Immunotherapy in Esophageal Cancer

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Introduction

Esophageal cancer is a highly aggressive malignancy which accounted for approximately 17,290 cases and 15,850 deaths in the United States in 2018 [1]. Given that 50% of patients present with overt metastatic disease and the majority of patients initially treated for locoregional disease will develop recurrence, most patients will undergo systemic therapy during their disease course [2]. Chemotherapy remains the core treatment for metastatic disease and improves survival over best supportive care. However, the prognosis for patients with esophageal cancer remains poor as the majority of patients will develop chemotherapy resistance and treatment options beyond first- and second-line therapy are limited. With the exception of the addition of trastuzumab to first-line therapy for Her2-positive disease [3] and ramucirumab as monotherapy [4] or in combination with paclitaxel [5] as second-line treatment, clinical trials evaluating targeted therapies have been disappointing. Thus, there is a critical need to improve outcomes for those diagnosed with this virulent disease.

In recent years, immunotherapy has emerged as a novel treatment strategy that has transformed outcomes in several cancers with a historically poor prognosis such as melanoma and lung cancer. In 2011, ipilimumab, an anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) antibody, became the first immune checkpoint inhibitor to be approved by the US Food and Drug Administration (FDA) for the treatment of advanced melanoma [6, 7]. More recently, antibodies that target the programmed death (PD-1) and PD-ligand-1 (PD-L1) pathways have undergone evaluation in multiple other solid tumors which has resulted in FDA approval of these agents in melanoma, non-small cell lung cancer, renal cell carcinoma, urothelial carcinoma,

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squamous cell carcinoma of the head and neck, classical Hodgkin's lymphoma, hepatocellular carcinoma, and microsatellite unstable (MSI) or mismatch repair protein-deficient (dMMR) cancers (irrespective of primary site, the first site-agnostic approval for any anti-cancer therapy).

There has been similarly strong interest in the evaluation of immune checkpoint inhibitors in esophageal cancer, and, in a landmark approval, the FDA approved pembrolizumab in September 2017 for patients with advanced gastric and gastro-esophageal (GE) junction adenocarcinoma whose tumors express PD-L1 and who have received two or more prior chemotherapy regimens.

This chapter will outline the biologic rationale for the use of immunotherapeutic strategies in the treatment of cancer and discuss the accumulating data regarding their use in esophageal cancer.

CTLA-4 and PD-1/PD-L1/PD-L 2 Pathways in Cancer

CTLA-4 is a protein receptor that was implicated as a negative regulator of T cell activation in the mid-1990s [8, 9]. When expressed on the cell surface of CD4+ and CD8+ T lymphocytes, it has higher affinity for the costimulatory receptors B7-1 and B7-2 present on antigen-presenting cells (APCs) than for the T cell costimulatory receptor CD28 [10]. Expression of CTLA-4 is upregulated by the degree of T cell receptor activation and cytokines such as interleukin-2 and interferon gamma, which form a feedback inhibition loop on activated T effector cells. Activation leads to downregulation of the immune response triggered by APCs. CTLA-4 was implicated in the immune surveillance of cancer in sarcoma and colon adenocarcinoma mouse models, in which inhibition of CTLA-4 led to tumor shrinkage [11]. Ipilimumab was subsequently the first immune checkpoint inhibitor approved, based on a phase III study demonstrating that it improved survival in patients with metastatic malignant melanoma [6].

PD-1 is a transmembrane protein expressed on T cells, B cells, and NK cells. Like CTLA-4, it is also an inhibitory immune checkpoint molecule [12]. It has two ligands, PD-L1 and PD-L2. PD-L1 is expressed on multiple tissue types, including tumor cells, while PD-L2 is mostly expressed on APCs. When PD-L1 expressed on tumor cells binds to PD-1 on activated T cells, an inhibitory signal is delivered to the T cell, which inhibits apoptosis of the tumor cell [13]. Unlike CTLA-4, which functions in T cell activation, the PD-1/PD-L1/PD-L2 pathway is thought to protect cells from attack by T cells [14].

CTLA-4 Inhibitors in Esophageal Cancer

By blocking the interaction between CTLA-4 and its ligands, CTLA-4 inhibition promotes antitumor responses through T cell activation and tumor infiltration. Two anti-CTLA-4 antibodies, ipilimumab and tremelimumab, have been evaluated in esophageal cancer. The results presented below suggest very modest single-agent

activity for these drugs, and, indeed, further evaluation of this class of drug as monotherapy in esophageal cancer is not being undertaken.

Tremelimumab

Tremelimumab was the first immune checkpoint inhibitor to be evaluated in esophagogastric (EG) cancer when a phase II study investigated its role as second-line therapy in patients with metastatic gastric, GE junction, and esophageal adenocarcinomas [15]. Tremelimumab was administered every 3 months at a dose of 15 mg/kg. Of 18 patients who were enrolled, 15 had received 1 prior line of therapy, and 3 had received 2 lines of therapy. At the end of the first cycle of treatment, four patients (22%) had stable disease. One of these patients had incremental reduction in tumor burden and achieved a partial response (PR) after 8 cycles that was sustained after 33 months of follow-up. Median PFS was 2.83 months and median OS was 4.83 months. Encouragingly, however, 12-month OS was 33%. Of note, the dose of tremelimumab utilized in this study was lower than the 10 mg/kg every 4 weeks dose currently being evaluated in ongoing studies, although it is unclear if a dose-relationship curve exists for these drugs.

Ipilimumab

A phase II study subsequently evaluated ipilimumab monotherapy in patients with advanced gastric or GE junction adenocarcinoma [16]. Patients who had achieved at least stable disease after first-line fluoropyrimidine/platinum chemotherapy were randomized to ipilimumab, 10 mg/kg every 3 weeks for four doses followed by 10 mg/kg every 12 weeks for up to 3 years, or best supportive care (BSC), which mainly consisted of continuation of fluoropyrimidine maintenance. The primary endpoint of the study was immune-related progression-free survival (irPFS). In 114 patients accrued, there was disappointingly no improvement in irPFS (2.92 vs. 4.90 months) or median overall survival (OS; 12.7 vs. 12.1 months) with ipilimumab. Grade 3/4 treatment-related adverse events (TRAEs) occurred more frequently in the patients who received ipilimumab vs. those who received active BSC (23% vs. 9%) and included diarrhea, fatigue, and hypothyroidism.

PD-1 and PD-L1 Checkpoint Inhibitors in Esophageal Cancer

Based on prolonged overall survival (OS) in phase III trials and durable responses in phase II studies, antibodies inhibiting PD-1 (pembrolizumab and nivolumab) and PD-L1 (avelumab, durvalumab, and atezolizumab) have now been approved in several malignancies, and these drugs continue to be extensively evaluated in EG cancer. The following section provides a summary of the current data for PD-1 and PD-L1 blockade in this disease.

Pembrolizumab

The KEYNOTE-012 study was a phase Ib multicenter, open-label, multi-cohort study which evaluated the benefit of pembrolizumab (10 mg/kg every 2 weeks) in patients with PD-L1-positive recurrent or metastatic gastric and GE junction tumors [17]. PD-L1 positivity was defined as ≥1% membrane staining of tumor or contiguous mononuclear inflammatory cells. The PD-L1 positivity rate was 40% based on this criterion (65 of 162 tumors). Thirty-nine patients were enrolled on the study, 68% of whom had received ≥2 prior therapies for metastatic disease and 49% of whom were from Asia. Of 36 patients evaluable for response by central assessment, 8 (22%) had an objective response, all PRs. At the time of analysis, median duration of response (DOR) was 40 weeks, and four of the responders had ongoing response. Median PFS was 1.9 months, and median OS was 11.4 months, while the 6- and 12- month OS rates were 66% and 42%, respectively. Grade 3/4 TRAEs occurred in five patients (13%; six events), consisting of fatigue, pemphigoid, hypothyroidism, peripheral sensory neuropathy, and one case of grade 4 pneumonitis.

The similarly designed phase Ib KEYNOTE-028 trial enrolled a cohort of patients with advanced esophageal cancer [18, 19]. This study evaluated pembrolizumab (10 mg/kg every 2 weeks) in 23 patients with PD-L1-positive esophageal carcinoma, 17 with squamous cell carcinoma (SCC), 5 with adenocarcinoma, and 1 with mucoepidermoid carcinoma. Of the 90 patients screened, 41% had PD-L1-positive tumors. Most patients (87%) had received ≥2 prior therapies. The objective response rate (ORR) was 30%, all PRs, and two patients had stable disease. Five of seven responses were ongoing at the time of data analysis with a median DOR of 40 weeks. The 6- and 12-month PFS rates were 30.4% and 21.7%, respectively. Grade 3 TRAEs occurred in four patients including lymphopenia, anorexia, liver disorder, and generalized rash.

The promising activity of pembrolizumab in gastric/GE junction tumors led to the KEYNOTE-059 study, a large phase II study that enrolled such patients into several cohorts. Cohort 1 investigated pembrolizumab 200 mg every 3 weeks in patients who had received ≥2 prior therapies. Patients in cohort 2 received pembrolizumab 200 mg in addition to cisplatin 80 mg/m^2 and fluoropyrimidine (5-fluorouracil [5-FU] 800 mg/m^2 or capecitabine 1000 mg/m^2) in the first-line setting every 3 weeks for 6 cycles followed by pembrolizumab plus fluoropyrimidine maintenance for up to 2 years or until disease progression.

KEYNOTE-059 cohort 1 enrolled 259 patients, and data has been presented in abstract form [20]. In this heavily pretreated population (51.7% received 2 prior lines of therapy and 29% and 19.3% had received 3 or ≥4 prior lines of therapy, respectively), the ORR was 11.6% after a median follow-up of 5.8 months. The complete response (CR) rate was 2.3% and 9.3% of patients had a PR. The median DOR was 8.4 months. Patients treated in the third-line setting had an ORR of 16.4% vs. 6.4% in patients who had received ≥4 prior therapies. The median PFS and OS in the intention-to-treat population were 2.0 and 5.6 months, respectively, and the 12-month OS rate was 23.4%. ORRs were improved in the approximately 60% of patients with tumors that were PD-L1 positive (defined when the combined positive

score or CPS [the sum of the percentage of PD-L1 staining tumor cells, lymphocytes, and macrophages divided by the percentage of PD-L1 staining tumor cells] is ≥1%) vs. PD-L1 negative (15.5% vs. 6.4%), and the median DOR was 16.3 months in the PD-L1-positive group vs. 6.9 months in the PD-L1-negative group. When patients who received pembrolizumab in the third-line setting were stratified by PD-L1 status, the ORR was 22.7% in those who had PD-L1-positive tumors vs. 8.6% in those with PD-L1-negative tumors. Treatment was well tolerated with 2.3% of patients experiencing a grade 3/4 TRAE and grade 3/3 immune-related AEs occurring in 4.6%.

These results suggest that pembrolizumab has promising, albeit modest, activity in pretreated advanced gastric and GE junction adenocarcinoma and led to US FDA approval of pembrolizumab in September 2017 for patients with advanced gastric/GE junction adenocarcinoma whose tumors express PD-L1, as determined by the PD-L1 IHC 22C3 pharmDx Kit (Dako) companion test by CPS, and who have received ≥2 prior chemotherapy regimens. This accelerated approval is contingent on the results of a confirmatory trial.

Preliminary efficacy and safety data from cohort 2 of the KEYNOTE-059 study have also been presented in abstract form. This arm enrolled 25 patients to the combination of fluoropyrimidine/cisplatin and pembrolizumab as first-line therapy. The safety profile was encouraging. At a median follow-up of 14.7 months, grade 3/4 TRAEs occurred in 76% of patients, most commonly neutropenia and stomatitis. Three patients experienced grade 3 immune-related AEs (rash and nephritis). There were no treatment-related deaths. The ORR was 60% and 20% of patients had stable disease (for a disease control rate of 80%). The ORR was 69% in patients with PD-L1-positive tumor vs. 38% in patients with PD-L1-negative tumor. The median DOR was 4.6 months. Median PFS and OS were 6.6 and 20.8 months, respectively. While the small number of patients and relatively early follow-up preclude any specific conclusions, these early data suggest that combination pembrolizumab and cisplatin/fluoropyrimidine has a manageable toxicity profile and encouraging antitumor activity.

Nivolumab

The largest study to date evaluating nivolumab in EG adenocarcinoma is the ATTRACTION-2 trial [21]. This was a randomized phase III East Asian study of 493 patients who had received ≥2 prior chemotherapy regimens. Patients were randomized 2:1 to nivolumab vs. placebo. The study revealed a very modest improvement in PFS (1.61 vs. 1.45 months, hazard ratio or HR 0.60, $p < 0.0001$) and OS (5.26 vs. 4.14 months, HR 0.63, $p < 0.0001$) in patients who received nivolumab. The 12-month OS rate was a landmark 26.6% vs. 10.9% in favor of nivolumab in a chemorefractory population. The ORR was 11.2% (vs. 0% in the placebo group), with a median DOR to nivolumab of 9.53 months. An exploratory analysis retrospectively assessed PD-L1 expression status in approximately 40% of patients ($n = 192$); 13.5% ($n = 26$) of tumors were assessed to be PD-L1 positive using the

28-8 pharmDx assay (Dako) and by assessing PD-L1 staining only in tumor cells. Similar OS was observed (5.22 months vs. 6.05 months in patients with PD-L1-positive vs. PD-L1-negative tumors) irrespective of PD-L1 positivity (<1% vs. ≥1% of tumor cells). The HRs for OS favored nivolumab over placebo in both PD-L1-positive and PD-L1-negative groups, suggesting an OS benefit regardless of PD-L1 expression status. Based on this study, nivolumab received regulatory approval in Japan for use in all patients irrespective of PD-L1 status in October 2017.

When comparing outcomes from the nivolumab ATTRACTION-2 study with cohort 1 of the pembrolizumab KEYNOTE-059, we observe near identical results for OS, PFS, and ORR as outlined in Table 15.1. Taken together, both of these studies confirm activity for anti-PD-1 blockade in EG adenocarcinomas and would suggest no difference in activity between Asian and non-Asian patients.

As further evidence, CheckMate 032 was a phase I/II open-label study which demonstrated a comparable degree of benefit from nivolumab in a Western population of patients. This study evaluated the safety and activity of nivolumab alone or in combination with ipilimumab in advanced and metastatic solid tumors and enrolled 160 heavily pretreated patients (79% had received ≥2 regimens) with advanced chemotherapy-refractory gastric, esophageal, or GE junction cancer. Patients were enrolled sequentially to three different arms: 3 mg/kg of nivolumab every 2 weeks (N3), 1 mg/kg of nivolumab plus 3 mg/kg of ipilimumab (N1 plus I3), and 3 mg/kg of nivolumab plus 1 mg/kg of ipilimumab (N3 plus I1) every 3 weeks for 4 cycles followed by nivolumab 3 mg/kg every 2 weeks until disease progression or intolerable toxicity. Preliminary results have been presented in abstract form [22] now published - https://www.ncbi.nlm.nih.gov/pubmed/30110194.

Results from the 59 patients enrolled in the N3 cohort suggest similar activity to pembrolizumab and nivolumab in an Asian population, as outlined in Table 15.1. The ORR was 12%, with a median time to response of 1.6 months and DOR of 7.1 months in the responders. In this study, PD-L1 positivity was assessed using a cutoff of ≥1% tumor staining on immunohistochemistry (assessed by the Dako 28-8 pharmDx assay).

Finally, a Japanese open-label, single-arm, multicenter phase II study has evaluated nivolumab in patients with esophageal SCC [23]. This study enrolled 65 patients who had received a median of three prior therapies. Of 65 patients enrolled, 64 were evaluable for the primary endpoint of ORR, and all patients were assessable for safety. Eleven patients (17%) had an ORR. The median PFS and OS were 1.5 and 10.8 months, respectively. The toxicity profile was manageable, and there were no treatment-related deaths.

Avelumab

Avelumab is the anti-PD-L1 antibody that has undergone the most extensive evaluation to date.

The phase Ib JAVELIN study [24] enrolled patients with GE junction and gastric adenocarcinoma. This study enrolled patients to two cohorts. The first evaluated

Table 15.1 Published or completed trials in metastatic EG carcinoma current as of March 2018

		Phase	Line of therapy	Patients	ORR	Median PFS (months)	Median OS (months)	1-year OS
Pembrolizumab	KEYNOTE-012[13]	Ib	≥2	*n* = 39 PD-L1+ *n* = 39	22%	1.9	11.4	42%
	KEYNOTE-059[16] Cohort 1	II	≥2	*n* = 259 PD-L1+ *n* = 148 PD-L1− *n* = 109	11.6% 15.5% 6.4%	2.0	5.6	23.4%
	KEYNOTE-059[17] Cohort 2 (pembro + chemo)	II	1st	*n* = 25 PD-L1+ *n* = 16 PD-L1− *n* = 8	60% 68.8% 37.5%	6.6	20.8	NS
Nivolumab	CheckMate 032[19] N 3 mg	I/II	≥2	*n* = 59 PD-L1+ *n* = 16 PD-L1− *n* = 26	12% 19% 12%	1.4	6.2	39%
	ATTRACTION-2[22]	III	≥2	*n* = 493	11.2%	1.65	5.32	26.6%
Nivolumab + ipilimumab	CheckMate 032[19] N 1 mg + I3 mg	I/II	≥2	*n* = 49 PD-L1+ *n* = 10 PD-L1− *n* = 32	24% 40% 22%	1.4	6.9	35%
	CheckMate 032[19] N 3 mg + I1 mg	I/II	≥2	*n* = 52 PD-L1+ *n* = 13 PD-L1− *n* = 30	8% 23% 0%	1.6	4.8	24%
Avelumab	JAVELIN Solid Tumor[23]	Ib	≥2	*N* = 20 PD-L1+ *n* = NS PD-L1− *n* = NS	15% 20% 0%	11.6 weeks 36 weeks 11.6 weeks	NS	NS
Durvalumab[24]	N/A	I	≥2	*n* = 16	25%	NS	NS	NS

NS not stated, ORR overall response rate, mPFS median progression-free survival, mOS median overall survival, OS overall survival, N nivolumab, I ipilimumab

patients who had progressed following first-line therapy (n = 20), and the second enrolled patients whose disease had not progressed on first-line therapy to maintenance therapy (n = 55). Both groups received avelumab 10 mg/kg every 2 weeks. In patients who received second-line avelumab, the ORR was 15% (3/20). PD-L1 expression (≥1% cutoff) was evaluable in 12/20 patients. Median PFS was 36 weeks (95% CI 6.0, 36.0) for patients with PD-L1-positive tumors and 11.6 weeks (2.1, 21.9) for those with PD-L1-negative tumors. In the cohort who received maintenance avelumab, the ORR was 7.3% (4/55, 1 complete response), and 47.3% had stable disease. The disease control rate was 54.5%. PD-L1 expression was evaluable in 43/55 patients, and median PFS for PD-L1-positive and PD-L1-negative status was 17.6 weeks (95% CI 5.9, 18.0) and 11.6 weeks (2.1, 21.9) respectively.

The small numbers here preclude a definitive conclusion. Activity appears to be generally comparable to anti-PD-1 antibodies and has paved the way for two phase III studies; the top-line results of one of which is discussed below.

Durvalumab

Durvalumab is a PD-L1 inhibitor also being evaluated in EG cancer. Data presented in abstract form reported an acceptable safety profile with early evidence of clinical activity in multiple tumor types. The ORR was 7% (2/28 patients) in the gastroesophageal cohort with a disease control rate of 25% at 12 weeks [25].

A phase Ib/II study is currently enrolling patients with GE junction or gastric adenocarcinomas in the second- and third-line setting to single-agent durvalumab, single-agent tremelimumab, or the combination of both (NCT02340975).

Combination Immune Checkpoint Inhibition

Data for combination immune checkpoint blockade in EG carcinoma comes from the CheckMate 032 study [22] now published - https://www.ncbi.nlm.nih.gov/pubmed/30110194. As discussed above, this study enrolled patients into three cohorts, two of which evaluated combination ipilimumab and nivolumab. Forty-nine patients received N1 + 13, and 52 patients received N3 + I1. Almost half of patients in both cohorts had received ≥3 lines of therapy. The highest ORR of all three cohorts was 24%, reported in the N1 + I3 group. The ORR for the N3 + I1 group was 8%. Median OS was 6.9 months in the N1 + I3 and 4.8 months in the N3 + I1 group. In both groups, the ORR was higher in patients with PD-L1-positive tumors: 40% vs. 22% in the N1 + I3 group and 23% vs. 0% in the N3 + I1 group. Grade ≥ 3 toxicities were highest in patients who received N1 + I3 (35%). The most common G3/4 toxicities were diarrhea and elevated transaminases.

It is important that these results are interpreted with caution both because of the small numbers and also because patients were enrolled sequentially and not in a randomized fashion. Nevertheless, several hypothesis-generating observations arise. The ORR for the N3 + I1 arm (8%) was certainly not superior to that observed in the N3 arm

(12%)—and the KEYNOTE-059 and ATTRACTION-2 studies. In addition, despite a higher ORR (40%; the highest reported in any immunotherapy study in EG cancer) in the N1 + I3 arm than the N3 arm (12%), the 18-month OS rate was similar between the groups (28% vs. 25%). Of note, the 18-month OS was 13% in the N3 + I1 cohort. Based on the superior ORR (at the expense of significant additional toxicity, which is discussed below), the N1 + I3 dose was selected for study in the phase III CheckMate 649 trial.

Phase III Studies

Based on the results outlined in this chapter, numerous phase III studies are ongoing or planned, both in the metastatic and adjuvant settings, as noted in Table 15.2. Studies evaluating single-agent therapy include the KEYNOTE-061 study which is a randomized study investigating second-line therapy with pembrolizumab vs. paclitaxel in patients with advanced gastric or GE junction adenocarcinoma. In a recent press release, it was reported that the primary endpoints of OS and PFS were not met in patients whose tumors are PD-L1 positive [26]. We await presentation of the data. The KEYNOTE-063 study (NCT03019588) is a similarly designed study evaluating pembrolizumab vs. paclitaxel in an Asian population.

The KEYNOTE-181 trial (NCT02564263) is investigating pembrolizumab vs. physician's choice of paclitaxel, docetaxel, or irinotecan in metastatic adenocarcinoma or SCC of the esophagus and Siewert type I GE junction adenocarcinoma following progression of disease on first-line therapy. ONO-4538 is a phase III, randomized, open-label study (NCT02569242) evaluating nivolumab vs. paclitaxel or docetaxel in patients with advanced esophageal cancer who have progressed following standard therapies.

Finally, the JAVELIN 300 study also evaluated avelumab in the third-line setting in a phase III study which randomized patients to avelumab vs. physician's choice chemotherapy with paclitaxel or irinotecan (NCT02625623). In a recent press release, it was reported that the trial did not meet its pre-specified primary endpoint of improved OS for avelumab vs. chemotherapy, and again we await presentation of the data.

The KEYNOTE-062 study (NCT02494583) is investigating pembrolizumab monotherapy vs. 5-FU/cisplatin vs. 5-FU/cisplatin plus pembrolizumab as first-line therapy for patients with advanced PD-L1-positive, Her2-negative gastric or GE junction adenocarcinoma [27]. This trial has accrued, and results are anticipated. The CheckMate 649 trial (NCT02872116) is a phase III study which is currently enrolling patients with advanced gastric or GE junction tumor (irrespective of PD-L1 status) and randomizing them to ipilimumab/nivolumab vs. fluoropyrimidine/oxaliplatin plus nivolumab vs. fluoropyrimidine/oxaliplatin in the first-line setting [28]. The JAVELIN 100 (NCT02625610) study is evaluating an alternative strategy of avelumab administered as switch maintenance therapy compared with continuation of first-line chemotherapy after 12 weeks of induction 5-FU/oxaliplatin or capecitabine/oxaliplatin. This trial is a randomized, open-label, multicenter phase III study which will enroll 466 patients with GE junction and gastric carcinoma. Patients must have at least stable disease following 12 weeks of first-line therapy to be eligible for enrollment.

Table 15.2 Selected ongoing studies evaluating immune checkpoint inhibition +/− combinatorial strategies in EG adenocarcinoma current as of March 2018

Drug	Trial identifier	Phase	Neoadjuvant/adjuvant	1st line metastatic	2nd line metastatic	3rd line metastatic	Status
Pembrolizumab	KEYNOTE-062 (NCT02494583)	III		Pembro vs. pembro + cisplatin/5-FU vs. cisplatin/5-FU			Accrued
	KEYNOTE-061 (NCT02370498)	III			Pembro vs. paclitaxel in gastric or GE junction AC		Completed
	KEYNOTE-181 (NCT02564263)	III			Pembro vs. taxane or irinotecan in AC or SCC esophagus or Siewert I AC of GE junction		Recruiting
	KEYNOTE-180 (NCT02559687)	II				Pembro in AC or SCC esophagus or Siewert I AC of GE junction	Ongoing
	(NCT02918162)	II	Pembro + platinum/5-FU doublet or triplet for 3 cycles pre-op and 3 cycles post-op, followed by maintenance pembro X 1 year				Recruiting
	PROCEED (NCT03064490)	II	Pembro + carbo/taxol + radiation, followed by adjuvant pembro X 3 cycles				Recruiting
	(NCT02954536)	II		Pembro +trastuzumab + platinum/5-FU			Recruiting

Nivolumab	CheckMate 649 (NCT02872116)	III		Nivo + Ipi vs. Nivo + FOLFOX vs. FOLFOX			Recruiting
	ONO-4538 (NCT02569242)	III			Nivo vs. taxane		Recruiting
	CheckMate 577 (NCT02743494)	III	Nivo vs. placebo as adjuvant therapy in pts with residual disease after multi-modal therapy				Recruiting
	FRACTION-GC NCT02935634	II Adaptive			Tx-naïve and Tx- experienced tracks Nivo + Ipi vs. Nivo + anti-LAG Other combinations will open Any line of therapy		Recruiting
	CheckMate 906 (NCT03044613)	Ib	Induction Nivo x2 cycles (arm A) vs. induction Nivo + Ipi x1 cycle (arm B) prior to CRT + Nivo followed by surgery in pts with stage II/III esophageal or GE junction cancer				Recruiting

(continued)

Table 15.2 (continued)

Drug	Trial identifier	Phase	Neoadjuvant/adjuvant	1st line metastatic	2nd line metastatic	3rd line metastatic	Status
Avelumab	JAVELIN 100 (NCT02625610)	III		Oxaliplatin/5-FU followed by maintenance avelumab vs. maintenance chemotherapy or BSC			Recruiting
	JAVELIN 300 (NCT02625623)	III				Avelumab + BSC vs. taxane or irinotecan +BSC or BSC alone	Ongoing
Durvalumab	(NCT02340975)	Ib/II			Durvalumab vs. tremelimumab vs. durvalumab + tremelimumab	Durvalumab + tremelimumab	Recruiting
	(NCT02658214)	Ib		Durvalumab + tremelimumab + oxaliplatin/5-FU			Recruiting
	NCT 02639065	II	Durvalumab every 4 weeks × 12 months in pts with residual disease after multi-modal therapy				Recruiting

5-FU indicates 5-fluorouracil; BSC best supportive care; carbo carboplatin; FOLFOX folinic acid, 5-fluorouracil, and oxaliplatin; Ipi ipilimumab; Nivo nivolumab; Pembro pembrolizumab; AC adenocarcinoma; SCC squamous cell carcinoma; GE gastroesophageal; pts patients; CRT chemoradiation; Tx treatment

Neoadjuvant and Adjuvant Therapy

Given the activity of immune checkpoint inhibitors in the advanced disease setting, the role of these agents in the perioperative setting in patients with stage II and III disease is now being investigated.

The CheckMate 577 is a global phase III study evaluating adjuvant nivolumab vs. placebo in patients with locally advanced esophageal or GE junction carcinoma who have persistent disease (defined as $ypT_{any}N+$ or $ypT1\text{-}4N_{any}$) following preoperative chemoradiation and surgery with clear margins [29]. The optimal treatment strategy for patients who do not achieve a pathologic complete response is unclear, and the current standard of care is surveillance following trimodality therapy. Thus, there is an unmet need in this patient population as the risk of disease relapse is high, particularly in patients with node-positive disease at surgery [30].

The KEYNOTE-585 study (NCT03221426) is a phase III study enrolling patients with ≥T3 and/or node-positive gastric and GE junction adenocarcinoma to perioperative chemotherapy (either a fluoropyrimidine/cisplatin doublet or the FLOT regimen of 5-FU/leucovorin/oxaliplatin/docetaxel) with or without pembrolizumab.

In Asian countries, postoperative adjuvant chemotherapy with tegafur-gimeracil-oteracil potassium (S-1) or oxaliplatin/capecitabine (CapeOx) is the standard of care in patients with pathologic stage II/III gastric and GE junction cancer. The ATTRACTION-05 study is a randomized phase III trial randomizing East Asian patients with stage II/III disease to adjuvant nivolumab or placebo in combination with physician's choice of S-1 or CapeOx [31].

Several phase I/II studies with various designs are assessing the safety and efficacy of nivolumab, pembrolizumab, durvalumab, and atezolizumab in the neoadjuvant setting, administered either sequentially or concurrently with neoadjuvant chemoradiation. See Table 15.2 for a list of selected adjuvant and neoadjuvant studies.

Ramucirumab and PD-1 or PD-L1 Inhibition

Targeted therapies against the vascular endothelial growth factor (VEGF) pathway elicit effects on tumor antigenicity and intratumoral T cell infiltration. These immunomodulating effects provide a rationale for combining anti-angiogenic therapies with immunotherapies [32–34]. Preclinical studies suggest that simultaneous blockade of the VEGFR-2 and PD-1/PD-L1 pathways induces synergistic antitumor effects by inhibiting tumor angiogenesis and promoting access of cytotoxic T cells to tumors while preventing exhaustion of T cells [35–37].

Ramucirumab is a monoclonal antibody against VEGFR2, which is approved as a single agent and in combination with paclitaxel for second-line therapy in EG adenocarcinoma. A multi-cohort phase Ia/b study was the first to evaluate the simultaneous targeting of both PD-1 and VEGFR2 in EG adenocarcinoma [38]. Forty-one patients with advanced gastric or GE junction adenocarcinomas were

enrolled to three cohorts: previously treated with chemotherapy (cohorts A and B) or chemotherapy-naive (cohort A2). Ramucirumab was administered at 8 mg/kg on days 1 and 8 (cohorts A and A2) or 10 mg/kg on day 1 (cohort B) with pembrolizumab 200 mg every 3 weeks. The response rate in cohorts A and B was 7%. PFS and OS rates at 6 months were 22.4% and 51.2%, respectively. Eighteen patients were enrolled to the A2 cohort with an ORR of 17%. Any grade toxicity was 80%, with a grade 3/4 toxicity rate of 24%, most commonly colitis (7%) and hypertension (7%).

Preliminary results from a phase Ib expansion of cohort A2 (treatment-naïve) reported an ORR of 25% (7/28 patients; 6 had PD-L1-positive tumors). An additional 12 patients (43%) had stable disease for a disease control rate (DCR) of 68%. The median PFS was 5.3 months and median OS was not reached. The most common grade 3 toxicity was hypertension [39].

Results from an ongoing multi-cohort phase I study evaluating ramucirumab plus durvalumab in patients with metastatic gastric or GE junction adenocarcinoma, who have progressed after one or two prior lines of therapy, reported an ORR of 17% (5/29 patients) and DCR of 55%. All responders had PD-L1 tumor expression ≥25%. The combination appears safe with hypertension the most common grade 3/4 TRAE reported [40].

While the safety profile in both studies is encouraging, the ORR observed with pembrolizumab/ramucirumab is modest when compared to that achieved with standard-of-care chemotherapy in the first-line setting. Furthermore, although the ORR of 17% achieved with durvalumab/ramucirumab compares relatively favorably to that observed with single-agent PD-1/PD-L1 therapy in the chemorefractory setting, the ORR seen with paclitaxel/ramucirumab in the second-line setting (28%) was substantially higher [5]. Ultimately, these likely represent sufficient data to justify further evaluation of this combinatorial strategy, although the increasingly crowded therapeutic environment and the awaited results of several potentially practice-changing phase III studies make the optimal setting for such evaluation unclear at this time.

Trastuzumab and PD-1 Inhibition

Trastuzumab has been shown to have immune-mediated mechanisms of action [41], and a preclinical study demonstrated that Her2-targeted therapy in combination with anti-PD-L1 therapy enhanced tumor growth inhibition, increasing the rates and durability of therapeutic response [42].

Our group at Memorial Sloan Kettering Cancer Center is currently evaluating pembrolizumab in combination with fluoropyrimidine/platinum and trastuzumab as first-line therapy in patients with metastatic Her2-positive EG adenocarcinoma with the rationale that dual Her2 and PD-1 blockade will result in enhanced antibody-dependent cell-mediated cytotoxicity (ADCC), NK cell degranulation, and synergistic activity in combination with fluoropyrimidine and platinum.

Immune-Related Toxicity from Checkpoint Inhibitors

In stimulating the immune system with immune checkpoint blockade, the goal is to achieve a hyper-activated T cell response directed toward tumor cells. However, this response can affect normal tissues and result in inflammatory side effects, termed immune-related adverse events (irAEs). The underlying mechanism has not been fully elucidated but is thought to relate to the role that immune checkpoints play in maintaining immunologic homeostasis [30]. IrAEs can affect any organ system but most commonly involve the skin, gastrointestinal tract, endocrine glands, and liver. Pulmonary, central nervous system, renal, ocular, pancreatic, cardiovascular, musculoskeletal, and hematologic immune-related toxicities occur less frequently [43, 44].

To date, the irAEs that have been observed in trials of checkpoint inhibitors in EG carcinoma have been similar to published data in other disease types with no new safety signals observed [45]. IrAEs are more likely to occur in patients treated with CTLA-4 blockade than those treated with PD-1 and PD-L1 blockade [45]. With respect to EG carcinoma, the highest rate of adverse events in any trial to date was observed in the CheckMate 032 study in patients who received the combination of nivolumab 1 mg/kg and ipilimumab 3 mg/kg [22]. Table 15.3 summarizes the grade 3–4 adverse events reported in studies of checkpoint inhibition in EGC to date.

Biomarkers of Response

The results of the discussed studies uniformly suggest that benefit from immune checkpoint inhibitors is modest in an unselected population. Most studies report a median PFS of less than 2 months, even in the setting of encouraging OS, suggesting that most patients develop rapid progression on these treatments. Therefore, the identification of biomarkers to select patients most likely to benefit from these expensive and potentially toxic agents is a priority.

Approximately 40–60% of gastric and GE junction cancers are PD-L1 positive [17, 20]. There has been significant effort to investigate if PD-L1 expression by IHC can be used as a biomarker to select patients for immune-directed therapy with PD-1/PD-L1 inhibition, and pembrolizumab is approved by the FDA only in patients with PD-L1-positive tumors, as determined by the PD-L1 IHC 22C3 pharmDx Kit (Dako) companion test, and who have received ≥2 prior chemotherapy regimens. However, PD-L1 has been demonstrated to be an imperfect biomarker in EG cancer and many other cancers. Although PD-L1-positive tumors appear more likely to respond to treatment with anti-PD-1 and anti-PD-L1 antibodies, many of the studies above report responses and disease control even in patients with PD-L1-negative tumors. There appear to be key differences between PD-L1 expression in EG carcinoma and lung cancer and melanoma, and its role as a biomarker does not appear to be generalizable between tumor types. In EG cancer, expression of PD-L1

Table 15.3 Grade 3–4 toxicity from checkpoint inhibitors in EGC clinical studies

		Fatigue	Rash	Pruritus	Diarrhea	Colitis	Elevated LFTs	Pneumonitis	Endocrinopathies	Nephritis	Myelotoxicity
Pembrolizumab	KEYNOTE-012	5%	3% (pemphigoid)	0%	0%	0%	0%	3%	3% (hypothyroidism)	0%	0%
	KEYNOTE-059 Cohort 1	2.3%	0.8%	0%	1.2%	1.2%	0%	0.8%	0.4% hypothyroidism 0.4% thyroiditis	0%	0%
	KEYNOTE-059 Cohort 2	8%	8%	0%	0%	0%	0%	0%	0%	4%	64% (low neutrophils) 8% (low platelets) 8% (anemia)
Nivolumab	CheckMate 032 N3 mg	2%	0%	0%	2%	0%	8%	0%	0%	0%	0%
	ATTRACTION-2	0.6%	0%	0%	0.6%	0%	0.9%	0%	0%	0%	0%
Nivolumab + Ipilimumab	CheckMate 032 N1 mg + I3 mg	6%	0%	2%	14%	0%	24%	0%	0%	0%	0%
	CheckMate 032 N3 mg + I1 mg	0%	0%	0%	2%	0%	6%	0%	0%	0%	0%
Avelumab	JAVELIN Solid Tumor	2.7%	0%	0%	0%	0%	0%	0%	0%	0%	2.7% (low platelets) 2.7% (anemia)
Tremelimumab	N/A	0%	0%	0%	0%	5.5%	5.5%	0%	0%	0%	0%

principally occurs on infiltrating myeloid cells at the invasive margin and much less frequently on cancer cells [46, 47]. One study reported only a 12% rate of tumor cell membranous expression, while 44% of immune stromal cells expressed PD-L1 [46]. It remains unclear if membranous versus stromal PD-L1 expression affects response in EG cancer. Of note, rates of PD-L1 staining on tumor cells and immune cells are higher in tumors that are Epstein-Barr virus (EBV) positive and MSI high [48].

Testing of PD-L1 status is also a complex issue as there are currently several antibodies available for PD-L1 testing which have not been directly compared against each other. In addition, expression is heterogeneous, and the optimal cutoff is uncertain, and concordance among pathologists is also more difficult to achieve when measuring PD-L1 positivity on immune cells. This is highlighted by the discrepancy in PD-L1 positivity rates reported between the KEYNOTE-012 and KEYNOTE-059 studies (40% and 60%, respectively) and the ATTRACTION-2 study which reported a 13.5% PD-L1 positivity rate. The lower PD-L1 positivity rate in the ATTRACTION-2 study is at least in part because only tumor cells were evaluated for PD-L1 staining (unlike the CPS used in the pembrolizumab studies, which includes both tumor cells and peri-tumoral mononuclear cells). Similarly, the difference in PD-L1 positivity rate between the KEYNOTE-012 and KEYNOTE-059 studies—despite the use of the same antibody and the CPS—can be explained because later studies have mandated rapid processing of cell blocks for central PD-L1 testing. In light of the current uncertainty regarding the utility of PD-L1 as a biomarker, ongoing studies are enrolling patients irrespective of PD-L1 status.

A mononuclear inflammatory cell density score (0–4) was assessed in the KEYNOTE-012 study as part of a clinical trial PD-L1 assay which scored expression separately in tumor cells and mononuclear inflammatory cells. Of 35 patients who had biopsies available to be assessed with this assay, 4 of 9 (44%) patients who had a mononuclear cell density score of 3 had a PR, compared with 4 of 26 (15%) of patients with a score of ≤ 2. While the number of patients whose tumors were analyzed is small, the data is provocative.

The KEYNOTE-012 also investigated the potential use of an interferon-γ signature that may correlate with an increased magnitude of benefit from immune checkpoint inhibitors. In the KEYNOTE-001 study a six-gene (*CXCL9*, *CXCL10*, *IDO1*, *IFNG*, *HLA-DRA*, and *STAT1*) signature of interferon-γ-related genes was associated with response to pembrolizumab in patients with melanoma [49]. In KEYNOTE-012, an interferon-γ composite score was calculated using gene expression profiling of RNA isolated from tumor samples. Only 30 tumor samples were evaluable. There was a trend toward treatment response in patients with a higher interferon-γ signature score ($p = 0.070$) [17]. An 18-gene T cell-inflamed gene expression signature, derived using pretreatment tissue samples from previous pembrolizumab studies across several cancer types, significantly predicted ORR and survival in patients treated with pembrolizumab [50, 51]. In the KEYNOTE-059 study, this gene expression signature was significantly associated with improved response to pembrolizumab ($p = 0.014$) in 144 patients who had pretreatment testing of tumor tissue [20]. These results suggest that this gene signature may be a

meaningful predictor of treatment response. Further evaluation is attractive as it may be more reproducible and robust as a biomarker than PD-L1.

The Cancer Genome Atlas (TCGA) has characterized molecular subtypes of gastric and esophageal cancer, and an active area of investigation is correlation of response to immune checkpoint inhibition with the different subtypes identified. The four subtypes identified in gastric cancer are EBV positive, MSI, genomically stable (GS), and chromosomal instability (CIN) [52]. Esophageal adenocarcinomas strongly resemble the chromosomal instability variant of gastric adenocarcinoma. The EBV and MSI subtypes show elevated mutation rates. It is speculated that most patients who respond to single-agent checkpoint inhibitors may have these subtypes and patients with the genomically stable and chromosomally unstable subtypes may require combination immunotherapeutic strategies. Of note, MSI-high tumors occur very rarely in esophageal cancer, and squamous cell esophageal carcinomas show frequent genetic amplifications [53].

While the MSI subgroup accounted for 22% of gastric cancer patients in TCGA analysis, this subgroup is rarely seen in esophageal and GE junction cancers. In addition, this analysis was restricted to patients with operable tumors, and the incidence of MSI tumors in the metastatic setting appears to be much lower [54]. The presence of MSI is associated with an elevated mutation rate and has been identified as predictive of response to PD-1 inhibition.

Finally, it is now well recognized that PD-1 inhibitors are active in dMMR/MSI-high colorectal cancer, and Le et al. also reported significant activity in other mismatch repair-deficient gastrointestinal cancers, including gastric cancer [55]. In the first tissue site-agnostic approval, the FDA granted accelerated approval in May 2017 to pembrolizumab for adult and pediatric patients with unresectable or metastatic, MSI solid tumors that had progressed on one standard therapy. The approval was based on data from 149 patients with MSI cancers enrolled across 5 single-arm clinical trials [56], and 9 of these patients had EG carcinoma. In this group, ORR was 56% with five out of the nine patients achieving a PR. Given this approval, testing for MSI via PCR or MMR status by immunohistochemistry is now standard, along with Her2 and PD-L1 testing. In addition, the increasing use of next-generation sequencing assays will also identify patients with MSI tumors. Furthermore, high somatic mutational burden may be of value in predicting response to PD-1 inhibitors, and only melanoma, lung, and bladder cancers demonstrate a more mutated profile than esophagogastric cancers [57]. Elevated tumor mutation burden may occur independent of MSI disease and may be utilized in the future as a biomarker of response [54].

Future Directions

At the time of the writing of this manuscript, we are rapidly approaching the end of the era of evaluating single-agent immunotherapy or even single-agent PD-1 blockade combined with chemotherapy. The next decade of evaluation will involve combination immunotherapeutic strategies to try to increase the proportion of patients

who benefit but also the magnitude of benefit obtained. Studies evaluating chemotherapy in combination with immune checkpoint inhibition are at an advanced stage, and selected studies are described in Table 15.2.

There are multiple ongoing or planned phase I/II studies investigating immune checkpoint inhibitors in combination with other immunotherapy drugs, targeted therapies, or locoregional approaches (such as radiation or ablative procedures).

An interesting combinatorial strategy that is being investigated in other cancers is the combination of immune checkpoint inhibition with locoregional therapy aiming to generate an abscopal effect which refers to response in gross tumor sites outside of a radiation field. The hypothesis is that lysis of tumor cells by a locoregional treatment results in the release of intracellular antigens which are then recognized by an activated immune system and resultant anti-cancer effect. This has previously been observed in patients with melanoma who were receiving ipilimumab and then received palliative radiation [58]. A number of studies are currently evaluating this strategy in microsatellite-stable/MMR-proficient colorectal cancer.

Other immunomodulators, vaccines, and targeted therapies are also being evaluated in combination with immune checkpoint inhibitors. Many of these studies are specifically enrolling patients with EG carcinoma but also include studies that are enrolling EG patients in dose-expansion cohorts.

Conclusions

The evaluation of immune checkpoint inhibitors both in solid tumors and more recently in EG cancer has occurred at a rapid pace. The ATTRACTION-2 (nivolumab) and KEYNOTE-059 (pembrolizumab) studies have now confirmed activity of single-agent anti-PD-1 antibodies in the chemorefractory setting, resulting in regulatory approval (pembrolizumab in the United States and nivolumab in Japan) for this indication. While this is positive progress in a disease that continues to have a dismal prognosis, benefit is modest with single-agent therapy. It is therefore important to harness the knowledge that we have gained to date in order to move forward with innovative immunotherapeutic strategies to further improve outcomes for patients with EG cancer. The results of the ongoing phase III studies are awaited with eager anticipation, and it is hoped that they will establish new treatment paradigms in this disease. Finally, these drugs are not without both clinical and financial toxicities, with responses rates observed in a small albeit significant population of patients. Therefore, it is imperative that we attempt to identify patients most likely to benefit from these therapies, through ongoing correlative efforts and the next generation of studies evaluating combinatorial strategies.

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