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Immune checkpoint inhibitors for the treatment of gastric cancer

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Um agradecimento especial à minha família e amigos...

Immune checkpoint inhibitors in gastric cancer

Abstract

Gastric cancer (GC) is the fifth most incident and the fourth deadliest cancer worldwide. GC is a heterogeneous disease from the histological and molecular standpoints. This malignancy is mostly diagnosed at advanced stages of the disease, where the available therapeutic interventions are not effective.

The emergence of immunotherapy has transformed the landscape of cancer treatment, including GC, and currently immune checkpoint inhibitors have been approved for the treatment of patients with recurrent/metastatic GC. This review summarizes the main clinical trials evaluating the use of immune checkpoint inhibitors in GC. It also highlights the potential of biomarkers for patient selection for GC immune checkpoint inhibition therapy, including PD-L1 expression and tumour mutational burden, and characteristics of the GC molecular classification, such as microsatellite instability status and Epstein-Barr virus infection, as predictors of response to blockade of the PD-1/PD-L1 axis.

Keywords: Gastric cancer; Molecular classification; Immune checkpoint inhibitors; Biomarkers for immunotherapy

Introduction

Gastric cancer (GC) is among the cancers with the most impact in all societies. It is the fifth most incident malignant disease worldwide, with a geographically heterogeneous incidence.[1] The highest rates are observed mainly in Eastern Asia (especially in Korea, Mongolia, Japan, and China), Europe (central and eastern), and South America, and the lowest incidence rates in Africa and Northern America. [1, 2] GC is also the fourth main cause of death by cancer in the world.[1] The great majority of cases experience a late diagnosis, mostly due to the lack of solid and global screening strategies and to the lack of specific symptoms.[3] Consequently, a significant proportion of patients present with advanced stage tumours.[4] The prognosis for advanced stages of this disease continues to be dire, and the 5-year survival is 25-30%.[5-7]

There are various approaches to GC therapy, including perioperative, adjuvant, and palliative chemotherapy, and tumour endoscopic/surgical resection, none of which is fully effective.[4] Targeted therapy has been also introduced for a particular subset of GCs that overexpress human epidermal growth factor receptor 2 (HER2).[7-9] More recently, there has been a great deal of attention on immunotherapy for various types of cancers, which can also be used for GC patients.[10]

For this review, PubMed searches for data published recently in the literature were performed. Additionally, PubMed searches for recent clinical trials were done, using search terms, such as “gastric cancer”, “immune checkpoint inhibitors”, “PD-1”, “PD-L1”, “microsatelite instability”, “EBV”, and “tumour mutational burden”. Only articles in published English were selected.

Gastric Cancer Heterogeneity

GC comprises various types of tumours. The great majority arise in glandular structures and are classified as adenocarcinomas.[11] Additionally, mesenchymal tumours and B-cell lymphomas can also be found, although to a much lesser extent.[12, 13] Herein GC will be used as synonym of gastric adenocarcinoma.

GC is a heterogeneous disease from both histological and molecular standpoints. Histologically, and according to the Laurén's classification, there are two main GC subtypes.[14] The intestinal subtype is usually diagnosed in older patients, most frequently in males, and appears in the distal part of the stomach, with a frequently exophytic growth pattern. The main histological characteristic of these tumours is the formation of glands and the synthesis of extracellular mucins. The diffuse subtype affects younger patients of both sexes equally, and arises mainly, although not exclusively, in the gastric body, frequently with *linitis plastica* growth pattern. Histologically, diffuse GC is characterized by the loss of cellular cohesion and the presence of isolated cells that contain high quantities of intracytoplasmic mucins ("signet ring cells").[14] An additional and rarer GC subtype, comprises tumours that present characteristics of both the intestinal and diffuse subtypes, and is denominated mixed type GC. According to the World Health Organization (WHO), GC comprises five main histological subtypes: papillary, tubular, poorly cohesive (characterized by "signet ring cells"), mucinous (when mucinous pools exceeds 50% of the tumour), and mixed adenocarcinomas. Other less common variations exist, such as the squamous cell, adenosquamous, hepatoid, and medullary carcinomas.[11, 15] Despite the heterogeneity referred above, histological subtypes have not provided significant contribution to therapeutic decisions.[16]

From the molecular standpoint, GC is also heterogeneous. The so-called "Singapore-Duke" classification considers three GC subtypes.[17] The proliferative GC subtype that corresponds to the intestinal type, presenting high *TP53* mutations, high CNA (copy number alterations), and oncogenic activation. The mesenchymal GC subtype that corresponds to the diffuse type, having low CNA, low number of *TP53* mutations, and a high activity of the epithelial-mesenchymal transition pathway, similarly to stem cells. Finally, the metabolic GC subtype, is characterized by low number of *TP53* mutations, high activity of the spasmolytic-polypeptide-expressing metaplasia (SPEM) pathway and high activity of metabolic pathways.[17, 18]

The Cancer Genome Atlas (TCGA) classification considers four GC subtypes.[19] The EBV-infected (EBV+) subtype constitutes 9% of all GCs and is more frequent in men and in younger patients. These tumours appear mainly in the upper part of the stomach, specifically, in the *gastric fundus*. Histologically, EBV+ GC is moderate to poorly differentiated, usually with dense lymphocytic infiltration.[20] Molecular characteristics of EBV+ GC include extreme hypermethylation, *CDKN2A* methylation, but without *MLH1* methylation, and *PIK3CA* and *ARID1A* mutations. The EBV+ GC subtype is also characterized by amplification of *JAK2*, *ERBB2*, and PD-L1/2, the latter with an important role as targets of immunotherapy in the treatment of GC. An additional characteristic of this tumour subtype is the enhancement in immune cell signalling pathways.[21]

Microsatellite unstable (MSI) tumours make up to 15-30% of all GC, and are more frequent in females and older patients. They arise mainly in the lower part of the stomach, particularly in the gastric antrum. The histology of MSI GC is similar to that of the intestinal subtype. MSI tumours are diploid and hypermutated, with mutations in *ARID1A*, *PIK3CA*, *PTEN*, *ERBB2*, *ERBB3*, *EGFFR*, *KRAS*, *RNF43*, and *MHC1*. *TP53* mutations are frequent and *MLH1* is hypermethylated. MSI tumours are enriched in mitotic and DNA damage pathways.[21]

Genomically stable (GS) tumours constitute 20% of all GC. GS tumours affect both sexes equally and have an early onset diagnosis compared to other subtypes of GC. They are mainly located in the antrum and comprise histologically diffuse tumours. GS tumours are characterized by mutations in *CDH1*, *ARID1A*, and *RHOA*, by *CLDN18-ARHGAP* fusion, and by high cell adhesion and angiogenesis pathways expression. In this subtype, *TP53* mutations are not common.[21]

Chromosomally unstable (CIN) GC comprises 50% of the cases.[20] CIN tumours originate in the gastric fundus and esophago-gastric junction. Histologically, CIN tumours can be of the intestinal subtype, when associated with gains in copy number of 8q, 17q and 20q, and of the diffuse subtype, when associated with gains of 12q and 13q.[20] CIN tumours are aneuploid and harbour genomic amplifications in: *RTKs and KRAS*, which are mutually exclusive; transcription factors, including *KLF5*, *GATA4*, *GATA6*, and *OCT1*; cell cycle

mediators, including *CCNE1*, *CCND1*, and *CDK6.* Mutations in *HER2*, *BRAF*, *EGFR*, *MET*, *FGFR2*, and *RAS* have also been identified. Unlike the other subtypes, CIN GC shows a high frequency of *TP53* mutations.[21]

The Asian Cancer Research Group (ACRG) classification also considers four GC subtypes, which unlike the TCGA classification, may predict disease progression or disease prognostic.[18, 21-23] Epithelial-to-mesenchymal transition/microsatellite stable (EMT/MSS) comprises 15% of all GC, it is located in the gastric body, and is histologically similar to the diffuse subtype. EMT/MSS presents EMT gene-expression signature and loss of *CDH1* expression. This subtype corresponds to the worst prognostic. The MSI subtype represents 23% of tumours, present in the antrum, and is closer to histological intestinal subtype. A MSI gene-expression signature, together with loss of *MLH1* expression and hypermutation is observed in this GC subtype that is associated to a better prognosis. The MSS/TP53+ and the MSS/TP53- subtypes constitute respectively 26% and 36% of GCs. These subtypes are distinguished by the activation of *TP53*, in which MSS/TP53+ has an intact *TP53* gene. There are overlaps between the GC subtypes of the TCGA and ACRG classifications and their major characteristics are summarized in Figure 1. Overall, the characteristics associated to specific GC subtypes can potentially provide novel therapeutic targets as well as new means for patient stratification.[21]

Gastric Cancer Immunotherapy using Immune Checkpoint Inhibitors

Immunotherapy has revolutionized cancer treatment. Among the different forms of immunotherapy, immune checkpoint inhibitors are the best studied and the most used therapeutic agents.[24] Malignant tumours frequently use mechanisms of immune suppression and tolerance in order to prevent immune destruction. The idea underlying immune checkpoint blockade therapy is the removal of signals that inhibit T-cell activation and effector functions, which in turn allows the establishment of an efficient anti-tumour response.[24]

The pioneer immune checkpoint inhibitor was ipilimumab, an anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) monoclonal antibody, originally approved for the treatment of unresectable/metastatic melanoma.[25] Following this breakthrough, other molecules for immune checkpoint inhibition followed, including nivolumab, pembrolizumab, and avelumab, which target the programmed cell death 1 (PD-1)/ programmed cell death ligand 1 (PD-L1) axis.[26-28] In the context of GC, some of these immune checkpoint inhibitors were approved as third line therapy in advanced or recurrent GC, while their use as first/second line options has been and still is under evaluation. Results of major clinical trials are summarized in Table 1.[26-31]

Nivolumab is a human monoclonal IgG4 kappa antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2. Nivolumab was initially approved in Japan for treatment of several different types of cancer.[32] The randomised, double-blind, placebo-controlled, phase III ATTRACTION-2 trial assessed the efficacy and safety of nivolumab in patients with unresectable advanced/recurrent gastric or gastro-esophageal junction (GEJ) cancer, who had been previously treated with two or more chemotherapeutic regimens.[27] The trial enrolled 493 Asian patients from Japan, South Korea, and Taiwan, who were randomly assigned to receive nivolumab or placebo. The results showed that nivolumab improved the overall survival (OS) in patients with GC refractory to standard chemotherapy; the median OS was 5.26 months in the nivolumab group, contrasting with 4.14 months in the placebo group. In this study, the 1-year OS rate was 26.2% in patients receiving nivolumab, in comparison with 10.9% in patients receiving placebo. The updates of the trial showed that the 2-year and the 3-year OS rates were, respectively, 10.6% and 5.6% for nivolumab, and 3.2% and 1.9% for placebo, and confirmed the long-term efficacy of nivolumab.[33, 34] Following the first results of the ATTRACTION-2 trial, nivolumab was also approved in various Asian countries as a third-line or later option in patients with unresectable advanced or recurrent gastric or GEJ cancer.[33] One of the limitations of the trial was that the patient population consisted only of Asian patients.[35, 36]

The KEYNOTE-59, open-label, phase II trial, evaluated the safety and efficacy of monotherapy with pembrolizumab, a humanized monoclonal IgG4 kappa antibody that binds to the PD-1 receptor, blocking its interaction with PD-L1 and PD-L2.[26] The trial involved a cohort of 259 patients from 16 international locations with previously treated advanced GC or GEJ cancer.[26] The objective response rate (ORR) associated with pembrolizumab treatment was 11.6%, 2.3% of the patients had complete response, and the median OS was 5.6 months. These results favoured further developments of the use of pembrolizumab monotherapy in patients with advanced gastric or GEJ cancer with ≥ 2 previous lines of treatment. Based on the outcomes of this trial, the FDA approved pembrolizumab for treatment of recurrent and locally advanced or metastatic GC.[37]

An additional immune checkpoint inhibitor is avelumab, a human IgG1 lambda monoclonal antibody that binds PD-L1 and blocks its interactions with PD-1 and B7.1 receptors. The use of avelumab as third line therapy was investigated in the JAVELIN Gastric 300 phase III trial, which enrolled 371 patients with recurrent locally advanced or metastatic gastric or GEJ cancer that were randomized to receive avelumab or the physician's choice of third-line chemotherapy.[28] Although the safety profile of avelumab was better than that of chemotherapy, the trial was not successful in meeting the primary or secondary end points; the median OS was 4.6 in the avelumab group and 5 months in the chemotherapy group; the PFS was 1.4 vs. 2.7 months and the ORR was 2.2% vs. 4.3% in the avelumab vs. chemotherapy arms, respectively. Therefore, in the third line setting, the use of avelumab as a single agent did not improve OS or PFS in comparison chemotherapy.

The CheckMate-032 phase I/II trial tested the efficacy of nivolumab and ipilimumab in patients with chemotherapy-refractory metastatic gastric or GEJ cancer, in 160 patients from centres in the United States and Europe.[31] Patients were treated with nivolumab, or with combinations of nivolumab plus ipilimumab. The ORR, median PFS and OS were, respectively, 12%, 1.4 months, and 6.2 months in the patients receiving nivolumab, 24%, 1.4 months, and 6.9 months in those receiving nivolumab 1mg/kg and ipilimumab 3mg/kg, and 8%, 1.6 months, and 4.8 months with the combination of nivolumab 3mg/kg and ipilimumab

1mg/kg. Although the combination of nivolumab 1mg/kg plus ipilimumab 3mg/kg had a numerically higher ORR than that of patients treated with nivolumab monotherapy, the median OS was similar in these patient groups. Nevertheless, the study demonstrated a reasonable safety profile and long-lasting responses. Aditionally, the clinical benefits of nivolumab monotherapy was similar to those of the ATTRACTION-2, suggesting consistent therapeutic benefit across patients from Asian and Western countries.

Although now established as a third line therapy, immune checkpoint inhibitors have not been so successful in earlier lines of therapy for GC. The open-label, phase III KEYNOTE-061 trial enrolled 592 patients with advanced gastric or GEJ cancer that progressed on first-line chemotherapy, with a PD-L1 combined positive score (CPS) ≥ 1.[29] Patients were randomized to receive pembrolizumab or paclitaxel. Pembrolizumab did not significantly improve the OS compared with paclitaxel (9.1 vs 8.3 months) or PFS (1.5 vs. 4.1 months), although it had a better safety profile.[29]

The following randomized, controlled, phase III KEYNOTE-062 trial enrolled 763 patients with untreated, locally advanced, unresectable or metastatic gastric or GEJ cancer, with a PD-L1 CPS ≥ 1.[30] Patients were randomized to receive pembrolizumab, pembrolizumab plus chemotherapy, or chemotherapy plus placebo. The OS of patients with PD-L1 CPS ≥ 1 treated with pembrolizumab was noninferior to that of patients treated with chemotherapy (10.6 vs 11.1 months). Interestingly, in patients with PD-L1 CPS ≥ 10 pembrolizumab extended OS vs chemotherapy (17.4 vs 10.8), but without statistical significance. Pembrolizumab plus chemotherapy was not superior to chemotherapy for OS in patients with CPS ≥ 1 (12.5 vs 11.1 months) or with CPS ≥10 (12.3 vs 10.8 months), or for PFS in patients with CPS ≥ 1 (6.9 vs 6.4 months).[30]

The ongoing randomized, phase II/III ATTRACTION-4 evaluates nivolumab plus chemotherapy vs. placebo plus chemotherapy as first-line treatment in HER2-negative, advanced or recurrent gastric or GEJ cancer Asian patients.[38] The results of the double-blind phase III part, demonstrated a statistically significant improvement in PFS in patients receiving nivolumab plus chemotherapy in comparison with the other study arm (10.5 vs 8.3

months), reaching one of the primary endpoints of the trial. However, no differences in OS, which was the other primary study endpoint, were observed between the two groups (17.5 vs 17.2 months). Similarly, the randomized, open-label, phase III CheckMate-649 trial compared nivolumab plus chemotherapy with chemotherapy alone as first line treatment for patients with advanced or metastatic gastric or GEJ cancer. The first results of the trial that enrolled 1581 patients from geographic locations worldwide, demonstrated that in patients with tumors with PD-L1 CPS ≥ 1 there was statistically significant benefit in OS those treated with nivolumab plus chemotherapy vs those treated with chemotherapy alone (14.0 vs 11.3 months). In particular, in patients with tumors expressing PD-L1 with CPS ≥ 5, nivolumab plus chemotherapy showed statistically significant improvements in both OS and PFS in comparison with chemotherapy alone. Based on the results of the CHECKMATE-649 trial, the European Medicines Agency validated[39] and the U.S. FDA accepted for priority review[40] the application of nivolumab combined with chemotherapy as first-line treatment in metastatic GC, GEJ cancer, and oesophageal adenocarcinoma.

Biomarkers for Immune Checkpoint Inhibition in Gastric Cancer

The use of biomarkers for patient selection for immune checkpoint inhibition therapy aims to increase its efficacy, while reducing useless therapeutic exposure and health-related costs. In this section, we will summarize knowledge on biomarkers that are presently being tested in clinical trials addressing immune checkpoint blockade in GC.

PD-L1 expression

PD-L1 expression is the most widely studied biomarker for patient selection for PD-1/PD-L1 therapy. The usefulness of PD-L1 expression as a biomarker has been reported in various large clinical trials that assessed PD-1 and/or PD-L1 inhibitors in melanoma, non-small cell lung cancer, and urothelial carcinoma.[41-43] In these studies, higher expression levels of PD-L1, as evaluated by immunohistochemistry, were predictive of response to therapy with PD-1

and/or PD-L1 inhibitors. However, for other cancer types, including renal-cell carcinoma and hepatocellular carcinoma, PD-L1 expression did not show to be a good biomarker.[44, 45]

In GC, between 25% and 65% of tumours express PD-L1, and multiple mechanisms have been associated with PD-L1 upregulation, including *PDL1* gene amplification, structural variations in the 3'UTR of *PDL1*, polymorphisms in *PDL1* promoter, activation of oncogenic PI3K signalling, and cytokine- and chemokine-mediated regulation.[46] [47] PD-L1 expression in GC has been associated with high density of tumour-infiltrating lymphocytes, with MSI, and with EBV infection.[48, 49]

The relationship between PD-L1 expression and prognosis in GC is controversial, and while some studies reported increased PD-L1 expression associated with adverse prognosis, others have shown a relationship with better patient outcome, or report that PD-L1 expression is not a prognostic factor.[48-50] Several meta-analyses have been now been performed to examine the clinicopathological and prognostic significance of PD-L1 expression. A meta-analysis that included 10 studies and 1901 GC patients from Asia indicated that PD-L1 expression was associated with a shorter OS.[46] The expression of PD-L1 was also associated with tumour size, and lymph node metastasis, but not with age or gender, tumour differentiation, invasion depth, or tumour stage. A more recent meta-analysis including 15 studies and 3218 patients from China, South Korea, Japan, and Germany, showed that PD-L1 expression was associated with a decrease in the 3-year and 5-year survival rates [51]. In the subgroup analyses of ethnicity, PD-L1 expression in Asian patients was also associated with a decrease in the 3-year and 5-year survival rates. PD-L1 expression was associated with lymph-node metastasis but not with tumour staging. These results point to the possibility of using PD-L1 expression as GC biomarker for PD-1/PD-L1 targeted therapy.

It is important, however, to mention that major problems exist regarding the comparisons between studies, namely the use of different antibodies, assays or devices for PD-L1 immunohistochemistry, as well as differences in scoring criteria.

In the 3-year update of the ATTRACTION-2 trial, PD-L1 was retrospectively analysed using the PD-L1 IHC 28-8 pharmDx assay. In the 192 patients that had available tumour tissue, no differences were found regarding the efficacy of nivolumab compared to that of placebo in patients' OS.[27, 34] In CHECKMATE-032, the benefits of nivolumab or the combinations of nivolumab with ipilimumab were observed, no matter the imunohistochemical status of PD-L1 the independently.[31] Finally, in the JAVELIN Gastric 300 that used the PD-L1 IHC 73-10 pharmDx assay, no differences were identified in the outcomes of avelumab treatment between patients with PD-L1-positive or -negative tumours. In these trials, however, the scoring of PD-L1 was performed using a tumour proportion score (TPS) of ≥ 1%, which considers expression of PD-L1 in 1% or more of tumour cells.[52]

The trials that assessed pembrolizumab in GC, showed efficacy of this immune checkpoint inhibitor in PD-L1 positive tumours, using the FDA-approved PD-L1 IHC 22C3 pharmDx assay, which scores PD-L1 expression in tumour cells, lymphocytes and macrophages (CPS).[26, 29, 30, 52] In KEYNOTE-59, the ORR and the median response duration of patients with PD-L1 positive tumours were 22.7% and 8.1 months, while the responses were significantly lower, 8.6% and 6.9 months, respectively, in patients with tumours that were PD-L1 negative.[26] In KEYNOTE-61, the ORR for patients treated with pembrolizumab vs. paclitaxel was 16% vs. 14% in patients with CPS ≥1 tumours, but in subgroup analysis, ORR was 25% vs 9% in the PD-L1 CPS ≥10 subgroup, and 2% vs 10% in the PD-L1 CPS <1 subgroup.[29] In KEYNOTE-062, the OS for patients treated with pembrolizumab was 10.6 months in those with PD-L1 CPS ≥1 tumours, and a prolonged, though not statistically tested, OS of 17.4 months was observed in patients with PD-L1 CPS ≥10 tumours.[30]

Very recently, and to better define CPS specificity as predictor of clinical outcome, Wainberg *et al.* comprehensively studied in *post-hoc* analyses the efficacy of pembrolizumab in patients with CPS ≥ 10 in the three trials mentioned above.[53] In KEYNOTE-059 (median follow-up 6 months), median OS was 8 months, objective response rate (ORR) was 17%, and median duration of response (DOR) was 21 months. In KEYNOTE-061 (median follow-up 9 months), median OS (pembrolizumab vs. chemotherapy) was 10 vs. 8 months, median

PFS was 3 vs. 3 months, objective response rate ORR was 25% vs. 9%, and median DOR was not reached vs. 7 months. In KEYNOTE-062 (median follow-up 11 months), median OS (pembrolizumab vs. chemotherapy) was 17 vs. 11 months, median PFS was 3 vs. 6 months, ORR was 25% vs. 38%, and median DOR was 19 vs. 7 months. This shows that more favourable clinical outcomes are consistently observed in first-, second-, and third-lines of pembrolizumab therapy in patients with PD-L1 CPS ≥ 10 tumours, and suggest that PD-L1 expression could be used to identify patients who would benefit from PD-1/PD-L1 targeted therapy.

Microsatellite Instability

The accumulation of mutations in microsatellite regions of the genome, which are repeated sequences of nucleotides where DNA-polymerase is more prone replication errors, is known as microsatellite instability (MSI). MSI is generally caused by a deficiency in mismatch repair (MMR) systems.[54, 55]

About 20% of gastric tumours have the MSI phenotype.[19] Patients with MSI GC have better prognosis than those with microsatellite stable (MSS) tumors.[56, 57] In a meta-analysis that included 48 studies and 18.612 patients, of which 9.2% had MSI tumours, a relationship was found between MSI tumours and female sex, older age, Laurén's intestinal histology, absence of lymph-node metastases, and stages I-II (TNM). Patients with MSI tumours also had better OS than patients with MSS GC.[57]

MSI and MMR deficiency in tumour cells may lead to higher levels of mutations and the appearance of immunogenic neoantigens, leading to easier recognition by immune cells. This may facilitate the action of immune checkpoint inhibitors, as these types of tumours exhibit a high density of immune cells. Accordingly, in comparison with MSS GC, MSI gastric tumours have higher numbers of PD-L1-positive tumour and immune cells, and increased number of tumour-infiltrating lymphocytes.[49, 58, 59]

In KEYNOTE-59, 67% of the enrolled patients were assessed for GC MSI, of which 4% had MSI-high tumours. In patients with MSI-high GC, the ORR to pembrolizumab treatment was

57.1%, contrasting with an ORR of 9% for patients with non-MSI-high GC.[26] In CHECKMATE-32, and in all study arms, there were significantly better responses in patients with MSI-high GC compared to non-MSI-high patients.[31] The ORRs for MSI-high vs. non-MSI-high were for nivolumab: 29% vs. 11%; for nivolumab 1mg/kg plus ipilimumab 3mg/kg: 50% vs. 19%; and for nivolumab 3mg/kg plus ipilimumab 1mg/kg: 50% vs. 5%. The OS for MSI-high vs. non-MSI-high were for nivolumab: 57% vs. 33%; for nivolumab 1mg/kg plus ipilimumab 3mg/kg: 50% vs. 32%; and for nivolumab 3mg/kg plus ipilimumab 1mg/kg: 50% vs. 23%.

In *post-hoc* analysis of the patients enrolled in KEYNOTE-61, those with MSI tumours had superior responses to pembrolizumab (47%, regardless of the PD-L1 CPS), compared to 17% in the paclitaxel group.[29] In CHECKMATE 62, OS was enhanced in patients with MSI tumours with PD-L1 CPS ≥1, and overall outcomes proved more efficient in the MSI-high population. The predictive value of PD-L1 CPS ≥10 remained constant, regardless of the MSI status, which demonstrates the independent value of both biomarkers.

A recent meta-analysis of randomized clinical trials evaluated the role of MSI as a positive predictive factor for PD-1 immunotherapy as first- or second-line regimens in patients with advanced GC.[60] The study included data from KEYNOTE-061, CHECKMATE-649, JAVELIN Gastric 100, and KEYNOTE-062[29, 30, 61, 62], and provided evidence of improved survival and response in advanced GC patients with MSI-high tumours who received anti-PD-1 blockade, with significantly greater OS compared with patients with MSS tumours.

Also recently, a *post hoc* analysis of 1614 patients, 84 of which had MSI-high gastric or GEJ cancer, and enrolled in the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 trials, assessed the anti-tumour effects of pembrolizumab vs. chemotherapy, irrespectively of the line of therapy.[63] Results from this study showed that pembrolizumab alone, or combined with chemotherapy, was associated with prolonged OS and PFS and with durable responses in comparison to chemotherapy alone, suggesting the MSI-high status as biomarker for patient selection, irrespectively of the line of therapy in which it is received.

EBV infection

As discussed above, the TCGA identified EBV-positive tumours as a distinct GC subgroup.[19] Among other features, these tumours are characterized by rich lymphocytic infiltrates, containing CD8-positive cytotoxic T cells and high number of mature dendritic cells, and are enriched in immune cell signalling pathways.[48, 64] Furthermore, about 15% of EBV-positive GC have amplification of the PD-L1- and PD-L2-encoding genes, and have PD-L1 expression in both tumour cells and immune cells.[19, 49, 65] These features suggest that EBV-positive GC may be which may potentially more susceptible to PD-1/PD-L1 blockade.
A case report described response to avelumab treatment in one EBV-positive GC patient.[66] In a clinical trial that evaluated the impact of toripalimab (an anti-PD-1 antibody) on 55 advanced GC patients, of the four EBV-positive patients, one case had partial response, two cases had stable disease, and one case had progressive disease.[22] In a prospective phase 2 clinical trial of 61 patients with metastatic GC that had been treated with pembrolizumab, while the general ORR was 24.6%, in the six patients with EBV-positive tumours the ORR was 100%, all responding to pembrolizumab.[67] In this trial, there were also seven patients with MSI-high GC and, in this group, the ORR was 85.7%. These finding suggest that EBV-positive GC patients may derive benefit from pembrolizumab therapy. Large prospective clinical trials are needed to evaluate EBV-positivity as a biomarker for GC immune checkpoint therapy.

Tumour Mutational Burden (TMB)

During cancer initiation and progression, tumour cells acquire thousands of different mutations. Nonsynonymous mutations will cause tumours to express neoantigens, which are tumour cell specific and will distinguish them from normal cells.[68] Epitopes of these mutant proteins can be expressed at the cancer cell surface, thus rendering these cells recognizable as foreign by T cells. It has been shown that the tumour mutational burden (TMB), and consequently the neoantigen formation potential in a certain tumour, will determine the effectiveness of the response to immunotherapy, as highly mutated cells can be

distinguished and, therefore, targeted more proficiently.[68, 69] Accordingly, melanoma and non-small cell lung cancer, the two tumour types with highest prevalence of somatic mutations,[70] have excellent responses to immune checkpoint blockade.[71-74] Interestingly, in a study that evaluated almost 9900 samples of 35 cancer types, no significant correlations between TMB and PD-L1 expression within most cancer subtypes were observed, suggesting that each may be used as a biomarker for predicting the response to immune checkpoint blockade.[75]

The relationship between TMB and response to therapy with pembrolizumab has been analysed in a study that involved multiple cohorts of patients with different types of solid tumours.[76] Objective responses were observed in 29% of patients with TMB-high status (≥10 mutations per megabase), in comparison to only 6% in patients with non-TMB-high. Noteworthy, TMB had predictive value, regardless of the tumour PD-L1 expression and of the MSI status.

In a study of metastatic gastrointestinal cancer patients from China, including 57 GC patients, treated with immune checkpoint inhibitors, patients with higher TMB had longer OS than those with lower TMB.[77] In another study of 63 South Korean patients with advanced GC treated with pembrolizumab or nivolumab, responders had significantly higher TMB than non-responders with stable disease.[36] In survival analysis, patients with high TMB had longer PFS. While in univariate analysis, TMB, MSI, response to treatment, and ECOG performance status were all significantly associated with PFS, in multivariate analysis, both TMB-high and the ECOG ≤1 remained independent predictors of longer PFS.

In a clinical trial that analysed toripalimab therapy (a PD-1 antibody) in advanced GC, patients with TMB-high had significant higher OS (14.6 months) than those with TMB-low (4.0 months) with patients.[78] Patients with TMB-high vs. TMB-low also had enhanced ORR (33.3% vs. 7.1%), and a numerically longer PFS, but without statistical significance. The analysis of the TMB in patients of KEYNOTE-061 showed that TMB ≥10 mut/Mb had a positive association with ORR, PFS, and OS in patients treated with pembrolizumab but not with paclitaxel.[79] Interestingly, the OS benefits of pembrolizumab vs. paclitaxel in TMB ≥10

mut/Mb remained, when patients with MSI-high tumours were excluded. Taken together, and although needing more consolidated data, the TMB appears to be a promising biomarker for GC immunotherapy.

Conclusions

The outcome of GC, in particular of advanced disease stages, remains poor. Immunotherapy based on immune checkpoint inhibition in advanced GC has shown promising benefits, in particular when patients who will derive most benefit from this type of therapy are selected. The heterogeneity of GC and the identification of GC subtypes with distinct molecular profiles, has offered the opportunity to discover not only new GC therapeutic targets but also novel markers of response to immune checkpoint blockade. Nevertheless, further research is needed, as there is still a lot of uncertainty regarding drug and biomarker effectiveness. Future approaches should also consider additional biomarkers to identify patients who could better respond to the different inhibitors, thus contributing to improve the negative prognosis associated with advanced GC.

Conflicts of Interest

The author has no conflicts of interest.

Figure legend

Figure 1. Main features of The Cancer Genome Atlas (TCGA) and of the Asian Cancer Research Group (ACRG) classifications of Gastric Cancer.

Table 1. Summary of results of selected clinical trials that evaluate the use of immune checkpoint inhibitors in gastric cancer.

Trial name [Reference]	Phase/ Line	Agent	Target	PD-L1	Treatment arms	Number patients	Primary endpoint	OS (months)	PFS (months)	ORR (%)
ATTRACTION-2 [27]	III/ ≥3L	Nivolumab	PD-1	Unselected	Nivolumab	330	OS	5.26	1.6	11.2
					Placebo	163		4.14	1.5	0
KEYNOTE-59 [26]	II/ ≥3L	Pembrolizumab	PD-1	Unselected	Pembrolizumab	259	ORR	5.6	2	11.6
JAVELIN Gastric 300 [28]	III/ 3L	Avelumab	PD-L1	Unselected	Avelumab	185	OS	4.6	1.4	2.2
					CT[1]	186		5.0	2.7	4.3
CHECKMATE-032 [31]	I-II/ ≥3L	Nivolumab	PD-1	Unselected	Nivolumab	59	ORR	6.2	1.4	12
					Nivolumab1 + ipilimumab3	49		6.9	1.4	24
					Nivolumab3 + ipilimumab1	52		4.8	1.6	8
KEYNOTE-61 [29]	III/ 2L	Pembrolizumab	PD-1	Positive	Pembrolizumab	196	OS, PFS	9.1	1.5	16
					Paclitaxel	199		8.3	4.1	14
KEYNOTE-62 [30]	III/ 1L	Pembrolizumab	PD-1	Positive	Pembrolizumab	256	OS, PFS	10.6 CPS≥1; 17.4 CPS≥10	2.0	14.8
					Pembrolizumab + CT[2]	257		12.5 CPS≥1; 12.3 CPS≥10	6.9	48.6
					CT[2]	250		11.1 CPS≥1; 10.8 CPS≥10	6.4	37.2
CHECKMATE-649 [61]	III/ 1L	Nivolumab	PD-1	Positive	Nivolumab + CT[3]	473	OS, PFS	14.4	7.7	-
					CT[3]	482	in CPS ≥ 5	11.1	6.1	-
ATTRACTION-4 [38]	II/III 1L	Nivolumab	PD-1	Unselected	Nivolumab + CT[4]	362	PFS, OS	17.5	10.5	57.5
					Placebo + CT[4]	362		17.2	8.3	47.8

Abbreviations: 1L, first line; 2L, second line; 3L, third line; ≥3L, third line or later; CPS, combined positive score; CT, chemotherapy; CT[1] included paclitaxel or irinotecan; CT[2] included cisplatin plus fluorouracil or capecitabine; CT[3] included capecitabine plus oxaliplatin or fluorouracil, leucovorin and oxaliplatin; CT[4] included S-1 plus oxaliplatin or capecitabine plus oxaliplatin; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

FIGURE 1

	TCGA
MSI	• 15-30% • Females and older patients • Gastric antrum • Intestinal histological subtype • *MLH1* hypermethylation • Mitotic pathways
GS	• 20% • Gastric antrum • Diffuse histological subtype • *CDH1*, *ARID1A*, and *RHOA* mutations • *CLDN18-ARHGAP* fusion • High cell adhesion
CIN	• 50% • Gastric fundus and esophagogastric junction • *TP53* mutations • *RTKs* and *KRAS* amplification
EBV	• 9% • Males and younger patients • Gastric fundus • *PD-L1/L2* amplification • *CDKN2A* methylation • *PIK3CA* and *ARID1A* mutations • Immune cell signalling

	ARCG
MSI	• 23% • Gastric antrum • Intestinal histological subtype • MSI gene-expression signature • *MLH1* expression loss • Better prognosis
EMT/MSS	• 15% • Gastric body • Diffuse histological subtype • EMT gene-expression signature • *CDH1* expression loss • Worse prognosis
MSS/TP53 -	• 36% • Intestinal histological subtype • Absent *TP53* activity • Intermediate prognosis
MSS/TP53 +	• 26% • Intestinal histological subtype • Intermediate prognosis

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Ethical approval for this study (Ethical Committee N° NAC 207) was provided by the Ethical Committee NAC of Geneva University Hospitals, Geneva, Switzerland on 12 February 2015.

In addition, for studies or case reports conducted on human participants you must state clearly in the text that you obtained written informed consent from the study participants; please also look at the latest version of the Declaration of Helsinki. Similarly, for experiments involving animals you must state the care of animal and licensing guidelines under which the study was performed and report these in accordance with the ARRIVE (Animals in Research: Reporting In Vivo Experiments) statement. If ethics clearance was not necessary, or if there was any deviation from these standard ethical requests, please state why it was not required. Please note that the editors may ask you to provide evidence of ethical approval. If you have approval from a National Drug Agency (or similar) please state this and provide details, this can be particularly useful when discussing the use of unlicensed drugs.

Patient's Privacy

The protection of a patient's right to privacy is essential. Please collect and keep copies of patients' consent forms on which patients or other subjects of your experiments clearly grant permission for the publication of photographs or other material that might identify them. If the consent form for your research did not specifically include this, please obtain it or remove the identifying material.

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Data Reporting

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A financial disclosure questionnaire must be completed by the corresponding author and all co-authors at initial submission. Co-authors will receive a link to complete the questionnaire via email. Please ensure each co-author's email address is properly listed at the 'Add/Edit/Remove Authors' submission step in Editorial Manager, to avoid delays in reaching co-authors.

The primary purpose of the disclosure section is to determine whether authors have received any commercial financial support that could create a conflict of interest. In addition to monetary interests, a potential for conflict of interest can exist whether or not an individual believes that a relationship (such as dual commitments, competing interests, or competing loyalties) affects his or her scientific judgment. Please review ICMJE Uniform Requirements for Manuscripts Submitted to Biomedical Journals at the following link: http://www.icmje.org/conflicts-of-interest.

In addition to completing the financial disclosure questionnaire authors must clearly state all relevant conflicts of interest in the Acknowledgements section of the submitted manuscript.

Retractions

Porto Biomedical Journal is a member of the Committee on Publication Ethics (COPE), and also refers to the ICMJE advice on Scientific Misconduct, Expressions of Concern, and Retraction as well as on Overlapping Publications.

Article Types

Original articles
These should describe fully, but as concisely as feasible, the results of original clinical, laboratory or biomedical research. *Special note regarding case studies:* Case studies will be considered for publication only in the Letters to the Editor section of the Journal. The average Original Article fills 7 pages in the printed journal, although manuscripts that exceed this may be occasionally accepted for publication at the Editors' discretion. In general, an Original Article should not exceed 3500 words, not including the abstract, figure legends, and references. Abstracts should be 250 words or less. If possible, each figure legend should be held to 60 words or less. Each Original Article may be accompanied by no more than 8 graphic presentations (tables and/or figures)-for example, 3 tables + 5 figures. (Additional text, tables, or figures can be designated as "supplemental" material, which will be included in the PBJS Online Repository. Please note: Original Article manuscripts that are determined to significantly exceed these limits, or that do not include all of the elements listed below, may be returned to the authors for revision prior to review.

Letters to the Editor
Letters to the Editor are brief reports of clinical or laboratory observations, substantiated by controlled data but limited in scope, and without sufficient depth of investigation to qualify as Original Articles. These may include a brief description of a particular condition that provides insights into diagnosis and clinical management or images that impart important clinical information. Like Original Articles, these manuscripts are subject to peer review. A Letter to the Editor must:

1) Be brief. The average Letter to the Editor fills 2 pages in the printed journal, although manuscripts that exceed this may be occasionally accepted for publication at the Editors'

discretion. In general, a Letter to the Editor should not exceed 1000 words, not including the figure legend(s) and references. If possible, the figure legend(s) should be held to 60 words or less. Please note: Letter to the Editor manuscripts that are determined to significantly exceed these limits may be returned to the authors for shortening prior to review.

2) Have a short, relevant title. Please see the suggestions that appear above (under "A. Original Articles").

3) Have a complete title page (see section A1).

4) Be accompanied by a short summary that encapsulates the report's findings for a clinically oriented audience (see above).

5) Begin with the salutation "To the Editor:"

6) Close with the author's name(s), academic degree(s), institutions(s), and location(s).

7) Have no more than nine references.

8) List the references as complete bibliographic citations following the closure of the letter (see section above for formatting).

9) Present lists of Key words, as relevant (see sections above).

10) Be limited to a total of 2 figures and/or tables. (Additional figures or tables may be placed in the article's Online Repository; please see the relevant section below.)

Correspondence and replies

Correspondence concerning recent publications in the Journal will be considered for publication and accepted based on their pertinence, their scientific quality, and available space in the Journal. If the correspondence is considered acceptable, a response will be requested from the authors of the referenced PBJ article. Upon review and approval by the Editor, the Correspondence and relevant Reply will both be published together. Both Correspondence and Reply manuscripts must:

1) Be no longer than 500 words.

2) Have a short, relevant title, distinct from the title of the referenced article. Please note that all Replies should have the title "Reply to [Corresponding author's name]."

3) Have a complete title page (see section above).

4) List the references as complete bibliographic citations at the end of the letter with the journal article being discussed as the first reference (see section above). The total number of references should be no more than seven. Replies should include the Correspondence to which they are replying as one of the references.

5) Have no more than one graphic presentation (table or figure). (See the section on Graphic Presentations below).

6) Begin with the salutation "To the Editor:" and close with the author's name(s), academic degree(s), institutions(s), and location(s).

Review articles
Definitive, in-depth, state-of-the-art reviews of clinical and research subjects. Unsolicited reviews are not generally published in PBJ. Before submitting any unsolicited reviews, please forward an outline to the Editor for consideration.
Systematic reviews and meta-analyses should follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (seehttp://www.prisma-statement.org/). A PRISMA flow diagram (http://www.prisma-statement.org/documents/PRISMA%202009%20flow%20diagram.pdf) should be used to describe the steps of the systematic review, and a complete PRISMA checklist (http://www.prisma-statement.org/documents/PRISMA%202009%20checklist.pdf) should be provided during submission.

Clinical Guidelines
Official recommendations from professional organizations on issues related to clinical practice and health care delivery. PBJ is most interested in publishing the primary guideline documents but will also consider synopses of guidelines when the primary document is published elsewhere. Synopses should focus on those issues of most relevance to generalist clinicians. Manuscripts must:

1) Have an equal or less than 275 words, structured abstract (use the following subheadings: Description, Methods and Recommendations)

2) Include the name of the responsible organization in the title and identify the article as a clinical guideline.

3)Primary Guideline Reports: PBJ is flexible with length, reference, and other format requirements given the variability in the format of guidelines developed by different organizations. However, if guidelines are lengthy (more than 4000 words), we may require the production of an executive summary document with the full document published as a digital-only appendix. A concise table or concise graphic summarizing the recommendations and other key points is desirable.

Guideline Synopses
Text of synopses include the following sections and subheads:

Rationale, Guideline Focus, Target Population, Guideline Development Process, Evidence Review and Grading, Comments and Modification, Clinical Recommendations, Research Recommendations, Applicability and Implementation Issues, and Summary. Guideline Group members followed by key references should be listed at the end.

Rostrum articles
Opinion articles about subjects of particular interest and/or debate may be accepted for peer review after preliminary review by the Editor. Proposals for rostrum articles may be emailed to the Editorial Office; they will be evaluated based on level of interest, novelty, and the current needs of the Journal.

MANUSCRIPT PREPARATION AND FORMATTING INSTRUCTIONS

Manuscripts must be written in clear, grammatical English (see English Language Assistance above). Manuscripts not conforming to Journal format will be returned to authors for modification. Please double space the entire main body document and number each page. Do not add line numbers as the system will generate those when the PDF is built.

Title page, footnotes, abbreviations, and abstract pages must be included in the main body file. Please do not upload separate copies of these documents.

Acceptable document file types for text and tables include .DOC and .DOCX; do not submit a PDF.

Page 1:

Title Page. The following elements are required for every submission:

Title. Include a descriptive title of the work; the title should not be a sentence. No proprietary or brand names for drugs or agents may be used in article titles. Please, include the study design in the title; for instance, "randomised controlled trial", or "systematic review". Titles should be as informative and complete as possible.

Authors. The full first name, middle initials, and family name of each author, as well as the name(s) of the department(s) and institution(s) to which the work should be attributed.

Address for Correspondence. A current email and full mailing address for the corresponding author must be provided.

Page 2:

Abstract. Original articles should include a structured abstract of no more than 300 words using the following headings: Background; Methods; Results; and Conclusions. They should briefly describe, respectively, the problem being addressed in the study, how the study was performed, the salient results, and what the authors conclude from the results. Conventional non-systematic, reviews should include an unstructured abstract of no more than 250 words.

Main Body: *Introduction*. The introduction contains a statement of the purpose of the work, the problem that stimulated it, and a brief summary of relevant published investigations.

Methods. Avoid detailed description of previously published methods and cite the appropriate reference. Include appropriate ethical and statistical information.

Results. The results should be concise, avoiding redundant tables and figures illustrating the same data.

Discussion. This section should follow the results and is used to interpret results, with minimal recapitulation of findings.

Acknowledgments: The acknowledgements section should be headed 'Acknowledgements relating to this article' and contain the following distinct statements in separate paragraphs:

- Assistance with the study. Acknowledgements should be made only to those who have made a substantial contribution to the study. Authors are responsible for

obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions. If there was no assistance state: 'Assistance with the study: none.'

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- Presentation (for original articles only). Presentations of preliminary data at, for example, international meetings should be acknowledged separately. If preliminary data was not previously presented please state: Presentation: none.

References: Use the Vancouver reference system as adopted by the U.S. National Library of Medicine ensuring that all journal titles conform to Index Medicus approved abbreviations. Number references consecutively in the order in which they are first mentioned in the text. Identify references in the text, tables and legends using superscripted Arabic numerals that are placed after the punctuation. References cited only in tables or in legends to figures should be numbered in accordance with the sequence established by the first identification in the text of the particular table or illustration.

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Authors must confirm that the details of these references are accurate and complete. In the full list of references give the names and initials of all authors. If there are more than six, cite only the first three names followed by et al. The authors' names are followed by the title of the article: the title of the journal (italics) abbreviated according to the style of Index Medicus: the year of publication: the volume number (in bold): the first and last page numbers in full followed by a full stop. Titles of books should be followed by the town and country of publication, the publisher, the year and inclusive page numbers. See the following examples:

Journal articles:

Pollard BJ, Bryan A, Bennett D et al. Recovery after oral surgery with halothane, enflurane, isoflurane or propofol anaesthesia. Br J Anaesth 1994; 72:559–566.

Books:

Korttila K. Recovery period and discharge. In: White P, ed. Outpatient Anaesthesia. New York, USA: Churchill Livingstone Inc, 1990: 369–395.

Chapter in a book:

Pessayre D, Feldmann G, Haouzi D, Fau D, Moreau A, Neumann M. Hepatocyte apoptosis triggered by natural substances (cytokines, other endogenous molecules and foreign toxins). In Cameron RG, Feuer G (editors): Apoptosis and its Modulation by Drugs. Handbook of Experimental Pharmacology. Berlin: Springer-Verlag; 2000, pp. 59-108.

Electronic articles:

Margolis PA, Stevens R, Bordley WC, Stuart J. From concept to application: the impact of a community-wide intervention to improve the delivery of preventive services to children. Pediatrics [online serial] 2001; 108:e42.

http://www.pediatrics.org/cgi/content/full/108/3/e42. [Accessed 20 September 2001].

Tables: References to tables should be made in order of appearance in the text and should be in Arabic numerals in parentheses, e.g. (Table 1). Each table should be typed on a separate sheet in 1.5 spacing. Tables should not be submitted as photographs. Each table should have a brief title as a heading. Vertical rules should not be used. Place explanatory matter in footnotes, not in the heading. Authors are discouraged from using abbreviations in tables. If abbreviations are necessary then please explain them in the table's footnotes. Be sure that each table is cited in the text. If you use data from another published or unpublished source, obtain permission and acknowledge the source fully.

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SCALE FOR THE ASSESSMENT OF NARRATIVE REVIEW ARTICLES – SANRA
Immune checkpoint inhibitors in gastric cancer

1. Justification of the article's importance for the readership (2)

Page 2 of the manuscript: "GC is also the fourth main cause of death by cancer in the world.[1] The great majority of cases experience a late diagnosis, mostly due to the lack of solid and global screening strategies and to the lack of specific symptoms.[3] Consequently, a significant proportion of patients present with advanced stage tumours.[4] The prognosis for advanced stages of this disease continues to be dire, and the 5-year survival is 25-30%.[5-7]"

2. Statement of concrete aims or formulation of questions (2)

Page 1: "This review summarizes the main clinical trials evaluating the use of immune checkpoint inhibitors in GC. It also highlights the potential of biomarkers for patient selection for GC immune checkpoint inhibition therapy, including PD-L1 expression and tumor mutational burden, and characteristics of the GC molecular classification, such as microsatellite instability status and Epstein-Barr virus infection, as predictors of response to blockade of the PD-1/PD-L1 axis."

3. Description of the literature search (2)

Page 2: For this review, PubMed searches for data published recently in the literature were performed. Additionally, PubMed searches for recent clinical trials were done, using search terms, such as "gastric cancer", "immune checkpoint inhibitors", "PD-1", "PD-L1", "microsatelite instability", "EBV", and "tumour mutational burden". Only articles in published English were selected.

4. Referencing (2)

Page 6: "Following the first results of the ATTRACTION-2 trial, nivolumab was also approved in various Asian countries as a third-line or later option in patients with unresectable advanced or recurrent gastric or GEJ cancer.[48] One of the limitations of the trial was that the patient population consisted only of Asian patients.[50, 51]"

Page 10: "In GC, between 25% and 65% of tumours express PD-L1, and multiple mechanisms have been associated with PD-L1 upregulation, including *PDL1* gene amplification, structural variations in the 3'UTR of *PDL1*, polymorphisms in *PDL1* promoter, activation of oncogenic PI3K signalling, and cytokine- and chemokine-mediated regulation.[46] [47] PD-L1 expression in GC has been associated with high density of tumour-infiltrating lymphocytes, with MSI, and with EBV infection.[48, 49]"

5. Scientific reasoning (2)

Page 6: “In the context of GC, some of these immune checkpoint inhibitors were approved as third line therapy in advanced or recurrent GC, while their use as first/second line options has been and still is under evaluation. Results of major clinical trials are summarized in Table 1.[41-46]”

Page 12: MSI and MMR deficiency in tumour cells may lead to higher levels of mutations and the appearance of immunogenic neoantigens, leading to easier recognition by immune cells. This may facilitate the action of immune checkpoint inhibitors, as these types of tumours exhibit a high density of immune cells. Accordingly, in comparison with MSS GC, MSI gastric tumours have higher numbers of PD-L1-positive tumour and immune cells, and increased number of tumour-infiltrating lymphocytes.[49, 58, 59]

6. Appropriate presentation of data (2)

Page 7: “The CheckMate-032 phase I/II trial tested the efficacy of nivolumab and ipilimumab in patients with chemotherapy-refractory metastatic gastric or GEJ cancer, in 160 patients from centres in the United States and Europe.[46] Patients were treated with nivolumab, or with combinations of nivolumab plus ipilimumab. The ORR, median PFS and OS were, respectively, 12%, 1.4 months, and 6.2 months in the patients receiving nivolumab, 24%, 1.4 months, and 6.9 months in those receiving nivolumab 1mg/kg and ipilimumab 3mg/kg, and 8%, 1.6 months, and 4.8 months with the combination of nivolumab 3mg/kg and ipilimumab 1mg/kg. Although the combination of nivolumab 1mg/kg plus ipilimumab 3mg/kg had a numerically higher ORR than that of patients treated with nivolumab monotherapy, the median OS was similar in these patient groups.”

Page 15: “In another study of 63 South Korean patients with advanced GC treated with pembrolizumab or nivolumab, responders had significantly higher TMB than non-responders with stable disease.[36] In survival analysis, patients with high TMB had longer PFS. While in univariate analysis, TMB, MSI, response to treatment, and ECOG performance status were all significantly associated with PFS, in multivariate analysis, both TMB-high and the ECOG ≤1 remained independent predictors of longer PFS.

In a clinical trial that analysed toripalimab therapy (a PD-1 antibody) in advanced GC, patients with TMB-high had significant higher OS (14.6 months) than those with TMB-low (4.0 months) with patients.[78] Patients with TMB-high vs. TMB-low also had enhanced ORR (33.3% vs. 7.1%), and a numerically longer PFS, but without statistical significance.”