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The evolving landscape of therapeutic drug development for hepatocellular carcinoma

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ABSTRACT

Currently, only one drug, sorafenib, is FDA approved for the treatment of advanced hepatocellular carcinoma (HCC), achieving modest objective response rates while still conferring an overall survival benefit. Unlike other solid tumors, no oncogenic addiction loops have been validated as clinically actionable targets in HCC. Outcomes of HCC could potentially be improved if critical molecular subclasses with distinct therapeutic vulnerabilities can be identified, biomarkers that predict recurrence or progression early can be determined and key epigenetic, genetic or microenvironment drivers that determine best response to a specific targeting treatment can be uncovered.

Our group and others have examined the molecular heterogeneity of hepatocellular carcinoma. We have developed a panel of patient derived xenograft models to enable focused pre-clinical drug development of rationally designed therapies in specific molecular subgroups. We observed unique patterns, including synergies, of drug activity across our molecularly diverse HCC xenografts, pointing to specific therapeutic vulnerabilities for individual tumors. These efforts inform clinical trial designs and catalyze therapeutic development. It also argues for efficient strategic allocation of patients into appropriate enriched clinical trials. Here, we will discuss some of the recent important therapeutic studies in advanced HCC and also some of the potential strategies to optimize clinical therapeutic development moving forward.

1. Introduction

Hepatocellular carcinoma (HCC) remains a major global health problem [1]. It is the fifth most common cancer in men, seventh in women and the third most common cause of cancer deaths worldwide [2]. In 2008, approximately 749,000 new cases of HCC were diagnosed and 695,000 deaths were attributed to HCC. There is distinct geographical variation with the majority of the cases (85%) occurring in developing countries in East Asia and sub-Saharan Africa and lower incidence rates in Australia, Northern Europe and America [3,4].

The pathogenesis of HCC is composed of a multistep progression involving chronic inflammation, hyperplasia, dysplasia and finally malignant transformation. Cirrhosis is present in 80% to 90% of patients with HCC. The main risk factors for development of HCC are therefore related to the formation and progression of cirrhosis. Chronic hepatitis B (HBV) infection is the predominant etiological agent accounting for approximately half of all cases of HCC. HBV is endemic in high incidence regions across China and Africa. HBV also accounts for a large proportion of HCC cases among Asian Americans. Hepatitis C infection (HCV) confers a 15–20 fold increased risk of HCC and accounts for the majority of cases in Japan, United States and parts of Europe. HCC related to HCV has become the fastest-rising cause of cancer-related death in the United States. Metabolic causes leading to non-alcoholic fatty liver disease are also an increasing concern. The other risk factors for HCC can be classified as toxins

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(aflatoxin B1, alcohol), metabolic diseases (non-alcoholic fatty liver disease, diabetes), hereditary diseases (hemochromatosis) and immune related diseases (autoimmune hepatitis and primary biliary cirrhosis) [5,6].

Despite decades of research in HCC, prognosis still remains poor. Only 20% of the patients with HCC are amenable to curative strategies such as resection, transplantation or local therapy with radiofrequency ablation [7]. Another 20% have multifocal lesions for which locoregional modalities such as transarterial chemoembolization (TACE) [8,9] or selective internal radiotherapy (SIRT) [10–12] can be employed. The majority of patients are not candidates for curative treatment or loco-regional approaches and will receive systemic therapy if they have adequate hepatic reserves and good functional status [7,13]. Due to the underlying liver dysfunction, many patients do not receive any anti-cancer therapy and are palliated with symptom control and best supportive care.

Substantial efforts have been made to molecularly characterize HCC and rationally develop targeted therapeutics in HCC. Unlike other solid tumors, there are no oncogenic addiction loops that have successfully completed the journey from bench to bedside as validated therapeutic targets in HCC [14]. Despite that, only one drug, sorafenib, is FDA approved for the treatment of advanced HCC, achieving modest objective response rates while still conferring an overall survival benefit. In this review, we describe the current landscape of drug development in HCC in light of its molecular heterogeneity, present the available evidence in support of stratified therapy for HCC and discuss potential strategies to accelerate this process by optimizing clinical trials design.

2. Current therapeutic landscape in advanced HCC

2.1. Chemotherapy in HCC

The impact of systemic chemotherapy is limited in HCC patients because of cirrhotic livers and potentially poor hepatic reserves. Specific complications of cirrhosis such as thrombocytopenia also compromise effective delivery of systemic chemotherapy. Several phase II trials with various chemotherapy agents such as doxorubicin, gemcitabine and capecitabine have reported modest results. Among these agents, anthracyclines such as doxorubicin appear to have the most activity, with response rate of 20% and a median survival of 4 months [15–20].

2.2. Combination chemotherapy in HCC

Combination chemotherapy is employed in HCC to obtain a radiological response and can still be employed for quicker palliation. A retrospective multi-center series of 210 patients reported that gemcitabine with oxaliplatin led to an objective response rate of 21% (WHO criteria) and disease control rate of 62%. In addition, 10% of patients had responses that made secondary "curative-intent" surgical therapies possible.

The phase 3 EACH study randomized 371 Asian patients with advanced HCC to open-label FOLFOX4 regimen (5-fluorouracil and leucovorin plus oxaliplatin) or single-agent doxorubicin, crossover was not permitted [21]. Objective response rate (8.2% vs. 2.7%) and disease control rate (52% vs. 32%) were superior with FOLFOX4. The study's pre-specified final analysis, conducted after 266 deaths in the intent-to-treat population, showed a trend toward better median overall survival (the primary end point) among patients treated with FOLFOX4, compared with doxorubicin (6.40 vs. 4.97 months; hazard ratio (HR) 0.79; $p = 0.07$ using a stratified log-rank test). Statistical significance ($p = 0.0425$) was achieved at the post hoc analysis conducted after additional follow-up of 7 months and 305 deaths (HR, 0.79; $p = 0.04$). However, there have been statistical concerns raised regarding the validity of this post-hoc analysis.

The combination of chemotherapy with immunotherapy has also been evaluated. The only randomized phase III study by Yeo et al. reported a response rate of 21% with PIAF (cisplatin, doxorubicin, interferon, and fluorouracil) and a median overall survival of 8.7 months in patients with unresectable HCC. However, PIAF did not result in a significant survival benefit compared to doxorubicin and had significantly more toxicities [22].

2.3. Sorafenib

Sorafenib is the first and only FDA approved drug for use in advanced HCC. It inhibits multiple receptors, namely VEGFR 1–3, PDGFR-B, c-KIT and Fms-related tyrosine kinase-3(FLT-3) [23,24]. Sorafenib has been shown to inhibit angiogenesis, induce apoptosis and inhibit the mTOR pathway in preclinical studies [25]. FDA approval was based on the pivotal phase III Sorafenib Hepatocellular Carcinoma Assessment Randomised Protocol (SHARP) trial. Llovet et al. randomized 602 patients (mainly from Europe) with unresectable advanced HCC with Child-Pugh "A" score without prior systemic therapy to sorafenib 400 mg BD ($n = 299$) or placebo ($n = 303$) [26]. Compared to placebo, sorafenib significantly prolonged time to progression (TTP) from a median of 2.8 months to 5.5 months (HR 0.58) and overall survival (OS) from a median of 7.9 months to 10.7 months (HR 0.69; 95% CI 0.55–0.87; $p < 0.001$). This randomized trial clearly established the survival benefit of sorafenib in advanced HCC. Notably, there was no difference in the median time to symptomatic progression (TTSP), a co-primary end-point. A parallel study was performed in 271 Asian patients with advanced HCC by Cheng et al. which also showed a statistically significant improvement of overall survival (HR 0.68; 95% CI 0.50–0.93; $p = 0.014$). However, outcomes in both arms were poorer with a median overall survival of 4.2 months in the placebo arm and 6.5 months with sorafenib therapy respectively. Median time to progression (TTP) was 2.8 months in the sorafenib arm compared to 1.4 months in the placebo arm. Akin to the SHARP study, there was no significant difference in the time to symptomatic progression [27]. The shorter time to progression and median overall survival in the Asian study were postulated to be due to the presence of more unfavorable prognostic factors including higher incidence of hepatitis B infections (73% vs. 12%) and more advanced disease with a higher proportion of extra-hepatic metastasis.

Of note, both trials required Child-Pugh class A score as an inclusion criteria. There are no randomized data regarding efficacy of sorafenib in Child-Pugh B patients. A phase II study by Abou-Alfa that included patients with Child-Pugh B status

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suggests similar drug exposures (as measured by AUC of sorafenib) [28,29]. Child-Pugh B status patients tend to have greater risk of encephalopathy or ascites, although these might be related to the underlying liver disease or be drug related. The GIDEON expanded access program also provides information regarding sorafenib in Child-Pugh B status patients [29].

2.4. Combining sorafenib with chemotherapy

Sorafenib was combined with doxorubicin in a randomized phase II trial by Abou-Alfa et al. [30]. Ninety-six patients with advanced HCC and Child-Pugh A disease were randomized to receive intravenous doxorubicin 60 mg/m^2 every 21 days and sorafenib or placebo 400 mg BD per day. Outcomes favored the combination arm (sorafenib and Doxorubicin) with an overall survival of 13.7 months compared to 6.5 months in the Doxorubicin group, and progression free survival (PFS) 6 months in the combination arm versus 2.7 months in the doxorubicin arm. Toxicity profiles appeared manageable. This study suggests possible synergy of doxorubicin and sorafenib. An ongoing phase 3 trial is evaluating sorafenib and doxorubicin versus sorafenib alone (NCT01015833).

A single arm phase II study (SECOX) by Yau et al. evaluated the efficacy of sorafenib combined with capecitabine and oxaliplatin [31]. The investigators reported an objective response rate of 16% and median overall survival of 11.8 months. A phase III trial underway comparing this SECOX combination with sorafenib monotherapy is currently ongoing.

2.5. Moving beyond sorafenib

Drug development in HCC seeks to capitalize on molecular studies which point to several key signaling pathways in HCC. These include the epidermal growth factor (EGF)/EGF receptor (EGFR), vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR), platelet-derived growth factor (PDGF)/PDGF receptor (PDGFR), fibroblast growth factor (FGF)-2, insulin-like growth factor (IGF)/IGF receptor (IGFR), and the Ras/Raf/mitogen-extracellular activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK), Wnt/β-catenin, and phosphatidylinositol-3- kinase (PI3K)/phosphatase and tensin homolog deleted on chromosome ten (PTEN)/Akt/mammalian target of rapamycin (mTOR) signaling pathways [32]. Beyond tumor factors, HCC is a vascular angiogenic tumor. Targeting angiogenesis in HCC focuses on several key mediators including vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), angiopoietin (ANG) and fibroblast growth factor (FGF).

To translate molecular studies towards therapeutic advances beyond sorafenib, drug development in HCC has taken 2 parallel tracks. In the first line setting, novel treatment options are developed either as monotherapy for head to head comparison with sorafenib or as combination therapy together with sorafenib. In the second line setting, novel therapeutics are being developed as monotherapy usually with placebo as the control arm.

2.6. Targeting angiogenesis (VEGFR, PDGFR, FDGFR)

VEGF, PDGF and FGF are paramount mediators of neovascularization and invasiveness in HCC [33–35]. Upregulation of VEGF expression has been demonstrated in oncogenesis and activation of VEGFR, in particular VEGFR-2 drives angiogenesis [36]. The overexpression of PDGF has also been shown to promote angiogenesis and increase the potential for metastasis [37,38]. FGF is highly expressed in HCC. It promotes cell proliferation via an autocrine mechanism [39,40] and has been shown to augment the angiogenic effect of VEGF [40].

Although originally identified as a Raf kinase inhibitor, sorafenib's activity in HCC is believed to be due to inhibition of VEGFR [41].Other inhibitors of VEGFR have also been developed in HCC including sunitinib, linifanib, brivanib and ramucirumab. We have summarized HCC studies using these agents in Table 1.

Sunitinib is an oral multi-kinase inhibitor of VEGFR, PDGFR and the stem cell factor receptor (KIT) approved for treatment of advanced renal cell carcinoma (RCC) and gastrointestinal tumor (GIST) [42,43]. Preclinical studies by our group confirmed its angiogenic activity with suppression of five HCC xenografts [44]. Modest activity was reported in several phase 2 trials but when tested in a randomized phase III trial (SUN 11700) by Cheng A et al., sunitinib at 37.5 mg daily had a more adverse events and inferior survival compared to sorafenib alone [45]. Thus, this study was terminated early. Of note, the median overall survival outcomes observed with sorafenib were replicated once again in this large global phase 3 trial.

Linifanib (ABT-689) is another potent and selective mutikinase inhibitor that targets both VEGFR and PDGFR [46]. We reported a phase II open label multicenter study of ABT-689 in 44 patients with advanced HCC [47]. Linifanib dosed at 0.25 mg/kg daily in patients with Child Pugh A disease and every other day in patients with Child Pugh B disease resulted in a response rate of 8.7% and OS of 295 days for CP A patients. A phase III study comparing linifanib versus sorafenib in advanced HCC has shown equivalence in overall survival between the two drugs although linifanib fared better in ORR and TTP (ASCO GI 2013).

Bevacizumab, a recombinant humanized monoclonal antibody against all isoforms of VEGF disrupts the autocrine and paracrine signaling mediated by VEGFR by blocking the binding of VEGF to its receptors [48]. Bevacizumab has been shown to decrease vessel density and prolong time to progression in HCC xenografts [49]. A phase 2 trial of bevacizumab monotherapy dosed at 5 mg/kg or 10 mg/kg once every 2 weeks in 46 patients reported a response rate of 13%, median progression free survival (PFS) of 6.9 months and median OS of 12.4 months [50]. There are currently no phase III trials evaluating Bevacizumab as monotherapy but it has been combined with chemotherapy in several phase 2 trials. These are summarized in Table 2.

Ramucirumab (IMC-1121B) is a recombinant human monoclonal antibody against VEGFR 2. A phase II trial by Zhu et al. dosed ramucirumab at 8 mg/kg every 2 weeks as first-line monotherapy in 42 patients with advanced HCC, reporting response rates of 10% with a median PFS of 4 months and median OS of 12 months [51]. An ongoing

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Table 1
Summary of VEGFR inhibitors.

Agent	Study	Phase	Comparator	Response	PFS (months)	OS (months)	Adverse events
Sorafenib	SHARP Llovet et al. [26]	III ($n = 602$)	Placebo	2% PR No CR	5.5 vs. 2.8 (TTP)	10.7 vs. 7.9	Diarrhea (39%), HFS (21%)
	Cheng et al. [27]	III ($n = 271$)	Placebo	–	2.8 vs. 1.4 (TTP)	6.5 vs. 4.2	HFS (45%), diarrhea (26%), rash (20%), fatigue (20%)
	Abou Alfa et al. [28]	II ($n = 137$)	–	8% PR/MR 34% SD	4.2 (TTP)	9.2	G3/4: HFS (5.1%), diarrhea (8%), fatigue (9.5%)
Sorafenib + Doxorubicin	Abou Alfa et al. [30]	II ($n = 96$)	Placebo + Doxorubicin	–	6.4 vs. 2.8 (TTP)	13.7 vs. 6.5	–
Sorafenib + Erlotinib	SEARCH [85]	III ($n = 720$)	Sorafenib + Placebo	DCR 44% vs. 52.5%	3.2 vs. 4.0 (TTP)	9.5 vs. 8.5	Similar toxicity profiles compared to single agents
Sunitinib	Cheng et al. [45]	III ($n = 1074$)	Sorafenib	–	3.6 vs. 2.9	8.1 vs. 10	G3/4: Sunitinib: thrombocytopenia (19%), neutropenia (16%) Sorafenib: skin disorders (21%)
	Zhu et al. [138]	II ($n = 34$)	–	2.9% PR 38.5% SD	3.9	9.8	Deranged LFT (18%), lymphopenia (15%), neutropenia, thrombocytopenia, fatigue (12%), HFS (6%)
	Faivre et al. [139]	II ($n = 37$)	–	2.7% PR 35% SD	3.7	8	G3/4: Thrombocytopenia (37.8%), neutropenia (24.3%), asthenia (13.5%), anemia (10%), HFS (10%)
Linifanib (ABT-869)	Toh et al. [47]	II ($n = 44$)	–	9.1% RR	3.7 (TTP)	9.7	G3/4: hypertension (25%), fatigue (14%)
Brivanib	Park et al. [55]	II ($n = 55$)	–	1.8% CR 5.5% PR 40% SD	2.7	10	G3/4: fatigue (16%), AST elevation (19%), hyponatremia (41%)
Cediranib (AZD2171)	Alberts et al. [140]	II ($n = 28$)	–	0% RR	2.8 (TTP)	5.8	G3/4: fatigue (46%), anorexia (25%), hypertension (21%), elevated AST (18%)
TSU-68	Kanai et al. [141]	II ($n = 35$)	–	5.7% PR 42.8% SD	2.1 (TTP)	13.1	Diarrhea, anorexia, abdominal pain, malaise, edema, transaminitis
Pazopanib	Yau et al. [142]	I ($n = 28$)	–	73% PR/SD	–	–	Diarrhea, skin hypopigmentation, AST elevation
Lenalidomide	Safran et al. [143]	II ($n = 13$)	–	15% PR/CR	–	–	–
Ramucirumab	Zhu et al. [51]	II ($n = 43$)	–	10% RR 60% SD 70% DCR	4	12	G3/4: fatigue (10%), GI bleed (7%), hypertension (12%), infusion-related (5%), headache (2%)
Axitinib	NCT01210495	II	–	–	–	–	–
Valatinib+ Doxorubicin	Yau et al. [144]	I/II ($n = 27$)	26% PR	–	5.4	7.3	–
Dovitinib	NCT01232296	II	Sorafenib		–	–	–

RR: overall response rate, MR: minor response, PR: partial response, SD: stable disease, CR: complete response, DCR: disease control rate, PFS: progression-free survival, TTP: time to progression, OS: overall survival, AEs: adverse events, HFS: hand–foot syndrome, BGIT: bleeding gastrointestinal tract, G3/4: grade 3 and 4.

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Table 2
Summary of bevacizumab studies.

Agent	Study	Phase	Comparator	Response	PFS (months)	OS (months)	Adverse events
Bevacizumab	Siegel et al. [50]	II ($n = 46$)	–	13% RR	6.9	1 year OS: 53% 2 years OS: 28% 3 years OS: 23%	G3/4 hypertension (15%), thrombosis (6%), hemorrhage (11%)
Bevacizumab + gemcitabine + oxaliplatin	Zhu et al. [145]	II ($n = 33$)	–	20% RR 27% SD	5.3	9.6	Neutropenia, leukopenia, transaminitis, hypertension, fatigue
Bevacizumab + Capecitabine	Hsu et al. [146]	II ($n = 45$)	–	9% RR 52% DCR	2.7	5.9	G3/4: diarrhea (4%), BGIT (9%), HFS (9%)
Bevacizumab + Capecitabine + Oxaliplatin	Sun et al. [147]	II ($n = 40$)	–	20% PR 78% DCR	6.8	9.8	Neurotoxicity, fatigue
Bevacizumab + Erlotinib	Thom-as et al. [84]	II ($n = 40$)	–	25% RR	9.8	17	Fatigue (20%), BGIT (13%), hypertension (15%)

RR: overall response rate, MR: minor response, PR: partial response, SD: stable disease, CR: complete response, DCR: disease control rate, PFS: progression-free survival, TTP: time to progression, OS: overall survival, AEs: adverse events, HFS: hand–foot syndrome, BGIT: bleeding gastrointestinal tract, G3/4: grade 3 and 4.

phase III trial (REACH) is investigating the efficacy of ramucirumab in sorafenib refractory patients (NCT01140347).

2.7. Inhibitors that also target the FGF pathway

Emerging data from pre-clinical murine models and correlative studies in HCC patients suggest that resistance to angiogenic agents is attributable to upregulation of FGF induced by VEGFR inhibition driven hypoxia [33,34,52,53]. Several novel tyrosine kinase inhibitors (TKIs) that inhibit the FGF pathway such as brivanib, dovitinib and TSU-68 are being evaluated in HCC.

Our group evaluated the antineoplastic role of brivanib, a selective dual inhibitor of VEGFR and FGFR in six HCC xenograft models [54]. The use of brivanib has led to decreased phosphorylated VEGFR-2 at Tyr (1054/1059), inhibition of cell proliferation and tumor growth and increased apoptosis. In the first line setting, a phase 2 trial evaluated brivanib at 800 mg daily in 55 patients, reporting an objective response rate of 25% by mRECIST for HCC, median PFS of 2.7 months (95% CI: 1.4–3 months) and median OS of 10 months (95% CI: 6.8–15.2 months) [55]. In the second line setting after failure of one prior angiogenic inhibitor, a phase 2 trial reported median PFS of 2.7 months and OS of 9.8 months [56]. However, in both settings, subsequent phase III trials were negative. The BRISK-FL trial comparing brivanib against sorafenib in the first line setting failed to meet its primary overall survival end-point. Similarly, brivanib did not improve OS as 2nd-line therapy compared with placebo in the BRISK-PS trial.

2.8. C-met inhibition

C-met is a receptor tyrosine kinase [57] with hepatocyte growth factor (HGF), also known as scatter factor (SF), as the natural ligand [58]. C-met activation leads to activation of downstream effector pathways (PI3K/AKT/MTOR, RAF/MEK/ERK) promoting cell survival, invasion and proliferation [59–61]. Overexpression of c-met has been demonstrated in 20%–48% of human HCC samples in various clinical trials and is associated with higher proliferation and worse prognosis [62].

Much excitement regarding c-met inhibition emerged from the 2012 American Society of Clinical Oncology meeting when 2 trials of c-met inhibitors reported promising results. Tivantinib (ARQ 197) is a selective non-adenosine triphosphate-competitive c-met inhibitor. In a randomized phase 2 trial of tivantinib vs. placebo is 2nd line HCC, time-to-tumor progression was increased from 6 weeks to 6.9 weeks (HR 0.64; $p = 0.04$) in the ITT population but OS was unchanged [62]. However, in the pre-defined c-met high subgroup analysis, median OS was 7.2 months in the tivantinib arm ($n = 22$) and 3.8 months ($n = 15$) in the placebo arm to (HR 0.38; $p = 0.01$), median TTP was 2.9 months in the tivantinib arm and 1.5 months in the placebo arm (HR 0.43; $p = 0.03$). With the caveat that the observations were based on small numbers, these results are highly encouraging. In the next trial, cabozantinib (XL184), a dual c-MET and VEGFR inhibitor was evaluated in a randomized discontinuation study [63]. Out of 41 patients, 3 had PR and overall disease control rate was 71%. The median PFS was 4.2 months. A phase II trial comparing this agent to placebo in the second line setting is currently ongoing (NCT00988741). Our group has also demonstrated significant anti-tumor and anti-angiogenic activities of foretinib, a VEGFR-2 and c-met inhibitor in patient derived HCC xenograft models. Foretinib induces G2/M cell cycle arrest, inhibits HGF, induces cell migration and proliferation and induces apoptosis in both orthotopic and ectopic HCC models. Mouse survival was also significantly prolonged in the orthotopic model [64]. A phase 1/2 open label, multicenter trial is ongoing (NCT00920192). Based on these results, c-met inhibition appears to be the most promising target currently in advanced HCC.

2.9. Targeting the PI3K/AKT/MTOR pathway

The PI3K/AKT/MTOR pathway plays an important role in HCC etiology [65]. Dysregulation of the PI3K/AKT/MTOR pathway has been reported in 40%–60% of human HCC [65–69]. Activation occurs primarily through PIK3CA mutations and PTEN loss and is associated with a poor prognosis [66,70,71]. Downregulation of PTEN expression occurs in 20%–30% of HCC patients and is frequently due to the loss of

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heterozygosity of PTEN [72,73] or PTEN promoter methylation [74]. Activation of this pathway regulates phosphorylated p70S6 serine–threonine kinase (S6K1) and translational repressor protein PHAS-1/4E-BP which in turn regulates expression of VEGF, c-myc, and cyclin D1. In addition, 4E-BP1 and S6K1 have been associated with higher tumor grade. This led to the advent of developing drugs that can target this pathway at different levels. The most promising of which are MTOR inhibitors. Zhu AX et al. reported a phase 2 trial of everolimus in 28 patients with advanced HCC. The disease control rate was 44% and median overall survival was 8.4 months with the use of everolimus [75]. A phase III study EVOLVE-1 is underway investigating the efficacy of everolimus versus placebo in patients who have either progressed or are intolerant of sorafenib (NCT01035229). Several studies have suggested increased efficacy with combination therapy, rapamycin plus sorafenib [76], rapamycin plus AZD6244 [77] and rapamycin plus bevacizumab [78]. The above encouraging results form the future landscape for further clinical investigation.

2.10. Targeting EGFR

EGFR belongs to the ERB family of tyrosine kinase receptors. Ligand binding by TGF-α and EGF leads to dimerization and activation of downstream signaling pathways (PI3K/AKT/MTOR, RAS/RAF/MEK/ERK) [79–81]. EGFR is commonly upregulated in HCC and has been demonstrated to play an important role in oncogenesis in preclinical studies via activation of the Ras/RAF/MEK/ERK and PI3K/AKT/MTOR pathways. Targeting EGFR can be achieved with extracellular neutralizing monoclonal antibodies such as cetuximab or panitumumab or intracellulary via receptor tyrosine kinase inhibitors such as gefitinib and erlotinib. These agents, as monotherapy reported dismal results with regards to response rates and survival [82]. The studies evaluating these agents in HCC are summarized in Table 3.

In the phase II study by Phillip et al. of 38 patients treated with erlotinib at 150 mg daily, 3 (9%) had PR and 59% had disease control. The median OS was 13 months [83]. Another phase II study by Thomas et al. evaluated the efficacy and safety of erlotinib in 40 patients. There were no complete or partial responses but 17 (42.5%) achieved stable disease. PFS at 16 weeks was 43% with a median OS of 10.75 months [84]. The randomized, double-blind, placebo-controlled phase III trial of sorafenib plus erlotinib (SEARCH) as upfront treatment of HCC was reported at the European Society for Medical Oncology meeting in 2012. The addition of erlotinib did not improve OS (the primary end point) or PFS [85].

Erlortinib was also combined with bevacizumab in advanced HCC in a phase 2 trial by Thomas et al. [86]. The objective response rate was 25% with an impressive median PFS of 9.8 months and OS of 17 months. There is an ongoing phase 2 trial evaluating the efficacy of this combination compared to sorafenib in the first line treatment of patients with advanced HCC (NCT00881751).

Lapatinib is a dual inhibitor of EGFR and HER-2/NEU tyrosine kinase. In 2 single arm trials, lapatinib in advanced HCC achieved not more than 5% responses and median OS of 6.2 months [87] and 12.6 months [88].

2.11. Targeting the RAF/MEK/ERK pathway

The mitogenic Ras/Raf/MEK/ERK signaling pathway plays a crucial role in cell proliferation, survival and apoptosis [89]. Proteins encoded by HBV and HCV genome have been demonstrated to activate the Ras/Raf/MEK/ERK pathway [90–93]. Repression of the pathway by overexpression of negative regulators such as Ras inhibitors Sprouty and its related proteins and Raf kinase inhibitor protein (RKIP) have also been reported [94–98]. Fifty percent of the biopsies of HCC demonstrated overexpression of the Raf-1 and 100% had increased Raf-1 protein activation [99]. Pathway activity as evaluated by ERK activation is a poor prognostic factor in HCC [90]. We have reported the activity of a selective MEK1/2 inhibitor AZD6244 (selumetinib) on human HCC xenografts with induction of apoptosis and reduction in tumor progression in vitro [100]. In a multicenter, single arm phase II study by O Neil et al. Selumetinib was administered orally at a dose of 100 mg twice a day. Median PFS of 1.4 months and median OS of 4.2 months in patients with advanced HCC were observed but no radiologic responses were reported with

Table 3
Summary of EGFR tyrosine kinase inhibitors.

Agent	Study	Phase	Comparator	Response	PFS (months)	OS (months)	Adverse events
Erlotinib	Philip et al. [83]	II ($n = 38$)	–	8% PR 59% DCR	32% (6 months PFS)	13	Diarrhea (13%), skin toxicity (8%)
	Thomas et al. [84]	II ($n = 40$)	–	0% PR/CR 43% DCR	43% (16 weeks PFS)	10.8	Well tolerated
Gefitinib	O'Dwyer [148]	II ($n = 31$)	–	3% PR 22% SD	2.8	6.5	G3/4 neutropenia (10%), G3 rash (6%), G3 diarrhea (1%)
Lapatinib	Ramanathan et al. [87]	II ($n = 40$)	–	5% RR	2.3	6.2	Well tolerated
	Bekaii et al. [88]	II ($n = 26$)	–	0% RR 40% SD	1.9	12.6	Diarrhea (73%), nausea (54%), rash (42%)
Cetuximab	Zhu et al. [149]	II ($n = 30$)	–	0% CR/PR 17% SD	1.4	9.6	G3 AST elevation (3%), hypomagnesaemia (3%), fever without neutropenia (3%)
	Gruenwald et al. [150]	II ($n = 32$, 27 evaluable)	–	0% CR/PR 44.4% SD	2 (TTP)	–	Well tolerated

RR: overall response rate, MR: minor response, PR: partial response, SD: stable disease, CR: complete response, DCR: disease control rate, PFS: progression-free survival, TTP: time to progression, OS: overall survival, AEs: adverse events, HFS: hand–foot syndrome, BGIT: bleeding gastrointestinal tract, G3/4: grade 3 and 4.

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the use of selumetinib [101]. There are currently two phase 1/2 studies evaluating combination therapy with MEK inhibitors, AZD6244 and sorafenib (NCT01029418) and RDEA119 and sorafenib (NCT00785226).

2.12. Targeting the Jak/Stat pathway

Multiple cytokines and growth factors participate in the activation of this pathway which in turn leads to cell proliferation and apoptosis [102]. HCC cells expressed phosphorylated Jak and Tyk2 tyrosine kinases and had increased activation of Stat [94]. Thus, inhibitors of Jak/Stat signaling are promising therapeutic agents in the treatment of HCC and this requires further validation studies.

2.13. Targeting epigenetic regulation of HCC

2.13.1. Targeting epigenetic dysregulation

Epigenetic dysregulation of the HCC genome via cytosine-guanine dinucleotide (GpG) hypermethylation or histone deacetylation repression of gene expression downregulates tumor suppressor genes [103,104]. Preclinical studies suggest apoptotic activity of histone deacetylase inhibitors (HDAC inhibitors) in HCC [105–107]. Belinostat is a novel histone deacetylase inhibitor. A phase I/II study by Yeo W et al. investigating belinostat in patients with advanced HCC reported a disease control rate of 47.6%, median PFS of 2.6 months (95% CI: 1.55–3.17 months) and a median overall survival of 6.6 months (95% CI: 4.53–11.60 months) [108].

2.13.2. Other pathways under investigation

A subset of HCC is characterized by activation of the IGF-signaling pathway which contributes to invasiveness and cell proliferation [109–111]. Of the HCC, 16%–40% overexpress IGF-II and 30% overexpress IGF-R [112,113]. Activation of the IGF pathway results in the activation of downstream signaling pathways including Ras/Raf/MEK/ERK, PI3K/Akt/mTOR and Jak/Stat [114]. They thus serve as attractive targets for multi-blockade regimens. MK-0646, a monoclonal antibody against IGFR has been shown to decrease downstream effector proteins (phosphorylated AKT and S6 kinase) and tumor proliferation [115]. In a phase 2 study, the use of another monoclonal antibody IMC-A12 in a patient with refractory HCC resulted in stabilization of disease for 9 months [116]. This pathway can potentially serve as a target for personalized treatment of HCC.

The WNT/β-catenin signaling cascade is another pathway that plays a role in hepatocarcinogenesis. β-catenin gene mutation has been demonstrated in 20%–40% of HCC samples [117–119] and is associated with nuclear translocation and activation of target genes that regulate proliferation and apoptosis. Inhibition of this pathway in HCC with either CTNNB1 or AXIN mutations with antibodies against WNT or with inhibitors of upstream events such as the Fzd-Dvl complex formation and B-catenin/Lef/Tcf transcriptional complex remains a viable therapeutic option [120].

HCC is thought to be auxotrophic for arginine due to lack of expression of argininosuccinate synthetase (ASS) [121]. This dependence on exogenous arginine makes them susceptible to arginine depleting agents [121–123]. A phase 2 study has reported the antitumor activity of pegylated recombinant human arginase in vivo and in vitro [124].

Early preclinical studies have also demonstrated that hedgehog pathway signaling is involved in the pathogenesis of HCC [125–127].

Some studies have shown specific CD8(+) T-cell responses against tumor associated antigens (TAA) in patients with HCC and a clinical benefit with infiltration of T cells, suggesting a possible role for immunotherapy [128]. Toll-like receptor 3 (TLR3) activation has been shown to increase cell death, promote NK activation and cytotoxicity in vitro and in vivo. TLR3 expression also correlates with improved survival of HCC patients (hazard ratio of survival 2.1; $p = 0.002$) and serves as a potential target for immunotherapy [129]. Ongoing immunocompetent HCC mouse studies are encouraging for single agent TLR3 agonist efficacy against HCC (unpublished data).

Recent studies have also demonstrated that a subpopulation of cancer cells possess stem cell properties, called cancer stem cells (CSCs) and these are responsible for growth and metastasis of HCC [130–133]. Therapeutic targeting of CSCs may provide a novel strategy that is more effective than the current drugs targeting the bulk mature cancer cells in treatment of HCC.

Collectively, molecular characterization of HCC and its microenvironment offers a host of potential targets for rational therapeutic intervention to halt tumor progression.

2.13.3. Combining targeted therapies with sorafenib

The next step beyond sorafenib monotherapy involves exploring the synergism of combining it with other targeted agents. Martell et al. reported the results of a phase 1 study of tivantinib, an oral, selective MET inhibitor with sorafenib with an overall response rate of 10% and PFS of 3.5 months [134]. Another phase 2 study by Lim et al. evaluated the role of combining a MEK inhibitor (BAY 86-9766) with sorafenib in unresectable HCC [135]. Disease control rate was 43% and median PFS was 4.1 months. In another study, Kelley et al. reported the results of the combination treatment of temsirolimus with sorafenib in treatment naive HCC patients and in this study, 10% had partial response and 52% stable disease [136].

3. Common themes

Two major themes have emerged from intense investigations in HCC over the last decade. First, large phase 3 trials in unselected populations commenced on marginal early phase data, were uniformly negative. Second, given its vast molecular heterogeneity, it is unlikely that targeted therapy will benefit all patients with HCC. Thus, a strategy of stratified medicine is advocated in HCC. This approach requires segmenting the population by identifying predictive biomarkers and evaluating therapy in subgroups of patients predicted to benefit from specific therapy.

4. Strategies/Methodology

4.1. Novel trial designs

This new paradigm of stratified medicine poses substantial statistical challenges. In the past, drug approval was based on the rejection of a single null hypothesis evaluated in

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all eligible patients with a particular cancer. For targeted therapy in a heterogeneous tumor like HCC, this will be inefficient or completely ineffective. When based on preliminary data, treatment effect is anticipated to be strongest in a marker defined subgroup; several novel trial designs have been proposed.

4.1.1. Sequential testing strategies

A sequential testing approach evaluates the primary hypothesis in the overall population first and then in a prospectively planned subset, or in the marker-defined subgroup first, and subsequently in the entire population [137]. Both sequences can appropriately control for the type I error rates provided the marker has sufficiently large prevalence to allow adequate power to test the treatment effect in the subgroups.

4.1.2. Marker by treatment interaction design

In this design, only patients with a valid marker result are randomized with the randomization stratified by marker status. Sample size is prospectively specified separately within each marker-based subgroup, allowing conclusions to be drawn in each subgroup. Accrual to the more prevalent subgroup will be completed prior to the less common subgroup.

4.1.3. Adaptive accrual design

Similar to the previous design, the trial begins with accrual to marker positive and marker negative subgroups. However, an interim futility threshold is prespecified. At interim analysis, if the treatment effect in either subgroup fails to exceed a futility boundary, accrual to that subgroup is terminated. Accrual to the remaining subgroup continues but now also including the planned number of patients that were initially intended to be included from the terminated subgroup such that the planned total sample size remains the same. While this strategy terminates accrual of patients unlikely to benefit from treatment early, it could lead to substantial increase in the number of patients that have to be screened and the accrual duration particularly if the selected subgroup is of low prevalence.

4.1.4. Adaptive allocation design

This is a very complex design which uses a Bayesian hierarchical framework that integrates outcome from accumulated data from the trial to adaptively assign subsequent patients to experimental treatments based on the biomarker status. It is workable for any number of distinct drug treatments under an umbrella protocol although more treatment arms will exponentially increase its complexity.

The employment of the adaptive accrual and adaptive allocation designs in HCC is challenging because they require a rapid and reliable end point. Objective response rates are sporadic even in efficacious treatment like sorafenib and progression free survival correlates poorly with overall survival. Almost all ongoing HCC trials have a significant component of biomarker studies incorporated into them. Until now, finding a reliable biomarker that can predict treatment response and thus, treatment choice for the individual patient in HCC remains elusive. Beyond the substantial statistical and endpoint issues, logistics and timeliness of data flow are also challenging.

5. Conclusions

There is clearly an unmet need in HCC beyond sorafenib. The development of targeted therapeutics for HCC remains an uphill task. Over the past decades, only sorafenib proved to have modest clinical benefit. The underlying mechanisms of the pathogenesis of HCC remain complex and elusive and this is further complicated by the heterogeneity of HCC tumors and its stroma. Currently, there are many ongoing studies that seek to leverage on molecular profiling of HCC to develop potential predictive biomarkers of HCC that inform personalized targeted treatment. The optimal choice of a trial design takes into context known characteristics of the predictive biomarker.

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