Immune Checkpoint Inhibitor Therapy in Locally Advanced MSI GI Malignancies

Emily C. Harrold, MB, BCh, BAO[1]; Fergus Keane, MB, BCh, BAO[1]; and Andrea Cercek, MD[1]

[1]Memorial Sloan Kettering Cancer Center, New York, New York

Corresponding author:
Andrea Cercek, MD
Gastrointestinal Oncology Service
Department of Medicine
Memorial Sloan Kettering Cancer Center
300 East 66th Street
New York, NY 10065
Tel: (646) 888-4189
Email: cerceka@mskcc.org

Abstract: Locally advanced gastrointestinal (GI) malignancies have conventionally been treated in a multimodal fashion that combines (neo)adjuvant chemotherapy with or without radiation and definitive surgical resection. Clinical data have demonstrated the reduced responsiveness of GI malignancies with microsatellite instability (MSI) to both adjuvant and neoadjuvant systemic chemotherapy when compared with microsatellite stable (MSS) disease. The elevated tumor mutational burden associated with MSI tumors of all types sensitizes these tumors to the effects of immune checkpoint blockade in the metastatic setting, which led to tumor-agnostic approval of immune checkpoint inhibitors in this context. The recent demonstration of greater sensitivity and high pathologic complete response rates to neoadjuvant immunotherapy in locally advanced GI malignancies may ultimately establish a novel treatment paradigm and herald potential nonoperative management of this distinct subgroup of GI malignancies. This article provides an overview of immune checkpoint inhibitor therapy in locally advanced MSI GI malignancies. It also covers the clinical significance of MSI status across the GI cancer spectrum, the available data demonstrating differential responses of MSI and MSS disease to conventional chemotherapy, and the biological rationale for novel strategies utilizing immunotherapy in the neoadjuvant, adjuvant, and nonoperative settings.

Keywords
Gastrointestinal, GI malignancies, immune checkpoint inhibitors, MSI, neoadjuvant

Introduction

The advent of immunotherapy has radically altered the treatment paradigm for metastatic tumors with microsatellite instability (MSI) and mismatch repair deficiency (dMMR). We have seen unprecedented survival advances with the addition of immunotherapy to standard treatment algorithms in the setting of inoperable disease. We are increasingly witnessing the utility of checkpoint inhibitors in the neoadjuvant setting, with the potential omission of conventional treatment modalities, like chemotherapy and radiation, and their associated morbidity and heralding an era of nonoperative management

in the setting of MSI locally advanced disease. The use of checkpoint inhibitors has expanded rapidly across the gastrointestinal (GI) cancer spectrum (Figure) since the initial MSI pan-cancer approval of pembrolizumab (Keytruda, Merck) in 2017.

MSI Tumors and Immunotherapy

MSI, which describes a clonal change in the number of repeated DNA nucleotide units in microsatellites,[1] is the consequence of impairment of the DNA base repair system. Microsatellites are short, repetitive DNA sequences consisting of nucleotide, dinucleotide, or higher order nucleotide repeats[2]; these regions are prone to accumulation of mutations owing to the inefficiency of DNA polymerases during DNA synthesis, with high rates of insertions and deletions (indels) frequently leading to frameshift mutations and altered protein function and expression.[3] The number and pattern of indels can distinguish microsatellite stable (MSS) tumors from MSI tumors.[4] The high neoantigen load associated with MSI tumors is enriched for mutations encoding proteins that are immunogenic, sensitizing them to immune checkpoint blockade (ICB).[5] This efficacy of ICB in MSI tumors led to the first tumor-agnostic approval of ICB in patients with metastatic disease.[6-8] Although the terms *MSI* and *dMMR* are often used interchangeably, MSI refers to the phenotypic consequence of deficient mismatch repair.

Checkpoint Inhibitor Mechanism of Action and Rationale for Neoadjuvant Application

The checkpoint inhibitors that are currently in widespread use are antibodies that block either the cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) or programmed death 1 (PD-1) pathway. They enhance the innate immune response, allowing for antitumor T-cell responses. Application of a CTLA-4 antibody releases the inhibitory effect of CTLA-4 translocation to the cell surface, leading to enhanced T-cell activation and resultant tumor regression.[9] The PD-1/programmed death ligand 1 (PD-L1) interaction between T cells and tumor cells leads to inhibition of the antitumor T-cell responses.[10] Application of PD-1 or PD-L1 antibodies (PD-1 inhibitors, PD-L1 inhibitors) similarly inhibits the negative regulators of immune activation, allowing for potent antitumor responses.[11] More recently, the role of lymphocyte activation gene 3 (LAG-3) in suppressing T cell activation and cytokine secretion has been explored.[12] The significant synergy of LAG-3 with PD-1[13] that was identified in preclinical studies has been effectively exploited in the treatment of metastatic melanoma,[14] and more recently in early-stage colorectal cancer (CRC).[15]

Neoadjuvant immunotherapy can be viewed as in situ cancer vaccination.[16] Effective 'vaccination' of the immune system against cancer, which typically is achieved before surgical resection, requires the presence of a sufficient volume of tumor-associated neoantigens. Liu and colleagues provide data from 2 tumor models that demonstrate the significance of timing of immunotherapy before surgical resection, along with greater induction of CD8-positive T cells when immunotherapy is applied in the neoadjuvant setting.[17]

Locally Advanced GI Tumors and the Impact of MSI

Locally advanced GI cancers are typically managed with definitive surgical resection with or without (neo) adjuvant radiation and systemic therapy with curative intent. Neoadjuvant therapy for locally advanced disease has been demonstrated to improve survival,[18,19] enhance tumor resectability,[20] reduce local recurrence,[21] and, in some circumstances, allow for nonoperative management.[22] From an investigational perspective, neoadjuvant therapy allows for the correlation of clinical, pathologic, and radiologic responses. Additionally, access to biological specimens allows for elucidation of the molecular mechanisms underpinning both response and resistance. Thus, neoadjuvant systemic therapy is the standard of care across several GI malignancies.

Conventional treatment strategies have been demonstrated to be less effective in MSI tumors. Although the data were conflicting initially,[23] MSI in the setting of locally advanced CRC was demonstrated to predict a lack of benefit of adjuvant 5-fluorouracil (5-FU).[24-27] Fortunately, the addition of adjuvant oxaliplatin to 5-FU in the setting of stage III CRC appeared to mitigate this detrimental effect.[28] Differential responses to neoadjuvant chemotherapy were also seen by microsatellite status among patients with rectal cancer treated with total neoadjuvant therapy, with disease progression occurring in 25% of dMMR/MSI rectal tumors treated with neoadjuvant chemotherapy[29] and none of the MSS rectal tumors in the same study. This study also included an analysis of patient-derived tumoroids. Notably, the dMMR rectal cancer tumoroids demonstrated significant resistance to leucovorin, 5-FU, and oxaliplatin (FOLFOX) compared with tumoroids with MMR proficiency (pMMR) (50% inhibitory concentration [IC50]=1.97 [95% CI, 1.49-2.54] vs 5.02 [95% CI, 3.86-6.63]). Responses to neoadjuvant chemoradiotherapy have also been demonstrated to vary by microsatellite status in a National Cancer Database analysis of more than 5000 patients with locally advanced rectal cancer.[30]

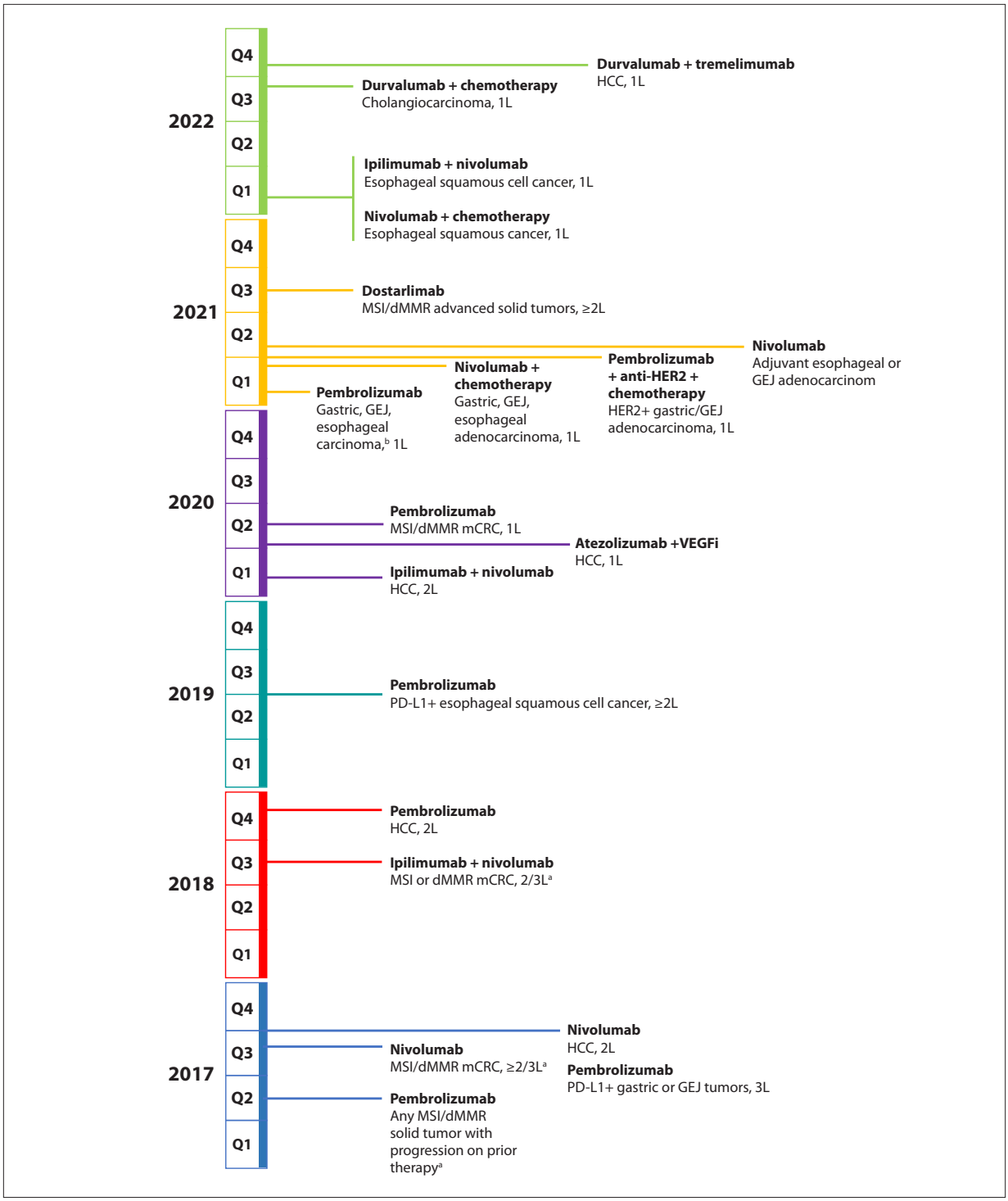

Figure. Timeline of FDA approvals of immunotherapy used in GI malignancies.
1L, first line; 2L, second line; 3L, third line; dMMR, mismatch repair deficiency; FDA, US Food and Drug Administration; GEJ, gastroesophageal junction; GI, gastrointestinal; HCC, hepatocellular carcinoma; HER2+, human epidermal growth factor receptor 2–positive; mCRC, metastatic colorectal cancer; MSI, microsatellite instability; PD-L1+, programmed death ligand 1–positive; VEGFi, vascular endothelial growth factor inhibitor.
[a]In the setting of metastatic CRC, patients had to have received oxaliplatin, irinotecan, and a fluoropyrimidine.
[b]Metastatic or locally advanced unresectable.

Colorectal Cancer

Approximately 15% of CRCs are MSI.[31] The likelihood of MSI status in CRC varies according to the stage of disease[32]; it is approximately 20% in stage I to II, 12% in stage III, and 4% to 5% in stage IV. About one-third of MSI CRC arises from an underlying pathogenic variant in the DNA mismatch repair genes (*MLH1, MSH2, MSH6, PMS2, EPCAM)*. Pathogenic germline variants in the DNA mismatch repair genes are the hallmark of Lynch syndrome, a pan-cancer predisposition syndrome characterized by near-universal MSI status of the associated cancers.[33] Lynch syndrome carries a lifetime risk of CRC of up to 80%, depending on the underlying germline variant. However, most MSI CRCs (80%-90%) are sporadic and arise from MLH1 hypermethylation,[34] which is associated with high CpG island methylation phenotype (CIMP+).

A systemic review evaluating the prognostic impact of MSI status in CRC included 32 studies and 7642 CRC cases, of which 1277 were MSI.[35] Patients with MSI CRC had better prognoses, even when the data were restricted to clinical trial patients and patients with locally advanced disease. The combined overall survival (OS) hazard ratio (HR) associated with MSI was 0.65 (95% CI, 0.59-0.71; heterogeneity P=.16; I^2=20%). When the analysis was restricted to trial patients only, the HR was 0.69 (95% CI, 0.56-0.85), and when restricted to patients with locally advanced disease only, the HR was 0.67 (95% CI, 0.58-0.78). Importantly, patients with MSI tumors did not appear to derive benefit from adjuvant 5-FU in the locally advanced disease setting (HR, 1.24; 95% CI, 0.72-2.14). This is corroborated by several other studies[36-38] and is further confirmed in the more recent analysis of the prospective FOxTROT trial of neoadjuvant FOLFOX in locally advanced CRC.[39,40] In this study, the response to neoadjuvant chemotherapy was significantly less (P<.001) in dMMR than in pMMR tumors, with a rate of moderate or greater histological tumor regression of 7% (8/115) vs 23% (128/553), respectively. Reductions in 2-year recurrence were also seen only in patients with pMMR tumors (rate ratio [RR], 0.69; 95% CI, 0.50-0.97; P=.043) with no apparent reduction in 2-year recurrence among patients with dMMR tumors (RR, 0.86; 95% CI, 0.42-1.76; P=.68). Essentially, about 95% of the patients with dMMR tumors who received neoadjuvant chemotherapy showed no or little response to therapy.

Metastatic CRC

Le and colleagues published their groundbreaking phase 2 study in 2015, evaluating the PD-1 inhibitor pembrolizumab in treatment-refractory metastatic cancer.[5] This study included 32 patients with CRC, of whom 11 (9 colon, 2 rectal) had dMMR disease and 21 (198 colon, 3 rectal) had MMRp disease. The objective response rate (ORR) and immune-related progression-free survival (PFS) rate in this heavily pretreated cohort was 40% and 78% for dMMR CRC vs 0% and 11% for pMMR CRC. The OS was not reached for the dMMR CRC cohort. The expanded KEYNOTE-016 study demonstrated an improved response rate, disease control rate, median PFS, and median OS with pembrolizumab when the dMMR CRC group was compared with the pMMR group.[41] The KEYNOTE-177 trial established the superiority of pembrolizumab vs chemotherapy in the first-line metastatic setting.[42] The phase 2 CheckMate 142 trial evaluated the efficacy of single-agent treatment with the PD-1 inhibitor nivolumab (Opdivo, Bristol Myers Squibb) vs a combination of nivolumab and the CTLA-4 inhibitor ipilimumab (Yervoy, Bristol Myers Squibb) in patients with chemotherapy-refractory MSH-H metastatic CRC.[43] This trial demonstrated a response rate of 31% to the single-agent regimen and 55% to the combination regimen. Pembrolizumab and nivolumab were approved in the first-line setting based on these results, with subsequent approval of ipilimumab/nivolumab after progression on regimens containing 5-FU, oxaliplatin, and irinotecan (Figure).

Early-Stage CRC

Colon. The NICOLE study (NCT04123925) evaluated the utility of 2 doses of preoperative nivolumab (Day 1 and Day 15) in patients with locally advanced colon cancer (T3 and T4).[44] Surprisingly, no major pathologic responses were demonstrated in the 3 patients with dMMR tumors, although completed results have not yet been published. The ATOMIC trial (NCT02912559), which has closed to recruitment, is evaluating the impact of the addition of atezolizumab (Tecentriq, Genentech) to standard-of-care adjuvant chemotherapy in patients with resected stage III CRC.[45]

Neoadjuvant Therapy in CRC. In early 2022, Hu and colleagues published the results of their single-center, open-label, noncomparative, randomized phase 2 study evaluating the combination of immunotherapy with the PD-1 inhibitor toripalimab with or without celecoxib.[46] All 34 patients enrolled in the study had an R0 resection (>1-mm resection margin). Furthermore, 15 of 17 patients (88%; 95% CI, 64-99) in the toripalimab/celecoxib group and 11 of 17 patients (65%; 95% CI, 38-86) in the toripalimab monotherapy group had a pathologic complete response (pCR). Later in the same year, 2 studies of immunotherapy alone in the neoadjuvant setting were presented that had the potential to radically alter the treatment of both MSI colon and MSI rectal cancer, and possibly lead to the complete omission of standard

treatment modalities. Chalabi and colleagues initially presented the NICHE study in 2020, demonstrating a 100% pathologic response rate and a 60% pCR rate among 20 patients with dMMR CRC treated with a single dose of ipilimumab and 2 doses of nivolumab before surgery.[47] The expansion of this study was presented at the 2022 European Society for Medical Oncology (ESMO) Congress, demonstrating a 95% major pathologic response among 112 locally advanced dMMR CRCs (cT3 and/or node-positive disease) treated with the same regimen preoperatively.[48] A pCR was noted in 67% of patients in this study.

Subsequently, Ludford and colleagues reported the results of their phase 2 study evaluating pembrolizumab in localized unresectable or high-risk resectable MSI tumors.[49,50] This study included 27 patients with CRC. Among the 17 patients who underwent surgery, the pCR rate was comparable to that of the NICHE studies, at 65%. Xiao and colleagues reported a larger real-world cohort of patients with CRC receiving neoadjuvant immunotherapy (n=73).[51] At the time of publication, 50 patients had undergone surgery, and the pCR rate was 57.1%.

Building on the success of the NICHE-2 study, the NICHE-3[15] study was presented at ESMO 2023. Patients with MSI early-stage CRC received 2 preoperative doses of a LAG-3 inhibitor and a PD-1 inhibitor. A major pathologic response was noted in 89% of patients, with a pCR seen in 79% of patients. All patients proceeded to surgery without delays, and the toxicity profile was manageable.

On the basis of these data, the AZUR2 phase 3 global multicenter trial comparing perioperative dostarlimab with standard-of-care surgery followed by adjuvant chemotherapy for T4N0 or stage III dMMR/MSI CRC is actively recruiting patients.[52]

Rectum. The VOLTAGE-A study evaluated the addition of nivolumab monotherapy after neoadjuvant chemoradiotherapy and before surgical resection, in a small cohort of patients with MSI rectal tumors (n=5) and compared outcomes to a larger cohort of MSS rectal tumors.[53] A pCR rate of 60% (3/5) was demonstrated in the MSI group. Similarly, a US multisite phase 2 trial was designed to evaluate the combination of ipilimumab/nivolumab and short-course radiation in the neoadjuvant treatment of MSI rectal cancer (NCT04751370). The UNION study from China, results from which were presented at ESMO 2023, evaluated the addition of the PD-1 inhibitor camrelizumab to chemotherapy after short-course radiotherapy.[54] It is critical to note that these studies, as well as others, are primarily focused on assessing the incorporation of ICB into standard treatment rather than the exclusion of conventional treatment modalities.

A New Paradigm

Cercek and colleagues initiated a prospective phase 2 study in which the PD-1 inhibitor dostarlimab (Jemperli, GSK) was administered every 3 weeks for 6 months in patients with dMMR/MSI rectal cancer.[55] All the patients had a clinical CR, which was a composite of endoscopic, histologic (biopsies only), and radiologic findings. No patients have required salvage radiotherapy or surgery. The study is ongoing, with updated data presented at the 2023 Japanese Society of Medical Oncology Annual Meeting demonstrating a continued 100% CR in 23 patients who completed 6 months of dostarlimab. The AZUR1 global multicenter trial is ongoing to validate the initial MSKCC trial's findings.[56]

Taken together, these data from NICHE-2,[48] NICHE-3,[15] the trial by Cercek and colleagues,[47] and the trial by Ludford and colleagues[49] offer a rationale for a potential nonoperative approach in a biomarker-selected cohort of patients with rectal cancer with the omission of conventional treatment modalities, including radiotherapy and surgery.

Esophagogastric Cancers

dMMR/MSI status has been reported in anywhere up to 12% of gastric cancers.[57,58] Similar to MSI status in CRC, it is associated with better OS in locally advanced disease despite a poorer pathologic response to chemotherapy.[59] In both the MAGIC[60] and CLASSIC[61] clinical trials, dMMR/MSI status was determined to be a favorable prognostic factor but a potentially negative predictive factor for receipt of perioperative chemotherapy in patients with localized, resectable gastric cancer. Furthermore, a meta-analysis of 4 randomized controlled trials that evaluated the prognostic value of MSI status, namely MAGIC, CLASSIC, ARTIST, and ITACA-S, determined that MSI/dMMR status was associated with better OS and disease-free survival (DFS) outcomes at 5 years compared with MSS/pMMR status (77.5% vs 59.3% and 71.8% vs 52.3%, respectively).[57] Again, this supported the finding that dMMR/MSI gastric cancer is biologically distinct from MSS disease. MAGIC, which established perioperative chemotherapy as a standard for gastric cancer, found that patients with MSI status had a favorable prognosis vs those with MSS status in the surgery-only treatment arm (median OS not reached vs 20.3 months respectively), but less favorable survival outcomes in the chemotherapy-plus-surgery arm, with a median OS of 9.6 months in those with dMMR/MSI tumors vs 22.5 months in those with pMMR/MSS tumors,[62] indicating that patients with MSI disease are poorly served by receipt of perioperative therapy in resectable gastric cancer. The CLASSIC trial, a phase 3 study of 1035 Asian patients with resectable

(stage II-IIIb) gastric cancer, demonstrated a lack of 5-year survival benefit from adjuvant capecitabine and oxaliplatin in those with MSI status.[63]

In the metastatic setting, the CheckMate 649 clinical trial randomized patients to receive FOLFOX or capecitabine/oxaliplatin (XELOX; n=833), FOLFOX or XELOX plus nivolumab (n=789), or ipilimumab plus nivolumab (N=409).[64] This trial demonstrated a significant improvement in OS across all patients with the addition of nivolumab (13.8 vs 11.6 months; HR, 0.79), and led to the approval of first-line therapy with immunotherapy plus chemotherapy for patients in the metastatic setting. In contrast, the analysis of chemotherapy vs ipilimumab/nivolumab did not demonstrate a significant survival difference for the whole study population (11.9 vs 11.7 months; HR, 0.91). However, subgroup analysis of patients with dMMR/MSI tumors demonstrated a marked improvement in the ORR among those who received dual ICB when compared with chemotherapy; additionally, among the patients with MSI tumors, an OS of 38.7 months was noted in the chemotherapy/nivolumab group vs 12.3 months in the chemotherapy-only group (HR, 0.38). Furthermore, patients with dMMR/MSI tumors receiving ipilimumab and nivolumab had a survival advantage, with OS not reached vs 10.0 months in the ipilimumab/nivolumab arm vs the chemotherapy-only arm, respectively (HR, 0.28). Although acknowledging that these analyses included small numbers of patients with dMMR tumors (n=21, chemotherapy alone; n=23, chemotherapy plus immunotherapy; n=11, ipilimumab plus nivolumab), they again highlight the predictive significance of microsatellite status for consideration of immune checkpoint inhibitors in this biomarker-defined subgroup.

Both the KEYNOTE-585[65] and MATTERHORN[66] studies were designed to evaluate the addition of immunotherapy to perioperative chemotherapy for resectable gastric/gastroesophageal junction (GEJ) tumors. Patients in KEYNOTE-585 were randomized to receive pembrolizumab in conjunction with perioperative cisplatin and 5-FU, and patients in the MATTERHORN study were randomized to receive durvalumab (Imfinzi, AstraZeneca) and perioperative chemotherapy with 5-FU, leucovorin, oxaliplatin, and docetaxel (FLOT). Provisional analyses of both trials reported in June 2023 demonstrated a statistically significant improvement in pCR with the addition of immunotherapy. Unfortunately, an updated analysis of the KEYNOTE-585 data presented at ESMO 2023 demonstrated that despite a nonsignificant improvement in event-free survival, this was not associated with improved OS. Event-free survival and OS analyses are ongoing in the MATTERHORN trial. Analyses of these study cohorts by MSI status will be critical in defining which patients are most likely to benefit from this strategy.

The potential for a neoadjuvant ICB alone for patients with localized gastric or GEJ dMMR/MSI tumors has been supported by the French GERCOR NEONIPIGA trial.[67] This phase 2 study evaluated neoadjuvant ipilimumab at 1 mg/kg once every 6 weeks for 2 cycles, plus nivolumab at 240 mg flat dose every 2 weeks for 6 cycles, followed by surgery and adjuvant nivolumab at 480 mg every 4 weeks in patients with locally advanced gastric/GEJ adenocarcinoma. Of the 32 patients enrolled, 29 underwent resection, which was R0 in all patients. The pCR rate was 58.6%. In addition, the 3 patients who did not have surgery all had complete endoluminal responses with tumor-free biopsies and normal imaging. These results compare favorably with the pCR rate observed in a biomarker-unselected cohort of patients who received neoadjuvant standard-of-care chemotherapy with FLOT for locally advanced gastric cancer, where pCR rates are in the order of 7% to 20%.[18] The aforementioned prospective study by Ludford and colleagues that evaluated neoadjuvant pembrolizumab in dMMR/MSI tumors included only 1 patient with gastric cancer, in whom a clinical CR was observed as the best response.[50]

The prevalence of dMMR/MSI status in esophageal tumors is reported to be less than 2% in most series,[68,69] although one series reported a prevalence of MSI status in 6.5% (5 of 76) of Barrett esophagus–associated adenocarcinomas.[70] dMMR/MSI status has been less well defined in esophageal squamous cell carcinoma, although cases have been reported in some small series.[71,72]

In the adjuvant setting, the CheckMate 577 trial, which was unselected for MSI status, demonstrated that the addition of adjuvant nivolumab in patients with esophageal cancer with residual disease after neoadjuvant chemoradiotherapy was associated with a statistically significant improvement in DFS. The recently presented results of the VESTIGE study evaluating adjuvant combination immunotherapy following preoperative chemotherapy in patients with gastric/GEJ/lower esophageal tumors were negative for an improvement in DFS (NCT03443856).[73]

Most ongoing trials in esophageal and gastric/GEJ cancers are evaluating the utility of immune checkpoint inhibitors in combination with chemotherapy or radiotherapy and are unselected by MSI status.

Pancreatic Cancer

dMMR/MSI status is present in approximately 1% to 2% of cases of pancreatic cancer.[74] Some series suggest that MSI pancreatic cancers have a more favorable prognosis than their MSS counterparts[75]; however, their response to

ICB has traditionally appeared to be more modest than that observed in other MSI GI cancers. Several reasons for this are postulated, including a low tumor mutational burden (TMB)[76] and a paucity of neoantigens.[77] Le and colleagues demonstrated the efficacy of PD-1 blockade in dMMR/MSI tumors, including 8 patients with pancreatic cancer.[5,6] An ORR of 62% and a disease control rate of 75% were observed, including 2 CRs, 3 partial responses (PRs), and 1 case of stable disease. In stage IV disease, the KEYNOTE-158 basket trial evaluated pembrolizumab in patients with MSI non-CRCs.[78,79] This study enrolled 351 patients of varying primary tumor types, including 22 patients with pancreatic cancer. An ORR of 18.2% was observed in the pancreatic cancer group, including 1 CR and 3 PRs. The median PFS was 2.1 months (1.9-3.4) and the median OS was 4 months (2.1-9.8). Although the response rates in this group were less favorable than those observed in other groups, it is notable that the duration of response at the time of data cut-off (37.5 months from the time of the first dose of pembrolizumab) was not reached, indicating that durable responses are achieved in a subset of patients.

The prospective study by Ludford and colleagues of neoadjuvant pembrolizumab in 35 dMMR/MSI tumors included 2 patients with pancreatic cancer.[49] Both patients achieved stable disease as the best response, neither patient achieved a pCR, and adaptive progression was identified in both. Most recently, Coston and colleagues reported on the outcomes of 32 patients with dMMR/MSI pancreatic cancer.[80] Of the 16 patients with nonmetastatic disease who underwent locoregional management (resection and adjuvant therapy or definitive chemoradiation), the recurrence rate at a median follow-up of 25 months was notably low, at 19%. This included 6 patients who received perioperative immunotherapy, including 1 who received neoadjuvant immunotherapy alone, 1 who received adjuvant immunotherapy alone, and 1 who received immunotherapy both before and after resection. Of the 2 patients who received neoadjuvant immunotherapy and proceeded with resection, both had a pCR. In the metastatic setting, an ORR to immunotherapy of 75% was observed, including a CR rate of 20% and a disease control rate of 60%. The median PFS was not reached. In contrast, responses to cytotoxic chemotherapy were comparatively modest, with an ORR of 30% and a disease control rate of 60%. The remaining data on pancreatic cancer rely predominantly on case reports. In one case, a patient who received pembrolizumab for dMMR locally advanced unresectable pancreatic cancer experienced shrinkage of the tumor that made it eligible for resection. Although the patient declined resection, a durable response was observed 1 year after discontinuation of pembrolizumab.[81]

Biliary Tract Cancers

The proportion of patients with dMMR/MSI biliary tract cancers is approximately 2%,[82] with a higher frequency in intrahepatic and extrahepatic cholangiocarcinoma compared with gallbladder adenocarcinoma.[83,84] The TOPAZ-1 trial established cisplatin, gemcitabine, and durvalumab as a standard of care in unselected advanced biliary tract cancers.[85] Notably, MSI status was available for only 50% of patients enrolled. Of the 333 patients enrolled, 5 (1.5%) were identified as having dMMR/MSI tumors.

Regarding ICB monotherapy, 1 patient with biliary tract cancer was included in Le and colleagues' landmark paper underpinning the role of pembrolizumab in dMMR/MSI tumors.[6] The best response in this patient was a PR.[6] Furthermore, 22 patients with biliary tract cancers were enrolled in the KEYNOTE-158 trial.[8,79] Subgroup analysis demonstrated an ORR of 40.9%, with a median PFS of 4.2 months (95% CI, 2.1 to not reached) and a median OS of 24.3 months. Notably, CRs were observed in 3 patients and PRs in 6 patients. These data support a role for ICB in locally advanced MSI biliary tract cancers, although prospective data are lacking.

Hepatocellular Carcinoma

Data on the prevalence of dMMR/MSI status in hepatocellular carcinoma (HCC) are limited. One large analysis based on data from The Cancer Genome Atlas demonstrated a prevalence of 2.9%,[86] which is consistent across datasets.[87] The incorporation of ICB into the treatment paradigm of unresectable HCC is now well established in several different contexts, including in combination with the anti–vascular endothelial growth factor inhibitor bevacizumab,[88] in combination with transarterial chemoembolization with bevacizumab in the EMERALD-1 study,[89,90] and as dual immune checkpoint inhibitor therapy with durvalumab plus a single dose of tremelimumab in the HIMALAYA trial.[91] In general, studies evaluating ICB in HCC do not stratify by MMR/MSI status. A small number of case studies describe remarkable responses to ICB in locally advanced, MSI hepatocellular carcinoma[87,92]; however, both prospective and retrospective data are lacking.[82,92]

We have identified 2 actively recruiting trials that are evaluating neoadjuvant ICB in HCC and are not specific for dMMR/MSI status (NCT04658147, NCT04123379).

Small Bowel Cancer

Data regarding ICB in small bowel cancer are limited,

which is unsurprising given its relative rarity.[93] MSI status and high TMB appear to be enriched in small bowel adenocarcinoma.[94] Furthermore, 38.5% of cases of dMMR small bowel cancer arise owing to an underlying germline variant in the DNA mismatch repair genes. The risk of recurrence in early-stage disease is lower in dMMR small bowel tumors than in pMMR small bowel tumors.[95] Notably, PD-L1 expression, which has been used as a potential biomarker for the efficacy of immunotherapy, has been demonstrated to vary by disease location within the small bowel.[96]

Two small bowel cancers were included in the original study of pembrolizumab in metastatic noncolorectal dMMR tumors by Le and colleagues, and the OS in this noncolorectal cohort was not reached.[6] The phase 2 ZEBRA trial looked specifically at the efficacy of pembrolizumab in advanced small bowel cancers treated in the second-line setting.[97] Two of 4 patients with MSI disease achieved a PR. In another small study evaluating avelumab (Bavencio, EMD Serono/Pfizer), 1 of 8 patients was found to have MSI disease and responded to avelumab for 18 months.[98] Similarly, 2 patients with localized duodenal adenocarcinoma were included in a study of neoadjuvant pembrolizumab by Ludford and colleagues.[50] Neither of these patients had undergone surgery at the time of publication. One patient had a complete radiologic response as the best response, and the other achieved stable disease with subsequent progression.

Anal Cancer

Anal cancer accounts for less than 3% of GI malignancies, but the incidence is rising.[99] Between 80% and 90% of patients with squamous anal cancer are positive for human papillomavirus, which is associated with increased tumor immunogenicity.[100] In the metastatic setting, efficacy has been demonstrated for both nivolumab and pembrolizumab in refractory anal cancer, although PD-L1 status rather than microsatellite instability status has typically been used to stratify patients. Morris and colleagues reported a response rate of 24% in 37 patients, including 2 CRs and 7 PRs.[101] Ott and colleagues reported a comparable response rate of 17% among patients with squamous histology (n=24; 4 PRs, 10 cases of stable disease).[102] The KEYNOTE-158 study included 112 patients with metastatic and/or unresectable squamous cell anal cancer.[8] MSI status is not available for this cohort, but the response rate was reported as 15% among patients with PD-L1–positive disease. In the setting of localized anal cancer, data are more limited. A phase 3 study from the National Cancer Institute was designed to explore the impact of the addition of nivolumab post-completion of definitive chemoradiotherapy with the primary endpoint of DFS (NCT03233711). This trial has closed to accrual and results are awaited. In a similar approach, the RADIANCE trial was designed to evaluate the effect of the addition of durvalumab to standard-of-care chemoradiotherapy; this trial has recently closed to accrual.[103]

Future Challenges

The unprecedented success of immunotherapy across the spectrum of MSI metastatic GI malignancies has led to the exploration of its utility in the locally advanced disease setting.[16] Numerous ongoing trials are exploring the use of immunotherapy in both the neoadjuvant and adjuvant settings. The potential for nonoperative management of a subset of MSI locally advanced GI malignancies is provocative and may emerge as a novel standard of care.[47,55]

Although a clear precedent has been established for the omission of surgery in the management of MSS rectal cancer,[22] and this precedent can be applied to the MSI setting, surveillance of the colon in the event of nonoperative management is challenging. Additionally, radiologic interpretation of CRs in the absence of routine use of MRI may necessitate the development of clear predefined radiologic parameters and potential integration of other modalities, such as circulating tumor DNA. An additional consideration moving forward will be defining the most appropriate disease-specific endpoints for neoadjuvant studies in patients with dMMR/MSI locally advanced tumors. The appropriate endpoints may differ for a tumor where nonoperative management is desired (eg, rectal cancer) vs where organ preservation is less of a priority for patient quality of life (eg, colon cancer), which will not necessitate a long-term colostomy.

In gastric and GEJ tumors, the data are indicative of MSI/dMMR status as a predictor of poor responses to 5-FU–based chemotherapy. Future studies will focus on whether ICB alone or in combination with chemotherapy will better serve patients with this disease. However, esophageal tumor heterogeneity and the apparent necessity of incorporation rather than omission of chemotherapy based on current data will lead to additive toxicity, both on an individual patient level and from a financial toxicity perspective. The lack of OS advantage conferred with the addition of pembrolizumab to perioperative chemotherapy in the KEYNOTE-585 study, despite higher pCR rates in this cohort, is disappointing. Still, the relative proportion of MSI patients in this study requires evaluation.

Specific to pancreatic cancer, the challenge of small tumor content makes accurate analyses of tissue challenging. This challenge may be even greater in locally advanced disease, where tissue samples are often dependent on tumor acquisition at the time of endoscopic ultrasound,

and tumor content may be low. A recent study by Coston and colleagues demonstrated a discrepancy rate between MMR and MSI testing methods in 26% of evaluable cases, highlighting this limitation and the necessity of orthogonal testing in this disease.[80]

Although the College of American Pathologists guidelines for determination of MSI status to determine eligibility for immune checkpoint inhibitor therapy are prescriptive for endometrial, colorectal, gastric/GEJ, and small bowel cancers, no such standard testing modality has been established for identifying patients with pancreatic tumors likely to benefit from treatment with ICB.[104]

Additionally, in tumors with low rates of dMMR/MSI status where ICB has become an important component of standard therapy in unselected populations, such as HCC and biliary tract cancers, future studies may focus on stratifying patients by their MMR/MSI status to definitively determine response rates to ICB, whether administered alone or in combination.

Refinement of patient selection to identify patients most likely to benefit from exposure to ICB in the locally advanced disease setting is critical. Importantly, not all trials in the setting of GI cancer were stratified according to MSI status. Biomarkers beyond MSI[105] will be required to further refine patient selection. The pan-cancer approval of pembrolizumab in 2017 was predicated on the identification of MSI status of the tumor, a designation that varies depending on the testing method used.[106] The optimal method of MSI status determination is not clearly defined, with orthogonal testing encouraged.[104] Retrospective analysis of TMB suggested a predictive role in relation to ICB efficacy.[107,108] The KEYNOTE-158 study led to the approval of TMB as a biomarker for patient selection for treatment, with a TMB of 10 or more mutations per megabase being designated as TMB-high.[109] However, what is increasingly becoming clear is that the spectrum of benefit derived from exposure to ICB in hypermutated tumors varies,[110] and that the influence of TMB is lost when other factors such as MMR status or the presence of pathogenic mutations in polymerase ε (*POLE*) or polymerase δ1 (*POLD1*) are used to stratify study cohorts. Furthermore, the optimal cutoff that is predictive of response to immune checkpoint blockage may vary by tumor type.[111] Similarly, PD-L1 expression has also been employed as a predictive biomarker, but in isolation is an imperfect biomarker.[112]

Although the data herein demonstrate a clear role for ICB in dMMR/MSI GI tumors, resistance to ICB is evident in a proportion of patients. Several patient- and tumor-related factors are also thought to influence outcomes. Regarding patient factors, the influence of sex on the effectiveness of ICB has been described in several studies, with some contrasting outcomes reported in different series.[113,114] Specific to dMMR/MSI tumors, a limited number of studies have addressed gender-based differences. One study of resectable dMMR/MSI gastric cancer demonstrated gender-based prognostic differences, with the favorable prognosis typically associated with MSI status only observed in female patients.[115] Whether the magnitude of benefit from ICB differs between males and females with dMMR/MSI cancers remains unknown, however. With respect to tumor-related factors, several recent studies have indicated that in MSI/dMMR CRC, hepatic metastatic disease confers a poor OS after ICB treatment compared with nonhepatic sites of metastasis.[116] The resistance mechanisms underlying this phenomenon have not yet been fully characterized. Whether there are similar clinical factors in the early-disease setting that are predictive of poorer response is not yet fully defined.

Chalabi and colleagues demonstrated in an exploratory analysis of patients who achieved a pCR in the NICHE-2 study that patients with Lynch syndrome–associated tumors had a higher rate of pCR in response to dual ICB compared with patients with sporadic (non-Lynch) colorectal tumors (78% vs 58%; P=.056).[48] This finding again demonstrated that certain tumor-specific features may affect the response to ICB. Differential responses to ICB among patients with Lynch syndrome–associated tumors vs sporadic tumors had not been reported in the prior pivotal studies in the metastatic disease setting, and ongoing validation of this finding is critical. Additionally, responses may vary by the underlying Lynch syndrome germline variant,[117] which may be an important factor to integrate into clinical trial design.

Furthermore, consideration of the co-mutation profile of dMMR/MSI tumors may also be important in predicting the response to ICB. In the setting of sporadic metastatic CRC, *BRAF* V600E mutation status may influence responsiveness to ICB,[43,118] and the analysis of the influence of *BRAF* status will be important in the neoadjuvant and perioperative settings. Similarly, *ARID1A* alterations may be important. In one large cohort, among 9 tumor types with at least a 5% prevalence of *ARID1A* alterations, MSI status, and high TMB were more frequently observed in *ARID1A*-altered vs *ARID1A* wild-type tumors (P<.001).[119] The median PFS after ICB was 11 months in *ARID1A*-altered tumors compared with 4 months in *ARID1A* wild-type tumors (P=.006). Multivariate analysis demonstrated that the PFS benefit observed in *ARID1A*-altered tumors was independent of MSI status. These patient- and tumor-related factors are important considerations, particularly in the setting of locally advanced dMMR/MSI tumors, where the ultimate cure remains the key endpoint. Future trials may incorporate stratification of patients based on such features.

There are multiple unresolved questions regarding the use of immunotherapy in the setting of locally

advanced GI cancers. Critically, predictors of resistance to immunotherapy require further exploration.[120] Despite unprecedented responses seen with neoadjuvant ICB in clinical studies by both Cercek and colleagues and Chalabi and colleagues, there are still patients who do not respond. The pCR rate in the NICHE-2 and NICHE-3 studies was not 100%. Differential response by tumor site of origin may be important, and our experience of ICB in the metastatic setting necessitates caution as we await confirmation of the durability of responses. These studies were conducted in small biomarker-defined cohorts, and longer-term follow-up is required. Additionally, the optimal neoadjuvant regimen and the appropriate duration of treatment have not yet been established.

Nonetheless, current data underline the importance of MSI as both a prognostic and predictive biomarker and necessitate the inclusion of microsatellite status as a stratification factor in future trial design in locally advanced GI cancers.

Disclosures

Dr Harrold has served as a consultant to Pfizer Ireland and has received educational grants from Merck and Amgen. Dr Keane has no disclosures. Dr Cercek has received research funding from GSK and Seagen/Pfizer and has served as an advisor to Bayer, Merck, GSK, Roche, Seagen/Pfizer, Amgen, AbbVie, and Agenus.

References

1. de la Chapelle A, Hampel H. Clinical relevance of microsatellite instability in colorectal cancer. *J Clin Oncol*. 2010;28(20):3380-3387.
2. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. *Nat Rev Clin Oncol*. 2010;7(3):153-162.
3. Ellegren H. Microsatellites: simple sequences with complex evolution. *Nat Rev Genet*. 2004;5(6):435-445.
4. Maruvka YE, Mouw KW, Karlic R, et al. Analysis of somatic microsatellite indels identifies driver events in human tumors. *Nat Biotechnol*. 2017;35(10):951-959.
5. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509-2520.
6. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409-413.
7. Diaz L, Marabelle A, Kim T, et al. Efficacy of pembrolizumab in phase 2 KEYNOTE-164 and KEYNOTE-158 studies of microsatellite instability high cancers. *Ann Oncol*. 2017;28:v128-v129.
8. Marabelle A, Le DT, Ascierto PA, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair–deficient cancer: results from the phase II KEYNOTE-158 study. *J Clin Oncol*. 2020;38(1):1-10.
9. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. *Annu Rev Immunol*. 2001;19(1):565-594.
10. Baumeister SH, Freeman GJ, Dranoff G, Sharpe AH. Coinhibitory pathways in immunotherapy for cancer. *Annu Rev Immunol*. 2016;34(1):539-573.
11. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359(6382):1350-1355.
12. He Y, Rivard CJ, Rozeboom L, et al. Lymphocyte-activation gene-3, an important immune checkpoint in cancer. *Cancer Sci*. 2016;107(9):1193-1197.
13. Woo S-R, Turnis ME, Goldberg MV, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012;72(4):917-927.
14. Tawbi HA, Schadendorf D, Lipson EJ, et al; RELATIVITY-047 Investigators. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N Engl J Med*. 2022;386(1):24-34.
15. Verschoor Y, van den Berg J, Balduzzi S, et al. LBA31 Neoadjuvant nivolumab plus relatlimab (anti-LAG3) in locally advanced MMR-deficient colon cancers: the NICHE-3 study [ESMO abstract LBA31]. *Ann Oncol*. 2023;34(2)(suppl).
16. Petricevic B, Kabiljo J, Zirnbauer R, Walczak H, Laengle J, Bergmann M. Neoadjuvant immunotherapy in gastrointestinal cancers – the new standard of care? *Semin Cancer Biol*. 2022;86(2):834-850.
17. Liu J, O'Donnell JS, Yan J, et al. Timing of neoadjuvant immunotherapy in relation to surgery is crucial for outcome. *OncoImmunology*. 2019;8(5):e1581530.
18. Al-Batran S-E, Homann N, Pauligk C, et al; FLOT4-AIO Investigators. Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): a randomised, phase 2/3 trial. *Lancet*. 2019;393(10184):1948-1957.
19. Cedermark B, Dahlberg M, Glimelius B, Påhlman L, Rutqvist LE, Wilking N; Swedish Rectal Cancer Trial. Improved survival with preoperative radiotherapy in resectable rectal cancer. *N Engl J Med*. 1997;336(14):980-987.
20. Bosset J-F, Calais G, Mineur L, et al. Enhanced tumorocidal effect of chemotherapy with preoperative radiotherapy for rectal cancer: preliminary results—EORTC 22921. *J Clin Oncol*. 2005;23(24):5620-5627.
21. Cammà C, Giunta M, Fiorica F, Pagliaro L, Craxì A, Cottone M. Preoperative radiotherapy for resectable rectal cancer: a meta-analysis. *JAMA*. 2000;284(8):1008-1015.
22. Garcia-Aguilar J, Patil S, Gollub MJ, et al. Organ preservation in patients with rectal adenocarcinoma treated with total neoadjuvant therapy. *J Clin Oncol*. 2022;40(23):2546-2556.
23. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet*. 2000;355(9217):1745-1750.
24. Benatti P, Gafà R, Barana D, et al. Microsatellite instability and colorectal cancer prognosis. *Clin Cancer Res*. 2005;11(23):8332-8340.
25. Aggarwal N, Quaglia A, McPhail MJW, Monahan KJ. Systematic review and meta-analysis of tumour microsatellite-instability status as a predictor of response to fluorouracil-based adjuvant chemotherapy in colorectal cancer. *Int J Colorectal Dis*. 2022;37(1):35-46.
26. Guastadisegni C, Colafranceschi M, Ottini L, Dogliotti E. Microsatellite instability as a marker of prognosis and response to therapy: a meta-analysis of colorectal cancer survival data. *Eur J Cancer*. 2010;46(15):2788-2798.
27. Webber EM, Kauffman TL, O'Connor E, Goddard KA. Systematic review of the predictive effect of MSI status in colorectal cancer patients undergoing 5FU-based chemotherapy. *BMC Cancer*. 2015;15(1):156.
28. Zaanan A, Cuilliere-Dartigues P, Guilloux A, et al. Impact of *p53* expression and microsatellite instability on stage III colon cancer disease-free survival in patients treated by 5-fluorouracil and leucovorin with or without oxaliplatin. *Ann Oncol*. 2010;21(4):772-780.
29. Cercek A, Dos Santos Fernandes G, Roxburgh CS, et al. Mismatch repair–deficient rectal cancer and resistance to neoadjuvant chemotherapy. *Clin Cancer Res*. 2020;26(13):3271-3279.
30. Hasan S, Renz P, Wegner RE, et al. Microsatellite instability (MSI) as an independent predictor of pathologic complete response (PCR) in locally advanced rectal cancer: a National Cancer Database (NCDB) analysis. *Ann Surg*. 2020;271(4):716-723.
31. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology*. 2010;138(6):2073-2087.e3.
32. Battaglin F, Naseem M, Lenz H-J, Salem ME. Microsatellite instability in colorectal cancer: overview of its clinical significance and novel perspectives. *Clin Adv Hematol Oncol*. 2018;16(11):735-745.
33. Latham A, Srinivasan P, Kemel Y, et al. Microsatellite instability is associated with the presence of Lynch syndrome pan-cancer. *J Clin Oncol*. 2019;37(4):286-295.
34. Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. *Nat Rev Cancer*. 2017;17(2):79-92.
35. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol*. 2005;23(3):609-618.
36. Jin Z, Sinicrope FA. Prognostic and predictive values of mismatch repair deficiency in non-metastatic colorectal cancer. *Cancers (Basel)*. 2021;13(2):300.
37. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a pre-

dictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol*. 2010;28(20):3219-3226.
38. Sargent DJ, Shi Q, Yothers G, et al. Prognostic impact of deficient mismatch repair (dMMR) in 7,803 stage II/III colon cancer (CC) patients (pts): a pooled individual pt data analysis of 17 adjuvant trials in the ACCENT database [ASCO abstract 3507]. 2014;32(15)(suppl).
39. Morton D, Seymour M, Magill L, et al; FOxTROT Collaborative Group. Preoperative chemotherapy for operable colon cancer: mature results of an international randomized controlled trial. *J Clin Oncol*. 2023;41(8):1541-1552.
40. Morton D. FOxTROT: an international randomised controlled trial in 1053 patients evaluating neoadjuvant chemotherapy (NAC) for colon cancer [ESMO abstract 5561]. *Ann Oncol*. 2019;30(5)(suppl).
41. Le DT, Uram JN, Wang H, et al. Programmed death-1 blockade in mismatch repair deficient colorectal cancer [ASCO abstract 103]. *J Clin Oncol*. 2016;34(15)(suppl).
42. Andre T, Shiu K-K, Kim TW, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high/mismatch repair deficient metastatic colorectal cancer: the phase 3 KEYNOTE-177 study [ASCO abstract LBA4]. *J Clin Oncol*. 2020;38(18)(suppl).
43. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017;18(9):1182-1191.
44. Avallone A, De Stefano A, Pace U, et al. 491P Neoadjuvant nivolumab in early stage colorectal cancer [ESMO abstract 491P]. *Ann Oncol*. 2020;(31)(4)(suppl).
45. Sinicrope FA, Ou F-S, Zemla T, et al. Randomized trial of standard chemotherapy alone or combined with atezolizumab as adjuvant therapy for patients with stage III colon cancer and deficient mismatch repair (ATOMIC, Alliance A021502). [ASCO abstract e15169]. *J Clin Oncol.* 2019; 37(15)(suppl).
46. Hu H, Kang L, Zhang J, et al. Neoadjuvant PD-1 blockade with toripalimab, with or without celecoxib, in mismatch repair-deficient or microsatellite instability-high, locally advanced, colorectal cancer (PICC): a single-centre, parallel-group, non-comparative, randomised, phase 2 trial. *Lancet Gastroenterol Hepatol*. 2022;7(1):38-48.
47. Chalabi M, Fanchi LF, Dijkstra KK, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med*. 2020;26(4):566-576.
48. Chalabi M, Verschoor YL, van den Berg J, et al. Neoadjuvant immune checkpoint inhibition in locally advanced MMR-deficient colon cancer: the NICHE-2 study [ESMO abstract LBA7]. *Ann Oncol*. 2022;33(7)(suppl).
49. Ludford K, Ho WJ, Thomas JV, et al. Neoadjuvant pembrolizumab in localized microsatellite instability high/deficient mismatch repair solid tumors. *J Clin Oncol*. 2023;41(12):2181-2190.
50. Ludford K, Ho WJ, Thomas JV, et al. Neoadjuvant pembrolizumab in localized microsatellite instability high/deficient mismatch repair solid tumors. *J Clin Oncol*. 2023;41(12):2181-2190.
51. Xiao B-Y, Zhang X, Cao T-Y, et al. Neoadjuvant immunotherapy leads to major response and low recurrence in localized mismatch repair–deficient colorectal cancer. *J Natl Compr Canc Netw*. 2023;21(1):60-66.e5.
52. Starling N, Neumann K, Colwell B, et al. AZUR-2, a phase III, open-label, randomized study of perioperative dostarlimab monotherapy vs standard of care in previously untreated patients with T4N0 or stage III dMMR/MSI-H resectable colon cancer [ASCO abstract TPS240]. *J Clin Oncol*. 2024;42(3)(suppl).
53. Yuki S, Bando H, Tsukada Y, et al. Short-term results of VOLTAGE-A: nivolumab monotherapy and subsequent radical surgery following preoperative chemoradiotherapy in patients with microsatellite stable and microsatellite instability-high locally advanced rectal cancer [ASCO abstract 4100]. *J Clin Oncol*. 2020;38(15)(suppl).
54. Zhang T, Tao K, Lin Z, et al. Neoadjuvant short-course radiotherapy followed by camrelizumab plus chemotherapy versus long-course chemoradiotherapy followed by chemotherapy in locally advanced rectal cancer: a randomized phase III trial (UNION) [ESMO abstract LBA25]. *Ann Oncol*. 2023;34(2)(suppl).
55. Cercek A, Lumish M, Sinopoli J, et al. PD-1 blockade in mismatch repair–deficient, locally advanced rectal cancer. *N Engl J Med*. 2022;386(25):2363-2376.
56. Cercek A, Bachet J-B, Capdevila J, et al. Phase II, single-arm, open-label study of dostarlimab monotherapy in previously untreated patients with stage II/III dMMR/MSI-H locally advanced rectal cancer [ASCO abstract TPS3639]. *J Clin Oncol*. 2023;41(16)(suppl).
57. Pietrantonio F, Miceli R, Raimondi A, et al. Individual patient data meta-analysis of the value of microsatellite instability as a biomarker in gastric cancer. *J Clin Oncol*. 2019;37(35):3392-3400.
58. Tran-Minh M-L, Lehmann-Che J, Lambert J, et al; for NORDICAP. Prevalence and prognosis of microsatellite instability in oesogastric adenocarcinoma, NORDICAP 16-01. *Clin Res Hepatol Gastroenterol*. 2021;45(4):101691.
59. Vos EL, Maron SB, Krell RW, et al. Survival of locally advanced MSI-high gastric cancer patients treated with perioperative chemotherapy: a retrospective cohort study. *Ann Surg*. 2023;227(5):798-805.
60. Cunningham D, Allum WH, Stenning SP, et al; MAGIC Trial Participants. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med*. 2006;355(1):11-20.
61. Bang Y-J, Kim Y-W, Yang H-K, et al; CLASSIC trial investigators. Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): a phase 3 open-label, randomised controlled trial. *Lancet*. 2012;379(9813):315-321.
62. Smyth EC, Wotherspoon A, Peckitt C, et al. Mismatch repair deficiency, microsatellite instability, and survival: an exploratory analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial. *JAMA Oncol*. 2017;3(9):1197-1203.
63. Choi YY, Kim H, Shin S-J, et al. Microsatellite instability and programmed cell death-ligand 1 expression in stage II/III gastric cancer: post hoc analysis of the CLASSIC randomized controlled study. *Ann Surg*. 2019;270(2):309-316.
64. Janjigian YY, Shitara K, Moehler M, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. *Lancet*. 2021;398(10294):27-40.
65. Bang Y-J, Van Cutsem E, Fuchs CS, et al. Phase 3 KEYNOTE-585 study of chemotherapy (chemo) + pembrolizumab (pembro) vs chemo + placebo as neoadjuvant/adjuvant treatment for patients (pts) with gastric or gastroesophageal junction (G/GEJ) cancer [ESMO abstract 784TiP]. *Ann Oncol*. 2018;29(8)(suppl).
66. Janjigian YY, Van Cutsem E, Muro K, et al. MATTERHORN: phase III study of durvalumab plus FLOT chemotherapy in resectable gastric/gastroesophageal junction cancer. *Future Oncol*. 2022;18(20):2465-2473.
67. André T, Tougeron D, Piessen G, et al. Neoadjuvant nivolumab plus ipilimumab and adjuvant nivolumab in localized deficient mismatch repair/microsatellite instability-high gastric or esophagogastric junction adenocarcinoma: the GERCOR NEONIPIGA phase II study. *J Clin Oncol*. 2023;41(2):255-265.
68. Quaas A, Rehkaemper J, Rueschoff J, et al. Occurrence of high microsatellite-instability/mismatch repair deficiency in nearly 2,000 human adenocarcinomas of the gastrointestinal tract, pancreas, and bile ducts: a study from a large German comprehensive cancer center. *Front Oncol*. 2021;11:569475.
69. The Cancer Genome Atlas Research Network. Integrated genomic characterization of oesophageal carcinoma. *Nature*. 2017;541(7636):169-175.
70. Farris AB III, Demicco EG, Le LP, et al. Clinicopathologic and molecular profiles of microsatellite unstable Barrett esophagus-associated adenocarcinoma. *Am J Surg Pathol*. 2011;35(5):647-655.
71. Kagawa Y, Yoshida K, Hirai T, et al. Microsatellite instability in squamous cell carcinomas and dysplasias of the esophagus. *Anticancer Res*. 2000;20(1A):213-217.
72. Naidoo R, Ramburan A, Reddi A, Chetty R. Aberrations in the mismatch repair genes and the clinical impact on oesophageal squamous carcinomas from a high incidence area in South Africa. *J Clin Pathol*. 2005;58(3):281-284.
73. Smyth E, Knödler M, Giraut A, et al. VESTIGE: adjuvant immunotherapy in patients with resected esophageal, gastroesophageal junction and gastric cancer following preoperative chemotherapy with high risk for recurrence (N+ and/or R1): an open label randomized controlled phase-2-study. *Front Oncol*. 2020;9:1320.
74. Luchini C, Brosens LAA, Wood LD, et al. Comprehensive characterisation of pancreatic ductal adenocarcinoma with microsatellite instability: histology, molecular pathology and clinical implications. *Gut*. 2021;70(1):148-156.
75. Yamamoto H, Itoh F, Nakamura H, et al. Genetic and clinical features of human pancreatic ductal adenocarcinomas with widespread microsatellite instability. *Cancer Res*. 2001;61(7):3139-3144.
76. Evans RA, Diamond MS, Rech AJ, et al. Lack of immunoediting in murine pancreatic cancer reversed with neoantigen. *JCI Insight*. 2016;1(14):e88328.
77. Macherla S, Laks S, Naqash AR, Bulumulle A, Zervos E, Muzaffar M. Emerging role of immune checkpoint blockade in pancreatic cancer. *Int J Mol Sci*. 2018;19(11):3505.
78. Sidaway P. MSI-H: a truly agnostic biomarker? *Nat Rev Clin Oncol*. 2020;17(2):68-68.
79. Maio M, Ascierto PA, Manzyuk L, et al. Pembrolizumab in microsatellite instability high or mismatch repair deficient cancers: updated analysis from the phase II KEYNOTE-158 study. *Ann Oncol*. 2022;33(9):929-938.
80. Coston T, Desai A, Babiker H, et al. Efficacy of immune checkpoint inhibi-

tion and cytotoxic chemotherapy in mismatch repair-deficient and microsatellite instability-high pancreatic cancer: Mayo Clinic experience. *JCO Precis Oncol*. 2023;7(7):e2200706.
81. Cox RE Jr, Mahipal A, Chakrabarti S. A patient with locally advanced mismatch-repair-deficient pancreatic ductal adenocarcinoma successfully treated with neoadjuvant immunotherapy. *Cureus*. 2021;13(4):e14640.
82. Bonneville R, Krook M, Kautto E, et al. Landscape of microsatellite instability across 39 cancer types [published online October 3, 2017]. *JCO Precis Oncol*. doi:10.1200/PO.17.00073.
83. Valle JW, Kelley RK, Nervi B, Oh D-Y, Zhu AX. Biliary tract cancer. *Lancet*. 2021;397(10272):428-444.
84. Goeppert B, Roessler S, Renner M, et al. Low frequency of mismatch repair deficiency in gallbladder cancer. *Diagn Pathol*. 2019;14(1):36.
85. Oh D-Y, He AR, Qin S, et al. A phase 3 randomized, double-blind, placebo-controlled study of durvalumab in combination with gemcitabine plus cisplatin (GemCis) in patients (pts) with advanced biliary tract cancer (BTC): TOPAZ-1 [ASCO GI abstract 378]. *J Clin Oncol*. 2022;40(4)(suppl).
86. Cortes-Ciriano I, Lee S, Park W-Y, Kim T-M, Park PJ. A molecular portrait of microsatellite instability across multiple cancers. *Nat Commun*. 2017;8(1):15180.
87. Kawaoka T, Ando Y, Yamauchi M, et al. Incidence of microsatellite instability-high hepatocellular carcinoma among Japanese patients and response to pembrolizumab. *Hepatol Res*. 2020;50(7):885-888.
88. Finn RS, Qin S, Ikeda M, et al; IMbrave150 Investigators. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med*. 2020;382(20):1894-1905.
89. Sangro B, Kudo M, Qin S, et al. P-347 A phase 3, randomized, double-blind, placebo-controlled study of transarterial chemoembolization combined with durvalumab or durvalumab plus bevacizumab therapy in patients with locoregional hepatocellular carcinoma: EMERALD-1. *Ann Oncol*. 2020;31:S202-S203.
90. Imfinzi plus bevacizumab met primary endpoint for progression-free survival in liver cancer eligible for embolization in EMERALD-1 phase III trial [press release]. Cambridge, UK; AstraZeneca; November 9, 2023.
91. Abou-Alfa GK, Chan SL, Kudo M, et al. Phase 3 randomized, open-label, multicenter study of tremelimumab (T) and durvalumab (D) as first-line therapy in patients (pts) with unresectable hepatocellular carcinoma (uHCC): HIMALAYA [ASCO abstract 379]. *J Clin Oncol*. 2022;40(4)(suppl).
92. Ando Y, Yamauchi M, Suehiro Y, et al. Complete response to pembrolizumab in advanced hepatocellular carcinoma with microsatellite instability. *Clin J Gastroenterol*. 2020;13(5):867-872.
93. Raghav K, Overman MJ. Small bowel adenocarcinomas—existing evidence and evolving paradigms. *Nat Rev Clin Oncol*. 2013;10(9):534-544.
94. Schrock AB, Devoe CE, McWilliams R, et al. Genomic profiling of small-bowel adenocarcinoma. *JAMA Oncol*. 2017;3(11):1546-1553.
95. Latham A, Shia J, Patel Z, et al. Characterization and clinical outcomes of DNA mismatch repair-deficient small bowel adenocarcinoma. *Clin Cancer Res*. 2021;27(5):1429-1437.
96. Pedersen K, Smyrk TC, Harrington S, McWilliams RR. Programmed death-ligand 1 (PD-L1) expression in small bowel adenocarcinomas (SBA) [ASCO GI abstract 3619]. *J Clin Oncol*. 2015;33(15)(suppl).
97. Pedersen KS, Foster NR, Overman MJ, et al. ZEBRA: a multicenter phase II study of pembrolizumab in patients with advanced small-bowel adenocarcinoma. *Clin Cancer Res*. 2021;27(13):3641-3648.
98. Cardin DB, Gilbert J, Whisenant JG, et al. Safety and efficacy of avelumab in small bowel adenocarcinoma. *Clin Colorectal Cancer*. 2022;21(3):236-243.
99. Eng C, Ciombor KK, Cho M, et al. Anal cancer: emerging standards in a rare disease. *J Clin Oncol*. 2022;40(24):2774-2788.
100. Balermpas P, Martin D, Wieland U, et al. Human papilloma virus load and PD-1/PD-L1, CD8$^+$ and FOXP3 in anal cancer patients treated with chemoradiotherapy: rationale for immunotherapy. *OncoImmunology*. 2017;6(3):e1288331.
101. Morris VK, Salem ME, Nimeiri H, et al. Nivolumab for previously treated unresectable metastatic anal cancer (NCI9673): a multicentre, single-arm, phase 2 study. *Lancet Oncol*. 2017;18(4):446-453.
102. Ott PA, Piha-Paul SA, Munster P, et al. Safety and antitumor activity of the anti-PD-1 antibody pembrolizumab in patients with recurrent carcinoma of the anal canal. *Ann Oncol*. 2017;28(5):1036-1041.
103. Martin D, Balermpas P, Gollrad J, et al. RADIANCE - radiochemotherapy with or without durvalumab in the treatment of anal squamous cell carcinoma: a randomized multicenter phase II trial. *Clin Transl Radiat Oncol*. 2020;23:43-49.
104. Bartley AN, Mills AM, Konnick E, et al. Mismatch repair and microsatellite instability testing for immune checkpoint inhibitor therapy: guideline from the College of American pathologists in collaboration with the association for molecular pathology and fight colorectal cancer. *Arch Pathol Lab Med*. 2022;146(10):1194-1210.
105. Kim JH, Kang GH. Molecular and prognostic heterogeneity of microsatellite-unstable colorectal cancer. *World J Gastroenterol*. 2014;20(15):4230-4243.
106. Ziegler J, Hechtman JF, Ptashkin R, et al. MiMSI - a deep multiple instance learning framework improves microsatellite instability detection from tumor next-generation sequencing [preprint]. *bioRxiv*. 2020. doi.org/10.1101/2020.09.16.299925.
107. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014;371(23):2189-2199.
108. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med*. 2017;377(25):2500-2501.
109. Marabelle A, Fakih M, Lopez J, et al. Association of tumour mutational burden with outcomes in patients with select advanced solid tumours treated with pembrolizumab in KEYNOTE-158. *Ann Oncol*. 2019;30:v477-v478.
110. Rousseau B, Foote MB, Maron SB, et al. The spectrum of benefit from checkpoint blockade in hypermutated tumors. *N Engl J Med*. 2021;384(12):1168-1170.
111. McGrail DJ, Pilié PG, Rashid NU, et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. *Ann Oncol*. 2021;32(5):661-672.
112. Sankar K, Ye JC, Li Z, Zheng L, Song W, Hu-Lieskovan S. The role of biomarkers in personalized immunotherapy. *Biomark Res*. 2022;10(1):32.
113. Conforti F, Pala L, Bagnardi V, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol*. 2018;19(6):737-746.
114. Grassadonia A, Sperduti I, Vici P, et al. Effect of gender on the outcome of patients receiving immune checkpoint inhibitors for advanced cancer: a systematic review and meta-analysis of phase III randomized clinical trials. *J Clin Med*. 2018;7(12):542.
115. Quaas A, Biesma HD, Wagner AD, et al. Microsatellite instability and sex differences in resectable gastric cancer - a pooled analysis of three European cohorts. *Eur J Cancer*. 2022;173:95-104.
116. Saberzadeh-Ardestani B, Jones JC, Hubbard JM, et al. Association between survival and metastatic site in mismatch repair-deficient metastatic colorectal cancer treated with first-line pembrolizumab. *JAMA Netw Open*. 2023;6(2):e230400-e230400.
117. Valle L. Lynch syndrome: a single hereditary cancer syndrome or multiple syndromes defined by different mismatch repair genes? *Gastroenterology*. 2023;165(1):20-23.
118. Sahin IH, Goyal S, Pumpalova Y, et al. Mismatch Repair (MMR) gene alteration and BRAF V600E mutation are potential predictive biomarkers of immune checkpoint inhibitors in MMR-deficient colorectal cancer. *Oncologist*. 2021;26(8):668-675.
119. Okamura R, Kato S, Lee S, Jimenez RE, Sicklick JK, Kurzrock R. *ARID1A* alterations function as a biomarker for longer progression-free survival after anti-PD-1/PD-L1 immunotherapy. *J Immunother Cancer*. 2020;8(1):e000438.
120. Huang AC, Orlowski RJ, Xu X, et al. A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma. *Nat Med*. 2019;25(3):454-461.