8-1-2022

# Immune Checkpoint Inhibitors in Luminal Gastrointestinal Malignancies: Going Beyond MSI-H/dMMR, TMB and PD-L1

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**Short running title:** Checkpoint inhibitors in luminal GI malignancies

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**Author contributions:** All authors conceptualized the manuscript. DSL was responsible for writing and editing the text, AES reviewed and edited, and BB wrote, reviewed, and edited the text.

**Financial disclosures:** DSL has no conflicts to disclose. AES is a consultant for Targeted Diagnostics and Therapeutics, Inc. which provided research funding that, in part, supported this work and has a license to commercialize inventions related to this work. AES is supported by the Defense Congressionally Directed Medical Research Programs #W81XWH-19-1-0263), West Pharmaceutical Services, Inc., The Courtney Ann Diacont Memorial Foundation, and Lorraine and David Swoyer. BB reports research funding to institution from Amgen, Inc., Boehringer Ingelheim., Bicycle Therapeutics., KAHR Medical., Syros Pharmaceuticals., Tarveda Therapeutics., all outside the submitted work.

**Information pertaining to writing assistance:** No funded writing assistance was used.

**Ethical disclosure:** This review article did not include any individual patient information and did not require input from an Institutional Review Board. The authors have no ethical disclosures to report.

**Data sharing statement:** This review article did not include any individual patient information. Therefore, there is no process by which data sharing needs delineation.

**Word count:** 6564

**Figure number:** 1

**Table number:** 2

**Abstract**

In luminal gastrointestinal (GI) tumors, immune checkpoint inhibitors (ICIs) targeting PD-1, PD-L1, and CTLA-4 have been investigated in multiple settings. The indications for these drugs are primarily dependent on specific biomarkers that imply immunogenicity: overexpression of PD-L1, tumor mutational burden (TMB), loss of mismatch repair proteins (dMMR) and/or microsatellite instability-high (MSI-H) status. Although these markers can be both predictive and prognostic, there is variability in how they are measured and used to guide therapies. Moreover, the use of ICIs can be further refined with a better understanding of the tumor microenvironment and interactions with other available therapies. The purpose of this review is to characterize luminal GI tumors' responses to ICIs considering known predictive biomarkers and discuss emerging therapeutic approaches using ICIs.

**Lay abstract**: Immune checkpoint inhibitors (ICIs) are medications that help the natural immune system fight cancer cells, preventing their growth. In tumors of the gastrointestinal tract, mounting research has shown that ICIs are useful in treatment regimens. However, this depends on certain characteristics of individual cancers like how many mutations they have, if they are missing certain enzymes, and other considerations. ICIs can also be paired with standard—and non-standard—treatments like chemotherapy, radiation, and other targeted therapy to increase their effectiveness against cancer. This article discusses how ICIs are used in gastrointestinal tract cancers according to the available evidence in the medical literature, and it explores the directions of the research on the forefront of immunotherapy.

**Tweetable abstract:** Immune checkpoint inhibitors have become ubiquitous in the treatment of many #cancers. This review explores their role in luminal GI tumors, from biomarker-driven therapy to novel drug combinations. #ICIs #oncology

**Keywords**: immune checkpoint inhibitors, immunotherapy, esophageal cancer, colorectal cancer, gastric cancer, PD-L1, tumor mutational burden, mismatch repair, microsatellite instability, tumor microenvironment

## Introduction

Immune checkpoint inhibitors (ICIs), as the name implies, refer to immunotherapy drugs that block immune checkpoints. These immune checkpoints represent a natural defense mechanism to prevent autoimmunity. However, tumors often hijack these checkpoints to suppress infiltration of cytotoxic T lymphocytes (CTLs), which serves to avoid tumor immunity and leads to unhindered growth. Notable checkpoints include programmed death-1 (PD-1) and cytotoxic T-lymphocyte associated protein-4 (CTLA-4) expressed on CTLs and PD-L(ligand)-1 expressed on antigen presenting cells and tumor cells. Their overexpression leads to immune suppression and underscore the promising role of ICIs. These cognate proteins represent best understood checkpoints with drugs targeting each, though other checkpoints are quickly entering the clinical space.

The first ICI, ipilimumab, was approved by the U.S. Food and Drug Association (FDA) in 2011.[1] In the decade since, this method of reinvigorating the immune system to target cancer cells has become widely used, incorporating newly developed drugs into the treatment paradigms of many different cancers. In luminal gastrointestinal (GI) tumors, targeting of PD-1 and PD-L1 has achieved particular successes in cancers that express certain biomarkers. The FDA has approved the use of pembrolizumab and nivolumab for specific indications in luminal GI tumors (Figure 1), but further evidence is emerging for related agents such as avelumab, atezolizumab, and others.

Cancer treatment has been moving away from histology-specific cytotoxic chemotherapy and towards tumor-specific molecular and immunologic therapies that promise higher efficacy and fewer off-target effects. In the case of ICIs, three biomarkers are primarily used to predict responses to therapy: high microsatellite instability (MSI-H) or deficient mismatch repair (dMMR), high tumor mutational burden (TMB-H), and PD-L1 expression. In fact, the FDA's first-ever drug approval based solely on a biomarker, independent of tumor location, was for pembrolizumab in patients whose tumors exhibit dMMR or MSI-H.[2] Among luminal GI cancers, ICIs now carry FDA approval for treatment of colorectal cancer (CRC), gastric cancer (GC), and esophageal cancer (EC) in multiple settings—in most cases attendant to these predictive biomarkers (Table 1).

Though these biomarkers are certainly predictive of responses to ICIs in solid tumors, they have prognostic value as well.[3-8] For instance, patients with MSI-H GC and gastroesophageal junction (GEJ) cancers undergoing neoadjuvant chemotherapy enjoy longer overall survival (OS), than their microsatellite stable (MSS) counterparts, even after relapse.[3] Similar trends are seen in patients with stage II and III MSI-H CRC.[4] Additionally, a retrospective review of 516 patients with stage II and III CRC revealed that patients who were TMB-H were more likely to have N0 disease and an improved 5-year relapse free survival (RFS) compared with TMB-L patients, though there was significant overlap between TMB-H and MSI-H in the cohort.[6] Meanwhile, Yang et al. performed a systematic review and found that CRC patients whose tumors overexpress PD-L1 had significantly shorter overall survival (OS), disease free survival (DFS), and RFS, possibly related to adverse histopathologic features.[5]

At face value, these findings imply that dMMR/MSI-H and TMB-H can be considered positive prognostic markers, whereas PD-L1 overexpression confers a negative prognosis. However, many factors make interpretation of this data much more complex. One such factor is a significant degree of overlap between MSI-H and TMB-H. As many as 83% of MSI-H solid tumors are also TMB-H, and this co-occurrence is particularly strong in GI tumors.[9] Furthermore, although indications for use of ICIs generally combine dMMR and MSI-H, thus implying they are inseparable characteristics of tumors, the rate of discordance between the two can range between 1-10%.[10-14] It has been hypothesized that this variability is related to the use of older methods of measurement of both MSI-H and MMR. A recent retrospective examination of over 3000 patients with CRC revealed a 1.6% discordance rate, mainly due to misclassifications of immunohistochemistry (IHC). Once re-analyses and expert reviews were performed, a “true” discordance rate of 0.4% remained.[15] In other words, over three-quarters of discordant cases were reclassified after further evaluation.

These findings highlight the importance of dual MSI-H and MMR IHC in order to identify truly discordant cases, so as not to inappropriately restrict available therapies. Moreover, there remains significant variability among measure cutoffs discerning TMB-H from TMB-L and what constitutes PD-L1 overexpression. Such inconsistencies make consolidation of the broader

literature into an integrated clinicopathologic understanding more difficult. However, despite these impediments in interpretation, the majority of approved indications for ICIs in luminal GI cancers are based on the three aforementioned biomarkers.

With regards to treatment approaches that involve ICIs, ongoing research couples these agents with those targeting both well-established and investigational pathophysiological mechanisms, including but not limited to traditional chemotherapy, radiation, anti-angiogenesis agents, and vaccines. Additionally, although the chief concentration of historical cancer research has been cytotoxicity, as a more complete understanding of cancer has developed, some of the field's attention has shifted towards the tumor microenvironment (TME). Of particular interest to oncologists using ICIs is the effect of TME immune cells on cancer, which can suppress or promote cancer growth, survival, metastasis, and more. However, in order to maximize ICI utility, one cannot ignore the other components of the TME, as the interdependence of these mechanisms cannot be understated.

The purposes of this review are to highlight the biomarkers that currently guide treatment of luminal GI cancers with ICIs, detail the physiology that explains their utility, characterize the efficacy of ICIs based on unique tumor characteristics, mention the limitations of current evidence in the field, and explore forward-looking endeavors using ICIs at the cutting edge of science.

## Microsatellite instability-high and mismatch repair-deficient tumors

Microsatellites are short repeated sequences of fewer than 10 nucleotide base pairs distributed throughout the genome, mostly near coding regions.[16, 17] They are particularly prone to DNA replication errors that are normally corrected by the cell's DNA mismatch repair (MMR) mechanisms.[18] However, when MMR is functionally deficient (dMMR) due to the lost activity of enzymes such as MLH1, MSH2, MSH6, PMS1, and PMS2, microsatellites accumulate mutations leading to instability.[19]

Subsequently, the rate of mutations in dMMR cells can reach 1000 times that of normal cells, providing a basis for carcinogenesis in sporadic and inherited dMMR tumors.[20] MSI-H tumors

are generally defined as having ≥ 30-40% of markers of instability (e.g. mononucleotide repeats), whereas MSI-L tumors do not meet this threshold and are phenotypically similar to microsatellite stable (MSS) tumors.[21] This phenomenon has been associated with increased expression of neoantigens, higher PD-L1 expression, and TMB-H, all of which make MSI-H tumors more immunogenic, and thus susceptible to checkpoint blockade.[22, 23] Among luminal GI cancers, approximately 15% of colorectal cancers, 6-9% of gastric adenocarcinomas, and 5% of esophageal adenocarcinomas are MSI-H, most of which are sporadic cases rather than related to Lynch syndrome.[24, 25]

For patients with dMMR/MSI-H advanced CRC, the KEYNOTE-177 trial demonstrated that PD-1 blockade with pembrolizumab was associated with an overall response rate (ORR) of 43.8% and median progression free survival (mPFS) of 16.5 months. The median duration of response (DOR) and OS were not reached after more than 44 months of follow-up.[26] The updated analysis revealed that mPFS was improved with ICI therapy when compared with chemotherapy, and there were lower rates of treatment-related adverse events. Additionally, while there was no significant difference in OS, the authors noted a trend towards improved survival that was especially notable in the setting of a 60% effective crossover rate to treatment with ICI.[27] Nivolumab was examined in the phase 2 CheckMate-142 study in a similar population, finding overall responses in approximately one-third of patients and a 12-month OS of 73.4%, though no comparator group was enrolled.[28] Overman et al. also explored the use of nivolumab with low-dose ipilimumab in another cohort of pretreated patients, finding that ORR was 55%, 12-month PFS was 71%, and 12-month OS was 85%.[29]

Within the broader KEYNOTE-158 trial, 24 patients with dMMR/MSI-H gastric cancer were treated with pembrolizumab. An ORR of 45.8% was noted in this population, mPFS was 11.0 months, and mDOR and mOS were not reached. Additionally, KEYNOTE-062 added that for patients with dMMR/MSI-H untreated tumors which also demonstrated PD-L1 CPS ≥ 1, ORR to pembrolizumab was 57.1%, mPFS was 11.2 months, and mOS was not reached at a follow-up of nearly 30 months. These response rates were improved with the addition of concurrent chemotherapy and numerically higher when compared with tumors that overexpressed PD-L1 but were MSS.[30]

Unfortunately, to date there are no studies specifically investigating the efficacy of ICIs in patients with dMMR/MSI-H esophageal adenocarcinoma, though this is probably because a smaller proportion of patients with this cancer are MSI-H when compared with other luminal GI cancers. This is supported by the observation in the ATTRACTION-3 trial that of 112 evaluable patients (40% of study population), none were MSI-H.[31] However, the available data indicate ICIs generate robust responses both in the frontline and pretreated settings for patients with advanced, recurrent, and metastatic dMMR/MSI-H CRC and GC. In fact, responses of dMMR/MSI-H GC to both ICI monotherapy and ICI plus chemotherapy seem to be more robust than responses to ICIs in PD-L1 overexpressing GC in phase III studies. Taken together, the overall response rates of MSI-H GC and CRC compare favorably against those of PD-L1 overexpressing gastric and esophageal cancers, though caution must be used when comparing across both trials and diseases (Table 1).

**Tumor mutational burden**

It is no coincidence that some of the first evidence demonstrating the activity of ICIs was in patients with melanoma and non-small cell lung cancer (NSCLC). It is thought that these malignancies derive their carcinogenesis and immunogenicity from the mutagenic effects of environmental toxins—ultraviolet light and smoking, respectively.[32, 33] Hypermutation then results in the expression of numerous tumor-specific neoantigens that are immunogenic and can induce an anti-tumor response.

As previously described, TMB-H status overlaps significantly with dMMR/MSI-H. More specifically, MSI-H has been found to occur primarily as a subset of TMB-H. Conversely, in one large genomic analysis, only 16% of TMB-H tumors were found to be MSI-H.[9] This means that the majority of TMB-H tumors may not be related to deficiencies in mismatch repair, owing their increased mutational burden to other causes, such as mutations in DNA polymerase epsilon (*POLE*), *TOP2A*, and yet unrecognized mechanisms. Taken a step further, these close associations imply that responses of TMB-H tumors may be expected to be similar to those of dMMR/MSI-H tumors, and that is in fact what has borne out in the available medical literature.

Although clinical evidence is somewhat lacking for use of ICIs in TMB-H luminal GI tumors, a subgroup analysis of KEYNOTE-158 participants (14% of whom had anal cancer) laid the groundwork for FDA approval of pembrolizumab for any solid tumor with TMB ≥ 10 mut/Mb.[34, 35] Schrock et al. examined the efficacy of PD-1/PD-L1 inhibitors specifically in patients with MSI-H metastatic CRC, finding that TMB-H was predictive of both improved ORR and PFS.[36] However, the optimal cutoff determined in this retrospective review (and used by the authors) was 37-41 mut/Mb, which is significantly higher than the aforementioned 10 mut/Mb. Additionally, as with KEYNOTE-158 this review included only patients known to be MSI-H, and little is known about TMB-H/MSS patients. In a phase Ib/II study of the PD-1 inhibitor toripalimab in patients with advanced gastric cancer that used a TMB cutoff of 12 mut/Mb, TMB-H patients had an ORR of 33% compared with 7.1% in TMB-L patients, and OS was significantly improved (14.6 vs. 4 months).[37]

There is currently no published phase III evidence illustrating TMB's ability to predict the efficacy of ICIs in luminal GI cancers, making it especially difficult to compare to dMMR/MSI-H and PD-L1 in this setting. Even more notably, there is a notable dearth of studies evaluating TMB in patients with esophageal cancer, as most have focused on PD-L1 expression in these patients instead of other predictive biomarkers. However, overall trends in CRC and GC indicate similar responses of TMB-H tumors as compared with MSI-H tumors. Considering the significant overlap between these two markers, this finding is consistent with the medical community's understanding of the relationships between hypermutation, the generation of neoantigens, immunogenicity, and efficacy of ICIs. Still, few data exist to elucidate the relationships among these characteristics, and a significant hurdle remains in the absence of cutoff standards across studies.

**Programmed death-ligand 1 expression**

Of the three biomarkers used to direct ICI therapy in luminal GI tumors, PD-L1 is perhaps the most widely studied. The role of PD-1 on T cells is primarily to limit autoimmunity through inhibitory regulation. It is highly expressed on regulatory T cells (Tregs), which are enriched in tumors. There, they suppress local inflammation and cytotoxic killing of cancer cells.[38]

Additionally, malignant cells have been shown to upregulate PD-L1 expression both through innate and adaptive immune resistance mechanisms.[39] By leveraging the endogenous immune system's inhibitory signaling, tumors can escape T cell-mediated immune surveillance. Conversely, by "removing the brake" on this immune checkpoint by blocking PD-1 or PD-L1 with ICIs, treating oncologists can reinstate a more normal anti-tumor immune environment and induce tumor killing.

As is the case with determination of TMB, there is no standard for measuring PD-L1 expression, which has resulted in variable techniques used across studies. Often, choice of PD-L1 testing is determined by FDA approved companion therapies. These issues are perhaps illustrated best by a 2018 systematic review, in which eight antibodies were found to be used across 26 studies to determine PD-L1 expression in lung tumors via immunohistochemistry (IHC). The authors detail a wide range of PD-L1 cutoffs used in the studies (1-50%), discordance between biopsies and surgically resected tissue samples, various rates of concordance and agreement among tests, and differences in the types of cells tested.[40] This last factor highlights the difference between TPS and CPS, the former of which considers only tumor cells that are positive for PD-L1 and the latter of which considers all cells that are PD-L1 positive including tumor cells and immune cells. At least one study compared TPS and CPS in patients with gastric cancer and determined that CPS is likely more sensitive at least as a prognostic marker.[41] CPS also appears to outperform TPS in its ability to predict response to immune checkpoint blockade.[42]

Unlike studies of dMMR/MSI-H and TMB-H in luminal GI cancers, studies using PD-L1 as a predictive biomarker are dominated by investigations of esophageal cancer (EC). In KEYNOTE-590, patients with advanced EC received either pembrolizumab plus chemotherapy or chemotherapy alone. Those with CPS $\geq$ 10 experienced a mPFS of 7.5 months, mOS of 13.5 months, and 24-month OS rate was 31%, though all patients enjoyed significant benefit of ICI therapy regardless of CPS stratification.[43] This benefit seemed to be especially strong in patients with squamous cell carcinoma (SCC). CheckMate-648 was similarly designed and included three arms: chemotherapy alone, nivolumab plus chemotherapy, and nivolumab plus ipilimumab. In the patients with PD-L1 expression of 1% or greater, both patient cohorts treated with nivolumab resulted in longer OS than chemotherapy alone, and the majority of the efficacy appeared to be

driven by PD-L1 overexpression.[44] These findings are concordant with those of ATTRACTION-3, which found that patients with pre-treated esophageal SCC experienced an ORR of 19% and mOS of 10.9 months with nivolumab, though in this case the improved outcomes were irrespective of PD-L1 overexpression (using a cutoff of 1%).[31] Together, these imply that PD-L1 positivity may confer some predictive value in EC patients with SCC, but the histology itself is also a main driver of response to ICIs.

In KEYNOTE-181, patients with pretreated esophageal SCC or adenocarcinoma (AC) and CPS ≥ 10 who received pembrolizumab had an ORR of 21.5%, mDOR of 9.3 months, mPFS of 2.6 months, mOS of 9.3 months, and 43% of patients were alive at 12 months.[45] Meanwhile, ORR in patients with CPS < 10 was 11.9% for patients with SCC and 3.3% for patients with AC, further illustrating the compounding predictive ability of histology and PD-L1 status. CheckMate-577 is the only adjuvant trial in this group, investigating a course of nivolumab in patients with resected EC and GEJ tumors. Although this study found significantly prolonged DFS with nivolumab in all enrolled patients, the effect was emphasized in a subset of patients with CPS ≥ 5 who experienced a mDFS of 29.4 months compared with 16.3 months in those with CPS < 5.[46]

In gastric cancer, KEYNOTE-062 investigated the use of pembrolizumab in the first-line treatment of advanced disease. Patients with PD-L1 CPS ≥ 1 experienced an ORR of 15% with pembrolizumab alone and 49% with pembrolizumab plus chemotherapy.[30] The effect was slightly increased in patients with CPS ≥ 10, revealing ORR of 25% and 53%, respectively. Median OS was 10.6 months in patients receiving pembrolizumab monotherapy who had CPS ≥ 1 and 17.4 months in those who had CPS ≥ 10. Although the available evidence across studies is not yet mature, this is consistent with observations in esophageal SCC that progressively higher PD-L1 expression connotes improved responses to ICIs.[47] However, contrasting evidence was presented in ATTRACTION-4, as patients with advanced HER2-negative GC experienced prolonged PFS but not improved survival when nivolumab was added to chemotherapy, even when taking into account PD-L1 expression.[48] Furthermore, JAVELIN Gastric 100 did not show superior OS with avelumab maintenance following induction chemotherapy for advanced disease, but only 12% of patients in this trial had PD-L1 ≥ 1%, suggesting that further investigation in that population is needed.[49]

In colorectal cancer, response to ICIs based on PD-L1 expression is much more muted, though evidence is also much scarcer. Twenty-three patients from KEYNOTE-028 with metastatic CRC and PD-L1 expression ≥ 1% were evaluated for responses to pembrolizumab, and ORR was demonstrated to be only 4%.[50] Additionally, the authors of CheckMate-142 reported that the subset of MSI-H patients with tumor cell PD-L1 expression ≥ 1% did not have markedly different ORR or disease control rate (DCR) compared with their counterparts who had PD-L1 < 1%, though there was a trend towards improved responses in those with higher immune cell PD-L1 expression.[28] A similar lack of effect of PD-L1 expression was demonstrated in CRC patients treated with regorafenib and nivolumab, and PD-L1 ≥ 10% did not correlate either with PFS or OS in patients with metastatic CRC receiving regorafenib and avelumab.[51, 52] Therefore, although PD-L1 expression has been shown to have prognostic value in CRC, its predictive value is likely much more limited—especially when compared with dMMR/MSI-H and TMB-H.

Overall, compared with response rates of dMMR/MSI-H tumors in phase III trials to ICI monotherapies (approaching 50%), luminal GI tumors that overexpress PD-L1 have more moderate response rates nearer to 20% (Table 1). However, the significant variability in biomarker measure cutoffs and study methodology limits any ability to draw firm conclusions from this observation. Moreover, while the addition of chemotherapy to either pembrolizumab or nivolumab can increase the ORR to these agents by double or more in EC and GC patients whose tumors overexpress PD-L1, its effects on both PFS and OS—while positive—may be at least partially dependent on the degree of PD-L1 overexpression and the underlying histology.[30, 31, 43, 45, 53]

## Improving ICI efficacy: combination therapies and advanced monitoring

The use of ICIs in luminal GI tumors is historically tied to dMMR/MSI-H, TMB-H, and PD-L1 expression. However, as ICIs have solidified their roles in treatment paradigms and demonstrated effectiveness as monotherapy and in combination with conventional regimens, there has been rising interest in adding them to other established therapies as well as emerging treatments. This has led to use of these agents, both within the confines of their predictive biomarkers, and outside

of them. Ongoing trials using ICIs in combination with established regimens of chemotherapy and radiation, agents targeting angiogenesis, investigational medications, alternative immune mechanisms, and biomarker guidance are listed in Table 2 and are summarized below.

Standard-of-care chemotherapy and radiation

Studies such as KEYNOTE-590, KEYNOTE-062, and CheckMate-649 have demonstrated the additive role for ICIs when combined with standard-of-care chemotherapy in advanced and metastatic luminal GI cancers. As evidence has mounted for combination therapies with ICIs, further studies evaluating this approach have been developed, even moving from advanced disease into the neoadjuvant and adjuvant settings. One such investigation is the NICHE trial, which recruited 41 patients with early-stage CRC to receive neoadjuvant nivolumab and ipilimumab. The investigators noted pathologic responses in 100% of dMMR tumors (including 60% complete pathologic responses) and 27% of MMR-proficient (pMMR) tumors, which compares favorably against evidence for neoadjuvant chemotherapy in the FOXTROT trial.[54] Interestingly, 8 of 17 patients in the pMMR group also received celecoxib as a way to inhibit $PGE_2$ and thus increase ICI effectiveness, which may be a mechanism adopted by future studies if effective. Other ongoing trials in esophageal cancer (e.g. NCT03604991, NCT0400604) and gastric cancer (e.g. NCT04354662) are similarly using ICIs with traditional neoadjuvant therapies to evaluate improved responses to these combinations.

Likewise, ICIs are being added in the adjuvant setting. There is a precedent for such an approach in other solid tumors. In renal cell carcinoma, KEYNOTE-564 showed that adding pembrolizumab after nephrectomy significantly improved two-year DFS (HR 0.68) and OS (HR 0.58).[55] Additionally, in resected non-small cell lung cancer (NSCLC), the addition of atezolizumab after platinum-based adjuvant chemotherapy was recently shown to improve DFS, primarily in patients whose tumors expressed PD-L1 (HR 0.66).[56] It is worth noting that there is already published evidence for adjuvant ICI treatment in esophageal and GEJ cancer, demonstrated in CheckMate-577, though unlike in NSCLC, it appears the benefit was unrelated to PD-L1 expression and the patients had pathologic evidence of residual disease.[46] In CRC, the ATOMIC trial

(NCT02912559) is currently investigating the addition of atezolizumab to FOLFOX for patients with stage III disease and who are dMMR/MSI-H.[57]

Finally, there are almost innumerable studies that use ICIs in the advanced/metastatic setting. Of course, as with any newer therapy, this is where much of the evidence already lies for use of ICIs in luminal GI tumors. Of particular interest is the AVETUXIRI trial (NCT03608046), which attempts to leverage the theoretical immunogenic activity of cetuximab to induce tumor cell death by combining it with avelumab and irinotecan. Interim analysis revealed that in MSS metastatic CRC, DCR was approximately 60% regardless of RAS mutation status, and further investigation is ongoing.[58] Other notable studies include ARION (NCT03777813), which is currently evaluating the addition of durvalumab to chemoradiation in esophageal cancer, and INEC (NCT03544736), which is similarly evaluating nivolumab in esophageal cancer in multiple settings including unresectable disease.

Agents targeting angiogenesis

Adding ICIs to established chemotherapy and radiation regimens is an approach that seeks to compound the effects of both therapies. However, while there is some preclinical evidence that the efficacy of cytotoxic therapy is additive to immunotherapy, other data conversely support a sub-additive effect—and there exists no firm basis for synergism of the two approaches.[59] Therefore, using novel approaches that modify the tumor microenvironment (TME) may produce outsized clinical benefits by comparison, as this has the potential to truly modify the underlying physiology of anti-tumor immunity.

One such method is the time-tested targeting of the vascular makeup of cancers. Tumors recruit blood vessels to facilitate both local growth and metastatic spread of cells, but the vascularization of tumors is not typical. However, instead of maximizing tissue oxygenation, tumor angiogenesis promotes hypoxia, acidosis, impaired entry of systemic anticancer drugs, and incapacitation of T cell-mediated cytotoxicity.[60] Moreover, TME production of factors such as vascular endothelial growth factor (VEGF) and angiopoietin 2 (ANG2) inhibit immune surveillance by modulating adhesion molecules and inhibiting dendritic cell maturation, among other mechanisms.[60-62]

Furthermore, blockade of VEGFR has been shown to inhibit regulatory T cell proliferation in colorectal and other cancers, thus reversing a tumor's adaptive immune escape.[62, 63] Therefore, by normalizing the vascularization of tumors and subsequently disrupting the pro-cancer TME, inherent immune responses can be reinstated, potentially augmenting the effects of immunotherapeutics.

Perhaps one of the most recognizable of these approaches in solid tumors is the combination of atezolizumab and bevacizumab, an anti-VEGF agent, which has revolutionized first-line therapy of hepatocellular carcinoma.[64] This highlights the use of monoclonal antibodies that target angiogenesis. In fact, bevacizumab has been broadly studied in CRC, combined with standard therapies such as FOLFOX and FOLFIRI.[65-67] Newer investigations such as NIVACOR (NCT04072198) attempt to add immune checkpoint inhibition to this archetype, seeking to not only achieve rapid responses with FOLFOXIRI plus bevacizumab and nivolumab, but also control disease in the longer-term with maintenance bevacizumab and nivolumab.[68] In dMMR CRC, the COMMIT study (NCT02997228) uses a similar paradigm with FOLFOX, atezolizumab, and bevacizumab, and still more trials (e.g. NCT05035381, NCT04988191) use other ICIs and chemotherapies.[69] Like bevacizumab, ramucirumab inhibits the functioning of VEGF, but through the inhibition of the VEGF receptor 2 (VEGFR2). Ramucirumab has established uses in combination with chemotherapy, but it is also being investigated in combination with pembrolizumab for patients with metastatic GC (NCT04632459).

The VEGF/VEGFR axis can be targeted with tyrosine kinase inhibitors (TKIs) as well. The benefit of these medications is that they can be taken orally, which is convenient for patients. However, many of these are notably "dirty" drugs with off-target effects due to both their primary activity (against VEGFR) well as multikinase inhibitory functions. Cabozantinib, for instance, inhibits VEGFR2, MET, RET, and AXL, and though its activity against other kinases is leveraged deliberately in other cancers with particular mutations, these unintentional foci of activity can lead to significant side effects.[70] However, despite its limitations, the evidence for the "TKI + ICI" model is beginning to surface, with some encouraging results. In fact, cabozantinib was evaluated in combination with durvalumab in the CAMILLA trial (NCT03539822) in both gastric/esophageal and colorectal cancer cohorts. Of 30 patients available for efficacy analysis

presented in a preliminary report (which included a small number of patients with hepatocellular carcinoma), ORR was 26.7%, DCR was 83.3%, and median OS was 9.1 months, with PD-L1 CPS ≥ 5 showing improved efficacy and survival.[71]

Likewise, in the phase II REGOMUNE trial, 43 patients with MSS CRC received regorafenib and avelumab, and although there were pathological signs of response (such as increases in $CD8^+$ T cell infiltration of tumors), no patients experienced an objective response.[51] The authors noted that the PFS and OS observed in the patients receiving TKI + ICI were numerically higher than historical data for regorafenib monotherapy, but altogether it is not clear if this pathologic response translates reliably to the clinical setting without further investigation. Regorafenib has also been combined with nivolumab in Japanese patients with GC and MSS CRC, an approach that achieved an objective response in approximately 40% of both cohorts.[52] Not only was this response more impressive than historical responses to either agent as monotherapy—noting caution with cross-trial comparisons—but there was also a trend towards improved PFS in GC patients with PD-L1 CPS ≥ 1, and responses were even seen in patients who were resistant to prior PD-L1 therapy. Unfortunately, when a retrospective evaluation of 17 patients at a single U.S. center was performed, no such responses were found, bringing into question the selection of patients and differences in outcomes between patients of Asian and non-Asian descent.[72, 73]

Other notable trials of comparable regimens hope to add to the therapeutic landscape. LEAP-005 (NCT03797326) is a phase 2, multicohort study evaluating the safety and efficacy of pembrolizumab and lenvatinib in patients with pre-treated solid tumors. In LEAP-005's 31 patients with GC, ORR to this regimen was 10% and DCR was 48%, and in CRC patients ORR was 22%, which led to expansion of both cohorts.[74, 75] This has led to the phase III LEAP-017 trial comparing pembrolizumab and lenvatinib to later-line therapy for metastatic CRC with regorafenib or TAS-102 (NCT04776148). NCT04976634 adds belzutifan, a HIF-2a inhibitor, to the pembrolizumab + lenvatinib regimen, attempting to capitalize on the cooperative effects of reversing TME hypoxia and inhibiting adaptive angiogenesis.

<u>Other targeted therapies</u>

In addition to standard chemotherapies and agents targeting the VEGF/VEGFR axis, researchers are now also adding ICIs to regimens that include alternative targeted therapies. Primarily, this involves previously established targets. One example of this is a phase II trial of perioperative atezolizumab plus XELOX and trastuzumab in HER2-positive gastric and GEJ cancers (NCT04661150). Another is a Chinese trial using camrelizumab, a PD-1 inhibitor, in combination with chemotherapy and an oral HER2 agent in the first-line treatment of GC (NCT05070598). Of note, the interplay between the expression of HER2 and PD-L1 in gastroesophageal cancers is not entirely clear, as some data point towards a positive correlation between the two, some a negative correlation, and some indicate no correlation at all.[76] This might suggest the expectation of additive, rather than synergistic, effects of combination HER2-directed therapies and immunotherapies.

Other more familiar therapies being matched with ICIs target MEK and deficiency in homologous recombination (HRD). Although there exists data for the use of the MEK inhibitor trametinib in combination with BRAF inhibition in metastatic CRC, a newer investigation hopes to evaluate its use in combination with nivolumab with or without ipilimumab (NCT03377361).[77] This is based on encouraging preclinical evidence that MEK inhibition protects $CD8^+$ T cells from apoptosis and synergistically induces durable tumor responses.[78] However, it is worth mentioning that the phase III IMblaze 370 trial combining atezolizumab with the MEK inhibitor cobimetinib did not demonstrate improved survival over regorafenib in pre-treated CRC patients, bringing into doubt the efficacy of this approach.[79] Meanwhile, the phase I/II RiME trial (NCT03995017) is evaluating the PARP inhibitor rucaparib and ramucirumab with or without nivolumab in GC and EC, in both molecularly unselected patients as well as those selected by HRD testing. Finally, newer targeted therapies are being investigated with ICIs, as well. For instance, the NEDD8 inhibitor pevonedistat is being investigated in combination with pembrolizumab (NCT04800627), as it has already shown anticancer activity in hematologic malignancies and is known to downregulate inducible Tregs while also polarizing helper T cells toward the Th1 phenotype, thus potentially augmenting ICI activity.[80, 81]

In summary, new antineoplastics continue to enter clinical testing and are being explored in novel combinations. Although this discussion only highlights a few that are being combined with ICIs,

it demonstrates the almost limitless permutations possible. Undoubtedly, there will be more investigations following this approach, with some attempting to find additive benefits with minimal compounding toxicities and others aspiring to exploit mechanistic synergies.

Novel immune-directed therapies

Another familiar therapy that is being extended into the immunotherapy paradigm—though it is not an immunotherapy itself—is temozolomide (TMZ). The activity of TMZ in pMMR CRC is limited to patients with high *MGMT* promotor methylation, and even in those cases, responses are relatively short lived.[82] However, its use in combination with pembrolizumab is founded on its ability to induce inactivation of MMR enzymes, thus increasing TMB and expression of neoantigens and making tumors sensitive to immune checkpoint blockade (NCT03519412).[83] This is a unique example of using inherent resistance to chemotherapy to potentially induce an immune-sensitive state, without adding a "true" secondary immunotherapy.

Of course, the combination of immunotherapies is being investigated as well, using well-known ICIs with experimental immunotherapeutic agents. Although immune checkpoint inhibition has not yet expanded broadly beyond PD-1/PD-L1 and CTLA-4 in clinical practice, several other checkpoints are increasingly showing promise as antineoplastic targets. Checkpoints are named because they provide inhibitory signals to immune responses through mostly cell surface ligands and receptors including PD-1, CTLA-4, LAG3, and TIGIT, among others.[84, 85] A detailed exploration of checkpoint signaling mechanisms is beyond the scope of this review, but two of these warrant particular mention in the treatment of luminal GI cancers. Lymphocyte activation gene 3 (LAG3), also known as CD223, is expressed on NK and T cells and interacts with MHC class II—with a higher affinity than CD4—and facilitates negative T cell regulation, augments immunosuppression by Tregs, and contributes to T cell exhaustion.[86, 87] To take advantage of this complementary immune mechanism, in one arm of the NCI-MATCH trial (NCT02465060), the LAG3 inhibitor relatlimab is being paired with nivolumab in LAG3-expressing tumors. TIGIT (T cell immunoglobulin and ITIM domain) is another inhibitory receptor that interacts with tumor ligands, such as CD155, CD112, and CD113, to mediate inhibitory immune signaling.[88] Exploiting this mechanism, SKYSCRAPER-08 (NCT04540311) is evaluating atezolizumab and

tiragolumab, a monoclonal antibody directed against TIGIT, with chemotherapy in patients with EC.

Still more immune targeted therapies are being investigated, including the CCR4 antagonist FLX475 in locally advanced and metastatic GC (NCT04768686) and the addition of fecal microbiota transplant to leverage the effects of the gut microbiome to modulate PD-1 responsiveness of CRC (NCT0472932), based on evidence from phase 1 data in melanoma.[89] As these complex interactions begin to be more fully understood, new therapeutic opportunities continue to present themselves at a breakneck pace.

Vaccines

An approach that has not yet reached wide clinical practice for any malignancy, vaccines pose a unique opportunity to direct the immune system to target cancer- and TME-related antigens. There are a wide variety of methods to accomplish this (e.g. peptide vaccines, vector-based antigen-specific vaccines, whole-cell vaccines, and others), but, regardless of the design, ICIs are thought to be able to augment these responses by ensuring immune activity after vaccine-induced tumor infiltration of effector T cells.[90, 91] The phase I study of Nous-209 (NCT04041310), an off-the-shelf neoantigen viral-vectored vaccine that targets shared frameshift peptides commonly expressed in dMMR/MSI-H tumors, in combination with pembrolizumab in patients with dMMR/MSI-H CRC, GC, and GEJ cancer, finding acceptable tolerability and proving immunogenicity.[92] Other vaccines being investigated with ICIs include VB-111 (NCT04166383), an adenovirus vector vaccine directed towards blood vessels that induces apoptosis and results in an anti-angiogenesis effect, and an mRNA vaccine targeting KRAS-mutated CRC (NCT 03948763).[93] Further notable ongoing trials can be found in Table 2.

Emerging biomarkers

The final noteworthy effort partnered with ICIs in luminal GI cancers is not a therapy at all. Rather, it is the advanced monitoring afforded by circulating tumor DNA (ctDNA) measurement, which has so far shown promise for post-operative risk stratification and early detection of

recurrence.[94-96] The most widely studied ctDNA approaches are either tumor-informed, which uses each patient's known mutations as markers of disease, or tumor-agnostic, which uses broader sequencing approaches.[97] In the GALAXY study whose results were first reported in 2022, ctDNA dynamics were tested comparing 4-week and 12-week biomarker measurements with 6-month PFS in patients with colorectal cancer in the adjuvant setting. Notably, 6-month PFS rate was 45% in the patients who retained measurable ctDNA over the two measurements, whereas those who converted from detectable to undetectable had a 6-month PFS rate of 100%.[98] Furthermore, clearance rate was significantly higher in patients who received adjuvant chemotherapy compared with those who did not. Although the role for ctDNA measurement in the decision to use adjuvant therapy is becoming clearer in the early-stage setting, the use of this biomarker in advanced disease and specifically with ICIs is more uncertain.

In a broad study across solid tumors, ctDNA levels were determined to be both prognostic and predictive of benefit to pembrolizumab.[99] Similarly, a retrospective study of patients with urothelial cancer in a phase III trial of adjuvant atezolizumab revealed that those with detectable ctDNA at the beginning of therapy had poorer prognosis and benefited from ICI therapy, with hazard ratios for DFS and OS approaching 0.6.[100] Additionally, there have been attempts to correlate ctDNA-based measurements of traditional predictive biomarkers like TMB with responses to ICIs in other malignancies.[101, 102] Further investigations are being performed to elucidate the usefulness of ctDNA monitoring while patients are on immunotherapy, such as the BESPOKE study (NCT04761783), which includes CRC patients, and the CALIBRATION study (NCT03653052) in EC patients.

Beyond ctDNA, the Immunoscore® has been applied to CRC prognostic and predictive models, and it may be an additional data point that helps guide treatment with ICIs. The score is based on tumor densities of $CD3^+$ and $CD8^+$ T cells in the tumor core and invasive margin, and it has demonstrated both prognostic abilities—even independent of MMR/MSI status—and predicted benefit from adjuvant chemotherapy.[103, 104] It has not yet been tested systematically in patients during or prior to immunotherapy. However, in a retrospective study of 12 patients with dMMR tumors, $CD3^+$ and $CD8^+$ T cell density scores were correlated with better rates of objective responses and duration of disease control with pembrolizumab, implying that the Immunoscore®

could be used as a supplemental measure before considering ICI administration.[105] In this context, one current investigation (NCT04262687) employs the Immunoscore® to help identify MSS metastatic CRC patients who may benefit from ICI therapy, and then treats those patients with a pembrolizumab-inclusive regimen.

## Conclusions and future perspectives

There is a growing body of evidence supporting the use of ICI therapy in esophageal cancer, gastric cancer, and colorectal cancer in multiple settings. In colorectal cancer, response to ICIs seems to be primarily driven by dMMR/MSI-H and TMB-H rather than PD-L1 expression, though the latter can be used as a negative prognostic indicator. Gastric cancer exhibits similar responses to ICIs based on dMMR/MSI-H and TMB-H designation, but evidence also points to a significant predictive effect of PD-L1 expression that is closer to that of esophageal cancer. Whereas there is minimal data surrounding the predictive abilities of dMMR/MSI-H and TMB-H in esophageal cancer, response to ICIs is driven at least in part by PD-L1 expression in addition to histology, such that esophageal squamous cell carcinomas respond to ICIs somewhat more profoundly than adenocarcinomas.

Although some clear patterns have arisen from mounting clinical evidence, differences in methodologies limit the full understanding of the underlying biology of these tumors' responses to ICIs. As previously explored, primary limitations include the myriad ways these biomarkers are measured and how their cutoffs are determined. dMMR/MSI-H definitions are clearer than those of TMB-H and PD-L1 overexpression, which vary both in choices of tests and cutoffs—sometimes by wide margins. Selection bias is also a shortcoming, as multiple studies indicate that Asian populations tend to have more significant responses to immune checkpoint blockade.[31, 43, 45] The cause of this is yet unclear, as preliminary investigations, at least in esophageal SCC, have not revealed differences in immune cell infiltrates, immune subtypes, or T cell-related gene signatures between Asian and non-Asian patients.[106] However, this is a persistent trend that brings into question the portability of evidence between these two populations, necessitating subgroup analyses especially in larger, phase III studies. An additional challenge is the varying degrees to

which PD-L1 therapy is applied as monotherapy, in combination with anti-CTLA-4 agents, or with chemotherapy.

Beyond the widely studied PD-1/PD-L1 targeted agents, several other approaches to accomplish immune checkpoint blockade, with new agents and through augmentation of known entities, have come to the forefront of cancer research. Perhaps one of the most widely studied domains is anti-angiogenesis agents, but combination therapies with chemotherapy, radiation, and existing targeted therapies are also common. More novel approaches focus on other immune checkpoints such as LAG3 and TIGIT, either in isolation or in combination with known PD-1/PD-L1 directed therapies. Meanwhile, some investigators are using vaccines to elicit or augment T cell responses, while relying on ICIs to maximize their anti-tumor effects. Finally, biomarkers such as ctDNA and the Immunoscore® may be able to further refine patient selection for ICI therapy (and evaluate effectiveness of that therapy) beyond the abilities of more established biomarkers such as dMMR/MSI-H, TMB, and PD-L1.

Overall, the prototypical immunologic biomarkers used to predict responses to ICIs in luminal GI cancers are intricately interconnected. With more evidence rapidly accruing, GI oncologists will be able to hone their use of these powerful anti-neoplastic agents and more completely appreciate the nuances of their characteristics, based on the underlying biology of individual patients' cancers.

## Executive summary

- Biomarkers of immunogenicity are used to determine utility of ICIs in luminal GI cancers
    - Currently, approved indications for ICIs in luminal GI malignancies are primarily based on biomarkers that predict tumor response: dMMR/MSI-H, TMB-H, and PD-L1 expression.
    - In addition to predictive abilities, these biomarkers have prognostic value: overexpression of PD-L1 generally portends poorer prognoses while dMMR/MSI-H and TMB-H may connote better prognoses in select circumstances.

    - There is significant overlap between MSI-H and TMB-H solid malignancies, especially among GI tumors, largely because MSI-H status appears to occur primarily as a subset of TMB-H status.
    - Responses to ICIs appear to be stronger in dMMR/MSI-H tumors compared with those expressing PD-L1, according to evidence primarily in gastric and colorectal cancers.
    - There is FDA approval for the use of pembrolizumab in solid tumors with TMB $\geq$ 10. However, the optimal cutoff for TMB is unclear as studies vary widely in their cutoffs delineating between TMB-H vs. TMB-L.
    - CPS has been favored as a measure of PD-L1 expression in recent studies evaluating esophageal and gastric cancers, as it outperforms TPS in its ability to predict responses to ICIs. Its role as a predictive biomarker in CRC is less well established.
- Efforts to improve ICI efficacy involve both therapeutic combinations and advanced biomarker testing
    - Combination therapies
        - ICIs have contributed to increased responses to standard-of-care therapies like chemotherapy and radiation in the advanced disease setting, and this has led to investigations in the neoadjuvant and adjuvant settings. One promising example is the use of combined immunotherapy prior to resection of early-stage CRC, which showed 100% rate of pathologic response in dMMR patients.
        - Targeting of angiogenesis can modify the TME to allow native immune regulation to proceed and potentiate responses to ICIs. Agents that target the VEGF/VEGFR axis include monoclonal antibodies (e.g. bevacizumab) and TKIs (e.g. lenvatinib).
        - Other targeted therapies that are being investigated in combination with ICIs include agents that target HER2, MEK, PARP, and NEDD8.
        - Experimental therapies targeting immune checkpoints LAG3 and TIGIT are being investigated with ICIs that are already used in clinical settings, as these are hypothesized to have synergistic effects.

- Advanced biomarkers
    - The use of advanced biomarker testing using ctDNA is still under investigation, although gaining traction, and its role for monitoring disease status during or after ICIs is promising.
    - The Immunoscore® is a measure of tumor immune cells that may predict responses to ICIs.
- Research is quickly moving beyond using ICIs only pursuant to known predictive biomarkers with narrow relevance, turning instead to novel combinations and biomarkers that promise to maximize these powerful therapies' clinical utility.

**Figure and table legends:**

**Figure 1. Approved immune checkpoint inhibitors by site of luminal GI cancers**

The FDA has approved pembrolizumab and nivolumab, both PD-1 inhibitors, for multiple indications in patients with luminal GI cancers. Most of these indications are in advanced, metastatic, or recurrent disease, and many are attendant to predictive biomarkers. Figure created with BioRender.com.

dMMR = deficient mismatch repair, MSI-H = microsatellite instability-high, TMB-H = tumor mutational burden-high, SCC = squamous cell carcinoma, CPS = combined positive score, GEJ = gastroesophageal junction, AC = adenocarcinoma, CT = chemotherapy

**Table 1. FDA approved indications and efficacy data for PD-1 inhibitors pembrolizumab and nivolumab in luminal GI cancers**

* = in months unless otherwise stated, ** other studies included KEYNOTE-012/-016/-028 but efficacy outcomes listed only for KEYNOTE-158/-164, SCC = squamous cell carcinoma, GEJ = gastroesophageal junction, AC = adenocarcinoma, 1L = first-line, CT = chemotherapy, CRT = chemoradiotherapy, dMMR = deficient mismatch repair, MSI-H = microsatellite instability-high, CRC = colorectal cancer, ORR = objective/overall response rate, mDOR = median duration of response, mOS = median overall survival, mPFS = median progression-free survival, CPS = combined positive score, mDFS = median disease-free survival, TMB = tumor mutational burden

**Table 2. Select ongoing studies of immune checkpoint inhibitors in luminal GI cancers with novel combinations**

a = includes gastroesophageal junction (GEJ) tumors, ICI = immune checkpoint inhibitor, CRC = colorectal cancer, ctDNA = circulating tumor DNA

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**Reference annotations:**

9. * A genomic analysis that broadly characterizes TMB across tumor types and specifically found a high degree of overlap between MSI-H and TMB-H in gastrointestinal cancers compared with others.

29. * The phase II CheckMate-142 study included a cohort of patients with previously treated dMMR/MSI-H CRC, treating them with ipilimumab plus nivolumab. This cohort had numerically improved ORR and 12-month OS, implying an additional benefit of combination ICI therapy over monotherapy.

35. ** KEYNOTE-158 directly led to the approval of pembrolizumab for any solid tumors with dMMR/MSI-H status, making it the first time the FDA approved a drug for a broad, biomarker-specific indication.

41. * This study showed that, in 191 patients with GC, CPS was a more accurate prognostic factor than TPS. Other evidence supports a similar benefit to CPS with regards to its superior ability to predict responses to ICIs.

46. ** CheckMate-577 was the first evaluation of adjuvant therapy after neoadjuvant chemoradiation for esophageal and GEJ cancers, finding improved DFS with this approach.

54. ** The NICHE trial is the first to evaluate short-term, neoadjuvant combination immunotherapy in dMMR CRC patients, revealing a promising 100% rate of pathological response in this population and indicating a possible benefit to this new approach.

74. * A cohort of LEAP-005 with colorectal cancer treated with lenvatinib and pembrolizumab has an ORR of 22% and has led to many other investigations of combination TKI + ICI therapy.

104. * A high Immunoscore® is correlated with better prognoses in patients with stage III CRC, and these patients also derive the most benefit from chemotherapy.