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Review

Advancements in Immunotherapy for Advanced Gastric Cancer

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ABSTRACT

Among all types of cancer, globally, stomach cancer is regarded as the fourth-leading cause of fatalities and among the five most common kinds of cancer overall. This article summarizes data and results of clinical trials conducted worldwide in recent years for the treatment of gastric cancer, focusing on immunotherapy for advanced cases of gastric cancer, including chimeric antigen receptor T-cell immunotherapy (CAR-T) and immune checkpoint inhibitors. The article selects several molecular loci and immune drugs with high international recognition and discussion, discusses the therapeutic effects of different targets of molecular therapies and the safety and efficacy of drug treatment in different modalities, and explores new directions for future gastric cancer treatment by comparing experimental data to prove the advantages of immunotherapy and the shortcomings that still exist. In addition, the article refers to the cancer genome atlas (TCGA) study report on molecular typing of gastric cancer in 2014 and the latest experimental data at home and abroad, introduces the relevant studies and controversies of microsatellite instability (MSI) typed gastric cancer and discusses the feasibility of future immunotherapy research for gastric cancer patients who meet the new typing treatment conditions.

Keywords: Gastric cancer; Immunotherapy; Adoptive cellular immunotherapy; Immune checkpoint inhibitors; Microsatellite instability

INTRODUCTION

The fifth most prevalent cancer globally and the fourth leading cause of cancer mortality is gastric cancer, which includes stomach cancer and adenocarcinoma of the gastroesophageal junction.[1] Most Chinese stomach cancer patients are detected at the advanced stage of cancer and cannot be treated surgically.[2] Currently, most Chinese patients with advanced gastric cancer receive SOX (Tegafur and Oxaliplatin) or XELOX (Oxaliplatin and Capecitabine) chemotherapy regimens, but the overall patient response rate has not been shown to improve significantly.[3] The proposal and development of immunotherapy can provide a new treatment direction for patients with advanced gastric cancer, and the announcement of the new cancer genome atlas (TCGA) staging in 2014 suggests the feasibility of targeting gastric cancer lesions based on their unique molecular loci, thereby reducing treatment side effects.[4]

In recent years, numerous studies have demonstrated that, based on the unique molecular locations of gastric cancer lesions, inducing the body to produce an active or passive

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immune response to eliminate gastric cancer cells can improve the therapeutic effect and prognosis of patients on the one hand, and reduce the toxicity of drugs and the harm caused by the treatment to the patient's body on the other.

Currently, immunotherapy for gastric cancer mainly includes: innate immunotherapeutics, tumor vaccines, adoptive cellular immunotherapies, monoclonal antibody therapies, and immune checkpoint inhibitors, among which adoptive cellular immunotherapies and immune checkpoint inhibitor therapies are the most researched and clinically effective [Figure 1].[5]

This article introduces immunotherapy for advanced gastric cancer through different sites of gastric cancer molecules and introduces the existing research results of gastric cancer with microsatellite instability (MSI).

The main types of immunotherapies include CAR-T cell therapy, adoptive cell therapy, cancer vaccine, vascular endothelial growth factor (VEGF) inhibitor, immune checkpoint inhibitor like programmed cell death protein 1 (PD-1), programmed cell death-ligand 1 (PD-L1), T cell immunoglobulin domain and mucin domain-3 (TIM3), lymphocyte activation gene-3 (LAG3), CD28.

ADOPTIVE CELLULAR IMMUNOTHERAPIES

Adoptive cellular immunotherapy refers to the *in vitro* modification of the patient's immune cells to make them highly specific and capable of killing tumor cells. The modified cells are then infused back into the patient's body to stimulate the body's immune response and achieve the therapeutic goal. The main focus here being on specific immunotherapies, also known as chimeric antigen receptor T-cell immunotherapy (CAR-T).

In CAR-T treatment, the patient's T cells are separated and given the chimeric antigen receptor (CAR) and then reconstituted *in vitro* using genetic engineering techniques that allow the cells to simultaneously identify tumor cells and stimulate T lymphocytes [Figure 2].[6] CAR-T cells are developed before being reintroduced into the body, allowing the immune system to attack specific cells expressing the desired antigen, regardless of the major histocompatibility complex (MHC).[7]

Four structural domains make up CAR domains: a spacer area, a transmembrane structural domain, an electrochemical structural region for signaling, and an extracellular structural domain for the identification of antigens. The latest CAR-T cells are now in their fifth generation.[8]

Universal CAR-T cells (UCAR-T) are the most recent CAR-T cells under investigation. The UCAR-T cells, as opposed to the earlier autologous CAR-T cells, have chosen to obtain T cells from a healthy population for gene editing. The advantages of this treatment are that the T cells are not impacted by the patient's physical condition and can be provided quickly, in large quantities, and at a significantly lower cost. In associated studies, some UCAR-T-treated patients saw improvements, but others experienced graft-versus-host disease (GVHD) and host-versus-graft reaction (HvGR). Researchers suggest using genetic approaches to alter or delete rejection-related genes like T cell receptor (TCR) and human leukocyte antigen (HLA) to resolve these problems and prevent GVHD or HvGR.[9]

Currently, the main targets of CAR-T therapy for gastric cancer include claudin18.2 (CLDN18.2), human epidermal growth factor receptor (HER-2), epithelial cell adhesion molecule (EpCAM), carcinoembryonic antigen (CEA), mucin1 (MUC1), mesothelin (MSLN), folate receptor 1 (FOLR1), *etc*. The most popular and researched targets are CLDN18.2, HER-2, EpCAM, CEA, and MUC1.

CLDN18.2 targets

Cellular tight junction protein 18.2 is expressed predominantly in epithelial cells, whose main function is to regulate endothelial barrier permeability. Since CLDN18.2 is overexpressed in 70% of primary and metastatic gastric cancers, inhibiting CLDN18.2 expression specifically is expected to be able to treat stomach cancer that has spread or progressed.[10]

Based on the identification of a single-stranded CLDN18.2 fragment of human origin, Jiang and colleagues developed CAR-T cells in 2019 using the hu8E5 or hu8E5-2I target type. Following medical care, further investigations revealed incomplete or total tumor eradication in a gastric cancer model with patient-derived tumor xenograft (PDX) that was CLDN18.2 positive. What’s more, CAR-T cells could persist and effectively infiltrate into tumor cells in mice while it was found that mice did not exhibit significant detrimental effects on their normal organs, including their gastric tissue. The results demonstrate that the therapy has a good safety profile.[11]

The results of research using CAR-T cells directed against CLDN18.2 (CT041) to treat advanced cancer patients are orally reported in the European Society of Medical Oncology (ESMO) Congress 2021.[12] The 28 late gastric/oesophageal junction cancer patients had an objective response rate (ORR) of 57.1%, 18 of whom had received at least a second-line treatment before the trial. The disease control rate (DCR) for these 18 patients was 83.3%,

with an ORR of 61.1%. According to the study's findings, CT041 has a strong clinical track record for treating patients with late gastrointestinal and oesophageal cancer.[13]

An interim study of CT041 published by Qi *et al.* in 2022 (NCT03874897) provided initial results. The trial recruited patients with CLDN18.2-positive gastrointestinal tumors who had received other treatments, then re-treated them with CLDN18.2-targeted CAR-T cells (CT041). Overall, grade 3 or higher hematologic toxicity was observed in all patients, but no patient experienced neurotoxicity, cytokine release syndrome of level 3 or greater, treatment-related mortality, or dose-limiting toxicity. Patients had an ORR of 48.6%, a DCR of 73.0%, and a 6-month remission rate of 44.8%. In these investigations, stomach cancer patients had a 6-month overall survival rate of 81.2% and ORR and DCR were 57.1% and 75.0%, respectively.[14] The findings of this trial demonstrate that CT041 is an effective and secure therapeutic option for CLDN18^{+} people with gastric cancer who have previously had rigorous therapy.

Clinical investigations targeting CAR-T cells with CLDN18.2 are still ongoing, and together with published experimental data, CLDN18.2 is a potential target for CAR-T treatments, which might lead to substantial advancements in CAR-T therapy research.

HER-2 targets

The epidermal growth factor receptor (EGFR) family of proteins includes the transmembrane protein HER-2, which has tyrosine-protein kinase activity. HER-2 overexpression stimulates many signaling pathways that promote the growth of cells and tumor development. Although HER-2 is infrequently seen in ordinary human tissues, it is overexpressed in several cancers and can be used as an indicator to predict a patient's prognosis.[10]

The ScFV and HER-2 monoclonal antibodies with a hinge region that selectively identifies and binds tumor-associated cell antigens (TAA) make up the extracellular antigen-binding region of HER-2. HER-2 on the tumor surface can also bind to IL-13R2 on the cell barrier via CAR-T cells, further increasing CAR-T cell activity and functionality.

Most HER-2-aimed CAR-T cell trials for stomach cancer are now in the pre-clinical stage. While studies show that HER-2-focused CAR-T treatment is effective for treating late gastric cancer, the treatment's safety has not yet been established. For instance, Song *et al.* used a lentiviral carrier to transfect CD137-anti-HER2ScFV into cells and discovered the CAR-T cells had significant and robust anticancer activity *in vivo* against patient-derived basic gastric cancer cells and HER-2-positive gastric cancer embryonic cells and eliminating HER-2-positive gastric cancer cells *in vitro*.[15]

Although additional research is required to validate the medication's safety, CAR-T has so far demonstrated tentative effectiveness in patients with HER-2-positive gastric cancer.

EpCAM targets

Many distinct epithelial cells and the majority of malignant tumor cells contain the extracellular protein EpCAM, additionally referred to as CD326, on their surfaces, which affects cell signaling, proliferation, differentiation, and migration. More than 90% of people with stomach cancer have elevated levels of EpCAM, which are evenly expressed on the tumor cells' whole surface.[8] EpCAM could be a unique target for CAR-T based on a number of its properties. Additionally, it has been demonstrated that EpCAM expression levels may be utilized to gauge and track the development and prognosis of tumors.

CAR-T cell therapy targeting EpCAM in patients with diverse EpCAM-positive tumors is being evaluated for safety and efficacy in three large clinical trials (NCT02725125, NCT03013712, and NCT03563326).

At the 2022 ESMO Annual Meeting, Prof. Weijia Fang shared a study on CAR-T therapy targeting EpCAM for advanced gastric and colorectal cancers. The research included eight EpCAM-positive patients having advanced gastrointestinal tumors with one or more target lesions, among which there were four gastric cancers and four colorectal cancers. Among all the patients enrolled, 6 were given a medium-low dose and 2 received a high dose.

The results of the study showed that dose-limited toxicity (DLT) and immune effector cell-associated neurotoxicity syndrome (ICANS) did not appear in any one of these patients, and the most common Grade III and higher adverse reactions were haematotoxic effects, including reduced lymphocyte, white blood cell, and platelet counts. One stomach cancer patient had a grade 1-2 cytokine storm (CRS) and one patient with gastric cancer had a grade 3 CRS following the infusion. Both of them returned to normal after treatment.

In terms of efficacy, the disease control rate of the eight patients achieved 75% disease control, one patient had more than 30% tumor regression, experienced partial remission (PR), and had stable disease status for 32 weeks. After days 7-28 of reinfusion, circulating tumor cell (CTC) counts in the patients' blood decreased and cytokine levels increased significantly in all patients.[16]

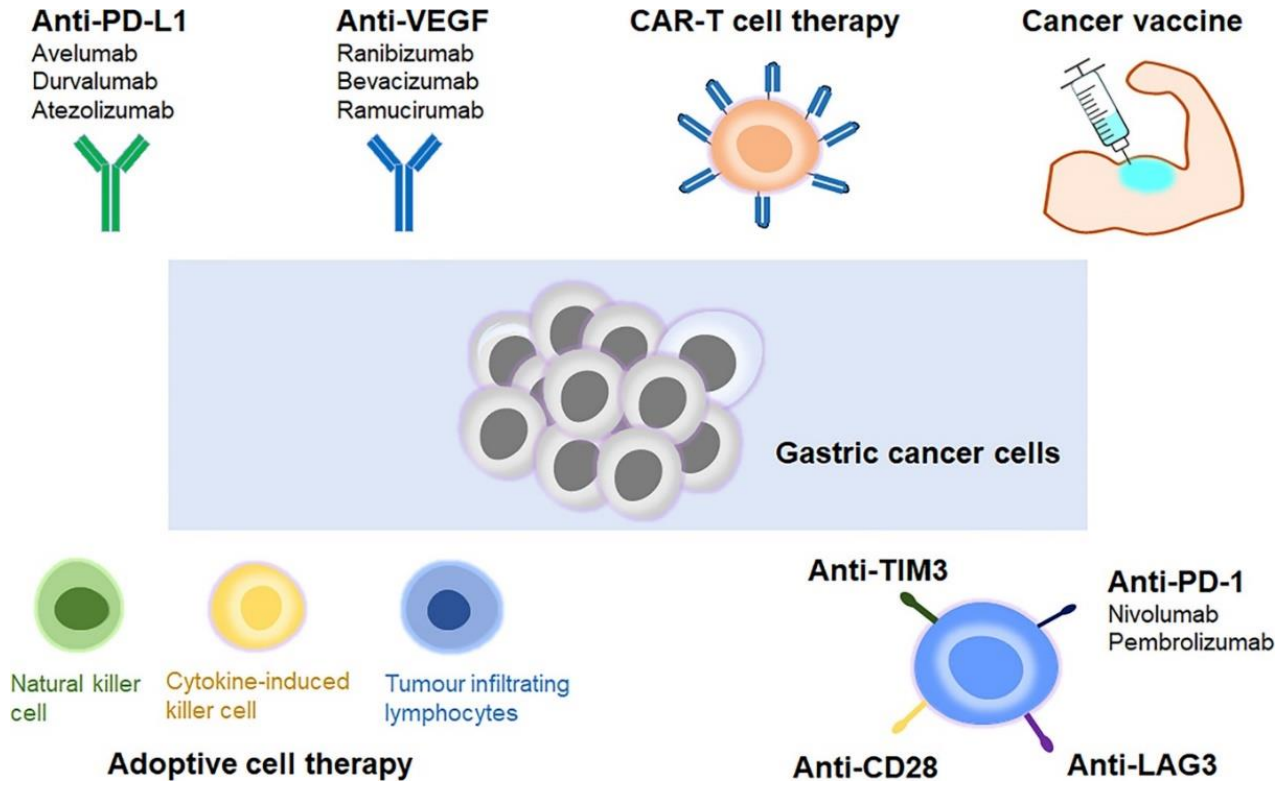

Figure 1. *Different types of immunotherapies in advanced gastric cancer. (Referring to Jin* et al.*)*[5]

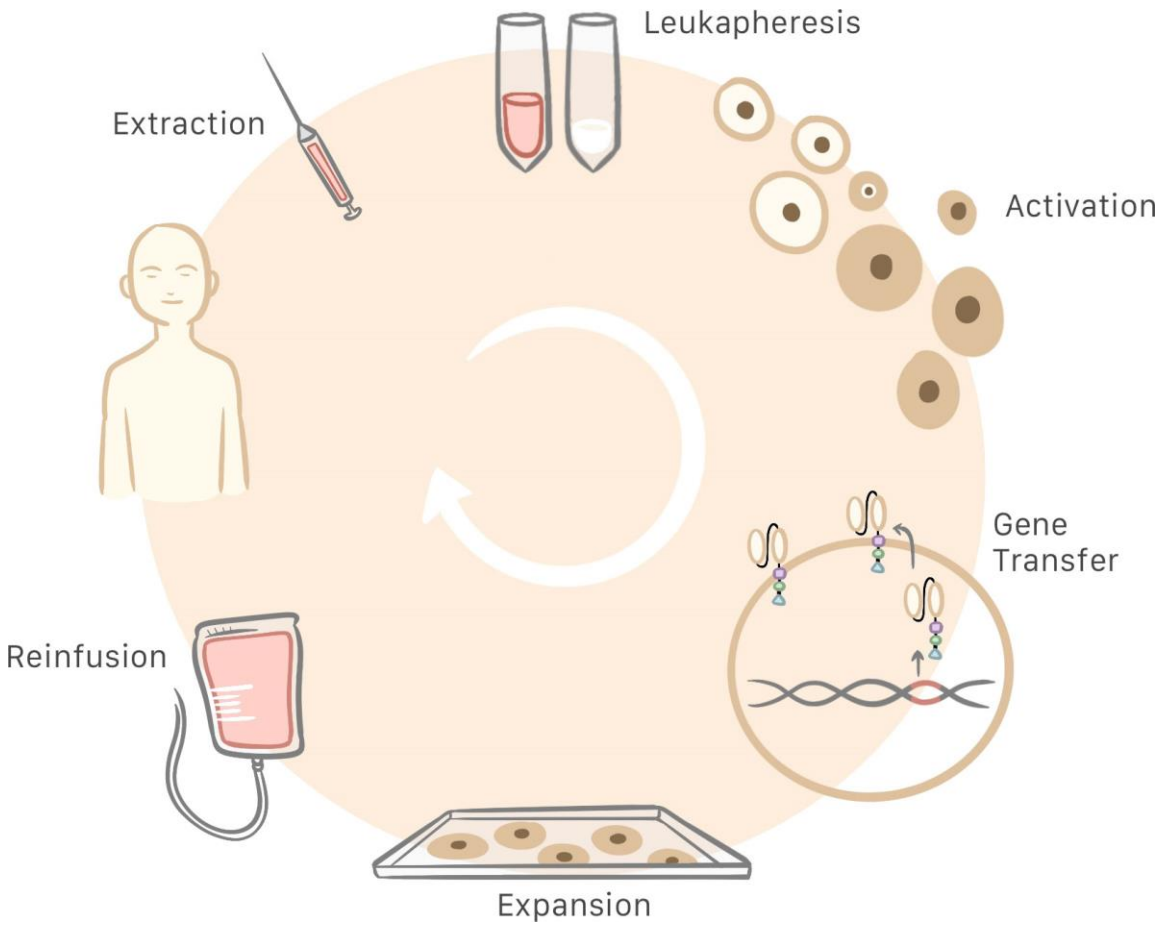

Figure 2. *CAR-T cell therapy progress. (Referring to Domínguez-Prieto et al.)*[6]

In ASCO 2023, researchers presented the latest data from the study with the trial newly enrolling 6 patients with gastric cancer. Of all patients, 2 with PR were seen in the low and medium dose groups and 1 patient with PR underwent successful radical gastric cancer surgery after 28 weeks of treatment while all of the patients in the group were well tolerated. There were 2 patients with DLT in the high-dose group, but no ICANS were observed in any of them.

The apparent effectiveness and tolerability of CAR-T therapy targeting EpCAM indicate that EPCAM is a promising new target for advanced gastric cancer.

CAR-T cells with dual chimeric antigen receptors

Given the specificity of CLDN18.2 and HER-2 in gastric cancer expression, in 2022, Meng *et al.* created the cell CHbbz, which is directed against both HER-2 and CLDN18.2, using a lentiviral infection method. The purpose is to investigate its efficacy against advanced gastric cancer and its effect on off-target effects. The results showed that CHbbz had specific dose-dependent killing activity against CLDN18.2$^+$ HER-2$^+$ tumor cells *in vitro* (P<0.001); In a nude mouse xenograft model inoculated with CH-AGS, only CHbbz produced *in vivo* tumor suppressive activity with tumor cells and tumor infiltration of T cells (P<0.001). In addition, CHbbz was produced only against tumor cells carrying both CLDN18.2$^+$ HER-2$^+$ and tumor-killing potential (P<0.001).[17] The trial confirmed the effectiveness of the cells with dual chimeric antigen receptors for the curative use of positive gastric cancer patients, while the dual chimeric CAR-T cells also produced killing activity only on dual positive tumor cells, reducing the damage to normal tissue cells and decreasing the incidence of off-target effects.

Problems with CAR-T treatment

While novel CAR-T therapeutic targets are developing and the technology is constantly evolving, the problems that arise during the treatment process cannot be ignored. For example, the treatment is limited by the tumor microenvironment (TME), prone to off-target effects, and a series of adverse effects such as cytokine storm brought about by the treatment.

TME

TME is a microenvironment that cancer cells change to encourage cancer cells to develop and consists mainly of inflammatory cells, fibroblasts, and cytokines. Unlike blood tumors where T cells can easily reach their targets, solid tumors exist deep within the body and are unconducive for T cells to transit and infiltrate. On one hand, the extracellular matrix, of which tumor-associated fibroblasts are a major component, and the structurally irregular tumor vasculature, together form a physical barrier that prevents CAR-T cells from homing and infiltrating.[18] On the other hand, tumor cells, tumor stromal cells, and tumor-associated immune cells in TME secret chemokines while the chemokines bind to their corresponding receptors and attract immune cells, which could promote or inhibit tumor growth. In contrast, CAR-T cells lack receptors on their surface that match tumor-secreted chemokines, resulting in their poor homing ability to tumors.[19,20]

The tumor microenvironment also contains various immunosuppressive factors, such as prostaglandin E2 (PGE2), transforming growth factor-β (TGF-β), interleukin6 (IL6), and IL10. It can prevent the activation of T cells and tumor cell death. In addition, the microenvironment contains immune-suppressive cells including T cells with regulatory function (Treg) and suppression cells derived from myeloid-derived suppressor cells (MDCS), which release chemicals like Arg-1 and reactive oxygen species (ROS), limiting the CAR-T cells' ability to destroy cancerous cells.[21]

By controlling inhibitory cell surface receptors such as programmed death ligands through tumor-negative regulatory systems, tumor cells can also suppress cellular function; the gluconeogenesis of tumor cells leads to hypoxia, low pH, and nutrient deprivation in the tumor microenvironment, which is also detrimental to CAR-T cell survival.[22]

It has been discovered that increasing CAR-T cell activity can overcome the cancer microenvironment's therapeutic limitations, as by adding functional chemokine receptors to CAR-T cells to improve T cell targeting and penetration into solid tumors.[23] To increase the anti-cancer efficacy, target CAF, activate CAF-CAR-T cells, and alter T cells such that they co-express a variety of immunostimulatory factors and *in vivo* persistence of CAR-T cells. Engineer CAR-T cells to bind PD-L1ScFV antibody sequences by using monoclonal antibody and gene editing technologies to improve their tumor-specific recognition ability.[24] CAR-T cells are combined with immune checkpoint inhibitors in a therapy known as combined blockade of immune checkpoint PD-1 therapy to prevent the body's T-cells from being suppressed and to increase the anticancer benefits of CAR-T cells [Figure 3]. The approach is currently being used to treat various solid tumors including neuroblastoma and ovarian cancer, and has a high potential for clinical application.[25-27]

Off-target effects

CAR-T treatment carries significant risks, in particular side effects. Targets selected for CAR-T therapy, such as HER-2, are also expressed in normal cells, whereas tumor cells

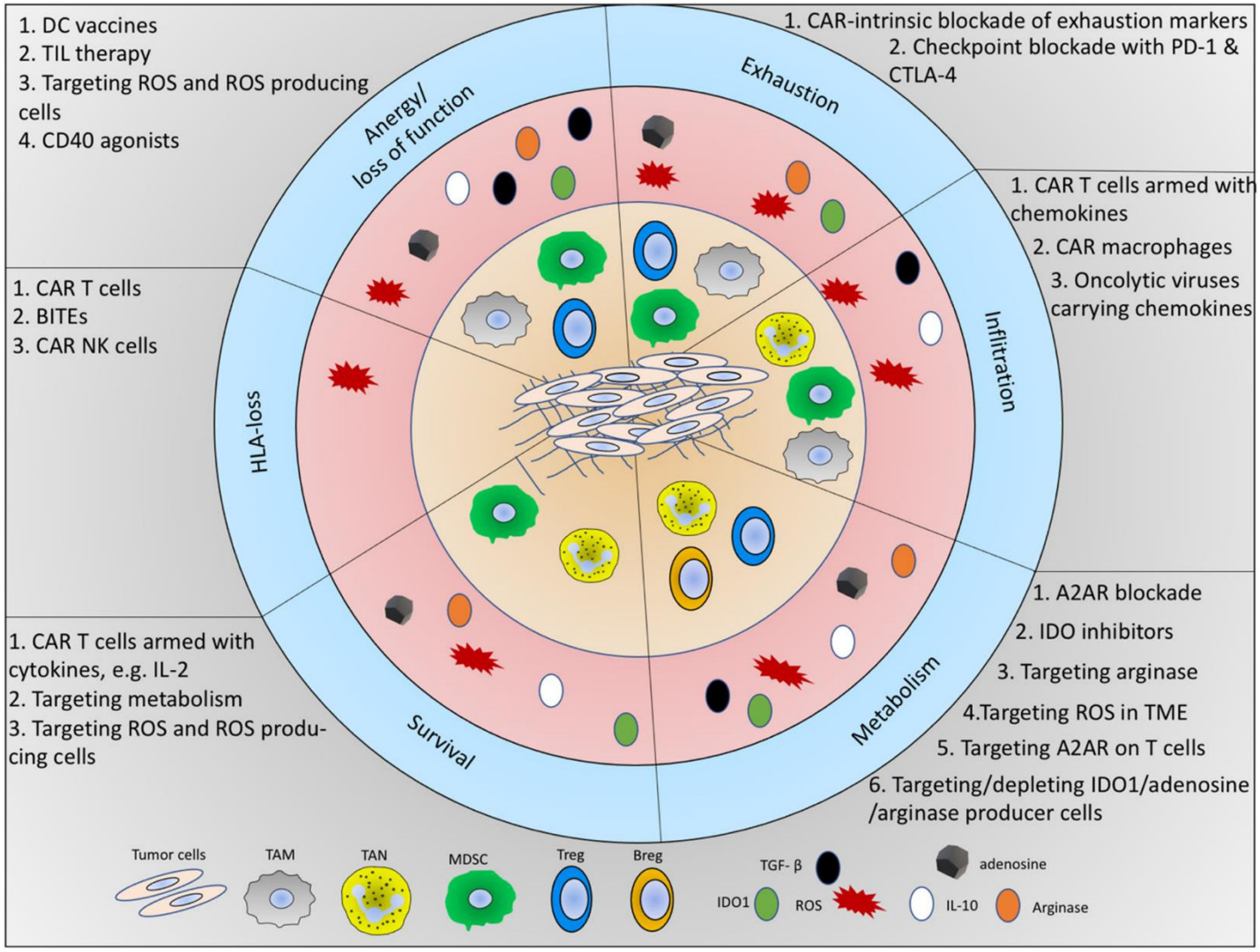

Figure 3. *Diagrammatic review of the TME's immunosuppressive cells and molecules, anti-immunosuppression processes, and immunotherapeutic approaches to combat immunosuppression. (Referring to Balta et al.)*[27]

The innermost (orange area) are tumor cells and different types of immunosuppressive immune cells in the TME, which include tumor-associated macrophage (TAM), tumor-associated neutrophile (TAN), MDSC, Treg and regulatory B cell (Breg). The outer circle (red area) is the immunosuppressive molecules. This region includes TGF-β, ROS, IL-10, IDO1, arginase, and adenosine. And then the blue area outside indicates the mechanism of inhibition on tumor-fighting immune cells like $CD4^+$ T cells, $CD8^+$ T cells, and NK cells. The outermost gray area lists the latest immunotherapeutic strategies corresponding to the different mechanisms.

have no specific target. As a result, CAR-T cells not only kill tumor cells but also damage normal organs, tissues, and cells to varying degrees.[28] The ideal target antigen is TSA, which is expressed only on the surface of tumor cells and can't be found on normal cells. The success of hematologic oncology CAR-T therapy is due to the specific antibodies that appear only at the tumor site. However gastric cancer lacks the specific antigen that appears only on tumor cells.

Dual chimeric receptors were developed by the researchers to limit the ability of CAR-T cells to target two antigens present on tumor cells at once. This limitation prevents CAR-T cells from fully activating until they bind to both targets simultaneously. This lessened side effects and increased the limitation of CAR-T cell attachment to tumor cells.[17] However, this method applies only to patients with tumors expressing two molecular loci at the same time and is more restrictive. Tandem CAR-T is similarly being

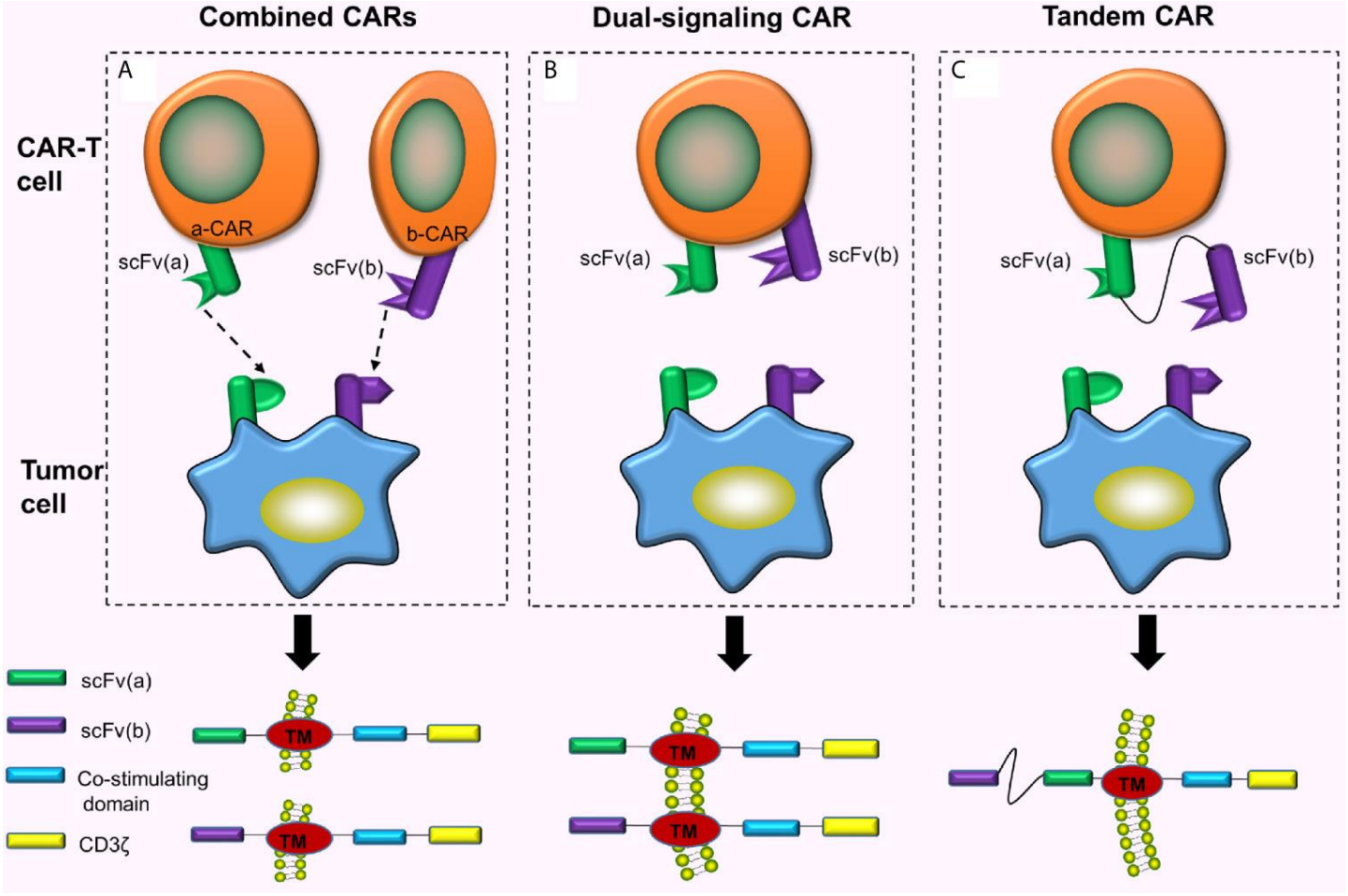

Figure 4. *Combined CARs, Dual-singling CAR, and Tandem CAR. (A) Simultaneous or sequential use of 2 or more different CAR-T cells; (B) Two distinct CARs are expressed simultaneously and separately on a comparable T cell surface; (C) Two distinct scFvs join and create CARs simultaneously. (Referring to Miao et al.)* [29]

investigated. This type of CAR-T cell connects two targets in tandem, which provides a better role in tumor killing and preventing tumor escape than parallel CAR-T [Figure 4].[29]

Cytokine storm (CRS)

CRS, which is also known as cytokine release syndrome, is the most severe side effect that may be caused by CAR-T cells infused back into patients, usually occurring 6-20 days after treatment.[30] When activated by binding to the target antigen, CAR-T cells clear the corresponding tumor cells and stimulate the body's immune system through a dual intracellular signaling domain-co-stimulatory molecule signal, which stimulates the cells to release large amounts of inflammatory factors, such as IFNγ, TNF-α, interleukins. Eventually, the cytokines induce an inflammatory response, activate the immune system, and damage the body's tissues and organs. Severe cytokine storms can even induce respiratory failure, fever, tachycardia, and capillary infiltration syndrome, which can be life-threatening to patients.[31]

The amount of activated CAR-T cells, target antigen density, and the patient's tumor burden all influence how severe CRS is. CRS can be classified into 5 grades according to the severity of the body's response, with grade 3 and above being considered severe CRS, which can be life-threatening and requires close attention and timely intervention. Interleukins (IL-6,10,13), TNF-α, and C-reactive protein are currently considered to be used to predict the severity of CRS.

CAR-T therapy as a novel therapeutic approach has shown greater potential and clinical application in recent experimental studies, and the proposal of new targets such as HER-2 and CLDN-18.2 has also pushed CAR-T therapy towards a new process. However, due to uncontrollable factors such as high treatment costs and small patient samples, currently, the application of CAR-T therapies is limited to clinical trials, while factors such as tumor microenvironment and the broad use of CAR-T treatments are also constrained by cytokine burden. Therefore, there are

still many difficulties to be discovered and overcome before CAR-T therapies are truly used in the clinic.

IMMUNE CHECKPOINT INHIBITOR THERAPY

The immunosuppressive molecule PD-1 also referred to as CD279, is primarily expressed in various immune cells. In 1992, PD-1 expression was first detected in apoptotic T cells by Ishida.[32] In 1999, Dong *et al.* identified and reported the T-cell regulatory function of human B7-H1 and its involvement in tumor immune escape.[33,34] Subsequently, in 2000, Freeman *et al.* demonstrated an interaction and interconnection between B7-H1 and PD-1.[35] After this, as the interaction between them continued to be demonstrated, B7-H1 was named PD-L1 and the pathways of action and biological activities between the two were gradually elucidated and formally applied in tumor research.

PD-1 currently has two ligands, PD-L1 and PD-L2, which are structurally similar. However, PD-L1 can be widely expressed on T cells, B cells, dendritic cells, monocytes, macrophages, various tumor cells, and some lymph node tissues. PD-L2 expression is relatively limited and PD-L1 is typically regarded as the primary ligand of PD-1.[36] Inhibiting T cell proliferation and differentiation while promoting T cell death are all effects of the interaction between PD-1 and PD-L1.[37]

Gastric cancer evades the killing effect by modulating immune checkpoints. According to available research results, PD-L1 expression levels are closely associated with gastric cancer screening, tumor staging, differentiation and patient prognosis, and activation of the PD-1/PD-L1 pathway has been linked to decreased cytotoxic T lymphocyte (CTL) activity, despite the fact that the exact mechanisms are yet unknown. Immune checkpoint drugs like nivolumab and pembrolizumab, that target the PD-1 pathway, have shown promising results in treating patients with late combined gastroesophageal adenocarcinoma. Immune checkpoint drugs prevent inhibitory signaling between T cells and tumor cells by binding to the corresponding target, which protects the differentiation of T cells and promotes body immunity [Figure 5].[29]

Monotherapy

In 2018, findings of the phase III clinical trial (NCT02267343) for advanced stomach cancer based on the ATTRACTION-02 research, investigating the safety and efficacy of the PD-1 checkpoint inhibitor nivolumab in patients with incurable advanced or recurrent gastric/gastrointestinal adenocarcinoma in Asian countries, were announced at the ESMO Congress. After two years of follow-up surveys, it was evident that patients receiving nivolumab treatment had better overall survival than those receiving placebo treatment. Nivolumab-treated patients' median overall survival (mOS) was 5.26 months compared to 4.14 months for placebo-treated patients [hazard ratio (HR)=0.63, 95% confidence interval (CI): 0.51-0.78, $P<0.0001$].[38] In patients who have undergone treatment, nivolumab was reported to have a superior therapeutic impact and has the potential to transform into a new clinical treatment agent.

Subsequently, with the joint promotion of multiple research results, in March 2020, the National Medical Products Administration (NMPA) formally consented to utilize nivolumab monotherapy as the third-line chemotherapy for gastric cancer. In China, nivolumab was also the first immunotherapy medicine to be approved for the treatment of stomach cancer. The Guidelines for the Diagnosis and Treatment of Gastric Cancer, issued in 2022, also recommend nivolumab as a Class I treatment for advanced metastatic gastric cancer in the third line of therapy.[39]

Similar to nivolumab, the clinical trial KEYNOTE-059 examined pembrolizumab's efficacy in patients who had previously been treated and were diagnosed with advanced cancer of the gastroesophageal junction. The trial selected patients underwent at least two different treatments and both of them were unsuccessful, and the results showed that in those subjects who were PD-L1 positive and had a positive score CPS ≥1, pembrolizumab attained an ORR of 12%, and the median overall survival was 5.8 months.[40]

The results showed that in patients with advanced gastroesophageal junction adenocarcinoma who had been treated with at least two different regimens before, pembrolizumab had better results against the tumor and the drug had a longer duration of remission, better overall outcomes, and a satisfactory patient prognosis. Therefore, for patients with PD-L1 CPS ≥1, as a third-line therapy, pembrolizumab monotherapy has been authorized. In addition, in the 2022 edition of the Chinese Society of Clinical Oncology (CSCO) Guidelines, as a Tier 3 recommended agent for the first-line treatment of patients with this kind of cancer, pembrolizumab monotherapy is included.[39]

As a third-line therapy for advanced gastric cancer, PD-1 immune checkpoint inhibitors are not a mainstream choice in clinical gastric cancer treatment. Meanwhile, studies have proven that the therapeutic effect achieved with immune checkpoint inhibitors alone is much lower than expected, therefore, in the current clinical practice treatment, there is a preference for combining immune checkpoint inhibitors with other drugs or therapies.

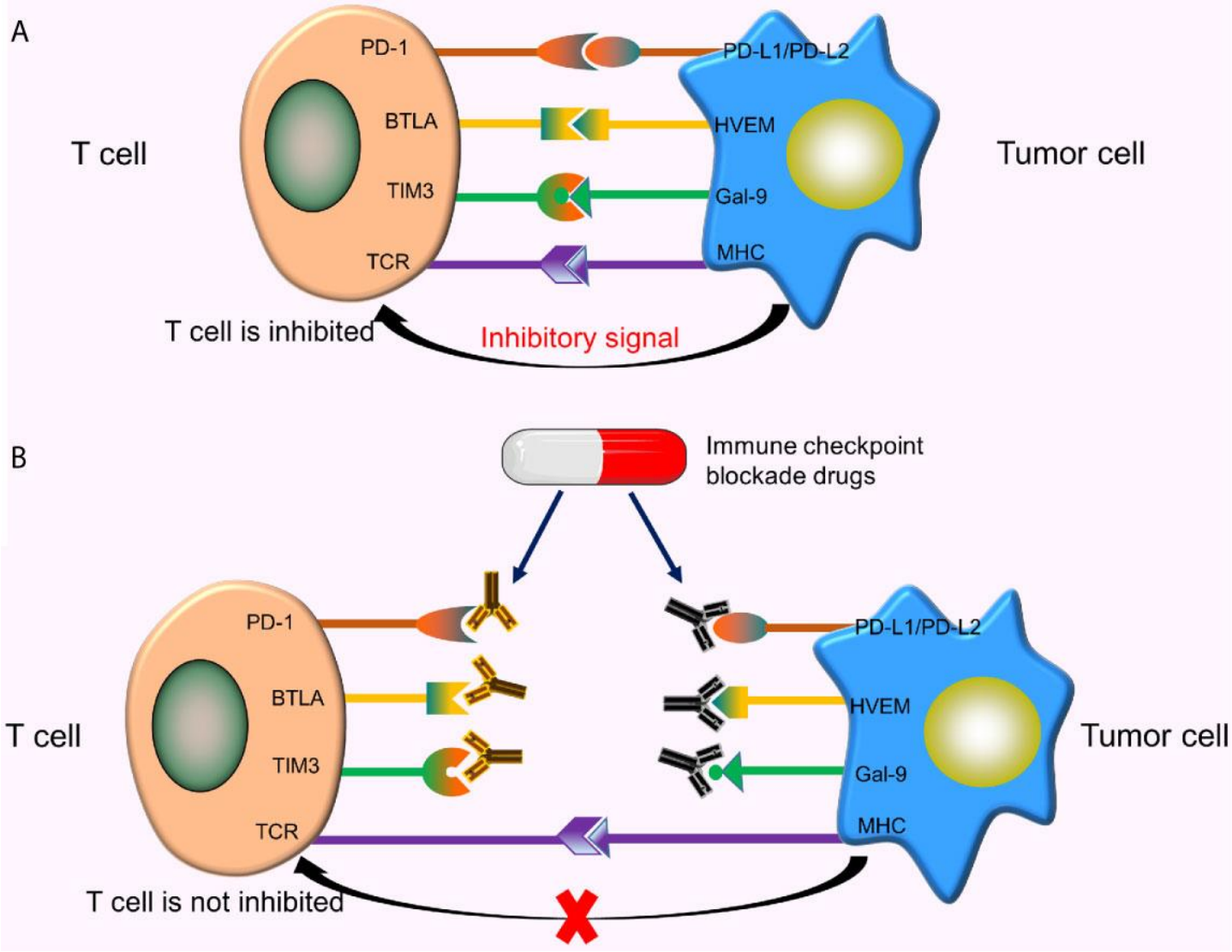

Figure 5. *Immune checkpoints and immune checkpoint inhibitors. (Referring to Miao et al.)* [29] *(A) Prior to administration, the immune checkpoints PD-1, B and T lymphocyte attenuator (BTLA), and TIM3 on T cells can respectively bind to the corresponding ligands PD-L1/PD-L2, herpes virus entry mediator (HVEM) and galectin-9 (Gal-9) on tumor cells, leading to inhibitory signals from the tumor cells to the T cells and inhibiting T cell activation; (B) With the use of immune checkpoint inhibitor drugs, the drugs can bind to targets specific to the surface of T cells or tumor cells, thereby preventing tumor cells from interfering with T cell activation by generating inhibitory signals to the T cells.*

Single-agent combined chemotherapy

Currently, the combination of PD-1 monoclonal antibody and first-line chemotherapy is the most commonly used regimen for multi-protocol combination therapy.

The Checkmate-649 trial compared chemotherapy alone with nivolumab in conjunction with chemotherapy [FOLFOX (Oxaliplatin, Calcium Folinate, and Fluorouracil) or XELOX] in order to assess the effectiveness and security of this treatment. Among all patients with PD-L1 CPS ≥5, the median OS in the combination therapy group was 14.4 months versus 11.1 months in the chemotherapy alone group (HR=0.71, *P*<0.0001), according to data presented by the researchers at the ESMO 2021 meeting. More importantly, comparing the progression-free survival (PFS) data, nivolumab with chemotherapy was found to work better together, improving both the PFS benefit and the overall outcome.[41] Therefore, starting in 2021, Nivolumab in conjunction with FOLFOX/XELOX for two years straight is recommended by the CSCO recommendations as the first-line therapeutic choice for patients with advanced gastric cancer who are HER-2 negative and have a PD-L1 CPS of at least 5.[39]

At the 2023 American Society of Clinical Oncology (ASCO) GI meeting, researchers updated three-year follow-up data for the global population and Chinese subgroup of subjects in the Checkmate-649 trial. A total of 1,581 subjects were selected for the trial with at least 36 months of follow-up, and the results demonstrated that both the PD-L1 CPS-5 group and all randomized patients benefited in terms of OS and PFS improvements when nivolumab was used in conjunction with chemotherapy regimens. In the PD-L1 CPS-5 group, the ORR was 60% (*vs.* 45%) with a median duration of response (mDOR) of 9.6 months (*vs.* 7.0 months) in the nivolumab combination treatment group compared to the chemotherapy group. While among all randomized patients, the nivolumab combination therapy group had an ORR of 58% (*vs.* 46%) and a median DOR of 8.5 months (*vs.* 6.9 months) compared with the chemotherapy group.[42] Therefore, it can be found that nivolumab combination therapy showed a better treatment effect in both the PD-L1 CPS≥5 group and in randomized patients. It means that nivolumab combination therapy had a therapeutic advantage in the whole population of HER-2 negative patients.

For the clinical treatment of cancer, the KEYNOTE-062 study was a phase III clinical trial that compared the various treatment outcomes of two regimens that is pembrolizumab in conjunction with chemotherapy against chemotherapy alone. According to the 2022 ASCO GI conference, individuals with CPS at least 1 level had a 2-year OS of 24.5% and a median PFS of 6.9 months in the pembrolizumab combination chemotherapy group compared to 18.8% and 6.5 months in the chemotherapy alone arm (HR=0.84, 95%CI: 0.70-1.01). Additionally, in patients with CPS more than 10, the median PFS was 5.8 months and 6.2 months, respectively, with a 2-year OS of 28.3% and 21.1% in the groups receiving combination therapy and chemotherapy alone (HR=0.71, 95%CI: 0.52-0.96).[43] Contrary to chemotherapy given alone, combined treatment did not significantly increase patients' chances of survival in cases of stomach cancer.

In 2023, at the ESMO meeting, Merck Sharp & Co. presented detailed data from the Keynote-859 trial, a new trial also studying pembrolizumab in combination with a chemotherapy regimen like Keynote-062. The difference against Keynote-062 is that the new trial Keynote-859 changed the chemotherapy agent to an oxaliplatin-based regimen. They enrolled 1,579 individuals, with a 31-month median follow-up period. The trial's findings revealed that, compared to the placebo group receiving combination chemotherapy, the pembrolizumab arm's mOS was 12.9 months (*vs.* 11.5 months), median PFS of 6.9 months (*vs.* 5.6 months), ORR of 51.3% (*vs.* 42.0%) (*P*=0.00009), and median DOR of 8.0 months (*vs.* 5.7 months). In addition to this, there were 785 grade 3-5 treatment-related adverse events in the pembrolizumab combination therapy group (59.4%) and 787 in the placebo group (51.1%), with approximately 1.0% and 2.0% of patients, respectively, dying from treatment-related adverse events.[44] This trial showed significant results when pembrolizumab was used in combination with chemotherapy and other approaches, illustrating the potential of pembrolizumab for the development of clinical combination therapy, and further studies on pembrolizumab can be expected in the future.

Clinical data from the Orient-16 trial were presented at the ESMO Congress in 2021. The median PFS and median OS for the sintilimab and chemotherapy (XELOX) versus chemotherapy alone arms, respectively, were 7.1 months *vs.* 5.7 months (HR=0.0636, *P*<0.0001), and 18.4 months *vs.* 12.3 months (HR=0.766, *P*=0.0090).[45] Data show that sintilimab has a substantial therapeutic impact on gastric cancer when combined with chemotherapy. For patients with advanced gastric cancer who are HER-2 negative and have a PD-L1 CPS≥5, sintilimab in conjunction with chemotherapy (FOLFOX/XELOX) was successfully included in the CSCO recommendations' 2022 edition.[39]

The efficacy of immune drugs was shown in several of the big studies mentioned above by combining them with chemotherapy *vs.* chemotherapy alone. In terms of clinical use, immunotherapy and chemotherapy combined with immune checkpoint inhibitors like PD-1 immune checkpoint inhibitors are the most frequent therapies for advanced gastric cancer. However, PD-1 immunosuppressants are restrictive and selective for patients, and some patients who do not meet the conditions cannot use them. Therefore, in the subsequent research, more exploration is needed to discover other immune checkpoints besides PD-1, such as CTLA4, to reduce the restriction of treatment and provide more options for patients' treatment.

Dual immunotherapy combined with chemotherapy

At the 2022 ASCO GI meeting, the findings of a phase II clinical trial using the immunosuppressant candonilimab (AK104), which target PD-1 and CTLA-4, combined with chemotherapy (XELOX) as the first-line therapy for locally advanced adenocarcinoma that is unresectable, were released. AK104 patients had an ORR of 68.1%, a DCR of 92.3%, and a mOS of 17.41 months (95% CI: 12.35 to NE), according to study data. However, in the nivolumab combination therapy group, the average mOS for the people was just 13.8 months, and a comparison between the two can clearly reveal better data in the AK104 group, indicating that AK104 has a better prognostic improvement, longer patient survival and better overall treatment effect.[46]

According to studies on drug safety, approximately 62.5% of patients in the AK104 combination chemotherapy treatment group experienced at least grade 3 treatment-related adverse events (TRAES). Compared to the 60.0% TRAES rate in the single-agent immune combination trial Checkmate-649 and the 59.8% TRAES rate in the Orient-16 trial, the AK104 combination treatment group adverse reactions occurrence probability was slightly greater. Among them, in terms of TRAES that may lead to discontinuation, the incidence rate in the AK104 combination group was 6.3%, compared with 38.0% and 11.6% in the Checkmate-649 and Orient-16 studies, respectively, and the incidence rate of AK104 was significantly lower, indicating that the probability of adverse reactions leading to discontinuation of AK104 was lower, and the safety of AK104 can be assured.[46]

Also targeting two specific targets for treatment, a related drug study of the dual immune combination trial Checkmate-649 was forced to discontinue the trial due to severe adverse effects in patients treated with the combination of nivolumab and ipilimumab. In contrast, the bispecific antibody drug AK104, which has fewer adverse effects and a higher safety profile, has become a focus for further development of immunotherapy in the future, and a phase III clinical study on AK104 combination therapy (NCT05008783) is currently underway.

Recently, a hospital also reported the outcome of a case of AK104 for an advanced gastric adenocarcinoma patient who was HER-2 (1+) with liver and lung metastases. The patient achieved complete remission (CR) after treatment while the adverse effects such as grade 1-2 hematological toxicity and infusion reactions were experienced during treatment. But the toxicities were significantly milder compared to the combination of PD-1 inhibitors with CTLA-4 inhibitors, and the overall safety of treatment was satisfactory.[47]

The results of the available studies indicate that dual-targeted antibody inhibitor immunotherapy is more effective than single immunotherapy for gastric cancer, while the safety profile is higher than that of dual single immunosuppressant combination, and the overall treatment safety and possible adverse reaction rates are within acceptable limits, making dual antibody immunosuppressants of higher clinical value and research potential. However, the specific dose and target population selection for the clinical application of the drug remains to be studied.

MSI-TYPE GASTRIC CANCER

Overview of MSI

Microsatellites (MS) refer to certain short tandem repeats (1-6 nucleotides) scattered throughout the human genome that are more prone to mutation, with a mutation rate of 10^{-7}-10^{-3} per generation. The MMR system is involved in the identification and repair of mismatched bases generated during DNA replication or genetic recombination. When the MMR system is defective, mismatched base pairs created during DNA replication are not repaired in a timely manner, which in turn causes mutations in genes, resulting in microsatellites becoming less stable and further leading to the deletion of the associated expressed proteins.

MSI-type gastric cancer is thought to affect 8% to 25% of all stomach cancer patients globally, however survey data from different countries and studies vary.[48] There are many reasons for the differences between study data, and it is currently believed that the most influential are geographical differences between samples, differences in gastric cancer staging among investigated individuals, and differences in the detection methods used by different research institutions.

MSI prognostic marker controversy

In a meta-analysis, Polom *et al.* screened 48 studies from multiple databases including PubMed, Cochrane, and Ovid up to 2016 and found an overall survival risk ratio of 0.69 (HR=0.69, CI: 0.56-0.86, P<0.001). Based on a study of 21 of these trials, it was found that MSI patients had a relatively better prognosis than patients without MSI.[49] And in a 2017 post hoc analysis report about the MAGIC trial, it was shown that among those gastric cancer patients who received only surgery, those with MSI-H fraction had better treatment and prognosis outcomes relative to subjects with MSS/MSI-L fraction, but the data analysis revealed no statistically significant difference in efficacy between the two groups (HR=0.35, CI: 0.11-1.11, P=0.08); the non-MSI-H group had a better prognosis in patients who received both surgical treatment and chemotherapy.[50]

For the time being, MSI cannot be properly employed as a predictive biomarker for stomach cancer due to the retrospective nature of the majority of existing research, which lack timeliness, and the small sample size included in the study, which lacks representativeness and reliability. More significantly, research has demonstrated that a number of variables, including the tumor stage and adjuvant treatment, may have an impact on the prognosis of MSI-type gastric cancer.

Clinical management of MSI gastric cancer

Chemotherapy sensitivity controversy of MSI-H gastric cancer

In a post hoc evaluation of the MAGIC experiment from 2017, Smyth randomized gastric cancer patients into two groups, one receiving surgery alone and the other receiving adjuvant chemotherapy in the perioperative period. Comparing the experimental data from the two groups, it was shown that patients in the surgery-only group had better treatment-related prognostic indicators, suggesting that adjuvant chemotherapy offered no improvement in the recovery of patients with MSI-H gastric cancer.[51] The 5-year disease-free survival rate for patients with MSI gastric cancer was 85.7% in the surgery alone group and 83.9% in the surgery combined with chemotherapy group, according to a post hoc analysis of the CLASSIC trial in 2019. There was no significant difference between the two data sets, suggesting that chemotherapy was not effective in improving the prognosis of patients undergoing surgery.[52] Polom *et al.* compiled data from four large clinical trials (MAGIC, CLASSIC, ARTIST and ITACA-S) related to the perioperative treatment of patients with MSI-type gastric cancer and performed a meta-analysis of these data. According to the findings, patients in the surgery-alone group with MSI-H staging had superior 5-year disease-free survival rates and longer overall survival times than those with MSS/MSI-L gastric cancer. However, this result was the opposite in the group that underwent both surgery and chemotherapy, and subjects with non-MSI-H fraction in the combined treatment group had significantly better outcomes and prognosis.[52]

However, in a recent Korean study with locally progressive MSI-H gastric cancer, researchers made a different finding, showing that among MSI-H gastric cancer patients, subjects who received postoperative adjuvant chemotherapy with 5-fluorouracil had higher 5-year survival rates (94.8% *vs.* 78.3%, P=0.022) and 5-year disease-free survival rates (87.0% *vs.* 72.9%, P=0.044) than the surgery-alone group.[53] The results of this trial suggest that postoperative adjuvant chemotherapy has a greater impact on MSI-H patients with locally progressive gastric cancer, and patients benefit more from it.

Since most of the current studies on MSI are retrospective and lack timeliness, and most meta-analyses lack sufficient sample data, the results obtained are not representative of the majority of the population, and the results obtained from different studies even appear to be contradictory. There is no clear conclusion about the sensitivity of MSI-H gastric cancer to chemotherapy and whether MSI-H gastric cancer patients can benefit from chemotherapy.

Immunotherapy for MSI gastric cancer

Immunotherapy is now a popular research component in the field of oncology treatment, and clinics are encouraging the use of different targeted agents for patients with different molecular subtypes of gastric cancer to achieve specific treatment. Currently, only a few gastric cancer patients can benefit from specific immunotherapy, therefore, it is crucial to determine which types of cancer patients can benefit from immunotherapy. Research on immunotherapy for MSI-H gastric cancer continues, with most studies currently focusing on advanced gastric cancer, mainly due to the fact that most individuals with stomach cancer are already in an advanced stage when they are detected.

Keynote-059 is a phase III clinical trial that enrolled patients with metastatic gastric/gastroesophageal union cancer who failed second-line chemotherapy and treated them with pembrolizumab. The final data showed that the MSI-H group achieved an ORR of 57.1% and a DCR of 71.4% in all subjects, while the corresponding figures for the non-MSI-H group were only 9.0% and 22.2%. By comparison, it was evident that the MSI-H group had significant therapeutic and prognostic effects relative to the non-MSI-H group, indicating that patients with MSI-H typing have higher drug sensitivity to immunotherapy.[54] Similarly, the final results of Keynote-158, a phase II multicohort trial, showed more favorable treatment outcomes after treatment with pembrolizumab in certain gastric cancer patients who did not respond to chemotherapy.[55] Related similar studies include the Keynote-061 and Keynote-062 trials, in which Keynote-061 aimed to compare the effect of pembrolizumab with paclitaxel and found that the ORR in the pembrolizumab group was 46.7%, while the other group was only 16.7%, and comparing the data of the two groups, it can be concluded that pembrolizumab immunotherapy has a better therapeutic effect and clinical performance relative to the chemotherapy approach.[56]

Keynote-062 trial focused on comparing and studying the difference in efficacy between monotherapy with pembrolizumab and combination therapy with chemotherapy. The results showed that in patients with MSI-H gastric cancer, the ORR was 57.1% in the monotherapy group, 64.7% in the combination therapy group, and 36.8% in the chemotherapy alone group. It was obvious that the combination therapy group had the best efficacy, indicating that in the application of immunosuppressive drugs, the combination therapy can bring out the maximum efficacy of the drugs and achieve the best prognosis.[43]

Thanks to these findings demonstrating the efficacy of pembrolizumab, the 2022 edition of the CSCO Guidelines

for the Treatment of Gastric Cancer formally recommends pembrolizumab monotherapy as a Class II recommended agent for patients with dMMR/MSI-H gastric cancer.[39]

DISCUSSION

The development of immunotherapy for stomach cancer has accelerated recently, but it has also encountered additional challenges. The research ideas can be broken down into three main categories. First, due to the repeated failures of the current exploratory in first-line treatment (KEYNOTE-811 study), neoadjuvant therapy (KEYNOTE-585 study), and adjuvant therapy (ATTRACTION-5 study) for advanced gastric cancer, the emphasis of future research will be shifted to optimizing the immune checkpoint inhibitor therapy, which is currently more widely used, increase the efficacy of PD-1/PD-L1 antibody-based immune checkpoint inhibitors and universalize the detection rate of MMR status for patients in clinic. Second, although immunotherapy is more effective against gastric cancer in the Chinese population, and the effectiveness increases as PD-L1 expression rises, the cut-off value of PD-L1 expression is yet unknown. Therefore, in order to increase the accuracy of screening patients for immunotherapy, it is crucial to further investigate the characteristics of the immune microenvironment of gastric cancer and even look for improved biomarkers that can be used to predict the success of PD-1/PD-L1 antibodies. Third, its crucial to keep exploring for new targets in order to develop immunotherapy drugs for gastric cancer. Recently, CLDN18.2 has emerged as the most promising star target in the field of immunotherapy for the disease, and more than 50 different drugs targeting CLDN18.2 are currently undergoing research and development in China. In the future, it is worth looking forward to whether mono-antibody therapy, dual-antibody therapy, chemotherapy plus immunotherapy, or CAR-T therapy would maximize the immunotherapeutic efficacy for gastric cancer.

The treatment of gastric cancer has progressed inconsistently when compared to immunotherapy for other solid tumors, but with the emergence of some creative research and results from fundamental research, we believe that immunotherapy has great potential and advantages in the treatment of gastric cancer, and will improve patient survival.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

AUTHOR CONTRIBUTIONS

Min Jiang drafted the manuscript. Zhengmao Lu undertook the guidance and revised the article. Rui Zheng, Ling Shao and Ning Yao collected the data. All authors read and approved the final manuscript.

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