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REVIEW

THE EMERGING ROLE OF IMMUNOTHERAPY IN GASTROESOPHAGEAL CANCER: STATE OF ART AND FUTURE PERSPECTIVE

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ABSTRACT

The introduction of immunotherapy in the therapeutic algorithm of gastroesophageal cancer is still a debated issue. Recent findings from randomized clinical trials documented the efficacy of adjuvant nivolumab in improving disease free survival (DFS) in resectable esophageal and gastroesophageal junction cancer patients with residual pathologic disease after neoadjuvant chemoradiation (CheckMate 577). Consistently, the combination of pembrolizumab and doublet chemotherapy with 5-fluorouracil plus cisplatin improved first-line treatment outcomes in metastatic esophageal squamous cancer; moreover the major benefit was observed in tumor expressing PD-L1 combined positive score (CPS) > 10 (Keynote 590). Finally, the addition of nivolumab to first-line oxaliplatin and 5-fluorouracil-based chemotherapy improved overall survival, progression free survival and response rate in patients with metastatic gastric/gastroesophageal junction cancer with PD-L1 positive score (PD-L1 CPS ≥ 5) (CheckMate 649). Moving forward, the research focused on the identification of predictive biomarkers of response to immunotherapy, to refine the patients' selection and maximize the treatment benefit. Microsatellite instability has been shown to predict higher response to checkpoint inhibitors as highlighted by subgroup analyses of the pivotal studies. For what concerns microsatellite stable tumors, the expression of PD-L1, the positivity for Epstein-Barr virus and a high tumor mutational burden are now regarded as the most promising and reliable predictive markers for immunotherapy as far as now. Therefore, the anti-PD1 agents nivolumab and pembrolizumab proved to confer

an improvement in the outcome of gastroesophageal cancer patients but the real magnitude of benefit of immunotherapy in this disease setting is under definition. Biomarker-focused research will allow clinicians to define the optimal therapeutic algorithm in the different patients populations.

KEY WORDS

Immunotherapy; biomarker; gastric cancer; PD-L1; microsatellite instability.

IMPACT STATEMENT

Immune-checkpoint inhibitors proved to confer a meaningful benefit in the setting of gastric/gastroesophageal junction cancer, nevertheless a refinement of patients selection according to predictive biomarkers could maximize the treatment benefit.

INTRODUCTION

Gastroesophageal cancer (GC) is a highly aggressive tumor that ranks at the sixth place for the incidence of new cancers worldwide (about 1,033,000 cases) and represents the third most common cause of cancer-related death, resulting in approximately 780,000 deaths yearly (1). The epidemiology of GC widely varies according to the geographical region, and, specifically, in Europe the incidence is estimated at 81,600 and 51,500 cases in men and women, respectively, and the number of deaths is rated at 62,000 in men and 40,300 in women (2). In Italy 14,500 new diagnoses were estimated in 2020 and about 8,500 deaths were estimated in 2021, respectively (3).

The cornerstone of potentially curative treatment in non-metastatic disease is radical surgery, combined with peri-operative or adjuvant chemotherapy according to International Guidelines (4-10). Nowadays, both the adjuvant and peri-operative chemotherapy schedules are evidence-based and guideline-endorsed treatments, although in Asia the preferred approach is surgery plus adjuvant chemotherapy, whereas outside of Asia peri-operative chemotherapy is the most frequent choice (11). Despite the improvements in the disease management thanks to the development of multimodality treatment strategies, more than half patients still relapse and die from their disease. Nowadays, GC/gastroesophageal junction cancer (GEJC) remains one of the most lethal malignancies with 5-year survival rates of about 22% and less than 4% for localized and metastatic disease, respectively (2). In the setting of metastatic GC/GEJC the choice of the optimal first-line chemotherapy is based upon, on the one side, the extension and molecular characteristics of tumor, mainly the presence of HER2 overexpression/amplification, and on the other side, the clinical conditions and comorbidities of patients (11). The doublet combination with platinum derivative and fluoropyrimidine is considered a standard of care with or without trastuzumab (in case of HER2 overexpressed/amplified tumors). In the second-line setting and in later treatment lines, the combination of taxanes plus ramucirumab, ramucirumab monotherapy or irinotecan represent the main choices, even though with poor survival outcomes (12-17). The research progresses led to a deeper understanding of the molecular characterization of GC, providing the opportunity to classify tumors into different subtypes on the basis of their genomic profile, with the most common TCGA classification, furtherly described in the **table I** (18).

The immunotherapy revolution deeply changed the therapeutic management and the prognosis of patients in several cancer settings, such as non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma (RCC), urothelial cancer and head and neck tumors (19). The principal therapeutic weapon is represented by immune checkpoint inhibitors (ICIs) targeting the programmed cell death receptor 1 and its ligand (PD1 and PD-L1). In fact, tumors upregulate the inhibitory checkpoints of the immune system, while ICIs release the brakes and reactivate T-cells activity in order to promote the anti-tumor immune reaction(20). In the setting of GC/GEJC, several studies have been conducted or are ongoing to explore and define the potential role of ICIs. In this review we aim at depicting a comprehensive picture of the current scenario and of the future perspectives.

LOCALIZED DISEASE

In the setting of localized or locally advanced disease, eligible for curative radical surgery, few data have been collected on the role of immunotherapy. First of all, in stage II/III esophageal or GEJC patients treated with chemoradiotherapy followed by surgery and with evidence of residual disease, adjuvant treatment with nivolumab for 1 year provided a statistically significant and clinically meaningful advantage in disease-free survival (DFS) (median DFS 22.4 *vs* 11.0 months, HR 0.69, 95% CI 0.56-0.86, p ≤ 0.001) over placebo in the phase III CheckMate 577 trial. Disease-free survival favored nivolumab across multiple prespecified subgroups, and the benefit was more pronounced in the squamous histotype (median DFS 29.7 *vs* 11.0 months, HR 0.61, 95% CI 0.42-0.88) although maintained also in the adenocarcinoma subtype (median DFS 19.4 *vs* 11.1 months, HR 0.75, 95% CI 0.59-0.96), potentially opening a new therapeutic scenario (21).

Moving forward, clinical trials are ongoing in order to provide evidence-based results. In details, the randomized, open-label, phase II DANTE study (NCT03421288) is investigating the combination of the anti-PD-L1 agent atezolizumab to peri-operative FLOT regimen (5fluorouracil, oxaliplatin and docetaxel), followed by adjuvant atezolizumab, versus standard peri-operative FLOT in GC or GEJC (Siewert I-III) cT2 or higher, any N or node positive, without any biological selection and HER2 status not assessed. The randomization is stratified per microsatellite instability (MSI) status while PD-L1 expression is performed but does not represent a stratification factor. The study completed the recruitment and the presented safety results showed that the chemo-immuno regimen was safe and feasible in the peri-operative setting of GC/GEJC, while activity and efficacy results are not available yet (22). Similarly, the randomized, double-blind, phase III KEYNOTE-585 study (NCT03221426) is investigating pembrolizumab or placebo combined with peri-operative chemotherapy, followed by pembrolizumab or placebo maintenance in T3 or higher or N positive GC/GEJC patients. The initial chemotherapy schedule was cisplatin plus 5fluorouracil or capecitabine, but the study was amended to include a cohort with FLOT after the results of the FLOT4 trial (9). The trial will assess the status of MSI and PD-L1 as exploratory biomarkers, though neither MSI status nor PD-L1 represent stratification factors. The two above-described trials are investigating immunotherapy in an unselected population. However, the results of the recent pivotal trials conducted in the metastatic setting highlighted how predictive biomarkers of response to immunotherapy are crucial to select patients with predicted enhanced response to ICIs. Particularly, as discussed above, agnostic tumors with MSI-high status are highly responsive to immunotherapy, thus clinical trials are ongoing in this peculiarly selected subpopulation (23, 24). The rationale relies in the results of proof of concept studies that showed how pre-operative immunotherapy could achieve a pathologic major or complete response in potentially resectable mismatch repair deficient (dMMR)/MSI-high tumors and eventually provide a chance of cure even regardless

SUBTYPE	FREQUENCY	CHARACTERISTICS
Chromosomal Instability (CIN)	50%	• Intestinal histology • TP53 mutation • High frequency of tyrosine kinase/RAS pathway activation
Genomically Stable (GS)	20%	• Diffuse histology • CDH1, RHOA mutations • CLDN18-ARHGAP fusion • alterations in cellular adhesion molecules genes
Epstein-Barr Virus (EBV)	9%	• PIK3CA mutation • PD-L1/2 overexpression • EBV-CIMP • CDKN2A silencing • Immune cell signalling
Microsatellite Instability (MSI),	22%	• Hypermutation • Gastric-CIMP • MLH1 silencing • Mitotic pathways

Table I. *Description of The Cancer Genome Atlas Classification (TCGA) of gastric cancer.*

of surgery. In details, in the phase II NICHE study, a window of opportunity treatment with 1 cycle of ipilimumab plus nivolumab in resectable colorectal cancer patients obtained no meaningful response in pMMR cases while a major or complete pathological response in all but one dMMR ones (25). This was confirmed by a case series of localized MSI-high GC or colon cancer patients achieving a high rate of pCR after immunotherapy (26). On this basis, two trials are ongoing to test immunotherapy in MSI-high GC/GEJC patients eligible for radical surgery. The first one is the GERCOR NEONIPIGA trial (NCT04006262) that is aimed at enrolling 32 patients to receive a 12-week preoperative combo-immunotherapy with nivolumab plus ipilimumab and, after radical surgery, postoperative nivolumab up to 1 year. The second one is the Italian, multicenter, single-arm, multicohort, phase II INFINITY study (NCT04817826) aimed at investigating the safety and activity of the ICIs combination durvalumab (1500 mg q4w for 3 cycles) plus tremelimumab (300 mg single dose) as preoperative or potentially definitive treatment in dMMR/MSI-high/Epstein-Barr (EBV) negative GC/GEJC patients. The Cohort 1 is enrolling up to 18 patients and its primary endpoint is the rate of pCR at surgery after neoadjuvant immunotherapy, while Cohort 2 will investigate a non-operative-management strategy in patients achieving complete clinical response at radiological, tissue and liquid biopsy level after immunotherapy (**figure 1**) (27).

UNTREATED METASTATIC DISEASE

In the setting of first-line treatment for advanced/metastatic GC/GEJC, the first study was the non-randomized, multicohort, phase II KEYNOTE-059 study that investigated pembrolizumab in combination with standard cisplatin-fluoropyrimidine chemotherapy irrespectively of PD-L1 expression in Cohort 2 and pembrolizumab monotherapy in patients with PD-L1 combined positive score (CPS) ≥ 1 in Cohort 3. Overall, 25 and 31 patients were enrolled in Cohort 2 and 3, respectively: the ORR was 60.0% (95% CI, 38.7-78.9) and 25.8% (95% CI 11.9-44.6), median duration of response was 4.6 and 9.6 months, and median overall survival (OS) was 13.8 and 20.6 months, respectively, with a globally manageable tolerability profile (28).

On this basis, the randomized, phase III KEYNOTE-062 trial was designed and conducted in treatment naïve advanced GC/GEJC Asian and non Asian patients selected for PD-L1 expression CPS ≥ 1. A total of 763 patients were randomized 1:1:1 to pembrolizumab monotherapy versus pembrolizumab plus standard cisplatin-fluoropyrimidine chemotherapy versus placebo plus chemotherapy. The complex statistical design of the study compared pembrolizumab to placebo plus chemotherapy, showing the non-inferiority (primary endpoint) (median OS 10.6 *vs* 11.1 months, HR 0.91, 99.2% CI 0.69-1.18) but not the superiority of pembrolizumab as compared to chemotherapy. Nevertheless, it should be pointed out that at least half of fit-for-a-trial patients treated with pembrolizumab died earlier than with chemotherapy and that the accepted confidence interval for inferiority margin worse than chemotherapy was wide, besides the absence of an improvement in quality of life. Moreover, the addition of pembrolizumab to chemotherapy failed to condition an improvement

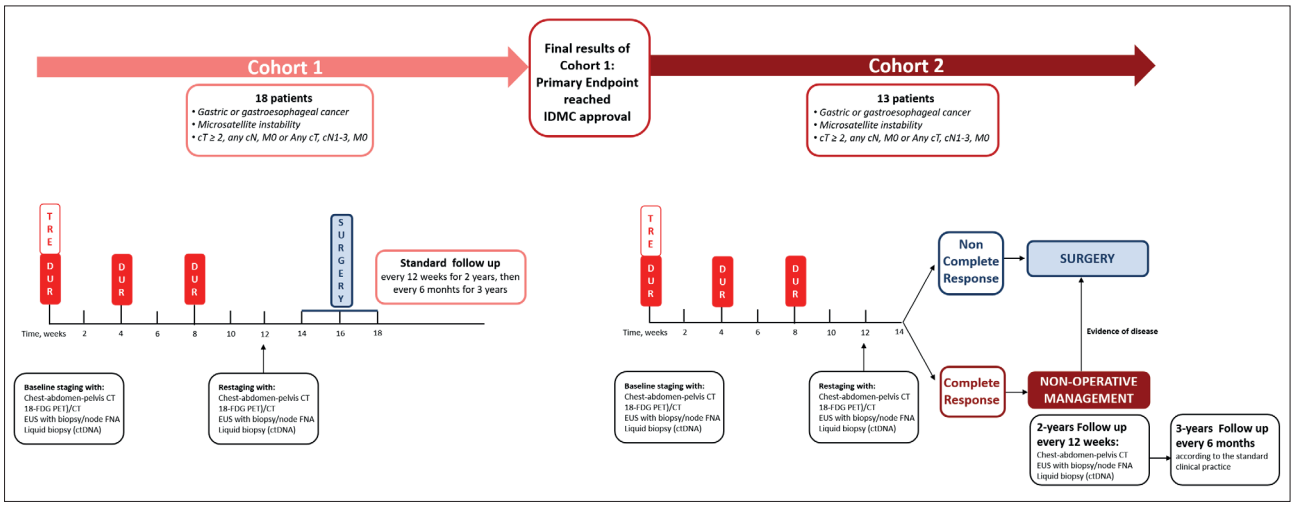

Figure 1. *Study Diagram.*

in terms of OS over standard chemotherapy alone both in patients with CPS ≥ 1 (median OS 12.5 *vs* 11.1 months, HR 0.85, 95% CI 0.70-1.03, p = 0.05) and CPS ≥ 10 (median OS 12.3 *vs* 10.8 months, HR 0.85, 95% CI 0.62-1.17, p = 0.16) (29).

Afterwards, the randomized, open-label, phase III JAVELIN Gastric 100 study enrolled Asian and non Asian advanced or metastatic GC/GEJC patients (independently from PD-L1 expression) who achieved disease control after a 12-week first-line therapy with platinum/fluoropyrimidine, and compared the switch maintenance with avelumab versus the continuation of the standard treatment. Avelumab failed to achieve the superiority in terms of OS (median OS 10.4 *vs* 10.9 months, HR 0.91, 95% CI 0.74-1.11, p = 0.1779) in the overall trial population and in the PD-L1 positive on tumor cells subgroup. The possible caveats could be found in the duration of "induction" first-line chemotherapy (12 weeks only) and in the selection of patients (with TPS instead of CPS), since in an exploratory analysis stratifying patients for PD-L1 CPS ≥ 1 and < 1, a survival advantage was reported (median OS 14.9 *vs* 11.6 months, HR 0.72, 95% CI 0.49-1.05), even though not confirmed with the cutoff of CPS ≥ 10 (30).

Recently, three pivotal studies have been presented, with potentially practice-changing results. First, the randomized, open-label, phase III CheckMate 649 trial enrolled previously untreated, advanced or metastatic, HER2 negative, Asian and non Asian GC/GEJC patients, regardless of PD-L1 expression, who were randomized to ipilimumab plus nivolumab, nivolumab plus XELOX/FOLFOX or XELOX/FOLFOX. Afterwards, the combo-immuno arm was closed and the primary population was amended to cases with PD-L1 CPS ≥ 5. The combination of nivolumab to standard first-line chemotherapy succeeded in significantly improving OS (median OS 14.4 *vs* 11.1 months, HR 0.71, 95% CI 0.59-0.86, p < 0.0001) and progression-free survival (PFS) (7.7 *vs* 6.0 months, HR 0.68, 95% CI 0.56-0.81, p < 0.0001) over chemotherapy alone in patients with CPS ≥ 5. Consistently, the OS outcome was significantly improved with the addition of nivolumab to first line in patients with CPS ≥ 1 and in the overall study population, with a manageable safety profile, thus the Food and Drug Administration (FDA) approved this schedule independently from the expression of PD-L1 (31). Nevertheless, it should be remarked that the magnitude of benefit, in terms of delta of OS improvement and HR, progressively decreased from CPS ≥ 5 to CPS ≥ 1 to overall, and, notably, there was an enrichment of patients with CPS ≥ 5 in the two latter populations (about 70% in CPS ≥ 1 and 60% in all patients randomized). Therefore, the results in patients with CPS < 1 or between 1 and 5 would possibly provide interesting insights on the real benefit of immunotherapy in the different subgroups of patients. On the other hand, the immunotherapy combination ipilimumab plus nivolumab failed to significantly improve OS over chemotherapy in the CPS ≥ 5 subgroup and the curves showed the typical crossing, suggesting that a chemotherapy-free regimen should not be the choice for the upfront treatment in metastatic GC/GEJC (32).

Second, the randomized, placebo-controlled, phase II/III ATTRACTION-4 study randomized only Asian advanced/metastatic GC/GEJC patients to nivolumab or placebo plus oxaliplatin and capecitabine or S1 irrespectively of the expression of PD-L1. While the phase II part of the study showed promising results for the chemo-immunotherapy combination, the phase III part reported a statistically significant improvement in terms of PFS (median PFS 10.4 *vs* 8.3, HR 0.68, 95% CI 0.51-0.90, p = 0.0007) while no significant benefit in OS (median OS 17.4 *vs* 17.1, HR 0.90, 95% CI 0.75-1.08, p = 0.257) (33, 34). It could be argued that the different results obtained in CheckMate 649 and ATTRACTION-4 studies, very similar for design and treatment schedule, may be partially explained by the different selection of patients (according or independently to PD-L1 CPS) and by the variable weight of further treatment lines, especially with immunotherapy, higher in the Asian population.

Finally, the randomized, placebo-controlled, phase III Keynote-590 trial compared pembrolizumab plus cisplatin/5-fluorouracil versus placebo plus cisplatin/5-fluorouracil chemotherapy in patients with previously untreated advanced unresectable or metastatic esophageal or gastroesophageal junction carcinoma either adenocarcinoma or squamous cell carcinoma. The study demonstrated that the combination of immunotherapy to the standard first-line chemotherapy provides a statistically significant benefit in terms of OS irrespectively of CPS status, although the magnitude of benefit was higher in patients selected for CPS ≥ 10 or squamous histology and the highest in squamous cell carcinoma with CPS ≥ 10. These results are clinically relevant and conditioned the approval of European Medical Association for patients with untreated advanced esophageal carcinoma with CPS ≥ 10 independently on histology (35). The main results of the pivotal studies are reported in **table II**.

	ATTRACTION-2 N = 330 *VS.* 163	JAVELIN GASTRIC 300 N = 185 *VS.* 186	KEYNOTE-061 N = 296 *VS.* 296	JAVELIN GASTRIC 100 N = 249 *VS.* 250	KEYNOTE-062 N = 256 *VS.* 250 N = 257 *VS.* 250	CHECKMATE 649 N = 789 *VS.* 792	ATTRACTION-4 N = 362 *VS.* 362	KEYNOTE-590 N = 373 *VS* 376
Setting	≥ 3L Unselected GC/GEJC	3L Unselected GC/GEJC	2L CPS ≥ 1 (n = 395) GC/GEJC	1L Maintenance GC/GEJC	1L CPS ≥ 1 GC/GEJC	1L All pts/CPS ≥ 5 GC/GEJC	1L Unselected GC/GEJC	1L Unselected Esophageal/GEJC
Treatment arms	Nivolumab *vs.* Placebo	Avelumab *vs.* CT(inv's choice)	Pembrolizumab *vs.* Paclitaxel	Avelumab *vs.* CT	A: Pembrolizumab *vs.* CT B: Pembrolizumab+CT vs. CT+Placebo	Nivolumab+CT *vs.* CT	Nivolumab+CT *vs.* CT	Pembrolizumab +CT*vs.* CT+Placebo
Response rate	11% *vs.* 0%	2.2% *vs.* 4.3%	15.8% *vs.* 13.6%	13.3% *vs.* 14.4	14.8% *vs.* 37.2% 48.6% *vs.* 37.2%	60% *vs.* 45%	57.5% *vs.* 47.8%	ITT: 45% *vs.* 29.3%
Median PFS, mos	1.61 *vs.* 1.45	1.4 *vs.* 2.7	1.5 *vs.* 4.1	3.2 *vs.* 4.4	2.0 *vs.* 6.4 6.9 *vs.* 6.4	7.7 *vs.* 6.9 7.7 *vs.* 6.0	10.5 *vs.* 8.3	CPS ≥ 10:7.5 *vs.* 5.5 SCC: 6.3 *vs.* 5.8 ITT: 6.3 *vs.* 5.8
Median OS, mos	5.26 *vs.* 4.14	4.6 *vs.* 5.0	9.1 *vs.* 8.3	10.4 *vs.* 10.9	10.5 *vs.* 11.1 12.5 *vs.* 11.1	13.8 *vs.* 11.6 14.4 *vs.* 11.1	17.5 *vs.* 17.2	CPS ≥ 10:13.5 *vs.* 9.4 SCC: 12.6 *vs.* 9.8 ITT: 12.4 *vs.* 9.8
HR (95%CI)	0.63 (0.51-0.78)	1.1 (0.9-1.4)	0.82 (0.66-1.03)	0.91 (0.74-1.11)	0.91 (0.69-1.18) 0.85 (0.70-1.03)	0.80 (0.68-0.94) 0.71 (0.59-0.86)	0.90 (0.75-1.08).	CPS ≥ 10:0.62 (0.49-0.78) SCC:0.72 (0.60-0.88) ITT:0.73 (0.62-0.86)

Table II. *Summary of the main results of the pivotal clinical trials conducted on immunotherapy in the setting of gastroesophageal cancer.*

GC: gastric cancer; GEJC: gastroesophageal junction cancer; CPS: combined positive score; CT: chemotherapy; ITT: intention-to-treat population; SCC: squamous cell carcinoma; PFS: progression-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval.

PRETREATED METASTATIC DISEASE

In the setting of metastatic GC/GEJC patients refractory to previous treatments, landmark clinical trials have been conducted on the role of immunotherapy, providing conflicting results. First, the randomized, double-blind, phase III ATTRACTION-2 trial investigated the anti-PD1 agent nivolumab (at the dose of 3 mg/kg q14) versus best supportive care in patients with advanced GC/GEJC pretreated with 2 or more lines of therapy, irrespectively of the expression of PD-L1. The study showed a statistically significant improvement in OS for immunotherapy (median OS 5.3 *vs* 4.1 months, HR 0.63, 95% CI 0.51-0.78, p < 0.0001), that displayed an overall response rate (ORR) of 11.4%, with a manageable safety profile. Comparable results were reported in PD-L1 tumor cell (TPS) < and ≥ 1%, that represented about 86.5% and 13.5% of the overall population, with a median OS of 6.0 *vs* 4.2 and 5.2 *vs* 3.8 months, with nivolumab versus best supportive care, respectively. The trial enrolled an only Asian population, therefore nivolumab was approved in third-line setting of GC/GEJC in Asia, while no data are available for non Asian patients, and this is crucial taking into consideration the different tumor biology and the variable sensitivity to immunotherapy in the two populations (36).

Second, the randomized, open-label, phase III JAVELIN Gastric 300 study compared the anti-PD-L1 agent avelumab (at the dose of 10 mg/kg q14) with physician's choice chemotherapy (paclitaxel or irinotecan or best supportive care in patients unfit for chemotherapy) as third-line therapy in advanced GC/GEJC patients both Asian and non Asian. The trial failed to demonstrate a significant benefit in terms of OS with immunotherapy versus standard of care treatment (median OS 4.6 *vs* 5.0 months, HR 1.1, 95% CI 0.9-1.4, p = 0.81), even though with a more favorable safety profile. Negative results were obtained even for the secondary endpoints of PFS and ORR and no differences to remark were found in the subgroup analyses (37).

Third, in the single-arm, multi cohort, open-label, phase II KEYNOTE-059 study, 259 Asian or non Asian patients with GC/GEJC pretreated with 2 or more previous lines were enrolled in Cohort 1 and received the anti-PD1 agent pembrolizumab (200 mg flat dose q21). In this setting, the ICI monotherapy conferred a 11.0% ORR overall and 15.5% *vs* 6.4% in patients with PD-L1 CPS ≥ 1 and < 1, respectively. Therefore, the benefit of immunotherapy was higher in PD-L1 positive patients with durable responses (median duration of response 16.3 months) and median OS of 5.8 months *vs* 4.6 months in PD-L1 negative ones (38). On this basis, pembrolizumab received the approval of FDA for previously treated PD-L1 positive GC patients.

Finally, the randomized, open-label phase III KEYNOTE-061 trial randomized Asian or non Asian GC/GEJC patients having progressed to the first-line treatment to pembrolizumab versus paclitaxel independently from the expression of PD-L1, even though the study was furtherly amended to include only patients with PD-L1 CPS ≥ 1. In patients with CPS ≥ 1, representing about 2/3 of the overall population, pembrolizumab failed to reach a statistically significant improvement in terms of OS over the standard second-line chemotherapy that lacked the combination with the biologic agent ramucirumab (median OS 9.1 *vs* 8.3 months, HR 0.82, 95% CI 0.66-1.03, p = 0.0421) (39). Looking at the curves, about half patients treated with pembrolizumab died before than what occurred with chemotherapy, since curves crossed at 8 months, and the apparent benefit for immunotherapy shown by the tails of the curves may be jeopardized by the limited numbers of patients. Nevertheless, the post-hoc analysis about the stratification for PD-L1 expression provided meaningful results, since in the subgroup analyses for PD-L1, in patients with negative PD-L1 (CPS < 1) pembrolizumab provided worse results than paclitaxel, while in patients with CPS ≥ 10 pembrolizumab was superior to chemotherapy (median OS 10.4 *vs* 8.0 months, HR 0.64, 95% CI 0.41-1.02) (39).

The main results of the pivotal studies are reported in **table II**.

BIOMARKERS

In light of the results of the studies conducted on ICIs in several tumor settings, the research focused on the identification of specific and reliable predictive biomarkers of response to immunotherapy, with the aim of refining the patients' selection and maximizing the treatment benefit. The highest burden of evidence collected on this topic concerns the expression of PD-L1, the status of MSI, the positivity for EBV, the Tumor Mutational Burden (TMB), but the research is going further and new biomarkers are under investigation.

MICROSATELLITE INSTABILITY

The status of MSI-high is a well-established good prognostic factor for prolonged survival in early-stage colorectal cancer patients, and a potential predictive marker of lack of benefit from adjuvant fluoropyrimidine monotherapy in stage II disease (40). In the setting of resectable GC, an Individual Patient Data pooled analysis combining the results of four large international randomized trials (MAGIC, CLASSIC, ARTIST and ITACA-S) was performed and confirmed the powerful positive prognostic effect of MSI-high status in surgically resected GC patients and the predicted lack of benefit of peri-operative or adjuvant chemotherapy after surgery in this molecular subgroup (41). Recently, the key role of MSI status has been established as a powerful predictive marker for responsiveness to immunotherapy since advanced tumors with MSI-high or dMMR status, across different primary sites of origin, proved to be highly responsive to immunotherapy, even more of the other well-known immune-sensitive cancers (23, 24). In fact, the FDA granted an accelerated approval to pembrolizumab for adult and pediatric patients with agnostic unresectable/metastatic MSI-high or dMMR cancers. The explanation lies in the high mutational load of MSI-high tumors, with elevated amount of neoantigens eliciting and boosting the anti-tumor immune response (18, 42). In the specific setting of GC/GEJC, the exploratory analyses of the pivotal clinical trials KEYNOTE-059 and -061 and -062 showed that patients with MSI-high GC had a dramatic benefit in terms of response and survival outcomes from immunotherapy. In details, in Cohort 1 of KEYNOTE-059, the ORR with pembrolizumab monotherapy was 11.6% overall, while 57.1% in MSI-high patients vs 9.0% in MSS ones (38). In KEYNOTE-061 study, patients with MSI-high tumors, irrespectively of PD-L1 CPS, had a median OS not reached (95% CI 5.6 months-not reached) with pembrolizumab *vs* 8.1 months (2.0-16.7) with paclitaxel, and 7/15 patients (47%) achieved an objective response with pembrolizumab *vs* 2/12 (17%) with paclitaxel (39). Finally, in KEYNOTE-062 trial, in the MSI-high subgroup median OS was not reached (95% CI, 10.7-not reached) *vs* 8.5 months (95% CI, 5.3-20.8), median PFS was 11.2 months (95% CI, 1.5-NR) *vs* 6.6 months (95% CI, 4.4-8.3), and ORR was 57.1% *vs* 36.8% with pembrolizumab versus standard chemotherapy, respectively (29). This was confirmed in a meta-analysis including 9 clinical trials and more than 2000 patients, in which MSI-high GC/GEJC treated with anti-PD1 ICIs achieved a higher ORR and disease control rate than MSS ones (43) and even in another meta-analysis of the pivotal first and subsequent treatment lines clinical trials described above (44). Therefore, the significant benefit of immunotherapy in terms of survival outcome in GC/GEJC patients selected for MSI-high status was shown both in first and subsequent treatment lines, as confirmed by the subgroup analysis of the CheckMate 649 study, even though only a minor part of advanced GC/GEJC are MSI-high (about 4%).

EPSTEIN-BARR VIRUS

The recent studies highlighted how the positivity for the EBV in the setting of GC/GEJC represents a powerful biomarker of response to immunotherapy with ICIs, although present in a very limited proportion of advanced GC/GEJC patients, less than 5% (42, 45). For this reason, the available clinical data derive from case reports or series and this biomarker has never been tested in randomized clinical trials. EBV positive is one of the TCGA subtypes, as identified on molecular profiling analyses, characterized by extensive DNA hypermethylation, mutations of PIK3CA and amplifications of CD274 and PDCD1LG2 genes, encoding for PD-L1 and PD-L2, respectively, as well as activation of immune signaling pathways (18). Although EBV-positive tumors are endowed with a low tumor mutational burden, they are characterized by a high expression of immune checkpoints such as PD1 and CTLA-4 and by an elevated histological lymphocytic infiltration (46). Consistently with MSI-high GC/GEJC, EBV-positive ones are endowed with better outcomes after radical surgery than the other subtypes, likely related to the host immune response, and even improved prognosis in the metastatic setting (47). Therefore, results have been obtained on the enhanced sensitivity of EBV-positive tumors to immunotherapy with anti-PD1 and anti-PD-L1 agents, with impressive responses with pembrolizumab in previously treated GC patients (48). Nevertheless, the evidence collected is limited by the low prevalence of this condition, that impairs the opportunity to design and conduct dedicated clinical trials (42, 46).

PD-L1

The first and most investigated biomarker for response to anti-PD1/PD-L1 agents is the expression

of PD-L1, based on the mechanism of activity of ICIs. The clinical significance of the expression of PD-L1 on tumor cells and/or on the immune cells infiltrating the tumor assessed by immunohistochemistry (IHC) was identified in the initial clinical trial investigating the anti-PD1 agent nivolumab and, since then, it has been widely studied in several tumor settings with variable results (19). The rate of PD-L1 expression is highly variable across histologies and the different studies, namely in tumors with enhanced response to immunotherapy, such as NSCLC, melanoma and RCC, it ranges between 14% and 100% and, conversely, in cancers with reduced sensitivity to ICIs, like colorectal cancer or sarcoma, a comparable expression is shown, underlining the potential limitations of this biomarker (49, 50). Furthermore, other crucial limitations of PD-L1 expression may be found in the variability in the methods of assessment and in the tumor heterogeneity. In fact, each anti PD1/PD-L1 ICI has its own companion antibody (*e.g.*, Dako, Leica platform, Ventana Medical System), the scoring systems are not homogeneous for the target cells assessed, whether only tumor cells (Tumor Proportion Score - TPS) or both tumor cells and immune cells infiltrating the neoplastic stroma (Combined Positive Score - CPS), and the definition of the cutoff of positivity is uncertain (51). Additionally, the intra- and inter-tumor heterogeneity should be considered, with potential differential expression between primary tumor and metastases, as well as the possible dynamics of increase and decrease of the expression during the natural history of cancer (52, 53).

In the specific setting of GC/GEJC, the score of reference is the CPS, since it was validated by a comparison with the TPS in the frame of the Cohort 1 of the KEYNOTE-059 study (54). While PD-L1 positive tumors according to TPS ≥ 1% accounted for 12.5% overall with minimal enrichment of responses, CPS ≥ 1 ones represented 57.6% of the total, with meaningful enrichment of responses, besides reaching a high rate of inter and intra-pathologist agreement for the definition of CPS (54). Therefore, the CPS score is currently used in the definition of the study populations, study endpoints and stratification factors of the pivotal clinical trials, as increased PD-L1 expression corresponds to an enhanced tumor response to immunotherapy, even though this does not apply to all cases, with some PD-L1 patients benefitting from ICIs and PD-L1 positive ones not (48). Moreover, the optimal positivity cutoff to discriminate the responsiveness to immunotherapy has not been defined yet (*e.g.* 1, 5, 10). In fact, as discussed above, in the KEYNOTE-061 second-line study, no significant benefit in terms of OS was shown in patients with CPS ≥ 1 (median OS 9.1 *vs* 8.3 months, HR 0.81, 95% CI 0.66-1.00) while an increased benefit was seen with CPS ≥ 5 (10.4 *vs* 8.3 months, HR 0.72, 95% CI 0.53-0.99) and higher with CPS ≥ 10 (10.4 *vs* 8.0 months, HR 0.64, 95% CI 0.41-1.02) (39). Consistently, in the KEYNOTE-062 trial, pembrolizumab was superior in OS to standard chemotherapy in first-line in patients with CPS ≥ 10 (median OS 17.4 *vs* 10.8 months, HR 0.69, 95% CI 0.49-0.97) even though this endpoint could not be formally analyzed due to the statistical design of the study, while the results were negative even for the CPS ≥ 10 subgroup in the pembrolizumab plus chemotherapy versus chemotherapy arm (29). In a recent comprehensive analysis of the pembrolizumab-based trials (KEYNOTE-059, -061 and -062), a consistent improvement was observed in terms of the clinical and survival outcome with pembrolizumab across the different lines of treatment in patients with CPS ≥ 10 (55). Conversely, the cutoff CPS ≥ 5 was chosen for the nivolumab-based CheckMate 649 study, where, as speculated above, the magnitude of benefit of the addition of nivolumab to first-line chemotherapy progressively decreased from the subgroup of CPS ≥ 5, to CPS ≥ 1 to the overall population, possibly suggesting that in the CPS ≥ 1 and whole population the real benefit could have been conditioned by those with CPS ≥ 5, even though the results in patients with CPS < 1 or between 1 and 5 are not available to support this hypothesis (31).

TUMOR MUTATIONAL BURDEN

The tumor mutational burden (TMB) is a recently-defined potential biomarker of response to immunotherapy with ICIs. TMB is defined as the total number of non-synonymous mutations per coding area of tumor genome, as measured as mutations per megabase (mut/Mb). The genomic alterations that occur in tumor cells are able to generate tumor-specific antigens (neoantigens), that are processed and presented on the tumor cells membrane, thus allowing to elicit the anti-tumor immune response after the activation of T cells (56, 57). The potential association of TMB with sensitivity to immunotherapy relies on the rationale that the production of neoantigens is increased in tumors with high TMB,

therefore boosting the response of the immune system (58). The role of TMB as a stratification marker to predict the response to anti-PD1/PD-L1 immune agents has been investigated in several tumor settings, mainly NSCLC and melanoma, showing promising yet still not conclusive results (59-61). In the specific setting of GC/GEJC, the effect of TMB to predict the response to pembrolizumab was explored in the negative second-line KEYNOTE-061 trial. The tissue TMB resulted to be statistically significantly associated with the clinical outcomes in the overall population treated with pembrolizumab, not stratified for MSI and PD-L1 status, but not with paclitaxel, and the results were maintained after adjusting for CPS. Nevertheless, after the exclusion of MSI-high patients, those endowed with the highest TMB (> 175 mut/exome), the effect was reduced, thus leaving unanswered questions about the effective role of TMB as a predictive marker (62).

FUTURE PERSPECTIVES

On the basis of the clinically significant results obtained by the recent pivotal clinical trials conducted on the topic of the integration of immunotherapy to the therapeutic algorithm of gastroesophageal carcinoma, the research is ongoing with the aim to optimizing the potential benefit by exploring novel combinations with immunotherapy.

First, the combination of pembrolizumab to trastuzumab was investigated in the setting of HER2 positive disease, by exploiting the potential synergy between the two drugs, since in preclinical models trastuzumab proved to upregulate PD-1 expression and induce an immune-sensitive gene expression signature, conversely pembrolizumab may augment HER2-specific T cell response and potentiate the activity of effector T cells. The biological proof was obtained first in a single-arm phase II study and afterwards confirmed in the first interim analysis of the randomized phase III Keynote-811 trial, where the addition of pembrolizumab to the standard first-line trastuzumab plus chemotherapy in HER2 positive advanced GC or GEJC conditioned a significant improvement in ORR with deeper and more durable responses (63, 64).

Second, in order to foster the immune response in generally poorly immunogenic tumors, such as MSS gastrointestinal cancers, the combination of ICI and anti-angiogenic agents was investigated, relying on the rationale that they could enhance immune activation besides remodeling tumor neoangiogenesis. In details, in preclinical studies, regorafenib showed to reduce tumor-associated macrophages and T regulatory cells. The phase Ib REGONIVO EPOC1603 study reported that the regorafenib plus nivolumab regimen is endowed with a manageable safety profile and encouraging antitumor activity in GC and colorectal cancer asian patients, to be potentially furtherly investigated in a larger population (65).

Finally, based on the results obtained in several tumor settings, the combination of the ICI pembrolizumab plus the multikinase inhibitor lenvatinib was explored in GC, on the rationale that lenvatinib proved to reduce the tumor-associated macrophages and increase the anti-tumor activity of PD-1 inhibitors thanks to the up-regulation of the interferon gamma signalling pathway. In details, an open-label single-arm phase II trial showed that this combination is endowed with promising anti-tumor activity and manageable safety profile in previously treated advanced GC patients (66). A randomized phase III Trial is ongoing to investigate the addition of the pembrolizumab-lenvatinib combo to the standard first-line chemotherapy in advanced GC patients (NCT04662710).

CONCLUSIONS

In conclusion, immunotherapy with ICIs entered the treatment scenario of GC/GEJC, since the anti-PD1 agents nivolumab and pembrolizumab proved to confer an improvement in the patients outcome. Nevertheless, the real magnitude of benefit of immunotherapy in this disease setting is under definition since the results of the landmark studies conducted so far showed that the selection of patients according to the predictive biomarkers of response to ICIs plays a key role in order to maximize the therapeutic efficacy. The next future will provide clinicians further data both on the definition of the optimal therapeutic algorithm in the different patients populations and on the investigation of combination regimens between chemotherapy, immune agents and possibly targeted therapies.

ETHICS

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Conflicts of interests

Monica Niger received travel expenses from Celgene, speaker honorarium from Accademia della Medicina and consultant honoraria from EMD Serono, Basilea Pharmaceutica, Incyte and MSD Italia; Federica Morano received honoraria from Servier; Filippo Pietrantonio received honoraria from Amgen, Sanofi, Bayer, Servier, Merck-Serono, Lilly, MSD, Astrazeneca and research grants from Bristol-Myers Squibb and Astrazeneca; Maria Di Bartolomeo served on advisory board fo Myland Italy, received research grant from Lilly, payments for lectures including service on speakers bureaus from BMS, MSD, Lilly. The remaining authors declare no conflicts of interest.

Availability of data and materials

The data underlying this article can be shared just before a reasonable request to the corresponding author.

Authors' contribution

All the authors contributed equally to conception, data collection, analysis and writing of this paper.

Ethical approval

N/A.

Consent to participate

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