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Immunotherapy for GI Cancers

Who Benefits, Who Does Not, and Promising Strategies to Expand Use

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KEYWORDS

• Microsatellite instability • Tumor mutational burden • DNA mismatch repair • Gastrointestinal neoplasms • B7-H1 antigen • CTLA-4 antigen • Cell cycle checkpoints • Biomarkers

KEY POINTS

- The applicability of immunotherapy uniformly across all gastrointestinal (GI) cancers has been unproven.
- Immunotherapy has proved highly effective in patients with high-frequency microsatellite instability, regardless of site of primary tumor origin.
- Patients with microsatellite stable GI cancer, mainly those with colorectal cancer, seem to be primarily resistant to immune checkpoint blockade.
- Tumor mutational burden is a predictive biomarker, which can guide clinicians to consider immune checkpoint inhibitors.
- The identification of predictive biomarkers will be imperative for future successes of immunotherapy in GI cancers.

INTRODUCTION

Gastrointestinal (GI) cancers comprise a markedly heterogeneous group of malignancies, with the highest incidence and mortality rates worldwide, responsible for almost 5 million new cases and 3.5 million deaths in 2018 [1]. Apart from squamous cell carcinoma of the anal canal (SCCA), surgical resection is the cornerstone of the curative therapy in localized disease, but multimodality treatment with use of systemic chemotherapies and/or radiotherapy has been making significant contributions to the rising survival rates observed in GI cancers in the last decades [2]. Nevertheless, the 5-year survival rates of patients with metastatic GI cancers remain dismal, ranging from 1% in liver and intrahepatic bile duct tumors to 11% in colorectal cancer (CRC) [2].

The treatment of metastatic GI cancers is highly challenging and depends on the context of the tumor of origin. The applicability of genome-guided personalized therapy has been incipient in GI oncology. After the incorporation of *HER2* status in the therapeutic management of esophagogastric (EG) adenocarcinomas [3] and *RAS* and *BRAF* mutations in CRC [4,5], only recently we have seen the applicability of *FGFR* fusions [6] and *IDH1* mutations in cholangiocarcinoma [7], and mutations in genes related to homologous recombination DNA damage response in pancreatic cancer (PC) [8,9]. Therefore, new advances

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in personalized treatments in GI oncology are urgently needed.

Immunotherapy has ushered in a new era in cancer therapy. Immune checkpoints are membrane receptors expressed in T cells with an important inhibitory role, counterbalancing the immune response, and thereby avoiding tissue damage and autoimmunity [10]. CTLA-4 (cytotoxic T-lymphocyte antigen-4) and PD-1 (programmed cell death-1 protein) are the main examples of immune checkpoints. One of the mechanisms of immune evasion by tumor cells is the upregulation of ligands of these inhibitory receptors [11]. By acting on these autoregulatory mechanisms, the immune checkpoint inhibitors (ICIs) anti-CTLA-4 and anti–PD-1/PD-L1 (PD-1 ligand) unleash the immune cells to act on the tumor cells. Immune checkpoint blockade has changed the landscape of systemic therapy of several solid and hematological tumors, bringing hope to patients with GI cancer.

In this comprehensive review, we describe the current state of the art of immunotherapy in GI malignancies, the role of the emerging predictive biomarkers, and the promising strategies being investigated to overcome the resistance to immunotherapy (Table 1).

ESOPHAGOGASTRIC TUMORS

The efficacy of ICIs in EG tumors seems to be associated with PD-L1 expression, which may be expressed by the combined positive score (CPS). CPS is the ratio of the number of all PD-L1–expressing cells (tumor cells, lymphocytes, macrophages) to the total number of viable tumor cells × 100 [12]. It is estimated that 55% to

TABLE 1
Who Benefits?

Setting	Population	ICI
Esophagogastric tumors		
Squamous cell carcinoma		
2nd line	PD-L1 CPS ≥10	Pembrolizumab
2nd line	Irrespective of PD-L1 status	Nivolumab
Adenocarcinoma		
3rd line	PD-L1 CPS ≥1	Pembrolizumab
3rd line	Irrespective of PD-L1 status	Nivolumab[a]
Hepatocellular carcinoma		
1st line	Child A	Atezolizumab + Bevacizumab
2nd line		Nivolumab
2nd line		Pembrolizumab
2nd line		Ipilimumab + Nivolumab
Colorectal cancer		
1st line	MSI-H or dMMR	Pembrolizumab
3rd line		Nivolumab
3rd line		Pembrolizumab
3rd line		Ipilimumab + Nivolumab
Any malignancy		
2nd line	MSI-H or dMMR	Pembrolizumab
2nd line	TMB ≥10	Pembrolizumab

Abbreviations: CPS, combined positive score; dMMR, deficient mismatch repair; ICI, immune checkpoint inhibitor; MSI-H, high-frequency microsatellite instability; PD-L1, programmed cell death-ligand 1; TMB, tumor mutational burden.

[a] Not approved by the Food and Drug Administration.

66% of the patients with EG adenocarcinoma will present PD-L1 CPS ≥1 [13,14], and more than 20% with CPS ≥10 [15].

Immunotherapy alone or associated with chemotherapy was compared with the standard of care systemic therapy in the first-line setting for advanced EG adenocarcinoma in the KEYNOTE-062, a randomized study with 763 patients, who were HER2-negative and had PD-L1 CPS ≥1 [15]. The 3-arm study compared pembrolizumab versus pembrolizumab plus chemotherapy (cisplatin plus infusional 5-fluorouracil [5-FU] or capecitabine) versus chemotherapy alone (no immunotherapy). Primary endpoints were overall survival (OS) in PD-L1 CPS ≥1 and CPS ≥10, and progression-free survival (PFS) in CPS ≥1. Pembrolizumab plus chemotherapy was not superior to chemotherapy for OS, either in CPS ≥1 or CPS ≥10 population (12.5 months vs 11.1 months, hazard ratio [HR] 0.85, 95% confidence interval [CI] 0.70–1.03, $P = .046$ for CPS ≥1; and 12.3 months vs 10.8 months, HR 0.85, 95% CI 0.62–1.17, $P = .158$ for CPS ≥10). Nevertheless, pembrolizumab met the criteria for noninferiority in the comparison with chemotherapy for OS in CPS ≥1 (HR 0.91, 99.2% CI 0.69–1.18, noninferiority margin: 1.2), and the ICI showed superiority to chemotherapy in OS in CPS ≥10 population (17.4 months vs 10.8 months, HR 0.69, 95% CI 0.49–0.97) in a post hoc analysis. KEYNOTE-062 findings show that pembrolizumab may be effective as front-line therapy in subgroups of patients with EG adenocarcinoma, and the identification of predictive biomarkers in addition to PD-L1 might be useful in the selection of patients to immunotherapy.

In second-line therapy of EG adenocarcinomas, KEYNOTE-061 addressed the benefit of pembrolizumab versus paclitaxel in patients who had progressed on prior platinum and fluoropyrimidine combination chemotherapy [13]. Coprimary endpoints were OS and PFS in patients with CPS ≥1. A total of 395 of 592 (66.7%) patients had CPS ≥1, whose OS was not significantly improved by pembrolizumab compared with paclitaxel: 9.1 months versus 8.3 months (HR 0.82, 95% CI 0.66–1.03, 1-sided $P = .042$). An updated follow-up was recently presented (92.6% of the overall population have died), and demonstrated that the greater the PD-L1 expression, the greater the benefit of pembrolizumab versus paclitaxel for OS: HR 0.81 (95% CI 0.66–1.00), HR 0.72 (95% CI 0.53–0.99), and HR 0.69 (95% CI 0.46–1.05), in CPS ≥1, CPS ≥5, and CPS ≥10 subgroups, respectively [16]. Likewise, overall response rate (ORR) increased according to PD-L1 expression in pembrolizumab versus paclitaxel arms, respectively: 16.3% versus 13.6% (CPS ≥1), 20.0% versus 14.3% (CPS ≥5), and 24.5% versus 9.5% (CPS ≥10). Exploratory analysis of KEYNOTE-061 also demonstrated a strong association between tissue TMB (tumor mutational burden) and response to pembrolizumab [17]. The clinical utility of TMB was assessed using the prespecified cutoff of 175 mutations/exome. In the subgroup of patients with ≥175 mutations/exome (n = 76), ORR was 30.0% for pembrolizumab versus 11.1% for paclitaxel, and OS was 16.4 months versus 8.1 months (HR: 0.46, 95% CI 0.27–0.81), respectively. This analysis suggests that tissue TMB is a significant and independent predictor beyond PD-L1 status in EG adenocarcinoma. Interestingly, there was low correlation between tissue TMB and PD-L1 CPS in both treatment arms.

In later lines of therapy, pembrolizumab demonstrated activity in cohort 1 of the 3-cohort phase II KEYNOTE-059 trial, with 259 patients who had experienced progressive disease after 2 or more lines of therapy [14]. Primary endpoints were ORR and safety. Fifty-five percent of the patients (n = 143) had PD-L1 CPS ≥1, who presented ORR and median duration of response of 15.5% and 16.3 months, compared with 6.4% and 6.9 months in PD-L1–negative tumors. Based on cohort 1 of KEYNOTE-059, pembrolizumab was approved by the Food and Drug Administration (FDA) for the treatment of patients with advanced EG adenocarcinoma whose tumors express PD-L1 CPS ≥1 and had disease progression on or after 2 or more prior lines of therapy, including fluoropyrimidine- and platinum-containing chemotherapy and if appropriate, HER2/neu-targeted therapy [18].

Another study confirmed the benefit of immune checkpoint inhibition with anti–PD-1 monotherapy as had been suggested by the previously mentioned KEYNOTE-059. In the randomized clinical trial ATTRACTION-2, which compared nivolumab with placebo in 493 patients with advanced EG who had failed to 2 or more previous chemotherapy regimens, regardless of PD-L1 expression, nivolumab was associated with an increase in median OS, the primary endpoint for the study: 5.3 months versus 4.1 months (HR 0.63, 95% CI 0.51–0.78, $P<.0001$) [19,20]. On the other hand, avelumab, an anti–PD-L1 monoclonal antibody, did not improve OS over chemotherapy in JAVELIN Gastric 300 trial, a phase III study that randomized 371 patients with advanced EG tumors to avelumab versus physician's choice of chemotherapy

(paclitaxel or irinotecan) [21]. OS was 4.6 months versus 5.0 months (HR 1.1, 95% CI 0.9–1.4), respectively.

The phase 3 KEYNOTE-181 study compared pembrolizumab versus chemotherapy (paclitaxel, docetaxel, or irinotecan) as second-line therapy for 628 patients with advanced squamous cell carcinoma (SCC) (64% of the overall population) and adenocarcinoma of the esophagus (36% of the overall population) [22]. Primary end points were OS in the SCC with PD-L1 CPS $\geq$10 in the intention-to-treat population. In overall population CPS $\geq$10, OS was 9.3 months for pembrolizumab versus 6.7 months for chemotherapy (HR 0.69, 95% CI 0.52–0.93, P = .0074). In CPS $\geq$10 SCC, OS was 10.1 months versus 6.7 months (HR 0.61, 95% CI 0.44–0.85), and in CPS $\geq$10 adenocarcinoma was 6.6 months versus 6.9 months (HR 0.87, 95% CI 0.49–1.55). Here, clinical responses for SCC to pembrolizumab for patients with advanced esophageal cancers appeared better than those with adenocarcinomas. Based on these data, the FDA approved pembrolizumab for the treatment of patients with recurrent locally advanced or metastatic SCC of the esophagus whose tumors express PD-L1 CPS $\geq$10, with disease progression after 1 or more prior lines of systemic therapy [18].

ATTRACTION-3 study has also addressed the role of immune checkpoint inhibition in patients with esophageal SCC who had failed to first-line platinum- and fluoropyrimidine-based therapy [23]. Nivolumab was compared with chemotherapy (paclitaxel or docetaxel) in a population of 419 patients, regardless of PD-L1 expression, in a phase III design. OS, the primary endpoint, was significantly improved in the nivolumab group compared with the chemotherapy group: 10.9 months versus 8.4 months (HR 0.77, 95% CI 0.62–0.96, P = .019). Based on ATTRACTION-3 data, FDA approved nivolumab for patients with unresectable, advanced, recurrent or metastatic esophageal SCC after prior fluoropyrimidine- and platinum-based chemotherapy, regardless of PD-L1 status [24].

HEPATOCELLULAR CARCINOMA

Immunotherapy has provided important treatment options beyond targeted kinase inhibitors for patients with advanced hepatocellular carcinoma (HCC). In a phase 1/2 dose-escalation and expansion trial totaling 262 patients (CheckMate-040), nivolumab demonstrated an ORR of 23% in patients not previously exposed to sorafenib, and 21% in those previously exposed to this agent [25].

KEYNOTE-224, a phase II single-arm trial, demonstrated that pembrolizumab is effective and tolerable in patients with advanced HCC (limited to Child-Pugh A) previously exposed to sorafenib [26]. In this study, patients presented an ORR of 17%, with expected safety profile similar for other tumor settings. These initial findings prompted the design of KEYNOTE-240, a phase III study that compared pembrolizumab plus best supportive care (BSC) versus placebo plus BSC in 413 patients with advanced HCC in second-line setting following progression on sorafenib [27]. Primary endpoints were OS and PFS (1-sided significance thresholds, P = .0174 (final analysis) and P = .002 (first interim analysis), respectively). Median OS was 13.9 months for pembrolizumab versus 10.6 months for placebo (HR 0.78, 95% CI 0.61–0.99, P = .0238). Median PFS was 3.0 months versus 2.8 months, respectively, at the first interim analysis (HR 0.77, 95% CI 0.60–0.98, P = .0186) and 3.0 months versus 2.8 months, at final analysis (HR 0.71, 95% CI 0.57–0.90, P = .0022). Thus, both OS and PFS did not reach statistical significance per specified criteria.

The combination of ipilimumab plus nivolumab was also evaluated in the multicohort CheckMate-040, and it showed an ORR twice that of nivolumab monotherapy in a population of sorafenib-treated patients (31% and 14% [25], respectively) [28]. These findings prompted the approval of the combination therapy by the FDA in the following doses: nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks for 4 doses, followed by nivolumab monotherapy thereafter [24]. In addition to the combination therapy, the FDA has also approved both nivolumab alone and pembrolizumab alone for the treatment of patients with advanced HCC who had failed to sorafenib, based on the findings of CheckMate-040 and KEYNOTE-224 studies, respectively [18,24].

The promising data revealed by the second-line studies elicited the conception of clinical trials addressing the efficacy of ICIs in patients with treatment-naïve advanced HCC. CheckMate-459 was a large phase III study with 743 patients comparing nivolumab versus sorafenib in first-line setting [29]. Median OS, the primary endpoint, was 16.4 months for nivolumab and 14.7 months for sorafenib (HR 0.85, 95% CI 0.72–1.02, P = .0752), and it did not meet the predefined threshold of statistical significance (HR 0.84, P = .0419). ORR was 15% for nivolumab versus 7% with sorafenib. Interestingly, PD-L1 status did appear to be associated with likelihood for response to ICI: 28% of ORR in patients expressing PD-L1 $\geq$1%, compared with 12% in PD-L1–negative patients.

Nivolumab presented a more favorable safety profile, with 22% versus 49% of grade 3/4 treatment-related adverse events (TRAEs).

The combination of ICIs with anti-angiogenic inhibitors was addressed in the first-line setting by the IMbrave150 trial, a phase III study that compared the efficacy of the anti–PD-L1 inhibitor atezolizumab plus bevacizumab versus sorafenib in 501 previously untreated patients with HCC with advanced disease [30]. The coprimary end points were OS and PFS. At the time of the primary analysis (August 29, 2019), the median survival had not been reached by the immunotherapy arm. OS at 12 months was 67.2% with atezolizumab plus bevacizumab versus 54.6% with sorafenib (HR 0.58, 95% CI 0.42–0.79, P<.001). PFS was 6.8 months versus 4.3 months (HR 0.59, 95% CI 0.47–0.76, P<.001). ORR was 27% versus 12%, according to independent assessment with Response Evaluation in Solid Tumors (RECIST) 1.1 (P<.001), and 33% versus 13% according to HCC-specific modified RECIST (P<.001). Grade 3 or 4 AEs occurred in 57% versus 55%. Grade 3 or 4 hypertension occurred in 15% of patients in the atezolizumab plus bevacizumab group versus 12% in the sorafenib group. No new or unexpected adverse events were observed with the combination therapy. Atezolizumab plus bevacizumab also delayed deterioration of patient-reported quality of life (median time to deterioration, 11.2 months vs 3.6 months). This landmark trial provided the basis for the FDA approval of atezolizumab plus bevacizumab in the first-line setting of unselected patients with advanced disease and this combination has become the new standard of care for treatment-naïve patients [31].

PANCREATIC CANCER

In an unselected population of microsatellite stable (MSS) PC, the efficacy of immune checkpoint inhibition has been disappointing, even in those patients with higher levels of PD-L1 expression. In KEYNOTE-028, a nonrandomized multicohort phase Ib trial of pembrolizumab in 475 patients with PD-L1–positive advanced solid tumors, 20 PC patients were included, and none of them presented objective response [32]. In another phase I clinical trial with 207 patients with advanced solid tumors, none of the 14 patients with PC had objective response to nivolumab [33]. Phase II study with 27 PC patients with locally advanced or metastatic disease also demonstrated no objective responses to ipilimumab [34]. Phase II randomized clinical trial with PC patients previously submitted to one first-line therapy compared the combination of durvalumab plus tremelimumab versus durvalumab monotherapy [35]. In the first phase of the study, from the 64 randomized patients, ORR was 3% for patients receiving combination therapy and 0% for patients receiving monotherapy. The ORR did not meet the prespecified threshold of 10% to start the expansion phase of the study.

The benefits of immunotherapy in PC, to date, are restricted to fewer than 2% of patients with PC who harbor deficient mismatch repair (dMMR) or high-frequency microsatellite instability (MSI-H) [36,37]. In the phase II KEYNOTE-158 study of pembrolizumab in patients with previously treated, advanced non-colorectal dMMR or MSI-H cancer, there were 22 patients (9.4% of the overall population) with PC [38]. Four patients (18.2%) presented objective response, of whom 1 was complete. Patients with MSI-H pancreatic cancers are approved by the FDA for use of anti–PD-1 monotherapy.

Interestingly, the rates of predictive biomarkers for immunotherapy (MSI-H, PD-L1, and TMB) seem to be associated with *BRCA1/2* mutations in PC. In a study with 2824 patients with PC, *BRCA1/2* mutations were associated with higher MSI-H frequency (4.8% in *BRCA*-mutated vs 1.2% in *BRCA* wild-type, $P = .002$), elevated PD-L1 expression (22% vs 11%, P<.001) and higher TMB (mean 8.7 mutations/Mb vs 6.5 mutations/Mb, P<.001) [39].

BILIARY TRACT CANCER

dMMR/MSI-H tumors occur in approximately 1% to 5% of all biliary tract cancers (BTCs) and occur with different incidences according to site of origin: gallbladder carcinoma and extrahepatic cholangiocarcinoma with 5% each, and intrahepatic cholangiocarcinoma and ampullary carcinoma with 10% [36,37,40].

Basket trials that evaluated the role of anti–PD-1 inhibitors in advanced solid tumors demonstrated encouraging results of immune checkpoint inhibition in MSS BTC, but prospective studies specifically addressing this population revealed modest activity in unselected patients. In KEYNOTE-158, the 22 patients with MSI-H BTC enrolled presented an OS of 24.3 months, and 9 (41%) of them presented objective response [38]. In KEYNOTE-028 trial, from the 23 patients with PD-L1–positive BTC accrued, 17% reached objective response, but with a modest median PFS of 1.8 months, and median OS of 6.2 months [32].

A nonrandomized, phase 1 Japanese study compared nivolumab alone or in combination with

cisplatin plus gemcitabine in patients with unresectable or recurrent BTC [41]. In the nivolumab monotherapy cohort, only 1 of 30 patients had an objective response. Combined therapy cohort showed better activity, reaching 36% of ORR. Nivolumab was also evaluated in a phase II study with 54 patients with advanced refractory BTC [42]. From the 46 response-evaluable patients, 10 (22%) had investigator-assessed objective response, with a disease control rate of 59%. Objective response was observed in 21% of those with intrahepatic cholangiocarcinoma, 40% with extrahepatic cholangiocarcinoma, and 15% with gallbladder cancer. Interestingly, 9 of the 10 responders were PD-L1–positive, and all of them had proficient MMR (pMMR) tumors. From the 42 PD-L1-tested tumors, 18 (43%) had PD-L1 overexpression, which was associated with prolonged PFS (HR 0.23, 95% CI 0.10–0.51, $P<.001$). However, a central independent review found an ORR of 11%, with a disease control rate of 50%.

A phase I trial with 30 patients with previously treated advanced BTC evaluated the efficacy and safety of bintrafusp alfa (M7824), a bifunctional fusion protein composed of the extracellular domain of the transforming growth factor (TGF)-βRII receptor (a TGF-β "trap") fused to a PD-L1 monoclonal antibody, and showed promising activity [43]. The ORR, PFS, and OS were 20%, 2.5 months, and 12.7 months, respectively. The efficacy was irrespective of PD-L1 expression and MSI status. However, the drug demonstrated a concerning safety profile. Nineteen (63%) patients experienced TRAEs, most commonly rash (17%) and fever (13%). Eleven (37%) patients had grade ≥3 TRAEs, and 3 patients had grade 5 events (septic shock n = 1; interstitial pneumonitis n = 2). M7824 was designated an orphan drug by the FDA and it is currently under investigation in patients with BTC in phase II/III studies as first-line and second-line therapy (NCT04066491 and NCT03833661).

COLORECTAL CANCER

Activity of ICIs in patients with CRC remains limited to those with dMMR tumors. A pivotal pilot phase II trial (KEYNOTE-164) assessed the activity of ICIs in patients with CRC according to MMR status [44]. Patients with metastatic solid tumors were divided in groups dMMR CRC (n = 10) and pMMR CRC (n = 18). The immune-related ORR was 40% and 0%, respectively. This study was 1 of the 5 studies [44–48] that led to the accelerated approval of pembrolizumab for adult and pediatric patients with unresectable or metastatic, previously treated MSI-H/dMMR solid tumors following prior treatment [44–48].

The activity of ICIs as monotherapy and as combination therapy for patients with advanced MSI-H CRC tumors has been tested in other trials. One of the largest initiatives was the CheckMate-142 trial, a 6-cohort phase II study designed to evaluate the efficacy of nivolumab monotherapy or nivolumab in combination with other anticancer drugs in patients with MSI-H and non–MSI-H metastatic CRC (NCT02060188). Enrolled patients must have failed at least 1 previous line of treatment, including a fluoropyrimidine, oxaliplatin, and/or irinotecan. Of the 74 MSI-H patients enrolled in cohort 1 (nivolumab monotherapy), 31% had a response [49]. Cohort 3 consisted of MSI-H patients treated with the combination of nivolumab plus ipilimumab (n = 119). Sixty-five patients (55%) had a response, including 3% with a complete response [50]; median PFS was not reached; and 83% of patients had durable responses lasting ≥6 months. Based on the aforementioned studies, the FDA incorporated the indication of nivolumab and the combination of nivolumab plus ipilimumab in the management of patients with MSI-H metastatic CRC (mCRC) with progression or intolerance of fluoropyrimidine, oxaliplatin, and/or irinotecan [51].

Promising neoadjuvant therapy in colon cancer has been explored in a pilot phase II study NICHE [52]. Patients with resectable, early-stage colon cancer, received 1 cycle of nivolumab (3 mg/kg, day 1 and 15) plus ipilimumab (1 mg/kg, day 1). Of 14 evaluable patients, major pathologic responses (<5% viable tumor cells) were observed in all 7 patients with dMMR (100%), with 4 patients in pathologic complete response (pCR) (57%). No major pathologic responses were seen in pMMR tumors. Likewise, in a retrospective study with 121 patients with metastatic dMMR CRC treated with ICIs, 14 were submitted to surgical resection of the primary and/or metastatic tumor after the use of immunotherapy [53]. Interestingly, pCR was noted in the resected specimens of 13 patients despite the presence of residual tumor on preoperative imaging in 12 of those patients. The high pCR rates to ICIs demonstrated by the 2 studies open an avenue toward the development of new therapeutic applications of immunotherapy in patients with dMMR CRC so that future clinical trials might explore the nonoperative management of primary and metastatic lesions.

Disappointingly, a randomized phase II study of atezolizumab added to fluoropyrimidines plus bevacizumab was not beneficial as maintenance therapy versus chemotherapy alone (NCT02291289) [54]. The median

PFS was 7.13 months (experimental arm) versus 7.39 months (HR 0.92, $P = .480$), and the median OS was 21.91 months versus 22.05 months, respectively (HR 0.86, $P = .283$).

IMblaze370 was the first completed phase III study evaluating the role of ICI, with or without MEK inhibition in CRC [55]. Treatment-refractory patients were recruited to 1 of 3 arms (2:1:1): (1) atezolizumab plus the MEK inhibitor cobimetinib, (2) atezolizumab monotherapy, or (3) regorafenib. The enrollment of MSI-H patients was capped at 5% of the overall population. The primary endpoint of OS was 8.8 months for atezolizumab plus cobimetinib (HR 1.00, 95% CI 0.73–1.38, $P = .99$), 7.1 months for atezolizumab (HR 1.19, 95% CI 0.83–1.71, $P = .34$), and 8.5 months for the control arm of regorafenib. The median PFS was 1.9 months, 1.9 months, and 2.0 months, and ORR was 3%, 2%, and 2%, respectively. Likewise, there was no statistically significant difference in OS and PFS between the atezolizumab arms.

KEYNOTE-177 was a phase III study comparing pembrolizumab versus standard of care (mFOLFOX6 or FOLFIRI $\pm$ bevacizumab or cetuximab) as first-line therapy of patients with MSI-H or dMMR metastatic CRC [56]. Primary end points were PFS and OS. At data cutoff (February 19, 2020), 307 patients were randomized, and OS data were not presented. Patients receiving chemotherapy could crossover to pembrolizumab arm after confirmed progressive disease. Pembrolizumab was superior to chemotherapy for PFS: 16.5 months versus 8.2 months (HR 0.60, 95% CI 0.45–0.80, $P = .0002$). ORR was 43.8% versus 33.1%. Duration of response data remarkably favored pembrolizumab, with a 24-month response duration of 83% versus 35%. Grade 3 to 5 TRAEs were 22% versus 66%. Interestingly, a subgroup analysis suggested that the benefit derived from immunotherapy might be different by *RAS* status. *RAS* wild-type patients derived a large benefit from pembrolizumab for PFS (HR 0.44, 95% CI 0.29–0.67), whereas *RAS*-mutated patients did not (HR 1.19, 95% CI 0.68–2.07). An interaction test was not presented. A potential association between *RAS* mutations and benefit derived from ICIs in the MSI-H population should be more carefully analyzed in the ensuing data of KEYNOTE-177 trial.

Previous studies and the KEYNOTE-177 trial revealed that approximately 30% of the patients with metastatic MSI-H CRC are primarily resistant to anti–PD-1 inhibitors. It is not clear which are the mechanisms of primary resistance to anti–PD-1 inhibitors in this enriched population and it is not possible to identify these patients as resistance biomarkers have not been identified. Retrospective data suggest that MSI-H patients with higher TMB present higher response rates to immunotherapy. In a series with 22 MSI-H patients treated with ICIs, TMB showed the strongest association with ORR and PFS [57]. Optimal predictive cut-point for TMB was estimated between 37 and 41 mutations/Mb. All 13 TMB-high cases presented objective response, whereas 6 of 9 TMB-low cases had progressive disease. Likewise, phase II randomized clinical trial comparing durvalumab plus tremelimumab versus BSC in refractory CRC showed that MSS patients with TMB $\geq$28 mutations/Mb in cfDNA (21% of MSS patients) derived meaningful benefit in OS from immunotherapy (HR 0.34, 90% CI 0.18–0.63, $P = .004$) [58].

There is a strong association between MSI status and TMB. Study analyzing tumor samples from 6004 patients with advanced CRC showed that 99.7% of MSI-H patients have TMB-high ($\geq$12 mutations/mb), compared with 2.9% of MSS patients [59]. Patients with POLE and POLD1 mutations, which seem to be found in approximately 1% of patients with CRC, present MSS status but they are typically hypermutated, and case reports have showed impressive responses to immunotherapy [60,61]. On the other hand, it is unclear if the recent FDA approval of pembrolizumab for patients with TMB-high ($\geq$10 mutations/mb) based on KEYNOTE-158 findings, regardless of site of origin, might be translated to patients with CRC [18].

Therefore, it is possible that TMB might be an important predictive factor to select which patients with MSI-H CRC have a higher sensitivity to immunotherapy. Molecular analyses from resistant patients in the KEYNOTE-177 trial will be imperative to clarify the presence of resistance markers to immunotherapy in MSI-H CRC.

MSI-H is the most powerful predictive biomarker for immunotherapy in CRC. Strategies to overcome the supposed innate resistance of MSS disease have been pursued in the past few years. The combination of anti–PD-1 inhibitors with regorafenib has been promising based on recent studies. REGONIVO is an open-label, dose-escalation, and dose-expansion phase Ib trial of regorafenib plus nivolumab for gastric and CRC [62]. It is hypothesized that tumor-associated macrophages (TAM) may play a role in the resistance to PD-1 blockade in MSS CRC. In tumor models, regorafenib reduced TAM through inhibition of colony-stimulating factor 1 receptor [63,64], and a synergistic effect of tyrosine-kinase and anti–PD-1 inhibitors is supposed to have an antitumor activity in MSS disease. An encouraging number of 9 of 25 patients with CRC presented objective response (36% ORR) in the initial

study. The combination of regorafenib 80 mg plus nivolumab presented a manageable safety profile and should be explored in larger populations of CRC. Based on this rationale, REGOMUNE phase II trial evaluated the combination of regorafenib plus avelumab, an anti–PD-L1 inhibitor, in a treatment-refractory MSS CRC population [65]. From the 48 enrolled patients, 40 had at least 1 imaging tumor assessment, and 12 (30%) presented tumor shrinkage. The median PFS and OS were 3.6 months and 10.8 months, respectively. Correlative studies revealed that patients with low TAM infiltration and high tumor infiltration by CD8+ T cells (6 of 24 patients, 25%) derived larger benefit from the combination therapy, with a median PFS of 5.3 months versus 1.9 months ($P = .037$), and a median OS not reached versus 5.3 months ($P = .02$).

ANAL CANCER

The efficacy of ICIs in anal cancer was first evaluated in NCI9673, a multicentre phase II single-arm trial evaluating the role of nivolumab in 37 patients with treatment-refractory metastatic disease [66]. The primary endpoint was ORR. Patients received a median of 6 doses of nivolumab. Nine patients (24%) presented objective responses (7 partial and 2 complete responses) and 17 patients (47%) had stable disease. One of the 2 patients who were positive for human immunodeficiency virus (HIV) had a partial response. Median PFS was 4.1 months, with a 6-month PFS of 38%. Median OS was 11.5 months, with an estimated 1-year OS of 48%. No grade 3 or 4 adverse events occurred in the HIV-positive patients.

Pembrolizumab has been evaluated in advanced anal cancer population also in KEYNOTE-158 (NCT02628067) [67]. Thirteen (11.6%) of 112 patients included presented objective response (8 partial and 5 complete responses). Two or more prior therapies had been completed in 73.2% of the patients. Median PFS was 2.0 months and median OS was 12.0 months. Responses occurred in 15% of 75 patients with PDL1 CPS 1 and in 7% of 30 patients with PD-L1 CPS less than 1. Here, PD-L1 does not appear to be a robust predictive marker associated with response to ICI.

Axalimogene filolisbac (AXAL) is an immunotherapeutic vaccine using *Listeria monocytogenes* (Lm) as bacterial vector, and it has been developed to secrete the Lm-listeriolysin O fusion protein targeting human papillomavirus (HPV)-positive tumors. AXAL has been investigated in combination with standard of care radiation therapy and concurrent 5-FU and mitomycin-C in a phase I study in patients with high-risk locally advanced SCCA (BrUOG 276) [68]. Nine out of 11 patients had complete remission with a well-tolerated safety profile. Phase II trial evaluated the efficacy and safety of AXAL in patients with surgically unresectable or metastatic SCCA [69]. Thirty-six patients were treated, of whom 29 patients were evaluable for response. One patient had a prolonged partial response (3.4% ORR). The 6-month PFS rate was 15.5%. Grade 3 adverse event were noted in 10 patients, with most being cytokine-release symptoms. Despite being safe and well-tolerated, ADXS11-001 study did not meet either primary endpoint (ORR $\geq$10% or 6-month PFS rate $\geq$20%) to proceed to the second stage of the study.

Adoptive T-cell therapies has also been evaluated in SCCA. Phase I/II clinical trial of T cells genetically engineered to express a T-cell receptor (TCR) that targets an HLA-A*02:01-restricted epitope of E6 (E6 TCR T Cells) for patients with metastatic HPV16-positive carcinoma evaluated 16 patients, of whom 4 were patients with anal cancer [70]. Two patients with anal cancer showed partial responses lasting 3 months and 6 months after treatment. The patient with a 6-month response had complete regression of 1 tumor and partial regression of 2 tumors that were resected on progression.

DISCUSSION

Despite sharing epidemiologic, clinical, and therapeutic aspects, the GI cancers comprise such a diverse group of malignancies from a molecular and genetic perspective, with a markedly heterogeneous molecular landscape that imposes different scenarios and obstacles to the use of immunotherapy in the unselected population. The small subset of patients with GI cancer who harbor dMMR or MSI-H is the only group that has demonstrated high sensitivity to immune checkpoint inhibition. Anti–PD-1/PD-L1 and anti-CTLA-4 inhibitors have exhibited modest activity in the unselected population of GI cancers, composed predominantly by MSS tumors.

It is not clear why certain tumors are more sensitive to immunotherapy. Based on the target of anti–PD-1/PD-L1 therapies, it is logical to evaluate the benefit of these monoclonal antibodies according to the expression of PD-L1. Nevertheless, objective responses and prolonged survival can also be reached in patients with lower expression of PD-L1 or even with no expression. The threshold in the definition of PD-L1 positivity is also unclear. Cutoffs of 1% [71], 5% [72], and 50% [73] have been used in clinical trials, although it is not possible to state which value has greater accuracy.

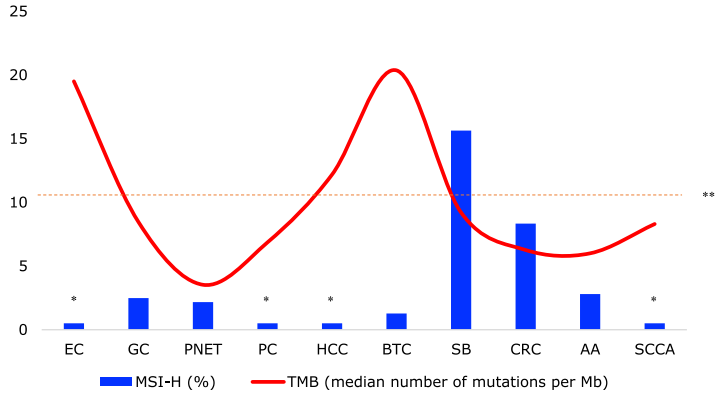

FIG. 1 Rates of MSI-H and TMB in GI cancers. *MSI-H rate less than 1%. **Dashed line: The threshold of TMB 10 mutations/Mb approved by the FDA for the use of pembrolizumab irrespective of the tumor origin. AA, appendiceal adenocarcinoma; EC, esophageal cancer; GC, gastric cancer; PNET, pancreatic neuroendocrine tumors; SB, small bowel carcinoma; SCCA, squamous cell carcinoma of the anal canal. (*Data from* Refs. [37,82–86])

In EG tumors, both SCC and adenocarcinoma, the benefit of PD-1 inhibition with pembrolizumab differs according to the PD-L1 expression, but with different cutoffs by histology. In SCC, the efficacy in second-line therapy has been demonstrated if PD-L1 CPS $\geq$10, and in adenocarcinoma in third-line therapy if PD-L1 CPS $\geq$1.

Patients with genomic instability, such as those with germline or somatic abnormalities in DNA MMR pathways, tend to present a higher number of nonsynonymous mutations, and thereby high TMB. In the phase II trial evaluating pembrolizumab in cohorts of patients according to MMR status, a mean of 1782 somatic mutations per tumor in dMMR patients was found, compared with 73 per tumor in pMMR patients [44]. There is a high concordance rate between MSI-H and TMB. In a study with more than 62,000 tumor samples analyzed, 83% of MSI-H patients had high TMB [74]. However, the converse was not true. Only 16% of the samples with high TMB were classified as MSI-H. Similar to PD-L1 expression, the cutoff to dichotomize TMB in high and low is also uncertain, with studies using both greater than 100 [75] and $\geq$178 [76] nonsynonymous mutations as thresholds. The recent approval by the FDA of pembrolizumab for those patients with TMB $\geq$10 mutations per Mb was based on the KEYNOTE-158 findings [38], which showed an ORR of 29% in the 102 patients (13%) who had tumors identified as TMB-H, defined as TMB $\geq$10 mutations per Mb. The median duration of response was not reached, with 57% of patients having response durations $\geq$12 months and 50% of patients having response durations $\geq$24 months [18]. Most of the population of patients with GI cancer present TMB <10 and low rate of MSI-H positivity (Fig. 1). In the face of the cost and potential benefit of ICIs, it is imperative to find biomarkers with higher accuracy to better select patients, which is a field of intensive research.

The association of biomarkers (eg, MSI-H and TMB) may be useful to better select patients, because sensitive patients presented higher median TMB (54 mutations/Mb) compared with resistant ones (29 mutations/Mb) in a cohort of 22 patients with MSI-H metastatic CRC treated with PD-1/PD-L1 inhibitors [77]. The association of MSI-H with immunoscore may also be potentially useful in the identification of immunotherapy-sensitive patients. MSI-H patients are associated with high immunoscore, but 20% of patients with MSS CRC also present immunoscore high, and might be sensitive to immunotherapy [78–80]. Studies searching for predictive biomarkers in circulating tumor DNA (ctDNA) have an interesting rationale. It seems that ctDNA reflects more accurately the intratumoral and intertumoral heterogeneity and might demonstrate the dynamics of antitumoral immune response with better accuracy [81].

SUMMARY

The applicability of immunotherapy in an unselected population of GI cancers has been challenging. Immune checkpoint inhibition has proved highly effective in the restricted population of MSI-H patients, and it has become the standard of care of first-line therapy

of MSI-H metastatic CRC. MSS patients seem to be primarily resistant to ICI, apart from some patients with high TMB, mainly those hypermutated with *POLE* and *POLD1* mutations. PD-L1–positive EG tumors, HCC, and anal cancer have demonstrated moderate sensitivity to ICI. The identification of mechanisms of resistance and predictive biomarkers to ICI will be imperative for the development of therapeutic strategies, which are under intensive investigation.

CLINICS CARE POINTS

- Every patient with metastatic GI cancer eligible to systemic therapy must be tested for MSI status and TMB.
- Immunotherapy should be considered as first-line systemic therapy of patients with MSI-H CRC, and for every patient with MSI-H GI cancer who had progressed following prior treatment.
- Patients with advanced GI cancers with TMB $\geq$10 should be considered to immunotherapy.
- Patients with advanced HCC Child-Pugh A should be preferentially treated with atezolizumab plus bevacizumab in first-line therapy.
- Immunotherapy should be considered to patients in second-line of esophageal SCC, and to patients in third-line of EG adenocarcinoma with PD-L1 CPS $\geq$1.
- Patients with advanced anal cancer who had failed first-line therapy should be offered immunotherapy.
- Immunotherapy remains experimental in PC and in biliary tract tumors.

DISCLOSURE

The authors have nothing to disclose.

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