Immunotherapy for advanced gastric and esophageal cancer: preclinical rationale and ongoing clinical investigations

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Abstract: Gastric and esophageal cancers represent a major global cancer burden and novel approaches are needed. Despite recent improvements in outcomes with trastuzumab and ramucirumab the prognosis for advanced disease remains poor, with a median overall survival of 1 year. Comprehensive genomic characterization has defined molecular subgroups and potentially actionable genomic alterations, but the majority of patients do not yet benefit from molecularly directed therapies. Breakthroughs in immune checkpoint blockade have provided new therapeutic avenues in melanoma, and continue to expand into other tumor types, with ongoing investigations in gastrointestinal (GI) malignancies. The frequency of programmed death ligand 1 (PD-L1) overexpression, a putative response biomarker, approaches forty percent in gastric cancers. Translational studies and molecular classification suggest gastric and esophageal cancers are candidate malignancies for immune checkpoint inhibition trials and early clinical data is promising. Here we review the mechanisms, preclinical, and early clinical data supporting the role for immune checkpoint blockade in gastric and esophageal cancer.

Keywords: Immunotherapy; gastric; esophageal; cancer; programmed death ligand 1 (PD-L1); checkpoint; programmed cell death protein 1 (PD-1)

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Introduction

Despite therapeutic advances in oncology, the prognosis of late stage gastric and esophageal carcinoma remains exceedingly poor. Gastric cancer is the second leading cause of global cancer-related death, with an estimated 723,000 deaths in 2012 (1). Nearly 1 million new gastric cancers are diagnosed annually making this the fifth most common malignancy overall (1). Esophageal cancer affected an additional 456,000 people in 2012 and caused approximately 400,000 deaths, making it the sixth most common cause of cancer-related death and eighth most common cancer globally (1). While the overall incidence gastric cancer is on the decline, the prevalence of esophageal cancer is rising (2-4).

The majority of gastric and esophageal cancer patients present with advanced disease and evidence-based therapeutic options are limited. First line systemic therapy for metastatic disease is largely based on a platinum/5-fluoropyrimidine backbone, which produces moderate survival benefits in patients with good performance status (5). The addition of an anthracycline or taxane to platinum/5-fluoropyrimidine regimens may provide additional survival benefit in select patients (5-7). In Her2 amplified adenocarcinoma incorporation of the anti-Her2 monoclonal antibody, trastuzumab, significantly improves survival, and is the first molecularly targeted agent to improve outcomes in advanced gastric and esophageal cancers (8). The recently approved vascular endothelial growth factor receptor 2 (VEGFR-2) antibody ramucirumab has also been shown to improve survival in patients with gastric and gastroesophageal junction (GEJ) adenocarcinoma who progressed on first line therapy (9). While ramucirumab and trastuzumab are meaningful additions to the gastroesophageal armamentarium, overall...
survival outcomes remain poor and novel approaches are needed.

Immunotherapy has caused a paradigm shift in the treatment of melanoma and its use continues to expand to include other tumor types (10-12). With increasing clinical experience, biomarker analyses, and improvements in preclinical models, the potential role for immunotherapy in gastric and esophageal cancers is emerging. The major approaches to harnessing immunotherapeutic anticancer effects have come from the development of inhibitory antibodies which modulate immune checkpoint, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death ligand 1 (PD-L1). Here we review the basic immunotherapeutic mechanisms of CTLA-4 and PD-L1, existing preclinical data, and available clinical results incorporating immunotherapy into the treatment of advanced gastric and esophageal cancers.

**Immunotherapeutic mechanisms**

Numerous co-stimulatory and inhibitory molecules interact to form a network of activating and inhibitory pathway “checkpoints” which serve to regulate the human immune system. This molecular interplay allows for uninterrupted pathogen-fighting capabilities while simultaneously preventing autoimmunity and persistent immune response (13). Many of these pathways converge on T lymphocytes, which play a central role in triggering adaptive immune responses to both foreign pathogens as well as neoplastic cells. However, in cases of malignancy, tumor cells frequently escape immune detection by hijacking elements of these checkpoint pathways thereby inhibiting T cell effector function. Ultimately this results in reduced tumor surveillance and tumor recognition (14). The development of antibodies to immune checkpoints, known collectively as immune checkpoint inhibitors, has now translated to improved patient outcomes in several malignancies (11,15).

CTLA-4 is a ubiquitous T-cell receptor belonging to the immunoglobulin superfamily. CTLA-4 shares many similarities with the T-cell co-stimulatory protein CD28, and like CD28, is activated upon binding with CD80 (B7-1) or CD86 (B7-2) (16). In fact, CTLA-4 has been shown to compete with CD28 for CD80 and CD86 binding (17). However, unlike CD28, which stimulates T cells, the effects of CTLA-4 activation differ between T-cell subsets. In CD4+ helper T cells activated CTLA-4 down modulates activity, whereas in CD4+ T regulatory cells (T<sub>reg</sub>) CTLA-4 up-regulates function (18). The net effect of endogenous CTLA4 activation is immune tolerance (19) (Figure 1).

Similarly, the T-cell surface receptor PD-1, also a member of the immunoglobulin superfamily, inhibits T cell function upon binding to its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) (20) (Figure 1). The PD-1 ligands are also members of the B7 family, although the inhibitory pathway that PD-1 participates in is thought to be mutually exclusive to that of CTLA-4 (21). PD-L1 is expressed on T cells, B cells, NK cells, dendritic cells, monocytes/macrophages, mast cells, and various tumor types where it is thought to play a role in tumor immune escape (22) (Figure 1). It has been suggested that while CTLA-4 may play a significant role in early immune response, primarily occurring in lymphoid tissues, PD-1 whose expression is up regulated after T cell activation in peripheral tissues may be more involved in late immune response (23). Although CTLA-4 inhibition highlighted the power of immune checkpoint modulation, therapeutic focus is shifting towards the use of PD-1 and PD-L1 blockade, which offer benefits of potentially fewer side effects and perhaps improved outcome data.

**Preclinical observations in gastric and esophageal cancers**

**Distribution of PD-L1/PD-L2**

PD-L1 is broadly expressed in many human tissues and organs. In addition to immune cells PD-L1 has been identified on endothelial cells, mesenchymal stem cells, cells of the eye and placenta (22). In contrast, PD-L2 expression is restricted to lymphoid tissues and has only been observed on macrophages and dendritic cells, suggesting non-redundant roles for these two ligands (24). Varying levels of PD-L1 and PD-L2 are expressed on a majority of human cancer cells including: melanoma, renal cell carcinoma (RCC), multiple myeloma, breast, bladder, colon, and lung cancer (22,25,26). Melanoma, RCC, and non-small cell lung cancer (NSCLC) tumor series have shown high levels of PD-L1 expression by both immunohistochemical and RNA analysis, ranging from 66-100% (27-29).

Until recently, few studies had attempted to quantify PD-L1 and PD-L2 expression in gastric and esophageal cancers. Work by Ohigashi et al. using immunohistochemical and RT-PCR approaches to examine expression from 41 esophageal squamous cell cancer (ESCC) patients found that 43.9% of samples had either PD-L1 or PD-L2
overexpressing tumor cells (30) (Table 1). Similarly, PD-L1 expression was detected in 42.2% of gastric adenocarcinoma samples (n=102) and was undetectable in normal gastric tissue controls and only weakly detectible in gastric adenomas using an IHC approach (31). A recent Chinese series (n=111) suggested PD-L1 positivity in 63% (70/111) of gastric adenocarcinoma resection specimens (32) (Table 1). Data from the phase Ib KEYNOTE-012 trial corroborated the above results and found a 40% rate of PD-L1 overexpression in advanced gastric adenocarcinomas (33). Few studies have yet to specifically address rates of PD-1 and PD-L1 positivity in GEJ adenocarcinomas, the predominant location and histology in US patients. Although more studies will be necessary to substantiate these findings in gastric and esophageal cancers, PD-L1 expression levels are comparable to cancers in which anti-PD-L1 directed therapies have demonstrated early success.

**PD-1 expression and tumor infiltrating lymphocytes (TILs)**

The presence of lymphocytes in close tumor proximity has been used as a crude surrogate for immune responsiveness to tumor presence. Multiple large studies in melanoma, colorectal, ovarian, and breast have shown a correlation between increased immune infiltrates and favorable outcomes (34-37). Previous work has also correlated a higher density of TILs with improved outcomes in GI malignancies (38). Recently, work by Turcotte et al. defined the presence of endogenous CD8+ tumor infiltrating T-cells in a small series of patients with advanced gastrointestinal (GI) malignacies, including gastric cancer. They were able to demonstrate that naturally occurring CD8+ TILs can recognize specific autologous tumor-derived cell lines (39). However, despite the presence of TILs in the tumor microenvironment, tumor regression of late stage gastric...

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**Figure 1** Immune checkpoint blockade in central and peripheral immune compartments. (A) Expression of CTLA-4 is upregulated on T cells in lymphoid tissues following activation via MHC/TCR and M7/CD28 mediated signaling. Once activated, CTLA-4 inhibits T cell function leading to immune tolerance. In the presence of blocking antibodies this tolerance can be broken, allowing for enhanced antitumor response; (B) PD-1, also expressed on T lymphocytes, inhibits the action of T lymphocytes upon binding to its ligands PD-L1/2; this process likely occurs in the tumor microenvironment, between PD-L1/2 expressing tumor cells and PD-1 expressing T lymphocytes; (A,B) blocking antibodies to either PD-1 or its ligands allows for T cell activation, enhancing anti-tumor effects peripherally. CTLA-4, cytotoxic T-lymphocyte antigen 4; PD-1, programmed death 1; PD-L1, programmed death ligand 1; APC, antigen presenting cell; MHC, major histocompatibility complex; TCR, T cell receptor.
and esophageal cancers is rarely seen suggesting endogenous mechanisms are likely inadequate. Preclinical models have suggested that there are greater TIL numbers in earlier stage disease, and that advanced GI malignancies are less immunogenic due to selection of the least immunogenic cancer cell clones during disease progression (40,41). Several studies have identified up regulation of PD-1 on TILs in both RCC and hepatocellular carcinoma and correlated increased PD-1 expression with worse prognosis (42,43). In gastric cancer, PD-1 expression on CD8⁺ lymphocytes is significantly higher than that of normal gastric mucosa and peripheral blood (44). Further studying the relationship of TIL density to stage and immunotherapy response may help refine the optimal disease setting in which to pursue immune checkpoint inhibition in gastric and esophageal cancer.

**PD-L1/PD-L2 expression and patient outcomes**

In many cancers increased PD-L1 and PD-L2 expression correlate with worse prognosis, and ongoing investigation is needed to determine the prognostic power of PD-L1 expression in gastric and esophageal cancers (45-50). Increased PD-L1 expression in both gastric and esophageal cancer is associated with nodal metastases, advanced stage, and worse outcomes (31,32). Jiang et al. demonstrated a positive correlation between expression of B7-H4, another B7 family member, and gastric cancer invasiveness and metastasis. The median overall survival is significantly reduced in gastric cancer patients with higher B7-H4 expression (51). Similarly, higher levels of PD-L1 and PD-L2 expression have been shown to be negative prognostic markers in esophageal cancer, especially in cases in which both ligands are expressed (30). Higher tumor B7-H4 levels, detected by IHC, were associated with worse prognosis and inversely correlated with CD3⁺ and CD8⁺ T-cells in 112 ESCC samples (52). PD-L1 overexpression, particularly at higher levels, may also serve as a predictive response biomarker in gastric cancer. Updated analysis from the KEYNOTE-012 phase I study suggests a trend toward improved overall response rate (ORR), progression free survival (PFS) with higher levels of PD-L1 overexpression (33).

Further support for the predictive power has come from lambrolizumab melanoma and NSCLC cohorts suggesting increased tumor PD-L1 expression correlates with response rate (53,54).

**Previous gastroesophageal immunotherapies**

The role for immune modulating therapies in gastric cancer has been a subject of multiple prior investigations, largely in Asian patients. Non-specific immune potentiators such as polysaccharide-K, OK-432, and BCG have been previously investigated dating back to 1975 (55-60). The pleiotropic
immune modulator protein-bound polysaccharide (PSK), derived from the CM-101 strain of the fungus *Coriolus versicolor*, has been shown to increase leukocyte activation, shift the Th1:Th2 balance and inhibit tumor growth in several cancer models (61-63). In Japanese gastric cancer patients undergoing gastrectomy the addition of PSK to mitomycin/5-FU adjuvant therapy improved the five year disease free survival (DFS) (70.7% vs. 59.4%) and 5-year OS (73% vs. 60%) (57). The sclerosant OK-432 (penicillin-killed lyophilized Streptococcus pyogenes) induces IL-12, stimulates NK and T-cells favoring a Th1 response, and may improve the function of antigen presenting dendritic cells (64-68). In a small Japanese trial the combination of OK-432 with 5-FU/leucovirin and cisplatin was safe an produced a response rate of 40%, however, a larger adjuvant trial comparing S-1 vs. S-1 and OK-432 failed to demonstrate a survival difference (58,69). Similarly, the non-specific immune upregulation following BCG has translated to some anti-tumor responses without a reliable improvement in overall survival in combination studies (55,70). More recently, a small Chinese trial investigating cytokine-induced natural killer cells given after adjuvant 5-FU based chemotherapy for resected gastric cancer demonstrated a trend toward improved OS and a 6-month improvement in median DFS (34.1 vs. 40.4 months) (71). Retrospective analysis of this data suggested that benefits might be restricted to intestinal type histology (71). The combination of cytotoxic chemotherapy with non-specific immune modulators (chemoimmunotherapy) has largely been restricted to Asian patients and the lack of reproducible survival improvements has limited clinical adoption.

**Early checkpoint inhibitor clinical experience**

The first clinical success with immune checkpoint blockade was observed in patients with metastatic melanoma treated with the anti-CTLA-4 monoclonal antibody (mAb) ipilimumab (15). Subsequently, ipilimumab, and another anti-CTLA-4 mAb, tremelimumab, have shown promising results in phase I-III clinical trials in several cancer types including, gastric/GEJ carcinomas (72). Several anti-PD-1 mAbs including nivolumab, pembrolizumab (MK-3475), and pidilizumab have been developed and early data with these agents has shown significant response rates in melanoma, NSCLC, RCC, and diffuse large B-cell lymphoma (73-75). PD-L1 blocking antibodies have also demonstrated favorable outcomes in early trials (12).

Gastric and esophageal cancers have represented a small minority of patients in early phase immune checkpoint inhibitor trials. In the multicenter phase I trial of the anti-PD-L1 mAb BMS-936559 only 7 of 207 enrolled patients had gastric cancer. The gastric cancer cohort were assigned to the safety arm as opposed to the efficacy arm, and limited efficacy data in gastric cancer is available (12). In a second line gastroesophageal-specific phase II trial (n=18) with tremelimumab (anti-CTLA4 mAb) the observed response rate (RR) was 5%, below the observed response rate to second-line cytotoxic chemotherapy (76). Although this trial failed to meet its pre-specified RR endpoint several patients achieved stable disease (SD) and one patient achieved a partial response (PR), which is quite impressive given the aggressive natural history of advanced gastric and esophageal cancer. Further support comes from the interim analysis of the anti-PD-L1 mAbs MPDL3280A and MEDI4736 (77,78). In the MEDI4736 gastroesophageal cohort (n=16) two heavily pretreated patients remained on study over 24 weeks in the early reporting, beyond the median PFS for second line gastric and esophageal cancer therapies (78). In the most recent ESMO conference preliminary data from the phase IB anti-PD-1 antibody pembrolizumab trial (KEYNOTE-012) in advanced gastric cancer was presented. Patients with PD-L1 positive advanced gastric adenocarcinoma (IHC positive in >1% cells) received pembrolizumab 10 mg/kg every 2 weeks until progression or toxicity. A total of 39 patients were enrolled after screening 162 samples for PD-L1 (65 positive samples, 40% IHC+) (33). An updated analysis of this trial has suggested an ORR of 22% and a median response duration of 24 weeks in this heavily pre-treated population (33). There was a positive correlation with PD-L1 positivity and PFS (P=0.032). Results of this trial have prompted the planned KEYNOTE-059 phase II trial of cisplatin/5-FU in combination with pembrolizumab (33). The toxicity profile and early efficacy signals have prompted expansion of immune checkpoint inhibitors in advanced gastric and esophageal cancers (Table 2).

**Conclusions and future directions**

Advanced gastric and esophageal cancers carry a poor prognosis with limited therapeutic options, and few major therapeutic advances. While improving molecular characterization will continue to identify subsets of patients who may benefit from genotype-directed targeted therapies, a majority of patients do not yet benefit and therefore further therapies are needed.
The recently published Cancer Genome Atlas (TCGA) gastric cancer analysis has provided molecular rationale for division of gastric adenocarcinoma into four distinct molecular subtypes (79). Interestingly, the EBV-positive gastric cancer subgroup demonstrated high levels of PD-L1/L2 overexpression highlighting a molecularly defined patient population possibly most likely to derive benefit from immune checkpoint blockade (79). Early translational efforts have suggested comparable rates of PD-1 and PD-L1 expression in gastric and esophageal cancers, strengthening the argument that immune checkpoint inhibitors warrant further clinical investigation. Development and validation of predictive biomarkers for response to immune checkpoint blockade will help to refine the optimal location for immunotherapy in gastric and esophageal cancers. Some recent biomarker analyses suggest that PD-L1 directed therapy is most effective in patients with higher pre-treatment CTLA4 expression, absence of fractalkine (CX3CL1) in pre-treatment biopsies, and T-helper type 1 gene expression patterns (80). Interesting preclinical work continues to expand immunotherapy combination approaches including low dose chemosensitization with alkylating agents (81). Irradiation is known to induce antigen presentation and upregulate PD-L1 expression (82-84). The frequent use of adjuvant chemoradiation and high recurrence rates despite adjuvant therapy may make the use of anti-PD-L1 therapies an interesting adjunct to adjuvant therapy, a concept currently under investigation in NSCLC. Here we have presented a review of the current landscape of immunotherapy in gastric and esophageal cancer with attention to translational studies and early clinical investigations.

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None.

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**Footnote**

*Conflicts of Interest*: The authors have no conflicts of interest to declare.

**References**


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**Table 2** Ongoing clinical investigations targeting immune checkpoint blockade in gastric and esophageal cancer

<table>
<thead>
<tr>
<th>Study population</th>
<th>Histology</th>
<th>Number of samples</th>
<th>PD-L1 positive (%)</th>
<th>Outcome</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Esophageal</td>
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<td>44.0</td>
<td>Worse outcomes</td>
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<td>Adenocarcinoma</td>
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<td>42.2</td>
<td>Nodal mets, advanced stage</td>
<td>(31)</td>
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<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>111</td>
<td>63.0</td>
<td>Advanced stage, worse outcome</td>
<td>(32)</td>
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<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>243</td>
<td>43.6</td>
<td>Improved DFS, lower stage</td>
<td>(49)</td>
</tr>
</tbody>
</table>

PD-L1, programmed death ligand 1; DFS, disease free survival.


