Oncol Rev (2011) 5:177–184
DOI 10.1007/s12156-011-0082-3

REVIEW

Can we develop effective combination antiangiogenic therapy for patients with hepatocellular carcinoma?

Justin B. Wenger · Napoleon Santos · Yanxia Liu · Jennifer Dallas · Sukanthini Subbiah · Steven Hochwald · Emina H. Huang · Duyen T. Dang · Carmen J. Allegra · Hendrik Luesch · Long H. Dang

Received: 9 April 2011 / Accepted: 18 July 2011 / Published online: 9 August 2011
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Abstract Antiangiogenic therapy has shown promise in the treatment of patients with hepatocellular carcinoma (HCC). Bevacizumab, sorafenib, and sunitinib showed efficacy in patients with HCC; and sorafenib is approved by the FDA for treatment of this cancer. In practice, the clinical benefit of these agents has been heterogeneous; and in patients who do respond, the benefit is modest and/or short-lived. Recent advances in the molecular understanding of tumor angiogenesis along with the rapid development of targeted drug discovery have made it possible to explore novel combination therapy for HCC. We review the clinical trial results, discuss possible molecular mechanisms of resistance, and suggest novel combinations with antiangiogenic therapy.

Keywords HIF-1α · HIF-2α · Antiangiogenic therapy · Hepatocellular carcinoma

J. B. Wenger and N. Santos contributed equally to this work.

J. B. Wenger · N. Santos · J. Dallas · S. Subbiah · C. J. Allegra · L. H. Dang (✉)
Division of Hematology/Oncology,
Department of Internal Medicine,
University of Florida Shands Cancer Center,
University of Florida, 1600 SW Archer Road,
Gainesville, FL 32610, USA
e-mail: long.dang@medicine.ufl.edu

Y. Liu · H. Luesch (✉)
Department of Medicinal Chemistry,
University of Florida, 1600 SW Archer Road,
Gainesville, FL 32610, USA
e-mail: luesch@cop.ufl.edu

S. Hochwald · E. H. Huang
Department of Surgery, University of Florida,
Gainesville, USA

D. T. Dang
Department of Internal Medicine,
University of Michigan, Ann Arbor, MI, USA

Introduction

Liver cancer is the fifth most common malignancy and the third most common cause of cancer-related mortality worldwide [1]. Even though more than 80% of the cases occur in developing countries, its incidence continues to increase in developed countries largely due to the Hepatitis C epidemic [2]. Hepatocellular carcinoma (HCC) represents the major histologic subtype and accounts for an estimated 70–85% of the cases [3]. Because of the asymptomatic nature of early HCC, most patients present with unresectable disease [4]. Presently, curative rates for patients with advanced disease are dismal due to the resistant nature of this malignancy to systemic chemotherapy. Unfortunately, local interventions such as chemoembolization and ablation have failed to significantly extend life expectancy [5]. And even in those who undergo surgical resection, intrahepatic recurrence occurs in 70% of the patients [6].

Recently, antiangiogenic therapy has shown promise in the treatment of patients with HCC. This novel class of agents was designed to inhibit the vascular endothelial growth factor (VEGF) pathway; as VEGF is the most prevalent and dominant proangiogenic growth factor in the tumor microenvironment [7–10]. FDA approved agents that directly target the VEGF pathway include the anti-VEGF antibody bevacizumab and the multitargeted tyrosine kinase inhibitors (sunitinib, sorafenib, and pazopanib),

which inhibit VEGFRs along with other receptor tyrosine kinases [11–16].

At the present time, the factors/pathways that mediate resistance to antiangiogenic therapy are unknown. In preclinical models, there is evidence of compensatory pathways that mediate resistance at the levels of the tumor blood vessels, microenvironment, and tumor epithelial cells [17–21]. Antiangiogenic therapy may induce HIF-1α and HIF-2α due to tumor blood vessel constriction, decrease in blood flow, and induction of intratumoral hypoxia [22, 23]. HIF-1α and HIF-2α, in turn, transactivate gene programs important for inducing tumor angiogenesis, cancer cell growth/survival, and energy metabolism; mediating resistance. Like many other solid tumors, overexpression of HIF-1α has been shown to occur in HCC and its expression has been correlated with resistance to therapy and confers a poor prognosis [24]. This overexpression is triggered by tissue hypoxia, aberrant growth factor receptor signaling, and mutations in oncogenes and tumor suppressor genes.

Clinical trials with targeted agents in HCC (Table 1)

Sorafenib

Sorafenib is a small molecule, multi-targeted tyrosine kinase inhibitor (TKI) that mediates its antiangiogenic effect through inhibition of VEGFR-2/3 and PDGFR-β, which direct the formation of new vasculature. Sorafenib has been approved by the FDA for the treatment of hepatocellular carcinoma and renal cell cancer.

The primary controlled studies indicating the potential of single-agent sorafenib in the treatment of advanced HCC are the SHARP (Sorafenib HCC assessment randomized protocol) and Asian-Pacific trials. Sorafenib monotherapy in the SHARP trial—a double-blind, placebo-controlled, phase III study— proved efficacious in prolonging both median overall survival (OS) and time to radiologic progression by 3 months. The sorafenib group recorded an OS of 10.7 months as compared to 7.9 months for the placebo control [15]. Patients in the sorafenib-arm showed a median time to radiographic progression of 5.5 months while the placebo-arm was 2.8 months for the same parameter [15]. Although sorafenib was therapeutically beneficial in extending median OS and time to radiographic progression, there was no significant difference in the time to symptomatic progression between the sorafenib and placebo groups. Only 2% of the patients in the sorafenib-arm achieved a partial response (PR); without any complete responses (CR) in either the sorafenib/placebo groups. However, the disease control rate was substantially higher in the sorafenib-arm versus the control-arm (43 to 32%) [15].

Single-agent sorafenib in the Asian-Pacific trial patients demonstrated a significant increase in median OS (6.5 months) as compared to the median OS in the control group (4.2 months). There was an increase in median time to disease progression (TTP, 2.8 months) in the sorafenib treatment group in comparison to the placebo group (TTP, 1.4 months) [25]. The clinical findings in the SHARP and Asian-Pacific studies indicate the potential therapeutic benefits of single-agent sorafenib in treating advanced HCC.

In addition to its single-agent efficacy, sorafenib has also shown a benefit when combined with doxorubicin. In a phase I study of combined sorafenib/doxorubicin, all four patients with metastatic HCC maintained stable disease state for more than 1 year of treatment [26]. In a randomized, double-blind, phase II trial, sorafenib/doxorubicin

Table 1 Single-agent and combination therapy trials in HCC

Agent	Phase	ORR/PFS[a]/OS[a]	Comments	References
Sorafenib	III	NS/5.5/10.7	SHARP trial; time to radiologic progression	Llovet et al [15]
	III	3.3%/2.8/6.5	Asian-Pacific trial; time to progression	Cheng et al. [25]
Sorafenib + Doxorubicin	I	NS/–/–	Noted that drug combination is well tolerated	Richly et al [26]
	II	8%/4.2/9.2		Keating et al. [27]
Bevacizumab	II	13%/6.9/12.4	Serious bleeding occurred in about 11% of patients	Siegel et al [32]
Bevacizumab + Gemcitabine/Oxaliplatin	II	20%/5.3/9.6	GEMOX-B Trial	Zhu et al [33]
Bevacizumab + Erlotinib	II	25%/8.9/15.6		Thomas et al [34]
Sunitinib	II	NS/–/–	European-Asian trial; trial did not proceed to second phase due to low response rate	Faivre et al [35]
	II	2.9/3.9/9.8		Zhu et al [36]

ORR objective response rate, *PFS* progression free survival, *OS* overall survival, *NS* no statistical significance between groups

[a] In months (values converted from those reported in the literature)

combination failed to prolong median TTP; however, it prolonged median OS and progression-free survival (PFS) when compared to doxorubicin alone [27].

Sorafenib combined with transarterial chemoembolization (TACE) is currently under clinical investigation [28, 29]. It is theorized that one of the reasons for the high local recurrence rate after TACE is because of increased expression of vascular growth factors and HIF-1α [30, 31]. A rational solution to this problem is to administer anti-angiogenic agents concurrently or shortly after these procedures.

Bevacizumab

Bevacizumab is a recombinant monoclonal anti-VEGF antibody that has been approved by the FDA for the treatment of advanced breast, non-squamous non-small cell lung, and colorectal cancers when combined with chemotherapy. Bevacizumab is also approved for the treatment of renal cell cancer in combination with IFN-α.

Siegal et al. [32] tested the efficacy of single-agent bevacizumab in metastatic HCC in a phase II trial. Forty-six patients were enrolled in this study; 12 patients received 5 mg/kg of bevacizumab and 34 patients received 10 mg/kg of bevacizumab every two weeks until disease progression. Of the 46 eligible patients, 13% had an objective response and 65% were progression-free at 6 months. Overall survival at 1, 2, and 3 years was 58, 28, and 23%, respectively; and median PFS was 6.9 months. In addition, bevacizumab therapy resulted in substantially decreased tumor enhancement upon dynamic contrast-enhanced MRI and circulating VEGF concentrations. Therefore, single-agent bevacizumab has shown promise in patients with advanced HCC.

In the phase II GEMOX-B clinical trial, bevacizumab was combined with an active chemotherapy regimen (gemcitabine and oxaliplatin) in HCC [33]. In the first cycle (14 days), 10 mg/kg of bevacizumab was administered on day 1 followed by 10 mg/kg on days 1 and 15 in subsequent cycles (28 days). Gemcitabine (1000 mg/m^2) and oxaliplatin (85 mg/m^2) were administered on days 2 and 16. Thirty patients were assessed for efficacy in this study; 20% of the patients demonstrated an objective response, and 27% achieved stable disease (SD). Median OS was 9.6 months and median PFS was 5.3 months.

In another phase II trial, bevacizumab was also tested in combination with erlotinib [34]. Forty patients received 10 mg/kg of bevacizumab every 14 days and 150 mg of oral erlotinib daily for 28 day cycles; anti-tumor activity was analyzed every 2 cycles via RECIST criteria. Primary end-point for this study was 16-week PFS with secondary end-points of response rate (RR) and median PFS. The 16-week PFS was 62.5% and 10 patients had a partial response (PR); median PFS was 39 weeks and the median OS was 68 weeks. Overall, results from these phase II trials suggest that bevacizumab in combination with other agents may be additive, warranting randomized trials.

Sunitinib

Sunitinib is a multi-targeted TKI that inhibit VEGFR-1/2/3, PDGFR-α/β, c-kit, FLT3, and RET kinases. Sunitinib has received FDA approval for the treatment of renal cell carcinoma and gastrointestinal stromal tumors. There have been two phase II clinical trials focusing on the efficacy of sunitinib monotherapy in advanced HCC.

The European/Asian trial included 37 patients receiving 50 mg daily sunitinib on a 4 weeks on and 2 weeks off schedule (6 week/cycle; 4/2 schedule) [35]. The primary end-point and determinant of efficacy in this trial was overall RR via RECIST criteria with an expected RR of 15%. However, only one patient displayed PR; therefore, outcome was well below the desired primary end-point of 15% with an actual RR of 2.7%. Thirteen patients had SD over 3 months. In addition to less than expected response rates, there was severe, adverse events leading to the death of four patients from GI perforations, encephalopathy, and hepatorenal syndrome.

In another phase II study by Zhu et al. [36], 34 patients with unresectable HCC were treated with sunitinib 37.5 mg daily for 28 days followed by 14 days of rest in 6-week cycles (4/2 schedule). The primary end-point of this trial was PFS. Even though the 3-month PFS rate failed to meet the authors' predefined targeted end point of 59%, results were nonetheless encouraging showing an objective RR of 2.9%, median PFS of 3.9 months and median overall survival of 9.8 months. Adverse events were modest. Grade 3 and 4 toxicities occurred in less than 20% of the patients in any particular category and most commonly included myelosuppression, fatigue and transaminase abnormalities. Moreover, analysis of response of vessel integrity to single-agent sunitinib demonstrated a drastic reduction in vascular permeability; and measurement of angiogenic plasma proteins and inflammatory cytokines showed that these biomarkers are significantly altered by sunitinib.

Mechanism of resistance to antiangiogenic therapy

Although the inhibition of tumor blood vessel formation has shown clinical efficacy, treatment resistance inevitably develops through a variety of mechanism. First, resistance can occur at the tumor endothelial cell level; evidence has shown that tumor endothelial cells may harbor unstable genomes [37]. These aberrant genomes may lead to an increase in genetic alterations and the propagation of

mutations in subsequent endothelial cell lines. These altered cell lines may acquire resistance to molecularly targeted antiangiogenic therapy.

Another form of resistance may evolve from the redundancy of angiogenic signaling pathways; tumor dependence on a particular proangiogenic factor can be altered while being subjected to antiangiogenic agents [38]. For instance, treatment with anti-VEGF agents may result in vascular rearrangement and recruitment of excessive pericytes through the overexpression of PDGF-β. The vascular remodeling that results from pericytic over-coverage renders the neovasculature less responsive to VEGF for growth dependance effectively circumventing a blocked signaling pathway with a heavier dependence on an alternate mechanism.

Thirdly, antiangiogenic therapy may lead to the susceptibility of a tumor for metastatic spread. It has been shown that inhibition of both VEGFR and PDGFR results in an increased propensity for metastatic dissemination because the newly created hypoxic microenvironment selects for highly aggressive, invasive tumor cells [21]. Blagosklonny [39] elegantly hypothesized that antiangiogenic therapy may lead to increased intratumoral hypoxia and select for highly aggressive, metastatic tumor cells.

Tumors can also evade antiangiogenic therapy by acquiring the ability to grow without neovasculature. For instance, tumor cells may grow via a process known as "vascular cooption," which is characterized by neoplastic cells growth around pre-existing, normal tissue vasculature; these tumor cells acquire oxygen and nutrients from normal tissue blood vessels [40]. Antiangiogenic mechanisms inhibit sprouting angiogenesis which relies on the need for endothelial cell proliferation and migration. Tumor cells can evade these pruning effects via intussusception; a process characterized by the splitting of pre-existing vasculature into new blood vessels without a need for VEGF expression and endothelial cell proliferation [40].

A final obstacle for antiangiogenic therapy is vasculogenic mimicry, a process by which tumor cells form blood-conducting vessels without the need for endothelial cell proliferation [41].

Rationale for development of more effective antiangiogenic therapy in HCC

Traditional strategies to design combination therapy in clinical trials are to empirically combine agents with proven clinical efficacy. These strategies have been successful in deriving effective combination treatment. With the wealth of data available from molecular studies on tumor angiogenesis, we can now rationalize novel combinations which can be readily tested in preclinical models and then clinical trials.

Treatment with the currently available antiangiogenic agents including sunitinib, sorafenib, pazopanib, axitinib, or bevacizumab would disrupt endothelial tubes and/or pericytes through the inhibition of VEGF/VEGFR and PDGF/PDGFR signaling pathways, respectively. Pericytes are vascular smooth muscle cells that provide structural support and stabilization for sprouting endothelial tubes during vascular development. These support cells are responsible for sustaining tumor angiogenesis via paracrine production of VEGF. During tumor angiogenesis, cancer cells and endothelial cells synthesize and secrete PDGF-β, which acts in a paracrine fashion, stimulating pericytes to express VEGF. Due to their function in the propagation of tumor angiogenesis, pericytes serve as an important therapeutic target in the treatment of highly vascular cancers. Inhibiting both pericytes and endothelial cells would then lead to more significant antiangiogenic effect than inhibiting endothelial cells alone. As such, selection of clinically available agents with the most potent antiangiogenic effect would involve disrupting both the VEGF/VEGFR and PDGF/PDGFR pathways. This would include agents like sorafenib, sunitinib, pazopanib, and axitinib.

Despite maximal inhibition of VEGF/VEGFR and PDGF/PDGFR pathways, resistance reliably develops due to a process known as "reactive resistance" [39]. Effective antiangiogenic therapy works by creating a hypoxic condition devoid of nutrients for sustained cellular growth/survival. However, intratumoral hypoxia leads to the induction of compensatory pathways that mediate resistance at the levels of the tumor blood vessels, microenvironment, and tumor epithelial cells [17, 18]. In preclinical models, the hypoxia-inducible factors, HIF-1α and HIF-2α, have been shown to be major mediators of reactive resistance [21, 42] (Fig. 1). HIF-1α and HIF-2α induction would lead to the modulation of >200 genes important for inducing tumor angiogenesis, cancer cell growth/survival, and energy metabolism; mediating resistance [43, 44]. In addition to mediating reactive resistance, HIF-1α and HIF-2α also mediate primary resistance due to their induction through dysregulated growth signaling by inflammatory mediators, growth factors, and mutations in cancer genes [45–54]. Like many other solid tumors, overexpression of HIF-1α and HIF-2α has been shown to occur in HCC and may mediate resistance to therapy [24].

Thus, combining agents that inhibit HIF-1α and HIF-2α with effective antiangiogenic agents, such as sorafenib, sunitinib, pazopanib, or axitinib would counteract reactive resistance and other driving forces of carcinogenesis. In combination with antiangiogenic agents, inhibiting both HIF-1α and HIF-2α would further disrupt tumorigenesis through two potential mechanisms. The first mechanism of

synergy is by modulation of additional angiogenic pathways that complement those affected by the currently available antiangiogenic agents [18]. We recently reported that inhibition of HIF-1α and HIF-2α in cancer cells improved the antiangiogenic effect of sunitinib due to the inhibition of the proangiogenic factor, ANGPTL4, and induction of the antiangiogenic factor, TSP1 [42]. The second mechanism of synergy is through the direct effects of inhibiting HIF-1α and HIF-2α on tumor epithelial cell growth. HIF-1α and HIF-2α can be induced in cancer cells through dysregulated growth signaling by inflammatory mediators, growth factors, and mutations in cancer genes. In addition to tumor angiogenesis, HIF-1α and HIF-2α are responsible for a multitude of cellular processes that regulates growth/survival, invasion, and metastatic potential of tumor cells [55]. Altogether, inhibiting both HIF-1α and HIF-2α would effectively counteract the development of resistance to antiangiogenic therapy; since the effects of HIF inhibition would be seen in both endothelial cells and tumor cells.

To date, high-throughput small-compound screens and mechanistic studies have identified several classes of anticancer agents that disrupt HIF-1α function, including inhibition of its transcriptional activity and HIF-1α protein synthesis or stability [56–59]. Based on partial structural and functional similarities between HIF-1α and HIF-2α, it is possible that many of the already identified HIF-1α inhibitors would inhibit HIF-2α. In addition, systematic efforts are currently ongoing to identify compounds that are effective at inhibiting both HIF-1α and HIF-2α for

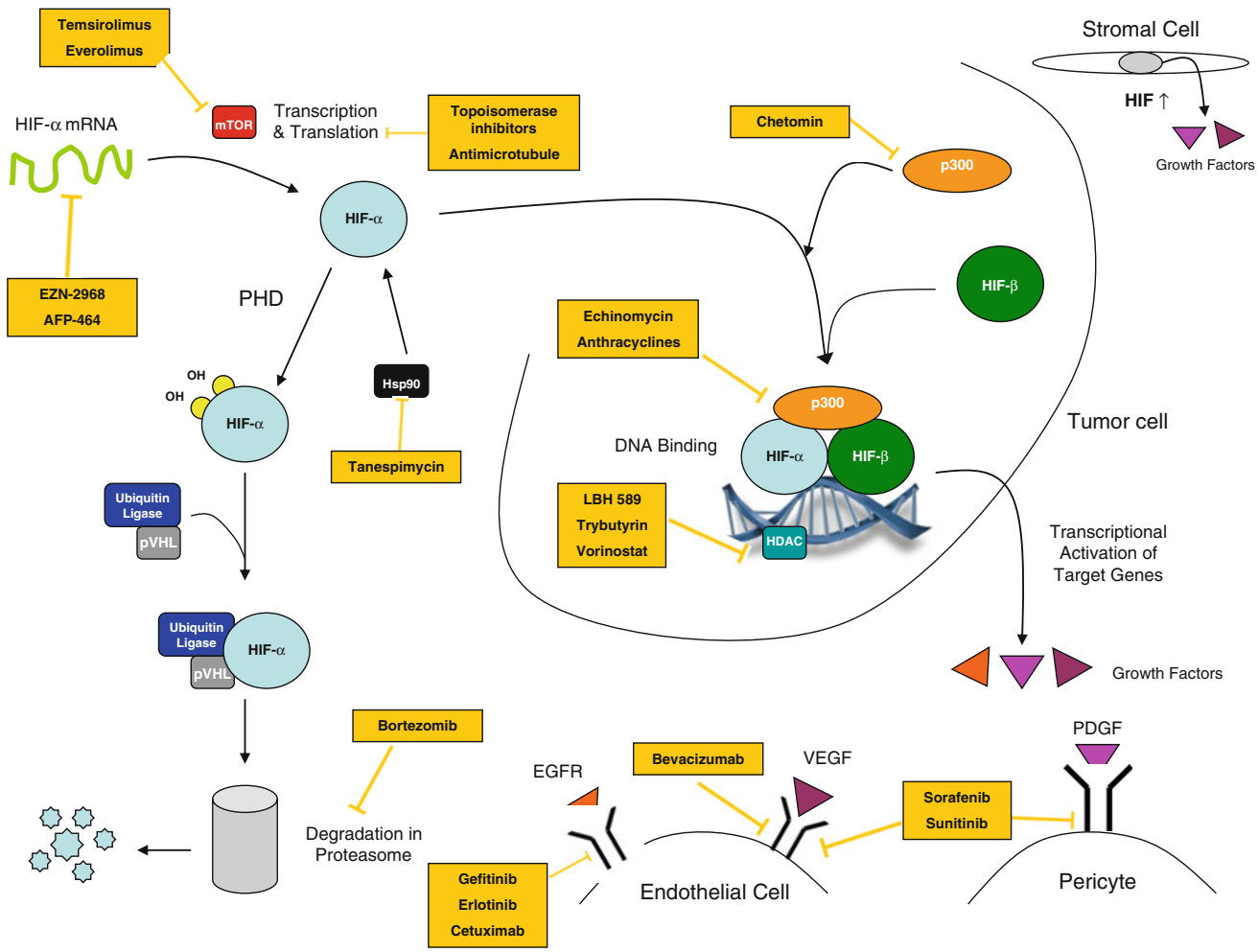

Fig. 1 Diagram depicting the tumor microenvironment and mechanism of known HIF inhibitors. HIF-α is overexpressed in cancer cells and stromal cells, promoting tumor angiogenesis by inducing the production of VEGF and other proangiogenic factors. HIF inhibitors work by disrupting HIF-α protein synthesis, stability, or transcriptional activity. *PHD* prolyl hydroxylase, *HIF-α* hypoxia-inducible factor-α, *HIF-β* hypoxia-inducible factor-β, *pVHL* von Hippel-Lindau protein, *HDAC* histone deacetylase, *HSP90* heat shock protein 90, *mTOR* mammalian target of rapamycin, *p300* transcriptional coactivator p300, *EGFR* epidermal growth factor receptor, *VEGF* vascular endothelial growth factor, *PDGF* platelet-derived growth factor

Table 2 HIF inhibitors currently in clinical trials

Agent	Target	Phase/status
AFP464	AhR	I, R
Carboxyamido-triazole	CCB in VEGF	I, II, ANR, C
SU5416	c-MET	I, ANR, C
Alvocidib (flavopiridol)	Cyclin-dependent kinase	II, C
Decitabine	Cytosine nucleoside/DNMT	I, ANR, C, #
XL-647	EGFR	I, C
Lonafarnib	Farnesyl-OH-transferase	II, C
Valproic acid	HDAC	I, C, R, #
MS-275	HDAC	I, C
Belinostat	HDAC	I, C, R, #
Tributyrin	HDAC	I, C
Trastuzumab	Her2/neu	I, C, #
Vandetanib	Her2/neu; EGFR	I, II, ANR, C, R
Romidepsin (FK228)	Histone deacetylase	II, C, #
Vorinostat (SAHA)	Histone deacetylase	I, R, #
Panobinostat (LBH589)	Histone deacetylase	I, R, #
Tanespimycin	HSP	I, II, ANR, C, EBI, NYR
Taxoprexin	Microtubule	II, C
Docetaxel	Microtubule	II, C, T, #
Ixabepilone	Microtubule	I, II, C, #
Patupilone	Microtubule	II, C
XL-765	mTOR	I, R
Temsirolimus	mTOR	I, II, NYR, R, #
Everolimus	mTOR	I, II, III, R, #
Pazopanib	Multitarget TKI	I, R, #
Imatinib	PDGFR; cKIT	I, II, ANR, C, T, #
Bortezomib	Proteasome	I, II, ANR, C, #
EZN-2968	RNA	I, R,
PX-12	Thioredoxin	I, II, C
Topotecan	Topoisomerase	I, II, ALL, #
Vatalanib	VEGF	I, II, ANR, C, R

Phase I–III status: *ANR* active not recruiting, *C* completed, *EBI* enrollment by invitation, *NYR* not yet recruited, *R* recruiting, *T* terminated, # FDA approved

cancer treatment [44, 60]. Many of these HIF inhibitors are currently in phase I and II clinical trials (Table 2 and Fig. 1).

Antiangiogenic therapy has revolutionized the treatment of HCC. Sorafenib has recently been approved for the treatment of advanced HCC; with other antiangiogenic agents, bevacizumab and sunitinib, showing promising activity. Future clinical development in HCC should include trials with HIF inhibitors, particularly in combination with antiangiogenic drugs. Appropriate doses and sequencing of these agents would need to be tested in preclinical models to help with clinical trial design.

Acknowledgments This study was funded by the National Comprehensive Cancer Network (NCCN) from general research support provided by Pfizer, Inc, NIH Grant K22CA111897, and NIH Grant R21CA115809.

Conflict of interest The authors declare no conflict of interests.

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