Review Article

The efficacy-associated biomarkers for immune checkpoint inhibitors in gastrointestinal cancer: a literature review

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Background and Objective: Immune checkpoint inhibitors (ICIs) have been widely applied and studied in the treatment of gastrointestinal (GI) cancers, and have achieved good results. However, in clinical practice, it has been observed that only some patients respond well to ICIs, and some patients may experience various degrees of adverse reactions during the treatment. Timely evaluation of the potential therapeutic effects and adverse reactions of ICIs for patients has important clinical significance. This review aimed to summarize recent progress regarding efficacy-associated biomarkers for ICIs in GI cancer.

Methods: The literature on ICI treatment in GI cancers was searched in the PubMed, Embase, and Cochrane Library databases for publications up to April 2023.

Key Content and Findings: Clinical practice and research has gradually revealed some biomarkers related to the treatment of GI cancers with ICIs, which can be roughly divided into three types: biomarkers that predict the effectiveness of ICIs treatment, biomarkers associated with resistance to ICIs, and biomarkers associated with immune related adverse events (irAEs). This review article provides a literature review on biomarkers related to the efficacy of ICIs in the treatment of GI cancers.

Conclusions: According to existing clinical research results, there are multiple biomarkers that can be used for predicting and monitoring the efficacy and risk of adverse events of ICIs in the treatment of digestive system malignant tumors.

Keywords: Immune checkpoint inhibitors (ICIs); gastrointestinal cancers (GI cancers); biomarkers

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Introduction

Malignant tumors of the gastrointestinal (GI) tract include esophageal cancer (EC), gastric cancer (GC), colorectal cancer (CRC), liver cancer, pancreatic cancer (PC), and biliary cancer. According to global cancer statistics, there were approximately 5.1 million new cases and approximately 3.61 million deaths from digestive system malignancies in 2020 (1). In addition, the incidence and mortality of

Table 1 The search strategy summary

Items	Specification
Date of search	April 24, 2023
Databases and other sources searched	PubMed, Embase, Cochrane Library
Search terms used	biomarker, digestive system cancers, gastrointestinal cancers, immune checkpoint inhibitors, esophageal cancer, gastric cancer, colorectal cancer, liver cancer, hepatocellular carcinoma, pancreatic cancer, programmed cell death protein 1, programmed cell death ligand-1
Timeframe	Up to April 24, 2023
Inclusion criteria	Clinical studies including randomized clinical trial, observative studies, and case reports published in English would be included
Selection process	Two investigators independently reviewed literatures, and if disagreement exists, a third investigator would make final decision

GI tumors are on the rise, and some cancers, such as GC and CRC, are increasingly affecting younger patients (2). At present, the treatment for malignant tumors of the digestive system mainly includes surgery, radiotherapy, and chemotherapy, with surgery remaining the first choice if the tumor is surgically resectable offering a chance of cure. And even though older CRC patients with pT4 disease are more prone to severe postoperative complications, there is no consensus that age affects survival outcomes. The prognosis of older patients may be confounded by differences in stage at presentation, tumor site, preexisting comorbidities, and type of treatment received (3). Systemic therapy also plays an important role in the treatment of malignant tumors of the digestive system. Systemic therapy can shrink the tumor and provide surgical opportunities when used neoadjuvantly, reduce the risk of relapse and recurrence when given in adjuvant setting, and even achieve clinical cure for some patients. In recent years, there has been significant progress in systemic therapy, especially in the use of immune checkpoint inhibitors (ICIs). At present, it has shown strong anti-tumor activity in the treatment of many tumors, such as melanoma, non-small cell lung cancer, renal cancer, bladder cancer, and triple negative breast cancer. ICIs may be the preferred choice when aiming for sustained efficacy outcomes, while targeted therapies are primarily considered for patients in need of a relatively rapid objective response. Interestingly enough, when immunotherapy is administered to melanoma of unknown primary patients, it is likely to result in improved outcomes when contrasted with the melanoma of known primary subset. This may be attributed to their higher immunogenicity, as evidenced by immunologically mediated primary site regression (4). ICIs have become the most promising approach for immunotherapy by inhibiting tumor cells from escaping immune surveillance, recognition, and subsequent cytotoxic T-cell mediated damage to tumor cells (5). In recent years, ICIs have also been widely applied and studied in the treatment of GI tumors, and have achieved good results in all five major digestive system tumor types. However, in clinical practice, it has been observed that only some patients have a good response to ICIs, whereas others may experience various degrees of adverse reactions during the treatment process. Therefore, timely evaluation of the potential therapeutic effects and adverse reactions of ICIs for patients has important clinical significance and can help to screen potential patients who can achieve good therapeutic effects while avoiding serious adverse reactions as much as possible.

The use of biomarkers in blood that predict response to therapy, and treatment safety have been studied extensively. This article provides a literature review on the biomarkers related to the efficacy of ICIs in the treatment of malignant tumors of the digestive system. We present this article in accordance with the Narrative Review reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-843/rc).

Methods

For this review, we searched PubMed, Embase, Cochrane Library with strategies listed in *Table 1*. In short, we used "biomarker", "digestive system cancers", "gastrointestinal cancers", "immune checkpoint inhibitors", "esophageal cancer", "gastric cancer", "colorectal cancer", "liver

cancer", "hepatocellular carcinoma", "pancreatic cancer", "programmed cell death protein 1", and "programmed cell death ligand-1" as search terms to find relevant literatures (Appendix 1). Two investigators independently reviewed literatures, if disagreement exists, a third investigator would make final decision. Only literatures for original clinical studies including randomized clinical trial, observative studies, and case reports published in English would be included.

The application of ICIs in digestive system cancer

At present, among digestive system malignancies, ICIs have been used for the treatment of EC, GC, CRC, hepatocellular cancer (HCC), PC, and bile duct cancer. In the treatment of EC/GC, CTLA-4 inhibitors have not yet shown satisfactory results (6-8). However, programmed cell death protein 1 (PD-1) and its ligand (PD-L1) inhibitors have shown satisfactory outcomes in numerous studies. The ATTRACTION series of studies confirmed that nivolumab alone or in combination with chemotherapy can modestly improve overall survival (OS), progression-free survival (PFS), and other outcomes in patients with EC, gastroesophageal junction cancer, and GC (9-11). The CheckMate577 study confirmed the value of nivolumab as an adjuvant therapy for stage II–III patients who have undergone surgical treatment (12). The CheckMate 649 study further demonstrated the value of nivolumab combined with chemotherapy as a first-line treatment for advanced gastric, gastroesophageal junction, and esophageal adenocarcinoma (EAC) patients (13). Pembrolizumab has also shown a certain therapeutic effect on EC in a series of KEYNOTE studies. The results of the phase III KEYNOTE 181 study showed that pembrolizumab significantly prolonged OS compared to chemotherapy in patients with PD-L1 positive advanced EC (14). The phase III KEYNOTE 590 study showed that pembrolizumab combined with chemotherapy can significantly prolong patients' OS and PFS (15). The KEYNOTE-811 study showed that for human epidermal growth factor receptor 2 (HER2) positive unresectable or metastatic GC/gastric esophageal junction adenocarcinoma patients, those receiving pembrolizumab + trastuzumab + chemotherapy had significantly better objective response rate (ORR) and complete response rates (CRRs) than the placebo + trastuzumab + chemotherapy group (16). In CRC patients, the benefits of ICIs treatment are mainly limited to those with microsatellite instability-high (MSI-H) or defective mismatch repair (dMMR) (17). The phase II CheckMate-142 study showed that nivolumab combined with low-dose ipilimumab can achieve a satisfactory ORR and disease control rate (DCR) in the treatment of metastatic CRC, with 69% [95% confidence interval (CI): 53% to 82%] and 84% (95% CI: 70.5% to 93.5%), respectively, and a CRR of 13% (18). The results of the phase III KEYNOTE 177 study showed that pembrolizumab significantly prolonged the median PFS and achieved satisfactory overall response (complete or partial response) in MSI-H metastatic CRC patients compared to chemotherapy (19). The relevant guidelines have identified pembrolizumab as the first line standard treatment for MSI-H or dMMR CRC patients (20). In patients with advanced liver cancer, nivolumab can achieve a DCR of 55% and an ORR of 12%, with a median remission period of 9.9 months, indicating that nivolumab has good efficacy (21). However, in CheckMate 459 study, predefined threshold of statistical significance for OS with nivolumab was not met, although nivolumab demonstrated clinical benefit (22). The results of the phase II KEYNOTE-224 study showed that pembrolizumab significantly prolonged OS and PFS compared to placebo for patients with advanced hepatocellular carcinoma (HCC) previously treated with sorafenib (23). Although immunotherapy has shown good efficacy in some tumors, mono-immunotherapy has not improved the survival prognosis of PC patients. The latest research shows that an ICI-based combination treatment scheme can increase the sensitivity of PC to ICI treatment, thus achieving a certain anti-tumor effect (24). In addition, the response rate of PD-1 inhibitor pembrolizumab combined with gemcitabine and albumin-bound paclitaxel in metastatic PC also improved, with the median PFS and OS of 9.1 and 15.0 months, respectively (25). In biliary cancer, whether using nivolumab or pembrolizumab alone or nivolumab combined with chemotherapy, a certain therapeutic effect can still be achieved (26-28). Besides, in an open-label, single-center, phase 3 study, the authors found that the addition of durvalumab improved OS and PFS in patients with advanced biliary tract cancer (29). Overall, ICIs have broad application prospects in malignant tumors of the digestive system and have achieved good results in some tumors. Moreover, the combination of ICIs and other treatment strategies has been shown to be safe and have potential for durable response in some patients.

ICI treatment-related biomarkers

As mentioned earlier, ICIs only have good therapeutic effects in some patients. Based on the mechanism and characteristics of drug action, the efficacy of ICIs can be predicted through long-term clinical research of various biomarkers.

PD-1/PD-L1

PD-L1 is a specific ligand of PD-1, and its binding with PD-1 can activate the immunosuppressive signaling pathway, weaken the cytotoxicity of T cells to tumor cells, and lead to immune escape. PD-1/PD-L1 inhibitors exert a therapeutic effect on tumors by inhibiting this pathway (5). Therefore, PD-L1 positive patients may have better therapeutic effects than PD-L1 negative patients (detailed in the following sections), but the research results have been inconsistent. The ATTRACTION-3 study showed that nivolumab significantly prolonged the OS of patients with advanced esophageal squamous cell carcinoma (ESCC) who had previously received treatment, and this benefit was independent of the tumor PD-L1 score (10). The reasons for the inconsistency in the predictive value of PD-L1 may include the following: (I) differences in PD-L1 evaluation (PD-L1 combined positive score (CPS) *vs.* tumor proportion score (TPS) *vs.* PD-L1 staining of tumor cells vs immune cells) among different studies; (II) lack of a unified threshold for determining PD-L1 positivity across various trials; (III) the expression level of PD-L1 exhibits certain dynamic changes.

Mismatch repair (MMR) defects and MSI

MMR proteins play a key role in the repair of DNA replication errors. One or more dMMR proteins can lead to a decline in DNA repair ability, which can cause spontaneous mutation accumulation of the genome, that finally is very likely to cause MSI (30). Under normal circumstances, MSI has three states, namely highly unstable (MSI-H), low unstable (MSI-L) and stable (MSS). Immune cell PD-L1 expression is significantly higher in MSI-H CRC as compared to MSI-L tumors, with no differences among the different MSI-H molecular subtypes. The recommended screening for defective, DNA MMR includes immunohistochemistry (IHC) and/or MSI test. However, distilling the biological and technological heterogeneity of MSI testing as usable data in clinical practice poses certain difficulties. According to a previous study, somatic mutations may lead to different results in IHC testing of MMR mechanisms for a given germline mutation (31). The dMMR state often corresponds to MSI-H (32). In clinical practice, MMR status is often evaluated by either immunohistochemical detection of four proteins (MLH1, MSH-2, MSH-6, and PMS-2), or detection of MSI status by polymerase chain reaction (PCR) at specific sites. In most cases, there is high consistency between the two methods (33). Some studies have confirmed that MSI-H and dMMR are associated with ICIs in the treatment of digestive system malignancies (34,35).

Tumor mutational burden (TMB)

TMB refers to the number of non-synonymous mutations in the tumor genome. Previously, it was believed that if a tumor had a higher TMB, it could expose more new antigens to the immune system, making it more likely to respond to ICI treatment (36). However, as a biomarker, TMB also has many problems. For example, TMB is not strictly positively correlated with the production of new antigens, and not all mutations can produce new antigens; the evaluation method of TMB and how to divide the high and low thresholds are not clear. At present, because the TMB predicted by exome sequencing and gene panel is not completely equal, the threshold division criteria of TMB detected by different methods have not been unified, which is not conducive to the promotion of TMB indicators.

Application of biomarkers in the treatment of digestive system cancer with ICIs

EC

EC can be histologically divided into ESCC and EAC. A small sample (n=44) study on ESCC patients showed that approximately 44% of patients had PD-L1 or PD-L2 expression, and positive PD-L1 and PD-L2 expression were associated with poor prognosis (37). Research has found that PD-L1 positive expression can be observed in approximately 20% of EC patients (38). The increased expression of PD-L1 is associated with lymph node metastasis, advanced disease, and poor prognosis. The combination of PD-L1 and tumor-infiltrating lymphocytes (TILs) status can be used as a predictive biomarker for ICIs targeted therapy in patients with surgically resected EC (39).

The ATTRACTION-3 study showed that nivolumab

significantly prolonged the OS of patients with advanced ESCC who had previously received treatment, but this benefit was independent of the TPS of tumor PD-L1 (10). Janjigian *et al.* included 37 patients (including metastatic HER2 positive EC, gastroesophageal junction cancer, and GC patients) who received the regimen of pembrolizumab + trastuzumab + capecitabine-oxaliplatin and the DCR reached 100%. According to the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1) standard, the original tumor volume of 35 patients shrank by 20–100% (the remaining 2 patients could not be evaluated). The median PFS was 13 months, and the median OS was 27.3 months (40). The mechanism study found that HER2 positive tumors may recruit more immune cells through chemokines, so as to achieve higher infiltration of T cells and monocytes and higher expression of PD-L1 (41).

In the pembrolizumab KEYNOTE-181 trial of an Asian population, for ESCC patients irrespective of PD-L1 expression level, the median OS in the pembrolizumab group was 10.0 months (95% CI: 8.0–12.2), whereas the median OS of patients with a PD-L1 CPS ≥10 treated with pembrolizumab was 12.5 months (95% CI: 9.1–14.9), suggesting that patients with PD-L1 high expression have a lasting clinical response to pembrolizumab (42). In the KEYNOTE-590 Chinese subgroup study, compared with chemotherapy alone, patients with unresectable locally advanced or metastatic EC can experience significant survival benefits after receiving pembrolizumab combined with chemotherapy, and patients with PD-L1 CPS ≥10 ESCC have greater benefits (15). The above studies all suggest that PD-L1 has certain predictive value in the treatment of ESCC patients with ICIs. It should be noted that the main significance of these studies is to suggest that PD-L1 positive patients are more likely to benefit from ICIs treatment than PD-L1 negative patients, and there is still no correlation between the level of expression and response in PD-L1 positive tumors. On the contrary, some trials have shown no correlation PD-L1 positivity and therapy response. In the ESCORT study of camrelizumab, the expression level of PD-L1 has no significant correlation with the objective remission rate and DCR (43). Yang *et al.* concluded that patients with low or negative PD-L1 expression can also benefit from ICIs immunotherapy (44). In addition, the number of patients receiving ICIs dual drug combination therapy or ICIs combined with surgical/chemotherapy regimens is gradually increasing, and the predictive effect of PD-L1 is also affected. Therefore, the predictive value of PD-L1 may be limited at this time.

GC

Research has shown that MSI-H/dMMR is present in 8–25% of GC patients, and MSI-H type GC is associated with better prognosis compared to MSI-L tumors (45). The Epstein-Barr virus (EBV) positive GC subgroup was shown to have an increased expression of PD-L1 in tumors and immune cells, as well as enriched interferon-γ (IFN-γ); $CD8^+$ TILs were enriched. Therefore, this group was viewed as the most effective potential group for ICIs treatment (46). A meta-analysis of GC patients showed that PD-L1 expression is associated with shorter OS (47). In the ATTRACTION-2 and CheckMate032 trials, it was shown that the survival benefits of ICIs treatment in GC patients were not related to the PD-L1 status (9,48). Therefore, the significance of PD-L1 as a predictive biomarker for GC still needs further study. In a phase II study of pembrolizumab, there was a positive correlation between the TMB level and the efficacy of ICIs. A higher TMB value was associated with a higher overall response rate (49). A recent study found that high TMB may be a predictive marker for OS in patients with advanced GC who receive toripalimab as a monotherapy. Patients with TMB-high (TMB-H) had a superior OS of 14.6 months as compared to TMB-low (TMB-L) with OS 4.0 months [hazard ratio (HR) =0.48, 96% CI: 0.24–0.96, P=0.038] (50). Although there is evidence that TILs can help determine prognosis, the exact predictive value of TILs in immunotherapy for GC is still unclear. A previous study has shown that high-density TILs are closely related to PD-L1 expression and MSI-H in GC, but different types of lymphocytes have different prognoses and significance (51). In addition, invasive edge or central infiltration may have different densities of T cells and may lead to different outcomes (52). Therefore, further exploration is needed regarding the use of TILs as biomarkers to predict the efficacy of ICIs in GC.

In a multicenter, stage Ib/II clinical study of advanced GC (NCT02915432), 58 chemotherapy resistant advanced GC patients who received toripalimab treatment were defined as TMB-H according to the top 20% of TMB evaluated by whole exome sequencing (WES), whereas the remaining patients were defined as TMB-L. This study found that the TMB-H group as discussed previously had better indicators such as ORR, OS, and PFS compared to the TMB-L group (50). Folprecht *et al.* also believe that high TMB may be a biomarker for predicting the efficacy of PD-1 antibodies in advanced GC (53).

In 2020, a study used 425 gene panels to conduct blood

tests on 46 patients with advanced GC who received PD-1 monoclonal antibody treatment. The results showed that patients with a decrease in max variant allele frequency (maxVAF) of more than 25% after treatment had longer median PFS and higher ORR, and patients without circulating tumor DNA (ctDNA) detected in serum also had longer median PFS compared to those with ctDNA (54). EBV-associated GC (EBVaGC) is an independent subtype with an average onset age of 58 years, of which 71% of cases are male and often occur in the proximal part of the stomach (cardia and body) (55). EBVaGC patients have stronger immune infiltration, manifested as higher $CD8^+$ T cells and fewer $CD204^+$ macrophages (56,57). A 2020 study further demonstrated a strong correlation between EBV positivity and PD-L1 expression level (58). Other studies have yielded different results. An observational study showed that only 16.7% of EBV positive GC patients had partial remission after receiving PD-L1 single drug treatment (59). In another study, 100% (n=6) of patients with EBV positive GC who were treated with camrelizumab did not respond to treatment, and 83% (n=5) of patients died at the end of the study (60). Currently, research on the impact of EBV on ICI treatment is limited. In studies where EBV positivity has been strongly correlated with better ICI treatment efficacy, most EBV positive patients were PD-L1 positive (56-60), so the results may be affected by PD-L1 positivity.

The *HER2* gene encodes a transmembrane receptor-like HER2 protein, which is amplified or overexpressed in 7–34% of GC patients (61). Satoh *et al.* found in a subgroup analysis based on ATTRACTION-2 that the ORR of GC patients receiving nivolumab who had a history of trastuzumab (HER2 targeted inhibitor) was higher than that of patients without a history of trastuzumab (62). For HER2 positive GC, the anti HER2 + ICI + chemotherapy regimen also achieved good results. For example, in the KEYNOTE-811 study, 433 HER2 positive GC/gastroesophageal junction cancer patients were divided into two groups—pembrolizumab + trastuzumab + chemotherapy and placebo + trastuzumab + chemotherapy. The results showed that the ORR of the ICI treatment group was significantly higher than that of the placebo group, and the continuous responsivity of both 6 and 9 months was higher in the ICI treatment group, whereas the incidence of adverse events of the two groups was similar (63).

In the GC KEYNOTE-059 trial, an 18 gene panels of T cell inflammatory gene expression was used for scoring, and the scores of responders were significantly higher than those of non-responders (64).

A study by Fu *et al.* has also shown that an increase in FOXP3 Tregs in gastric tumors is often accompanied by a decrease in $CD8^+$ T cell infiltration, which is associated with poor prognosis in patients (65). Murakami *et al.* found that among patients with unresectable GC, those with a low neutrophil/lymphocyte ratio had a longer median survival time (66).

CRC

Research has found that in advanced CRC, the MSI-H/dMMR status is associated with a more significant therapeutic effect of PD-1 inhibitors (67). Based on the characteristics of TMB in CRC, Zang *et al.* found a significant correlation between TMB-H and dMMR (68). Fabrizio *et al.* found that TMB's utility to distinguish CRC subgroups responsive to ICIs exceeded that of dMMR (69). In the REGONIVO trial, a study including 25 CRC patients showed that the OS time of patients in the TMB-H group was significantly higher than that in the TMB-L group (70). All of the above demonstrate that TMB can serve as an independent molecular marker for ICIs in treating CRC patients. Approximately 53% of CRC patients have positive expression of PD-L1, but its use as a predictive biomarker for ICIs treatment still needs further confirmation (71). In the CRC group of MSI-H/dMMR, PD-L1 failed to predict the efficacy of nivolumab alone or in combination with CTLA-4 inhibitors. In addition, there was no correlation between PD-L1 expression and patient PFS and OS in the CRC group of the KEYNOTE-164 study (35).

Other studies have shown that gut microbiota can serve as a barrier against pathogen invasion, and stimulate T cells to transport to CRC tumor tissue. At the same time, specific gut bacteria can affect the therapeutic effect of ICIs, making gut microbiota a predictive factor for CRC immunotherapy (72). It is also suggested that cytokine receptors such as interleukin 2 receptor subunit beta (IL2RB) are also related to the treatment of CRC with ICIs (73). Another study showed that FOXP3 expression in CRC patients is associated with PD-1, and high FOXP3 expression indicates poor efficacy (74).

In the VOLTAGE-A study, 37 patients with locally advanced rectal cancer who achieved microsatellite stability after sequential use of preoperative chemotherapy and radiotherapy, five courses of nivolumab, and radical surgery were analyzed. It was found that the higher the ratio of

infiltrating $CD8^+$ T cells to effector Tregs cells in tumor tissue, the better the efficacy. In addition, PD-L1 positivity, Ki-67 expression of tumor infiltrating $CD8^+$ T cells, and CMS1 and CMS3 typing of CRC also predicted high response to chemoradiotherapy (75). In a prospective phase 2 study, all 12 patients (100%) with dMMR stage II or III rectal adenocarcinoma had a clinical complete response when treated with dostarlimab, with no evidence of tumor on imaging examination (76). In terms of gut microbiota and metabolites, Wang *et al.* found that in patients with metastatic CRC who were treated with regorafenib and toripalimab monoclonal antibody, patients with higher fecal *Clostridium* abundance before treatment had shorter PFS (77). Chen *et al.* reported a case of late-stage CRC with POLE F367S mutation. After receiving pembrolizumab monotherapy, the median PFS reached 49 months, the tumor burden significantly decreased, and the microsatellite status remained stable (78). The characteristics of POLE mutated CRC include increased infiltration of CD8+lymphocytes and the presence of cytotoxic T cell markers, which are similar to immunogenic MSI-H cancer. Based on tumor immunogenicity, POLE mutations suggest a relatively better prognosis for this CRC subgroup. The evaluation of POLE mutations helps to stratify the risk of CRC, and patients with POLE mutations may benefit more from ICI therapy (79). Droeser *et al.* found a correlation between the expression of PD-L1 and the improvement of OS in patients with MMR gene normal CRC (80). In Berntsson *et al.*'s study, high expression of PD-L1 did not predict the prognosis and efficacy of CRC (81). Kong *et al.* believe that the expression of PD-L1 in different regions has different effects on disease-free survival (DFS) in CRC, and the high expression of PD-L1 in TILs is significantly correlated with DFS prolongation in CRC patients (82). Therefore, the expression of PD-L1 in specific regions (such as TIL PD-L1) may be a potential predictive indicator of the effectiveness of ICI treatment in CRC patients (82).

The KEYNOTE016 clinical trial conducted in 2015 evaluated the efficacy of pembrolizumab in CRC patients with dMMR and complete MMR function (pMMR), as well as in non-CRC patients with dMMR. The results showed that at 20 weeks, the ORR of dMMR CRC patients and other dMMR non-CRC patients was 40% (4/10) and 71% (5/7), respectively, with PFS rates of 78% and 67%. The ORR of CRC patients with pMMR was 0%, and the PFS rate was only 11% (83). In a phase II clinical trial, it was found that 74 metastatic MSI-H/dMMR CRC patients had an ORR of 31.1% against nivolumab, with a 12-month PFS rate of 50.4% and an OS rate of 73.4%, respectively (84). The same research group also conducted a study on the combined treatment of nivolumab and ipilimumab in 119 previously treated MSI-H/dMMR CRC patients. The results showed that the patient's ORR was 55%, and the 12-month PFS and OS rates were 71% and 85%, respectively (85).

Liver cancer

Research shows that about 20% of HCC patients have PD-L1 overexpression, and PD-L1 expression will inhibit the function of T cells in the liver tumor microenvironment (TME), which is related to poor prognosis (86,87). However, in the KEYNOTE-240 study, the effect of pembrolizumab second-line treatment on advanced HCC was not related to PD-L1 or the expression of immune cells (23). The proportion of patients with TMB-H or dMMR/MSI-H in HCC is relatively low, and TMB is not associated with predicted new antigen ratios or immunogenic expression patterns. Therefore, the value of TMB as a predictive marker is not yet clear (88). Based on the important role of gut microbiota in innate and adaptive immune regulation, as well as the presence of the gut-liver axis, there is increasing evidence that gut microbiota affects immunotherapy for HCC (89). Zheng *et al.* showed that there are significant differences in the diversity and composition of gut microbiota between responders and non-responders in patients with HCC receiving ICIs treatment, and to some extent, dynamic changes in gut microbiota characteristics can predict the efficacy of immunotherapy (90).

A phase II trial analysis of 29 patients with advanced HCC treated with pembrolizumab showed that baseline plasma transforming growth factor-β (TGF-β) cytokine levels were significantly higher in non-responders than in responders, and that a baseline plasma TGF-β level <200 pg/mL is an effective predictor of OS and PFS, indicating that baseline plasma TGF-β is a reliable biomarker to predict the response to pembrolizumab (91). A clinical study using nivolumab to treat advanced liver cancer found that the lower PD-1 positive rate of peripheral blood B cells before treatment and the higher PD-L1 positive rate of monocytes after treatment were related to the disease control of HCC (92). A trial of combining anti CTLA-4 treatment and local treatment in patients with liver cancer found that $CD4^+PD\text{-}1^+$ cells in peripheral blood monocytes of patients with effective treatment showed high expression rate (93).

Dharmapuri *et al.* found that the neutrophil/lymphocyte ratio and platelet/lymphocyte ratio significantly decreased after nivolumab treatment in liver cancer patients compared to before treatment (94). In a study targeting 42 patients with unresectable primary liver cancer, ctDNA was more closely associated with tumor burden and could more sensitively predict treatment outcomes compared to alpha fetoprotein (AFP) (95). A prospective phase II clinical trial targeting patients receiving pembrolizumab treatment for advanced HCC showed that changes in ctDNA levels at baseline and post baseline were associated with OS and PFS (96). Further research is needed to explore ctDNA as a biomarker for predicting the efficacy of liver cancer immunotherapy (97). Winograd *et al.* have shown that the presence of PD-L1$^+$ circulating tumor cells is associated with better immunotherapy efficacy, and it is also a biomarker for overall poor prognosis. Compared to patients who failed treatment, all patients for whom treatment was effective expressed PD-L1$^+$ circulating tumor cells (98). A study examining 34 proteins in baseline plasma of 34 patients receiving atezolizumab/bevacizumab treatment found that plasma interleukin 6 (IL-6) and IFN-α level is a significant predictor of non-responders, and PFS and OS are significantly inferior for individuals with high IL-6 levels compared to those with low IL-6 levels (99). A study of 297 liver cancer patients treated with atezolizumab/bevacizumab found that compared to patients with lower levels of AFP, patients with high levels of AFP (≥100 ng/dL) had poorer prognosis and shorter PFS (100). Another recent study on liver cancer patients treated with atezolizumab/bevacizumab found that AFP decrease ≥75% and AFP increase ≤10% were used as the boundary values for identifying responders and obtaining disease controllers, both of which were related to longer OS and PFS, especially in patients with a history of hepatitis B (101). However, the CheckMate 040 study found that although baseline AFP <400 μg/L was associated with increased OS compared to baseline AFP ≥400 μg/L, the ORR and DCR of both were similar (102).

Zhu *et al.* found that after pembrolizumab was used to treat HCC patients, the ORR of patients with PD-L1 CPS ≥1% in various types of cells was 25%, and the proportion of patients with PFS prolongation was as high as 83%, yet there was no significant correlation between TPS and treatment response (103). In another study, after immunohistochemical detection of HCC patients treated with nivolumab, it was found that the median OS of patients with PD-L1 CPS ≥1% was significantly longer than that of patients with PD-L1 CPS <1% (104). In addition, a global multicenter trial showed that the PD-L1 positive rate in HCC patients treated with tislelizumab reached 23.1% for ORR and 57.7% for DCR in the ≥1% group (105). However, Liu *et al.* found that the expression of PD-L1 was negatively correlated with patients' OS and DFS (106). In a phase I/II clinical trial, it was found that patients with a PD-L1 positive rate of <10% had an ORR of 19% and a DCR of 63%, whereas in patients with a PD-L1 positive rate of ≥10%, both ORR and DCR were 0 (107).

Based on results of studies mentioned before, we found that PD-L1 was correlated with better response rate in some studies and worse in others. The underlying reason might include as follows: difference of endpoint or outcome assessment, different types of immunotherapies, difference in patients between studies.

Pancreatic carcinoma

Pancreatic ductal adenocarcinoma (PDAC) is the most common subtype of PC, accounting for 90% of all PC cases (108). PC usually lacks T cell infiltration, which may lead to poor effect of single ICIs treatment. In PDAC, TMB has been shown to be positively correlated with PD-L1 expression. The average TMB of PC patients is relatively low, and about 1% of patients have TMB-H (109). Since PDAC is a malignant tumor with a complex immune mechanism, its related TMB has become the focus of immunotherapy (110).

Biliary tract cancer

Biliary carcinoma is a tumor rich in fibrous stroma, and its TME contains a large number of immunosuppressive cells. Cholangiocarcinoma (CCA) with high abundance of CD8$^+$ T cell infiltration and high expression of immune checkpoint molecules can be called an immune "hot" tumor, which has a high response rate to ICIs treatment. However, a previous study found that the response rate of CCA to single drug ICIs treatment was low, suggesting that most CCA cases were immune "cold" tumors lacking T-cell infiltration (111). In the phase II clinical study of KEYNOTE 158, among 104 patients with advanced CCA (61 patients with PD-L1 positive), the ORR was only 5.8% (6/104), of which 6 patients were in partial response (including 1 patient with PD-L1 negative), 17 patients had stable disease, and the effective time was 6.2 months to >15 months (2 patients). In these studies, there was no statistically significant difference in PFS time and median

survival time between PD-L1 positive and negative patients (112,113). In a phase II clinical study of nivolumab in the treatment of patients with advanced CCA, the ORR of 46 patients was 22% (10/46), which was partial response, and the DCR was 59% (27/46). For evaluable patients, the disease PFS time was 3.68 months, with a median OS of 14.24 months. PD-L1 positivity in tumors was associated with prolonged PFS. However, all the responders had MMR-proficient disease (26). In a clinical study on nivolumab in Japan, the ORR of a single drug was only 3% (1/30), with a median OS 5.2 months and a disease PFS of 1.4 months (28). In one study, 652 CCA specimens were analyzed, and the positive expression rate of PD-L1 was 8.6% (56/652). Among known biomarkers, PD-L1 positive tumors had higher TMB (10.7%, 6/56) and increased MSI-H (7.1%, 4/56) than PD-L1 negative tumors; meanwhile, the mutation rates of *BRAF*, *BRCA2*, *RNF43*, and *TP53* in PD-L1 positive tumors were also higher (114). The above research suggests that some patients with CCA may have unrecognized biomarkers. Liddell *et al.* analyzed the clinical data of 47 patients with advanced biliary tract tumors, all of whom received at least one type of ICIs treatment. Biomarker analysis revealed improved PFS (but not OS) in patients with multiple tumors >5 mutations per megabase (115). Another study found that when compared with patients with other diseases of early stage, *KRAS* and *TP53* mutations were more often detected in patients with advanced-stage biliary tract cancer. Specifically, CCA patients with both *KRAS* and *TP53* mutations have better response to immunotherapy and prognosis compared with patients with single *KRAS* mutations. However, CCA had more mutations of KRAS signaling associated genes than gall bladder cancer, which lead to poor immunotherapy outcomes. Based on these findings, the authors developed a genomic signature including 11 genes with good ability to predict prognosis and immunotherapy outcomes in both CCA and gall bladder cancer(116). Yoon *et al.* found that in patients treated with immunotherapy, tumors with KRAS alteration and chromosomal instability were often resistant to immunotherapy, leading to no benefit from PD-1/PD-L1 blockade. Low TIL density in tumors with these characteristics was associated with immune-suppressive TMEs, whereas high intratumoral TIL density predicted a favorable response to immunotherapy (117).

Conclusions and future perspectives

According to existing clinical research results, there are multiple biomarkers that can be used for predicting and monitoring the efficacy and adverse events of ICIs in the treatment of digestive system malignant tumors. At the same time, there may be some new biomarkers in different malignant tumor tissues and circulating blood, which warrant further exploration in future research, in order to provide better means for selecting suitable patients for ICIs treatment and monitoring efficacy and adverse events. Further, predictive models based on the biomarkers discussed in this review are worth investigating in the future, so as to provide accurate prediction on efficacy and safety in ICIs treatment.

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Footnote

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References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Arnold M, Abnet CC, Neale RE, et al. Global Burden of 5 Major Types of Gastrointestinal Cancer. Gastroenterology 2020;159:335-349.e15.
3. Osseis M, Nehmeh WA, Rassy N, et al. Surgery for T4 Colorectal Cancer in Older Patients: Determinants of Outcomes. J Pers Med 2022;12:1534.
4. Boussios S, Rassy E, Samartzis E, et al. Melanoma of unknown primary: New perspectives for an old story. Crit Rev Oncol Hematol 2021;158:103208.
5. Blank C, Brown I, Peterson AC, et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res 2004;64:1140-5.
6. Bang YJ, Cho JY, Kim YH, et al. Efficacy of Sequential Ipilimumab Monotherapy versus Best Supportive Care for Unresectable Locally Advanced/Metastatic Gastric or Gastroesophageal Junction Cancer. Clin Cancer Res 2017;23:5671-8.
7. Ralph C, Elkord E, Burt DJ, et al. Modulation of lymphocyte regulation for cancer therapy: a phase II trial of tremelimumab in advanced gastric and esophageal adenocarcinoma. Clin Cancer Res 2010;16:1662-72.
8. Shitara K, Ajani JA, Moehler M, et al. Nivolumab plus chemotherapy or ipilimumab in gastro-oesophageal cancer. Nature 2022;603:942-8.
9. Kang YK, Boku N, Satoh T, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;390:2461-71.
10. Kato K, Cho BC, Takahashi M, et al. Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol 2019;20:1506-17.
11. Kang YK, Chen LT, Ryu MH, et al. Nivolumab plus chemotherapy versus placebo plus chemotherapy in patients with HER2-negative, untreated, unresectable advanced or recurrent gastric or gastro-oesophageal junction cancer (ATTRACTION-4): a randomised, multicentre, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2022;23:234-47.
12. Kelly RJ, Ajani JA, Kuzdzal J, et al. Adjuvant Nivolumab in Resected Esophageal or Gastroesophageal Junction Cancer. N Engl J Med 2021;384:1191-203.
13. 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;398:27-40.
14. Kojima T, Shah MA, Muro K, et al. Randomized Phase III KEYNOTE-181 Study of Pembrolizumab Versus Chemotherapy in Advanced Esophageal Cancer. J Clin Oncol 2020;38:4138-48.
15. Sun JM, Shen L, Shah MA, et al. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study. Lancet 2021;398:759-71.
16. Chung HC, Bang YJ, S Fuchs C, et al. First-line pembrolizumab/placebo plus trastuzumab and chemotherapy in HER2-positive advanced gastric cancer: KEYNOTE-811. Future Oncol 2021;17:491-501.
17. Nguyen M, Tipping Smith S, Lam M, et al. An update on the use of immunotherapy in patients with colorectal cancer. Expert Rev Gastroenterol Hepatol 2021;15:291-304.
18. Lenz HJ, Van Cutsem E, Luisa Limon M, et al. First-Line Nivolumab Plus Low-Dose Ipilimumab for Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: The Phase II CheckMate 142 Study. J Clin Oncol 2022;40:161-70.
19. André T, Shiu KK, Kim TW, et al. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. N Engl J Med 2020;383:2207-18.
20. Benson AB, Venook AP, Al-Hawary MM, et al. Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw

2021;19:329-59.
21. Kudo M, Matilla A, Santoro A, et al. CheckMate 040 cohort 5: A phase I/II study of nivolumab in patients with advanced hepatocellular carcinoma and Child-Pugh B cirrhosis. J Hepatol 2021;75:600-9.
22. Yau T, Park JW, Finn RS, et al. Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol 2022;23:77-90.
23. Finn RS, Ryoo BY, Merle P, et al. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. J Clin Oncol 2020;38:193-202.
24. Kamath SD, Kalyan A, Kircher S, et al. Ipilimumab and Gemcitabine for Advanced Pancreatic Cancer: A Phase Ib Study. Oncologist 2020;25:e808-15.
25. Weiss GJ, Blaydorn L, Beck J, et al. Phase Ib/II study of gemcitabine, nab-paclitaxel, and pembrolizumab in metastatic pancreatic adenocarcinoma. Invest New Drugs 2018;36:96-102.
26. Kim RD, Chung V, Alese OB, et al. A Phase 2 Multi-institutional Study of Nivolumab for Patients With Advanced Refractory Biliary Tract Cancer. JAMA Oncol 2020;6:888-94.
27. 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;38:1-10.
28. Ueno M, Ikeda M, Morizane C, et al. Nivolumab alone or in combination with cisplatin plus gemcitabine in Japanese patients with unresectable or recurrent biliary tract cancer: a non-randomised, multicentre, open-label, phase 1 study. Lancet Gastroenterol Hepatol 2019;4:611-21.
29. Oh DY, He AR, Qin S, et al. 78P Updated overall survival (OS) from the phase III TOPAZ-1 study of durvalumab (D) or placebo (PBO) plus gemcitabine and cisplatin (+ GC) in patients (pts) with advanced biliary tract cancer (BTC). Ann Oncol 2022;33:S1462-3.
30. Bateman AC. DNA mismatch repair proteins: scientific update and practical guide. J Clin Pathol 2021;74:264-8.
31. Adeleke S, Haslam A, Choy A, et al. Microsatellite instability testing in colorectal patients with Lynch syndrome: lessons learned from a case report and how to avoid such pitfalls. Per Med 2022;19:277-86.
32. Zhang CM, Lv JF, Gong L, et al. Role of Deficient Mismatch Repair in the Personalized Management of Colorectal Cancer. Int J Environ Res Public Health 2016;13:892.
33. Hatch SB, Lightfoot HM Jr, Garwacki CP, et al. Microsatellite instability testing in colorectal carcinoma: choice of markers affects sensitivity of detection of mismatch repair-deficient tumors. Clin Cancer Res 2005;11:2180-7.
34. Chao J, Fuchs CS, Shitara K, et al. Assessment of Pembrolizumab Therapy for the Treatment of Microsatellite Instability-High Gastric or Gastroesophageal Junction Cancer Among Patients in the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 Clinical Trials. JAMA Oncol 2021;7:895-902.
35. Le DT, Kim TW, Van Cutsem E, et al. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: KEYNOTE-164. J Clin Oncol 2020;38:11-9.
36. Johnson DB, Frampton GM, Rioth MJ, et al. Targeted Next Generation Sequencing Identifies Markers of Response to PD-1 Blockade. Cancer Immunol Res 2016;4:959-67.
37. Derks S, Nason KS, Liao X, et al. Epithelial PD-L2 Expression Marks Barrett's Esophagus and Esophageal Adenocarcinoma. Cancer Immunol Res 2015;3:1123-9.
38. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther 2015;14:847-56.
39. Yagi T, Baba Y, Ishimoto T, et al. PD-L1 Expression, Tumor-infiltrating Lymphocytes, and Clinical Outcome in Patients With Surgically Resected Esophageal Cancer. Ann Surg 2019;269:471-8.
40. Janjigian YY, Maron SB, Chatila WK, et al. First-line pembrolizumab and trastuzumab in HER2-positive oesophageal, gastric, or gastro-oesophageal junction cancer: an open-label, single-arm, phase 2 trial. Lancet Oncol 2020;21:821-31.
41. Triulzi T, Forte L, Regondi V, et al. HER2 signaling regulates the tumor immune microenvironment and trastuzumab efficacy. Oncoimmunology 2019;8:e1512942.
42. Cao Y, Qin S, Luo S, et al. Pembrolizumab versus chemotherapy for patients with esophageal squamous cell carcinoma enrolled in the randomized KEYNOTE-181 trial in Asia. ESMO Open 2022;7:100341.
43. Huang J, Xu J, Chen Y, et al. Camrelizumab versus investigator's choice of chemotherapy as second-line therapy for advanced or metastatic oesophageal squamous cell carcinoma (ESCORT): a multicentre, randomised,

open-label, phase 3 study. Lancet Oncol 2020;21:832-42.
44. Yang H, Wang K, Wang T, et al. The Combination Options and Predictive Biomarkers of PD-1/PD-L1 Inhibitors in Esophageal Cancer. Front Oncol 2020;10:300.
45. Di Bartolomeo M, Morano F, Raimondi A, et al. Prognostic and Predictive Value of Microsatellite Instability, Inflammatory Reaction and PD-L1 in Gastric Cancer Patients Treated with Either Adjuvant 5-FU/LV or Sequential FOLFIRI Followed by Cisplatin and Docetaxel: A Translational Analysis from the ITACA-S Trial. Oncologist 2020;25:e460-8.
46. Derks S, Liao X, Chiaravalli AM, et al. Abundant PD-L1 expression in Epstein-Barr Virus-infected gastric cancers. Oncotarget 2016;7:32925-32.
47. Gu L, Chen M, Guo D, et al. PD-L1 and gastric cancer prognosis: A systematic review and meta-analysis. PLoS One 2017;12:e0182692.
48. Janjigian YY, Bendell J, Calvo E, et al. CheckMate-032 Study: Efficacy and Safety of Nivolumab and Nivolumab Plus Ipilimumab in Patients With Metastatic Esophagogastric Cancer. J Clin Oncol 2018;36:2836-44.
49. Kim ST, Cristescu R, Bass AJ, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med 2018;24:1449-58.
50. Wang F, Wei XL, Wang FH, et al. Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD-1 antibody in phase Ib/II clinical trial NCT02915432. Ann Oncol 2019;30:1479-86.
51. Kawazoe A, Kuwata T, Kuboki Y, et al. Clinicopathological features of programmed death ligand 1 expression with tumor-infiltrating lymphocyte, mismatch repair, and Epstein-Barr virus status in a large cohort of gastric cancer patients. Gastric Cancer 2017;20:407-15.
52. Pagès F, Mlecnik B, Marliot F, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 2018;391:2128-39.
53. Folprecht G. Tumor mutational burden as a new biomarker for PD-1 antibody treatment in gastric cancer. Cancer Commun (Lond) 2019;39:74.
54. Jin Y, Chen DL, Wang F, et al. The predicting role of circulating tumor DNA landscape in gastric cancer patients treated with immune checkpoint inhibitors. Mol Cancer 2020;19:154.
55. Camargo MC, Kim KM, Matsuo K, et al. Anti-Helicobacter pylori Antibody Profiles in Epstein-Barr virus (EBV)-Positive and EBV-Negative Gastric Cancer. Helicobacter 2016;21:153-7.
56. Zhang M, Hu S, Min M, et al. Dissecting transcriptional heterogeneity in primary gastric adenocarcinoma by single cell RNA sequencing. Gut 2021;70:464-75.
57. Kim SY, Park C, Kim HJ, et al. Deregulation of immune response genes in patients with Epstein-Barr virus-associated gastric cancer and outcomes. Gastroenterology 2015;148:137-147.e9.
58. Liu X, Choi MG, Kim K, et al. High PD-L1 expression in gastric cancer (GC) patients and correlation with molecular features. Pathol Res Pract 2020;216:152881.
59. Xie T, Liu Y, Zhang Z, et al. Positive Status of Epstein-Barr Virus as a Biomarker for Gastric Cancer Immunotherapy: A Prospective Observational Study. J Immunother 2020;43:139-44.
60. Sun YT, Guan WL, Zhao Q, et al. PD-1 antibody camrelizumab for Epstein-Barr virus-positive metastatic gastric cancer: a single-arm, open-label, phase 2 trial. Am J Cancer Res 2021;11:5006-15.
61. Lei YY, Huang JY, Zhao QR, et al. The clinicopathological parameters and prognostic significance of HER2 expression in gastric cancer patients: a meta-analysis of literature. World J Surg Oncol 2017;15:68.
62. Satoh T, Kang YK, Chao Y, et al. Exploratory subgroup analysis of patients with prior trastuzumab use in the ATTRACTION-2 trial: a randomized phase III clinical trial investigating the efficacy and safety of nivolumab in patients with advanced gastric/gastroesophageal junction cancer. Gastric Cancer 2020;23:143-53.
63. Janjigian YY, Kawazoe A, Yañez P, et al. The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. Nature 2021;600:727-30.
64. Fuchs CS, Doi T, Jang RW, et al. Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer: Phase 2 Clinical KEYNOTE-059 Trial. JAMA Oncol 2018;4:e180013.
65. Fu J, Xu D, Liu Z, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology 2007;132:2328-39.
66. Murakami Y, Saito H, Shimizu S, et al. Neutrophil-to-Lymphocyte Ratio as a Prognostic Indicator in Patients With Unresectable Gastric Cancer. Anticancer Res 2019;39:2583-9.
67. Bai R, Lv Z, Xu D, et al. Predictive biomarkers for cancer

immunotherapy with immune checkpoint inhibitors. Biomark Res 2020;8:34.
68. Zang YS, Dai C, Xu X, et al. Comprehensive analysis of potential immunotherapy genomic biomarkers in 1000 Chinese patients with cancer. Cancer Med 2019;8:4699-708.
69. Fabrizio DA, George TJ Jr, Dunne RF, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. J Gastrointest Oncol 2018;9:610-7.
70. Fukuoka S, Hara H, Takahashi N, et al. Regorafenib Plus Nivolumab in Patients With Advanced Gastric or Colorectal Cancer: An Open-Label, Dose-Escalation, and Dose-Expansion Phase Ib Trial (REGONIVO, EPOC1603). J Clin Oncol 2020;38:2053-61.
71. Li Y, He M, Zhou Y, et al. The Prognostic and Clinicopathological Roles of PD-L1 Expression in Colorectal Cancer: A Systematic Review and Meta-Analysis. Front Pharmacol 2019;10:139.
72. Mager LF, Burkhard R, Pett N, et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. Science 2020;369:1481-9.
73. Alderdice M, Craig SG, Humphries MP, et al. Evolutionary genetic algorithm identifies IL2RB as a potential predictive biomarker for immune-checkpoint therapy in colorectal cancer. NAR Genom Bioinform 2021;3:lqab016.
74. Loddenkemper C, Nagorsen D, Zeitz M. Foxp3 and microsatellite stability phenotype in colorectal cancer. Gut 2008;57:725-6.
75. Tojo M, Horie H, Koinuma K, et al. Programmed cell death ligand 1 expression on monocytes is inversely correlated with tumour response to preoperative chemoradiotherapy for locally advanced rectal cancer. Colorectal Dis 2022;24:1140-9.
76. Cercek A, Lumish M, Sinopoli J, et al. PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. N Engl J Med 2022;386:2363-76.
77. Wang F, He MM, Yao YC, et al. Regorafenib plus toripalimab in patients with metastatic colorectal cancer: a phase Ib/II clinical trial and gut microbiome analysis. Cell Rep Med 2021;2:100383.
78. Chen J, Lou H. Complete Response to Pembrolizumab in Advanced Colon Cancer Harboring Somatic POLE F367S Mutation with Microsatellite Stability Status: A Case Study. Onco Targets Ther 2021;14:1791-6.
79. Boussios S, Ozturk MA, Moschetta M, et al. The Developing Story of Predictive Biomarkers in Colorectal Cancer. J Pers Med 2019;9:12.
80. Droeser RA, Hirt C, Viehl CT, et al. Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer 2013;49:2233-42.
81. Berntsson J, Eberhard J, Nodin B, et al. Expression of programmed cell death protein 1 (PD-1) and its ligand PD-L1 in colorectal cancer: Relationship with sidedness and prognosis. Oncoimmunology 2018;7:e1465165.
82. Kong P, Wang J, Song Z, et al. Circulating Lymphocytes, PD-L1 Expression on Tumor-infiltrating Lymphocytes, and Survival of Colorectal Cancer Patients with Different Mismatch Repair Gene Status. J Cancer 2019;10:1745-54.
83. Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. BMJ 2018;362:k3529.
84. Marcus L, Lemery SJ, Keegan P, et al. FDA Approval Summary: Pembrolizumab for the Treatment of Microsatellite Instability-High Solid Tumors. Clin Cancer Res 2019;25:3753-8.
85. Overman MJ, Lonardi S, Wong KYM, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J Clin Oncol 2018;36:773-9.
86. Jung HI, Jeong D, Ji S, et al. Overexpression of PD-L1 and PD-L2 Is Associated with Poor Prognosis in Patients with Hepatocellular Carcinoma. Cancer Res Treat 2017;49:246-54.
87. Kim HD, Song GW, Park S, et al. Association Between Expression Level of PD1 by Tumor-Infiltrating CD8(+) T Cells and Features of Hepatocellular Carcinoma. Gastroenterology 2018;155:1936-1950.e17.
88. Dhanasekaran R, Nault JC, Roberts LR, et al. Genomic Medicine and Implications for Hepatocellular Carcinoma Prevention and Therapy. Gastroenterology 2019;156:492-509.
89. Schwabe RF, Greten TF. Gut microbiome in HCC - Mechanisms, diagnosis and therapy. J Hepatol 2020;72:230-8.
90. Zheng Y, Wang T, Tu X, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. J Immunother Cancer 2019;7:193.
91. Feun LG, Li YY, Wu C, et al. Phase 2 study of pembrolizumab and circulating biomarkers to predict anticancer response in advanced, unresectable hepatocellular carcinoma. Cancer 2019;125:3603-14.
92. Hung YP, Shao YY, Lee JM, et al. Potential of circulating immune cells as biomarkers of nivolumab treatment

efficacy for advanced hepatocellular carcinoma. J Chin Med Assoc 2021;84:144-50.
93. Agdashian D, ElGindi M, Xie C, et al. The effect of anti-CTLA4 treatment on peripheral and intra-tumoral T cells in patients with hepatocellular carcinoma. Cancer Immunol Immunother 2019;68:599-608.
94. Dharmapuri S, Özbek U, Lin JY, et al. Predictive value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in advanced hepatocellular carcinoma patients treated with anti-PD-1 therapy. Cancer Med 2020;9:4962-70.
95. Zhao W, Qiu L, Liu H, et al. Circulating tumor DNA as a potential prognostic and predictive biomarker during interventional therapy of unresectable primary liver cancer. J Gastrointest Oncol 2020;11:1065-77.
96. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nat Cancer 2020;1:873-81.
97. von Felden J, Craig AJ, Garcia-Lezana T, et al. Mutations in circulating tumor DNA predict primary resistance to systemic therapies in advanced hepatocellular carcinoma. Oncogene 2021;40:140-51.
98. Winograd P, Hou S, Court CM, et al. Hepatocellular Carcinoma-Circulating Tumor Cells Expressing PD-L1 Are Prognostic and Potentially Associated With Response to Checkpoint Inhibitors. Hepatol Commun 2020;4:1527-40.
99. Myojin Y, Kodama T, Sakamori R, et al. Interleukin-6 Is a Circulating Prognostic Biomarker for Hepatocellular Carcinoma Patients Treated with Combined Immunotherapy. Cancers (Basel) 2022;14:883.
100. Hatanaka T, Kakizaki S, Hiraoka A, et al. Prognostic impact of C-reactive protein and alpha-fetoprotein in immunotherapy score in hepatocellular carcinoma patients treated with atezolizumab plus bevacizumab: a multicenter retrospective study. Hepatol Int 2022;16:1150-60.
101. Zhu AX, Dayyani F, Yen CJ, et al. Alpha-Fetoprotein as a Potential Surrogate Biomarker for Atezolizumab + Bevacizumab Treatment of Hepatocellular Carcinoma. Clin Cancer Res 2022;28:3537-45.
102. Sangro B, Melero I, Wadhawan S, et al. Association of inflammatory biomarkers with clinical outcomes in nivolumab-treated patients with advanced hepatocellular carcinoma. J Hepatol 2020;73:1460-9.
103. Zhu AX, Finn RS, Edeline J, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol 2018;19:940-52.
104. Qin S, Ren Z, Meng Z, et al. Camrelizumab in patients with previously treated advanced hepatocellular carcinoma: a multicentre, open-label, parallel-group, randomised, phase 2 trial. Lancet Oncol 2020;21:571-80.
105. Desai J, Deva S, Lee JS, et al. Phase IA/IB study of single-agent tislelizumab, an investigational anti-PD-1 antibody, in solid tumors. J Immunother Cancer 2020;8:e000453.
106. Liu CQ, Xu J, Zhou ZG, et al. Expression patterns of programmed death ligand 1 correlate with different microenvironments and patient prognosis in hepatocellular carcinoma. Br J Cancer 2018;119:80-8.
107. Shen L, Guo J, Zhang Q, et al. Tislelizumab in Chinese patients with advanced solid tumors: an open-label, non-comparative, phase 1/2 study. J Immunother Cancer 2020;8:e000437.
108. Wang S, Zheng Y, Yang F, et al. The molecular biology of pancreatic adenocarcinoma: translational challenges and clinical perspectives. Signal Transduct Target Ther 2021;6:249.
109. Ammannagari N, Atasoy A. Current status of immunotherapy and immune biomarkers in gastro-esophageal cancers. J Gastrointest Oncol 2018;9:196-207.
110. Kieler M, Unseld M, Bianconi D, et al. Challenges and Perspectives for Immunotherapy in Adenocarcinoma of the Pancreas: The Cancer Immunity Cycle. Pancreas 2018;47:142-57.
111. Loeuillard E, Conboy CB, Gores GJ, et al. Immunobiology of cholangiocarcinoma. JHEP Rep 2019;1:297-311.
112. Piha-Paul SA, Oh DY, Ueno M, et al. Efficacy and safety of pembrolizumab for the treatment of advanced biliary cancer: Results from the KEYNOTE-158 and KEYNOTE-028 studies. Int J Cancer 2020;147:2190-8.
113. Marabelle A, Fakih M, Lopez J, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol 2020;21:1353-65.
114. Mody K, Starr J, Saul M, et al. Patterns and genomic correlates of PD-L1 expression in patients with biliary tract cancers. J Gastrointest Oncol 2019;10:1099-109.
115. Liddell SS, Chakrabarti S, Wintheiser GA, et al. Tumor Mutational Burden Is a Potential Predictive Biomarker for Response to Immune Checkpoint Inhibitors in Patients With Advanced Biliary Tract Cancer. JCO Precis Oncol 2022;6:e2200003.
116. Chen X, Wang D, Liu J, et al. Genomic alterations in

biliary tract cancer predict prognosis and immunotherapy outcomes. J Immunother Cancer 2021;9:e003214.
117. Yoon JG, Kim MH, Jang M, et al. Molecular Characterization of Biliary Tract Cancer Predicts Chemotherapy and Programmed Death 1/Programmed Death-Ligand 1 Blockade Responses. Hepatology 2021;74:1914-31.

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Supplementary

Appendix 1 Detailed search strategy example

PubMed

#1 "biomarker"[Mesh]
#2 "digestive system cancers" [Mesh] OR "gastrointestinal cancers" [Mesh] OR "esophageal cancer" [Mesh] OR "gastric cancer" [Mesh] OR "colorectal cancer" [Mesh] OR "liver cancer" [Mesh] OR "hepatocellular carcinoma" [Mesh] OR "pancreatic cancer"
#3 "Immune checkpoint inhibitor" [Mesh] OR "programmed cell death protein 1" [Mesh] OR "programmed cell death ligand-1"
#4 #1 and #2 and #3

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