Molecular therapies and precision medicine for hepatocellular carcinoma

Josep M. Llovet[1,2]*, *Robert Montal*[2], *Daniela Sia*[1] *and Richard S. Finn*[3]

Abstract | The global burden of hepatocellular carcinoma (HCC) is increasing and might soon surpass an annual incidence of 1 million cases. Genomic studies have established the landscape of molecular alterations in HCC; however, the most common mutations are not actionable, and only ~25% of tumours harbour potentially targetable drivers. Despite the fact that surveillance programmes lead to early diagnosis in 40–50% of patients, at a point when potentially curative treatments are applicable, almost half of all patients with HCC ultimately receive systemic therapies. Sorafenib was the first systemic therapy approved for patients with advanced-stage HCC, after a landmark study revealed an improvement in median overall survival from 8 to 11 months. New drugs — lenvatinib in the frontline and regorafenib, cabozantinib, and ramucirumab in the second line — have also been demonstrated to improve clinical outcomes, although the median overall survival remains ~1 year; thus, therapeutic breakthroughs are still needed. Immune-checkpoint inhibitors are now being incorporated into the HCC treatment armamentarium and combinations of molecularly targeted therapies with immunotherapies are emerging as tools to boost the immune response. Research on biomarkers of a response or primary resistance to immunotherapies is also advancing. Herein, we summarize the molecular targets and therapies for the management of HCC and discuss the advancements expected in the near future, including biomarker-driven treatments and immunotherapies.

Liver cancer is the second leading cause of cancer-related death globally[1]. Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancers and can be caused by chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), alcohol abuse, and metabolic syndrome related to diabetes and obesity[2,3]. In developed countries, surveillance programmes lead to early HCC diagnosis in 40–50% of patients, at a stage amenable to potentially curative treatments[2,4,5]. Patients with intermediate-stage HCC are treated with locoregional therapies, whereas those with advanced-stage disease can benefit from systemic treatments[2]. Overall, ~50% of patients receive systemic therapies at some point during the disease course[2,4,5]. In a breakthrough study[6], the multi-target tyrosine kinase inhibitor (TKI) sorafenib, which has anti-angiogenic and anti-proliferative effects, extended the median overall survival of patients with advanced-stage HCC from 8 to 11 months and had a manageable toxicity profile. Sorafenib was the sole systemic therapy approved for the treatment of HCC between 2007 and 2016. In the past year or so, however, improvements in patient outcomes have been demonstrated in randomized phase III trials with lenvatinib[7] in the frontline and regorafenib[8], cabozantinib[9], and ramucirumab[10] in the second line after disease progression on sorafenib; regorafenib is currently FDA approved in the second-line setting. In addition, immunotherapy with nivolumab — a monoclonal antibody targeting the inhibitory immune-checkpoint molecule programmed cell death protein 1 (PD-1) — led to promising response rates and survival durations in a phase I–II study involving patients previously treated with sorafenib[11] and has been granted accelerated approval by the FDA. By contrast, several kinase inhibitors (for example, sunitinib, brivanib, and erlotinib), doxorubicin, and radioembolization with yttrium 90 (^{90}Y) -microspheres failed to improve overall survival in patients with unresectable HCC[12].

Indeed, HCC is a highly therapy resistant and thus difficult to treat cancer; although systemic therapies have clinical benefits, the improvements in patient outcomes have been modest and incremental. Thus, novel therapies for HCC remain an unmet medical need. In this regard, important insights into the biology of the disease have been obtained through genomic, transcriptomic, and epigenomic studies[2,3]. In this Review, we analyse the molecular targets and therapies for the management of HCC and highlight the advancements in biomarker-driven treatments and immunotherapies that are expected in the near future.

[1]*Mount Sinai Liver Cancer Program, Division of Liver Diseases, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA.*

[2]*Liver Cancer Translational Lab, Barcelona Clinic Liver Cancer (BCLC) Group, Liver Unit, Hospital Clinic Barcelona, IDIBAPS, University of Barcelona, Barcelona, Spain.*

[3]*Department of Medicine, Division of Hematology/Oncology, Geffen School of Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, USA.*

**e-mail: Josep.Llovet@mssm.edu*

https://doi.org/10.1038/s41571-018-0073-4

Key points

- The global incidence of hepatocellular carcinoma (HCC) is increasing and might reach 1 million cases per year during the next decade.
- Next-generation sequencing studies have established the landscape of molecular aberrations associated with HCC; although the most common mutations (in the *TERT* promoter, *CTNNB1*, and *TP53*) are not clinically actionable, ~25% of HCCs harbour potentially targetable driver alterations.
- In phase III studies, survival benefits for patients with advanced-stage HCC have been demonstrated with five systemic therapies: sorafenib and lenvatinib in the first-line setting and regorafenib, cabozantinib, and ramucirumab in the second-line setting. Promising results have also been obtained with nivolumab in phase II studies in the second-line setting.
- Prolonging the outcome of patients with advanced-stage HCC to beyond 1 year is an unmet medical need; refining the identification of patients with tumours responsive or intrinsically resistant to immunotherapy and optimizing combinations with molecularly targeted therapies are major avenues for research.
- Proof-of-concept and biomarker-based trials of molecularly targeted agents should be implemented in both intermediate-stage and advanced-stage disease settings.

The molecular landscape of HCC

Molecular drivers

HCC development is a complex multistep process, with 70–80% of cases occurring in the context of established liver cirrhosis[2,3]. The natural history of HCC in patients with cirrhosis progresses through a sequence of clinicopathological events starting with the appearance of pre-cancerous cirrhotic nodules (so-called dysplastic nodules), which can ultimately transform into HCC[3]. Overall, one-third of patients with cirrhosis will develop HCC during their lifetime, with different rates per year observed according to aetiology[2]. The median time between development of cirrhosis and the development of HCC is ~10 years[13]. In the non-cirrhotic liver, HCC can arise principally on a background of HBV infection or nonalcoholic steatohepatitis and more rarely through the malignant transformation of hepatocellular adenoma, a monoclonal and typically benign lesion[14]. Malignant transformation from adenomas occurs in <10% of cases and has been associated with *TERT* and *CTNNB1* mutations[14]. Mature hepatocytes have been identified as the cell of origin for most HCCs; however, a subset of ~20% of HCCs with progenitor cell markers, such as epithelial cell adhesion molecule (EPCAM) and cytokeratin 19 (CK19), can arise from either progenitor cells or dedifferentiated mature hepatocytes[15].

HCC results from the accumulation of somatic genomic and epigenomic alterations in the tissue of origin over time. In HCCs, an average of 40–60 somatic alterations are detected in protein-coding regions of the genome[2,16]. Most of these alterations occur in 'passenger' genes that are not directly implicated in neoplasia, but a few genomic alterations are considered to be 'drivers' involved in activating key signalling pathways for hepatocarcinogenesis. The identification of recurrently mutated genes and copy number alterations through integration of data from whole-exome sequencing (WES) studies and single-nucleotide polymorphism (SNP) array analyses has enabled deciphering of these pivotal pathways, which include telomere maintenance, cell cycle control, WNT–β-catenin signalling, chromatin modification, receptor tyrosine kinase (RTK)–RAS–PI3K cascades, and oxidative stress[16–21] (TABLE 1). Unfortunately, most of the clonal, 'trunk' mutations and prevalent drivers (*TERT*, *CTNNB1*, *TP53*, *AXIN1*, *ARID1A*, and *ARID1B*) detected in HCCs are not clinically actionable[16] — at least at present. Indeed, reports of WES studies indicate that only ~25% of HCCs harbour alterations that are potentially targetable with existing drugs[16]. DNA methylation profiling also enabled the discovery of *IGF2* overexpression and *CDKN2A* silencing as epigenetic mechanisms of HCC tumorigenesis[22].

Molecular classifications

Integrative molecular analyses involving genomic, transcriptomic, and/or epigenomic profiling of thousands of surgically resected tumours have provided the basis for the molecular classification of HCC subtypes[21,23–26]. These distinct molecular classes reflect different biological backgrounds with potential implications in patient prognostication and selection for therapies. Specifically, two major molecular subtypes of HCC, each encompassing ~50% of patients with this disease, have been proposed: a proliferation class and non-proliferation class[3,27,28] (FIG. 1).

As their designation suggests, HCCs of the proliferation class are characterized by activation of signalling pathways involved in cell proliferation and survival, such as the PI3K–AKT–mTOR, RAS–MAPK, and MET cascades[21,23,24]. Chromosomal instability seems to be a driving force in these tumours, with a particular enrichment of *TP53* inactivation and *FGF19* and/or *CCND1* amplifications[29]. Our group and others[3,27,28] have proposed that two subclasses exist within the proliferative class: a WNT–TGFβ group (also known as S1 tumours) characterized by non-canonical activation of WNT; and a progenitor cell group (also known as S2 tumours) characterized by overexpression of *EPCAM*, *AFP*, and *IGF2*, and a unique DNA hypermethylation signature[30] (FIG. 1a). Overall, the proliferation class of HCC is associated with HBV-related aetiology and poor clinical outcomes.

The non-proliferation class is more heterogeneous than the proliferative class and might consist of at least three HCC subclasses[3,21] (FIG. 1). One clear subclass has been delineated and is characterized by activation of the canonical WNT signalling pathway, often owing to mutation of *CTNNB1* (encoding β-catenin)[31], and is also associated with higher rates of *TERT* promoter mutations. From the clinical standpoint, non-proliferation class tumours are associated with alcohol-related and HCV-related aetiologies and better outcomes.

These proposed molecular classes have been confirmed and further characterized in the comprehensive molecular analysis of 363 patients with HCC — the largest cohort published to date — reported by The Cancer Genome Atlas (TCGA) Research Network[18]. The integration of up to 5 other platforms — DNA copy number, DNA methylation, mRNA expression, microRNA (miRNA) expression, and reverse phase protein array (RPPA) assays — for 196 tumours yielded 3 subtypes, including a poor prognosis iClust1 subtype with a gene expression profile that closely resembles that of the progenitor cell subclass tumours and a lower-grade

Table 1 | **Recurrent somatic genetic alterations detected in HCCs**

Altered pathway	Altered gene	Effect of alteration	Percentage (range)
Mutations			
Telomere maintenance	*TERT* promoter	Activating	54 (44–59)
Cell cycle control	*TP53*	Loss of function	28 (23–31)
	ATM	Loss of function	4 (2–5)
	RB1	Loss of function	4 (3–5)
	CDKN2A	Loss of function	2 (1–3)
WNT–β-catenin signalling	*CTNNB1*	Activating	29 (23–36)
	AXIN1	Loss of function	7 (5–10)
	APC	Loss of function	2 (1–3)
Chromatin modifiers	*ARID1A*	Loss of function	8 (4–12)
	ARID2	Loss of function	7 (3–10)
	KMT2A	Loss of function	3 (0–4)
	KMT2B	Loss of function	3 (0–4)
	KMT2C	Loss of function	3 (2–5)
	BAP1	Loss of function	2 (0–5)
	ARID1B	Loss of function	1 (1–3)
RTK–RAS–PI3K signalling	*RPS6KA3*	Unclassified	4 (3–6)
	PIK3CA	Activating	2 (1–4)
	KRAS	Activating	1 (0–1)
	NRAS	Activating	1 (0–1)
	PDGFRA	Unclassified	1 (0–2)
	EGFR	Activating	1 (0–2)
	PTEN	Loss of function	1 (0–2)
Oxidative stress	*NFE2L2*	Activating	4 (3–6)
	KEAP1	Activating	3 (2–5)
Hepatocyte differentiation	*ALB*	Unclassified	9 (5–13)
	APOB	Unclassified	8 (1–10)
JAK–STAT signalling	*IL6ST*	Unclassified	2 (1–3)
	JAK1	Unclassified	1 (0–3)
TGFβ signalling	*ACVR2A*	Loss of function	3 (1–5)
IGF signalling	*IGF2R*	Unclassified	1 (0–2)
Copy number alterations			
Cell cycle control	*MYC*	High-level focal amplification	12 (4–18)
	CCND1	High-level focal amplification	7 (5–7)
	CDKN2A	Homozygous deletion	5 (4–6)
	RB1	Homozygous deletion	5 (4–6)
	TP53	Homozygous deletion	2 (0–2)
RTK–RAS–PI3K signalling	*VEGFA*	High-level focal amplification	5 (1–8)
	FGF19	High-level focal amplification	6 (5–6)
Telomere maintenance	*TERT*	High-level focal amplification	5 (1–6)

Mutation frequencies are reported for a total of 1,289 patients included in multiple whole-exome sequencing studies; *TERT* promoter mutations were assessed using Sanger sequencing ($n = 1,213$ patients). Copy number alterations were detected using single-nucleotide polymorphism (SNP) arrays ($n = 704$ patients). HCC, hepatocellular carcinoma; RTK, receptor tyrosine kinase; STAT, signal transducer and activator of transcription.

iClust2 subtype that shares molecular and pathological characteristics (for example, *CTNNB1* mutations and less frequent microvascular invasion) with the non-proliferation class. The third TCGA cluster, iClust3, generated a *TP53* signature associated with chromosomal instability and poor prognosis.

Beyond tumour cell-intrinsic molecular aberrations, an altered tumour microenvironment (TME) is now

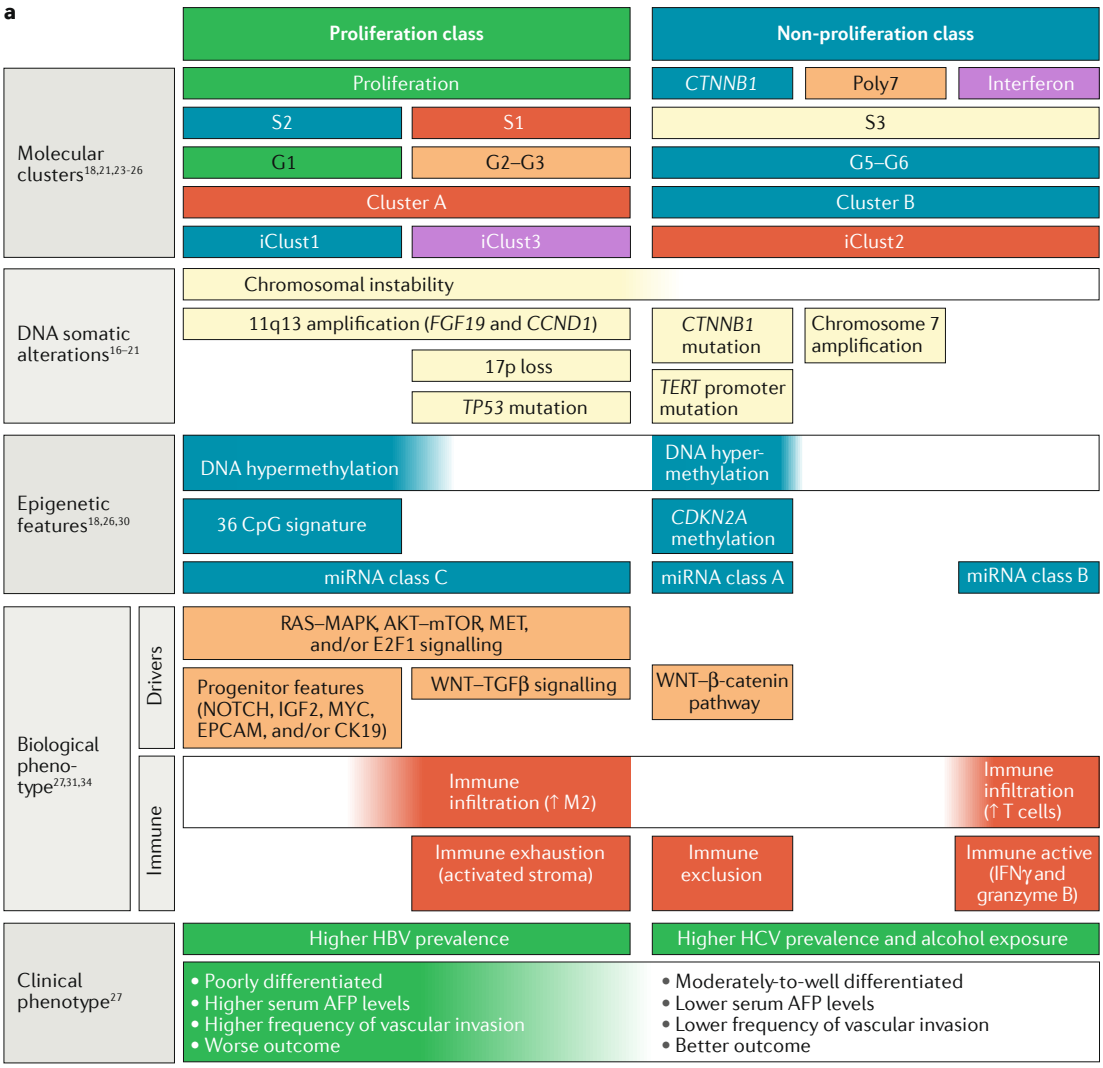
a
Proliferation class
Non-proliferation class
Molecular clusters[18,21,23–26]
Proliferation
CTNNB1
Poly7
Interferon
S2
S1
S3
G1
G2–G3
G5–G6
Cluster A
Cluster B
iClust1
iClust3
iClust2
DNA somatic alterations[16–21]
Chromosomal instability
11q13 amplification (FGF19 and CCND1)
CTNNB1 mutation
Chromosome 7 amplification
17p loss
TERT promoter mutation
TP53 mutation
Epigenetic features[18,26,30]
DNA hypermethylation
DNA hyper-methylation
36 CpG signature
CDKN2A methylation
miRNA class C
miRNA class A
miRNA class B
Biological pheno-type[27,31,34]
Drivers
RAS–MAPK, AKT–mTOR, MET, and/or E2F1 signalling
Progenitor features (NOTCH, IGF2, MYC, EPCAM, and/or CK19)
WNT–TGFβ signalling
WNT–β-catenin pathway
Immune
Immune infiltration (↑ M2)
Immune infiltration (↑ T cells)
Immune exhaustion (activated stroma)
Immune exclusion
Immune active (IFNγ and granzyme B)
Clinical phenotype[27]
Higher HBV prevalence
Higher HCV prevalence and alcohol exposure
• Poorly differentiated
• Higher serum AFP levels
• Higher frequency of vascular invasion
• Worse outcome
• Moderately-to-well differentiated
• Lower serum AFP levels
• Lower frequency of vascular invasion
• Better outcome
b
HCC immune classes
Immune class (~30% of HCCs)
Immune intermediate class (45% of HCCs)
Immune excluded class (~25% of HCCs)
Immune subtypes
Active immune (~20% of HCCs)
Exhausted immune (~10% of HCCs)
Gene expression and enrichment for signatures
↑ T cells, cytotoxic cells, TLS, macrophages, and PD-1 signalling,
↓ T cells, B cells, and cytotoxic cells
IFNγ, GZMB, and PRF1
Activated stroma
↑ PTK2
Signatures of response to immunotherapy
TGFβ
CCL4
T cell exhaustion
DNA structural alterations
• Copy number variations
• Mutations
↓ Chromosomal aberrations
↑ Chromosomal aberrations
CTNNB1
Protein immunohistology
↑ Immune cell infiltration, PD-1–PD-L1+, and TLS
↓ Immune cell infiltration, PD-1–PD-L1−, and TLS
Epigenetic aberrations
192 immune-related genes differentially methylated

Fig. 1 | **Integrative molecular and immunological classification of HCC.** **a** | Hepatocellular carcinomas (HCCs) can be classified into two major transcriptome-based phenotypic classes that are also associated with characteristic somatic genetic alterations, epigenetic features, biological phenotypes (activated oncogenic and immune signalling pathways), and clinical characteristics. First, the proliferation class, which is associated with a poor prognosis, chromosomal instability, and activation of classic oncogenic signalling pathways (such as the RAS–MAPK and AKT–mTOR pathways). Data from genomic profiling studies indicate that a subset of tumours within the proliferation class might have a progenitor cell phenotype (S2) characterized by high levels of α-fetoprotein (AFP), overexpression of epithelial cell adhesion molecule (EPCAM), cytokeratin 19 (CK19), and/or IGF2 and a unique hypermethylation profile (36 CpG signature)[30]. The other subset of proliferation class tumours (S1) is defined by activated WNT–TGFβ signalling and an immune exhausted tumour microenvironment. Second, the non-proliferation class tumours, which have a less aggressive course with slower disease progression and thus a better prognosis than proliferation class tumours; a subset of non-proliferation class tumours (*CTNNB1*) is characterized by WNT–β-catenin pathway activation, predominantly via *CTNNB1* mutation. The poly7 and interferon subclasses need to be further characterized[21]. **b** | Immune-based classification of HCCs according to the immune status in the tumour microenvironment is shown. This novel classification defines three tumour classes on the basis of molecular data and immune-related parameters: the immune class, the immune intermediate class and the immune excluded class, each of which might require different immunotherapy approaches tailored to the immune microenvironment. E2F1, transcription factor E2F1; HBV, hepatitis B virus; HCV, hepatitis C virus; M2, M2-like macrophages; miRNA, microRNA; NOTCH, neurogenic locus NOTCH homologue protein; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1; TLS, tertiary lymphoid structures.

recognized as a key enabling factor in the development of HCC[32,33]. In fact, HCC is a prototypical inflammation-associated cancer attributable to viral hepatitis or steatohepatitis (alcoholic or nonalcoholic). Multiple cell types interact with hepatocytes in the chronically inflamed liver, including lymphocytes, macrophages, stellate cells, and endothelial cells. In this regard, a novel molecular classification of HCC based upon immune status has been proposed[34] (FIG. 1b). Through analyses of inflammatory gene-expression profiles, infiltrates, and regulatory molecules, 30% of HCCs could be classified into an 'immune class', with high levels of immune cell infiltration, expression of PD-1 and/or programmed cell death 1 ligand 1 (PD-L1), activation of IFNγ signalling, markers of cytolytic activity (such as granzyme B and perforin 1), and an absence of *CTNNB1* mutations[34]. Within this class, two distinct 'active immune' and 'exhausted immune' subclasses, characterized by markers of an adaptive T cell response or exhausted immune response, respectively, have been identified[34]. The exhausted immune tumours express many genes regulated by TGFβ, which mediate immunosuppression and T cell exhaustion. An 'immune excluded class' accounting for ~25% of HCCs was characterized by T cell exclusion from the TME and *CTNNB1* mutations[34]. The immune exhausted class mostly overlaps with the proliferative WNT–TGFβ subclass, whereas the immune excluded class overlaps with the *CTNNB1* mutated non-proliferative class. Our group is currently exploring whether the immune active class is associated with responsiveness to immune-checkpoint inhibitors and whether, conversely, the immune exhausted and/or the immune excluded classes are associated with primary resistance to these agents.

Clearly, further research is needed to translate the current knowledge of HCC biology into prognostic and predictive biomarkers in order to guide clinical decision-making and, ultimately, improve patient outcomes. In this regard, analysing the molecular landscape of tumour tissues obtained from patients with advanced-stage HCC, predominantly through tumour-tissue and liquid biopsy procedures, is of crucial relevance because these are the patients who are actually treated with systemic therapies in clinical trials. Notably, the fact that systemic drugs with demonstrated survival benefits in patients with HCC (sorafenib, regorafenib, lenvatinib, cabozantinib, and ramucirumab) share an — at least partially — anti-angiogenic mechanism of action highlights the importance of this hallmark of cancer, which is mainly promoted by endothelial cells[35]. Indeed, angiogenic signalling is prominent in all subclasses of HCC[36,37]. Understanding how the distinct angiogenic signalling pathways interact with the immune component of HCCs and how mechanisms of resistance to anti-angiogenic agents arise could potentially reveal novel therapeutic strategies.

Clinical management of HCC

Several HCC staging systems have been proposed during the past four decades[38–41]; however, the Barcelona Clinic Liver Cancer (BCLC) staging classification is the most widely recognized clinical algorithm used for patient stratification and treatment allocation[4,5,42]. As mentioned previously, in developed countries, 40–50% of patients with HCC are diagnosed at early stages (BCLC stage 0–A), when potentially curative treatments (resection, liver transplantation, or local ablation) are possible[4]. These treatments can result in median overall survival durations >60 months[4]. Nevertheless, up to 70% of patients undergoing HCC resection or ablation present with disease recurrence within 5 years[2], and no adjuvant therapies tested to date are able to prevent this complication[43]. Patients with intermediate-stage disease (BCLC stage B) with preserved liver function (Child–Pugh class A without any ascites) can benefit from transarterial chemoembolization (TACE), as reported in two randomized studies comparing this approach with best supportive care[44,45] and one meta-analysis[46], with estimated median overall survival durations of 25–30 months. No combination of kinase inhibitors (such as sorafenib or brivanib)[47–49] with TACE has been shown to provide additive improvements in patient outcomes. Nevertheless, most patients with HCC (>50%) will eventually receive systemic treatments: patients with disease progression after TACE or those who are diagnosed with advanced-stage HCC (BCLC stage C) can benefit from sorafenib[6]. More recently, first-line lenvatinib[7] and second-line regorafenib[8], cabozantinib[9], and ramucirumab[10] have also been demonstrated to provide survival benefits for patients with advanced-stage disease. In clinical trials, the median overall survival durations achieved with these therapies are around 1 year. Nivolumab is another new option in the second-line setting on the basis of the promising response rates and durations observed in the phase I–II trial of this agent[11]. Patients with end-stage disease (BCLC stage D) should be considered for nutritional and psychological support and appropriate management of pain. In 2018,

international guidelines[4] have been revised to provide updated recommendations on the treatment of HCC based on levels of evidence, encompassing all major treatments tested in this cancer (FIG. 2).

Molecular targeted therapies

First-line treatments

Most patients with HCC are diagnosed at advanced-disease stages, at which the natural history of the disease carries a dismal prognosis. In this setting, conventional systemic chemotherapy lacks survival benefits. Phase III trials of doxorubicin alone, the PIAF regimen (cisplatin, IFNα2b, doxorubicin, and fluorouracil), and the FOLFOX4 regimen (fluorouracil, leucovorin (folinic acid), and oxaliplatin) all had negative results, in some instances with substantial toxicity[50–52]. Randomized studies also failed to prove any clinical effects of anti-oestrogen therapies or vitamin D derivatives[53,54].

Sorafenib. In 2007, results of the phase III SHARP trial[6] demonstrated survival benefits with sorafenib versus placebo (median overall survival 10.7 months versus 7.9 months; HR 0.69, 95% CI 0.55–0.87; $P < 0.001$), thus representing a breakthrough in the management of advanced-stage HCC. A similar magnitude of benefit was observed in another phase III study of sorafenib conducted in parallel in Asian patients, mostly with HBV-related HCC[55]. In these trials, treatment was generally associated with manageable adverse events (AEs), such as diarrhoea (grade 3 in 8–9%), hand–foot skin reactions (grade 3 in 8–16%), fatigue (grade 3 in 3%), and hypertension (grade 3 in 2%). Intolerance to sorafenib (treatment discontinuation owing to AEs) typically occurs in 10–15% of patients[6,55]. The severity of toxicities — particularly hand–foot syndrome — has been associated with better survival outcomes in cohort studies[56]. A meta-analysis of the two phase III trials testing sorafenib revealed a consistent survival benefit across all clinical subgroups[57]. The greatest magnitude of the benefit was observed in patients with tumour confined to the liver, those who were HCV-positive, or those with a low neutrophil-to-lymphocyte ratio[57].

Sorafenib is indicated for patients with well-preserved liver function (Child–Pugh class A) and BCLC stage C disease or BCLC stage B disease that has progressed after locoregional therapy. Of note, the median overall survival of patients with BCLC stage B HCC treated with sorafenib is 15–20 months according to the findings of post-marketing studies[58,59]. Similarly, surveys conducted in >3,000 patients to evaluate the safety and tolerability

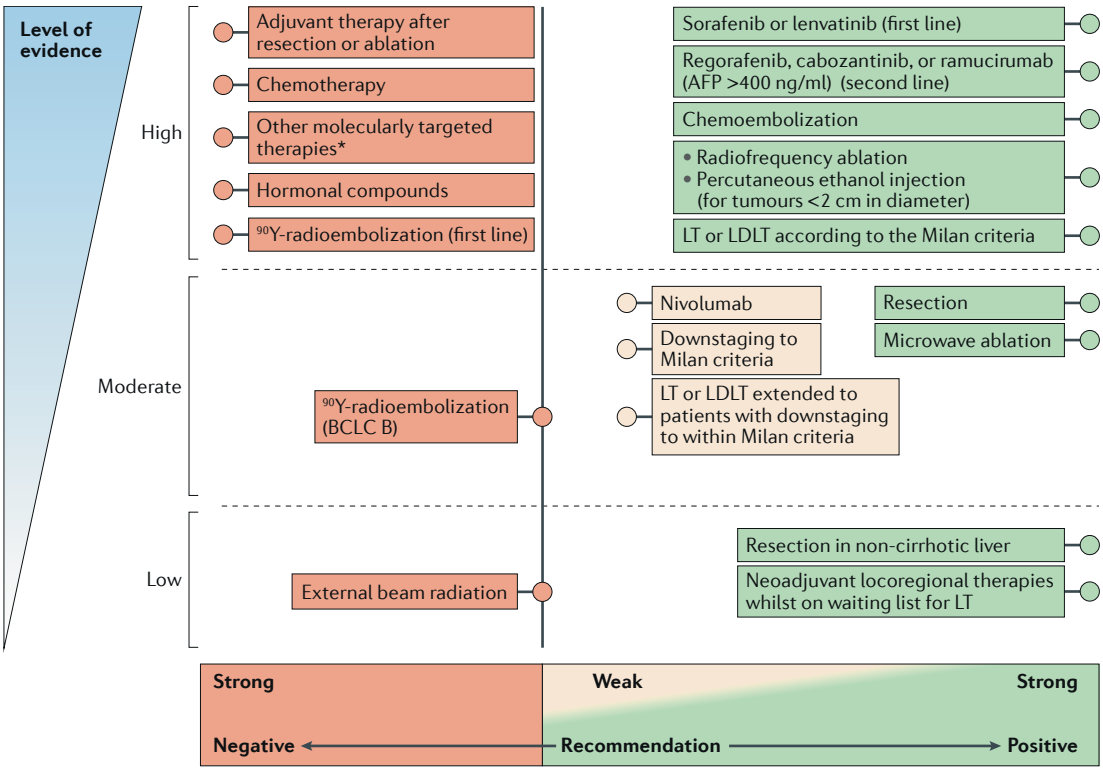

Fig. 2 | **Hepatocellular treatments recommended in international EASL guidelines[4].** Treatment recommendations from the European Association for the Study of the Liver (EASL) international guidelines are illustrated according to levels of evidence and strength of recommendation (on the basis of adaptation of the GRADE system)[4]. Treatments endorsed in the international guidelines (strong positive recommendation) are shown in green[4,5]. Treatments for which more evidence is needed (weak positive recommendations) are shown in orange, whereas those not endorsed (strong negative recommendation) are shown in red. The Milan criteria for liver transplantation are a single tumour ≤5 cm or three nodules ≤3 cm in diameter. AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; LDLT, living donor liver transplantation; LT, orthotopic liver transplantation. *Other molecularly targeted therapies include sunitinib, linifanib, brivanib, tivantinib, erlotinib, and everolimus. Figure adapted with permission from REF.[4], Elsevier.

of sorafenib in clinical practice reported median overall survival durations of 13.6 months for the Child–Pugh class A group and 5.2 months for a Child–Pugh class B group[60,61].

From the mechanistic standpoint, the efficacy of sorafenib probably results from a balance between targeting cancer cells and cells of the TME: this agent can inhibit up to 40 kinases, including mainly angiogenic RTKs (including VEGF receptors (VEGFRs) and PDGF receptor-β (PDGFRβ)) and drivers of cell proliferation (such as RAF1, BRAF, and KIT)[62]. Unfortunately, at least partially owing to this pharmacological complexity, no predictive biomarkers of a response to sorafenib have been identified; however, the companion biomarker study conducted within the SHARP trial showed a nonsignificant trend towards a greater survival benefit of sorafenib in patients with tumours harbouring high levels of KIT and low plasma HGF concentrations[63].

The efficacy of sorafenib in the advanced-stage setting has led to testing of this drug at earlier clinical stages. In the phase II SPACE and phase III TACE 2 placebo-controlled trials involving patients with intermediate-stage HCC[47,48], sorafenib plus TACE was safe, but the combination did not improve time to progression (TTP) in a clinically meaningful manner. Similarly, in the adjuvant setting after surgical resection or local ablation (phase III STORM trial)[43], sorafenib did not improve recurrence-free survival (RFS) compared with that observed with placebo. A thorough molecular analysis of resected tumours from this trial enabled the design of a multi-gene signature that could be used to identify patients who benefited from adjuvant sorafenib treatment[64]; however, this biomarker test requires prospective validation.

The successful SHARP trial[6] provided a framework for trial design that has been implemented in subsequent phase III studies[65]. The main traits of this design are the selection of an adequate target population: patients with well-preserved liver function (Child–Pugh class A), to minimize the risk of liver failure and death as a result of cirrhosis, and patients with either advanced-stage (BCLC stage C) or intermediate-stage (BCLC stage B) disease that has progressed following TACE, to provide clear results for this clinical stage. Moreover, overall survival was established as the most robust end point to assess efficacy in this population. Surrogate end points, such as TTP, have been associated with inconsistent results and are currently being revisited[12]. In this regard, use of the modified Response Evaluation Criteria in Solid Tumors (mRECIST), which are based on the concept of viable tumour, generally provides greater sensitivity in the assessment of response than the standard RECIST guidelines[66]; in phase III trials of sorafenib, objective response rates (ORRs) were 10–15% by mRECIST versus 2–6% by RECIST[67].

Several phase III trials have failed to demonstrate the superiority of a number of agents over sorafenib in the frontline setting (FIG. 3a). These therapies include brivanib (a selective VEGFR and FGF receptor (FGFR) TKI)[68], sunitinib (a multi-target TKI with activity against VEGFRs, PDGFRs, and KIT)[69], linifanib (a VEGFR and PDGFR TKI)[70], and erlotinib (an EGFR inhibitor)[71]. The reasons for the disappointing phase III trial results include overinterpretation of marginal antitumour efficacy in small phase II studies, considerable liver toxicity, flaws in trial design, and the lack of biomarker-based enrichment[12].

Moreover, the results of the phase III SARAH[72] and SIRveNIB[73] superiority trials of internal radiation with ^{90}Y resin microspheres versus sorafenib in patients with advanced-stage HCC (including >30% with main portal vein thrombosis) did not fulfil the primary overall survival end points. In these studies[72,73], median overall survival was 8.0–8.8 months in the ^{90}Y-microsphere arms compared with 9.9–10.0 months in the sorafenib arms, resulting in nonsignificant detriments in survival with radioembolization (HR 1.12–1.15) (FIG. 3a). Per-protocol subgroup analyses did not reveal any survival advantages[72,73]. The authors of both trials highlighted the better response rates and quality of life (QOL) outcomes with radioembolization, thus suggesting this treatment as an alternative to sorafenib for selected patients. However, the indication of a therapy should be based upon the primary end point; therefore, the conclusion that the frontline treatment strategy can be decided on the basis of secondary end points is not sound. In addition, QOL outcomes typically have a negative correlation with time on therapy, which is clearly longer with sorafenib versus the one-time treatment with ^{90}Y-microsphere radioembolization. Two additional phase III trials (STOP-HCC and SORAMIC) comparing combinations of ^{90}Y glass microspheres plus sorafenib versus sorafenib alone have been initiated (NCT01556490 and NCT01126645). Preliminary results from the SORAMIC trial presented in abstract form in April 2018 indicate that this combination does not improve survival[74].

Lenvatinib. Lenvatinib, an oral inhibitor of the VEGFRs, FGFR1–FGFR4, RET, KIT, and PDGFRα[75], has been tested in phase II and phase III trials in patients with advanced-stage HCC[7,76]. In the phase III trial[7], lenvatinib was found to be non-inferior to sorafenib in terms of overall survival (median 13.6 months versus 12.3 months; HR 0.92, 95% CI 0.79–1.06) (FIG. 3a). Importantly, the ORR in the lenvatinib group according to mRECIST was 24.1% when evaluated by investigators but reached 40.6% (versus 18% by RECIST) upon masked independent imaging review[7]. Of note, patients with ≥50% liver occupation, obvious invasion of the bile duct, and/or invasion at the main portal vein were excluded from this study[7]. In a subgroup analysis, patients with baseline serum α-fetoprotein (AFP) levels of >200 ng/ml had a greater benefit from lenvatinib than sorafenib (HR 0.78, 95% CI 0.63–0.98). The frequency of grade ≥3 treatment-related AEs was higher with lenvatinib than with sorafenib (57% versus 49%). The most common treatment-emergent AEs of any grade associated with lenvatinib were hypertension (42%), diarrhoea (39%), decreased appetite (34%), and decreased bodyweight (31%); 9% and 7% of patients treated with lenvatinib and sorafenib, respectively, discontinued treatment owing to treatment-related AEs. Fatal AEs related to lenvatinib treatment, including hepatic failure, cerebral haemorrhage, and respiratory

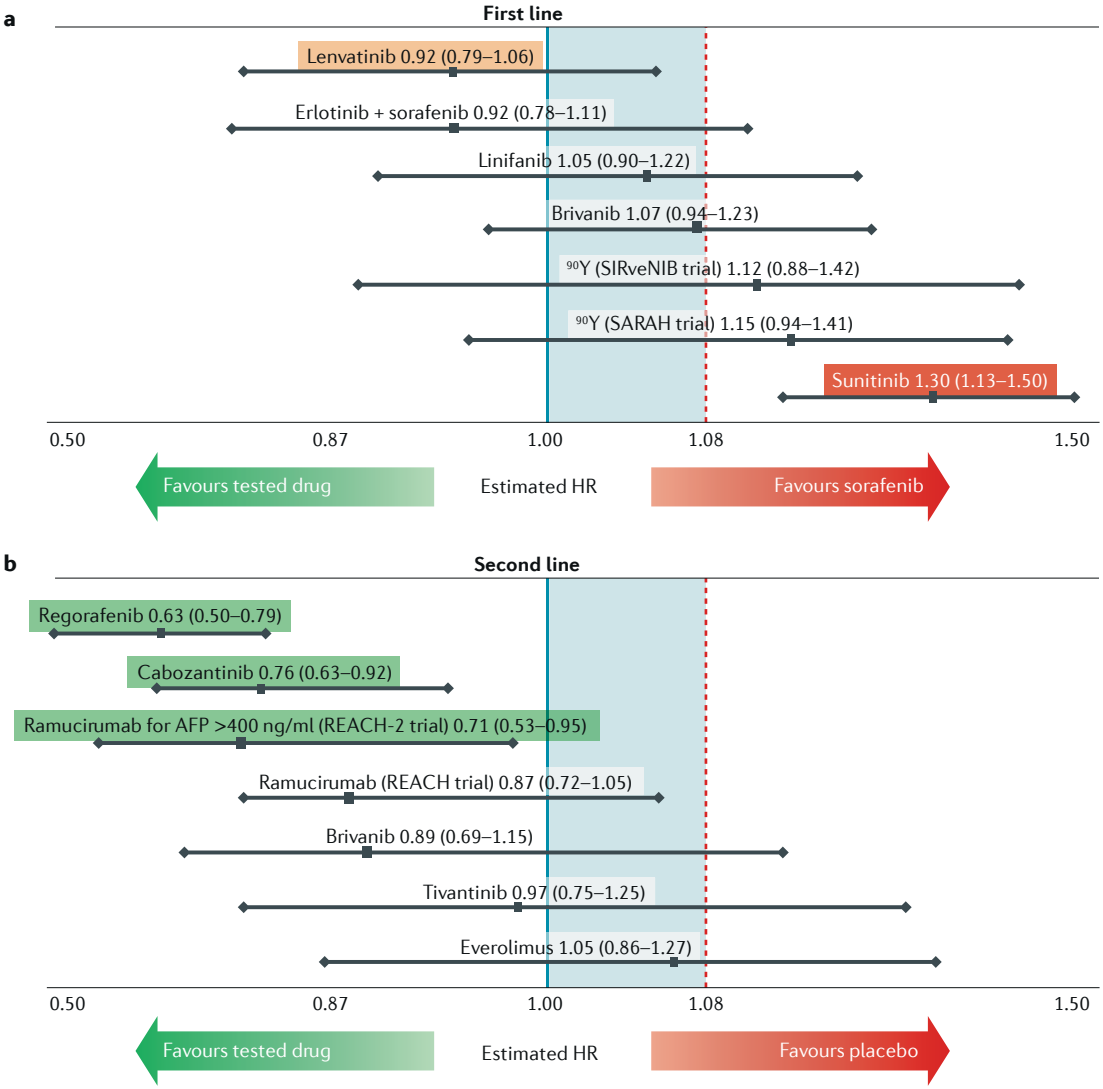

Fig. 3 | **Overall survival outcomes of phase III clinical trials testing molecularly targeted therapies or radioembolization with ^{90}Y in patients with advanced-stage HCC.** The figure illustrates the estimated overall survival hazard ratios (HRs) and 95% confidence intervals (in parentheses) for the experimental drug (or combination) versus either sorafenib in the first-line setting (part **a**) or placebo in the second-line setting (part **b**). Green-shaded text indicates positive results from trials with a superiority design. The orange-shaded text indicates a positive result from a trial with a non-inferiority design. Black text with no shading and red-shaded text represent negative results with an HR confidence interval crossing or not crossing 1, respectively. Second-line treatment with ramucirumab did improve overall survival when tested in patients with high serum α-fetoprotein (AFP) levels (≥400 ng/ml)[10]. The blue lines and red hashed lines indicate the upper limits for superiority and non-inferiority, respectively. HCC, hepatocellular carcinoma.

failure, occurred in 2% of patients versus 1% of patients in the sorafenib arm.

On the basis of these results, lenvatinib can be considered as an alternative first-line treatment option to sorafenib for patients with advanced-stage HCC (except those with main portal vein thrombosis or >50% liver involvement) or intermediate-stage disease after progression following TACE; FDA and European Medicines Agency (EMA) approvals are pending. Data from QOL studies suggest a similar overall profile for both drugs[7]. No cost-effectiveness studies comparing both drugs have been reported to date. Similarly, no biomarkers predicting responses to either agent have been reported.

Second-line therapies

Since the approval of sorafenib in 2007, perhaps the largest unmet clinical need for patients with HCC has been in the second-line setting after disease progression on sorafenib. With therapies that improve overall survival without inducing high ORRs, such as sorafenib, identifying patients who are no longer benefiting from treatment is inherently challenging owing to difficulties in relating radiographic tumour measurements with clinical outcomes. Furthermore, in the pivotal phase III SHARP trial of sorafenib[6], patients were allowed to remain on treatment beyond radiological progression, ultimately adding additional layers of complexity. The decision to move novel therapies into phase III trials in the

second-line setting has generally been based on findings from single-arm studies with small cohorts of patients; ultimately, most of the randomized phase III trials did not meet their end points, including studies of agents targeting the mTOR[77], VEGF[78] and/or FGF[79], or HGF–MET[80] signalling pathways (FIG. 3b). Since 2017, however, we have witnessed the reporting of positive results from three phase III trials in patients who had disease progression on, or were intolerant of, sorafenib[8–10], as well as promising data from two phase II studies of different anti-PD-1 antibodies[11,81]. The results of these studies are now providing the clinicians with a number of second-line treatment options in the absence of comparative studies. Thus, treatment choices will need to be based on the sound data that are available and clinical judgement. Given the increasingly rapid pace of approvals, data on sequencing of the available agents is also lacking. As in other diseases, clinical factors that can influence second-line treatment choices include the first-line therapy used, the duration of response to that therapy, how treatment was tolerated, the clinical condition of the patient upon progression, and the expected efficacy and AEs of the available treatments.

Regorafenib. Regorafenib has structural similarities to sorafenib, but the inhibitory profiles of these drugs differ slightly, with regorafenib having greater potency against the VEGFR kinases and a broader activity, for example, against angiopoietin 1 receptor (TIE2), KIT, and RET[82]. A small, single-arm phase II study of regorafenib provided some evidence of antitumour activity in the second-line setting[83]; however, the efficacy signals were not dissimilar from those obtained with other agents studied in this space. Nevertheless, the data led to the first positive phase III trial in patients with advanced-stage HCC for nearly a decade and the subsequent FDA approval of second-line regorafenib. The results of this global trial (RESORCE)[8] demonstrated an improvement in the median overall survival of patients who had HCC progression on sorafenib from 7.8 months with placebo to 10.6 months with regorafenib (HR 0.63, 95% CI 0.50–0.79; $P < 0.0001$) (FIG. 3b). Unlike other studies in this setting[77–80], this trial required that patients not only have documented progression on sorafenib (according to RECIST) but also to have tolerated sorafenib for a minimum period of time (≥400 mg daily for at least 20 of the 28 days before discontinuation)[8]. Regorafenib also significantly improved secondary end points, including TTP (HR 0.44, 95% CI 0.36–0.55; $P < 0.0001$) and progression-free survival (PFS) (HR 0.46, 95% CI 0.37–0.56; $P < 0.0001$). ORRs were higher with regorafenib versus placebo by both mRECIST and RECIST (10.6% versus 4.1% and 6.6% versus 2.6%, respectively). A subsequent evaluation of overall survival from the start of sorafenib treatment to death on study demonstrated a median duration of 26 months for regorafenib-treated patients versus 19 months for those in the placebo arm[84]. Toxicities were manageable in this sorafenib-tolerant population and were similar to those observed with sorafenib, including hand–foot skin reaction, diarrhoea, and hypertension.

Given the similarities between the two molecules, the exact mechanism of the benefit from regorafenib after progression on sorafenib is not clear. Besides continued suppression of VEGFR signalling and anti-angiogenic effects, regorafenib has been hypothesized to directly inhibit pathways regulating tumour cell growth, proliferation, and metastasis and to modify the TME[82].

Cabozantinib. Cabozantinib is a small-molecule multi-target TKI with an inhibitory profile that is unique among the molecules evaluated in phase III studies in patients with HCC to date; in addition to activity against VEGFRs, this drug also potently inhibits MET and AXL[85,86]. Of note, the HGF receptor MET has been implicated in the pathogenesis of HCC and sorafenib resistance[87]. Cabozantinib was initially evaluated in both patients with untreated HCC and those with progression on, or intolerance of, sorafenib in a randomized phase II discontinuation study, resulting in an overall median PFS of 5.5 months without substantial radiographical responses (2 of 41 patients had a partial response)[86]. CELESTIAL[9] was a global, randomized, placebo-controlled, phase III trial of cabozantinib in patients who had HCC progression on prior sorafenib. Unlike in other studies, patients who had received up to two prior therapies for advanced-stage HCC were eligible for enrolment in CELESTIAL[9]. This trial was stopped after a second interim analysis of data from the entire study population revealed a median overall survival of 10.2 months in the cabozantinib group versus 8.0 months in the placebo group (HR 0.76, 95% CI 0.63–0.92; $P = 0.0049$) (FIG. 3b). Approximately 72% of patients had received only prior sorafenib treatment, and in this subpopulation, median overall survival was 11.3 months with cabozantinib versus 7.2 months with placebo (HR 0.70, 95% CI 0.55–0.88)[9]. Cabozantinib did not have a notable ORR (4% by RECIST), but did improve PFS and TTP[9]. AEs with cabozantinib were as seen in earlier studies of this agent; the most frequent grade 3–4 AEs were hand–foot syndrome (in 17% of patients) and hypertension (in 16%)[9]. Six grade 5 treatment-related AEs occurred with cabozantinib versus one with placebo[9].

Ramucirumab. Unlike the small-molecule TKIs discussed so far, ramucirumab is an antagonistic anti-VEGFR2 monoclonal antibody. On the basis of encouraging activity observed in a pilot study[88], ramucirumab was compared with placebo in the phase III REACH trial involving patients with advanced-stage HCC and prior sorafenib treatment[78]. The study was negative for its primary end point of overall survival in the intention-to-treat population, although a subgroup of patients with a baseline serum AFP levels ≥400 ng/ml had a significant improvement in median overall survival from 4.2 months with placebo to 7.8 months with ramucirumab (HR 0.67, 95% CI 0.51–0.90; $P = 0.006$). This observation paved the way for a second phase III trial of ramucirumab in the second-line setting (REACH-2; NCT02435433), this time incorporating biomarker-based enrichment for patients with baseline AFP concentrations ≥400 ng/ml. Results of this trial were reported in abstract form at the 2018 ASCO Annual Meeting[10] and indicate a superior median overall

survival duration of 8.5 months with ramucirumab versus 7.3 months with placebo (HR 0.71, 95% CI 0.53– 0.95; $P=0.0199$) (FIG. 3b) and a manageable safety profile (grade ≥3 hypertension and hyponatraemia in 12.2% and 5.6%, respectively). Thus, ramucirumab becomes the first agent with a demonstrated clinical benefit for a biomarker-selected population of patients with HCC. AFP is a plasma glycoprotein that is produced in the liver, predominantly during early fetal development, but also in few tumour types, including HCC, hepatoblastoma, and non-seminomatous germ cell tumours of the ovary and testis[89]. Of note, ~40% of patients with advanced-stage HCC have serum levels of AFP ≥400 ng/ml, and this feature is associated with poor prognosis[63]. Some studies have linked high AFP levels with higher microvessel densities and VEGFA expression in HCCs[90].

Immune-checkpoint inhibitors. The impact of treatments targeting immune checkpoints on oncology practice cannot be overstated: agents that target cytotoxic T lymphocyte protein 4 (CTLA-4), PD-1, or its ligand PD-L1 have revolutionized the management of many tumour types. A detailed description of the therapeutic mechanisms is beyond the scope of this Review, but in general, they involve blockade of negative feedback pathways of the immune system that mediate immunosuppression in the setting of malignancies[91,92]. For example, CTLA-4 is constitutively expressed in regulatory T cells but is also upregulated in cytotoxic T cells after T cell priming and is a dominant negative signalling molecule[93]. Monoclonal antibodies to CTLA-4, such as ipilimumab and tremelimumab, have been proven to block this negative feedback response and can lead to deep and durable responses in patients with cancer[93]. Similarly, PD-1 is a receptor expressed by T cells that provides negative regulatory signals predominantly during the effector phase of T cell responses. In the context of cancer pathogenesis, PD-1 on T cells can engage with its two known ligands, PD-L1 and PD-L2, in the TME to suppress anticancer immunity[94]. Monoclonal antibodies to either PD-1 (nivolumab and pembrolizumab) or PD-L1 (atezolizumab, avelumab, and durvalumab) are approved for the treatment of various malignancies[95].

HCC develops in an inflammatory milieu, and various studies have revealed a role for immune tolerance in the development of this cancer[96], hinting at the potential of immune-checkpoint inhibition as an effective treatment strategy. Results of an initial phase II study of tremelimumab in a small cohort of patients with advanced-stage HCC ($n=20$) demonstrated an ORR of 17.6% and a median TTP of 6.5 months[97]. Despite these signs of clinical efficacy, some safety concerns were raised, owing to transient but substantial increases in serum transaminase levels[97]. Notably, however, 43% of the patients enrolled had Child–Pugh class B liver disease[97].

More recently, nivolumab has been demonstrated to have single-agent activity in the much larger CheckMate 040 trial population, including patients with or without prior exposure to sorafenib[11]. In the phase I–II CheckMate 040 study[11], a total of 262 eligible patients were treated with nivolumab, including 48 in the dose-escalation phase and another 214 in the dose-expansion cohort. Considering all patients included in the dose-expansion phase, the investigator-assessed ORR was 20%, with 3 complete responses and 39 partial responses[11]. Most impressive, though, was the duration of response of 9.9 months among the patients who had an objective response[11]. Overall survival for patients in the second-line setting was 15.6 months[98]. Given the unmet needs in the second-line setting, the FDA granted accelerated approval to nivolumab for patients with advanced-stage HCC previously treated with sorafenib on the basis of the efficacy and safety data reported for a subpopulation comprising 154 sorafenib-treated patients included in CheckMate 040. In this subgroup, the ORR confirmed through blinded independent central review was 14.3% by RECIST 1.1 and 18.2% by mRECIST and the median duration of response was 16.6 months[99]. The toxicity data from the second-line population of CheckMate 040 seems manageable, with the most frequent AEs being fatigue, musculoskeletal pain, pruritus and rash, and diarrhoea. Treatment-emergent grade 3–4 AEs included elevations in serum aspartate transaminase (AST), alanine transaminase (ALT), and bilirubin levels in 18%, 11%, and 7% of patients, respectively[99]. Importantly, no patient had on-treatment hepatic failure, and only 11% of patients had to discontinue treatment owing to AEs. As for other indications, patients with HCC need to be monitored closely during immune-checkpoint inhibition, as this class of agents can affect essentially any organ system. A confirmatory open-label, randomized phase III trial comparing sorafenib to nivolumab in the frontline setting is ongoing (CheckMate 459; NCT02576509); patient accrual is complete and the results are eagerly awaited.

Pembrolizumab seems to have similar activity to nivolumab in patients with HCC. In KEYNOTE-224 (REF.[100]), a single-arm study of pembrolizumab for second-line treatment after frontline sorafenib, the ORR in 104 patients was 16.3%, including 1 complete response and 16 partial responses, and median overall survival was 12.9 months. Toxicities included fatigue, AST elevations, diarrhoea, and itching; seven patients discontinued treatment owing to AEs[81]. Longer-term follow-up data from this study are awaited, as are the results of KEYNOTE-240, a randomized, placebo-controlled phase III trial of pembrolizumab[101].

Durvalumab, an anti-PD-L1 monoclonal antibody, has also been tested in a phase I–II trial that included a dose-expansion cohort of patients with HCC[102]. In this study[102], durvalumab had an acceptable safety profile and demonstrated antitumour activity (ORR 10%).

The challenges to the development of immune-checkpoint inhibitors in patients with HCC are similar to those faced with other targeted therapies, most importantly, relating to the identification of predictive biomarkers of response. In other malignancies, several biomarkers have been proposed, including PD-L1 and/or PD-1 expression by immunohistochemistry (IHC)[103], a high tumour mutational burden[104], and tumour T cell infiltration[105]. To date, data presented on nivolumab and pembrolizumab therapy for HCC have not shown any correlation between PD-L1 expression or underlying aetiology of cirrhosis and clinical benefit[11,106].

The FDA has approved pembrolizumab for the treatment of microsatellite instability-high or mismatch repair-deficient advanced-stage cancers. This indication is agnostic to tumour histology and therefore includes HCC; however, the incidence of these defects in HCC is estimated to be low (~3%)[107].

Combination strategies. The development of systemic therapies for HCC continues to benefit from knowledge gained in other tumour types. Combined CTLA-4 and PD-1 or PD-L1 blockade has been shown to improve survival outcomes, most notably in patients with melanoma[108]. In HCC, this approach is now being pursued in a phase III trial of durvalumab in combination with tremelimumab in the frontline setting (NCT03298451). The control arms of this trial include single-agent sorafenib and single-agent durvalumab. The trial is based on a phase I–II study evaluating the durvalumab–tremelimumab combination[109], which resulted in a confirmed ORR of 15% among 40 evaluable patients included in the phase I component. AEs were manageable and most commonly included fatigue, ALT and AST elevations, and pruritus; no unexpected toxicities were observed[109].

The combination of molecularly targeted therapies with immunotherapies is another area of active interest. Again borrowing from experiences in other diseases, impressive responses have been seen in patients with renal cell carcinoma (RCC) using the combination of lenvatinib and pembrolizumab (two drugs that have meaningful activity as single agents in this disease), resulting in a 'breakthrough therapy' designation from the FDA. In a study involving patients with non-HCC malignancies, those with RCC had an ORR to the lenvatinib and pembrolizumab combination of 63%; the median PFS and overall survival durations had not been reached at the time of presentation[110]. Toxicities were in keeping with those of the single agents, and no new safety signals were observed. This combination is now in development for the frontline treatment of HCC (NCT03006926), as is the combination of regorafenib and pembrolizumab (NCT03347292). These studies are building on the fact that these drugs have single-agent activity in patients with advanced-stage HCC, and as multi-target TKIs of VEGFRs and other kinases, lenvatinib and regorafenib have potential effects on the TME that might promote a response to immunotherapy[36,37]. Along those lines, monoclonal antibodies to VEGFA (bevacizumab) or VEGFR2 (ramucirumab) are being pursued in combination with PD-1 or PD-L1 inhibitors. Indeed, the combination of bevacizumab and atezolizumab is now being compared with sorafenib in a phase III study in the frontline setting (NCT03434379) and the FDA has granted this combination breakthrough designation on the basis of an ORR of 65% in 23 patients[111]. Early phase studies evaluating the safety and efficacy of ramucirumab plus durvalumab in patients with HCC are underway (NCT02572687).

Proof of concept for precision medicine

As the above sections highlight, promising and robust clinical trial results have been presented in the past 2 years that are changing the treatment options for patients with advanced-stage HCC. All of the successful phase III studies yielded positive results without enriching for a biomarker-selected population, with the exception of REACH-2 (REF.[10]). Despite the rapidly changing approach in other areas of oncology towards the development of molecularly targeted therapies in biomarker-selected populations[112], this strategy is lacking in HCC. Nevertheless, attempts are being made at investigating this approach in patients with this disease (FIG. 4; TABLE 2).

MET

The MET RTK has nonmalignant roles in liver physiology but has been implicated in the development of HCC. For example, elevated expression of MET and its ligand HGF has been associated with poor prognosis and resistance to sorafenib[87]. Subgroup analyses of a phase II study testing the small-molecule MET inhibitor tivantinib in 107 patients previously treated with sorafenib revealed a correlation of high MET expression by IHC (≥2+ in ≥50% of tumour cells) with an unfavourable prognosis but improved survival with tivantinib versus placebo[113]. This concept was then tested in a prospective, randomized, phase III study in the second-line setting in patients with MET-high HCC. This study did not meet its primary end point of an improvement in overall survival with tivantinib versus placebo[80] (FIG. 3b). The placebo group of patients with MET-high HCC had a median overall survival of 9.1 months[80]. This survival duration is the longest ever reported for patients with advanced-stage HCC in the context of a second-line phase III trial, raising the question of whether or not a high level of MET expression is a negative prognostic marker in this setting. Alternatively, the assay and the cut-off used for defining MET-driven HCC might not have been appropriate. In addition, tivantinib has been postulated to have a mechanism of action that is independent of MET inhibition[114]. Nevertheless, studies evaluating the activity of more-specific MET inhibitors as single agents and in combination with immunotherapy (for example, the small-molecule MET inhibitor capmatinib alone (NCT01737827) or in combination with the anti-PD-1 antibody spartalizumab (NCT02795429)) are ongoing in patients with HCC. The relative contribution of MET inhibition by cabozantinib to the proven efficacy of this agent in the second-line treatment of HCC remains to be determined.

The FGF19–FGFR4 axis

The FGF family consists of at least 5 RTKs and a large number of cognate ligands (at least 22) that have long been pursued as targets for anticancer treatments[115]. While FGFR2 alterations are being pursued as therapeutic targets in several cancers[116,117], in HCC, FGFR4 — the predominant FGFR expressed in the liver[118] — has been identified as a potentially important target. FGF19 can bind to and activate FGFR4 and induce hepatocyte proliferation[119]. *FGF19* amplification occurs in ~5–10% of HCC and has been shown to be an oncogenic driver implicated in sorafenib resistance[120] and a potential predictive marker of response to FGFR kinase inhibitors[121–123]. Specific FGFR4 kinase inhibitors are moving through the clinical development pathway, including BLU-554 (NCT02508467)[124], H3B-6527

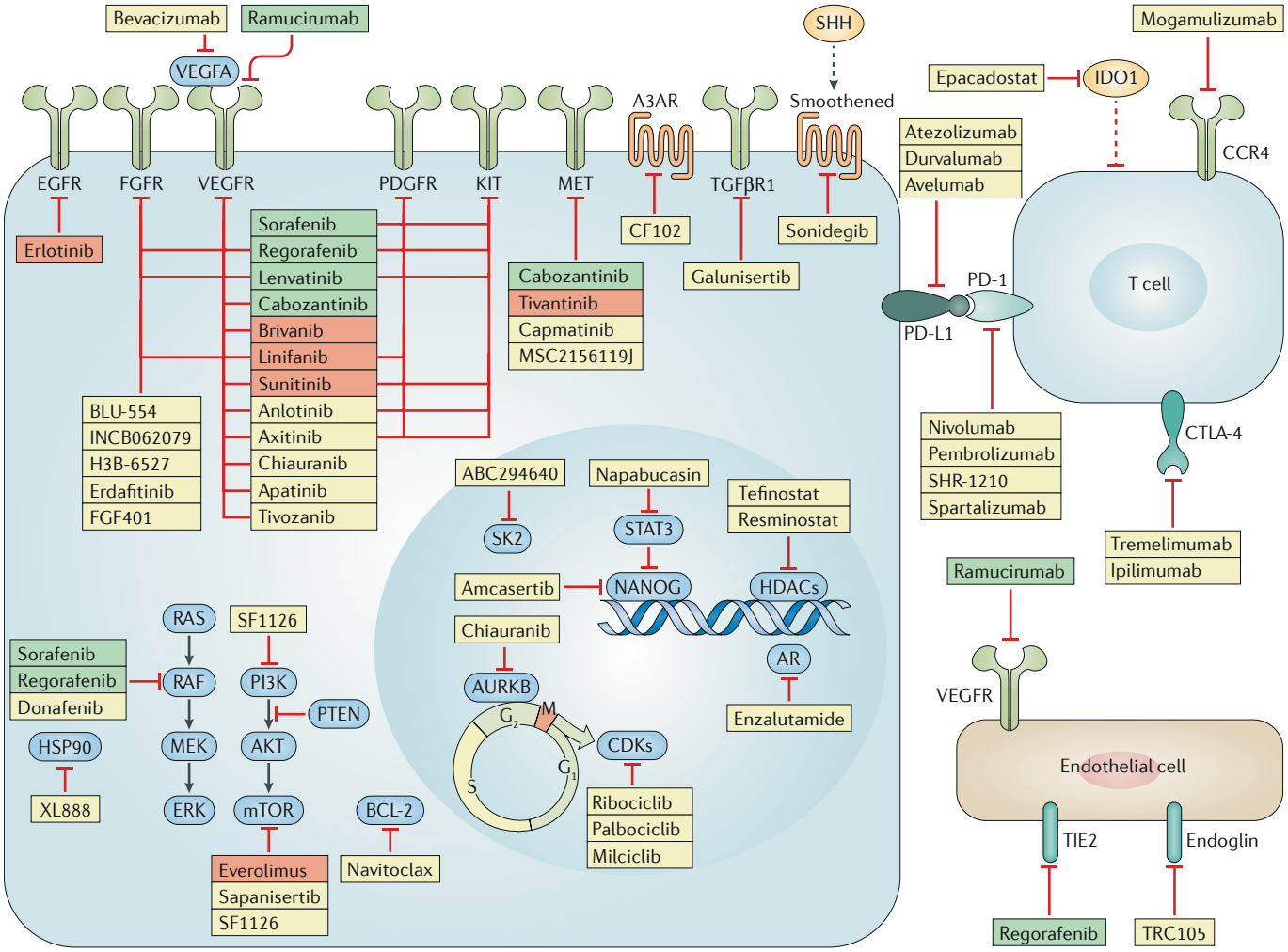

Fig. 4 | **Molecularly targeted therapies for HCC and their target signalling pathways.** Green boxes indicate drugs with positive results from phase III trials (sorafenib, regorafenib, lenvatinib, cabozantinib, and ramucirumab). Red boxes indicate drugs with negative results from phase III trials (everolimus, sunitinib, linifanib, erlotinib, brivanib, and tivantinib). Drugs in yellow boxes are currently in development for hepatocellular carcinoma (HCC) in either phase I, phase II, or phase III clinical trials (TABLE 2). The dashed lines indicate indirect activities. A3AR, adenosine receptor A3; AR, androgen receptor; AURKB, Aurora kinase B; CCR4, CC-chemokine receptor 4; CDKs, cyclin-dependent kinases; CTLA-4, cytotoxic T lymphocyte protein 4; FGFR, FGF receptor; HDACs, histone deacetylases; HSP90, heat shock protein 90; IDO1, indoleamine 2,3-dioxygenase 1; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1; PDGFR, PDGF receptor; SHH, sonic hedgehog protein; SK2, sphingosine kinase 2; STAT3, signal transducer and activator of transcription 3; TGFβR1, TGFβ type 1 receptor; TIE2, angiopoietin 1 receptor; VEGFR, VEGF receptor.

(NCT02834780)[125], and FGF401 (NCT02325739). All these agents are being evaluated using a biomarker-based approach, primarily on the basis of IHC for FGF19, FGFR4, and, in some cases, β-klotho, a transmembrane protein that enhances FGF19–FGFR4 interaction and signalling. BLU-554 has progressed furthest in clinical development, and preliminary data in patients with advanced-stage HCC have shown a response rate of 16% to this agent in an FGFR4-driven group (defined by ≥1% tumour expression of FGF19 by IHC) versus 0% in the FGFR4-negative group[126]. Responses occurred regardless of *FGF19*-amplification status, and toxicities were generally low grade, including diarrhoea, nausea, vomiting, and elevated AST and/or ALT levels (transaminase elevations had an increased tendency to be of grade 3–4). Mature data are awaited while this drug class moves through development as single agents and potentially in combination with other agents, particularly immune-checkpoint inhibitors (as in NCT02325739).

Intracellular kinases

Clearly, most efforts in HCC drug development have been focused on RTKs. However, several clinical studies have examined intracellular kinases as targets on the basis of preclinical and laboratory evidence. mTOR is a central kinase involved in signalling downstream of many RTKs implicated in HCC tumorigenesis[127,128]. Everolimus, an allosteric inhibitor of mTOR complex 1 (mTORC1), has been evaluated in a phase III study as a second-line treatment of HCC[77] but yielded negative results in an unselected patient population. A second-generation of mTOR pathway inhibitors (dual mTORC1 and mTORC2 inhibitors

Table 2 | **Ongoing trials of targeted therapies for HCC**

Drug	Targets	Clinical stage and treatment setting	Enrichment biomarker	Study phase (comparator)	Primary end point	ClinicalTrials.gov reference
Anti-angiogenic agents						
Ramucirumab	VEGFR2	Advanced; second line	AFP >400 ng/ml	III (placebo)	OS	NCT02435433
Apatinib	VEGFR2	Advanced; second line	None	III (placebo)	OS	NCT02329860
		Adjuvant	None	II	RFS	NCT03261791
Anlotinib	VEGFRs, KIT, and PDGFRs	Advanced; second line	None	II	PFS	NCT02809534
Tivozanib	VEGFRs	Advanced; first line	None	I–II	PFS	NCT01835223
TRC105	Endoglin	Advanced; first line (plus sorafenib)	None	I–II	MTD	NCT02560779
Immune modulators						
Nivolumab	PD-1	Advanced; first line	None	III (sorafenib)	OS	NCT02576509
		Adjuvant	None	III (placebo)	RFS	NCT03383458
		Intermediate (plus TACE)	None	I	AEs	NCT03143270
Pembrolizumab	PD-1	Advanced; second line	None	III (placebo)	PFS	NCT02702401
		Neoadjuvant	None	II	RFS	NCT03337841
		Advanced; first line (plus sorafenib)	None	I–II	ORR	NCT03211416
Durvalumab ± tremelimumab	PD-L1 and CTLA-4	Advanced; first line	None	III (sorafenib)	OS	NCT03298451
SHR-1210	PD-1	Advanced; second line	None	II	ORR	NCT02989922
Avelumab	PD-L1	Advanced; second line	None	II	ORR	NCT03389126
Nivolumab ± ipilimumab	PD-1 and CTLA-4	Neoadjuvant	None	II	AEs	NCT03222076
Mogamulizumab + nivolumab	CCR4 and PD-1	Advanced; second line	None	I–II	MTD	NCT02705105
Pembrolizumab + epacadostat	PD-1 and IDO1	Advanced; second line	None	I–II	DLTs	NCT02178722
Spartalizumab	PD-1	Advanced; first line (plus sorafenib)	None	I	AEs	NCT02988440
Cell cycle inhibitors and anti-proliferative agents						
Donafenib	RAF	Advanced; first line	None	III (sorafenib)	OS	NCT02645981
Palbociclib	CDK4 and CDK6	Advanced; second line	RB^+	II	TTP	NCT01356628
Milciclib	CDKs	Advanced; second line	None	II	AEs	NCT03109886
Ribociclib	CDK4 and CDK6	Intermediate (plus TACE)	RB^+	II	PFS	NCT02524119
Chiauranib	AURKB, VEGFRs, KIT, and PDGFRs	Advanced; second line	None	I	PFS	NCT03245190
Capmatinib	MET	Advanced; second line	MET^+	II	TTP	NCT01737827
MSC2156119J	MET	Advanced; second line	MET^+	I–II	DLTs	NCT02115373
Galunisertib	TGFβR1	Advanced; first line (plus sorafenib)	None	II	OS	NCT02178358
BLU-554	FGFR4	Advanced; second line	$FGF19^+$ (by IHC)	I–II	MTD	NCT02508467
INCB062079	FGFR4	Advanced; second line	*FGF19* amplification	I–II	AEs	NCT03144661
H3B-6527	FGFR4	Advanced; second line	None	I	DLTs	NCT02834780
Erdafitinib	FGFRs	Advanced; second line	*FGF19* amplification	I	RP2D	NCT02421185
Sapanisertib	mTOR	Advanced; first line	None	I–II	MTD	NCT02575339
SF1126	PI3K and mTOR	Advanced; second line	None	I	MTD	NCT03059147
Miscellaneous						
Enzalutamide	AR	Advanced; second line	None	II	OS	NCT02528643
		Advanced; first line (plus sorafenib)	None	I–II	PFS	NCT02642913

Table 2 (cont.) | **Ongoing trials of targeted therapies for hepatocellular carcinoma**

Drug	Targets	Clinical stage and treatment setting	Enrichment biomarker	Study phase (comparator)	Primary end point	ClinicalTrials.gov reference
Miscellaneous (cont.)						
CF102	A3AR	Advanced; second line	None	II	OS	NCT02128958
Tefinostat	HDACs	Advanced; second line	None	I–II	MTD	NCT02759601
Resminostat	HDACs	Advanced; first line (plus sorafenib)	None	I–II	DLTs	NCT02400788
Amcasertib	Cancer stemness kinase inhibitor (target unknown)	Advanced; second line	None	II	DCR	NCT02232633
		Advanced; first line (plus sorafenib)	None	I–II	DLTs	NCT02279719
Napabucasin	STAT3	Advanced; first line (plus sorafenib)	None	I	DLTs	NCT02358395
Sonidegib	Smoothened antagonist (inhibitor of Hedgehog signalling)	Advanced; second line	None	I	DLTs	NCT02151864
ABC294640	SK2	Advanced; second line	None	II	ORR	NCT02939807
Targeted therapy combinations						
Atezolizumab + bevacizumab	PD-L1 and VEGFA	Advanced; first line	None	III	OS	NCT03434379
Galunisertib + nivolumab	TGFβR1 and PD-1	Advanced; second line	AFP > 200 ng/ml	I–II	MTD	NCT02423343
Apatinib + SHR-1210	VEGFR2 and PD-1	Advanced; second line	None	I–II	OS	NCT02942329
Spartalizumab ± capmatinib	PD-1 and MET	Advanced; second line	None	I–II	DLTs	NCT02795429
FGF401 ± spartalizumab	FGFR4 and PD-1	Advanced; second line	$FGFR4^+$ and KLB^+	I–II	DLTs	NCT02325739
Pembrolizumab + lenvatinib	PD-1 plus VEGFR2 and VEGFR3	Advanced; second line	None	I	DLTs	NCT03006926
Regorafenib + pembrolizumab	VEGFRs, FGFRs, KIT, PDGFRs, and RAF plus PD-1	Advanced; first line	None	I	AEs	NCT03347292
Cabozantinib + nivolumab	MET and VEGFRs plus PD-1	Neoadjuvant	None	I	AEs	NCT03299946
Avelumab + axitinib	PD-L1 plus VEGFRs, KIT, and PDGFRs	Advanced; first line	None	I	AEs	NCT03289533
Ramucirumab + durvalumab	VEGFR2 and PD-L1	Advanced; second line	AFP > 1.5 × ULN	I	DLTs	NCT02572687
XL888 + pembrolizumab	HSP90 and PD-1	Advanced; second line	None	I	RP2D	NCT03095781
Navitoclax + sorafenib	BCL-2 plus VEGFRs, KIT, PDGFRs, and RAF	Advanced; second line	None	I	MTD	NCT02143401

Data were obtained in January 2018 from the ClinicalTrials.gov database. Keyword searches for "hepatocellular carcinoma" were used to identify active clinical trials that started in the past 5 years to investigate systemic targeted therapies alone or in combination with other treatments with proven survival benefits for patients with hepatocellular carcinoma (HCC). A3AR, adenosine receptor A3; AEs, adverse effects; AFP, α-fetoprotein; AR, androgen receptor; AURKB, Aurora kinase B; CCR4, CC-chemokine receptor 4; CDK, cyclin-dependent kinase; CTLA-4, cytotoxic T lymphocyte protein 4; DCR, disease control rate; DLTs, dose-limiting toxicities; FGFR, FGF receptor; HDACs, histone deacetylases; HSP90, heat shock protein 90; IDO1, indoleamine 2,3-dioxygenase 1; IHC, immunohistochemistry; KLB, β-klotho; MTD, maximum tolerated dose; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1; PDGFRs, PDGF receptors; PFS, progression-free survival; RB, retinoblastoma-associated protein; RFS, relapse-free survival; RP2D, recommended phase II dose; SK2, sphingosine kinase 2; STAT3, signal transducer and activator of transcription 3; TACE, transarterial chemoembolization; TGFβR1, TGFβ type 1 receptor; TTP, time to progression; ULN, upper limit of normal; VEGFR, VEGF receptor.

and mTOR–PI3K inhibitors) with a broader inhibitory action against PI3K–AKT signalling has been developed, and these agents are currently being investigated in early clinical trials (for example, NCT03059147)[127,129].

In contrast to everolimus, development of refametinib, a small-molecule MEK inhibitor, has been pursued in a biomarker-selected population. In a retrospective analysis of a single-arm phase II study evaluating refametinib plus sorafenib in patients with advanced-stage HCC, the best clinical responses were seen in patients with *RAS* mutations[130]. Two subsequent studies (NCT01915589 and NCT01915602) aimed to prospectively select patients on the basis of the presence of *KRAS* or *NRAS* mutations detected in serum circulating tumour DNA have been conducted using BEAMing technology; however, only 59 of 1,318 samples (4.4%) had detectable *RAS*

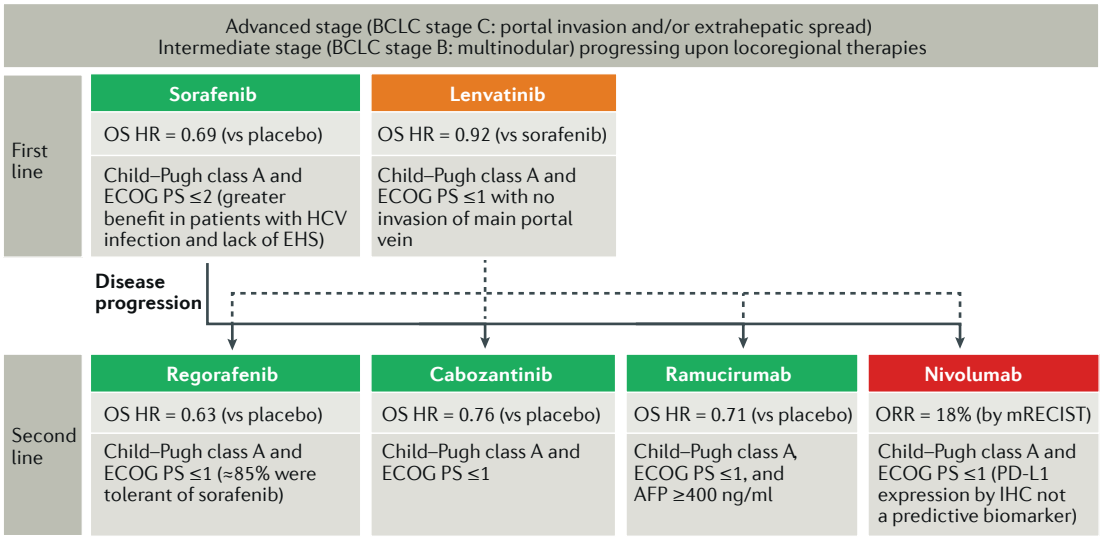

Fig. 5 | **Treatment strategy for advanced HCC.** Drugs in green have positive results from phase III trials with a superiority design (sorafenib in the first-line setting and regorafenib and cabozantinib in the second-line setting). Ramucirumab improved overall survival in the phase III REACH-2 trial[10], which involved patients with high serum α-fetoprotein (AFP) levels (≥400 ng/ml). Drugs in orange have positive results from phase III trials with a non-inferiority design (lenvatinib in the first-line setting). Drugs in red have received accelerated approval from the FDA on the basis of promising efficacy results in phase II trials (nivolumab in the second-line setting). Key details of the patient populations are provided. BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; EHS, extrahepatic spread; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IHC, immunohistochemistry; mRECIST, modified Response Evaluation Criteria in Solid Tumors; ORR, objective response rate; OS, overall survival; PD-L1, programmed cell death 1 ligand 1.

mutations[131]. A phase II combination trial enriched for RAS mutations testing refametinib plus sorafenib led to a median overall survival of 12.7 months in 16 patients[131].

Future prospects

Molecular characterizations have uncovered the most frequently mutated drivers (the *TERT* promoter, *TP53*, and *CTNNB1*), chromosomal aberrations (loss of 1q and 8p and high-level gains of 11q13 and 6p21), and deregulated pathways (RAS–MAPK, WNT, mTOR, or IGF2 signalling, among others)[2,3,16,20] associated with HCC (FIG. 1; TABLE 1). Nonetheless, the advancements in the understanding of these molecular drivers have not yet been translated into biomarker-driven trials of precision medicine. In HCC, before the recently published REACH-2 trial[10], all effective drugs in phase III trials were multi-kinase inhibitors with no known predictive biomarkers (FIG. 5). Similarly, positive data from studies of immune-checkpoint inhibitors have not been accompanied by companion diagnostic tools. Thus, an urgent need exists to implement genome-based HCC therapies and to understand predictors of response to immunotherapies or identify agents that are able to boost immune response in primary resistant tumours.

Implementing driver-based therapies

Molecular studies have already made great contributions to the understanding of HCC biology, but this knowledge has not been translated into clinical practice[132]. Strategic efforts are needed to foster precision medicine in this field. The co-development of predictive biomarkers together with novel targeted therapies is essential to overcome this issue[133]. In this regard, owing to the difficulties associated with the acquisition of biological samples of advanced-stage HCC, liquid biopsy — analyses of tumour cell-derived DNA and mRNA in cell-free plasma or circulating tumour cells — is envisioned as a useful tool to guide therapeutic decision-making in the near future[134,135].

A new drug development pathway has been established, consisting of positive proof-of-concept phase II trials — leading to accelerated approval — followed by phase III randomized studies versus the standard of care to support conventional approval. In addition, 'monster' phase I trials have emerged in the field[136], consisting of studies including 1,000–2,000 patients, which include multiple amendments for establishing the final well-selected population that will define the target patient cohort. This clear strategy is based upon the following concepts of precision medicine. First, driver mutations lead to oncogenic addiction loops; therefore, molecular therapies blocking these oncogenic drivers achieve substantial responses (in general, ORRs of ~50%) and survival advantages. Second, clonal founder or trunk mutations can be assessed with single biopsy samples. Currently, >25 molecular therapies in oncology have been approved for use based upon a predictive biomarker of efficacy[112]. The percentage of patients with tumours harbouring a biomarker that guides therapies approved by regulatory agencies ranges from 0% (for example, in those with HCC or prostate or pancreatic cancer) to >40% (in those with melanoma and gastrointestinal stromal tumours)[137]. However, the percentage of patients with a genomic alteration with compelling clinical evidence of an association with a response is much higher (>40% in those with non-small-cell lung, endometrial, breast, or thyroid cancer, and approaching 20% in those with HCC), although the corresponding drug is not

yet standard of care owing to a lack of strong evidence[137]. In HCC, the landscape of mutations and targetable drivers has been defined, and ~25% of them are considered potentially actionable[16]. Unfortunately, therefore, most trunk mutations and prevalent drivers in HCC[138] (affecting the *TERT* promoter, *CTNNB1*, *TP53*, *AXIN1*, *ARID1A*, and *ARID1B*) are not directly actionable at present[16]. Thus, driver-based trials are scarce in this field. A few studies, for instance, assessing refametinib plus sorafenib in patients with *RAS*-mutated HCC[131] or FGFR4 inhibitors in patients with overexpression and/or amplification of FGF19 (REFS[124,125]), have shown promise, whereas others failed (tivantinib in patients with MET-positive HCC)[80]. According to the molecular pathogenesis and known pathways in HCC, drugs that block the effects of *CTNNB1* mutations are expected to be relevant to precision medicine approaches.

Immunotherapies — new opportunities

Increased understanding of the mechanisms that govern tumour–host interactions has accelerated the development of novel immunotherapies for cancer. Indeed, several immune-checkpoint inhibitors obtained regulatory approval for the treatment of melanoma and lung, renal, and bladder cancers[139]. Despite this unprecedented success, responses typically occur in a minority of patients, ranging from 20% to 50% depending on the tumour type. In a small proportion of patients, immunotherapy can cause severe and potentially permanent autoimmune AEs[140]; therefore, the identification of candidate biomarkers to target patients who are most likely to benefit is becoming crucial. Unfortunately, only PD-L1 expression by IHC has been approved as a companion diagnostic (for lung cancer) or complementary test (for melanoma and bladder cancer) for anti-PD-1 treatments[141]. The FDA has also approved pembrolizumab for the treatment of solid tumours with microsatellite instability (<5% of all cancers)[142], although only 40% of patients with microsatellite instability-high disease respond to treatment. In patients with HCC, responses to nivolumab do not seem to be associated with PD-L1 expression on tumour cells[11], highlighting the urgent need for alternative biomarkers. TCGA investigators observed that 22% of HCCs have lymphocyte infiltration[18], which is consistent with the findings of a previous study[34] describing the immune class of HCCs, in ~27% of patients, characterized by high infiltration of immune cells, expression of PD-1 and PD-L1, and active IFNγ signalling[34] (FIG. 1b). In the same study[34], an immune exclusion phenotype was observed in ~25% of HCCs, characterized by *CTNNB1* mutations, lower immune infiltration (on the basis of immune-specific gene signatures), and overexpression of *PTK2*, an oncogenic pathway associated with poor T cell infiltration into tumours[143]. These data are consistent with findings in melanoma showing that activation of the β-catenin (*CTNNB1*) pathway is associated with T cell exclusion and resistance to immunotherapy[144], suggesting that the immune exclusion class of HCC encompasses patients with ineffective or suboptimal responses to immunotherapies. Importantly, if the results of the ongoing phase III CheckMate 459 trial comparing nivolumab to sorafenib are positive, this immune-checkpoint inhibitor will become the standard-of-care frontline therapy; thus, biomarker-driven identification of responders will not only improve therapeutic decision-making in the advanced-stage setting but also help to move immunotherapies to earlier clinical stages. Conversely, if the study fails to hit the primary end point, a clear understanding of the biomarkers for predicting a response or primary resistance to these agents will be essential for future efforts to establish immunotherapy as a treatment strategy for patients with HCC.

Conclusions

The global disease burden of HCC is increasing and might surpass an incidence of 1 million cases annually in the near future. In this regard, primary and secondary prevention policies along with improved implementation of surveillance programmes will be essential to reduce the morbidity and mortality associated with this disease. In fact, few patients with HCC (<10%) are cured. Thus, the majority of patients ultimately develop advanced-stage HCC, at which point only systemic therapies are effective in delaying the natural history of the disease (FIG. 5); however, the median overall survival of these patients remains ~1 year with the use of efficacious multi-kinase inhibitors. Immune-checkpoint inhibitors are now entering HCC clinical practice on the basis of promising early data. New phase III studies are expected to demonstrate even more promising outcomes with these agents in the frontline. Similarly, combinations of molecularly targeted therapies and immunotherapies are emerging as tools to boost responses of the immune system against HCC-derived neoantigens. Hopefully, these strategies might raise the bar for systemic HCC therapy by extending median overall survival beyond 2 years, particularly if predictors of responsiveness are identified. In this scenario, systemic therapies might start competing with locoregional therapies, such as chemoembolization, for intermediate-stage HCC.

Published online 30 July 2018

1. Torre, L. A. et al. Global cancer statistics, 2012. *CA. Cancer J. Clin.* **65**, 87–108 (2015).
2. Llovet, J. M. et al. Hepatocellular carcinoma. *Nat. Rev. Dis. Prim.* **2**, 16018 (2016).
3. Zucman-Rossi, J., Villanueva, A., Nault, J. C. & Llovet, J. M. Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* **149**, 1226–1239 (2015).
4. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J. Hepatol.* https://doi.org/10.1016/j.jhep.2018.03.019 (2018).
5. Bruix, J., S. M. A. A. for the S. of L. D. Management of hepatocellular carcinoma: an update. *Hepatology* **53**, 1020–1022 (2011).
6. Llovet, J. et al. Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* **359**, 378–390 (2008).
7. Kudo, M. et al. A randomised phase 3 trial of lenvatinib versus sorafenib in firstline treatment of patients with unresectable hepatocellular carcinoma. *Lancet* **391**, 1163–1173 (2018).
8. Bruix, J. et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* **389**, 56–66 (2017).
9. Abou-Alfa, G.K. et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N. Engl. J. Med.* **379**, 54–63 (2018).
10. Zhu, A. X. et al. REACH-2: A randomized, double-blind, placebo-controlled phase 3 study of ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma (HCC) and elevated baseline alpha-fetoprotein (AFP) following first-line sorafe [abstract]. *J. Clin. Oncol.* **36**, 4003 (2018).
11. El-Khoueiry, A. B. et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* **389**, 2492–2502 (2017).
12. Llovet, J. M. & Hernandez-Gea, V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. *Clin. Cancer Res.* **20**, 2072–2079 (2014).

13. Sangiovanni, A. et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* **126**, 1005–1014 (2004).
14. Nault, J.-C. et al. Molecular classification of hepatocellular adenoma associates with risk factors, bleeding, and malignant transformation. *Gastroenterology* **152**, 880–894.e6 (2017).
15. Sia, D., Villanueva, A., Friedman, S. L. & Llovet, J. M. Liver cancer cell of origin, molecular class, and effects on patient prognosis. *Gastroenterology* **152**, 745–761 (2016).
16. Schulze, K. et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat. Genet.* **47**, 505–511 (2015).
17. Charles Nault, J. et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat. Commun.* **4**, 2218 (2013).
18. Wheeler, D. A. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell* **169**, 1327–1341 (2017).
19. Ahn, S.-M. et al. Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. *Hepatology* **60**, 1972–1982 (2014).
20. Totoki, Y. et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat. Genet.* **46**, 1267–1273 (2014).
21. Chiang, D. Y. et al. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. *Cancer Res.* **68**, 6779–6788 (2008).
22. Martinez-Quetglas, I. et al. IGF2 is up-regulated by epigenetic mechanisms in hepatocellular carcinomas and is an actionable oncogene product in experimental models. *Gastroenterology* **151**, 1192–1205 (2016).
23. Hoshida, Y. et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res.* **69**, 7385–7392 (2009).
24. Boyault, S. et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* **45**, 42–52 (2007).
25. Lee, J.-S. et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat. Med.* **12**, 410–416 (2006).
26. Toffanin, S. et al. MicroRNA-based classification of hepatocellular carcinoma and oncogenic role of miR-517a. *Gastroenterology* **140**, 1618–1628 (2011).
27. Llovet, J. M., Villanueva, A., Lachenmayer, A. & Finn, R. S. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. *Nat. Rev. Clin. Oncol.* **12**, 408–424 (2015).
28. Hoshida, Y. et al. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. *Semin. Liver Dis.* **30**, 35–51 (2010).
29. Wang, K. et al. Genomic landscape of copy number aberrations enables the identification of oncogenic drivers in hepatocellular carcinoma. *Hepatology* **58**, 706–717 (2013).
30. Villanueva, A. et al. DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma. *Hepatology* **61**, 1945–1956 (2015).
31. Lachenmayer, A. et al. Wnt-pathway activation in two molecular classes of hepatocellular carcinoma and experimental modulation by sorafenib. *Clin. Cancer Res.* **18**, 4997–5007 (2012).
32. Hoshida, Y. et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N. Engl. J. Med.* **359**, 1995–2004 (2008).
33. Pikarsky, E. et al. NF-κB functions as a tumour promoter in inflammation-associated cancer. *Nature* **431**, 461–466 (2004).
34. Sia, D. et al. Identification of an immune-specific class of hepatocellular carcinoma, based on molecular features. *Gastroenterology* **153**, 812–826 (2017).
35. Weis, S. M. & Cheresh, D. A. Tumor angiogenesis: molecular pathways and therapeutic targets. *Nat. Med.* **17**, 1359–1370 (2011).
36. Khan, K. A. & Kerbel, R. S. Improving immunotherapy outcomes with anti-angiogenic treatments and vice versa. *Nat. Rev. Clin. Oncol.* **15**, 310–324 (2018).
37. Fukumura, D., Kloepper, J., Amoozgar, Z., Duda, D. G. & Jain, R. K. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. *Nat. Rev. Clin. Oncol.* **15**, 325–340 (2018).
38. Yau, T. et al. Development of Hong Kong Liver Cancer staging system with treatment stratification for patients with hepatocellular carcinoma. *Gastroenterology* **146**, 1691–1700.e3 (2014).
39. [No authors listed]. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* **28**, 751–755 (1998).
40. Sobin, L. H. & Compton, C. C. TNM seventh edition: what's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer. *Cancer* **116**, 5336–5339 (2010).
41. Kudo, M., Chung, H. & Osaki, Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). *J. Gastroenterol.* **38**, 207–215 (2003).
42. Llovet, J. M., Brū, C. & Bruix, J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin. Liver Dis.* **19**, 329–338 (1999).
43. Bruix, J. et al. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet. Oncol.* **16**, 1344–1354 (2015).
44. Llovet, J. M. et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* **359**, 1734–1739 (2002).
45. Lo, C.-M. et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* **35**, 1164–1171 (2002).
46. Llovet, J. M. & Bruix, J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* **37**, 429–442 (2003).
47. Lencioni, R. et al. Sorafenib or placebo plus TACE with doxorubicin-eluting beads for intermediate stage HCC: the SPACE trial. *J. Hepatol.* **64**, 1090–1098 (2016).
48. Meyer, T. et al. Sorafenib in combination with transarterial chemoembolisation in patients with unresectable hepatocellular carcinoma (TACE 2): a randomised placebo-controlled, double-blind, phase 3 trial. *Lancet Gastroenterol. Hepatol.* **2**, 565–575 (2017).
49. Kudo, M. et al. Brivanib as adjuvant therapy to transarterial chemoembolization in patients with hepatocellular carcinoma: a randomized phase III trial. *Hepatology* **60**, 1697–1707 (2014).
50. Qin, S. et al. Randomized, multicenter, open-label study of oxaliplatin plus fluorouracil/leucovorin versus doxorubicin as palliative chemotherapy in patients with advanced hepatocellular carcinoma from Asia. *J. Clin. Oncol.* **31**, 3501–3508 (2013).
51. Abou-Alfa, G. K. et al. Doxorubicin plus sorafenib versus doxorubicin alone in patients with advanced hepatocellular carcinoma. *JAMA* **304**, 2154 (2010).
52. Yeo, W. et al. A randomized phase III study of doxorubicin versus cisplatin/interferon α-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J. Natl Cancer Inst.* **97**, 1532–1538 (2005).
53. Chow, P. et al. High-dose tamoxifen in the treatment of inoperable hepatocellular carcinoma: a multicenter randomized controlled trial. *Hepatology* **36**, 1221–1226 (2002).
54. Dalhoff, K. et al. A phase II study of the vitamin D analogue Seocalcitol in patients with inoperable hepatocellular carcinoma. *Br. J. Cancer* **89**, 252–257 (2003).
55. Cheng, A.-L. et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* **10**, 25–34 (2009).
56. Reig, M. et al. Early dermatologic adverse events predict better outcome in HCC patients treated with sorafenib. *J. Hepatol.* **61**, 318–324 (2014).
57. Bruix, J. et al. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: analysis of two phase 3 studies. *J. Hepatol.* **67**, 999–1008 (2017).
58. Iavarone, M. et al. Field-practice study of sorafenib therapy for hepatocellular carcinoma: a prospective multicenter study in Italy. *Hepatology* **54**, 2055–2063 (2011).
59. Ganten, T. M. et al. Sorafenib in patients with hepatocellular carcinoma—results of the Observational INSIGHT Study. *Clin. Cancer Res.* **23**, 5720–5728 (2017).
60. Marrero, J. A. et al. Observational registry of sorafenib use in clinical practice across Child-Pugh subgroups: the GIDEON study. *J. Hepatol.* **65**, 1140–1147 (2016).
61. Kudo, M. et al. Regional differences in sorafenib-treated patients with hepatocellular carcinoma: GIDEON observational study. *Liver Int.* **36**, 1196–1205 (2016).
62. Wilhelm, S. M. et al. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol. Cancer Ther.* **7**, 3129–3140 (2008).
63. Llovet, J. M. et al. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin. Cancer Res.* **18**, 2290–2300 (2012).
64. Pinyol, R. et al. Molecular predictors of recurrence prevention with sorafenib as adjuvant therapy in hepatocellular carcinoma: biomarker study of the STORM phase III trial. *J. Hepatol.* **66**, S12–S13 (2017).
65. Llovet, J. M. et al. Design and endpoints of clinical trials in hepatocellular carcinoma. *J. Natl Cancer Inst.* **100**, 698–711 (2008).
66. Lencioni, R. et al. Objective response by mRECIST as a predictor and potential surrogate end-point of overall survival in advanced HCC. *J. Hepatol.* **66**, 1166–1172 (2017).
67. Montal, R., Lencioni, R. & Llovet, J. M. Reply to: mRECIST for systemic therapies: more evidence is required before recommendations could be made. *J. Hepatol.* **67**, 196–197 (2017).
68. Johnson, P. J. et al. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J. Clin. Oncol.* **31**, 3517–3524 (2013).
69. Cheng, A.-L. et al. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J. Clin. Oncol.* **31**, 4067–4075 (2013).
70. Cainap, C. et al. Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. *J. Clin. Oncol.* **33**, 172–179 (2015).
71. Zhu, aX. et al. SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. *J. Clin. Oncol.* **33**, 559–566 (2014).
72. Vilgrain, V. et al. Efficacy and safety of selective internal radiotherapy with yttrium-90 resin microspheres compared with sorafenib in locally advanced and inoperable hepatocellular carcinoma (SARAH): an open-label randomised controlled phase 3 trial. *Lancet Oncol.* **18**, 1624–1636 (2017).
73. Chow, P. K. H. et al. SIRveNIB: selective internal radiation therapy versus sorafenib in Asia-Pacific patients with hepatocellular carcinoma. *J. Clin. Oncol.* **36**, 1913–1921 (2018).
74. Ricke, J. et al. The impact of combining Selective Internal Radiation Therapy (SIRT) with sorafenib on overall survival in patients with advanced hepatocellular carcinoma: the SORAMIC trial palliative cohort. *J. Hepatol.* **68**, S102 (2018).
75. Matsui, J. et al. Multi-kinase inhibitor E7080 suppresses lymph node and lung metastases of human mammary breast tumor MDA-MB-231 via inhibition of vascular endothelial growth factor-receptor (VEGF-R) 2 and VEGF-R3 kinase. *Clin. Cancer Res.* **14**, 5459–5465 (2008).
76. Ikeda, K. et al. Phase 2 study of lenvatinib in patients with advanced hepatocellular carcinoma. *J. Gastroenterol.* **52**, 512–519 (2017).
77. Zhu, A. X. et al. Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. *JAMA* **312**, 57–67 (2014).
78. Zhu, A. X. et al. Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol.* **16**, 859–870 (2015).
79. Llovet, J. M. et al. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. *J. Clin. Oncol.* **31**, 3509–3516 (2013).
80. Rimassa, L. et al. Second-line tivantinib (ARQ 197) versus placebo in patients (Pts) with MET-high hepatocellular carcinoma (HCC): results of the METIV-HCC phase III trial. *J. Clin. Oncol.* **35** (Suppl. 15), 4000 (2017).
81. Zhu, A. X. et al. KEYNOTE-224: Phase II study of pembrolizumab in patients with previously treated

advanced hepatocellular carcinoma. *J. Clin. Oncol.* **35** (Suppl. 4), TPS504 (2017).
82. Wilhelm, S. M. et al. Regorafenib (BAY 73–4506): A new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int. J. Cancer* **129**, 245–255 (2011).
83. Bruix, J. et al. Regorafenib as second-line therapy for intermediate or advanced hepatocellular carcinoma: Multicentre, open-label, phase II safety study. *Eur. J. Cancer* **49**, 3412–3419 (2013).
84. Finn, R. S. et al. Outcomes with sorafenib followed by regorafenib or placebo for HCC: additional analyses from the phase 3 RESORCE trial. *J. Hepatol.* https://doi.org/10.1016/j.jhep.2018.04.010 (2018).
85. Yakes, F. M. et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol. Cancer Ther.* **10**, 2298–2308 (2011).
86. Kelley, R. K. et al. Cabozantinib in hepatocellular carcinoma: Results of a phase 2 placebo-controlled randomized discontinuation study. *Ann. Oncol.* **28**, 528–534 (2017).
87. Goyal, L., Muzumdar, M. D. & Zhu, A. X. Targeting the HGF/c-MET pathway in hepatocellular carcinoma. *Clin. Cancer Res.* **19**, 2310–2318 (2013).
88. Zhu, A. X. et al. A phase II and biomarker study of ramucirumab, a human monoclonal antibody targeting the VEGF receptor-2, as first-line monotherapy in patients with advanced hepatocellular cancer. *Clin. Cancer Res.* **19**, 6614–6623 (2013).
89. Terentiev, A. A. & Moldogazieva, N. T. Alpha-fetoprotein: a renaissance. *Tumor Biol.* **34**, 2075–2091 (2013).
90. Shan, Y. F. et al. Angiogenesis and clinicopathologic characteristics in different hepatocellular carcinoma subtypes defined by EpCAM and α-fetoprotein expression status. *Med. Oncol.* **28**, 1012–1016 (2011).
91. Topalian, S. L., Drake, C. G. & Pardoll, D. M. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* **27**, 451–461 (2015).
92. Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **12**, 252–264 (2012).
93. Boutros, C. et al. Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. *Nat. Rev. Clin. Oncol.* **13**, 473–486 (2016).
94. Ribas, A. Releasing the brakes on cancer immunotherapy. *N. Engl. J. Med.* **373**, 1490–1492 (2015).
95. Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* **168**, 707–723 (2017).
96. Iñarrairaegui, M., Melero, I. & Sangro, B. Immunotherapy of hepatocellular carcinoma: facts and hopes. *Clin. Cancer Res.* **24**, 1518–1524 (2017).
97. Sangro, B. et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J. Hepatol.* **59**, 81–88 (2013).
98. El-Khoueiry, A. B. et al. Impact of antitumor activity on survival outcomes, and nonconventional benefit, with nivolumab (NIVO) in patients with advanced hepatocellular carcinoma (aHCC): subanalyses of CheckMate-040. *J. Clin. Oncol.* **36** (Suppl. 4), 475 (2018).
99. US Food & Drug Administration. FDA grants accelerated approval to nivolumab for HCC previously treated with sorafenib (FDA, 2017).
100. Zhu, A. X. et al. KEYNOTE-224: Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib. *J. Clin. Oncol.* **36** (Suppl. 4), 209 (2018).
101. Finn, R. S. et al. KEYNOTE-240: Randomized phase III study of pembrolizumab versus best supportive care for second-line advanced hepatocellular carcinoma. *J. Clin. Oncol.* **35** (Suppl. 4), TPS503 (2017).
102. Wainberg, Z. A. et al. Safety and clinical activity of durvalumab monotherapy in patients with hepatocellular carcinoma (HCC). *J. Clin. Oncol.* **35** (Suppl. 4), 4071 (2017).
103. Patel, S. P. & Kurzrock, R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. *Mol. Cancer Ther.* **14**, 847–856 (2015).
104. Rizvi, N. A. et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124–129 (2016).
105. Tumeh, P. C. et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568–571 (2014).
106. Crocenzi, T. S. et al. Nivolumab (nivo) in sorafenib (sor)-naive and -experienced pts with advanced hepatocellular carcinoma (HCC): CheckMate 040 study. *J. Clin. Oncol.* **35** (Suppl. 15), 4013 (2017).
107. Le, D. T. et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* **357**, 409–413 (2017).
108. Wolchok, J. D. et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N. Engl. J. Med.* **377**, 1345–1356 (2017).
109. Kelley, R. K. et al. Phase I/II study of durvalumab and tremelimumab in patients with unresectable hepatocellular carcinoma (HCC): phase I safety and efficacy analyses. *J. Clin. Oncol.* **35** (Suppl. 15), 4073 (2017).
110. Lee, C.-H. et al. A phase 1b/2 trial of lenvatinib plus pembrolizumab in patients with renal cell carcinoma. *Ann. Oncol.* **28** (Suppl. 5), 295–329 (2017).
111. Genentech. FDA grants breakthrough therapy designation for Genentech's TECENTRIQ in combination with avastin as first-line treatment for advanced or metastatic hepatocellular carcinoma (HCC). *Genentech* https://www.gene.com/media/press-releases/14736/2018-07-17/fda-grants-breakthrough-therapy-designat?utm_source=F&utm_medium=P&utm_term=15538&utm_content=TecentriqHCCBTD&utm_campaign=TecentriqHCCBTD (2018).
112. Collins, D. C., Sundar, R., Lim, J. S. J. & Yap, T. A. Towards precision medicine in the clinic: from biomarker discovery to novel therapeutics. *Trends Pharmacol. Sci.* **38**, 25–40 (2017).
113. Santoro, A. et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *Lancet Oncol.* **14**, 55–63 (2013).
114. Basilico, C. et al. Tivantinib (ARQ197) displays cytotoxic activity that is independent of its ability to bind MET. *Clin. Cancer Res.* **19**, 2381–2392 (2013).
115. Babina, I. S. & Turner, N. C. Advances and challenges in targeting FGFR signalling in cancer. *Nat. Rev. Cancer* **17**, 318–332 (2017).
116. Javle, M. et al. Phase II study of BGJ398 in patients with FGFR-altered advanced cholangiocarcinoma. *J. Clin. Oncol.* **36**, 276–282 (2017).
117. Konecny, G. E. et al. Second-line dovitinib (TKI258) in patients with FGFR2-mutated or FGFR2-non-mutated advanced or metastatic endometrial cancer: a non-randomised, open-label, two-group, two-stage, phase 2 study. *Lancet Oncol.* **16**, 686–694 (2015).
118. Jeong Lee, H. et al. Fibroblast growth factor receptor isotype expression and its association with overall survival in patients with hepatocellular carcinoma. *Clin. Mol. Hepatol.* **21**, 60–70 (2015).
119. Wu, X. et al. FGF19-induced hepatocyte proliferation is mediated through FGFR4 activation. *J. Biol. Chem.* **285**, 5165–5170 (2010).
120. Gao, L. et al. FGF19/FGFR4 signaling contributes to the resistance of hepatocellular carcinoma to sorafenib. *J. Exp. Clin. Cancer Res.* **36**, 1–10 (2017).
121. Sawey, E. T. et al. Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by oncogenomic screening. *Cancer Cell* **19**, 347–358 (2011).
122. Finn, R. S. et al. Gains in FGF19 are predictive of response to the fibroblast growth factor receptor (FGFR) small molecule tyrosine kinase inhibitor BGJ 398 in vitro [abstract 3858]. *Cancer Res.* **72** (Suppl. 8), 3858 (2012).
123. Guagnano, V. et al. FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective Pan-FGFR inhibitor. *Cancer Discov.* **2**, 1118–1133 (2012).
124. Hagel, M. et al. First selective small molecule inhibitor of FGFR4 for the treatment of hepatocellular carcinomas with an activated FGFR4 signaling pathway. *Cancer Discov.* **5**, 424–437 (2015).
125. Joshi, J. J. et al. H3B-6527is a potent and selective inhibitor of FGFR4 in FGF19-driven hepatocellular carcinoma. *Cancer Res.* **77**, 6999–7013 (2017).
126. Harris, J. BLU-554 associated with improved response in HCC. *OncLive* https://www.Onclive.com/Conference-Coverage/Ilca-2017/blu554-Associated-With-Improved-Response-in-hcc (2017).
127. Matter, M. S., Decaens, T., Andersen, J. B. & Thorgeirsson, S. S. Targeting the mTOR pathway in hepatocellular carcinoma: current state and future trends. *J. Hepatol.* **60**, 855–865 (2014).
128. Villanueva, A. et al. Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* **135**, 1972–1983 (2008).
129. Janku, F., Yap, T. A. & Meric-Bernstam, F. Targeting the PI3K pathway in cancer: are we making headway? *Nat. Rev. Clin. Oncol.* **15**, 273–291 (2018).
130. Lim, H. Y. et al. A phase II study of the efficacy and safety of the combination therapy of the MEK inhibitor refametinib (BAY 86–9766) plus sorafenib for Asian patients with unresectable hepatocellular carcinoma. *Clin. Cancer Res.* **20**, 5976–5985 (2014).
131. Lim, H. Y. et al. Phase II studies with refametinib or refametinib plus sorafenib in patients with ras-mutated hepatocellular carcinoma. *Clin. Cancer Res.* https://doi.org/10.1158/1078-0432.CCR-17-3588 (2018).
132. Sia, D. & Llovet, J. M. Liver cancer: Translating '–omics' results into precision medicine for hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 571–572 (2017).
133. de Gramont, A. et al. Pragmatic issues in biomarker evaluation for targeted therapies in cancer. *Nat. Rev. Clin. Oncol.* **12**, 197–212 (2014).
134. Siravegna, G., Marsoni, S., Siena, S. & Bardelli, A. Integrating liquid biopsies into the management of cancer. *Nat. Rev. Clin. Oncol.* **14**, 531–548 (2017).
135. Xu, R. et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat. Mater.* **16**, 1155–1162 (2017).
136. Mullard, A. Reining in the supersized Phase I cancer trial. *Nat. Rev. Drug Discov.* **15**, 371–373 (2016).
137. Hyman, D. M., Taylor, B. S. & Baselga, J. Implementing genome-driven oncology. *Cell* **168**, 584–599 (2017).
138. Torrecilla, S. et al. Trunk mutational events present minimal intra- and inter-tumoral heterogeneity in hepatocellular carcinoma. *J. Hepatol.* **67**, 1222–1231 (2017).
139. Smyth, M. J., Ngiow, S. F., Ribas, A. & Teng, M. W. L. Combination cancer immunotherapies tailored to the tumour microenvironment. *Nat. Rev. Clin. Oncol.* **13**, 143–158 (2016).
140. Weber, J. S., Yang, J. C., Atkins, M. B. & Disis, M. L. Toxicities of immunotherapy for the practitioner. *J. Clin. Oncol.* **33**, 2092–2099 (2015).
141. Masucci, G. V. et al. Validation of biomarkers to predict response to immunotherapy in cancer: Volume I — pre-analytical and analytical validation. *J. Immunother. Cancer* **4**, 1–25 (2016).
142. Poh, A. First tissue-agnostic drug approval issued. *Cancer Discov.* **7**, 656 (2017).
143. Jiang, H. et al. Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. *Nat. Med.* **22**, 851–860 (2016).
144. Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231–235 (2015).

Acknowledgements
The work of J.M.L. is supported by grants from the European Commission Horizon 2020 programme (HEPCAR, proposal number 667273–2), the US Department of Defense (CA150272P3), the US National Cancer Institute (P30 CA196521), the Samuel Waxman Cancer Research Foundation, the Spanish National Health Institute (SAF 2016–76390), Asociación Española Contra el Cáncer (AECC), and the Generalitat de Catalunya (AGAUR, SGR-1162 and SGR-1358). The work of D.S. is supported by the Gilead Sciences Research Scholar Program in Liver Disease.

Author contributions
All authors made substantial contributions to each stage of the preparation of this manuscript for publication.

Competing interests
J.M.L. is a consultant to Bayer HealthCare, Bristol-Myers Squibb (BMS), Celsion, Eisai, Eli Lilly, Exelixis, and Ipsen and has active research funding from Bayer HealthCare, BMS, and Eisai. R.S.F. is a consultant to Bayer HealthCare, BMS, Eisai, Eli Lilly, Merck, Pfizer, and Roche. R.M. and D.S. declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Reviewer information
Nature Reviews Clinical Oncology thanks G. Gores, J.-L. Raoul, and other anonymous reviewer(s) for their contribution to the peer review of this work.