet chemotherapy for 2 cycles (16 patients without EGFR mutation), observing a RR (67% *vs* 19%) and an OS (51 *vs* 20.9 mo) that were numerically higher in the patients treated with erlotinib. The pathological response was higher in the erlotinib group (67% *vs* 38%), although this difference was not statistically significant and there was no pCR in this group[62]. Finally, the phase II trial EMERGING-CTONG 1103 included 72 patients with stage IIIA EGFR-mutated NSCLC, randomly assigned to erlotinib (42 d neoadjuvant and 12 mo adjuvant) *vs* cisplatin plus gemcitabine (2 cycles neoadjuvant and 2 cycles adjuvant). There were no significant differences in RR (54.1% *vs* 34.3%; *P* = 0.092) or OS (45.8 *vs* 39.2 mo; HR: 0.77, 95%CI: 0.41-1.45; *P* = 0.417), and pCR was not observed in either arm[63]. Final analysis of OS was recently reported, with similar results (42.2 *vs* 36.9 mo; HR: 0.83, 95%CI: 0.47-1.47; *P* = 0.513)[64].

The small sample sizes and heterogeneity of these phase II trials do not allow us to draw definitive conclusions regarding efficacy. There was a remarkable lack of pCR in these studies; in addition, the response rates appear to be lower than those observed with EGFR TKIs as first-line treatment[31,33,34,36], which could be due to the short preoperative treatment duration in these trials. Although neoadjuvant treatment with these agents appears feasible, there remain many unanswered questions, such as the risk of disease flare after stopping EGFR TKI treatment[65]; randomised trials with larger sample sizes are needed to provide more data on the safety and efficacy of these agents in potentially-resectable disease. Currently underway is the phase III clinical trial NeoADAURA (ClinicalTrials.gov number, NCT04351555), which compares osimertinib for 9 wk with or without chemotherapy for 3 cycles *vs* chemotherapy alone for 3 cycles, as neoadjuvant treatment, in patients with stage II-IIIB EGFR-mutated NSCLC, followed by adjuvant osimertinib for 3 yr. This trial could provide more information on the optimal duration of preoperative treatment, the role of chemotherapy in this scenario, and the need for adjuvant treatment.

Table 3 Clinical trials of pidermal growth factor receptor tyrosine kinase inhibitors in the management of unresectable pidermal growth factor receptor-mutated non-small-cell lung cancer

Clinical trial	Type of study	Sample size	Primary outcome	Stage	Treatment	TKI duration	RR	PFS	OS
RECEL, Xing *et al*[70]	Phase II	40	PFS	III unresectable	Erlotinib + RT *vs* cisplatin-etoposide + RT	2 yr	70% *vs* 61.9%; $P = 0.744$	24.5 *vs* 9 mo; HR: 0.104; $P < 0.001$	Not reported
Lee *et al*[71]	Phase II	59 (12 with EGFR mutation)	RR, toxicity and OS	III unresectable	EGFR mutation: erlotinib x 3 → erlotinib+RT → erlotinib x 6 *vs* erlotinib x 3 → cisplatin-irinotecan+RT Native/unknown EGFR: cisplatin-irinotecan × 3 → cisplatin-irinotecan+RT *vs* cisplatin-irinotecan+RT → cisplatin-irinotecan x 3	33 wk	EGFR mutation: 71.4% *vs* 80% Native/unknown EGFR: 70% *vs* 73.9%	EGFR mutation: 11.6 *vs* 8.1 mo Native/unknown EGFR: 9 *vs* 12.3 mo	EGFR mutation: 39.3 *vs* 31.2 mo Native/unknown EGFR: 16.3 *vs* 25.3 mo Mutated *vs* native EGFR: 74.8 *vs* 25.3 mo, $P = 0.034$
LOGIK0902/OLCSG0905, Saeki *et al*[73]	Phase II	20	2-yr OS	III unresectable	Gefitinib cisplatin-docetaxel+RT	8 wk	85%	2-yr PFS 36.9%	2-yr OS 90%

HR: Hazard ratio; OS: Overall survival; PFS: Progression-free survival; RR: Response rate; RT: Radiotherapy; TKI: Tyrosine kinase inhibitors.

EGFR TKIS AS TREATMENT FOR UNRESECTABLE LOCALLY-ADVANCED DISEASE

The phase III PACIFIC trial demonstrated that maintenance with durvalumab after chemoradiotherapy, in patients with unresectable stage III NSCLC, significantly prolonged DFS and OS[66,67], positioning it as the standard treatment for these patients. Despite the good outcomes with this treatment strategy, approximately 44% of patients had progression and died from the disease[67]. Therefore, there is a need for new biomarker-guided therapies that would allow us to appropriately select the best treatment for each patient.

The PACIFIC trial subgroup analysis suggests that patients with EGFR mutation may benefit less from chemoradiotherapy followed by durvalumab[66]. This is probably due to the biology of EGFR-mutated NSCLC, which is associated with a higher risk of metastasis, meaning these patients obtain a greater benefit from local treatment such as chemoradiotherapy[68,69]. Thus, the optimal treatment in this patient subgroup is unknown.

Preclinical studies suggest that EGFR TKIs have a radiosensitizing effect[21,22]. This has prompted several clinical trials to evaluate the role of these targeted agents in unresectable locally-advanced disease (Table 3). The phase II trial RECEL compared erlotinib (for 2 yr) or cisplatin plus etoposide, concomitantly with radiotherapy in 40 patients with unresectable stage III EGFR-mutated NSCLC, and demonstrated that erlotinib plus radiotherapy significantly prolonged DFS (24.5 *vs* 9 mo; HR: 0.104, 95%CI: 0.028-0.389; $P < 0.001$) compared to chemoradiotherapy[70]. A different phase II study by Lee *et al* included 59 patients with unresectable stage III NSCLC, of whom

12 had an EGFR-activating mutation. Patients with mutated EGFR were randomised to erlotinib for 3 cycles, followed by erlotinib plus radiotherapy, followed by erlotinib for 6 cycles, *vs* erlotinib for 3 cycles followed by chemoradiotherapy with cisplatin plus irinotecan; patients with native/unknown EGFR status were randomised to cisplatin plus irinotecan for 3 cycles before or after chemoradiotherapy with cisplatin plus irinotecan. Patients with mutated EGFR had a significantly longer OS (74.8 *vs* 25.3 mo, $P = 0.034$) than patients with native EGFR[71]. Gefitinib has also been assessed, in the phase II trial LOGIK0902/OLCSG0905[72], which included 20 patients with unresectable stage III EGFR-mutated NSCLC who were treated with gefitinib for 8 wk followed by chemoradiotherapy with cisplatin plus docetaxel, and found a RR of 85%, a 2-year DFS rate of 36.9%, and a 2-year OS of 90%[73].

Although these phase II studies show encouraging results, they must be confirmed in phase III clinical trials. Currently, the phase III LAURA trial is underway, comparing osimertinib until progression *vs* placebo, as maintenance treatment after standard chemoradiotherapy[74].

CONCLUSION

Adjuvant treatment with first- and second-generation EGFR TKIs, in patients with resected EGFR-mutated NSCLC, has demonstrated a benefit in DFS, which does not translate to OS. Surprisingly, in the ADAURA trial, the third-generation EGFR TKI osimertinib prolonged DFS in these patients; however, certain limitations of the design of this study and its early termination based on a benefit in DFS only, raise questions about its use as a standard adjuvant treatment. The OS data from the ADAURA trial and the results of the ALCHEMIST trial will confirm if EGFR TKIs have a role as adjuvant treatment.

These targeted therapies are also undergoing evaluation in potentially-resectable and unresectable locally-advanced disease, with encouraging results; however, we must await the results of the phase III trials NeoADAURA and LAURA, which should provide more data on the safety and efficacy of EGFR TKIs in these situations.

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ORIGINAL ARTICLE

Retrospective Study

A biomarker study in Peruvian males with breast cancer

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Abstract

BACKGROUND

Breast cancer (BC) frequency in males is extremely low and tumor features vary from its female counterpart. Breast cancer clinical and pathological features differ by race in women. Tumor infiltrating lymphocyte (TIL) levels, mismatch repair (MMR) protein loss, androgen receptor (AR) expression, and PIK3CA gene mutations are predictive biomarkers of response to biological therapy in female BC. There is limited information about clinical and pathological features as well as predictive biomarkers in males of non-Caucasian races with BC.

AIM

To investigate clinicopathological features and biomarkers of BC tumors in males and their prognostic value in Peruvian population.

METHODS

This study looked at a single-institution series of 54 Peruvian males with invasive BC who were diagnosed from Jan 2004 to June 2018. Standard pathological features, TIL levels, MMR proteins, AR immunohistochemistry staining, and PIK3CA gene mutations were prospectively evaluated in cases with available

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Institutional review board statement: This study was reviewed and approved by the Instituto Nacional de Enfermedades Neoplasicas Institutional Review Board. Personal and filiation data including identity of every patient was protected with an added code in the excel table. It is a retrospective case series that does not have any not activity or contact with the patients.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: We have no financial relationships to disclose.

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paraffin material. Percentage of AR and estrogen receptor (ER) positive cells was additionally calculated by software after slide scanning. Statistical analyses included association tests, intraclass correlation test and Kaplan Meier overall survival curves.

RESULTS

The median age was 63 years and most cases were ER-positive (85.7%), HER2 negative (87.2%), Luminal-A phenotype (60%) and clinical stage II (41.5%) among our male breast tumors. Median TIL was 10% and higher levels tended to be associated with Luminal-B phenotype and higher grade. AR-positive was found in 85.3% and was correlated with ER (intraclass index of 0.835, $P < 0.001$). Loss of MMR proteins was found in 15.4% and PIK3CA mutation (H1047R) in 14.3% (belonged to the Luminal-A phenotype). Loss of MMR proteins was associated with AR-negative ($P = 0.018$) but not with ER ($P = 0.43$) or TIL ($P = 0.84$). Early stages ($P < 0.001$) and lower grade ($P = 0.006$) were associated with longer overall survival. ER status, phenotype, AR status, TIL level, MMR protein loss nor PIK3CA mutation was not associated with survival ($P > 0.05$).

CONCLUSION

Male BC is usually ER and AR positive, and Luminal-A. MMR loss and PIK3CA mutations are infrequent. Stage and grade predicted overall survival in our South American country population.

Key Words: Male breast neoplasm; Androgen receptor; Tumor-infiltrating lymphocyte; Mismatch repair protein; PIK3CA mutation

Core Tip: Most male breast cancers were estrogen receptor-positive, HER2-negative, androgen receptor (AR)-positive, and Luminal A phenotype. Loss of mismatch repair (MMR) and PIK3CA mutations was found in around 15% of the cases. AR was correlated with ER expression and without loss of MMR proteins. Stage and grade are prognostic features in Peruvian male breast cancer.

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INTRODUCTION

Male breast cancer (BC) represents less than 1% of mammary carcinomas. Male BC has a higher expression rate of estrogen receptor (ER), higher proliferative activity, and generally more aggressive behavior than in females. The etiological attribution of inheritance for BC is more prevalent among males than females, but only a small fraction is attributed to BRCA2 mutation, and even less to BRCA1[1-5].

Combination of immunohistochemical biomarkers predicts response to therapy and allows us to classify molecular subtypes in female BC[6]. Molecular subtypes distribution and clinical features differ regarding racial populations, for instance, high triple negative breast cancer (TNBC) prevalence and young age at diagnosis are described in Latin-American women[6]. However, only a few small reports have evaluated frequency of molecular BC subtypes in Latin-American males [7,8].

Androgen-receptor (AR) expression[9], tumor-infiltrating-lymphocytes (TIL)[10], loss of mismatch-repair (MMR) proteins (biomarker of the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome)[11] and PI3K mutations[12] have been associated with response to target therapy in female BC. There is a need to develop epidemiological and therapeutic information in the male counterpart. This work aims

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to evaluate the prevalence of clinical and molecular biomarkers of BC tumors in Peruvian males, as well as their impact on survival.

MATERIALS AND METHODS

Study design and Patients

Fifty-four male BC cases who were histologically diagnosed at the Instituto Nacional de Enfermedades Neoplasicas between 2004 and 2018 were included. Clinical information was obtained from the patients' medical records. Live status of patients with not accurate follow-up was obtained from the Peruvian national registry (https://www.reniec.gob.pe) through the Epidemiology Department of the institution. The institutional review board waived informed consent and approved this retrospective case series.

Routine pathological examination

TIL assessment on Hematoxylin and Eosin-stained slide was possible in 42 available samples. TIL and histological grade was prospectively evaluated by three experienced pathologists (JS, ET and HG), who were blinded to the clinical data, following international recommendations[7].

Immunohistochemistry staining

Tissue samples were fixed in 10% buffered formalin to obtain 4 μm paraffinized histological sections from 35 available tissue paraffin blocks. Sections were transferred onto adhesive slides and were dried at 60 ℃ for 30 min. After incubation with the primary antibodies, immunodetection was performed using biotinylated anti-mouse immunoglobulin, followed by peroxidase-labeled streptavidin. The labeled streptavidin biotin kit was used, and 3,3'-diaminobenzidine chromogen was used as a substrate. Immunohistochemical staining for androgen-receptor protein (AR antibody, Dako), estrogen-receptor protein (ER antibody, Zhongshan Bio), Mut L Protein Homolog protein (MLH1 antibody, Dako), DNA mismatch repair protein Msh2 (MSH2 antibody, Dako), MutS Protein Homolog 6 protein (MSH6, Dako) and Postmeiotic Segregation Increased 2 protein (PMS2 antibody, Dako) were carried out according to the manufacturer's instructions. Normal prostatic tissue was used as a positive control of AR. Phosphate-buffered saline was used to replace the primary antibody and served as the negative control.

Tumors that had more than 10% of cells exhibiting a moderate or strong intensity of AR expression were considered positive[7]. Additionally, slides of AR and ER staining were scanned in BX63 Olympus (Tokyo, Japan) and the analysis was performed through Visiopharm software in 34 male BC cases. Negative and positive cells were marked in blue and green by TissueMorph Software, (Visopharm, Hoerlson, Denmark), and the proportion of cell count was obtained through the ratio of AR and ER on number of positive overall cells in 5 high power fields (Figure 1) under supervision of a pathologist as previously described (JS)[7]. Non-malignant stromal cells were used as internal positive controls for MLH1, MSH2, MSH6, and PMS2, while the loss of MMR was considered when lacking nuclear staining for at least one was found[13].

Determination of PIK3CA mutation

Tumor DNA from paraffin-embedded samples was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen; Hilden, Germany) in the available 14 tumor samples. TaqMan-based real-time PCR analysis was conducted using a LightCycler® 96 Real-Time PCR System (Roche Applied Science, Mannheim, Germany) to detect the three ''hot spot'' PIK3CA mutations (H1047R, E545K and E542K). Custom TaqMan primers and probes were designed for the PIK3CA mutations (PI3KCA 760: c.1624 G (VIC) >A (FAM), PI3KCA 763: c.1633 G (VIC) >A (FAM), PI3KCA 775: c.3140 A (VIC) >G (FAM), ThermoFisher scientific). The thermal cycler protocol was as follows: 10 min at 96 C, 39 cycles at 60 ℃ for 2 min, 98 ℃ for 30 s, and 60 ℃ for 1 min. All samples were analyzed in a single assay for each mutation. The mutational threshold was determined by measuring the WT HDx FFPE reference standards (Horizon Diagnostics, Cambridge, UK), and was 1%. In contrast, the threshold of reagents inducing false positives was assumed to be 0.5% of the mutation frequency.

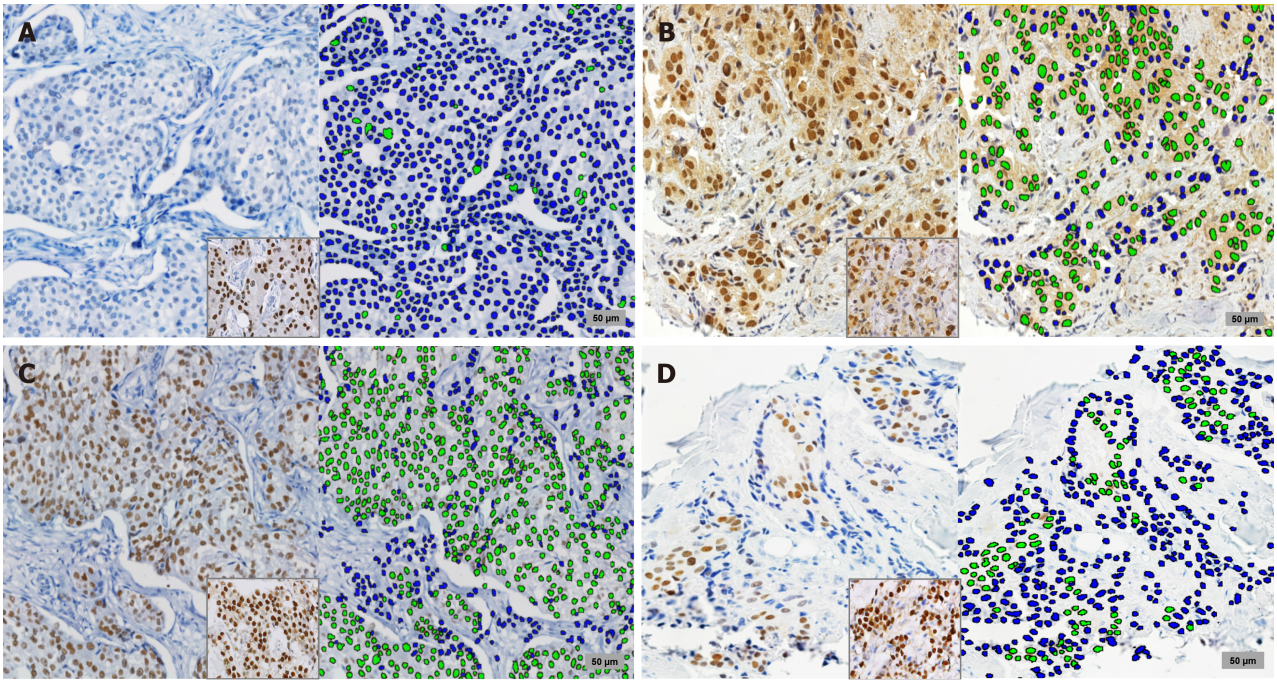

Figure 1 Immunohistochemical staining and automated scoring. Sections show positive staining for androgen receptor (A and B) and estrogen receptor (C and D) from the same tissue areas and the positive control. In the left, sections show images of the immunohistochemical studies (× 20). In the right, the automated analysis is presented, where negative nuclei are highlighted in blue and positive nuclei in green.

Statistical analyses

Associations of clinical-pathological variables were performed by the Chi-square test or the Fisher's exact test. Associations of ordinary variables were performed by the Kruskal-Wallis test. Identification of co-expression of AR and ER was performed by the intraclass correlation test. Overall survival (OS) analysis was estimated using the Kaplan–Meier method. Log-rank or Breslow tests were used to find differences between categories, according to the case. A $P < 0.05$ was considered significant. Analyses were performed using the SPSS statistical package (version 26.0.0, IBM SPSS Statistical).

RESULTS

Patient's characteristics

There were included 54 male BC patients and their median age was 63 years. Twenty-eight (51.9%) tumors were located on the left side, concurrent in-situ component in 41.4% and the most frequent clinical stage was II (41.5%) (Table 1). A family cancer history was found in 12 (22.2%) cases. Regarding treatment, mastectomy, tumorectomy and no primary resection were performed in 72.2%, 7.4% and 20.4%, respectively. Axillary dissection, sentinel node biopsy and no study of nodes were performed in 59.3%, 5.5% and 35.2%, respectively. Neoadjuvant and adjuvant chemotherapy was administrated in 22.2 and 35.2% of the resected cases, while adjuvant radiation was used in 44.4%. Tamoxifen was used in ER-positive cases. Recurrence was found in 28.9% (11/38) of the cases who underwent a curative surgical treatment.

Biomarkers, clinical-pathological features, and the correlations

Most cases were ER-positive (85.7%), HER2-negative (87.2%) and belonged to Luminal-A phenotype (Table 1). ER status was not associated with MMR-loss (P = 0.43). No association between ER percentage and MMR-loss was found (P = 0.22).

Positive AR status was found in 85.3% of patients and the two TNBC cases were negative for AR. MMR-loss was associated with AR-negative (75% *vs* 10%, P = 0.018). A lower median percentage of AR-positive cells was found in cases with MMR-loss (5% *vs* 70%, P = 0.02).

Evaluation of similar areas for ER and AR found that a median of 3738 cells (52.9%) (range 0%-87%) from 7577 cells (range 2139-11883 cells) were positive for ER, and 3795

Table 1 Clinical-pathological features and overall survival impact in male breast cancer

Features	*n*	%	OS-5yr	*P* value
Age				
Median (yr)	63			0.105[a]
< 63	25	53.7	56.0	
≥ 63	29	46.3	69.0	
Clinical stage (*n* = 53)				< 0.001[a]
I	2	3.8	100.0	
II	22	41.5	86.4	
III	20	37.7	50.0	
IV	9	17.0	22.2	
Histological grade (*n* = 46)				0.006[a]
1	7	15.2	85.7	
2	26	56.5	73.1	
3	13	28.3	30.8	
sTIL (*n* = 42)				0.397[b]
< 10%	12	28.6	50.0	
≥ 10%	30	71.4	66.7	
ER status (*n* = 49)				0.567[a]
Positive	42	85.7	66.7	
Negative	7	14.3	57.1	
PgR status (*n* = 49)				0.305[a]
Positive	43	87.8	67.4	
Negative	6	12.2	50.0	
HER2 status (*n* = 47)				0.088[b]
Positive	6	12.8	33.3	
Negative	41	87.2	70.7	
Ki67 (*n* = 40) (median 5%)				0.232[a]
0%-5%	22	55.0	59.1	
> 5%	18	45.0	66.7	
Phenotype (*n* = 45)				0.356[a]
Luminal A	27	60.0	63.0	
Luminal B	13	28.9	76.9	
TNBC	5	11.1	40.0	
AR (*n* = 35)				0.294[a]
0%-9%	5	14.3	60.0	
≥ 10%	30	85.7	63.3	
Mismatch repair loss (*n* = 26)				0.501[a]
No	20	76.9	65.00	
Yes	4	15.4	75.00	
Undetermined	2	7.7	100.00	
PIK3CA mutation H1047R (*n* = 14)				0.844[b]
Positive	2	14.3	50.0	

Negative	12	85.7	75.0

[a]*P*: Breslow.
[b]*P*: Log Rank. OS: Overall survival; TIL: Tumor infiltrating lymphocyte; TNBC: Triple negative breast cancer.

cells (55%) (range 0%-87.8%) from 7366 (range 2520-12135 cells) were positive for AR. Intraclass correlation coefficient of the means for AR and ER was 0.835 ($P < 0.001$) (Figure 2). A lower median of AR-positive tumor cell count proportion was also found in cases with MMR-loss (8% *vs* 65%, $P = 0.018$). No association between median of ER-positive tumor cell count proportion and MMR-loss was found ($P = 0.163$).

Median TIL was 10% and higher levels tended to be associated with Luminal-B ($P = 0.058$) (Figure 2). TIL ≥ 50% was associated with higher grade ($P = 0.039$), but not to age ($P = 0.44$), clinical stage ($P = 0.59$), MMR-loss (20% *vs* 15%, $P = 0.72$) nor PIK3CA mutation status ($P = 0.53$).

Loss of MMR protein expression was found in 4 (15.4%) cases. It was not associated with age ($P = 0.69$), clinical-stage ($P = 0.68$), grade ($P = 0.53$), molecular subtypes ($P = 0.91$) or PIK3CA mutation status ($P = 0.41$).

PIK3CA mutations were found in 2 (14.3%) of the 14 evaluated cases and both were positive for H1047R (1321 copies/microL-15.07% and 1019 copies/microL-18.06%). Both cases belonged to the Luminal-A phenotype.

The association of prognostics and clinical-pathological features

Longer OS was associated with early stage ($P < 0.001$) and lower grade ($P = 0.006$). Survival was not associated with ER status ($P = 0.305$), phenotype ($P = 0.152$), AR status ($P = 0.613$), TIL level ($P = 0.397$), MMR protein loss ($P = 0.501$) nor PIK3CA mutation status ($P = 0.844$).

DISCUSSION

We found a high frequency of AR expression and low frequency of both MMR-loss and PIK3CA mutations in our BC series in male Peruvian population.

Expression of AR was found in 85% of our male BC cases which is in the previously described range (40%–90%)[1], however we didn't find its association with prognosis. Androgen-receptor is a key driver of proliferation and cell survival, and although some retrospective male BC series describe its association with better prognosis[3] others like ours not[4]. Additionally, recent basic research and clinical trials describe that AR can predict activity of targeting drugs[9].

Our findings of high rates of ER-positive status and Luminal phenotype have been extensively described in Caucasian series[2,5]. Large sample size studies describe that ER-positive status would have a favorable prognostic effect (similar to the female BC) and predicts response to endocrine modulation[5,14,15]. The fact that we did not find an association with OS could be because of the small size of our series.

There was absence of HER2 enriched phenotype in our series and HER2- positive status was very infrequent. This corroborates previously published information that HER2 overexpression is less frequent than in female series, but it is expected to behave as a predictive feature to anti-HER2 therapies[2,5].

Our findings of 15% of cases with MMR loss have not previously been described in male BC. The HNPCC syndrome is an autosomal dominant genetic disorder characterized by predisposition to extracolonic malignancies at various sites including BC, is detected by loss of MMR proteins and is associated with high response to anti-PDL1 therapy. Studies with small sample size in female BC describe the lack or reduced expression of hMSH2 and hMLH1 in less than 20%[11,16]. Boyd *et al*[17] described a case of male BC belonging to a large HNPCC kindred that harbors a germline mutation of the MLH1. And recently, Piscuoglio *et al*[2] developed a pathway and network analysis of 241 genes in a series of 59 male BC and described an enrichment of mutations affecting DNA repair-related genes. We found that MMR-loss was associated with the previously described marker of endocrine response[3,4], the lower AR expression. Furthermore, this association appears to be specific for this steroid marker and not for ER, despite AR and ER expression were co-related. Haricharan *et al*[18] suggest that alteration in DNA damage repair genes could produce endocrine resistance because of the finding that defects in MMR pathway genes doesn't allow an accurate CDK4 suppression by endocrine therapy.

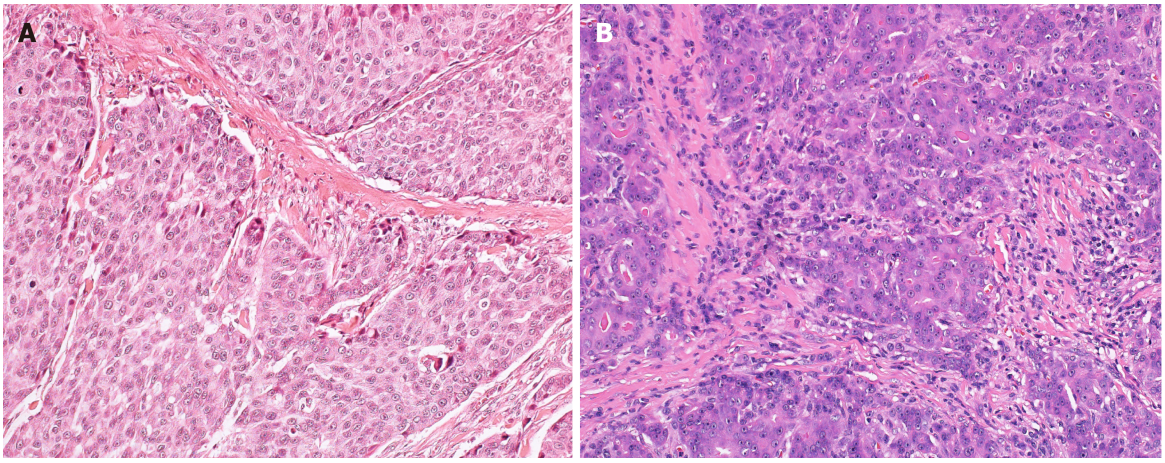

Figure 2 Evaluation of tumor infiltrating lymphocytes on hematoxylin-eosin staining (× 20). A: A Luminal-A breast cancer with low TIL level; B: A Luminal-B breast cancer with high TIL level.

PIK3CA mutations were found in only 2 of 14 evaluated male BC cases that is lower than previously reported by our group in women with BC[19,20]. Piscuoglio *et al*[2] sequenced 241 genes in 59 male BC and found that most recurrent mutations affected the PIK3CA gene (20%). However, these rates would be lower than their female counterparts that have been detected in approximately 30%-40% of female BC[21]. In vitro and translational research in tumor samples from clinical trials found that PIK3CA mutations reduce sensitivity to anti-HER2 drugs[22] and endocrine modulation[19,20]. Additionally, a recent study demonstrates that Alpelisib, a drug targeting the PIK3CA pathway, has activity in breast cancer cases with presence of the mutation [12].

TIL is a well-described prognostic and predictive biomarker for anti-PDL1-therapy in female BC[10]. Vermeulen *et al*[22] found that high TIL density evaluated by a method different than the currently recommended in international guidelines was associated with Luminal-B HER2-positive subtype and longer OS in a 1483 male BC series. We also found a trend of higher TILs in Luminal-B subtype but not a relationship with longer OS (P = 0.378). Contrary to the female counterpart or other malignancies, we did not find that higher TIL levels were associated with AR or ER percentage nor with MMR-loss.

Finally, our finding that early stage and lower grade features achieve significant association with better prognosis is consistent with previous reports, and supports the current management of this entity[2,5].

The weakness of our study was that several patients were lost of follow-up; however, obtaining information about their live status from the national registry allowed us to build OS curves. The number of included cases is also small because it is a very unusual entity, representing less than 0.5% of their female counterpart (18552 female new BC cases diagnosed in our center during the same period). Our strongness is the prospective evaluation of biomarkers by pathologists and biologist authors. Our results serve to complement male BC knowledge in the South American population, which has been under-studied.

CONCLUSION

We conclude that early-stage and low-grade features identify favorable prognoses in male BC. Most Peruvian BC cases are ER-positive, HER2-negative, AR-positive and Luminal-A tumors. MMR loss and PIK3CA mutations are infrequent, and MMR loss was associated with AR negative.

ARTICLE HIGHLIGHTS

Research background

Information about clinicopathological features associated with treatment response and

prognosis has been extensively described for female breast cancer, and differences regarding races has been described. Breast cancer in males is much less frequent, has important clinicopathological differences and is less studied than their female breast cancer counterpart.

Research motivation

Discussion and new information about features and biomarkers of Breast cancer (BC) in males have been included in recent cancer-related meetings, and more than 30000 articles have been published in the last two years. However, very few of them have evaluated a South American population.

Research objectives

To describe rates of currently accepted biomarkers for prognosis and for prediction of treatment response in Peruvian males with BC.

Research methods

Clinical files and tumor slides were reviewed. Tumor-infiltrating lymphocytes, mismatch repair proteins (MMR), PIK3CA gene mutations, estrogen (ER) and, androgen receptors (AR) were prospectively evaluated in available paraffin material.

Research results

In our series of 54 Peruvian males with invasive breast cancer, we found that most cases were Luminal-A phenotype (60%), ER-positive (85.7%), AR-positive (85.3%), and the median of tumor-infiltrating lymphocytes was 10%. MMR loss was found in 15.4% and PIK3CA mutation (H1047R) in 14.3% (all in the Luminal-A group). MMR loss was associated with AR-negative (P = 0.018). Longer overall survival was associated with early stages (P < 0.001) and lower grade (P = 0.006).

Research conclusions

Most breast cancer tumors in Peruvian males are ER and AR-positive, and MMR loss and PIK3CA mutations are infrequent. MMR loss was associated with hormone receptor-negative.

Research perspectives

Biomarkers identified for women with breast cancer need to be validated for the male counterpart. Research in race disparities needs to be extended also for males of non-Caucasian races. Association between MMR loss and activity of AR pathways requires further evaluation.

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ORIGINAL ARTICLE

Retrospective Study

Jejunostomy in the palliative treatment of gastric cancer: A clinical prognostic score

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Author contributions: Ramos MFKP and Pereira MA performed the study design, data retrieval, statistical analysis, critical analysis, the draft of the manuscript; Dias AR and Sakamoto E performed the data retrieval and manuscript review; Ribeiro Jr U, Zilberstein B and Nahas SC performed the critical analysis and manuscript review.

Institutional review board statement: The study was approved by the hospital ethics committee and registered online (https://plataformabrasil.saude.gov.br; CAAE: 31626220.8.0000.0068).

Informed consent statement: Informed consent was waived by the local Ethics Committee because of the retrospective nature of the study.

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Abstract

BACKGROUND

Clinical stage IV gastric cancer (GC) may need palliative procedures in the presence of symptoms such as obstruction. When palliative resection is not possible, jejunostomy is one of the options. However, the limited survival of these patients raises doubts about who benefits from this procedure.

AIM

To create a prognostic score based on clinical variables for 90-d mortality for GC patients after palliative jejunostomy.

METHODS

We performed a retrospective analysis of Stage IV GC who underwent jejunostomy. Eleven preoperative clinical variables were selected to define the score categories, with 90-d mortality as the main outcome. After randomization, patients were divided equally into two groups: Development (J1) and validation (J2). The following variables were used: Age, sex, body mass index (BMI), American Society of Anesthesiologists classification (ASA), Charlson Comorbidity index (CCI), hemoglobin levels, albumin levels, neutrophil-lymphocyte ratio (NLR), tumor size, presence of ascites by computed tomography (CT), and the number of disease sites. The score performance metric was determined by the area under the receiver operating characteristic (ROC) curve (AUC) to define low and high-risk groups.

RESULTS

Of the 363 patients with clinical stage IVCG, 80 (22%) patients underwent

conflicts of interest that might be relevant to the contents of this manuscript.

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jejunostomy. Patients were predominantly male (62.5%) with a mean age of 62.4 years old. After randomization, the binary logistic regression analysis was performed and points were assigned to the clinical variables to build the score. The high NLR had the highest value. The ROC curve derived from these pooled parameters had an AUC of 0.712 (95%CI: 0.537–0.887, P = 0.022) to define risk groups. In the validation cohort, the diagnostic accuracy for 90-d mortality based on the score had an AUC of 0.756, (95%CI: 0.598–0.915, P = 0.006). According to the cutoff, in the validation cohort BMI less than 18.5 kg/m^2 (P < 0.001), CCI ≥ 1 (P = 0.001), ASA III/IV (P = 0.002), high NLR (P = 0.012), and the presence of ascites on CT exam (P = 0.004) were significantly associated with the high-risk group. The risk groups showed a significant association with first-line (P = 0.012), second-line chemotherapy (P = 0.009), 30-d (P = 0.013), and 90-d mortality (P < 0.001).

CONCLUSION

The scoring system developed with 11 variables related to patient's performance status and medical condition was able to distinguish patients undergoing jejunostomy with high risk of 90 d mortality.

Key Words: Stomach neoplasms; Gastric cancer; Palliative surgery; Jejunostomy; Gastric cancer with outlet obstruction; Stage IV gastric cancer

Core Tip: This is a retrospective study to evaluate the outcomes of jejunostomy in clinical stage IV gastric cancer patients, and create a scoring system based on clinical variables to identify the best candidates for this approach and avoid futile procedures. We analyzed 80 patients divided into a development and validation cohort. The score had an accuracy of 75.6% in the validation cohort, and was able to properly identify the cases with high risk of 90-d mortality.

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INTRODUCTION

Gastric cancer (GC) represents the third leading cause of cancer mortality worldwide [1]. Surgical treatment remains the standard of care for patients with resectable GC. Nevertheless, many patients at the time of diagnosis have already locally unresectable tumors or signs of systemic disease (clinical stage IV GC patients). As a result, palliative procedures are indicated in the presence of symptoms, including bleeding, perforation, or obstruction.

Palliative gastrectomy is the preferred treatment option for locally advanced GC with gastric outlet obstruction whenever it is feasible. However, if the tumor cannot be resected, the placements of endoscopic stents or gastrojejunostomy are additional options for distal lesions, according to the patient's life expectancy[2,3]. Unfortunately, some tumors may be too large or located in the upper third of the stomach, not allowing performing the gastrojejunostomy proximal to the tumor. In this scenario, a nasoenteric tube or jejunostomy are the main alternatives. Although jejunostomy enables the use of enteral nutrition, the patient persists with the obstruction and does not tolerate a full oral diet, with negative impact on quality of life.

Most studies evaluate the outcomes concerning placing a feeding jejunostomy at the time of gastroesophageal resection[4,5]. There are few reports concerning the influence of clinical and treatment variables on the outcome of palliative jejunostomy. Questions about improving survival and quality of life, in contrast to the morbidity and mortality rates in these cases, remain unanswered. Consequently, there are still doubts about

who benefits from jejunostomy.

Thus, the aim of this study was to analyze the clinical characteristics and prognosis of stage IV patients who underwent palliative jejunostomy due to obstructive GC, and create a prognostic score for mortality based on variables related to survival and worse outcomes.

MATERIALS AND METHODS

We conducted a retrospective study of patients with stage IV who underwent jejunostomy for obstructive GC at the Cancer Institute between 2009 and 2020. The indications for jejunostomy and inclusion in the study were: Adenocarcinoma histology, obstructive GC that could not be resected, diffuse or proximal gastric lesion that could not be submitted to gastrojejunostomy.

Abdominal and pelvis computed tomography (CT), endoscopy, and laboratory tests were assessed preoperatively in all patients. The clinical data collected from our prospectively-maintained database included body mass index (BMI), laboratory blood test, the clinical performance by the American Society of Anesthesiologists (ASA) classification[6], the presence of comorbidities using the Charlson Comorbidity index (CCI)[7] (without the inclusion of age and GC as comorbidity), and *Lauren* histological type. GCs were staged according to the TNM 8th edition.

All patients were operated in a high-volume center by specializes surgeons. Postoperative complications (POC) were graded according to Clavien-Dindo's classification[8] and major POC were defined as Clavien III-V.

Palliative chemotherapy (CMT) consisted of a doublet containing fluoropyrimidine (capecitabine or 5-fluorouracil) and a platin (oxaliplatin or cisplatin) as the preferred systemic regimen for the first line. Irinotecan and cisplatin chemotherapy was chosen in some cases to avoid the use of infusion pumps or in those patients with difficulty swallowing capecitabine tablets. Paclitaxel or irinotecan was used in the second line. In our center monoclonal antibodies (trastuzumab or ramucirumab), as well as immunotherapy, are not usually available for GC treatment[9-11].

Postoperative follow-up was performed every month or in shorter period if necessary. Absence in appointments for more than 12 mo was considered as a loss of follow-up. The study was approved by the hospital ethics committee (NP1681/20) and registered online (https://plataformabrasil.saude.gov.br/; CAAE: 31626220.8.0000.0068).

Prognostic scoring system-predicted mortality

To create a scoring system, patients were randomized into two groups (1:1) by computer using the statistical software (SPSS). The score was developed with half of the patients and further evaluated in a validation cohort with the remaining patients. The score was built with 90-d mortality as the main outcome.

As predictors, 11 preoperative clinical variables were selected and classified in a dichotomous way to define the scoring system categories. Clinical and baseline variables—generally used in clinical practice that reflect the general patient´s status—and oncological variables related to the GC, that would have an impact on the prognosis and survival of patients undergoing palliative care, were selected to compose the score[12-15]. The following variables were used: Age, sex, BMI, ASA, CCI, hemoglobin (Hb) levels, albumin levels, neutrophil-lymphocyte ratio (NLR), tumor size, presence of ascites by tomography, and number of disease sites.

The points assigned to each category were determined by binary logistic regression analysis, and the score was calculated for each patient. The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the performance metric of the score, as explained below.

Statistical analysis

Statistical analyses were carried out using SPSS software, version 20.0 (SPSS Inc, Chicago, IL). Chi-square or Fisher's Exact test, and *t*-test were used to evaluate categorical and continuous variables, respectively. Factors related to 90-d mortality were analyzed by binary logistic regression analysis, and the odds ratios (ORs) with 95% confidence interval (95%CI) were calculated. The score performance metric was determined by the AUC. An AUC > 0.7 was considered to indicate high diagnostic accuracy, given that at least 70% of the patients were classified correctly as the high-risk group for 90-d mortality. The optimal cutoff values were determined by maximizing Youden's index (sensitivity + specificity - 1). Survival was estimated using

the method of Kaplan–Meier, and the log-rank test was employed for comparisons between the curves. Overall survival (OS) was calculated from the date of surgery until the date of death. *P* value less than 0.05 was considered statistically significant.

RESULTS

During the referred period, 363 patients with clinical stage IV GC underwent surgical procedures. Among these, jejunostomy was performed in 80 (22%) patients. The clinical characteristics of all jejunostomy patients are summarized in Supplementary Table 1.

Patients were predominantly male (62.5%) with a mean age of 62.4 (SD ± 12.8, range 24-84.6) years, and a mean BMI of 21.0 (SD ± 3.9). Most tumors affected the middle third of the stomach (67.4%), with a median diameter of 7.2 cm on the longest axis.

The presence of disease in one or more sites, in addition to the primary, was observed in 70% of the cases: 63.7% had peritoneal metastasis and 17.5% had distant metastasis.

After randomization, jejunostomy patients were divided equally into two groups: (J1) score development group; and (J2) score validation group.

Prognostic index and risk groups

Considering the J1 group, the 90-d mortality rate was 52.5%. To develop the predictive score of mortality in 90-d, variables related to the worst outcome were selected for the design of the model. After the binary logistic regression analysis (Supplementary Table 2), 11 variables were included in the model, and points were assigned as shown in Table 1, for a maximum score value of 100. The following variables with the respective defined categories were used: Age (< 65 *vs* ≥ 65 years), sex (female *vs* male), BMI (< 18.5 kg/m^2 *vs* ≥ 18.5 kg/m^2), ASA (I/II *vs* III/IV), CCI (0 *vs* ≥ 1), Hb levels (≤ 11 g/dL *vs* > 11 g/dL, which represents the lower limit between mild and moderate anemia for men and women), albumin levels (< 3.5 g/dL *vs* ≥ 3.5 g/dL), NLR (< 2.5 *vs* ≥ 2.5), tumor size (≤ 7 cm *vs* > 7 cm), number of disease sites (one *vs* two or more, with represents locoregional, distant, or peritoneal) and the presence of ascites by tomography (absent *vs* present).

The highest score value was assigned to the high NLR category and the lowest value to the low levels of Hb and BMI. After, a value was assigned to each of the 40 patients, and the performance metric of the score was assessed through the construction of the ROC curve (Figure 1A). The AUC was 71.2% (AUC 0.712, 95%CI: 0.537–0.887, *P* = 0.022), with an estimated value of 65.5 for optimal cutoff.

Based on the cutoff, the J1 patients were divided in two risk groups for 90-d mortality: Low-risk (score < 65.5), with 14 (35%) patients; and high-risk (score ≥ 65.5) with 26 (65%) patients. The characteristics of the groups are shown in Table 2.

Sex, age, stage, ASA, and the presence of comorbidities showed no statistically significant differences between the groups. High NLR (*P* = 0.001), albumin levels lower than 3.5 g/dL (*P* = 0.026), and tumor size greater than 7 cm (*P* = 0.001) were related to the high-risk group**.** The risk groups showed a significant association with both 30 and 90-d mortality (*P* = 0.013 and *P* < 0.001, respectively).

Internal validation

Using the previously constructed scoring system, the score value was calculated for each patient in the validation cohort (J2 group, *n* = 40). The rate of 90-d mortality in this group was 50%.

As shown in Figure 1B, the risk score had superior diagnostic accuracy for 90-d mortality in the validation cohort (AUC 0.756, 95%CI: 0.598–0.915, *P* = 0.006). Using the previously established cutoff value (65.5 points), 21 (52.5%) and 19 (47.5%) patients were classified as low-risk and high-risk groups, respectively (Table 3).

BMI less than 18.5 kg/m^2 (*P* < 0.001), CCI ≥ 1 (*P* = 0.001), ASA III/IV, (*P* = 0.002), high NLR (*P* = 0.012), and the presence of ascites on CT exam (*P* = 0.004) were significantly associated with the high-risk group.

Considering the postoperative outcomes, there were no differences in POC rate and length of hospital stay between the groups. However, mortality at 30 and 90 d were significantly higher in the high-risk group, demonstrating the performance of the score in predicting mortality based on the adopted cutoff value (*P* = 0.049 and *P* = 0.027, respectively). Besides, the rate of patients who received first and second-line palliative treatment was higher in the low-risk group (*P* = 0.012 and *P* = 0.009, respectively).

Table 1 Variables and points for each category-risk score for 90-d mortality

Variables	Category	Points
Sex	Female	7
Age (years)	≥ 65	4
BMI (kg/m^2)	BMI < 18.5	1
CCI	CCI ≥ 1	7
ASA classification	III/IV	9
Hb (g/dL)	Hb ≤ 11	1
Alb (g/dL)	Alb < 3.5	3
NLR	NLR ≥ 2.5	50
Presence of ascites on CT	Present	5
Tumor size (cm)	> 7 cm	5
Number of disease sites	Two or more	8
Total		**100**

CCI: Charlson's comorbidity index; CT: Computed tomography; NLR: Neutrophil lymphocyte ratio; ASA: American Society of Anesthesiologists; BMI: Body mass index; Hb: Hemoglobin; Alb: Albumin.

Survival outcomes–J1 and J2 groups

The median follow-up time for all patients was 2.1 mo (interquartile range = 0.6-5.9, mean of 4.4 mo). At the time of this study, 8 patients were alive, and 72 patients died (J1 = 36 and J2 = 36). The median OS for the entire jejunostomy cohort was 2.7 mo, compared to a median OS of 8 mo for the other clinical stage IV GC patients undergoing other surgical procedures (resection, bypass, or diagnostic laparoscopy) (P < 0.001) (Supplementary Figure 1).

Regarding the risk groups in J1 patients (development cohort), the median OS for high and low-risk was 1.0 mo and 4.3 mo, respectively (P < 0.001) (Figure 2A). For the J2 patients (validation cohort), the median OS was 1.4 mo for the high-risk compared to 5.7 mo in the low-risk group (P = 0.059) (Figure 2B).

DISCUSSION

In the present study, we performed an analysis of a cohort of stage IV GC, not amenable to surgical resection, who underwent palliative jejunostomy, and we developed a prognostic score for 90-d mortality. Jejunostomy represented the therapeutic choice in 22% of stage IV GC patients. We provided a simple and feasible scoring system with variables easily available in the clinical assessment of patients. The score demonstrated an accuracy of 75.6% in the validation cohort and was associated with mortality rates.

As is widely known, palliative gastric resection is the best option for obstructive lesions, but some patients are unable to undergo this procedure. Proximal and bulky tumors also do not allow gastrojejunostomy to be performed to restore gastrointestinal continuity. Thus, in these cases, jejunostomy or nasoenteric tube are often indicated as a palliative measure to allow maintenance of nutritional enteral support[16]. Once the nutritional emergency is solved, patients may receive palliative CMT and resume oral intake in cases with good response.

The nasoenteric tube causes discomfort in the nasopharynx and oropharynx besides its visual uncomfortable aspect. On the other hand, jejunostomy involves performing an invasive surgical procedure on a patient who is already frail and debilitated[17].

Since these patients have a restricted prognosis, and particular clinical and oncological conditions, the rationale of developing the score was to take into account characteristics that can impact survival to properly identify the patients that are likely to benefit from the procedure.

Building a score involves the appropriate choice of variables since a wide variety of characteristics must be considered in GC cases for the management decision. In our

Table 2 Characteristics of jejunostomy patients according to the risk group-score development cohort (*n* = 40)

Variables	J1-score development cohort		*P* value
	Low-risk	High-risk	
	n = 14 (%)	*n* = 26 (%)	
Sex			0.507
Female	6 (42.9)	14 (53.8)	
Male	8 (57.1)	12 (46.2)	
Age (years)			0.954
mean (SD)	62.0 (15.0)	61.7 (12.9)	
BMI (kg/m^2)			0.101
mean (SD)	20.4 (2.7)	22.4 (3.9)	
CCI			0.885
0	10 (71.4)	18 (69.2)	
≥ 1	4 (286)	8 (30.8)	
ASA class			0.507
I/II	8 (57.1)	12 (46.2)	
III/IV	6 (42.9)	14 (53.8)	
Hemoglobin (g/dL)			0.456
mean (SD)	11.1 (1.9)	10.5 (2.0)	
Albumin (g/dL)			0.123
mean (SD)	3.7 (0.6)	3.3 (0.6)	
NLR			0.001
mean (SD)	3.03 (1.58)	7.46 (5.22)	
Tumor size (cm)			0.18
mean (SD)	6.9 (2.1)	8.3 (3.4)	
Presence of ascites on CT			0.48
Absent	6 (42.9)	7 (26.9)	
Present	8 (57.1)	19 (73.1)	
Lauren type			1
Intestinal	4 (28.6)	6 (23.1)	
Diffuse/mixed	9 (64.3)	17 (65.4)	
Undetermined	1 (7.1)	3 (11.5)	
Number of disease sites			0.408
One (only locoregional)	6 (42.9)	7 (26.9)	
Two or more	8 (57.1)	19 (73.1)	
POC			0.075
No POC/Clavien 1-2	14 (100)	19 (73.1)	
Clavien 3-5	0 (0)	7 (26.9)	
Length of hospital stay (days)			0.452
Median (IQR)	4.5 (1.7-6.3)	5 (2.7-9.5)	
Palliative treatment-1st line			0.257
No	6 (42.9)	16 (61.5)	
Yes	8 (57.1)	10 (38.5)	

30-d mortality			0.013
No	13 (92.9)	13 (50)	
Yes	1 (7.1)	13 (50)	
90-d mortality			< 0.001
No	12 (85.7)	7 (26.9)	
Yes	2 (14.3)	19 (73.1)	

SD: Standard deviation; IQR: Interquartile range; POC: Postoperative complication; CCI: Charlson's comorbidity index; CT: Computed tomography; NLR: Neutrophil lymphocyte ratio; ASA: American Society of Anesthesiologists; BMI: Body mass index.

model, we included 11 common usage variables related to the survival outcomes with broad external validity. The variables were categorized in a dichotomous way, which facilitates their use and reproducibility. Additionally, to provide an internal validation, the cohort was randomized into two samples: One for the construction of the model, and the second for the validation of the model.

Considering the variables that support the score, the impact of the NLR on the outcome was noteworthy, since the highest score was achieved by this parameter. The NLR represents a prognostic marker used in different solid tumors, including GC, and its correlation with survival is widely reported. Furthermore, the incidence of POC and their relation to NLR is also described in GC patients[12,18].

The rationale for this result is likely complex. Neutrophils present a pro-tumor behavior, as they promote angiogenesis, damage DNA, inhibit T-cell activity against tumor cells, and facilitate the metastatic process. Inversely, lymphocytes exert an anti-tumor function when they recognize tumor cell antigens, promoting cytolytic activity against these cells. Thus, the high NLR reflects a proinflammatory systemic status, leading to a protumoral environment that allows rapid tumor progression and development[19]. Therefore, NLR may also reflect a more advanced disease stage.

Regarding the outcome used to assign the score, we chose the 90-d mortality because it is considered a good parameter to reflect the value of a palliative procedure in patients with limited survival. Another outcome option to consider would be the ability to receive palliative CMT in the postoperative setting. However, this variable reflects not only the patient's performance after the procedure, but also their decision to perform palliative CMT. Some patients choose to adopt only best support care. Even so, in the validation court, low-risk patients received more frequently first and second lines of CMT.

Regarding complications related to jejunostomy, most of the reports are related to its performance after major gastrointestinal procedures for postoperative nutritional support[4,5,20,21]. Common surgical complications include loss or obstruction of the tube and local leakage of its content. The findings of some studies have shown that jejunostomy placed during GC resection is associated with increased complications. The analysis of this research included only palliative patients already with limited survival. Therefore, it is difficult to assess the impact of possible complications related to the procedure.

As previously mentioned, it is noteworthy that our score could distinguish low-risk patients, which received significantly more palliative CMT and had better median OS. Wang *et al*[22] analyzed 545 palliative procedures for GC treatment. Of these, 77 were considered as intubation procedures (17 gastrostomies and 60 jejunostomies), with a surgical mortality rate of 23.4%. The median OS in this group was 3.8 mo, and patients who underwent intubation procedures receive less chemotherapy compared to resected patients. In the study performed by Schmidt *et al*[23], the intubation group accounted for 12 out of 110 patients. These patients, combined with 52 other patients who underwent surgical procedures that did not involve the removal of the tumor, had a median OS of 9.2 mo. In our study, the median OS for the entire population was 2.7 mo. These results highlight the limited survival of these patients and the need for a good selection of patients for palliative jejunostomy.

Thus, with the application of the score, we were able to distinguish patients with a more limited survival: A high-risk group, with 1 mo and 1.4 mo in J1 and J2 cohorts, respectively; compared to the low-risk group, with 4.3 and 5.7 mo in J1 and J2 cohorts, respectively. This difference was significant in the development cohort but not in the validation cohort. Perhaps, the presence of one outlier with extremely high survival may have influenced the result in the validation cohort. As the score was developed to

Table 3 Characteristics of jejunostomy patients according to the risk group-score validation cohort (*n* = 40)

Variables	J2-score validation cohort		*P* value
	Low-risk	High-risk	
	n = 21 (%)	*n* = 19 (%)	
Sex			0.721
Female	6 (28.6)	4 (21.1)	
Male	15 (71.4)	15 (78.9)	0.072
Age (years)			
mean (SD)	59.7 (12.4)	66.7 (11.4)	
BMI (kg/m^2)			0.074
mean (SD)	21.4 (3.5)	19.0 (4.7)	
CCI			0.001
0	21 (100)	11 (57.9)	
≥ 1	0 (0)	8 (42.1)	
ASA class			0.002
I/II	16 (76.2)	5 (26.3)	
III/IV	5 (23.8)	14 (73.7)	
Hemoglobin (g/dL)			0.345
mean (SD)	11.4 (2.3)	10.6 (2.4)	
Albumin (g/dL)			0.047
mean (SD)	3.6 (0.6)	3.1 (0.6)	
NLR			0.012
mean (SD)	3.65 (3.45)	8.81 (7.64)	
Tumor size (cm)			0.731
mean (SD)	6.6 (2.6)	6.9 (3.4)	
Presence of ascites on CT			0.004
Absent	14 (66.7)	4 (21.1)	
Present	7 (33.3)	15 (78.9)	
Lauren type			0.753
Intestinal	5 (23.8)	7 (36.8)	
Diffuse/mixed	13 (61.9)	9 (47.4)	
Undetermined	3 (14.3)	3 (15.8)	
Number of disease sites			0.385
One (only locoregional)	7 (33.3)	4 (21.1)	
Two or more	14 (66.7)	15 (78.9)	
POC			1
No POC/Clavien 1-2	19 (90.5)	17 (89.5)	
Clavien 3-5	2 (9.5)	2 (10.5)	
Length of hospital stay (days)			0.455
Median (IQR)	3 (3-7.5)	4 (4-8)	
Palliative treatment-1st Line			0.012
No	5 (23.8)	12 (63.2)	
Yes	16 (76.2)	7 (36.8)	

30-d mortality			0.049
No	18 (85.7)	11 (57.9)	
Yes	3 (14.3)	8 (42.1)	
90-d mortality			0.027
No	14 (66.7)	6 (31.6)	
Yes	7 (33.3)	13 (68.4)	

SD: Standard deviation; IQR: Interquartile range; POC: Postoperative complication; CCI: Charlson's comorbidity index; CT: Computed tomography; NLR: Neutrophil lymphocyte ratio; ASA: American Society of Anesthesiologists; BMI: Body mass index.

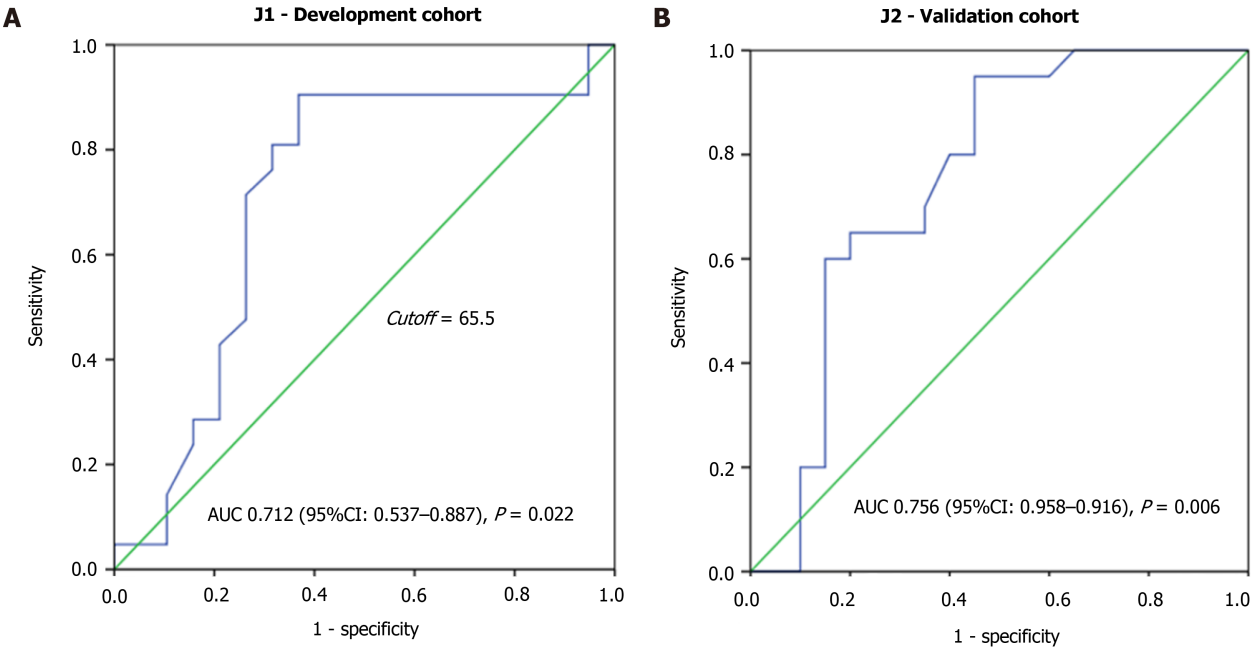

Figure 1 Receiver operating characteristic for the diagnostic accuracy of risk-score. A: Development cohort in predicting 90-d mortality in patients with stage IV gastric cancer (GC) undergoing jejunostomy; B: Validation cohort in predicting 90-d mortality in patients with stage IV GC undergoing jejunostomy. The area under the receiver operating characteristic curve (AUC) was 71.2% in the development group, with an estimated value of 65.5 for optimal cutoff. In the validation cohort the AUC was 75.6%, demonstrating a good diagnostic accuracy for 90-d mortality. AUC: Area under the receiver operating characteristic curve.

predict 90-d mortality, it did not affect the performance of the model. Furthermore, the score performance was higher in the validation cohort compared to the development group (AUC 0.756 *vs* AUC 0.712, respectively).

Some limitations in the present study should be mentioned. Unfortunately, due to its retrospective design, other outcomes related to the effectiveness of nutritional therapy provided by jejunostomy have not been evaluated. Analysis of weight curve, performance status, and quality of life could provide other relevant information. Also, the number of cases is a limitation. Even in a referral center, jejunostomy has a limited role in cancer treatment. This leads to a low volume of reports in the literature concerning its results, and maintains doubts as to its real benefit in cancer care and survival of these patients.

Accordingly, we believe that our series can provide data to assist in the decision of choosing the best way to maintain enteral nutrition. Since our score is easy to perform, it can be applied in other centers—including retrospectively—to evaluate their reproducibility and impact on the oncological outcomes of jejunostomy in these patients.

CONCLUSION

The scoring system developed with 11 variables related to patient's performance status and medical condition was able to distinguish patients submitted to jejunostomy with

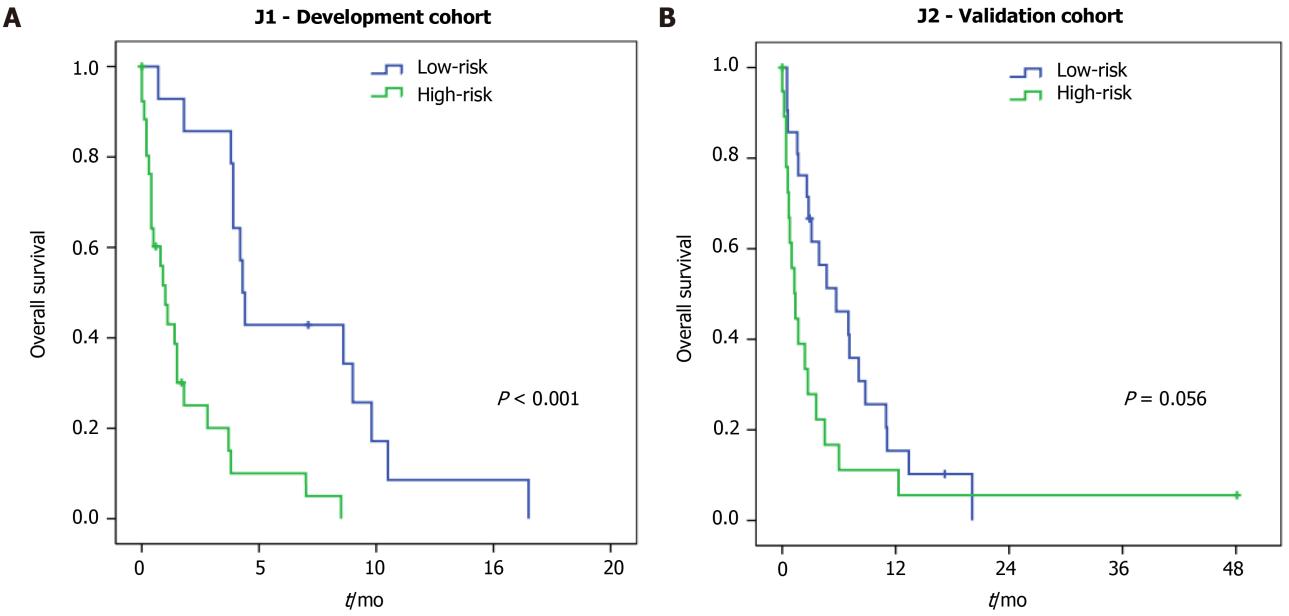

Figure 2 Overall survival of patients with stage IV gastric cancer who underwent jejunostomy according to the risk group. A: Score development cohort; B: Score validation cohort.

a high risk of 90 d mortality. In addition, the score identified patients who were able to receive more CMT in the validation cohort.

ARTICLE HIGHLIGHTS

Research background

Palliative gastrectomy is the initial treatment option for locally advanced gastric cancer (GC) with gastric outlet obstruction whenever it is feasible. Unfortunately, in some cases, nasoenteric tube or jejunostomy becomes the therapeutic alternative to allow maintenance of nutritional enteral support.

Research motivation

The limited survival of these patients raises doubts about who benefits from jejunostomy.

Research objectives

This study aimed to create a prognostic score for 90-d mortality for stage IV GC patients who underwent jejunostomy based on the clinical variables related to survival.

Research methods

We conducted a retrospective analysis of 80 stage IV patients who underwent jejunostomy for obstructive GC. To create a scoring system, patients were randomized into two groups (1:1) by computer using statistical software. The score was developed with half of the patients and further evaluated in a validation cohort with the remaining patients. The score was developed with 90-d mortality as the main outcome.

Research results

We provided a simple and feasible score system with 11 variables easily available in the clinical assessment of patients. The score demonstrated an accuracy of 75.6% in the validation cohort and was associated with the mortality rates in patients who underwent jejunostomy.

Research conclusions

The scoring system developed with variables related to patient's performance status and medical condition was able to distinguish patients submitted to jejunostomy with a high risk of 90 d mortality.

Research perspectives

The results of our series may contribute to identifying stage IV GC with unresectable tumors who can obtain better results with the jejunostomy. In addition, the score may contribute to the selection of patients who were able to receive chemotherapy, and thereby improving their survival.

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ORIGINAL ARTICLE

Observational Study

Novel molecular panel for evaluating systemic inflammation and survival in therapy naïve glioma patients

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Author contributions: Gandhi P conceived the concept, study design, interpretation of data, initial draft, and final review; Shrivastava R performed the data acquisition, analysis, and initial draft; Garg N and Sorte SK performed the patient enrollment, and clinical data and its interpretation.

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Abstract

BACKGROUND

Inflammation is crucial to tumor progression. A traumatic event at a specific site in the brain activates the signaling molecules, which triggers inflammation as the initial response within the tumor and its surroundings. The educated immune cells and secreted proteins then initiate the inflammatory cascade leading to persistent chronic inflammation. Therefore, estimation of the circulating inflammatory indicators kynurenine (KYN), interleukin-6 (IL-6), tissue-inhibitor of matrix-metalloproteinase-1 and human telomerase reverse transcriptase (hTERT) along with neutrophil-lymphocyte ratio (NLR) has prognostic value.

AIM

To assess the utility of chosen inflammatory marker panel in estimating systemic inflammation.

METHODS

The chosen markers were quantitatively evaluated in 90 naive, molecularly subtyped plasma samples of glioma. A correlation between the markers and confounders was assessed to establish their prognostication power. Follow-up on the levels of the indicators was done 3-mo post-surgery. To establish the validity of circulating KYN, it was also screened qualitatively by dot-immune-assay and by immunofluorescence-immunohistochemistry in tumor tissues.

RESULTS

Median values of circulating KYN, IL-6, hTERT, tissue-inhibitor of matrix-metalloproteinase-1 and NLR in isocitrate-dehydrogenase-mutant/wildtype and within the astrocytic sub-groups were estimated, which differed from controls, reaching statistical significance ($P < 0.0001$). All markers negatively correlated

with mortality ($P < 0.0001$). Applying combination-statistics, the panel of KYN, IL-6, hTERT and NLR achieved higher sensitivity and specificity (> 90%) than stand-alone markers, to define survival. The inflammatory panel could discriminate between WHO grades, and isocitrate-dehydrogenase-mutant/wildtype and define differential survival between astrocytic isocitrate-dehydrogenase-mutant/wildtype. Therefore, its assessment for precise disease prognosis is indicated. Association of KYN with NLR, IL-6 and hTERT was significant. Cox-regression described KYN, IL-6, NLR, and hTERT as good prognostic markers, independent of confounders. Multivariate linear-regression analysis confirmed the association of KYN and hTERT with inflammation marker IL-6.There was a concomitant significant decrease in their levels in a 3-mo follow-up.

CONCLUSION

The first evidence-based study of circulating-KYN in molecularly defined gliomas, wherein the tissue expression was found to be concomitant with plasma levels. A non-invasive model for assessing indicators of chronic systemic inflammation is proposed.

Key Words: Circulating; Glioma; Inflammatory marker; Kynurenine; Non-invasive; Prognostic

Core Tip: The current study is the first-ever analysis of the circulatory levels of kynurenine, interleukin-6, tissue-inhibitor of matrix-metalloproteinase-1 and human telomerase reverse transcriptase along with neutrophillymphocyteratio in a sizeable cohort of molecularly classified glioma samples. The ability of this panel to differentiate survival in glioma subgroups has been assessed. Evaluation of this inflammatory panel of potential biomarkers using the minimally invasive blood sample, for prognostication and targeted personalized therapy in glioma, is suggested.

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INTRODUCTION

The role of inflammation and its partakers in glioma, especially the molecular markers secreted at the tumor initiation stage, is still not completely worked out. Since the brain is unique in comparison to other organ systems, being confined within the blood-brain barrier, it presents a local innate immune response to an inflammatory stimulus, like tumor antigens[1], injury, or oncogene over-expression, by the production of inflammatory molecules and/or metabolites[2]. Consequently, inflammation can be a cause or consequence of actively proliferating cells, an event that also initiates the recruitment of immune cells at the tumor site[3]. The stimuli, if persistent, can manifest as chronic inflammation, aberrant cell proliferation, and increased angiogenesis leading to the escape of specific molecules and cells into peripheral circulation[4]. Therefore, clinical management of inflammation associated with glioma initiation and progression is clinically recognized.

One of the significant inflammatory metabolites in the brain is kynurenine (KYN) of the KYN pathway; it is produced and secreted by endothelial cells and pericytes of the blood-brain barrier, which have been stimulated by inflammation. In the absence of effective immune regulation, chronic inflammation is generated by aggressively proliferating cells. Excess KYN production is triggered by inflammation in the tumor environment (TE), which leads to local immune tolerance, and the inflammatory signals generated by the tumor results in KYN being transduced across the intact blood-brain barrier to be detected in systemic circulation[5,6]. Over-activation of KYN

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was first described in glioblastoma (GB) cell lines and tumor tissues as early as 2012 by Opitz and colleagues[7]. Excessive production of this metabolite was linked to increased tumor immunity and decreased survival as recorded by Adams *et al*[8] in cultured cells and 18 GB patient samples[8].

The tumor-infiltrating immune cells - neutrophils and lymphocytes, are also markers of systemic inflammation[9]. Their involvement as local inflammation indicators in glial tumors has been documented by Zadora *et al*[10]. Neutrophil recruitment initiates cytokine secretion, which strengthens the initial *in situ* neuro-inflammatory response by activating more neutrophils and macrophages. Brain tumors, including glioma, express high levels of different cytokines, specifically interleukin-6 (IL-6), involved in multiple pathways of tumor pathology[11]. During gliomagenesis, IL-6 executes a more critical role than other interleukins since it predominately triggers the pro-inflammatory cascade. In the last few years, a couple of blood-based investigations analyzing the expression of IL-6 in different grades of glioma suggested that IL-6 expression may indicate disease prognosis[12,13].

Involved in this scenario of brain inflammation is another set of proteins, the tissue-inhibitor of matrix-metalloproteinases (TIMPs), known to perform multiple functions, including regulating inflammation in the TE. In this context, there is a lone study by Lin *et al*[14] reporting plasma levels of TIMP-1 to be associated with an inflammatory response in glioma, proposing a role of this molecule in prognosis[14].

In this landscape of inflammation partakers in the TE, the association between increased chronic inflammation and telomerase activity is often overlooked. The human telomerase reverse transcriptase (hTERT) is an enzyme active in the immune system cells and regulates inflammation; however, its activity beyond a critical limit initiates uncontrolled proliferation. Several studies suggest that TERT activates the nuclear factor kappa-B target genes such as IL-6, which is crucial to inflammation and cancer progression[15-17]. Research groups, including ours, have demonstrated that hTERT executes many vital functions independent of its telomere maintenance, namely angiogenesis, inflammation, and stemness in glioma[18,19]. These findings suggested that the feedback loop of the hTERT signaling pathway may reinforce the inflammatory signaling *via* cytokine secretion, leading to the development of chronic inflammation in the TE.

Mechanistically, a traumatic event at a specific site in the brain leads to the secretion of inflammatory molecules such as IL-6 and KYN, which trigger the inflammatory cascade leading to over-activation of telomerase. The abnormal hTERT physiology is responsible for immune system dysfunction, followed by persistent chronic inflammation leading to a malignant phenotype. Further, this unresolved chronic inflammation also begins contributing to disease progression. The current exploratory study is the first ever analysis of the circulatory levels of KYN, IL-6, neutrophil-lymphocyte ratio (NLR), hTERT, and TIMP-1 in a sizeable cohort of glioma samples categorized according to their histological grade and isocitrate-dehydrogenase (IDH) expression. The ability of this panel to differentiate survival in glioma subgroups has been assessed.

MATERIALS AND METHODS

Inclusion criteria

Patients with symptoms of recurrent seizures and headache radiologically diagnosed and histopathologically confirmed as glioma grade II, III, or IV.

Exclusion criteria

Patients less than 18 years of age or patients histopathologically confirmed as glioma grade I.

Subjects enrolled for observational study

Ninety treatment-naive samples, presenting with clinical symptoms like recurrent seizures, headache, increased intracranial pressure, and radiological diagnosis of glial tumor, were collected from the in-patient ward of the Neurosurgery department. Informed consent in writing was taken from all the participants of the study cohort (IRB/21/Res/11). Forty-five healthy subjects without any recent clinical history of inflammation or autoimmune disease, were taken as controls for blood samples. Whole blood was collected just before surgery; plasma and serum were separated and preserved at -80°C until further testing for target proteins was undertaken. A follow-up sample of all patients was done 3 mo post-surgery. According to the established lab

protocol, the systemic levels of the marker KYN were correlated with its *in situ* tumor expression by immunofluorescence-immunohistochemistry for validation (Supplementary File Method A).

Dot immune-binding assay

Qualitative screening of circulating markers was done using 20μL of serum after removing high abundance proteins. Subsequently, the samples were loaded onto nitrocellulose membrane and incubated overnight at 4°C. Then the membrane was probed with KYN, IL-6, TIMP-1, and hTERT antibody (1:2000 dilution, Monoclonal, Santa Cruz Biotechnology, United States) for 2 h followed by host-specific respective alkaline-phosphatase-conjugated antibody tagging (Santa Cruz Biotechnology, United States; 1:2500 dilutions) for 1 h. The label was detected using an alkaline-phosphatase substrate (BCIP, Sigma Aldrich, United States), which produced a visible signal corresponding to the concentration of the target protein.

Quantification of circulatory markers

Quantification of the biomarkers in plasma samples was done by enzyme-linked immunosorbent assay (ELISA) for KYN (Creative Diagnostics, United States), TIMP-1 (R&D systems, United States), and hTERT (Elabscience, United States) according to details provided in the instruction manuals. The plasma concentrations were noted in ng/mL for KYN and TIMP-1, and ng/L for hTERT. IL-6 test results were computed from the patients' clinical reports and compared with the baseline values earlier established for this marker in the lab[13]. The standard reference range of each marker was defined and set as per kit insert. Complete blood counts of all enrolled subjects were recorded from the pre-surgery blood profile; the neutrophil and lymphocyte counts were extrapolated into the mathematical formula and calculated[18] to establish the NLR in patients and controls.

Statistical analysis

Non-parametric Kruskal-Wallis test was used to assess the difference between samples of different histological grades for plasma values of all biomarkers. Mann Whitney test was applied on the groups stratified according to IDH status and for marker panel KYN, TIMP-1, NLR, IL-6, and hTERT, to differentiate their significance. Analysis of area under the curve for receiver operating characteristic was performed to define the cut-off values and sensitivity of the markers between the grades and within subgroups that attained ≥ 80% specificity. To establish the diagnostic accuracy of the markers, CombiROC software (https://combiroc.eu) was used, and the predictive probability of the inflammatory panel for prognostication was subjected to receiver operating characteristic analysis. To discern the influence of covariates on plasma maker levels, univariate analysis was carried out to identify the confounding factors, namely, age, site, extent of resection, therapy, KYN, TIMP-1, NLR, IL-6 and, hTERT. Parameters attaining a level of significance in this analysis were entered into the multivariate functionally to create the final model. Furthermore, the Cox proportional regression model was used to compute hazard ratios with 95% confidence interval (CI) to establish the independent status of prognostic markers.

Overall survival (OS), defined as the time from randomization to death from any cause, was considered as a direct measure of clinical benefit to the patient. Patients alive or lost to follow-up were treated as censored. Curves defining total survival period with reference to the identified biomarkers were drawn on Kaplan-Meier estimates and differences compared between IDH-mutant/wildtype (IDH-*m/w*) subgroups for statistical significance using the log-rank test. Spearman's rho coefficient was applied to calculate the correlation of all five markers with OS within histological grades, between IDH-*m/w*, astrocytic-*m/w*, and in terms of KYN, TIMP-1, NLR, IL-6, and hTERT. Multivariate linear regression was performed to describe the association of the four markers with inflammation. Paired *t*-test was performed for pre-operative and 3-mo follow-up samples for the panel. The *P* values of all statistical tests were two-sided ($P < 0.05$).

RESULTS

Plasma samples from healthy controls ($n = 45$) and glioma patients ($n = 90$, IDH-*m:* $n = 60$ inclusive of astrocytic and oligo-component and IDH-*w*, astrocytic $n = 30$) formed the study cohort. Details of patients, relevant demographics, and clinical data are presented in Table 1.

Table 1 Demographic features and concentrations of molecular markers of recruited subjects

No.	Factors	Controls	IDH-mutant	IDH-wildtype
1	Sample size, (*n*)	(*n* = 45)	60	30
2	Sex, M/F	M = 30; F = 15	M = 51; F = 9	M = 25; F = 5
3	Age (yr)	25 (21-50)	35.0 (13-75)	51.5 (25-76)
4	Site	-	Frontal = 37/Non-frontal = 23	Frontal = 12/Non-frontal = 18
5	EOR	-	STR = 38; GTR = 14	STR = 30; GTR = 0
6	OS	-	28.5 (1-158)	6.5 (1-38)

Values presented as median with range. M: Male; F: Female; EOR: Extent of resection; STR: Sub-total resection; GTR: Gross total resection; OS: Overall survival; IDH: Isocitrate dehydrogenase.

Qualitative screening

The presence/absence of KYN, IL-6, TIMP-1, and hTERT in circulation was screened on nitrocellulose membrane by dot immune-binding assay. A colored signal (blue) indicated immune-complex formation and presence of the inflammatory molecule KYN (Figure 1) .The signal intensity of each marker corresponded to the pathological grading of the tumor.

Quantification of biomarkers by sandwich ELISA

Median values of plasma KYN in grade-II, III, and IV were 69.86ng/mL, 112.15ng/mL, 237.318 ng/mL, respectively. Levels of circulating KYN, IL-6, TIMP-1, and hTERT emerged as substantially higher ($P < 0.0001$) with increasing histological grade when the Kruskal-Wallis test was applied. The statistical difference between IDH-*m/w* groups (Table 2) using Mann Whitney test was highly significant for KYN ($P < 0.0001$), NLR ($P = 0.0002$), TIMP-1 ($P = 0.0405$) and hTERT ($P < 0.0001$).

The association of plasma levels (preoperative) of all markers yielded a positive correlation with the grade; however, the best value was observed for IL-6 ($r = 0.64$, $P < 0.0001$, Table 3), while the most significant inverse correlation of KYN ($r = 0.6154$, $P < 0.0001$) was attained with OS (Table 3). When the samples were molecularly stratified, there was an inverse correlation of all biomarkers with survival outcome, being worse for IDH-*w* ascompared to IDH-*m*. The Spearman coefficient for inflammatory markers KYN, TIMP-1, NLR, IL-6, and hTERT was significant and positive for tumor grade, but there was an inverse association with OS, suggesting a poor prognosis with increasing systemic levels of these markers in therapy naïve glioma patients.

The tissue expression of KYN was concomitant with its plasma levels and increased with increasing histological grade (Supplementary Figure 1).

Determination of independence in prognostication

The relation of OS with KYN, TMIP-1, IL-6, NLR, hTERT, age, site, the extent of resection and therapy was calculated using univariate and multivariate Coxregression models to identify the plausible prognostic factors (Table 4). Univariate analysis delineated shorter patient survival to be associated with KYN, IL-6, NLR, TIMP-1, hTERT ($P = 0.0001$), and age ($P = 0.0004$). When multivariate Cox-regression model was applied, higher levels of KYN ($P = 0.0003$), IL-6 ($P = 0.0004$), NLR ($P = 0.0001$) and hTERT ($P = 0.0026$) were found to independently define prognosis. Based on these results, it was assumed that TIMP-1 could not be considered a sensitive marker for inflammation. So, the final maker panel of four markers, KYN, IL-6, NLR, and hTERT, was taken further for validation.

AUROC

The cut-off thresholds based on area under the curve for the four circulatory biomarkers were KYN: > 22.89, IL-6: > 62.5, NLR: > 2.775, and hTERT: > 1.309, at more than 70% sensitivity and 80% specificity, when comparing controls *vs* glioma patients. Levels of KYN, IL-6, NLR, and hTERT could significantly differentiate between histological grades, low and high grade and IDH-*m/w*, with more than 80% sensitivity (Supplementary Table 1).

Based on the AUROC analysis, optimal cut-off points were determined for the best balance of sensitivity and specificity and the highest value of likelihood ratio to predict survival through log-rank analysis, and thereafter Kaplan Meier curves were con-

Table 2 Concentrations of molecular markers of recruited subjects of glioma patients in terms of isocitrate dehydrogenase mutant and wildtype glioma

No.	Factors	Controls (*n* = 45)	IDH-mutant (*n* = 60)	IDH-wildtype (*n* = 30)	*P* value
1	KYN (ng/mL)	5.009 (0.052-34.43)	77.155 (1.56-387.44)	237.318 (53.43-894.95)	< 0.0001[c]
2	IL-6 (pg/mL)	37.009 (6.715-62.49)	68.62 (21.85-209.73)	197.672 (59.217-803.711)	< 0.0001[c]
3	NLR	1.59 (0.87-2.8)	3.67 (1.23-11.5)	5.585 (3.1-18)	0.0002[c]
4	TIMP-1 (ng/mL)	38.03 (10.26-73.99)	82.54 (25.09-131.30)	107.01 (37.175-185.09)	0.0405[a]
5	hTERT (ng/mL)	1.029 (0.075-1.58)	1.364 (0.1-6.886)	2.185 (0.565-8.84)	< 0.0001[c]

[a]*P* < 0.05.
[c]*P* < 0.001.
Median with ranges of all circulatory markers in controls, isocitrate dehydrogenase (IDH)-*m* and IDH-*w*. KYN: Kynurenine; IL-6: Interleukin-6; NLR: Neutrophil-lymphocytes ratio; TIMP-1: Tissue inhibitor metalloproteases-1; hTERT: Human telomerase reverse transcriptase; IDH: Isocitrate dehydrogenase.

Table 3 Spearman coefficient correlation of circulatory markers with overall survival, histological grade and Kynurenine

No.	Circulatory markers	Spearman r	95%CI	*P* value
	Correlation with OS			
1	KYN (ng/mL)	-0.6154	-0.7324 to -0.463	< 0.0001[c]
2	IL-6 (pg/mL)	-0.5531	-0.6854 to -0.3855	< 0.0001[c]
3	NLR	-0.5696	-0.698 to -0.4058	< 0.0001[c]
4	TIMP-1 (ng/mL)	-0.4831	-0.6312 to -0.3011	< 0.0001[c]
5	hTERT (ng/mL)	-0.4386	-0.5960 to -0.2489	< 0.0001[c]
	Correlation with grade			
6	KYN (ng/mL)	0.5409	0.3705 to 0.6761	< 0.0001[c]
7	IL-6 (pg/mL)	0.6400	0.4944 to 0.7507	< 0.0001[c]
8	NLR	0.4982	0.3190 to 0.6430	< 0.0001[c]
9	TIMP-1 (ng/mL)	0.2614	0.05118 to 0.4495	0.0128[a]
10	hTERT (ng/mL)	0.3000	0.06441 to 0.4834	0.0102[a]
	Correlation with KYN			
11	IL-6 (pg/mL)	0.4919	0.3115 to 0.638	< 0.0001[c]
12	NLR	0.4084	0.214 to 0.5717	< 0.0001[c]
13	TIMP-1 (ng/mL)	0.4718	0.2877 to 0.6223	< 0.0001[c]
14	hTERT (ng/mL)	0.3504	0.1485 to 0.5243	0.0007[c]

[a]*P* < 0.05.
[c]*P* < 0.001.
An extremely significant inverse correlation of kynurenine, interleukin-6, neutrophil-lymphocytes ratio, tissue inhibitor metalloproteases-1 and human telomerase reverse transcriptase was obtained with respect to overall survival. A significant positive correlation of interleukin-6, neutrophil-lymphocytes ratio, tissue inhibitor metalloproteases-1 and human telomerase reverse transcriptase was obtained with respect to grades and kynurenine circulatory levels. OS: Overall survival; KYN: Kynurenine; IL-6: Interleukin-6; NLR:Neutrophil lymphocytes ratio; TIMP-1: Tissue inhibitor metalloproteases-1; hTERT: Human telomerase reverse transcriptase; CI: Confidence interval.

structed for the four biomarkers (Figure 2A-D). OS was even better in patients with IDH-*m* astrocytic tumors than their IDH-*w* counterparts (Figure 2E).

Circulating concentrations of KYN, NLR, IL-6 and hTERT were set at 22.89 ng/mL, 2.775, 62.5 pg/mL and 1.309 ng/L, respectively. Patients with values exceeding these thresholds were observed to have a shorter survival period. The OS was defined for KYN (18 *vs* undefined months, HR 3.176 with 95%CI: 1.626-6.206, *P* = 0.0007), NLR (20 mo *vs* 48 mo, HR 0.4167 with 95%CI: 0.204-0.8512, *P* = 0.0025), IL-6 (20 mo *vs* 80 mo,

Table 4 Univariate and multivariate Cox regression analysis of variables

No.	Variable	Univariate		Multivariate	
		HR (95%CI)	*P* value	HR (95%CI)	*P* value
1	KYN (ng/mL)	1.0037 (1.0026-1.0048)	0.0001[c]	1.0024 (1.0011-1.0037)	0.0003[c]
2	IL-6 (pg/mL)	1.0032 (1.0021-1.0042)	0.0001[c]	1.0022 (1.0010-1.0035)	0.0004[c]
3	NLR	1.2437 (1.1621-1.3312)	0.0001[c]	1.2085 (1.1138-1.3114)	0.0001[c]
4	TIMP-1 (ng/mL)	1.0150 (1.0075-1.0225)	0.0001[c]	1.0046 (0.9975-1.0118)	0.2042
5	hTERT (ng/mL)	1.2681 (1.1498-1.3987)	0.0001[c]	1.2303 (1.0749-1.4080)	0.0026[b]
5	Age(yr)	1.0245 (1.0108-1.0383)	0.0004[c]	1.0090 (0.9938-1.0233)	0.2165
6	EOR	1.3379 (1.8805-3.0328)	0.1726	NA	NA
7	Site	0.9527 (0.6221-1.4589)	0.8236	NA	NA
8	Therapy	0.8507 (0.2201-1.3578)	0.906	NA	NA

[b]$P < 0.01$.

[c]$P < 0.001$; indicated a significant association.

Results are presented as estimated hazards ratio with 95% confidence interval for specific variables. Variables with a significant P value (< 0.05) in univariate Cox-regression model were selected and analyzed using multivariate Cox-regression. CI: Confidence interval; EOR: Extent of resection; HR: Hazard ratio; hTERT: Human telomerase reverse transcriptase; KYN: Kynurenine; IL-6: Interleukin-6; NA: Not assessed; NLR: Neutrophil-lymphocytes ratio; TIMP-1: Tissue inhibitor metalloproteases-1.

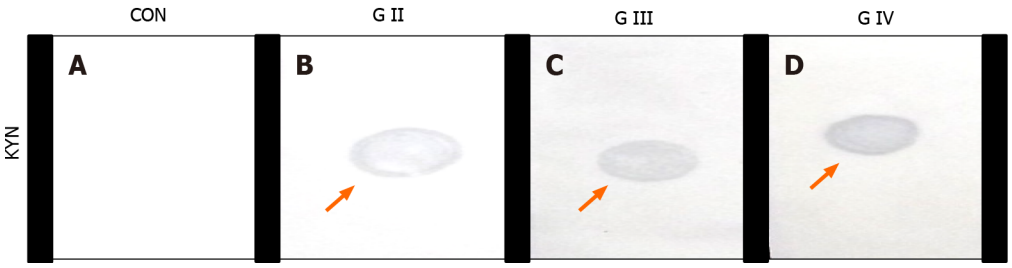

Figure 1 Qualitative screening of kynurenine by dot–immune binding assay in control, grade II, grade III, and grade IV samples; red arrow indicates the presence of immune-complex. A: Control; B: Grade II; C: Grade III; D: Grade IV. KYN: Kynurenine; CON: Control; G: Grade.

HR 0.25 with 95%CI: 0.1318-0.4741, $P = 0.0008$), and hTERT (18 mo *vs* 48 mo, HR 0.21 with 95%CI: 0.1178-0.4741, $P = 0.0006$).

On follow-up, paired t-test between pre-and 3-mo post-surgery levels of KYN ($P = 0.0148$), IL-6 ($P = 0.0107$), NLR ($P = 0.0038$), and hTERT ($P = 0.0016$) showed a significant difference, and lower values post-intervention indicate that inflammation has plausibly reduced on debulking of the tumor (Table 5).

CombiROC

The predicting accuracy of these chosen candidate markers to ascertain systemic inflammation status in glioma patients was increased substantially in combination as a panel rather than standalone markers. Applying CombiROC enhanced the sensitivity of the biomarker panel. The sensitivity of KYN, IL-6, NLR, and hTERT was 86.11%, 72.22%, 77.78%, and 80% individually, which increased to 94.4% in combination with 96.7% specificity and an area under the curve of 0.983 as seen in combination VII (Figure 2F).

Based on the above results and multivariate linear regression, it can be inferred that there exists anassociation between the tumor secreted inflammatory molecules (Figure 3); therefore, these markers can be evaluated to assess systemic inflammation and prognosis.

Table 5 Difference in preoperative and postoperative concentrations of markers in blood samples collected in a 3-mo follow-up

No	Markers	Preoperative concentration	3-mo postoperative concentration	*P* value	rs value
1	KYN (ng/mL)	126.1 (19.410-387.440)	75.405 (16.340-227.400)	0.0148[a]	0.4509
2	IL-6 (pg/mL)	75.65 (23.96-209.73)	60.03 (21.85-140.77)	0.0107[a]	0.1850
3	NLR	4.30 (1.43-15.00)	2.93 (0.80-6.90)	0.0038[b]	0.7260
4	hTERT (ng/L)	2.27 (1.16-6.86)	1.33 (0.78-4.78)	0.0016[b]	0.6574

[a]$P < 0.05$.
[b]$P < 0.01$.
Concentration presented as median with ranges. hTERT: Human telomerase reverse transcriptase; IL-6: Interleukin-6; KYN: Kynurenine; NLR: Neutrophil-lymphocytes ratio.

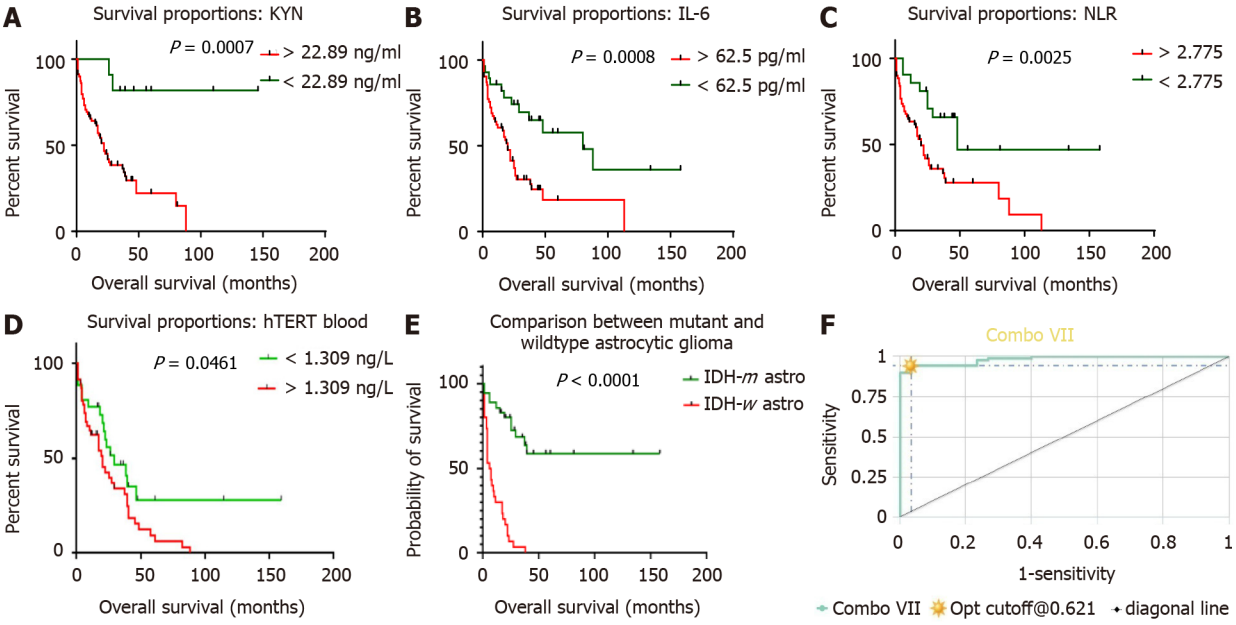

Figure 2 Survival curves of circulatory markers kynurenine, interleukin-6, neutrophil-lymphocyte ratio and human telomerase reverse transcriptase(red indicates worse prognosis while green represents favorable prognosis), differential survival between astrocytic isocitrate dehydrogenase-*mutant* and isocitrate dehydrogenase-*wildtype* gliomas, combination receiver operating characteristic of the panel of kynurenine, interleukin-6, neutrophil-lymphocyte ratio, and human telomerase reverse transcriptase to achieve increased sensitivity and specificity for predicting overall survival. A: Kynurenine; B: Interleukin-6; C: Neutrophil-lymphocyte ratio; D: Human telomerase reverse transcriptase; E: Differential survival between astrocytic isocitrate dehydrogenase-mutant and wildtype gliomas; F: Combination receiver operating characteristic of the panel of kynurenine, interleukin-6, neutrophil-lymphocyte ratio, and human telomerase reverse transcriptase to achieve increased sensitivity and specificity for predicting overall survival. KYN: Kynurenine; IL-6: Interleukin-6; hTERT: Human telomerase reverse transcriptase; NLR: Neutrophil- lymphocyte ratio; IDH-*m* astro: Isocitrate dehydrogenase mutant astrocytic; IDH-*w* astro: Isocitrate dehydrogenase wildtype astrocytic.

DISCUSSION

The link between a tumor and inflammation is a well-established fact and is one of the major attributes of malignancy[19]. Inflammation in the TE mediates all aspects of glial oncogenesis, including *in situ* progressive development of vasculature and tissue remodeling[20]. Thus, by and large, the inflammatory molecules orchestrate the extrinsic and intrinsic stimuli, thereby initiating and contributing to tumor progression [21].

Although KYN is an important inflammatory metabolite in the glial-onco-transformation process, there are limited studies on the role of this molecule in glioma, with three research groups recording the ratio of KYN and tryptophan in a very small number of patients. In reference is the study on the plasma of 18 GB patients by Adams *et al*[8], who presented data to show that because of activation of the KYN pathway, the KYN/TRP-ratio was significantly higher in GB as compared to healthy volunteers. The other group of investigators presented a different opinion on KYN as a marker, stating that there was no significant difference in the levels before and after

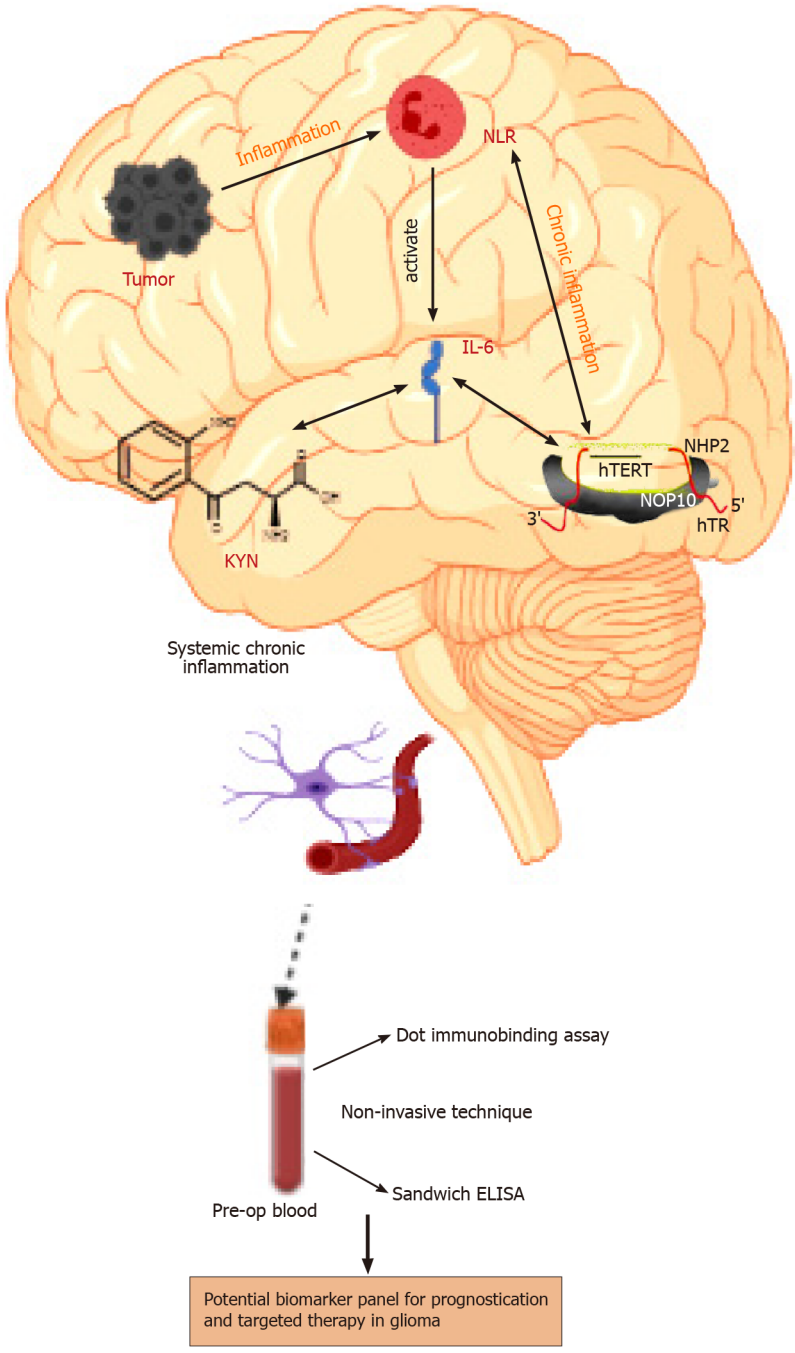

Figure 3 A non-invasive model for evaluating chronic systemic inflammation and overall survival in glioma. KYN: Kynurenine; IL-6: Interleukin-6; NLR: Neutrophil lymphocytes ratio; hTERT: Human telomerase reverse transcriptase; ELISA: Enzyme-linked immunosorbent assay.

surgical intervention, as seen in 10 GB samples[22]. However, the research by Lenzen *et al*[23] documented significantly decreased serological values of KYN after a vaccine treatment with heat shock protein-peptide complex-96, but they did not provide any baseline data of KYN for comparison.

Therefore, the present therapy-naive dataset is unique as it establishes KYN levels in circulation for glioma, with values screened qualitatively at baseline and recorded quantitatively at two time points, pre-and post-surgery. This marker was able to differentiate between IDH-*m/w* groups with high significance (Table 2), along with histological grades and OS (Figures 1 and 2).

When a chronic inflammatory response is generated within the tumor, the KYN pathway proteins trigger the over-expression of pro-inflammatory cytokine IL-6[8]. We noted a raised expression of the inflammatory marker IL-6 in our enrolled glioma patients. Similarly, limited systemic studies available on GB reveal that aberrant production of this circulating cytokine is directly associated with tumor growth and poor survival, demonstrating IL-6 as an independent prognostic factor for survival[13,

24,25]. These studies suggest that the cytokine plays an important role in the *in situ* inflammatory response within the tumor.

Despite the established association between telomerase activity and inflammation, the related molecular pathway in glioma has not been elucidated. Elevated telomerase activity for the sustained proliferation of tumor cells begins a smoldering response of acute followed by chronic inflammation[26,27]. The work of Shervington and Patel[28] in glioma subtypes suggests a noteworthy variation of TERT protein expression levels in GB when compared with control. Two case reports presented from our lab also correlate hTERT expression to proliferation, stemness, and survival in glioma subtypes[13,29]. In a first-of-its-kind investigation, the correlation between tissue and blood concentrations of hTERT marker was also established by us in glioma[30]. Findings of the current analysis are also in line, and there was a significant difference of this marker among grades, IDH-*m*/*w*, and a positive association with KYN and IL-6.

In the present cohort analysis, the NLR score emerged as a good indicator of prognosis and survival in the molecularly typed sub-groups. Concurrence of this result can be found in studies conducted on smaller groups of GB patients[31,32] which show that there is a significant association of NLR with OS[33-36]. Our previous work on diffuse gliomas, along with the current study on IDH characterized gliomas, provides substantial laboratory-based evidence that NLR is a marker of inflammation and can be used as a prognosticator in the clinical setting[18].

There is an increasing acceptance of the proactive role of glial tumor cells in brain inflammation, leading to suppression of innate immune response *via* secretion of matrix metalloproteases[37,38]. In a study conducted earlier in the decade, Sreekantreddy and his co-workers established GBM-specific up-regulation of serum TIMP-1 by ELISA[39]. Similarly, while working with 36 patients, it was suggested by Crocker *et al*[40] that serum TIMP-1 levels may serve as an independent predictor of survival in glioma subtypes[40]. In an extensive study on low-grade glioma patients, Zeng *et al*[41] established that plasma TIMP-1 expression was significantly correlated with OS and relapse-free survival in these patients[41]. However, our results are partially in contention with the above studies. The plasma levels of TIMP-1 were up-regulated in our patients compared to controls, but the marker did not reach significance as an independent prognosticator.

CONCLUSION

The systemic levels of target molecules discussed herein can be considered to represent the cascade of events leading to chronic systemic inflammation in glioma, as depicted in Figure 3. The present work is a one of its kind experiment-based qualitative and quantitative estimation of circulating KYN in a substantial sized cohort of molecularly defined gliomas. KYN is able to differentiate between histological grades, IDH-*m/w*, in terms of patient survival, along with IL-6, hTERT, and NLR, thus advocating assessment of this inflammatory panel of potential biomarkers using the minimally invasive blood sample for targeted therapy and prognostication in glioma. The study needs to be replicated in a bigger cohort and conducted as a multicentric study to establish clinical utility of this panel.

ARTICLE HIGHLIGHTS

Research background

Chronic persistent inflammation is a hallmark of glioma and a major contributor to the disease progression. Currently there are no serological or molecular markers that are routinely evaluated before deciding treatment and in the follow-up period for monitoring survival and therapeutic efficacy.

Research motivation

A non-invasive inflammatory marker panel is essential to define survival and plan for a better clinical outcome.

Research objectives

The objective of this investigation was to assess the utility of the non-invasive biomarker panel to estimate systemic inflammation and whether it can define and differentiate survival in glioma subgroups.

Research methods

Dot-immune assay for screening of the expression of molecular makers followed by estimation of circulatory levels by enzyme-linked immunosorbent assay are easy, cost-effective and sensitive methods even in resource limited settings. The expression of marker kynurenine (KYN) has been validated by immunofluorescence-immunohisto-chemistry *in situ*.

Research results

The molecular marker panel of KYN, interleukin-6, human telomerase reverse transcriptase and neutrophil-lymphocyte ratio were negatively correlated with mortality ($P < 0.0001$) and achieved higher sensitivity and specificity (> 90%) than stand-alone markers, to define survival. It could discriminate between WHO-grades, isocitrate-dehydrogenase-*mutant/wildtype* and define differential survival between astrocytic isocitrate-dehydrogenase-*mutant/wildtype*. Association of KYN with neutrophil-lymphocyte ratio, interleukin-6, and human telomerase reverse transcriptase was significant. Cox-regression described KYN, interleukin-6, neutrophil-lymphocyte ratio, and human telomerase reverse transcriptase, as good prognostic markers, independent of confounders. Multivariate linear-regression analysis confirmed a concomitant significant decrease in the levels of the markers, in a 3-mo follow-up.

Research conclusions

This is a first of its kind evidence-based study of a non-invasive panel that can estimate chronic systemic inflammation, wherein the identified over-expressed molecular markers can be targeted to design a personalized therapy.

Research perspectives

The study model when replicated in a bigger sized multicentric cohort can pave way for the use of the inflammatory molecular screen for routine patient care.

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CASE REPORT

Gastric myeloid sarcoma: A case report

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Abstract

BACKGROUND

Myeloid sarcoma (MS) is a rare hematologic malignancy defined as an extramedullary tumor of immature granulocytic cells. It can occur as primary or *de novo* and be associated with myelodysplasia or myeloproliferative neoplasms. The most frequent locations are the skin, lymph nodes and bones. The case of a patient with a diagnosis of primary granulocytic *de novo* gastric MS is reported.

CASE SUMMARY

A 19-year-old female patient with MS, whose abdominal computed tomography showed a bulky tumor of 16.5 cm in the gastric chamber with infiltration in the retroperitoneal, pancreatic and bile duct region; the histological study showed gastric mucosa diffusely infiltrated by mononucleated cells and the immunohistochemistry expressed myeloperoxidase. After receiving induction chemotherapy based on the 3 + 7 regimen (daunorubicin/cytarabine), the patient developed severe hematological toxicity and neutropenic typhlitis which required a prolonged medical treatment. She presented a rapid disease progression. Although she received supportive treatment, the patient died.

CONCLUSION

Gastric primary *de novo* MS is a rare and aggressive course neoplasm, fostering knowledge is very important to decide its management and to promote more approaches focused on understanding this pathology and its particularities in our population.

Key Words: Myeloid sarcoma; Granulocytic sarcoma; Stomach; Chemotherapy; Peru; Case report

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Core Tip: This case report describes a gastric primary *de novo* myeloid sarcoma (MS) which is a very rare hematological neoplasm with poor prognosis in a young and symptomatic patient. After receiving chemotherapy, she presented severe toxicity (neutropenic typhlitis) and rapid disease progression. This case highlights the importance of detecting gastric primary MS as a rare form of extramedullary myeloid leukemia presentation. Moreover, management of gastric primary MS could lead to interventions to avoid deterioration of gastrointestinal system during treatment. There is limited information of management and outcomes regarding gastric primary MS. Furthermore, there is very limited data about *de novo* MS in Peruvian patients.

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INTRODUCTION

Myeloid sarcoma (MS) is defined as a myeloblast tumor produced in an anatomical place different from the bone marrow that destroys the original architecture of the local tissue. These tumors are also known as granulocytic sarcomas, chloromas or extramedullary myeloid tumors[1,2]. MS occurs more frequently in males and young individuals; its location is variable, and cerebral, mammary, testicular, gastrointestinal involvement has been reported, among other visceral organs, and it appears more frequently in the skin, lymph nodes and bones[3].

MS occurrence is frequently associated with acute myeloid leukemia (AML), affecting between 2.5% and 9% of patients. When the disease is detected without clinical signs of leukemia and in association with a negative bone marrow biopsy, it is classified as *de novo* MS[2], the incidence of which is 2 cases per million adults[2,4]. Gastrointestinal presentation is uncommon and shows nonspecific symptoms related to the effect of tumor mass[3,5].

CASE PRESENTATION

Chief complaints

The patient was a 19-year-old female without a relevant history.

History of present illness

The patient was admitted to the hospital with nausea, vomiting and early satiety of 5 mo of evolution associated with an episode of hematemesis.

Physical examination

The patient showed low body weight, a regular general condition, distended abdomen, ascites, and a poorly defined bulky mass located in the upper hemiabdo-men.

Laboratory examinations

Laboratory tests at admission revealed moderate anemia (Hb: 9 g/dL), hypoalbuminemia (albumin: 2.5 g/dL), grade 4 hyperbilirubinemia (total bilirubin: 2.5 mg/dL, indirect bilirubin: 1.8 g/dL), grade 2 hypertransaminasemia (aspartate aminotransferase: 90 IU/L, alanine aminotransferase: 100 IU/L), and elevated alkaline phosphatase (360 IU/L).

Imaging examinations

Abdominal computed tomography showed a bulky tumor of 16.5 cm in the gastric chamber, which infiltrated the retroperitoneal, pancreatic and bile duct regions, with dilation of the latter. In addition, peritoneal thickening, free fluid, splenomegaly and

bilateral hydronephrosis were observed (Figure 1). Upper digestive endoscopy was reported at the level of the infiltrated gastric mucosal body with decreased contractility, thickened gastric folds, and infiltration of the duodenal bulb throughout its length. The colonoscopy showed no lesions.

Pathology

The histological study showed gastric mucosa diffusely infiltrated by mononucleated cells, which were intermediate in size with eosinophilic cytoplasm and blast-like nuclei (Figure 2). Immunohistochemistry indicated that these cells expressed myeloperoxidase (MPO) (+), CD117 (+) CD34 (+), CD20 (-), CD3 (-), CD68 (-), CD38 (-), CD30 (-), DTT (-), and Ki67: 80% (Figure 3). Bone marrow analysis by cytomorphology and flow cytometry was negative for infiltration by myeloid blasts. The bone marrow karyotype was 46 XX, and it was not possible to demonstrate the presence of the AML1-ETO, CBFB-MYH11, NPM1 mut A, and FLT3-ITD genes or mutations in exons 8 and 17 of the c-kit gene in bone marrow and the primary tumor.

FINAL DIAGNOSIS

This case final diagnosis is primary gastric *de novo* granulocytic MS.

TREATMENT

The patient began induction treatment based on the 3 + 7 regimen (daunorubicin 60 mg/m^2 for 3 d + citarabine 200 mg/m^2 for 7 d). She developed severe hematological toxicity and neutropenic typhlitis requiring antibiotic and antifungal coverage, parenteral nutritional support and a prolonged stay.

OUTCOME AND FOLLOW-UP

In the reassessment of the disease 30 d after treatment, the patient reached a partial response. A second cycle of 3 + 7 regimen treatment was scheduled, and regular tolerance and rapid disease progression were observed, thus she received supportive treatment; however, the patient died.

DISCUSSION

The case of a 19-year-old woman with a final diagnosis of gastric granulocytic type MS is presented. The symptoms were similar to those reported in other cases of gastric MS, which was conditioned by the extensive intra-abdominal involvement of the neoplasm (Figure 1)[2,6,7]. The differential histopathological diagnosis was based on morphological and immunohistochemical characteristics, which included non-Hodgkin lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma, lymphoblastic lymphoma, and Ewing's sarcoma, among others[3,8]. The present case had a blast type histology. Classically, MS expresses myeloid markers such as CD13, CD33 and MPO, but these markers are not present in all cases. For example, the expression of MPO, one of the most frequent markers, has been reported to be between 83.6% and 64.1% in different series[9]. On the other hand, the expression of CD68, a marker related to lymphocyte lineage and lysosome leakage, was positive in our patient; its presence has been reported in up to 100% of patients with MS[8]. Other markers, such as CD33, CD13 and related to line B, are less consistent in these patients[4,8,9]. It can be distinguished from other round cell neoplasms, such as Ewing sarcoma or neuroendocrine tumors, with specific markers, such as CD99-specific neuronal enolase and CD99, respectively. It has been reported that the anomaly of the nucleus binding factor (translocation between chromosomes 8 and 21) is the most frequent in patients with *de novo* MS; this has been reported in 38% of cases in an North American study[8]. In contrast, translocation 8; 21 was only found in 3% of patients in an Italian series[9]. This translocation was not found in the present case, and there is probably a different frequency of it in our population.

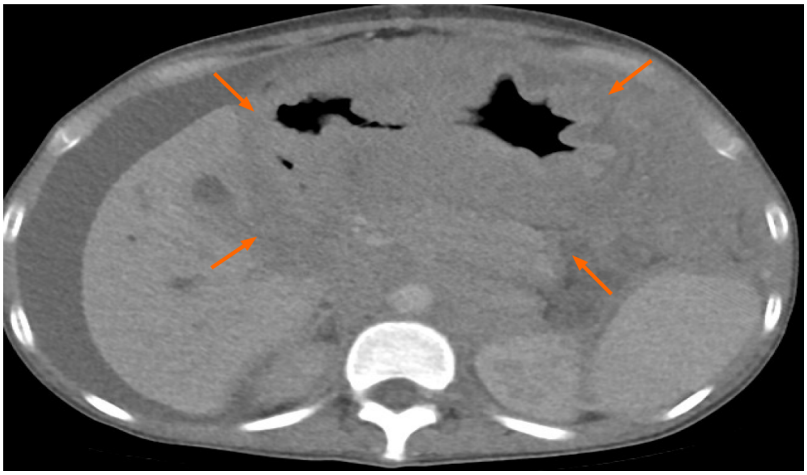

Figure 1 Abdominal computed tomography scan used a part of diagnosis. Computed tomography scan showing extensive intra-abdominal bulky tumor of 16.5 cm at diagnosis with the presence of ascitis.

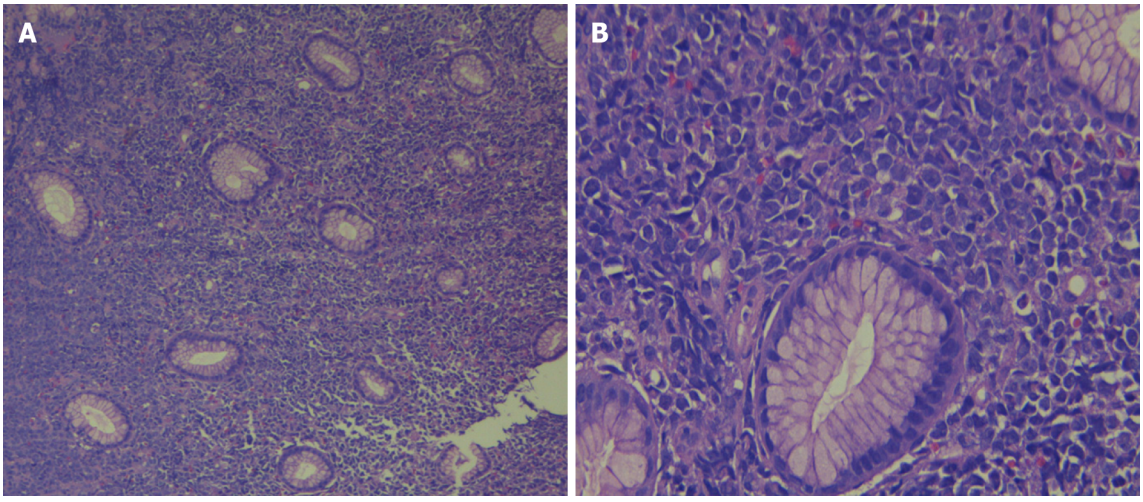

Figure 2 Hematoxylin-eosin staining of gastric myeloid sarcoma. A: There is mucosa with diffuse infiltration of monomorphic medium cells (× 10 magnification); B: At higher magnification (× 40 magnification) the cells show eosinophilic cytoplasm and nucleus with fine chromatin (blast).

Due to the rarity of MS, there are no prospective studies that guide its mana-gement [3,4,8,10], with induction therapy and postadmission therapy for AML being the only current alternatives to treatment. The prognosis of these patients is poor, and a 12-month survival has been reported for patients with MS without treatment and progression within 1 year to leukemia[3]. However, retrospective series such as that of Pileri *et al*[4] and Kawamoto *et al*[9] report that systemic treatment, including daunorubicin and cytarabine, offers longer progression-free survival than local treatments (surgery and radiotherapy). In addition, promising results have been achieved with hematopoietic progenitor (TPH) transplantation; Movassaghian *et al*[10] reported a median OS of 16.7 mo in a retrospective series of 22 patients with allogeneic post-PHT MS. However, despite this, the majority of the patients had disease progression in less than 6 mo[5,7].

CONCLUSION

In conclusion, gastric primary *de novo* MS is a rare neoplasm with an aggressive course. The differential diagnosis depends on the histological and immunohistochemical characteristics. Chemotherapy is the standard treatment, and important results have been reported with bone marrow transplantation. However, further collaborative studies are necessary to understand this pathology and its particularities in our population.

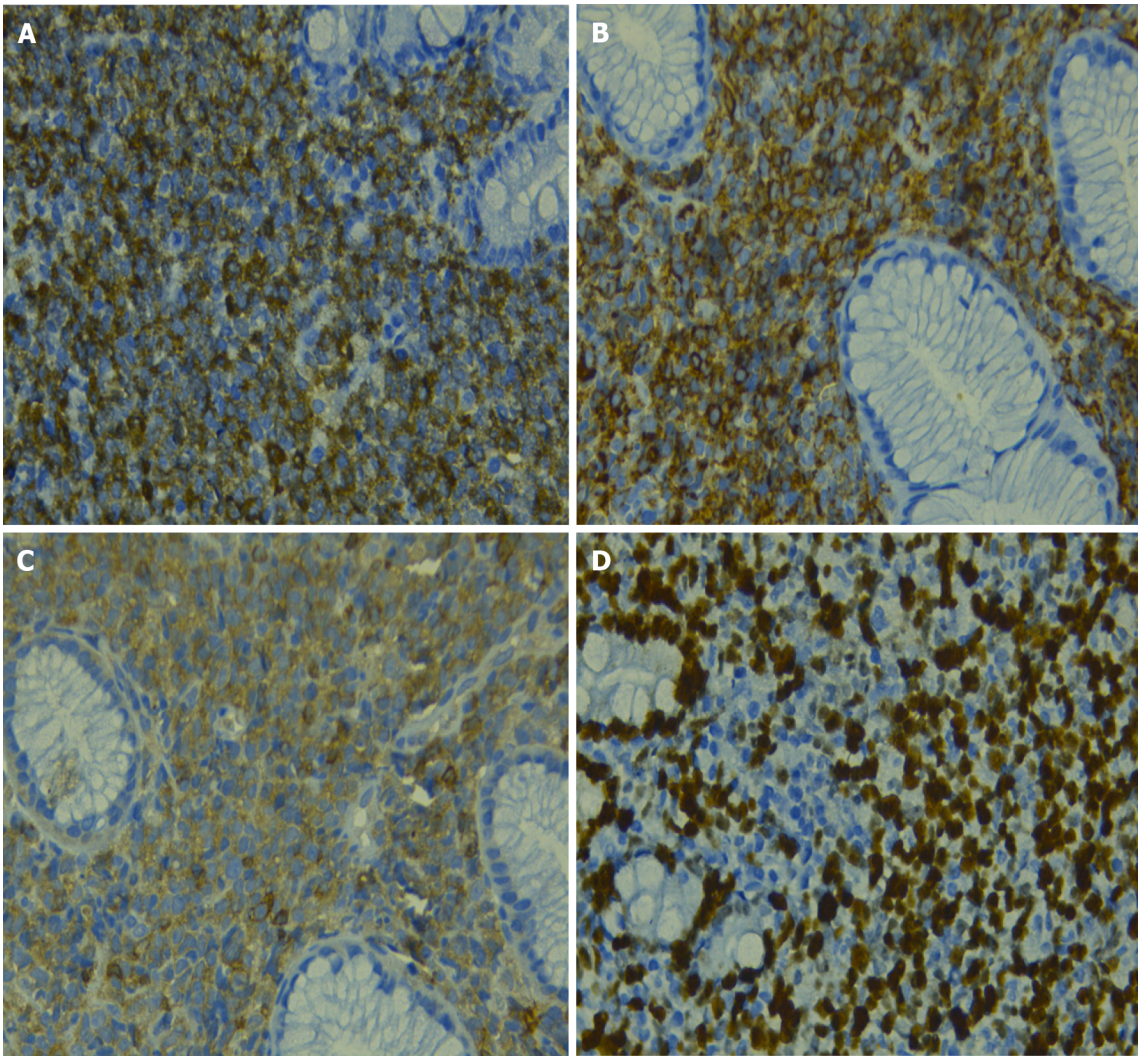

Figure 3 Immunohistochemistry. A: Positive staining for myeloperoxidase (× 40 magnification); B: Positive staining for CD34 (× 40 magnification); C: Positive staining for CD117 (× 40 magnification); D: Strong staining for Ki-67 (proliferation index), around 80% (× 40 magnification).

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