REVIEW

Molecularly targeted therapy for advanced hepatocellular carcinoma - a drug development crisis?

Kiruthikah Thillai, Paul Ross, Debashis Sarker

Kiruthikah Thillai, Paul Ross, Debashis Sarker, Department of Medical Oncology, Guy's and St Thomas NHS Trust, London SE1 9RT, United Kingdom

Kiruthikah Thillai, Debashis Sarker, Division of Cancer Studies, King's College London, Guy's Hospital, London SE1 9RT, United Kingdom

Paul Ross, Debashis Sarker, Institute of Liver Studies, King's College Hospital NHS Trust, London SE5 9RS, United Kingdom

Correspondence to: Dr. Debashis Sarker, Division of Cancer Studies, King's College London, Guy's Hospital Campus, Great Maze Pond, London Bridge, London SE1 9RT, United Kingdom. debashis.sarker@kcl.ac.uk
Telephone: +44-20-71884260
Fax: +44-20-71880919

Abstract

Hepatocellular carcinoma is the fastest growing cause of cancer related death globally. Sorafenib, a multi-targeted kinase inhibitor, is the only drug proven to improve outcomes in patients with advanced disease offering modest survival benefit. Although comprehensive genomic mapping has improved understanding of the genetic aberrations in hepatocellular cancer (HCC), this knowledge has not yet impacted clinical care. The last few years have seen the failure of several first and second line phase III clinical trials of novel molecularly targeted therapies, warranting a change in the way new therapies are investigated in HCC. Potential reasons for these failures include clinical and molecular heterogeneity, trial design and a lack of biomarkers. This review discusses the current crisis in HCC drug development and how we should learn from recent trial failures to develop a more effective personalised treatment paradigm for patients with HCC.

Key words: Hepatocellular carcinoma; Molecular targets; Genomics; Sorafenib; Tyrosine kinase inhibitors

Core tip: This review discusses the current drug therapy landscape for advanced hepatocellular carcinoma, in particular the reasons for failure of several clinical trials of molecularly targeted therapy and future directions of research to address these problems.

Thillai K, Ross P, Sarker D. Molecularly targeted therapy for advanced hepatocellular carcinoma - a drug development crisis?

INTRODUCTION

Hepatocellular cancer (HCC) is the sixth most prevalent cancer worldwide and accounts for over 745000 deaths a year[1]. Despite the implementation of screening programs for high-risk individuals, the majority of patients present with incurable disease. Median overall survival for advanced disease remains poor at less than 12 mo and there is an urgent need for more effective treatments[2]. Global epidemiological patterns vary depending on the prevalence of risk factor. Incidence rates are highest in East Asia in areas where hepatitis B and C are endemic[3]. However, improved management of early viral hepatitis in Japan has seen a reduction in new HCC cases[4]. By contrast the upward trends of HCV, obesity and metabolic syndrome in North America and Europe contribute to HCC being the fastest growing cause of cancer related mortality in these regions[5]. Resection, radiofrequency or microwave ablation, and liver transplantation comprise the mainstay of treatment for early disease offering the only chance of cure, but only one third of patients present with disease suitable for these treatments[6]. Loco-regional therapy with trans-arterial chemoembolization (TACE) can lead to sustained disease control for intermediate stage HCC[7,8]. Sorafenib, a multi-targeted tyrosine kinase inhibitor (TKI), remains the only systemic therapy that is effective in advanced disease offering marginal survival benefit without significant improvement in cancer related symptoms or quality of life[2]. After many years of disappointing results with chemotherapy, sorafenib was thought to herald a new era in HCC treatment with great optimism for molecularly targeted therapies. Disappointingly, several negative first and second line phase III clinical trials ensued. However, the combination of recent extensive genomic studies and biomarker based clinical trials, provide hope for the development of a more personalised treatment paradigm. This review discusses the current concepts and management of advanced HCC with a particular focus on the failure of molecular targeted therapy beyond sorafenib and outlines how this should be addressed.

Current therapy for advanced disease

Despite only marginal benefits with chemotherapy reported in single arm studies, lack of alternative treatments meant its use was routine prior to the advent of sorafenib. Challenges with toxicities (especially in patients with underlying liver disease) led to chemotherapy being reserved for patients with good performance status and preserved hepatic function. Single agents such as doxorubicin, cisplatin and fluorouracil offer response rates of 10%[9-11]. This increases to 20% with combination regimens, none of which impact survival[9,12]. The recently reported EACH trial, a phase III study conducted in China, Taiwan, Korea and Thailand randomly assigned 371 patients with advanced disease to receive either combined oxaliplatin and fluorouracil/leucovorin (FOLFOX4) or doxorubicin[13]. The trial failed to demonstrate a significant survival difference between each arm, although a trend towards improved outcomes with FOLFOX4 was noted (median overall survival was 6.4 mo for FOLFOX4 and 4.97 mo for doxorubicin; P = 0.7; HR = 0.8; 95%CI: 0.63-1.02).

The search for more efficacious treatments eventually led to two large randomised phase III trials that reported a significant survival benefit with sorafenib in close succession. The first, conducted in a European, Australian and American population, demonstrated a median overall survival (OS) of 10.7 mo for patients treated with sorafenib (400 mg BD) compared with 7.9 mo for placebo (HR = 0.69; 95%CI: 0.55-0.87; P < 0.001)[2]. The latter, conducted in the Asian-Pacific region reported that patients treated with sorafenib led to a median overall survival of 6.5 mo compared with 4.2 mo (HR = 0.68; 95%CI: 0.50-0.93; P = 0.014)[14]. The survival advantages in both trials were modest and neither study established any improvement in cancer symptoms or quality of life. Yet this benefit was sufficient for sorafenib to become the new standard of care for patients with advanced disease. Data extracted from the prospectively maintained GIDEON database (Global Investigation of Therapeutic Decisions in Hepatocellular Carcinoma and of its Treatment with Sorafenib) showed that in 3202 patients treated with HCC, adverse events were comparable between patients with Child-Pugh A and Child-Pugh B cirrhosis[15]. Yet the frequency of serious adverse events was higher in the Child-Pugh B group (60.4% for Child-Pugh B and 36.0% for Child-Pugh A) and median overall survival was shorter 5.2 mo (4.6-6.3) for Child-Pugh B and 13.6 mo (12.8-14.7) for Child-Pugh A (Table 1).

Four separate phase III trials exploring different multi-targeted TKIs have now failed to show superior outcomes to sorafenib. HCCs are vascular tumours and both VEGF and angiopoietin-2 (Ang2) were independent prognostic markers during the SHARP trial and have been associated with tumour growth and metastatic spread[16]. The success of sorafenib was thought to be predominantly related to its anti-angiogenic properties and subsequent studies aimed to identify more potent anti-angiogenic drugs. Sunitinib, a multi-kinase inhibitor targeting VEGFR, PDGFR, c-KIT and FLT-3 has been approved for use in gastro-intestinal stromal tumours and renal cell carcinomas and was more potent that sorafenib in preclinical models[17,18]. Phase II studies showed modest benefit in HCC at best although did highlight potential biomarkers such as interleukin-6, stromal-derived factor1alpha and soluble c-KIT, as changes in tumour vascular permeability and circulating inflammatory molecules were associated with poorer outcome[19-21]. Adverse events in these phase II studies were concerning with liver related toxicities including encephalopathy and hepato-renal syndrome and 5%-10%

Table 1 First line trials with molecular targeted therapies in advanced hepatocellular cancer

Trial	Drugs	Design	*n*	Median survival	HR	*P* value	Ref.
ASIA-PACIFIC	Sorafenib *vs* placebo	Superiority	150	6.5	0.68	0.01	[14]
			76	4.2			
SHARP	Sorafenib *vs* placebo	Superiority	229	10.7	0.69	0.001	[2]
			303	7.9			
SUNITINIB	Sunitinib *vs* sorafenib	Superiority	530	7.9	1.3	0.001	[22]
			544	10.2			
BRISK-FL	Brivanib *vs* sorafenib	Non-inferiority	577	9.5	1.06	0.31	[28]
			578	9.9			
LIGHT	Linifanib *vs* sorafenib	Non-inferiority	514	9.1	1.04	0.52	[24]
			521	9.8			
SEARCH	Sorafenib/erlotinib *vs* sorafenib/placebo	Superiority	362	9.5	0.92	0.48	[29]
			358	8.5			

of patients died from treatment related causes. The daily dose of 50 mg that is routinely used in other tumour types was deemed too high for patients with HCC where it precipitated liver toxicities including portal hypertension, encephalopathy, oesophageal variceal bleeding, ascites and thrombocytopenia. A subsequent head-to-head phase Ⅲ study of 1074 patients randomised to either sunitinib or sorafenib patients terminated early due to both futility and safety concerns[22]. The most frequent grade 3/4 adverse events in the sunitinib group were thrombocytopenia (29.7%) and neutropenia (25.7%) and in the sorafenib group were hand-foot syndrome (21.2%). Overall survival was also significantly lower in the sunitinib arm (7.9 mo *vs* 10.2 mo *P* = 0.0014). Temporary treatment discontinuation was more frequent with sunitinib (76.6% *vs* 58.7%). The failure of sunitinib was likely related to a combination of inadequate dosing, toxicities and trial design, and highlights the need for caution in over-interpretation of phase Ⅱ data and decision to move to Phase Ⅲ trials.

Pre-clinical studies identified linifanib as a more potent dual vascular epidermal growth factor receptor (VEGFR) and platelet derived growth factor receptor (PDGFR) inhibitor than sorafenib (IC_{50} = 25 nmol for linifanib and IC_{50} = 57 nmol sorafenib) and VEGFR (IC_{50} = 8 nmol for linifanib and IC_{50} = 90 nmmol for sorafenib)[23]. A single arm phase Ⅱ trial in the first line setting resulted in a median overall survival of 9.7 mo (10.4 mo in patients with Child-Pugh-A status), which led to a non-inferiority phase Ⅲ trial with sorafenib[24]. The study of 1035 patients failed to reach its end-point with an overall survival of 9.1 mo for linifanib and 9.8 mo for sorafenib (HR = 1.04; 95%CI: 0.89-1.22; *P* = 0.001)[25]. Toxicities of hypertension and hepatic toxicities including encephalopathy were also higher in the linifanib arm.

A single arm first line phase Ⅱ study of 55 patients treated with brivanib, an ATP competitive inhibitor of several kinases including VEGFR2 (IC_{50} = 25 nmol), FGFR-1 (148 nm) and VEGFR1 (380 nmol), resulted in a median overall survival of 10.0 mo[26,27]. Phase Ⅱ studies confirmed that brivanib was well tolerated and one patient had a completed response, three had a partial response and twenty-two had stable disease. Yet BRISK-FL, the subsequent phase Ⅲ direct comparison

trial of brivanib and sorafenib, failed to establish a significant survival benefit (9.5 mo for brivanib *vs* 9.9 mo for sorafenib; HR = 1.06; *P* = 0.31)[28]. Due to the trial design, in order to demonstrate non-inferiority, brivanib needed to produce a hazard ratio between 1 and 1.08, which it narrowly failed to reach. The BRISK-FL trial highlighted the difficulties in extracting comprehensive survival data from non-randomised phase Ⅱ trials. Grade 3/4 toxicities for sorafenib and brivanib were hyponatraemia (9% and 23% respectively), elevated liver enzymes (17% and 14%), fatigue (7% and 15%) and hand-foot reaction (15% and 2%). Even if this trial had met its end-point of non-inferiority, the significant toxicity and economic profiles were not more favourable than sorafenib, and thus would have been of little meaningful clinical benefit.

Erlotinib, an epidermal growth factor receptor (EGFR) TKI was tested in a first-line phase Ⅲ trial in combination with sorafenib compared to placebo/sorafenib in a study of 720 patients with advanced disease[29]. The combination had not previously been tested in phase Ⅱ trials, with two single arm phase Ⅱ studies demonstrating modest disease control[29-31]. The combined treatment did not improve overall survival (9.5 mo compared with 8.5 mo for sorafenib alone HR = 0.92; *P* = 0.2). Toxicities in the combination arm were also higher resulting in a reduced median treatment duration that may have contributed to its diminished efficacy. This trial demonstrates both the danger of proceeding to large-scale phase Ⅲ trials without a clear signal of efficacy from earlier phase studies and the difficulties in combining therapies for HCC (especially for drugs that have overlapping toxicities). Robust HCC-specific phase Ⅰ/Ⅱ studies are needed to identify optimal dosing of combination regimens (Table 2).

FGF has been pursued as a potential target in HCC and recent data suggests the FGF signalling pathway may play a key role in the development of resistance to anti-VEGF therapies by activating alternative proangiogenic signalling pathways[32]. Forty-six patients who had not responded to prior anti-angiogenic therapies were treated with brivanib in a single arm phase Ⅱ study[33]. The results were promising with a median overall survival of 9.7 mo. A subsequent phase Ⅲ trial that was conducted in parallel to the BRISK-FL trial compared brivanib with placebo

Table 2 Second line trials with molecular targeted therapies in advanced hepatocellular cancer						
Trial	Drugs	Design	*n*	Median survival	HR	*P* value
BRISK-PS	Brivanib *vs* placebo	Superiority	263	9.4	0.89	0.33
			132	8.2		
EVOLVE-1	Everolimus *vs* placebo	Superiority	362	7.6	1.05	0.68
			184	7.3		
REACH	Ramucirumab *vs* placebo	Superiority	277	9.2	0.87	0.14
			276	7.6		

as second line treatment failed to meet its end point[34]. Patients treated with brivanib had a median overall survival of 9.7 mo compared with 8.2 mo in the placebo arm (*P* = 0.3). Yet significant improvements were seen in the secondary end points of overall response rate (10% for brivanib *vs* 2% for placebo *P* = 0.003), disease control rate (61% *vs* 40% *P* ≤ 0.001) and alpha-feto protein reduction in 74% of patients with elevated baseline levels (> 50% reduction seen in 54% *vs* 7%). These indicate that brivanib has anti-tumour activity despite the negative primary outcome. Furthermore, despite stratification the placebo cohort had fewer patients with macro-vessel invasion and a numerically lower median AFP level. The unexpectedly long survival of patients in the placebo cohort has been cited as one of the reasons for treatment failure. As expected, there were also higher rates of treatment discontinuation and elective patient withdrawal from the brivanib arm, which may have reduced efficacy in this group.

Mammalian target of rapamycin (mTOR) is upregulated in many solid tumours including HCC and appears to have a critical role in pathogenesis[35,36]. A second line study with the mTOR inhibitor everolimus, offered no survival advantage over placebo (7.6 mo for everolimus *vs* 7.3 mo; HR = 1.05; *P* = 0.68)[37]. Ramucirumab is a fully human monoclonal antibody against vascular endothelial growth factor receptor 2 (VEGFR2), which also failed to improve survival compared with placebo (median overall survival for ramucirumab was 9.2 mo compared with 7.6 mo; HR = 0.86, *P* = 0.13) in the REACH trial[38]. However, a pre-planned sub-group analysis revealed that in patients with elevated baseline alpha-feto protein (AFP) of more than 400 ng/mL, ramucirumab extended both overall and progression free survival. Grade 3 toxicities that occurred more frequently in the ramucirumab arm included hypertension (12% compared with 4%) and fatigue (5% compared with 2%), but its toxicity profile is otherwise favourable compared to the multi-targeted TKIs. Due to this data, a phase Ⅲ trial with second line ramucirumab in a select population with AFP > 400 ng/mL is ongoing.

REASONS FOR THE FAILURE OF PHASE Ⅲ TRIALS

Clinical and molecular heterogeneity
So far all phase Ⅲ trials have unexpectedly failed to reach their end-points. There are several reasons for this.

In the majority of patients with HCC, the cancer arises predominantly as a consequence of liver injury secondary to a variety of causes. It is clear that underlying liver pathology affects both outcome and treatment response, suggesting trials need to be stratified according to aetiology as well as Child-Pugh status, histological grade and stage[39]. Whilst patients with hepatitis B had longer overall survival and shorter time to progression following treatment with sorafenib in the SHARP trial, these results may have been confounded by the imbalance in numbers between patients with hepatitis B and C[2]. Without prior stratification, it is difficult to analyse the survival between sub-groups, highlighting the need for careful trial design.

Limited understanding of oncogenic drivers mean all recent negative phase Ⅲ trials were for "all comers", yet there is marked molecular heterogeneity amongst HCC tumours. Extensive genomic studies have revealed multiple genetic aberrations with more than 30 somatic mutations per tumour[40,41]. The challenge lies in distinguishing which are oncogenic drivers and which are bystander passenger mutations. Once drivers are identified, trials can be tailored to pertinent pathways. However, several studies have challenged the idea that single biopsies can represent the mutational landscape of the whole cancer. With highly mutated tumours such as HCC, the key is finding the so-called "trunk" mutations that exist in all tumour sites[42]. Even if a driver is found, inhibiting pathways may induce resistant mutations. Whilst "liquid" biopsies evaluating circulating DNA are under evaluation, further research is needed to validate these techniques before their use in the clinical setting[43]. One of the barriers to drug development is that many previous HCC trials did not mandate a tissue diagnosis, relying on clinical criteria alone. Several studies have now highlighted histological changes following treatment with loco-regional therapy such as TACE. In a prospective analysis of 80 nodules found in explant livers following transplantation for HCC, 14 cases of mixed hepatocholangiocellular tumours were found in patients who had received TACE whilst none were seen in the treatment-naive group, implying differentiation into a cholangiocellular phenotype for some patients[44]. Furthermore, the lack of histology arguably impedes both predictive and prognostic biomarker development. For example, a phase Ⅱ trial with the selective non ATP competitive c-MET inhibitor tivantinib, did not offer a survival advantage in patients with advanced HCC but a post study sub-group analysis revealed that the overall survival was longer in patients

with high baseline expression of c-MET (overall survival was 7.2 mo for tivantinib and 3.8 mo for placebo HR = 0.38, *P* = 0.01)[45]. A phase Ⅲ trial for patients with tumours over-expressing c-MET in the second line setting is on going (NCT01755767). Therefore, several agents that have failed in phase Ⅲ trials may still be efficacious in sub-groups of patients, emphasising the urgent need for tissue collection and more sophisticated trial designs that accommodate molecular stratification.

Underlying liver cirrhosis

Another challenge when treating patients with HCC is the presence of underlying liver cirrhosis. Historically, clinical trials were reserved for patients with good hepatic reserve so that competing liver morbidity does not overshadow outcomes from malignancy. Yet even in patients with preserved baseline hepatic function, reaching the optimal maximum tolerated dose in patients can be limited by hepatotoxicity. Treatment duration in these trials may have been insufficient to elicit a response. Liver dysfunction and co-existing cirrhosis may affect drug metabolism and due to the consequent changes in the pharmacokinetic and pharmacodynamics profiles of drugs, there is now a trend to conduct HCC-specific phase trials rather than extrapolate results from "all-comer" phase 1 studies conducted in patients with normal or near normal liver function.

There are no approved therapies in patients who progress on sorafenib and who retain well preserved liver function and good performance status. Many centres use cytotoxic chemotherapy (usually with FOLFOX due to results of the EACH trial) despite the lack of clear evidence supporting its use. Due to the lack of effective second-line therapy, patients are encouraged to enter clinical trials of novel agents. By definition, patients suitable for second line trials are more likely to have less aggressive disease than the wider HCC population in whom performance status often deteriorates rapidly on progression and is associated with decompensation of liver function. In a number of the recent second-line phase Ⅲ trials comparing novel therapies to placebo, there has been unexpected prolonged survival in the placebo cohort, potentially diminishing the survival differences between groups. Although the trend for overall survival favoured brivanib in the second line BRISK-PS trial, the results were non-significant suggesting the study was not sufficiently powered to detect benefits with brivanib against a placebo controlled population in whom survival was unexpectedly long[34].

Novel direct-acting antivirals (DAA) that target HCV-encoded proteins necessary for viral replication, can offer patients with hepatitis C sustained virological responses (SVR). The increasing use of these novel agents are expected to have a future impact on the incidence of HCV related HCC. Yet the presence of advanced fibrosis will continue to pose a risk for oncogenesis, even in the absence of a detectable viral load, and screening high risk individuals is still required[46]. The development of molecular predictive biomarkers could help identify patients that require ongoing surveillance. Furthermore, biomarker based stratification could be used to enrich HCC chemoprevention trials[47].

Response evaluation

Finally, response criteria in trials must be chosen carefully. Traditional endpoints such as tumour shrinkage relate to chemotherapy treatments and may not be applicable when assessing the benefits of targeted treatments, which can be cytostatic rather than cytoreductive[48]. Drugs that have been deemed failures in phase Ⅲ studies may have therapeutic activity in HCC, but insufficient potency to improve conventional end-points in phase Ⅲ trials[49]. Furthermore liver disease can elicit an inflammatory response, which can be mistaken for progression resulting in premature cessation of treatment. Thus the use of traditional imaging has been highlighted as insufficient in assessing response in HCC whereby functional imaging provides more useful information. RECIST criteria that is routinely used to measure disease response in many solid tumours, has been recognised as insensitive in HCC. In the SHARP trial, despite an improvement in overall survival, only 2% of patients treated with sorafenib underwent a response by RECIST criteria. The RECIST response criteria were amended to incorporate tumour necrosis induced by treatment. The modified RECIST (mRECIST) measures arterially enhancing lesions that are more representative of residual viable tumour[50,51]. Large multi-centre clinical trials in patients with HCC pose unique challenges and future study designs must accommodate these in order to exploit the true potential of novel agents in this disease[52,53].

THE GENETIC BACKGROUND OF HCC

In malignancies such as melanoma, key driver mutations have now been identified, leading to the use of effective targeted therapy that directly translates to improved patient survival[54]. Despite the presence of more than 40 somatic mutations, there does not appear to be solitary frequent genetic defects in the majority of HCC tumours[40,41,55,56]. Polyclonality has been noted in patients with HCC reflecting a complex genetic landscape. The recently proposed concept of "trunk *vs* branch" heterogeneity can be applied to HCC, whereby key mutations that drive tumorigenesis exist in both primary and secondary lesions (trunk) and need to be distinguished from those that are only present in a minority of tissue (branch)[42]. The question remains as to whether the vast number of genetic alterations in HCC reflect multiple "trunk" mutations that would each require inhibition, or if the majority are mere passenger alterations that do not need treating. Recent advances in high throughput sequencing have uncovered several mechanisms of genetic changes, including somatic mutations, copy number alterations, HBV integration and somatic changes of retrotransposons[55,57]. Whole genome sequencing of 88 primary HCC tumours with

matched adjacent liver tissue revealed the predominant oncogenic mutation was beta catenin (15.9%) which is mutually exclusive with the most frequently mutated tumour suppressor gene Tp53 (35.2%) echoing results from previous genomic studies[41,55,58,59]. Further mutations have been found in ARID1 and 2 (both of which regulate chromatin remodelling pathways) and rare mutations in RPS6KA3 which codes for RSK2 (a serine threonine kinase of the MAPK pathway)[60]. A larger study of 503 HCC liver genomes revealed 30 driver genes implicating 11 core pathways in tumorigenesis. Recurrent focal amplifications were seen in 25% of cases, including telomerase reverse-transcriptase (TERT) and CCND1-FGF19. Key oncogenic pathways included TP53-RB, Wnt and mTOR-PIK3CA[61]. Frequently altered in HCC, somatic TERT mutations have also been found in pre-cancerous cirrhotic nodules and hepatic adenomas, suggesting they play a pivotal role in malignant transformation. Sequencing of the promoter region of tissue taken from 305 HCCs revealed recurrent TERT mutations in 179 samples (59%) at two common mutually exclusive hot spots[62]. Yet despite a greater understanding of the role of TERT in HCC, its potential as a druggable target remains unknown. A small early phase Ⅱ study of a telomerase derived peptide, GV1001, failed to elicit any responses, although the trial was not enriched for TERT mutated tumours[63].

HCC can be classified into two distinct sub-groups based on genetic aberrations[64-67]. The proliferative subclass is characterised by activation of RAS, mTOR and IGF signalling and has been associated with poor outcomes. This group can be further divided into those with Wnt/transforming growth factor (TGF)-β activation and the progenitor cell group that have higher progenitor cell, epithelial cell adhesion molecules and type 1 cytoskeletal 19 markers. By comparison, the non-proliferative group is more heterogeneous with less shared mutations. The Wnt/beta catenin and JAK/STAT signalling pathways are the most frequently affected pathways, with alterations in as many as 50%-62.5% and 45% of cases respectively[66,68,69]. Several distinct protein-altering JAK1 mutations have been identified, the majority of which affect the kinase domain[55,70]. HCC development is often attributed to chronic inflammation triggered by both viral infection and cell necrosis and the JAK/STAT pathway has been identified as a promoter of carcinogenesis in a sub-set of HCC *via* cytokine-induced JAK/STAT pathway activation[55,71].

Copy number analyses using array based comparative genomic hybridization (aCGH) have revealed recurrent amplifications in genes for p53, Wnt signalling, proliferation pathways with recurrent deletions of genes involved in the immune response, chromatin remodelling and NF-kβ pathways[72,73]. Furthermore, the DNA virus hepatitis B (HBV), a leading cause of HCC, integrates into the host genome affecting gene expression. Deep sequencing of HCC samples on a background HBV found direct genetic disruption, aberrations of viral promoter-driven transcription, viral-human transcription and copy number changes confirming theories that alternate aetiologies

lead to distinct genetic alterations[74,75]. Whole exome sequencing of 243 liver tumours revealed mutational signatures that appeared to correlate with specific risk factors for HCC development including CTNNB1 (alcohol) and TP53(HBV)[76]. In addition, different mutations were associated with varying clinical outcomes. Early stage disease harboured TERT promoter mutations whereas FGF, CCDN1, TP53 were associated with more aggressive pathology.

Conclusions from these extensive genetic studies have highlighted not only the heterogeneity of HCC tumours but also the significant differences in key oncogenic drivers of HCC compared with many other solid malignancies. In breast, colorectal and lung for example, MAPK and PI3K as well as EGFR activated pathways dominate progression in distinct cohorts[77-79]. However, for HCC Wnt/β-catenin and JAK/STAT pathways have consistently been identified as responsible for key oncogenic signalling. These differences are likely to explain the failures of therapies in HCC that have provided benefit in other malignancies. Comprehensive genetic mapping will undoubtedly aid drug development for HCC but a major challenge is that the majority of pathways found remain "undruggable" and interacting protein kinases must be targeted instead (Figure 1). A selection of key pathways and novel agents recently or currently under investigation are discussed below.

EMERGING TARGETS IN DRUG DEVELOPMENT

MEK inhibition

The RAF/MEK/ERK pathway plays a pivotal role in several cellular process including proliferation, apoptosis and migration[80,81]. Although RAS and RAF mutations are uncommon in HCC, there is evidence that this pathway is activated in the majority of HCC tumours. Selumetinib, a potent selective MEK 1/2 inhibitor, was assessed in a single arm phase 2 trial in 19 patients who had not received prior systemic therapy. There were no responses and time to progression was short (8 wk). The trial was subsequently terminated at the interim analysis[82]. Examination of pre and post treatment tissue revealed that four out of five patients achieved significant inhibition of phospho-ERK1/2 in tumours suggesting the failure of selumetinib was not due to lack of target inhibition. A small study assessing in combination with sorafenib resulted in three partial responses and six with stable disease. Whilst these numbers were small and therefore difficult to interpret, it suggests that perhaps this combination should be assessed further[83]. A phase Ⅱ study assessing the efficacy and safety of combination inhibition using sorafenib and the MEK inhibitor refametinib, resulted in a median time to progression of 122 d and median OS of 290 d[84]. Toxicities however were significant with rash, diarrhoea, elevated liver enzymes and vomiting and the majority of patients required dose reductions. Interestingly the best responders harboured a RAS mutation and a proof of concept phase Ⅱ trial using this combination for
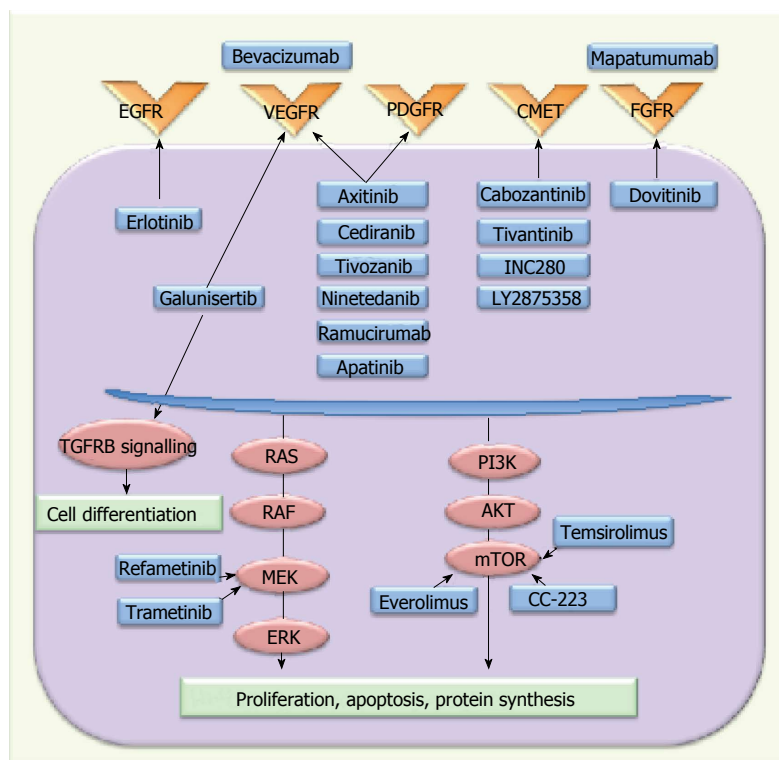
Figure 1 Novel compounds under investigation and their predominant targets. EGFR: Epidermal growth factor receptor; VEGFR: Vascular endothelial growth factor receptor; PDGFR: Platelet derived growth factor receptor; FGFR: Fibroblast growth factor receptor; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; mTOR: Mammalian target of rapamycin.

patients with RAS mutations is on going (NCT01915602). Crucially, this study is one of the first attempts to select a specific cohort of HCC patients based on molecular genotype utilising cfDNA to detect mutations in RAS. The study raises a number of important issues regarding feasibility and cost given the incidence of RAS mutation is approximately 3%-5%, requiring a large cohort of patients to be prescreened to identify the small group with aberrant genotype (Table 3).

Anti-angiogenic therapy

HCC is a hyper vascular tumour enriched with high levels of angiogenesis due to the presence of growth factors such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF)[85]. A meta-analysis assessing the prognostic value of VEGF expression confirmed that tissue and serum VEGF levels seemed to predict poor disease free and overall survival[86]. Biomarker data from the SHARP trial also demonstrated that VEGF and angiopoietin-2 [(Ang2) a further critical molecule in angiogenesis] were independent prognostic markers but not predictive of response[16]. Sorafenib has anti-angiogenic properties and its success fuelled the search for more potent, selective anti-angiogenics. Yet several negative clinical trials have questioned the emphasis on VEGF inhibition in HCC, supporting theories that multiple mechanisms may be in play. As discussed the VEGF inhibitors, sunitinib, linifanib and brivanib failed to prove non-inferiority compared with sorafenib. Some commentators have therefore argued that an antiangiogenic monotherapy "ceiling" has been reached, and combination strategies will be required to extend survival beyond this[87]. Trials of sorafenib

in combination with other antiangiogenic therapy (bevacizumab), chemotherapy (doxorubicin or FOLFOX) or other molecularly targeted therapy (*e.g.*, everolimus and temsirolimus) are on-going. In order to ensure optimal results with these agents, the development of predictive biomarkers is needed to select patients who are most likely to benefit.

HGF/c-MET pathway

In vitro studies suggest that c-Met may play a role in proliferation, angiogenesis and metastatic spread in HCC and the hepatocyte growth factor (HGF)-cMET axis is therefore an attractive target. Whilst HGF expression in HCC tumours is low compared with surrounding liver tissue, over-expression of cMET has been observed in nearly a quarter of HCC cases and there is some evidence to suggest c-MET expression is a poor prognostic marker[88-90]. Biomarker data from the SHARP trial revealed that HGF levels correlated with tumour size[16]. There is also evidence of an interaction between c-MET and both EGFR and VEGF[91]. Preliminary data from c-MET inhibition with cabozantinib is promising and as previously discussed a phase III trial with tivantinib in patients with high levels of MET expression is on going[92].

FGFR inhibition

Fibroblast growth factors are trans membrane receptor kinases that signal downstream pathways including the RAS-RAF-MAPK. FGF3/4 is expressed in normal tissue including benign hepatocytes[93]. Gene array studies and Immunohistochemical expression assays have shown overexpression of FGF3 and FGF4 in HCC tumours that mediate proliferation, cell death and alpha

Table 3 Novel agents currently under evaluation in clinical trials

Drug	Phase	Target	Enriched population	Trial identifier	Study location
Tivantinib	III	MET/tubulin	High MET expression	NCT01755767	North America, Europe
Axitinib	II	VEGFR/c-KIT/PDGFR	No	NCT01334112	North America
Tivozanib	I / II	VEGFR	No	NCT01835223	North America
Nintedanib	I / II	VEGFR/FGFR/PDGFR	No	NCT00987935	Asia
Ramucirumab	III	VEGFR2	AFP > 400	NCT02435433	North America, Asia, Europe
Apatinib	III	VEGFR2	No	NCT02329860	Asia
Cabozantinib	III	MET	No	NCT01908426	North America, Asia, Europe
INC280	II	MET	MET aberration	NCT01737827	Asia
LY2875358	I / II	MET/VEGFR	No	NCT01287546	North America
Refametinib	II	MEK	RAS mutations	NCT01915602	North America, Asia, Europe
Trametinib	I / II	MEK1/2	No	NCT02292173	North America
Dovitinib	II	VEGFR, FGFR	No	NCT01232296	Asia
Temsirolimus	I , II	mTOR	No	NCT01687673	North America
Cc-223	I , II	mTOR	No	NCT01177397	North America, Europe
Galunisertib	II	TGFRβ	No	NCT02423343	North America
Mapatumumab	I / II	TRAIL-R1	No	NCT01258608	North America, Europe
Nivolumab	I	PD1	No	NCT01658878	North America, Europe, Asia
Lenvatinib	III	VEGF	No	NCT01761266	North America, Europe, Asia
Enzalutamide	II	Androgen receptors	No	NCT02528643	TBC
OMP-54F28	I	Wnt signalling	No	NCT02069145	North America

feto protein (AFP) levels[94]. Brivanib, in addition to its anti-angiogenic properties as discussed above, is an ATP competitive inhibitor of FGF1-3. Although it failed to improve survival in the first and second line setting, further multi-kinase inhibitors that also target FGFR are currently underway. The lack of response to brivanib may be partly explained by its use in an unspecified population and biomarkers may aid selection of patients likely to respond to inhibition. Lenvatinib, an oral multi-targeted tyrosine kinase inhibitor of VEGFR-1, FGFDR1-4, PDGFRβ, RET and KIT is currently under evaluation in a non-inferiority study with sorafenib following a phase II trial which resulted in a median time to progression of 12.8 mo (95%CI: 7.23-14.7) and median OS of 18.7 mo NCT01761266[95]. The REFLECT phase III trial comparing sorafenib to lenvatinib has recently been completed. This trial has attempted to learn the lessons from the previous high profile failures described in this article by utilising stricter criteria for trial entry, excluding poor prognosis groups such as those patients with greater than 50% liver involvement, bile duct invasion, or main branch portal venous infiltration.

Dovitinib, an FGFR, VEGFR and PDGFR TKI demonstrated efficacy in xenograft mouse models and is currently under investigation in a phase II trial[96,97]. FGF19, located on chromosome 11q13, a region amplified in 10%-15% of HCC tumours, is a potential predictive biomarker for FGF inhibitors and FGF19 targeted antibodies are under investigation in *in vitro* models[97]. *In vivo* studies with murine models suggest that dual targeting with FGFR and mTOR inhibition impaired tumour growth unlike treatment with the FGFR inhibitor alone providing support for combination trials[98].

TGF-β signalling
TGF-β signalling plays a role in the micro-tumour en-vironment promoting epithelial-mesenchymal transition (EMT), dysplastic nodule formation and subsequent HCC development[99-101]. Patients with higher levels of TGF-β signalling are associated with larger less differentiated tumours with higher levels of AFP[102]. It remains unclear whether TGF-β plays a role in a sub-group of patients, or in the carcinogenesis of all HCCs due to its dual role in tumour suppression in normal tissue and tumour promotion in HCC. TGF-β inhibitors modulate EMT leading to reduced tumour growth in pre-clinical models. Galunisertib, a selective TGF-β TKI is currently under investigation in a phase II trial (NCT02178358).

Immunotherapy
Recent years have seen a resurgence in the use of immunotherapy, led partly by the success of anti-CTL4 antibodies in solid tumours such as melanoma and more recently antibodies targeting the programmed death (PD) receptor and its ligand[103,104]. Immunotherapy works by enhancing anti-tumour response, an important mechanism in HCC as the surrounding micro-tumour environment is rich in immune cells. Tremelimumab, a fully human IgG2 monoclonal anti-CTL4 antibody was assessed in a phase II study of 24 patients with HCC on a background of HCV. The drug had a good safety profile and a partial response of 17.6% and disease control rate of 76.4%. Time to progression was 6.48 m (95%CI: 3.95-9.14). Changes were also seen in the predominant variants of HCV as well as a reduction in viral loads. These early reports are promising and suggest that immunotherapy may have the dual benefit of treating both HCC and underlying viral hepatitis. Anti-programmed death ligand 1 (PDL1) inhibitors are checkpoint inhibitors that block T cell activation when bound by PD ligands 1 and 2. Patients with tumours that over-express PD-L1 are associated with a poorer prognosis. In a recently reported phase I / II dose

escalation study, patients received 0.1 to 10.0 mg/kg of the anti-PDL1 agent nivolumab intravenously for up to 2 years. 2 patients had a complete response (CR) and a further 7 patients had a partial response (PR)[105]. The overall survival rate at 6 mo was 72%. Although these results are from a very small early phase trial, they are highly encouraging and a number of trials using checkpoint inhibitors are now planned in both first and second line settings.

CONCLUSION

The era of personalised medicine and treatment stratification has yet to impact clinical practice of HCC and the failure of several clinical trials has been disappointing. Nevertheless our understanding of this unique disease has improved significantly with the benefit of genomic sequencing and biomarker data from clinical trials. Proof of concept studies such as the ongoing phase Ⅱ trial with refametinib for RAS mutated cancers and tivantinib for c-MET positive tumours are a step forward in designing adequate trials to maximise potential benefit of novel agents in pre-determined sub groups. Molecular testing, improved clinical trial design and the development of predictive biomarkers should finally see an improvement in survival for this global disease.

REFERENCES

1   Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2   Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

3   El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124]

4   Osaki Y, Nishikawa H. Treatment for hepatocellular carcinoma in Japan over the last three decades: Our experience and published work review. *Hepatol Res* 2015; **45**: 59-74 [PMID: 24965914 DOI: 10.1111/hepr.12378]

5   Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013; **47** Suppl: S2-S6 [PMID: 23632345 DOI: 10.1097/MCG.0b013e3182872f29]

6   Lencioni R, Chen XP, Dagher L, Venook AP. Treatment of intermediate/advanced hepatocellular carcinoma in the clinic: how can outcomes be improved? *Oncologist* 2010; **15** Suppl 4: 42-52 [PMID: 21115580 DOI: 10.1634/theoncologist.2010-S4-42]

7   Llovet JM, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862]

8   Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164-1171 [PMID: 11981766 DOI: 10.1053/jhep.2002.33156]

9   Yeo W, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo

FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ. A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005; **97**: 1532-1538 [PMID: 16234567 DOI: 10.1093/jnci/dji315]

10 Okada S, Okazaki N, Nose H, Shimada Y, Yoshimori M, Aoki K. A phase 2 study of cisplatin in patients with hepatocellular carcinoma. *Oncology* 1993; **50**: 22-26 [PMID: 7678453 DOI: 10.1159/000227142]

11 Tetef M, Doroshow J, Akman S, Coluzzi P, Leong L, Margolin K, Morgan RJ, Raschko J, Shibata S, Somlo G. 5-Fluorouracil and high-dose calcium leucovorin for hepatocellular carcinoma: a phase II trial. *Cancer Invest* 1995; **13**: 460-463 [PMID: 7552810 DOI: 10.3109/07357909509024907]

12 Parikh PM, Fuloria J, Babu G, Doval DC, Awasthy BS, Pai VR, Prabhakaran PS, Benson AB. A phase II study of gemcitabine and cisplatin in patients with advanced hepatocellular carcinoma. *Trop Gastroenterol* 2005; **26**: 115-118 [PMID: 16512457]

13 Qin S, Bai Y, Lim HY, Thongprasert S, Chao Y, Fan J, Yang TS, Bhudhisawasdi V, Kang WK, Zhou Y, Lee JH, Sun Y. 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* 2013; **31**: 3501-3508 [PMID: 23980077 DOI: 10.1200/JCO.2012.44.5643]

14 Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. 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* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]

15 D'Angelo S, Germano D, Zolfino T, Sansonno D, Giannitrapani L, Benedetti A, Montesarchio V, Attili A, Buonadonna A, Barni S, Gasbarrini A, Burlone ME, Cillo U, Marenco S, Villa E, Giovanis P, Proserpio I, Saitta C, Magini G, Cengarle R, Fava G, Cuttone F, Calvani N, Angelico M, Di Costanzo F, Noto A, Poggi G, Marignani M, Cascinu S, Amoroso D, Palmieri V, Massa E, Crocè LS, Picardi A, Tumulo S, Erminero C, Lencioni R, Lorusso V. [Therapeutic decisions and treatment with sorafenib in hepatocellular carcinoma: final analysis of GIDEON study in Italy]. *Recenti Prog Med* 2015; **106**: 217-226 [PMID: 25994538 DOI: 10.1701/1868.20406]

16 Llovet JM, Peña CE, Lathia CD, Shan M, Meinhardt G, Bruix J. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2290-2300 [PMID: 22374331 DOI: 10.1158/1078-0432.CCR-11-2175]

17 Huynh H, Ngo VC, Choo SP, Poon D, Koong HN, Thng CH, Toh HC, Zheng L, Ong LC, Jin Y, Song IC, Chang AP, Ong HS, Chung AY, Chow PK, Soo KC. Sunitinib (SUTENT, SU11248) suppresses tumor growth and induces apoptosis in xenograft models of human hepatocellular carcinoma. *Curr Cancer Drug Targets* 2009; **9**: 738-747 [PMID: 19754358 DOI: 10.2174/156800909789271530]

18 Proceedings of a Bridge to a Consensus on Hepatocellular Carcinoma Management. The 2nd Asia Pacific Primary Liver Cancer Expert Meeting. July 1-3, 2011, Osaka, Japan. *Oncology* 2011; **81** Suppl 1: 1-164 [PMID: 22371967]

19 Zhu AX, Sahani DV, Duda DG, di Tomaso E, Ancukiewicz M, Catalano OA, Sindhwani V, Blaszkowsky LS, Yoon SS, Lahdenranta J, Bhargava P, Meyerhardt J, Clark JW, Kwak EL, Hezel AF, Miksad R, Abrams TA, Enzinger PC, Fuchs CS, Ryan DP, Jain RK. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. *J Clin Oncol* 2009; **27**: 3027-3035 [PMID: 19470923 DOI: 10.1200/JCO.2008.20.9908]

20 Faivre S, Raymond E, Boucher E, Douillard J, Lim HY, Kim JS, Zappa M, Lanzalone S, Lin X, Deprimo S, Harmon C, Ruiz-Garcia A, Lechuga MJ, Cheng AL. Safety and efficacy of sunitinib

in patients with advanced hepatocellular carcinoma: an open-label, multicentre, phase II study. *Lancet Oncol* 2009; **10**: 794-800 [PMID: 19586800 DOI: 10.1016/S1470-2045(09)70171-8]

21   **Strosberg JR**, Weber JM, Choi J, Campos TL, Valone TL, Han G, Schell MJ, Kvols LK. A phase II clinical trial of sunitinib following hepatic transarterial embolization for metastatic neuroendocrine tumors. *Ann Oncol* 2012; **23**: 2335-2341 [PMID: 22317769 DOI: 10.1093/annonc/mdr614]

22   **Cheng AL**, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J Clin Oncol* 2013; **31**: 4067-4075 [PMID: 24081937 DOI: 10.1200/JCO.2012.45.8372]

23   **Chang AY**, Wang M. In-vitro growth inhibition of chemotherapy and molecular targeted agents in hepatocellular carcinoma. *Anticancer Drugs* 2013; **24**: 251-259 [PMID: 23187461 DOI: 10.1097/CAD.0b013e32835ba28]

24   **Toh HC**, Chen PJ, Carr BI, Knox JJ, Gill S, Ansell P, McKeegan EM, Dowell B, Pedersen M, Qin Q, Qian J, Scappaticci FA, Ricker JL, Carlson DM, Yong WP. Phase 2 trial of linifanib (ABT-869) in patients with unresectable or metastatic hepatocellular carcinoma. *Cancer* 2013; **119**: 380-387 [PMID: 22833179 DOI: 10.1002/cncr.27758]

25   **Cainap C**, Qin S, Huang WT, Chung IJ, Pan H, Cheng Y, Kudo M, Kang YK, Chen PJ, Toh HC, Gorbunova V, Eskens FA, Qian J, McKee MD, Ricker JL, Carlson DM, El-Nowiem S. Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2015; **33**: 172-179 [PMID: 25488963 DOI: 10.1200/JCO.2013.54.3298]

26   **Huynh H**, Ngo VC, Fargnoli J, Ayers M, Soo KC, Koong HN, Thng CH, Ong HS, Chung A, Chow P, Pollock P, Byron S, Tran E. Brivanib alaninate, a dual inhibitor of vascular endothelial growth factor receptor and fibroblast growth factor receptor tyrosine kinases, induces growth inhibition in mouse models of human hepatocellular carcinoma. *Clin Cancer Res* 2008; **14**: 6146-6153 [PMID: 18829493 DOI: 10.1158/1078-0432.CCR-08-0509]

27   **Park JW**, Finn RS, Kim JS, Karwal M, Li RK, Ismail F, Thomas M, Harris R, Baudelet C, Walters I, Raoul JL. Phase II, open-label study of brivanib as first-line therapy in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2011; **17**: 1973-1983 [PMID: 21349999 DOI: 10.1158/1078-0432.CCR-10-2011]

28   **Johnson PJ**, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Aviña J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, Decaens T, Tak WY, Jeng LB, Liu D, Ezzeddine R, Walters I, Cheng 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* 2013; **31**: 3517-3524 [PMID: 23980084 DOI: 10.1200/JCO.2012.48.4410]

29   **Zhu AX**, Rosmorduc O, Evans TR, Ross PJ, Santoro A, Carrilho FJ, Bruix J, Qin S, Thuluvath PJ, Llovet JM, Leberre MA, Jensen M, Meinhardt G, Kang YK. SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2015; **33**: 559-566 [PMID: 25547503 DOI: 10.1200/JCO.2013.53.7746]

30   **Philip PA**, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, Donehower RC, Fitch T, Picus J, Erlichman C. Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. *J Clin Oncol* 2005; **23**: 6657-6663 [PMID: 16170173]

31   **Thomas MB**, Chadha R, Glover K, Wang X, Morris J, Brown T, Rashid A, Dancey J, Abbruzzese JL. Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. *Cancer* 2007; **110**: 1059-1067 [PMID: 17623837]

32   **Bergers G**, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 2008; **8**: 592-603 [PMID: 18650835 DOI: 10.1038/nrc2442]

33   **Finn RS**, Kang YK, Mulcahy M, Polite BN, Lim HY, Walters I, Baudelet C, Manekas D, Park JW. Phase II, open-label study of brivanib as second-line therapy in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2090-2098 [PMID: 22238246 DOI: 10.1158/1078-0432.CCR-11-1991]

34   **Llovet JM**, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, Kang YK, Assenat E, Lim HY, Boige V, Mathurin P, Fartoux L, Lin DY, Bruix J, Poon RT, Sherman M, Blanc JF, Finn RS, Tak WY, Chao Y, Ezzeddine R, Liu D, Walters I, Park JW. 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* 2013; **31**: 3509-3516 [PMID: 23980090 DOI: 10.1200/JCO.2012.47.3009]

35   **Villanueva A**, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, Tovar V, Roayaie S, Minguez B, Sole M, Battiston C, Van Laarhoven S, Fiel MI, Di Feo A, Hoshida Y, Yea S, Toffanin S, Ramos A, Martignetti JA, Mazzaferro V, Bruix J, Waxman S, Schwartz M, Meyerson M, Friedman SL, Llovet JM. Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* 2008; **135**: 1972-1983 [PMID: 18929564 DOI: 10.1053/j.gastro.2008.08.008]

36   **Matter MS**, Decaens T, Andersen JB, Thorgeirsson SS. Targeting the mTOR pathway in hepatocellular carcinoma: current state and future trends. *J Hepatol* 2014; **60**: 855-865 [PMID: 24308993 DOI: 10.1016/j.jhep.2013.11.031]

37   **Zhu AX**, Kudo M, Assenat E, Cattan S, Kang YK, Lim HY, Poon RT, Blanc JF, Vogel A, Chen CL, Dorval E, Peck-Radosavljevic M, Santoro A, Daniele B, Furuse J, Jappe A, Perraud K, Anak O, Sellami DB, Chen LT. Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. *JAMA* 2014; **312**: 57-67 [PMID: 25058218 DOI: 10.1001/jama.2014.7189]

38   **Zhu AX**, Park JO, Ryoo BY, Yen CJ, Poon R, Pastorelli D, Blanc JF, Chung HC, Baron AD, Pfiffer TE, Okusaka T, Kubackova K, Trojan J, Sastre J, Chau I, Chang SC, Abada PB, Yang L, Schwartz JD, Kudo M. 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* 2015; **16**: 859-870 [PMID: 26095784 DOI: 10.1016/S1470-2045(15)00050-9]

39   **Arzumanyan A**, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013; **13**: 123-135 [PMID: 23344543 DOI: 10.1038/nrc3449]

40   **Guichard C**, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, Clément B, Balabaud C, Chevet E, Laurent A, Couchy G, Letouzé E, Calvo F, Zucman-Rossi J. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet* 2012; **44**: 694-698 [PMID: 22561517 DOI: 10.1038/ng.2256]

41   **Cleary SP**, Jeck WR, Zhao X, Chen K, Selitsky SR, Savich GL, Tan TX, Wu MC, Getz G, Lawrence MS, Parker JS, Li J, Powers S, Kim H, Fischer S, Guindi M, Ghanekar A, Chiang DY. Identification of driver genes in hepatocellular carcinoma by exome sequencing. *Hepatology* 2013; **58**: 1693-1702 [PMID: 23728943 DOI: 10.1002/hep.26540]

42   **Gerlinger M**, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, Varela I, Phillimore B, Begum S, McDonald NQ, Butler A, Jones D, Raine K, Latimer C, Santos CR, Nohadani M, Eklund AC, Spencer-Dene B, Clark G, Pickering L, Stamp G, Gore M, Szallasi Z, Downward J, Futreal PA, Swanton C. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 2012; **366**: 883-892 [PMID: 22397650 DOI: 10.1056/NEJMoa1113205]

43   **Crowley E**, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; **10**: 472-484 [PMID: 23836314 DOI: 10.1038/nrclinonc.2013]

44   **Zen C**, Zen Y, Mitry RR, Corbeil D, Karbanová J, O'Grady

J, Karani J, Kane P, Heaton N, Portmann BC, Quaglia A. Mixed phenotype hepatocellular carcinoma after transarterial chemoembolization and liver transplantation. *Liver Transpl* 2011; **17**: 943-954 [PMID: 21491582 DOI: 10.1002/lt.22314]

45   **Santoro A**, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, Van Vlierberghe H, Trojan J, Kolligs FT, Weiss A, Miles S, Gasbarrini A, Lencioni M, Cicalese L, Sherman M, Gridelli C, Buggisch P, Gerken G, Schmid RM, Boni C, Personeni N, Hassoun Z, Abbadessa G, Schwartz B, Von Roemeling R, Lamar ME, Chen Y, Porta C. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *Lancet Oncol* 2013; **14**: 55-63 [PMID: 23182627 DOI: 10.1016/S1470-2045(12)70490-4]

46   **Li DK**, Chung RT. Impact of hepatitis C virus eradication on hepatocellular carcinogenesis. *Cancer* 2015; **121**: 2874-2882 [PMID: 26079399 DOI: 10.1002/cncr.29528]

47   **Goossens N**, Hoshida Y. Hepatitis C virus-induced hepatocellular carcinoma. *Clin Mol Hepatol* 2015; **21**: 105-114 [PMID: 26157746 DOI: 10.3350/cmh.2015.21.2.105]

48   **Gschwind A**, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer* 2004; **4**: 361-370 [PMID: 15122207]

49   **Llovet JM**, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 698-711 [PMID: 18477802 DOI: 10.1093/jnci/djn134]

50   **Goh V**, Sarker D, Osmany S, Cook GJ. Functional imaging techniques in hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2012; **39**: 1070-1079 [PMID: 22434049 DOI: 10.1007/s00259-012-2096-x]

51   **Prajapati HJ**, Spivey JR, Hanish SI, El-Rayes BF, Kauh JS, Chen Z, Kim HS. mRECIST and EASL responses at early time point by contrast-enhanced dynamic MRI predict survival in patients with unresectable hepatocellular carcinoma (HCC) treated by doxorubicin drug-eluting beads transarterial chemoembolization (DEB TACE). *Ann Oncol* 2013; **24**: 965-973 [PMID: 23223331 DOI: 10.1093/annonc/mds605]

52   **Llovet JM**, Hernandez-Gea V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. *Clin Cancer Res* 2014; **20**: 2072-2079 [PMID: 24589894 DOI: 10.1158/1078-0432.CCR-13-0547]

53   **Llovet JM**. Liver cancer: time to evolve trial design after everolimus failure. *Nat Rev Clin Oncol* 2014; **11**: 506-507 [PMID: 25091613 DOI: 10.1038/nrclinonc.2014.136]

54   **Chapman PB**, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011; **364**: 2507-2516 [PMID: 21639808 DOI: 10.1056/NEJMoa1103782]

55   **Kan Z**, Zheng H, Liu X, Li S, Barber TD, Gong Z, Gao H, Hao K, Willard MD, Xu J, Hauptschein R, Rejto PA, Fernandez J, Wang G, Zhang Q, Wang B, Chen R, Wang J, Lee NP, Zhou W, Lin Z, Peng Z, Yi K, Chen S, Li L, Fan X, Yang J, Ye R, Ju J, Wang K, Estrella H, Deng S, Wei P, Qiu M, Wulur IH, Liu J, Ehsani ME, Zhang C, Loboda A, Sung WK, Aggarwal A, Poon RT, Fan ST, Wang J, Hardwick J, Reinhard C, Dai H, Li Y, Luk JM, Mao M. Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. *Genome Res* 2013; **23**: 1422-1433 [PMID: 23788652 DOI: 10.1101/gr.154492.113]

56   **Villanueva A**, Llovet JM. Liver cancer in 2013: Mutational landscape of HCC--the end of the beginning. *Nat Rev Clin Oncol* 2014; **11**: 73-74 [PMID: 24395088 DOI: 10.1038/nrclinonc.2013.243]

57   **Lee JS**, Thorgeirsson SS. Comparative and integrative functional genomics of HCC. *Oncogene* 2006; **25**: 3801-3809 [PMID: 16799621]

58   **Laurent-Puig P**, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F,

Monges G, Thomas G, Bioulac-Sage P, Zucman-Rossi J. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology* 2001; **120**: 1763-1773 [PMID: 11375957]

59   **de La Coste A**, Romagnolo B, Billuart P, Renard CA, Buendia MA, Soubrane O, Fabre M, Chelly J, Beldjord C, Kahn A, Perret C. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci USA* 1998; **95**: 8847-8851 [PMID: 9671767]

60   **Li M**, Zhao H, Zhang X, Wood LD, Anders RA, Choti MA, Pawlik TM, Daniel HD, Kannangai R, Offerhaus GJ, Velculescu VE, Wang L, Zhou S, Vogelstein B, Hruban RH, Papadopoulos N, Cai J, Torbenson MS, Kinzler KW. Inactivating mutations of the chromatin remodeling gene ARID2 in hepatocellular carcinoma. *Nat Genet* 2011; **43**: 828-829 [PMID: 21822264 DOI: 10.1038/ng.903]

61   **Totoki Y**, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M, Tsuji S, Donehower LA, Slagle BL, Nakamura H, Yamamoto S, Shinbrot E, Hama N, Lehmkuhl M, Hosoda F, Arai Y, Walker K, Dahdouli M, Gotoh K, Nagae G, Gingras MC, Muzny DM, Ojima H, Shimada K, Midorikawa Y, Goss JA, Cotton R, Hayashi A, Shibahara J, Ishikawa S, Guiteau J, Tanaka M, Urushidate T, Ohashi S, Okada N, Doddapaneni H, Wang M, Zhu Y, Dinh H, Okusaka T, Kokudo N, Kosuge T, Takayama T, Fukayama M, Gibbs RA, Wheeler DA, Aburatani H, Shibata T. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat Genet* 2014; **46**: 1267-1273 [PMID: 25362482 DOI: 10.1038/ng.3126]

62   **Nault JC**, Zucman-Rossi J. TERT promoter mutations in primary liver tumors. *Clin Res Hepatol Gastroenterol* 2015 Aug 31; Epub ahead of print [PMID: 26336998 DOI: 10.1016/j.clinre.2015.07.006]

63   **Greten TF**, Forner A, Korangy F, N'Kontchou G, Barget N, Ayuso C, Ormandy LA, Manns MP, Beaugrand M, Bruix J. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. *BMC Cancer* 2010; **10**: 209 [PMID: 20478057 DOI: 10.1186/1471-2407-10-209]

64   **Chiang DY**, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, LeBlanc AC, Donovan DJ, Thung SN, Solé M, Tovar V, Alsinet C, Ramos AH, Barretina J, Roayaie S, Schwartz M, Waxman S, Bruix J, Mazzaferro V, Ligon AH, Najfeld V, Friedman SL, Sellers WR, Meyerson M, Llovet JM. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. *Cancer Res* 2008; **68**: 6779-6788 [PMID: 18701503 DOI: 10.1158/0008-5472]

65   **Boyault S**, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P, Zucman-Rossi J. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 2007; **45**: 42-52 [PMID: 17187432]

66   **Lachenmayer A**, Alsinet C, Savic R, Cabellos L, Toffanin S, Hoshida Y, Villanueva A, Minguez B, Newell P, Tsai HW, Barretina J, Thung S, Ward SC, Bruix J, Mazzaferro V, Schwartz M, Friedman SL, Llovet JM. Wnt-pathway activation in two molecular classes of hepatocellular carcinoma and experimental modulation by sorafenib. *Clin Cancer Res* 2012; **18**: 4997-5007 [PMID: 22811581]

67   **Llovet JM**, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. *Nat Rev Clin Oncol* 2015; **12**: 436 [PMID: 26099984]

68   **Wong CM**, Fan ST, Ng IO. beta-Catenin mutation and overexpression in hepatocellular carcinoma: clinicopathologic and prognostic significance. *Cancer* 2001; **92**: 136-145 [PMID: 11443619]

69   **Huang H**, Fujii H, Sankila A, Mahler-Araujo BM, Matsuda M, Cathomas G, Ohgaki H. Beta-catenin mutations are frequent in human hepatocellular carcinomas associated with hepatitis C virus infection. *Am J Pathol* 1999; **155**: 1795-1801 [PMID: 10595907]

70   **Xie HJ**, Bae HJ, Noh JH, Eun JW, Kim JK, Jung KH, Ryu JC, Ahn YM, Kim SY, Lee SH, Yoo NJ, Lee JY, Park WS, Nam SW. Mutational analysis of JAK1 gene in human hepatocellular carcinoma. *Neoplasma* 2009; **56**: 136-140 [PMID: 19239328]

71   **He G**, Karin M. NF-κB and STAT3 - key players in liver inflammation and cancer. *Cell Res* 2011; **21**: 159-168 [PMID: 21187858 DOI: 10.1038/cr.2010.183]

72   **Wang K**, Lim HY, Shi S, Lee J, Deng S, Xie T, Zhu Z, Wang Y, Pocalyko D, Yang WJ, Rejto PA, Mao M, Park CK, Xu J. Genomic landscape of copy number aberrations enables the identification of oncogenic drivers in hepatocellular carcinoma. *Hepatology* 2013; **58**: 706-717 [PMID: 23505090 DOI: 10.1002/hep.26402]

73   **Jia D**, Wei L, Guo W, Zha R, Bao M, Chen Z, Zhao Y, Ge C, Zhao F, Chen T, Yao M, Li J, Wang H, Gu J, He X. Genome-wide copy number analyses identified novel cancer genes in hepatocellular carcinoma. *Hepatology* 2011; **54**: 1227-1236 [PMID: 21688285 DOI: 10.1002/hep.24495]

74   **Toh ST**, Jin Y, Liu L, Wang J, Babrzadeh F, Gharizadeh B, Ronaghi M, Toh HC, Chow PK, Chung AY, Ooi LL, Lee CG. Deep sequencing of the hepatitis B virus in hepatocellular carcinoma patients reveals enriched integration events, structural alterations and sequence variations. *Carcinogenesis* 2013; **34**: 787-798 [PMID: 23276797 DOI: 10.1093/carcin/bgs406]

75   **Jiang Z**, Jhunjhunwala S, Liu J, Haverty PM, Kennemer MI, Guan Y, Lee W, Carnevali P, Stinson J, Johnson S, Diao J, Yeung S, Jubb A, Ye W, Wu TD, Kapadia SB, de Sauvage FJ, Gentleman RC, Stern HM, Seshagiri S, Pant KP, Modrusan Z, Ballinger DG, Zhang Z. The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. *Genome Res* 2012; **22**: 593-601 [PMID: 22267523 DOI: 10.1101/gr.133926.111]

76   **Schulze K**, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J, Soysouvanh F, Calatayud AL, Pinyol R, Pelletier L, Balabaud C, Laurent A, Blanc JF, Mazzaferro V, Calvo F, Villanueva A, Nault JC, Bioulac-Sage P, Stratton MR, Llovet JM, Zucman-Rossi J. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015; **47**: 505-511 [PMID: 25822088 DOI: 10.1038/ng.3252]

77   **Fang JY**, Richardson BC. The MAPK signalling pathways and colorectal cancer. *Lancet Oncol* 2005; **6**: 322-327 [PMID: 15863380]

78   **Rosell R**, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, Porta R, Cobo M, Garrido P, Longo F, Moran T, Insa A, De Marinis F, Corre R, Bover I, Illiano A, Dansin E, de Castro J, Milella M, Reguart N, Altavilla G, Jimenez U, Provencio M, Moreno MA, Terrasa J, Muñoz-Langa J, Valdivia J, Isla D, Domine M, Molinier O, Mazieres J, Baize N, Garcia-Campelo R, Robinet G, Rodriguez-Abreu D, Lopez-Vivanco G, Gebbia V, Ferrera-Delgado L, Bombaron P, Bernabe R, Bearz A, Artal A, Cortesi E, Rolfo C, Sanchez-Ronco M, Drozdowskyj A, Queralt C, de Aguirre I, Ramirez JL, Sanchez JJ, Molina MA, Taron M, Paz-Ares L. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; **13**: 239-246 [PMID: 22285168 DOI: 10.1016/S1470-2045(11)70393-X]

79   **Baselga J**. Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. *Oncologist* 2011; **16** Suppl 1: 12-19 [PMID: 21278436 DOI: 10.1634/theoncologist.2011-S1-12]

80   **Schulze A**, Lehmann K, Jefferies HB, McMahon M, Downward J. Analysis of the transcriptional program induced by Raf in epithelial cells. *Genes Dev* 2001; **15**: 981-994 [PMID: 11316792]

81   **Samatar AA**, Poulikakos PI. Targeting RAS-ERK signalling in cancer: promises and challenges. *Nat Rev Drug Discov* 2014; **13**: 928-942 [PMID: 25435214 DOI: 10.1038/nrd4281]

82   **O'Neil BH**, Goff LW, Kauh JS, Strosberg JR, Bekaii-Saab TS, Lee RM, Kazi A, Moore DT, Learoyd M, Lush RM, Sebti SM, Sullivan DM. Phase II study of the mitogen-activated protein kinase 1/2 inhibitor selumetinib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 2350-2356 [PMID: 21519015 DOI: 10.1200/JCO.2010.33.9432]

83   **Huynh H**, Ngo VC, Koong HN, Poon D, Choo SP, Toh HC, Thng CH, Chow P, Ong HS, Chung A, Goh BC, Smith PD, Soo KC.

AZD6244 enhances the anti-tumor activity of sorafenib in ectopic and orthotopic models of human hepatocellular carcinoma (HCC). *J Hepatol* 2010; **52**: 79-87 [PMID: 19910069 DOI: 10.1016/j.jhep.2009.10.008]

84   **Lim HY**, Heo J, Choi HJ, Lin CY, Yoon JH, Hsu C, Rau KM, Poon RT, Yeo W, Park JW, Tay MH, Hsieh WS, Kappeler C, Rajagopalan P, Krissel H, Jeffers M, Yen CJ, Tak WY. 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* 2014; **20**: 5976-5985 [PMID: 25294897 DOI: 10.1158/1078-0432.CCR-13-3445]

85   **Faivre S**, Zappa M, Vilgrain V, Boucher E, Douillard JY, Lim HY, Kim JS, Im SA, Kang YK, Bouattour M, Dokmak S, Dreyer C, Sablin MP, Serrate C, Cheng AL, Lanzalone S, Lin X, Lechuga MJ, Raymond E. Changes in tumor density in patients with advanced hepatocellular carcinoma treated with sunitinib. *Clin Cancer Res* 2011; **17**: 4504-4512 [PMID: 21531821 DOI: 10.1158/1078-0432.CCR-10-1708]

86   **Zhan P**, Qian Q, Yu LK. Prognostic significance of vascular endothelial growth factor expression in hepatocellular carcinoma tissue: a meta-analysis. *Hepatobiliary Surg Nutr* 2013; **2**: 148-155 [PMID: 24570933 DOI: 10.3978/j.issn.2304-3881.2013.06.06]

87   **Abou-Alfa GK**, Venook AP. The antiangiogenic ceiling in hepatocellular carcinoma: does it exist and has it been reached? *Lancet Oncol* 2013; **14**: e283-e288 [PMID: 23725711 DOI: 10.1016/S1470-2045(13)70161-X]

88   **Kiss A**, Wang NJ, Xie JP, Thorgeirsson SS. Analysis of transforming growth factor (TGF)-alpha/epidermal growth factor receptor, hepatocyte growth Factor/c-met,TGF-beta receptor type II, and p53 expression in human hepatocellular carcinomas. *Clin Cancer Res* 1997; **3**: 1059-1066 [PMID: 9815784]

89   **Tavian D**, Salvi A, De Petro G, Barlati S. Stable expression of antisense urokinase mRNA inhibits the proliferation and invasion of human hepatocellular carcinoma cells. *Cancer Gene Ther* 2003; **10**: 112-120 [PMID: 12536199]

90   **Wu F**, Wu L, Zheng S, Ding W, Teng L, Wang Z, Ma Z, Zhao W. The clinical value of hepatocyte growth factor and its receptor--c-met for liver cancer patients with hepatectomy. *Dig Liver Dis* 2006; **38**: 490-497 [PMID: 16627020]

91   **Jo M**, Stolz DB, Esplen JE, Dorko K, Michalopoulos GK, Strom SC. Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. *J Biol Chem* 2000; **275**: 8806-8811 [PMID: 10722725]

92   **Xiang Q**, Chen W, Ren M, Wang J, Zhang H, Deng DY, Zhang L, Shang C, Chen Y. Cabozantinib suppresses tumor growth and metastasis in hepatocellular carcinoma by a dual blockade of VEGFR2 and MET. *Clin Cancer Res* 2014; **20**: 2959-2970 [PMID: 24700742 DOI: 10.1158/1078-0432.CCR-13-2620]

93   **Sandhu DS**, Baichoo E, Roberts LR. Fibroblast growth factor signaling in liver carcinogenesis. *Hepatology* 2014; **59**: 1166-1173 [PMID: 24716202]

94   **Miura S**, Mitsuhashi N, Shimizu H, Kimura F, Yoshidome H, Otsuka M, Kato A, Shida T, Okamura D, Miyazaki M. Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma. *BMC Cancer* 2012; **12**: 56 [PMID: 22309595 DOI: 10.1186/1471-2407-12-56]

95   **Yamamoto Y**, Matsui J, Matsushima T, Obaishi H, Miyazaki K, Nakamura K, Tohyama O, Semba T, Yamaguchi A, Hoshi SS, Mimura F, Haneda T, Fukuda Y, Kamata J, Takahashi K, Matsukura M, Wakabayashi T, Asada M, Nomoto K, Watanabe T, Dezso Z, Yoshimatsu K, Funahashi Y, Tsuruoka A. Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. *Vasc Cell* 2014; **6**: 18 [PMID: 25197551 DOI: 10.1186/2045-824X-6-18]

96   **Tai WT**, Cheng AL, Shiau CW, Liu CY, Ko CH, Lin MW, Chen PJ, Chen KF. Dovitinib induces apoptosis and overcomes sorafenib resistance in hepatocellular carcinoma through SHP-1-mediated inhibition of STAT3. *Mol Cancer Ther* 2012; **11**: 452-463 [PMID:

22180308 DOI: 10.1158/1535-7163.MCT-11-0412]

97   **Sawey ET**, Chanrion M, Cai C, Wu G, Zhang J, Zender L, Zhao A, Busuttil RW, Yee H, Stein L, French DM, Finn RS, Lowe SW, Powers S. Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by Oncogenomic screening. *Cancer Cell* 2011; **19**: 347-358 [PMID: 21397858 DOI: 10.1016/j.ccr.2011.01.040]

98   **Chesselet MF**, Robbins E. Characterization of striatal neurons expressing high levels of glutamic acid decarboxylase messenger RNA. *Brain Res* 1989; **492**: 237-244 [PMID: 2568874 DOI: 10.1038/bjc.2014.638]

99   **Maherali N**, Hochedlinger K. Tgfbeta signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and cMyc. *Curr Biol* 2009; **19**: 1718-1723 [PMID: 19765992 DOI: 10.1016/j.cub.2009.08.025]

100   **Yu W**, Huang C, Wang Q, Huang T, Ding Y, Ma C, Ma H, Chen W. MEF2 transcription factors promotes EMT and invasiveness of hepatocellular carcinoma through TGF-β1 autoregulation circuitry. *Tumour Biol* 2014; **35**: 10943-10951 [PMID: 25087096 DOI: 10.1007/s13277-014-2403-1]

101   **Giannelli G**, Villa E, Lahn M. Transforming growth factor-β as a therapeutic target in hepatocellular carcinoma. *Cancer Res* 2014; **74**: 1890-1894 [PMID: 24638984 DOI: 10.1158/0008-5472.CAN-14-0243]

102   **Coulouarn C**, Factor VM, Thorgeirsson SS. Transforming growth factor-beta gene expression signature in mouse hepatocytes predicts clinical outcome in human cancer. *Hepatology* 2008; **47**: 2059-2067 [PMID: 18506891 DOI: 10.1002/hep.22283]

103   **Hodi FS**, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**: 711-723 [PMID: 20525992 DOI: 10.1056/NEJMoa1003466]

104   **Postow MA**, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor D, Salama AK, Taylor M, Ott PA, Rollin LM, Horak C, Gagnier P, Wolchok JD, Hodi FS. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med* 2015; **372**: 2006-2017 [PMID: 25891304 DOI: 10.1056/NEJMoa1414428]

105   **Anthony B**. El-Khoueiry, Phase I/II safety and antitumor activity of nivolumab in patients with advanced hepatocellular carcinoma (HCC): CA209-040. ASCO, 2013

Baishideng®