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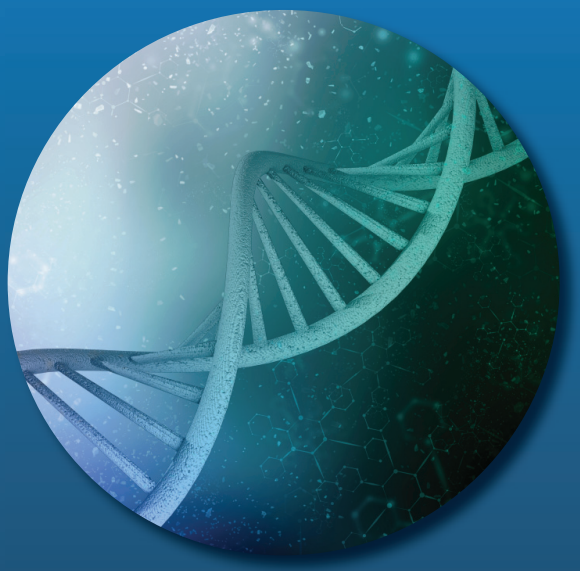

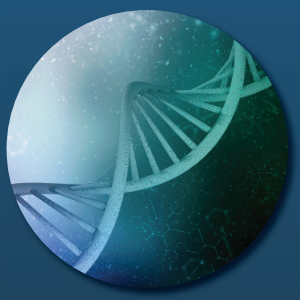

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Oncology and Translational Medicine (OTM) is an international, open-access, peer-reviewed academic journal that aims to promote research in oncology and translational medicine as well as levels of service in clinical practice. It publishes the results of novel research investigations which bridge the laboratory and clinical settings including risk assessment, cellular and molecular characterization, prevention, detection, diagnosis and treatment of human cancers with the overall goal of improving the clinical care of oncology patients. OTM covers the types of Original research, Review articles, Consensus and Guideline, Perspectives, Letters to the Editor, Editorials, Commentary, Personage Column, Case Report, etc.

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Review Article

Oncology and Translational Medicine

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Eosinophil disorders: an update on diagnosis and management

Chuan-Yi M. Lu*

Abstract

Eosinophilia can be seen in almost all medical subspecialty patients. Delay in diagnostic workup and treatment is associated with significant morbidity and mortality. Clinical vigilance and timely referral for diagnostic evaluation are critical. Causes of hypereosinophilia (HE) are diverse and can be grouped under 3 categories: primary (neoplastic), secondary (reactive), and idiopathic. Advances in molecular genetic diagnostics have led to elucidation of the genetic basis for many neoplastic hypereosinophilic disorders. One common molecular feature is formation of a fusion gene, resulting in the expression of an aberrantly activated tyrosine kinase (TK). The World Health Organization endorsed a biologically oriented classification scheme and created a new major disease category, namely, myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions. Rearrangement of other TK genes and activating somatic mutation(s) in TK genes have also been reported in eosinophilic neoplasms. Diagnostic evaluation of HE involves a combination of clinical, histopathologic, and immunophenotypic analyses, as well as molecular genetic testing, including next-generation sequencing–based mutation panels. The management of primary HE is largely guided by the underlying molecular genetic abnormalities. Good knowledge of recent advances in HE is necessary to ensure timely and accurate diagnosis and to help optimize patient care.

Keywords: Eosinophil; Eosinophilia; Hematological malignancy; Molecular genetics; Precision diagnostics; Targeted therapy; Tyrosine kinase

1. Introduction

Eosinophils—specialized myeloid cells (eosinophilic granulocytes) in peripheral blood and tissue—originate from multipotent hematopoietic stem cells and play a crucial rule in host innate defense.[1] In healthy subjects, the eosinophil count in peripheral blood ranges from 0.05 to 0.5 $\times$ 10^9/L (50–500 per μL). Eosinophilia is defined as blood eosinophil count greater than 0.5 $\times$ 10^9/L and can be further categorized as mild (up to 1.5 $\times$ 10^9/L), moderate (1.5–5.0 $\times$ 10^9/L), and severe (>5.0 $\times$ 10^9/L).[2–4] The definition of eosinophilia also includes relative eosinophilia (>6% in white blood cell differential counts). The term hypereosinophilia (HE) is defined as marked blood eosinophilia (≥1.5 $\times$ 10^9/L) that is documented on at least 2 occasions with a minimum time interval of 2 weeks (Figure 1). In patients with certain myeloid leukemias, such as chronic myeloid leukemia and some variants of acute myeloid leukemia, HE is defined as persistent blood eosinophilia ≥1.5 $\times$ 10^9/L and ≥10% eosinophils in peripheral blood differential counts.

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Mature eosinophils are present in normal bone marrow aspirates, with the proportion of eosinophils in nucleated cell differential counts ranging from <1% to 6%. Eosinophils are also found in the healthy thymus, spleen, lymph nodes, uterus, and the entire gastrointestinal tract distal to the esophagus; however, the physiological counts of eosinophils in these organs vary. The term tissue HE has also been proposed, for example >20% eosinophils in the bone marrow for marrow HE, although the defining criteria remain to be widely accepted.[4]

Mild blood eosinophilia is seen in 4% to 10% of individuals in primary care settings, whereas HE—much less common—is seen in approximately 0.3% of the general patient population.[1,5,6] Hypereosinophilia can be transient, episodic, or persistent (chronic). Hypereosinophilia is seen in almost all medical subspecialties and can be observed in patients with allergic, infectious, and inflammatory conditions, as well as various solid tumors and many types of hematological malignancies. The causes of HE are diverse and can be grouped into 3 categories: primary (neoplastic or clonal), secondary (reactive), and idiopathic (Table 1).[3] Secondary HE (reactive, HE_R) is due to an underlying condition of nonneoplastic or paraneoplastic origin, in which the eosinophils themselves are benign (nonneoplastic). Primary HE (neoplastic/clonal), on the other hand, is caused by clonal stem cell disorders of myeloid or lymphoid origin, in which the eosinophils arise from the malignant clone and eosinophilia may be the initial manifestation of disease. If neither a secondary nor primary etiology can be identified and no organ dysfunction is detected, HE is considered idiopathic (also referred to as HE of undetermined significance). Of note, the World Health Organization Classification[7,8] specifies that HE must persist for ≥6 months for a case to be classified as idiopathic HE. In addition to these 3 categories, a rare hereditary (familial, HE_{FA}) variant of HE with autosomal dominant inheritance has been reported. The hereditary form of HE typically has a benign clinical course, with only a few reports of associated organ dysfunction.[3,4]

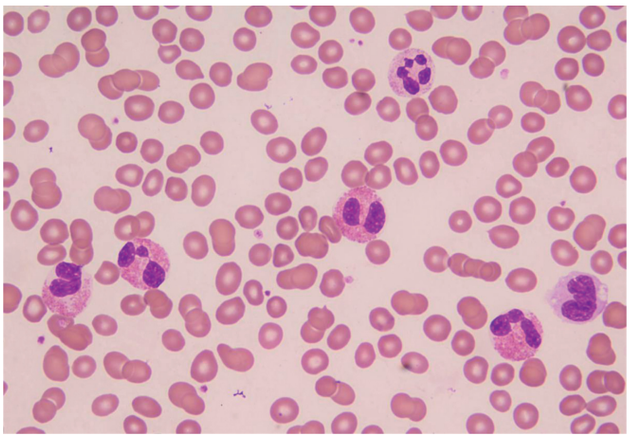

Figure 1. Peripheral blood smear from a 61-year-old man shows marked eosinophilia (absolute eosinophil count, 6.83 × 10^9/L). The white cells in this image include 4 eosinophils, 1 segmented neutrophil, and 1 monocyte (Wright stain, ×1000).

In patients with HE, eosinophils can infiltrate tissues, degranulate, and/or release proinflammatory and prothrombotic mediators and cytokines, causing organ damage. When organ dysfunction accompanies HE (of any type), the case is labeled as hypereosinophilic syndrome (HES), in which "syndrome" applies to organ damage that can be attributed to the eosinophilic infiltrate. Therefore, the terms HE and HES differ and should not be used interchangeably. Similar to HE, HES can be further categorized based on its underlying etiology: primary (neoplastic) HES, secondary (reactive) HES, and idiopathic HES (unknown etiology).[3,4] If tissue HE is present and causes organ damage, but the criteria for blood HE are not fulfilled, the term tissue/organ-restricted HES can be used. In addition, a lymphocyte variant of HE/HES (LV-HE/HES) has also been recognized. This variant is a hypereosinophilic condition resulting from excess production of eosinophilic cytokines secreted by immunophenotypically aberrant T cells (eg, $CD3^+/CD4^-/CD8^-$ or $CD3^-/CD4^+$).[3,4,9]

Hypereosinophilia affects many organ systems, most commonly the skin (40%–70%), lungs (25%–40%), and gastrointestinal tract (15%–35%), followed by the heart (5%–20%) and nervous system (5%–20%).[10] The most critical organ that may be affected is the heart as cardiac involvement can be life-threatening. Hypereosinophilic syndrome involving cardiac damage may manifest as myocardial infarction, acute heart failure, or thromboembolism, or rarely as endomyocardial fibrosis, restrictive cardiomyopathy, or valvular insufficiency.[11]

Table 1

Common causes of hypereosinophilia*[3]

• Secondary HE (reactive HE)†	
Nonneoplastic conditions	-Allergy and hypersensitivity (eg, asthma)
	-Drug reactions (allergic or toxic)
	-Parasitic infections (eg, helminthiasis)
	-Scabies and other infestations
	-Collagen vascular disorders (autoimmune)
	-Skin diseases (eg, atopic dermatitis)
	-Chronic inflammatory disorders (eg, IBD)
	-Metabolic condition (eg, adrenal insufficiency)
	-Chronic graft-versus-host disease
	-Eosinophilic myalgia syndrome
	-Lymphoid variant HE/HES
Neoplastic conditions (paraneoplastic)	-Solid tumors
	-T-cell lymphoma/leukemia
	-Hodgkin lymphoma
	-B-cell ALL with t(5;14)/IL-3–immunoglobulin H
	-Langerhans cell histiocytosis
• Primary HE (neoplastic HE)‡	-CEL-NOS
	-Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (*PDGFRA*, *PDGFRB*, *FGFR1*, *JAK2*, *FLT3*, *ABL1*, etc)
	-MPN with eosinophilia (eg, CML and JAK2 $V617F^+$ MPN)
	-AML with inv(16) or t(16;16)/*CBFB-MYH11*
	-MDS with eosinophilia
	-MDS/MPN with eosinophilia
	-ASM with eosinophilia
• Idiopathic HE (HE_{US})	Defined as HE that lasts ≥6 mo and without an underlying reactive or neoplastic cause
• Familial HE (HE_{FA})	Rare inherited condition with an autosomal dominant inheritance; pathogenesis unknown; benign clinical course

*When organ damage/dysfunction accompanies and attributable to HE, the case is denoted as HES (ie, secondary HES, primary HES, and idiopathic HES).

†HE is triggered by eosinopoietic cytokines in most cases.

‡Eosinophils are clonal cells (neoplastic eosinophils).

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ASM, aggressive systemic mastocytosis; CEL-NOS, chronic eosinophilic leukemia not otherwise specified; CML, chronic myeloid leukemia; HE, hypereosinophilia; HE, hypereosinophilia (absolute eosinophil count in blood >1.5 × 10^9/L on 2 occasions that are at least 2 weeks apart); HES, hypereosinophilic syndrome; HE_{US}, HE of undetermined significance; IBD, inflammatory bowel disease; MDS/MPN, myelodysplastic/myeloproliferative overlap neoplasm; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NOS, not otherwise specified.

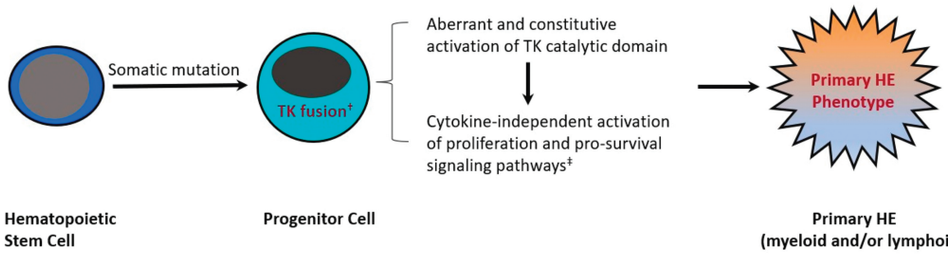

Figure 2. Schematic illustration of molecular pathogenesis in hypereosinophilia (HE) with TK gene fusions. The aberrant constitutive activation of TK catalytic domain drives disease initiation and development. The hematological neoplasms of primary HE can be myeloid and/or lymphoid in differentiation and can manifest as chronic or acute leukemia. †TK gene fusion involves *PDGFRA* (eg, *FIP1L1::PDGFRA*, *BCR::PDGFRA*), *PDGFRB* (for example, *ETV6::PDGFRB*), *FGFR1* (eg, *ZMYM2::FGFR1*, *CNTRL::FGFR1*, *BCR::FGFR1*), *JAK2* (eg, *PCM1::JAK2*, *BCR::JAK2*, *ETV6::JAK2*), *FLT3* (eg, *ETV6::FLT3*), or *ABL1* (for example *ETV6::ABL1*). ‡The proliferation and pro-survival pathways involved may include SRC, STAT5, PI3K, and RAS/MAPK signaling pathways, depending on the type of TK fusion gene involved.

Secondary HE and HES are the most common (45%–50%), including cases with eosinophilic disease restricted to a single organ system (eg, skin, lungs, intestines, etc), followed by 10% to 20% primary HE/HES (myeloid or lymphoid), 15% to 25% LV-HE/HES, 5% to 10% idiopathic HE/HES, and rare familial HE.

2. Pathogenesis

Reactive eosinophilia is induced by eosinophilic cytokines such as interleukin 5 (IL-5), IL-3, or granulocyte-macrophage colony-stimulating factor. These eosinophilic cytokines are primarily produced by activated T cells, mast cells, stromal cells, and tumor cells. Other cytokines and chemokines, such as transforming growth factors, platelet-derived growth factors (PDGFs), and CC/CXC chemokine receptor ligands, can modulate eosinophil function.[1] Some tumors and certain acute leukemias can lead to increased eosinopoietic cytokines and cause reactive eosinophilia. For example, B-cell acute lymphoblastic leukemia t(5;14)(q31;q32) translocation is associated with eosinophilia due to the overproduction of IL-3 by leukemic blasts. In addition, inhibition of eosinophil migration from blood into tissues could explain the transient HE reported in some patients under certain drug treatments (eg, dupilumab, a dual inhibitor of IL-4 and IL-13).[12]

Primary (neoplastic) eosinophilia can be associated with various hematolymphoid neoplasms and is typically caused by rearrangements in certain oncogenic target genes (typically tyrosine kinase [TK] genes), including *PDGFRA*, *PDGFRB*, *FGFR1*, *JAK2*, *FLT3*, and *ABL1* (Figure 2). These neoplasms are now grouped under "myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions."[3,13] Certain gene mutations are also associated with eosinophilic myeloid/lymphoid neoplasms. Genetic alterations in eosinophilic signaling pathways cause an increase in the number of neoplastic eosinophils. The resulting eosinophilia may be an initial manifestation of an underlying hematolymphoid malignancy, warranting further clinical investigation and monitoring.

Eosinophils produce, store, and release a slew of biologically active substances, including cytotoxic proteins, lipid mediators, chemotactic proteins (chemokines), and cytokines (many have cytotoxic properties, such as eosinophil-derived cationic protein, major basic protein 1 and 2, peroxidase, and neurotoxin).[1] Under various physiological conditions and pathologies, eosinophils migrate into certain target organs and, once activated, release their products in affected tissue sites, thereby promoting local inflammation, tissue remodeling, and sometimes tissue damage. Tissue fibrosis and/or thrombosis with end-organ damage develop in a subset of these patients, resulting in the diagnosis of HES. Organ dysfunction in HES may be reversible or irreversible, depending on the magnitude and duration of HE, underlying etiology, presence of certain comorbidities, and response to therapy. A critical manifestation of HES is a thromboembolic state, which may include stroke, intracavitary thrombi in the heart, and vascular (arterial and/or venous) thrombosis. In addition, endomyocardial fibrosis, chronic tissue inflammation, and skin ulcerations have been observed in HES. Associated organ damage may develop in all HES variants, independent of the underlying etiology. It should be noted that eosinophil-related organ involvement cannot be accurately predicted based on the absolute eosinophil count in the blood. In fact, HE does not always cause organ involvement or tissue damage; conversely, a mild eosinophilia value may be associated with significant organ involvement.[14]

3. Diagnosis

Clinician vigilance, especially in primary care settings, and timely referral are critical to avoid delays in the diagnostic workup and treatment of HE. The first step in the HE workup is to exclude secondary (reactive) eosinophilia, followed by the exclusion of paraneoplastic HE and LV-HE (Table 1, Figure 3).[3,4] A detailed medical history—taking into account allergies, travel, medications, food, domestic pets, and so on—physical examination, and clinical evaluation are essential. Routine laboratory examinations should be performed, including complete blood count with white blood cell differentiation, serum chemistry including metabolic panel and serum tryptase level, inflammatory markers (eg, fibrinogen and C-reactive protein), autoantibodies, serum immunoglobulin E, and vitamin B_{12}. Diagnostic imaging (X-ray, computed tomography, magnetic resonance imaging, and/or positron emission imaging, if indicated) and additional laboratory testing (eg, parasitology and allergy testing, flow cytometry, and serology) are often needed. The detection of an aberrant T-cell immunophenotype by flow cytometry and clonal TCR gene rearrangement analysis are often required for the diagnosis of LV-HE. However, the detection of an isolated clonal TCR rearrangement in the absence of an abnormal T-cell phenotype is not sufficient for the diagnosis of LV-HE/HES. Referral to a hematology and/or oncology specialist is required when a solid tumor or hematolymphoid malignancy is suspected.

4. Differential diagnosis

The most important differential diagnosis is secondary (reactive) versus primary (neoplastic/clonal) HE. The features suggestive of primary HE include constitutional symptoms, such as fever, weight

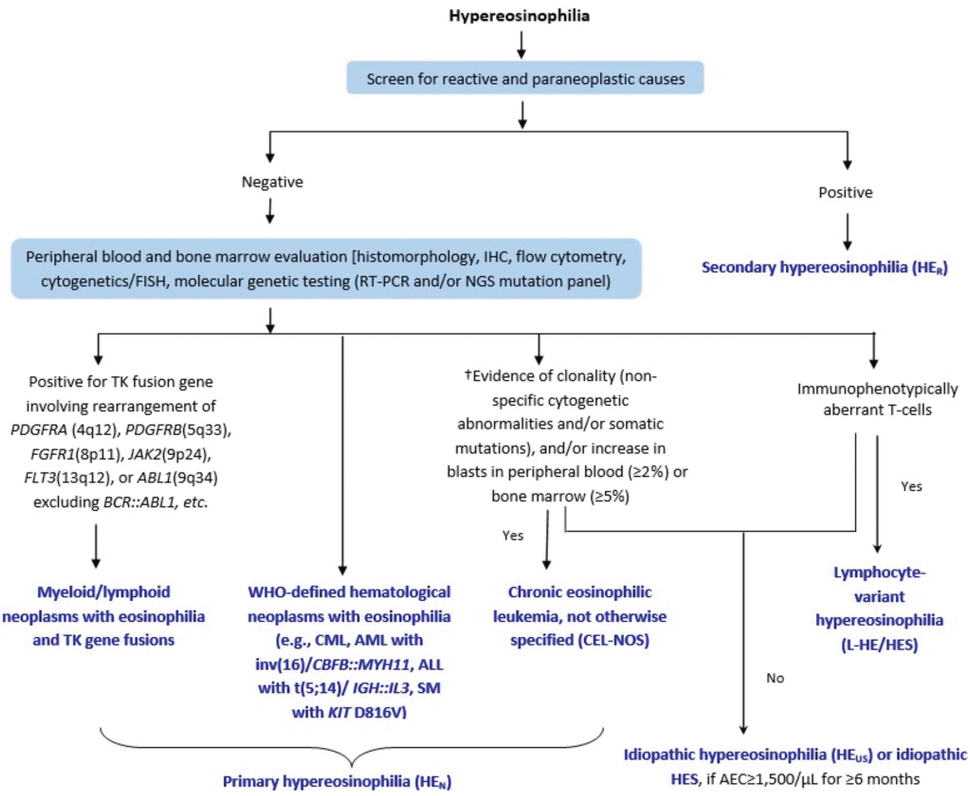

Figure 3. Diagnostic algorithm for patients with hypereosinophilia (absolute eosinophil count >1.5 × 10^9/L for ≥2 weeks). †Nonspecific cytogenetic abnormalities include +8, +15, –7, del(5q), i(17q), and complex karyotype, and nonspecific somatic mutations include mutations in *ASXL1*, *TET2*, *EZH2*, *DNMT3A*, *STAT5B*, and so on. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CEL-NOS, chronic eosinophilic leukemia, not otherwise specified; CML, chronic myeloid leukemia, *Bcr-Abl1*–positive; IHC, immunohistochemistry; NGS, next-generation sequencing RT-PCR, reverse transcription–polymerase chain reaction; SM, systemic mastocytosis; TK, tyrosine kinase.

loss, fatigue, cough, dyspnea, and so on; persistent and worsening eosinophilia that is refractory to steroids; leukocytosis (neutrophilia, monocytosis, and/or basophilia); the presence of both mature and immature and/or dysplastic cells in peripheral blood; anemia; abnormal platelet count (thrombocytopenia or thrombocytosis); and/or increase in serum vitamin B_{12} (eg, >1000 pg/mL) or tryptase (eg, >12 ng/mL), among other abnormal laboratory findings. Patients with primary or HE/HES may develop hepatomegaly or splenomegaly. Bone marrow aspiration and core biopsy should always be offered if primary HE is suspected. Abnormal bone marrow is considered a strong indicator of eosinophilic neoplasms and should be a major consideration in distinguishing patients with primary (clonal) HE from those with reactive or idiopathic hypereosinophilic disorders.[3,15]

Routine cytogenetic analyses can detect translocations involving 5q31-33 (*PDGFRB*), 8p11 (*FGFR1*), and 9p24. 1 (*JAK2*) and t(12;13)/*ETV6::FLT3*, but not 4q12 (*PDGFRA*) rearrangement. *FIP1L1::PDGFRA* is typically associated with a cryptic 4q12 deletion (an 800-kb interstitial deletion). Fluorescence in situ hybridization and/or reverse transcription–polymerase chain reaction should be performed to detect *FIP1L1::PDGFRA* fusion, particularly in certain clinical situations.[3] Of note, cytogenetically cryptic *PDGFRB* rearrangement has been reported, especially with variant partner genes other than *ETV6*.[16] The t(9;12)/*ETV6::ABL1* is usually cryptic on routine G-banded chromosome analysis[17]; therefore, fluorescence in situ hybridization and/or reverse transcription–polymerase chain reaction should be performed if clinically indicated.[3]

Next-generation sequencing (NGS)–based targeted mutation panels have become available and are used to study eosinophilic myeloid neoplasms.[3,18] Somatic mutations are uncommon in cases associated with *PDGFRA*, *PDGFRB*, or *PCM1::JAK2* rearrangement, but significantly more frequent in *FGFR1* rearranged cases; for example, up to 80% of *FGFR1*-rearranged individuals harbor *RUNX1* mutations. Mutations in *ASXL1*, *TET2*, *EZH2*, *SETBP1*, *CBL*, *NOTCH1*, *SCRIB*, *STAG2*, and *SH2B3* have also been reported in primary HE/HES. An NGS myeloid panel should be considered for HE cases if a secondary cause is excluded, the karyotype is normal or of no diagnostic significance (eg, loss of Y chromosome in elders), and HE is considered either a result of a myeloid neoplasm or idiopathic. The utilities of NGS myeloid panel in an HE workup include detecting rare activating mutation(s) in a TK gene (eg, *PDGFRA*) that can be targeted by TK inhibitor (TKI) and predicting outcome in "idiopathic" HES (ie, if mutation is detected, the outcome would be closer to that of chronic eosinophilic leukemia not otherwise specified [CEL-NOS] except for *SF3B1* mutation).[3] Rare activating mutations have been identified in *PDGFRA* and *STAT5B* (eg, N642H).[19] In addition, detection of myeloid neoplasm–associated mutation(s) helps differentiate truly idiopathic HE versus CEL-NOS.

5. Treatment overview

The clinical treatment of HE and HES depends on the disease etiology and subtypes. The overall goals of treatment are to decrease

the absolute eosinophil count, to ameliorate symptoms, and to prevent disease progression. Even in the absence of a known cause, HES must be promptly treated to reduce the potential morbidity resulting from organ damage.[3,13] In particular, emergency treatment with high-dose intravenous glucocorticoid is indicated if there are extremely high eosinophil counts (eg, absolute eosinophil count >100,000/μL), signs and symptoms of leukostasis, and/or evidence of potentially life-threatening complications (eg, acute heart failure, thromboembolic events).

For patients with mild eosinophilia (absolute eosinophil count $<1.5 \times 10^9$/L) and without symptoms or evidence of organ dysfunction, a watch-and-wait approach with close follow-up may be undertaken, especially if the absolute eosinophil count fluctuates.

The HE_R is best managed by treatment and (if possible) eradication of the underlying disease or pathology. If eradication is not possible, symptomatic therapy may be sufficient to control problems related to eosinophilia and eosinophil activation. Organ damage can often be prevented by the administration of corticosteroids (eg, prednisone) and/or other anti-inflammatory drugs. Additional drugs, such as cyclophosphamide, may be required in patients with severe eosinophilic granulomatosis and polyangiitis.

Glucocorticoids are the first-line treatment for patients with LV-HE/HES or idiopathic HE/HES, and allogeneic hematopoietic stem cell transplantation (allo-HSCT) can be pursued for the latter. Approximately 10% to 20% of patients with LV-HE/HES eventually progress to overt T-cell lymphoma; following up patients with LV-HE/HES is therefore strongly recommended. Adverse effects of long-term corticosteroid therapy can pose a clinical challenge. If indicated, corticosteroid-sparing agents should be considered.

Novel agents, such as anti–IL-5 monoclonal antibodies (eg, mepolizumab, reslizumab) and anti–IL-5 receptor antibodies (eg, benralizumab), have been shown to be effective corticosteroid-sparing agents for HES and organ-restricted inflammatory conditions with eosinophilia (eosinophil-associated single-organ diseases, such as eosinophilic asthma, eosinophilic bronchitis or pneumonia, eosinophilic esophagitis or gastroenteritis, etc).[2,13] Use of anti–IL-5 and anti–IL-5 receptor antibodies for the treatment of other HE types remain investigational.

Patient with primary (neoplastic) hypereosinophilia (HE_N) and hypereosinophilic syndrome (HES_N) do not respond to corticosteroids or other anti-inflammatory agents. For patients with HE and rearrangements of *PDGFRA* or *PDGFRB* with or without organ dysfunction, imatinib is the treatment of choice.[2,3,13] It should be noted that patients with rare *PDGFRA* mutations (eg, T674I, D842V, or tandem S601P and L629P) are often resistant to imatinib.

As to patients with HE and *FGFR1* rearrangement, intensive chemotherapy is the treatment of choice.[2,13,20] Variable short-term responses to midostaurin (a multitarget kinase inhibitor) and ponatinib have been reported. Allo-HSCT is the only option for achieving long-term remission or cure; however, pemigatinib, a highly selective inhibitor of FGFR, has shown promising results in clinical trials. In addition, in 1 study, 78% of patients with *FGFR1* rearrangement had an *RUNX1* mutation, suggesting that RUNX1 could be a novel therapeutic target.[21]

Patients with *PCM1::JAK2* may respond to ruxolitinib (a JAK2 inhibitor), but the response is usually short-term, with relapse within 1 to 2 years.[20] Allo-HSCT should be explored early for eligible patients because the utility of ruxolitinib is in disease cytoreduction and as a bridge to HSCT.

Patients diagnosed with CEL-NOS are typically treated with hydroxyurea or interferon α. Therefore, allo-HSCT should be explored for eligible patients. In addition, empirical imatinib treatment and investigational clinical trials can be considered.

Patients diagnosed with HE and acute myeloid leukemia or acute lymphoblastic leukemia/lymphoblastic lymphoma (T- or B-cell type) often require high-dose chemotherapy in addition to selective TKIs.

Monitoring of patients with HE/HES should be individualized. Regular eosinophil counts are recommended with additional testing, depending on the treatment protocol and the end organs involved. The frequency of monitoring is variable, ranging from weekly for patients with continued symptoms and eosinophilia despite therapy to every 6 months for stable patients on chronic low-dose glucocorticoids.

6. Prognosis

The prognosis of eosinophilic disorders varies significantly, depending on the underlying etiology, diagnostic classification, and whether there is critical organ/tissue involvement. Clinical features that suggest a better prognosis include the absence of cardiac or neurologic involvement, lower eosinophil counts, steroid responsiveness, and treatability with TKIs (eg, imatinib). Of the neoplastic HE/HES, after the introduction of imatinib therapy, myeloid/lymphoid neoplasms with *PDGFRA* or *PDGFRB* rearrangement are associated with long-term survival. However, the prognosis of other neoplastic HE/HES (eg, HES with *FGFR* rearrangement) remains to be poor due to lack of specific and effective therapy.

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The author declares that he has no conflict of interest with regard to the content of this report.

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References

[1] Valent P, Degenfeld-Schonburg L, Sadovnik I, et al. Eosinophils and eosinophil-associated disorders: immunological, clinical, and molecular complexity. *Semin Immunopathol*. 2021;43(3):423–438.
[2] Shomali W, Gotlib J. World Health Organization–defined eosinophilic disorders: 2019 update on diagnosis, risk stratification, and management. *Am J Hematol*. 2019;94(10):1149–1167.
[3] Mattis DM, Wang SA, Lu CM. Contemporary classification and diagnostic evaluation of hypereosinophilia. *Am J Clin Pathol*. 2020;154(3):305–318.
[4] Valent P, Klion AD, Roufosse F, et al. Proposed refined diagnostic criteria and classification of eosinophil disorders and related syndromes. *Allergy*. 2023;78(1):47–59.
[5] Andersen CL, Siersma VD, Hasselbalch HC, et al. Eosinophilia in routine blood samples and the subsequent risk of hematological malignancies and death. *Am J Hematol*. 2013;88(10):843–847.
[6] Schulte C, Krebs B, Jelinek T, Nothdurft HD, von Sonnenburg F, Löscher T. Diagnostic significance of blood eosinophilia in returning travelers. *Clin Infect Dis*. 2002;34(3):407–411.
[7] Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Revised 4th ed. Lyon, France: IARC; 2017.

[8] Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. *Leukemia*. 2022;36(7):1703–1719.
[9] Shi M, Rech KL, Otteson GE, et al. Prevalence and spectrum of T-cell lymphoproliferative disorders in patients with hypereosinophilia: a reference laboratory experience. *Ann Diagn Pathol*. 2020;44:151412.
[10] Ogbogu PU, Bochner BS, Butterfield JH, et al. Hypereosinophilic syndrome: a multicenter, retrospective analysis of clinical characteristics and response to therapy. *J Allergy Clin Immunol*. 2009;124(6): 1319–1325.e3.
[11] Cheung CC, Constantine M, Ahmadi A, Shiau C, Chen LYC. Eosinophilic myocarditis. *Am J Med Sci*. 2017;354(5):486–492.
[12] Caminati M, Olivieri B, Dama A, et al. Dupilumab-induced hypereosinophilia: review of the literature and algorithm proposal for clinical management. *Expert Rev Respir Med*. 2022;16(7):713–721.
[13] Stella S, Massimino M, Manzella L, et al. Molecular pathogenesis and treatment perspectives for hypereosinophilia and hypereosinophilic syndromes. *Int J Mol Sci*. 2021;22(2):486.
[14] Bjerrum OW, Siersma V, Hasselbalch HC, Lind B, Andersen CL. Association of the blood eosinophil count with end-organ symptoms. *Ann Med Surg (Lond)*. 2019;45:11–18.
[15] Wang SA, Hasserjian RP, Tam W, et al. Bone marrow morphology is a strong discriminator between chronic eosinophilic leukemia, not otherwise specified and reactive idiopathic hypereosinophilic syndrome. *Haematologica*. 2017;102(8):1352–1360.
[16] Jawhar M, Naumann N, Knut M, et al. Cytogenetically cryptic ZMYM2-FLT3 and DIAPH1-PDGFRB gene fusions in myeloid neoplasms with eosinophilia. *Leukemia*. 2017;31(10):2271–2273.
[17] Cessna MH, Paulraj P, Hilton B, et al. Chronic myelomonocytic leukemia with ETV6-ABL1 rearrangement and *SMC1A* mutation. *Cancer Genet*. 2019;238:31–36.
[18] Wang SA, Tam W, Tsai AG, et al. Targeted next-generation sequencing identifies a subset of idiopathic hypereosinophilic syndrome with features similar to chronic eosinophilic leukemia, not otherwise specified. *Mod Pathol*. 2016;29(8):854–864.
[19] Cross NCP, Hoade Y, Tapper WJ, et al. Recurrent activating *STAT5B N642H* mutation in myeloid neoplasms with eosinophilia. *Leukemia*. 2019;33(2):415–425.
[20] Reiter A, Gotlib J. Myeloid neoplasms with eosinophilia. *Blood*. 2017; 129(6):704–714.
[21] Strati P, Tang G, Duose DY, et al. Myeloid/lymphoid neoplasms with FGFR1 rearrangement. *Leuk Lymphoma*. 2018;59(7):1672–1676.

Review Article

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Review of surgical strategies in gastric cancer

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Abstract

Successful and effective management of gastric cancer involves a multidisciplinary approach.

Accurate and appropriate staging of gastric cancer is mandatory to define the optimum treatment strategy. Safe surgery achieving R0 resection is considered potentially curative. Surgery remains the cornerstone of multimodal therapy. There is a well-defined role for neoadjuvant and adjuvant chemotherapy regimens. Newer medications and chemotherapy combinations have improved tumor response rates, which have translated into more effective surgery and reduced local recurrences and improved long-term survivals.

Keywords: Gastric cancer; Lymphadenectomy; Surgical treatment

1. Surgical strategy in gastric cancer

1.1. Introduction

Recently, the incidence of gastric and gastroesophageal junction cancers has progressively increased. This incidence remains higher in East Asia than in the Western world.[1] The peak incidence of these cancers occurs in the seventh decade of life. In India, gastric cancer is the second most frequent cause of cancer-related mortality and the fifth most common cancer in the younger population (15–44 years).[2]

Almost 90% of gastric cancer cases are sporadic. Risk factors include smoking, male sex, *Helicobacter pylori* infection, atrophic gastritis, previous partial gastrectomy, and a diet high in salt and processed meat.[3] Obesity and reflux are associated with a higher incidence of Barrett esophagus and tumors of the cardioesophageal (CO) junction and proximal stomach.[3] Approximately 10% of gastric cancers are familial or have a genetic basis and associated with a specific genetic predisposition (1%–3%).[1,3]

2. Diagnosis

Gastric cancer is typically diagnosed after an investigation of constitutional symptoms, such as weight loss, fatigue, and fever. Patients present with features of gastric outlet obstruction when the tumor is obstructing or with dysphagia from a growth obstructing the CO junction. Anemia is a common condition in gastric cancer cases, particularly in older adults. Advanced stages of the disease manifest with abdominal lumps, hepatomegaly resulting from liver metastases, ascites due to peritoneal disease, jaundice, and lymphadenopathy. In rare instances, gastric cancer is incidentally detected during screening endoscopy.

Upon suspicion of gastric pathology, particularly with a high index of suspicion for cancer, upper gastrointestinal (GI) endoscopy (esophagogastroduodenoscopy) is mandatory to confirm the diagnosis. This procedure allows identifying the tumor, defining its precise location (with implications for surgical therapy); enables the collection of biopsies; and provides insights into the condition of the stomach. Therapeutic endoscopy effectively manages bleeding gastric ulcers through various methods, such as injection, clipping, argon coagulation, and contact diathermy. Gastric cancer is conventionally classified into 2 main variants based on the Lauren Classification (1965): intestinal and diffuse.[3] The intestinal variant has a well-differentiated histology, contrasting with the diffuse variant. The intestinal variety has a lower incidence of lymph node and distant metastases, rendering it prognostically superior to the diffuse variety on a stage-for-stage basis. Upon confirmation of the diagnosis, patients require comprehensive staging of the disease to determine the precise treatment approach.

2.1. Staging of gastric cancer

2.1.1. Computed tomography scan

Comprehensive computed tomography (CT) of the chest, abdomen, and pelvis is mandatory and crucial in the staging and planning of the treatment strategies. The CT results

- define the tumor's size, location, and its relationship with adjacent structures;
- identify the infiltration or abutment of surrounding structures, providing complete anatomical details about the tumor (T stage);
- identify lymph nodes, aiding with the assessment of lymph node involvement (N stage);
- identify peritoneal, hepatic, and omental disease along with retroperitoneal and para-aortic nodes (M stage); and
- identify any pulmonary metastases.

3. Positron emission tomography/CT scan

Positron emission tomography/CT is used as a complementary tool to CT staging. Positron emission tomography/CT assists in detecting fluorodeoxyglucose-avid nodes and indeterminate lesions in the

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liver, lungs, and peritoneum, enhancing understanding of their nature. However, positron emission tomography/CT often shows disease progression in mucinous tumors and diffuse gastric cancers.

4. Endoscopic ultrasound

Endoscopic ultrasound, an invasive technique, is used to accurately stage very small and early mucosal and submucosal lesions and define lymph nodes. Endoscopic ultrasound–directed biopsy and tissue sampling further contribute to the diagnosis.[4]

5. Staging and diagnostic laparoscopy

Diagnostic laparoscopy[5] with peritoneal washings is recommended in the following scenarios:

- locally advanced gastric cancers to exclude peritoneal metastases,
- before the commencement of neoadjuvant chemotherapy, and
- in patients considered for cytoreductive surgery, because it aids in determining the peritoneal carcinomatosis index.

Standard staging of gastric cancer—TNM (T—tumor, N—lymph nodes, and M—metastasis)

staging[6] guidelines following the AJCC Cancer Staging System, eighth edition, is followed (Table 1).

5.1. Treatment strategy

The treatment of gastric cancer is based on TNM staging, with well-defined protocols guiding the therapeutic strategies. Accurate TNM staging, based on the aforementioned modalities, is fundamental. Treatment usually involves a consensus among a multidisciplinary team, which includes a surgeon, a medical oncologist, and a specialized GI radiologist.

The stage of the tumor depends on the T, N, and M stages of the disease, as determined by staging evaluations.

5.2. Classification of gastric cancer based on stage

The local staging (T stage) of stomach cancer depends on the tumor invasion across the gastric wall.

The risk of lymph node metastases in gastric cancer is highly dependent on the T stage. Hence, a deeper infiltration into the gastric wall (T stage) corresponds to an increased probability of regional lymph node metastases.

The risk of lymph node metastases in T1A gastric tumors is minimal.

(1) Very early gastric cancer (T1A tumors): These tumors are confined to the mucosa and do not extend into the submucosa (T1B). The tumor does not extend beyond the lamina propria or the muscular mucosa (Table 1).
(2) Early gastric cancer (T1B tumors): These tumors extend into, but not beyond, the submucosa. The presence or absence of regional lymph node metastasis is not a significant factor. Hence, early gastric cancer encompasses T1B tumors, whether N0 or N+.
(3) Locally advanced gastric cancer: These include T3/T4 and N0/N+ tumors.
(4) Metastatic disease: This includes cancer that has disseminated to distant sites (M+ disease).

5.3. Endoscopic therapy

For T1A tumors, endoscopic therapy is advisable.[7] These superficial lesions do not extend to the submucosa. Endoscopic mucosal resection and endoscopic submucosal dissection are viable procedures. The lesions eligible for these procedures include the following:

- well-differentiated tumors.
- tumors <2 cm
- nonulcerated tumors
- lesions without perineurial invasion or lymphovascular invasion
- if R0 resection en bloc can be achieved rather than piecemeal resection

Post–endoscopic resection aims to identify any unfavorable prognostic factors in histology, where formal surgical resection with complete lymphadenectomy is recommended.

5.4. Surgery

Surgery remains the cornerstone of curative treatment for gastric cancer. Essentially, nearly all tumors, excluding those categorized as very early (described previously) and those suitable for endoscopic therapy, as well as cases of metastatic disease, require surgery as potentially curative therapy. Surgery may be performed initially, particularly for most T1/T2 tumors, or after neoadjuvant chemotherapy in locally advanced gastric cancer.

Table 1

Staging of gastric cancer—TNM staging[6]

Primary tumor T stage	Regional lymph nodes	Distant metastases
Tx: primary tumor cannot be assessed T0: no evidence of primary tumor	NX: regional nodes cannot be assessed N0: no regional node metastases	M0: no distant metastases M1: distant metastases including positive peritoneal cytology
Tis: carcinoma in situ or Intra epithelial Tumor with no invasion into the lamina propria T1: tumor not extending beyond the submucosa 1A: tumor invades up to lamina propria or the muscular mucosa (tumor restricted to the mucosa) 1B: tumor invades the submucosa T2: tumor invades the muscular propria	N1: metastases in 1–2 nodes N2: metastases in 3–6 regional nodes N3: metastases in >6 nodes (7 nodes) N3A: 7–15 regional lymph nodes N3B: >16 regional nodes	
T3: tumor invades the subserosal connective tissue without invasion of the visceral peritoneum or adjoining structures T4: tumor invades the serosa (visceral peritoneum) or adjacent structures T4A: tumor invades serosa (visceral peritoneum) T4B: tumor invades adjacent structures		

AJCC Cancer Staging System, eighth edition.[6]

Table 2

Lymph node stations in gastric cancer[8]

1	Right cardiac nodes
2	Left cardiac nodes
3	Nodes along the lesser curvature of the stomach
4	Nodes along the greater curvature of the stomach
5	Suprapyloric nodes
6	Infrapyloric nodes
7	Nodes along the left gastric artery
8	Nodes along the common hepatic artery
9	Nodes around the coeliac axis
10	Nodes along the splenic hilus
11	Nodes along the splenic artery
12	Hepatoduodenal ligament nodes
13	Nodes posterior to the head of the pancreas
14	Nodes at the root of the mesentery
15	Nodes in the transverse mesocolon
16	Para-aortic nodes

The guidelines mentioned previously are not absolute. Ultimately, treatment needs to be tailored to an individual patient's needs, defined by a variety of factors:

- age, medical comorbidities, general health condition
- presence of compelling obstruction or bleeding from the tumor
- tolerance and response to chemotherapy

Surgery involves excising the primary tumor with en bloc regional lymph node dissection to remove the complete set of draining lymph nodes. Resection of the stomach tumor includes the removal of the stomach with adequate margins and an appropriate reconstruction to restore the continuity of the GI tract. A 5-cm margin is considered adequate.

5.5. Extent of lymphadenectomy

For the potentially curative surgical treatment of stomach adenocarcinoma, radical lymphadenectomy, along with gastric visceral resection, is mandatory. The lymph nodes of the stomach are mapped along the major vascular pedicles and accordingly labeled (lymph node stations). The lymphatics within the regional nodes typically run parallel to the major vascular pedicles, supplying and draining from the stomach. Lymphadenectomy in gastric resection is defined according to the lymph node stations that have been removed. Lymph nodes draining from the stomach are classified according to their location[8] (Table 2).

Lymph node stations 1–12 and 14 are conventionally considered regional nodes,[8] and anything other than these are considered distant nodes.[8] Lymph nodal stations 110, 111, and 112 are located within the lower esophagus, paraesophageal nodes, and lower posterior mediastinal nodes.[8,9] These nodes are more important in cancers of the CO junction and lower esophagus and can be classified, with additional subdivisions, within each lymph nodal group.[8–10] The algorithm is illustrated in Figure 1.

Typically, the standard lymphadenectomy for gastric cancer is referred to as D2 lymphadenectomy. D2 lymph node dissection involves the removal of all lymph nodes along the arteries supplying the stomach (left gastric, right gastric, gastroepiploic, splenic, common hepatic arteries up to the porta, and the right pericardiac

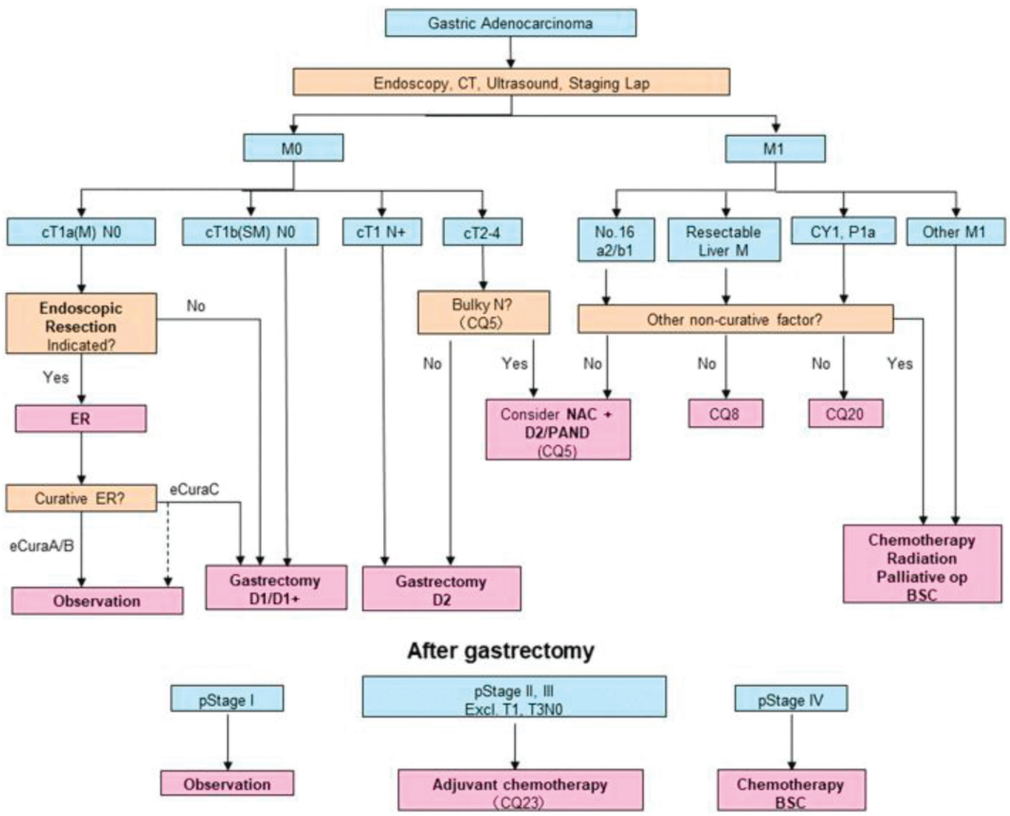

Figure 1. Algorithm for management of gastric adenocarcinoma. Adapted from the Japanese Gastric Cancer Association.

Table 3

Extent of lymph nodal dissection in gastric adenocarcinoma[9,10]

	D0	D1	D1+	D2
Total gastrectomy	< D1	1 TO 7	All D1 + 8a,9,11p	All D1 + 8a,9,11p,11d,12a
Distal gastrectomy	< D1	1,3,4sb,4d,5,6,7	All D1 + 8a,9	All D1 + 8a,9,11p,12a
Proximal gastrectomy	< D1	1,2,3a,4sa,4sb,7	All D1 + 8a,9,11p	

nodes). The lymph nodes along with all the pedicles are systematically removed. This is the classic D2 lymph node dissection, which is the optimum extent of lymph node clearance. Any dissection less than D2 is termed D1, and this typically includes the removal of lymph nodes along the perigastric regions but not along the vascular supply of the stomach. D1 lymphadenectomy is widely considered a suboptimal oncological procedure, except in certain cases of very early gastric cancer (T1A), where its application may suffice because of the minimal risk of lymph node metastases. D1+ resection is typically pursued by encompassing the resection of all D1 nodes and some nodes extending beyond D1 but not reaching a classic D2 resection. A minimum of 15 lymph nodes should be present in the surgical specimen to ensure an adequate lymphadenectomy. This is required to complete the pathological staging. Comparisons between D2 and D1 lymphadenectomy have been the subject of major clinical trials for stomach cancer.[11–13] However, the survival benefits of D2 lymphadenectomy remain unclear. Nonetheless, D2 lymphadenectomy remains the standard of care, correlating with fewer locoregional recurrences and increased long-term survival among patients with resected gastric cancer.[14] Extended lymphadenectomy, beyond D2 (also called D3 lymphadenectomy), which includes the removal of the para-aortic nodes and nodes from the root of the mesentery (stations 1–16), has not been proven to improve oncological outcomes and only adds to the morbidity of the surgical procedure.[9] However, extended lymphadenectomy may improve outcomes in well-selected cases and has become popular in Japan.[8,9] The magnitude and extent of lymphadenectomy in the different types of gastrectomies are highlighted in Table 3.[9,10]

5.5.1. Technical aspects of gastric cancer surgery

The surgery for gastric cancer includes the removal of the stomach along with the en bloc removal of the regional draining lymph nodes (D2 gastrectomy). A 5-cm margin or greater is required for optimal oncological resection. Depending on the location of the tumor, gastric resection is performed. Thus, the gastric resection may involve the following:

(1) Partial/hemigastrectomy: Part of the stomach is removed, typically when the growth is limited to the distal stomach. Distal gastrectomy includes the removal of the antrum, along with the distal body and lesser curvature.
(2) Subtotal gastrectomy: This procedure is performed for midbody or larger tumors in the distal stomach/antrum. A substantial part of the stomach is removed, retaining small portion of fundus with adequate tumor-free margin.
(3) Total gastrectomy: The entire stomach is removed from the CO junction to the first part of the duodenum. This is performed in the following scenarios:
   - when tumors are large and involve most of the stomach
   - for proximally located tumors where proximal transaction occurs at the CO junction or in the distal esophagus
   - for certain mucinous tumors or poorly differentiated tumors of the diffuse variant, considering disease biology in these cases
(4) Proximal gastrectomy: The procedure involves the removal of the proximal stomach. Reconstruction is achieved by anastomosing the CO junction or the distal esophagus to the midstomach.

All the above procedures are performed via an abdominal approach, either as a conventional open procedure or as a minimally invasive (laparoscopic or robotic) procedure. Open surgery remains the conventional method for resecting gastric cancer. However, laparoscopic surgery is increasingly being performed and has equivalent postoperative outcomes. Laparoscopic surgery has the additional advantages of reduced pain and early mobilization. The lymph node yields in open and laparoscopic surgeries remain similar.[15]

The Merendino procedure involves the resection of the proximal stomach with the CO junction and interposition of the jejunal segment between the resected esophagus and stomach. The procedure involves the construction of 3 anastomoses while preserving the natural gastric conduit and preventing the need for total gastrectomy.[16]

Tumors located at the CO junction or in proximal gastric cancer (Siewert type 3) represent a surgical challenge. The resection of these tumors with an adequate margin also involves the removal of the distal esophagus with the lymph nodes in the lower posterior mediastinum. These surgical procedures require an abdominal and a thoracic approach for the operation. Typically, the stomach is mobilized via the abdomen. Depending on the tumor location, either left or right thoracotomy is required to resect the esophagus and enable anastomosis. In advanced tumors, wherein a total gastrectomy with resection of the CO junction and the distal esophagus is performed, the continuity of the GI tract is restored by performing an esophageal and jejunal anastomosis in the left chest. The operation is performed through a single in-continuity abdominal incision extending into the left chest (eighth intercostal space) after division of the rib. The internal surgical division of the diaphragm enables access to the left chest and posterior mediastinum to mobilize the distal esophagus and complete the anastomosis.

5.6. Reconstruction of GI tract after stomach resection

Upon resection of the stomach, the continuity of the GI tract is restored by surgically joining part of the residual stomach to the jejunum.

To date, various surgical techniques for this procedure have been described. Broadly, gastrojejunostomy (Billroth 2 gastrectomy) is performed. Following total gastrectomy, esophagojejunostomy is performed. A feeding jejunostomy may be added to the operation to enable early resumption of enteral nutrition following surgery while the anastomosis heals. Some surgeons often choose to insert a feeding tube across the anastomosis for enteral nutrition during surgery.

Anastomoses can be performed using sutures (hand-sewn) or mechanical stapling devices (staplers).

5.7. Multimodality therapy for stomach carcinoma

Perioperative chemotherapy plays a defined role in the treatment of adenocarcinoma of the stomach.

All patients with stage 1B gastric cancer and above require perioperative chemotherapy (either preoperative, postoperative, or both) to achieve the best oncological outcomes. Patients with node-positive disease or T3/T4 disease (irrespective of nodal status) require preoperative chemotherapy.

Preoperative chemotherapy helps with the following:

(1) downstaging the primary tumor and reducing tumor burden;
(2) decreasing the volume of lymph nodal disease;
(3) eradicating micrometastatic disease;
(4) allowing for initially unresectable disease to become resectable: in locally advanced gastric cancers, where the tumor is initially unresectable owing to infiltration into the surrounding structures, chemotherapy helps reduce the tumor burden and enables surgery. However, in patients who do not respond to chemotherapy and whose tumors do not reduce to become resectable, surgery is not recommended; this is regarded as a poor prognostic sign and indicator of aggressive disease. Second- and third-line chemotherapy regimens, including targeted and immunotherapies, should be explored for such patients;
(5) enabling surgery with higher rates of R0 resection; and
(6) allowing natural disease biology to unfold: should a patient develop progressive disease while on chemotherapy, surgery is not advisable, and this is regarded as a poor prognostic factor.

Most patients with gastric adenocarcinoma require chemotherapy. Gastric cancer surgery is associated with increased morbidity. Recovery can be slow, and patients often do not recover in time for the commencement of adjuvant chemotherapy.

Therefore, preoperative chemotherapy allows for the very early initiation of systemic treatment, which prevents distant failures and metastases. However, for successful chemotherapy administration, patient performance status must be good to tolerate chemotherapy. Chemotherapy-related toxicity can be troublesome and may result in treatment discontinuity, leading to adverse oncological outcomes.

Patients who present with near-complete obstruction related to the tumor or serious anemia and bleeding related to the tumor may not be eligible for chemotherapy at the commencement of the cancer treatment. These patients require complete surgical resection of the tumor to alleviate the obstruction and/or anemia; upon recovery, they will be eligible for adjuvant chemotherapy.

The administration of perioperative chemotherapy is the standard of care and has been confirmed in multiple randomized trials, including the MAGIC (Medical Research Council Adjuvant Gastric Infusional Chemotherapy), FNCLCC (Fédération Nationale des Centres de Lutte contre le Cancer),[17] and FLOT-4 (Fluorouracil plus Leucovorin, Oxaliplatin, and Docetaxel) studies.[3]

In patients where an initial resection is performed, adjuvant chemotherapy is required for those with node-positive disease, T3/T4 tumors in node-negative disease, <15 nodes sampled, poor prognostic factors including lymphovascular invasion and perineurial invasion, poorly differentiated tumors, and R+ resection (margin-positive resections). Chemotherapy should be commenced as soon as patients have recovered from their surgery.

6. Surgery for gastric primary cancer in the metastatic disease setting

As a rule, stage 4 metastatic stomach cancer should only be treated with palliative intent. Cure is not possible to achieve.

7. Relief of obstruction and bleeding

Antral lesions causing obstruction are treated using palliative gastrojejunostomy. This allows the restoration of swallowing and improves the patient's quality of life. In palliative gastric resection, removal of a part of the stomach is usually not advocated unless in specific circumstances when the tumor is seriously bleeding or obstructing; in these cases, resection can be performed with minimal morbidity.

8. Resection of primary gastric cancer and metastasis in M1 stomach cancer

Stomach and metastatic disease resections are not the standard of care. These procedures are purely experimental. The REGATTA multicenter trial failed to show any survival benefits of these procedures.[18] This trial reported a high complication rate from total gastrectomy, which was substantially higher than that from distal gastrectomy. More than 50% of patients in the REGATTA trial underwent palliative total gastrectomy, which may have led to the aforementioned conclusion.[18] Furthermore, the trial was terminated early owing to its futility, in addition to other limitations in patient selection and the choice of chemotherapy administered.[19] The role of palliative gastric resection in the metastatic setting remains controversial, although benefits may be observed in some patients with obstruction and serious hemorrhage. The reluctance to perform gastric resection in the metastatic setting is also due to the high morbidity and mortality observed in this group of patients.[20,21] In a propensity score–matched analysis of a large SEER database, Li et al[19] determined the survival benefit of gastrectomy in the metastatic setting and advocated for its judicious use in a select group of patients. This survival advantage was particularly observed in patients with metastases limited to a single site, as opposed to those with metastases in 2 or more sites.[19] Palliative gastrectomy in the metastatic setting may be particularly beneficial after the administration of systemic chemotherapy. A specific group of patients exhibited nearly 2-fold improved overall survival when subjected to this combined approach, in comparison to those who received chemotherapy alone.[19,22] Hence, a pragmatic and judicious case-based decision should be made for patients with metastatic gastric cancer to address the role of palliative gastrectomy. The improvements and refinements in surgical techniques, instrumentation, and perioperative care, including anesthesia and intensive care, that the combined procedure, contrary to the best supportive care or chemotherapy only, may be safely offered to a specific group of patients who will experience extended survival.

9. Resection of stomach cancer metastases

Isolated case reports on the resection of metastases derived from gastric cancer have evidenced favorable outcomes. Therefore, the careful selection of cases is crucial. Favorable results have been documented in instances involving the resection of ovarian,[17] pulmonary,[23] and hepatic metastases.[24,25] Most patients with metastases present with an extensive burden of such condition, which does not permit individual metastases resection. Isolated metastases may be resected in select cases with good performance status, a long disease-free interval (from the treatment of the primary tumor), and a low metastatic burden.

10. Multivisceral resection in stomach cancer

Multivisceral resection for gastric cancer may be required for certain patients with locally advanced gastric cancer. Most patients undergo upfront downstaging chemotherapy before surgery. Multivisceral resection may include the removal of the stomach along with the colon, pancreas, left lobe of the liver, spleen, and diaphragm. These extended resections can be safely performed with acceptable morbidity and mortality rates to achieve R0 resection. The oncological benefits of these resections have been demonstrated.[26]

11. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in gastric cancer surgery

Approximately 7% to 10% of the total of patients who undergo curative gastric cancer resection test positive on peritoneal cytology. These patients are deemed to have peritoneal carcinomatosis.

Approximately 50% of all recurrences after gastric cancer surgery occur peritoneally. Hence, the concept of surgically removing these peritoneal metastases (cytoreduction), along with the administration of intraperitoneal chemotherapy, is considered to be beneficial. The use of higher temperatures (42°C) to instill chemotherapy directly into the peritoneal cavity is cytotoxic. Hence, cytoreductive surgery with hyperthermic intraperitoneal chemotherapy may be beneficial and improve survival in some patients.[27] This procedure is indicated for patients with limited peritoneal disease (peritoneal carcinomatosis index <7).

12. Role of radiation therapy in stomach carcinoma

Radiation therapy has been used with limited benefits for stomach cancer, in both the neoadjuvant and adjuvant settings. However, these drugs are typically used only in combination with chemotherapy. Preoperative chemoradiotherapy improves survival in patients with potentially curable esophageal or esophagogastric junction cancer, with no substantial increase in complications.[28] In the adjuvant setting chemoradiation is also an advantage, with a 5-year survival of up to 40% achieved in the INT-0116 trial.[29] Chemoradiotherapy is not routinely administered in most cases. However, specific indications include heavy nodal burden, the sampling of <25 nodes, D1 gastrectomy, and R+ resection. The overall algorithm for the treatment of gastric cancer is summarized in various guidelines.[10]

13. Endoscopic management of gastric cancer obstruction and bleeding

Endoscopy and endoscopic ultrasound play defined roles in the management of very early gastric cancers.[7] Endoscopic ultrasound–directed advanced endoscopic techniques, using endoscopic mucosal resection and endoscopic submucosal dissection, can resect certain gastric mucosal lesions.[7,30] In addition to the potentially curative application of endoscopic resection, advanced endoscopy plays an important role in the effective management of gastric outlet obstruction and bleeding due to gastric cancer. Endoscopic balloon dilatation, endoscopic placement of covered self-expanding metal stents (SEMSs), and endoscopic ultrasound–guided gastroenterostomy are some of the advanced endoscopic techniques that produce effective results.[31] Of these techniques, the placement of a covered SEMS seems to be an effective modality for patients who undergo upfront chemotherapy and are awaiting surgery and for those who need palliation for gastric outlet obstruction. The presence of distal small bowel obstruction owing to peritoneal carcinomatosis should be excluded before SEMS placement. Both covered and uncovered stents may be used for the management of gastric outlet obstruction owing to their advantages and disadvantages. Although covered stents prevent tumor ingrowth, they can migrate and obstruct the ampulla of Vater, leading to biliary obstruction.[32] Contrastingly, uncovered stents do not migrate but are prone to obstruction over time because of tumor ingrowth.[31] However, this may not be a major problem in patients with gastric cancer following stent placement; patients either proceed to surgery after a period of chemotherapy or the stent may be purely for palliation when survival is limited. Typically, stents can be successfully deployed in 90% of patients with malignant gastric outlet obstruction, with good clinical outcomes in 80% of patients.[33] A recent systematic review and meta-analysis comparing endoscopic stenting and surgical gastroenterostomy for malignant gastric outlet obstruction evidenced that whereas those receiving endoscopic stenting experience an initial shortened hospital stay, surgical gastroenterostomy has lower reintervention and blockage rates in the long term, achieves good outcomes, and may even contribute to increased overall survival compared with endoscopic stenting.[34] Surgical gastroenterostomy can also be achieved through minimally invasive techniques and should be offered as a palliative modality where applicable; it has advantages over endoscopic therapies[34] and has been effectively used for the management of bleeding gastric tumors. Whereas ideal gastrectomy may be a more definitive therapy for bleeding gastric cancer in both curative and palliative settings, bleeding may be endoscopically controlled to facilitate gastrectomy (through temporary control of bleeding) or may be an effective palliation in patients who are too sick or too advanced to qualify for resection, or where resection can be performed (high tumors invading the distal esophagus needing a combined thoracoabdominal procedure in the metastatic setting). Tumor bleeding is responsible for approximately 5% of all upper GI hemorrhages, of which 38% to 58% are associated with upper GI cancer.[35] Endoscopic therapy should be the first-line treatment approach for patients presenting with upper GI bleeding of any cause, including gastric cancer. Mechanical or ablative therapies may be used to control bleeding at the discretion of the endoscopist. Mechanical therapies include heme clips, balloon tamponade, and band ligation, whereas ablation therapies include the application of a heater probe, monopolar or bipolar diathermy, argon plasma coagulation, and laser coagulation.[35,36] External radiation therapy may be used as an effective palliative therapy to control gastric cancer–related bleeding, achieve symptomatic relief, and reduce the need for recurrent transfusions.[37]

14. Conclusions

The successful and effective management of gastric cancer involves a multidisciplinary approach.

The accurate and appropriate staging of gastric cancer is mandatory for defining the optimal treatment strategy. Surgery to achieve R0 resection is considered potentially curative; hence, surgery remains the cornerstone of multimodal therapy. Neoadjuvant and adjuvant chemotherapy regimens have well-defined roles in the treatment of gastric cancer. Novel medication and chemotherapy combinations have increased tumor response rates, which has translated into more effective surgery, reduced local recurrence, and improved long-term survival.

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The authors declare that they have no conflict of interest with regard to the content of this report.

Author contributions

All authors contributed to data acquisition and interpretation and reviewed and approved the final version of this manuscript.

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References

[1] Waddell T, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D, European Society for Medical Oncology (ESMO); European Society of Surgical Oncology (ESSO); European Society of Radiotherapy and Oncology (ESTRO). Gastric cancer: ESMO-ESSO-ESTRO clinical practice guidelines for diagnosis, treatment, and follow-up. *Ann Oncol*. 2013; 24(Suppl 6):vi57–63.

[2] Shrikhande SV, Sirohi B, Barreto SG, et al. Indian Council of Medical Research consensus document for the management of gastric cancer. *Indian J Med Paediatr Oncol*. 2014;35, 4:239–243.

[3] Smyth EC, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D, ESMO Guidelines Committee. Gastric cancer: ESMO clinical practice guidelines for diagnosis, treatment, and follow-up. *Ann Oncol*. 2016; 27(Suppl 5):v38–49.

[4] Bhutani MS, Hawes RH, Hoffman BJ. A comparison of the accuracy of echo features during endoscopic ultrasound (EUS) and EUS-guided fine-needle aspiration for diagnosis of malignant lymph node invasion. *Gastrointest Endosc*. 1997;45(6):474–479.

[5] Leake PA, Cardoso R, Seevaratnam R, et al. A systematic review of the accuracy and indications for diagnostic laparoscopy before curative-intent resection of gastric cancer. *Gastric Cancer*. 2012;15(Suppl 1):S38–47.

[6] Ji X, Bu ZD, Yan Y, et al. The 8th edition of the American Joint Committee on Cancer tumour-node-metastasis staging system for gastric cancer is superior to the 7th edition: results from a Chinese mono-institutional study of 1663 patients [published online November 22, 2017]. *Gastric Cancer*. 2018;21(4):643–652.

[7] Pimentel-Nunes P, Dinis-Ribeiro M, Ponchon T, et al. Endoscopic submucosal dissection: European Society of Gastrointestinal Endoscopy (ESGE) guideline [published online August 28, 2015]. *Endoscopy*. 2015; 47(9):829–854.

[8] Degiuli M, de Manzoni G, Di Leo A, et al. Gastric cancer: current status of lymph node dissection. *World J Gastroenterol*. 2016;22(10): 2875–2893.

[9] Solsky I, In H. Surgical treatment for gastric Cancer. *Gastrointest Endosc Clin N Am*. 2021;31(3):581–605.

[10] Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2018 (5th edition) [published online February 14, 2020]. *Gastric Cancer*. 2021;24(1):1–21.

[11] Bonenkamp JJ, Hermans J, Sasako M, et al, Dutch Gastric Cancer Group. Extended lymph-node dissection for gastric cancer. *N Engl J Med*. 1999;340(12):908–914.

[12] Cuschieri A, Fayers P, Fielding J, et al. Postoperative morbidity, and mortality after D1 and D2 resections for gastric cancer: preliminary results of the MRC randomized controlled surgical trial. The Surgical Cooperative Group. *Lancet*. 1996;347(9007):995–999.

[13] Degiuli M, Sasako M, Ponti A, et al, Italian Gastric Cancer Study Group. A randomized clinical trial comparing survival after D1 or D2 gastrectomy for gastric cancer. *Br J Surg*. 2014;101(2):23–31.

[14] Songun I, Putter H, Kranenbarg EM, Sasako M, van de Velde CJ. Surgical treatment of gastric cancer: 15-year follow-up results of the randomized nationwide Dutch D1D2 trial [published online April 19, 2010]. *Lancet Oncol*. 2010;11(5):439–449.

[15] Quan Y, Huang A, Ye M, et al. Comparison of laparoscopic versus open gastrectomy for advanced gastric cancer: an updated meta-analysis [published online July 28, 2015]. *Gastric Cancer*. 2016;19(3):939–950.

[16] Nallasamy N, Ambikapathi T, Ooi WK, Hayati F. Merendino procedure as an alternative to total gastrectomy in the treatment of gastrointestinal stromal tumors of the gastroesophageal junction: a case series. *Formos J Surg*. 2022;55:154–157.

[17] Rosa F, Marrelli D, Morgagni P, et al. Krukenberg tumors of gastric origin: the rationale of surgical resection and perioperative treatments in a multicenter Western experience. *World J Surg*. 2016;40(4):921–928.

[18] Fujitani K, Yang HK, Mizusawa J, et al, REGATTA Study Investigators. Gastrectomy plus chemotherapy versus chemotherapy alone for advanced gastric cancer with a single non-curable factor (REGATTA): a phase 3, randomised controlled trial [published online January 26, 2016]. *Lancet Oncol*. 2016;17(3):309–318.

[19] Li Q, Zou J, Jia M, et al. Palliative gastrectomy and survival in patients with metastatic gastric cancer: a propensity score–matched analysis of a large population-based study. *Clin Transl Gastroenterol*. 2019; 10(5):1–8.

[20] Yazıcı O, Özdemir N, Duran AO, et al. The effect of the gastrectomy on survival in patients with metastatic gastric cancer: a study of ASMO [published online January 18, 2016] [published correction appears in *Future Oncol* 2016;12(6):881]. *Future Oncol*. 2016;12(3):343–354.

[21] Hartgrink HH, Putter H, Klein Kranenbarg E, Bonenkamp JJ, van de Velde CJ, Dutch Gastric Cancer Group. Value of palliative resection in gastric cancer. *Br J Surg*. 2002;89(11):1438–1443.

[22] Huang KH, Wu CW, Fang WL, et al. Palliative resection in noncurative gastric cancer patients. *World J Surg*. 2010;34(5):1015–1021.

[23] Shiono S, Sato T, Horio H, et al, Metastatic Lung Tumor Study Group of Japan. Outcomes and prognostic factors of survival after pulmonary resection for metastatic gastric cancer [published online November 8, 2012]. *Eur J Cardiothorac Surg*. 2013;43(1):e13–16.

[24] Kodera Y, Fujitani K, Fukushima N, et al. Surgical resection of hepatic metastasis from gastric cancer: a review and new recommendation in the Japanese gastric cancer treatment guidelines. *Gastric Cancer*. 2014;17(2):206–212.

[25] Cheon SH, Rha SY, Jeung HC, et al. Survival benefit of combined curative resection of the stomach (D2 resection) and liver in gastric cancer patients with liver metastases [published online February 27, 2008]. *Ann Oncol*. 2008;19(6):1146–1153.

[26] Brar SS, Seevaratnam R, Cardoso R, et al. Multivisceral resection for gastric cancer: a systematic review [published online July 23, 2011]. *Gastric Cancer*. 2012;15(Suppl 1):S100–S107.

[27] Yang XJ, Huang CQ, Suo T, et al. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy improves survival of patients with peritoneal carcinomatosis from gastric cancer: results of phase III randomized clinical trial [published online March 23, 2011]. *Ann Surg Oncol*. 2011;18(6):1575–1581.

[28] van Hagen P, Hulshof MC, van Lanschot JJ, et al, CROSS Group. Preoperative chemoradiotherapy for oesophagal or junctional cancer. *N Engl J Med*. 2012;366(22):2074–2084.

[29] Macdonald JS, Smalley SR, Benedetti J, et al. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Engl J Med*. 2001;345(10):725–730.

[30] Bestetti AM, de Moura DTH, Proença IM, et al. Endoscopic resection versus surgery in the treatment of early gastric cancer: a systematic review and meta-analysis. *Front Oncol*. 2022;12:939244.

[31] Tringali A, Giannetti A, Adler DG. Endoscopic management of gastric outlet obstruction disease [published online May 30, 2019]. *Ann Gastroenterol*. 2019;32(4):330–337.

[32] van den Berg MW, Walter D, Vleggaar FP, Siersema PD, Fockens P, van Hooft JE. High proximal migration rate of a partially covered "big cup" duodenal stent in patients with malignant gastric outlet obstruction [published online December 11, 2013]. *Endoscopy*. 2014; 46(2):158–161.

[33] van Hooft JE, Uitdehaag MJ, Bruno MJ, et al. Efficacy and safety of the new WallFlex enteral stent in palliative treatment of malignant gastric outlet obstruction (DUOFLEX study): a prospective multicenter study [published online January 18, 2009] [published correction appears in *Gastrointest Endosc* 2010;72(3):674]. *Gastrointest Endosc*. 2009; 69(6):1059–1066.

[34] Hong J, Chen Y, Li J, et al. Comparison of gastrojejunostomy to endoscopic stenting for gastric outlet obstruction: an updated systematic review and meta-analysis [published online October 30, 2021]. *Am J Surg*. 2022;223(6):1067–1078.

[35] Kim YI, Choi IJ. Endoscopic management of tumor bleeding from inoperable gastric cancer [published online March 27, 2015]. *Clin Endosc*. 2015;48(2):121–127.

[36] Ofosu A, Ramai D, Latson W, Adler DG. Endoscopic management of bleeding gastrointestinal tumors [published online May 31, 2019]. *Ann Gastroenterol*. 2019;32(4):346–351.

[37] Lee JA, Lim DH, Park W, Ahn YC, Huh SJ. Radiation therapy for gastric cancer bleeding. *Tumori*. 2009;95(6):726–730.

Review Article

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OPEN

Immunotherapy for hepatocellular carcinoma: molecular pathogenesis and clinical research progress

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Abstract

The treatment of hepatocellular carcinoma (HCC) is advancing rapidly in the 21st century. Although there are various treatment methods, the most promising breakthrough seems to be in immunotherapy. Recent guidelines from the American Society of Clinical Oncology and the European Association for the Study of the Liver have recommended immunotherapies with strong antitumor effects for HCC treatment. Emerging systemic therapeutic strategies, such as immune checkpoint inhibitors combined with targeted therapy or local treatment, are among the most promising for improving overall and tumor-free survival times in patients with HCC. This review analyzes the molecular mechanisms of existing immune checkpoint inhibitors, vaccines, and chimeric antigen receptor–T cells; summarizes the latest progress in relevant clinical research; and outlines future trends and opportunities for HCC immunotherapy.

Keywords: Immunotherapy; Hepatocellular carcinoma; Immune checkpoint inhibitor; Cancer vaccine

1. Introduction

Hepatocellular carcinoma (HCC) is the fourth most common cancer and the second most common cause of cancer-related deaths in China.[1] These data underscore the urgent need for further research and development to combat this disease. Significant advancements have been made in the treatment of both early and advanced stages of HCC; options such as radiofrequency ablation, surgical resection, and liver transplantation can be adopted; and neoadjuvant and postoperative adjuvant therapies have been explored and shown promising results.[2,3] Targeted therapy, immunotherapy, and combined treatment have exhibited significant effects for advanced HCC (aHCC).[4] Owing to the study of immune checkpoints and the tumor immune microenvironment (TIM), immunotherapy for HCC has shown great potential. Immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR)–T cells, and vaccines are currently the focal points of research.[5] This review summarizes the molecular pathogenesis of immunotherapy for HCC and provides an overview of the latest clinical research progress, with the goal of promoting standardized and effective clinical treatments for HCC.

2. TIM and immune tolerance of HCC

The TIM in HCC plays a significant role in HCC progression and determines the effectiveness of immunotherapy. TIM is composed of various immune-related cells and cytokines that can promote or inhibit tumor progression or mediate immune escape.[6,7] $CD8^+$ cytotoxic T cells and natural killer (NK) cells perform immune surveillance by inducing the apoptosis of target cells, whereas mature dendritic cells (DC) limit tumor incidence and progression. In contrast, regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs), cancer-associated fibroblasts, and M2-polarized macrophages exhibit both anti-inflammatory and protumor effects. Tregs suppress protective immune responses against invading pathogens or tumors, leading to disease progression, and MDSCs boost their suppressive activity to maintain an immunosuppressive state.[8,9]

Understanding the composition and function of TIM in HCC is crucial for developing effective immunotherapies that can overcome the immune evasion tactics used by tumors. This highlights the need to identify new TIM targets for potential therapies and to develop strategies to effectively manipulate immune tolerance in HCC.[10]

3. ICIs and molecular mechanism

One possible mechanism of immunosuppression in the TIM of HCC is the activation of immune checkpoint pathways, which inhibit the activation of effector immune cells. This phenomenon may be due to the overexpression or aberrant activation of inhibitory receptors, such as PD-1 and CTLA-4 on the surface of T cells. Although several ICIs have been investigated in clinical trials, only the PD-1/PD-l1 and CTLA-4 inhibitors have shown significant clinical benefits. These inhibitors block the interactions between inhibitory receptors and their ligands, leading to the reactivation of T cells and the enhancement of antitumor immune responses (Figure 1).

PD-1, also known as CD279, belongs to the immunoglobulin superfamily and plays a role in the immune system by regulating T-cell activity. Its ligands, PD-L1 and PD-L2, serve as immunosuppressive molecules that reduce T-cell proliferation and activity. In HCC, both PD-1 and its ligands are widely expressed in tumors and immune

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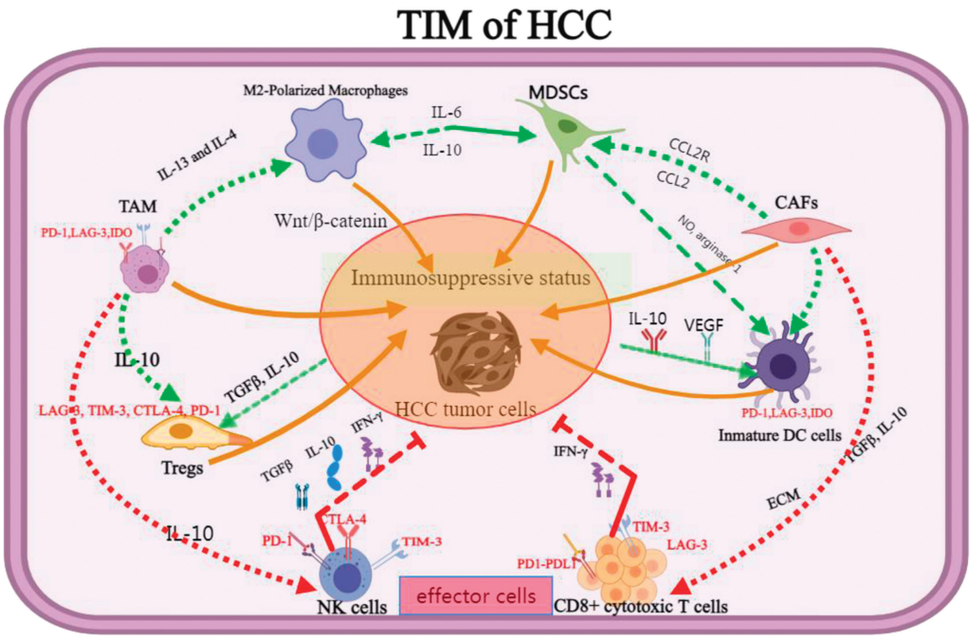

Figure 1. Molecular mechanisms underlying the interactions between tumor and immune cells in the tumor microenvironment.

cells, which can lead to immune escape of tumor cells through the suppression of T-cell function. Research has shown that PD-1 is upregulated in exhausted T cells that are unable to eliminate cancer cells because of chronic stimulation. Blocking inhibitory receptors on these T cells can restore their function and enhance the immune response against the tumor.[11,12] ICIs targeting PD-1 have shown exciting results in patients with melanoma and non–small cell lung carcinoma, and PD-L1 may be degraded by OTUB1 through the endoplasmic reticulum–associated pathway.[13] Blocking the PD-1/PD-L1 inhibitory pathway can activate T cells in the TIM, leading to the release of inflammatory cytokines and cytotoxic granules to eliminate tumor cells.[14]

CTLA-4 was first cloned in 1987 as a novel molecule expressed on activated T cells, mainly Tregs, and plays a negative role in the immune system. The expression of CTLA-4 can be induced to increase by TGF-β. CTLA-4 and CD28 both can bind to B7 protein but produce completely different reactions.[15] The specific CTLA-4 antibody, ipilimumab, has improved the long-term prognosis of patients with unresectable malignant melanoma.[16]

In addition to PD-1 and CTLA-4, several new immune checkpoints have been discovered. Among these, the tumor necrosis factor receptor superfamily (TNFRSF) acts as a co-stimulatory receptor for T cells. This receptor is highly expressed on activated $CD4^+$ and $CD8^+$ effector T cells, as well as on nonactivated Tregs, and its primary function is to promote immune activation. Two members of this family, ox40 and cd27, have been found to enhance T-cell proliferation.[17,18] This discovery may offer a new approach for HCC treatment. TIGIT, a class of T-cell immunoreceptors with immunoglobulin and ITIM domains, inhibits both innate and adaptive immunity. TIGIT is expressed in tumor-infiltrating lymphocytes (TILs) and is associated with PD-1 expression. Combining TIGIT and PD-1 blockade significantly enhanced the proliferation of $CD8^+$ TILs. CD155 (PVR) and CD112 (PVRL2, nectin-2) are the 2 main ligands of TIGIT, and their high expression is common in HCC cells.[19,20]

TIM-3 is an inhibitory immune checkpoint receptor expressed by IFN-γ–producing $CD4^+$ and $CD8^+$ T cells. TIM-3 inhibition enhances the antitumor effects of PD-1 blockers. When TIM-3 binds to its ligands, such as Galectin-9, it mediates apoptosis of effector T cells.[21,22] High mobility group box-1 protein (HMGB1) is another ligand that can weaken the antitumor effects of DNA vaccines and cytotoxic chemotherapy, while also inhibiting innate and antitumor immune responses.[23] CD47, which belongs to the immunoglobulin superfamily, is widely expressed in both normal and diseased tissues. Signal regulatory protein α (SIRPα) is one of its main ligands.[24] HCC cells may use the CD47/SIRPα axis to escape immune surveillance and inhibit tumor-associated macrophage phagocytosis, blocking the CD47/SIRPα axis and improving the adaptive immune response.[25,26]

Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) are enzymes that inhibit the proliferation and activity of T cells. TDO promotes EMT, contributing to the invasion and metastasis of HCC and leading to a poor prognosis.[27] IDO in tumor cells promotes the expansion, recruitment, and activation of MDSCs.[28,29] Clinical trials of BMS-986205 in combination with nivolumab (NIVO) are underway.[30] Lymphocyte-activating gene 3 (LAG-3) is a suppressive molecule that can bind to MHC-II and FGL1 in HCC, leading to the generation of immunosuppressive effectors that mediate immune escape. The FGL1/LAG-3 pathway may be a potential target for cancer immunotherapy.[31,32]

4. Principles of immune checkpoint–based therapy

The interaction between immune and nonimmune cells in the context of ICI therapy has been elucidated over time, and studies have demonstrated that TILs and other immune cells in the TIM can exert influence on ICI therapy. However, one type of suppressive immune cell in the TIM is MDSCs, which derive from immature myeloid cells

and can induce immunosuppressive cytokines that impede $CD4^+$ $CD8^+$ T-cell proliferation, promote macrophage differentiation into an immune-inhibitory phenotype, inhibit NK cell cytotoxicity, and induce Tregs. The enhancement of tumor growth, angiogenesis, and metastasis can hinder the efficacy of ICI therapy.[33,34] In contrast, M1-polarized macrophages exhibit antitumor activity, whereas M2-polarized macrophages promote tumorigenesis. M2-polarized macrophages upregulate multiple negative immune checkpoints, such as PD-L1, LAG-3, and CTLA-4, to sustain the immunosuppressive state and inhibit T-cell activation and proliferation, thus hindering the effectiveness of ICI therapy.[35,36] In addition, Tregs express CTLA-4 and TIM-3 on their cell surfaces, which exert an immunosuppressive effect by suppressing the activity of effector T cells.

Immune checkpoints play essential regulatory roles in the immune cells. When activated by T-cell receptors costimulatory complex, $CD8^+$ T lymphocytes secrete death-inducing granules that trigger cell death or apoptosis. However, the immunosuppressive microenvironment induces the expression of PD-1, TIM-3, and LAG-3 in effector CD8+ T cells, which inhibits their activation. $CD4^+$ T helper I cells can be classified into T_H1 and T_H2 subclasses, which mediate proinflammatory and anti-inflammatory functions, respectively. The expression of multiple immune checkpoints, such as CTLA-4 and PD-1, inhibits their activity.[37,38] NK cells recognize tumor-associated surface proteins and eliminate malignant cells. Unfortunately, PD-1, CTLA-4, LAG-3, and TIM-3 are expressed on the surface of NK cells, which hampers their antitumor responses. Targeting PD-1/PD-L1 using ICI can improve the antitumor response of NK cells.[39,40]

The TME contains various nonimmune mesenchymal cells that interact with immune cells, including cancer-associated fibroblasts, hematopoietic stem cells, endothelial cells, and DCs. These cells can secrete immunosuppressive cytokines such as TGF-β, IL-4, IL-10, CSF-1, and VEGF, or express immune checkpoints to maintain an immunosuppressive state.[37,41] Recent studies have demonstrated the presence of an immune barrier in the TME of HCC that exhibits primary resistance to immunotherapy-based regimens. Multiple immune checkpoints, including LAG-3, FGL1, TIGIT, NECTIN2, and CD155, are overexpressed during HCC immune escape, and WNT/CTNNTB1 mutations further facilitate this mechanism. Epigenetic mechanisms, such as DNA methylation dysregulation, have also been implicated in immune tolerance in the TME, contributing TME-related resistance to ICIs. Understanding the interaction between these immune cells and the precise role of immune checkpoints could improve the antitumor immune effectors of ICIs, although further research is required to fully understand the molecular mechanisms underlying immunotherapy based on immune checkpoints.

5. Immune checkpoint drugs and clinical treatment strategy

5.1. Monotherapy and limitations

NIVO is a human anti–PD-1 IgG4 monoclonal antibody. In 2017, a clinical research (CheckMate 040) was conducted to evaluate the safety and efficacy of NIVO compared with sorafenib (SOR) in patients with aHCC.[42] The positive results of this study prompted the Food and Drug Administration to accelerate the approval of NIVO as a second-line treatment for aHCC. Subsequently, a phase III trial (CheckMate 459) was conducted to compare NIVO and SOR as first-line treatments for aHCC. Although the results did not show significant difference, NIVO demonstrated a favorable therapeutic effect in patients with aHCC.[43] In a real-world cohort study conducted by Choi et al., NIVO was compared in patients with HCC with Child-Pugh A and B, and the results revealed that patients with Child-Pugh A had better objective response rate (ORR) and disease control rate (DCR) and longer overall survival (OS) compared with those with Child-Pugh B (42.9 vs 11.3 weeks; 95% confidence interval). However, it is worth mentioning that most patients in this study were infected with hepatitis B virus, which has a low prevalence.[44] Further results from a cohort phase trial (CheckMate 040) showed that the ORR of NIVO therapy reached 12%, the disease control rate reached 55%, and the duration of response was 9.9 months, indicating that the treatment was still effective in patients with Child-Pugh B.[45]

Pembrolizumab, an anti–PD-1 IgG4 antibody, was initially evaluated as a second-line treatment for HCC in patients who progressed after SOR in the KEYNOTE-240 trial. The experimental group received intravenous injection of 200 mg pembrolizumab every 3 weeks, leading to an OS of 13.9 months compared with 10.6 months for the control group. Although neither progression-free survival (PFS) nor OS reached their preset end points, long-term observations demonstrated better therapeutic effects of pembrolizumab.[46] Atezolizumab is an engineered IgG1 monoclonal antibody targeting PD-L1. Few clinical studies have focused on single-agent treatments; however, combinations of targeted drugs, such as first-line and second-line therapies, have shown significant efficacy. In particular, the combination of atezolizumab with the VEGF-targeting agent bevacizumab has been successful, making it a promising drug for first-line treatment of HCC.[47] Tislelizumab is another anti–PD-1 antibody designed to escape FcγR1-mediated effector function. A global phase III clinical trial (RATIONALE301) aimed to compare tislelizumab with SOR as a first-line treatment.[48] The clinical study is currently ongoing and evaluates single-agent treatment for aHCC as a first-line therapy.

There are several analogs of anti–PD-1 monoclonal antibodies available, including sintilimab, camrelizumab, toripalimab, and pembrolizumab. In addition, anti–PD-L1 monoclonal antibodies, such as durvalumab and envafolimab, as well as anti–CTLA-4 antibody remelimumab, have been successively introduced. However, the efficacy of a single agent is significantly lower than that of combination therapy. Combining immunotherapy and remodeling the tumor microenvironment are novel ways to improve the effectiveness of ICIs.

5.2. Combination therapy

Evidence from many phase II/III trials demonstrates that combination therapy has superior safety and efficacy compared with single-agent treatments.[49]

5.2.1. Combination of VEGF or VEGFR inhibition and ICIs

Angiogenesis is a crucial factor in the growth of liver cancer. Targeting angiogenic factors such as VEGF or VEGFR can lead to antiangiogenesis and tumor regression. TKIs, including SOR, are antiangiogenic targets that enhance antitumor immunity by increasing M1 polarization and reversing the function of MDSCs, thereby promoting immunoactivity in the TME. In the Imcourage 150 trial, atezolizumab and bevacizumab demonstrated the highest clinical efficacy in the first-line treatment of aHCC. Patients in the atezolizumab-bevacizumab group had significantly longer OS compared with those in the SOR group. The median PFS (mPFS) was 6.8 versus 4.3 months.[50] The ORIENT-32 phase III trial, which used sintilimab and IBI305, showed improved OS and PFS.[51] Camrelizumab plus apatinib (VEGFR-2) achieved potent efficacy in aHCC both in first-line and second-line settings, with mPFS values of 5.7 and 5.5 months, respectively.[52,53]

5.2.2. Combination of TKIs and ICIs

TKIs target multiple signaling pathways, and when combined with ICI treatment, they can achieve a synergistic effect. The LEAP clinical trial, which investigated the combination of pembrolizumab and lenvatinib, represents a first-line treatment option for aHCC. Recent

data from a phase Ib trial demonstrated an ORR of 46%, a median OS (mOS) of 22 months, and an mPFS of 9.3 months.[54] The COSMIC-312 trial, which investigated the combination of atezolizumab and cabozantinib, also explored a first-line treatment option for patients with unresectable HCC who have not received systemic treatment previously. The results indicated an improvement in PFS and a trend toward longer OS.[55] Furthermore, a phase I trial investigating the combination of camrelizumab and apatinib reported an ORR of 50%. Phase III trials are currently ongoing and have already achieved the predefined standard of primary end points.[56,57]

5.2.3. Combination of anti–PD-1/PD-L1 and CTLA-4 inhibitors

The response rate increased to approximately 30% when CTLA-4 and PD-1/PD-L1 were both blocked, as evidenced by relevant data from CheckMate 040 (NIVO and ipilimumab).[58] Other studies with a similar design are currently underway, like the HIMALAYA study (tremelimumab and durvalumab), which represents a common first-line therapy for patients with aHCC. The statistical results indicated an ORR of 24% and an mOS of 18.7 months.[59]

5.2.4. Combination of triplet drugs

Double immunotherapy has shown to be more effective than single-drug therapy; however, the mOS remains to be less than 20 months. In an attempt to improve both the ORR and OS, triple-drug combination therapy has been explored. TRIPLET-HCC (NCT05665348) is a multicenter, randomized, open-label phase II and III trial that investigated the triple combination of ipilimumab plus atezolizumab/bevacizumab compared with the double atezolizumab/bevacizumab alone. In the TRIPLET-HCC trial, ipilimumab was administered at a dose of 1 mg/kg per injection for the initial 4 cycles. The aims of TRIPLET-HCC were to evaluate the potential synergistic effect of anti–PD-L1 + anti–CTLA-4 + anti-VEGF, and assess whether it can enhance both the ORR and OS. These results were highly anticipated.[60] Other triplet combinations, such as ICIs + chemotherapy (restricted to the Asian population) and anti-PD1 + anti-VEGF + alternative immune targets like TIGIT, LAG3, or IL-27, have also been explored in multiple global clinical studies.[61]

5.2.5. Combination of locoregional therapies and ICIs

Local therapies for HCC encompass transarterial embolization, transarterial chemoembolization (TACE), and radiofrequency ablation. These local therapies are designed to target and eradicate tumors, stimulate the release of immunogenic substances, and modulate the immune microenvironment. The hypoxic reaction caused by TACE leads to the release of proangiogenic cytokines and the demise of immunogenic cells. Therefore, the combination therapy of TACE with ICIs, abbreviated with TACE-SOR-ICIs, has been used to manage TACE-refractory HCC, resulting in a notable extension of OS and mPFS.[62]

5.3. Current ICI treatment strategies and protocols

The first-line treatment strategy includes atezolizumab plus bevacizumab, which was approved in May and October 2020 for the treatment of unresectable HCC that has not previously received systemic treatment.[50] Currently, multiple guidelines recommend this program as a priority first-line systematic treatment. Although the preset mOS of sintilimab combined with IBI305 (bevacizumab analogs) has not yet been achieved, it has shown significantly better results than SOR, with an mPFS of 4.6 months and an ORR of 21%.[51] Based on these findings, sintilimab combined with IBI305 was approved in June 2021 as a first-line treatment for nonresectable or metastatic HCC by the National Medical Products Administration.

Two studies 117 (lenvatinib combined with NIVO) and Keynote 524 (lenvatinib combined with pembrolizumab) confirmed that ICIs combined with lenvatinib yield better tumor response as first-line treatments.[63,64] The 2022 Chinese Society of Clinical Oncology liver cancer guidelines recommend apatinib combined with camrelizumab as the first-line treatment for aHCC, and lenvatinib combined with pembrolizumab or NIVO is recommended by level III expert. Camrelizumab, tirelizumab, and pembrolizumab have all been approved as second-line treatments for aHCC. Several studies on the combination of targeted therapy and dual immunotherapy for liver

Table 1

Main phase III clinical trials of ICIs for HCC

Name	Trail	Phase	Control group	Primary end point	ORR%	Median OS	Median PFS
KEYNOTE-240	Pembrolizumab	III	Placebo	mOS, mPFS	18.3	13.9	3.3
CheckMate 459	Nivolumab	III	Sorafenib	mOS	NA	16.4	NA
IMbrave150	Atezolizumab + bevacizumab	III	Sorafenib	mOS, mPFS	27.3	19.2	6.9
COSMIC-312	Cabozantinib + atezolizumab	III	Sorafenib	mOS, mPFS	11	15.8	6.8
Keynote-394	Pembrolizumab (MK-3475)	III	Placebo	mOS, mPFS	12.7	14.6	NA
03713593 LEAP-002	Lenvatinib + pembrolizumab	III	Lenvatinib	mOS, mPFS	28.1	26.3	8.3
04720716	IBI310 + sintilimab	III	Sorafenib	mOS, ORR		Recruiting	
04194775 CS1003	Nofazinlimab + lenvatinib	III	Lenvatinib and placebo	mOS, mPFS		Active, not recruiting	
03298451 (HIMALAYA)	Durvalumab + tremelimumab	III	Durvalumab	mOS	20.1	16.43	3.8
03412773 (RATIONALE 301)	Tislelizumab	III	Sorafenib	mOS	14.3	15.9	2.2
04723004	Boripalimab + bevacizumab	III	Sorafenib	mOS, mPFS		Active, not recruiting	
04560894	SCT-I10A + SCT510	III	Sorafenib	mOS, mPFS		Recruiting	
03794440 (ORIENT-32)	Sintilimab + IBI305	III	Sorafenib	mOS, mPFS		Unknown	
04523493	Toripalimab + lenvatinib	III	Lenvatinib + placebo	mOS, mPFS		Recruiting	
03764293	SHR-1210 + apatinib	III	Sorafenib	mOS, mPFS		Active, not recruiting	
NCT03867084 (KEYNOTE-937)	Pembrolizumab	III	Placebo	RFS, mOS		Active, not recruiting	
NCT03383458 (CheckMate 9DX)	Nivolumab	III	Placebo	RFS		Active, not recruiting	

HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; mOS, median overall survival; mPFS, median progression-free survival; NA, not available; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RFS, recurrence free survival.

Table 2

Main clinical trials of CAR–T cells for HCC

NCT no.	Interventions	Status	Phase	Measures	Results
NCT05620706	GPC3 CAR–T cells	Recruiting	I	AE/SAE, ORR	NA
NCT05003895	CAR–T cell + cyclophosphamide	Recruiting	I	OS, BOR	NA
NCT05323201	fhB7H3.CAR–Ts + fludarabine	Recruiting	I/II	AE/SAE, ORR	NA
NCT05155189	TCCAR031	Recruiting	I	TEAEs/AESIs	NA
NCT03884751	CARGPC3 T cells	Completed	I	OS, PFS	NA
NCT04951141	Anti-GPC3 CAR–T cells	Recruiting	I	OS, ORR	NA
NCT02905188	GLYCAR–T cells	Completed	I	Limiting toxicity	NA
NCT03198546	GPC3 and/or TGF-β targeting CAR–T cells	Recruiting	I	Limiting toxicity	NA

AE, adverse event; AESI, adverse event of special interest; BOR, best of response; CAR, chimeric antigen receptor; HCC, hepatocellular carcinoma; NA, not available; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; SAE, serious adverse event; TEAE, treatment emergent adverse events.

cancer are currently underway, including NIVO combined with regorafenib (GOING study), and tirelizumab combined with sitravatinib (BGB-900-104 study).[65–67] The results of the main phase III clinical trials of ICIs for HCC are listed in Table 1.

6. Immunotherapy based on liver cancer vaccine

6.1. Tumor peptides vaccine

Liver vaccines use tumor antigen peptides to initiate antitumor immunity, which aims to eliminate or control tumors. Although a vaccine alone may not significantly augment immune function, the combined use of ICIs can potentially enhance the efficacy of tumor immunotherapy. Common target antigens for vaccines encompass the following: AFP, glypican-3, multidrug resistance-associated protein 3 (MRP3), human telomerase reverse transcriptase, and cancer testicular antigen. These antigens have the ability to activate T cells and elicit the secretion of IL-12 and γ-interferon. Some phase I trials have reported that peptide vaccines promote the infiltration of CTLs into tumors, thereby specifically inducing cytotoxic T cells to eliminate tumor cells.[68] Notably, these vaccines have also demonstrated good tolerability.

6.2. DC vaccines

DCs play a critical role in adaptive immune responses, functioning as a potent class of antigen-presenting cells. They are essential for the induction of T- and B-cell–mediated cellular and humoral immunity. Because of their unique immunological functions, DC vaccines have garnered significant attention. These vaccines can be classified into 3 groups based on their preparation strategies and research timelines. Early DC vaccines used antigens such as long peptides or proteins along with adjuvants that facilitated DC maturation. The remaining 2 categories involve targeting DCs in vivo and in vitro induction of DCs loaded with antigens, followed by reinfusion. Various animal experiments have demonstrated that DC vaccines, either alone or in combination with ICIs, can instigate more effective CTL-mediated cytotoxicity and effectively suppress tumor growth. Preclinical studies have investigated DC vaccine-based immunotherapies, such as tumor antigen pulsing, ICI combination therapy, and DC-derived exosomes, with the aim of optimizing the efficacy in anti-HCC treatment.[69]

6.3. In situ vaccines

Antigens are acquired from deceased or moribund tumor cells within the tumor microenvironment. This elicits a profound immune response and repetitive sequences of immune initiation, immune effects, and tumor cell demise. The release of antigens consequently stimulates immune reactivation and re-effectiveness, which ultimately optimizes the antitumor immune response. In recent times, researchers from Southeast University have conceived in situ cancer vaccines using nanotechnology that holds the potential to effectively engage the body's immune response.[70]

6.4. DNA vaccine and RNA vaccine

These vaccines are based on similar principles. However, DNA vaccines require entry into the nucleus to complete transcription before protein translation and expression can occur in the cytoplasm. On the other hand, mRNA vaccines can synthesize proteins directly in the cytoplasm, requiring one less step than DNA vaccines. Clinical data from phase Ib/IIa trials have demonstrated effective T-cell responses, thereby increasing expectations for the future of personalized immunotherapy.[71]

7. Immunotherapy based on CAR–T-cell therapy

T cells express T-cell receptors or CARs on their surfaces to enhance the specificity and reactivity of immune cells through gene modification. CAR–T cell therapy involves genetic modification of T cells to recognize tumor-specific antigens, which facilitates T-cell activation signals and equips T cells with the ability to eradicate tumors. Potential substrates for CAR–T cell therapy in HCC include GPC3, melanoma antigen gene 3 (MAGE3), human telomerase reverse transcriptase (hTERT), and AFP.

Two phase I studies were conducted to treat adults with HCC using an infusion of GPC3-targeted CAR–T cells after lymphatic clearance of cyclophosphamide and fludarabine. Of the 13 patients, 9 achieved complete remission, and none experienced grade 3 or 4 neurotoxicity. The OS rates were 10.5% at 3 years, 42% at 1 year, and 50.3% at 6 months. One patient with persistently stable disease remains alive after 44.2 months.[72,73] Currently, there are ongoing trials for combined therapies, such as dual-target CAR–T-cell therapy and combination therapy of CAR–T cell with other treatments. However, the effectiveness of CAR–T cells in the treatment of HCC is limited because of several factors. These factors include a lack of highly specific tumor antigen maintenance; limited and unstable transport, proliferation, and activity of CAR–T cells; targeting effects; and cytokine release syndrome. Consequently, the exploration of CAR–T cells for the treatment of HCC is currently restricted to phase I clinical trials and animal experiments, which have low clinical value. The results of the main ongoing clinical trials of CAR–T cells for HCC are listed in Table 2.

8. Perspectives

Immunotherapy for HCC is predominantly driven by ICIs along with tumor vaccines, whereas CAR–T-cell therapy is also gaining recognition. Numerous clinical guidelines have emphasized the increasing significance of immunotherapy. Various types of immune and mesenchymal cells present in the tumor microenvironment play a direct or indirect role in antitumor immunity, thereby forming the molecular basis for tumor immunosuppression and the induction of immune tolerance. Rebuilding the immune microenvironment could potentially enhance the effectiveness of immunotherapy and overcome its limitations.

Dual immunotherapy might hold promise as a second-line treatment option. Emerging immunotherapy strategies, such as oncolytic viruses, tumor vaccines, combinations of single ICIs, or combination therapies with ICIs + TKIs or bispecific antibodies, are actively being reached and offer new options for liver cancer treatment. However, given to the heterogeneity of HCC, future immunotherapies for HCC necessitate more precise and individualized treatment strategies.

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References

[1] Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66(2):115–132.

[2] Papaconstantinou D, Hewitt DB, Brown ZJ, Schizas D, Tsilimigras DI, Pawlik TM. Patient stratification in hepatocellular carcinoma: impact on choice of therapy. *Expert Rev Anticancer Ther*. 2022;22(3):297–306.

[3] Stefanini B, Tonnini M, Serio I, Renzulli M, Tovoli F. Surveillance for hepatocellular carcinoma: current status and future perspectives for improvement. *Expert Rev Anticancer Ther*. 2022;22(4):371–381.

[4] Edeline J, Meyer T, Blanc JF, Raoul JL. New challenges facing systemic therapies of advanced HCC in the era of different first-line immunotherapy-based combinations. *Cancers (Basel)*. 2022;14(23):5868.

[5] Lu L, Zhan M, Li XY, et al. Clinically approved combination immunotherapy: current status, limitations, and future perspective. *Curr Res Immunol*. 2022;3:118–127.

[6] Liu Y, Xun Z, Ma K, et al. Identification of a tumour immune barrier in the HCC microenvironment that determines the efficacy of immunotherapy. *J Hepatol*. 2023;78(4):770–782.

[7] Qin H, Sheng W, Zhang G, et al. Comprehensive analysis of cuproptosis-related prognostic gene signature and tumor immune microenvironment in HCC. *Front Genet*. 2023;14:1094793.

[8] Hao X, Sun G, Zhang Y, et al. Targeting immune cells in the tumor microenvironment of HCC: new opportunities and challenges. *Front Cell Dev Biol*. 2021;9:775462.

[9] Khanam A, Kottilil S. New therapeutics for HCC: does tumor immune microenvironment matter? *Int J Mol Sci*. 2022;24(1):437.

[10] Dong Y, Wong JSL, Sugimura R, et al. Recent advances and future prospects in immune checkpoint (ICI)–based combination therapy for advanced HCC. *Cancers (Basel)*. 2021;13(8):1949.

[11] Kalathil SG, Lugade AA, Miller A, Iyer R, Thanavala Y. PD-1+ and Foxp3+ T cell reduction correlates with survival of HCC patients after sorafenib therapy. *JCI Insight*. 2016;1(11):e86182.

[12] Zhang Y, Zhang H, Wei M, et al. Recombinant adenovirus expressing a soluble fusion protein PD-1/CD137L subverts the suppression of CD8+ T cells in HCC. *Mol Ther*. 2019;27(11):1906–1918.

[13] Zhu D, Xu R, Huang X, et al. Deubiquitinating enzyme OTUB1 promotes cancer cell immunosuppression via preventing ER-associated degradation of immune checkpoint protein PD-L1. *Cell Death Differ*. 2021;28(6):1773–1789.

[14] Fan Z, Wu C, Chen M, et al. The generation of PD-L1 and PD-L2 in cancer cells: from nuclear chromatin reorganization to extracellular presentation. *Acta Pharm Sin B*. 2022;12(3):1041–1053.

[15] Safaeifard F, Goliaei B, Aref AR, et al. Distinct dynamics of migratory response to PD-1 and CTLA-4 blockade reveals new mechanistic insights for potential T-cell reinvigoration following immune checkpoint blockade. *Cell*. 2022;11(22):3534.

[16] Afzal MZ, Shirai K. Immune checkpoint inhibitor (anti–CTLA-4, anti–PD-1) therapy alone versus immune checkpoint inhibitor (anti–CTLA-4, anti–PD-1) therapy in combination with anti-RANKL denosumuab in malignant melanoma: a retrospective analysis at a tertiary care center. *Melanoma Res*. 2018;28(4):341–347.

[17] Silke J, Brink R. Regulation of TNFRSF and innate immune signalling complexes by TRAFs and cIAPs. *Cell Death Differ*. 2010;17(1):35–45.

[18] Safaya S, Alfarhan M, Sulaiman A, Alsulaiman A, Al-Ali A. TNFSF/TNFRSF cytokine gene expression in sickle cell anemia: up-regulated TNF-like cytokine 1A (TL1A) and its decoy receptor (DcR3) in peripheral blood mononuclear cells and plasma. *Cytokine*. 2019;123:154744.

[19] Davern M, Fitzgerald M-C, Buckley CE, et al. PD-1 and TIGIT blockade differentially affect tumour cell survival under hypoxia and glucose deprived conditions in oesophageal adenocarcinoma; implications for overcoming resistance to PD-1 blockade in hypoxic tumours. *Transl Oncol*. 2022;19:101381.

[20] Lee YH, Lee HJ, Kim HC, et al. PD-1 and TIGIT downregulation distinctly affect the effector and early memory phenotypes of CD19-targeting CAR T cells. *Mol Ther*. 2022;30(2):579–592.

[21] Flecken T, Sarobe P. Tim-3 expression in tumour-associated macrophages: a new player in HCC progression. *Gut*. 2015;64(10):1502–1503.

[22] Yan W, Liu X, Ma H, et al. Tim-3 fosters HCC development by enhancing TGF-β–mediated alternative activation of macrophages. *Gut*. 2015;64(10):1593–1604.

[23] Nakayama C, Tanoue K, Idichi T, et al. Implications of PD-1, Tim-3, and TIGIT expression for cancer immunity and pancreatic cancer prognosis. *Anticancer Res*. 2022;42(7):3373–3380.

[24] Weiskopf K. Cancer immunotherapy targeting the CD47/SIRPα axis. *Eur J Cancer*. 2017;76:100–109.

[25] Li F, Lv B, Liu Y, et al. Blocking the CD47-SIRPα axis by delivery of anti-CD47 antibody induces antitumor effects in glioma and glioma stem cells. *Oncoimmunology*. 2017;7(2):e1391973.

[26] Barkal AA, Weiskopf K, Kao KS, et al. Engagement of MHC class I by the inhibitory receptor LILRB1 suppresses macrophages and is a target of cancer immunotherapy. *Nat Immunol*. 2018;19(1):76–84.

[27] Li L, Wang T, Li S, et al. TDO2 promotes the EMT of hepatocellular carcinoma through Kyn-AhR pathway. *Front Oncol*. 2021;10:562823.

[28] Ebokaiwe AP, Ushang OR, Ogunwa TH, Kikiowo B, Olusanya O. Quercetin attenuates cyclophosphamide induced-immunosuppressive indoleamine 2,3-dioxygenase in the hippocampus and cerebral cortex of male Wister rats. *J Biochem Mol Toxicol*. 2022;36(11):e23179.

[29] Chen X, Zang Y, Li D, et al. IDO, TDO, and AHR overexpression is associated with poor outcome in diffuse large B-cell lymphoma patients in the rituximab era. *Medicine (Baltimore)*. 2020;99(21):e19883.

[30] Li H, Bullock K, Gurjao C, et al. Metabolomic adaptations and correlates of survival to immune checkpoint blockade. *Nat Commun*. 2019;10(1):4346.

[31] Guo M, Yuan F, Qi F, et al. Expression and clinical significance of LAG-3, FGL1, PD-L1 and CD8+ T cells in hepatocellular carcinoma using multiplex quantitative analysis. *J Transl Med*. 2020;18(1):306.

[32] Wang J, Sanmamed MF, Datar I, et al. Fibrinogen-like protein 1 is a major immune inhibitory ligand of LAG-3. *Cell*. 2019;176(1–2):334–347.e12.

[33] Tomiyama T, Itoh S, Iseda N, et al. Myeloid-derived suppressor cell infiltration is associated with a poor prognosis in patients with hepatocellular carcinoma. *Oncol Lett*. 2022;23(3):93.

[34] Deng X, Li X, Guo X, et al. Myeloid-derived suppressor cells promote tumor growth and sorafenib resistance by inducing FGF1 upregulation and fibrosis. *Neoplasia*. 2022;28:100788.

[35] Wang L, Yi X, Xiao X, Zheng Q, Ma L, Li B. Exosomal miR-628-5p from M1 polarized macrophages hinders m6A modification of circFUT8 to suppress hepatocellular carcinoma progression. *Cell Mol Biol Lett*. 2022; 27(1):106.

[36] Gou Y, Yang D, Tian T, et al. The transcription of *ZIP9* is associated with the macrophage polarization and the pathogenesis of hepatocellular carcinoma. *Front Immunol*. 2022;13:725595.

[37] Suthen S, Lim CJ, Nguyen PHD, et al. Hypoxia-driven immunosuppression by Treg and type-2 conventional dendritic cells in HCC. *Hepatology*. 2022;76(5):1329–1344.

[38] Riaz F, Wei P, Pan F. Fine-tuning of regulatory T cells is indispensable for the metabolic steatosis-related hepatocellular carcinoma: a review. *Front Cell Dev Biol*. 2022;10:949603.

[39] Sajid M, Liu L, Sun C. The dynamic role of NK cells in liver cancers: role in HCC and HBV associated HCC and its therapeutic implications. *Front Immunol*. 2022;13:887186.

[40] Mahgoub S, Abosalem H, Emara M, Kotb N, Maged A, Soror S. Restoring NK cells functionality via cytokine activation enhances cetuximab-mediated NK-cell ADCC: a promising therapeutic tool for HCC patients. *Mol Immunol*. 2021;137:221–227.

[41] Xie BH, Yang JY, Li HP, et al. Dendritic cells transfected with hepatocellular carcinoma (HCC) total RNA induce specific immune responses against HCC in vitro and in vivo. *Clin Transl Oncol*. 2014;16(8):753–760.

[42] El-Khoueiry AB, Sangro B, Yau T, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet*. 2017;389(10088):2492–2502.

[43] Yau T, Park JW, Finn RS, et al. Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): a randomised, multicentre, open-label, phase 3 trial. *Lancet Oncol*. 2022;23(1):77–90.

[44] Choi WM, Lee D, Shim JH, et al. Effectiveness and safety of nivolumab in Child-Pugh B patients with hepatocellular carcinoma: a real-world cohort study. *Cancers (Basel)*. 2020;12(7):1968.

[45] Kudo M, Matilla A, Santoro A, et al. CheckMate 040 cohort 5: a phase I/II study of nivolumab in patients with advanced hepatocellular carcinoma and Child-Pugh B cirrhosis. *J Hepatol*. 2021;75(3):600–609.

[46] Finn RS, Ryoo B-Y, Merle P, et al; KEYNOTE-240 Investigators. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, phase III trial. *J Clin Oncol*. 2020;38(3):193–202.

[47] Fulgenzi CAM, Cheon J, D'Alessio A, et al. Reproducible safety and efficacy of atezolizumab plus bevacizumab for HCC in clinical practice: results of the AB-real study. *Eur J Cancer*. 2022;175:204–213.

[48] Qin S, Finn RS, Kudo M, et al. RATIONALE 301 study: tislelizumab versus sorafenib as first-line treatment for unresectable hepatocellular carcinoma. *Future Oncol*. 2019;15(16):1811–1822.

[49] Ma X, Zhang Y, Wang S, Wei H, Yu J. Immune checkpoint inhibitor (ICI) combination therapy compared to monotherapy in advanced solid cancer: a systematic review. *J Cancer*. 2021;12(5):1318–1333.

[50] Finn RS, Qin S, Ikeda M, et al, IMbrave150 Investigators. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med*. 2020;382(20):1894–1905.

[51] Ren Z, Xu J, Bai Y, et al, ORIENT-32 Study Group. Sintilimab plus a bevacizumab biosimilar (IBI305) versus sorafenib in unresectable hepatocellular carcinoma (ORIENT-32): a randomised, open-label, phase 2–3 study. *Lancet Oncol*. 2021;22(7):977–990.

[52] Xu J, Shen J, Gu S, et al. Camrelizumab in combination with apatinib in patients with advanced hepatocellular carcinoma (RESCUE): a nonrandomized, open-label, phase II trial. *Clin Cancer Res*. 2021;27(4):1003–1011.

[53] Meng X, Wu T, Hong Y, et al. Camrelizumab plus apatinib as second-line treatment for advanced oesophageal squamous cell carcinoma (CAP 02): a single-arm, open-label, phase 2 trial. *Lancet Gastroenterol Hepatol*. 2022;7(3):245–253.

[54] Taylor MH, Schmidt EV, Dutcus C, et al. The LEAP program: lenvatinib plus pembrolizumab for the treatment of advanced solid tumors. *Future Oncol*. 2021;17(6):637–648.

[55] Kelley RK, Oliver JW, Hazra S, et al. Cabozantinib in combination with atezolizumab versus sorafenib in treatment-naive advanced hepatocellular carcinoma: COSMIC-312 phase III study design. *Future Oncol*. 2020;16(21):1525–1536.

[56] Zhang Z, Zhou Y, Hu K, Li Z, Wang Z, Huang Y. Complete response of early stage hepatocellular carcinoma in a patient treated with combination therapy of camrelizumab (SHR-1210) and apatinib. *Dig Liver Dis*. 2019;51(10):1488–1490.

[57] Xia Y, Tang W, Qian X, et al. Efficacy and safety of camrelizumab plus apatinib during the perioperative period in resectable hepatocellular carcinoma: a single-arm, open label, phase II clinical trial. *J Immunother Cancer*. 2022;10(4):e004656.

[58] Yau T, Kang YK, Kim TY, et al. Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: the CheckMate 040 randomized clinical trial. *JAMA Oncol*. 2020;6(11):e204564.

[59] Kelley RK, Sangro B, Harris W, et al. Safety, efficacy, and pharmacodynamics of tremelimumab plus durvalumab for patients with unresectable hepatocellular carcinoma: randomized expansion of a phase I/II study. *J Clin Oncol*. 2021;39(27):2991–3001.

[60] Merle P, Blanc JF, Edeline J, et al. Ipilimumab with atezolizumab-bevacizumab in patients with advanced hepatocellular carcinoma: the PRODIGE 81-FFCD 2101-TRIPLET-HCC trial. *Dig Liver Dis*. 2023;55(4):464–470.

[61] Trojaniello C, Vitale MG, Ascierto PA. Triplet combination of BRAF, MEK and PD-1/PD-L1 blockade in melanoma: the more the better? *Curr Opin Oncol*. 2021;33(2):133–8. doi:10.1097/CCO.0000000000000709. PMID: 33399314.

[62] Zheng L, Fang S, Wu F, et al. Efficacy and safety of TACE combined with sorafenib plus immune checkpoint inhibitors for the treatment of intermediate and advanced TACE-refractory hepatocellular carcinoma: a retrospective study. *Front Mol Biosci*. 2021;7:609322.

[63] Sun X, Zhang Q, Mei J, Yang Z, Chen M, Liang T. Real-world efficiency of lenvatinib plus PD-1 blockades in advanced hepatocellular carcinoma: an exploration for expanded indications. *BMC Cancer*. 2022;22(1):293.

[64] Wu W-C, Lin T-Y, Chen M-H, et al. Lenvatinib combined with nivolumab in advanced hepatocellular carcinoma-real-world experience. *Invest New Drugs*. 2022;40(4):789–797.

[65] Kuo YH, Yen YH, Chen YY, et al. Nivolumab versus regorafenib in patients with hepatocellular carcinoma after sorafenib failure. *Front Oncol*. 2021;11:683341.

[66] Xia J, Gelfond J, Arora SP. Second-line treatment with nivolumab, cabozantinib, regorafenib, or best supportive care in patients with advanced hepatocellular carcinoma: analysis at a Hispanic-majority NCI-designated cancer center. *J Gastrointest Oncol*. 2021;12(6):2943–2951.

[67] Zhao J, Yu X, Huang D, et al. SAFFRON-103: a phase 1b study of the safety and efficacy of sitravatinib combined with tislelizumab in patients with locally advanced or metastatic non–small cell lung cancer. *J Immunother Cancer*. 2023;11(2):e006055.

[68] Berretta M, Cobellis G, Franco R, et al. Features of microvessel density (MVD) and angiogenesis inhibitors in therapeutic approach of hepatocellular carcinoma (HCC). *Eur Rev Med Pharmacol Sci*. 2019;23(22):10139–10150.

[69] Bai X, Zhou Y, Yokota Y, et al. Adaptive antitumor immune response stimulated by bio-nanoparticle based vaccine and checkpoint blockade. *J Exp Clin Cancer Res*. 2022;41(1):132.

[70] Wang Y, Zhao Q, Zhao B, et al. Remodeling tumor-associated neutrophils to enhance dendritic cell–based HCC neoantigen nano-vaccine efficiency. *Adv Sci (Weinh)*. 2022;9(11):e2105631.

[71] Jacobson JM, Zahrieh D, Strand CA, et al, Cancer Prevention Network. Phase I trial of a therapeutic DNA vaccine for preventing hepatocellular carcinoma from chronic hepatitis C virus (HCV) infection. *Cancer Prev Res (Phila)*. 2023;16(3):163–173.

[72] Siddiqui RS, Sardar M. A systematic review of the role of chimeric antigen receptor T (CAR–T) cell therapy in the treatment of solid tumors. *Cureus*. 2021;13(4):e14494.

[73] Cao G, Zhang G, Liu M, et al. GPC3-targeted CAR–T cells secreting B7H3-targeted BiTE exhibit potent cytotoxicity activity against hepatocellular carcinoma cell in the in vitro assay. *Biochem Biophys Rep*. 2022;31:101324.

Review Article

Oncology and Translational Medicine

OPEN

Recent advances in conversion therapy schemes for stage IV gastric cancer

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Abstract

"Conversion therapy" is a treatment modality that involves the use of radiotherapy, chemotherapy, targeted therapy, immunotherapy, and other therapeutic methods to transform initially late-stage tumors that cannot be cured into treatments that can achieve an R0 curative effect. However, selecting an appropriate conversion therapy scheme remains a challenge, and there are currently few relevant studies on this topic. This article reviews successful cases of conversion therapy and clinical studies on treatment schemes, at domestic and international levels, over the past few years to offer a broad range of treatment options for patients.

Keywords: Conversion therapy; Immunotherapy; Stage IV gastric cancer; Surgery

1. Introduction

Approximately 1 million new cases of gastric cancer are diagnosed worldwide each year.[1] Among all countries, China has the second highest incidence of malignant tumors, with approximately 80% of these being advanced gastric cancers (AGCs), of which approximately 30% are unresectable.[2] Stage IV gastric cancer, particularly unresectable gastric cancer, exhibits high heterogeneity and diverse biological behaviors. It can metastasize to different areas, including the peritoneum, liver, and lymphatic system, leading to poor prognosis. Although palliative treatment is the prime choice for patients with late-stage gastric cancer, administration of systemic chemotherapy, targeted therapy, or immunotherapy has resulted in tumor regression and positive response or an increased possibility of "curative surgical resection," thus prolonging their total survival time. Therefore, experts from various countries have proposed a "conversion therapy" strategy for unresectable gastric cancer. Conversion therapy assesses the patient's condition through auxiliary examinations, such as abdominal computed tomography, endoscopic ultrasound, tumor markers, and laparoscopic exploration, and uses treatment methods, such as radiotherapy, chemotherapy, targeted therapy, and immunotherapy, to treat initially untreatable late-stage tumors into treatable tumors that can achieve an R0 curative effect. This approach thereby extends the patient's progression-free survival (PFS) and overall survival (OS) periods. However, this topic needs further exploration as research results from different periods and regions may be inconsistent. Moreover, there are many controversies regarding the associated factors, such as the selection of conversion therapy schemes, operation timing, postoperative complications, and conversion rates, making gastric cancer conversion therapy a hotspot but challenging to be implemented at present.

2. Attempts and evolution of conversion therapy

Conversion therapy was first reported by Nakajima et al[3] in 1997. After treating 30 patients with initially unresectable gastric cancer with a FLEP (fluorouracil, leucovorin, cisplatin, and etoposide) regimen for 2 cycles, the overall response rate was 50%. The 5-year survival rate of the 9 R0 resection cases was 55.6%, and the median survival time of the 10 R1/R2 resection cases was 6.5 months. In 2012, Satoh et al[4] conducted a study of 51 patients who received preoperative oxaliplatin plus cisplatin (SP) chemotherapy and postoperative S-1 monotherapy. The results showed that the 2-year OS rate of the 26 R0 resection patients was 75%, and the median survival time was 19.2 months. In a study by Yamaguchi et al,[5] 249 patients with stage IV gastric cancer were treated, of whom 84 underwent surgery after chemotherapy. The median survival increased from 24.7 to 31.0 months, and the median survival of patients who underwent R0 resection was 41.3 months. These results suggest that conversion therapy is a feasible treatment for cancer. For patients with stage IV unresectable gastric cancer, selecting an appropriate conversion treatment scheme and achieving a good therapeutic response can increase opportunities for subsequent curative surgery and improve patient prognosis.

3. Selection of conversion therapy regimens

3.1. Chemotherapy

3.1.1. Systemic chemotherapy

Currently, first-line chemotherapy regimens for AGC primarily rely on fluoropyrimidine drugs combined with platinum and/or taxane drugs to form 2- or 3-drug regimens. Sym et al[6] evaluated the efficacy and safety of the DXP (capecitabine + cisplatin + docetaxel) regimen in patients with locally invasive or peritoneal metastases from unresectable gastric cancer. The results showed a high surgical conversion rate (74%), suggesting that the DXP regimen might

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provide a surgical opportunity for patients with unresectable, locally advanced, or peritoneal lymph node metastases. The phase II clinical trial AIOFLOT3 conducted by the German Al-Batran team[7] showed that, in patients with locally advanced or gastroesophageal junction adenocarcinoma and limited metastasis, the median survival time of patients who received surgery after chemotherapy with 5-fluorouracil + oxaliplatin + docetaxel (FLOT) was 31.3 months, significantly higher than that of nonsurgical patients (15.9 months), indicating that the FLOT sequential chemotherapy-surgery approach improved patient survival. Hence, treatment of limited metastatic gastric cancer with chemotherapy followed by surgery is considered to provide the patients with good survival benefits. In a Japanese study,[8] 27 patients with initially unresectable locally advanced gastric cancer were treated with S-1 plus cisplatin (SP) chemotherapy, followed by surgical resection. The R0 resection rate was 48.1%. Therefore, the SP regimen can be a potential preoperative conversion therapy for AGC; however, further validation is needed in terms of patient selection and other aspects. Chemotherapy alone remains the main treatment modality for conversion therapy for AGC, and there is no significant difference in the selection of treatment regimens; however, the optimal treatment choice should still be based on a comprehensive consideration of the patient's condition.

3.1.2. Neoadjuvant intraperitoneal-systemic chemotherapy

Peritoneal metastasis is common in stage IV gastric cancer, occurring in 14% to 43% of patients, 35% of whom have synchronous metastases, with a median survival time of 4 to 6 months.[9] The prognosis is worse than that of other types of metastases owing to the presence of the blood-peritoneum barrier, which limits the effectiveness of traditional systemic chemotherapy.[10] Intraperitoneal (IP) chemotherapy provides a potential therapeutic advantage for peritoneal metastasis over systemic chemotherapy, by producing high local concentrations of chemotherapeutic drugs in the peritoneal cavity. This advantage is evident in the area under the curve of exposure between IP and plasma chemotherapy.[11]

Ishigami et al[12] reported 100 patients with gastric cancer with peritoneal metastasis who underwent radical surgery after IP injection plus paclitaxel (PTX) combined with oral S-1 and intravenous PTX chemotherapy. Of 64 patients who underwent surgical treatment, 44 achieved R0 resection (69%). The median survival time was 34.6 months in patients who underwent surgery and 14.3 months in those who did not undergo surgical treatment. Okabe et al[13] pointed out that sequential R0 resection after successful conversion chemotherapy and disappearance of peritoneal dissemination significantly prolonged median survival time (43.2 months) compared with non-R0 resection patients (12.6 months) and nonsurgical patients (10.3 months). Thus, IP chemotherapy may be an efficient treatment choice for patients with gastric cancer and peritoneal metastasis; however, more clinical studies are needed to confirm the selection of treatment strategies, improve the R0 resection rate, and obtain better treatment effects.

3.2. Targeted therapy

3.2.1. Molecular targeted therapy

Hayano et al[14] studied 11 patients with HER-2–positive stage IV gastric cancer who received trastuzumab plus capecitabine or S-1 plus cisplatin chemotherapy as first-line treatment. The study showed that trastuzumab chemotherapy had a good therapeutic response, with a response rate of 63.6%, conversion rate of 54.5%, and R0 resection rate of 36.3%. The 3-year survival rate of patients who underwent R0 resection surgery was 100% (median OS [mOS], 51.8 months), which is extremely high compared with that of patients with stage IV gastric cancer treated with chemotherapy alone. Zhang et al[15] conducted a retrospective study of 32 in-hospital patients with HER-2–positive stage IV gastric cancer who received trastuzumab combined with chemotherapy and surgery. The objective response rate (ORR) was 65.6%, and the mOS and OS were 25.1 and 30.2 months, respectively. Targeted therapy for HER-2 and an adequate selection of patients with stage IV GC would provide more benefits through conversion therapy.

Trastuzumab has definite efficacy in conversion therapy for patients with HER-2–positive gastric cancer, and postconversion therapy pathological recurrence of HER-2–negative gastric cancer occurs; however, the specific mechanism has not been reported, which will affect the choice of subsequent treatment. In addition, HER-2 positivity accounts for approximately only 10% of gastric cancer cases; therefore, the selection of targeted therapy for non-HER-2–positive gastric cancer is still under exploration.

3.2.2. Antiangiogenic-targeted, HER-2–negative gastric cancer

Apatinib is a highly selective inhibitor of the vascular endothelial growth factor receptor 2 (VEGFR-2) that was developed independently in China. In a randomized controlled trial of the third-line treatment for AGC, apatinib showed good tumor suppression and tolerability.

Xu et al[16] reported the clinical results of 33 patients with unresectable AGC treated with apatinib combined with chemotherapy as conversion therapy. The results showed that, after the addition of apatinib to PTX and S-1 chemotherapy (PS regimen) before surgery, the ORR was 73.3%, disease control rate (DCR) was 93.3%, and conversion and R0 resection rates were 60.0% and 56.7%, respectively. For patients who received conversion therapy, the 1- and 3-year OS incidence rates were 100% and 52.9%, respectively. Wu and Fang[17] reported the clinical results of 66 patients with AGC receiving PTX + S-1 + apatinib conversion therapy, and the ORR after conversion therapy was 71.2%. The R0 resection rate was 70.8%. The follow-up results showed that the 1-year OS rate in the surgically treated group was 93.8%.[17] Ye et al[18] reported a single-arm exploratory clinical study on “apatinib mesylate combined with tegio and oxaliplatin for conversion therapy in advanced gastric cancer.” The results indicated that the ORR was 69.2%, and the R0 resection rate was 42.9%. The addition of apatinib to chemotherapy has a higher ORR and R0 resection rate and provides a new choice for patients with initially unresectable GC.

Ramucirumab (RAM) is a human immunoglobulin G monoclonal antibody that exerts its effects by specifically antagonizing VEGFR-2. The REGARD study was a phase III clinical trial that evaluated the use of RAM as second-line treatment for AGC.[19] The results showed that the mOS in the RAM group was significantly longer than that in the placebo group (5.2 vs 3.8 months) ($P = 0.047$).

Fukuda et al[20] reported a case of clinically diagnosed stage IV HER-2–positive gastric cancer with liver metastasis (cT4a N3 M1) in which a serious infusion reaction occurred during the initial administration of trastuzumab. The patient then received S-1 + cisplatin (SP) as first-line chemotherapy; however, after 6 courses of SP, the primary tumor progressed in size. Therefore, second-line chemotherapy with RAM and PTX was initiated. After 6 courses of RAM + PTX, the primary tumor size decreased significantly. The patient underwent total gastrectomy with D2 lymph node dissection, and the pathological diagnosis was stage IB (ypT2 N0 M0). This is a case of a patient with advanced stage IV gastric cancer who benefited from R0 resection and long-term survival after secondary chemotherapy with RAM, again demonstrating the importance of conversion therapy in gastric cancer treatment and the efficacy of RAM in conversion therapy. Namikawa et al[21] reported a case of late-stage gastric cancer initially diagnosed as

cT4 N2 M0, which received S-1 + oxaliplatin as first-line chemotherapy; however, owing to liver metastasis, the treatment plan was changed to RAM + PTX as second-line treatment. After 4 cycles of PTX and RAM treatment, the liver metastases completely disappeared. The patient underwent total gastrectomy with D2 lymph node dissection, and survival was significantly prolonged after 13 months of supportive care.

In conclusion, conversion therapy after second- or third-line systemic treatment may be the treatment of choice for patients with initially unresectable gastric cancer, challenging the established concept of palliative care only after first-line treatment failure and bringing hope to patients with more advanced stage gastric cancer. Although currently limited to single case reports with no domestic evidence of domestic cases, further case-control studies with larger sample sizes are needed to validate the role of RAM in conversion surgery after full-line treatment of AGC. With the increasing use of antiangiogenic drugs in gastric cancer treatment, an increasing number of studies are focusing on combining antiangiogenic drugs with chemotherapy as a first-line treatment for conversion therapy; RAM will also provide more treatment options for patients.

3.3. Immunotherapy

With the continuous accumulation of evidence for immunotherapy in AGC, authoritative guidelines from both domestic and international societies, such as the European Society for Medical Oncology and the Chinese Society of Clinical Oncology, recommend immunotherapy as a third-line treatment option.[22,23] However, the efficacy of third-line immunotherapy is not high, with a short PFS, and the clinical treatment demand remains unmet. Subsequently, the synergistic mechanism of immunotherapy combined with other treatment methods has been revealed, with clinical trials gradually being conducted for combining immunotherapy with chemotherapy or targeted therapy, to be used as first-line treatment, or conversion therapy for AGC. Thus, immunotherapy brings new hope for the treatment of AGC.

Toyota et al[24] reported successful conversion therapy for late-stage gastric cancer with chylous ascites after third-line nivolumab treatment; no recurrence was observed within 7 months without adjuvant chemotherapy. Matsumoto et al[25] reported a case of fourth-stage gastric cancer treated with third-line nivolumab that achieved a pathological complete response and conversion therapy with R0 resection. Kumamoto et al[26] reported a case of stage IV esophagogastric junction cancer treated with third-line nivolumab, achieving a pathological complete response and successful conversion surgery. A pathological biopsy revealed microsatellite instability–negative, Epstein-Barr virus–negative, and a positive score of 2 for the combination of programmed cell death 1 (PD-1) and PD-1 ligand. The patient was followed up for 3 months without recurrence.

The above case reports included first-line fluoropyrimidine + platinum, second-line RAM + PTX, and third-line nivolumab monotherapy after disease progression in the first- and second-line treatments. Following third-line immunotherapy, the conditions improved, with a reduction in the primary focus and the disappearance of metastatic lesions. R0 resection was performed to achieve a complete pathological response. Notably, the aforementioned case reports were not from a specific population that benefits the most from gastric cancer immunotherapy, thus providing new directions for research on immunotherapy and conversion therapy.

Consequently, the CheckMate 649 trial,[27] which presented its results at the 2020 European Society for Medical Oncology Congress, demonstrated for the first time the efficacy of immune checkpoint inhibitors in combination with chemotherapy as a first-line treatment for metastatic gastric cancer (mGC) or adenocarcinoma of the esophagogastric junction (AEG). Nivolumab plus chemotherapy (CapeOX or FOLFOX) was effective in treating HER2-negative unresectable AEG or mGC that was not subjected to prior treatment. In all randomized patients with gastric cancer, the mOS of nivolumab + chemotherapy was 13.8 months compared with 11.6 months with chemotherapy alone ($P = 0.0002$). Nivolumab + chemotherapy for first-line treatment of unresectable AEG or mGC significantly improved OS and PFS compared with chemotherapy alone. Meanwhile, with the release of the results of ORIENT-15 and ORIENT-16 studies, China's CSCO guidelines recommend the combination of Sintilimab and chemotherapy as the first-line treatment for mGC/AEG cancer.[28]

With progress in immunotherapy as the first-line treatment for AGC, there is also an increasing amount of research into conversion therapy. Yin et al reported 17 patients with advanced gastric adenocarcinoma at the junction of the esophagus and stomach[29] who were treated with the SOX regimen combined with a PD-1 inhibitor. The results showed that 8 patients completed surgery, with an ORR of 100% and an R0 resection rate of 100%, suggesting the efficacy of immunotherapy in conversion therapy. Subsequently, Deng et al[30] conducted a prospective single-arm study on the use of carilizumab + apatinib, albumin-bound PTX, and tegio in the conversion treatment of AGC. Among the 17 patients with AGC, 8 underwent surgical treatment, and all underwent R0 resection, with a conversion rate of 47.1% and an mOS of 23.63 months. The ORR was 47.1% in all the patients who received treatment, and the disease control rate was 82.4%. In this study, anti–PD-1 and antiangiogenic therapies were combined with traditional chemotherapy, and the overall efficacy was comparable to that of traditional chemotherapy or combined IP chemotherapy. Therefore, to further improve the effectiveness of conversion therapy, it is important to identify the population that may benefit from immunotherapy.

T-cell infiltration, visceral metastasis, tumor burden, and varying degrees of systemic immune suppression in the early stages of cancer may result in a better response to immunotherapy. Unlike radiotherapy or chemotherapy, the use of immunotherapy in advance aims to enhance overall immunity against tumor antigens and eliminate micrometastases, which would otherwise become a source of recurrence. Therefore, the application of immune checkpoint inhibitors as a first-line treatment or neoadjuvant therapy may help enhance the antitumor effects of immunotherapy. Whether conversion therapy and postoperative adjuvant therapy can be achieved by immunotherapy is of substantive significance. Moreover, the aforementioned case reports are not representative of the advantages of gastric cancer immunotherapy, as even nonadvantaged populations may benefit from immunotherapy, providing a theoretical basis for the feasibility of using immune checkpoint inhibitors in conversion therapy.

4. Discussion

In recent years, there have been several studies on conversion therapy for gastric cancer.

Rivera et al[31] initiated phase II clinical trials in 2010, using a therapeutic approach that involved administering a combination of erlotinib + cisplatin as adjuvant chemotherapy followed by concurrent chemoradiotherapy with the same combination. The results revealed a mortality rate of 24% during the combined radiotherapy phase, with 20% of the patients dying within 30 days postsurgery. The R0 resection rate was only 29.4%. Although the FLEEOX regimen demonstrated significant conversion and R0 resection rates, its pronounced adverse effects rendered it intolerable for many patients. Moreover, the severe accumulation of toxic adverse effects of chemotherapy drugs significantly affects patients' quality of life. Hence, safety should be considered when enhancing treatment efficacy.

Unlike neoadjuvant therapy, which can be evaluated based on the pathologic response rate, and adjuvant therapy, which can be evaluated based on the ORR, PFS, and OS, there is currently no unified efficacy evaluation standard for conversion therapy. Therefore, the conversion and R0 resection rates were chosen as the evaluation criteria for this study as patients who undergo surgery and achieve R0 resection have significantly longer OS and PFS than those who do not undergo surgery. Table 1 lists some small sample retrospective studies in gastric cancer conversion therapy in chronological order. Considering that there is no unified definition or inclusion criteria for conversion therapy among researchers, the data in Table 1 do not reflect the superiority or inferiority of the treatment regimens. However, overall, it can be seen that, with the passage of time and an improved understanding of gastric cancer treatment, we have gradually progressed from traditional chemotherapy to targeted therapy and immunotherapy while improving efficacy and ensuring safety. There are 3 main aspects of cases with poor efficacy: (1) patients were staged late at enrollment and were diagnosed with completely unresectable cases with a poor pathological response after systemic treatment; the majority could only undergo palliative surgery; (2) to achieve the fastest and maximum tumor shrinkage, researchers often choose a combination of multiple chemotherapeutic drugs or concurrent chemoradiotherapy, which patients often cannot tolerate and have poor safety; (3) after systematic treatment, the patients' imaging evaluation meets the surgical criteria; however, their general condition is poor, making it difficult for them to tolerate surgery or leading to more postoperative complications. Not only does this fail to extend OS, but it also fails to guarantee the quality of life. Therefore, there is still a long way to go before conversion therapy regimens can be selected, and finding a safe and effective personalized treatment regimen is the focus of clinical research.

In 2016, Yoshida et al[41] categorized stage IV gastric cancer into 4 types based on the extent of metastasis. For patients with type I, type II, and some cases of type III, conversion therapy followed by surgery has the potential for survival benefits. However, for patients with type IV disease, the clinical focus should be on symptom improvement and enhanced quality of life. Currently, both the domestic and international focus is primarily on conversion therapy for type II patients. However, no unified treatment regimen or relevant randomized controlled trials or meta-analyses exist. Thus, the selection of conversion therapy regimens for gastric cancer is controversial. This review aimed to consolidate the current domestic and international clinical research to provide theoretical support for clinical physicians and confirms that a 3-drug intravenous chemotherapy regimen based on PTX lays the foundation for conversion therapy in stage IV gastric cancer, with a conversion rate of approximately 65% and an R0 resection rate of 75%, under safe and controlled conditions. Concurrent systemic and neoadjuvant peritoneal chemotherapy may further improve the conversion rate of patients with gastric cancer with peritoneal metastases. Patients with HER-2–positive gastric cancer treated with a combination of trastuzumab have better efficacy, with a conversion rate of 70% and an R0 resection rate of greater than 50%, without increasing surgical complications. For patients with HER-2–negative gastric cancer, the combination with apatinib can increase tumor shrinkage with tolerable adverse effects. Considering the complex tumor microenvironment of patients with AGC, the combination of immune checkpoint inhibitors and antiangiogenic drugs may play an important role in the conversion treatment of gastric cancer.

5. Summary and prospects

With the development of chemotherapy, targeted therapy, and immunotherapy, the survival rate of patients with stage IV gastric cancer has improved; however, the overall treatment effectiveness is still far from favorable. Therefore, converting unresectable into resectable gastric cancer remains the best option for patients to achieve "cure." However, the selection of conversion therapy regimens remains a major challenge. Currently, all treatment strategies are based on chemotherapy, and the efficacy and regimen of chemotherapy have been extensively studied, with no significant differences. The most widely recognized regimens are SOX and FLOT. The controversy lies primarily in the use of immune and targeted therapies. Based on recent clinical trials and the wave of immunotherapy, many researchers have used immunotherapy, targeted therapy, and chemotherapy for gastric cancer conversion therapy to provide patients with the strongest initial treatment, thereby achieving the best treatment effectiveness and obtaining high R0 resection and pathological response rates. With the publication of clinical trial results, the immunotherapy + targeted therapy + chemotherapy treatment regimen has increased R0 resection and pathologic complete response (PCR) rates in newly diagnosed patients. However, the selection of subsequent treatment regimens for patients who cannot undergo surgery or who experience postoperative recurrence remains a significant problem. Moreover, most current research involves prospective single-arm

Table 1

In recent years, there have been several studies on conversion therapy for gastric cancer

Time	Researchers	Scheme	Conversion rate	R0 resection rate
2014	Kitayama et al[32]	S-1 + paclitaxel + intraperitoneal infusion of paclitaxel	53.1% (34/64)	65.0% (22/34)
2015	Fukuchi et al[33]	SP scheme or S-1 + paclitaxel	26.5% (40/151)	68.7% (44/64)
2015	Ratosa et al[34]	CF scheme combined synchronous radiotherapy	63.3% (57/90)	87.7% (50/57)
2015	Mitsui et al[35]	Trastuzumab + DCS scheme	100% (16/16)	56. 3% (9/16)
2016	He et al[36]	5-Fluorouracil + etoposide + oxaliplatin + epirubicin + calcium folinate	74.3% (78/105)	85.9% (67/78)
2017	Sato et al[37]	DCS scheme	33.0% (30/100)	84. 8% (28/33)
2017	Al-Batran et al[7]	FLOT scheme	60% (36/60)	81.6% (29/36)
2017	Ishigami et al[38]	PHOENIX-GC scheme	64% (64/100)	68.7% (44/64)
2020	Sawasaki et al[39]	SOX + albumin paclitaxel + itraconazole scheme	57.1% (12/21)	66.7% (8/12)
2021	Xu et al[16]	Apatinib + S-1 + paclitaxel	60.0% (18/30)	94. 4% (17/18)
2021	Ye et al[18]	Apatinib + SOX	67. 6% (46 /68)	93.5% (43/46)
2021	Yin et al[29]	PD-1 inhibitor combination with SOX	44.4% (8/17)	100% (8/8)
2022	Deng et al[30]	Cindilimab + tegio + albumin paclitaxel + apatinib	60% (33 /55)	66. 7% (22 /33)
2022	Li et al[40]	Apatinib + camrelizumab + SOX	76% (19/25)	89.5% (17/19)

CF, cyclophosphamide fluorouracil; DCS, docetaxel/cisplatin/S-1; FLOT, 5-fluorouracil + oxaliplatin + docetaxel; PD-1, programmed cell death 1; PHOENIX, is a study that focuses on (hyperthermic intraperitoneal chemotherapy, HIPEC); SOX, oxaliplatin teysuno; SP, S-1 plus cisplatin.

studies, and few control groups are available to provide experimental results. In addition, the current advantages of gastric cancer immunotherapy have been observed in a relatively small proportion of patients who exhibit microsatellite instability–high/mismatch repair–deficient, high PD-1 ligand expression, and high tumor mutation burden (TMB) and epstein-barr virus (EBV) positivity. Therefore, when genetic testing and immunohistochemistry have not been completed preoperatively, the suitability of immunotherapy requires a large amount of experimental data.

In addition to trastuzumab-targeted therapy for HER-2–positive gastric cancer, other targeted therapies mainly focus on antiangiogenic therapy. Pathological angiogenesis is indispensable for rapid growth and metastasis of malignant tumors. Malignant tumor angiogenesis is regulated by various factors. For example, the vascular endothelial growth factor family and its receptors (VEGFR) can promote the differentiation and migration of vascular endothelial cells, increase vascular permeability and diameter, induce tumor angiogenesis, and lead to excessive and disordered blood vessel growth, as well as malignant proliferation of tumor cells.[42] Vascular endothelial growth factor and VEGFR are highly expressed in gastric cancer tissues and are associated with the proliferation and invasion of gastric cancer cells.[43] Based on previous experimental research results and theoretical support, it can be seen that antiangiogenic targeted therapy is a feasible treatment method in gastric cancer conversion therapy, with manageable adverse reactions and no reported adverse events so far. At present, most studies are limited to apatinib, and the use of antiangiogenic drugs, such as RAM and anlotinib, may increase in future clinical trials.

Immune cells are important components of the tumor microenvironment and play indispensable roles in tumor growth, differentiation, invasion, and escape. Activated immunosuppressive cells can also promote the secretion of various cytokines and chemokines, such as VEGF,[44] and the interaction between the two can jointly regulate the tumor microenvironment. On the one hand, antiangiogenic agents suppress negative immune responses by increasing the proportion of tumor immune cells and reducing the expression of multiple immune checkpoints; on the other hand, immune checkpoint inhibitor therapy can reshape the immune microenvironment and promote vascular normalization.[45,46] In addition, vascular normalization can improve the efficiency of drug delivery. Therefore, immune combination with antiangiogenic drugs is a feasible option in gastric cancer conversion therapy. However, there is still a long way to go for personalized precision treatment of stage IV gastric cancer conversion therapy and the selection of conversion therapy regimens.

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The authors indicated no potential conflicts of interest.

Author contributions

S.L. visualized and wrote the manuscript. W.L. reviewed the manuscript. K.Z. complex drawing and organization of tables.

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References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67(1):7–30.
[2] Chen W, Sun K, Zheng R, et al. Cancer incidence and mortality in China, 2014. *Chin J Cancer Res*. 2018;30(1):1–12.
[3] Nakajima T, Ota K, Ishihara S, et al. Combined intensive chemotherapy and radical surgery for incurable gastric cancer. *Ann Surg Oncol*. 1997;4(3):203–208.
[4] Satoh S, Okabe H, Teramukai S, et al. Phase II trial of combined treatment consisting of preoperative S-1 plus cisplatin followed by gastrectomy and postoperative S-1 for stage IV gastric cancer. *Gastric Cancer*. 2012;15(1):61–69.
[5] Yamaguchi K, Yoshida K, Tanahashi T, et al. The long-term survival of stage IV gastric cancer patients with conversion therapy. *Gastric Cancer*. 2018;21(2):315–323.
[6] Sym SJ, Chang HM, Ryu MH, et al. Neoadjuvant docetaxel, capecitabine and cisplatin (DXP) in patients with unresectable locally advanced or metastatic gastric cancer. *Ann Surg Oncol*. 2010;17(4):1024–1032.
[7] Al-Batran SE, Homann N, Pauligk C, et al. Effect of neoadjuvant chemotherapy followed by surgical resection on survival in patients with limited metastatic gastric or gastroesophageal junction cancer: the AIO-FLOT3 trial. *JAMA Oncol*. 2017;3(9):1237–1244.
[8] Inoue K, Nakane Y, Kogire M, et al. Phase II trial of preoperative S-1 plus cisplatin followed by surgery for initially unresectable locally advanced gastric cancer. *Eur J Surg Oncol*. 2012;38(2):143–149.
[9] Thomassen I, van Gestel YR, van Ramshorst B, et al. Peritoneal carcinomatosis of gastric origin: a population-based study on incidence, survival and risk factors. *Int J Cancer*. 2014;134(3):622–628.
[10] Sugarbaker TA, Chang D, Koslowe P, Sugarbaker PH. Patterns of spread of recurrent intraabdominal sarcoma. *Cancer Treat Res*. 1996;82:65–77.
[11] Imano M, Peng YF, Itoh T, et al. A preliminary study of single intraperitoneal administration of paclitaxel followed by sequential systemic chemotherapy with S-1 plus paclitaxel for advanced gastric cancer with peritoneal metastasis. *Anticancer Res*. 2012;32(9):4071–4075.
[12] Ishigami H, Yamaguchi H, Yamashita H, Asakage M, Kitayama J. Surgery after intraperitoneal and systemic chemotherapy for gastric cancer with peritoneal metastasis or positive peritoneal cytology findings. *Gastric Cancer*. 2017;20(Suppl 1):128–134.
[13] Okabe H, Ueda S, Obama K, Hosogi H, Sakai Y. Induction chemotherapy with S-1 plus cisplatin followed by surgery for treatment of gastric cancer with peritoneal dissemination. *Ann Surg Oncol*. 2009;16(12):3227–3236.
[14] Hayano K, Watanabe H, Ryuzaki T, et al. Prognostic benefit of conversion surgery for HER2 positive stage IV gastric cancer; a case series study of eleven patients treated with trastuzumab-based chemotherapy. *Surg Case Rep*. 2020;6(1):219.
[15] Zhang Y, Xu X, Hu C, et al. Trastuzumab in combination with chemotherapy for HER2-positive metastatic gastric cancer patients underwent conversion therapy. *Transl Cancer Res*. 2022;11(7):2145–2156.
[16] Xu Z, Hu C, Yu J, et al. Efficacy of conversion surgery following apatinib plus paclitaxel/S1 for advanced gastric cancer with unresectable factors: a multicenter, single-arm, phase II trial. *Front Pharmacol*. 2021;12:642511.
[17] Wu Z, Fang H. Efficacy of paclitaxel and S-1 combined with apatinib in the conversion therapy for unresectable advanced gastric cancer. *J BUON*. 2021;26(4):1485–1490.
[18] Ye ZS, Zeng Y, Wei SH, et al. Safety and short-term efficacy of apatinib combined with oxaliplatin and S-1 in the conversion treatment for gastric cancer with peritoneal metastasis [in Chinese]. *Zhonghua Wei Chang Wai Ke Za Zhi*. 2021;24(3):240–247.
[19] Fuchs CS, Tomasek J, Yong CJ, et al, REGARD Trial Investigators. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet*. 2014;383(9911):31–39.
[20] Fukuda K, Arigami T, Tokuda K, et al. Successful conversion surgery for stage IV gastric cancer with liver metastases after second-line chemotherapy with ramucirumab and paclitaxel: a case report. *Surg Case Rep*. 2022;8(1):58.
[21] Namikawa T, Marui A, Yokota K, et al. Successful conversion surgery for advanced gastric cancer with multiple liver metastases following ramucirumab plus paclitaxel combination treatment. *In Vivo*. 2021;35(5):2929–2935.

[22] Shan YP, Liang J, Lyu YL, Wang L, Zhang XL, Shan BE. Efficacy and safety of immune checkpoint inhibitors therapy on advanced gastric cancer or gastroesophageal junction cancer: a meta-analysis. *Cancer Prev Treat*. 2021;48(2):166–172.
[23] Han L, Shi Y, Dai GH. Current status and prospect of second- and later-line drug therapy for advanced gastric cancer. *Natl Med J*. 2021; 101(5):369–373.
[24] Toyota S, Orita H, Fukuyama Y, et al. Successful conversion surgery following chylous ascites after nivolumab for advanced gastric cancer. *In Vivo*. 2020;34(2):583–585.
[25] Matsumoto R, Arigami T, Matsushita D, et al. Conversion surgery for stage IV gastric cancer with a complete pathological response to nivolumab: a case report. *World J Surg Oncol*. 2020;18(1):179.
[26] Kumamoto T, Tomita T, Hojo Y, et al. Pathological complete response and successful conversion surgery after nivolumab therapy for stage IV oesophagogastric junction cancer. *In Vivo*. 2021;35(4):2247–2251.
[27] Janjigian YY, Shitara K, Moehler M, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. *Lancet*. 2021;398(10294):27–40.
[28] Lu Z, Wang J, Shu Y, et al. ORIENT-15 study group. Sintilimab versus placebo in combination with chemotherapy as first line treatment for locally advanced or metastatic oesophageal squamous cell carcinoma (ORIENT-15): multicentre, randomised, double blind, phase 3 trial. *BMJ*. 2022;377:e068714.
[29] Yin YP, Zhang P, Cai M, Chen JH, Li W, Li G, Wang Z, Wang GB, Tao KX. Primary exploration of therapeutic efficacy and safety of PD-1 inhibitor plus SOX neoadjuvant therapy for advanced esophagogastric junction adenocarcinoma. *J Abdom Surg*. 2021;34(03):199-202+210.
[30] Deng W, Zhang YQ, Jing C, Yang XB, Bai ZG, Zhang J. Camrelizumab, apatinib, nab-paclitaxel, and S-1 in combination for the conversion therapy of advanced gastric cancer. *Inter J Surg*. 2022;49(04):242–247.
[31] Rivera F, Galán M, Tabernero J, et al, Spanish Cooperative Group for Digestive Tumour Therapy (TTD). Phase II trial of induction irinotecan-cisplatin followed by concurrent irinotecan-cisplatin and radiotherapy for unresectable, locally advanced gastric and oesophageal-gastric junction adenocarcinoma. *Cancer Chemother Pharmacol*. 2011;67(1):75–82.
[32] Kitayama J, Ishigami H, Yamaguchi H, et al. Salvage gastrectomy after intravenous and intraperitoneal paclitaxel (PTX) administration with oral S-1 for peritoneal dissemination of advanced gastric cancer with malignant ascites. *Ann Surg Oncol*. 2014;21(2):539–546.
[33] Fukuchi M, Ishiguro T, Ogata K, et al. Prognostic role of conversion surgery for unresectable gastric cancer. *Ann Surg Oncol*. 2015;22(11): 3618–3624.
[34] Ratosa I, Oblak I, Anderluh F, et al. Preoperative treatment with radiochemotherapy for locally advanced gastroesophageal junction cancer and unresectable locally advanced gastric cancer. *Radiol Oncol*. 2015;49(2):163–172.
[35] Mitsui Y, Sato Y, Miyamoto H, et al. Trastuzumab in combination with docetaxel/cisplatin/S-1 (DCS) for patients with HER2-positive metastatic gastric cancer: feasibility and preliminary efficacy. *Cancer Chemother Pharmacol*. 2015;76(2):375–382.
[36] He Q, Li Y, Ma L, Ji X, Li G. Application of FLEEOX preoperative chemotherapy via intra-arterial and intravenous administration in treatment of unresectable locally advanced gastric cancer. *J Gastrointest Surg*. 2016;20(8):1421–1427.
[37] Sato Y, Ohnuma H, Nobuoka T, et al. Conversion therapy for inoperable advanced gastric cancer patients by docetaxel, cisplatin, and S-1 (DCS) chemotherapy: a multi-institutional retrospective study. *Gastric Cancer*. 2017;20(3):517–526.
[38] Ishigami H, Fujiwara Y, Fukushima R, et al. Phase III trial comparing intraperitoneal and intravenous paclitaxel plus S-1 versus cisplatin plus S-1 in patients with gastric cancer with peritoneal metastasis: PHOENIX-GC trial. *J Clin Oncol*. 2018;36(19):1922–1929.
[39] Sawasaki M, Tsubamoto H, Nakamoto Y, Kakuno A, Sonoda T. S-1, oxaliplatin, nab-paclitaxel and itraconazole for conversion surgery for advanced or recurrent gastric cancer. *Anticancer Res*. 2020;40(2): 991–997.
[40] Li S, Yu W, Xie F, et al. Neoadjuvant therapy with immune checkpoint blockade, antiangiogenesis, and chemotherapy for locally advanced gastric cancer. *Nat Commun*. 2023;14(1):8.
[41] Yoshida K, Yamaguchi K, Okumura N, Tanahashi T, Kodera Y. Is conversion therapy possible in stage IV gastric cancer: the proposal of new biological categories of classification. *Gastric Cancer*. 2016;19(2): 329–338.
[42] Ramjiawan RR, Griffioen AW, Duda DG. Anti-angiogenesis for cancer revisited: is there a role for combinations with immunotherapy? *Angiogenesis*. 2017;20(2):185–204.
[43] Zhao WX, Liu ZF, Li XL, Li Z. Correlations of serum homocysteine, VEGF and gastrin 17 with gastric cancer and precancerous lesions. *Eur Rev Med Pharmacol Sci*. 2019;23(10):4192–4198.
[44] Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci*. 2020; 77(9):1745–1770.
[45] Liu M, Wang X, Wang L, et al. Targeting the IDO1 pathway in cancer: from bench to bedside. *J Hematol Oncol*. 2018;11(1):100.
[46] Lopes-Coelho F, Martins F, Pereira SA, Serpa J. Anti-angiogenic therapy: current challenges and future perspectives. *Int J Mol Sci*. 2021; 22(7):3765.

Original Research

Oncology and Translational Medicine

OPEN

Risk evaluation of splenic hilar lymph node metastasis and survival analysis of patients with advanced gastric cancer

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Abstract

Background: There is no consensus regarding the influence of prophylactic no. 10 lymph node (LN) dissection in patients with advanced gastric cancer (AGC). We aimed to evaluate whether patients with AGC could benefit from no. 10 LN dissection and to explore the clinicopathological indicators of no. 10 LN metastasis.

Methods: We analyzed the data of 218 patients with AGC who underwent standard D2 lymphadenectomy (SD2; n = 108) or modified D2 lymphadenectomy (MD2; n = 110) between January 2017 and January 2021. In addition, we examined factors influencing no. 10 LN metastasis in the SD2 group.

Results: Differentiation, tumor location, and no. 4 positive LNs were significantly correlated with no. 10 LN metastasis ($P < 0.05$). Borrmann classification, differentiation, depth of invasion, LN metastasis (N), and tumor size were found to correlate with survival in univariate analyses. Age, sex, extent of gastrectomy, tumor location, and extent of lymphadenectomy were not associated with survival ($P > 0.05$). The median survival times were 72.23 and 68.56 months for the SD2 and MD2 groups, respectively ($P = 0.635$). Postoperative major morbidity and mortality rates were 37.96% and 3.70% in the SD2 group, and 23.64% and 1.82% in the MD2 group, respectively.

Conclusions: Based on our findings, prophylactic no. 10 lymphadenectomy may be recommended in patients with AGC who exhibit positive no. 4 LN status, poor differentiation, and tumors located on the greater curvature.

Keywords: Advanced gastric cancer (AGC); Complication; No. 10 lymphadenectomy; Survival time

1. Introduction

Gastric cancer is the fourth most common cause of death from cancer and the fifth most commonly diagnosed cancer type, accounting for 5.6% of all new cancers reported worldwide.[1] Advanced gastric cancer (AGC) is prone to lymph node (LN) metastasis, a deeper tumor invasion that has a higher tendency for LN involvement.[2] Lymph node metastasis often occurs at the primary site and is the most important mechanism of tumor spread, as confirmed in one study using carbon particles.[3] Surgical resection of the primary tumor with curative lymphadenectomy remains the criterion standard of treatment for AGC. Advanced gastric cancer sometimes metastasizes to the splenic hilar LN (no. 10 LN), which is defined as the N2 station according to current guidelines. As no. 10 LN's special position is located in the ligament of the spleen, it is technically difficult to completely remove the no. 10 LN without some surgical complications, such as bleeding, infection, pancreatic fistula, damaged immune system, and postoperative thrombosis.[4–6] Several studies have reported that patients who undergo complete lymphadenectomy for no. 10 LN have shown no survival benefits.[7,8] Owing to the relatively low rate of no. 10 LN metastasis and high complication rates, surgeons must weigh the risks of postoperative complications and benefits to decide whether no. 10 LN dissection should be performed during surgery. This study analyzed the clinicopathological factors to confirm the indicators of no. 10 LN metastasis and survival in patients with AGC.

2. Patients and methods

We enrolled 218 patients who underwent D2 curative proximal or total gastrectomy for advanced gastric carcinoma between January 2017 and January 2021. Sex, age, Borrmann classification, differentiation, depth of invasion (T), LN metastasis (N), extent of gastrectomy, tumor location, and tumor size were retrospectively correlated with survival.

Standard D2 lymphadenectomy (SD2) included the removal of the following lymphatic fat tissues: no. 1/2/3/4/5/6/7/8a/9/10/11/12a for total gastrectomy and no. 1/2/3/4/7/8a/9/10/11 for proximal gastrectomy.

Modified D2 lymphadenectomy (MD2) included the removal of the following lymphatic fat tissues: no. 1/2/3/4/5/6/7/8a/9/11/12a for total gastrectomy and no. 1/2/3/4/7/8a/9/11 for proximal gastrectomy.

According to a computer-generated random indicator, patients with AGC were assigned in a randomized manner to the SD and MD groups. Patients in both groups were matched for sex, age, Borrmann classification, differentiation, T, N, extent of gastrectomy, tumor location, and tumor size. We also compared these clinicopathological parameters between the positive no. 10 LN and

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negative no. 10 LN in the SD2 group. Complications were compared between groups SD2 and MD2.

2.1. Follow-up assessments

All enrolled patients in our hospital had complete personal follow-up files and an explicit pathological diagnosis. Patients with obvious no. 10 LN metastasis direct invasion of the spleen or preoperative chemoradiotherapy were excluded. All patients underwent follow-up assessments every 3 months for the first 2 years postoperatively, at 6 months during the third year, and at 12 months thereafter. Outpatient or inpatient reviews and telephone or e-mail were used for follow-up.

2.2. Ethics

This study was performed in accordance with the Declaration of Helsinki and was approved by the institutional review board of Xuzhou Central Hospital. Written informed consent was obtained from all the patients.

2.3. Statistical analysis

All statistical analyses were performed using SPSS software (version 13.0; SPSS Inc, Chicago, IL). Relevant clinical and pathological factors were compared between the 2 groups using the χ^2 test. The relationship between clinicopathological factors and metastasis to the no. 10 LN was evaluated using the χ^2 test. Morbidity and mortality comparisons between the 2 groups were also conducted using the χ^2 test. Overall survival was analyzed using the Kaplan-Meier method, and statistical significance was calculated using the log-rank test. Multivariate logistic regression analysis was conducted. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1 Clinicopathological factors of the AGC patients

All the patients underwent curative gastrectomy with D2 LN dissection. There were 132 male and 86 female patients with a median age of 54.5 years (range, 33–82 years). Of the 218 patients, 192 were younger than 70 years, and 26 were older than 70 years. Among the Borrmann types, 74 were types I and II, and 144 were types III and IV. Ninety patients had well and moderately differentiated tumors, whereas 128 had poorly differentiated tumors. At the depth of invasion, 115 patients were in stages T2 and T3, and 103 were in the T4 stage. One hundred forty-one patients had N1 and N2 stages, and 77 patients had N3 stages. All patients underwent gastrectomy: 74 of the subtotal group and 144 of the total group. A total of 108 patients had tumors located in the lesser curvature, and 110 had tumors located in the greater curvature. In 128 patients, lesion was ≤5 cm, and in 90, lesion was >5 cm. The SD2 group included 108 patients, and the MD2 group included 110 patients, respectively (Table 1).

Table 1

Comparison of patients with advanced gastric cancer between the SD2 and MD2 groups

Items	SD2 (108)	MD2 (110)	*P*
Sex			0.699
Male	64	68	
Female	44	42	
Age, y			0.713
>70	12	14	
≤70	96	96	
Borrmann type			0.635
I + II	35	39	
III + IV	73	71	
Differentiation			0.477
Well/moderately	42	48	
Poorly	66	62	
Depth of invasion (T)			0.582
T2/T3	56	59	
T4	54	49	
Lymph node metastasis (N)			0.145
N1/N2	75	66	
N3	33	44	
Gastrectomy			0.850
Subtotal	36	38	
Total	72	72	
Tumor location			0.615
Lesser curvature	44	64	
Greater curvature	54	56	
Tumor size, cm			0.348
≤5	60	68	
>5	48	42	

3.2 Influencing factors of no. 10 LN metastasis in the SD2 group

Table 2 shows that differentiation, tumor location, and no. 4 positive LN were significantly correlated with no. 10 LN metastasis ($P < 0.005$). The incidence of metastasis to the no. 10 LN differed significantly between the tumor location at the lesser and greater curvatures ($P = 0.022$). The poorer the tumor differentiation, the more prone the patient was to LN metastasis ($P = 0.018$). Of the 108 patients with AGC in the SD2 group who underwent SD2 lymphadenectomy, 45 had no. 4 LN metastasis. Of the 63 patients with negative no. 4 LN, only 2 patients had positive no. 10 LN metastasis (Table 2).

3.3 Univariate and multivariate analysis results

Borrmann classification, differentiation, depth of invasion, LN metastasis, and tumor size were found to correlate with survival in univariate analyses. Age, sex, extent of gastrectomy, tumor location, and extent of lymphadenectomy were not associated with survival ($P > 0.05$). The median survival times were 72.23 and 68.56 months for the SD2 and MD2 groups, respectively ($P = 0.635$). Although the patients in the MD2 group had worse survival than those in the SD2 group, the difference was not statistically significant (Fig. 1). Moreover, the Cox proportional regression hazard model showed that differentiation, depth of invasion, and LN metastasis were independent prognostic factors (Table 3) (Figs. 2–4).

3.4 Complications between the 2 groups

As shown in Table 4, the morbidity and mortality rates of the patients in the SD2 group were higher than those of the patients in the MD2 group. Postoperative major morbidity and mortality rates were 37.96% and 3.70% in the SD2 group and 23.64% and 1.82% in the MD2 group, respectively ($P = 0.011$) (Table 4).

4. Discussion

Currently, surgical resection with LN dissection is the only curative treatment for AGC. Some articles reported that there was approximately 10% of AGC patients who had developed no. 10 LN metastasis.[9–11] Some surgeons performed SD2 lymphadenectomy to complete no. 10 LN dissection, but the complications have prevented them from rethinking this surgery type.[12,13] Moreover,

Table 2

The influencing factors of no. 10 lymph node metastasis in the SD2 group

Items	No. 10 Lymph node		*P*
	Positive	Negative	
Sex			0.782
Male	8	50	
Female	6	44	
Age, y			0.275
≤70	9	73	
>70	5	21	
Borrmann type			0.357
I + II	4	39	
III + IV	10	55	
Differentiation			0.018
Well/moderately	3	52	
Poorly	11	42	
Depth of invasion (T)			0.115
T2/T3	3	41	
T4	11	53	
Lymph node metastasis (N)			0.482
N1/N2	4	36	
N3	10	58	
Gastrectomy			0.737
Subtotal	5	38	
Total	9	56	
Tumor location			0.022
Lesser curvature	2	44	
Greater curvature	12	50	
Tumor size, cm			0.097
≤5	3	38	
>5	11	56	
No. 4 lymph node			<0.001
Positive	12	33	
Negative	2	61	

Table 3

Univariate analysis and Cox multivariate analysis to identify independent prognostic factors

Items	Median Survival, mo	Univariate *P*	Multivariate *P*
Sex		0.426	
Male	58.42		
Female	61.93		
Age, y		0.342	
≤70	61.06		
>70	50.58		
Borrmann type		0.030	0.070
I + II	67.05		
III + IV	58.21		
Differentiation		0.001	0.001
Well/moderately	66.12		
Poorly	51.74		
Depth of invasion (T)		0.001	0.012
T2/T3	67.16		
T4	50.35		
Lymph node metastasis (N)		0.003	0.049
N1/N2	67.03		
N3	52.53		
Gastrectomy		0.541	
Subtotal	61.78		
Total	56.97		
Tumor location		0.645	
Lesser curvature	60.25		
Greater curvature	60.18		
Tumor size, cm		0.043	0.112
≤5	64.92		
>5	56.55		
Lymphadenectomy extent			
SD2	72.23	0.635	
MD2	68.56		

patients with no. 10 LN metastasis had worse survival than patients without metastasis, but they could not gain survival benefits from no. 10 LN dissection.[8,14] Considering the safety of surgery and patients' quality of life, however, there is no clear consensus as to whether AGC patients without preoperative metastasis to no. 10 LN could avoid prophylactic no. 10 LN dissection. Thus, the aim of this study was to evaluate the impact of prophylactic no. 10 LN

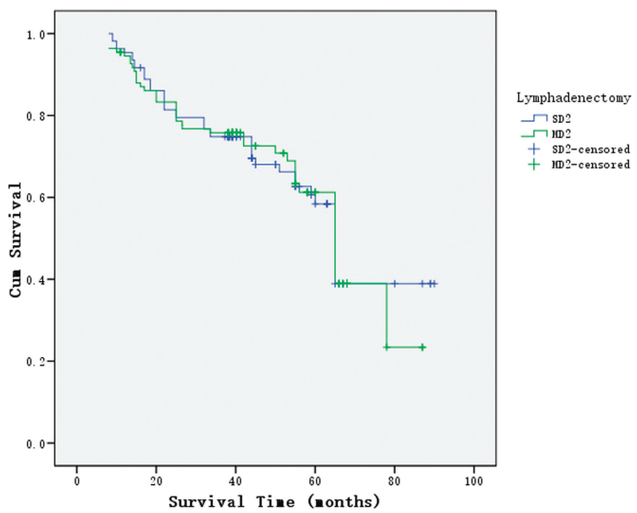

Figure 1. The comparison of survival time between SD2 and MD2 groups.

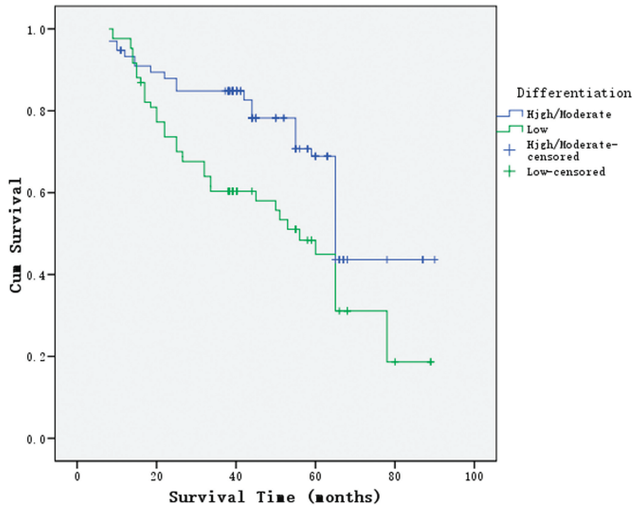

Figure 2. The comparison of survival time between different degrees of differentiation.

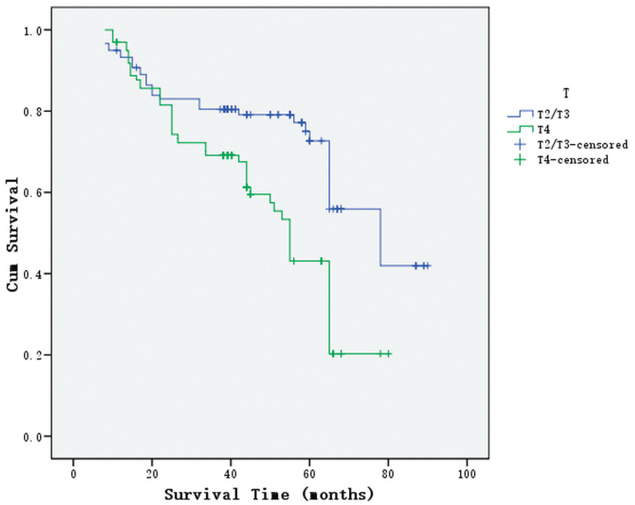

Figure 3. The comparison of survival time between different stages of T.

clearance on the prognosis and postoperative complications in patients with AGC.

As commonly acknowledged, location is a key factor correlated with no. 10 LN, especially those in the upper and greater curvatures of the stomach.[15,16] There are reasonable explanations for these findings. The lymphatic flow of the greater curvature, located along the upper body, drains to the splenic hilar LNs via the short gastric, left gastroepiploic, and posterior gastric arteries. Tammaro et al[17] reported that no. 10 LN dissection should be considered for tumors localized in the upper two-thirds of the stomach, which is in good accordance with the current study. Kusano et al[18] reported that the rate of positive no. 10 LN located at the greater curvature was 17.0%, higher than that at the lesser curvature (10%). Meanwhile, our study revealed that the no. 10 LN located at the greater curvature was 11.1%, higher than that at the lesser curvature (1.1%). The results suggested that no. 10 LN easily occurred in gastric cancer at the greater curvature, suggesting that tumor location is a predictor of AGC.

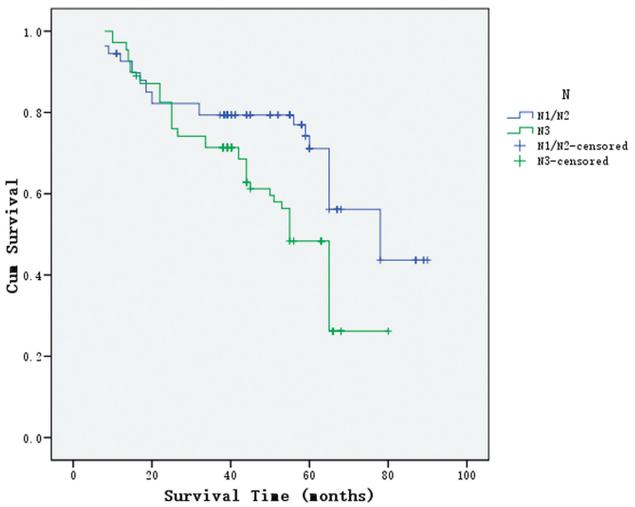

Figure 4. The comparison of survival time between different stages of N.

Table 4

The morbidity and mortality of patients with advanced gastric cancer between SD2 and MD2 group

Items	MD2	SD2	*P*
Morbidity	26 (23.64%)	41 (37.96%)	
Wound abscess	2	3	
Intra-abdominal abscess	3	5	
Pancreatic fistula	3	7	
Bleeding	3	8	
Pleural fluid	8	10	
Postoperative thrombosis	2	5	
Others	5	3	
Mortality	2 (1.82%)	4 (3.70%)	
Cardiac and respiratory failure	1	0	
Bleeding	1	2	
Others	0	2	
Total	28	45	0.011

To date, some researchers believe that there exists a close negative correlation between the degree of differentiation and no. 10 LN metastasis.[19,20] In general, the poorer the differentiation degree, the higher the rate of no. 10 LN. Our study showed that the number of no. 10 LN in the group with well/moderate differentiation (2.8%) was lower than that in the group with poor differentiation (10.2%). Wu et al[21] explained that positive no. 10 LN was correlated with a higher level of matrix metalloproteinase 3 expression, which was induced by poorer differentiation in patients with AGC. Consequently, lower differentiation should be a criterion for patients with AGC who could benefit from no. 10 LN resection.

The depth of invasion and tumor size can predict tumor staging to a certain degree. Some studies have reported that the above 2 are potential no. 10 LN influencing factors.[22,23] In our study, the positive rate of no. 10 LN in tumor size equal to or larger than 5 cm was 10.2%, whereas the rate in tumor size less than 5 cm was 2.8%. In our study, the no. 10 rate of the T4 stage was 10.2%, higher than that of the T2 and T3 stages (2.8%). Some reported that no. 10 LN metastasis was observed in 3.4% of patients with tumors smaller than 5 cm and in 21.3% of patients with tumors larger than 5 cm.[24] However, there was no statistically significant difference between the depth of invasion and tumor size in no. 10 LN metastasis in our study. Aoyagi et al[14] also reported that the T stage was not associated with splenic hilar LN metastasis.

In addition, in the present study, no. 10 LN was not associated with the Borrmann type. The no. 10 LN rates in patients with AGC with the types I/II and III/IV were 3.7% and 7.4%, respectively. Other studies reported that no. 10 LN metastasis occurred more easily in patients with infiltrative AGC than in those with local AGC, possibly because of the tumor growth pattern.[25] However, in our study, we failed to confirm the correlation between no. 10 LN and the Borrmann type.

In our study, we also found that when no. 4 LNs were positive, no. 10 LN positive detection rate was as high as 26.7%; when no. 4 LNs were negative, no. 10 LN positive detection rate was only 3.2%. The difference between them was statistically significant ($P < 0.001$). Therefore, we can take the no. 4 positive LNs as sentinel LNs. This can be explained as follows: the no. 10 LN is located near the greater curvature and occurs through no. 4 LN located along the greater curvature via lymphatic drainage.[26] Bian et al[27] reported that for a certain group of patients with AGC, avoiding unnecessary no. 10 lymphadenectomy could lead to less invasive trauma and tissue damage. As for the cases that were no. 4 LN negative, no. 10 LN was still positive. Cancer cells will have to pass through the lesser curvature LNs, then LNs 7, 9, and 11, finally reaching LN 10. This is one of the possible

mechanisms of explaining the phenomenon of skip metastasis, but the rate is too low to confirm, and the incidence of this type of skip metastasis was only 3.2% in our study and was also very low in other reports.[28]

Moreover, multivariate analysis revealed that differentiation, invasion depth, and LN metastasis were independent prognostic factors ($P < 0.05$). Patients with these characteristics have poor survival. These results are consistent with those of other statistical reports.[29–31] Regarding the extent of lymphadenectomy, Sano et al[32] enrolled 505 patients and showed no survival difference between the SD2 and MD2 groups in patients with AGC. In 2014, some scholars analyzed 8 randomized controlled trials and found no significant difference in the overall 5-year survival rate between the 2 groups.[33] In our study, we found no significant difference in survival rates between the 2 groups; the median survival times were 72.23 and 68.56 months for the SD2 and MD2 groups, respectively ($P = 0.635$). Although the patients in the MD2 group had worse survival than those in the SD2 group, the difference was not statistically significant. A recent study also showed no statistically significant difference in 5-year survival between patients with and without splenic hilar LN metastasis in the greater curvature group.[34] Huang et al[35] retrospectively compared patients who did and did not undergo no. 10 LN dissection in the spleen-preserving surgery group and found that the 3-year disease-free survival time was significantly better in the dissection group; however, the overall survival time was not statistically significant.

The mortality and morbidity rates of the 2 groups are summarized in Table 4. The results of our study are consistent with those previously reported. The overall postoperative morbidity in the SD2 group was significantly higher ($P = 0.022$). Postoperative major morbidity and mortality rates were 37.96% and 3.70% in the SD2 group, and 23.64% and 1.82% in the MD2 group, respectively. We speculate that the fragile texture of the spleen and its special anatomical location may increase the risk of postoperative complications.

This study has several limitations. First, as a retrospective study, it included a relatively small number of patients from a single center, which included a possible selection bias. Therefore, further randomized studies are required to compare the outcomes of SD2 with those of MD2. Second, there were 2 false-negative cases in our study, namely, patients with negative no. 4 LN results but positive no. 10 LN results. Consequently, it becomes imperative to ascertain the optimal sensitivity and specificity of better indicators of no. 10 LN, and large-scale, well-designed studies are needed to explore the role of no. 10 LN clearance in patients with AGC. The unique nature of this study provided new insights into the extent of lymphadenectomy in patients with AGC.

5. Conclusion

For patients with AGC without high-risk factors, no. 10 lymphadenectomy may not be recommended, whereas for those with positive no. 4LN, poor differentiation, and tumors involving greater curvature should undergo an SD2 lymphadenectomy.

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Conflicts of interest statement

The authors declare that they have no conflict of interest with regard to the content of this report.

Author contributions

All authors contributed to data acquisition and data interpretation and reviewed and approved the final version of the manuscript.

Data availability statement

Not applicable.

Ethical approval

Not applicable.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424.

[2] Lawson JD, Sicklick JK, Fanta PT. Gastric cancer. *Curr Probl Cancer*. 2011;35:97–127.

[3] McCulloch P, Nita ME, Kazi H, Gama-Rodrigues JJ. Withdrawn: extended versus limited lymph nodes dissection technique for adenocarcinoma of the stomach. *Cochrane Database Syst Rev*. 2012;1:CD001964.

[4] Yao XX, Sah BK, Yan M, Chen MM, Zhu ZG. Radical gastrectomy with combined splenectomy: unnecessary. *Hepatogastroenterology*. 2011;58(107–108):1067–1070.

[5] Schmid A, Thybusch A, Kremer B, Henne-Bruns D. Differential effects of radical D_2-lymphadenectomy and splenectomy in surgically treated gastric cancer patients. *Hepatogastroenterology*. 2000;47(32):579–585.

[6] Biffi R, Chiappa A, Luca F, et al. Extended lymph node dissection without routine spleno-pancreatectomy for treatment of gastric cancer: low morbidity and mortality rates in a single center series of 250 patients. *J Surg Oncol*. 2006;93(5):394–400.

[7] Zhu GL, Sun Z, Wang ZN, et al. Splenic hilar lymph node metastasis independently predicts poor survival for patients with gastric cancers in the upper and/or the middle third of the stomach. *J Surg Oncol*. 2012;105(8):786–792.

[8] Shin SH, Jung H, Choi SH, et al. Clinical significance of splenic hilar lymph node metastasis in proximal gastric cancer. *Ann Surg Oncol*. 2009;16(5):1304–1309.

[9] Jeong O, Jung MR, Ryu SY. Clinicopathological features and prognostic impact of splenic hilar lymph node metastasis in proximal gastric carcinoma. *Eur J Surg Oncol*. 2019;45(3):432–438.

[10] Kosuga T, Ichikawa D, Okamoto K, et al. Survival benefits from splenic hilar lymph node dissection by splenectomy in gastric cancer patients: relative comparison of the benefits in subgroups of patients. *Gastric Cancer*. 2011;14(2):172–177.

[11] Chen XL, Yang K, Zhang WH, et al. Metastasis, risk factors and prognostic significance of splenic hilar lymph nodes in gastric adenocarcinoma. *PloS One*. 2014;9(6):e99650.

[12] Kristinsson SY, Gridley G, Hoover RN, Check D, Landgren O. Long-term risks after splenectomy among 8,149 cancer-free American veterans: a cohort study with up to 27 years follow-up. *Haematologica*. 2014;99(2):392–398.

[13] Zakeri S, Gorji N, Akhtari M, Moeini R. Splenectomy may have more complications than currently proven. *Med Hypotheses*. 2018;112:43–46.

[14] Aoyagi K, Kouhuji K, Miyagi M, Imaizumi T, Kizaki J, Shirouzu K. Prognosis of metastatic splenic hilum lymph node in patients with gastric cancer after total gastrectomy and splenectomy. *World J Hepatol*. 2010;2(2):81–86.

[15] Yu W, Choi GS, Chung HY. Randomized clinical trial of splenectomy versus splenic preservation in patients with proximal gastric cancer. *Br J Surg*. 2006;93(5):559–563.

[16] Sasada S, Ninomiya M, Nishizaki M, et al. Frequency of lymph node metastasis to the splenic hilus and effect of splenectomy in proximal gastric cancer. *Anticancer Res*. 2009;29(8):3347–3351.

[17] Tammaro V, Carlomagno N, Lombari P, Tedesco G, Renda A. Il ruolo prognostico di splenectomia e linfoadenectoma nella chirurgia del carcinoma gastrico [Prognostic value of splenectomy and lymph-node dissection during gastric cancer resection]. *Chir Ital*. 2006;58(2):163–170.

[18] Kusano T, Shiraishi N, Shiroshita H, Etoh T, Inomata M, Kitano S. Poor prognosis of advanced gastric cancer with metastatic suprapancreatic lymph nodes. *Ann Surg Oncol*. 2013;20(7):2290–2295.

[19] Zu H, Wang H, Li C, Xue Y. Clinicopathologic characteristics and prognostic value of various histological types in advanced gastric cancer. *Int J Clin Exp Pathol*. 2014;7(9):5692–5700.

[20] Lee JH, Choi IJ, Han HS, et al. Risk of lymph node metastasis in differentiated type mucosal early gastric cancer mixed with minor undifferentiated type histology. *Ann Surg Oncol*. 2015;22(6):1813–1819.

[21] Wu ZY, Li JH, Zhan WH, He YL, Wan J. Effect of lymph node micrometastases on prognosis of gastric carcinoma. *World J Gastroenterol*. 2007;13(30):4122–4125.

[22] Xu CY, Shen JG, Shen JY, Chen WJ, Wang LB. Ulcer size as a novel indicator marker is correlated with prognosis of ulcerative gastric cancer. *Dig Surg*. 2009;26(4):312–316.

[23] Shida A, Fujioka S, Kawamura M, et al. Prediction of lymph node metastasis in patients with submucosa-invading early gastric cancer. *Anticancer Res*. 2014;34(8):4471–4474.

[24] Sun Z, Wang Q, Yu X, et al. Risk factors associated with splenic hilar lymph node metastasis in patients with advanced gastric cancer in Northwest China. *Int J Clin Exp Med*. 2015;8(11):21358–21364.

[25] Lazăr D, Tăban S, Sporea I, et al. Gastric cancer: correlation between clinicopathological factors and survival of patients (III). *Rom J Morphol Embryol*. 2009;50(3):369–379.

[26] Toriumi T, Terashima M. Disadvantages of complete no. 10 lymph node dissection in gastric cancer and the possibility of spleen-preserving dissection: review. *J Gastric Cancer*. 2020;20(1):1–18.

[27] Bian S, Xi H, Wu X, et al. The role of no. 10 lymphadenectomy for advanced proximal gastric cancer patients without metastasis to no. 4sa and no. 4sb lymph nodes. *J Gastrointest Surg*. 2016;20(7):1295–1304.

[28] Dresner SM, Lamb PJ, Bennett MK, Hayes N, Griffin SM. The pattern of metastatic lymph node dissemination from adenocarcinoma of the esophagogastric junction. *Surgery*. 2001;129(1):103–109.

[29] Dittmar Y, Rauchfuss F, Dondorf F, Ardelt M, Scheuerlein H, Settmacher U. Extended pathohistological criteria for assessment of the long-term prognosis of gastric cancer [in German]. *Zentralbl Chir*. 2016;141(4):433–441.

[30] Zhu Z, Sun X, Wang J, et al. Histopathology-based prognostic score is independent prognostic factor of gastric carcinoma. *BMC Cancer*. 2014;14:663.

[31] Komatsu S, Ichikawa D, Miyamae M, et al. Histological mixed-type as an independent prognostic factor in stage I gastric carcinoma. *World J Gastroenterol*. 2015;21(2):549–555.

[32] Sano T, Sasako M, Mizusawa J, et al, Stomach Cancer Study Group of the Japan Clinical Oncology Group. Randomized controlled trial to evaluate splenectomy in Total gastrectomy for proximal gastric carcinoma. *Ann Surg*. 2017;265(2):277–283.

[33] De Manzoni G, Baiocchi GL, Framarini M, et al. The SIC-GIRCG 2013 consensus conference on gastric Cancer. *Updates Surg*. 2014;66(1):1–6.

[34] Yura M, Yoshikawa T, Otsuki S, et al. The therapeutic survival benefit of splenic hilar nodal dissection for advanced proximal gastric cancer invading the greater curvature. *Ann Surg Oncol*. 2019;26(3):829–835.

[35] Huang CM, Lin JX, Zheng CH, Li P, Xie JW, Lin BJ. Effect of negative lymph node count on survival for gastric cancer after curative distal gastrectomy. *Eur J Surg Oncol*. 2011;37(6):481–487.

Original Research

Oncology and Translational Medicine

OPEN

Integrated bioinformatics analysis identifies immune-related epithelial-mesenchymal transition prognostic biomarkers and immune infiltrates in patients with lung adenocarcinoma

Yu Huang[a], Peng Zhang[a], Shu-Chang Zhou[b], Qing-Xu Liu[a,*]

Abstract

Background: Lung cancer, particularly lung adenocarcinoma (LUAD), is highly lethal. Understanding the critical interaction between epithelial-mesenchymal transition (EMT) and the immune status of patients is imperative for clinical assessment.

Methods: We conducted bioinformatics analysis to identify potential immune-related EMT (iEMT) prognostic genes and explored the immune status in LUAD. Using data from The Cancer Genome Atlas and GSE68465, differentially expressed genes, were identified, and a risk model was constructed. Cluster analysis was conducted using the Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathways.

Results: Our findings revealed 69 differentially expressed iEMT genes, with risk values demonstrating independent prognostic significance for both The Cancer Genome Atlas and GSE68465 samples. The risk value was positively correlated with tumor stage. Immune cell infiltration analysis showed a significant decrease in resting dendritic cells and an increase in CD4 memory T cells in high-risk groups with poor survival prognoses. The immunotherapy analysis revealed weak immunotherapeutic effects in the high-risk group.

Conclusions: This study provides insights into potential aberrant differential iEMT genes and risk models and explores immune landscapes that inform personalized immunotherapy in patients with LUAD.

Keywords: Immune cell infiltration; Immune-related EMT genes; Lung adenocarcinoma; Prognosis; Tumor immune microenvironment

1. Introduction

Lung adenocarcinoma (LUAD) is the primary pathological subtype of non–small cell lung cancer (NSCLC) and is characterized by high heterogeneity and aggressiveness.[1] Recent advances in surgical techniques and immunotherapy have resulted in improved survival rates for patients with LUAD; however, local and distant metastases remain significant causes of treatment failure.[2] Therefore, a better understanding of the pathogenesis of LUAD and mechanisms underlying metastasis is critical for improving treatment outcomes.

Epithelial-mesenchymal transition (EMT) plays a crucial role in promoting the mesenchymal features and invasive potential of epithelial cells, driving cancer progression. Epithelial-mesenchymal transition is associated with the development, metastasis, and drug resistance in LUAD.[3–8] During EMT, epithelial markers such as E-cadherin are downregulated, whereas mesenchymal markers such as N-cadherin and Snail are upregulated, leading to malignant characteristics, such as increased metastatic capacity of tumor cells.[7,8]

Epithelial-mesenchymal transition plays a vital role in the tumor microenvironment (TME).[9] Cancer-associated fibroblasts are key factors promoting the invasion and metastasis of LUAD cells. In the early stages of EMT, cytokines or chemokines secreted by tumor cells attract various stromal and immune cells to create a niche for tumor progression, invasion, and metastasis.[10] The TME is a complex ecosystem consisting of tumor cells, immune cells (including macrophages, polymorphonuclear cells, mast cells, natural killer cells, dendritic cells [DCs], and T and B lymphocytes), and nonimmune cells (including endothelial cells and stromal cells), which interact subtly with each other and ultimately determine the natural course of the tumor.[11] Immune cellular components are particularly crucial in determining the fate of a tumor and its invasive and metastatic abilities.

Epithelial-mesenchymal transition can significantly alter the anti-tumor immune microenvironment (TIME).[12] Multiple immune cells can infiltrate tumors, and their composition and organization within the TIME strongly impact clinical outcomes in patients with cancer. Mesenchymal cell subtypes enriched with EMT-related gene features are associated with poor prognosis. Epithelial-mesenchymal transition markers have been linked to significant reductions in the infiltration of tumors by $CD4^+$ and $CD8^+$ T cells, increased expressions of immunosuppressive cytokines such as interleukin 10 and transforming growth factor β, and overexpression of the suppressive immune checkpoint molecule cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) in NSCLC studies.[9] One study demonstrated the high expression of immune checkpoints and other druggable immune targets such as programmed cell death 1 (PD-1), programmed cell death 1 ligand

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(PD-L1), CTLA-4, OX40L, and PD-L2 in tumors with the highest mesenchymal EMT scores.[13] In addition, the EMT phenotype has been shown to be related to PD-L1 overexpression in LUAD cells, and patients with EMT-phenotype LUAD may benefit from PD-1/PD-L1–blocking immunotherapy.[14] Although several studies have investigated the effect of EMT on the TIME, the impact of EMT on immune cell infiltration in the TIME and its function in LUAD remains unclear.

Therefore, investigating TIME alterations driven by EMT is critical for understanding LUAD development. Through bioinformatics analysis of The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) data sets, we observed that CDH1, ANGPTL4, SHC1, FGF2, S100P, KRT16, and WFDC2 could serve as risk genes for the survival prognosis of patients with LUAD. Furthermore, immune analysis revealed that the aforementioned risk genes were associated with significant immune alterations and were consistent with the survival prognosis of patients with LUAD.

2. Materials and methods

2.1. Downloading RNA-seq data and identifying expression of immune-related EMT genes

RNA-seq data for LUAD were obtained from TCGA database. The GSE68465 RNA-seq expression data set for LUAD was retrieved from the GEO database. Immune-related genes were downloaded from the InnateDB (https://www. innatedb. com/) and ImmPort (https://immport. org/shared/home) databases. Epithelial-mesenchymal transition–related genes were obtained from the EMTome database (www.emtome.org). The intersection of immune and EMT genes has been identified as the immune-related EMT (iEMT) gene.

2.2. Data processing and cluster analysis

In this study, the ComBat function of the Sva package was used to correct for batch effects in RNA sequencing data. Differentially expressed genes (DEGs) were analyzed using R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria), of the Limma package, with |logFC| >1 and false discovery rate <0.05. Differentially expressed iEMT genes were identified by intersecting the DEGs and iEMT genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted using clusterProfiler.

2.3. Risk model construction

We obtained the intersection data set by taking the common genes between the key node genes and DEGs from the TCGA and GSE68465 data sets. We then merged the TCGA intersection data set with patient survival data and used univariate and multivariate Cox analyses to construct a risk model. The risk value is calculated as follows: CDH1 × 0.189 + ANGPTL4 × 0.145 + SHC1 × 0.22 + FGF2 × 0.349 + S100P × 0.05 + KRT16 × 0.08 – WFDC2 × 0.1.

Based on the above formula, the intersection data set GSE68465 was used to obtain the patient risk values. Survival analysis was performed using the survival package, and the receiver operating characteristic (ROC) curve was validated using the survivalROC package. Heat maps were plotted using the heat map package for risk model genes, patient risk values, and patient survival statuses. Finally, the risk model was visualized using the RMS package to plot a nomogram of the patients' risk values against the risk genes.

2.4. Clinical trait analysis

We used the survival package to perform Cox regression analysis of the patients' age, sex, T stage, and risk values. Clinical traits were analyzed based on the patients' risk values. Heat maps of clinical traits versus risk values were generated using the ComplexHeatmap package. The numbers of high- and low-risk groups in each of the four stages were counted, followed by the calculation of the proportion of high- and low-risk groups in each stage.

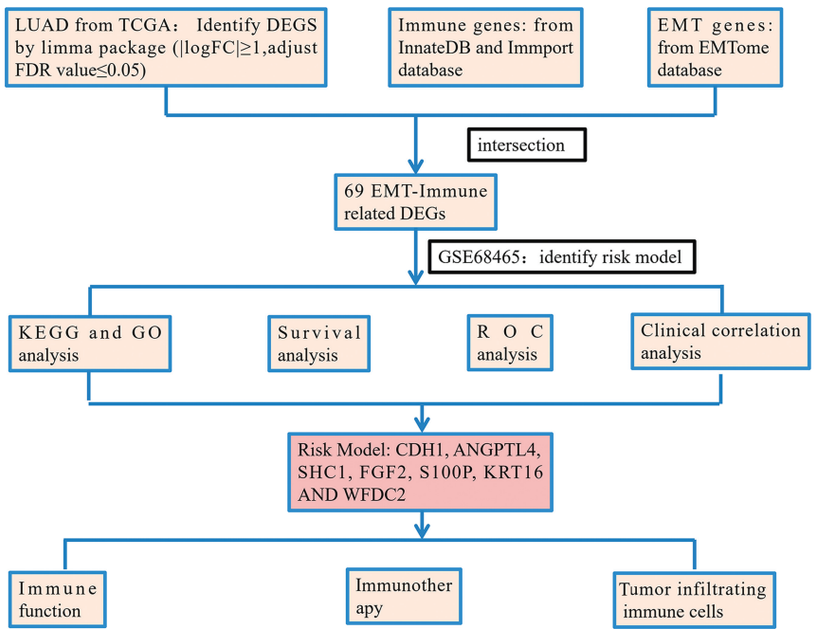

Figure 1. Workflow diagram of text and data mining tools used for the identification of prognostic iEMT genes. FC, fold change; FDR, false discovery rate; iEMT, immune-related epithelial-mesenchymal transition.

2.5. Immune status analysis

In TCGA data set, the number of immune cells in patient samples was determined based on the patient's risk value using the Limma package. To determine the differences in the numbers of various immune cells between the high- and low-risk groups, data on the number of immune cells were converted into a ggplot2 input file using the reshape2 package. For survival analysis, patients were divided into high- and low-risk groups based on the immune cell content in different samples. Based on the immune function scores, each patient was assigned to either a high- or low-risk group for survival analysis. The percentage of immune typing in the high- and low-risk groups was calculated using the immune typing of patients in TCGA data set. Immunoscore data predicting CTLA-4 and PD-1 responsiveness were obtained from the TCIA database (https://tcia.at/patients), and differences in the predictors of CTLA-4 and PD-1 responsiveness were analyzed between high- and low-risk groups to predict the effectiveness of CTLA-4 and PD-1 responsiveness.

2.6. Statistical analysis

The variance was analyzed using the Limma package. For GO and KEGG analyses, we used the ClusterProfiler package. Plotting was performed using the ggplot package, ROC curves were generated using the survROC package, and heat maps were created using the pheatmap package. The data batch was corrected using the Sva package, and survivor and Cox regression analyses were performed using the survival package. All analyses were performed using the statistical software R version 4.2.1.

3. Results

3.1. Identification of differentially expressed iEMT genes and cluster analysis

Epithelial-mesenchymal transition plays a critical role in tumorigenesis and invasion, and the TME, particularly immune-related TME, can induce EMT. To explore the role of the iEMT in LUAD, we

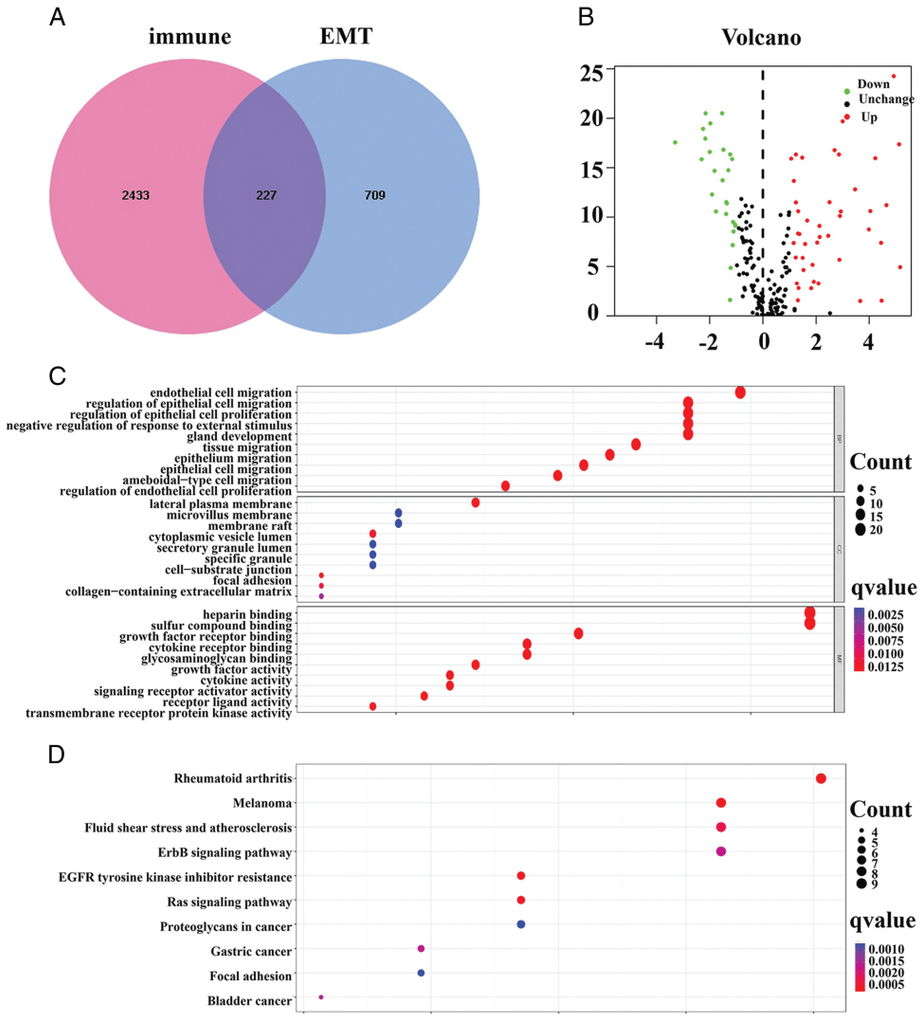

Figure 2. Differentially expressed iEMT genes and enrich analysis. A, The Venn diagram of iEMT genes. B, The volcano of different expressions of iEMT genes. logFC ≤−1 shows green points; logFC ≥1 shows red points. C, GO analysis of different expressions of iEMT genes. D, KEGG analysis of different expressions of iEMT genes. GO, Gene Ontology; iEMT, immune-related epithelial-mesenchymal transition; KEGG, Kyoto Encyclopedia of Genes and Genomes.

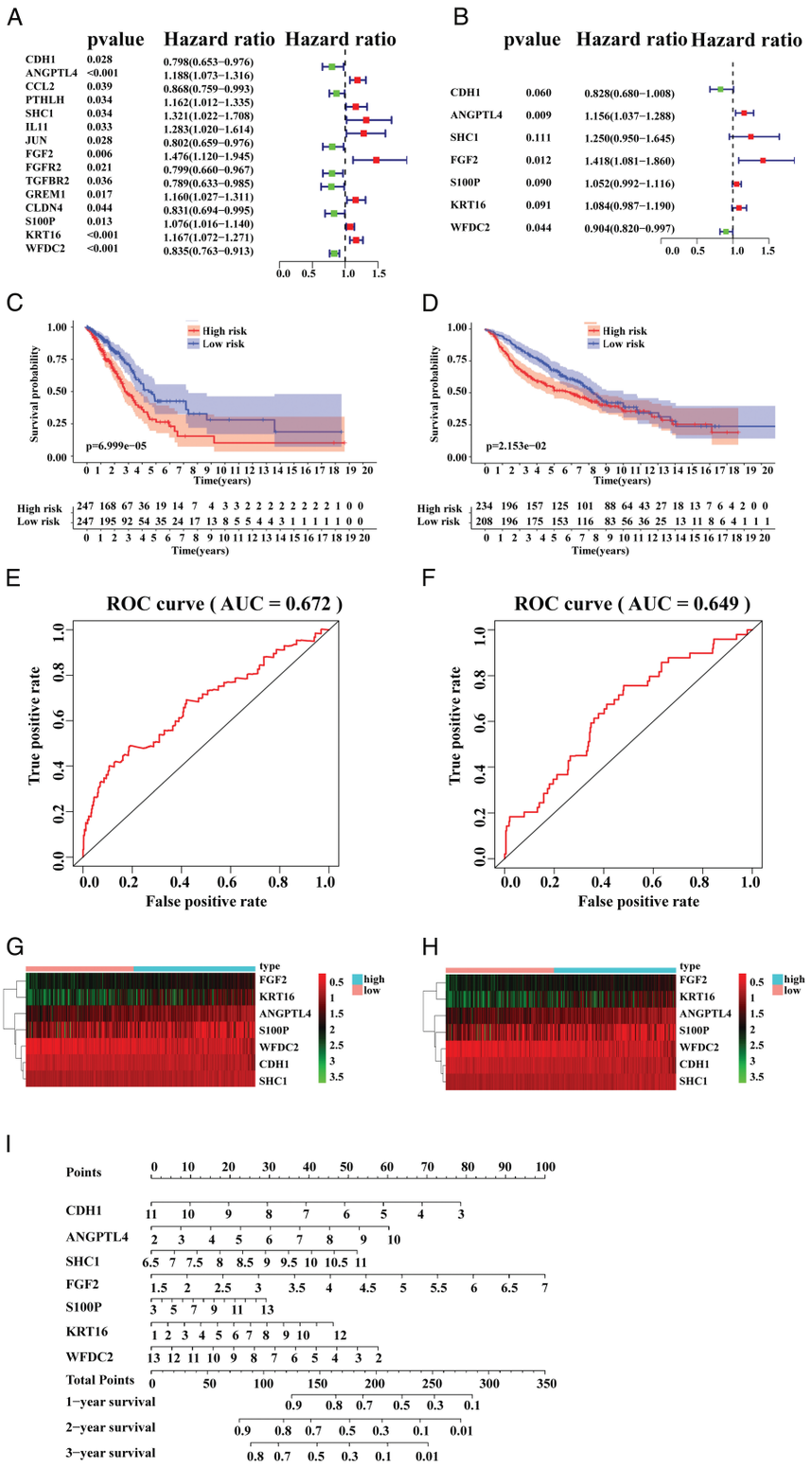

Figure 3. Risk model construction and evaluation. A, univariate Cox analysis by TCGA data. B, multifactorial Cox analysis by TCGA data. C, Survival analysis based on TCGA samples. D, Survival analysis based on GSE68465 samples. E, The area under ROC curves based on the TCGA sample. F, The area under ROC curves based on GSE68465 samples. G, Risk gene expression in TCGA. H, Risk gene expression in GSE68465. I, Nomogram based on the survival status of the samples and the coefficient values of risk genes. ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas.

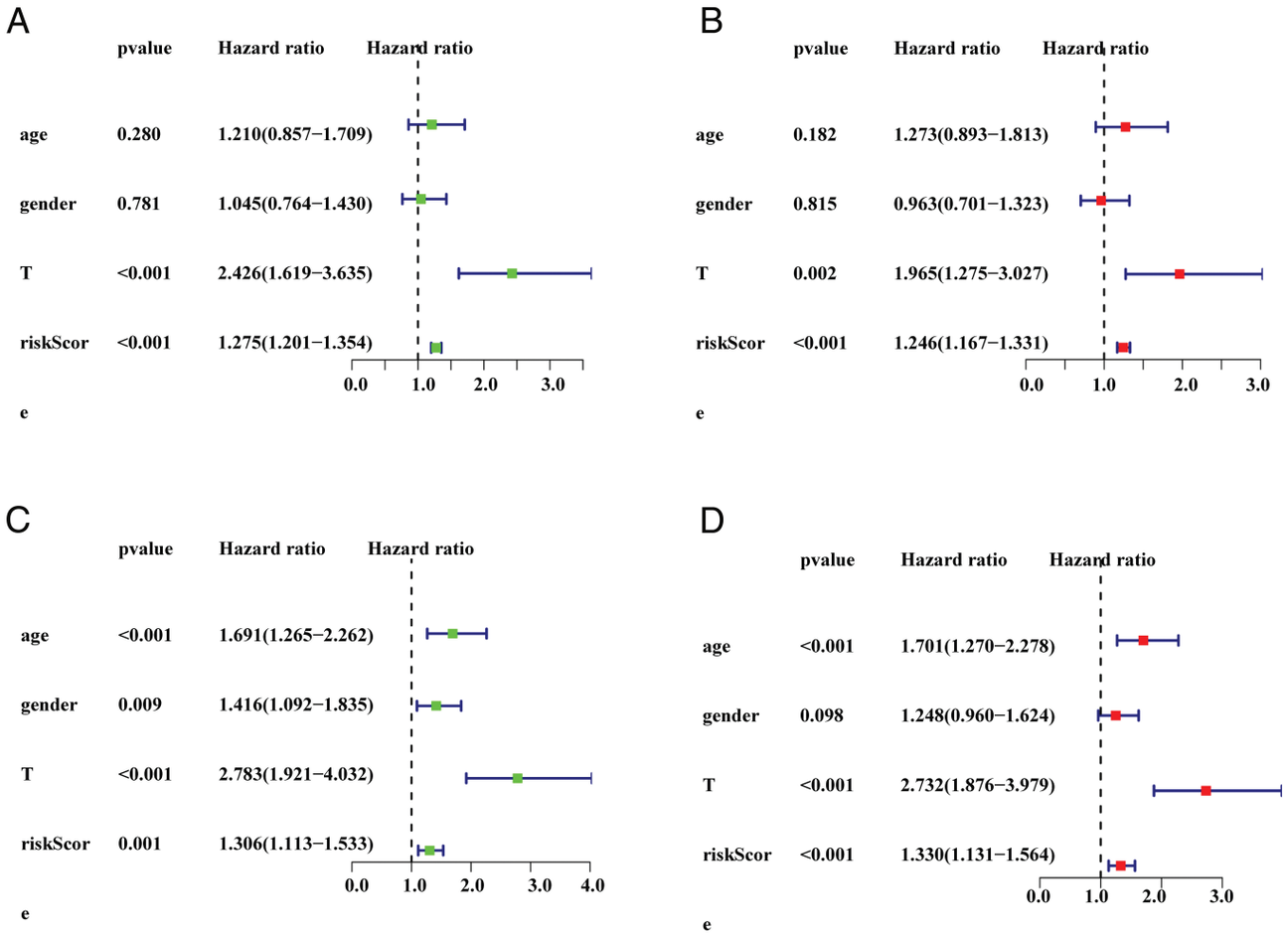

Figure 4. Clinical characteristics analysis. A, Univariate prognostic analysis in TCGA samples. B, Multifactorial prognostic analysis in TCGA samples. C, Univariate prognostic analysis in GSE68465 samples. D, Multifactorial prognostic analysis in GSE68465 samples. TCGA, The Cancer Genome Atlas.

analyzed public databases as outlined in Figure 1. We obtained 2660 unique immune genes from the InnateDB and Import databases and 936 unique EMT genes from the EMTome database. The intersection of these 2 gene sets yielded 227 iEMT genes (Fig. 2A). Subsequently, we intersected the iEMT gene set with LUAD data from TCGA and performed differential expression analysis to identify 69 differentially expressed iEMT genes (Fig. 2B). The results of GO analysis revealed that the DEGs were primarily associated with biological processes such as amoeboid-type cell migration, changes in cellular composition involving the collagen-containing extracellular matrix, and molecular functions associated with receptor-ligand activity (Fig. 2C). The KEGG analysis showed that the DEGs were clustered mainly in the gastric cancer and focal adhesion signaling pathways (Fig. 2D).

3.2. Evaluation of the risk model

The iEMT genes and GSE68465 LUAD RNA-seq were intersected with the key nodal genes, and the resulting intersected genes of iEMT were subjected to univariate Cox analysis by combining them with survival status. The analysis revealed that CDH1, ANGPTL4, CCL2, PTHLH, SHC1, IL11, JUN, FGF2, FGFR2, TGFBR2, GREM1, CLDN4, S100P, KRT16, and WFDC2 had significant impacts on prognosis ($P < 0.05$) (Fig. 3A). Multivariate Cox analysis further suggested that CDH1, ANGPTL4, SHC1, FGF2, S100P, KRT16, and WFDC2 had independent prognostic values (Fig. 3B). A risk model was constructed using the following equation: CDH1 × 0.189 + ANGPTL4 × 0.145 + SHC1 × 0.22 + FGF2 × 0.349 + S100P × 0.05 + KRT16 × 0.08 – WFDC2 × 0.1.

Using the risk model, researchers calculated the risk values of the GSE68465 and TCGA samples and divided them into high- and low-risk groups according to the median risk value. Survival analysis revealed that the risk model was effective in predicting the prognosis of patients in both GSE68465 (Fig. 3C) and TCGA (Fig. 3D) samples ($P < 0.05$). Further validation of the risk model using ROC curves showed that the areas under the ROC curve were 0.672 for the TCGA sample (Fig. 3E) and 0.649 for the GSE68465 sample (Fig. 3F), indicating significant predictive significance. Heat maps were plotted to show the expression of risk genes in the high- and low-risk groups in both the TCGA and GSE68465 samples (Figs. 3G, H). Finally, we constructed a nomogram based on the survival status of the samples and the coefficient values of the risk genes (Fig. 3I), which showed that the survival rate of patients could be directly deduced from the expression of risk genes.

3.3. Clinical traits and risk values

Because there was no tumor stage in the GSE68465 samples, we selected age, sex, T stage, and risk value for univariate and multivariate

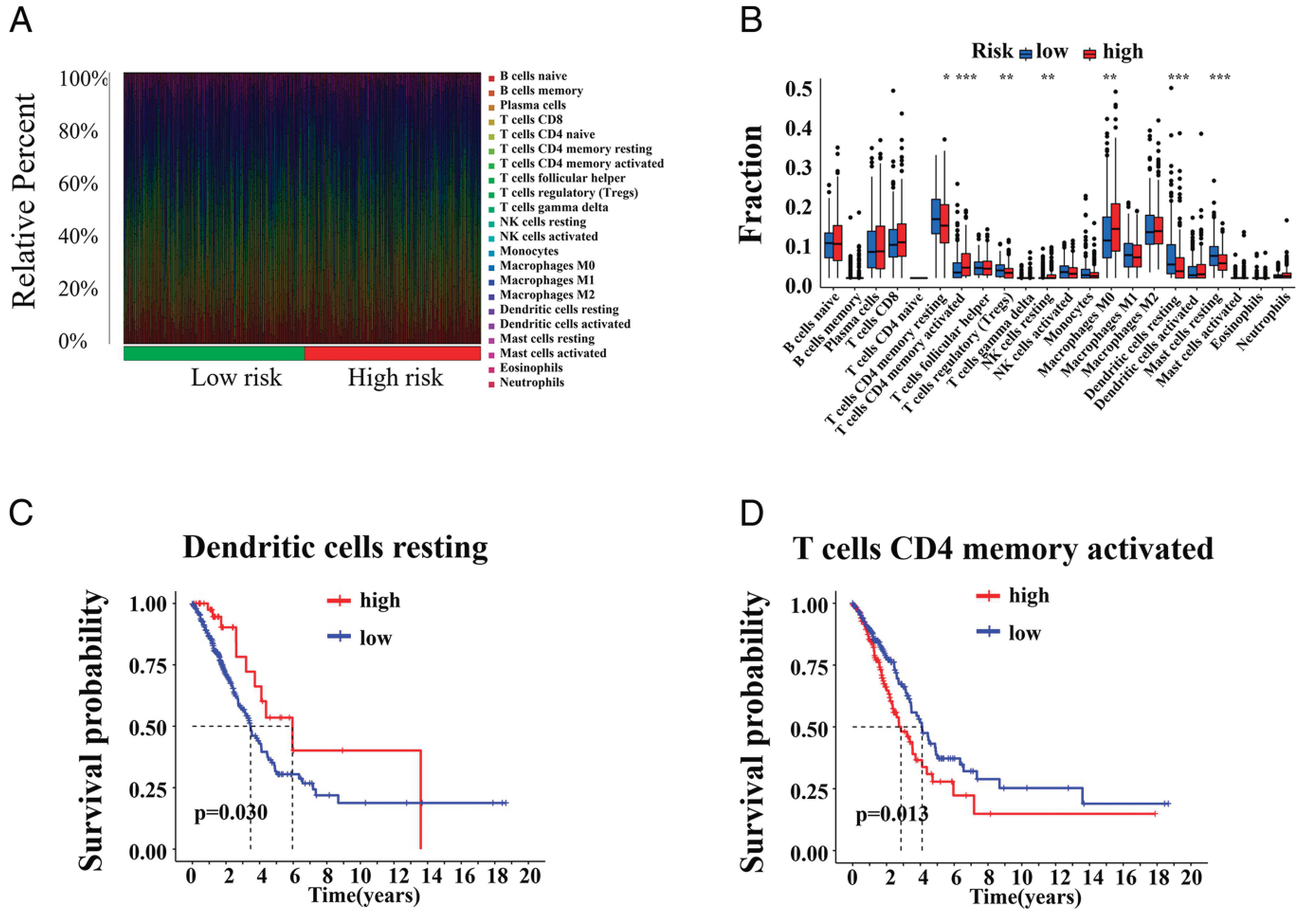

Figure 5. Immune cell infiltration analysis. A, Different immune infiltration in high- and low-risk groups. B, Boxplot of immune cells in high- and low-risk groups. C, Survival analysis for overall survival based on resting dendritic cells. D, Survival analysis for overall survival based on activated $CD4^+$ memory.

independent prognostic analyses and observed that T stage and risk value were independent prognostic factors in the TCGA samples (Figs. 4A, B), whereas age, T stage, and risk value were independent prognostic factors in the GSE68465 samples (Figs. 4C, D). This indicated that the risk value was an independent prognostic factor for patient survival in both TCGA and GSE68465 data sets.

3.4. Immune cell infiltration

Based on the risk values of the samples, immune cell infiltration was plotted, as shown in Figure 5A. By examining the high- and low-risk expression of immune cells in TCGA samples using a boxplot, we discovered that there was a notable decline in resting DCs and mast cells, whereas $CD4^+$ memory T cells in the high-risk group showed a significant increase (Fig. 5B). In addition, survival analysis utilizing both immune infiltration and sample survival data revealed that those with lower expression of resting DCs had unfavorable survival prognoses (Fig. 5C), whereas individuals with higher expression of activated $CD4^+$ memory T cells had poor prognoses (Fig. 5D), consistent with previous research findings.

3.5. Correlation analysis of immune function

The function of immune cells was assessed using relevant markers, and a boxplot was generated through differential analysis of high- and low-risk groups based on patient risk values. The results revealed significantly limited functions in activated DCs, HLA, immature DCs, T-helper cells, and mast cells in the high-risk group, whereas natural killer cells displayed significantly higher functions in the same group (Fig. 6A). Survival analysis of immune function and status in TCGA samples indicated low numbers of activated DCs (Fig. 6B), HLA (Fig. 6C), and mast cells (Fig. 6D). These findings indicate a significant decrease in immune function in the high-risk group and suggest that reduced immune function is associated with a poor survival prognosis.

3.6. Immunotherapy analysis

Data on CTLA-4 and PD-1 responsiveness in TCGA samples were downloaded from The Cancer Imaging Archive database, and the samples were divided into high- and low-risk groups to analyze CTLA-4 and PD-1 responsiveness in these 2 groups. The results revealed that the low-risk group had a more favorable immunotherapeutic effect than the high-risk group, regardless of the condition (Fig. 7), implying that a higher risk leads to a weaker immunotherapeutic effect.

4. Discussion

Despite the significant improvement in patient survival in various metastatic cancer types, such as NSCLC, melanoma, renal cancer, bladder cancer, head and neck squamous cell carcinoma, and lymphoma,

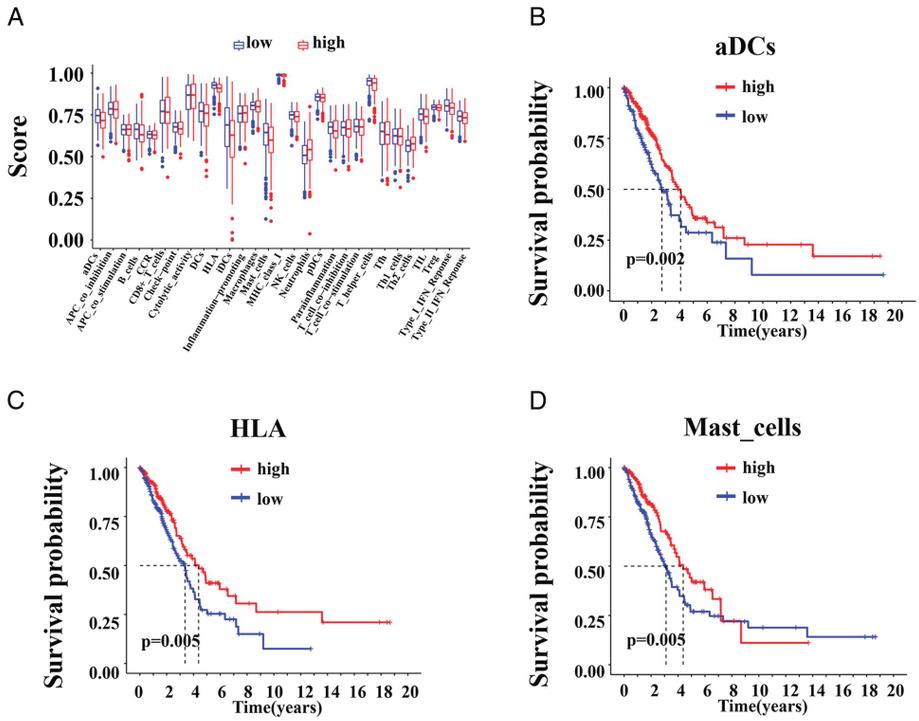

Figure 6. Immune function analysis. A, Boxplot of immune cell function in high- and low-risk groups. B, Survival analysis for overall survival based on aDC function. C, Survival analysis for overall survival based on HLA function. D, Survival analysis for overall survival based on mast cell function. aDC, activated dendritic cells.

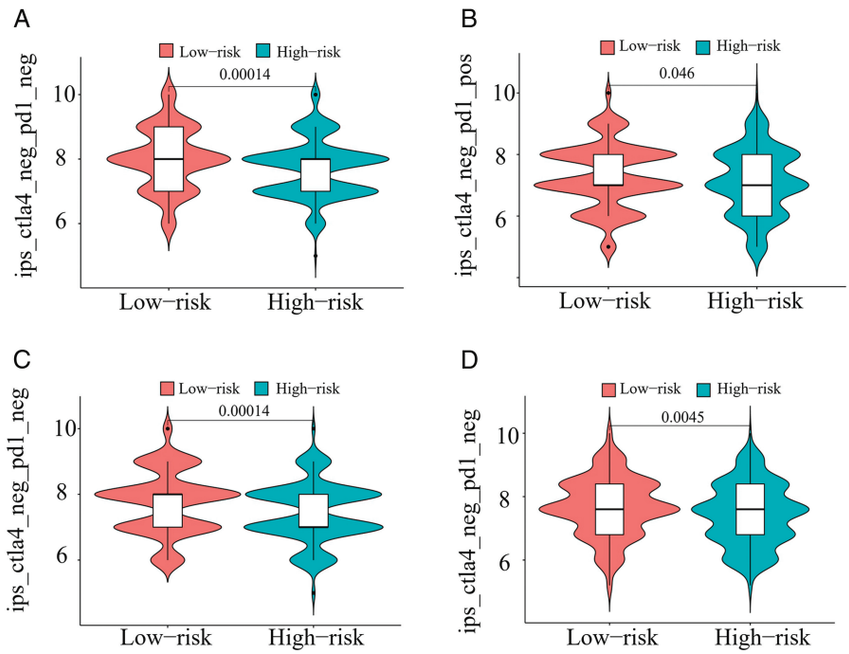

Figure 7. Immunotherapy analysis. A, Immunotherapy analysis of CTLA-4 negative and PD-1 negative in high- and low-risk groups. B, Immunotherapy analysis of CTLA-4 negative and PD-1 positive in high- and low-risk groups. C, Immunotherapy analysis of CTLA-4 positive and PD-1 negative in high- and low-risk groups. D, Immunotherapy analysis of CTLA-4 positive and PD-1 positive in high- and low-risk groups. CTLA-4, cytotoxic T-lymphocyte-associated protein 4.

with the use of immune checkpoint blockers on T cells (anti–PD-1, anti–PD-L1, and anti–CTLA-4), a notable proportion of patients still experience poor treatment outcomes.[15] In addition, even in patients with positive responses, there are notable differences in treatment regimens and expectations owing to changes in the TME during tumor progression and treatment.[16] Epithelial-mesenchymal transition is a key process of immune resistance and is also a potent driver for activating immunosuppressive networks within the TME. Targeted EMT may provide important prospects for immunotherapy approaches to current advanced tumors.[17] Studies have revealed that LUAD can be inhibited by modulating the TME using new immune checkpoint siglec-15 antibodies,[18] with TP53 mutations serving as biomarkers for immune checkpoint blockade therapy in LUAD because of their association with the TME.[19,20] Nonetheless, no systematic studies have explored the key iEMT molecules and subsequent alterations in the TME, emphasizing the significance of understanding immune-related molecular alterations in the TME and targeting specific TME areas in patients with LUAD.

Our results confirmed that the risk model constructed using CDH1, ANGPTL4, SHC1, FGF2, S100P, KRT16, and WFDC2 accurately predicted the prognosis of patients with LUAD. Based on this risk model, our results suggest that the risk values were significantly correlated with the tumor, T, and M stages of patients with LUAD, wherein a higher tumor stage corresponded to higher risk values. This further demonstrated the reliability of the proposed model. Furthermore, the risk model revealed alterations in the TME and differences in immune function in the high-risk group. Among them, CDH1 can promote the self-renewal of LUAD stem cells through the PI3K signaling pathway, thus exerting a procarcinogenic effect.[21] In breast invasive lobular carcinoma, deletion of CDH1 and activation of Pik3ca induce tumor formation and immune cell infiltration.[22] However, the role of CDH1 in immune cell infiltration in LUAD has not been reported. Several studies have reported that ANGPTL4 is an immune gene in LUAD and is strongly associated with patient prognosis.[23,24] Studies have demonstrated that ANGPTL4 is positively associated with the T stage in NSCLC patients with lymph node metastasis and negatively correlated with patient prognosis,[25] suggesting that it may be associated with lymph node metastasis in patients. Our results suggest that ANGPTL4 regulates the TIME. Further investigation of the correlation between ANGPTL4 and TIME is required. FGF2 regulates tumor-associated macrophages, especially M2-type macrophages, and FGF2 knockdown slows tumor growth after radiotherapy.[26] Another study demonstrated that API5 upregulates FGF2 via the FGFR1/PKCδ/ERK signaling pathway, thereby mediating immune escape.[27] S100P is an immune-related prognostic gene in LUAD.[28,29] S100P is also a driving gene that significantly alters the subtype of M1-type macrophages and natural killer cells in the TME, resulting in tumor-associated macrophage migration and M2 polarization, suggesting that it significantly alters the TIME in LUAD, which is consistent with our findings. However, KRT16 has been studied less frequently in this field. KRT16 regulates innate immunity in response to epidermal barrier breach.[30] In LUAD, KRT16 expression was positively associated with lymph node metastasis and significantly altered EMT. Further investigations of TIME alterations induced by KRT16 in LUAD and their mechanisms are necessary.

The therapeutic efficacy of immune checkpoint blockers on T cells in patients with LUAD was verified by downloading the CTLA-4 and PD-1 responsiveness data from The Cancer Imaging Archive database. Our results showed a better responsiveness to immunotherapy in the low-risk group in all cases. This indicates that alterations in the TME of patients with LUAD lead to significant differences in the efficacy of immunotherapy, suggesting that targeted therapy for risk genes may alter the TME and improve patient prognosis.

In conclusion, our results highlight that the risk model constructed using CDH1, ANGPTL4, SHC1, FGF2, S100P, KRT16, and WFDC2 could accurately predict the prognosis of patients with LUAD, present alterations in the TME, and differences in immune function in the high-risk group and provide a novel target for research in the treatment of LUAD.

Acknowledgments

Not applicable.

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Conflicts of interest statement

The authors declare that they have no conflict of interest with regard to the content of this report.

Author contributions

All authors contributed to data acquisition and interpretation, and reviewed and approved the final version of this article.

Data availability statement

Our data are available in public repositories. Immune genes were obtained from the InnateDB (https://www. innatedb. com) and ImmPort (https://immport. org/shared/home) databases. Epithelial-mesenchymal transition–related genes were obtained from the EMTome database (www.emtome.org).

Ethical approval

Not applicable.

References

[1] Cooper WA, Lam DC, O'Toole SA, Minna JD. Molecular biology of lung cancer. *J Thorac Dis*. 2013;5(Suppl 5):S479–S490.
[2] Zappa C, Mousa SA. Non–small cell lung cancer: current treatment and future advances. *Transl Lung Cancer Res*. 2016;5(3):288–300.
[3] Feng J, Zhang X, Zhu H, Wang X, Ni S, Huang J. FoxQ1 overexpression influences poor prognosis in non–small cell lung cancer, associates with the phenomenon of EMT. *PloS One*. 2012;7(6):e39937.
[4] Byers LA, Diao L, Wang J, et al. An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. *Clin Cancer Res*. 2013;19(1):279–290.
[5] Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell*. 2008; 14(6):818–829.
[6] Roche J. The epithelial-to-mesenchymal transition in cancer [published correction appears in *Cancers* 2018;10:52]. *Cancers (Basel)*. 2018; 10(3):79.
[7] Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. *Nat Rev Cancer*. 2018;18(2):128–134.
[8] Chaffer CL, San Juan BP, Lim E, Weinberg RA. EMT, cell plasticity and metastasis. *Cancer Metastasis Rev*. 2016;35(4):645–654.
[9] Chae YK, Chang S, Ko T, et al. Epithelial-mesenchymal transition (EMT) signature is inversely associated with T-cell infiltration in non–small cell lung cancer (NSCLC). *Sci Rep*. 2018;8(1):2918.
[10] Dominguez C, David JM, Palena C. Epithelial-mesenchymal transition and inflammation at the site of the primary tumor. *Semin Cancer Biol*. 2017;47:177–184.
[11] Altorki NK, Markowitz GJ, Gao D, et al. The lung microenvironment: an important regulator of tumour growth and metastasis. *Nat Rev Cancer*. 2019;19(1):9–31.

[12] Nan X, Wang J, Liu HN, Wong STC, Zhao H. Epithelial-mesenchymal plasticity in organotropism metastasis and tumor immune escape. *J Clin Med*. 2019;8(5):747.
[13] Mak MP, Tong P, Diao L, et al. A patient-derived, pan-cancer EMT signature identifies global molecular alterations and immune target enrichment following epithelial-to-mesenchymal transition. *Clin Cancer Res*. 2016;22(3):609–620.
[14] Kim S, Koh J, Kim MY, et al. PD-L1 expression is associated with epithelial-to-mesenchymal transition in adenocarcinoma of the lung. *Hum Pathol*. 2016;58:7–14.
[15] Seidel JA, Otsuka A, Kabashima K. Anti–PD-1 and anti–CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations. *Front Oncol*. 2018;8:86.
[16] Petitprez F, Meylan M, de Reyniès A, Sautès-Fridman C, Fridman WH. The tumor microenvironment in the response to immune checkpoint blockade therapies. *Front Immunol*. 2020;11:784.
[17] Terry S, Savagner P, Ortiz-Cuaran S, et al. New insights into the role of EMT in tumor immune escape. *Mol Oncol*. 2017;11(7):824–846.
[18] Xiao X, Peng Y, Wang Z, et al. A novel immune checkpoint siglec-15 antibody inhibits LUAD by modulating mϕ polarization in TME. *Pharmacol Res*. 2022;181:106269.
[19] Lin X, Wang L, Xie X, et al. Prognostic biomarker TP53 mutations for immune checkpoint blockade therapy and its association with tumor microenvironment of lung adenocarcinoma. *Front Mol Biosci*. 2020;7:602328.
[20] Sun H, Liu SY, Zhou JY, et al. Specific TP53 subtype as biomarker for immune checkpoint inhibitors in lung adenocarcinoma. *EBioMedicine*. 2020;60:102990.
[21] Ye T, Li J, Sun Z, et al. Cdh1 functions as an oncogene by inducing self-renewal of lung cancer stem-like cells via oncogenic pathways. *Int J Biol Sci*. 2020;16(3):447–459.
[22] An Y, Adams JR, Hollern DP, et al. Cdh1 and Pik3ca mutations cooperate to induce immune-related invasive lobular carcinoma of the breast. *Cell Rep*. 2018;25(3):702–714.e6.
[23] Chen S, Zhang J, Li Q, et al. A novel secreted protein-related gene signature predicts overall survival and is associated with tumor immunity in patients with lung adenocarcinoma. *Front Oncol*. 2022;12:870328.
[24] Zhu J, Wang M, Hu D. Identification of prognostic immune-related genes by integrating mRNA expression and methylation in lung adenocarcinoma. *Int J Genomics*. 2020;2020:9548632.
[25] Sun L, Zhang Z, Yao Y, Li WY, Gu J. Analysis of expression differences of immune genes in non–small cell lung cancer based on TCGA and ImmPort data sets and the application of a prognostic model. *Ann Transl Med*. 2020;8(8):550.
[26] Im JH, Buzzelli JN, Jones K, et al. FGF2 alters macrophage polarization, tumour immunity and growth and can be targeted during radiotherapy. *Nat Commun*. 2020;11(1):4064.
[27] Noh KH, Kim SH, Kim JH, et al. API5 confers tumoral immune escape through FGF2-dependent cell survival pathway. *Cancer Res*. 2014;74(13):3556–3566.
[28] Wang Z, Chen X. Establishment and validation of an immune-associated signature in lung adenocarcinoma. *Int Immunopharmacol*. 2020;88:106867.
[29] Shi J, Wang Z, Guo J, et al. Identification of a three-gene signature based on epithelial-mesenchymal transition of lung adenocarcinoma through construction and validation of a risk-prediction model. *Front Oncol*. 2021;11:726834.
[30] Lessard JC, Piña-Paz S, Rotty JD, et al. Keratin 16 regulates innate immunity in response to epidermal barrier breach. *Proc Natl Acad Sci U S A*. 2013;110(48):19537–19542.

Original Research

Oncology and Translational Medicine

OPEN

Analysis of *EGFR* gene mutations in lung adenocarcinoma in Karamay, Xinjiang, China

Yu-Hua Ma, Yuan-Xin Li, Min Jiang, Jing Gao, Yi-Ning Lai, Kamila Kulaixijiang, Min Zhu*, Fei Liang*

Abstract

Background: To investigate the mutation types and mutation rate of the epidermal growth factor receptor (*EGFR*) gene in patients with lung adenocarcinoma and the clinical features of lung adenocarcinoma with *EGFR* gene mutations in Karamay, Xinjiang, China.

Methods: Paraffin-embedded tissue samples of adenocarcinoma patients were collected in the Karamay Central Hospital from March 2016 to June 2019, and mutations in exon 18–21 of the *EGFR* gene were detected by the allele-specific amplification polymerase chain reaction (Amplification Refractory Mutation System–PCR) method. The relationships between the mutation types, mutation incidence, and clinical features were analyzed.

Results: Of the 170 patients with lung adenocarcinoma, 83 had *EGFR* mutations. The total mutation rate of *EGFR* in patients with lung adenocarcinoma was 48.8%, which included mutations in exons 18 (1.2% [2/170]), 19 (19.4% [33/170]), 20 (2.4% [4/170]), and 21 (20.6% [35/170]). Intriguingly, there was a case with 9 mutations in exons 20 and 21. The mutations in exon 19 of *EGFR* resulted in the deletion of codons 746 to 750. The main mutation in exon 21 was L858R (91.4% [32/35]). There was no significant difference in exons 19 and 21 mutation rates ($P > 0.05$). The mutation rate of *EGFR* in female patients was significantly higher than that in male patients ($P < 0.05$) but had no correlation with the age, smoking status, and clinical stage of patients with non–small cell lung cancer ($P > 0.05$). The *EGFR* mutation rate may be related to the degree of tumor differentiation.

Conclusions: Among patients with lung adenocarcinoma in Kelamayi (city in Xinjiang), *EGFR* mutations were more frequently detected in female patients, and the main sites of mutations were exons 19 and 21.

Keywords: *EGFR* gene; Gene mutation; Lung adenocarcinoma

1. Introduction

In 2018, there were an estimated 18.1 million new cases of cancer worldwide, with 9.6 million deaths. However, the number of new cases in China was 3,804,000, and the number of death cases was 2,296,000, accounting for more than 20% of the global number.[1] Data show that the incidence and mortality of lung cancer rank first both globally and in China, accounting for 11.6% and 18.4% (global),[1] and 20% and 27.3% (China) of the total population of cancer cases, respectively.[2] More than 80% of lung cancer is non–small cell lung cancer. Moreover, approximately 80% of patients with non–small cell lung cancer are diagnosed in the middle and advanced stages; 24% have regional lymph node metastasis,[3] and 55% to 57% of new non–small cell lung cancer cases have been found to have distant metastasis.[3,4] According to the data published in the United States from 2008 to 2014, the 5-year total survival rate related to non–small cell lung cancer was only 22.7%, whereas that of patients with regional lymph node metastasis was 33.4%, and that of patients with distant metastasis was only 5.5%.[3] With the development of tumor molecular biology, research on epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) has become a hotspot. As a small-molecule EGFR inhibitor, it provides a new scheme for targeted drug use in patients with lung cancer. A number of large phase III randomized controlled clinical studies, such as First-SIGNAL, WJT0G3405, NEJ002, OPTIMAL, ENSURE, and EURTAC, have successively confirmed that the efficacy of EGFR-TKIs in patients with lung cancer with *EGFR* mutations is significantly better than that of traditional chemotherapy. The median progression-free survival for EGFR-TKI treatment can reach approximately 9 to 13 months, the objective response rate is approximately 60% to 85%, and adverse reactions and quality of life are significantly better than those associated with chemotherapy.[5,6] *EGFR* kinase activation mutations are the most important predictors of EGFR-TKI efficacy. *EGFR* mutations mainly occur in exons 18 to 21. Among these, deletion mutations in exon 19 and L858R point mutations in exon 21 are the most common subtypes of *EGFR* mutations, accounting for 90% of all mutation types.[7] Clinical studies have shown that different mutations have different sensitivities to TKI treatment.[8,9] It has been reported that the *EGFR* mutation rate is 10% in White patients with lung cancer and 30% in Asian patients.[10] It has also been reported that the incidence of *EGFR* mutations is higher

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Table 1

Analysis of *EGFR* mutation types and rates in 170 patients with lung adenocarcinoma

Exon	Mutation type	No. mutations	Mutation rate (%)
18	G719X	2	1.2
19	Deletion	33	19.4
20	T790M	4	2.4
21	L858R and L861Q	35	20.6
Composite mutation	20 and other	9	5.3
Total		83	48.8

in female and nonsmoking patients.[11] *EGFR* mutations also differ in different regions of China.[12,13] The purpose of this study was to investigate: (1) the *EGFR* mutation type and rate and (2) the clinicopathological characteristics, in patients with lung adenocarcinoma in Karamay, Xinjiang, China.

2. Materials and methods

2.1 Patient selection

A total of 170 patients with lung adenocarcinoma were selected from the Central Hospital of Karamay, Xinjiang, from June 2016 to June 2019. The cohort included 82 male and 88 female patients and 124 nonsmoking patients, with a median age of 64 years (range, 34–89 years). All tumor tissues were paraffin-embedded. Two pathologists diagnosed adenocarcinoma of the lung, with 98 cases of stage I/II and 72 cases of stage III/IV.

2.2 Methods

Amplification Refractory Mutation System was used for real-time quantitative PCR amplification of exons 18, 19, 20, and 21 of the *EGFR* gene. An *EGFR* mutation detection kit (The cobas EGFR Mutation Test v2 reagent batch no. 180828; Roche, USA) with a cobas z 480 analyzer was used to detect 45 mutations in exons 18 to 21 by PCR amplification. All procedures were conducted according to the manufacturer's instructions. To determine the effectiveness of the run, a mutant control and a negative control were included in each response. Amplification conditions and parameters: reaction cycle parameters: 95°C for 5 minutes, 1 cycle; 95°C for 25 seconds, 64°C for 20 seconds, 72°C for 20 seconds, 10 cycles; 93°C for 25 seconds, 60°C for 35 seconds, 72°C for 20 seconds, 36 cycles.

2.3 Statistical analysis

SPSS 20.0 statistical software (IBM Corp, Armonk, NY) was used for analysis, and the rates were compared using the χ^2 test or Fisher exact probability test. Statistical significance was set at $P < 0.05$.

3. Results

3.1 *EGFR* gene mutation

Among the 170 lung adenocarcinoma patients, 83 patients had *EGFR* gene mutations, with a total mutation rate of 48.8%. The mutation rates of exons 18, 19, 20, and 21 were 1.2% (2/170), 19.4% (33/170), 2.4% (4/170), and 20.6% (35/170), respectively. There were 9 cases of composite mutation; 6 cases of T790 mutations combined with others, including 19 (3 cases), 20 (1 case), 21 (2 cases), 1 case of L858R combined with L768I, 1 case of Del19 combined with E20ins, and 1 case of L858R combined with L861Q. All mutations in exon 19 resulted in the deletion of codons 746 to 750. Of the mutations in exon 21, 91.4% (32/35) were L858R, and there was no significant difference between the mutation rates of exons 19 and 21 ($\chi^2 = 0.735$, $P = 0.786$). These mutations (19 and 21) accounted for 91.6% (33 + 35 + 8/83) of the total mutations (Table 1).

3.2 Relationship between *EGFR* gene mutation and clinical features

The mutation rate of the *EGFR* gene was 59.1% (52/88) in female patients compared with 37.8% (31/82) in male patients ($\chi^2 = 7.697$, $P = 0.006$), 38.9% (14/36) in patients 64 years or younger and 31% (13/42) in patients older than 64 years ($\chi^2 = 0.539$, $P = 0.463$), and 43.5% (20/46) in smokers and 50.8% (63/124) in nonsmokers, with no significant difference ($\chi^2 = 0.721$, $P = 0.396$). The subgroup analysis revealed that the mutation rate in male smokers was 42.86% (18/42), and that in nonsmokers was 32.5% (13/40), with no significant difference ($\chi^2 = 0.935$, $P = 0.334$). In contrast, the mutation rate in female smokers was 50% (2/4), and that in nonsmokers was 59.5% (50/84), with no difference between the 2 groups ($P = 1$). The mutation rates were 59.61% (31/52), 52.83% (28/53), 35.18% (19/54), and 55.55% (5/9) in the highly differentiated, moderately differentiated, poorly differentiated, and undifferentiated groups, respectively. This difference was statistically significant ($\chi^2 = 7.90$, $P = 0.048$). The *EGFR* gene mutation rate of patients with clinical stage I + II was 51.0% (52/102), and that of patients with stage III + IV was 45.6% (31/68); the difference was not statistically significant ($\chi^2 = 0.091$, $P = 0.763$). Therefore, the results of this study suggest that in Karamay, Xinjiang, mutation of the *EGFR* gene is correlated with sex and degree of tissue differentiation in patients with lung adenocarcinoma, but not with age, smoking status, or clinical stage (Table 2).

4. Discussion

The *EGFR* gene consists of 28 exons encoding 1186 amino acids and is located in human chromosome regions 7p13–q22. Major *EGFR* mutations occur in 4 consecutive exons (18, 19, 20, 21). In Asian patients

Table 2

The relationship between *EGFR* mutation and clinicopathologic features in 170 patients with lung adenocarcinoma

Clinical features	Case no.	*EGFR* mutations	χ^2	*P*
Sex			7.697	0.006
Male	82	31		
Female	88	52		
Age, y			0.578	0.447
≤64	87	40		
>64	83	43		
Smoker			0.721	0.396
Yes	46	20		
No	124	63		
Male			0.935	0.334
Yes	42	18		
No	40	13		
Female			0.341	1.0
Yes	4	2		
No	84	50		
Degree of tissue differentiation			7.90	0.048
Highly differentiated	52	31		
Moderately differentiated	53	28		
Poorly differentiated	56	19		
Undifferentiated	9	5		
Clinical stages			0.960	0.327
I, II	102	52		
III, IV	68	31		

with non–small cell lung cancer, the mutation rate of the *EGFR* gene is 30%.[10] The mutation rate in lung adenocarcinoma can exceed 50%.[13,14] The mutation rate of each exon also differs, but the main focus is usually on deletion mutations in exon 19 and L858R mutations in exon 21.[12] In our study, the *EGFR* mutation rate was 48.8% (close to 50%), which was similar to that reported for lung adenocarcinoma in the Han nationality in Xinjiang (45.7%).[15] In our study, exons 19 and 21 mutations accounted for 91.6% (33 + 35 + 8/83) of the total mutations, which was consistent with a previous report.[12] Our study also showed that the mutation rate in exon 19 was not significantly different from that in exon 21 ($P > 0.05$), which is consistent with related reports.[16] Notably, it has been reported that patients with exons 19 and 21 mutations benefit significantly from EGFR-TKI treatment over conventional chemotherapy and that compared with exon 21 L858R mutations patients with *EGFR* 19 exon deletions experience longer progression-free survival and overall survival and a higher response rate to EGFR-TKI treatment.[12]

Studies have shown that *EGFR* mutation rate is significantly correlated with sex, smoking, and histological classification.[15–17] Our study showed that *EGFR* mutations were significantly associated with sex but not with smoking status. *EGFR* mutations have a higher occurrence rate in women, possibly due to female hormonal influences. In contrast, *EGFR* mutations had no significant correlation with smoking status, which may be related to the fact that most women in this area do not smoke. In this study, 4 women smoked, and 2 of them had exon 19 deletion mutations. There was no significant correlation between smoking status and mutation rate in the female subgroup. The male subgroup analysis also found no significant association between smoking status and the mutation rate. This may be related to the small number of patients enrolled; it may also be related to the correlation and overlap between smoking status and sex, which is inconsistent with Xiaofeng and colleagues' view that smoking is the main influencing factor.[17] We believe that sex is the main influencing factor.

Studies have shown that *EGFR* mutations are not significantly associated with age[13,16,17] or clinical stage.[16] In contrast, the correlation with the degree of differentiation[14,18,19] is quite different. In our study, there was no significant correlation between *EGFR* mutations and age, smoking behavior, or clinical stage. At present, some studies suggest that the *EGFR* mutation rate is higher in highly differentiated tumors, whereas others suggest that the *EGFR* mutation rate is not significantly correlated with the degree of differentiation. Our results showed that the *EGFR* mutation rate was significantly higher in patients with highly differentiated tumors ($\chi^2 = 7.90$, $P = 0.048$). Although the classification of pathological types of lung adenocarcinoma has also been updated, the World Health Organization 2016 Classification of Lung Cancer introduces the 2011 International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society classification systems for adenocarcinoma. The core purpose of the classification is to remove the traditional concept of bronchiolar/alveolar carcinoma and introduce the classification of adherent type adenocarcinoma, which is divided into in situ adenocarcinoma, microinvasive adenocarcinoma, and invasive adenocarcinoma according to the degree of invasion. In addition, the traditional mucous-type bronchiolar/alveolar carcinoma is named mucinous adenocarcinoma as an independent new subtype of lung cancer. Infiltrating adenocarcinoma has solid and micropapillary (low differentiation) and acinar and papillary (moderate differentiation) types. The adherent type corresponds to high medium differentiation. Lai et al[18] also found that the expression of *EGFR* mutational antibodies was more common in the mastic dominant type, acinar dominant type, and papillary dominant type, but less common in the solid dominant type and invasive mucinous adenoma, which is consistent with our results. However, some studies[19] have suggested that *EGFR* mutations are not related to the degree of differentiation, so further research and exploration are needed.

5. Conclusion

This study showed that the *EGFR* gene mutation in patients with lung adenocarcinoma in Karamay, Xinjiang, is unique, and the mutation rate is higher in female patients. The most common mutations were exon 19 deletion mutation and exon 21 L858R mutation, and the mutation rate is not significantly associated with age, smoking status, or tumor stage, which may be related to the degree of tumor differentiation. *EGFR* gene mutations are significantly related to the sensitivity to EGFR-TKI drug therapy. There is a consensus among clinicians to select EGFR-TKI drugs for chemotherapy based on *EGFR* mutations. This study provides information on the *EGFR* mutation rate in patients with lung adenocarcinoma in Karamay, Xinjiang, and can provide a reference for clinicians to carry out scientific research.

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Conflicts of interest statement

The authors declare that they have no conflict of interest with regard to the content of this report.

Author contributions

All authors contributed to data acquisition and data interpretation and reviewed and approved the final version of this manuscript.

Data availability statement

Not applicable.

Ethics approval

Patients' personal information, medical records, and other contents involved in this article are used with the consent of patients and treated confidentially. Personnel unrelated to this study were not allowed to view and use these data.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424.

[2] Chen W, Zheng R, Zhang S, et al. Cancer incidence and mortality in China, 2013. *Cancer Lett*. 2017;401:63–71.

[3] Howlader N, Noone AM, Krapcho M, et al. eds. *SEER Cancer Statistics Review, 1975–2017*. National Cancer Institute. Posted to the SEER website. April 2020. Available at: https://seer.cancer.gov/csr/1975_2017/ Accessed August 11, 2023.

[4] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7–33.

[5] Zhang Z, Stiegler AL, Boggon TJ, Kobayashi S, Halmos B. EGFR-mutated lung cancer: a paradigm of molecular oncology. *Oncotarget*. 2010;1(7):497–514.

[6] Jamal-Hanjani M, Wilson GA, McGranahan N, et al. TRACERx Consortium. Tracking the evolution of non–small-cell lung cancer. *N Engl J Med*. 2017;376(22):2109–2121.

[7] Chan SK, Gullick WJ, Hill ME. Mutations of the epidermal growth factor receptor in non–small cell lung cancer—search and destroy. *Eur J Cancer*. 2006;42(1):17–23.
[8] Yang JC, Wu YL, Schuler M, et al. Afatinib versus cisplatin-based chemotherapy for *EGFR* mutation–positive lung adenocarcinoma (LUX-lung 3 and LUX-lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol*. 2015;16(2):141–151.
[9] Mok TS, Wu Y-L, Ahn M-J, et al. AURA3 Investigators. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med*. 2017;376(7):629–640.
[10] Sequist LV, Bell DW, Lynch TJ, Haber DA. Molecular predictors of response to epidermal growth factor receptor antagonists in non–small-cell lung cancer. *J Clin Oncol*. 2007;25(5):587–595.
[11] Buonerba C, Iaccarino S, Dolce P, et al. Predictors of outcomes in patients with EGFR-mutated non–small cell lung cancer receiving EGFR tyrosine kinase inhibitors: a systematic review and meta-analysis. *Cancers (Basel)*. 2019;11(9):1259.
[12] Sheng M, Wang F, Zhao Y, et al. Comparison of clinical outcomes of patients with non–small-cell lung cancer harbouring epidermal growth factor receptor exon 19 or exon 21 mutations after tyrosine kinase inhibitors treatment: a meta-analysis. *Eur J Clin Pharmacol*. 2016; 72(1):1–11.
[13] Jiang WR, Cai YJ, Miao YX, et al. Correlation between EGFR gene mutations and clinical characteristics among lung cancer patients in Shanghai. *Lab Med (Chinese)*. 2017;32(1):30–34.
[14] Kang L, Zheng J, Zhu X. Relationship between *EGFR* mutations and pathological classification and specimen of lung adenocarcinoma. *Zhongguo Fei Ai Za Zhi*. 2017;20(6):382–388.
[15] Wang XL, Gu GM, Yuan J, Liu CL. Research on polymorphrim of epidemal growth factor receptor gene in Uighur patients with lung adenocarcinoma. *J Clin Med Pract (Chinese)*. 2014;18(9):31–33.
[16] Kong YY, Lu XL, Jiang M, et al. Analysis of *EGFR* mutations in non–small cell lung cancer in Jiangxi. *Exp Lab Med (Chinese)*. 2016;34(5):557–660.
[17] Li XF, Liu X, Zhang GJ, Wang YY. Detection of EGFR gene mutation in lung adenocarcinoma by ARMS and its clinicial significance. *J Mod Lab Med (Chinese)*. 2018;33(2):42–45.
[18] Lai YM, Feng Q, Sun Y, et al. Expression of epidermal growth factor receptor mutation specific antibodies in lung adenocarcinoma: evaluation of sensitivity, specificity and relationship to histologic subtypes. *Zhonghua Bing Li Xue Za Zhi*. 2016;45(9):606–611.
[19] Fan LL, Tian JJ, Zhang XB, Zhang Y, Du XB. Research on characteristics of epidermal growth factor receptor gene mutation in Qiang ethnic patients with lung adenocarcinoma in Beichuan, Mianyang. *J Cancer Control Treat (Chinese)*. 2019;32(9):837–840.