Molecular pathogenesis and systemic therapies for hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) remains one of the most prevalent and deadliest cancers. The poor outcome associated with HCC is dramatically changing due to the advent of effective systemic therapies. Here we discuss the molecular pathogenesis of HCC, molecular classes and determinants of heterogeneity. In addition, effective single-agent and combination systemic therapies involving immunotherapies as standard of care are analyzed. Finally, we propose a flowchart of sequential therapies, explore mechanisms of resistance and address the need for predictive biomarkers.

Hepatocellular carcinoma (HCC) accounts for more than 90% of liver cancer cases, but the life expectancy of patients with HCC has improved with the implementation of targeted and immune therapies1–3. Although the main features of the molecular pathogenesis, drivers and molecular and immune classes of HCC have been identified4–6, it presents few actionable mutations (only 25% of tumors harbor one druggable target)3,5. As a result, the understanding of oncogenic drivers and molecular classes has not yet been translated into clinical decision making. Recent findings on immune cell populations, tumor heterogeneity7,8 and etiology-specific pathogenic traits9 might help to overcome this unmet need, favoring the emergence of precision oncology strategies for this cancer.

Advanced HCC is chemo- and radio-resistant10, which limits the available therapeutic options for patients. In 2007, the approval of the tyrosine kinase inhibitor (TKI) sorafenib11, the first systemic treatment for HCC, radically changed prospects. Several single-agent systemic regimes were subsequently approved as first (lenvatinib)12 and second (regorafenib13, cabozantinib14 and ramucirumab15) line therapies. The year 2020 marked the start of a third era dominated by combination regimes involving immunotherapies16,17, ignited by the demonstrated superiority of the atezolizumab and bevacizumab combination versus sorafenib in all clinical endpoints18, including overall survival (OS), progression-free survival (PFS) and objective response, and in patient-reported outcomes. This has opened the path to exploring combinations of immunotherapies with TKIs or monoclonal antibodies specific to vascular endothelial growth factor (VEGF)A, combinations of two immunotherapies such as anti-programmed cell death protein 1/ PD-ligand 1 (PD-1 and PD-L1 respectively) with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) inhibitors such as durvalumab plus tremelimumab, and even triple combinations. In addition, two immune-based regimes ( pembrolizumab19 and nivolumab plus ipilimumab20) received accelerated approval from the Food and Drug Administration (FDA) based on the reporting of positive phase II clinical trials. Current ongoing trials with systemic regimes are expected to further impact the clinical benefits of patients at early to intermediate stages21.

In this Review, we provide an integrated description of the molecular pathogenesis of HCC, critical oncogenic drivers and molecular and immune classes, and the recent developments in systemic therapies. In addition, we discuss how this knowledge could be translated into precision oncology by providing a perspective on the role of systemic therapies in HCC, their current status in the management of the disease and the optimal transition from loco-regional to systemic regimes. We further dissect the evidence supporting the use of the approved molecular and immune treatments and provide insights on how to navigate through these regimes. Finally, we conduct a critical analysis on emerging clinical trials, biomarkers and trial design for future investigations16,22.

Molecular pathogenesis

HCC develops in ~80% of cases in the setting of a severely damaged cirrhotic liver that already gathers molecular alterations1. In addition, several etiological (hepatitis C virus (HCV) and hepatitis B virus (HBV) infection, alcohol use disorder and non-alcoholic steatohepatitis (NASH)) and environmental (aflatoxin, aristolochic acid and tobacco) factors have been identified with distinct specific paths to cancer development1. Specific molecular and immune classes have been defined, which integrate the current molecular knowledge of this cancer6. In this regard, immune11 and epigenetic mechanisms7,24–26 might have major consequences in understanding the onset, evolution and treatment of this malignancy. The main molecular alterations and pathogenic processes involved in HCC development have been extensively reviewed elsewhere1,4,6,13.

Hepatocarcinogenic process and drivers. Most HCCs develop in patients with cirrhosis1. These neoplasms progress through a sequence of well-defined histopathological phases, starting with the

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emergence of dysplastic nodules, which can ultimately transform into HCC (Fig. 1). Genetic and epigenetic oncogenic alterations likely occur within hepatocytes, a cell type that, despite its differentiated features, is a facultative stem cell. Although mature hepatocytes are the main cells of origin for HCC, liver stem cells and transit-amplifying cell populations have been also implicated in liver oncogenesis. In preclinical models, hepatic oncogenesis is favored by cell death with compensatory regeneration of hepatocytes, and blocking apoptosis reduces HCC formation. Replicative stress within regenerating hepatocytes induces genetic lesions favoring transformation and cancer progression, especially in the context of inflammation and fibrosis. In human NASH, auto-aggressive CD8+ T cells induce hepatocyte cell death, promote NASH pathogenesis and impair immune surveillance, thereby favoring HCC occurrence and progression.

On the other hand, the sequential accumulation of somatic genomic and epigenetic alterations has been shown to play a key role in liver carcinogenesis. Single-nucleotide polymorphisms that predispose to liver disease, including PNPLA3 (rs738409), TM6SF2 (rs585542926) and HSD17B13 (rs72613567) (which encode proteins involved in metabolism), increase the risk of HCC. Aflatoxin B1 and aristolochic acid are environmental genotoxic compounds that induce somatic mutations in HCC. Aflatoxin B1, an Aspergillus metabolite found in maize and nuts, synergistically promotes HCC in patients with HBV infection. Aristolochic acid, found in Chinese herbal teas, causes abundant T to A oncogenic transversions. On average, HCC tumors have 60–70 somatic mutations. The majority are ‘passenger mutations’ and do not directly participate in the carcinogenetic process, but some mutations occur in the so-called ‘driver genes’ and activate signaling pathways that are key for liver carcinogenesis (Table 1). The most prevalent somatic mutation in HCC (60%) affects the promoter region of the gene encoding telomerase reverse transcriptase (TERT), a master regulator of telomere length. Additionally, integration of HBV or adenovirus in the TERT promoter has been reported. TERT-activating mutations occur in 20% of dysplastic nodules, making this molecular feature a putative gatekeeper of HCC. Subpopulations of hepatocytes expressing high levels of telomerase exist distributed throughout all liver zones, which may contribute to hepatocarcinogenesis by preventing cellular senescence, thereby providing a mutation-prone source of replicating cells in chronic liver injury. Other epigenetic (for example, hypermethylation of TSPYL5) and genetic alterations (for example, chromosome 8q loss) found in dysplastic nodules have been suggested as cancer gatekeepers. The second most frequently altered gene in HCC is CTNNB1 (~30%), a gene that encodes β-catenin and is a critical effector of the WNT pathway. WNT–β-catenin signaling is largely limited to zone 3 of the hepatic lobule, and hepatocarcinogenesis involving β-catenin mutations likely occurs in this hepatic zone. Other key mutations occur in TP53 (~25%) and AXIN1 (~10%) or in genes encoding epigenetic regulators, such as BAP1, ARID1A, ARID1B and ARID2. Mutations in conventional targets for TKIs, such as those encoded by PDGFRα, MET, EGFR and PIK3CA, are rare (~3%).

Strikingly, of the 34 most commonly reported genes in HCC (Table 1), only 6 have been proven targetable by an FDA-approved drug and another eight are under evaluation in early-phase trials. Some examples include the high-level focal amplification of the 11q13 locus containing FGFR19 (refs. 42,43), which has led to proof-of-concept studies demonstrating anti-tumoral activity with fibroblast growth factor (FGF) receptor (FGFR) inhibitors.
in HCCs with \textit{FGF19} overexpression\textsuperscript{41}. Also, the high-level focal amplification in chromosome 6p21 includes the \textit{VEGFA} gene, which has a 5% reported prevalence in HCC\textsuperscript{45} (Table 1). Drugs targeting VEGFA (such as bevacizumab) or VEGF receptor (VEGFR)\textsubscript{2} (such as ramucirumab) have been approved, but there is no specific information on whether they are more efficacious in tumors with these amplifications. Other alterations such as those in the insulin growth factor (IGF) pathway are also prevalent in HCC\textsuperscript{46} (Table 1), but drugs blocking them are still in early clinical trials\textsuperscript{47,48}. Finally, although targeting non-enzymatic mutations has proven difficult (for example, \textit{CTNNB1} exon 3 mutations), newer therapeutic approaches such as proteolysis-targeted chimeras (PROTACs)\textsuperscript{49}, which induce targeted protein degradation by the ubiquitin–proteasome pathway, are promising.

### Table 1 | Key oncogenic drivers and pathways de-regulated in HCC

| Altered pathway                        | Altered gene | Type of alteration         | Percent prevalence in HCC (range) |
|----------------------------------------|--------------|-----------------------------|----------------------------------|
| Telomere maintenance                   | \textit{TERT}\textsuperscript{a} | Promoter-activating mutation | 55 (44–59)                       |
|                                        | \textit{TERT}\textsuperscript{a} | High-level focal amplification | 6 (1–9)                          |
|                                        | \textit{TERT}\textsuperscript{a} | Viral insertion             | 3 (1–5)                          |
|                                        | \textit{CDKN2A} | Loss-of-function mutation    | 27 (18–31)                       |
|                                        | \textit{CDKN2A} | Homozygous deletion         | 2 (0–2)                          |
|                                        | \textit{ATM} | Loss-of-function mutation    | 4 (2–5)                          |
|                                        | \textit{RB1} | Loss-of-function mutation    | 4 (3–5)                          |
|                                        | \textit{CDKN2A} | Homozygous deletion         | 5 (4–6)                          |
|                                        | \textit{MYC} | High-level focal amplification | 12 (4–18)                       |
|                                        | \textit{CCND1} | High-level focal amplification | 7 (5–7)                          |
| WNT-β-catenin signaling                | \textit{CTNNB1}\textsuperscript{a} | Activating mutation         | 29 (23–36)                       |
|                                        | \textit{AXIN1} | Loss-of-function mutation    | 7 (4–10)                         |
|                                        | \textit{APC} | Loss-of-function mutation    | 2 (0–3)                          |
| Chromatin remodeling                   | \textit{ARID1A} | Loss-of-function mutation    | 8 (4–12)                         |
|                                        | \textit{ARID2} | Loss-of-function mutation    | 7 (3–10)                         |
|                                        | \textit{KMT2A} | Loss-of-function mutation    | 3 (0–4)                          |
|                                        | \textit{KMT2C} | Loss-of-function mutation    | 3 (2–5)                          |
|                                        | \textit{KMT2B} | Loss-of-function mutation    | 2 (0–4)                          |
|                                        | \textit{BAP1} | Loss-of-function mutation    | 2 (0–5)                          |
|                                        | \textit{ARID1B} | Loss-of-function mutation    | 1 (0–3)                          |
| Ras-PI3K-mTOR                           | \textit{RPS6KA3} | Unclassified                | 4 (3–6)                          |
|                                        | \textit{PIK3CA}\textsuperscript{a} | Activating mutation         | 2 (1–4)                          |
|                                        | \textit{KRAS}\textsuperscript{a} | Activating mutation         | 1 (0–1)                          |
|                                        | \textit{NRAS} | Activating mutation         | 0 (0–1)                          |
|                                        | \textit{PDGFRA}\textsuperscript{a} | Mutation                   | 1 (0–4)                          |
|                                        | \textit{EGFR}\textsuperscript{a} | Activating mutation         | 1 (0–2)                          |
|                                        | \textit{PTEN} | Loss-of-function mutation    | 1 (0–2)                          |
| FGF signaling\textsuperscript{a}       | \textit{FGF19} | High-level focal amplification | 6 (5–6)                          |
| VEGF pathway\textsuperscript{a}        | \textit{VEGFA} | High-level focal amplification | 5 (1–8)                          |
| Oxidative stress                       | \textit{NFE2L2}\textsuperscript{a} | Activating mutation         | 4 (2–6)                          |
|                                        | \textit{KEAP1}\textsuperscript{a} | Activating mutation         | 3 (2–5)                          |
| Hepatocyte differentiation             | \textit{ALB} | Mutation                    | 9 (5–13)                         |
|                                        | \textit{APOB} | Mutation                    | 8 (1–10)                         |
| JAK-STAT                               | \textit{IL6ST} | Mutation                    | 2 (0–3)                          |
|                                        | \textit{JAK1}\textsuperscript{b} | Mutation                   | 1 (0–3)                          |
| TGF-β signaling\textsuperscript{b}    | \textit{ACVR2A} | Loss-of-function mutation    | 4 (1–10)                         |
| IGF signaling\textsuperscript{b}      | \textit{IGF2R} | Mutation                    | 1 (0–2)                          |

\textsuperscript{a}Mutations frequencies are reported for a total of 1,339 patients included in multiple whole-exome-sequencing studies\textsuperscript{3,41,60,170,171} (modified and updated from ref. \textsuperscript{5}); \textit{TERT} promoter mutations were assessed using Sanger sequencing (\(n = 1,517\) patients)\textsuperscript{172}. Copy-number alterations were detected using single-nucleotide polymorphism arrays (\(n = 857\) patients)\textsuperscript{3,41,45,60,170}. Viral integrations were detected using viral capture and DNA sequencing (\(n = 645\) patients). \textit{STAT}, signal transducer and activator of transcription. \textsuperscript{a}Targetable by an FDA-approved drug. \textsuperscript{b}Targetable by a drug in testing phases. \textsuperscript{c}Targetable using mTOR inhibitors in testing phases.
Molecular and immune HCC classes. The molecular landscape of each tumor results from the accumulation of genomic and epigenomic alterations and is shaped by the tumor microenvironment. Based on genomic, transcriptomic and epigenomic data, distinct HCC molecular and immune subtypes have been identified\(^{35,50-55}\).

Molecular classes. The most extended HCC molecular classification distinguishes between the proliferation class and the non-proliferation class. HCCs of the proliferation class (~50% of cases) are associated with high levels of α-fetoprotein (AFP), poor clinical outcome and HBV-related etiology. These tumors present activation of signaling pathways involved in cell proliferation and survival, including mitogen-activated protein (MAP) kinase (MAPK) signaling, phosphoinositide 3 kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) signaling and MET signaling\(^{35,50-54}\), and are enriched in TP53 mutations and focal chromosomal amplifications in the 11q13 locus, including FGF19 and CCND1 (refs. 40,46), and harbor high chromosomal instability\(^{55}\). Proliferation class tumors can be subdivided into those with transforming growth factor (TGF)-β-non-canonical WNT activation (S1 or WNT–TGF-β subclass)\(^{54}\) and those with progenitor cell features, overexpression of epithelial cell adhesion molecule (EPCAM), AFP and IGF2 (S2 subclass)\(^{35}\). A methylation-based signature with prognostic value has been reported to identify a subset of HCCs of the S2 subclass\(^{46}\).

The non-proliferation HCC class is more heterogeneous. It is associated with alcohol- and HCV-related cases and with better clinical outcome. A subgroup of tumors of this class are dominated by canonical WNT signaling\(^{68}\) (CTNNB1 subclass).

A recent publication analyzing NASH-related HCC reported an enrichment in bile and fatty-acid signaling, oxidative stress and inflammation; a higher proportion of the WNT–TGF-β subclass; and higher immunosuppressive features\(^{59}\). At the genomic level, mutations in the ACVR2A gene (10%) were significantly more frequent in HCCs associated with this etiology\(^{60}\).

Immune classes. Around ~35% of tumors belong to the ‘inflamed class’ and present high immune cell infiltration, high cytolytic activity, increased levels of PD1 and PD-L1, activation of interferon signaling and low burden of broad chromosomal alterations, which recapitulate the characteristics of ‘hot tumors’, and which include a small subgroup of tumors dominated by high interferon signaling coexisting with CTNNB1 mutations\(^{35,50-55}\). In principle, this class includes tumors with the highest immune infiltration, a more diverse T-cell repertoire and enrichment in signatures predicting response to immune checkpoint inhibitors (ICIs). Non-inflamed (‘cold’) tumors are characterized by T-cell exclusion and either TP53 mutations (intermediate class) or activation of canonical WNT signaling through CTNNB1 mutations (excluded class)\(^{54,55}\). Whether inflamed HCCs or other immune-related biomarkers are associated with response to immune checkpoint blockers is currently being investigated\(^{61,62}\).

Cancer evolution and molecular heterogeneity. The changing tumor microenvironment imposes a constant selective pressure that leads to intratumor heterogeneity (ITH), a key feature of solid malignancies\(^{43,44}\). Evidence of ITH in HCC via multi-regional DNA sequencing of tumors\(^{30,38,60,64}\) reveals the presence of trunk alterations, for example in TP53, CTNNB1 and TERT, during early stages of hepatocarcinogenesis (Fig. 1). However, while driver mutations may be positively selected during tumor evolution, putative passenger mutations are also inadvertently introduced\(^{67,68}\). Recent studies have focused on the characterization of non-genetic clonal diversity\(^{49-51}\), but the faithful integrated modeling of tumor evolution still remains a challenge to guide molecular targeted therapies. From the epigenetic perspective, genome-wide DNA-methylation studies across normal livers, cirrhotic tissues, dysplastic nodules and HCC point to epigenetic factors as key regulators during the transition between dysplastic nodules and HCC\(^{52,53}\).

Furthermore, single-cell studies have provided unique insights into tumor evolution\(^{69,70}\). For instance, single-cell RNA sequencing (scRNA-seq) has revealed new T-cell subtypes associated with HCC or with responses to treatment\(^{71,72}\) and uncovered substantial transcriptomic diversity of cancer stem cell populations within HCC\(^{66}\). Single-cell analysis coupled with regional neo-epitope profiling and viral antigen burden evidenced regional clonal immune responses contributing to ITH in HCC\(^{10}\). Another scRNA-seq analysis of patients with HCC undergoing immunotherapy revealed that VEGF expression was associated with higher transcriptomic diversity, tumor microenvironment reprogramming and worse OS and response to therapy\(^{71}\). Longitudinal multi-region analysis following therapy by scRNA-seq provided a molecular portrait of the immune cell landscape of early-relapse HCC\(^{71}\).

Translating molecular knowledge into precision oncology. With the exception of elevated serum AFP levels predicting response to ramucirumab\(^{14}\), approved systemic agents lack appropriate biomarkers to identify responders\(^{54}\). Three factors hamper the translation of precision oncology into HCC decision making. First, the most prevalent molecular alterations (TERT, CTNNB1 and TP53 mutations) are currently undruggable (Table 1)\(^{64,60}\), with only 20–25% of tumors hosting a driver actionable mutation\(^{64}\). This differs markedly from other cancers, such as melanoma or gastrointestinal stromal tumors\(^{24,41}\). For instance, in a study of ~10,000 solid tumors, patients with gastrointestinal stromal tumors, thyroid cancer, breast cancer, melanoma or glioma received specific targetted therapies against actionable aberrations in ~60–75% of cases, compared to only 5% of HCC cases\(^{24}\). Second, HCC is clinically diagnosed using non-invasive imaging criteria according to guidelines\(^{83-87}\). Despite clear calls for access to tissue specimens for research purposes in randomized clinical trials (RCTs)\(^{12,24,25}\), systematic collection of tissues to develop biomarkers has been scarce. Finally, the substantial intratumoral heterogeneity, present in up to 25% of cases\(^{10,66}\), is the third obstacle to the identification of useful biomarkers\(^{56}\).

Few RCTs have been designed enriched for biomarker-based populations, and none have been based on molecular or immune HCC classes. In the REACH-2 trial, ramucirumab showed better significant outcome in patients with serum AFP levels >400 ng ml\(^{-1}\) (around 40% of the advanced HCC second-line population) than placebo\(^{12}\). Other biomarker-related studies yielded negative or inconclusive results, including the trial of tivantinib, a non-specific MET and tubulin inhibitor tested in HCCs with high tumor MET expression (~50% of advanced HCC cases) detected by immunohistochemistry\(^{88}\). In a phase II study enriched for RAS mutations, results of the combination of sorafenib and refametinib were inconclusive\(^{24}\). Fisogatinib, a specific inhibitor of the FGFR4 receptor (activated by oncogenic FGF19 in ~25% of HCCs\(^{62}\)), was tested in a proof-of-concept study leading to 16% of objective response\(^{42,44}\).

Systemic therapies

Systemic therapies have profoundly changed the landscape of HCC management. It is estimated that 50–60% of patients are treated with systemic therapies either because they are diagnosed at advanced stages of the disease or because they progress after surgical or loco-regional therapies\(^{1}\). In this section, we discuss the timings, selection and prospects of systemic therapies for patients with HCC.

Current systemic therapies for HCC. Since the initial study showing benefits of sorafenib treatment compared to placebo, in the last 15 years, we have witnessed the approval by the FDA, the European Medicines Agency and most Asian regulatory agencies of six regimens (atezolizumab plus bevacizumab\(^{35}\), sorafenib\(^{1}\), lenvatinib\(^{14}\), regorafenib\(^{1}\), cabozantinib\(^{16}\) and ramucirumab\(^{53}\)) based on phase
Fig. 2 | Molecular depiction of systemic therapies in HCC. Tumor cells, liver sinusoidal endothelial cells and lymphocytes are represented in relation to TKIs, immunotherapies and monoclonal antibodies approved in HCC based on phase III trial data. Therapy names in bold black indicate positive results based on phase III trials, either with a superiority design (atezolizumab plus bevacizumab, sorafenib, regorafenib, cabozantinib and ramucirumab) or with a non-inferiority design (lenvatinib). Therapy names in bold blue designate other FDA-approved drugs based on non-randomized phase II trials (pembrolizumab and nivolumab plus ipilimumab). Grey boxes indicate combination therapies.

III data (Fig. 2 and Table 2). Two additional regimes (pembrolizumab19 and nivolumab plus ipilimumab20,21) have been approved by the FDA based on the results of phase II trials. Recently, the combination of tremelimumab and durvalumab has been shown to be superior to sorafenib for OS94, whereas cabozantinib plus atezolizumab showed superiority over sorafenib in terms of PFS93. This unprecedented improvement in the treatment armamentarium of the disease has impacted the expected outcome of patients and early transitioning from loco-regional therapies to systemic therapies.

Placing systemic therapies in the context of HCC management. Tumor stage, liver dysfunction and performance status underpin clinical practice guidelines of HCC from scientific societies81,82,89,95–97. The Barcelona Clinic for Liver Cancer (BCLC) staging algorithm proposed in 1999 (ref. 98), endorsed by European and American hepatology–oncology-based guidelines99,100, classifies patients into five stages (BCLC 0, A, B, C or D) and allocates them into specific treatments1,85 (Fig. 3). In principle, patients with HCC at very early stage (BCLC 0, single HCC <2 cm) and early stage (BCLC A, with a single tumor or two to three tumors <3 cm in diameter) are considered for curative therapies such as resection, liver transplant (following Milan criteria)96 or ablation85–86,95–97. Downstaging, that is, reducing the tumor burden with therapies to meet the Milan criteria, is accepted in the United States100. Patients with preserved liver function and more advanced multifocal tumors confined to the liver are classified into BCLC B and treated with transarterial chemoembolization (TACE). These patients have median survival times of 26–30 months22. Patients with portal vein invasion or extrahepatic disease are classified into BCLC C, and systemic therapies are recommended. Around 50–70% of the patients receiving systemic therapies are progressing from surgery or loco-regional therapies, while 30–50% are treatment naive101 (Table 2).

Timing for systemic therapies in HCC. Deciding when to transition from loco-regional to systemic treatment in patients at the intermediate stage (BCLC B) is of paramount importance. A late decision to transition might jeopardize gains in OS because only Child–Pugh A class patients benefit from systemic therapies12. However, there is no consensus on when to halt local therapies22,102. Score-based selection of patients for treatment and retreatment with TACE has not been thoroughly validated and is not widely implemented102–105. Overall, the recommendation to transition can be made in case of progressive disease, impairment of liver function or occurrence of technical or other known contraindications for TACE during the ongoing therapy102,106. Lack of objective response after at least two treatment sessions of TACE is a clear predictor of poor survival106. The success of the first-line combination of atezolizumab plus bevacizumab leading to an objective response of 30% and median survival of ~19 months for all patients10, with even better outcomes for patients at the intermediate stage, also provides justification for moving to systemic agents when response to TACE is limited. Initial combinations of TACE with single-agent molecular therapies (that is, sorafenib or brivanib) did not yield positive results107–109, a feature that is expected to change with immunotherapy-based combination regimens (Fig. 3).

Selection of patients and expected outcomes. The seminal SHARP trial13 established the benchmark criteria for selection of patients for front-line systemic treatment, including Child–Pugh stage liver dysfunction, tumor burden and Eastern Cooperative Oncology Group performance status (ECOG PS). Table 2 summarizes the overall characteristics of patients included in phase III trials in first and second line. Although, in almost all studies, Child–Pugh A is dominant (97–100%), differences are observed in first versus second line regarding advanced tumor staging (BCLC C (~80% versus ~60%), ECOG PS 0 (~60–70% versus ~50–60%) and extrahepatic spread (50–60% versus 70–80%). Overall, the expected survival of patients treated with first-line systemic therapies ranges from ~19 months for atezolizumab plus bevacizumab10 to ~13–14 months for lenvatinib14 or sorafenib15. This improvement in OS from the initial ~11 months (sorafenib) to ~19 months (atezolizumab plus bevacizumab) reflects not only the higher efficacy of the treatment but also additional nuances, for instance, the increased applicability of effective second-line treatments (currently administered in
| Study name       | Treatment                                      | Inhibited molecules                         | BCLC (0/A/B/C; %) | Previous local therapies (%) | MVI (%) | EHD (%) | ECOG PS (0/1/2; %) | Child-Pugh A (%) | Median OS (months; HR, 95% CI) | Median PFS (months; HR, 95% CI) | ORR mRECIST; RECIST (%) |
|------------------|------------------------------------------------|---------------------------------------------|-------------------|-------------------------------|--------|--------|-------------------|-----------------|-------------------------------|-------------------------------|------------------------|
| **First-line therapies** |                                               |                                             |                   |                               |        |        |                   |                 |                               |                               |                        |
| IMbrave150 (refs. 18, 141) | Atezolizumab plus bevacizumab                   | PD-L1 (immune checkpoint), VEGF (angiogenesis) | −/2/15/82         | 48                            | 38     | 63     | 62/38/-          | 100             | 19.2 (0.66, 0.52–0.85)        | 6.9 (0.65, 0.53–0.81)        | 35.4; 29.8             |
| SHARP (IMbrave150, REFLECT) | Sorafenib                                      | VEGFR, PDGFR (angiogenesis), MAPK (BRAF)   | −/−/18/82         | 67                            | 36     | 53     | 54/38/8          | 95              | 10.7–13.4 a (0.69, 0.55–0.87) b | 3.7–4.3 a (NR) b           | NR; 2                   |
| REFLECT 14       | Lenvatinib                                      | VEGFR, PDGFR, FGFR (angiogenesis), KIT, RET | −/−/22/78         | 78                            | 23     | 61     | 64/36/-          | 99              | 13.6 (0.92, 0.79–1.06)         | 7.4 (0.66, 0.57–0.77)         | 24; 18.8               |
| **Second-line therapies** |                                               |                                             |                   |                               |        |        |                   |                 |                               |                               |                        |
| RESORCE 15       | Regorafenib                                     | VEGFR, PDGFR (angiogenesis), MAPK (BRAF)   | −/−<1/14/86       | 85                            | 29     | 70     | 65/35/-          | 98              | 10.6 (0.63, 0.5–0.79)          | 3.1 (0.46, 0.37–0.56)         | 11; 7                  |
| CELESTIAL 16     | Cabozantinib                                     | MET (proliferation), VEGFR (angiogenesis), RET | −/−/9/91          | 44c                           | 27     | 79     | 52/48/−          | 98              | 10.2 (0.76, 0.63–0.92)         | 5.2 (0.44, 0.36–0.52)         | NR; 4                  |
| REACH-2 (ref. 17) | Ramucirumab (AFP > 4 ng ml−1)                   | VEGFR2 (angiogenesis)                       | −/−/17/83         | 62a                           | 36     | 72     | 57/43/-          | 100             | 8.5 (0.7, 0.53–0.95)           | 2.8 (0.45, 0.34–0.6)         | NR; 5                  |
| KEYNOTE-240 (ref. 19) | Pembrolizumab                                | PD1 (immune checkpoint)                     | −/−/20/80         | NR                            | 13     | 70     | 58/42/-          | 100             | 13.9 (0.78, 0.61–1)           | 3 (0.78, 0.61–0.99)          | NR; 18                 |
| KEYNOTE-224 (ref. 19) | Pembrolizumab                                | PD1 (immune checkpoint)                     | −/−/24/76         | NR                            | 17     | 64     | 61/39/-          | 94              | 13.2                           | 4.9                           | 15; 18.3               |
| CheckMate 040 (ref. 21) | Nivolumab plus ipilimumab (arm A)             | PD1 and CTLA4 (immune checkpoints)          | 2/4/8/86          | ≥72                           | 36     | 80     | NR               | 100             | 22.8                           | NR                           | NR; 32                 |

*This range corresponds to the reported survival data in the SHARP (experimental arm), REFLECT and IMbrave150 (control arm) trials. *The HR corresponds to the phase III SHARP trial that compared sorafenib with placebo. *Includes only liver-directed non-radiation therapies. *Includes only surgical procedures and radiotherapy. CI, confidence interval; EHD, extrahepatic disease; MVI, macrovascular invasion; mRECIST, modified RECIST; NR, not reported; RECIST, response evaluation criteria in solid tumors.
Fig. 3 | Staging system and treatment allocation for HCC.  

(a) BCLC treatment algorithm with new systemic agents. The treatment strategy for the management of HCC is guided by the BCLC staging system, which consists of five stages depending on tumor burden features, liver function and performance status. Asymptomatic patients with low tumor burden and good liver function (BCLC 0 or A) should be treated with local curative treatments (resection, ablation or transplantation, depending on the presence of portal hypertension, number of nodules and liver function). Asymptomatic patients with multinodular disease and adequate liver function (BCLC B) should receive chemoembolization, and patients with portal thrombosis or extrahepatic spread (BCLC C) should be treated with systemic therapies. LT, liver transplantation; M1, distant metastasis; N1, lymph node metastasis; SBRT, stereotactic body radiation therapy; TARE, transarterial radioembolization. Adapted with permission from ref. 23, Wiley. #Based on high level of evidence studies. ##Based on low or moderate level of evidence studies.

(b) Treatment strategy for HCC with systemic therapies. Green, regulatory-body-approved regimes based on phase III studies. Orange, positive combinations versus sorafenib, but drugs are not yet approved. Yellow, treatments that received FDA accelerated approval based on phase II studies. *Around 70–80% of patients are expected to receive this regime. PD, progressive disease.
### Table 3 | Systemic therapies approved for HCC: adverse events and regulatory status

| Treatment (study name) | Treatment dose (baseline) | Overall prevalence | Prevalence of most common AE | Percent of patients undergoing dose reduction/interruption | Percent of patients undergoing treatment withdrawal | Percent of patients with adverse events leading to death | Strategy dose reduction | Regulatory approval |
|------------------------|---------------------------|--------------------|-----------------------------|----------------------------------------------------------|-----------------------------------------------------|--------------------------------------------------------|-------------------------|---------------------|
| **First-line therapies** |                           |                    |                             |                                                          |                                                     |                                                        |                         |                     |
| Atezolizumab plus bevacizumab (IMbrave150; ref. 18) | 1,200 mg + 15 mg per kg every 3 weeks | 36% | Hypertension 10%, increased AST 4%, proteinuria 3% | Reduction: not allowed Interruption: 50% | Withdrawal of atezolizumab or bevacizumab; 16% withdrawal of atezolizumab plus bevacizumab, 7% | 2% | Not recommended. | 2020 EU US AWJPa JP |
| Sorafenib (SHARP13 (IMbrave150, REFLECT)) | 400 mg every 12 h | 45% | Diarrhea 8%, HFS 8%, fatigue 4% | Reduction: 26% Interruption: 44% | 11% | NR | Reduce 1 level (400 mg q24h) if persistent G2 or G3. Discontinue if G4. | 2007 EU US AWJP |
| Lenvatinib (REFLECT14) | 12 mg, ≥60 kg every 24 h; 8 mg, <60 kg every 24 h | 57% | Hypertension 23%, weight loss 8%, increased BR 7% | Reduction: 37% Interruption: 40% | 9% | 2% | Reduce 1 level (8 or 4 mg q24h in ≥60 kg and <60 kg, respectively) if persistent G2 or G3. Discontinue if G4. | 2018 EU US AWJP |
| **Second-line therapies** |                           |                    |                             |                                                          |                                                     |                                                        |                         |                     |
| Regorafenib (RESORCE15) | 160 mg every 24 h | 50% | Hypertension 13%, HFS 13%, fatigue 9% | Reduction/interruption: 66% | 10% | 2% | Reduce 1 level (120 mg q24h) for persistent G2 and G3 AEs. Discontinue if G4. | 2017 EU US AWJP |
| Cabozantinib (CELESTIAL16) | 60 mg every 24 h | 68%b | HFS 17%, hypertension 16%, increased AST 12% | Reduction: 62% | 16% | 1% | Reduce 1 level (40 mg q24h) for persistent G2 and G3 AEs. Discontinue if G4. | 2018 EU US AWJP |
| Ramucirumab (REACH-2; ref. 17) | 8 mg per kg every 2 weeks | 57% | Hypertension 8%, liver injury or failure 4%, proteinuria 2% | Reduction: 5% Interruption: 35% | 11% | 2% | Reduce 1 level (6 mg per kg q2w) for G3 AEs. Discontinue if G4. | 2019 EU US AWJP |
| Pembrolizumab (KEYNOTE-240; ref. 18) | 200 mg every 3 weeks | 53%b | Increased AST 13%, increased BR 8%, increased ALT 6% | Reduction: not allowed Interruption: 30% | 17% | 3% | Not recommended. | 2018 US AWJP |
| Pembrolizumab (KEYNOTE-224; ref. 19) | 200 mg every 3 weeks | 26% | Increased AST 7%, increased ALT 4%, fatigue 4% | Reduction: not allowed Interruption: 25% | 5% | 1% | Not recommended. | 2018 US AWJP |
| Nivolumab plus ipilimumab (CheckMate 040 (arm A19)) | 1 mg per kg + 3 mg per kg every 3 weeks (4 doses) followed by nivolumab (240 mg every 2 weeks) | 53% | Increased AST 16%, increased lipase 12%, increased ALT 8%, fatigue 4% | Reduction: not allowed Interruption: NR | 22% | 2% | Not recommended. | 2020 US AWJP |

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*aNot in all Asian countries without Japan. *bAdverse events owing to all causes are shown. *cFDA approval in 2019. AEs, adverse events; ALT, alanine aminotransferase levels; AST, aspartate aminotransferase levels; AWJP, Asia without Japan; BR, bilirubin; EMA, European Medicines Agency; EU, European Union; G, grade; HFS, hand–foot syndrome; JP, Japan; NR, not reported; q24h, once every 24 hours; q2w, once every 2 weeks.
~30–50% of cases), better patient selection, treatment at earlier stages of the natural history of the disease and better management of adverse events and complications. Other clinical endpoints have also improved, such as PFS (ranging from 4 to 7 months), objective response rates (ORRs) (up to ~35% with the combination atezolizumab plus bevacizumab) and patient-reported outcomes.

In second-line therapies after progression on sorafenib, expected survival ranges from 10 to 11 months for regorafenib and cabo-zan tinib to 8 months for ramucirumab in patients with aggressive tumors (AFP levels >400 ng ml). Subgroup analysis showed that some treatment-related adverse events are associated with better survival, including skin toxicity for sorafenib or hypertension for cabozantinib or hypertension for lenvatinib, cabozantinib and ramucirumab (Table 3).

Although OS is recommended as the best endpoint for phase III trials testing systemic therapies, the fact that patients are exposed to effective second-line treatments in ~30–50% of cases has raised a concern about whether OS should be the sole endpoint in front-line research. Previous concerns about using PFS as the endpoint in HCC due to competing risk with cirrhosis-related death have been diminished by the universal selection of patients with Child–Pugh A in phase III investigations, thus reducing the 1-year risk of death due to decompensation to <5%. Nowadays, PFS has been proposed as the primary endpoint using restrictive rules supported by results of an RCT showing that a hazard ratio (HR) <0.6 for PFS is a good surrogate of OS benefit. Conversely, HRS >0.6 for PFS are considered to have uncertain association with survival benefit, particularly in cases of HR >0.7, where almost all RCTs have not shown any survival benefit of the tested drug.

**Evidence-based knowledge for systemic therapies in HCC**

The treatment of advanced HCC has been limited to sorafenib for almost a decade. The panorama of HCC therapy is constantly evolving, and now a plethora of first- and second-line therapies are available, either as monotherapy or in combination with different agents, including immunotherapies. Here we discuss the available options and how they continuously evolving.

**Single-agent, targeted, first-line therapies.** The seminal SHARP trial was a placebo-controlled, double-blinded study that randomized 602 patients to sorafenib or placebo. This was the first systemic therapy approved as a result of improvements in OS (10.7 versus 7.9 months). The magnitude of effect was confirmed in an Asia–Pacific trial. Sorafenib has been widely used globally, and subsequent studies suggested that it is more effective in liver-only disease, in HCV etiology and in patients with a low neutrophil/lymphocyte ratio. The initial recommended dose is 800 mg per day, but it may be reduced (30% of cases) or withdrawn (in 10–15% of cases) due to treatment-related adverse events (Table 3) —particularly hand–foot skin reaction, a feature that has been associated with better outcomes. Treatment-related death is less than 2%. In patients with Child–Pugh B, it is not well tolerated and leads to median OS of 5–6 months.

A second era of first-line studies started after the approval of sorafenib, lenvatinib, a multikinase inhibitor blocking FGFR1–FGFR4 (Fig. 2) was compared to sorafenib in the open-label REFLECT trial with a non-inferiority design and demonstrated comparable efficacy with an HR of 0.92 and median OS of 13.6 versus 12.3 months. The treatment dose is 8 or 12 mg daily depending on body weight (above or below 60 kg). The most common adverse events are hypertension, weight loss and fatigue, leading to treatment reduction in ~40% of patients and withdrawal in ~10% of patients. Subgroup analysis yielded better outcomes for lenvatinib in patients with high tumoral burden, aggressive disease and HBV infection. Lenvatinib is currently being tested in phase III in combination with pembrolizumab in patients at intermediate stages and in first line at advanced stages (Fig. 3).

Other regimes tested in first line resulted in negative results, such as brivanib (a selective VEGFR and FGFR TKI), sunitinib (a multi-target TKI with activity against VEGFRs, platelet-derived growth factor receptors (PDGFRs) and KIT) and linifanib (a VEGFR and PDGFR TKI), as well as the combinations of sorafenib with erlotinib (an epidermal growth factor receptor (EGFR) inhibitor), doxorubicin, pravastatin or TACE. The reasons behind these negative results are reviewed elsewhere. Systemic doxorubicin treatment resulted in a lack of survival benefits and was discarded from the treatment armamentarium of advanced HCC.

The STAHL trial tested the combination of TACE and sorafenib compared to sorafenib alone in advanced HCC in Asian patients and did not meet its primary endpoint, survival (9.3 versus 9.4 months, HR of 0.91). Only the SoraHAIC open-label trial reported superior efficacy of the combination of sorafenib and hepatic intra-arterial chemotherapy of oxaliplatin, fluorouracil and leucovorin (that is, FOLFOX) compared to sorafenib alone in advanced HCC with portal vein invasion. This treatment regime, which led to a significant increase in OS from 7.1 months to 13.3 months (HR, 0.35), has not been adopted by Western guidelines due to methodological concerns. Finally, the phase III trials SARAH and SiReNIB testing transarterial radioembolization with yttrium-90 for first-line advanced HCC reported negative results compared to sorafenib treatment. Based on these results, transarterial radioembolization is not recommended as an alternative to systemic therapy in the advanced setting.

**Second-line targeted therapies.** In second-line advanced HCC, regorafenib improved OS compared to placebo in the randomized, phase III RESORCE trial. Regorafenib is a multikinase inhibitor but with a broader range of angiogenic (including tunicam interna endothelial cell kinase 2 (TIE2)) and oncogenic targets than sorafenib (Fig. 2). A key eligibility criterion was the requirement for prior treatment with first-line sorafenib at a dosage of at least 400 mg per day for at least 20 days, which selected for patients with increased likelihood of tolerating regorafenib. In these patients, regorafenib improved OS beyond placebo with an HR of 0.63 (95% confidence interval, 0.50–0.79) and median OS of 10.6 versus 7.8 months (P<0.0001). The sequential treatment strategy of sorafenib followed by regorafenib yielded an OS of 26.0 months compared to 19.0 months for sorafenib followed by placebo. The most common grade 3 or 4 adverse events for regorafenib are hypertension, hand–foot skin reaction, fatigue and diarrhea. In a retrospective multicenter analysis, regorafenib was associated with higher rates of grade 3 or 4 adverse events and shorter OS and PFS in patients with Child–Pugh B hepatic dysfunction than in patients with Child–Pugh A (Table 3).

Cabozantinib is another multikinase inhibitor targeting anti-angiogenic pathways, MET, AXL, TYRO3 and MER, members of a family of proteins that contribute to a suppressed tumor immune microenvironment (Fig. 2). In the randomized, phase III CELESTIAL trial, cabozantinib improved OS over placebo for patients who had received one or two prior systemic therapies for HCC, with an HR of 0.76 and median OS of 10.2 months by comparison to 8.0 months for placebo (P=0.0055). Cabozantinib also prolonged PFS compared to placebo with median OS of 5.2 months and 1.9 months, respectively (HR, 0.44). The most frequent grade 3 or 4 adverse events were hand–foot skin reaction, hypertension, elevated transaminase levels, fatigue and diarrhea. Finally, although the VEGFR2-targeted antibody ramucirumab did not improve survival compared to placebo in an unselected patient population, ramucirumab improved OS over placebo in patients with elevated AFP levels (>400 ng ml) after progression on sorafenib. The median OS was 8.5 versus 7.3 months for ramucirumab and sorafenib, respectively (HR, 0.71). The most common grade 3 or higher adverse events were hyponatraemia and hypertension.
These positive clinical trials of regorafenib, cabozantinib and ramucirumab were preceded by a multitude of negative studies in first-line and second-line treatment settings. The success of the more recent trials is credited to these agents’ distinct inhibitor profiles but may also reflect contributions from more favorable therapeutic indices and evolving supportive care for underlying hepatic dysfunction.

ICI monotherapies. Single-agent ICIs targeting PD1 were evaluated in advanced HCC and showed a safety profile similar to that in other solid tumors. In the phase I–II trials CheckMate 040 and KEYNOTE-224, nivolumab and pembrolizumab resulted in an ORR ranging from 14% to 20% respectively. The confirmatory phase III trials for both agents failed to show a statistically significant improvement in OS. The KEYNOTE-240 trial compared pembrolizumab to best supportive care in second-line treatment after sorafenib therapy with median OS of 13.9 versus 10.6 months, but the pre-specified significant P value was not reached. Nonetheless, the Asian phase III trial comparing pembrolizumab to placebo (KEYNOTE-394; ref. 33) has yielded positive OS outcomes with a similar magnitude of benefit. In CheckMate 459, a randomized study of nivolumab versus sorafenib in first-line HCC, the median OS was 16.4 months versus 14.7 months, respectively, with an HR of 0.85 (ref. 135). While both phase III studies suffered from some statistical design limitations and from cross-over to ICI treatment in the control arm, an important conclusion was that single-agent ICIs may not have sufficient activity to show significant improvements in median OS in an unselected population.

In CheckMate 040, there was an association between membrane expression of PD-L1 on ≥1% of tumor cells and OS in the overall trial population. In a subset of 37 patients, associations with ORR or OS were noted for seven of ten evaluated inflammatory gene signatures. A recent study also identified a gene signature able to predict response to either nivolumab or pembrolizumab. If validated, such emerging biomarkers may be used for patient selection in the future.

ICI combination with anti-VEGF antibody. There is strong scientific rationale to combine ICIs with targeted therapies or other immune-oncology agents. Anti-angiogenic therapies targeting VEGF ligands can mitigate the local immunosuppressive effects of VEGF signaling and promote T-cell infiltration. Effectively, combining bevacizumab, a monoclonal antibody targeting VEGFA, with the anti-PD-L1 inhibitor atezolizumab (Fig. 2) demonstrated safety and ORRs of 36% of patients in a large phase Ib study, leading to the positive randomized, open-label, sorafenib-controlled phase III IMBrave150 trial of this combination with co-primary endpoints of OS and PFS. This regimen represents a paradigm shift in the management of HCC due to the absolute gains in survival and has become a new standard of care for first-line treatment of advanced HCC. This study was halted at the interim analysis due to positive results favoring the combination arm, with an HR of 0.58 (ref. 136). Follow-up mature survival analysis confirmed the survival benefit with a median of 19.2 months for the combination arm compared to 13.2 months for sorafenib. The trial required performance of an upper gastrointestinal endoscopy within the 6 months before randomization to exclude the presence of high-risk esophageal and/or gastric varices, given the increased risk of bleeding associated with bevacizumab. The co-primary endpoint of PFS was also positive, with an HR of 0.59. In addition, patient-reported outcomes were significantly better for the combination compared to sorafenib alone (time to deterioration, 11.2 months versus 3.6 months, respectively). Finally, the ORR was significantly better in the combination arm (27%–33% versus 12–13%). Thirty percent of patients treated with atezolizumab and bevacizumab experienced durable objective responses, including 8% with confirmed complete responses. The most common grade 3 and 4 adverse event was hypertension with rare events of bleeding in the study population.

Combinations of ICls with TKls. Multikinase inhibitors with anti-angiogenic activity as well as a diverse array of other kinase targets also hold the potential to modulate the tumor immune microenvironment in varying ways that could augment response to ICls. In a large phase Ib study, the combination of lenvatinib with pembrolizumab achieved objective responses in ~40% of patients, with median PFS of 8.6 months and OS of ~22 months. These findings prompted the ongoing randomized phase III trial of this combination compared to lenvatinib monotherapy (LEAP-002). Another ongoing approach is the combination of cabozantinib with atezolizumab. The interim analysis of the randomized phase III trial COSMIC-312, comparing the efficacy of cabozantinib plus atezolizumab versus sorafenib, revealed a significant improvement in PFS (HR, 0.63) but not in OS. Other combinations of targeted therapies and ICls are being investigated in earlier-phase trials for advanced stages of HCC (Table 2 and Fig. 3).

Immu-oncology combinations. Co-targeting CTLA4 synergizes with anti-PD1 activity through regulation of T-cell activation in lymph nodes and tissues. Preclinical studies have shown that anti-CTLA4 inhibition results in expansion of an ICOS’ helper T-cell-like CD4+ effector population in addition to engaging specific subsets of exhausted-like CD8+ T cells. In HCC, the combination of nivolumab and ipilimumab has shown promising efficacy with an ORR of 32% and median OS of 22.8 months in second-line therapy, which resulted in accelerated approval by the FDA. There were no new safety signals, but the higher dose of ipilimumab was associated with increased frequency of immune-mediated events. A phase III trial (CheckMate 9DW) of the combination of nivolumab and ipilimumab versus sorafenib or lenvatinib is ongoing. A similar promising signal of activity was seen in study 22 of durvalumab (anti-PD-L1 therapy) with a single loading dose of tremelimumab (anti-CTLA4 therapy); the combination resulted in an ORR of 24% and median OS of 18.7 months, along with a manageable safety profile. More recently, the phase III HIMALAYA trial has shown that durvalumab with a single, high priming dose of tremelimumab is able to significantly improve OS versus sorafenib as a first-line treatment (HR, 0.78; 16.4 versus 13.8 months).

Selection of first-line therapies and treatment sequencing. In principle, if a given treatment is not available or contraindicated for a specific BCLC stage (for instance, TACE for intermediate HCC), systemic treatment is recommended (Fig. 3). This concept is known as treatment stage migration. A bigger challenge is how to sequentially apply different systemic therapies. Among the systemic regimens approved (Figs. 2 and 3 and Table 2), only a few were compared face to face, and none of the approved single agents has been explored after progression with atezolizumab plus bevacizumab.

There is a general agreement that the standard of care in first-line advanced HCC is atezolizumab plus bevacizumab (Figs. 2 and 3). There are some restrictions for the use of this combination according to the inclusion criteria reported in the phase III trial: Child–Pugh class A and ECOG PS 0–1, in the absence of other organ or hematologic dysfunction, autoimmune disease, active co-infection with HCV or HBV, or untreated varices. Specifically, an upper gastrointestinal endoscopy (within 6 months prior) is required to discard high-risk varices. If present, endoscopic band ligation is recommended. If this decision is taken, it is advised to start systemic treatment after ~2–6 weeks according to institutional guidelines. In case of untreated varices, durvalumab plus tremelimumab can be considered. Other major contraindications are prior liver transplantation and treatment with immunosuppressive drugs due to the risk of graft rejection. In all these circumstances that are estimated to affect ~20% of patients, the treatment of choice in first line should be either sorafenib or lenvatinib. A recent meta-analysis concluded that immune therapies are more effective for viral
### Table 4 | Selected ongoing phase I–III trials for advanced HCC

| Agent(s) (targets) | Primary endpoint | Line of treatment | Phase | Sample size | NCT |
|--------------------|------------------|-------------------|-------|-------------|-----|
| **ICI combinations with targeted therapies** |
| Pembrolizumab (PD1), lenvatinib (VEGFR1–VEGFR3, PDGFR, FGFR1–FGFR4, RET) | OS, PFS | First | III | 750 | NCT03713593 |
| Atezolizumab (PD-L1), cabozantinib (VEGFR1–VEGFR3, MET, RET) | OS, PFS | First | III | 740 | NCT03755791 |
| AK105 (PD1), anlotinib (VEGFR1–VEGFR3, FGFR1–FGFR4, PDGFR, KIT receptor) | OS | First | III | 648 | NCT04344158 |
| Camrelizumab (PD1), apatinib (VEGFR2) | OS, PFS | First | III | 510 | NCT03764293 |
| Tislelizumab (PD1), lenvatinib (VEGFR1–VEGFR3, PDGFR, FGFR1–FGFR4, RET) | ORR | First | II | 66 | NCT04401800 |
| Pembrolizumab (PD1), sorafenib (VEGFR1–VEGFR3, PDGFR, RAF kinase, KIT receptor) | ORR, MTD | First | II | 12 | NCT03439891 |
| Pembrolizumab (PD1), regorafenib (VEGFR1–VEGFR3, PDGFR, RAF kinase, FGFR1, FGFR2) | ORR | Second | II | 119 | NCT04696055 |
| Atezolizumab (PD-L1), sorafenib (VEGFR1–VEGFR3, PDGFR, RAF kinase, KIT receptor), lenvatinib (VEGFR1–VEGFR3, PDGFR, FGFR1–FGFR4, RET) | OS | Second | III | 554 | NCT04770896 |
| PDR001 (PD1), INC280/capmatinib (MET) | ORR, DLT | Second | I/II | 90 | NCT02795429 |
| Tislelizumab (PD1), sitravatinib (TYRO3, AXL, MERTK, VEGFR2, KIT receptor, MET) | ORR, incidence of AEs/SAEs, Refractory to standard therapies | First or second | I/II | 104 | NCT03941873 |
| **ICI combinations with other ICIs** |
| Durvalumab (PD-L1) plus tremelimumab (CTLA4) | OS | First | III | 1,504 | NCT03298451 |
| Pembrolizumab (PD1) plus ipilimumab (CTLA4) | ORR | First | III | 650 | NCT04039607 |
| Pembrolizumab (PD1), relatlimab (LAG-3) | ORR | Second | II | 250 | NCT04567615 |
| **Triplet combinations involving ICIs and targeted therapies** |
| Atezolizumab (PD-L1), bevacizumab (VEGFA), tiragolumab (TIGIT), tocilizumab (IL-6R), SAR439459 (TGF-β), TPST-1120 (PPAR-α), RO7247669 (PD1, LAG-3) | ORR | First | I/II | 280 | NCT04524871 |
| Pembrolizumab (PD1), quavonlimab (CTLA4), lenvatinib (VEGFR1–VEGFR3, PDGFR, FGFR1–FGFR4, RET) | ORR, DLT, incidence of AEs/SAEs, hepatic AEs, discontinuation due to AEs | First | II | 110 | NCT04740307 |
| Pembrolizumab (PD1), ipilimumab (CTLA4), cabozantinib (VEGFR1–VEGFR3, MET, RET) | ORR, incidence of AEs/SAEs | First or second | I/II | 1,097 | NCT01658878 |
| **New immunologic targets** |
| Voyager V1 (VSV oncolytic virus), cemiplimab (PD1) | ORR | Second | II | 152 | NCT04291105 |
| Talimogene laherparepvec (T-VEC, HSV oncolytic virus), pembrolizumab (PD1) | DLT, ORR | Second | I/II | 206 | NCT02509507 |
| GNOS-PVO2 (personalized neoantigen), INO-9012 (IL-12), pembrolizumab (PD1) | Incidence of AEs, immunogenicity | Second | I/II | 24 | NCT04251117 |
| ET140203 T cells (AFP) | Incidence of AEs, DLTs, RP2D | Third+ | I/II | 50 | NCT04502082 |
| ECT204 T cells (GPC3) | Incidence of AEs, DLTs, RP2D | Third+ | I/II | 12 | NCT04864054 |
| **Other targeted therapies** |
| Icaritin (stem cells) | OS | First | III | 200 | NCT033236649 |
| CVM-1118 (vascular mimicry), sorafenib (VEGFR1–VEGFR3, PDGFR, RAF kinase, KIT receptor) | ORR | TKI naive | II | 40 | NCT03582618 |
| Sorafenib (VEGFR1–VEGFR3, PDGFR, RAF kinase, KIT receptor), YIV-906 (unknown) | PFS | First | II | 125 | NCT04000737 |

Continued
etologies than for non-viral etiologies\textsuperscript{11,189}. Collectively, these data suggest that differences in the tumor microenvironment, likely etiology related, can impact response to systemic therapies and underscore the importance of clinical annotation and stratification for the etiology of liver disease in clinical trials for HCC.

The main controversy is how to sequence therapies after progression to atezolizumab plus bevacizumab due to the lack of phase III investigations assessing the efficacy of second-line therapies in this scenario. Most updated guidelines support the view that sorafenib or lenvatinib should be offered first, thus maintaining the previously established evidence-based hierarchy before atezolizumab–bevacizumab becoming the first-line preferred treatment\textsuperscript{11,189,199}. The most valuable clinical variables for decision making are the magnitude of clinical benefit in OS; then PFS or ORR, patient comorbidities, patient quality of life and the drug adverse event profile; and finally local availability and/or reimbursement. A summary of these factors is detailed in Tables 2 and 3 to facilitate decision making. Reimbursement plays an important role in certain regions, and, in the absence of evidence that any agent is superior, sorafenib is the most commonly offered second-line agent after atezolizumab–bevacizumab\textsuperscript{190}. Following progression to lenvatinib or sorafenib, conventional second-line therapies can be administered. Specifically, regorafenib is indicated in patients that tolerate sorafenib, whereas cabozantinib and ramucirumab were assessed following progression to sorafenib, the latter indicated only in patients with AFP levels $>400$ ng ml\textsuperscript{-1}. There are no head-to-head comparisons between regorafenib, cabozantinib or ramucirumab, and their reported response rates after tyrosine kinase inhibition are similar\textsuperscript{11,17}. Dose modifications and grade 3 adverse events were reported less frequently for ramucirumab compared to other agents, indicative that ramucirumab may be better tolerated in older patients with cirrhosis or ECOG PS $>0$ (ref. \textsuperscript{3}). Pembrolizumab is approved by the FDA and can be considered in second-line scenarios in the United States, particularly if adverse events and comorbidities might be detrimental with other agents. The role of durvalumab plus tremelimumab in second line needs to be established. There are not enough data to recommend a specific therapy for patients with liver dysfunction (Child–Pugh B class).

New therapeutic strategies. Most therapeutic strategies in phase II–III trials involve ICIs in combination with TKIs, other ICIs or triplet combinations including all of the above (Table 4). Nonetheless, new therapeutic approaches are being explored in the setting of phase I–II investigations. The advent of single-cell genomic technologies\textsuperscript{191} has been instrumental to improve cell taxonomy\textsuperscript{152}, assess cellular interactions\textsuperscript{192}, and decipher cell–cell interactions\textsuperscript{193}. This has revealed that, for instance, cancer-associated fibroblasts (CAFs) are critical in tumor progression or are involved in chemoresistance by sustaining stemness in cancer\textsuperscript{194,195}. Clinical trials of new agents targeting cancer stem cells, such as icaritin and DKN-01, are ongoing for HCC (Table 4).

Tumor-infiltrating lymphocytes (TILs) are highly heterogeneous, and as many as 11 subsets of unique subpopulation of CD8$^+$FOXP3$^+$ cells have been identified using scRNA-seq and single-cell T-cell receptor sequencing in HCC\textsuperscript{196}. The degree of TIL burden correlates with predicted tumor neoantigen distribution\textsuperscript{197}, which suggests interaction(s) between cancer and cytotoxic immunity\textsuperscript{198}. Given that most biomarker studies use single-tissue biopsies as source material, these intratumoral differences in TIL burden could interfere with biomarker discovery and validation. This was addressed in a comprehensive multidimensional study of a small cohort of 12 patients with HCC\textsuperscript{199}. Integrated transcriptomic and immunohistochemistry data demonstrated that most patients (60–70%) had consistent signals in terms of immune activation throughout different areas of the same tumor nodule. A variety of approaches, including oncolytic viruses coupled with immune checkpoint inhibition as well as personalized neoantigen vaccines, are being studied to induce lymphocyte infiltration in the tumor microenvironment (Table 4). Single-cell technologies have been applied to study mechanisms of resistance in HCC, including patients with paired biopsies before and after treatment with combined durvalumab and tremelimumab\textsuperscript{197}.

Finally, a leading candidate target for both peptide vaccines and engineered T-cell receptor or chimeric antigen receptor (CAR) T-cell therapies is glyppican 3, a cell surface glycoprotein overexpressed in over 70% of HCC but marginally expressed in the cirrhotic liver\textsuperscript{191,196}. AFP is another candidate target for both vaccine and T-cell therapies based on its high expression prevalence of around 50% in advanced HCC, without significant expression in non-tumor liver\textsuperscript{191,196}. Current ongoing clinical trials testing CAR T-cell immunotherapy, T-cell receptor-engineered T cells, CAR natural killer cells or HCC vaccines, among others, have been extensively reviewed elsewhere\textsuperscript{190} (Table 4).

Future directions

There is a high expectation of the impact of ongoing phase III studies on clinical decision making for the next years at all stages of the disease (Fig. 2). Neoadjuvant and adjuvant therapies in HCC are still an unmet need, and future studies will explore their utility in depth. These advancements will have implications for the composition of multidisciplinary teams, as the presence of experts in managing systemic therapies will be routinely requested for management of early stages of HCC. In addition, there is a need to identify biomarkers predicting response to single ICIs or combinations. Post hoc analysis of PD-L1 expression did not predict response to single-agent
ICIs, while gene signatures are in need of further validation. Liquid biopsy has emerged as a non-invasive technology for biomarker discovery in HCC. Although there are reports correlating mutation and copy-number-alteration analysis of circulating tumor DNA with HCC tissue, further research is needed to validate these biomarkers for surveillance or treatment allocation. Finally, from the regulatory and reimbursement perspective, studies addressing the cost effectiveness of sequential expensive therapies would need to be considered. Overall, there is an expected shift in the landscape of management that should be accompanied by the identification of biomarkers to guide precision oncology and to adapt trial design and endpoints to the new clinical scenarios.

Received: 27 September 2021; Accepted: 25 February 2022; Published online: 28 April 2022

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Acknowledgements

We thank M. Piqué and F. Castet, members of J.M.L.’s laboratory, for their support in the production of this manuscript. J.M.L. is supported by grants from Cancer Research UK, the Fondazione AIRC and the Fundación Científica de la Asociación Española Contra el Cáncer (HUNTER, reference C9380/A26813), the NIH (RO1DK56621 and RO1DK128289), the Samuel Waxman Cancer Research Foundation, EIT Health (CRISH2, reference 18053), the Generalitat de Catalunya (AGAUR, SGR-1358), the Spanish National Health Institute (MICINN, FID2019-10537/RYC-100) and the Acadèmia de Ciències Mèdiques i de la Salut de Catalunya i de Balears (reference BECA_ACADEMIA21_001). X.W. is supported by grants (Z01 BC 010877, Z01 BC 010876, Z01 BC 010313 and ZIA BC 011870) from the intramural research program of the Center for Cancer Research, National Cancer Institute of the US.

Competing interests

J.M.L. received research support from Bayer HealthCare Pharmaceuticals, Eisai, Boehringer Ingelheim and Ipsen and consulting fees from Merck, Eli Lilly, Eisai, Bayer HealthCare Pharmaceuticals, Bristol Myers Squibb, Ipsen, Genentech, Roche, GlaxoSmithKline, Nucleix, Omega Therapeutics, Ilyon, Mina Alpha, Boston Scientific and AstraZeneca. H.L.R. has acted in an advisory capacity for Boston Scientific and SirteX, and received speaker fees from Eisai and Bayer. A.V. has received consulting fees from Boehringer Ingelheim, FirstWorld, Natera, Cambridge Healthcare Research and Genentech; advisory board fees from Bristol Myers Squibb, Gilead and NGM Pharmaceuticals; and research support from Eisai. R.K.K. received research support from Agios, AstraZeneca, Bayer, Bristol Myers Squibb, Eli Lilly, EMD Serono, Exelixis, Genentech–Roche, Merck, Partner Therapeutics, Novartis, QED, Relay Therapeutics, Surface Oncology and Taiho; and consulting and/or advisory fees from Exact Sciences, Genentech–Roche and Gilead. A.E.-K. has received research support from Astex, AstraZeneca and Fulgent; advisory or consulting fees from Bayer, Bristol Myers Squibb, Eisai, Merck, Exelixis, AstraZeneca, Roche–Genentech, Agenus, ABL Bio, QED, Gilead, CytoX, Pieris and EMD Serono. G.J.G., X.W. and R.P. have no competing interests.

Additional information

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Peer review information Nature Cancer thanks Michel Ducruex, Jan Tchorz and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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