Ther Adv Gastroenterol
2016, Vol. 9(6) 853–860
DOI: 10.1177/1756283X16658251

Antagonizing programmed death-1 and programmed death ligand-1 as a therapeutic approach for gastric cancer

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***Abstract*:** Malignant tumor cells are equipped with mechanisms that can help them escape the surveillance by host immune system. Immune checkpoint molecules can transduce coinhibitory signals to immunocompetent cells and exert immunosuppressive roles in antitumor immunity. Programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) are the two important checkpoint molecules with great potential in targeted cancer therapy. Several antibodies targeting PD-1 and PD-L1 have been approved for clinical use. In this review, we focus on the recent development of targeting PD-1 and PD-L1 in gastric cancer (GC) therapy.

Keywords: gastric cancer, immune checkpoint molecules, programmed death-1, programmed death ligand-1

Introduction

Gastric cancer (GC) is the fifth most commonly diagnosed malignant tumor and the second most common cause of cancer-related death worldwide. Approximately 984,000 new cases of GC and 841,000 deaths were reported in 2013. Almost half of the world's total new cases of GCs are reported to occur in China. Despite some advances, the overall prognosis of GC remains poor and novel therapeutic strategies are urgently needed. In this article, we aim to summarize the current data in clinical trials of blocking checkpoint molecules programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) in GC.

Developing immunotherapeutic strategies has become a hot area of focus in the treatment of GC. In general, cellular immunity is the major defense mechanism in humans to tackle malignancies. The strong growth potential and invasive nature of malignant tumors are at least partially attributed to the ability of the tumor cells to escape the host immune surveillance. GC patients always have some sort of functional deficiency in adaptive and innate antitumor immunity. As such, immunotherapy may hold a great potential for the treatment of GC.

Costimulatory and coinhibitory receptors, also known as checkpoint molecules, play a key role in T-cell immune response. Previous studies have shown that some checkpoint molecules such as PD-1 and its ligand PD-L1, as well as cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), can facilitate tumor cells to evade host immunosurveillance. These immune checkpoint molecules could inhibit the natural immune response to tumors [Sharma and Allison, 2015].

Expression profiles of programmed death-1/programmed death ligand-1 in gastric cancer

Upregulation of immune checkpoint molecules and suppression of antitumor T-cell effect have been observed in GC patients. PD-1 is a typical co-inhibitory receptor expressed on the membrane of T cells and other immune cells. PD-L1 and PD-L2 are both ligands for PD-1. Binding of these ligands to the receptor PD-1 could alter T-cell receptor signaling and reduce immune response of T cells.

Aberrant expression of PD-1 has been reported in T cells of GC patients, and in particular upregulation of PD-1 on T cells was suggested to be

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involved in immune evasion in GC patients. In this aspect, it was found that PD-1 expressing T cells in GC patients releases much less gamma interferon (γ-IFN) than the PD-1-negative T cells [Zong *et al.* 2014]. Compared with normal gastric mucosal epithelial cells, GC patients displayed a significantly increased expression of PD-1 on CD8+ T cells [Saito *et al.* 2013]. In another study, increased PD-1 expression was observed on both CD4+ and CD8+ T cells, and this was thought to be responsible for impaired cell-mediated immunity in GC patients after surgery [Takaya *et al.* 2015].

Further studies have demonstrated that PD-L1 is highly expressed on tumor cells in GC patients. By immunohistochemistry (IHC) and real-time polymerase-chain reaction, PD-L1 was found in 42.2% of 102 GC cases whereas it was undetectable in normal gastric mucosal tissue in healthy controls [Wu *et al.* 2006]. Increased expression of PD-L1 in GC patients was supported by other studies in which more than half of the patients with stages II and III GC displayed increased expression of PD-L1 by IHC assay [Zhang *et al.* 2015]. In addition, GC patients whose tumor diameter was greater than 5 cm had a higher positive rate of PD-L1 expression [Zhang *et al.* 2015]. In a very recent study, it was found that 53.8% of GC patients were positive for PD-1 expression which was mainly restricted to tumor-infiltrating lymphocytes [Böger *et al.* 2016]. The same study also demonstrated that approximately 30.1% of GC patients were positive for PD-L1 expression in the tumor cells [Böger *et al.* 2016]. In normal human cells, PD-L1 is widely expressed in many cell types, while PD-L2 expression is limited to lymphoid cells, macrophages and dendritic cells [Raufi and Klempner, 2015]. However, PD-L1 and PD-L2 are expressed on a majority of malignant tumors including GC, both at the mRNA and protein levels [Takaya *et al.* 2015].

Expression of programmed death ligand-1 and programmed death ligand-2 in different subtypes of gastric cancers

GC is molecularly heterogeneous and may represent more than one disease, each with distinct genetic profiles and different tumor biology. Therefore, correct typing of GC would be helpful in directing the appropriate molecular targeted therapy and predict the prognosis. In this aspect, understanding the relationship between the expression of PD-1 and PD-L1 with distinct GC molecular subtypes is important.

Classically, the pathology of GC can be divided into the intestinal and diffuse types based on the Lauren typing. Over the recent years, analysis of the molecular characteristics of GC by Cancer Genome Atlas Research Network has resulted in a classification of GC into four major genomic subtypes: Epstein-Barr virus (EBV)-infected tumors, microsatellite-instability (MSI) tumors, genomically stable tumors and chromosomally unstable tumors [Sunakawa and Lenz, 2015]. The expression profiles of PD-1 and PD-L1 differ among different molecular subtypes in GC patients. The EBV-positive GCs but not other types of GC tissue show abundant expression of PD-L1 in the tumor tissues. In a recent study using IHC staining, more than half of the EBV-positive GC patients showed positive PD-1 expression within tumor-infiltrating lymphocyte cells [Böger *et al.* 2016]. In the EBV-negative GC patients, PD-L1 expression within tumors cells was observed only in patients with the MSI subtype [Böger *et al.* 2016]. The study also found that in EBV-positive GC patients, multiple mechanisms may be involved in the upregulation of PD-L1 expression and PD-1-driven immune evasion may play an important role in the development of EBV-related GCs [Böger *et al.* 2016]. Similar results were obtained with respect to PD-L1 expression in EBV-positive and MSI GCs [Dong *et al.* 2016, Derks *et al.* 2016]. PD-L1 expression is more prevalent in EBV-positive and MSI-GC patients, making them the ideal primary candidates for PD-1-targeted therapy. Further clinical studies in distinct subtypes of GC patients are needed to evaluate the therapeutic efficacy of the immune-checkpoint inhibitors.

Implication of programmed death-1 and programmed death ligand-1 in gastric cancer prognosis

A number of studies have demonstrated that expression of PD-1 and PD-L1 in GC is closely linked to the prognosis of GC patients. In a recent study, the expression of PD-1 on CD8+ T cells isolated from the peripheral blood mononuclear cells and tumor tissues of GC patients was examined by multicolor flow cytometry, and the results showed that an increased number of PD-1 positive CD8+ T cells is closely related to the

impaired function of CD8+ T cells [Takano *et al.* 2016]. In another recent study, it was shown that increased PD-1 expression in T cells is a favorable prognostic marker in GC patients [Takaya *et al.* 2015]. In a study involving 105 GC patients, PD-L1 expression correlated with clinical staging, depth of invasion, degree of differentiation, and patient survival [Wang *et al.* 2015]. Compared to the PD-L1-negative GC patients, GC patients with positive PD-L1 expression in tumor cells often showed a poorer prognosis [Zhang *et al.* 2015], a reduced disease-free survival (DFS), and a poorer overall survival (OS) [Eto *et al.* 2015]. Finally, a recent phase I study revealed that overexpression of PD-L1 is inversely correlated with the overall response rate (ORR) and progression-free survival (PFS) in GC patients who had received curative resection [Bang *et al.* 2015].

Clinical trials of antiprogrammed death-1 therapies in gastric cancer

Blockade of the PD-1 and PD-L1 signaling by their antagonizing antibodies could restore T-cell activity against tumor cells. Hence, targeting the PD-1 and PD-L1 signaling pathways may be a promising strategy for tumor immunotherapy and molecular-targeted therapy for GC patients. So far, six new agents that can specifically target PD-1 and PD-L1 have been developed, including three antibodies against PD-1 (pembrolizumab, nivolumab, and pidilizumab) and three antibodies against PD-L1 (durvalumab, BMS-936559 and atezolizumab). Many studies have already demonstrated that anti-PD-1 and anti-PD-L1 antibodies could achieve long-term responses with acceptable safety profiles in patients with lung cancer [D'Incecco *et al.* 2015], melanoma [Topalian *et al.* 2014], kidney cancer [Choueiri *et al.* 2015], bladder cancer [Heo *et al.* 2015], triple-negative breast cancer [Emens *et al.* 2015], and chemoresistant Hodgkin disease [Ansell *et al.* 2015]. As overexpression of PD-1 and PD-L1 is frequently observed in GC patients, and a close correlation between the overexpression of PD-1 and PD-L1 and treatment response has been identified, GC patients are ideal candidates for PD-1 and PD-L1-based immunotherapy.

Nivolumab (BMS-936558/MDX-1105)

Nivolumab (Bristol-Myers Squibb, New York, USA) is the first humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) against PD-1. The CheckMate-032 study is an ongoing phase I/II, open-labelled, multitumor cohort study designed to investigate the safety and efficacy of nivolumab as a single agent or in combination with ipilimumab (also an mAb directed against CTLA-4) in heavily pretreated patients with advanced or metastatic solid tumors, including GC patients [ClinicalTrials.gov identifier: NCT01928394].

In a trial of 163 cases of GC, 59 cases of advanced GC, including carcinoma from the gastroesophageal junction (GEJ), irrespective of PD-L1 expression status, were assigned to nivolumab monotherapy and the remaining 104 cases were assigned to nivolumab and ipilimumab combination therapy. Most GC patients had received at least two prior chemotherapies. Nivolumab was administered at 3 mg/kg intravenously every 2 weeks until either disease progression, discontinuation due to toxicity, withdrawal of consents, or the end of the study. The primary endpoint is ORR, secondary endpoints include treatment-related adverse events (AEs) and serious adverse events (SAEs), PFS and OS [Le *et al.* 2016]. The results showed that the ORR reached 14% in the nivolumab monotherapy group, including one case (2%) of complete remission (CR), six cases (11%) of partial remission (PR) and 12 cases (21%) of stable disease (SD). Among these 59 patients, 23 (39%) showed positive PD-L1 expression in tumor tissues, and of whom the ORR was seen in four (17%). Thus, GC patients with positive PD-L1 expression in tumor tissue seemed to have a better short-term response to nivolumab-based therapy. Among the responders, median duration of response was 7.1 months and the median OS was 6.8 months. A total of 41 patients (69%) experienced treatment-related AEs. The most frequently seen AEs with nivolumab included fatigue, increased alanine aminotransferase, pyrexia, vomiting, decreased appetite, pruritus, diarrhea and nausea. No treatment-related death occurred. Based on this trial, nivolumab was efficacious and well tolerated in heavily pretreated GC patients [Le *et al.* 2016]. The final data of this study are pending.

Pembrolizumab (lambrolizumab, MK-3475)

Pembrolizumab (Merck, Darmstadt, Germany) is another humanized IgG4 mAb against PD-1. It does not induce antibody-dependent

cell-mediated cytotoxicity (ADCC) or complement-dependent cell-mediated cytotoxicity (CDC) due to the modified Fc structure. The antitumor effect, safety, and tolerability of pembrolizumab in patients with advanced GC were tested in a recent phase Ib study [ClinicalTrials.gov identifier: NCT01848834] [Bang *et al.* 2015]. In this trial, most patients had previously received at least two chemotherapies. Only patients with PD-L1-positive tumors were included. Pembrolizumab was administered at a dose of 10 mg/kg every 2 weeks for up to 24 months or until there was CR, disease progression or unacceptable toxicity. There were nine objective responses among 39 patients, with ORR in 22%, 6-month PFS in 24%, and OS in 69% of patients. The most common AEs were fatigue and hypothyroidism. Treatment-related grade $\geqslant$3 AEs were observed in three patients (one each for hypoxia, peripheral neuropathy and pneumonitis). Grade 3–4 AEs were observed in four (10%) patients. One treatment-related death occurred that was thought to be due to drug-related hypoxia. Hence, pembrolizumab showed promising efficacy with manageable toxicity in patients with advanced GC [Bang *et al.* 2015].

An ongoing phase II trial 'KEYNOTE-59' has been initiated to further evaluate the therapeutic efficacy and toxicity of pembrolizumab in patients with advanced GC, including patients with carcinoma of the gastroesophageal junction [ClinicalTrials.gov identifier: NCT02335411] [Fuchs *et al.* 2015]. Pembrolizumab is administered as a monotherapy to participants who are either previously treated or treatment naïve, or as a combination therapy (with cisplatin and 5-fluorouracil or capecitabine) in treatment-naïve patients. In three cohorts, pembrolizumab will be administered for 2 years or until disease progression or intolerable toxicity develops. In addition, treatment may be discontinued upon CR. The primary endpoint is ORR, and the secondary endpoints include PFS, OS, disease-control rate and duration of response [Fuchs *et al.* 2015]. The outcomes of this trial are pending.

Pidilizumab (CT-011)

Pidilizumab (Creech, Yavne, Israel) is the third anti-PD-1 mAb. Although multiple early-stage clinical studies have evaluated the efficacy and safety of pidilizumab in a variety of hematologic malignancies and solid tumors, no data are available for patients with GC so far.

Clinical trial of antiprogrammed death ligand-1 therapies in gastric cancer

BMS-936559 (MDX-1105)

BMS-936559 (Bristol-Myers Squibb) is a fully humanized IgG4 mAb against PD-L1. A phase I trial was conducted to evaluate the antitumor activity and safety of BMS-936559 in patients with selected solid tumors [ClinicalTrials.gov identifier: NCT00729664]. A total of 207 patients, including seven GC cases, were enrolled in this study. The expression status of PD-L1 was not reported in this study. BMS-936559 was administered at an escalating dose of 0.3–10 mg/kg intravenously every 2 weeks in 6-week cycles for up to 16 cycles, or until the patient achieved a CR or confirmed disease progression. The efficacy data for the seven GC patients are not yet available. Grade 3–4 treatment-related AEs occurred in 9% of all patients [Brahmer *et al.* 2012]. Additionally, as BMS-936559 could revitalize T cells in chronic infection, it may likely be used in patients with human immunodeficiency virus (HIV) infection in the near future [Dai *et al.* 2012]. A phase I study in patients with HIV infection has been planned to evaluate the safety, pharmacokinetics, and immune response to BMS-936559 [ClinicalTrials.gov identifier: NCT02028403].

Durvalumab (MEDI-4736)

Durvalumab (AstraZeneca, London, UK) is an engineered human anti-PD-L1 IgG1 isotype that functions by preventing the binding of PD-L1 to PD-1 and B7-1 (also termed CD80). The Fc region of this mAb was structurally modified to prevent either ADCC or CDC. Durvalumab is currently in a phase I study to evaluate the efficacy and safety in patients with solid tumors including GC [ClinicalTrials.gov identifier: NCT01693562]. No PD-L1 expression status was reported in this study. Durvalumab was administered at a dose of 10 mg/kg intravenously every 2 weeks for up to 12 months. Tumor-growth control was achieved in 20% of the cancer patients tested including 28 cases of GC. Two cases of heavily pretreated GC remained stable over 24 weeks, exceeding the current median PFS. Treatment-related AEs occurred in 34% of patients. The most common treatment-related AEs included diarrhea, fatigue, rash and vomiting. Grade 2 pneumonitis was seen in one patient. No colitis or hyperglycemia were reported. Thus, the

preliminary clinical efficacy of durvalumab with acceptable safety profile was achieved across a variety of tumors, including GC [Segal *et al.* 2014].

More recently, an ongoing phase II randomized study (the Platform study) has been initiated to evaluate the efficacy and safety of durvalumab as a maintenance therapy for patients with advanced GC and oesophageal cancer [EudraCT number: 2014-002169-30]. Impressively, this is the first clinical trial of anti-PD-L1 agent in the alternative maintenance-therapy setting in patients with advanced GC. The main inclusion criteria were as follows: completion of at least six cycles of first-line chemotherapy with platinum and fluoropyrimidine in all cases; HER2-positive patients who have received trastuzumab alongside chemotherapy, and patients with SD at the end of first-line chemotherapy. The enrolled patients are randomized to receive either durvalumab or capecitabine monotherapy, or observation alone. The main endpoint is PFS, and the secondary endpoints include PFS rate at 3, 6 and 12 months, OS, ORR and treatment-related AEs. According to the latest available data, 32 cases of GC have been registered for the study. Recruitment of participants is still ongoing [Cafferkey *et al.* 2016].

Atezolizumab (MPDL-3280A)

Atezolizumab (Roche Basel, Switzerland) is a fully humanized, engineered mAb of IgG1 isotype directed against PD-L1. A phase I dose-escalation study was conducted to evaluate the pharmacokinetics and safety of atezolizumab [ClinicalTrials.gov identifier: NCT01375842]. In this trial, atezolizumab monotherapy was administered intravenously every 3 weeks in patients with locally advanced or metastatic solid tumors, including one GC patient. The pharmacokinetics data support every-3-weeks dosing at 15 mg/kg or fixed-dose equivalent. In nonselected solid tumors, the ORR reached 21% (25/122). Impressively, the responders began to demonstrate tumor regression within several days of initial treatment; some had delayed responses after radiographic disease progression, and others even had a prolonged SD prior to tumor shrinkage. No treatment-related deaths occurred during therapy [Herbst *et al.* 2014].

Summary and perspectives

Immunotherapy has now become a promising approach in cancer therapy. Immune-checkpoint blockade has revolutionized the current clinical treatment approaches for a wide variety of malignant tumors, especially for melanoma where the antitumor immunotherapy has produced significant survival benefits. In this perspective, anti-PD-1 and anti-PD-L1 mAbs may hold a great potential in cancer treatment. Currently, mAbs against PD-1 and PD-L1 are being evaluated, either as monotherapy or in combination with other chemotherapies. Two mAbs, pembrolizumab and nivolumab, have already been approved by the US Food and Drug Administration for clinical use in melanoma patients with very promising outcomes. Generally, these agents are effective and well tolerated, with response rates ranging from 10% to 50% and grade 3–4 AEs of less than 10%.

Like in patients with other malignant tumors, GC patients have many aspects of immune-function deficiency; aberrations in immune checkpoints play an important role in tumorigenesis, infiltration and metastasis in GC. Overexpression of PD-1 and PD-L1 on T cells and tumor cells, respectively, may be responsible, in part, for immune evasion in GC patients. In addition, elevated expression of PD-1 and PD-L1 indicates a worse prognosis in GC patients. Several early-stage clinical trials have demonstrated promising antitumor effects and acceptable safety profiles of blocking the immune-checkpoint molecules in GC patients. Therefore, immunotherapy targeting immune checkpoints may open a new avenue for GC treatment.

It is increasingly recognized that GC is a heterogeneous disorder consisting of several distinct subtypes that differ in epidemiological, anatomical, pathological and molecular features. Thus, different types of GC may have different therapeutic targets. Undoubtedly, appropriate case selection based on different molecular signatures would be a crucial step towards successful treatment for GC. It has been shown that the expression of PD-1 and PD-L1 varies among different GC-molecular subtypes, and patients with PD-L1 overexpression will more likely benefit from anti-PD-L1 therapy, making PD-L1 a potential candidate biomarker and therapeutic target for GC patients. However, to date, only a small number of clinical trials in GC patients have the expression status of PD-1 or PD-L1 in the inclusion criteria. In the era of precision medicine, patients with GC (and other cancers as well) should be

carefully selected, based on their molecular phenotypes, for example, expression profiles of PD-1 and PD-L1, so that more specific therapeutic targeting can be achieved.

Anti-PD-1 antibody blocks interactions between PD-1 and its ligands PD-L1 and PD-L2, whereas anti-PD-L1 antibody blocks interactions between PD-L1 and both PD-1 and B7-1. The latter interaction also decreases T-cell activities *in vitro* and *in vivo*. Although the two types of new agents have not been compared head-to-head in randomized clinical trials, the efficacy for short-term treatment with anti-PD-1 mAb seems to be superior to that with anti-PD-L1 mAb.

Accurate assessment of the therapeutic efficacy is critically important in cancer immunotherapy. Response-evaluation criteria in solid tumors (RECIST), which relies on a single, largest dimension of tumor, is the routinely adopted guideline for measuring the changes of tumor burdens in response to anticancer agents. In most occasions, RECIST can also be used to evaluate the tumor response to immune-targeted treatment. However, immune-related responses that differ from RECIST have also been observed in clinical trials of immune-checkpoint blockade. For example, by RECIST criteria, some cancer patients receiving anti-PD-L1 mAb atezolizumab experienced initial tumor enlargement followed by delayed responses [Herbst *et al.* 2013, 2014]. Other pseudoprogression includes shrinkage of the target-tumor lesions in the presence of new lesions. Such a phenomenon may be due to either lymphocyte infiltration, edema or necrosis induced by immune-related treatment, or initial tumor growth up to a sufficient immune response achieved [Chiou and Burotto, 2015]. Consequently, an immune-related response criteria (irRC) has been designed specifically to evaluate the antitumor response of novel immunotherapeutic agents [Wolchok *et al.* 2009]. For the irRC, total index and measurable new lesions are taken into account, as opposed to RECIST or World Health Organization criteria in which measurement of the new lesions is not required. The association between the irRC and patient survival is important in the evaluation of patient response to immunotherapy.

Developing more specific and potent immune-checkpoint inhibitors and exploring their efficacy and safety in combination with other conventional therapies for GC patients are important areas of future research. So far, no phase III clinical trials on the immune-checkpoint blockers have been conducted in GC patients.

It is worth mentioning that gut microbiota composition may influence the efficacy of immune-checkpoint blockers, as demonstrated by two recent studies showing that the enteric microorganism was a vital contributor to the antitumor efficacy of immune-checkpoint inhibitors. Some patients can benefit from immune-checkpoint inhibitors, while others do not respond to the same therapies. Specific members of the gut microbiota may influence the antitumor effect of checkpoint inhibitors. In two recent studies, *Bacteroides* was shown to enhance the efficacy of CTLA-4 inhibitors and *Bifidobacterium* spp. was found to increase the antitumor effect of anti-PD-L1 therapy [Vétizou *et al.* 2015, Sivan *et al.* 2015]. Overall, the application of immune-checkpoint inhibitors in the treatment of solid tumors like GC warrants more extensive studies.

Acknowledgements

Xiaojun Liu and Zhongxia Yang contributed equally to this work and share the first authorship.

Funding

This work was funded by Natural Science Foundation of Gansu, China (No.: 145RJZA036). OL and LQ's work was partially supported by a Cancer Council NSW grant (ID: APP1070076) and NHMRC grant (ID: APP1047417).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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