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A Review of Emerging Biomarkers for Immune Checkpoint Inhibitors in Tumors of the Gastrointestinal Tract

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In recent years, immune checkpoint inhibition (ICI) therapy has made a tremendous improvement in the treatment of malignant tumors of gastrointestinal tract, especially for those with metastatic or recurrent lesions. However, while some patients benefit from ICI, others do not. In fact, predictive biomarkers can play a crucial role in screening patients who may benefit from a selected or targeted treatment, including immunotherapies such as programmed death-1/programmed death-1 ligand 1 (PD-1/PD-L1) inhibitors. A variety of techniques can be used to detect and quantify tumor biomarkers, each of which has a specific clinical application scenario and limitations. Cancer biomarkers in the gastrointestinal system involve an extremely complex network that requires careful interpretation and analysis. Different prognostic or predictive biomarkers are playing important roles in various tumor types, stages, and pathology/molecular subgroups, sometimes overlapping. Expression levels of biomarkers vary between different tumor types and even between the different lesions in the same tumor, depending on the heterogeneity of the patient, the tumor types, and the techniques of detection. The present systematic review comprehensively summarizes the potential biomarkers of immunotherapy, such as PD-1/PD-L1, total mutation burden (TMB), and tumor-infiltrating lymphocytes (TILs) in various gastrointestinal tumors, including tumors of the colon, stomach, esophagus, liver, and pancreas, to assist future application of immunotherapy and patient selection in clinical practice.

Keywords: Biomarkers • Gastrointestinal Neoplasms • Immune Checkpoint Inhibitors • Immunotherapy

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Background

Immunotherapy is changing the treatment landscape for a variety of tumors, of which studies on immune checkpoint inhibitors (ICIs) has been at the forefront. In non-small cell lung cancer, immunological checkpoint inhibitors have become one of the standard first-line and sequential-line treatment options. In the standards gastrointestinal system, emerging research on immunological checkpoint inhibitors is also striking [1]. The programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) pathway and the cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) pathway are the most well-known immunological checkpoint pathways, and their inhibitors, therefore, are the most widely used. To date, represented by PD-1/PD-L1 inhibitors, a series of immunological checkpoint inhibitors have been approved in various gastrointestinal tumors such as gastric cancer, liver cancer, and colorectal cancer [1,2].

Immunological checkpoint molecules play a key role in the process of cancer evasion, which helps tumor cells escape from immune surveillance, and the PD-1/PD-L1 pathway is one of the most critical for tumor cells to evade immune surveillance. PD-1 is widely expressed on the surface of tumor-infiltrating lymphocytes (TILs), B cells, natural killer cells, monocytes, and dendritic cells (DCs) [3,4]. As the major ligand of PD-1, PD-L1 mainly expresses on the surface of tumors in the tumor microenvironment, and can be detected in several types of tumors, such as lung cancer, gastric cancer, colorectal cancer, kidney cancer, and bladder cancer. PD-L1 expression in these tumor cells can be induced through multiple oncogene signaling pathways, or experiences an adaptive upregulation mediated by infiltrating inflammatory cytokines which are continuously secreted by immune cells [5]. The binding between PD-1 and its ligand PD-L1 inhibits the proliferation, activity, and survival of T cells in the tumor microenvironment, and reduces the expression of immune effector molecules such as interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), and interleukin-2 (IL-2), which drive T cell dysfunction, reducing its anti-tumor effect [6].

Conversely, overexpression of PD-L1 also endows tumor cells with the ability of anti-apoptosis [7] and stimulates the proliferation of tumor stem cells [8]. Therefore, the PD-1/PD-L1 pathway plays an important role in weakening anti-tumor immunity as well as strengthening tumor proliferation. Inhibition of the PD-1/PD-L1 pathway restores the killing activity of T cells against tumors and controls tumor survival, growth, and invasion. Correspondingly, the CTLA-4 pathway acts mainly on the T cell-APC system, affecting the massive activation of T cells and the function of immune effector cells. When it binds to CD80 and CD86 on the surface of antigen-presenting cells (APC), it also inactivates T cells. CTLA-4 antibodies can block the signal by competitively inhibiting CTLA-4, allowing more T cells to resume activation, proliferation, and tumor microenvironment infiltration, reversing regulation of the T cell (Treg)-mediated immunosuppressive state, and enhancing the anti-tumor activity of T cells [9].

However, not all patients can benefit from treatment with immune checkpoint inhibitors. Although PD-1/PD-L1 and CTLA-4 antibodies can significantly improve the objective response rate (ORR) and long-term survival of patients, some patients do not respond to these treatments [2]. After patients respond to immunotherapy, their therapeutic effects usually seem to be durable, and their long-term survival outcomes are often significantly better than those of non-responders [10]. Therefore, determining which subgroup of patients can get the optimal benefit from treatment of immune checkpoint inhibitors is becoming an urgent problem in the clinical scenarios of such therapies. Researchers have begun to focus on biomarkers related to treatment response and effectiveness as predictive biomarkers (defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention) [11], to provide guidance for clinical decision-making. How to accurately find biomarkers that can predict patients' benefits from immunotherapy will become one of the important research directions in the future [12]. At present, a variety of biomarkers have been extensively studied or have been used clinically, such as PD-L1 expression, total mutation burden (TMB), DNA mismatch-repair defect (dMMR)/high microsatellite instability (MSI), TILs, T cell recognition of tumor-specific neo-antigens, immune transcriptomic signature, diversity of T cell repertoire, gene expression profile, peripheral blood PD-L1 expression, and other cytokines/chemokines [13,14].

Gastrointestinal tumors with different primary lesions and different histopathological features also have different characteristics associated with their immune microenvironment and tumor microenvironment. Therefore, predictive biomarkers for immunotherapy may also vary between different types of gastrointestinal tumors. Systematic studies have been conducted on the predictive biomarkers of immunotherapy for gastrointestinal tumors, including the correlation, test consistency, and clinical significance, and it has been shown that there are still no perfect or well-established biomarkers in immunotherapy [12,15]. Therefore, the present systematic review comprehensively summarizes the potential biomarkers of immunotherapy, including PD-1/PD-L1, total mutation burden (TMB), and tumor-infiltrating lymphocytes (TILs), in different gastrointestinal tumors, such as tumors of the liver, pancreas, biliary tract, and colon, to provide help for the future application of immunotherapy and patient selection in clinical practice.

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Table 1. Published studies of immune checkpoint inhibitors for gastrointestinal cancers.

Tumor type	Target/Drug	Trail identifier	Phase	Size	Study design	Clinical efficacy
ESCC [77]	PD-1/Nivolumab	ONO-4538-07	II	64	Single arm	ORR=17% (11/64)
ESCC [78]	PD-1/Nivolumab	ATTRACTION-3	III	419	Randomized, open-lable	OS=11.6 months (nivolumab) vs 10.9 (chemotherapy)
ESCC [21]	PD-1/Pembrolizumab	KEYNOTE-180	II	121	Single arm	ORR=9.9% (12/121)
GC/GEJC [55]	PD-1/Nivolumab	ATTRACTION-2	III	493	Randomized, double-blind	ORR=11.2%(30/268); 1-year OS=26.2% (nivolumab) vs 10.9% (placebo)
GC/GEJC [79]	PD-1/Pembrolizumab	KEYNOTE-059	II	259	Single arm	ORR=15.5% (PD-L1+) vs 5.5% (PD-L1-)
GC/GEJC [80]	PD-1/Pembrolizumab	KEYNOTE-061	III	592	Randomized, open-label	OS=9.1months (nivolumad) vs 8.3 months (paclitaxel)
PD-L1+ GC/GEJC [16]	PD-1/Pembrolizumab	KEYNOTE-012	Ib	39	Single arm	ORR=22% (8/36)
dMMR/MSI-H CRC [41]	PD-1/Pembrolizumab	CT01876511	II	28	Single arm	ORR=40% (dMMR/MSI-H) vs 0% (pMMR CRC)
dMMR/MSI-H tumors [42]	PD-1/Pembrolizumab	CT01876511	II	86	Single arm	ORR=53%; CR=21%
dMMR/MSI-H CRC [45]	PD-1/Nivolumab	Checkmate 142	II	74	Single arm	ORR=31.1% (23/74)
dMMR/MSI-H CRC [46]	PD-1+CTLA-4/ Nivolumab+ipilimumab	Checkmate 142	II	119	Single arm	ORR=55% (65/119)
HCC [20]	PD-1/Nivolumab	Checkmate 040	I/II	214	Dose escalation and expansion	ORR=20% (42/214)
HCC [81]	PD-1/Pembrolizumab	KEYNOTE-224	II	104	Non-randomized, open-label	ORR=17% (18/104)
HCC [82]	PD-1/Pembrolizumab	KEYNOTE-240	III	413	Randoimzed, double-blind	OS=13.9months (pembrolizumab) vs 10.6 (placebo)
PDAC [83]	PD-L1/MDX-1105	NCT00729664	I	207	Dose escalation	ORR in PDAC=0% (0/14)
PDAC [84]	PD-1/Pembrolizumab	NCT02362048	II	77	Randomized, open-label	ORR=7.9%(with pembrolizumab) vs 0% (with acalabrutinib)

ESCC – esophageal squamous cell carcinoma; GC or GEJC – gastric or gastroesophageal junction cancer; CRC – colorectal cancer; HCC – hepatocellular carcinoma; PDAC – pancreatic ductal adenocarcinoma; PD-1 – programmed death-1; dMMR – DNA mismatch-repair defect; MSI-H – high microsatellite instability.

Pan-Tumor Biomarkers

Early research on the use of immunological checkpoint inhibitors in gastrointestinal tumors has been emerging and has shown remarkable efficacy (**Table 1**). At present, immunotherapy-related pan-tumor predictive biomarkers that are of great interest to researchers include PD-L1 expression, TMB, and MSI-H/dMMR states.

PD-L1 is the most widely studied predictive biomarker for immunotherapy and has mature application in several types of solid tumors such as non-small cell lung cancer [16]. In gastrointestinal tumors, the expression rate of PD-L1 is generally high, at about 64% in colorectal cancer [17], about 40-43.9% in gastric/esophageal cancer [18-20], and about 27% in hepatocellular carcinoma [21], but its predictive value is still controversial (**Table 2**). Some studies have shown that PD-L1

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Table 2. Controversial findings of the predictive value of PD-L1 expression for the efficacy of immunotherapy in gastrointestinal tumors.

Biomarker	Tumor	Study	Drug	Correlation
PD-L1 expression in tumor cells	HCC	CheckMate-040 [22]	Nivolumab	No significant correlation
PD-L1 expression in tumor cells	GC	Attraction-2 [15]	Nivolumab	No significant correlation
PD-L1 expression in tumor cells, lymphocytes, and macrophages	GC/GEJC	KEYNOTE-059 [16]	Pembrolizumab	Significantly relevant
PD-L1 expression in tumor cells, lymphocytes, and macrophages	HCC	KEYNOTE-224 [29]	Pembrolizumab	Significantly relevant
PD-L1 expression in tumor cells, lymphocytes, and macrophages	EC	KEYNOTE-180 [30]	Pembrolizumab	No significant correlation

HCC – hepatocellular carcinoma; GC or GEJC – gastric or gastroesophageal junction cancer; EC – esophageal cancer; PD-L1 – programmed death-1 ligand-1.

expression is associated with the efficacy of PD-1 inhibitors, but some studies have shown that regardless of PD-L1 expression levels, patients can benefit from treatment with PD-1 inhibitors [22,23]. However, PD-1/PD-L1 alone is insufficient to determine sensitivity to ICIs.

TMB is a quantitative tool for assessing the total number of mutant genes in the cancer genome. As tumors grow, somatic mutations accumulate, which do not exist in germline DNA [24]. TMB is measured by the non-synonym mutations of each mega base (Mb) in the coding region of the cancer genome [25]. The predictive value of TMB on the efficacy of immunotherapy has been widely verified in various tumor types, such as melanoma, non-small cell lung cancer, urothelial carcinoma, and small cell lung cancer [25]. A pooled analysis in 27 types of solid tumors showed a significant correlation between TMB and the ORR of PD-1 inhibitors ($P<0.001$) [26]. Another pooled analysis in 21 types of cancer including gastrointestinal tumors (N=151) also showed that TMB was significantly associated with the ORR and survival prognosis of PD-1/PD-L1 inhibitors. Patients with high TMB (≥20 mutation/Mb) had significantly better response (ORR: 58% vs 20%, $P=0.0001$), longer median PFS (12.8 months vs 3.3 months, $P\leq0.0001$), and improved median OS (not reached vs 16.3 months, $P=0.0036$) than patients with moderate or low TMB (<19 mutation/Mb) [27].

MSI is a molecular phenotype caused by genomic hypermutation; the MMR system includes enzymes that correct DNA mismatches generated during DNA replication (eg, MLH1, MSH2, and MSH6), thereby preventing transient mutations in differentiated cells from becoming permanent. In contrast, the dMMR status results in persist mutations in the entire genome, especially in the microsatellite region, which in turn leads to a hypermutation phenotype of MSI-high (MSI-H) [28-30]. Therefore, the dMMR state is equal to the MSI-H state from a biological perspective [31]. In certain gastrointestinal tumors, including gastric cancer and colorectal cancer, a high proportion of MSI-H patients (indicating dMMR status) can be observed [32]. Recent studies have shown that MSI-H/dMMR status is associated with significantly better PD-1 inhibitor treatment response and survival outcome, so the Food and Drug Administration (FDA) has approved nivolumab for MSI-H/dMMR-positive patients with metastatic colorectal cancer that progressed after receiving a fluoropyrimidine, oxaliplatin, and irinotecan regimen [33], or pembrolizumab for the sequential-line treatment of solid tumors with MSI-H/dMMR phenotype, without tumor type limitation [34]. However, clinical research showed that MS-stable tumors with high TMB significantly benefit from immunotherapy [35].

Combination of the expression of PD-L1 and presence or absence of TILs can classify tumors into 4 types: type I (PD-L1 positive with TILs), type II (PD-L1 negative with no TILs), type III (PD-L1 positive with no TIL), and type IV (PD-L1 negative with TILs). Among then, type I indicates adaptive immune resistance. Type I tumors are most likely to benefit from single-agent anti-PD-1/L1 blockade, as these tumors have evidence of pre-existing intratumor T cells that are turned off by PD-L1 engagement [36]. A clinical analysis of surgically resected esophageal cancer showed that a stratification based on PD-L1 expression and TIL status was significantly associated with overall survival [37]. Although there are no published studies on these 4 immune types and sensitivity to immune checkpoint inhibitor in gastrointestinal tumor, the classification may be able to predict the response.

The status of other immune effect cells, such as tumor-associated macrophages (TAM) and natural killer (NK) cells, can also contribute to the treatment effects of immune checkpoint inhibitors. There are 2 types of TAM: M1 TAM has anti-tumor activity, while M2 TAM promotes the proliferation of tumors [38]. A study has showed that in the tumor microenvironment of melanoma,

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CTLA-4 inhibitor responders have higher CD68+CD16+ M1-like TAM ratios at baseline and show decreased Treg infiltration after treatment [39]. As for NK cells, a clinical trial showed that patients with melanoma treated with Nivolumab and patients with tumor infiltration of NK cells after treatment have better clinical response [40].

Colon Cancer

Based on gene expression data, colorectal cancer can be classified into 4 distinct groups, known as the Consensus Molecular Subtypes (CMS). Among them, CMS1 subtype, also referred to as microsatellite instability/immune subgroup, is hypermutated, microsatellite unstable, and has strong immune activated states; it is observed in about 14% of colorectal cancer patients and is immunogenic and hypermutated [41]. Because of the immunogenicity of these tumors, patients in this subgroup may be responsive to immunotherapy.

Colorectal cancer can be divided into 2 groups when taking mutation patterns into consideration: tumors with a dMMR-MSI-H signature (>12 mutation per 10^6 DNA base tumor burden) and tumors with a pMMR-MSI-L signature (<8.24 mutations per 10^6 DNA base) [42]. Because of defects in MMR proteins, such as MLH1, MSH2, and MSH6, replication errors of microsatellites cannot be corrected, and they accumulated continuously, which changes the sequence length or base composition of microsatellites, resulting in tumors with high-level microsatellite instability and generating an enlarged neoantigen repertoire for T cell priming [33]. In advanced colorectal cancer, MSI-H/dMMR status has been shown to predict the efficacy of the PD-1 inhibitor pembrolizumab [43]. In a recent study, MSI-H/dMMR status was shown to be related to significantly better PD-1 inhibitor response [44]. Pembrolizumab has been approved by the FDA for MSI-H/dMMR solid tumors, including colorectal cancer and other solid tumors.

Positive PD-L1 expression (with a cut-off value of 10%) is seen in approximately 53% of colorectal cancer patients [45]. High expression of PD-1 and PD-L1 has been shown to be associated with better prognosis [46]; however, in colorectal cancer, PD-L1 does not precisely predict patient response to PD-1/PD-L1 inhibitors [47,48]. In MSI-H/dMMR type colorectal cancer, PD-L1 failed to predict the efficacy of PD-1 inhibitor nivolumab monotherapy or combination therapy (with CTLA-4 inhibitor) [48], or the effectiveness of the PD-1 inhibitor pembrolizumab [43].

In right colon cancer and left colon cancer, the mean TMB/mega base (MB) was relatively high, reported as 11.6/MB and 9.9/MB, respectively. About 12% of patients with right colon cancer can be divided into TMB-high patient subgroups (3% in left colon cancer). A recent study showed that TMB appeared to be an important independent biomarker within MSI-H metastatic colorectal cancer to stratify patients for likelihood of response [49].

In addition to immune cells markers, cytokines receptors such as IL2RB have been identified to be extensively linked to immune-checkpoints in colorectal cancer [50]. Furthermore, it has been proven that the gut microbiome not only acts as a barrier to bacterial invasion but also plays an important role in cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) blockade, programmed cell death protein 1 (PD-L1) mediation, and T cell stimulation, suggesting that the gut microbiome may be a predictive factor for immunotherapy of colorectal cancer [51].

Stomach Cancer

According to their molecular characteristics, gastric cancer can be divided into 4 subgroups [52]: 1) chromosomal instability (CIN), accounting for about 50% of gastric cancer, which is characterized by intestinal histology and related to more frequent TP53 mutation and RTK-RAS activation; 2) Epstein-Barr virus (EBV), accounting for about 9% of gastric cancer, usually presents with more common genetic alterations including PIK3CA mutation, PD-L1/2 overexpression, EBV-CIMP and CDKN2A silencing, and abnormalities in immune cell signaling; 3) gene stability (GS), accounting for about 20% of gastric cancer, which is characterized by diffuse histology and related to CDH1, RHOA mutations, CLDN18-ARHGAP fusion, and functional abnormalities in cells adhesion pathway; and 4) microsatellite instability (MSI), accounting for about 22% of gastric cancer, with hypermutation and CpG island methylator phenotype (CIMP) status, whose genetic alterations are often involved in MLH1 silencing and mitotic pathways. In these 4 subgroups, EBV and MSI subgroups are considered to be more "immunogenic" and may be more sensitive to immunotherapies [53].

MSI-H/dMMR are present in approximately 8-37% of gastric cancer patients [52,54,55], and compared to MSI-stable/lower tumors, MSI-H type gastric cancer is associated with a better prognosis and is a prognostic biomarker for better survival outcome [54,56]. The appearance of MSI-H/dMMR status is mainly due to epigenetic gene silencing caused by hypermethylation of MLH1 gene promoter [57]. In general, MSI-H/dMMR status is a predictive biomarker for anti-PD-1/PD-L1 drugs in gastric cancer.

PD-L1 overexpression occurs in approximately 25-65% of gastric cancer patients [20,45,58]. In gastric cancer, increased PD-L1 expression is associated with lymph node metastasis, late stage of the disease, and poor prognosis [20]. Currently, PD-L1 expression has been accepted as one of the conditions for the use of certain immunotherapies (eg, pembrolizumab)

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in clinical treatment of gastric cancer and may guide the application of other therapeutic approaches. However, this conclusion is still controversial because patients with gastric cancer can benefit from some immune checkpoint inhibitors, especially nivolumab, regardless of PD-L1 levels [13].

The average TMB in gastric adenocarcinoma is relatively high (9/MB), and about 11% of patients with gastric adenocarcinoma can be divided into the TMB-high patient subgroup [59]. It was thought that there is no correlation between TMB status and PD-L1 expression level in gastric cancer, but it is highly correlated with MSI/dMMR status [59]. Moreover, a recent clinical trial showed that high TMB may be a predictive marker for OS of advanced gastric cancer patients receiving a new PD-1 antibody, toripalimab, as a single agent [60].

TILs, including T cells, B cells, and natural killer cells, are considered to be signs of host immune response against tumor cells [61,62]. In gastric cancer, a high density of TILs has been confirmed to be significantly associated with PD-L1 expression and MSI-H [55,56]. Different types of infiltrating lymphocytes have different prognostic and predictive implications and may be predictive of anti-PD-1/PD-L1 treatment response [61].

Virus-associated tumors have significant immunogenic features. In gastric cancer, EBV and MSI subtypes have more significant immunological characteristics; in contrast, CIN and GS subtypes have weaker immunological characteristics [52]. In virus-related tumors (such as EBV-related gastric cancer, HPV-related cervical cancer, and HBV-related liver cancer), immunotherapy is usually more effective. For example, 80% of Merkel cell carcinoma is related to the high load of MCV virus; correspondingly, the efficacy of pembrolizumab for Merkel cell carcinoma is ≥50% [63].

Esophageal Cancer

Esophageal adenocarcinomas (EAC) and esophageal squamous cell carcinomas (ESCC) have distinct histopathology, epidemiology, and molecular characteristics. A comprehensive molecular analysis including 164 patients with esophageal cancer showed that ESCC is more similar to squamous cell carcinoma located in other organs, whereas EAC is more similar to the CIN subtype of gastric cancer [64].

MSI-H/dMMR status occurs in <10% of patients with esophageal squamous cell carcinoma [65], which may be associated with rapid disease progression in standard cytotoxic treatment and significantly shorter PFS in first-line chemotherapy; however, distinct from chemotherapy that may result in endogenous resistance, MSI-H/dMMR status during immunotherapy may be associated with more durable remission [66].

Positive PD-L1 expression (with a cut-off value of 5%) is seen in approximately 20% of patients with esophageal cancer (approximately 44% of squamous cell carcinoma patients with a positive cut-off value of 10%) [45]. Increased PD-L1 expression is associated with lymph node metastasis, later disease stage, and poor prognosis [20]. For immunotherapy, positive PD-L1 expression defined as PD-L1 combined positive score (CPS) ≥10 seems to be associated with significantly better response and improved survival compared with chemotherapy in different treatment lines [67,68]. A combination of PD-L1 and TIL status may serve as predictive biomarkers in PD-1/PD-L1-targeting therapy for patients with surgically resected esophageal cancer [37].

The average TMB of patients with esophageal cancer is relatively low (6.7/MB for EAC and 6.4/MB for ESCC). About 2.0% of patients with EAC and 0% of those with ESCC can be divided into TMB-high subgroups [59]. To date, there has been no large-scale study demonstrating the relationship between TMB and immunotherapy in esophageal cancer.

Liver Cancer

Hepatocellular carcinomas with different histopathological characteristics and stages also have different molecular features [69]. In hepatocellular carcinoma, the incidence of MSI-H/dMMR is relatively low, varying from 0% to 18% [70]. Moreover, dMMR does not involve the pathogenesis mechanism of hepatocellular carcinoma [70]. Therefore, no clinical study has confirmed the clinical significance of MSI-H as a predictive biomarker for immunotherapy in hepatocellular carcinoma.

Positive PD-L1 expression (with a cut-off value of 5%) can be seen in approximately 15% of hepatocellular carcinoma patients [45]. In the Checkmate-040 study, the objective response rates for hepatocellular carcinoma patients with PD-L1 ≥1% and PD-L1 <1% were 27% and 12%, respectively [22].

In hepatocellular carcinoma, TMB has been shown to be associated with the expression of PD-L1 (P<0.005) [59]. The average TMB of hepatocellular carcinoma patients is 7.2/MB, and about 3% of liver cancer patients can be divided into TMB-high patient subgroups [59]. However, there is no clear conclusion about the correlation between TMB and the efficacy of immunotherapy in hepatocellular carcinoma.

HBV and HCV infection increase the risk of hepatocellular carcinoma. Viral dynamics may have a predictive role for response to certain treatments. In patients with hepatocellular carcinoma receiving sorafenib and without prophylactic antiviral therapy, high HBV DNA levels have been shown to be an independent predictive factor for poor survival (P=0.005; HR 2.85) and

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disease progression (*P*=0.008; HR 87.4) [71]. However, there was no significant correlation between the changes in HBV and HCV viral loading levels and tumor response to immunotherapy.

A better understanding of the etiology of hepatocellular carcinoma may contribute to the development of more practical and accurate biomarkers. Potential biomarkers include [72]: 1) miRNAs, such as miR-4147 that can allele-specifically regulate PD-1 expression through interaction with the 3' UTR of *PD1* mRNA, significantly decrease PD-1 expression, and increased TNF-α and IFN-γ production, and downregulation of miR-4147 may predict a poor response to immune checkpoint inhibitor [73]; another miRNA, miR-802, may increase the expression of PD-1 and decrease the expression of IFN-γ and CD8+CD28+ T cell number, result in a downregulation of T cell function, and, inversely, upregulation of miR-802 may be a negative biomarker for immune checkpoint inhibitor treatment [74]; 2) enzymes and isozymes such as phosphoprotein 2 (GOLPH2) may help to predict tumor invasiveness [75]; 3) abnormal cytokine levels such as IL-6, IL-8, and IL-10 [76]; 5) molecular mutations and signatures involved in immune pathway and growth factors such as transforming growth factor beta (TGF-β) [77].

Pancreas Cancer

Pancreatic cancer with different histopathological types may have distinct molecular and immune characteristics and thus may affect treatment decisions [78]. In pancreatic cancer, the incidence of MSI-H/dMMR is 13-22%, which may be a predictive biomarker for response to anti-PD-1/PD-L1 drugs [79,80]. PD-L1 overexpression can be observed in approximately 19-40% of patients with pancreatic cancer and is associated with poor prognosis [45]. In pancreatic cancer, TMB has been demonstrated to be positively associated with PD-L1 expression (*P*<0.05) [59]. The average TMB in patients with pancreatic cancer is relatively low (5.0/MB), and approximately 1% of these patients can be classified as belonging to TMB-high patient subgroups [59,81]. Finally, T cell receptor repertoire profiling can serve as a biomarker of clinical response in pancreatic cancer patients receiving immunotherapy [82].

Conclusions

Modern personalized medicine now includes the screening of tumor tissue for predictive biomarkers for targeted therapy. Immunotherapy that includes PD-1/PD-L1 inhibitors has a role in the management of patients with advanced gastrointestinal malignancy. Prognostic and predictive biomarkers also have important roles in identifying and targeting molecular subgroups of tumors and identifying tumor heterogeneity between primary and secondary tumors.

This review has highlighted how the identification of biomarkers for gastrointestinal tumors has affected treatment decisions. For example, MSI-H/dMMR status is required before implementing treatment with pembrolizumab and nivolumab, and tumor PD-L1 expression is required before implementing treatment with pembrolizumab and atezolizumab. Some emerging biomarkers such as TMB contribute to the efficacy of PD-1/PD-L1 inhibitors. Continuing advances and methods of detecting biomarkers expressed by tumors of the gastrointestinal tract will guide future targeted immunotherapy.

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