Review Article

Systemic therapy for hepatocellular carcinoma

James J. Harding[1,2], Ghassan K. Abou-Alfa[1,2]

[1]Memorial Sloan-Kettering Cancer Center, New York, NY, USA; [2]Weill Medical College at Cornell University, New York, USA

Corresponding to: James J. Harding, MD. Memorial Sloan-Kettering Cancer Center, 300 East 66th Street, New York, NY 10065, USA. Email: hardinj1@mskcc.org.

Abstract: The prognosis is poor for patients with advanced hepatocellular carcinoma (HCC). Sorafenib is the only accepted standard of care for advanced disease. The benefits of this agent are modest and the precise mechanism of antitumor activity in HCC is unknown. Since the approval of sorafenib, there has been intense investigation into strategies that block angiogenic pathways. Unfortunately, the results of three randomized phase III trials that compared newer anti-angiogenic treatments to sorafenib failed to demonstrate their superiority or non-inferiority. Thus, there remains a critical need for both continued molecular characterization and aggressive drug development in hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma (HCC); hepatocarcinogenesis; sorafenib; anti-angiogenic therapy; drug development

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Introduction

Historically, several therapeutic strategies for the treatment of advanced hepatocellular carcinoma (HCC) have been studied; however, no approach has resulted in an improvement in patient outcomes (1). In the last decade, intensive investigation into the molecular pathogenesis of liver cancer has led to new mechanistic insight, particularly regarding the angiogenic dependence of HCC (2). This has resulted in the successful clinical development of the sorafenib, a multi-kinase inhibitor (3). The success of sorafenib has galvanized the global medical research community, and currently, there are approximately 60 small molecule targeted therapeutics in various stages of clinical development, and over 200 ongoing or completed advanced HCC specific clinical trials worldwide (www.clinicaltrials.gov). Despite these advancements, several critical questions and challenges remain for HCC treatment and drug development. In this manuscript, we will conduct a brief review of the molecular pathogenesis of HCC followed by a discussion of development of anti-angiogenic therapy in this disease. Remaining clinical and translational research questions as well as the challenges of clinical trial design in context of HCC will also be highlighted herein.

Molecular and cellular biology of hepatocellular carcinoma

Hepatocarcinogenesis is a complex, multistep process whereby recurrent hepatic injury results in the accumulation of aberrant genomic, chromosomal, and epigenetic events (4). Such events define the malignant phenotype; activate numerous developmental pathways and signal transduction cascades; disrupt cell-cycle checkpoints and normal apoptotic pathways; and lead to uncontrolled cellular proliferation, growth, survival, and angiogenesis (5).

The WNT/β-catenin pathway, a tightly regulated signaling cascade in normal embryogenesis and hepatocyte differentiation, is heavily dysregulated in HCC (*Figure 1*). Activating somatic mutations within in the gene encoding β-catenin, *CTNNB1* (~30%), or in mutually exclusive inactivating mutations in *AXIN1* (~15%) or *APC* (~2%) have been observed by numerous investigators (6-11). High level chromosomal imbalances also occur on several loci that contain genes known to modulate WNT signaling (i.e., *FZD3*, *WISP1*, *SIAH-1* and *AXIN2*) (12).

Furthermore, overexpression of *FZD7*, a component of Frizzled (i.e., the WNT receptor), is observed in up 90% of HCC human tumors (13). The functional consequences of global changes in this pathway as well as the individual contributions of each alteration to tumorigenicity require more detailed characterization. However, it is clear that a large subset (up to 50%) of HCC is characterized by functional WNT pathway activation, and that such aberrant signaling, in part, drives HCC proliferation and growth (14,15). Other developmental pathways are implicated in hepatocarcinogenesis and these include the hedgehog (16), notch (17), and the c-MET proto-oncogene/hepatocyte growth factor receptor (HGF) pathways (18,19).

Mitogen-activated signaling cascades are also critical in HCC biology; however, unlike other malignancies, driver mutations in these pathways do not occur at a high frequency (9-11). The phosphatidylinositide 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) (14,20), and classic mitogen-activated protein kinase (MAPK) (21-23) pathways are activated in HCC (*Figure 1*). Blockade of these individual signaling cascades suppresses tumor growth *in vitro* and *in vivo* (24). Importantly, overproduction of mitogens [i.e., vascular endothelial growth factor (VEGF), and platelet derived growth factor (PDGF)] by the tumor and the surrounding cirrhotic microenvironment serves to sustain the neoplastic clone, drive downstream signaling cascades, and stimulate neo-angiogenesis (2). Over-expression and/or activation of the receptor tyrosine kinases linked to theses oncogenic pathways, including the epidermal growth factor receptor (EGFR) (18), VEGFR-1/-2/-3 (25-27), PDGFR (19), insulin-like growth factor receptor (IGFR) (28), and fibroblast growth factor receptor (FGFR) (29) are frequent in HCC. Finally, impairment of negative regulators of growth factor-dependent signaling, such as decreased PTEN activity in the case of PI3K-AKT-mTOR pathway, serves to further deregulate normal signals for growth and cell survival (30).

Evasion of normal apoptotic mechanisms and cell-cycle checkpoints by HCC also promote cancer formation and progression. Transforming growth factor (TGF)-β, via the SMAD proteins and other downstream effectors, exhibits potent anti-proliferative properties in normal hepatocytes (*Figure 1*) (31). Alterations in this pathway, particularly loss of SMAD4, can result in escape of the growth inhibitory properties of TGF-β (32). In this setting, TGF-β paradoxically promotes growth, invasion, and angiogenesis, and induces epithelial-mesenchymal transition (31). *TP53*, a tumor suppressor gene and cell-cycle checkpoint, is inactivated by somatic mutation in up to 50% of HCC (9,10). Further, impairment of *RB1/p16* function, which limits cell replication in the setting of DNA damage, is suppressed by promotor hypermethylation and other mechanisms in a majority of tested tumors (33). Finally, alterations in epigenetic modifiers (*ARID1/2*, *MLL*, *MLL3* and others) (10,11) and mutations within non-coding regulator promoters (*TERT*) (10,34) are common and the implications of these changes are only now being explored.

Moving forward continued molecular characterization of HCC will likely clarify the consequences of the above alterations and give insight into new therapeutic targets and novel combination strategies. Although targeting WNT appears to be priority in HCC, "drugging" this pathway has been difficult and we are only now seeing these compounds entering phase I clinical trials. Agents predicted to impair HCC growth, specifically by blocking VEGF signaling and other related mitogen-activated signal transduction cascades, have been extensively studied. The ensuing discussion will focus on the successes, failures, and ongoing studies in this area.

Inhibition of angiogenesis

Sorafenib

Sorafenib is a small molecule that targets tumoral angiogenesis and neoplastic proliferation leading to tumor-cell apoptosis in preclinical models (35). Its anti-angiogenic effects are thought to be mediated by blockade of VEGFR-2/-3, PDGFR-β, and other receptor tyrosine kinases. The compound also appears to inhibit the RAF kinases, critical components of the MAPK pathway, in both biochemical and cellular experimental systems. Given that the molecular pathogenesis of HCC is dependent upon both exuberant angiogenesis mediated, in part, by VEGF (2), and aberrant MAPK signaling (21-23), strong preclinical rationale exists for sorafenib as a therapy in HCC. Several clinical trials established the utility of sorafenib in this disease, and as such, the European Commission and the United States Food and Drug Administration licensed it for the treatment of advanced HCC in 2007 (3,36-39). In the subsequent year, the State Food and Drug Administration of China and other international agencies approved sorafenib for the same indication.

The clinical efficacy of sorafenib in HCC was firmly established by a multicenter phase II study (3). One-

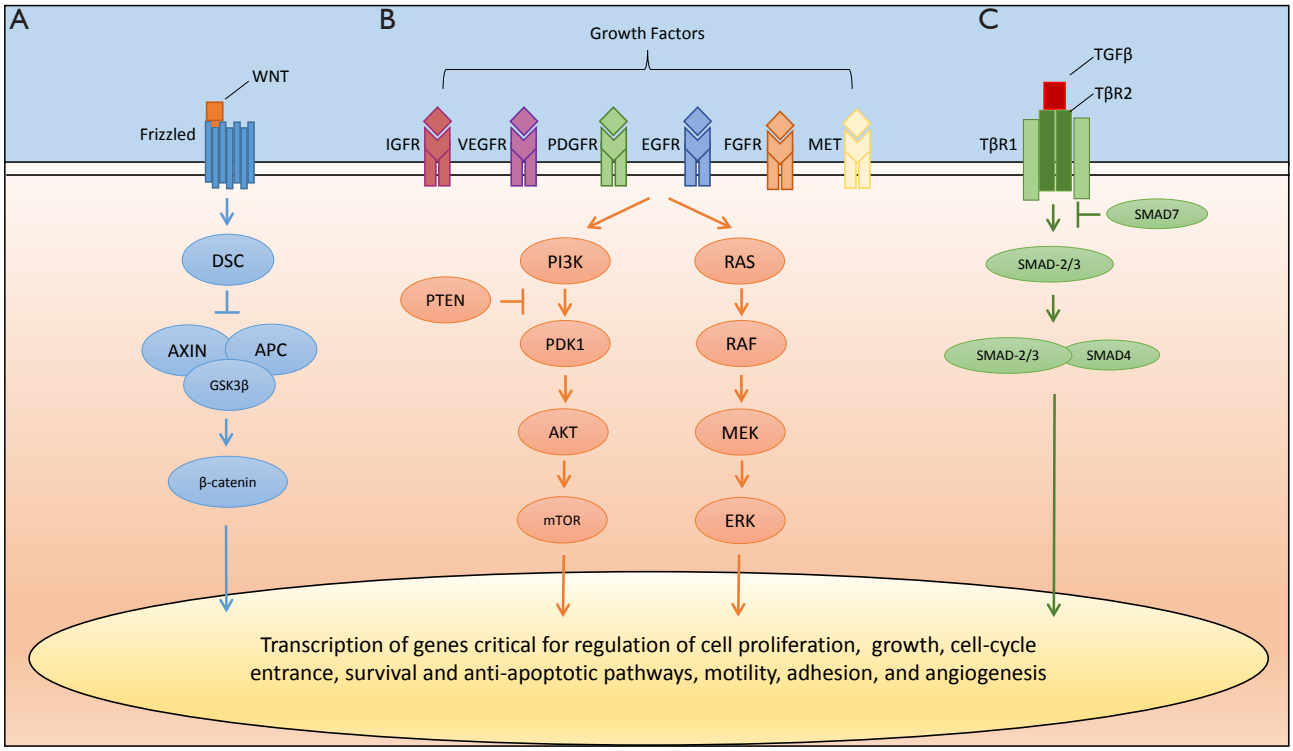

Figure 1 Schematic of signal transduction cascades relevant to hepatocellular carcinoma biology. The WNT/β-catenin, PI3K-AKT-mTOR, MAPK and TGF-β pathways are heavily disrupted in HCC. A. In canonical WNT/β-catenin signaling, engagement of the WNT receptor, Frizzled, leads to the activation of disheveled (DSC). Once activated DSC inhibits the β-catenin destruction complex, which is composed of Axin, adenomatosis polyposis coli (APC), glycogen synthase kinase 3β (GDK3β), and other regulatory molecules. In this setting β-catenin avoids ubiquitination and subsequent proteasome digestion thereby allowing it to translocate to the nucleus to activate numerous regulatory genes; B. MAPK and PI3K-AKT-mTOR pathway activation is complex and signal modulation between each pathway is well documented. In physiologic circumstances, external growth factors engage the appropriate receptor tyrosine kinase (RTK) embedded in the phospholipid bilayer at the cell surface. Ligand binding leads to dimerization of the RTK followed by transphosphorylation of the cytoplasmic components of the receptor. The phosphorylated cytoplasmic tail recruits a variety of accessory molecules. In the case of the MAPK pathway, sequential activation of RAS, RAF, MEK, and ERK ensues leading to the modification of a number of substrates (i.e., Cyclin D, Myc, Elk, etc.) that in turn regulate protein synthesis, transcription and entrance into the cell cycle. In the PI3K-AKT-mTOR pathway, activation of the RTK leads to sequential modification of phosphatidyl inositol residues in phospholipid bilayer. In the terminal step of this enzymatic process, PI3K generates phosphatidyl inositol (3-5) triphosphate (PIP3). PIP3 recruits AKT to the cell membrane and in association with PDK1 activates AKT. AKT then modulates the activity of a number of downstream substrates including mTOR, thus promoting angiogenesis, proliferation and cell survival. By reversing the effects of PI3K, PTEN is a negative regulator of this pathway. C. The end result of canonical TGF-β signaling in normal circumstances is to prevent proliferation. Isoforms of TGFβ engage the TGFβ receptor type 2 (TβR2) dimer at the cell surface. This in turn leads to recruitment and phosphorylation of the TGFβ receptor type-1 (TβR1). Subsequent phosphorylation of SMAD-2/3 proteins alters their conformational structure allowing complexing with SMAD4 and translocation to the nucleus. Here, the SMAD-2/3/4 complex causes the transcription of a number of genes necessary for apoptosis, cell-cycle arrest, and extracellular matrix formation. SMAD7, a product of TGFβ signaling is an important negative regulator of this pathway.

hundred and thirty-seven patients with systemic treatment-naïve, inoperable HCC and varying hepatic reserve (72% Child-Pugh A, 28% Child-Pugh B) received the agent. The primary objective of the study was to determine the objective response rate to sorafenib, and the predefined boundary to establish cytotoxic efficacy was set at a 7% confirmed response rate. Although only 2.2% of the study population achieved a confirmed objective response by

Table 1 SHARP and Asia-Pacific studies patient outcome and response metrics

Response metric	SHARP		Asia-Pacific	
	Placebo (n=303)	Sorafenib (n=299)	Placebo (n=76)	Sorafenib (n=150)
Response rate				
Complete response	-	-	-	-
Partial response	1%	2%	1.3%	3.3%
Stable disease	67%	71%	27.6%	54.0%
Progressive disease	-	-	54.0%	30.7%
Disease control rate	32%	43%	12%	53%
TTRP (months)	2.8	5.5	1.4	2.8
TTSP* (months)	4.1	4.9	3.4	3.5
Median OS (months)	7.9	10.7	4.2	6.5
1-year survival rate	33%	44%	-	-
Hazard ratio for survival	0.69 (CI: 0.55-0.87)		0.68 (CI: 0.50- 0.93)	

Abbreviations: TTRP, time to radiographic progression; TTSP, time to symptomatic progression; OS, overall survival; CI, confidence interval.

WHO criteria, 42% percent of the study population had extended disease control. The median overall survival was 9.2 months, which was encouraging when compared to historical controls. A second study composed exclusively of an Asian population obtained similar favorable results (37).

Subsequently, two pivotal, multicenter, double-blind, placebo-controlled, randomized phase III studies of sorafenib versus best supportive care in patients with advanced HCC demonstrated a statistically significant improvement in overall survival in favor of sorafenib (*Table 1*) (38,39). The SHARP (Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol) trial enrolled 602 patients with advanced HCC who had not received prior systemic therapy (39). The majority of the study population, which was recruited predominately from Europe and Australasia, had HCC with macroscopic vascular invasion, extrahepatic spread or both. Preserved liver function was a strict inclusion criterion of the study, and in fact, only 3.3% of participants had Child Pugh class B hepatic function. HCC etiologic factors were well distributed amongst participants with roughly 28%, 26%, and 18% of cases related to HCV, alcohol, and HBV, respectively. Patients were randomly assigned to receive sorafenib at 400 mg orally twice a day (n=299) or best supportive care (n=303). The co-primary endpoints of the study were overall survival and time to symptomatic progression. Sorafenib rarely resulted in tumor shrinkage; however, the agent was associated with an absolute increase in the disease control rate of 11% when compared with placebo. This cytostatic effect translated to a statistically significant longer time to radiographic progression and an absolute 11% increase in the 1-year survival rate. Median overall survival was 10.7 months in the sorafenib arm versus 7.9 months in the cohort receiving best supportive care (HR=0.69, 95% CI: 0.55-0.87). Predefined subset analysis indicated that the survival benefit of sorafenib was independent of performance status and disease burden.

Designed in parallel with SHARP, the Asia-Pacific study assessed the efficacy and tolerability of sorafenib in comparison with best supportive care in the patients with advanced HCC geographically localized to China, South Korean, and Taiwan (38). The study was therefore well positioned to assess the potential impact of known regional differences in HCC etiologic factors on responsiveness to treatment. By providing a closer representation of the worldwide HCC patient population, the Asia-Pacific study also minimizes theoretical confounding factors (e.g., environmental aflatoxin exposure, socioeconomic variables, etc.) that might be unique to Asia and not adequately represented by the SHARP study population. As expected and in contrast to SHARP, the Asia-Pacific study was enriched with patients with HBV-related HCC (73% of the total study population), and in general, was compromised of a greater proportion of patients with poorer ECOG performance status and greater disease burden. Despite these differences, the trial confirmed that sorafenib, when compared to best supportive care, was tolerable and led to a statistically significant improvement in disease control, time

to radiographic progression, and overall survival.

It is important to note that the magnitude of the overall survival benefit on the Asia-Pacific study was not as substantial as observed on the SHARP study—the median overall survival was only 6.5 and 4.2 months for patients receiving sorafenib and placebo, respectively. The inclusion of patients who were more ill prior to beginning therapy than those patients on the SHARP study might, partly or even fully, explain this slight survival difference. Another postulate is that the observed differential outcomes on the two trials were due to differing treatment patterns between Asia and Western countries. Aggressive local regional therapies might be more common in Asia, thus leading to the selection of patients on the Asia-Pacific study who are presenting later in the course of their disease. The inclusion criteria for the Asia-Pacific study; however, do not necessarily support this assertion. Alternatively and provocatively, specific viral etiologic factor might affect prognosis and influence the responsiveness of liver cancer to sorafenib.

In an unplanned subset analysis of the SHARP study, patients with HBV-related HCC (n=60) who were treated with sorafenib had a modest prolongation in median overall survival over placebo (9.7 *vs.* 6.1 months) but similar disease control rates (34.4% *vs.* 32.1%) and near equivalent time to progression (2.7 *vs.* 4.2 months) (40). In contrast, HCV-related HCC patients (n=167) treated with sorafenib appeared to derive much greater clinical benefit, with substantial improvements over placebo in overall survival (14.0 *vs.* 7.4 months), disease control rates (44.2% *vs.* 29.6%), and time to progression (7.6 *vs.* 2.8 months). Retrospective analysis of initial phase II study of sorafenib observed similar etiologic-dependent trends in survival (41). Patients who were infected with HCV lived longer (n=13, 12.4 months) than did patients infected with HBV (n=33, 7.3 months, P=0.29). Finally, the recently reported phase III study of first-line sunitinib indicates that there may in fact be differential outcomes relative to disease cause and ethnic origin, with median overall survival for HCV-associated HCC ranging from 18.3 months for patients with living outside of Asia to 7.9 months for patients living in Asia (42).

A caveat to drawing a firm conclusion on the matter of variable sensitivity to sorafenib is that sample size is small and *ad hoc* subgroup analyses are notoriously subject to confounding secondary to population imbalance. Certainly if differentially, antitumor activity exists, etiologic-dependent genomic differences in HCC might explain improved outcomes to sorafenib in patients with HCV-related HCC. *CTNNB1* mutations are more commonly observed in HCV-related but not in HBV-related HCC and are associated with a specific WNT gene expression profile (9,14,15). Sorafenib can modulate this gene signature, interfere with WNT signaling output, and lead to HCC growth suppression in preclinical models (15). Etiologic-dependent differences in outcome might also be explained by HCV core protein-induced upregulation of the sorafenib target CRAF, among other kinases (43). Finally, *in vitro* data suggest that sorafenib can directly inhibit HCV viral replication, though the clinical importance of this observation is debatable (44). Although more exploration is certainly required, it should be emphasized that the utility of sorafenib is not undercut by this observation and it remains an effective and life prolonging therapy for HCC, irrespective of etiologic factor.

Sorafenib combination strategies

In the attempt to improve upon the modest results observed with sorafenib, investigators have proposed combination strategies with cytotoxic chemotherapy and novel biologic agents. Prior to the approval of sorafenib, doxorubicin was evaluated as monotherapy or in combination with sorafenib in a randomized, double blind, phase II study (45). The trial enrolled 96 patients with treatment-naïve advanced HCC and Child-Pugh A liver function. The primary endpoint of the study was time to progression. Importantly, both time to progression, as determined by independent review, and progression-free survival were increased by approximately 4 months, and the median overall survival doubled in favor of combined therapy (13.7 *vs.* 6.5 months, P=0.006). Cardiac toxicity was notable, with a higher proportion of patients on the combination experiencing left ventricular systolic dysfunction (19% *vs.* 2%). Although the majority of such cases were asymptomatic, the median cumulative doxorubicin dose was limited to 165 mg/m^2.

The dramatic increase in survival over placebo was striking; however, the lack of sorafenib as a comparator arm limits the interpretation of the trial. Doxorubicin may contribute little to outcome. The observed benefit in the doxorubicin-sorafenib group may be due to the effects of sorafenib alone. Alternatively, the combination may be synergistic. Inhibition of the MAPK pathway by sorafenib may restore chemosensitivity by enhancing pro-apoptotic pathways and dampening multi-drug resistance (MDR) pathways. Anthracycline-induced cytotoxicity is mediated by the pro-apoptotic kinase ASK1 (46). Growth factor-

induced MAPK activation, via FGF, has been shown to abrogate ASK1 activity. Blockade of the RAF kinases by sorafenib might therefore augment the antitumor activity of doxorubicin. Furthermore, MAPK activation leads to the induction of MDR-1 pump (47). Sorafenib decreases ATP-binding cassette/MDR protein gene expression thereby restoring HCC sensitivity to doxorubicin *in vitro* (48). A randomized phase III study of sorafenib versus sorafenib and doxorubicin in the first-line setting (www.clinicaltrials.gov NCT01015833) and a phase II study of the regimen in second-line setting after sorafenib failure (www.clinicaltrials.gov NCT01840592) are currently underway.

Gemcitabine and oxaliplatin (GEMOX) therapy has established efficacy in HCC (49), and there is reason to believe that addition of sorafenib to gemcitabine might offer synergistic anti-tumor effects (48). GEMOX-sorafenib versus sorafenib was recently tested in a randomized phase II study (GONEXT) (50). The trial enrolled 95 patients with advanced HCC (CLIP 52% 2/3), excellent performance status (69% WHO PS 0), and Child-Pugh A liver function. The primary endpoint was 4-month progression—free survival of greater than or equal to 50%. The combination of GEMOX plus sorafenib resulted in a 4-month PFS rate of 61% compared to 54% in sorafenib monotherapy group. The combination was feasible and efficacy data were encouraging (ORR 16%, DCR 77%), though grade 3/4 neutropenia, fatigue, thrombocytopenia, diarrhea, and sensory neuropathy were common. More data will be required to define the role of this sorafenib combination strategy in HCC. In addition, several other trials are evaluating sorafenib in combination with other forms of cytotoxic chemotherapy.

In addition to its application with anti-angiogenic agents such as bevacizumab, sorafenib is being combined with antisense technologies; receptors tyrosine kinase inhibitors and monoclonal antibodies blocking EGFR, c-MET, FGFR and IGFR; multiple small molecule inhibitors of the MAPK and PI3K-AKT-mTOR pathways; histone deacetylase inhibitors; and novel immune-based therapies. The majority of these biologic combinations are still in early drug development and it is premature to comment on how they might improve upon sorafenib, though emerging data are promising and there remains enthusiasm for drug development in this area.

Erlotinib, an EGFR tyrosine kinase inhibitor, and sorafenib are the first novel pairing to reach later stages of clinical development. Although there is a theoretical benefit to blocking both EGFR and VEGFR in HCC, the addition of erlotinib to sorafenib did not produce additive or synergistic effects *in vitro* or *in vivo* (51). A phase I study that evaluated sorafenib and erlotinib in 17 patients with various solid tumors, included a single case of HCC (52). This patient received the recommended phase II dose and had a best overall response of stable disease with ~5% tumor growth on study. In an extension cohort of this trial, an additional evaluable HCC patient progressed after 75 days of combination therapy (53). The SEARCH trial confirmed that the addition of erlotinib to sorafenib provided no benefit in HCC (54). In this randomized, placebo controlled, double blind, phase III study the combination of sorafenib and erlotinib were compared to sorafenib alone in the first-line setting in 720 patients with advanced HCC. There was no statistically significant difference between study arms with regard to the primary endpoint of overall survival (combination 9.5 months, sorafenib 8.5 months, HR=0.93, 95% CI: 0.78-1.11).

Multi-targeted receptor tyrosine kinase inhibitors

Several small molecule, orally available, receptor tyrosine kinase inhibitors with the ability to inhibit VEGFR, and other kinases, have undergone extensive evaluation or are being tested in clinical trials of varying stages for the treatment of advanced HCC. These agents include sunitinib, axitinib, regorafenib, brivanib, linifanib, vandetanib, cediranib, pazopanib, TSU-68, vatalanib, and lenvantinib. Thus far, emerging results have been disappointing with the major phase III studies of anti-angiogenic therapy failing to improve upon sorafenib in the first-line setting, and no clear benefit over best supportive care of additional anti-angiogenic monotherapy in the second-line setting.

Sunitinib inhibits VEGFR-1/-2 with greater potency than sorafenib (55). Additionally, the agent targets PDGFR-α/β, c-KIT, FLT3, RET, and other kinases. Three separate phase II studies of sunitinib evaluated three different dosing schedules of the agent as a treatment for advanced HCC (56-58). A subsequent randomized phase III study of sunitinib, dosed continuously, versus sorafenib in patients with advanced HCC and Child Pugh Class A liver function was initiated and rapidly enrolled 1,073 patients (42). The study, powered to test the dual hypotheses of non-inferiority and superiority with regard to overall survival, was halted by an independent data monitoring committee due to futility and safety concerns. Median overall survival for the sunitinib cohort was 8.1 months as compared to

10 months in sorafenib arm (HR=1.31, 95% CI: 1.13-1.52, P=0.0019). Axitinib and regorafenib, which inhibit similar molecular targets to both sunitinib and sorafenib but exhibit a slightly different spectrum of toxicities, are now being evaluated as monotherapy after progression on sorafenib (www.clinicaltrials.gov NCT01334112, NCT01273662, NCT01210495, and NCT01774344).

Brivanib, a dual inhibitor of VEGFR and FGFR, demonstrated modest antitumor activity in both treatment-naïve and those patients who had failed prior anti-angiogenic therapy in two separate phase II studies (59,60). Based on these data, a large randomized phase III study compared brivanib to sorafenib in patients with systemic treatment-naïve, advanced HCC (61). This non-inferiority trial did not meet its primary endpoint; median overall survival with brivanib treatment was 9.5 *vs.* 9.9 months with sorafenib (HR=1.06, 95% CI: 0.93-1.22, P=0.3730). Albeit, antitumor activity and disease control rates were similar between each group. A randomized phase III study of brivanib after progression of disease on sorafenib versus best supportive care also failed to meet its primary endpoint of improved overall survival (62).

Linifanib, a selective inhibitor of VEGFR and PDGFR (63), also failed to improve upon the modest survival advantage of sorafenib (64). Early efficacy data were encouraging (65); however, these results did not translate into success in a large multicenter, randomized, phase III study of sorafenib versus linifanib as a first-line therapy for advanced HCC (64). Patient composition was similar to prior pivotal studies. Failing to meet the both pre-specified endpoints of superiority and non-inferiority, the median overall survival for linifanib was 9.1 *vs.* 9.8 months for sorafenib (HR=1.046, 95% CI: 0.896-1.221). A higher proportion of patients attained an objective response on linifanib (13% *vs.* 6.9%); however, serious adverse events were more common in this cohort than compared with sorafenib.

Cediranib, vandetanib, pazopanib, TSU-68, vatalanib, and lenvatinib have not reached later stages of clinical development. Cediranib, a pan-VEGFR inhibitor, has been associated with a high incidence of toxicity with minimal efficacy (66,67). Vandetanib, a small molecule inhibitor that blocks signaling through VEGFR and EGFR, is tolerable but has limited clinical activity (68). Pazopanib (69), TSU-68 (70), vatalanib (71), and lenvatinib (72) block VEGFR and other targets. Currently, these agents have an established safety profile, modest efficacy, and represent an important area of continued investigation.

Monoclonal antibodies

Over 20 separate clinical trials have assessed or are assessing bevacizumab, a monoclonal antibody directed against VEGF, in patients with advanced HCC. Evaluated regimens include monotherapy and combination therapy with chemotherapy, targeted agents, and embolization procedures. In general, completed studies have reported higher response rates than those observed with RTK inhibitors; however, adverse events such as arterial/venous thrombotic events and variceal hemorrhage (some fatal) are more common. A phase II study of bevacizumab monotherapy at two different doses in patients with advanced, liver-limited HCC demonstrated an objective response rate of 13% in 39 evaluable patients, with one patient obtaining a complete response (73). Grade 3 or 4 hypertension, hemorrhage and thrombosis occurred in 15%, 11% and 6% of the study group, respectively. One fatal esophageal hemorrhage due to varices occurred early in the course of the study. Subsequently, prophylactic variceal treatment was required prior to study enrollment. A second phase II study in advanced HCC with extrahepatic disease observed similar efficacy (ORR 14%) with bevacizumab monotherapy (74). It has not advanced to later stage development due to safety concerns regarding bleeding.

The addition of cytotoxic chemotherapy or targeted therapy to bevacizumab may augment antitumor activity. Response proportions (CR + PR) with various cytotoxic combinations range from 9-20%, with disease control rates reportedly as high as 78% (75-77). Bevacizumab and erlotinib may offer enhanced antitumor activity with a response rate of 24% and favorable patient outcomes with a median overall survival of 13.7 months (78,79). These results were not corroborated is a second study that reported minimal activity in a comparable patient population with similar disease assessment parameters and an identical dosing schedule (80). This observation serves to illustrate the heterogeneous nature of HCC and the potential for subtle differences in patient specific factors (i.e., disease burden, Child-Pugh class, etiologic factor) to either cloud interpretation of early stage trials or, as in the case of etiologic factor, potentially influence responsiveness to therapy. As seen above, it is also possible that erlotinib adds little to the effects of anti-angiogenic therapy. To clarify this issue, a multicenter, randomized phase II trial of bevacizumab combined with erlotinib (www.clinicaltrials.gov NCT00881751) versus sorafenib monotherapy is ongoing. Several other additional phase II studies are

evaluating bevacizumab with sorafenib, everolimus, temsirolimus, and other treatment modalities.

Ramucirumab, a monoclonal antibody blocking VEGFR-2, was recently assessed in a phase II study comprised of 43 patients with systemic treatment-naïve advanced HCC. The majority of study participants had extrahepatic disease with excellent hepatic function. The median progression-free survival was 4.3 months with a disease control rate was 50% (7% of patients had a partial response). The agent was tolerable, but like bevacizumab, severe hypertension and hemorrhage with drug-related deaths were reported. Based on these data a randomized phase III study of ramucirumab versus best supportive care in the second line setting is ongoing (www.clinicaltrials.gov NCT01140347). Several other novel anti-angiogenic monoclonal antibodies are entering early stage development in HCC (81). Such agents may offer a more favorable safety profile, with a lower incidence of hemorrhage, which might be ideal in the HCC patient population.

Critical questions in targeting angiogenic pathways

Several important considerations remain in the treatment of this heterogeneous malignancy and for future drug development. Perhaps the most critical question is to define (if possible) the mechanistic basis for the antitumor activity of sorafenib in HCC. As discussed above, three drugs, which were perceived to be more potent and precise inhibitors of angiogenic pathways than sorafenib, failed to demonstrate greater efficacy in the clinical setting. In addition to directly interrogating patient tumor samples, there are renewed efforts to develop preclinical animal models that adequately recapitulate the features of human disease (i.e., etiologic factor, cirrhotic background, etc.). Such approaches will be important for a mechanistic understanding of angiogenesis and translating basic science breakthroughs to the clinic and vise-versa.

Establishing biomarkers of responsiveness is also a priority. Molecular sub-categorization of tumors will identify the biologic profile that might make a patient's tumor more susceptible to a specific targeted therapy. Thus far, these attempts have been unsuccessful for sorafenib. Pretreatment serum-based response surrogates, such as VEGF, VEGFR-1, VEGFR-2, VEGFR-3, Ang-2, FGF, and several cytokines are not predictive of benefit to anti-angiogenic therapy (82). Trends toward enhanced survival from sorafenib were observed in patients with high circulating c-KIT or low hepatocyte growth factor (HGF, the ligand for c-MET) concentration at baseline. Oncogenic pathway activation as assessed by pretreatment phosphorylated-ERK, the downstream effecter of the MAPK pathway, was associated with longer time to progression on sorafenib (3). In contrast, activation of the transcriptional regulator c-Jun is associated with a poor response to sorafenib (83). These observations obviously require further validation and clarification. Other areas of intense biomarker exploration include the study of circulating tumor cells, HCC gene expression profiles, and importantly the application of next-generation sequencing technologies to define cancer genotypes that are more likely to response to targeted therapy (84,85).

Finally, defining the optimal method of radiographic assessment in HCC will be critical to assess early efficacy in phase I and II clinical trials. Thus far, anti-angiogenic therapy appears to suppress growth and disrupt the vasculature, but does not yielded dramatic tumor shrinkage. Clinical benefit occurs without tumor response. Thus, standard Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, which assesses the sum of one-dimensional measurement in multiple target lesions, may not adequately reflect the cytostatic effect of anti-angiogenic therapy on tumor viability (86). New response assessment tools have been developed to incorporate the concept of tumor viability, reflected by tissue density due to vascular enhancement. Modified RECIST incorporate decreased intra-tumoral enhancement to define a response. Limited data are available to indicate that this approach, which was never prospectively validated, is a superior surrogate to RECIST in the metastatic setting in response to anti-angiogenic therapy (87). Other proposed schemas include the ratio of tumor necrosis to tumor volume (41), volumetric measurement (86), and the application of functional MRI imaging such as dynamic-contrast enhanced (DCE), blood oxygen level dependent (BOLD), diffusion weighting, and image subtraction to assess for tumor response (41). Large prospective studies evaluating these techniques will be required before implementation of global standard.

Selected therapeutic strategies in late stage drug development

Given the multitude of drugs under evaluation in early stage clinical trials or with early safety and modest efficacy data available, an exhaustive review of each agent or each agent class will be forgone and the remaining discussion will focus

on those agents that are currently under investigation on active phase III clinical trials.

Targeting the HGF/c-MET axis

Overexpression of c-MET and its ligand HGF occur in up to 80% of human HCC tumors (19). Transgenic mice that overexpress MET in hepatocytes developed HCC and inactivation of this transgene leads to tumor regression, mediated by apoptosis and growths suppression (88). Downregulation of MET *in vitro* using RNA interference (89), micro-RNAs (90), of transfection of NK4 (an antagonist of HGF) (91) reduces the migratory and invasive capacity of HCC cells. Finally, blocking MET with several different multi-targeted TKIs induces in vitro HCC growth suppression, cell-cycle arrest and decreased viability as well as growth suppression and survival prolongation in vivo (92). Given these data, MET has emerged as a promising target in HCC.

Tivantinib, a selective MET receptor tyrosine kinase inhibitor, was evaluated at two doses in a randomized, placebo-controlled phase II in advanced HCC patients who had progressed after first-line therapy (93). This study reported two critical findings. First, a statistically significant difference in outcomes between high-MET expressing tumors in favor of tivantinib. For patients with high MET expressing tumors, tivantinib therapy resulted in a median time to progression of 2.7 months in comparison to 1.4 months for placebo (HR=0.43, 95% CI: 0.19-0.97) and a median overall survival of 7.2 compared with 3.8 months for placebo (HR=0.38, 0.18-0.81). Importantly, no such differences between the agent and placebo were observed in low-MET expression tumor. This strongly suggests that MET expression is a predictive biomarker for MET-directed targeted therapy in HCC. Second, in those patients on the placebo arm, high tumoral MET expression was associated with an improved overall survival when compared with low tumoral MET expression (3.8 *vs.* 9 months, HR=2.94, 95% CI: 1.16-7.43). This observation indicates that MET expression may also be prognostic in this disease. Given these data, tivantinib is being compared with placebo in double-blind, randomized phase III study in patients with advance HCC and high-MET expressing tumors in the second-line setting (clinicaltrials.gov NCT01755767).

Cabozantinib, an inhibitor of MET and VEGFR-2, has also shown promising efficacy data in a cohort of 41 patients with advanced HCC (94). In 78% of patients, tumor regression was observed by RECIST with a 5% confirmed partial response rate. Median progression-free survival for the cohort was estimated at 4.2 months. Unfortunately baseline MET expression has not been reported. A phase III study cabozantinib is in planning. Several other agents are entering HCC-specific clinical trials, and these include oral MET inhibitors such as foretinib, golvatinib and INC280, MET blocking monoclonal antibodies, and novel combination strategies.

Targeting the mammalian target of rapamycin pathway

The mTOR pathway plays a critical role in hepatocarcinogenesis, and in xenograft mouse models, blockade of this pathway results in HCC growth suppression and lengthening of survival (20). These observations, as well as retrospective data indicating enhanced survival among patients receiving sirolimus immunosuppression following liver transplantation for HCC, piqued interest in developing these compounds in this disease. A phase I/II study of everolimus established that 10 mg daily was a safe dose (95). The phase II portion, a two-stage efficacy design, did not meet its pre-specified boundary for expansion to the second stage. Of 25 evaluable patients, 1 (4%) had a partial response and 10 (40%) had stable disease. Median time to progression was 3.9 months and median overall survival was 8.4 months. Presently, everolimus is being investigated in the second line setting after sorafenib failure in the phase III, randomized, placebo-controlled EVOLVE-1 study (www.clinicaltirals.gov NCT01035229). Temsirolimus, AZD8055, as well as multiple combination strategies are ongoing.

Targeting metabolic pathways

The biosynthesis of the nonessential amino acid arginine occurs as part of the urea cycle and is dependent upon the enzymes argininosuccinate synthetase and argininosuccinate lyase. Messenger RNA encoding argininosuccinate synthetase is not present in subsets of hepatocellular carcinomas, therefore arginine must be extracted from the circulation (96). Pegylated arginine deiminase (ADI-PEG 20) is an arginine degrading enzyme isolated from *Mycoplasma* that is formulated with polyethylene glycol (molecular weight 20 kilodalton). In preclinical models, ADI-PEG 20 decreases HCC cell viability at low nanomolar concentrations, reduces serum arginine levels to undetectable levels, and prolongs survival in HCC xenograft mouse models. A phase I/II study demonstrated

an excellent safety profile in a patient population comprised with a high burden of disease and impaired hepatic function (~49% study population Child Pugh B or C) (97). The most common events were injection site reactions and isolated lab abnormalities such as elevated fibrinogen. Of 19 patients evaluable, 2 (10.5%) had complete response, 7 (36.8%) had a partial response and 7 (36.8%) had stable disease. The duration of response ranged from 37 to >680 days. Two subsequent randomized phase II studies that compared escalating doses demonstrated less marked antitumor efficacy (98,99). Glazer and colleagues reported a disease control rate of 63.1% and 2.6% objective response rate and a median overall survival of 11.4 months (98). This exclusively European patient population was composed predominately of HCV-associated (79%) HCC confined to the liver (84%) with otherwise excellent hepatic function (81%). In contrast, Yang and colleagues tested the agent in a heavily pretreated Asian population with HBV-associated (69%) extrahepatic (58%) hepatocellular carcinoma. In this study, no objective responses were noted and the median overall survival was 7.3 months. Currently, a double blind placebo controlled study of ADI-PEG 20 after prior systemic therapy is ongoing (www.clinicaltrials.gov NCT01287585).

Conclusions and future directions

Despite the availability of sorafenib as a standard of care for HCC, there is a substantial need to enhance the armamentarium of therapies in the metastatic setting. Presently, the global standard of care for a patient presenting with metastatic hepatocellular carcinoma is either clinical trial enrollment or sorafenib monotherapy. Although several, high-profile, phase III clinical trials have failed to improve on the current standard, the pipeline for drug development is robust, preliminary phase II data are promising for several agents, and the international research community is committed to continued collaboration to understand this complex disease. In the laboratory, interrogation of HCC genome may isolate novel targets. It is also likely that more trials will attempt to select molecular profiles that are predicted to respond to specific targeted therapy, as in the case of MET inhibition. Looking forward, there will certainly be a greater attention to immune based therapy. Tremelimumab, a CTLA-4 blocking antibody, demonstrated durable disease control in a recent phase II study in addition to exhibiting antiviral activity (100). Several trials evaluating other immune checkpoint modulators (i.e., anti-PD-1 and anti-PDL1) are ongoing or are being planned. Engineered viral stains, termed oncolytic immunotherapeutics, are capable of selectively targeting tumors by inducing both viral replication-dependent tumor death and tumor-specific immunity (101). This approach has shown promising activity as well. Finally, efforts will continue to target the WNT pathway, which is heavily disrupted in HCC. Hopefully, the international field will continue to witness meaningful progress for the treatment of patients with metastatic hepatocellular carcinoma.

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