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Review article

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Biomarkers for immunotherapy of hepatocellular carcinoma

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Abstract

Immune-checkpoint inhibitors (ICIs) are now widely used for the treatment of patients with advanced-stage hepatocellular carcinoma (HCC). Two different ICI-containing regimens, atezolizumab plus bevacizumab and tremelimumab plus durvalumab, are now approved standard-of-care first-line therapies in this setting. However, and despite substantial improvements in survival outcomes relative to sorafenib, most patients with advanced-stage HCC do not derive durable benefit from these regimens. Advances in genome sequencing including the use of single-cell RNA sequencing (both of tumour material and blood samples), as well as immune cell identification strategies and other techniques such as radiomics and analysis of the microbiota, have created considerable potential for the identification of novel predictive biomarkers enabling the accurate selection of patients who are most likely to derive benefit from ICIs. In this Review, we summarize data on the immunology of HCC and the outcomes in patients receiving ICIs for the treatment of this disease. We then provide an overview of current biomarker use and developments in the past 5 years, including gene signatures, circulating tumour cells, high-dimensional flow cytometry, single-cell RNA sequencing as well as approaches involving the microbiome, radiomics and clinical markers. Novel concepts for further biomarker development in HCC are then discussed including biomarker-driven trials, spatial transcriptomics and integrated 'big data' analysis approaches. These concepts all have the potential to better identify patients who are most likely to benefit from ICIs and to promote the development of new treatment approaches.

Sections

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Introduction

With almost one million new cases worldwide[1] and as the cause of an estimated 30,000 deaths in the USA in 2023 (ref. 2), liver cancer is a major health problem. The medical management of hepatocellular carcinoma (HCC), the most common form (~90%) of liver cancer, is complex owing to its heterogeneity and coexistence with other underlying liver diseases[3]. Liver cancers have several unique features including being the only solid tumour that can be routinely diagnosed without the need for a biopsy sample. Furthermore, organ transplantation remains a standard of care, given that >80% of patients will also have underlying liver disease and/or liver dysfunction[3].

Up until 2017, when regorafenib was approved as second-line therapy[4], systemic treatment options were limited to the tyrosine kinase inhibitor (TKI) sorafenib[5]. Currently, more than ten FDA-approved systemic therapies are available for patients with HCC[6], including several immune-checkpoint inhibitors (ICIs)[3]. These latter agents have led to an improvement in the median overall survival (OS) of patients in various clinical trials[4,6–10], although only a minority of patients derive durable benefit, and the lack of predictive biomarkers precludes identifying such patients prior to treatment[11]. Data from retrospective studies indicate a positive correlation between immune-related adverse events (irAEs) and improved OS in patients receiving ICIs[12,13], although this knowledge cannot help in identifying patients prior to treatment initiation. Thus, predictive biomarkers are urgently needed.

In this Review, we provide a summary of the current status of biomarker development in HCC. We also provide some insights into potential novel biomarkers and the most promising future research directions in this field.

Immunotherapy for HCC

HCC is the most common primary liver cancer, and frequently develops in the presence of chronic liver disease owing to hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, alcohol use disorders, or non-alcoholic steatohepatitis (NASH)[3]. NASH, which is typically associated with metabolic syndrome and diabetes mellitus, is the fastest growing aetiology of HCC, particularly in Western countries[14]. This aetiology confirms HCC as an inflammation-induced cancer. HCC is usually diagnosed based on imaging results in patients with liver cirrhosis[15]; thus, tumour biopsy sampling is unnecessary in most patients. Commonly used staging systems incorporate measures of tumour diameter, number of lesions and their locations, liver function (Child–Pugh classification)[16] and performance status (albumin–bilirubin (ALBI) score)[14]. Various curative treatment options can be offered for patients with small (up to 3 cm in diameter) and/or few (up to 3) lesions, including surgical resection, orthotopic liver transplantation and radiofrequency ablation, leading to excellent 5-year OS outcomes of >75%[17]. Transarterial chemoembolization (TACE) has been globally adopted as the standard of care for patients with intermediate-stage (multinodular disease limited to the liver, and preserved liver function[17]) HCC leading to a median OS >2.5 years[3,15,18–21]. Several different systemic therapies are recommended for patients with advanced-stage HCC, which is characterized by the presence of extrahepatic disease and/or rapid progression on locoregional therapies[3].

Currently, five different drugs and/or regimens have been shown to be effective as first-line therapy for patients with advanced-stage HCC. The SHARP trial was the first trial to demonstrate any OS benefit in this setting in 2008, with patients receiving sorafenib having a median OS duration of 10.7 months versus 7.9 months in those randomized to placebo (HR 0.69, 95% CI 0.55–0.87; P <0.001)[5]. A second broad-spectrum TKI, lenvatinib, was subsequently demonstrated to be non-inferior to sorafenib in this setting in 2018 (ref. 22). Since then, two large-cohort randomized phase III trials testing ICI–VEGFR-targeted therapy combination regimens have demonstrated an improvement in OS relative to sorafenib. The combination of the anti-PD-L1 antibody atezolizumab plus bevacizumab was tested in the IMbrave150 study in a cohort of 501 patients (Table 1). Patients were randomly assigned 2:1 to receive either atezolizumab plus bevacizumab, or sorafenib. This trial was terminated at the first interim analysis after a median follow-up duration of only 8.6 months owing to early evidence of improved OS (HR 0.58, 95% CI 0.42–0.79; P = 0.0006) and progression-free survival (PFS; HR 0.59, 95% CI 0.47–0.76; P < 0.0001)[7], leading to FDA approval in 2020. Longer follow-up (median 15.6 months) revealed a median OS duration of 19.2 months in the atezolizumab plus bevacizumab arm and 13.4 months in the sorafenib arm[4]. Confirmed objective response rates (ORRs) according to RECIST 1.1 were 27.3% in the atezolizumab plus bevacizumab arm versus 11.9% in the sorafenib arm, with complete responses in 5.5% and partial response (PRs) in 21.8% versus 0% and 11.9%, respectively[7].

Similar results were observed in the ORIENT-32 study (Table 1). This study enrolled primarily patients of Asian ethnicity and tested the combination of the anti-PD-1 antibody sintilimab plus IBI305 (a bevacizumab biosimilar) versus sorafenib as first-line therapy in patients with unresectable HBV-associated HCC. At a median follow-up duration of 10.0 months, median OS was not reached in the sintilimab plus IBI305 arm versus 10.4 months in the sorafenib arm (HR 0.57, 95% CI 0.43–0.75; P < 0.0001)[23].

Results from the HIMALAYA trial, in which 1,171 patients were randomly assigned to receive a single priming dose of the anti-CTLA4 antibody tremelimumab plus the anti-PD-1 antibody durvalumab, durvalumab monotherapy, or sorafenib monotherapy, were reported in 2022 (ref. 8) (Table 1). The primary end point of this trial was OS among patients receiving tremelimumab plus durvalumab, relative to those receiving sorafenib. Non-inferior OS for durvalumab monotherapy versus sorafenib was a secondary objective. Median OS with tremelimumab plus durvalumab was 16.4 months versus 13.8 months with sorafenib (HR 0.78, 96.02% CI 0.65–0.93; P = 0.0035) and 16.6 months with durvalumab alone; OS at 36 months was 30.7%, 20.2% and 24.7%, respectively. Median OS with durvalumab monotherapy was non-inferior to that with sorafenib (HR 0.86, 95.67% CI 0.73–1.03; non-inferiority margin 1.08). Direct comparisons of tremelimumab plus durvalumab versus durvalumab monotherapy was not part of the study design[8].

In the second-line setting, the phase III KEYNOTE-240 trial compared the efficacy of pembrolizumab versus placebo in a total of 413 patients (Table 1). Although the median OS duration was 13.9 months with pembrolizumab versus 10.6 months with placebo (HR 0.78, 95% CI 0.61–1.00; P = 0.024), this study did not meet the primary end points because OS and PFS did not reach statistical significance per prespecified criteria[10]. KEYNOTE-394 was a similar study but conducted in Asia and in this case pembrolizumab significantly improved OS over placebo plus best supportive care[24] (Table 1).

The combination of an anti-CTLA4 antibody plus an anti-PD-1 antibody in the second-line (post-sorafenib) setting was tested in the CheckMate 040 trial. In this study 148 patients received nivolumab plus ipilimumab administered according to one of three different doses and schedules (Table 1). At the time of publication, at a median follow-up duration of 30.7 months, the best ORR of 32% was observed with nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg) administered every 3 weeks for a maximum of four doses followed by nivolumab (240 mg), and the median duration of response was not reached, leading to FDA Accelerated Approval in March 2020 (ref. 25).

Table 1 | Overview of trials testing ICIs and ICI-based regimens in patients with hepatocellular carcinoma

Trial	Intervention	Outcomes	Biomarker analysis	Refs.
Phase III trials				
IMbrave150	Atezolizumab plus bevacizumab (*n*=336) vs sorafenib (*n*=165) as first-line therapy	ORR 27.3% vs 11.9%; DCR 73.6% vs 55.3%; mPFS 6.6 months vs 4.3 months (HR 0.65, 95% CI 0.53–0.81; *P*<0.001); mOS 19.2 vs 13.4 months (HR 0.66, 95% CI 0.52–0.85; *P*<0.001)	Blood and tumour biopsies	7,37
HIMALAYA	Tremelimumab plus durvalumab (*n*=393) vs durvalumab (*n*=389) vs sorafenib (*n*=389) as first-line therapy	ORR 20.1% vs 17.0% vs 5.1%; DCR 60.1% vs 54.8% vs 60.7%; mPFS 3.8 months vs 3.7 months vs 4.1 months (HR 0.90, 95% CI, 0.77 to 1.05[a]); mOS 16.4 months vs 16.6 months vs 13.8 months (HR 0.78, 96.02% CI 0.65–0.93; *P*=0.0035[a])	Tumour biopsies	8
CheckMate 459	Nivolumab (*n*=371) vs sorafenib (*n*=372) as first-line therapy	ORR 15% vs 7%; DCR 55% vs 58%; mPFS 3.7 months vs 3.8 months (HR 0.93, 95% CI 0.79–1.10); mOS 16.4 months vs 14.7 months (HR 0.85, 95% CI 0.72–1.02; *P*=0.075)	Tumour biopsies	9
KEYNOTE-240	Pembrolizumab (*n*=278) vs BSC (*n*=135) as second-line therapy	ORR 18.3% vs 4.4%; DCR 62.2% vs 53.3%; mPFS 3.0 months vs 2.8 months, HR, 0.78, 95% CI 0.61–0.99, *P*=0.019; mOS 13.9 months vs 11.6 months, HR 0.78, 95% CI 0.62–1.0, *P*=0.024	NA	10
ORIENT-32	Sintilimab plus IBI305 (a bevacizumab biosimilar; *n*=380) vs sorafenib (*n*=191) as first-line therapy	ORR 21% vs 4%; DCR 72% vs 64%; mPFS 4.6 months vs 2.8 months (HR 0.56, 95% CI 0.46–0.70; *P*<0.0001); mOS NR vs 10.4 months (HR 0.57, 95% CI 0.43–0.75; *P*<0.0001)	Tumour biopsies	23
IMbrave050	Atezolizumab plus bevacizumab (*n*=334) vs active surveillance (*n*=334) as adjuvant therapy	HR 0.7 (mPFS not reached)	PD-L1 expression	27
RATIONALE-301	Tislelizumab (*n*=342) vs sorafenib (*n*=332) as first-line therapy	ORR 14.3% vs 5.4%; mPFS 2.2 months vs 3.6 months (HR 1.1, 95% CI 0.92–1.33); mOS 15.9 months vs 14.1 months (HR 0.85, 95.003% CI 0.71–1.02)	NA	190
NCT03764293	Camrelizumab plus rivoceranib (*n*=272) vs sorafenib (*n*=271) as first-line therapy	ORR 25.4% vs 5.9%; DCR 78.3% vs 53.9%; 5.6 months vs 3.7 months (HR 0.52, 95% CI 0.41–0.65; *P*<0.0001); mOS 22.1 months vs 15.2 months (HR 0.62, 95% CI 0.49–0.80; *P*<0.0001)	NA	191
COSMIC-312	Atezolizumab plus cabozantinib (*n*=432) vs sorafenib (*n*=217) vs cabozantinib (*n*=188) as first-line therapy	ORR 11% vs 4% vs 6%; DCR 78% vs 65% vs 84%; mPFS 6.8 months vs 4.2 months (HR 0.63, 99% CI 0.44–0.91; *P*=0.0012) vs 5.8 months; mOS 15.4 months vs 15.5 months (HR 0.90, 96% CI 0.69–1.18; *P*=0.44) vs NR	NA	192
LEAP-002	Lenvatinib plus pembrolizumab (*n*=395) vs lenvatinib (*n*=399) as first-line therapy	ORR 26.1% vs 17.5%; mPFS 8.2 months vs 8.1 months (HR 0.83, 95% CI 0.71–0.98; *P*=0.047); mOS 21.2 months vs 19.0 months (HR 0.84, 95% CI 0.71–1.0; *P*=0.023)	NA	193
NCT02645981	Donafenib (*n*=328) vs sorafenib (*n*=321) as first-line therapy	ORR 4.6% vs 2.7%; DCR 30.8% vs 28.7%; mPFS 3.7 months vs 3.6 months (HR 0.91, 95% CI 0.76–1.1; *P*=0.057); mOS 12.1 months vs 10.3 months (HR 0.83, 95% CI 0.7–0.99; *P*=0.025)	NA	194
AHELP	Apatinib (*n*=267) vs placebo (*n*=133) as second-line therapy	ORR 11% vs 2%; DCR 61% vs 29%; mPFS 4.5 months vs 1.9 months (HR 0.47, 95% CI 0.37–0.60, *P*<0.0001); mOS 8.7 months vs 6.8 months (HR 0.79, 95% CI 0.62–0.1; *P*=0.048)	NA	195
KEYNOTE-394	Pembrolizumab (*n*=300) vs placebo (*n*=153) as second-line therapy	ORR 12.7% vs 1.3%; DCR 51.0% vs 47.1%; mPFS 2.6 months vs 2.3 months (HR 0.74, 95% CI 0.60–0.92; *P*=0.0032); mOS 14.6 months vs 13.0 months (HR 0.79, 95% CI 0.63–0.99; *P*=0.018)	NA	196
Phase I/II trials				
CheckMate 040	Nivolumab plus ipilimumab as second-line therapy (*n*=148)[b]	ORR 32% vs 27% vs 29%; DCR 54% vs 43% vs 49%; mOS 22.8 months vs 12.5 months vs 12.7 months	Tumour biopsies, blood samples	25
NCT02658019	Pembrolizumab as second-line therapy (n=29)	ORR 32%; DCR 46%; mPFS 4.5 months; mOS 13 months	NA	97
KEYNOTE-224	Pembrolizumab as first-line therapy (*n*=51)	ORR 16%; DCR 57%; mPFS 4.0 months; mOS 17 months	NA	197
CheckMate 040 Cohort 6	Nivolumab plus cabozantinib (*n*=36) versus nivolumab plus cabozantinib plus ipilimumab (*n*=35) as first-line or second-line therapy	ORR 17% vs 29%; DCR 81% vs 83%; mPFS 5.1 months vs 4.3 months; mOS 20.2 months vs 22.1 months	NA	198
RESCUE	Camrelizumab plus apatinib as first-line or second-line therapy (*n*=190)	ORR 34.3–22.5%; DCR 77.1–75.8%; mPFS 5.7–5.5 months	Tumour biopsies	199
Study 116	Lenvatinib plus pembrolizumab as first-line or second-line therapy (*n*=104)	ORR 36%, DCR 88%, mPFS 8.6 months, mOS 22 months	NA	200
NCT03298451	Tremelimumab plus durvalumab (*n*=75)[c] vs durvalumab (*n*=104) vs tremelimumab (*n*=69) vs tremelimumab plus durvalumab (*n*=84)[d]	ORR 24.0% vs 10.6% vs 7.2% vs 9.5%; DCR 45.3% vs 37.5% vs 49.3% vs 36.9%; mPFS 2.2 months vs 2.1 months vs 2.7 months vs 1.9 months; mOS 18.7 months vs 13.6 months vs 15.1 months vs 11.3 months	PBMCs, tumour biopsies	201

BSC, best supportive care; DCR, disease control rate; ICI, immune-checkpoint inhibitor; mOS, median overall survival; mPFS, median progression-free survival; NA, not applicable; NR, not reached; ORR, objective response rate; PBMCs, peripheral blood mononuclear cells. [a]Statistical comparisons are provided for tremelimumab plus durvalumab versus sorafenib. [b]Nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks for four doses followed by followed by nivolumab 240 mg every 2 weeks (arm A) vs nivolumab 3 mg/kg plus ipilimumab 1 mg/kg administered every 3 weeks for four doses (arm B) vs nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks (arm C). [c]Tremelimumab 300 mg plus durvalumab 1,500 mg. [d]Tremelimumab 75 mg plus durvalumab 1,500 mg.

Immune-related toxicities are common in patients with HCC receiving ICIs and are dependent on the agent or agents received[26]. One or more adverse events of grade 3–4 occurred in 56.5% of patients receiving atezolizumab plus bevacizumab in the IMbrave150 study, including hypertension in 15.2% and serum aspartate aminotransferase increases in 7%[7]. Similarly, at least one adverse event of grade 3–4 was detected in 50.5% of the patients receiving tremelimumab plus durvalumab in the HIMALAYA study, of whom 12.6% had events deemed immune-related by the investigators[8].

Initial data from the IMbrave050 trial, the first phase III trial testing ICI–VEGF-targeted therapy combinations in the adjuvant setting, were reported in April 2023. A total of 668 patients with HCC with a high risk of disease recurrence following tumour resection and/or ablation were randomized to receive atezolizumab plus bevacizumab versus active surveillance. A significant difference in recurrence-free survival, the primary end point of the study, was demonstrated in an interim analysis at a median follow-up duration of 17.4 months, and favoured the atezolizumab plus bevacizumab arm (HR 0.72, 95% CI 0.56–0.93; $P = 0.012$)[27]. Investigators described the safety of the combination as generally manageable.

To date, three groups have reported provocative data on the activity of ICIs in the neoadjuvant setting in patients with surgically resectable HCC. In one study[28], investigators tested the combination of perioperative nivolumab with or without ipilimumab in an open-label randomized phase II trial involving 27 patients. Safety (the primary end point) was deemed acceptable, with adverse events of grade 3–4 seen in 23% versus 43% of patients in the nivolumab and nivolumab–ipilimumab groups, respectively. No patient in either group had surgery delayed owing to adverse events, although seven did not undergo surgery owing to disease progression or study non-compliance. Among those who underwent surgery, major pathological responses (MPRs) (defined as ≥70% necrosis in the resected tumour area) were observed in three of nine patients (33%) who received nivolumab versus three of 11 patients (27%) who received nivolumab plus ipilimumab. Elsewhere, the combination of neoadjuvant cabozantinib plus nivolumab was tested in 15 patients with HCC in a phase Ib trial. This study reportedly met the primary end points of safety and feasibility. Most patients (12/15, 80%) had margin-negative resections, and five of these 12 patients (42%) had an MPR[29,30]. Finally, neoadjuvant therapy with the anti-PD-1 antibody cemiplimab was tested in 21 patients with resectable HCC. Of the 20 patients who had successful surgical resection, four met the primary end point of MPR and three had a PR[31]. A major advantage of the neoadjuvant approach is that investigators are provided with surgically resected tumour tissues from the majority of patients, thus enabling in-depth biomarker analysis (Fig. 1).

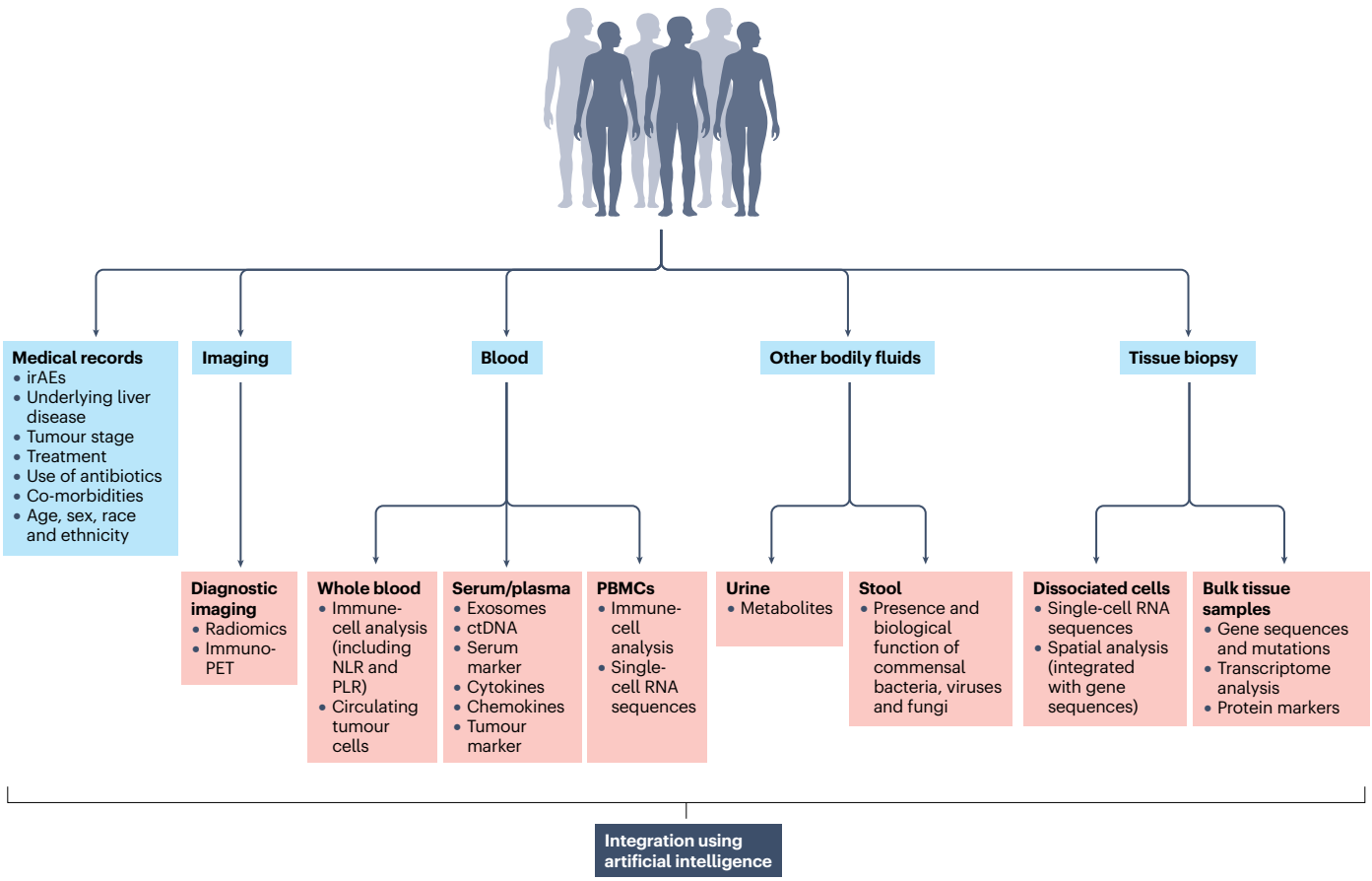

Fig. 1 | Materials needed for rigorous biomarker investigations. Successful biomarker studies require standardized, meticulous acquisition and documentation of clinical metadata in medical records. Clinical samples (including urine, blood or tissue samples) that are acquired either as part of clinical routine or through specific sampling can be stored and used for further downstream assays. Together with the available imaging data, integration of large datasets using artificial intelligence has the potential to optimize biomarker detection and assessment. ctDNA, circulating tumour DNA; irAEs, immune-related adverse events; NLR, neutrophil to lymphocyte ratio; PBMCs, peripheral blood mononuclear cells; PLR, platelet to lymphocyte ratio.

Box 1

Biomarker types

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of one or more biological processes, pathogenic processes, or biological responses to a therapeutic intervention[31].

- **Prognostic biomarker**
  - A prognostic biomarker is predictive of patient outcome regardless of treatment[32].
- **Predictive biomarker**
  - A predictive biomarker provides information about the effects of a specific therapeutic intervention, usually compared with another intervention[32]. A predictive biomarker generally applies only to a specific clinical setting.

Definition of biomarkers

A biomarker is defined as a characteristic that can be objectively measured and evaluated as an indicator of one or more physiological processes, pathogenic processes, or responses to a therapeutic intervention[32]. Biomarkers that can be used before treatment include prognostic biomarkers, which predict patient outcome regardless of treatment, whereas predictive biomarkers provide information on the effects of a specific therapeutic intervention, usually compared with another[33] (Box 1). Various biomarkers, including many related to antitumour immunity, have been tested in studies involving patients with HCC (Fig. 2).

Tumour-derived biomarkers

Immunohistochemical markers

Initial attempts to identify biomarkers predictive of a response to ICIs have focused on immunohistochemical analysis of tumour samples from patients receiving such agents. PD-L1, a natural ligand of PD-1, can be expressed on macrophages, B cells, dendritic cells and cancer cells, and facilitates the evasion of antitumour immunity[34]. Thus, PD-L1 expression was one of the first biomarkers tested in an attempt to predict responsiveness to ICIs[35]. PD-L1 expression on both cancer cells and tumour-infiltrating immune cells can be detected in formalin-fixed, paraffin-embedded tissue samples using immunohistochemistry. Indeed, PD-L1 staining seems to correlate with outcome to some extent in certain cancer types, such as non-small-cell lung cancer, but not in HCC. PD-L1 expression has been assessed in baseline biopsy samples obtained from patients with HCC enrolled in various clinical trials testing different ICIs, either as a single agent or in combination[7,9,25,36–38] (Table 2). A few studies evaluated the number of infiltrating $CD3^+$ or $CD8^+$ T cells using immunohistochemistry[37,39], with results suggesting a correlation between immune cell infiltration and response to ICIs. Finally, the presence of tumour-infiltrating $CD38^+CD68^+$ macrophages was reported to correlate with better outcomes in a cohort of 49 patients with HCC following treatment with ICIs[40].

Tumour mutational burden

Tumour mutational burden (TMB) refers to the number of non-synonymous mutations present in the tumour genome. Comprehensive genomic profiling of 315 cancer-related genes was used to evaluate the frequency of genomic biomarkers of ICI response in 755 patients with advanced-stage HCC. The median TMB was four mutations per megabase (mut/Mb); 52 samples (6.9%) had a TMB of ≥10 mut/Mb and only six (0.8%) had a TMB of ≥20 mut/Mb[41]. TMB is a potential pan-tumour biomarker of benefit from anti-PD-1 antibodies[42], although no clear relationship between TMB and either response rate or OS was detected in a subset of 130 patients enrolled in the IMbrave150 trial[37].

Molecular signatures

Tumour initiation and disease progression are known to be determined by tumour-intrinsic mechanisms that are inevitably linked with responsiveness to therapeutic interventions[43]. Gene expression analyses of tumour tissue samples have enabled the accurate molecular classification of HCC, including robust correlations with patient outcomes[44–46]. Thus, many studies have applied transcriptomics-based approaches to identify molecular signatures capable of predicting responsiveness to ICIs in patients with HCC.

Several such studies published over the past few years have focused on the retrospective characterization of the molecular features of tumour biopsy samples obtained from patients with advanced-stage HCC receiving ICIs. One such study demonstrated that IFNγ signalling and expression of MHC-related genes are key molecular features of HCCs that respond to anti-PD-1 antibodies[47]. These investigators developed an 11-gene signature based on assessments of samples from 28 patients who received ICIs as first-line therapy and another 55 who received ICIs in the second or third lines after a TKI. This signature was named IFNAP (interferon and antigen presentation) and comprises genes involved in IFNγ signalling (*STAT1* and *GBP1*), antigen presentation (*B2M*, *HLA-DRB5* and *HLA-DRA*) and chemotaxis (*CXCL9*). These results are encouraging, but need further validation by other investigators using larger and independent patient cohorts. Analysis of tumour biopsy samples obtained from patients enrolled in the phase I/II CheckMate 040 trial, in which patients received ipilimumab plus nivolumab, for candidate gene expression signatures demonstrated that PD-1 and PD-L1, biomarkers of inflammation, and inflammatory gene signatures are associated with improved survival outcomes and response rates[48]. In a single-arm study involving transcriptomic profiling of pretreatment core-needle or excisional biopsy samples obtained from 60 Korean patients with disease progression on sorafenib who received second-line pembrolizumab, responders showed elevated levels of intratumoural cytotoxic T cells[49]. Peripheral blood mononuclear cells (PBMCs) in both pretreatment and post-treatment samples from a subset of patients were also analysed using single-cell RNA sequencing (scRNA-seq). Data from this study consistently demonstrated that patients with a PR or stable disease had elevated circulating cytotoxic $CD8^+$ T cell levels, whereas those with progressive disease had an increased number of both $CD14^+$ and $CD16^+$ monocytes and activation of neutrophil-associated signalling pathways. Again, we emphasize that this study was exploratory, and the results will require further validation. In the largest retrospective study conducted thus far, investigators analysed tumour samples from 358 patients with HCC enrolled in the phase Ib GO30140 or phase III IMbrave150 trials, in which patients received the anti-PD-L1 antibody atezolizumab with or without bevacizumab, or sorafenib[37]. Pre-existing immunity (defined by high levels of PD-L1 expression, the presence of an effector T cell signature and higher intratumoural $CD8^+$ T cell density) was associated with improved clinical outcomes among patients receiving combination therapy. By contrast, lower levels of clinical benefit were associated

with high regulatory T cell to effector T cell ratios and expression of the oncofetal genes *GPC3* and *AFP*. Interestingly, tumours with a smaller diameter are more likely to respond to anti-PD-1 antibodies, although this association might also reflect upregulation of IFNγ signatures and higher levels of immune cell infiltration in many of these tumours[50].

In a USA-wide effort to determine why ICIs are effective in certain patients but not in others, the National Cancer Institute (NCI) Liver Cancer Program has established the Cancers of the Liver Accelerating Research of Immunotherapy by a Transdisciplinary Network (NCI-CLARITY) study (NCT04145141). In a retrospective arm of this study, the investigators analysed transcriptomic and genomic alterations in biopsy samples from a cohort of 86 patients with either HCC or intrahepatic cholangiocarcinomas (iCCAs), both before and following treatment with ICIs[51]. Data from this study suggest that a response to ICIs is determined by baseline tumour-intrinsic characteristics. Gene expression signatures related to more-differentiated tumour status were found in clusters associated with more favourable outcomes, whereas activation of signalling pathways associated with more aggressive disease, such as EpCAM signalling, were linked with inferior outcomes[51]. These data further reveal that the heterogeneous treatment responses seen in many cohorts might reflect the fact that certain tumours are highly infiltrated with $CD8^+$ T cells and others by B cells. The presence of B cells and tertiary lymphoid structures might serve as key determinants of responsiveness to ICIs, and thus clinical outcomes in patients with solid tumours[52,53]. These results reiterate the importance of the functional roles of tumour-infiltrating T cells and B cells in the context of a response to ICIs. These observations are consistent with those from many other studies, emphasizing the rationale to recommend tissue biopsy sampling before the patient starts to receive immune-based therapies in a research setting.

Several studies have attempted to characterize publicly available transcriptomics data from resected tumour specimens in order to determine the underlying roles of cellular immunity in patients' prognosis[54]. In an evaluation of transcriptomics data from 900 patients with HCC, investigators classified >90% of samples based on a 20-gene signature reflecting certain immunogenomic features as either

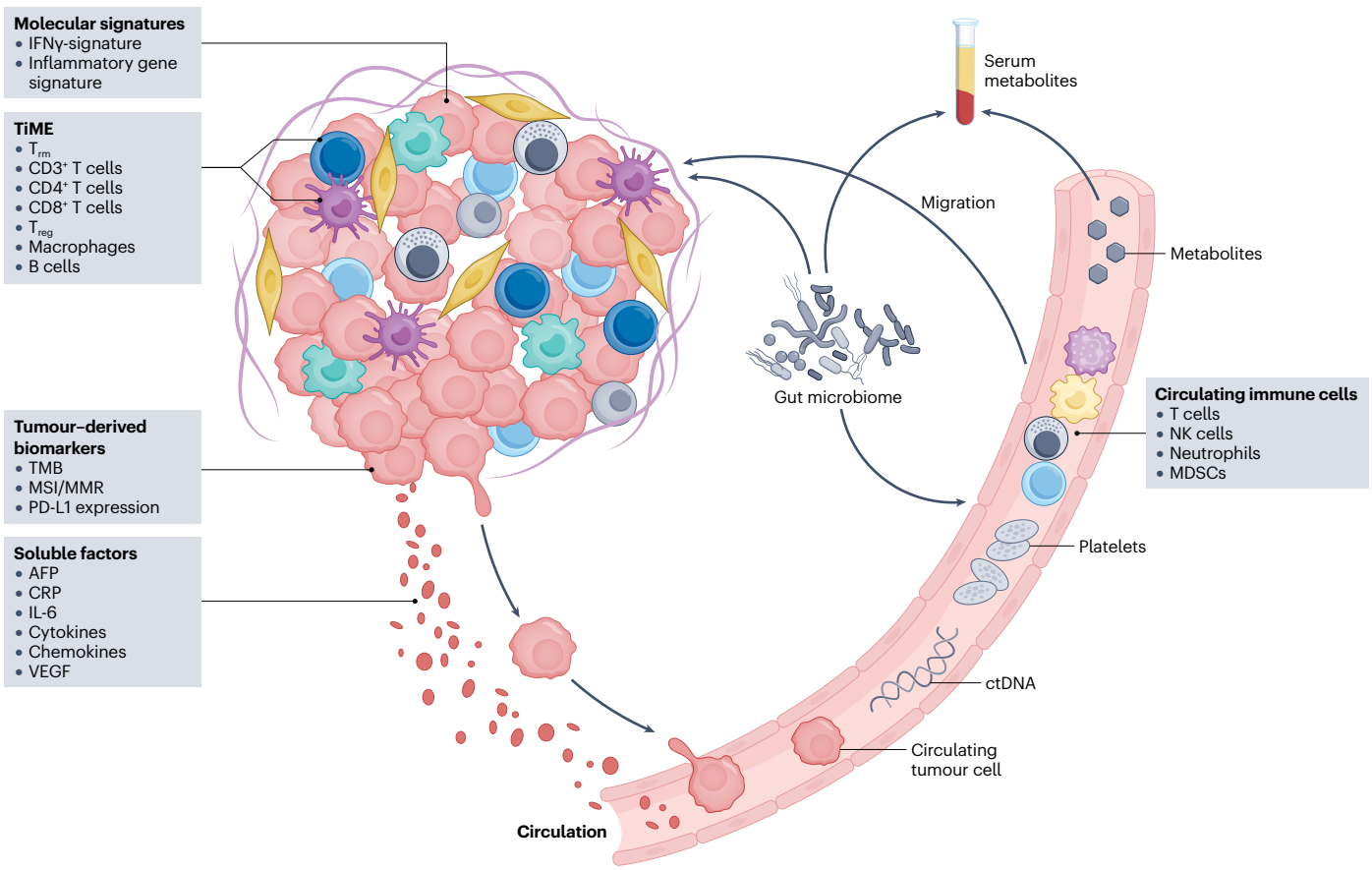

Fig. 2 | Overview of the sources of various biomarker candidates for immunotherapy of HCC. Analysis of tumour tissue samples enables the assessment of tumour-derived biomarkers such as tumour mutational burden (TMB), microsatellite instability/DNA mismatch repair (MSI/MMR) and/or selected proteins such as PD-L1. Gene expression signatures can also be derived from tumour tissue samples. Acquisition of tumour tissue samples also enables investigations of the tumour immune microenvironment (TiME), including lymphoid cells and myeloid cells. Tumour-derived or immune-related soluble factors as well as circulating tumour cells and circulating tumour DNA (ctDNA) can all be measured in peripheral blood samples, thus providing information on tumour biology in a non-invasive manner. The phenotypes and functions of peripheral immune cells and non-immune cells (such as platelets) can also potentially be used as biomarkers. The gut microbiome is able to directly influence both the tumour via the hepatic portal vein and to modulate the peripheral immune response. AFP, α-fetoprotein; CRP, C-reactive protein; MDSCs, myeloid-derived suppressor cells; NK cells, natural killer cells; T_{reg}, regulatory T cells; T_{rm}, tissue-resident memory T cells.

Table 2 | Exploratory biomarker analysis of samples obtained from clinical trials

Trial (treatment)	Biomarker	Results	Ref.
HIMALAYA (tremelimumab plus durvalumab)	PD-L1 TAP	No correlation with response	8
CheckMate 459 (nivolumab)	PD-L1 TC	PD-L1 TC was not associated with clinical benefit: mOS 16.1 months in patients with a PD-L1 TC of ≥1% vs 16.7 months for those with a PD-L1 TC of <1%	9
CheckMate 040 (nivolumab plus ipilimumab)	PD-L1 TC	Both patients with PD-L1$^+$ and PD-L1$^-$ tumours responded to treatment, although patients with a PD-L1 TC of <1% had an mOS of 10.4 months vs not evaluable in those with a PD-L1 TC of ≥1%	25
GO30140 and IMbrave150[a] (atezolizumab plus bevacizumab)	T_{eff} cell gene expression	Tumours with a high T_{reg} to T_{eff} cell ratio associated with reduced mPFS (5.6 vs 9.7 months) and mOS (19.17 vs NR); high levels of a T_{eff} gene expression signature associated with improved mPFS (8.8 vs 5.6 months); mOS was NR in both subgroups	37
	ABRS	Tumours with a high ABRS associated with superior mPFS (9.7 months vs 5.6 months); mOS NR in both subgroups	
	PD-L1 expression or PD-L1 TC	Tumours with high CD274 expression associated with superior mPFS (9.7 vs 5.5 months); mOS NR in both subgroups; no correlation observed between response and PD-L1 TC	
	CD8	Tumours with high levels of CD8$^+$ T cell infiltration associated with superior mPFS (11.0 vs 5.6 months); mOS NR in both subgroups	
	Myeloid inflammation signature	Tumours with high levels of a myeloid inflammation gene expression signature associated with improved mPFS (7.4 versus 5.7 months)	
KEYNOTE-224 (pembrolizumab)	PD-L1 CPS	22 of 52 patients had a PD-L1 CPS of >1; this cut-off was associated with response (P=0.021) and improved PFS (P=0.026)	38
	PD-L1 TPS	13 of 52 patients had a PD-L1 TPS of >1%; this cut-off was not associated with response or improved PFS	
NCT01853618 (tremelimumab plus ablation)	CD3, CD8	Increase in CD3$^+$ and CD8$^+$ T cell infiltration following treatment and in responders vs non-responders	39
	CD4, CD8, PD-1, HLA-DR, ICOS	Patients with a response to tremelimumab had increased levels of circulating CD4$^+$HLA-DR$^+$, CD4$^+$PD-1$^+$, CD8$^+$HLA-DR$^+$, CD8$^+$PD-1$^+$, CD4$^+$ICOS$^+$ and CD8$^+$ICOS$^+$ T cells relative to baseline (all P<0.05); patients with higher numbers of circulating CD4$^+$PD-1$^+$ cells were more likely to respond to tremelimumab (P<0.05); responders had a significant increase in tumour-infiltrating CD3$^+$ and PD-1$^+$ cells relative to non-responders (P<0.05)	117
	TCR genes	Longitudinal TCR sequencing revealed a significant reduction in peripheral clonality after one treatment cycle (P<0.05)	
CheckMate 040 (nivolumab)	PD-L1 TC	Both patients with PD-L1$^+$ and patients with PD-L1$^-$ tumours responded to treatment, although patients with a PD-L1 TC of ≥1% had longer OS vs those with a PD-L1 TC of <1% (28.1 vs 16.6 months; P=0.03)	48
	CD3, CD4, CD8, FOXP3	Increased CD3 and CD8 frequencies were associated with a trend towards improved OS	
	CD68, CD163	Macrophage infiltration at baseline was not associated with OS	
	Inflammatory gene expression signature	Higher median inflammatory signature scores were associated with a trend towards an association with PR vs SD (P=0.05); patients in the upper tertile had improved OS (P=0.01)	
	NLR	Patients in the lowest tertile had longer OS than those in other tertiles (P=0.015)	
NCT03163992 (pembrolizumab)	PD-L1 TPS	Tumours with a PD-L1 TPS of ≥1% were more likely to respond to treatment (ORR 21.0% vs 0%; P=0.042)	49
	NLR	Tumours with an NLR of ≤4 were more likely to respond to treatment (ORR 20.7% vs 0%; P=0.027)	
	RNA sequencing	Expression of genes with a role in TCR signalling was enriched in responders	
	scRNA-seq	Shifts towards cytotoxic CD8$^+$ T cells were more prevalent in patients with a response; increased numbers of both CD14$^+$ and CD16$^+$ monocytes and activation of neutrophil-associated pathways were more prevalent in non-responders	
NCT02658019 (pembrolizumab)	Cytokines and chemokines[b]	Baseline plasma TGFβ levels ≥200 pg/ml correlated with poor treatment outcomes: mPFS 2 months vs NR (P=0.005) and mOS 7 months vs NR (P=0.008)	97
	PD-L1 TC	PD-L1 TC did not correlate with response	
NCT02821754 and NCT01853618 (tremelimumab plus durvalumab)	ILCs	Tremelimumab plus durvalumab decreased the frequency of intratumoural ILC1s and increased the frequency of ILC3s (both P<0.05)	119
NCT03695952 (nivolumab or pembrolizumab)	CyTOF plus flow cytometry of PBMCs[c]	Higher frequencies of CXCR3$^+$CD8$^+$ T_{EM} cells and CD11c$^+$ APCs associated with superior response (P=0.0004 and P=0.0255) and PFS (P=0.00079 and P=0.0015), respectively	122

Table 2 (continued) | Exploratory biomarker analysis of samples obtained from clinical trials

Trial (treatment)	Biomarker	Results	Ref.
RESCUE (camrelizumab plus apatinib)	PD-L1 TPS	ORR 31.8% vs 18.1% among patients with a PD-L1 TPS of ≥1% vs <1%; PFS events observed in 63.6% vs 75.0% of patients, respectively, in an exploratory analysis of samples from 54 of 190 patients	199
NCT02519348 (tremelimumab plus durvalumab)	PD-L1 CPS	No correlation with response reported	201
	Whole-blood immune cell subsets[d]	Response was associated with an expansion of $Ki67^+CD8^+$ T cells occurring by day 15 of treatment; tremelimumab plus durvalumab caused the highest increase in circulating levels of this cell population	

ABRS, atezolizumab plus bevacizumab response signature; APC, antigen-presenting cell; CPS, combined-positive score (($PD\text{-}L1^+$ tumour cells, lymphocytes and/or macrophages)/total number of viable tumour cells ×100); CyTOF, cytometry by time of flight; ILC, innate lymphoid cell; mOS, median overall survival; mPFS, median progression-free survival; NLR, neutrophil to lymphocyte ratio; NR, not reached; ORR, objective response rate; OS, overall survival; PBMC, peripheral blood mononuclear cell; PFS, progression-free survival; PR, partial response; scRNA-seq, single-cell RNA sequencing; SD, stable disease; TAP, tumour area positivity score; TC, tumour cell ($PD\text{-}L1^+$ tumour cells/total number of viable tumour cells); TCR, T cell receptor; T_{eff}, effector T cells; T_{EM}, effector memory T cells; TPS, tumour-positive score (viable tumour cells with partial or complete PD-L1 membrane staining/all viable tumour cells in sample ×100); T_{reg}, regulatory T cells. [a]Values designated 'high' are above the median and values designated 'low' are below the median. [b]TGFβ, IL-1β, IL-12, 1L-18, IFNγ, IL-6, IL-8, IL-10, CXCL9, CCL4, CCL5, PD-1, PD-L1, PD-L2. [c]CyTOF: CD45, CD14, HLA-DR, CD19, CD45RO, CD3, CD8, IL-4, IgD, PD-1, CD4, KI67, CD95, CD161, TNF, CCR7, TIM-3, CD152, CXCR6, CD40, CD38, CD11c, IgM, CXCR5, CD56, CXCR3, CD32B, FOXP3, CD24, CD86, IFNγ, IL-17A, CD21, CXCR4, IgG-Fc, CCR5, Vα7.2, BAFF-R, CD16. [d]CD3 (T cells), CD19 (B cells), CD45 (natural killer cells), $CD45RA^+CD45RO^-CCR7^+CD4^+$ (naive), $CD45RA^-CD45RO^+CCR7^+CD4^+$ (central memory), $CD45RA^-CD45RO^+CCR7^-CD4^+$ (effector memory), $CD45RA^+CD45RO^-CCR7^+CD8^+$ (naive), $CD45RA^-CD45RO^+CCR7^+CD8^+$ (central memory), $CD45RA^-CD45RO^+CCR7^-CD8^+$ (effector memory), $CD4^+CD38^+$, $CD4^+HLA\text{-}DR^+$, $CD4^+Ki67^+$, $CD4^+FOXP3^+$, $CD8^+CD38^+$, $CD8^+HLA\text{-}DR^+$, $CD8^+Ki67^+$.

inflamed or non-inflamed[55]. The investigators speculated that tumours of the inflamed subtype would be immunologically 'hot' and that this feature might help to predict a response to ICIs. Elsewhere, investigators developed immune and stromal scores based on transcriptomics data from 371 patients with HCC[56]. These investigators demonstrated that such a score might help to determine both tumour purity and the extent of immune cell infiltration in the tumour microenvironment (TME), and incorporated these findings into a nine-gene prognostic signature. Similar strategies have been applied to iCCA[57].

Single-cell transcriptomics

Resolving the single-cell landscape of HCC will provide a detailed map of the community of cell types present in a given tumour and could help to identify novel biomarkers of response to ICIs. Such methods are unravelling the entire HCC cellular ecosystem, including the heterogeneous tumour cell populations and TMEs. scRNA-seq studies have started to reveal extensive intratumour heterogeneity (ITH) among malignant cells, with distinct molecular profiles and cellular states. ITH is associated with treatment failure and an inferior patient prognosis[58], and is a major barrier to effective cancer interventions[59]. In addition to cancer cells, heterogeneity among stromal cells and tumour-infiltrating immune cells in the surrounding TME has been uncovered using scRNA-seq. For example, within the $CD8^+$ T cell population, naive T cells ($CCR7^+$), central memory T cells ($IL\text{-}7R^+$), effector memory T cells ($GZMK^+$), tissue-resident memory T cells ($KLRD1^+$), effector T cells ($CX3CR1^+$), proliferative T cells ($MKI67^+$) and exhausted T cells ($PD\text{-}1^+$) have all been identified based on marker gene expression[60,61]. These different cellular states might all be related to varying responses to ICIs. A higher frequency of $TCF7^+CD8^+$ T cells is associated with favourable responses to ICIs in patients with melanoma[62], and higher levels of tumour-infiltrating $CD8^+$ T cells are related to prolonged OS in patients with HCC regardless of treatment received[58,63,64].

Single-cell analysis provides an opportunity to understand the mechanisms of resistance to ICIs in HCC. A robust association exists between tumour-infiltrating lymphocytes (TILs) and immunotherapy responses, with higher levels of TILs linked with better outcomes[62,65,66]. By contrast, an immunosuppressive immune landscape with limited numbers of TILs is usually associated with inferior outcomes. Furthermore, infiltration by other immune cell types (for example, tumour-associated macrophages (TAMs)) is associated with unfavourable responses to ICIs, as shown by several single-cell analyses[67–69]. In a study involving eight patients with HCC receiving anti-PD-1 antibodies, the investigators used spatial transcriptomics to identify a tumour–immune barrier structure comprising $SPP1^+$ macrophages and cancer-associated fibroblasts (CAFs) located at the tumour boundaries in samples from tumours that did not respond to anti-PD-1 antibodies[68]. Further single-cell analysis demonstrated that $SPP1^+$ macrophages can stimulate the surrounding CAFs and promote extracellular matrix remodelling and formation of the tumour–immune barrier, which restricts the infiltration of immune cells, thus limiting the efficacy of anti-PD-1 antibodies[68]. Similarly, pharmacological inhibition of SPP1 or macrophage-specific deletion of *Spp1* leads to enrichment with TILs and enhances the activity of anti-PD-1 antibodies in mouse models[68]. Intratumoural accumulation of monocytic myeloid-derived suppressor cells (MDSCs)[70] is also associated with reduced TIL levels[71]. Suppression of monocytic MDSCs using the bromodomain inhibitor i-BET762 combined with anti-PD-L1 antibodies can promote TIL infiltration and reinstate immunosurveillance, resulting in tumour shrinkage in mouse models of HCC[71]. Tumour-associated neutrophils (TANs) might also have a tumour-promoting role in liver cancers. In an scRNA-seq analysis of 189 samples derived from 124 patients with HCC, iCCA or other rare liver cancer subtypes and eight mice with experimentally induced liver tumours, the investigators identified various immune cell populations in which TANs were found to be associated with an unfavourable prognosis[72]. Further analysis demonstrated the roles of $CCL4^+$ TANs and $PD\text{-}L1^+$ TANs in recruiting macrophages and suppressing T cell cytotoxicity[72]. Interventions targeting these subtypes of TANs, in combination with ICIs, might be a promising treatment approach in patients with HCC[73].

Cancer cell-derived features could also be utilized as predictive biomarkers of responsiveness to ICIs. Single-cell scRNA-seq of tumour biopsy samples obtained from patients with HCC has revealed varying degrees of ITH, with more heterogeneous tumours associated with inferior OS. Such tumours were also found to have higher levels of *VEGFA* expression, which might reprogramme the TME towards a phenotype that supports cancer cell survival and/or metastatic dissemination[63]. A single-cell analysis of 46 tumour biopsy samples obtained from 37 patients with liver cancer identified SPP1 as a marker responsible for tumour evolution (quantified using a novel machine learning-based

consensus clustering approach) in ICI responders[58]. SPP1 is mainly expressed in malignant cells and TAMs, and therefore could be a target of interventions designed to improve the efficacy of ICIs. Cancer cells continuously interact with the TME, and these interactions shape the adaptive fitness of the malignant cells and vice versa. In a multiregional scRNA-seq analysis of material from seven primary liver tumours, we found stable communication networks between cancer cells and the TME that were linked with prognosis[74]. Specifically, ligand–receptor interactions between cancer cells and the TAMs via SPP1–PTGER4 and LGALS9–SLC1A5 were stable features associated with HCC aggressiveness, and these might serve as therapeutic targets for attempts to improve the efficacy of ICIs[74]. However, these putative functional links between these ligand–receptor interactions and responses to ICIs will require experimental validation. Collectively, data from the available scRNA-seq studies provide a deep mechanistic understanding of the HCC ecosystem and might facilitate the identification of novel biomarkers.

Spatial transcriptomics

Most transcriptomics analyses of HCC to date have involved either bulk tumour material or dissociated single cells. However, the spatial relationships among the various immune components within the TME are increasingly being recognized as determinants of clinical outcomes. For example, an analysis of HCC specimens from 15 patients who received neoadjuvant nivolumab plus cabozantinib identified the distance between T cells and immunosuppressive macrophages as a crucial predictor of pathological response[29]. Similarly, a spatial transcriptomics analysis demonstrated that immunosuppressive macrophages and CAFs were associated with exhausted T lymphocytes in samples from 113 patients with steatotic HCCs (comprising 23% of non-viral HCCs). Lipid accumulation was also associated with increased PD-L1 expression. Among 30 patients who received atezolizumab plus bevacizumab, seven presented with steatotic HCC, and these patients responded well to treatment, suggesting that intratumoural steatosis might have utility as an imaging biomarker[75]. Using a 37-plex immunohistochemistry approach, we demonstrated that TAMs also interact with mucosal-associated invariant T (MAIT) cells. MAIT cells are MR1-restricted innate-like T cells that recognize non-peptide antigens, including derivatives of microbiota-derived vitamin B_2 (riboflavin) precursors[76]. TAMs in the liver inhibit MAIT cell lytic function, cytokine secretion and migration into tumours[77]. HCCs often have high levels of immune cell heterogeneity, thus underscoring the need to interrogate transcriptomics immune networks in a diverse range of tumour regions in order to fully understand the TME. Two spatial transcriptomics analyses of tumour material from patients with HCCs have revealed intratumoural transcriptomics and immune heterogeneity within the TME as contributors to HCC progression[78,79]. These spatial analyses generally rely on the availability of surgical specimens, and underscore the challenges of trying to evaluate the HCC TME using fine-needle biopsy samples. Clinical trials testing systemic therapy in the neoadjuvant setting, which have the advantage of providing surgical specimens for biomarker analysis, might therefore be especially important for future attempts to interrogate the mechanisms of response and resistance to current systemic therapies.

Blood-derived biomarkers

Most clinically approved biomarkers require the analysis of tumour tissue samples (such as HER2 overexpression in breast cancer, which is a biomarker of responsiveness to certain HER2-targeted therapies[80]). In patients with HCC, use of tissue biomarkers can be difficult given that most patients are diagnosed based on imaging findings, meaning that tumour tissue is not generally available as part of routine clinical practice[81]. Biopsy sampling is now recommended in patients enrolled in clinical trials and in patients receiving treatment at tertiary academic centres[6]; nonetheless, limited access to tissue samples has hindered biomarker development. α-Fetoprotein (AFP) is a 70-kDa glycoprotein produced by the fetal liver and yolk sac during the first trimester of pregnancy. Serum AFP levels decline rapidly after birth, and then remain low over the entire lifespan. Poorly differentiated malignant HCC cells re-acquire the ability to synthesize and secrete AFP into the bloodstream, and serum levels of this protein are therefore routinely used as a biomarker for early detection, surveillance and diagnosis, and as a prognostic marker[82,83]. Elevated serum AFP levels have been shown to be associated with a poor prognosis in phase III trials testing various TKIs (sorafenib, lenvatinib, regorafenib and cabozantinib)[5,22,84,85] and the anti-VEGFR2 antibody ramucirumab[86]. An analysis of samples from the IMbrave150 trial cohort showed that serum AFP responses at 6 weeks provide a potential surrogate biomarker of prognosis in patients with HCC receiving atezolizumab plus bevacizumab[87]. A few smaller studies have investigated the role of serum AFP in patients receiving ICIs in real-world settings. A post-treatment decline in serum AFP levels was found to be associated with superior survival outcomes[88,89], and baseline serum AFP levels <400 μg/l were found to be associated with higher response rates[90]. In the CheckMate-040 trial, baseline serum AFP levels <400 μg/l were associated with longer OS compared with AFP levels ≥400 μg/l[48], similar to the experience in patients receiving TKIs. However, no associations were observed between serum AFP levels and either ORR or disease control rate[48].

CRAFITY score

The CRAFITY score was originally developed in a training set of 190 patients receiving anti-PD-1 or anti-PD-L1 antibodies as a simple score based on two variables: serum AFP and C-reactive protein (CRP), in which 1 point is assigned for a serum AFP level of >100 ng/ml and for a serum CRP level of >1 mg/dl[91]. In the pooled analysis (n = 292 including the training and validation sets), patients with 0 points (CRAFITY-low) had the longest median OS duration (27.6 months), followed by those fulfilling one criterion (CRAFITY-intermediate), who had a median OS of 11.3 months. Patients meeting both criteria had a median OS duration of 6.4 months, with patients with lower CRAFITY scores also having improved ORRs[91]. In a retrospective cohort study including 297 patients receiving atezolizumab plus bevacizumab in Japan, lower CRAFITY scores were significantly associated with both improved PFS (median 11.8 months versus 6.5 months versus 3.2 months for scores of 0, 1 and 2, respectively; P < 0.001) and OS (median not reached versus 14.3 months versus 11.6 months, respectively)[92]. Other studies have combined CRAFITY score with serum AFP decline after 6 months with similar results[93]. In conclusion, serum AFP is widely used as an inexpensive and non-invasive biomarker, although its role in combination with other serum markers[91–94] requires further investigation in order to improve predictive accuracy.

Immune mediators

Circulating immune mediators including cytokines and immune-checkpoint proteins have been evaluated as predictors of response to ICIs in patients with HCC. For example, higher serum IL-6 levels, along with older age, were independent predictors of disease progression in 64 patients with HCC receiving atezolizumab plus bevacizumab[95]. These results are supported by those of another

independent study[96]. Other examples include a correlation between high baseline plasma TGFβ levels and shorter OS in 28 patients receiving pembrolizumab monotherapy[97,98], which is supported by a meeting abstract from 2022 (ref. 98). Serum levels of soluble PD-L1 (sPD-L1) also seem to correlate with outcomes in patients with HCC, with higher levels consistently associated with shorter OS[99]. Data from patients with HCC remain scarce[100,101]. The reproducibility of results, which is affected by the absence of standardized reference levels for sPD-L1, and of pre-established cut-off levels for prognosis and response prediction, is another major challenge[102].

NLR and PLR

Neutrophil to lymphocyte and platelet to lymphocyte ratios (NLR and PLR) are established inflammatory cell ratios and potential predictive biomarkers in patients with various cancer types receiving anti-PD-1 antibodies[103]. In patients with HCC receiving anti-PD-1 antibodies, a decline in NLR is associated with a better response to treatment and improved survival outcomes[103,104]. In a retrospective study including data from 362 patients receiving ICIs, both NLR (≥5) and PLR (≥300) were described as independent negative prognostic factors[105]. Similar results were observed in a study conducted in China[106] and in a large cohort of patients receiving atezolizumab plus bevacizumab[107]. However, and despite being correlated with OS, NLR did not seem to be associated with any differences in ORR[107]. This lack of a consistent association with response raises concerns about the use of PLR and NLR as predictive biomarkers to guide the use of ICIs in patients with HCC.

Anti-drug antibodies

Anti-drug antibodies (ADAs) have also been assessed as a potential factor to define responses to ICIs in patients with HCC. Combined data from a training cohort of 61 patients and a validation cohort of 113 patients with HCC receiving first-line atezolizumab plus bevacizumab indicate ADA responses on day 1 of cycle 2 in 17% of patients[108]. Moreover, higher ADA levels correlated with a reduced ORR and inferior PFS in both cohorts. High ADA titres also resulted in decreased circulating concentrations of atezolizumab and impaired the proliferation of $CD8^+$ T cells. All of these studies have similar limitations: the lack of a control group to confirm that the biomarkers predict treatment response rather than being a prognostic parameter. Furthermore, given that ADAs are usually agent-specific, this effect might be surmountable by simply replacing atezolizumab with a different anti-PD-L1 antibody, although data supporting this approach are currently not available.

Immune cell profiling

High-dimensional single-cell analyses of the HCC immune TME have shed light on the complex immune phenotype composition of HCCs and the adjacent tumour-bearing liver tissues[58,60,109–112]. Data from these studies highlight the existence of parallel numerical and phenotypic changes in both the lymphocyte and myeloid cell compartments during carcinogenesis, although these observations are limited by tissue availability. In patients with advanced-stage, unresectable HCCs, tissue specimens are usually unavailable because such tumours can easily be diagnosed using imaging alone. Thus, high-dimensional immune profiling of PBMCs provides a prudent and easily accessible opportunity to study and monitor longitudinal changes in peripheral immune cell composition at baseline, in response to ICI treatment and upon disease progression. Technological advances during the past 5 years have enabled the routine measurement of up to 40 (or more) proteins simultaneously at the single-cell level (for example, through mass spectrometry-based cytometry by time-of-flight (CyTOF)[113], or high-dimensional conventional or spectral flow cytometry[114]). These novel single-cell technologies are likely to provide the level of depth and resolution required to generate clinically relevant immune cell signatures[115]. Once signatures have been established, high-throughput flow cytometry-based techniques that permit routine clinical testing can be developed.

To date, no robust PBMC-based biomarkers capable of predicting responses to ICIs in patients with HCC have been identified, although data from other tumour types such as advanced-stage melanoma suggest that this approach might be feasible[116]. A study in which patients with advanced-stage HCC received tremelimumab plus radiofrequency ablation, cryoablation or TACE provides some data supporting this possibility[117]. In this study, the baseline proportion of $CD4^+PD\text{-}1^+$ T cells in PBMCs was found to be higher in patients with a response to therapy than in non-responders. Conversely, other authors have reported lower levels of $PD\text{-}1^+$ B cells in patients with HCC with disease control on nivolumab monotherapy[118]. Beyond this finding, disease progression was associated with higher post-treatment PD-L1 levels on circulating monocytes[118]. High-resolution profiling of PBMCs also enables the identification of rare immune cell populations, such as innate lymphoid cells (ILCs). For example, an analysis of sorted and enriched ILCs involving high-dimensional flow cytometry as well as scRNA-seq identified an $NKp80^+$ (*KLRF1*) natural killer-like ILC1 subset that was expanded in patients with HCC receiving tremelimumab with or without durvalumab. Applying a gene signature corresponding to these $KLRF1^{high}$ ILCs revealed a subset of patients with improved PFS in a retrospective assessment of The Cancer Genome Atlas HCC data[119].

The liver contains several large populations of tissue-resident lymphocytes ranging from ILCs (including conventional natural killer cells and helper ILCs), innate-like T cells (natural killer T cells, γδ T cells and MAIT cells), and adaptive B and T lymphocytes (both $CD4^+$ and $CD8^+$ T cells)[120]. The degree to which changes in the peripheral blood reflect adaptive processes within tumour tissues remains to be determined and represents an attractive field for future research, especially in patients with HCC in whom repeat biopsy sampling is not feasible. This important consideration was addressed using high-plex CyTOF analysis to identify a highly polyfunctional $CD103^+$ subset of tissue-resident memory T (T_{RM}) cells. The ratio of these $CD103^+$ T_{RM} cells to poorly functional $PD\text{-}1^{high}$ exhausted T cells was associated with a superior outcome among the ten patients receiving nivolumab in this study[121]. Highlighting the importance of circulating immune cells and tissue-resident effector cells, investigators identified an association between $CXCR3^+CD8^+$ effector memory T (T_{EM}) cells and response to anti-PD-1 antibodies[122]. Also in this study, the presence of type 1 dendritic cells in PBMCs was associated with response to ICIs, whereas the presence of $CD14^+HLA\text{-}DR^-$ MDSCs was associated with inferior outcomes[122].

In summary, correlative studies investigating the peripheral immune cell compartment in patients with HCC receiving ICIs are scarce, and data from further studies are needed to advance our understanding of the roles of specific immune cell subsets and their responsiveness to ICIs. Following the advent of combination regimens (such as VEGF-targeted therapies plus ICIs) that target the TME beyond conventional T cells, comprehensive profiling of dynamic changes in both myeloid and lymphoid peripheral immune cell subsets at specific time points during treatment becomes important. Novel analytical pipelines incorporating multiple biomarkers potentially detected using machine learning approaches, clinical variables, baseline characteristics, including race and ethnicity, as well as HCC biology, will help

us to understand the increasing complexity of these high-dimensional datasets in a data-driven manner, and thus better ascribe phenotypic shifts in immune cell populations that contribute to responsiveness or resistance to ICIs[123].

Circulating tumour DNA

Liquid biopsy has emerged as an alternative solution for biomarker development in a diverse range of cancers including HCC. Unlike conventional blood biomarkers, liquid biopsy involves the analysis of tumour-derived components (such as circulating tumour cells (CTCs), circulating cell-free tumour DNA (ctDNA) and/or extracellular vesicles) in bodily fluids, mostly blood[124]. Owing to sampling being minimally invasive, this approach is amenable to longitudinal assessments, which could help in the clinical implementation of molecular monitoring of patients with HCC, as seen in those with haematological malignancies[125]. Nonetheless, thus far only a few studies, mostly involving ctDNA, have evaluated liquid biopsy for the prediction of response to ICIs in patients with HCC. ctDNAs are fragments of DNA (typically <145 bp in length) originating from necrotic or apoptotic tumour cells that can be detected in the bloodstream[126]. Analysis of ctDNA has been most widely investigated as a method of early HCC detection, particularly the analysis of specific ctDNA methylation marks[127,128]. Available data indicate correlations between mutations detected in ctDNA and prognosis[129] as well as response to sorafenib[130]. Data on biomarkers of response to ICIs include ctDNA profiling of samples from 85 patients with HCC who received atezolizumab plus bevacizumab[131]. This analysis included deep sequencing of a set of 25 genes known to be frequently mutated in HCC (such as those located in *TERT* promoter regions, *TP53* and *CTNNB1*). Patients with high levels of cell-free DNA (cfDNA) had lower response rates and inferior survival outcomes compared with those with lower cfDNA levels. This study also found a correlation between the presence of *TERT* promoter mutations in ctDNA and longer OS, although no robust correlations between any of the genes tested and responsiveness to ICIs were observed. Despite initial evidence suggesting that mutations in *CTNNB1* predict a lack of response to nivolumab[132], analysis of *CTNNB1* mutations in ctDNA failed to confirm this finding[130].

Circulating tumour cells

CTCs are cancer cells that become detached from the tumour tissue and circulate in the bloodstream[133]. CTCs are closely associated with tumour burden, tumour invasiveness and the likelihood of haematogenous metastasis; thus, the presence of CTCs is an independent unfavourable prognostic factor in various cancer types and has also been proposed as a predictive biomarker for ICI therapy[133,134]. In a small subset of ten patients with HCC receiving anti-PD-1 antibodies, all five responders had PD-L1$^+$ CTCs at baseline, compared with only one of five non-responders[135]. Prospective validation in a larger cohort is needed to better define the utility of PD-L1$^+$ CTCs as a prognostic and predictive biomarker in patients with HCC.

In summary, biomarkers that can be detected in peripheral blood including protein markers, ctDNA, CTCs and immune cells, have the great advantage of minimally invasive sampling. Nonetheless, no meaningful evidence currently exists that the predictive and prognostic accuracy is necessarily better than that of tissue sample analysis for any given biomarker.

Metabolites and secreted proteins

Metabolites are the biochemical by-products of cellular processes; thus, cancer metabolomics provides a promising new source of functional biomarkers, especially those found in serum and urine. For example, an integrated analysis of metabolite and gene expression profiles of tumour and non-malignant tissue samples demonstrated several stearoyl-CoA desaturase (SCD1) metabolites to be associated with disease progression in patients with HCC[136]. A similar analysis demonstrated that certain serum metabolites might have utility as non-invasive biomarkers to define prognostic molecular subtypes of HCC[137]. Candidate subtype-related serum metabolites include the microbial metabolites 4-ethylphenyl sulfate and *p*-cresol sulfate. However, the clinical utility of these biomarkers requires further and independent validation. Pan-cancer analysis of pre-diagnostic blood metabolites revealed several metabolites to be associated with the risk of most cancer types studied, including HCC[138]. An inverse association was observed between a sphingomyelin cluster and risk of HCC, although this cluster was positively associated with the risk of endometrial cancer. These results also require independent validation. Elsewhere, authors identified an eight-protein-based prognostic liver secretome signature (PLSec) that enables the accurate stratification of patients with advanced liver fibrosis for the long-term risk of HCC[139]. PLSec plus serum AFT was found to be a more accurate predictor of HCC risk than AFP alone in two separate cohorts of patients with advanced liver fibrosis owing to HCV infection previously treated with direct-acting antiviral therapy. These data are encouraging, although the clinical utility of this approach needs to be investigated further, especially in patients with HCC arising from other aetiological factors, such as HBV infection, alcohol intake or non-alcoholic fatty liver disease. Integrative transcriptomics and metabolomics analysis has revealed that HCC cell methionine metabolism might drive T cell exhaustion; therefore, reprogramming of tumour methionine metabolism might be a viable therapeutic strategy to promote antitumour immunity[140].

Investigators explored dynamic alterations in the gut microbiome and metabolome in prospectively obtained faecal and serum samples obtained both at baseline and at the time of disease progression from 35 patients with HCC receiving anti-PD-1 antibodies[141]. This study demonstrated that faecal samples from patients with a response to ICIs have higher baseline levels of α-diversity than those of non-responders. The authors also demonstrated that α-D-glucose is the only serum metabolite that differed between responders and non-responders after 3 months. More in-depth analysis revealed that a machine learning classifier based on serum metabolites is able to more readily identify patients with HCC who would derive benefit from ICIs at baseline (area under the curve (AUC) 0.79) than a classifier based on the content of the gut microbiome. These data are encouraging, but also preliminary and require further validation. Prospective studies in this area are currently limited, although the previously mentioned NCI-CLARITY study includes longitudinal stool sampling, which would permit comprehensive investigation of possible faecal biomarkers. Collectively, metabolomics is a promising method of discovering biomarkers capable of predicting responsiveness to ICIs. However, owing to the heterogeneous nature of HCC, the rapid evolution of the various technological platforms used to analyse metabolites, the limited cohort sizes of current studies and limited pretreatment biopsy sampling, knowledge in this area is currently limited.

Other novel biomarkers

Radiomics

Radiomics and artificial intelligence (AI) are rapidly growing areas of research that aim to convert large databases of digital medical images

into high-dimensional quantitative data. Radiomics features detected using MRI data can potentially provide information on differential diagnosis, histological grade, microvascular invasion status, gene expression, local and systemic therapeutic responses, and prognosis in patients with HCC[142]. A growing body of research supports the potential of AI tools to assess the biological characteristics of tumours and empower new prognostic, predictive and theranostic approaches in patients receiving ICIs[143,144]. Nonetheless, the number of published studies evaluating radiomics in patients with HCC receiving ICIs remains limited[145]. Various studies have used AI-based algorithms to predict PD-L2 expression[146], $CD3^+$ and $CD8^+$ T cell infiltration[145,147], and macrophage infiltration[148]. In a dataset comprising 58 patients with advanced-stage HCC, investigators established a radiomics nomogram and measured its ability to predict the activity of anti-PD-1 antibodies in patients by combining pretreatment contrast-enhanced CT images and clinical risk factors (tumour embolus and ALBI grade), resulting in a 'fusion radiomics score' with AUCs of 0.89 and 0.88 in the training and validation cohorts, respectively[149]. The rapidly increasing number of promising results offers proof of the concept that AI and radiomics could drive precision medicine approaches for a wide range of indications. Nonetheless, standardizing data collection as well as optimizing the methodological quality across different treatment centres are all necessary before these results can be translated into clinical practice[143].

ImmunoPET

The use of PET–CT in patients with HCC is not recommended given that ^{18}F-fluorodeoxyglucose uptake is observed in <40% patients[150,151]. Nonetheless, immunoPET, a novel molecular imaging modality combining the superior targeting specificity of a monoclonal antibody with the inherent sensitivity of PET, might be more effective in this regard. A variety of radionuclides and monoclonal antibodies have been used to develop immunoPET probes, which has been driven by advances in radiochemistry and conjugation strategies[152]. Radiolabelled agents targeting prostate-specific membrane antigen and GPC3 are currently under evaluation as diagnostic tools in patients with HCC[153–155].

Microbiome

The microbiome, which refers to the microbiota present within a host measured according to their collective genomes, is becoming increasingly recognized for its role in the regulation of immunity, as well as responses to various cancer treatments[156,157]. In patients with melanoma receiving anti-PD-1 antibodies, intestinal microbiota signatures have been shown to predict both clinical response and irAEs[158]. The gut microbiome and the liver interact bidirectionally via the gut–liver axis[159–161]. The gut microbiota and its metabolites can therefore both directly and indirectly modulate gene expression in hepatocytes, tumour cells and non-tumour cells, including immune cells[160,162]. Unlike in patients with melanoma, results from a meta-analysis of data from six studies including 1,056 patients demonstrated that antibiotic use and the subsequent reduction in microbiota diversity does not affect OS in patients with HCC receiving ICIs[163], although larger and better-controlled studies are clearly needed. For example, a post hoc analysis of data from the IMbrave150 trial indicates that antibiotic exposure within 30 days before or after treatment initiation has negative prognostic implications in patients receiving atezolizumab plus bevacizumab[164]. Finally, altering the gut microbiota via faecal microbiota transplantation has been shown to improve responsiveness to anti-PD-1 antibodies in patients with metastatic melanoma and resistance to these agents[165]. Whether or not similar approaches are effective in patients with HCC remains unknown.

Current biomarker use in clinical trials

The trials leading to the approval of current systemic therapies for patients with HCC were mostly designed to determine clinical benefit in unselected populations. However, the inclusion of exploratory biomarker analysis from these trials provides important information on the underlying mechanisms of response and resistance. An analysis of tumour samples from the phase Ib GO30140 trial and the IMbrave150 trial revealed an association between higher levels of effector T cell, regulatory T cell and myeloid cell inflammatory gene signatures, and improved levels of benefit from atezolizumab plus bevacizumab relative to atezolizumab alone[37] (Table 2). Increased myeloid signalling was also associated with benefit from VEGF-targeted therapies plus atezolizumab as compared with sunitinib alone in patients with renal cell carcinoma[166] and in preclinical models[37,167–169], providing further evidence that certain myeloid cell populations might be an important mechanism through which VEGF-targeted therapies can enhance responsiveness to ICIs. Higher tumour vessel density and higher levels of VEGFR2 expression are also associated with improved levels of benefit from bevacizumab plus atezolizumab over atezolizumab alone[37]. This biomarker analysis provides support for the hypothesis that patients harbouring HCC with higher levels of regulatory T cell proliferation, myeloid cell inflammation and/or angiogenesis might be particularly likely to derive benefit from VEGF-targeted–ICI combination therapies, as opposed to combinations of ICIs.

A biomarker analysis of samples from the phase III IMbrave150 trial found that tumours harbouring evidence of pre-existing T cell-mediated immunity (high PD-L1 levels, high $CD8^+$ T cell density, and enhanced expression of an effector T cell signature) are associated with improved benefit from bevacizumab plus atezolizumab relative to sorafenib. Conversely, *GPC3* and *AFP* expression are associated with reduced benefit from combination therapy[37]. These findings are consistent with a biomarker analysis of samples from the phase III CheckMate 459 study, in which patients received nivolumab versus sorafenib, which demonstrated an association between baseline expression of inflammation-associated genes and increased levels of benefit from nivolumab relative to sorafenib[170] (Table 2). Similarly, an analysis of samples from patients receiving nivolumab in the single-arm CheckMate 040 trial found an association between an inflammatory gene signature and improved ORR and OS[48] (Table 2). Collectively, data from these analyses suggest that an effector T cell gene expression signature enriches for benefit from anti-PD-1 or anti-PD-L1 antibodies in patients with HCC.

Genomic alterations in the WNT–β-catenin signalling pathway have been proposed as a source of resistance to anti-PD-1 antibodies in patients with HCC[171], although analyses of samples obtained from large prospective trial cohorts thus far do not support a role for genes related to this pathway as biomarkers associated with clinical benefit from these agents. Specifically, such alterations were not associated with survival outcomes among patients receiving nivolumab in the CheckMate 459 trial[170] and were not deemed prognostic in the IMbrave150 trial, although mutations in *CTNNB1* were associated with reduced levels of benefit from atezolizumab plus bevacizumab[37]. In another analysis, a correlation was detected between clinical responses among patients with HCC who received perioperative ICIs (nivolumab plus ipilimumab) followed by surgical resection and an increase in $CD8^+$ T cell infiltration, specifically with two effector T cell clusters ($CD3^+CD8^+CD45RO^+Eomes^+$ and $CD3^+CD8^+CD45RO^+Eomes^+CD57^+CD38^{low}$)[172].

Biomarker-driven trials

Clinical trials can be designed to enable the collection of patient data and identify various clinical and correlative biomarkers using samples derived from patients before, during and/or after treatment (Figs. 1,3). Once candidate biomarkers are identified, they can then also be used to stratify patients in subsequent clinical trials. A good example is provided by the REACH-2 trial, which led to the approval of ramucirumab for use in patients with HCC and elevated serum AFP levels (<400 ng/ml)[86]. Examples of other trial designs include basket trials (Fig. 4), which enrol patients with various different types of cancer to receive the same treatment. A phase I study in which patients with different GPC3-positive cancers (including, among others, HCC and Wilms tumour) will receive GPC3-targeted IL-15-expressing CAR T cells (NCT05103631) is such a study. Other designs include adaptive patient-specific trials including on-treatment second-biopsy sampling might also enable improved patient selection (Fig. 4c)

Multi-omics integrative analysis

Future studies will probably utilize numerous integrative analysis methods as they emerge to improve the performance of both hypothesis-driven and de novo biomarker discovery (as described in detail elsewhere[173]). Accordingly, the main goals one can foresee going forward are fourfold: developing biomarker signatures that enable more accurate administration of ICIs; developing biomarkers to guide the use

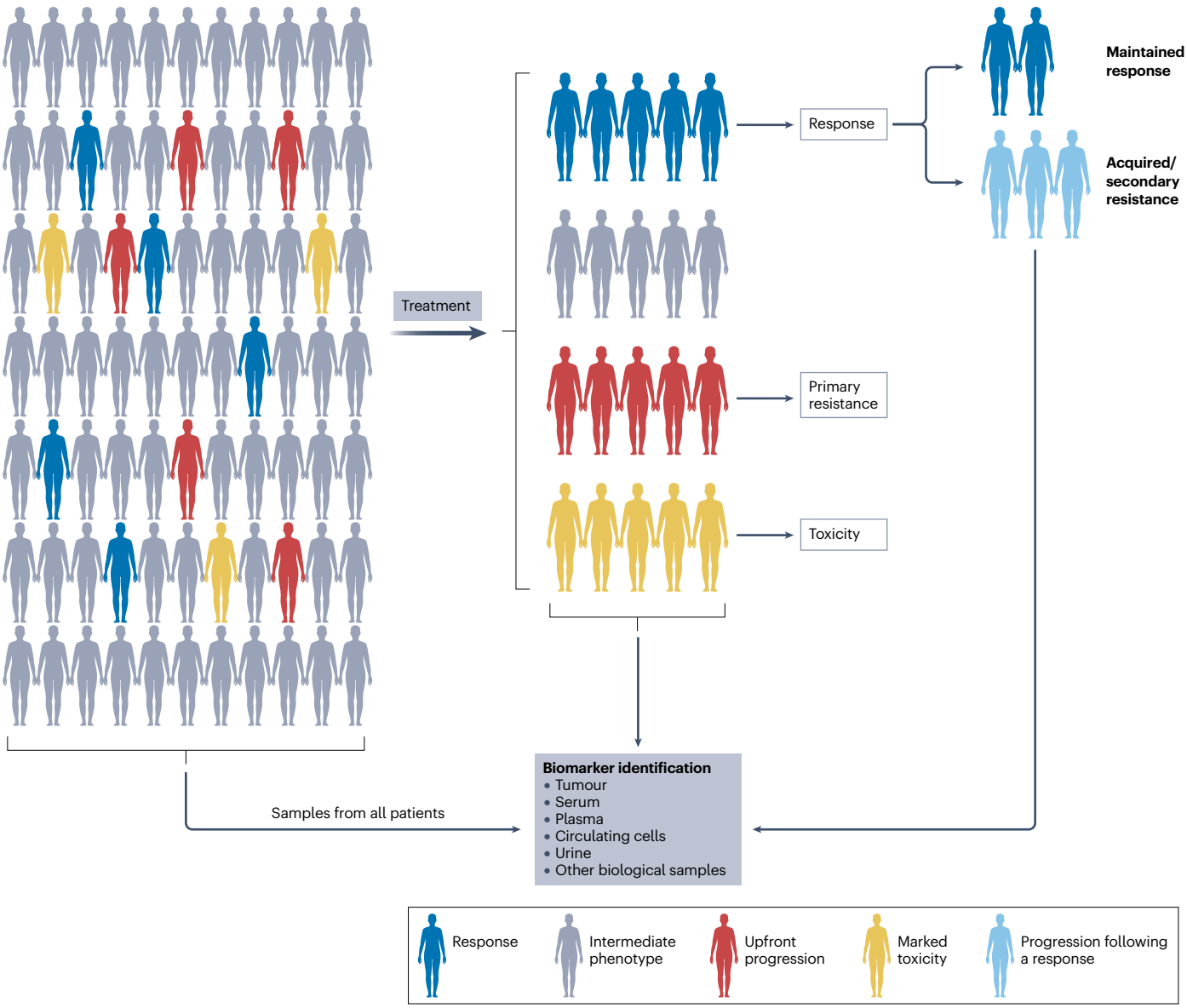

Fig. 3 | Trial design for biomarker identification studies. All patients enrolled in a clinical trial or biomarker study will undergo sampling to provide biological specimens at baseline to permit biomarker identification and/or monitoring. At follow-up points, either during or after the treatment, clinical response and other parameters are assessed, and another set of samples can be obtained. Patients with a response can undergo sampling at several time points to enable the identification of biomarkers associated with maintained responses and/or acquired resistance.

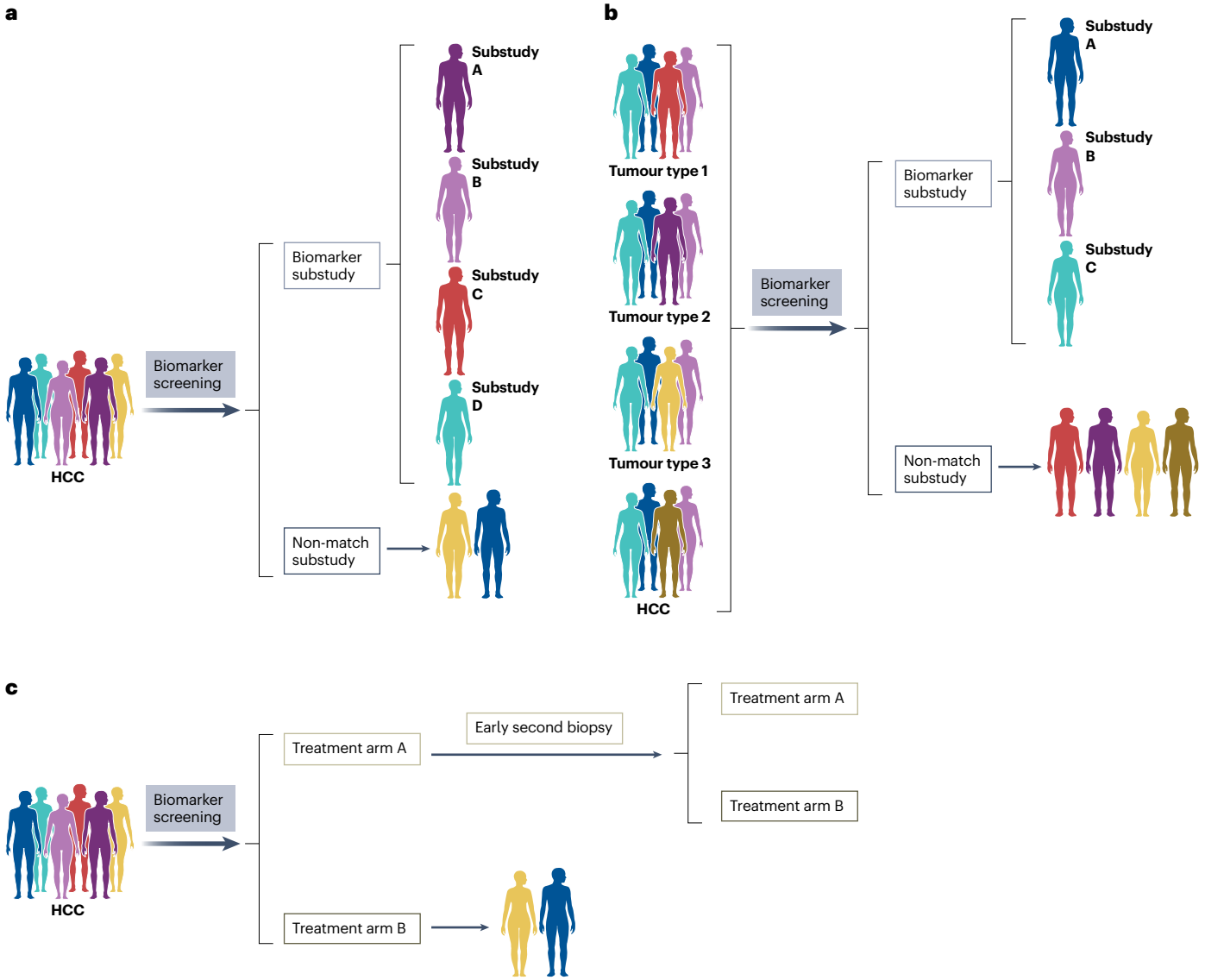

Fig. 4 | Trial designs for biomarker-driven clinical trials. a, Patients are screened for the presence of one or more predictive biomarkers. In case of a match, patients are included in substudies, in which they receive treatment predicated on the specific biomarker. If no biomarker is detected, patients are enrolled in a non-match study arm, or substudy. **b**, Basket trials involving patients with tumours of varying histologies (including hepatocellular carcinoma (HCC)) enable the identification of tumour-agnostic biomarkers. Subsequently, patients are enrolled into biomarker substudies to receive matched targeted therapies, or non-match substudies if no biomarker is detected. **c**, Patients are screened for the presence of a specific biomarker and, in case of a match, are included in the appropriate biomarker arm. Depending on the results from early on-treatment second biopsy samples, patients will be randomized to different treatment arms using an adaptive patient-specific approach.

of novel immunotherapies either as monotherapies or in combination; and, finally, identifying novel predictors of specific adverse events.

Current predictive biomarkers leave considerable room for improvement, as described in previous sections. To achieve substantial improvements, future biomarker development studies should include advanced multimodal integration of the effects of prior treatments, of tumour heterogeneity and clonal structure, the neoantigen expression patterns of the tumour, the TME and key clinically relevant characteristics relating to a patient's general health beyond those related to the tumour itself[173,174]. We especially foresee that scRNA-seq data collected from longitudinal series of patient samples will be crucial in providing a better understanding of mechanisms of treatment resistance and in enabling real-time assessments of the need for treatment modifications[175].

Beyond extended use of multi-omics data, future predictors should consider incorporating other forms of clinical data, such as histopathology, radiology images and health records, ideally by capitalizing on advances in deep learning and generative learning[176]. The effective integration of these different sources is likely to build upon relevant computational advances as they arise[173,177]. Such integrative treatment recommendations could be quite complex and call for

treatment decisions to be made by broad multidisciplinary molecular tumour boards, an approach that has already begun to demonstrate improvements in OS, albeit on a modest scale[177,178].

Overall, as ICIs or ICI-containing regimens are likely to continue to be first-line therapy in patients with HCC, predictors of response should be carefully tuned towards very high negative predictive values, thus ruling out the use of ICIs in favour of other treatments only in appropriate high-confidence cases. Such alternatives might also include treatments specifically designed to make tumours more immunogenic, and thus increase the activity of subsequent ICIs or ICI-containing combinations.

Obtaining molecular data from primary or metastatic tumour samples is often unfeasible and even when available, analysis of such samples can be difficult to complete within an acceptable clinical time frame. We envisage two main ways to bypass this hurdle in future. The first direction involves building integrative response predictors directly from pathology and radiology images without necessarily requiring any omics data collection[179] while concomitantly developing pathology-based predictors for multiple immune and targeted therapies[180]. The second avenue is more reliant on blood-based biomarkers. Here, in addition to the rapidly growing interest in and implementation of DNA and methylation-based liquid biopsies, we anticipate increased levels of interest in a wide array of metabolic and proteomics-based blood biomarkers, particularly the development of methods for analysing the transcriptomes of single immune cells obtained from liquid biopsy samples[181,182]. We cautiously note that implementing the vision described above will require extensive investment in careful data collection and public sharing on an unprecedented scale, although we are also confident that this effort will deliver worthwhile outcomes.

Future perspectives

The identification of biomarkers that are predictive of response to ICIs in patients with HCC will help to protect patients from exposure to treatment they do not benefit from, which can cause both medical and financial toxicities, although the research community needs to recognize that this is not an easy endeavour. One single biomarker is unlikely to be able to predict response with a sufficient level of accuracy and this applies especially in HCC, a disease occurring in the context of various chronic, infectious and/or metabolic underlying disease states that often occur in combination. An International Liver Cancer Association (ILCA) white paper on how to design, execute and interpret biomarker studies in HCC, with an emphasis on end points and measures of clinical efficacy was published in 2021 (ref. 183). For predictive biomarkers linked to experimental therapies, the ILCA proposes four different designs depending on when the biomarker is tested in relation to randomization for the investigational product.

In the long-term, we need to recognize that assays requiring tumour biopsy samples are much more difficult to implement clinically than blood-based assays. The number of liquid biopsy technologies available and the breadth of molecular information that can be obtained from blood are increasing rapidly. Some of these assays are being evaluated in other HCC-related indications, mostly relating to disease surveillance. For example, a large-cohort study evaluated viral exposure as a novel early detection tool in patients with a high risk of developing HCC[184]. This study demonstrated that molecular tracing of a patient's previous viral exposures using a signature detected in blood samples obtained decades before tumour development enables more accurate prediction of tumour development than serum AFP analysis.

cfDNA fragmentomics, which involves profiling of the fragment sizes of the DNA released into the bloodstream, is another example of a technology that has shown some promise for early detection of HCC. This fragmentation pattern reflects the different ways chromatin is organized in the nucleus of origin[185]. For a gene to be transcribed into mRNA, it must be localized in a region of open chromatin. Regions of open chromatin and regions of closed chromatin are differentially cleaved. Thus, the cfDNA 'fragmentome' can mirror the genomic and epigenetic characteristics of the cell of origin and can thus enable accurate detection of HCCs at an early stage[186]. Data published in 2021 confirm that this connection between chromatin folding and gene expression can be used as a proxy for dysregulated gene expression in tumour tissues[185]. Theoretically, such technology could facilitate the inference of tissue gene expression signatures using blood.

Another breakthrough technology currently under evaluation as a minimally invasive biomarker involves injectable biosensors. Specifically, a nanosensor library enabling the measurement of protease activity outperformed serum prostate-specific antigen in predicting the outcomes in patients with prostate cancer[187]. Besides new analytes, technological improvements have also increased the limits of nucleic acid detection. A rapid quantitative analysis of multiple small RNA sequences using nucleic acid toehold probe-based photonic resonator absorption microscopy can now yield detection limits in the femtomolar range[188,189]. This method could transform how we quantify nucleic acids in the blood and facilitate the implementation of liquid biopsy technologies. Most of these technologies have not yet been applied to studying response to systemic therapies in patients with HCC, although they give an indication of how quickly the field is moving forward and what to expect in terms of a blood-based biomarker in the upcoming years.

Conclusions

Only if academia, the pharmaceutical industry, regulatory agencies, patient advocates and patients agree that more and better clinical data from patients receiving ICIs are needed, will we be in a position to identify better biomarkers. We need to understand that a database for future AI-based interrogations should include, but not be limited to, information on type of treatment and response, race and ethnicity, underlying liver diseases, comorbidities, and results from tumour, blood and imaging studies. This process should start with improvements in clinical trial design (Fig. 3), although obtaining data from real-world cohorts will also be helpful. Finally, we should not underestimate that these studies will not only help predict response to specific therapies, but might also provide new insights into biological mechanisms and enable the development of even better future therapies for patients with HCC.

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References

1. International Agency for Research on Cancer. Estimated number of new cases in 2020, World, both sexes, all ages (excl. NMSC). *Cancer Today* https://gco.iarc.fr/today/online-analysis-table?v=2020&mode=cancer&mode_population=continents&population=900&populations=900&key=asr&sex=0&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&group_cancer=1&include_nmsc=0&include_nmsc_other=1 (2020).
2. Siegel, R. L., Miller, K. D., Wagle, N. S. & Jemal, A. Cancer statistics, 2023. *CA Cancer J. Clin.* **73**, 17–48 (2023).
3. Llovet, J. M. et al. Hepatocellular carcinoma. *Nat. Rev. Dis. Prim.* **7**, 6 (2021).
4. Cheng, A. L. et al. Updated efficacy and safety data from IMbrave150: atezolizumab plus bevacizumab vs. sorafenib for unresectable hepatocellular carcinoma. *J. Hepatol.* **76**, 862–873 (2022).
5. Llovet, J. M. et al. Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* **359**, 378–390 (2008).

6. Greten, T. F. et al. Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immunotherapy for the treatment of hepatocellular carcinoma. *J. Immunother. Cancer* **9**, e002794 (2021).
7. Finn, R. S. et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N. Engl. J. Med.* **382**, 1894–1905 (2020).
8. Abou-Alfa, G. K. et al. Tremelimumab plus durvalumab in unresectable hepatocellular carcinoma. *NEJM Evid.* **1** (8), https://doi.org/10.1056/EVIDoa2100070 (2022).
9. Yau, T. 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).
10. Finn, R. 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. Waldman, A. D., Fritz, J. M. & Lenardo, M. J. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat. Rev. Immunol.* **20**, 651–668 (2020).
12. Monge, C., Xie, C., Steinberg, S. M. & Greten, T. F. Clinical indicators for long-term survival with immune checkpoint therapy in advanced hepatocellular carcinoma. *J. Hepatocell. Carcinoma* **8**, 507–512 (2021).
13. Pinato, D. J. et al. Treatment-related toxicity and improved outcome from immunotherapy in hepatocellular cancer: evidence from an FDA pooled analysis of landmark clinical trials with validation from routine practice. *Eur. J. Cancer* **157**, 140–152 (2021).
14. Huang, D. Q., El-Serag, H. B. & Loomba, R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 223–238 (2021).
15. Marrero, J. A. et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. *Hepatology* **68**, 723–750 (2018).
16. Child, C. G. & Turcotte, J. G. Surgery and portal hypertension. *Major. Probl. Clin. Surg.* **1**, 1–85 (1964).
17. Reig, M. et al. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. *J. Hepatol.* **76**, 681–693 (2022).
18. Bruix, J., Chan, S. L., Galle, P. R., Rimassa, L. & Sangro, B. Systemic treatment of hepatocellular carcinoma: an EASL position paper. *J. Hepatol.* **75**, 960–974 (2021).
19. Kudo, M. et al. Management of hepatocellular carcinoma in Japan: JSH consensus statements and recommendations 2021 update. *Liver Cancer* **10**, 181–223 (2021).
20. Zhou, J. et al. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (2019 edition). *Liver Cancer* **9**, 682–720 (2020).
21. Vogel, A. et al. Hepatocellular carcinoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **29**, iv238–iv255 (2018).
22. Kudo, M. et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* **391**, 1163–1173 (2018).
23. Ren, Z. 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).
24. Qin, S. et al. Pembrolizumab plus best supportive care versus placebo plus best supportive care as second-line therapy in patients in Asia with advanced hepatocellular carcinoma (HCC): phase 3 KEYNOTE-394 study [abstract]. *J. Clin. Oncol.* **40** (4 Suppl.), 383 (2022).
25. Yau, T. et al. Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: the checkmate 040 randomized clinical trial. *JAMA Oncol.* **6**, e204564 (2020).
26. Sangro, B. et al. Diagnosis and management of toxicities of immune checkpoint inhibitors in hepatocellular carcinoma. *J. Hepatol.* **72**, 320–341 (2020).
27. Chow, P. et al. IMbrave050: phase 3 study of adjuvant atezolizumab + bevacizumab versus active surveillance in patients with hepatocellular carcinoma (HCC) at high risk of disease recurrence following resection or ablation [abstract]. *Cancer Res.* **83** (8 Suppl.), CT003 (2023).
28. Kaseb, A. O. et al. Perioperative nivolumab monotherapy versus nivolumab plus ipilimumab in resectable hepatocellular carcinoma: a randomised, open-label, phase 2 trial. *Lancet Gastroenterol. Hepatol.* **7**, 208–218 (2022).
29. Ho, W. J. et al. Neoadjuvant cabozantinib and nivolumab converts locally advanced HCC into resectable disease with enhanced antitumor immunity. *Nat. Cancer* **2**, 891–903 (2021).
30. Shu, D. H. et al. 12-chemokine gene signature identifies major pathologic response in patients with hepatocellular carcinoma treated with neoadjuvant nivolumab and cabozantinib [abstract]. *Cancer Res.* **82** (12 Suppl.), 1323 (2022).
31. Marron, T. U. et al. Neoadjuvant cemiplimab for resectable hepatocellular carcinoma: a single-arm, open-label, phase 2 trial. *Lancet Gastroenterol. Hepatol.* **7**, 219–229 (2022).
32. Biomarkers Definitions Working Group Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* **69**, 89–95 (2001).
33. McKean, W. B., Moser, J. C., Rimm, D. & Hu-Lieskovan, S. Biomarkers in precision cancer immunotherapy: promise and challenges. *Am. Soc. Clin. Oncol. Educ. Book.* **40**, e275–e291 (2020).
34. Han, Y., Liu, D. & Li, L. PD-1/PD-L1 pathway: current researches in cancer. *Am. J. Cancer Res.* **10**, 727–742 (2020).
35. Paver, E. C. et al. 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).
36. El-Khoueiry, A. B. 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).
37. Zhu, A. X. 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).
38. Zhu, A. X. 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).
39. Duffy, A. G. et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J. Hepatol.* **66**, 545–551 (2017).
40. Ng, H. H. M. 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).
41. Ang, C. et al. Prevalence of established and emerging biomarkers of immune checkpoint inhibitor response in advanced hepatocellular carcinoma. *Oncotarget* **10**, 4018–4025 (2019).
42. Yarchoan, M., Hopkins, A. & Jaffee, E. M. Tumor mutational burden and response rate to PD-1 inhibition. *N. Engl. J. Med.* **377**, 2500–2501 (2017).
43. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
44. Cancer Genome Atlas Research Network Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell* **169**, 1327–1341.e23 (2017).
45. Chaisaingmongkol, J. et al. Common molecular subtypes among Asian hepatocellular carcinoma and cholangiocarcinoma. *Cancer Cell* **32**, 57–70.e3 (2017).
46. Hoshida, Y. et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res.* **69**, 7385–7392 (2009).
47. Haber, P. K. et al. Molecular markers of response to anti-PD1 therapy in advanced hepatocellular carcinoma. *Gastroenterology* **164**, 72–88.e18 (2023).
48. Sangro, B. et al. Association of inflammatory biomarkers with clinical outcomes in nivolumab-treated patients with advanced hepatocellular carcinoma. *J. Hepatol.* **73**, 1460–1469 (2020).
49. Hong, J. Y. et al. Hepatocellular carcinoma patients with high circulating cytotoxic T cells and intra-tumoral immune signature benefit from pembrolizumab: results from a single-arm phase 2 trial. *Genome Med.* **14**, 1 (2022).
50. Huang, M. et al. The influence of immune heterogeneity on the effectiveness of immune checkpoint inhibitors in multifocal hepatocellular carcinomas. *Clin. Cancer Res.* **26**, 4947–4957 (2020).
51. Budhu, A. et al. Tumor biology and immune infiltration define primary liver cancer subsets linked to overall survival after immunotherapy. *Cell Rep. Med.* **4**, 101052 (2023).
52. Vanhersecke, L. et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. *Nat. Cancer* **2**, 794–802 (2021).
53. Fridman, W. H. et al. B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. *Nat. Rev. Clin. Oncol.* **19**, 441–457 (2022).
54. Yu, S. et al. Tumor-infiltrating immune cells in hepatocellular carcinoma: Tregs is correlated with poor overall survival. *PLoS ONE* **15**, e0231003 (2020).
55. Montironi, C. et al. Inflamed and non-inflamed classes of HCC: a revised immunogenomic classification. *Gut* **72**, 129–140 (2022).
56. Ge, P. L. et al. Prognostic values of immune scores and immune microenvironment-related genes for hepatocellular carcinoma. *Aging* **12**, 5479–5499 (2020).
57. Martin-Serrano, M. A. et al. Novel microenvironment-based classification of intrahepatic cholangiocarcinoma with therapeutic implications. *Gut* **72**, 736–748 (2023).
58. Ma, L. et al. Single-cell atlas of tumor cell evolution in response to therapy in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J. Hepatol.* **75**, 1397–1408 (2021).
59. Marusyk, A., Janiszewska, M. & Polyak, K. Intratumor heterogeneity: the Rosetta stone of therapy resistance. *Cancer Cell* **37**, 471–484 (2020).
60. Zhang, Q. et al. Landscape and dynamics of single immune cells in hepatocellular carcinoma. *Cell* **179**, 829–845.e20 (2019).
61. Zheng, L. et al. Pan-cancer single-cell landscape of tumor-infiltrating T cells. *Science* **374**, abe6474 (2021).
62. Sade-Feldman, M. et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* **175**, 998–1013.e20 (2018).
63. Ma, L. et al. Tumor cell biodiversity drives microenvironmental reprogramming in liver cancer. *Cancer Cell* **36**, 418–430.e6 (2019).
64. Bian, J. et al. T lymphocytes in hepatocellular carcinoma immune microenvironment: insights into human immunology and immunotherapy. *Am. J. Cancer Res.* **10**, 4585 (2020).
65. Llovet, J. M. et al. Immunotherapies for hepatocellular carcinoma. *Nat. Rev. Clin. Oncol.* **19**, 151–172 (2022).
66. Havel, J. J., Chowell, D. & Chan, T. A. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat. Rev. Cancer* **19**, 133–150 (2019).
67. Ho, D. W. et al. Single-cell RNA sequencing shows the immunosuppressive landscape and tumor heterogeneity of HBV-associated hepatocellular carcinoma. *Nat. Commun.* **12**, 3684 (2021).
68. Liu, Y. et al. Identification of a tumour immune barrier in the HCC microenvironment that determines the efficacy of immunotherapy. *J. Hepatol.* **78**, 770–782 (2023).

69. Nguyen, P. H. D. et al. Trajectory of immune evasion and cancer progression in hepatocellular carcinoma. *Nat. Commun.* **13**, 1441 (2022).
70. Hoechst, B. et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces $CD4^+CD25^+Foxp3^+$ T cells. *Gastroenterology* **135**, 234–243 (2008).
71. Liu, M. et al. Targeting monocyte-intrinsic enhancer reprogramming improves immunotherapy efficacy in hepatocellular carcinoma. *Gut* **69**, 365–379 (2020).
72. Xue, R. et al. Liver tumour immune microenvironment subtypes and neutrophil heterogeneity. *Nature* **612**, 141–147 (2022).
73. Geh, D. et al. Neutrophils as potential therapeutic targets in hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* **19**, 257–273 (2022).
74. Ma, L. et al. Multiregional single-cell dissection of tumor and immune cells reveals stable lock-and-key features in liver cancer. *Nat. Commun.* **13**, 7533 (2022).
75. Murai, H. et al. Multiomics identifies the link between intratumor steatosis and the exhausted tumor immune microenvironment in hepatocellular carcinoma. *Hepatology* **1**, 77–91 (2022).
76. Provine, N. M. & Klenerman, P. MAIT cells in health and disease. *Annu. Rev. Immunol.* **38**, 203–228 (2020).
77. Ruf, B. et al. Tumor-associated macrophages trigger MAIT cell dysfunction at the HCC invasive margin. *Cell* **186**, 3686–3705.e32 (2023).
78. Nguyen, P. H. D. et al. Intratumoural immune heterogeneity as a hallmark of tumour evolution and progression in hepatocellular carcinoma. *Nat. Commun.* **12**, 227 (2021).
79. Zhang, S. et al. Spatial transcriptomics analysis of neoadjuvant cabozantinib and nivolumab in advanced hepatocellular carcinoma identifies independent mechanisms of resistance and recurrence. Preprint at *bioRxiv* https://www.biorxiv.org/content/10.1101/2023.01.10.523481v1 (2023).
80. Slamon, D. J. et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**, 783–792 (2001).
81. Villanueva, A. Hepatocellular carcinoma. *N. Engl. J. Med.* **380**, 1450–1462 (2019).
82. Hu, X., Chen, R., Wei, Q. & Xu, X. The landscape of alpha fetoprotein in hepatocellular carcinoma: where are we? *Int. J. Biol. Sci.* **18**, 536–551 (2022).
83. Galle, P. R. et al. Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. *Liver Int.* **39**, 2214–2229 (2019).
84. Bruix, J. et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* **389**, 56–66 (2017).
85. Abou-Alfa, G. K. et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N. Engl. J. Med.* **379**, 54–63 (2018).
86. Zhu, A. X. et al. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* **20**, 282–296 (2019).
87. Zhu, A. X. et al. Alpha-fetoprotein as a potential surrogate biomarker for atezolizumab + bevacizumab treatment of hepatocellular carcinoma. *Clin. Cancer Res.* **28**, 3537–3545 (2022).
88. Shao, Y. Y. et al. Early alpha-foetoprotein response associated with treatment efficacy of immune checkpoint inhibitors for advanced hepatocellular carcinoma. *Liver Int.* **39**, 2184–2189 (2019).
89. Lee, P. C. et al. Predictors of response and survival in immune checkpoint inhibitor-treated unresectable hepatocellular carcinoma. *Cancers* **12**, 182 (2020).
90. Spahn, S. et al. Clinical and genetic tumor characteristics of responding and non-responding patients to PD-1 inhibition in hepatocellular carcinoma. *Cancers* **12**, 3830 (2020).
91. Scheiner, B. et al. Prognosis of patients with hepatocellular carcinoma treated with immunotherapy – development and validation of the CRAFITY score. *J. Hepatol.* **76**, 353–363 (2022).
92. Hatanaka, T. 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).
93. Teng, W. et al. Combination of CRAFITY score with alpha-fetoprotein response predicts a favorable outcome of atezolizumab plus bevacizumab for unresectable hepatocellular carcinoma. *Am. J. Cancer Res.* **12**, 1899–1911 (2022).
94. Sun, X. et al. Reductions in AFP and PIVKA-II can predict the efficiency of anti-PD-1 immunotherapy in HCC patients. *BMC Cancer* **21**, 775 (2021).
95. Myojin, Y. et al. Interleukin-6 is a circulating prognostic biomarker for hepatocellular carcinoma patients treated with combined immunotherapy. *Cancers* **14**, 883 (2022).
96. Yang, H. et al. High serum IL-6 correlates with reduced clinical benefit of atezolizumab and bevacizumab in unresectable hepatocellular carcinoma. *JHEP Rep.* **5**, 100672 (2023).
97. Feun, L. G. et al. Phase 2 study of pembrolizumab and circulating biomarkers to predict anticancer response in advanced, unresectable hepatocellular carcinoma. *Cancer* **125**, 3603–3614 (2019).
98. Feun, L. G. et al. Circulating biomarkers to predict antitumor response to immunotherapy in advanced unresectable hepatoma [abstract]. *Cancer Res.* **82** (12 Suppl.), 2771 (2022).
99. Li, X. S., Li, J. W., Li, H. & Jiang, T. Prognostic value of programmed cell death ligand 1 (PD-L1) for hepatocellular carcinoma: a meta-analysis. *Biosci. Rep.* **40**, BSR20200459 (2020).
100. Wang, T., Denman, D., Bacot, S. M. & Feldman, G. M. Challenges and the evolving landscape of assessing blood-based PD-L1 expression as a biomarker for anti-PD-(L)1 immunotherapy. *Biomedicines* **10**, 1181 (2022).
101. Lin, Z. F., Qin, L. X. & Chen, J. H. Biomarkers for response to immunotherapy in hepatobiliary malignancies. *Hepatobiliary Pancreat. Dis. Int.* **21**, 413–419 (2022).
102. Pallozzi, M. et al. Non-invasive biomarkers for immunotherapy in patients with hepatocellular carcinoma: current knowledge and future perspectives. *Cancers* **14**, 4631 (2022).
103. Dharmapuri, S. et al. Predictive value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in advanced hepatocellular carcinoma patients treated with anti-PD-1 therapy. *Cancer Med.* **9**, 4962–4970 (2020).
104. Hung, H. C. et al. Response prediction in immune checkpoint inhibitor immunotherapy for advanced hepatocellular carcinoma. *Cancers* **13**, 1607 (2021).
105. Muhammed, A. et al. The systemic inflammatory response identifies patients with adverse clinical outcome from immunotherapy in hepatocellular carcinoma. *Cancers* **14**, 186 (2021).
106. Mei, J. et al. Comparison of the prognostic value of inflammation-based scores in patients with hepatocellular carcinoma after anti-PD-1 therapy. *J. Inflamm. Res.* **14**, 3879–3890 (2021).
107. Wu, Y. L. et al. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios as prognostic biomarkers in unresectable hepatocellular carcinoma treated with atezolizumab plus bevacizumab. *Cancers* **14**, 5834 (2022).
108. Kim, C. et al. 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).
109. Chew, V. et al. Delineation of an immunosuppressive gradient in hepatocellular carcinoma using high-dimensional proteomic and transcriptomic analyses. *Proc. Natl Acad. Sci. USA* **114**, E5900–E5909 (2017).
110. Zheng, C. et al. Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. *Cell* **169**, 1342–1356.e16 (2017).
111. Sun, Y. et al. Single-cell landscape of the ecosystem in early-relapse hepatocellular carcinoma. *Cell* **184**, 404–421 e416 (2021).
112. Heinrich, B. et al. The tumour microenvironment shapes innate lymphoid cells in patients with hepatocellular carcinoma. *Gut* **71**, 1161–1175 (2022).
113. Spitzer, M. H. & Nolan, G. P. Mass cytometry: single cells, many features. *Cell* **165**, 780–791 (2016).
114. Monge, C. et al. Phase I/II study of PexaVec in combination with immune checkpoint inhibition in refractory metastatic colorectal cancer. *J. Immunother. Cancer* **11**, e005640 (2023).
115. Gohil, S. H., Iorgulescu, J. B., Braun, D. A., Keskin, D. B. & Livak, K. J. Applying high-dimensional single-cell technologies to the analysis of cancer immunotherapy. *Nat. Rev. Clin. Oncol.* **18**, 244–256 (2021).
116. Krieg, C. et al. High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. *Nat. Med.* **24**, 144–153 (2018).
117. Agdashian, D. et al. The effect of anti-CTLA4 treatment on peripheral and intra-tumoral T cells in patients with hepatocellular carcinoma. *Cancer Immunol. Immunother.* **68**, 599–608 (2019).
118. Hung, Y. P. et al. Potential of circulating immune cells as biomarkers of nivolumab treatment efficacy for advanced hepatocellular carcinoma. *J. Chin. Med. Assoc.* **84**, 144–150 (2021).
119. Heinrich, B. et al. Checkpoint inhibitors modulate plasticity of innate lymphoid cells in peripheral blood of patients with hepatocellular carcinoma. *Front. Immunol.* **13**, 849958 (2022).
120. Ruf, B., Heinrich, B. & Greten, T. F. Immunobiology and immunotherapy of HCC: spotlight on innate and innate-like immune cells. *Cell Mol. Immunol.* **18**, 112–127 (2021).
121. Barsch, M. et al. T-cell exhaustion and residency dynamics inform clinical outcomes in hepatocellular carcinoma. *J. Hepatol.* **77**, 397–409 (2022).
122. Chuah, S. et al. Uncoupling immune trajectories of response and adverse events from anti-PD-1 immunotherapy in hepatocellular carcinoma. *J. Hepatol.* **77**, 683–694 (2022).
123. Sidiropoulos, D. N. et al. Integrated T cell cytometry metrics for immune-monitoring applications in immunotherapy clinical trials. *JCI Insight* **7**, e160398 (2022).
124. Alix-Panabieres, C. & Pantel, K. Liquid biopsy: from discovery to clinical application. *Cancer Discov.* **11**, 858–873 (2021).
125. Schroers-Martin, J. G. et al. Molecular monitoring of lymphomas. *Annu. Rev. Pathol.* **18**, 149–180 (2023).
126. von Felden, J., Garcia-Lezana, T., Schulze, K., Losic, B. & Villanueva, A. Liquid biopsy in the clinical management of hepatocellular carcinoma. *Gut* **69**, 2025–2034 (2020).
127. Klein, E. A. et al. Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set. *Ann. Oncol.* **32**, 1167–1177 (2021).
128. Tran, N. H., Kisiel, J. & Roberts, L. R. Using cell-free DNA for HCC surveillance and prognosis. *JHEP Rep.* **3**, 100304 (2021).
129. Kaseb, A. O. et al. Molecular profiling of hepatocellular carcinoma using circulating cell-free DNA. *Clin. Cancer Res.* **25**, 6107–6118 (2019).
130. von Felden, J. et al. Mutations in circulating tumor DNA predict primary resistance to systemic therapies in advanced hepatocellular carcinoma. *Oncogene* **40**, 140–151 (2021).
131. Matsumae, T. et al. Circulating cell-free DNA profiling predicts the therapeutic outcome in advanced hepatocellular carcinoma patients treated with combination immunotherapy. *Cancers* **14**, 3367 (2022).

132. Harding, J. J. 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).
133. An, H. J., Chon, H. J. & Kim, C. Peripheral blood-based biomarkers for immune checkpoint inhibitors. *Int. J. Mol. Sci.* **22**, 9414 (2021).
134. Tamminga, M. et al. Circulating tumor cells in advanced non-small cell lung cancer patients are associated with worse tumor response to checkpoint inhibitors. *J. Immunother. Cancer* **7**, 173 (2019).
135. Winograd, P. 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).
136. Budhu, A. et al. Integrated metabolite and gene expression profiles identify lipid biomarkers associated with progression of hepatocellular carcinoma and patient outcomes. *Gastroenterology* **144**, 1066–1075.e1 (2013).
137. Pomyen, Y. et al. Tumor metabolism and associated serum metabolites define prognostic subtypes of Asian hepatocellular carcinoma. *Sci. Rep.* **11**, 12097 (2021).
138. Breeur, M. et al. Pan-cancer analysis of pre-diagnostic blood metabolite concentrations in the European Prospective Investigation into Cancer and Nutrition. *BMC Med.* **20**, 351 (2022).
139. Fujiwara, N. et al. A blood-based prognostic liver secretome signature and long-term hepatocellular carcinoma risk in advanced liver fibrosis. *Med* **2**, 836–850.e10 (2021).
140. Hung, M. H. et al. Tumor methionine metabolism drives T-cell exhaustion in hepatocellular carcinoma. *Nat. Commun.* **12**, 1455 (2021).
141. Wu, H. et al. Dynamic microbiome and metabolome analyses reveal the interaction between gut microbiota and anti-PD-1 based immunotherapy in hepatocellular carcinoma. *Int. J. Cancer* **151**, 1321–1334 (2022).
142. Gong, X. Q. et al. Progress of MRI radiomics in hepatocellular carcinoma. *Front. Oncol.* **11**, 698373 (2021).
143. Dercle, L. et al. Artificial intelligence and radiomics: fundamentals, applications, and challenges in immunotherapy. *J. Immunother. Cancer* **10**, e005292 (2022).
144. Dercle, L. et al. Emerging and evolving concepts in cancer immunotherapy imaging. *Radiology* **306**, 32–46 (2023).
145. Martinino, A. et al. Artificial intelligence in the diagnosis of hepatocellular carcinoma: a systematic review. *J. Clin. Med.* **11**, 6368 (2022).
146. Tao, Y. Y. et al. Radiomic analysis based on magnetic resonance imaging for predicting PD-L2 expression in hepatocellular carcinoma. *Cancers (Basel)* **15**, 365 (2023).
147. Chen, S. et al. Pretreatment prediction of immunoscore in hepatocellular cancer: a radiomics-based clinical model based on Gd-EOB-DTPA-enhanced MRI imaging. *Eur. Radiol.* **29**, 4177–4187 (2019).
148. Hectors, S. J. et al. MRI radiomics features predict immuno-oncological characteristics of hepatocellular carcinoma. *Eur. Radiol.* **30**, 3759–3769 (2020).
149. Yuan, G. et al. Development and validation of a contrast-enhanced CT-based radiomics nomogram for prediction of therapeutic efficacy of anti-PD-1 antibodies in advanced HCC patients. *Front. Immunol.* **11**, 613946 (2020).
150. Castilla-Lievre, M. A. et al. Diagnostic value of combining ^{11}C-choline and ^{18}F-FDG PET/CT in hepatocellular carcinoma. *Eur. J. Nucl. Med. Mol. Imaging* **43**, 852–859 (2016).
151. European Association for the Study of the Liver EASL clinical practice guidelines: management of hepatocellular carcinoma. *J. Hepatol.* **69**, 182–236 (2018).
152. Wei, W. et al. ImmunoPET: concept, design, and applications. *Chem. Rev.* **120**, 3787–3851 (2020).
153. Bell, M., Turkbey, E. B. & Escorcia, F. E. Radiomics, radiogenomics, and next-generation molecular imaging to augment diagnosis of hepatocellular carcinoma. *Cancer J.* **26**, 108–115 (2020).
154. Mena, E. et al. Functional imaging of liver cancer (FLIC): study protocol of a phase 2 trial of ^{18}F-DCFPyL PET/CT imaging for patients with hepatocellular carcinoma. *PLoS ONE* **17**, e0277407 (2022).
155. Rizzo, A. et al. PSMA radioligand uptake as a biomarker of neoangiogenesis in solid tumours: diagnostic or theragnostic factor? *Cancers* **14**, 4309 (2022).
156. Sepich-Poore, G. D. et al. The microbiome and human cancer. *Science* **371**, eabc4552 (2021).
157. McQuade, J. L., Daniel, C. R., Helmink, B. A. & Wargo, J. A. Modulating the microbiome to improve therapeutic response in cancer. *Lancet Oncol.* **20**, e77–e91 (2019).
158. McCulloch, J. A. et al. Intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-PD-1. *Nat. Med.* **28**, 545–556 (2022).
159. Schwabe, R. F. & Greten, T. F. Gut microbiome in HCC – mechanisms, diagnosis and therapy. *J. Hepatol.* **72**, 230–238 (2020).
160. Silveira, M. A. D., Bilodeau, S., Greten, T. F., Wang, X. W. & Trinchieri, G. The gut–liver axis: host microbiota interactions shape hepatocarcinogenesis. *Trends Cancer* **8**, 583–597 (2022).
161. Myojin, Y. & Greten, T. F. The microbiome and liver cancer. *Cancer J.* **29**, 57–60 (2023).
162. Ma, C. et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* **360**, eaan5931 (2018).
163. Zhang, L. et al. The association between antibiotic use and outcomes of HCC patients treated with immune checkpoint inhibitors. *Front. Immunol.* **13**, 956533 (2022).
164. Fulgenzi, C. A. M. et al. Effect of early antibiotic exposure on survival of patients receiving atezolizumab plus bevacizumab but not sorafenib for unresectable HCC: a sub-analysis of the phase III IMbrave150 study. *J. Clin. Oncol.* **41**, 597–597 (2023).
165. Davar, D. et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* **371**, 595–602 (2021).
166. McDermott, D. F. et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. *Nat. Med.* **24**, 749–757 (2018).
167. Lee, W. S., Yang, H., Chon, H. J. & Kim, C. Combination of anti-angiogenic therapy and immune checkpoint blockade normalizes vascular–immune crosstalk to potentiate cancer immunity. *Exp. Mol. Med.* **52**, 1475–1485 (2020).
168. Zhang, Y. et al. VEGFR2 activity on myeloid cells mediates immune suppression in the tumor microenvironment. *JCI Insight* **6**, e150375 (2021).
169. Kudo, M. Scientific rationale for combined immunotherapy with PD-1/PD-L1 antibodies and VEGF inhibitors in advanced hepatocellular carcinoma. *Cancers* **12**, 1089 (2020).
170. Neely, J. et al. Genomic and transcriptomic analyses related to the clinical efficacy of first-line nivolumab in advanced hepatocellular carcinoma from the phase 3 CheckMate 459 trial [abstract]. *Cancer Res.* **82** (12 Suppl.), 2145 (2022).
171. Ruiz de Galarreta, 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).
172. Kaseb, A. O. et al. Immunologic correlates of pathologic complete response to preoperative immunotherapy in hepatocellular carcinoma. *Cancer Immunol. Res.* **7**, 1390–1395 (2019).
173. Jiang, P. et al. Big data in basic and translational cancer research. *Nat. Rev. Cancer* **22**, 625–639 (2022).
174. Boehm, K. M., Khosravi, P., Vanguri, R., Gao, J. & Shah, S. P. Harnessing multimodal data integration to advance precision oncology. *Nat. Rev. Cancer* **22**, 114–126 (2022).
175. Cohen, Y. C. et al. Identification of resistance pathways and therapeutic targets in relapsed multiple myeloma patients through single-cell sequencing. *Nat. Med.* **27**, 491–503 (2021).
176. Echle, A. et al. Deep learning in cancer pathology: a new generation of clinical biomarkers. *Br. J. Cancer* **124**, 686–696 (2021).
177. Kato, S. et al. Real-world data from a molecular tumor board demonstrates improved outcomes with a precision N-of-One strategy. *Nat. Commun.* **11**, 4965 (2020).
178. Tamborero, D. et al. The molecular tumor board portal supports clinical decisions and automated reporting for precision oncology. *Nat. Cancer* **3**, 251–261 (2022).
179. Vanguri, R. S. et al. Multimodal integration of radiology, pathology and genomics for prediction of response to PD-(L)1 blockade in patients with non-small cell lung cancer. *Nat. Cancer* **3**, 1151–1164 (2022).
180. Hoang, D.-T. et al. Synthetic lethality-based prediction of cancer treatment response from histopathology images. *Cell* **3**, 2487–2502.e13 (2023).
181. Shi, A. et al. Plasma-derived extracellular vesicle analysis and deconvolution enable prediction and tracking of melanoma checkpoint blockade outcome. *Sci. Adv.* **6**, eabb3461 (2020).
182. Cao, Y. et al. Predicting tumor immune microenvironment and checkpoint therapy response of head & neck cancer patients from blood immune single-cell transcriptomics. Preprint at *bioRxiv* https://www.biorxiv.org/content/10.1101/2023.01.17.524455v1 (2023).
183. Singal, A. G. et al. International liver cancer association (ILCA) white paper on biomarker development for hepatocellular carcinoma. *Gastroenterology* **160**, 2572–2584 (2021).
184. Liu, J. et al. A viral exposure signature defines early onset of hepatocellular carcinoma. *Cell* **182**, 317–328.e10 (2020).
185. Lo, Y. M. D., Han, D. S. C., Jiang, P. & Chiu, R. W. K. Epigenetics, fragmentomics, and topology of cell-free DNA in liquid biopsies. *Science* **372**, eaaw3616 (2021).
186. Foda, Z. H. et al. Detecting liver cancer using cell-free DNA fragmentomes. *Cancer Discov.* **13**, 616–631 (2022).
187. Dudani, J. S., Ibrahim, M., Kirkpatrick, J., Warren, A. D. & Bhatia, S. N. Classification of prostate cancer using a protease activity nanosensor library. *Proc. Natl Acad. Sci. USA* **115**, 8954–8959 (2018).
188. Canady, T. D. et al. Digital-resolution detection of microRNA with single-base selectivity by photonic resonator absorption microscopy. *Proc. Natl Acad. Sci. USA* **116**, 19362–19367 (2019).
189. Zhao, B. et al. Digital-resolution and highly sensitive detection of multiple exosomal small RNAs by DNA toehold probe-based photonic resonator absorption microscopy. *Talanta* **241**, 123256 (2022).
190. Qin, S. et al. Final analysis of RATIONALE-301: randomized, phase III study of tislelizumab versus sorafenib as first-line treatment for unresectable hepatocellular carcinoma [abstract LBA36]. *Ann. Oncol.* **33** (Suppl. 7), S1402–S1403 (2022).
191. Qin, S. et al. Camrelizumab (C) plus rivoceranib (R) vs. sorafenib (S) as first-line therapy for unresectable hepatocellular carcinoma (uHCC): a randomized, phase III trial [abstract LBA35]. *Ann. Oncol.* **33** (Suppl. 7), S1401–S1402 (2022).
192. Kelley, R. K. et al. Cabozantinib plus atezolizumab versus sorafenib for advanced hepatocellular carcinoma (COSMIC-312): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* **23**, 995–1008 (2022).
193. Finn, R. S. et al. Primary results from the phase III LEAP-002 study: lenvatinib plus pembrolizumab versus lenvatinib as first-line (1L) therapy for advanced hepatocellular carcinoma (aHCC) [abstract LBA34]. *Ann. Oncol.* **33** (Suppl. 7), S1401 (2022).
194. Qin, S. et al. Donafenib versus sorafenib in first-line treatment of unresectable or metastatic hepatocellular carcinoma: a randomized, open-label, parallel-controlled phase II-III trial. *J. Clin. Oncol.* **39**, 3002–3011 (2021).
195. Qin, S. et al. Apatinib as second-line or later therapy in patients with advanced hepatocellular carcinoma (AHELP): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Gastroenterol. Hepatol.* **6**, 559–568 (2021).
196. Qin, S. et al. Pembrolizumab versus placebo as second-line therapy in patients from Asia with advanced hepatocellular carcinoma: a randomized, double-blind, phase III trial. *J. Clin. Oncol.* **41**, 1434–1443 (2023).

197. Verset, G. et al. Pembrolizumab monotherapy for previously untreated advanced hepatocellular carcinoma: data from the open-label, phase II KEYNOTE-224 trial. *Clin. Cancer Res.* **28**, 2547–2554 (2022).
198. Yau, T. et al. Nivolumab plus cabozantinib with or without ipilimumab for advanced hepatocellular carcinoma: results from cohort 6 of the CheckMate 040 trial. *J. Clin. Oncol.* **41**, 1747–1757 (2023).
199. Xu, J. et al. Camrelizumab in combination with apatinib in patients with advanced hepatocellular carcinoma (RESCUE): a nonrandomized, open-label, phase II trial. *Clin. Cancer Res.* **27**, 1003–1011 (2021).
200. Finn, R. S. et al. Phase Ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. *J. Clin. Oncol.* **38**, 2960–2970 (2020).
201. Kelley, R. K. et al. Safety, efficacy, and pharmacodynamics of tremelimumab plus durvalumab for patients with unresectable hepatocellular carcinoma: randomized expansion of a phase I/II study. *J. Clin. Oncol.* **39**, 2991–3001 (2021).

Author contributions

A.V., F.K., M.Y., L.M., E.R. and X.W.W researched data for the manuscript and made a substantial contribution to discussions of content. All authors wrote the manuscript, and A.V., F.K., B.R. M.Y., L.M. and E.R. edited and/or reviewed the manuscript prior to submission.

Competing interests

A.V. has acted as a consultant and/or adviser for Astra Zeneca, BMS, Eisai, FirstWorld, Genentech, Natera, NGM Pharmaceuticals, Pioneering Medicine and Roche; has received research support from Eisai; has stock options from Espervita; and is listed as an inventor on a patent related to early detection of HCC (PCT/US20/61441). M.Y. has acted as a consultant and/or adviser for AstraZeneca, Eisai, Exelixis, Genentech, Hepion and Replimune; has received research funding from Bristol-Myers Squibb, Genentech and Incyte; and holds equity in Adventris Pharmaceuticals. The other authors declare no competing interests.

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