

VOLUME 32 NUMBER 2 April 2026

pISSN 2287-2728
eISSN 2387-285X

CLINICAL and MOLECULAR HEPATOLOGY

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Review

Clinical applications of immunogenomics in hepatocellular carcinoma

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Liver cancer is one of the deadliest malignancies, with increasing incidence worldwide. Recent advances in immunotherapy have expanded the options for systemic therapy against advanced hepatocellular carcinoma (HCC), but there are no biomarkers currently available to predict which patients will respond, leading to suboptimal patient selection strategies. Understanding of the genetic and immunologic features of HCC is accelerating rapidly through the use of single cell and spatial transcriptomic techniques. However, there is a need to translate insights gained through these new studies to improve treatment options and improve patient selection. In this review we summarize knowledge of the immunogenomics of HCC, emphasizing recent advances, and discuss progress toward clinical translation. ([Clin Mol Hepatol 2026;32:511-535](#))

Keywords: Hepatocellular carcinoma; Immune checkpoint inhibitors; Precision medicine; Tumor microenvironment; Genomic medicine

INTRODUCTION

Liver cancer is an increasingly common and deadly health problem globally. There are nearly 900,000 new cases of liver cancer diagnosed each year and more than 750,000 liver cancer-related deaths, the 3rd highest mortality of any cancer type.¹ Hepatocellular carcinoma (HCC) accounts for the majority (~90%) of primary liver cancers.² Unlike other cancers, which occur sporadically, nearly all HCC occurs as the consequence of chronic liver disease (CLD), most commonly viral infection with hepatitis B (HBV) or hepatitis C (HCV), excess alcohol use, or metabolic dysfunction-associated steatohepatitis (MASH).³ Rates of HCC attributed to

HCV have fallen since the development of curative antiviral therapies; however, a concomitant rise in global obesity is fueling new cases of MASH. As a result, the incidence of HCC continues to climb.⁴

The last two decades have seen tremendous progress in developing systemic therapies that target the molecular drivers of HCC. Sorafenib was approved in 2007 as the first such therapy after investigators sought a multi-kinase inhibitor to disrupt inappropriate MAP kinase and pro-angiogenic signaling observed in many HCCs.⁵ Three additional small molecule kinase inhibitors have since been developed to target different pathways that are aberrantly activated in HCC.² A further breakthrough came in 2017

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Editor: Ju Hyun Shim, University of Ulsan, Korea

Received: Nov. 26, 2025 / Revised: Dec. 18, 2025 / Accepted: Dec. 26, 2025

with the approval of the immune checkpoint inhibitor (ICI) Nivolumab targeting programmed cell death protein 1 (PD-1).⁶ Dual immunotherapy using combinations of PD-1 / programmed death-ligand 1 (PD-L1) antibodies with a second ICI or anti-vascular endothelial growth factor (VEGF) has become first-line therapy on the basis of superiority to multi-kinase inhibitors in phase III trials.⁷⁻¹¹ The first approved combination in this class was atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) which improved overall survival to 19.2 months compared to 13.4 months with Sorafenib.¹¹ Subsequent studies have moved Tremelimumab (anti-cytotoxic T lymphocyte-associated antigen 4 [CTLA-4]) plus Durvalumab (anti-PD-L1) and Nivolumab (anti-PD-1) plus Ipilimumab (anti-CTLA-4) into the front line.^{7,10} Despite the growing armamentarium of systemic treatments for HCC, only 16–36% of patients respond. This is likely a consequence of HCC heterogeneity and the absence of predictive biomarkers that can identify which patients will benefit from a given treatment.¹²

In this review we provide a summary of current knowledge regarding the genetic and immune features of HCC and describe progress toward clinical translation of this knowledge for patient stratification and treatment.

HCC DEVELOPMENT AND GENETIC LANDSCAPE

HCC develops when homeostatic programs regulating hepatocyte proliferation break down, permitting uncontrolled growth.¹³ This is the consequence of years of CLD: most HCCs occur in cirrhotic livers, which is considered a premalignant state.³ HCC pathogenesis is generally modeled as a stepwise progression driven by sustained inflammation and mutagenesis. Healthy liver becomes cirrhotic, followed by the emergence of low-grade dysplastic nodules that progress to high-grade nodules, and finally malignant transformation into HCC.¹³⁻¹⁵

Malignant transformation is driven by genetic and epigenetic mechanisms. One of the hallmarks of this progression is the acquisition of oncogenic mutations. The number of somatic mutations overall is proportional to the stage of liver disease and progression of fibrosis. Advanced disease is also associated with greater chromosomal instability, with increasing frequency of insertions/deletions and copy number variation.¹⁶⁻¹⁸ Meanwhile, HCC undergo epigenetic remodeling with global DNA methylation changes, chromatin remodeling to facilitate oncogenic promoter-enhancer relationships, and translational regulation via microRNAs.¹⁹

A majority of mutations observed in HCC may not actually drive its pathogenesis, but rather give hepatocytes a replicative advantage in the regenerating liver during pre-cancerous chronic injury.²⁰ This is supported by the observation that the mutational frequencies for most individual genes are low.^{21,22} These first mutations have been termed “gatekeepers.” When cells harboring a gatekeeper mutation acquire additional driver mutations, it fuels rapid clonal expansion to form an HCC.²³ Studies have sought to identify the key mutations responsible for tumor initiation by comparing dysplastic nodules to early cancers.¹⁴ One such study screened livers for TERT promoter mutations. TERT mutation was present in only a small minority of dysplastic nodules (6% of low grade, 19% of high grade) but it was detected in the majority of early HCCs (61%).²⁴

Next-generation sequencing studies have extensively characterized the HCC genetic landscape, identifying frequently mutated genes, chromosomal anomalies, and affected molecular pathways.²⁵⁻²⁷ In the TCGA HCC Cancer Atlas, the most commonly mutated sites included the TERT promoter (44%), tumor suppressors TP53 (31%), and RB1 (4%), WNT genes CTNNB1 (27%) and AXIN1 (8%), and chromatin remodelers ARID1A (7%), ARID2 (5%), and BAP1 (5%), consistent with prior whole exome sequencing studies.^{25,27,28} These findings have been replicated in multiple studies including a recent Chinese report with deep se-

Abbreviations:

AA, aristolochic acid; AFB1, aflatoxin B1; AI, artificial intelligence; BCR, B cell receptor; BCLC, Barcelona Clinic Liver Cancer; CAFs, cancer-associated fibroblasts; CITE-seq, cellular indexing of transcriptomes and epitopes by sequencing; CLD, chronic liver disease; CRP, C-reactive protein; CTCs, circulating tumor cells; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; CTLs, cytotoxic T lymphocytes; ECM, extracellular matrix; EV, extracellular vesicle; HBV, hepatitis B; HCC, hepatocellular carcinoma; HCV, hepatitis C; HSCs, hepatic stellate cells; iCAF, inflammatory CAFs; ICI, immune checkpoint inhibitor; KCs, Kupffer cells; LRI, ligand-receptor interaction; LSECs, liver sinusoidal endothelial cells; MASH, metabolic dysfunction-associated steatohepatitis; MoMFs, monocyte-derived macrophages; myCAF, myofibroblast-type CAFs; NK, natural killer; NKT, natural killer T; NLR, neutrophil-to-lymphocyte ratio; ORR, objective response rate; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; scRNA-seq, single cell RNA sequencing; TCR, T cell receptor; TKIs, tyrosine kinase inhibitors; TMB, tumor mutational burden; TLS, tertiary lymphoid structures; TME, tumor microenvironment; VEGF, vascular endothelial growth factor

quencing on 494 HCCs that found a high frequency of mutations in TP53 (51%), CTNNB1 (21%), AXIN1 (12%), ARID1A (10%), and ARID2 (6%).²² Somatic copy-number alternations are also common, most frequently showing gains in chromosomes 1q and 8q and losses in 4q, 8p, 16p, 16q, and 17p.²⁹⁻³¹

Individual carcinogens and etiologies of CLD have been linked to characteristic mutational signatures²⁵; this is important to note as the epidemiology of liver disease is changing rapidly, and most seminal studies were performed on a population overwhelmingly composed of viral hepatitis.³² Some of the clearest mutational signatures come from hepatotoxins including aristolochic acid (AA) and aflatoxin B1 (AFB1). AA exposure, often through Chinese herbal medicines, is characterized by A:T > T:A transversions. Up to 78% of HCCs in Taiwan and 26% in China contain this exposure signature.^{16,31,33} AFB1, a mycotoxin encountered through aspergillus fungal contamination of food products, increases the risk of HCC, particularly in HBV-infected individuals. AFB1 exposure is linked to a characteristic mutation of TP53 at codon 249 and G:C>T:A translocations.^{34,35} HBV-associated HCC also has a unique signature, with changes due to Hep B viral integration. Deep whole genome sequencing of nearly 500 HCCs from China with 94% of tumors HBV-positive showed increased frequency of extrachromosomal circular DNA with frequent HBV integration.²² Direct HBV integration into the hepatocyte genome can cause substantial genetic instability with non-homologous chromosomal translocations and megabase-sized deletions.³⁶ It also contributes to direct insertional mutagenesis by integrating into exons or regulatory regions of oncogenes such as TERT and CCNE1 (cyclin 1) and long interspersed nuclear elements, which regulate miRNA expression. These insertional events are enriched in HCC where they confer a growth advantage over adjacent non-tumor tissue.³⁷ MASH, on track to become the dominant cause of cirrhosis and HCC globally,^{38,39} has a distinct mutational profile secondary to chronic lipotoxicity and chronic inflammation.⁴⁰ A recent study assessed the mutational profile of MASH-HCC compared to adjacent non-tumor tissue in 52 cases, finding an increased frequency of mutations in the tumor suppressor ACVR2A (10% MASH vs. 3% non-MASH) and a higher prevalence of C>T and C>A single nucleotide variants.⁴¹

The significance of these etiology-specific mutational

patterns to prognosis and treatment strategy is still being investigated. Emerging evidence will be discussed below.

Immune interactions in HCC

Single cell molecular technologies

Over the past decade, single cell RNA sequencing (scRNA-seq) and spatial transcriptomics have become the dominant tools for generating transcriptional profiles by providing a greater depth of information than was previously possible (Fig. 1). ScRNA-seq has the advantage of much greater resolution as compared to bulk sequencing methods that profile the average expression of all cells sampled. While informative of the sample, bulk sequencing is unable to detect heterogenous/clonal populations, quantify cell type frequencies, and reveal rare cell types. ScRNA-seq provides access to this data without the need for cell sorting or subtype enrichment.^{42,43} The related technology spatial transcriptomics provides RNA sequencing at up to single cell resolution while preserving spatial relationships – important context that has yielded many new insights into cellular niches and disease heterogeneity.^{44,45}

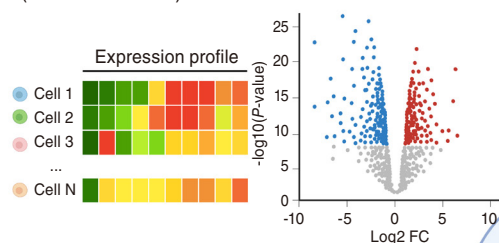
Computational tools can be applied to scRNA-seq to infer rich details of cell-cell interactions based on their expression of ligand and receptor.⁴⁶⁻⁴⁸ In the context of HCC, these technologies provide a high-resolution profile of the tumor microenvironment (TME) and the network of cellular interactions it contains. It is increasingly clear that this information is key to understanding mechanisms of HCC initiation, evolution, and resistance to treatment. Other single cell sequencing methods including T cell receptor (TCR) sequencing,^{49,50} cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq),⁵¹ and B cell receptor (BCR) sequencing^{52,53} combine surface protein profiling with transcriptomics to further delineate immune sub-populations and interactions within the TME. Insights from single cell transcriptomics have underscored the essential role of the TME, in addition to genetic and epigenetic factors, in controlling HCC development and progression.⁵⁴

Hepatic immune microenvironment

The liver is the first major organ to receive environmental antigens, microbes, and toxins that arrive from the gut via the portal circulation. To manage this foreign milieu, it contains a complex immune repertoire including liver-resident

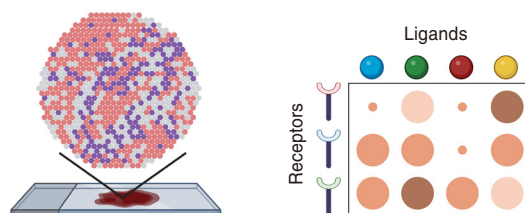
Single cell transcriptomics

Profile the expression patterns of individual cells to identify cell types, cellular heterogeneity, predicted cell to cell interactions, and drug resistance. Our group has previously used this technology to profile intratumoral HCC heterogeneity (PMID: 31941899).



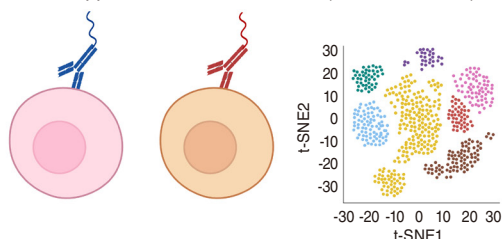
Spatial transcriptomics

Map transcriptomic profiles onto tissue to provide insights into the cross-talk and spatial arrangement of cells. Recent work in HCC has applied spatial transcriptomics to characterize fibroblast subtypes and their distinct spatial distribution (PMID: 39966876).



CITE-seq

Multi-modal analysis used to gain a more comprehensive profile of cell states by combining transcriptomics with surface markers. Also useful for identifying rare cells. One such example in HCC research is the identification of CD49f marking tumor initiating cells that create an immunosuppressive microenvironment (PMID: 39515328).



TCR/BCR sequencing

Multi-modal analysis with insight into the immune response and clonal diversity that can be used to monitor and predict functional immune response. Combining transcriptomic data with the TCR sequences, the functional clonality and origin of tumor infiltrating lymphocytes in HCC can be traced (PMID: 28622514).

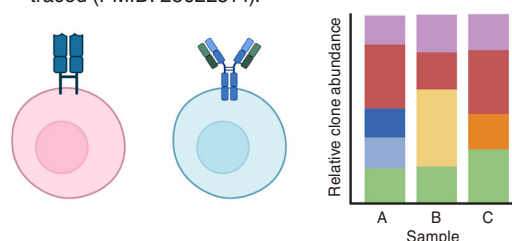


Figure 1. Overview of single cell technologies used in hepatocellular carcinoma (HCC). Single cell RNA sequencing can be used to profile the gene expression of individual cells for the full transcriptome. Analyses that can be performed include differential gene expression, cell clustering and dimensionality reduction, cell type identification, cell type composition analyses, and predictions of ligand receptor interactions. Spatial transcriptomics integrate histology with multi-cellular or single cell transcriptomics to enhance cellular interaction analysis of cells that colocalize in the same niche. CITE-seq enhances cell type identification, including rare cell types through the use of a DNA barcoded antibody that will bind to epitopes on the cell surface and be sequenced alongside the transcriptome to link the epitope profile to the transcriptional profile. TCR and BCR sequencing profiles the diversity of immune cells by analyzing the permutations of the V(D)J gene of the associated receptors. BCR, B cell receptor; CITE-seq, cellular indexing of transcriptomes and epitopes by sequencing; TCR, T cell receptor.

macrophages (Kupffer cells [KCs]), lymphoid cells, and stromal cells with immunoregulatory function including liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HSCs).⁵⁵ These cells endure constant exposure to environmental antigens and thus have evolved mechanisms to balance immune tolerance with active surveillance against harmful pathogens.⁵⁶

To grow within the liver, a nascent HCC needs to escape negative pressure from the immune system. This process, which has been described as “immunoediting,” can be diagrammed in 3 steps. During step 1, the immune system is dominant, eliminating new tumor cells that express novel antigens that are recognized as foreign. In step 2, equilibri-

um is reached as cancer growth equals the pace of immune-mediated killing. Finally, in step 3, the tumor evolves strategies to suppress immune responses, unleashing unrestricted growth.⁵⁷ Although the precise mechanisms by which this process occurs remain under investigation, many details have become clear. The relationship between tumor cells and individual immune populations is dynamic and heterogeneous with both pro- and anti-tumoral contributions (Fig. 2); however, studies have consistently observed better survival with increased immune infiltration.⁵⁸ Studies of tumor evolution show that eventually HCC develops mechanisms to evade anti-tumor immunity and continue tumor growth. Intra-tumoral heterogeneity is an important contrib-

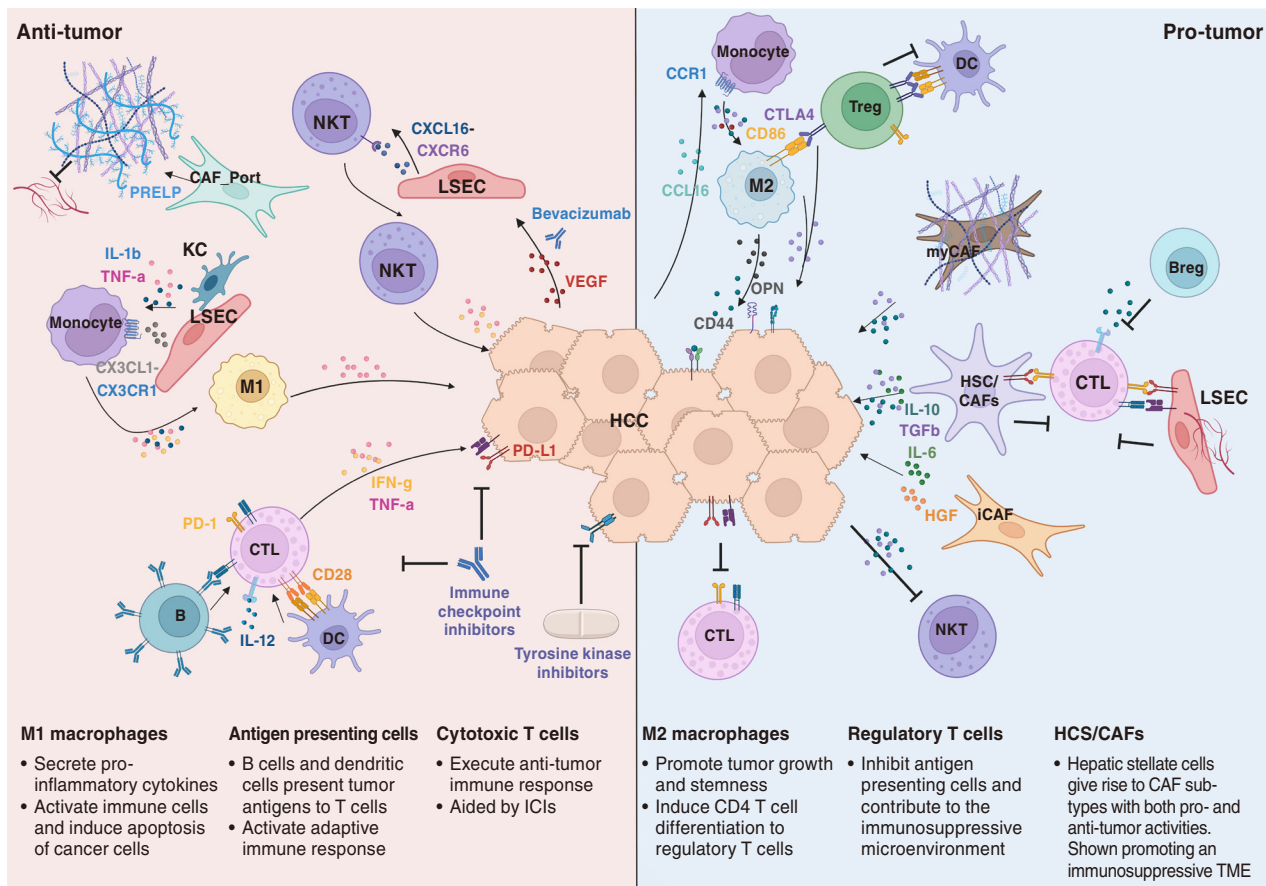


Figure 2. Anti-tumor and pro-tumor hepatocellular carcinoma (HCC) tumor microenvironment. The anti-tumor microenvironment is characterized by the presence and recruitment of cytotoxic T cells, functional antigen presenting cells, and M1 polarized macrophages amongst others that contribute to an immune response against the tumor. The cells in the microenvironment secrete inflammatory cytokines such as IFN-g and TNF-a to trigger inflammation and the immune response. This microenvironment is responsive to therapeutic interventions such as targeted PD-1/PD-L1/CTLA4/VEGF/tyrosine kinase inhibitors. In contrast, the pro-tumor microenvironment is composed of regulatory T and B cells, M2 polarized macrophages, and hepatic stellate cells that inhibit immune cells and the immune response. These immunosuppressive cells utilize cytokines including IL-10, TGF-b, and PD-L1. PRELP, Proline- and arginine-rich end leucine-rich repeat protein; IL-1b, interleukin 1 b; IL-6, interleukin 6; IL-10, interleukin 10; CX3CL1, C-X3-C motif chemokine ligand 1; CX3CR1, C-X3-C motif chemokine receptor 1; HGF, hepatocyte growth factor; iCAF, inflammatory cancer-associated fibroblast; TGFb transforming growth factor beta; Breg, regulatory B cell; M2, M2 macrophage, CCR1, C-C motif chemokine receptor 1; CCL16, C-C motif chemokine ligand 16.

utor to this process. Multiregional tumor sampling and spatial transcriptomics have shown that foci of poorly differentiated cells with reduced expression of neoantigens may be present even while other parts of the tumor remain immunologically active. These cells are the origin of treatment resistance and cancer recurrence.⁵⁹⁻⁶¹ The contributions of individual immune cells to the tumor-immune microenvironment are discussed below and summarized in Figure 2.

Macrophages

Macrophages cells in the liver originate from liver-resident KCs or from circulating monocyte-derived macro-

phages (MoMFs).⁶² MoMFs are recruited to the liver by KCs in response to innate immune activation via damage-associated molecular patterns, and pathogen-associated molecular patterns. Classically, macrophages are polarized into pro-inflammatory M1 by IL-1a, IL-1b, IFN-g, TNF-a or immunosuppressive M2 macrophages by IL-10, PD-L1, VEGF, and TGF-b.^{63,64} HCC cells can also influence the M2 polarization of macrophages via CCL16, chemotactic chemokine that promotes macrophage stemness, interacting with CCR1 of myeloid cells to recruit and polarize M2 macrophages.^{65,66}

Single-cell spatial profiling and ligand-receptor interac-

tion (LRI) analysis found that macrophages have the most frequent interactions with HCC of any cell type in the TME, serving to regulate the behavior of other immune cells.⁶⁷ For example, spatial transcriptomics revealed colocalization of VIM⁺ macrophages with Tregs resulting in immunosuppressive signaling through IL-1 β and IFN- γ during HCC progression.⁶⁸ SPP1⁺ M2 macrophages colocalize with HCC cancer stem cells communicating via an SPP1-CD44 axis, as well as integrins ITGAV, ITGA5, ITGB1, ITGB5 and excluding cytotoxic T lymphocytes (CTLs) from the local niche.^{69,70} Similar interactions were not detected between macrophages and non-tumor tissues.⁷⁰ Interestingly, patients with higher expression of markers associated with M2 polarized macrophages including SPP1, ANGPT2, and NCL had higher rates of resistance to ICIs, pointing to their immunosuppressive function.⁷¹

HSCs

HSCs are a mesenchymal cell population best known as the principal source of hepatic fibroblasts, including cancer-associated fibroblasts (CAFs).^{72,73} HSCs also have immunoregulatory functions and act as a signaling hub between other immune cell populations.^{74,75} ScRNA-seq has isolated subpopulations of HSCs with distinct functions including an inflammatory/immunoregulatory subset that is induced by chronic liver injury.⁷⁵⁻⁷⁷ In the context of chronic liver injury, HSCs promote an immunosuppressive TME through several mechanisms.^{78,79} Activated HSCs express PD-L1, inducing apoptosis of activated T and B cells and recruiting immunosuppressive regulatory T and myeloid-derived suppressor cells.⁸⁰⁻⁸⁴ Further, activated HSCs contribute to the immunosuppressive TME in HCC through their CX3CL1 expression interacting with infiltrating CX3CR1⁺ Ly6C⁺ macrophages which express genes related to retinol metabolism, as well as arginase-1 and IL27 which inhibit CD8⁺ T cells.^{85,86}

Within the TME, HSCs give rise to a heterogeneous population of CAFs with different, sometimes opposing, effects on HCC. The major CAF phenotypes that have been described in HCC are myofibroblast-type CAFs (myCAFs), defined by high expression of collagen I and other extracellular matrix (ECM) genes, inflammatory CAFs (iCAF), which release cytokines and growth factors, and vascular CAFs (vCAFs).^{73,78,79,87-90} Tumor-promoting roles have been identified for both myCAF and iCAF subtypes. These include

paracrine signaling to stimulate tumor cell proliferation, stemness, and metastasis,^{91,92} immunomodulatory functions such as recruitment of pro-tumor M2 macrophages and polarized neutrophils,^{88,93} and ECM remodeling around the tumor, creating a physical barrier to limit immune surveillance and increasing TME stiffness to promote HCC proliferation via mechanotransduction.^{78,89} A recent analysis of tumor biopsies from patients receiving anti-PD-1 therapy found SPP1⁺ macrophages recruit CAFs to the tumor margin and induce ECM deposition to form a tumor-immune barrier. SPP1⁺ knockout in a murine model improved response to immunotherapy.⁹⁴ A similar role has previously been described for CAFs in triple-negative breast cancer, suggesting a conserved program.⁹⁵ On the other hand, CAFs secrete the tumor suppressor prolargin (PRELP) into the TME, prompting more aggressive tumors to upregulate proteases to degrade it.⁹⁶ The competing roles for CAFs were described in a study that used scRNA-seq of human biopsies and murine HCC models to examine myCAF and iCAF activities in HCC. A protective role was identified for iCAFs in premalignant liver where they secrete hepatocyte growth factor to prevent hepatocyte death. In contrast, myCAFs produce type I collagen which activates YAP/TAZ and DDR1 pathways to promote tumor growth. Interestingly, an imbalance between myCAF and iCAF populations was observed in advanced liver disease and associated with increased risk for HCC development.⁸⁹

LSECs

LSECs are hepatic non-parenchymal cells with roles in immunity, primarily mediated through expression of innate immune receptors and signaling to macrophages and lymphocytes.⁹⁷⁻⁹⁹ LSECs contribute to an immunosuppressive TME, inducing CD8⁺ T cell exhaustion through PD-L1, ICAM1, and MHC1.¹⁰⁰ One preclinical study found that reversing pathologic LSEC capillarization with nano-targeted simvastatin delivery resulted in recruitment of natural killer T (NKT) cells and strengthened responses to anti-PD-L1 therapy.¹⁰¹

T cells

T cells are key effector cells of adaptive immunity whose function determines whether HCC is suppressed or permitted to grow. In advanced HCC and other malignancies, the presence of tumor-infiltrating T lymphocytes is correlated

with responsiveness to ICI therapy,¹⁰² although the presence of T cells alone is insufficient as a predictive biomarker because it lacks information about T cell functional state and niche signals that control their activity. Recent studies using single cell transcriptomics are providing a more nuanced view of T cell dynamics in HCC.⁵⁰ ScRNA-seq and TCR sequencing were used to comprehensively map T cell distribution in tumor and peripheral blood from six treatment naïve patients with HCC. Eleven subsets of T cells could be identified, including Tregs and dysfunctional “exhausted” CD8⁺ T cells. The main T cell subsets in tumor tissue were FOXP3⁺ and CTLA4⁺ CD4⁺ T cells and GNLY⁺, and CXCL13⁺ CD8⁺ T cell clusters whereas regulatory CD4⁺ T cells (Treg) and naïve CCR7⁺ T were found in peripheral blood. Notably, this study did not characterize how T cell distribution changed following ICI treatment.⁵⁰ TCR sequencing indicated that the majority of Tregs in the tumor tissue were unique and therefore had been recruited from the periphery, rather than evolving from CD4⁺ T cells in the tumor, while CTLs shared clones with other CD8⁺ clusters within the tumor such as CX3CR1⁺ effector and GZMK⁺ indicating a local origin.⁵⁰

High levels of Tregs and exhausted CD8⁺ T cells are features that portend poor prognosis and non-responsiveness to therapy; therefore studies have focused on the mechanisms that lead to their enrichment in the TME. Tregs are recruited to the TME by macrophage secreted ligands such as CCL17 and CCL22, and are activated by cytokines from macrophages and tumor cells including TGF- β and IL-10.⁵⁶ These tumor-infiltrating Tregs were also found to be enriched with markers such as CD177, not present in peripheral Tregs and CCR8.^{50,103} In experimental models, conditional knockouts of CD177 in tumor infiltrating Tregs reduced tumor progression and increased overall survival, and targeting CD177⁺ Tregs with monoclonal antibodies also achieved an inhibition of tumor growth and Treg infiltration.¹⁰³ Tregs interact with dendritic cells in a CTLA4-CD80/CD86 manner, decreasing antigen presentation and therefore disrupting CTL activation. This is associated with poor prognosis.⁵⁶ In the absence of Tregs and immunosuppressive cytokines, CTLs and the broader anti-tumor immune response can be activated by type 1 interferons and IL-12 from dendritic cells.⁵⁶ The PD-1 status of the CD8⁺ T cells negatively correlates with prognosis as those with higher levels of PD-1 are spatially localized with macro-

phages and away from GZMB⁺ CTLs and expressed exhaustion markers such as TIM3 and LAG3.^{67,104} LAYN is also overexpressed in exhausted CD8⁺ T cells that are LAG3 negative, as well as Tregs, suggesting it is a suppressive marker (supported by an inhibition of IFN- γ secretion) that is mutually exclusive of LAG3.⁵⁰

T cells crosstalk with macrophages has emerged as an important driver of immunosuppressive signaling. Analysis of LRIs in both healthy and diseased livers revealed that M2 macrophages begin to act on naïve CD4⁺ T cells to induce tolerigenic states well before the onset of HCC. Interestingly, the dominant signaling pathways changed with progression from early liver disease (MIF signaling) to cirrhosis (CCR1 and CCR5), and finally HCC (IL-6-CD4) indicating that there may be different therapeutic targets depending on stage of disease.¹⁰⁵ Further validation of this interaction between macrophages and naïve CD4⁺ T cells with spatial transcriptomics and histopathology implicated SPP1⁺ M2 macrophages in inducing differentiation of CD4⁺ T cells to Treg through the CD86-CTLA4 axis.^{67,106} Experimental mouse models have shown that the converse is true: disruption of an SPP1⁺ macrophage- CSF1-CSF1R axis restored normal CD4⁺ cytokine signaling and improved responsiveness to anti-PD-L1 treatment.¹⁰⁷ Furthermore, PD-L1⁺ macrophages spatially colocalize with CD8⁺ T cells and act to suppress them.^{104,108}

B cells

B cells can serve a supportive role in the HCC TME, largely through interactions with T lymphocytes. This includes antigen presentation to T cells, cytokine production to stimulate further antitumor adaptive immunity, promoting CTL survival, and helping to form tertiary lymphoid structures (TLS).^{109,110} TLS aid in the adaptive immune response, and their presence prior to ICI treatment has been identified as possible positive prognostic biomarker.^{111,112} TLS presence can vary within regions of the same tumor and may serve as a proxy for immune infiltration and interactions with cancer cells.⁶¹

Conversely, B cells contribute to chronic inflammation that drives progression of liver disease toward HCC. During malignant transformation, single cell analysis and lineage tracing show that B cells mature into immunosuppressive regulatory B cells (Bregs) and IgA⁺ plasma cells that suppress the anti-tumor activity of CD8⁺ T cells via IL-10 and

PD-L1.^{113,114} Deletion of Bregs via anti-CD20 antibodies reduces immunosuppressive signaling.^{115,116} Similarly, pharmacologic disruption of IgA⁺ B cell maturation reduced HCC incidence and accelerated tumor regression in an experimental model.¹¹⁴

NK/NKT cells

Natural killer (NK) cells are critical lymphocytes that contribute to both the innate and adaptive immune responses in HCC through their cytotoxic properties as well as inflammatory cytokine (IFN- γ and TNF- α) production. This response is initiated by NKG2D receptors recognizing MICA/MICB receptors on cancer cells, which the tumor cells also secrete to block and inhibit NK cell cytotoxicity and cytokine release.¹¹⁷ NK activity is also inhibited by secretion of TGF- β and IL-10 from myeloid-derived suppressor cells (MDSCs).¹¹⁸ Molecular markers of NK suppression and exhaustion as the TME becomes immunosuppressive include TIM-3, TIGIT, LAG-3, CTLA-4, and PD-1, in particular in cases of viral etiology.¹¹⁹

NKT cells are lymphocytes that express markers of the innate immune NK cells as well as TCR α and β chains.¹²⁰ Activated NKT cells release inflammatory cytokines (IFN- γ , IL-4, TNF- α , IL-5 and IL-13) which may contribute to anti-tumor immunity.^{121,122} As with other immune populations, exhausted NKT cells are observed in HCC,¹²³ and their overall contribution is likely to be influenced by context and the TME. One study using the mouse DEN-HCC model and NKT depleted mice found that the presence of NKT cells was associated with increased rates of HCC development, however, administration of naïve NKTs to mice with established tumors aided in HCC clearance.¹²⁴ There are ongoing efforts to capitalize on the anti-tumor capabilities of NKTs through chimeric antigen receptor NKT therapy for HCC.¹²⁵

Immune-based molecular classes of HCC

Efforts to integrate genomic and immunologic data for a comprehensive description of HCC behavior have led to several molecular classification systems (Fig. 3).⁵⁶ One of the earliest used microarray data from 91 patients to identify two classes of HCC, one “proliferative” class with poor prognosis and non-proliferative class.¹²⁶ Additional molecular classifications from Boyault, Chiang, and Hoshida each

delineated separate patient subsets based on transcriptional profiling, with common features emerging: subclasses defined by cell cycle activation, interferon signaling/immune infiltration, WNT/ β -catenin activation, and mutually exclusive TP53 and CTNNB1 mutations.¹²⁷⁻¹²⁹

More recent studies have built on the earlier signatures, adding layers of detail and including components of the TME in the classification. A large European and North American study of 956 patients identified an “immune class” of HCCs - 25% of biopsies with high inflammatory infiltrates, interferon signaling, and PD-L1 expression. The “immune class” could be further divided into tumors with “active” versus “exhausted” immune responses. Notably, as observed in the French cohort, CTNNB1 mutants were excluded from the inflamed group. The exhausted immune class was characterized by enrichment for immunosuppressive M2 macrophages, fibroblasts and TGF- β signaling. Importantly, the “active immune” profile was associated with improved overall survival in the TCGA dataset.¹³⁰ A recent follow up study expanded this categorization to define an “inflamed class” representing 37% of tumors overall and encompassing the immune class (22%). Other tumors were categorized based on immune activity as “intermediate,” with frequent TP53 mutations and “excluded,” with CTNNB1 mutants.¹³¹ Other studies have largely validated these signatures.^{130,132} A survey of liver cancers in Thailand raised the possibility that molecular classes should be refined by incorporating race/ethnicity. They defined three transcriptional signatures (C1-C3) that could be validated in external cohorts of Chinese and Asian American patients but were not observed in samples from European Americans. Furthermore, they described an immune-active subtype (“C2”) but found that it differed transcriptionally from other published immune classes from European cohorts.^{21,130,133}

Several recent studies have published classifications that integrate a multi-omics approach. One study incorporated proteomics into an analysis of a Chinese HBV-HCC cohort. Transcriptional and proteomics analyses were concordant at the pathway level, but differences were observed between individual gene-protein pairings, indicative of post-transcriptional regulation. Interestingly, proteomic analysis was more predictive of survival outcomes than transcriptomics.³¹ Another small study sorted tumors by metabolic profile into those with preserved “rich metabolisms” akin to differentiated hepatocytes and HCCs reliant on glycolysis;

Molecular classification

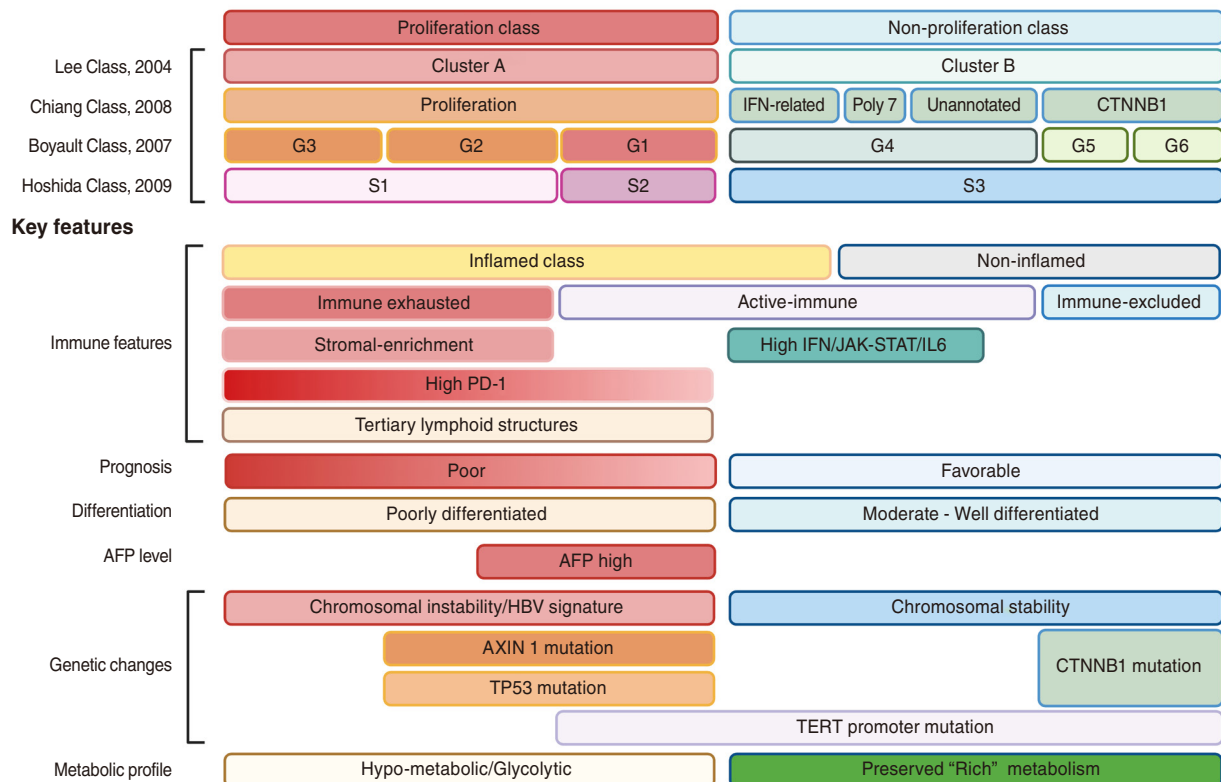


Figure 3. Graphical summary of the molecular classes of hepatocellular carcinoma. Associated immunologic and genetic features are outlined based on published classifications.

however, it was unclear if these profiles were driving tumor behavior or were a surrogate for differentiation status.¹³⁴ Collectively, these studies raise the possibility of using molecular phenotyping for prognosis and selection of candidates for immunotherapy, although further validation in prospective human trials is needed.

CLINICAL TRANSLATION OF IMMUNOGENOMICS IN HCC

Prognosis and patient stratification

For patients diagnosed with HCC, the AASLD and EASL recommend the Barcelona Clinic Liver Cancer (BCLC) strategy for prognosis rather than the traditional TNM staging system used in other cancers.^{135,136} The BCLC prognostic score is based on tumor burden, functional status (rated by ECOG tool), and underlying liver function.¹³⁷ Other multivariate models have been generated to describe HCC-

specific mortality but may have limited external validity for prognostication owing to regional variation, changes to the incidence of CLD, and advances in treatment options in recent decades.¹³⁸

Some data suggest that certain etiologies of liver disease could be associated with ICI-resistant HCCs, likely owing to disease-specific immunogenomic features. In MASH, chronic inflammation triggered by insulin-resistance, lipotoxicity, and endoplasmic reticulum stress is associated with maladaptive recruitment of CD8+ T cells which may actually promote disease progression by furthering inflammatory injury to hepatocytes.^{40,139,140} In both mouse and human MASH-HCC, studies have observed paradoxical increases in CD8+, PD1+ T cells, but loss of effector function, associated with impaired responses to ICI therapy.¹⁴¹ A meta-analysis of randomized clinical trials found significantly worse OS in ICI-treated HCCs in patients with non-viral etiologies of liver disease compared to viral hepatitis-associated HCC. Notably, there was no difference in overall survival (OS) between these groups when treated with tyrosine ki-

nase inhibitors (TKIs), suggesting immune responses to ICIs may be selectively impaired in MASH/non-viral HCCs or, conversely, that viral HCCs display enhanced ICI sensitivity.¹⁴² Attempts to reproduce this finding have been mixed^{143,144} and other studies have shown that molecular classes of HCC can distinguish responders and non-responders independent of underlying liver disease.¹⁴⁵ Given this uncertainty, the selection of systemic therapies in current treatment guidelines is not determined by the etiology of the liver disease.

Many studies have published single biomarkers or composite panels that correlate with overall survival. Perhaps the best established is the inverse correlation between AFP levels and survival, with elevated levels at cancer diagnosis associated with poor prognosis.^{146,147} Probing the epigenome, one report identified 36 DNA methylation markers that were associated with progenitor features and accurately predicted poor survival in a validation cohort.¹⁴⁸ Among molecular classes of HCC, the subtypes with high proliferation, WNT/TGF- β signaling, TP53 mutations, and an immune-exhausted profile are associated with the most aggressive tumors, while non-proliferation class, interferon signaling, and immune-active phenotype portend improved survival.^{2,22,31,126-130,133} Notably, except for serum AFP, these signatures require tumor biopsies which are not routinely performed for tumors with diagnostic imaging.^{135,136} Analysis of tumor components in peripheral blood samples, termed liquid biopsy, could be more readily integrated into current clinical workflows, but to date has not been validated for prognostication.¹⁴⁹

Precision medicine: prediction of response to therapy

Despite advances in the diagnosis and early treatment of HCC, more than 50% of patients will require systemic therapy.¹⁵⁰ First line options are dual immunotherapy combinations of Atezolizumab + Bevacizumab (anti-PD-L1, anti-VEGF), Durvalumab + Tremelimumab (anti-PD-L1, anti-CTLA-4) or Nivolumab + Ipilimumab (anti-PD-1, anti-CTLA-4), with alternative treatments including Sorafenib, Lenvatinib, or Durvalumab monotherapy.^{9-11,137} Unfortunately, the majority of patients do not respond to initial therapy. Only ~30% achieve an objective response to Atezolizumab-Bevacizumab, and rates are lower for monothera-

pies.^{9,151} Several studies have sought to identify biomarkers of response to ICIs (Table 1). In theory, tailored selection of the therapy based on such biomarkers could improve tumor response rates and reduce unnecessary side effects from treatment with drugs/combinations that are ineffective.

Blood-based biomarkers

The development of non-invasive biomarkers for cancer prognosis and monitoring is increasingly recognized as an important priority for all cancers.¹⁵² There has already been sustained interest in this area for HCC because liver biopsy is not routinely performed for diagnosis.¹²

AFP

The best studied biomarker for this purpose is AFP. AFP is fetal hepatocyte protein that is re-expressed in adults in response to hepatocyte injury, regeneration, and in 60–80% of HCCs.¹⁵³ Baseline serum AFP levels are readily available for HCC patients as AFP monitoring is incorporated into the AASLD HCC screening guidelines and newer diagnostic algorithms.^{135,154-156} High pre-treatment AFP levels (generally defined as >400 ng/mL) have been prognostic of shorter OS and used to stratify patients in clinical trials.¹⁵⁷⁻¹⁶⁵ More importantly, several studies have shown that a decrease in AFP following therapy may be a biomarker for treatment response.¹⁶⁶⁻¹⁶⁸ The strongest evidence for AFP comes from trials of the anti-VEGFR2 antibody Ramucirumab. An initial trial of Ramucirumab as second line therapy failed to provide a survival benefit; however, a follow-up trial (REACH-2) that enrolled only patients with AFP $\geq$ 400 ng/mL met its primary endpoint of improved OS.^{161,169} Additional prospective studies with universal definitions of high AFP levels are ultimately needed to validate its role as a prognostic biomarker.

Immune and inflammatory markers

Additional immunologic and inflammatory predictive biomarkers have been proposed but evidence is still limited. An elevated neutrophil-to-lymphocyte ratio (NLR), which has been studied as a surrogate marker for the dominance of immunosuppressive inflammation (neutrophils) over anti-tumoral adaptive immunity (lymphocytes), has been associated with poor response to ICIs across cancer types.^{170,171}

Table 1. Biomarkers for response to immune checkpoint inhibitors

Biomarker	Study type	ICI	Key results	Predictive vs. Response	Citation
Biopsy	RCT	Atezo + Beva	Pre-treatment immune activation including high IFN- γ , T effector signature, and increased intratumoral CD8 ⁺ T cells predicted favorable response to atezolizumab + bevacizumab.	Predictive	Zhu et al. (2022) ¹⁶⁶
Biopsy	RCT	Nivo	A 4 gene signature (CD274, LAG3, CD8A, STAT1) predicted ORR and OS. Tumor PD-1 and PD-L1 expression were also associated with improved OS.	Predictive	Sangro et al. (2020) ¹⁹⁶
Biopsy	Exploratory	Anti-PD1	Identified an 11 gene signature of response to anti-PD1 therapy (nivo, pembo, or tislelizumab). ICI-responders had high IFN and MHC-related gene expression. Pre-treatment with TKIs eliminated the response signature.	Predictive	Haber et al. (2023) ¹⁴⁵
Biopsy	Exploratory	Anti-PD1	The inflamed molecular class (high IFN, immune infiltration, and cytolytic activity) is associated with favorable response to ICIs.	Predictive	Montironi et al. (2023) ¹³¹
Biopsy	Exploratory	Variable	Two molecular classes of HCC with distinct immune profiles, one with CD8 ⁺ T and plasma cell enrichment (C2) and the other with B cell recruitment induced by ICIs (C1). Both had good response to ICI.	Predictive	Budhu et al. (2023) ¹⁹⁸
Biopsy	Exploratory	Variable	3 molecular classes with high (>60%) response rates to ICIs. Class 1 had an inflamed phenotype. The other two classes displayed an inhibitory TME.	Predictive	Chen et al. (2024) ²⁰¹
Biopsy	Exploratory	Atezo + Beva	Identified a signature that predicts response to Atezo + Beva. Features CD8 ⁺ Temra, CD8 ⁺ Tex T cells and CXCL10 ⁺ macrophages. ICI resistance is associated with immunosuppressive myeloid cells.	Predictive	Cappuyuns et al. (2023) ¹⁵¹ , Cappuyuns et al. (2025) ²⁰⁴
Biopsy	Exploratory	Nivo	Tumors responding to nivolumab + cabozantinib were enriched for adaptive immune cells including maturing B cells.	Response	Zhang et al. (2023) ²⁰⁸
Biopsy	Exploratory	Variable	Formation of tertiary lymphoid structures after neoadjuvant ICI therapy predicted longer relapse-free survival after resection.	Response	Shu et al. (2024) ¹¹¹
Biopsy	Exploratory	Anti-PD1	Intratumoral infiltration of CD103 ⁺ CTLs and their depletion from the peritumoral border was associated with better treatment response to anti-PD1 therapy.	Response	Huang et al. (2024) ²⁰⁹
Biopsy	Exploratory	Anti-PD1	Spatial transcriptomics showed mature TLS in patients treated with anti-PD1 were associated with improved outcomes.	Response	Tang et al. (2025) ²¹⁰
Blood	Retrospective	Variable	CRAFITY score (CRP and AFP) predicts OS in response to ICIs.	Predictive	Hatanaka et al. (2022) ¹⁷⁸ , Teng et al. (2022) ¹⁷⁹ , Ueno et al. (2024) ¹⁸⁰ , Wu et al. (2024) ¹⁸¹
Blood	Exploratory	Anti-PD1	A liquid biopsy signature of 13 proteins correlates well with the inflamed molecular class which is associated with good response to ICIs.	Predictive	Montironi et al. (2023) ¹³¹
Blood	Retrospective	Atezo + Beva	Baseline NLR ≥ 5 was associated with worse progression-free survival.	Predictive	Cheon et al. (2021) ¹⁷²
Blood	Retrospective	Anti-PD1	Stable NLR after initiating anti-PD1 therapy predicted poor OS.	Response	Du et al. (2024) ¹⁷³
Blood	Retrospective	Atezo + Beva	An AFP decrease $\geq 75\%$ identified ICI responders with modest sensitivity (0.59).	Response	Zhu et al. (2022) ¹⁶⁶

Biomarkers identified in RCTs, exploratory analyses and retrospective studies. Biomarkers that predict ICI responsiveness in treatment naive patients are labeled predictive whereas markers of response during/post treatment are labeled response.

CRP, C-reactive protein; CTLs, cytotoxic T lymphocytes; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; NLR, neutrophil-to-lymphocyte ratio; OR, overall survival; ORR, objective response rate; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; RCT, randomized controlled trial; TKIs, tyrosine kinase inhibitors; TLS, tertiary lymphoid structures; TME, tumor microenvironment.

Both elevated baseline NLR and lack of on-treatment improvement in NLR have correlated with worse responses to ICIs in recent retrospective analyses.^{172,173} Other soluble markers that may serve as proxies for overall immune tone and likelihood of a response to ICIs include IL-6, IL-137, and TGF- β .¹⁷⁴⁻¹⁷⁶

A composite of AFP and the inflammatory marker C-reactive protein (CRP) was used to create the CRAFTY score to predict responses to ICIs. In a retrospective cohort, CRAFTY score was predictive of OS and radiologic response to PD-1/PD-L1 ICIs with elevated AFP and CRP both independently associated with worse outcomes.¹⁷⁷ Several retrospective studies have now validated the CRAFTY score with other ICI combinations including atezolizumab-bevacizumab.¹⁷⁸⁻¹⁸¹

Liquid biopsy

Liquid biopsy is an umbrella term for analyses of tumor components including circulating tumor cells (CTCs), nucleic acids, and extracellular vesicles (EVs) that are collected from bodily fluids.¹⁴⁹ Liquid biopsy has emerged as a promising area for prognostic biomarker development because fluid collection (e.g., blood draw) is generally non-invasive and can be repeated for longitudinal monitoring and it has already been integrated into guidelines for some solid tumors, including breast cancer.^{182,183} Given the limited availability of tissue biopsies outside of the clinical trials setting, liquid biopsy is particularly appealing in HCC. Several studies have examined EpCAM+ CTCs using immunoaffinity platforms.¹⁴⁹ Abundance of these cells in peripheral blood correlates with baseline characteristics of higher BCLC stage, elevated AFP, metastatic disease, and decreased OS¹⁸⁴⁻¹⁸⁶ and their levels decline after locoregional therapies.¹⁸⁷ HCC-derived EV levels have similarly been used as a barometer for treatment response for patients receiving sorafenib +/- radiotherapy in the SORAMIC trial, with decreased EV levels after treatment. In retrospective analyses, a proteomic signature was developed for those with positive response to sorafenib.¹⁸⁸ CTCs have been interrogated by scRNA-seq which allows interrogation for rich tumor cell transcriptomic data, but their prognostic utility currently is limited by heterogeneity.¹⁸⁹ A proteomic signature was isolated from peripheral blood samples and correlated well with the tissue-based inflamed molecular class, with area under the receiver operating characteristic

curve (AUROC) 0.91.¹³¹ While prospective validation is needed, development of a non-invasive surrogate for tumor molecular class could make pre-treatment prognosis more accessible.

In summary, although there is a promising pipeline of biomarker candidates, high-quality prospective clinical trials are needed to validate their use for treatment selection.

Tissue-based biomarkers

Current FDA-approved biomarkers for primary response to ICIs include tumor PD-L1 expression and tumor mutational burden (TMB).¹⁹⁰ While these markers appear to have some efficacy in other tumor types, data does not support their use in HCC. PD-L1 expression assessed in biopsies collected as part of recent ICI clinical trials of nivolumab and atezolizumab did not correlate with therapeutic responses.^{6,11,12,191,192} Similarly, a high TMB has been linked to improved survival after treatment with ICIs across numerous cancer types.^{193,194} A survey of the CARIS cohort of 70,698 patients with microsatellite-stable cancers failed to detect an association between TMB and OS in HCC.¹⁹⁵ Thus, there is a clinical need to develop new biomarkers of response to immune-based therapies in HCC.

Bulk transcriptomics

Using biopsies collected through clinical trials, investigators have sought to identify the molecular signature of ICI-responsive HCCs. In one of the most comprehensive such studies, investigators performed retrospective studies on samples from multiple arms of the IMBrave150 trial including 209 patients in the phase 1b and phase 3 trials and 14 pre- vs. post-treatment biopsies. The treatment response to atezolizumab + bevacizumab was characterized by interferon signaling and infiltration of CD8 and CD4 T cells, Tregs, B cells and dendritic cells which were detected transcriptionally and confirmed on immunohistochemistry. Features of immune activation were absent from non-responders, which were instead characterized by high bile acid and lipid metabolism.¹⁹² Patients with high immune activation showed better survival when treated with ICI therapy compared to sorafenib, whereas there was no difference in OS in the cohort without this immune response signature.¹⁹² These findings are consistent with the results of smaller studies of biopsy data from human ICI trials. Investigators

in the Checkmate 040 trial of PD-1 inhibitor Nivolumab identified a 4 gene signature related to immune checkpoint receptors, CD8 T cells, and interferon signaling (CD274, LAG3, CD8A, and STAT1) predictive of objective response rate (ORR) and OS.¹⁹⁶ A smaller study of 28 patients treated with ICIs as first line and 55 who received them after TKIs had similar findings. Their analysis of transcriptomic data identified an 11-gene signature for ICI response composed of interferon signaling and MHC-related gene expression.¹⁴⁵ The characteristics of ICI responders share similarities with the published “immune class” of HCC.¹³⁰ In fact, in a recent study, investigators predicted that an expanded “inflamed” class (immune + immune-like classes) is likely to respond to ICIs.¹³¹ When the inflamed signature was applied to data from the GO30140 phase 1b and IMbrave150 phase III trials, it could predict the ICI response with an AUROC of 0.67.¹⁹⁷ An analysis of HCC and cholangiocarcinoma through the retrospective arm of a liver cancer ICI-response database established at the National Cancer Institute (NCI-CLARITY) also confirmed that published molecular classes could discriminate ICI responders and non-responders.¹⁹⁸ Despite promising results, these signatures have not been adopted in clinical practice. Liver biopsies are not routinely collected for most patients. Moreover, molecular signatures rely on assays that are slow and difficult to scale and standardize. Additionally, bulk transcriptomics fail to capture intra-tumoral heterogeneity which increases the risk of incomplete treatment responses or tumor recurrence. This challenge is reflected across the cancer spectrum where despite the availability of many correlative biomarkers, only breast cancer has seen a molecular signature applied to clinical practice for therapy selection.¹⁹⁹

Single cell and spatial sequencing

Single cell and spatial transcriptomics have enhanced recent efforts to predict ICI responders. Investigation of tumor cell composition from recent human clinical trials is identifying the importance of intra-tumoral heterogeneity and direct HCC-immune cell interactions, features that could not be easily appreciated through bulk transcriptomics.^{12,200} Some studies have reinforced established molecular features of favorable ICI response with improved clarity. Comprehensive scRNA-seq and immune profiling of tumor and peri-tumoral tissues from 36 patients treated

with ICIs identified an inflamed class with good prognosis. Two other clusters were enriched for an immunosuppressive TME with high MDSC and Treg infiltration, elevated T cell exclusion score and expression of markers CTLA4, CD274, and TIGIT; however, both displayed brisk responses to ICIs (67% and 100% respectively).²⁰¹ A recent investigation of the biology of response to atezolizumab + bevacizumab was able to resolve two distinct response populations, one immune-mediated and the other anti-angiogenic, using scRNA-seq. About 40% of responders had pre-treatment infiltration of CD8+ effector T cells, pro-inflammatory CXCL10+ macrophages and features of the inflamed TME previously associated with ICI response. The remaining 60% displayed TP53 mutations and downregulation of the VEGF co-receptor neuropilin-1 (NRP1) in pericytes and vascular endothelium, both features previously associated with response to bevacizumab.^{202,203} Interestingly, this group had similar levels of immune infiltration to the treatment non-responder group.²⁰⁴

One early study examined scRNA-seq on 46 HCC and 37 iCCA tumors and found that greater tumor cell heterogeneity was consistently predictive of worse outcome. Computational inference of cell lineages and receptor-ligand interactions indicated that tumor-lymphocyte interactions increase in more heterogeneous tumors with an associated shift toward proliferative pre-exhaustion T cells of CD4/CD8-c6/c7-MKI67-CXCL13 and pre-exhaustion T cells of CD8-c6-CXCL13. In their analyses, SPP1-CD44 was identified as a key mediator of tumor-immune communication.²⁰⁵

Several recent studies have emphasized the importance of spatial transcriptomics in understanding tumor behavior. Recruitment of CD4+ and CD8+ T cells to the tumor-immune interface and to TLS correlates with favorable molecular features and improved prognosis.^{206,207} Tumors responding to combined cabozantinib and nivolumab displayed activation of a B cell maturation program via the PAX5 module and increased peritumoral ECM remodeling by CAFs. Importantly, investigation of a single non-responder showed focal areas of immune exhaustion that could have been masked in bulk-transcriptomic analysis.²⁰⁸

Spatial analyses in preclinical and exploratory human trials have also identified multiple mediators of a local immunosuppressive niche that resist ICI activity. These include macrophage-mediated recruitment of CD103+ CTLs that

drive immunosuppressive inflammatory signaling,²⁰⁹ local tryptophan production by malignant cells that disrupts normal TLS maturation,²¹⁰ and formation of a barrier composed of CAFs and SPP1+ macrophages at the tumor-environmental interface that limits immune surveillance.⁹⁴

Microbiome

A healthy gut microbiome has been linked to improved ICI responses across many cancer types.²¹¹ Furthermore, for some solid tumors, certain microbial signatures have predicted benefit from ICI treatment.²¹²⁻²¹⁴ This relationship is expected to be particularly important in the liver where CLD and HCC are associated with dysbiosis and increased bacterial translocation via the portal circulation.^{215,216} Surprisingly, the data in HCC are so far less clear. Although antibiotic exposures have been linked to ICI resistance in other cancers, presumably through altered gut microbiota,^{217,218} a large retrospective analysis of 4,098 patients treated for advanced HCC with ICIs, TKIs, or placebo found reduced OS in all groups, but no effect specific to ICIs.²¹⁹ More investigation is needed in this area, particularly studies that directly monitor the fecal microbiome before and during treatment.

Artificial intelligence (AI)

AI is a set of computational tools capable of problem solving, analysis of complex datasets, and novel content generation without human supervision.²²⁰ These technologies are increasingly being incorporated into clinical and scientific practice, including oncology, where they are being tested for their utility across many domains including diagnosis, prognostication, and treatment selection.^{221,222}

Retrospective studies have shown that AI is capable of identifying prognostic signatures in computed tomography (CT), magnetic resonance imaging, or positron emission tomography images; moreover, these techniques can be applied to predict treatment responses.²²¹ In one early example, machine learning was applied to CT scans from patients with 5 solid tumor types, including HCC. They generated a radiomic signature that could discriminate inflamed vs. immune-exhausted molecular classes, levels of CD8+ T cell infiltration, and predict OS and progression-free survival.²²³ Similarly, investigators used AI analysis of H&E-stained histopathology slides to identify signatures of therapeutic response systemic therapy.²²⁴

Data for AI prognostication in HCC remains limited. Proof-of-concept analyses with machine learning have predicted treatment responses using transcriptional²²⁵ and immune-profiling²²⁶ data. A larger study used AI to analyze H&E slides, identifying histologic patterns correlated with a recently published transcriptional signature for response to atezolizumab + bevacizumab.¹⁹⁹ In an external validation set with 122 biopsies, AI-powered histopathology predicted progression-free survival, but not OS. Scores also correlated with transcriptional markers for immune activation and other established molecular changes associated with ICI response.¹⁹⁹ These preliminary studies are encouraging; however, challenges remain to improve clinical applicability. Standardized data and imaging collection protocols are needed across clinical trials to create the large, shared databases that are needed to train AI models.^{12,220} As discussed above, AI tools that rely on transcriptional or other omics techniques are likely to be limited by cost and tissue availability, whereas translation of AI radiomics may be feasible soon. Finally, before clinical application, AI tools will require validation in large, prospective clinical trials.

APPLICATION OF IMMUNOGENOMICS TO CLINICAL TRIALS

Clinical application of biomarkers for HCC will only be possible if randomized trials are performed to validate their use. While most trials for systemic therapies in HCC did not use biomarkers for patient stratification, blood and tissue samples collected in those studies have been used to identify biomarker candidates (Table 1). These biomarkers should be incorporated into future clinical trials to stratify patients or monitor for treatment response. One early success with a biomarker-driven approach to patient stratification is the REACH-2 trial (discussed in detail above), which demonstrated improved OS for patients with AFP ≥ 400 ng/mL treated with Ramucirumab after an initial trial in an unselected patient population was negative.¹⁶¹ Scientists and clinicians should build consensus around the most promising biomarkers to prioritize for inclusion in future studies, as well as the most effective trial designs to employ.

Traditional clinical trials were not designed to evaluate large numbers of biomarker and therapy combinations efficiently. New study designs such as umbrella and basket

trials were developed for precision oncology and may be better suited to accelerate biomarker evaluation.²²⁷ Umbrella trials enroll patients with a single cancer type and stratify them for treatment according to multiple biomarkers of interest. Basket trials use the opposite approach, testing a candidate drug against cancers with a shared mutation, regardless of underlying tumor type. The NCI-MATCH trial used a basket design, enrolling 1,593 patients into 27 studies based on shared driver mutations. More than a quarter (7/27) of these trials met a pre-specified endpoint for positive signal, warranting further investigation.²²⁸ MORPHEUS-Liver is an umbrella trial for immunotherapy in HCC with an adaptive design that permits the addition or removal of treatment arms based on continuing analysis of new data. Initial results from this trial suggest addition of an anti-TIGIT antibody (tiragolumab) to atezolizumab + bevacizumab may increase efficacy, even though this was not confirmed in the phase 3 clinical trial.²²⁹ Continued innovation in trial design will be critical to ensuring the successful clinical deployment of new discoveries in immunogenomics.

CONCLUSION

Rapid advances in sequencing technologies can now describe the immune and genetic features of HCC with unprecedented detail. While we have established a set of common genetic drivers in HCC, large international cohorts have demonstrated variation in mutation signatures and transcriptional patterns by disease etiology, environmental exposure, and genetic background. Single cell and spatial transcriptomics reveal further complexity at the level of the TME, where tumor-immune interactions vary by cell subtype and spatial orientation, relationships that were missed using bulk sequencing techniques.

Despite rapidly improving understanding of tumor biology and heterogeneity, clinical application of this knowledge has remained slow, hindered by limited access to liver biopsies, the need for standardized protocols, and inherent HCC heterogeneity. The development of novel biomarkers that capture this heterogeneity, particularly those that employ less invasive techniques such as liquid biopsy and radiomics rather than tissue biopsy, may speed clinical deployment. As the number of approved therapies for HCC

grows, there is a pressing need for prognostic biomarkers to guide personalized treatment selection. Current biomarker candidates need validation in large prospective trials with diverse patient enrollment and standardized protocols to accelerate clinical translation.

Authors' contributions

Concept and design: A.V., J.K.C. Writing and critical revision: A.V., D.C.C., J.K.C. Figures and visuals: D.C.C., J.K.C.

Conflicts of Interest

AV has received consulting fees from Genentech, Caris, Medpace, and Astra Zeneca. He has stock options from Espervita and Atzeio. He is listed as an inventor on a patent related to early detection of HCC (PCT/US20/61441), and he is funded by the NIH (1U01CA283931).

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