Current state and challenges of emerging biomarkers for immunotherapy in hepatocellular carcinoma (Review)

MO CHENG, XIUFENG ZHENG, JING WEI and MING LIU

Department of Medical Oncology, Gastric Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, P.R. China

Received March 22, 2023; Accepted August 29, 2023

DOI: 10.3892/etm.2023.12285

Abstract. Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer. According to the American Cancer Society, among patients diagnosed with advanced liver cancer, HCC has the sixth-highest incident rate, resulting in a poor prognosis. Surgery, radiofrequency ablation, transcatheter arterial chemoembolization, radiation, chemotherapy, targeted therapy and immunotherapy are the current treatment options available. Immunotherapy, which has emerged as an innovative treatment strategy over the past decade, is serving a vital role in the treatment of advanced liver cancer. Since only a small number of individuals can benefit from immunotherapy, biomarkers are required to help clinicians identify the target populations for this precision medicine. These biomarkers, such as PD-1/PD-L1, tumor mutational burden and circulating tumor DNA, can be used to investigate interactions between immune checkpoint inhibitors and tumors. The present review summarizes information on the currently available biomarkers used for immunotherapy and the challenges that are present.

Contents

Correspondence to: Dr Ming Liu, Department of Medical Oncology, Gastric Cancer Center, West China Hospital, Sichuan University, 37 Guoxue Lane, Wuhou, Chengdu, Sichuan 610041, P.R. China
E-mail: liuming629@wchscu.cn

Key words: hepatocellular carcinoma, immune checkpoint inhibitors, biomarkers, immunotherapy

1. Introduction

According to the 2020 global liver cancer epidemiology, liver cancer is responsible for 4.69% of all cases of cancer and 8.34% of all mortalities from cancer (1,2). Hepatocellular carcinoma (HCC) pathogenesis has been associated with infection by hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol abuse, non-alcoholic steatohepatitis, cirrhosis, and a family history of HCC, with cirrhosis caused by HBV being important as it generates 60% of all cases in China (3). Although surgical resection, radiofrequency ablation, transcatheter arterial chemoembolization (TACE), radiotherapy and chemotherapy are used as potentially curative treatments, the prognosis remains poor for patients with advanced (stage 2-4) disease (3,4). The emergence of cancer immunotherapies using immune checkpoint inhibitors (ICIs) has begun a new era of anti-tumor therapy during the past decade (5).

ICIs inhibit the activity of immune checkpoint proteins, such as PD-1, PD-L1 and CTLA-4, which restrict the immune response against tumors, thus reactivating antitumor activity (6). This immunotherapy has demonstrated promising results in patients with advanced, inoperable liver cancer and those undergoing radiofrequency ablation. For example, the IMbrve150 phase III trial demonstrated reductions in both tumor progression and mortality with the combined use of two ICIs, Atezolizumab and Bevacizumab, leading to Food and Drug Administration (FDA) approval for this drug combination as a first-line treatment for patients with unresectable or metastatic HCC (7). Additionally, the CheckMate040 and KEYNOTE-224 trials established Nivolumab and Pembrolizumab as second-line immunotherapies for liver cancer, although subsequent trials did not observe an improvement in overall survival (OS) (8). Details of the current immunotherapy clinical trials are provided in Table I. However, HCC is a heterogeneous disease with multiple immunological features and thus, despite encouraging results on specific forms of HCC, the use of immunotherapy does not guarantee clinical benefit for all patients with HCC (9). Data from randomized controlled trials indicate that only 10-30% of patients with advanced HCC who undergo immunotherapy achieve a complete response (CR) or partial response (PR) (7,10-13). A major contributing factor to this is the paucity of markers for the early diagnosis and treatment of HCC. The identification and application of predictive biomarkers that can accurately

distinguish patients that would benefit from immunotherapy could enable the use of precision treatment in HCC immunotherapy, allowing the proper allocation of medical resources and avoiding the exposure of non-responsive patients to treatment toxicity. Therefore, there is an urgent need for predictive markers, whether positive or negative prognostic markers, to screen individuals for immunotherapy suitability. The present review summarizes the currently known biomarkers for immunotherapy, as presented in Fig. 1.

2. Host-associated biomarkers

Hepatitis. HCC progression is known to be associated with HBV or HCV infection and liver cirrhosis. However, evidence from the CheckMate 040 and KEYNOTE-224 trials indicated that viral load or immune responses to HBV/HCV may not necessarily influence T cell activation and subsequent antitumor activity (14,15). Furthermore, the results of a meta-analysis revealed that neither HBV nor HCV affected the tumor immune microenvironment, and the presence or absence of viral infection was not an effective criterion for the selection of patients for programmed death 1 (PD-1)/programmed death-ligand 1 (PD-L1) immunotherapy (16).

Obesity. Obesity and being overweight are considered to be risk factors for numerous diseases, including cancer (17). Obesity caused by a high-fat diet impairs $CD8^+$ T cell infiltration and function, which alters the immune microenvironment in mice and enhances tumor growth (18). In contrast, another study revealed that patients with advanced HCC that had higher body mass indices (BMI; >25) appeared to have an improved prognosis following immunotherapy (19).

α-fetoprotein (AFP). In clinical practice, the serum AFP level represents a primary indicator used in diagnosis and for monitoring the effectiveness of liver cancer treatment (20). The AFP levels are increased in ~two-thirds of patients with HCC (21). The expression levels of certain immune checkpoint proteins, such as SIGLEC15, CTLA4, CD274, PDCD1LG2, PDCD1, TIGIT, LAG3 and HAVCR2, have been revealed to differ with regards to the AFP level (22). It has been suggested that AFP could be used as a prognostic biomarker for HCC immunotherapy. For example, Spahn *et al* (23) observed that baseline AFP concentrations <400 $\mu g\ l^{-1}$ before the start of treatment were associated with increased rates of PR or CR and reduced rates of progressive disease (PD). However, in the CheckMate 459 trial, patients with high baseline AFP levels (>400 ng/ml) had an increased overall survival (OS) (11). The objective response rate (ORR) was revealed to be positively associated with the early stages of AFP reduction therapy and PD-1 blockade, while progression-free survival (PFS) and OS were also increased (2,24). Therefore, the combination of AFP with other serum markers deserves further investigation to improve diagnostic accuracy. For example, previous studies have indicated that the C-reactive protein (CRP) and AFP in immunotherapy (CRAFITY) score, which combines CRP with AFP, can be used to predict treatment outcomes and treatment-associated adverse events in patients with HCC undergoing immunotherapy (25,26). However, there is still disagreement over whether AFP can serve as a prognostic biomarker for immunotherapy (25,27,28).

Blood inflammatory markers. Blood inflammatory biomarkers are both affordable and useful for the early identification of disease. It has been suggested that a neutrophil-lymphocyte ratio (NLR) ≥5 and a platelet-lymphocyte ratio (PLRs) ≥300 are independent prognostic factors for OS, predicting reduced OS, PFS, ORR and an increased risk of mortality in patients receiving immunotherapy (29,30). Similarly, a multicenter study revealed that the NLR could predict PFS in patients with unresectable HCC treated with Atezolizumab plus Bevacizumab, particularly in patients with modified albumin-bilirubin grade 1 or 2a (31).

Jeon *et al* (32) revealed that the numbers of classical monocytes (such as $CD14^+CD16$) increased on day 7 in patients with durable clinical benefit compared with that in patients with non-durable clinical benefit. The CRP level, an indicator of inflammation, has also been revealed to have good prognostic value in lung and renal cell cancer (33-35). The baseline CRAFITY score, developed by Scheiner *et al* (26), has been demonstrated to be effective for the assessment of patients receiving immunotherapy. Specifically, the median OS was revealed to be 27.6, 11.3 and 6.4 months in the CRAFITY-high (2 points), CRAFITY-intermediate (1 point) and CRAFITY-low (0 points) groups, respectively, and the best radiological response ratio [CR/PR/stable disease (SD)/PD] is stratified based on the CRAFITY score. This use of the score was also supported by a retrospective study conducted in Japan where the OS and PFS of 297 patients that received Atezolizumab and Bevacizumab treatment were associated with AFP and CRP (25). However, the prediction model is currently only applicable to patients that receive Atezolizumab and Bevacizumab; additional validation is required for other immunotherapy medications.

Gut microbiota. According to recent studies, the gut microbiota serves an important role in the development and occurrence of liver cancer (36-38). The underlying mechanism involves the gut-liver axis and is associated with dysbiosis, intestinal permeability and bacterial metabolites. Dysbiosis and intestinal permeability make it easier for bacterial metabolites to reach the liver. Bacterial products such as lipopolysaccharides (LPS) can cause inflammation and cancer in the liver (39,40). In addition, Toll-like receptor 4 (TLR-4), which is widely distributed on the surfaces of various liver cells and has been demonstrated to mediate hepatic carcinogenesis, is the specific recognition receptor for LPS (41). In a study by Chung *et al* (36) the stools of eight antibiotic-treated patients were collected for microbiota analysis. Patients receiving Nivolumab demonstrated no alterations in the diversity and composition of their gut microbiota. However, a skewed Firmicutes/Bacteroidetes ratio and a low Prevotella/Bacteroides ratio were revealed to predict a poor immunotherapy response in patients with liver cancer, while the presence of Akkermansia species suggested a positive prognosis (36). Another study on 167 patients with hepatobiliary cancer treated with immunotherapy, revealed that a number of bacteria, such as Lachnospiraceae bacterium-GAM79, were associated with an improved OS and PFS after treatment, while other bacteria, such as *Veillonella*,

Table I. Ongoing research on immunotherapy for hepatocellular carcinoma.

Treatment	Research project	Drug	Line of therapy	Number of patients	Stage	NCT	Early result
ICI monotherapy	KEYNOTE-394	Pembrolizumab + BSC vs. BSC	Second-line	453	III	NCT03062358	mOS, 14.6 vs. 13.0 months; and ORR, 12.7 vs. 1.3%
	RATIONALE-301	Tislelizumab vs. Sorafenib	First-line	674	III	NCT03412773	mOS, 15.9 vs. 14.1 months; ORR, 14.3 vs. 5.4%; and mPFS, 2.2 vs. 3.6 months
	KEYNOTE-224	Pembrolizumab	Second-line	107	II	NCT02702414	ORR, 18.3%; mPFS, 4.9 months; and mOS, 13.2 months
ICI double	HIMALAYA	Durvalumab + Tremelimumab (STRIDE) or Durvalumab vs. Sorafenib	First-line	1,504	III	NCT03298451	mOS, 16.4 vs. 16.56 vs. 13.8 months
	CheckMate 9DW	Nivolumab + Ipilimumab vs. Sorafenib or Lenvatinib	First-line	732	III	NCT04039607	Not published
ICI + VEGF/TKI	NA	Lenvatinib + Nivolumab	First-line	50	II	NCT03841201	ORR, 28%; mPFS, 9.0 months; and mOS, 27.1 months
	LEAP-002	Lenvatinib + Pembrolizumab vs. Lenvatinib	First-line	794	III	NCT03713593	mOS, 21.2 vs. 19.0 months; and mPFS, 8.2 vs. 8.0 months
	SHR-1210-III-310	SHR-1210 (Camrelizumab) + Apatinib vs. Sorafenib	First-line	543	III	NCT03764293	mOS, 22.1 vs. 15.2 months; mPFS, 5.6 vs. 3.7 months; and ORR 25.4 vs. 5.9%
	JUPITER-10	Toripalimab + Bevacizumab vs. Sorafenib	First-line	326	III	NCT04723004	Not published

Table I. Continued.

Treatment	Research project	Drug	Line of therapy	Number of patients	Stage	NCT	Early result
	EMERALD-2	Durvalumab + Bevacizumab vs. Durvalumab vs. placebo	NA	908	III	NCT03847428	Not published
	NA	Tislelizumab + Regorafenib vs. Regorafenib	First-line	125	II	NCT04183088	Not published
	IMbrave251	Atezolizumab + Lenvatinib or Atezolizumab + Sorafenib vs. Lenvatinib or Sorafenib	Second-line	554	III	NCT04770896	Not published
	GOING	Regorafenib vs. Nivolumab	Second-line	78	I/II	NCT04170556	mPFS, 6.1 vs. 6.7 months
	DEDUCTIVE	Tivozanib + Durvalumab	First-line	42	I/II	NCT03970616	mPFS, 7.3 months; and ORR, 27.8%
ICI + locoregional therapy	EMERALD-1	Durvalumab + TACE or Durvalumab + Bevacizumab + TACE vs. TACE	NA	724	III	NCT03778957	Not published
	NA	Y-90 TARE vs. Y-90 TARE + Atezolizumab + Bevacizumab	NA	128	II	NCT04541173	Not published

BSC, best supportive care; mOS, median overall survival; ORR, objective response rate; mPFS, median progression free survival; ICI, immune checkpoint inhibitor; TKI, tyrosine kinase inhibitor; NA, not available; TACE, trans arterial chemoembolization; TARE, trans arterial radioembolization; NCT, national clinical trial.

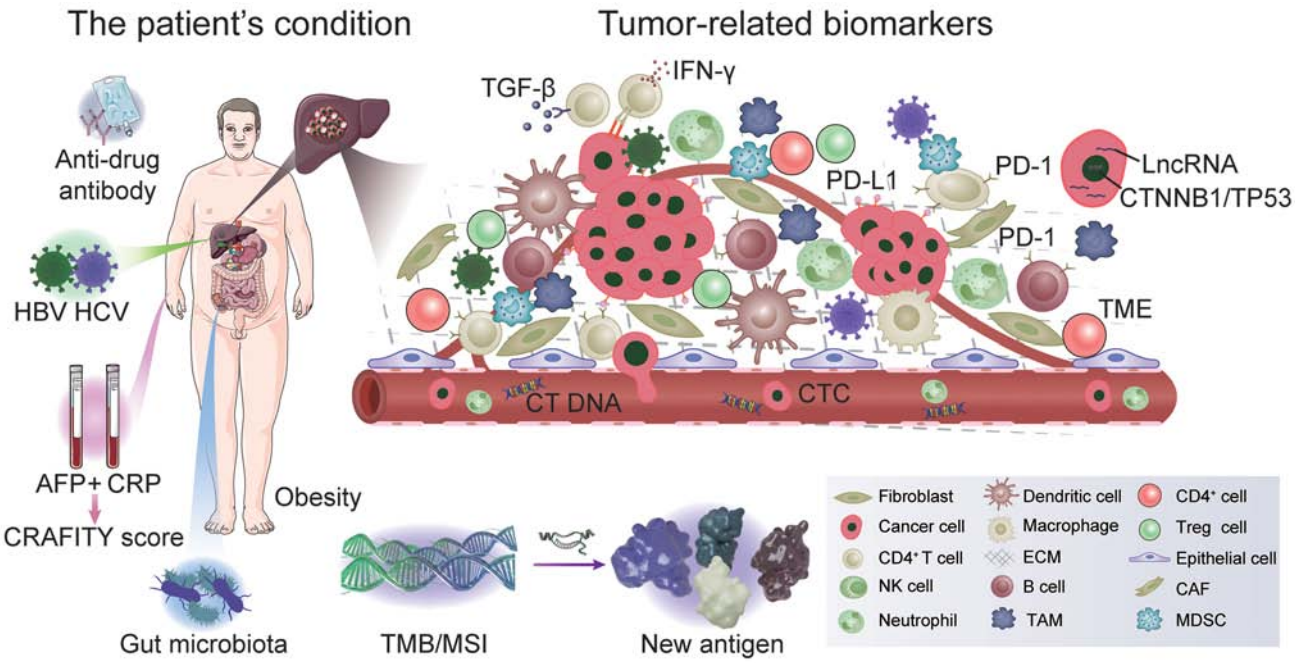

Figure 1. Potential biomarkers for predicting the response to immunotherapy in patients with liver cancer. HBV, hepatitis B virus; HCV, hepatitis C virus; TMB, tumor mutation burden; MSI, microsatellite instability; CTC, circulating tumor cell; TME, tumor microenvironment; AFP, α-fetoprotein; CRP, C-reactive protein; CRAFITY, CRP and AFP in immunotherapy; NK, natural killer; ECM, extracellular matrix; TAM, tumor-associated macrophages; Treg, regulatory T cell; ctDNA, circulation tumor DNA; CAF, cancer-associated fibroblast; MDSC, myeloid-derived suppressor cell; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; lncRNA, long non-coding RNA; CTNNB1, catenin β 1; TP53, tumor protein p53.

were associated with an increased risk of immune-associated side effects (37). There is also an association between the diversity of the gut microbiota and the levels of aspartate aminotransferase and alanine aminotransferase, which reflect liver function (38). Stool samples from patients that responded to anti-PD-1 therapy contained a greater taxonomic abundance compared with those of non-responders. The characterization of the dynamic changes in the gut microbiome can be useful for an earlier prediction of anti-PD-1 treatment outcomes in HCC. With the rapid development of microbial multi-omics, analysis of the gut microbiota has potential as a predictive biomarker for liver cancer immunotherapy. It has been reported that fecal microbiota transplantation from donors that achieved CR/PR on long-term anti-PD-1 therapy to patients failed to respond to immunotherapy can increase the intra-tumoral lymphocyte infiltration (42).

Anti-drug antibodies (ADAs). ICIs may be immunogenic and recognized by the human immune system, which could lead to the induction of the humoral immunity and subsequent adverse ADA responses (43). Different monoclonal antibodies are associated with different rates of ADA development, with Atezolizumab having the highest rate (~30%) compared with others (5-10%) (44). ADAs may affect the pharmacokinetics and pharmacodynamics of therapeutic antibodies, and may even neutralize the therapeutic antibodies (45). A cohort study by Kim *et al* (46) reported that increased ADA levels at the second Atezolizumab injection (day 1 of chemotherapy treatment cycle 2) may be associated with poor clinical outcomes. Reducing Atezolizumab exposure in patients with advanced HCC, Atezolizumab and Bevacizumab administration and an established ADA level >1,000 ng/ml can accurately predict the curative effect (46). Anti-Atezolizumab antibody-positive patients did not demonstrate a reduction in the frequency or severity of adverse events (44). However, a meta-analysis of 11 clinical trials, based on studies using Atezolizumab monotherapy or combination therapy, demonstrated that unadjusted descriptive analyses could not identify a clear association between the ADA status and the frequency or severity of adverse events. Furthermore, any ADA impact was not driven by neutralizing activity (47). The most distinctive feature of ADA assays is their lack of accurate quantification, as there is no reliable calibration reference standard for ADAs (48). Currently, there is no effective method for predicting which drugs may cause ADAs. Table II provides a brief overview of the host-associated biomarkers that are used for HCC immunotherapy.

3. Tumor-associated biomarkers

PD-1 and PD-L1. PD-1 is an immunosuppressive transmembrane protein that is expressed on the surface of cells such as T, B and myeloid cells. By binding to PD-L1, it inhibits T cell activation and proliferation, negatively stimulates T cells, blocks the T cell receptor, and negatively impacts how the immune system combats cancer (49,50). Inhibition of PD-1/PD-L1 prevents the interaction between PD-1 on T cells and PD-L1 on tumor cells, thus, restoring the T cell-mediated antitumor immune response (51). However, anti-PD-1/PD-L1 therapies are only effective in 20-40% of patients (52). Numerous studies have demonstrated that the expression level of PD-L1 on immune and tumor cells is associated with the anti-PD-1 treatment response in HCC (14,15,53,54). However, these studies varied in their detection techniques and methods

Table II. Host-associated biomarkers for HCC immunotherapy.

First author, year	Biomarker	Study design	Treatment	Patients	Outcome	Results	(Refs.)
Sangro *et al*, 2020	AFP, PLR, NLR and HBV/HCV	Retrospective	Nivolumab	AFP <400 μg/l (n=92); and AFP ≥400 μg/l (n=57)	OS	The median OS of patients with baseline AFP <400 μg/l was increased by 3.8 months compared with that of patients with baseline AFP ≥400 μg/l; patients with reduced NLR and PLR levels had an increased OS; and HBV/HCV replication was not associated with clinical deterioration or tumor response	(14)
Ho *et al*, 2020	Viral etiology	Meta-analysis	ICIs	-	-	There is no effect of viral etiology on the tumor immune microenvironment in HCC, and viral status should not be used as a criterion to select patients for PD-1/PD-L1 therapy.	(16)
Akce *et al*, 2021	BMI, NLR and sarcopenia	Retrospective	Anti-PD-1 antibody	n=57	OS	The BMI cut off value was 25; the NLR cut off value was 5.15; and sex-specific sarcopenia did not predict OS	(19)
Spahn *et al*, 2020	AFP and TMB	Retrospective	Anti-PD-1 antibody	n=99	PFS	AFP levels ≥400 μg/l were associated with reduced survival rates; and there is no difference in median TMB between responders and non-responders and no correlation between TMB and PFS	(23)
Zhu *et al*, 2022	AFP	Retrospective	Atezolizumab + Bevacizumab	n=208	PFS and OS	Patients with baseline AFP levels of >20 ng/ml, and a ≥75% decrease or ≤10% increase in AFP levels, measured 6 weeks after starting treatment,demonstrated an association with increased OS and PFS	(2)
Sun *et al*, 2021	AFP and PIVKA-II levels	Retrospective	PD-1 inhibitor	n=235	ORR, PFS and OS	Early reductions (>50% after 6 weeks) in AFP and PIVKA-II levels can be predictors of the efficacy of PD-1 inhibition in patients with HCC.	(24)
Dharmapuri *et al*, 2020	NLR and PLR	Retrospective	Nivolumab	n=103	OS	NLR <5 was associated with increased OS, and a combination of high NLR (≥5) and PLR (≥500) was associated with an eight-fold increased risk of mortality.	(29)

Table II. Continued.

First author, year	Biomarker	Study design	Treatment	Patients	Outcome	Results	(Refs.)
Muhammed *et al*, 2021	NLR and PLR	Retrospective	ICIs	n=362	PFS and OS	Patients with NLR ≥5 had reduced OS, PFS and ORR; and patients with PLR ≥300 reported reduced OS.	(30)
Scheiner *et al*, 2022	CRAFITY	Retrospective	Atezolizumab + Bevacizumab	n=102	Best radiological response (complete response, partial response, stable disease or progressive disease)	The CRAFITY score was associated with survival rates and radiological responses in patients receiving PD-(L)1 immunotherapy	(26)
Hatanaka *et al*, 2022	CRAFITY	Retrospective	Atezolizumab + Bevacizumab	0 points (n=147), 1 point (n=111) and 2 points (n=39)	PFS and OS	There were differences in the PFS and OS among CRAFITY score 0, 1 and 2 groups. The CRAFITY score is simple and can be used to predict treatment outcome.	(25)
Chung *et al*, 2021	Gut microbiome	Prospective	Nivolumab	n=8	Response rate	A skewed Firmicutes/Bacteroidetes ratio and a low Prevotella/Bacteroides ratio can serve as predictive markers for a lack of response to treatment, whereas the presence of Akkermansia species predicts a good response to treatment.	(36)
Mao *et al*, 2021	Gut microbiome	Retrospective	Anti-PD-1 antibody	n=65	PFS and OS	The gut microbiome was associated with the clinical response to anti-PD-1 immunotherapy in patients with types of hepatobiliary cancer.	(37)
Kim *et al*, 2022	ADA	Prospective	Atezolizumab + Bevacizumab	n=174	Response (complete response, partial response)	High ADA levels (≥1,000 ng/ml) may reduce Atezolizumab exposure and attenuate the anticancer efficacy of the drug.	(46)

HCC, hepatocellular carcinoma; AFP, α-fetoprotein; PLR, platelet-lymphocyte ratio; NLR, neutrophil-lymphocyte ratio; HBV/HCV, hepatitis B virus/hepatitis C virus; BMI, body mass index; TMB, tumor mutational burden; PIVKA-II, protein induced by vitamin K absence-II; CRAFITY, C-reactive protein and α-fetoprotein in immunotherapy; ADA, anti-drug antibodies; ICIs, immune checkpoint inhibitors; PD-1, programmed death 1; OS, overall survival; PFS, progression-free survival; ORR, objective response rate; PD-L1, programmed death-ligand 1.

used to measure PD-L1, so there is no universal standard for the detection and quantification of PD-L1 (55). The most commonly used methods for measuring PD-L1 are the tumor proportional score (TPS) and the combined positive score (CPS) (56).

The KEYNOTE-244 study retrospectively analyzed the association between PD-L1 expression levels and response to Pembrolizumab treatment, finding a treatment response to Pembrolizumab when PD-L1 was quantified using CPS but not when it was quantified using TPS (15). However, a study on the response to Nivolumab demonstrated different outcomes. Patients that tested positive for PD-L1 (TPS ≥1%) had an increased median OS (28.1 months) compared with those that tested negative for PD-L1 (median OS of 16.6 months) (13). Recently, a meta-analysis of nine cohort studies (seven PD-L1 and three PD-1) demonstrated that PD1/PDL-1 was a marker of poor survival rate regardless of OS, HR, CI, disease-free survival (DFS) and other evaluation methods (54). High PD-1/PD-L1 expression levels were associated with aging, multiple tumors, high α-fetoprotein levels and an advanced Barcelona Clinic liver cancer stage (14,53). In addition, PD-L1, as measured by CD274 (a PD-L1 messenger RNA) expression levels in the IMbrave150 trial, were revealed to be increased in patient with CR/PR compared with that in patients with SD/PD. Patients with high CD274 levels also demonstrated an increased PFS compared with those with low expression levels (54). However, PD-L1 expression levels are influenced by various factors. PD-L1 can be induced by IFN-γ, hypoxia or TLR-mediated pathways (57). Tumor heterogeneity and the tumor interstitium were observed to be the primary causes of inconsistent outcomes, followed by differences in detection methods (58). Thus, the value of the PD-L1 expression level as a predictive biomarker for immune checkpoint blockade therapy in HCC has been reduced.

Genetic characteristics. The CTNNB1 gene encodes the intracellular signaling transducer β-catenin, which is essential for embryonic development, cell fate determination, proliferation and migration (59). One of the key signaling pathways that control liver regeneration, homeostasis and tumorigenesis is the Wnt/β-catenin cascade (60,61). In a mouse model of HCC, activation of this pathway promoted immune evasion and conferred resistance to anti-PD-1 therapy (62,63). Similar outcomes were observed in liver cancer. Harding *et al* (64) reported that all 10 patients with mutations in components of the Wnt-β-catenin pathway demonstrated PD and a reduced median survival rate compared with patients without mutations. This implies that the Wnt-β-catenin pathway is a marker of immunotherapy sensitivity (62). Additionally, patients with HCC with mutations in CTNNB1 were revealed to have increased OS and PFS compared with patients with no mutations in CTNNB1. Thus, CTNNB1 may serve as an independent prognostic factor in HCC following immunotherapy (65,66).

Another dysregulated signaling pathway is the transforming growth factor-β (TGF-β) pathway which is involved in inflammation, fibrogenesis and immunomodulation in the HCC microenvironment (67). Increased TGF-β signaling may lead to T cell exhaustion through the upregulation of PD-1 signaling, while inhibition of TGF-β signaling may increase the anti-tumor immunity in HCC (68). Studies using mouse models have indicated that a combination of blocking TGF-β signaling and anti-PD-L1 antibodies could reduce TGF-β signaling, promote T cell infiltration into the tumor environment, and reshape the immune microenvironment, thus, stimulating effective anti-tumor immune responses and tumor regression (69,70).

Numerous studies are investigating cancerous genes in this era of precision medicine. Least absolute shrinkage and selection operator regression analysis of data from The Cancer Genome Atlas and International Cancer Genome Consortium dataset and the International Cancer Genome Consortium database revealed nine genes (ANP32B, BMI1, ASF1A, CDK5, BUB1, CBX3, CBX2, CDK1 and BCORL1) to be independent predictors of HCC prognosis (71). Another study identified 11 immune-associated genes, NDRG1, MAPT, FABP6, CACYBP, HSP90AA1, ISG20L2, NRAS, BRD8, OSGIN1, CD320 and PSMD14, that were used to predict immune cell infiltration and construct a prognostic index for the prediction of immunotherapy efficacy (72).

Tumor mutational burden (TMB) and microsatellite (MS) instability (MSI). The number of somatic mutations per DNA megabase (Mb), known as the TMB, is used to quantitatively evaluate the mutations carried by tumor cells (73). Greater numbers of neo-antigens, indicated by increased TMB, increases the likelihood that T cells will be recognized, which is clinically associated with improved ICI outcomes. Thus, the TMB is regarded as a reliable marker for estimating the effectiveness of immunotherapy in HCC. Data on 17 types of cancer were collected in a study by Samstein *et al* (74) confirming the initial finding that a high TMB is associated to immunotherapy effectiveness. Based in part on data from the KEYNOTE-158 study, the FDA approved the use of Pembrolizumab for solid tumors with 10 or more mutations/Mb in June 2020. However, there is not a fixed value of TMB for all types of cancer as the number of mutations defining TMB-high status varies with the type of cancer (74,75). Liver cancer has a median number of 4 mutations/Mb (n=755), with only 0.8% of patients having TMB-high tumors.

There are numerous studies on the role of the TMB in HCC (76,77). In a phase I clinical study, Xu *et al* (76) assessed the safety and efficacy of the combination of SHR-1210 (an anti-PD-1 antibody) and Apatinib in the treatment of patients with HCC. It was revealed that patients with a high-TMB had a worse prognosis compared with patients with a low TMB (mean, 8.53 vs. 1.44 mutations/Mb). Additionally, patients with a high-TMB had a reduced PFS with a reduction of 0.9 months compared with patients with a low TMB (76). However, only 1 patient (TMB, 15 mutations/Mb) in a fraction case series (total n=17) experienced a prolonged CR to ICI therapy. The TMB did not differ between responders and non-responders, highlighting the need for larger clinically annotated datasets to analyze outcome prediction (77).

Mismatch repair (MMR) in clinical practice is assessed largely by the reactions of four representative MMR-associated proteins (MLH1, MSH2, MSH6 and PMS2). One of the missing proteins is called DNA mismatch repair deficiency (dMMR) (78,79). MSI occurs during DNA replication, leading to alterations in the length or base composition of the MS, mainly as a result of dMMR. The MSI status of a tumor can

be categorized as stable (MSS), high instability (MSI-H) or low instability (80). Perbolizumab was given FDA approval in 2017 to treat MSI-H/dMMR solid tumors that are unresectable or metastatic, have progressed after prior therapy and for which there are no adequate alternative treatment options. The first pan-cancer marker identified, MSI-H/dMMR, is now being used to direct tumor immunotherapy, and has been demonstrated to have clinical value for the treatment of tumors (81,82). Even though the incidence of the MSI-H phenotype in HCC is low at only ~2%, inflammation-mediated MMR pathway dysfunction may be to blame for the accumulation of mutations observed during hepatitis-associated tumorigenesis (78,83,84). According to several reports, Pembrolizumab treatment completely reverses MSI in patients with advanced HCC (84,85). However, a study revealed that out of 50 patients, only one (2.0%) was identified as MSI-H with high TMB, $CD8^+$ lymphocyte infiltration, and low VEGF expression levels, and that patient did not experience as dramatic a response to Pembrolizumab treatment as suggested by other reports (86). MSI/dMMR is frequently used as a measure of the efficacy of immunotherapy for colorectal cancer (87). The most recent clinical study on neoadjuvant therapy for colorectal cancer included 12 patients with MSI-H/dMMR, and it revealed that all patients that finished treatment with checkpoint blockade had a clinically CR, without any reported adverse events of grade 3 or higher (88). However, another study that compared the OS of patients with resected colorectal cancer liver metastases between patients with MSS and MSI revealed that patients with MSI had a reduced OS, indicating a poor prognosis (89). The low proportion of patients with high TMB or MSI in HCC compared with gastric and colon cancer, and the sparse and contradictory information available, mainly from a small number of case reports or case series, make it impossible to determine predictive accuracy (78,86).

Tumor microenvironment (TME) components. The TME describes the area surrounding the tumor, containing various cell types, such as endothelial, immune cells and fibroblasts. Extracellular components, such as cytokines, the extracellular matrix, growth factors, hormones and peripheral blood vessels, are associated to the development and metastasis of tumors (90). In addition to these, the TME in liver cancer also contains pit cells, Kupffer cells, hepatic stellate cells, liver sinusoidal endothelial cells and hematopoietic stem cells (91). As $CD8^+$ lymphocytes are the most common T cell subset, the present review focuses on them. In several tumor types, high expression levels of $CD8^+$ tumor-infiltrating lymphocytes (TILs) are associated with a favorable prognosis (92). High intra-tumoral $CD8^+$ TIL levels have been associated with longer OS and DFS in a meta-analysis involving a total of 3,509 patients (93). Nevertheless, according to the experimental data from the CheckMate 040 trial, increased $CD3^+$ or $CD8^+$ tumor-infiltrating T cells were associated with improved survival rates and treatment responses, although this association was not apparent (94). Additionally, prognosis was not revealed to be associated to macrophage markers (14). Exhausted $CD8^+$ T cells also exhibit a lack of cytotoxicity, decreased release of proinflammatory cytokines, such as IL-2, IL-12, IFN-γ and TNF-α, increased expression levels of inhibitory receptors, such as PD-1 and CTLA-4, and transcriptional and epigenetic changes (95). Additionally, compared with other types of cancer, HCC has an increased concentration of PD-1(Hi) $CD8^+$ T cells that express exhaustion-associated inhibitory receptors, such as PD-1 and CTLA-4 on the surface of T cells, which is indicative of a poor prognosis (96). Immunohistochemistry (IHC) has demonstrated a strong association between an increased proportion of $CD38^+$ cells and an improved response to ICIs (97). An unfavorable prognosis was revealed to be predicted by the upregulation of the LDHA, BFSP1, PPAT, NR0B1 and PFKFB4 genes, as demonstrated by a tissue microarray analysis (98). Thus, the TME can be used as a biomarker for the precise identification of patients who are sensitive to immunotherapy. However, the clinical use of TME components as biomarkers to predict the response to immunotherapy in HCC appears challenging. There is a need for the standardization and validation of test methods, test timing and test interpretation.

Circulating biomarkers. Evaluation of the treatment of patients with liver cancer should be performed throughout the treatment course, with the need for convenient, rapid and reproducible methods. It is evident that repeated multiple invasive biopsies of tumor tissue are unacceptable to patients, and the detection of circulating tumor cells (CTCs) in the blood via liquid biopsy would be more convenient for clinical use. Peripheral blood can be used to detect circulating biomarkers such as exosomes, circulating tumor DNA (ctDNA), CTCs and metabolites (99). Single- or double-stranded DNA that responds to tumor heterogeneity forms ctDNA, which is derived from tumor cells (100). According to a study by Cabel *et al* (101), synchronous changes in the ctDNA levels and the tumor size at 8 weeks after immunotherapy were predictors of DFP and OS in non-small cell lung cancer (NSCLC) and colorectal cancer. However, the plasma contains only trace amounts of ctDNA, which also fluctuates dynamically, resulting in a fluctuating detection threshold and false negatives (102). Another possible circulating biomarker is CTCs. The potential of HCC-CTCs expressing PD-L1 as prognostic and predictive biomarkers was investigated in a study by Winograd *et al* (103), which revealed that PD-L1-positive CTCs were typical of advanced HCC. Immunotherapy led to a good therapeutic response in patients with PD-$L1^+$ CTCs (103). According to a different study, patients with 20% PD-L1-positive CTCs had an increased OS (median not reached vs. 8.9 months) and PFS (median 6.1 vs. 2.9 months) compared with patients with <20% PDL1-positive CTCs (76). These findings suggest that baseline ctDNA and high CTC levels might be used as predictors to select patients for immunotherapy and that dynamic changes in measured CTCs might be used as an indicator of treatment response in liver cancer, although this is still at an early stage. Further research is required on other circulating biomarkers such as extracellular vesicles and circulating RNA.

4. Combination of multiple biomarkers

There are a number of immunotherapy drugs applied in the treatment of liver cancer. Immunotherapy in combination with other anti-tumor treatments, such as TKI, VEGFR, TACE or double immunotherapy, is becoming more popular. It is challenging to identify biomarkers for the assessment

Table III. Tumor-associated biomarkers for HCC immunotherapy.

First author, year	Biomarker	Study design	Treatment	Patients	Outcome	Results	(Refs.)
Zhu *et al*, 2018	PD-L1	Prospective	Pembrolizumab	n=104	Response (complete response, partial response)	Using TPS scores, there was not a difference in the tumor response between patients with PD-L1 <1% and PD-L1 ≥1%.	(15)
Sangro *et al*, 2020	PD-L1 and PD-1	Retrospective	Nivolumab	PD-L1 <1% (n=159) and PD-L1 ≥1% (n=36)	OS	Tumor PD-1 and PD-L1 expression levels were associated with increased OS (P=0.05 and P=0.03,respectively).	(14)
Pinyol *et al*, 2019	Wnt-β-catenin pathway	Retrospective	Anti-PD-1 antibody	n=10	Median survival rate	Wnt-β-catenin was a marker of immuno-therapy sensitivity.	(62)
Ruiz de Galarreta *et al*, 2019	Wnt-β-catenin pathway	Mouse model	Anti-PD-1 antibody	-	-	β-catenin activation promoted immune escape and resistance to anti-PD-1, and may represent novel biomarkers for exclusion in patients with HCC.	(63)
Wang *et al*, 2015	Wnt-β-catenin pathway	Meta-analysis	-	β-catenin mutation (n=104) and control group (n=514)	OS	The meta-analysis revealed that the presence of β-catenin mutation, compared with the control group, was associated with an increased OS rate.	(65)
Chen *et al*, 2021	CTNNB1	Retrospective	ICIs	-	PFS	Univariate and multivariate Cox results demonstrated that only theCTNNB1-mutant was associated with the PFS of patients with HCC in the immunotherapy cohort.	(66)
Mariathasan *et al*, 2018	TGF-β	Mouse model	Anti-PD-1 antibody	-	-	TGF-β attenuates the tumor response to PD-L1 inhibition by contributing to the exclusion of T cells.	(69)
Xu *et al*, 2019	TMB	Prospective	SHR-1210 (an anti-PD-1 antibody) and Apatinib	n=43	PFS	Patients with a high TMB had a worse prognosis compared with patients with a low TMB. Additionally, patients with a high TMB had a reduced PFS with a reduction of 0.9 months compared with patients with a low TMB.	(76)
Ang *et al*, 2019	TMB and MSI	Retrospective	ICIs	n=17	Response	There were no genomic or TMB differences between patients that responded to treatment, had disease progression and had stable disease.	(77)
Kawaoka *et al*, 2020	MSI	Retrospective	Pembrolizumab	n=2	Response	CR with OS for >10 months was achieved in 1 patient, and the other patient did not respond to immunotherapy.	(84)

Table III. Continued.

First author, year	Biomarker	Study design	Treatment	Patients	Outcome	Results	(Refs.)
Xu *et al*, 2019	$CD8^+$ tumor-infiltrating lymphocytes	Meta-analysis	-	n=3,509	OS	The meta-analysis revealed that high levels of intra-tumoral $CD8^+$ tumor-infiltrating lymphocytes were associated with increased OS and DFS.	(93)
Ma *et al*, 2019	PD-1(Hi) and $CD8^+$ T cells	Retrospective	-	n=612	-	PD-1(Hi) or $TIM3^+$PD-1(Hi)$CD8^+$ T cells were associated with poor prognosis, and the latter was positioned in close proximity to PD-$L1^+$ tumor associated macrophages.	(96)
Ng *et al*, 2020	Intra-tumoral $CD38^+$ cells and $CD38^+$ $CD68^+$ macrophage density	Retrospective	ICIs	n=49	PFS and OS	IHC and mIHC/IF analyses revealed that an increased intra-tumoral $CD38^+$ cell proportion was strongly associated with an improved response to ICB. Patients with a high $CD38^+CD68^+$ macrophage density had an increased mOS (by 24 months) compared with patients with a low $CD38^+$ $CD68^+$ macrophage density.	(97)
Gu *et al*, 2021	Five immune-associated genes (LDHA, PPAT, BFSP1, NR0B1 and PFKFB4)	Retrospective	ICIs	n=365	-	ROC and Kaplan-Meier analyses indicated that the model could stratify patients into a low-risk and a high-risk group, wherein the high-risk group exhibited a worse prognosis and was less sensitive to immunotherapy compared with the low-risk group.	(98)
Winograd *et al*, 2020	CTCs	Prospective	ICIs	n=10	Response rate	There was a strong association between the presence of PD-$L1^+$ CTCs and a favorable treatment response in the subset of patients with HCC receiving immunotherapy.	(103)

HCC, hepatocellular carcinoma; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; CTCs, circulating tumor cells; CTNNB1, catenin β 1; MSI, microsatellite instability; ICIs, immune checkpoint inhibitors; OS, overall survival; PFS, progression-free survival; TPS, tumor proportional score; TMB, tumor mutational burden; CR, complete response; DFS, disease-free survival; $TIM3^+$, T cell immunoglobulin and mucin domain-containing protein 3; IHC, immunohistochemistry; mIHC/IF, multiplex IHC/immunofluorescence; mOS, median OS; ROC, receiver operating characteristic; ICB, immune checkpoint blocker.

of immunotherapy efficacy. A comprehensive treatment plan cannot be supported by a single biomarker (104). It is important to evaluate how different biomarkers interact, as is performed in the CRAFITY score, which combines CRP and AFP as aforementioned (105,106).

Analysis of the spatially distinct distribution of different immune cell types in the TME and the dynamic interactions between them has been demonstrated using multiplex IHC/immunofluorescence, which allows the simultaneous analysis of multiple immune parameters on the same paraffin-embedded tissue section (107). In HCC, Ng *et al* (97) revealed that the total $CD38^+$ cell ratio and $CD38^+CD68^+$ macrophage density were indicators of responsiveness to immune checkpoint blockade, and were an improvement on the PD-L1 score or $CD8^+$ T cell density. Additionally, the combined use of two markers can improve the prediction accuracy. In a recent study, the effects of TMB, gene expression profiling and PD-1, combined and alone, on the prognosis prediction in NSCLC were compared (108). It was revealed that the combination of at least two biomarkers was more accurate compared with the use of a single biomarker; however, combinations of three biomarkers were not revealed to be predictive (108). In patients with NSCLC, Hurkmans *et al* (109) investigated the interaction of PD-L1, CD8+ T cell infiltrates and human leukocyte antigens (HLA) class-I using IHC. The findings indicated that patients with an increased PFS had high tumor mutation loads, high infiltration of $CD8^+$ T cells or no loss of HLA class-I (109). In addition to the combination of immune drugs, new anti-tumor therapies such as photodynamic therapy and photothermal therapy can increase the immune response of tumor cells by changing the TME, and demonstrate synergistic effects (110). Comprehensive ranking based on the fundamental molecular and cellular pharmacological foundations and relevant mechanisms of action to hit multiple targets, as well as further investigation of the next-generation immunotherapies for patients with primary and acquired drug resistance, may improve the prediction of the optimal strategies (111,112). Currently, there are no data available on the combined prediction of immunotherapy efficacy by several indicators in liver cancer. Table III provides a brief summary of tumor-associated biomarkers used in HCC immunotherapy.

5. Conclusion

In recent years, more immune-associated drugs, including atezolizumab in combination with bevacizumab, pembrolizumab and nivolumab, have been administered in clinical settings. While progress has been made in the treatment of liver cancer, not all patients respond effectively to immunotherapy. An important problem that needs to be solved is how to identify patients who would be sensitive to immunotherapy to avoid exposure to drug toxicity and a waste of medical resources. Non-invasive biomarkers are necessary. The collection and detection of NLR, PLR, ctDNA, CTC and intestinal microorganisms is less traumatic to patients, easier to collect and can achieve dynamic detection. PD-1/PD-L1, genetic characteristics and the TME provide more information on tumor heterogeneity. However, the treatment of liver cancer is a combination of multiple treatment methods and various treatment modes. In metastatic melanoma, Pires da Silva *et al* (113) used conventional clinical parameters, factors such as the Eastern Cooperative Oncology Group Performance Status, presence/absence of liver and lung metastases, amongst others, to establish a model for predicting prognosis with validation in independent cohorts. The model successfully predicted the responses and survival rate outcomes of patients with metastatic melanoma after receiving immunotherapy (109,113,114). Furthermore, given the presence of tumor heterogeneity and the dynamic nature of the TME in liver cancer, as well as the complex interactions and regulation between the two, a single predictor is insufficient for the complexity of treatment methods. A combinatorial, precise and diverse strategy is thus necessary for immune biomarkers.

Acknowledgements

Not applicable.

Funding

This work was supported by the 1.3.5 Project for Disciplines of Excellence, West China Hospital, Sichuan University (grant no. ZYJC21043) and Sichuan Science and Technology Program (grant no. 23ZDYF2874).

Availability of data and materials

Not applicable.

Authors' contributions

MC conceived the topic for the present review and wrote the manuscript. JW and XZ were responsible for reviewing and editing the manuscript. ML revised the content of this review. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Zhu AX, Dayyani F, Yen CJ, Ren Z, Bai Y, Meng Z, Pan H, Dillon P, Mhatre SK, Gaillard VE, *et al*: Alpha-fetoprotein as a potential surrogate biomarker for atezolizumab + bevacizumab treatment of hepatocellular carcinoma. Clin Cancer Res 28: 3537-3545, 2022.
3. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J and Finn RS: Hepatocellular carcinoma. Nat Rev Dis Primers 7: 6, 2021.

4. Anwanwan D, Singh SK, Singh S, Saikam V and Singh R: Challenges in liver cancer and possible treatment approaches. Biochim Biophys Acta Rev Cancer 1873: 188314, 2020.
5. Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, Guo X, Kang B, Hu R, Huang JY, Zhang Q, *et al*: Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. Cell 169: 1342-1356.e16, 2017.
6. Wei SC, Duffy CR and Allison JP: Fundamental mechanisms of immune checkpoint blockade therapy. Cancer Discov 8: 1069-1086, 2018.
7. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, *et al*: Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 382: 1894-1905, 2020.
8. Zayac A and Almhanna K: Hepatobiliary cancers and immunotherapy: Where are we now and where are we heading? Transl Gastroenterol Hepatol 5: 8, 2020.
9. Ding X, He M, Chan A, Song QX, Sze SC, Chen H, Man MKH, Man K, Chan SL, Lai PBS, *et al*: Genomic and epigenomic features of primary and recurrent hepatocellular carcinomas. Gastroenterology 157: 1630-1645.e6, 2019.
10. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, *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 38: 193-202, 2020.
11. Yau T, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, Kudo M, Harding JJ, Merle P, Rosmorduc O, *et al*: Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): A randomised, multicentre, open-label, phase 3 trial. Lancet Oncol 23: 77-90, 2022.
12. Ren Z, Xu J, Bai Y, Xu A, Cang S, Du C, Li Q, Lu Y, Chen Y, Guo Y, *et al*: Sintilimab plus a bevacizumab biosimilar (IBI305) versus sorafenib in unresectable hepatocellular carcinoma (ORIENT-32): A randomised, open-label, phase 2-3 study. Lancet Oncol 22: 977-990, 2021.
13. Kudo M: Durvalumab plus tremelimumab in unresectable hepatocellular carcinoma. Hepatobiliary Surg Nutr 11: 592-596, 2022.
14. Sangro B, Melero I, Wadhawan S, Finn RS, Abou-Alfa GK, Cheng AL, Yau T, Furuse J, Park JW, Boyd Z, *et al*: Association of inflammatory biomarkers with clinical outcomes in nivolumab-treated patients with advanced hepatocellular carcinoma. J Hepatol 73: 1460-1469, 2020.
15. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, Verslype C, Zagonel V, Fartoux L, Vogel A, *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 19: 940-952, 2018.
16. Ho WJ, Danilova L, Lim SJ, Verma R, Xavier S, Leatherman JM, Sztein MB, Fertig EJ, Wang H, Jaffee E and Yarchoan M: Viral status, immune microenvironment and immunological response to checkpoint inhibitors in hepatocellular carcinoma. J Immunother Cancer 8: e000394, 2020.
17. Avgerinos KI, Spyrou N, Mantzoros CS and Dalamaga M: Obesity and cancer risk: Emerging biological mechanisms and perspectives. Metabolism 92: 121-135, 2019.
18. Ringel AE, Drijvers JM, Baker GJ, Catozzi A, García-Cañaveras JC, Gassaway BM, Miller BC, Juneja VR, Nguyen TH, Joshi S, *et al*: Obesity shapes metabolism in the tumor microenvironment to suppress anti-tumor immunity. Cell 183: 1848-1866.e26, 2020.
19. Akce M, Liu Y, Zakka K, Martini DJ, Draper A, Alese OB, Shaib WL, Wu C, Wedd JP, Sellers MT, *et al*: Impact of sarcopenia, BMI, and inflammatory biomarkers on survival in advanced hepatocellular carcinoma treated with anti-PD-1 antibody. Am J Clin Oncol 44: 74-81, 2021.
20. Bellissimo F, Pinzone MR, Cacopardo B and Nunnari G: Diagnostic and therapeutic management of hepatocellular carcinoma. World J Gastroenterol 21: 12003-12021, 2015.
21. Tsuchiya N, Sawada Y, Endo I, Saito K, Uemura Y and Nakatsura T: Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol 21: 10573-10583, 2015.
22. Cao W, Chen Y, Han W, Yuan J, Xie W, Liu K, Qiu Y, Wang X and Li X: Potentiality of α-fetoprotein (AFP) and soluble intercellular adhesion molecule-1 (sICAM-1) in prognosis prediction and immunotherapy response for patients with hepatocellular carcinoma. Bioengineered 12: 9435-9451, 2021.
23. Spahn S, Roessler D, Pompilia R, Gabernet G, Gladstone BP, Horger M, Biskup S, Feldhahn M, Nahnsen S, Hilke FJ, *et al*: Clinical and genetic tumor characteristics of responding and non-responding patients to PD-1 inhibition in hepatocellular carcinoma. Cancers (Basel) 12: 3830, 2020.
24. Sun X, Mei J, Lin W, Yang Z, Peng W, Chen J, Zhang Y, Xu L and Chen M: Reductions in AFP and PIVKA-II can predict the efficiency of anti-PD-1 immunotherapy in HCC patients. BMC Cancer 21: 775, 2021.
25. Hatanaka T, Kakizaki S, Hiraoka A, Tada T, Hirooka M, Kariyama K, Tani J, Atsukawa M, Takaguchi K, Itobayashi E, *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 16: 1150-1160, 2022.
26. Scheiner B, Pomej K, Kirstein MM, Hucke F, Finkelmeier F, Waidmann O, Himmelsbach V, Schulze K, von Felden J, Fründt TW, *et al*: Prognosis of patients with hepatocellular carcinoma treated with immunotherapy-development and validation of the CRAFITY score. J Hepatol 76: 353-363, 2022.
27. Guan R, Mei J, Lin W, Deng M, Li S and Guo R: Is the CRAFITY score a superior predictor of prognosis and adverse events in hepatocellular carcinoma patients treated with locoregional-immunotherapy? Hepatol Int 17: 1279-1288, 2023.
28. Yang M, Pan Y and Wang W: Prognostic significance of the CRAFITY score in hepatocellular carcinoma treated with immunotherapy: A systematic review and meta-analysis. BMC Cancer 23: 236, 2023.
29. Dharmapuri S, Özbek U, Lin JY, Sung M, Schwartz M, Branch AD and Ang C: 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 9: 4962-4970, 2020.
30. Muhammed A, Fulgenzi CAM, Dharmapuri S, Pinter M, Balcar L, Scheiner B, Marron TU, Jun T, Saeed A, Hildebrand H, *et al*: The systemic inflammatory response identifies patients with adverse clinical outcome from immunotherapy in hepatocellular carcinoma. Cancers (Basel) 14: 186, 2021.
31. Ochi H, Kurosaki M, Joko K, Mashiba T, Tamaki N, Tsuchiya K, Marusawa H, Tada T, Nakamura S, Narita R, *et al*: Usefulness of neutrophil-to-lymphocyte ratio in predicting progression and survival outcomes after atezolizumab-bevacizumab treatment for hepatocellular carcinoma. Hepatol Res 53: 61-71, 2023.
32. Jeon SH, Lee YJ, Kim HD, Nam H, Ryoo BY, Park SH, Yoo C and Shin EC: Dynamic changes in peripheral blood monocytes early after anti-PD-1 therapy predict clinical outcomes in hepatocellular carcinoma. Cancer Immunol Immunother 72: 371-384, 2023.
33. Riedl JM, Barth DA, Brueckl WM, Zeitler G, Foris V, Mollnar S, Stotz M, Rossmann CH, Terbuch A, Balic M, *et al*: C-reactive protein (CRP) levels in immune checkpoint inhibitor response and progression in advanced non-small cell lung cancer: A Bi-center study. Cancers (Basel) 12: 2319, 2020.
34. Klümper N, Saal J, Berner F, Lichtensteiger C, Wyss N, Heine A, Bauernfeind FG, Ellinger J, Brossart P, Diem S, *et al*: C reactive protein flare predicts response to checkpoint inhibitor treatment in non-small cell lung cancer. J Immunother Cancer 10: e004024, 2022.
35. Ramsey S: The role of the systemic inflammatory response as a biomarker in immunotherapy for renal cell cancer. Mol Diagn Ther 13: 277-281, 2009.
36. Chung MW, Kim MJ, Won EJ, Lee YJ, Yun YW, Cho SB, Joo YE, Hwang JE, Bae WK, Chung IJ, *et al*: Gut microbiome composition can predict the response to nivolumab in advanced hepatocellular carcinoma patients. World J Gastroenterol 27: 7340-7349, 2021.
37. Mao J, Wang D, Long J, Yang X, Lin J, Song Y, Xie F, Xun Z, Wang Y, Wang Y, *et al*: Gut microbiome is associated with the clinical response to anti-PD-1 based immunotherapy in hepatobiliary cancers. J Immunother Cancer 9: e003334, 2021.
38. Zhang L, Wu YN, Chen T, Ren CH, Li X and Liu GX: Relationship between intestinal microbial dysbiosis and primary liver cancer. Hepatobiliary Pancreat Dis Int 18: 149-157, 2019.
39. Schwabe RF and Greten TF: Gut microbiome in HCC-mechanisms, diagnosis and therapy. J Hepatol 72: 230-238, 2020.
40. Temraz S, Nassar F, Kreidieh F, Mukherji D, Shamseddine A and Nasr R: Hepatocellular carcinoma immunotherapy and the potential influence of gut microbiome. Int J Mol Sci 22: 7800, 2021.

41. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabanian H, Adeyemi A, Bataller R, *et al*: Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell 21: 504-516, 2012.
42. Peng X, Gong C, Zhang W and Zhou A: Advanced development of biomarkers for immunotherapy in hepatocellular carcinoma. Front Oncol 12: 1091088, 2023.
43. Vaisman-Mentesh A, Gutierrez-Gonzalez M, DeKosky BJ and Wine Y: The molecular mechanisms that underlie the immune biology of anti-drug antibody formation following treatment with monoclonal antibodies. Front Immunol 11: 1951, 2020.
44. Davda J, Declerck P, Hu-Lieskovan S, Hickling TP, Jacobs IA, Chou J, Salek-Ardakani S and Kraynov E: Immunogenicity of immunomodulatory, antibody-based, oncology therapeutics. J Immunother Cancer 7: 105, 2019.
45. Enrico D, Paci A, Chaput N, Karamouza E and Besse B: Antidrug antibodies against immune checkpoint blockers: Impairment of drug efficacy or indication of immune activation? Clin Cancer Res 26: 787-792, 2020.
46. Kim C, Yang H, Kim I, Kang B, Kim H, Kim H, Lee WS, Jung S, Lim HY, Cheon J and Chon HJ: Association of high levels of antidrug antibodies against atezolizumab with clinical outcomes and T-cell responses in patients with hepatocellular carcinoma. JAMA Oncol 8: 1825-1829, 2022.
47. Peters S, Galle PR, Bernaards CA, Ballinger M, Bruno R, Quarmby V, Ruppel J, Vilimovskij A, Wu B, Sternheim N and Reck M: Evaluation of atezolizumab immunogenicity: Efficacy and safety (Part 2). Clin Transl Sci 15: 141-157, 2022.
48. Myler H, Pedras-Vasconcelos J, Phillips K, Hottenstein CS, Chamberlain P, Devanaryan V, Gleason C, Goodman J, Manning MS, Purushothama S, *et al*: Anti-drug antibody validation testing and reporting harmonization. AAPS J 24: 4, 2021.
49. Sadreddini S, Baradaran B, Aghebati-Maleki A, Sadreddini S, Shanehbandi D, Fotouhi A and Aghebati-Maleki L: Immune checkpoint blockade opens a new way to cancer immunotherapy. J Cell Physiol 234: 8541-8549, 2019.
50. Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12: 252-264, 2012.
51. Kwok G, Yau TC, Chiu JW, Tse E and Kwong YL: Pembrolizumab (Keytruda). Hum Vaccin Immunother 12: 2777-2789, 2016.
52. Beaver JA, Hazarika M, Mulkey F, Mushti S, Chen H, He K, Sridhara R, Goldberg KB, Chuk MK, Chi DC, *et al*: Patients with melanoma treated with an anti-PD-1 antibody beyond RECIST progression: A US food and drug administration pooled analysis. Lancet Oncol 19: 229-239, 2018.
53. Ailia MJ, Heo J and Yoo SY: Navigating through the PD-1/PDL-1 landscape: A systematic review and meta-analysis of clinical outcomes in hepatocellular carcinoma and their influence on immunotherapy and tumor microenvironment. Int J Mol Sci 24: 6495, 2023.
54. Zhu AX, Abbas AR, de Galarreta MR, Guan Y, Lu S, Koeppen H, Zhang W, Hsu CH, He AR, Ryoo BY, *et al*: Molecular correlates of clinical response and resistance to atezolizumab in combination with bevacizumab in advanced hepatocellular carcinoma. Nat Med 28: 1599-1611, 2022.
55. Paver EC, Cooper WA, Colebatch AJ, Ferguson PM, Hill SK, Lum T, Shin JS, O'Toole S, Anderson L, Scolyer RA and Gupta R: Programmed death ligand-1 (PD-L1) as a predictive marker for immunotherapy in solid tumours: A guide to immunohistochemistry implementation and interpretation. Pathology 53: 141-156, 2021.
56. Doroshow DB, Bhalla S, Beasley MB, Sholl LM, Kerr KM, Gnjatic S, Wistuba II, Rimm DL, Tsao MS and Hirsch FR: PD-L1 as a biomarker of response to immune-checkpoint inhibitors. Nat Rev Clin Oncol 18: 345-362, 2021.
57. Dong ZY, Wu SP, Liao RQ, Huang SM and Wu YL: Potential biomarker for checkpoint blockade immunotherapy and treatment strategy. Tumour Biol 37: 4251-4261, 2016.
58. Kim MS, Xu A, Haslam A and Prasad V: Quality of biomarker defined subgroups in FDA approvals of PD-1/PD-L1 inhibitors 2014 to 2020. Int J Cancer 150: 1905-1910, 2022.
59. He S and Tang S: WNT/β-catenin signaling in the development of liver cancers. Biomed Pharmacother 132: 110851, 2020.
60. Perugorria MJ, Olaizola P, Labiano I, Esparza-Baquer A, Marzioni M, Marin JJG, Bujanda L and Banales JM: Wnt-β-catenin signalling in liver development, health and disease. Nat Rev Gastroenterol Hepatol 16: 121-136, 2019.
61. Llovet JM, Montal R, Sia D and Finn RS: Molecular therapies and precision medicine for hepatocellular carcinoma. Nat Rev Clin Oncol 15: 599-616, 2018.
62. Pinyol R, Sia D and Llovet JM: Immune exclusion-Wnt/CTNNB1 class predicts resistance to immunotherapies in HCC. Clin Cancer Res 25: 2021-2023, 2019.
63. Ruiz de Galarreta M, Bresnahan E, Molina-Sánchez P, Lindblad KE, Maier B, Sia D, Puigvehi M, Miguela V, Casanova-Acebes M, Dhainaut M, *et al*: β-catenin activation promotes immune escape and resistance to anti-PD-1 therapy in hepatocellular carcinoma. Cancer Discov 9: 1124-1141, 2019.
64. Harding JJ, Nandakumar S, Armenia J, Khalil DN, Albano M, Ly M, Shia J, Hechtman JF, Kundra R, El Dika I, *et al*: Prospective genotyping of hepatocellular carcinoma: Clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. Clin Cancer Res 25: 2116-2126, 2019.
65. Wang Z, Sheng YY, Gao XM, Wang CQ, Wang XY, Lu XU, Wei JW, Zhang KL, Dong QZ and Qin LX: β-catenin mutation is correlated with a favorable prognosis in patients with hepatocellular carcinoma. Mol Clin Oncol 3: 936-940, 2015.
66. Chen L, Zhou Q, Liu J and Zhang W: CTNNB1 alternation is a potential biomarker for immunotherapy prognosis in patients with hepatocellular carcinoma. Front Immunol 12: 759565, 2021.
67. Caja L, Dituri F, Mancarella S, Caballero-Diaz D, Moustakas A, Giannelli G and Fabregat I: TGF-β and the tissue microenvironment: Relevance in fibrosis and cancer. Int J Mol Sci 19: 1294, 2018.
68. Chen J, Gingold JA and Su X: Immunomodulatory TGF-β signaling in hepatocellular carcinoma. Trends Mol Med 25: 1010-1023, 2019.
69. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, Kadel EE III, Koeppen H, Astarita JL, Cubas R, *et al*: TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 554: 544-548, 2018.
70. Horn LA, Chariou PL, Gameiro SR, Qin H, Iida M, Fousek K, Meyer TJ, Cam M, Flies D, Langermann S, *et al*: Remodeling the tumor microenvironment via blockade of LAIR-1 and TGF-β signaling enables PD-L1-mediated tumor eradication. J Clin Invest 132: e155148, 2022.
71. Wu ZH, Yang DL, Wang L and Liu J: Epigenetic and immune-cell infiltration changes in the tumor microenvironment in hepatocellular carcinoma. Front Immunol 12: 793343, 2021.
72. Dai Y, Qiang W, Lin K, Gui Y, Lan X and Wang D: An immune-related gene signature for predicting survival and immunotherapy efficacy in hepatocellular carcinoma. Cancer Immunol Immunother 70: 967-979, 2021.
73. Klempner SJ, Fabrizio D, Bane S, Reinhart M, Peoples T, Ali SM, Sokol ES, Frampton G, Schrock AB, Anhorn R and Reddy P: Tumor mutational burden as a predictive biomarker for response to immune checkpoint inhibitors: A review of current evidence. Oncologist 25: e147-e159, 2020.
74. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, *et al*: Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 51: 202-206, 2019.
75. Marabelle A, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K, Chung HC, Kindler HL, Lopez-Martin JA, Miller WH Jr, *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 21: 1353-1365, 2020.
76. Xu J, Zhang Y, Jia R, Yue C, Chang L, Liu R, Zhang G, Zhao C, Zhang Y, Chen C, *et al*: Anti-PD-1 antibody SHR-1210 combined with apatinib for advanced hepatocellular carcinoma, gastric, or esophagogastric junction cancer: An open-label, dose escalation and expansion study. Clin Cancer Res 25: 515-523, 2019.
77. Ang C, Klempner SJ, Ali SM, Madison R, Ross JS, Severson EA, Fabrizio D, Goodman A, Kurzrock R, Suh J and Millis SZ: Prevalence of established and emerging biomarkers of immune checkpoint inhibitor response in advanced hepatocellular carcinoma. Oncotarget 10: 4018-4025, 2019.
78. Eso Y, Shimizu T, Takeda H, Takai A and Marusawa H: Microsatellite instability and immune checkpoint inhibitors: Toward precision medicine against gastrointestinal and hepatobiliary cancers. J Gastroenterol 55: 15-26, 2020.
79. Malapelle U, Parente P, Pepe F, De Luca C, Pisapia P, Sgariglia R, Nacchio M, Gragnano G, Russo G, Conticelli F, *et al*: Evaluation of micro satellite instability and mismatch repair status in different solid tumors: A multicenter analysis in a real world setting. Cells 10: 1878, 2021.

80. Cohen R, Hain E, Buhard O, Guilloux A, Bardier A, Kaci R, Bertheau P, Renaud F, Bibeau F, Fléjou JF, *et al*: Association of primary resistance to immune checkpoint inhibitors in metastatic colorectal cancer with misdiagnosis of microsatellite instability or mismatch repair deficiency status. JAMA Oncol 5: 551-555, 2019.
81. Marcus L, Lemery SJ, Keegan P and Pazdur R: FDA approval summary: Pembrolizumab for the treatment of microsatellite instability-high solid tumors. Clin Cancer Res 25: 3753-3758, 2019.
82. Prasad V, Kaestner V and Mailankody S: Cancer drugs approved based on biomarkers and not tumor Type-FDA approval of pembrolizumab for mismatch repair-deficient solid cancers. JAMA Oncol 4: 157-158, 2018.
83. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ, Reeser JW, Yu L and Roychowdhury S: Landscape of microsatellite instability across 39 cancer types. JCO Precis Oncol 2017: PO.17.00073, 2017.
84. Kawaoka T, Ando Y, Yamauchi M, Suehiro Y, Yamaoka K, Kosaka Y, Fuji Y, Uchikawa S, Morio K, Fujino H, *et al*: Incidence of microsatellite instability-high hepatocellular carcinoma among Japanese patients and response to pembrolizumab. Hepatol Res 50: 885-888, 2020.
85. Ando Y, Yamauchi M, Suehiro Y, Yamaoka K, Kosaka Y, Fuji Y, Uchikawa S, Kodama K, Morio K, Fujino H, *et al*: Complete response to pembrolizumab in advanced hepatocellular carcinoma with microsatellite instability. Clin J Gastroenterol 13: 867-872, 2020.
86. Mukai S, Kanzaki H, Ogasawara S, Ishino T, Ogawa K, Nakagawa M, Fujiwara K, Unozawa H, Iwanaga T, Sakuma T, *et al*: Exploring microsatellite instability in patients with advanced hepatocellular carcinoma and its tumor microenvironment. JGH Open 5: 1266-1274, 2021.
87. Buchler T: Microsatellite instability and metastatic colorectal cancer-a clinical perspective. Front Oncol 12: 888181, 2022.
88. Cercek A, Lumish M, Sinopoli J, Weiss J, Shia J, Lamendola-Essel M, El Dika IH, Segal N, Shcherba M, Sugarman R, *et al*: PD-1 blockade in mismatch repair-deficient, locally advanced rectal cancer. N Engl J Med 386: 2363-2376, 2022.
89. Turner KM, Delman AM, Wima K, Quillin RC, Shah SA, Ahmad SA, Patel SH and Wilson GC: Microsatellite instability is associated with worse overall survival in resectable colorectal liver metastases. Am J Surg 225: 322-327, 2023.
90. Wu T and Dai Y: Tumor microenvironment and therapeutic response. Cancer Lett 387: 61-68, 2017.
91. Oura K, Morishita A, Tani J and Masaki T: Tumor immune microenvironment and immunosuppressive therapy in hepatocellular carcinoma: A review. Int J Mol Sci 22: 5801, 2021.
92. Zheng X, Jin W, Wang S and Ding H: Progression on the roles and mechanisms of tumor-infiltrating T lymphocytes in patients with hepatocellular carcinoma. Front Immunol 12: 729705, 2021.
93. Xu X, Tan Y, Qian Y, Xue W, Wang Y, Du J, Jin L and Ding W: Clinicopathologic and prognostic significance of tumor-infiltrating CD8+ T cells in patients with hepatocellular carcinoma: A meta-analysis. Medicine (Baltimore) 98: e13923, 2019.
94. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, *et al*: Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): An open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet 389: 2492-2502, 2017.
95. Wang C, Singer M and Anderson AC: Molecular dissection of CD8(+) T-cell dysfunction. Trends Immunol 38: 567-576, 2017.
96. Ma J, Zheng B, Goswami S, Meng L, Zhang D, Cao C, Li T, Zhu F, Ma L, Zhang Z, *et al*: $PD1^{Hi}$ $CD8^{+}$ T cells correlate with exhausted signature and poor clinical outcome in hepatocellular carcinoma. J Immunother Cancer 7: 331, 2019.
97. Ng HHM, Lee RY, Goh S, Tay ISY, Lim X, Lee B, Chew V, Li H, Tan B, Lim S, *et al*: Immunohistochemical scoring of CD38 in the tumor microenvironment predicts responsiveness to anti-PD-1/PD-L1 immunotherapy in hepatocellular carcinoma. J Immunother Cancer 8: e000987, 2020.
98. Gu X, Guan J, Xu J, Zheng Q, Chen C, Yang Q, Huang C, Wang G, Zhou H, Chen Z and Zhu H: Model based on five tumour immune microenvironment-related genes for predicting hepatocellular carcinoma immunotherapy outcomes. J Transl Med 19: 26, 2021.
99. Maravelia P, Silva DN, Rovesti G, Chrobok M, Stål P, Lu YC and Pasetto A: Liquid biopsy in hepatocellular carcinoma: Opportunities and challenges for immunotherapy. Cancers (Basel) 13: 4334, 2021.
100. Cheng F, Su L and Qian C: Circulating tumor DNA: A promising biomarker in the liquid biopsy of cancer. Oncotarget 7: 48832-48841, 2016.
101. Cabel L, Riva F, Servois V, Livartowski A, Daniel C, Rampanou A, Lantz O, Romano E, Milder M, Buecher B, *et al*: Circulating tumor DNA changes for early monitoring of anti-PD1 immunotherapy: A proof-of-concept study. Ann Oncol 28: 1996-2001, 2017.
102. Peng Y, Mei W, Ma K and Zeng C: Circulating tumor DNA and minimal residual disease (MRD) in solid tumors: Current horizons and future perspectives. Front Oncol 11: 763790, 2021.
103. Winograd P, Hou S, Court CM, Lee YT, Chen PJ, Zhu Y, Sadeghi S, Finn RS, Teng PC, Wang JJ, *et al*: Hepatocellular carcinoma-circulating tumor cells expressing PD-L1 are prognostic and potentially associated with response to checkpoint inhibitors. Hepatol Commun 4: 1527-1540, 2020.
104. Cheng AL, Hsu C, Chan SL, Choo SP and Kudo M: Challenges of combination therapy with immune checkpoint inhibitors for hepatocellular carcinoma. J Hepatol 72: 307-319, 2020.
105. Lee JS and Ruppin E: Multiomics prediction of response rates to therapies to inhibit programmed cell death 1 and programmed cell death 1 ligand 1. JAMA Oncol 5: 1614-1618, 2019.
106. Lu S, Stein JE, Rimm DL, Wang DW, Bell JM, Johnson DB, Sosman JA, Schalper KA, Anders RA, Wang H, *et al*: Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade: A systematic review and meta-analysis. JAMA Oncol 5: 1195-1204, 2019.
107. Giraldo NA, Nguyen P, Engle EL, Kaunitz GJ, Cottrell TR, Berry S, Green B, Soni A, Cuda JD, Stein JE, *et al*: Multidimensional, quantitative assessment of PD-1/PD-L1 expression in patients with Merkel cell carcinoma and association with response to pembrolizumab. J Immunother Cancer 6: 99, 2018.
108. Kim H, Kwon HJ, Kim ES, Kwon S, Suh KJ, Kim SH, Kim YJ, Lee JS and Chung JH: Comparison of the predictive power of a combination versus individual biomarker testing in non-small cell lung cancer patients treated with immune checkpoint inhibitors. Cancer Res Treat 54: 424-433, 2022.
109. Hurkmans DP, Kuipers ME, Smit J, van Marion R, Mathijssen RHJ, Postmus PE, Hiemstra PS, Aerts JGJV, von der Thüsen JH and van der Burg SH: Tumor mutational load, $CD8^{+}$ T cells, expression of PD-L1 and HLA class I to guide immunotherapy decisions in NSCLC patients. Cancer Immunol Immunother 69: 771-777, 2020.
110. Yin S, Chen Z, Chen D and Yan D: Strategies targeting PD-L1 expression and associated opportunities for cancer combination therapy. Theranostics 13: 1520-1544, 2023.
111. Lemaire V, Shemesh CS and Rotte A: Pharmacology-based ranking of anti-cancer drugs to guide clinical development of cancer immunotherapy combinations. J Exp Clin Cancer Res 40: 311, 2021.
112. Marei HE, Hasan A, Pozzoli G and Cenciarelli C: Cancer immunotherapy with immune checkpoint inhibitors (ICIs): Potential, mechanisms of resistance, and strategies for reinvigorating T cell responsiveness when resistance is acquired. Cancer Cell Int 23: 64, 2023.
113. Pires da Silva I, Ahmed T, McQuade JL, Nebhan CA, Park JJ, Versluis JM, Serra-Bellver P, Khan Y, Slattery T, Oberoi HK, *et al*: Clinical models to define response and survival with anti-PD-1 antibodies alone or combined with ipilimumab in metastatic melanoma. J Clin Oncol 40: 1068-1080, 2022.
114. Rotte A: Predictive models for response and survival in patients treated with anti-PD-1 monotherapy or with anti-PD-1 and ipilimumab combination: Editorial commentary. Ann Transl Med 11: 227, 2023.