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Review Article

Predictive biomarkers and new developments of immunotherapy in gastric cancer: a 2023 update

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Abstract: Gastric cancer is an extremely common digestive tract tumor. The promotion and application of standardized therapy, treatment scheme optimization, and development of new targeted drugs and immunotherapies have improved gastric cancer survival somewhat. However, gastric cancer prognosis generally remains non-optimistic. Immune checkpoint inhibitors (ICI) have gradually become a new choice for gastric cancer treatment and can prolong the survival of some patients. Among them, high-microsatellite instability, Epstein-Barr virus-positive status, or high-tumor mutational burden patients with gastric cancer may be the potential population to benefit from immunotherapy. Nevertheless, there remains a lack of unified and effective predictive markers. Accordingly, this review mainly focused on the possible predictive biomarkers of anti-PD-1/PD-L1 in gastric cancer treatment. Furthermore, the application of anti-PD-1/PD-L1 therapy-related clinical trials on gastric cancer is discussed. The current findings suggest that immunotherapy is a promising application in gastric cancer treatment. Therefore, combining immunotherapy and other therapies may be the trend in the future. Nevertheless, exploring biomarkers to predict ICI response remains a major challenge.

Keywords: Gastric cancer, immunotherapy, immune checkpoint inhibitor, biomarker

Introduction

Gastric cancer is a malignant disease that is the fifteenth most frequent cancer by incidence and is ranked third for cancer mortality globally. According to 2018 statistics, approximately 783,000 people die from gastric cancer every year worldwide [1]. The most recent data revealed that the gastric cancer death rate in China was second among tumor-related deaths [2]. Chemotherapy remains the main advanced gastric cancer treatment. The recent ToGa trial demonstrated that trastuzumab + chemotherapy improved the survival of human epidermal growth factor receptor 2 (HER2)-positive patients with advanced gastric cancer [3]. Additionally, two phase III trials reported that ramucirumab prolonged the survival of patients with gastric cancer [4]. Despite the use of various new drugs in the past decade, the 5-year overall survival (OS) rate of metastatic gastric cancer is nevertheless only 6% [5].

Unlike traditional chemotherapy and targeted therapy, immunotherapy is an alternative cancer treatment and new anti-tumor approach. Immunotherapy has completely revolutionized tumor treatment by targeting the host's immune system. More than 1000 studies proved that immunotherapy is effective in various solid tumors, e.g., lung cancer and melanoma [6]. Accordingly, immunotherapy has been approved as standard treatment in different treatment lines of diverse tumors. The immune checkpoint inhibitors (ICI) that have been studied fall into three main categories: programmed cell-death-1 (PD-1), its ligand (PD-L1 or B7-H1), and cytotoxic Tlymphocyte-associated antigen 4 (CTLA-4) [7]. With continuously increasing clinical research, many researchers have focused

on gastric cancer immunotherapy. Recent studies proved that immunotherapy might be a new treatment method for gastric cancer. Here, we concentrate on the predictive biomarkers of immunotherapy for gastric cancer and the encouraging clinical data.

Predictive biomarkers of gastric cancer immunotherapy

Based on phase II trials, the US Food and Drug Administration (FDA) in 2017 permitted pembrolizumab for use in advanced solid tumors with mismatch repair defects (dMMR) or high microsatelliteinstability (MSI-H) that have progressed in previous treatment or have no other standard treatment methods. Subsequently, many researchers have gradually valued immunotherapy. However, different studies reported significantly different immunotherapy efficiency or tumor responses. Therefore, there is a need to find effective markers that enable better selection of patients for whom immunotherapy would be beneficial.

MSI-H

MSI-H status is considered a favorable prognostic marker for patients with resectable gastric cancer. Simultaneously, it is also a potential negative predictive factor in such patients, as they might not benefit from neoadjuvant/adjuvant chemotherapy [8]. MSI-H gastric cancer is a different subgroup proposed according to two large-scale genomic characterization-based molecular studies, where its incidence was 5.6-22.7% [9]. Compared with microsatellite stable (MSS) patients, patients with MSI-H resectable gastric cancer had a significantly higher survival rate but did not benefit from neoadjuvant/adjuvant chemotherapy [8, 10, 11]. MSI-H gastric cancer accounts for a very small proportion of gastric cancerandis related to female sex, older age, distal stomach location, earlier stage, Lauren intestinal type, and fewer lymphnode metastases [12]. A few genomic profiling studies demonstrated a significant association between MSI-H gastric cancers and high tumor-infiltrating lymphocyte (TIL) numbers and PD-L1 expression [13, 14]. In KEYNOTE-059 cohort 1, MSI-H patients had an objective response rate (ORR) and disease control rate (DCR) of 57% and 71% (n=7), respectively, while those of MSS patients were 9% and 22% (n=167), respectively [15]. Another study in Korea also reported promising results, where all patients with MSI-H gastric cancer received pembrolizumab alone and obtained a significant response. The phase II GERCOR NEONIPIGA study reported a higher pathological complete remission rate (pCR-R) in patients with dMMR/MSI-H resectable gastric and gastroesophageal junction cancer [16]. Based on these studies, MSI-H/dMMR is considered a positive predictor for immunotherapy response. Hence, a sensitive and reliable method to determine MSI status is very important for immunotherapy application in gastric cancer.

MSI status is determined by three standard procedures: immunohistochemistry (IHC), PCR, and next-generation sequencing (NGS). IHC is a widely available and low-cost MSI analysis method with relatively high sensitivity and specificity (> 90%). Pathologists typically evaluate MMR status and identify patients with Lynch syndrome based on MMR expression. PCR detection is also a standard approach to determine the MSI status. The National Cancer Institute recommended the Bethesda panel to test MSI status, which includes two mononucleotiderepeats (BAT-25 and BAT-26) and three dinucleotide repeats (D5S346, D2S123, D17S-250) [9]. These regions in tumor and normal DNA are amplified with multiplex PCR, then their size is evaluated with capillary electrophoresis. The identification of at least two microsatellite sites with size changes is rated as MSI-H. IHC and PCR results are highly consistent [17]. The NGS method is a substitute for determining MSI status [18]. One study reported that NGS and PCR demonstrated 95.8-100% consistency for detecting MSI status [19]. However, NGS is a form of bioinformatics analysis, which is costly and time-consuming. Therefore, its wide application in the clinic is limited.

Epstein-Barr virus (EBV)

The Cancer Genome Atlas states that EBV-positive gastric cancers are a unique gastric cancer type. The EBV-positive gastric cancer subgroup has unique clinicopathological characteristics (numerous TIL, male sex, earlier stage, relatively young), and this subtype has a good prognosis. EBV-positive advanced gastric cancer (EBVaGC) accounts for approximately 10% of gastric cancers [20]. Typically, EBVaGC and MSI-H gastric cancers are accompanied by

70-100% PD-L1-positive rates and more extensive CD8+ cytotoxic T cell infiltration [21, 22], which acts as an effective cytotoxic T cell that directly kills tumor cells. The standard EBVaGC detection method is in situ hybridization (ISH) detection of EBV-encoded RNA (EBER), which is the gold standard for detecting EBV status.

Compared with other molecular subtypes, EBV-positive gastric cancer has the best prognosis after radical resection [23]. Unlike patients with MSI-H gastric cancer, EBV-positive patients have the longest response to first-line chemotherapy containing platinum and fluorouracil compared to EBV-negative patients, and might even achieve complete remission (CR), which significantly improves the survival rate of such patients [24]. The KEYNOTE-061 study reported the first clinical indication regarding EBV status, where EBVaGC might be a good marker of immunotherapy effectiveness. In that study, all patients with EBV-positive gastric cancer achieved CR or partial remission (PR) [25], which were encouraging results. In another study, a molecular pathological analysis of 61 pembrolizumab-treated patients with advanced gastric cancer, demonstrated that PD-L1-positive status was highly correlated with EBV-positive status and MSI-H. This result suggested that EBVaGC might be a specific group that can clearly benefit from immunotherapy, where all EBV-positive patients achieved PR with a median remission period of 8.5 months [14].

A prospective observational study in China reported that patients with EBVaGC receiving immunotherapy achieved a favorable response. Of the nine patients enrolled in that study, three were PD-L1-positive and achieved PR, while five patients had stable disease (SD) and one patient had no assessable lesions but had significantly reduced ascites and tumor marker levels. Among these patients, the longest response time to immunotherapy was 18 months by the end of the last follow-up [26]. A recent study explored the effectiveness and potential biomarkers of immune checkpoint blockade (ICB) in EBVaGC with NGS and reported that the ORR of 22 immunotherapy-treated EBV-positive/pMMR patients was 54.5% (12/22) [27]. Currently, clinical studies on immunotherapy-treated EBV-positive patients with gastric cancer are ongoing, where such patients might benefit from immunotherapy.

PD-L1

PD-1/PD-L1 is a potential target of ICIs, and the tumor or immune cell PD-L1 protein expression level is a potential biomarker to predict immunotherapy sensitivity. However, the value of PD-L1 expression differs between tumors, where the results of relevant reports on PD-L1 expression in gastric cancer for predicting immunotherapy efficacy are inconsistent. One analysis that examined all clinical studies based on FDA-approved ICI-related drugs to evaluate PD-L1 as a predictor to estimate immunotherapy efficacy involved 45 drugs and 15 tumor types. The results demonstrated that PD-L1 predicted immunotherapy efficacy in only 28.9% of cases and nine drugs were associated with a specific PD-L1 threshold and concomitant diagnosis [28]. A meta-analysis that included 15 non-small cell lung cancer (NSCLC)-related randomized controlled trials involving 10,074 patients determined that the PD-L1 level might be a valuable predictor for patients with NSCLC treated with anti-PD-1/PD-L1 alone, but was not suitable for prediction regarding those receiving combined first-line treatment of chemotherapy + immunotherapy [29].

According to the KEYNOTE-059 phase II study, PD-L1-positive status (combined positive score [CPS] ≥ 1) predicted pembrolizumab effectiveness for advanced gastric/gastroesophageal junction cancer [15]. However, the JAVELIN Gastric 300 and ATTRACTION-2 clinical trials reported that PD-L1-positive status (CPS ≥ 1) did not have predictive value for nivolumab and avelumab efficacy for treating patients with advanced gastric cancer [30, 31]. The CheckMate 649 trial demonstrated significant survival improvement by including nivolumab in first-line chemotherapy. The survival benefit conferred by nivolumab was greater for patients with tumor CPS ≥ 5 (or even ≥ 10) [32]. The subgroup analysis of the ATTRACTION-2 phase III study demonstrated that PD-L1-positive status and low neutrophil-lymphocyte ratio (NLR, median ≤ 2.9) predicted better progression-free survival (PFS). In the nivolumab group, patients with PD-L1-positive status, low NLR, and normal sodium (Na, ≥ 135 mmol/L) achieved better treatment response and disease control rates, while tumor EBV infection and tumor mutational burden (TMB) were not

related to immunotherapy efficacy [33]. Why did various studies report inconsistent results regarding PD-L1 expression for predicting immunotherapy efficacy?

The greatest challenge for investigators and clinicians is the lack of consensus on the PD-L1 expression status evaluation criteria. PD-L1 expression is evaluated with the tumor proportional score (TPS) and CPS. For TPS, PD-L1 expression in tumors is evaluated by calculating the PD-L1-stained tumor cell-to-total living tumor cell ratio. In CPS evaluation, PD-L1 expression refers to the ratio of potential PD-L1 expression (including tumor andimmune cells)-to-total living tumor cells ratio. Most lung cancer-related clinical studies used the TPS. However, the TPS and CPS are also involved in numerous large clinical gastric cancer-related trials [15, 25, 30]. The CPS is more helpful than TPS for evaluating PD-L1 expression in gastric cancer, and can be utilized as a prognostic biomarker [34]. In gastric cancer, it is highly consistent to assess PD-L1 expression with 22C3 pharmDx and SP263 antibodies [35].

TMB

The somatic mutation number per megabase (Mb) of sequenced DNA, the TMB has become a new biomarker to forecast immunotherapy efficacy and is an independent prognosis predictor [36]. The TMB can be determined with NGS or whole-exomesequencing (WES) [37]. TMB was initially evaluated using WES, but WES has limited application for TMB detection due to the long sequencing duration and high cost of matching normal samples. Compared with WES, targeted sequencing panels using NGS are more widely used in the clinic due to the advantages of lower cost and higher mutation detection sensitivity [37]. Although several platforms published information using TMB as a biomarker, only two panels passed the regulatory channels: FoundationOne CDx analysis and MSK-IMPACT [38-40]. These panels were optimized to identify all types of molecular changes in cancer-related genes. Although an increasing number of studies demonstrated that panel-based TMB has possible clinical relevance as a immunotherapy response predictive biomarker, the test platforms used and the critical TMB values in these studies all differed, and there is no standardized and prospectively defined critical value [41]. The FoundationOne gene team's TMB cut-off value of 10 mutations/Mb is the only value that has been verified in a separate further study, where it could best distinguish the immunotherapy responders and non-responders among patients with NSCLC [42]. Based on the KEYNOTE-158 results, the FDA granted approval to use pembrolizumab in adult and child solid high-TMB (TMB-H) tumors [43].

Whether TMB can be used as a predictive immunotherapy indicator remains controversial. The CheckMate 227 study demonstrated that when the first-line treatment of nivolumab and ipilimumab was administered to people with NSCLC, the PFS of TMB-H patients (≥ 10 mutations/Mb) would increase irrespective of PD-L1 expression [44]. The KEYNOTE-158 study suggested that TMB-H patients achieved a higher ORR than low-TMB patients [45]. However, a recent study that involved 431 patients with different cancers reported that TMB could not be utilized as a prognostic marker for the immunotherapy response of all tumors, where the TMB could predict the melanoma and NSCLC immunotherapy response, but not that of renal cancer [46]. Small-sample studies demonstrated that TMB-H patients with gastric cancer had longer PFS [47]. The NCT02915432 clinical trial studied toripalimab safety and effectiveness in patients with advanced gastric cancer in China and reported that TMB-H patients had significant OS advantages compared with low-TMB patients [48]. The KEYNOTE-062 study evaluated the association between TMB status and first-line pembrolizumab ± chemotherapy versus chemotherapy efficacy, where the TMB correlated with first-line pembrolizumab clinical efficacy in patients with advanced gastric/gastroesophageal junction adenocarcinoma. However, excluding the MSI-H patients weakened the predictive effect of TMB [49]. The TMB results obtained from the circulating tumor DNA (ctDNA) extracted from blood samples (bTMB) demonstrated that bTMB could predict the survival rate of patients with NSCLC who received atezolizumab. Currently, several clinical trials are evaluating the prospective efficacy of bTMB in first-line treatment of patients with NSCLC [50-52]. Another study using MYSTIC phase III trial data described the analytic validation of a new bTMB algorithm. Using the new bTMB calcula-

tion method and ≥ 20 mut/Mb as the cut-off value of high bTMB, the results demonstrated that high bTMB predicted durvalumab + tremelimumab clinical efficacy in comparison with chemotherapy [53]. Briefly, the clinical implementation of TMB is challenging and more clinical studies are required to confirm the role of TMB to predict immunotherapy efficacy.

Other biomarkers

TIL and the gene expression profile (GEP) are two other biomarkers that have attracted research attention. Classically, TIL represent the tumor microenvironment (TME) αβ heterogeneous T cell population, which includes the CD4+ and CD8+ subgroups. The data from 85 ICI-treated patients with MSI-H metastatic colorectal carcinoma (mCRC) demonstrated that high TIL expression was associated with high TMB. Compared with patients with fewer TIL, the ORR of patients with more TIL (TILs-H) was higher, and TILs-H patients had significantly different survival results [54]. The GEP allows simultaneous evaluation of multiple parameters. Some genes have been incorporated into various genomes, such as the mRNA transcription level of genes related to inflammation, immune checkpoints, and even carcinogenesis. The GEP has uninterrupted output and has been used to advance the response characteristics of many different tumors. The main detection indicators in the majority of cases are the interferon (IFN) γ gene characteristics. However, GEP lacks the co-expression and location information of TME cells [55].

A meta-analysis demonstrated that the TMB, GEP, and PD-L1 IHC yielded similar areas under the curve to predictanti-PD-1/PD-L1 therapy response. In comparison with the TMB, PD-L1 IHC, or GEP, multiple immunohistochemistry/immunofluorescence and multimodal biomarker approaches appear to have a superior predictive role [56]. Patients with a 25% decrease in the maximum somatic variation allele frequency (maxVAF) had longer PFS and higher response rate to immunotherapy. Compared with patients in whom ctDNA could be detected after treatment, patients in whom ctDNA could not be detected after treatment had a longer median PFS (7.4 months vs. 4.9 months) [57]. In addition to the above biomarkers, a recently published study demonstrated that neutrophils are also related to tumor immunotherapy efficacy, wherea therapy-elicited systemic neutrophil response was positively related with lung cancer disease control [58]. A recent study demonstrated that multidimensional tumor infiltrating immune cell (TIIC) characteristics could predict the patients who would derive the greatest benefit from anti-PD-1/PD-L1 immunotherapy [59]. Additionally, the influenceof gut microbiota on immunotherapy and the use of artificial intelligence algorithms to automatically quantify radiological biomarkers for predicting immune efficacy are current research hotspots [60, 61], as evinced in some ongoing biomarker-related clinical trials. For example, an ongoing clinical study is verifying the accuracy of TIIC features to predict immunotherapy efficacy for gastric cancer and aims to include 300 patients (NCT05593419) [62] (**Figure 1** and **Table 1**).

Gastric cancer checkpoint inhibitors and clinical results

First-line treatment

There have been several studies on immunotherapy as first-line therapy for advanced gastric cancer (**Table 2**). For example, the KEYNOTE-059 cohort 2 study mainly assessed pembrolizumab-only efficacy for treating advanced gastric/gastroesophageal junctioncancer, while the KEYNOTE-059 cohort 3 study focused on pembrolizumab + chemotherapy efficacy [63], where the ORR of combination therapy was higher than that of pembrolizumab. The data of the KEYNOTE-659 cohort 1 phase IIb study demonstrated that the ORR of patients with CPS ≥ 1 was > 70%, the median PFS was 9.4 months, and OS was not achieved. That study reported that S-1 + oxaliplatin (SOX) combined with pembrolizumab demonstrated encouraging efficacy and controllable safety as first-line treatment for advanced gastric/gastroesophageal junction cancer [64]. Part 1 of the ATTRACTION-4 phase II trial evaluated nivolumab + SOX or capecitabine + oxaliplatin (CapeOX) safety and efficacy as first-line therapy for unresectable advanced or recurrent HER2-negative gastric/gastroesophageal junctioncancer [65], and reported that the ORR of nivolumab + SOX and CapeOX was 57.1% and 76.5% and the median PFS was 9.7 and 10.6 months, respectively. However, the OS rate of

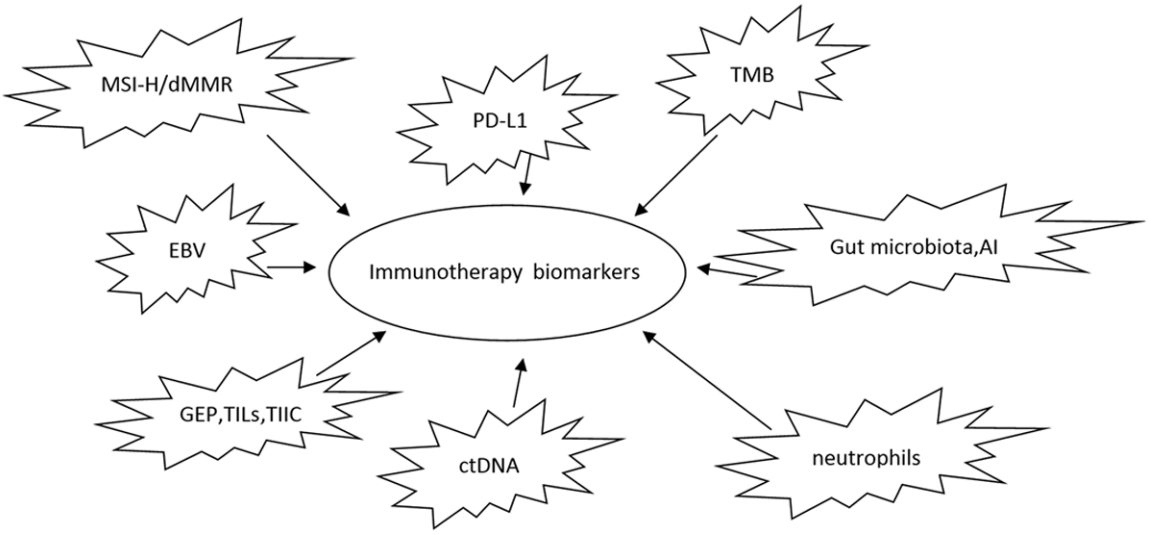

Figure 1. The potential biomarkers of immunotherapy in gastric cancer.

Table 1. Predictive biomarkers of gastric cancer immunotherapy

Biomarker	Agent	Phase	Signifcant association	Study or ClinicalTrial.gov, number or author	References
MSI-H/dMMR	Pembrolizumab	II	Better ORR	KEYNOTE-059	[15]
	pembrolizumab	II	Better pCR-R	GERCOR NEONIPIGA	[16]
EBV positive	Pembrolizumab	III	Better CR or PR	KEYNOTE-061	[25]
	Pembrolizumab	II	Better PR	Kim ST, et al	[14]
	ICI	NA	Better ORR	Xie T, et al	[26]
PD-L1	Pembrolizumab	II	Better ORR (CPS ≥ 1)	KEYNOTE-059	[15]
	Nivolumab	III	No	JAVELINGastric300	[30]
	Avelumab	III	No	ATTRACTION-2	[31]
	Nivolumab		Better OS (CPS ≥ 5)	CheckMate 649	[32]
TMB	Pembrolizumab	II	higher ORR	KEYNOTE-158	[45]
	ICI	NA	No	Wood MA, et al	[46]
	toripalimab	Ib/2	Better OS	NCT02915432	[48]
Other biomarkers					
TILs	ICI	NA	higher ORR	Loupakis F, et al	[54]
multimodal biomarker	ICI	Meta analysis	superior predictive role	Lu S, et al	[56]
ctDNA	ICI	NA	longer PFS	Jin Y, et al	[57]
TIIC			NA	NCT05593419	[62]

the two groups did not reach the median (no response, NR). The study progressed to the phase III trial to compare nivolumab + SOX/CapeOX and placebo + SOX/CapeOX efficacy as first-line treatment for advanced gastric cancer. The KEYNOTE-062 phase III clinical trial demonstrated that pembrolizumab efficacy on OS was not poor compared with standard chemotherapy for PD-L1-positive and HER2-negative advanced gastric/gastroesophageal junction cancer. However, in patients with PD-L1 CPS ≥ 10, the pembrolizumab group had significantly improved OS [66]. CheckMate 649 is an open-label, multicenter and omized phase III study on first-line treatment of untreated patients with advanced or metastatic gastric/gastroesophageal junction cancer. The experimental group was treated with nivolumab + ipilimumab or nivolumab + chemotherapy, and the control group underwent standard chemotherapy (oxaliplatin + fluoropyrimidine). The results demonstrated that the median OS of

Table 2. Clinical trials of the first-line treatment of ICIs in advance GC

Study number	Cancer type	Phase	Study design	Effect	References
KEYNOTE-059	GC, GEJC	II	Cohort 2: Pembrolizumab + chemotherapy Cohort 3: Pembrolizumab alone	ORR: 60% ORR: 25.8%	[63]
KEYNOTE-659	GC, GEJC	IIb	Cohort 1: Pembrolizumab + SOX	ORR: 73.9% (1 ≤ CPS < 10) ORR: 71.0% (CPS ≥ 10) PFS: 9.4 m OS: NR	[64]
ATTRACTION-4	GC, GEJC	II	Part 1: Nivolumab + SOX vs. nivolumab + CapeOX	ORR: 57.1% (sox) and 76.5% (CapeOX) PFS: 9.7 m (sox) and 10.6 (CapeOX) OS: NR	[62]
KEYNOTE-062	GC, GEJC	III	Pembrolizumab + chemotherapy vs. chemotherapy	OS (CPS ≥ 1): 12.5 vs. 11.1 m PFS (CPS ≥ 1): 6.9 vs. 6.4 m	[66]
CheckMate 649	GC, GEJC	III	Nivolumab + ipilimumab vs. nivolumab + chemotherapy vs. chemotherapy	OS: 11.2 m vs. 14.4 m vs. 11.1 m (CPS ≥ 5)	[65]
KEYNOTE-811	GC, GEJC	III	Pembrolizumab + trastuzumab + chemotherapy vs. chemotherapy	ORR: 74.4% vs. 51.9% Response depth: -65% vs. -49%	[68]
AIO INTEGA	GEJC	II	Trastuzumab and nivolumab + ipilimumab vs. Trastuzumab and nivolumab mFOLFOX6	Survival rate at 12 months: 70% vs. 57%	[69]

Table 3. Clinical trials of the second-line or later treatment of ICIs in advance GC

Study number	Cancer type	Phase	Study design	Effect	References
KEYNOTE-059	GC, GEJC	II	Pembrolizumab alone	ORR: 11.6% DCR: 27% OS: 5.6 m PFS: 2.0 m	[15]
ATTRACTION-2	GC, GEJC	III	Nivolumab vs. placebo	OS: 5.26 m vs. 4.14 m 1-year OS rates: 27.3% vs. 11.6% 2-year OS rates: 10.6% vs. 3.2%	[30]
KEYNOTE-061	GC, GEJC	III	Pembrolizumab vs. paclitaxel	negative results	[25]
JAVELIN Gastric 300	GC, GEJC	III	Avelumab vs. chemotherapy	negative results	[31]
CheckMate-032	GC, GEJC	II	Nivolumab 3 mg/kg vs. Nivolumab 1 mg/kg + ipilimumab 3 mg/kg vs. Nivolumab 3 mg/kg + ipilimumab 1 mg/kg	ORR: 12% vs. 24% vs. 8% 12-month OS rate: 39% vs. 35% vs. 24%	[74]

the nivolumab + chemotherapy group was longer than chemotherapy-alone group, respectively. In patients with PD-L1 CPS ≥ 5, the nivolumab + chemotherapy group had significantly improved OS as compared with the chemotherapy-alone group. Additionally, further analysis demonstrated that nivolumab + chemotherapy significantly improved the OS and PFS in the patients with PD-L1 CPS ≥ 1 and all randomly allotted patients [67]. Based on these results, many countries approved nivolumab or pembrolizumab plus chemotherapy as the first-line treatment for patients with advanced gastric cancer.

The KEYNOTE-811 phase III study evaluated pembrolizumab plus trastuzumab and chemotherapy efficacy as the first-line treatment for unresectable or metastatic HER2-positive gastric or gastroesophageal junction adenocarcinoma. The results demonstrated that the ORR was improved by 22.7% in the pembrolizumab group, which was statistically different. The pembrolizumab group also demonstrated greater reaction depth than the control group [68]. The CheckMate 649 cohort demonstrated that compared with chemotherapy alone, the OS rate of nivolumab + ipilimumab did not reach the predetermined significance limit in patients with PD-L1 CPS ≥ 5. Moreover, the PFS and ORR were not improved. However, patients with PD-L1 CPS ≥ 5 and all randomized patients had a more durable response to nivolumab + ipilimumab (median response duration: 13.2 vs. 6.9 months; 13.8 vs. 6.8 months) compared with chemotherapy [67]. The AIO INTEGA randomized clinical trial examined the efficacy of trastuzumab and PD-1 inhibitor + CTLA-4 inhibitor or FOLFOX in the first-line treatment of late-stage ERBB2-positive esophagogastric adenocarcinoma. The results demonstrated that the total survival rateat 12 months of FOLFOX treatment was 70% while the total survival rate of the ipilimumab group was 57% [69].

Currently, ongoing clinical trials are evaluating the safety and effectiveness of different ICIs as first-line treatment in patients with advanced gastric or gastroesophageal junction cancer. A phase II clinical trial will evaluate whether adding relatlimab based on nivolumab combined chemotherapy as first-line treatment would improve the effective rate in patients with gastric or gastroesophageal junction adenocarcinoma, but patient recruitment has not begun (NCT03662659) [70]. Furthermore, a prospective, single-arm research was designed to assess the efficacy and safety of another PD-1 inhibitor, tripletrumab, merged with oxaliplatin and teggio (SOX) in first-line treatment of unresectable locally advanced, recurrent, or metastatic gastric and gastroesophageal junction adenocarcinoma (NCT04202484) [71]. Lastly, the anlotinib + toripalimab as first-line treatment for advanced gastric or gastroesophageal junction cancer (APICAL-GE) trial is engaging patients (NCT04278222) [72].

Second-line or later treatment

Many clinical trials have evaluated immunotherapy in second-line treatment and above (**Table 3**). The KEYNOTE-059 cohort 1 studied the safety and efficacy of pembrolizumab alone as second-line treatment for advanced gastric cancer and gastroesophageal junction cancer [15]. The results demonstrated that PD-L1-positive patients (PD-L1 CPS ≥ 1) had higher ORR and median response time than PD-L1-negative patients. Based on these results, the FDA augmented the approval of pembrolizumab for treating people with recurrent locally advanced or metastatic gastric cancer and gastroesophageal junction adenocarcinoma who have received ≥ 2 previous treatments and are PD-L1-positive.

ATTRACTION-2 is the first phase III clinical study for Asians. It also evaluated nivolumab efficacy for treatingadvanced gastric/gastroesophageal junction cancer after ≥ 2 chemotherapy regimens [30]. Recently, the authors updated their 2-year follow-up data to state that the nivolumab group had a significantly longer median OS than the placebo group. Furthermore, the nivolumab group had higher 1- and 2-year OS rates than the placebo group. However, unlike the results of CheckMate-059, PD-L1 expression status did not affect nivolumab in terms of OS benefit. Patients who achieved a CR or PR had the most noticeable long-term survival benefit from nivolumab. Among these patients, the median OS was 26.6 months, and the 1- and 2-year OS rates were 87.1% and 61.3%, respectively. Based on that study, Japan approved nivolumab for treating unresectable advanced or recurrent gastric cancer post-chemotherapy.

Both the above positive studies compared immunotherapy with placebo, and immunotherapy appears hopeful for patients with gastric cancer undergoing ≥ 2 chemotherapy lines. However, the KEYNOTE-061 phase III study reported negative results [25]. Following a comparison of the efficacy of pembrolizumab and the standard chemotherapy drug paclitaxel as advanced gastric cancer second-line treatment. Compared with paclitaxel, pembrolizumab did not improve the OS rate in advanced or metastatic gastric cancer and gastroesophageal junction adenocarcinoma with PD-L1 CPS ≥ 1, which demonstrated that these results did not reach the main study end point. However, the updated results of the subsequent 2-year follow-up demonstrated that although the second-line pembrolizumab did not lead to a significant OS improvement vs. paclitaxel monotherapy, it outperformed the latter in terms of 24-month OS rate. Therefore, patients with higher PD-L1 expression benefited more from pembrolizumab [73].

JAVELIN Gastric 300 is a large, randomized phase III trial that mainly compared avelumab efficacy as third-line treatment for patients with advanced gastric cancer and gastroesophageal junction cancer; the control group was the chemotherapy scheme selected by doctors [31]. Similar to the above mentioned studies, the study also reported negative results. In the trial, 371 patients were randomly allocated the treatments, and the primary and secondary end points were not reached. Lastly, CheckMate-032 was targeted at patients with gastric cancer and gastroesophageal junction cancer who failed chemotherapy. The results demonstrated that the ORR of the nivolumab + ipilimumab group was higher than that of the nivolumab group, while there was no significant difference between the 12-month OS rates of the two groups [74].

Neoadjuvant immunotherapy

KEYNOTE-585 examined the neoadjuvant/adjuvant treatment of local gastric or gastroesophageal junction adenocarcinoma to assess the safety and efficacy of pembrolizumab + chemotherapy and placebo + chemotherapy in neoadjuvant treatment (NCT03221426) [75]. Meanwhile, the single-arm GERCOR NEONIPIGA phase II study assessed the efficacy of the perioperative strategy of using nivolumab and ipilimumab in neoadjuvant therapy, followed by nivolumab alone post-surgery for patients with resectable MSI/dMMR cancer. The primary end point was the pCR-R, and the secondary end point included disease-free survival (DFS), OS, and safety [76]. Seventeen patients (58.6%) achieved pCR and 29 patients underwent R0 resection [16]. Another phase Ib/IItrial examined pembrolizumab-containing trimodality therapy in patients with gastroesophageal junction adenocarcinoma and did not reach its main end point. However, patients with high TME PD-L1 expression had significantly higher pCR rates [77].

A single-arm phase II trial suggested that based on the XELOX protocol, adding sintilimab as neoadjuvant therapy achieved a promising pCR-R and major pathologic response (MPR) rate [78]. The NCT03878472 phase II clinical study will evaluate PD-1 antibody + apatinib efficacy as a new adjuvant treatment in resectable locally advanced gastric cancer based on the SOX protocol [79]. Furthermore, NCT0406-2656 will assess chemotherapy + immunotherapy efficacy during perioperative treatment, where the main end point is the rate of pathological late remission [80]. Moreover, the ICONIC trial will assess the efficacy of FLOT chemotherapy + avelumab to perioperatively treat patients with resectable esophageal and gastric cancer (NCT03399071) [81]. Similarly, a randomized controlled single-center phase II clinical trial will assess the effectiveness and safety of another PD-1 inhibitor, camrelizumab, in combination with chemotherapy in the perioperative period of locally advanced gastric or gastroesophageal junction adenocarcinoma. The study screened people who were more immunotherapy-sensitive by detecting the difference in T cell expression via single-cell RNA sequencing (NCT04367025) [82]. Another phase II immunotherapy study on neoadjuvant treatment of locally advanced resectable gastric and gastroesophageal junction adenocarcinoma has not begun recruiting participants, although the experimental drug (SHR1210) has been defined (NCT03939962) [83]. Furthermore, the new NCT04354662 study will mainly evaluate toripalimab + FLOT regimen efficacy as a new adjuvant therapy in patients with locally advanced resectable gastric cancer or gastroesophageal junction adenocarcinoma

[84]. Lastly, the PANDA trial will evaluate safety and pathological tumor regression grade following atezolizumab + chemotherapy in resectable gastric andgastroesophageal junction cancer (NCT03448835), and is recruiting patients [85].

Postoperative immunotherapy

The VESTIGE (NCT03443856) study will examine whether nivolumab + ipilimumab as an adjuvant treatment can improve the DFS rate of patients with stage IB-IVA gastric and esophagogastric junction adenocarcinoma who have completed neoadjuvant chemotherapy and have high recurrence risk after surgery (defined by ypN1-3 and/or R1 status) [86]. Additionally, the NCT05468138 study aims to prove whether the immunotherapy prognosis after D2 radical gastrectomy is better than standard postoperative adjuvant chemotherapy for patients with MSI-H gastric cancer, but has not begun recruitment [87].

Conclusion

Studying the effect of the TME on immunotherapy is necessary to better select patients who can benefit from immunotherapy. Although PD-L1, MSI-H, TMB, EBV, and TIL seem to predict immunotherapy efficacy in some patients with gastric cancer, they must be enhanced and verified in prospective studies. Currently, the predictive effect of a single indicator remains sub-optimal, and more precise molecular markers or marker combinations require further exploration to better guide clinical treatment strategies. According to the data reported, anti-PD-1 treatment demonstrated good efficacy and safety results compared with placebo in patients with gastric cancer who had failed previous treatment, and the anti-tumor effect was durable in patients with effective treatment. However, a phase III trial reported that anti-PD-1 treatment did not demonstrate obvious advantages when compared with standard second-line chemotherapy. Other ongoing randomized clinical trials might or might not prove the value of immunotherapy in advanced gastric cancer treatment in the future. Currently, the study of immunotherapy in perioperative and postoperative adjuvant treatment is in the research stage. The publication of more research results will clarify the role of immunotherapy in gastric cancer. Simultaneously, it is essential to evaluate patients' clinical response to immunotherapy according to their molecular classification to better apply individualized treatment and patient management. Finally, clinical research of diverse solid tumors demonstrates that combining immunotherapy and immunotherapy, and immunotherapy and chemotherapy, yields promising results. Immunotherapy holds great promise for treating gastric cancer, and combined immunotherapy might be the future trend. Future research should focus on how potential patients can be screened more accurately.

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Disclosure of conflict of interest

None.

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