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Review

Molecularly targeted therapy in hepatocellular carcinoma

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ABSTRACT

With an annual incidence of over 660,000 deaths, hepatocellular carcinoma (HCC) is the third leading cause of cancer death globally. This disease is often diagnosed at an advanced stage, when potentially curative therapies are not feasible. HCC is highly resistant to conventional systemic therapies and prognosis for advanced HCC patients remains poor. Given the clear need, clinical development of novel therapeutic agents in HCC has begun in earnest. Our recent knowledge of the molecular mechanisms responsible of tumor initiation and progression has identified several potential molecular targets in HCC. These targets are the receptor tyrosine kinase-activated pathways, which include the Raf/MEK/ERK, PI-3K/Akt/mTOR, and Jak/Stat. Sorafenib is the multikinase inhibitor that has shown modest survival benefits in advanced HCC in two randomized controlled trials, supporting the use of molecularly targeted therapies in treatment of HCC. A number of strategies including monoclonal antibodies and tyrosine kinase inhibitors such as erlotinib, sunitinib, vandetanib, cediranib, brivanib, foretinib, and dovitinib have been developed and tested in various phases of clinical trials. The successful development of these novel targeted agents in the future will be dependent on the selection of patient populations that are most likely to derive clinical benefit, optimization of the dose used and schedules, and investigation of combined therapies. This review describes evolving molecular targeted agents, their common adverse side effects, and its potential use in management of HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) is a major health problem, accounting for more than 660,000 new cases per year worldwide [1–3]. Despite the available treatment options, the incidence still nearly equals to the mortality rate. More than 80% of patients with HCC have inoperable disease with very poor prognosis [4]. Therefore, potentially curative treatment like locoregional ablation, surgical resection or liver transplantation can be achieved only in a minority of HCC patients [5]. Because of the frequent presence of recurrence, metastasis or the

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development of new primaries [6,7], 5-year survival rate is limited to 25–50% after surgery [8]. Thus, a large number of patients are eligible for systemic therapy. Most of the HCCs are developed in cirrhotic liver [9,10] and the cirrhosis severely compromises liver function. It is often the condition of the remaining liver that dictates the final treatment options. Therefore, HCC patients not only require effective agents to treat the tumor, but there is also significant unmet need for drugs that stabilize and treat the underlying liver disease.

Over the years, chemotherapy remained the only systemic approach for the treatment of HCC. Metaanalysis evaluating 37 randomized clinical trials of systemic and regional chemotherapies, hormonal therapy and immunotherapy in HCC patients demonstrated almost complete lack of efficacy [5,11]. Novel chemotherapeutic agents analyzed in recent clinical trials were associated with response rates varying between 0 and 23% and median survival below 9.0 months [12]. A regimen of cisplatin, IFN-alpha2b, doxorubicin and 5-FU increased the response rate

Table 1
Molecularly targeted agents that are approved or in development for the treatment of HCC and various cancers.

Compound	Indication	Stage of development	Mechanism of action
Sorafenib (Nexavar™)	Marketed for RCC and HCC; Treatment of GIST and various cancers	FDA approved for the treatment of advanced RCC and HCC; Clinical development for a range of solid tumor types	A multikinase inhibitor that targets Raf kinases and the VEGFR 1-3, PDGFR-β, c-kit, Flt3 and p38
Sunitinib (SUTENT)	Marketed for RCC and GIST; Treatment of various cancers, including HCC	FDA approved for the treatment of advanced RCC and imatinib-resistant or intolerant GIST; Clinical development for a range of solid tumor types, including breast, lung, colorectal and HCC	A multikinase inhibitor that targets VEGFR 1-3, PDGFR-α and β, FMS-like tyrosine kinase, c-KIT, RET and colony-stimulating factor receptor type 1
Brivanib (BMS-582664)	Treatment of various cancers, including breast, lung, CRC and HCC	Evaluated in various phases of clinical trials including Phase III development for the treatment of HCC	Dual tyrosine kinase inhibitor that targets VEGFR-2 and FGFR-1
Cediranib (AZD2171, Recentin)	Treatment of breast cancer, glioblastoma, NSCLC, CRC	Evaluated in various phases of clinical trials as single agent or in combination with selected chemotherapies	A tyrosine kinase inhibitor that targets VEGFRs
Dovitinib lactate (TKI258; CHIR-258)	Treatment of breast cancer, urothelial carcinoma, and other solid tumors	Phase II development for breast cancer and advanced urothelial carcinoma	A multikinase inhibitor that targets FGFR3, VEGFRs, FGFR1 and PDGFR
Foretinib (GSK-1363089, XL-880)	Treatment of HCC, gastric cancer, and various cancers	Phase I/II development for gastric cancer and HCC	A dual kinase inhibitor that targets c-Met and VEGFRs
TSU-68 (SU 6668)	Treatment of various solid tumors including CRC and HCC	Phase II development for HCC	A multikinase inhibitor that targets VEGFR, PDGFR and FGFR
Erlotinib (Tarceva)	Treatment of EGFR positive tumors including NSCLC, pancreastic cancer and HCC	FDA approved for treatment of pancreastic cancer and NSCLC. Phase II development for advanced HCC	A tyrosine kinase inhibitor that targets EGFR
Lapatinib	Treatment of various solid tumors including breast and lung cancer.	FDA approved for treatment of advanced metastatic breast cancer in conjunction with the chemotherapy drug capecitabine; Phase II development for advanced HCC	A dual tyrosine kinase inhibitor that targets EGFR and HER2
AZD6244	Use in combination with standard doses of selected chemotherapies or targeted agents for treatment of various cancers	Phase II development for malignant melanoma, metastatic pancreatic cancer, CRC, and advanced metastatic NSCLC	A kinase inhibitor that targets MEK
Gefitinib	Treatment of NSCLC and EGFR/Her2-dependent tumors	FDA limits use of gefitinib for NSCLC	A tyrosine kinase inhibitor that targets EGFR
ABT-869	Treatment of HCC and various tumors	Phase II development for HCC and Phase I for various solid tumors	A multikinase inhibitor that targets VEGF and PDGF receptor families
Axitinib (AG013736)	Treatment of breast cancer, RCC, pancreatic cancer, and several other tumor types	Phase III development for advanced pancreastic cancer; Phase II development for RCC, melanoma, thyroid carcinoma, NSCLC	A multikinase inhibitor that targets VEGFR1-3, PDGFR, and c-Kit
Motesanib diphosphate (AMG 706)	Treatment of thyroid and other solid tumors	Phase I development for thyroid cancer	A multikinase inhibitor that targets VEGFRs, PDGFR, and c-Kit
Vandetanib	Treatment of gliomas, NSCLC, biliary tract cancer	Phase II development for brain tumors and biliary tract cancer	A multikinase inhibitor that targets VEGFR-2, VEGFR-3, and EGFR
Pazopanib (GW-786034)	Treatment of RCC, multiple myeloma, NSCLC, and other solid tumors	Phase III development for RCC; Phase II development for multiple myeloma and NSCLC	A multikinase inhibitor that targets VEGFR-1 to 3, PDGFRs, and c-kit
Vatalanib	Treatment of GIST, CRC, lymphoma, pancreastic tumors and other solid tumors	Phase III development for GIST; Phase III development for metastatic CRC	A multikinase inhibitor that targets VEGFR-1 and -2 PDGFR-β, c-Kit, FLT-4 and c-FMS
XL-184	Treatment of NSCLC, glioblastoma, lymphoma, thyroid carcinoma	Phase II development for glioblastoma multiforme; A phase IB/II trial for NSCLC; Phase III development for thyroid carcinoma	A multikinase inhibitor that targets c-Met, VEGFR-2, c-Kit, and Tie-2
BIBF 1120	Treatment of prostate cancer, ovarian cancer, NSCLC	Phase II development for prostate cancer; Phase III development for ovarian cancer, NSCLC	A multikinase inhibitor that targets VEGFR, PDGFR and FGFR
Regorafenib (BAY 73-4506)	Treatment of RCC, HCC and other solid tumors	Phase II development for RCC	A dual tyrosine kinase inhibitor that targets VEGFR-2 and Tie2

from 10.5 to 20.9% and median survival from 6.8 to 8.7 months compared with doxorubicin alone, but the differences were not statistically significant and the combined regimen was associated with greater toxicity [13]. Because the lack of survival benefits of treatment with conventional drugs [14], new agents and novel therapeutic strategies are urgently needed.

The highly vascular nature of HCC reflects profound activation of angiogenic signaling pathways, many of which are activated through receptor tyrosine kinases (RTKs), resulting in activation of various signaling pathways including Raf, mitogen-activated protein extracellular kinase (MEK) and extracellular signal-regulated kinase (ERK) (Ras/Raf/MEK/ERK), janus kinase (Jak)/signal transducers and activators of transcription (Stat) (Jak/Stat), and phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) (PI3K/AKT/mTOR). These provide rational targets for innovative HCC therapies. Many targeted agents such as monoclonal antibodies (bevacizumab and cetuximab), small molecule tyrosine kinase inhibitors (Table 1) and serine-threonine kinase inhibitors (temsirolimus and everolimus) have already been tested in various phases of clinical trials and some of them have recently entered phase III studies. In late 2007, sorafenib, a targeted antiangiogenic agent, was approved for the treatment of unresectable HCC. As the first anti-cancer drug to improve survival in patients advanced HCC, sorafenib has paved the way for novel therapies in the treatment of patients with HCC. Sorafenib is now the standard care among patients with advanced HCC or those who have progressed from earlier stages [15].

2. Targeting angiogenesis and RTKs for the treatment of HCC

Many growth factors, RTKs, and the downstream signaling pathways of RTKs are deregulated in HCC. These have added an important new dimension for treatment, and the opportunity to design HCC-specific targeted treatment approaches. Targets include growth factor receptors [the epidermal growth factor receptor (EGFR), the fibroblast growth factor receptor (FGFR), the hepatocyte growth factor receptor (c-Met), the platelet growth factor receptor (PDGFR), insulin-like growth factor receptor (IGFR), and the VEGF receptor], and signal transduction pathways responsible for the proliferation, invasion, metastasis, or survival of tumor cells such as the Raf/MEK/ERK, PI3K/Akt/mTOR, and Jak/Stat. There is a strong rationale for using different antibodies or small molecule inhibitors, which are currently in clinical use or under early clinical or preclinical evaluation, to block these pathways at different levels. The following section will provide an overview of molecular targets identified in HCC and targeted agents, which are currently used or in the development for the treatment of HCC.

2.1. Targeting VEGF/VEGFR/FGFR/PDGFR pathways

VEGF, PDGF and basic FGF (bFGF; FGF-2) are three important pro-angiogenic factors that play an important role in the neovascularization, invasiveness, and metastatic potential of HCC [16–18]. The VEGFR-1 and -2 are expressed on endothelial cells and provide activating and survival signals to these cells [19]. Activation of VEGFR family RTKs and, in particular, of VEGFR-2 by VEGF plays a primary role in tumor angiogenesis. Elevated VEGF is associated with postoperative recurrence and poor prognosis in HCC [20,21]. VEGF expression is detected in dysplastic nodules, and increases during hepatocarcinogenesis (reviewed in [22]). PDGF overexpression has been linked to the increased metastatic potential of HCC [23]. PDGF is also angiogenic for microvascular sprouting endothelial cells, and PDGFRs are required for recruiting pericytes and smooth muscle cells around nascent vessel sprouts [24]. PDGFR inhibition has been shown to cause pericyte detachment from the endothelium, leaving endothelial cells more susceptible to VEGF inhibition [25]. High expression of bFGF is detected in patients with HCC [16,26]. It functions as a mitogen for HCC cell proliferation via an autocrine mechanism, and enhances the development and progression of HCC by binding to FGFR-1 [27]. Basic FGF stimulates the release and activity of collagenases, proteases, and integrins on the extracellular membrane to form nascent microvascular networks [28]. The expression of heparanase in connection with bFGF enhances growth, invasion, and angiogenesis of the tumor [29]. Indeed, in a clinical study of patients undergoing resection of HCC, a high preoperative serum bFGF level appeared to be predictive of invasive tumor and early postoperative recurrence [18]. Furthermore, bFGF has been shown to synergistically augment VEGF-mediated HCC development and angiogenesis [30]. Clinical and translational studies showed that FGF blockade may circumvent resistance to VEGFR modulating agents (reviewed in [31]), suggesting that it may play a role in "escape mechanisms" implicated in VEGF/VEGFR targeting. Accordingly, novel antiangiogenic therapies targeting FGF to synergize with VEGF-mediated antiangiogenesis may provide a mechanism to overcome resistance to VEGF-targeted agents in HCC. Given that VEGF, FGF and PDGF expression is correlated with metastatic potential of tumor cells and the degree of microvessel density [32–34], inhibitors of VEGF, FGF and PDGF signalings are promising therapeutic agents for HCC treatment.

2.1.1. Monoclonal antibody targeting VEGF

Bevacizumab (Avastin) is a humanized monoclonal antibody that neutralizes all isoforms of VEGF. In HCC xenograft model, bevacizumab significantly decreased vessel density and prolonged time to progression of tumor-bearing mice [35]. In a phase II clinical trial 46 patients with locally advanced HCC, objective clinical responses to bevacizumab were observed in 13% and disease stabilization lasting at least 6 months in 65% of patients. Median PFS was 6.9 months [36]. Most drug-related adverse events were hypertension, hemorrhage, and arterial thromboembolism. In another phase II study among 38 evaluable patients, bevacizumab induced 16% PR and 47% SD [37]. Currently, bevacizumab-based approaches are being tested in clinical trials, especially in combination with either conventional cytostatics or with other targeted agents in order to block several carcinogenic pathways. Our recent study showed that bevacizumab plus rapamycin treatment inhibited HCC growth to a greater degree than monotherapies [38]. A phase II trial conducted in patients with advanced HCC evaluates the efficacy of a combination of erlotinib and bevacizumab [39]. Ten patients achieved a partial response for a confirmed overall response rate of 25%. The median PFS was 39 weeks and the median overall survival was 68 weeks.

Even though blocking the VEGF pathway has been shown to prevent tumor progression in the short-term, eventual progression of disease in the presence of VEGF/VEGFR therapies has been observed in clinical studies [40–42]. A multitude of mechanisms of escape has been proposed that may enable tumors to evade and resist antiangiogenic therapy. Intrinsic anti-VEGF resistance has been shown to be associated with infiltration of the tumor tissue by bone marrow-derived cells. Intrinsic resistance can occur as a result of tumor cells using existing blood vessels in vasculature-rich organs or as a result of the absence of VEGF or VEGFR in metastatic tumors in certain organ sites [43]. The most immediate mechanism of acquired resistance is the increased reliance on alternative pro-angiogenic factors that do not use the VEGF pathway. Acquired resistance to anti-VEGFR-2 antibodies was shown in one study to be caused by the redundancy of angiogenesis stimulators, demonstrated by upregulation of FGF in a pancreatic tumor after anti-VEGFR antibody treatment. In clinical studies, bevacizumab was shown to increase circulating

levels of placental growth factor (PLGF) [44], stromal derived growth factor 1α, CXCR4, CXCL6, and neuropilin 1 [45] while sunitinib induced high levels of VEGF and PLGF that reverted to normal levels during drug-free periods [46]. Basic FGF and PDGF have been shown to synergistically promote tumor angiogenesis in mouse models [47], suggesting that two or more growth factors could act synergistically. These effects may contribute to the rapid vascular regrowth that has been observed in tumors after removal of VEGF inhibition [48]. Rapid vascular remodeling of tumor-associated vessels is thought to be another cause of resistance. Therefore, improvement of our understanding of the mechanism for angiogenic resistance may provide insights into predictive markers that can be used to determine which patients are most likely to benefit from angiogenic therapy. They also help to develop new approaches to overcome resistance, which might include the use of broad-acting and more potent antiangiogenic compounds, or combination treatments with drugs that overcome redundancies in signaling pathways.

2.1.2. Multikinase inhibitors that target angiogenesis

Sorafenib is a multikinase inhibitor targeting the Raf serine/threonine kinases and the VEGFR1-3, PDGFR-β, c-Kit, Flt3 and p38 tyrosine kinases [49,50], which block the VEGF and PDGF-dependent angiogenesis [49]. Sorafenib has been shown to stop tumor cells growth, induce apoptosis of HCC cell lines, and inhibit the growth of HCC xenografts through inhibition of angiogenesis, induction of apoptosis, and suppression of phospho-eIF4G and Mcl-1, and inactivation of the mTOR pathway [51,52]. A review of four phase I studies of sorafenib has demonstrated that sorafenib was generally well tolerated. Maximum-tolerated dose of sorafenib was 400 mg twice daily. Most drug-related adverse events at any grade included fatigue, anorexia, diarrhea, rash/desquamation, and hand-foot skin reaction [53]. Sorafenib has successfully undergone phase III trials [54,55]. Survival rates at 1 year were 44% in the sorafenib group and 33% in the placebo group. This significant survival benefit represents a 31% relative reduction in the risk of death [54]. The positive data obtained in the phase III trial have been validated in an Asian trial [55]. Despite obvious clinical efficacy of sorafenib, many patients turn out to be refractory to this therapy. Therefore, the approach of combining sorafenib with "conventional" means or other targeted agents of tumor therapy is most likely to find its way into clinical application in near future. A randomized phase II study of doxorubicin and sorafenib versus doxorubicin alone in the front-line setting improved median survival from 6.5 to 13.7 months, although the study was not designed to detect differences between treatments [56]. Other targeted agents such as mTOR inhibitors (everolimus or rapamycin) or MEK inhibitors also have a role in combination with sorafenib [51,57]. A phase III randomized, placebo controlled, double-blind trial of sorafenib plus erlotinib versus sorafenib plus placebo as first-line systemic treatment for unresectable advanced or metastatic Child-Pugh A HCC is ongoing (NCT00901901).

Recently, a new small molecule inhibitor called regorafenib (BAY 73-4506) was developed. The antiangiogenic activity found with regorafenib treatment is due to its distinct dual targeted VEGFR-2/Tie2 inhibition [58]. Regorafenib was recently tested in phase II clinical trial for the treatment of patients with metastatic renal cell carcinoma (RCC). This multicenter, open-label, single-arm study of regorafenib enrolled 49 previously untreated patients with metastatic or unresectable, predominantly clear-cell RCC. Regorafenib (160 mg) was administered once daily on a 3 weeks on/1 week off schedule. Treatment with regorafenib resulted in a 31% partial response rate and 50% stabilization rate in patients with metastatic RCC [58]. The most common drug-related adverse events were hand-foot skin reaction, fatigue, hypertension, mucositis, diarrhea, alopecia, rash, voice changes, anorexia, nausea, constipation and vomiting. Clinical trials are needed to determine the relative efficacy of regorafenib in comparison with sorafenib in patients with HCC.

Sunitinib malate targets receptor tyrosine kinases of VEGFR1-3, PDGFR-α, PDGFR-β, FMS-like tyrosine kinase 3, c-KIT, RET and colony-stimulating factor receptor type 1, some of which have been implicated in tumor growth angiogenesis and metastasis [59–64]. Sunitinib has demonstrated antiangiogenic efficacy [65,66] and is approved internationally for the treatment of advanced RCC and imatinib-resistant or -intolerant GIST. In preclinical study, sunitinib also suppressed tumor growth of five HCC xenografts [67]. The safety of sunitinib has been evaluated in two open-label phase II trials conducted in USA, Europe and Asia, respectively [68,69] (reviewed in [70]). Dose-limiting toxicities reported at the maximum-tolerated dose >75 mg/day were grade 3 fatigue, grade 3 hypertension, and grade 2 skin toxicity. At the dose of 50 mg/day, the main adverse effects were bleeding, sore mouth, edema, and thrombocytopenia [71]. The antitumor efficacy of sunitinib was demonstrated by a progression-free survival of 3.9 months and a disease-control rate of 52% [68]. In European and Asian populations, sunitinib was associated with a disease-control rate of 37.8%. One patient experienced a confirmed partial response [69], suggesting some antitumor activity for sunitinib but the objective response seemed to be modest [72]. The results of the available phase II trials indicate a more intense antitumoral effect than sorafenib when assessing the appearance of tumor necrosis rather than the tumor size. A dose of 50 mg/day is associated with major side effects such as bleeding and death [73], and a lower dose seems to reduce this incidence but does not really eliminate it [68]. Despite the lack of a direct comparison of sunitinib versus sorafenib in the same patient population in randomized phase II studies, a multinational randomized phase III trial to evaluate the safety and efficacy of sunitinib compared with sorafenib in patients with advanced HCC is actively recruiting [74]. This study will provide important information on the relative efficacy and safety profile of sunitinib in comparison with sorafenib. If the results show that sunitinib is lesser efficacy than sorafenib in HCC then combining sunitinib with either other targeted agents or chemotherapy represent alternative strategies, but these combinations will need to be examined carefully for tolerability and safety in specific Phase I studies in HCC.

Brivanib is an oral, dual inhibitor of VEGFR-2 and FGFR-1 tyrosine kinases. Brivanib inhibits both VEGF- and bFGF-stimulated human umbilical vascular endothelial cell growth *in vitro*. In preclinical study, brivanib significantly suppressed tumor growth in six HCC xenograft lines when dosed orally on a once-a-day schedule [75]. Furthermore, the brivanib-induced growth inhibition was associated with increased apoptosis, inhibition of angiogenesis and cell proliferation, and down-regulation of cell cycle regulators. In the phase I study of brivanib alaninate conducted in patients with advanced or metastatic solid tumors, grade 3/4 adverse events included fatigue, elevated transaminase, diarrhea, hypertension and platelets were observed [76]. A phase I study of brivanib alaninate in patients with renal cell carcinoma and HCC [77] has shown evidence of antitumor activity with two confirmed partial responses in patients receiving brivanib alaninate at ≥600 mg and six patients with stable disease at ≥6 months. The majority of adverse events were nausea, vomiting and diarrhea, fatigue, hypertension and reversible transaminitis. The most frequently reported grade 3/4 toxicities were fatigue and reversible transaminitis [77]. A phase II, open-label study conducted to evaluate the effects of brivanib alaninate 800 mg as either first-line or second-line therapy in patients with unresectable locally advanced or metastatic HCC has recently been reported [78]. The study included two patient cohorts. Cohort

A included patients who had received no prior systemic therapy for HCC and cohort B included patients who progressed following receipt of one prior antiangiogenic agent. Overall, 55 patients were included in cohort A, of which 64% were Asian; 46 patients were included in cohort B, 43 (94%) of whom had failed sorafenib therapy and 3 (6%) of whom had failed therapy with thalidomide. Interim analyses from this study have shown that 6 of 47 evaluable patients in cohort A had a partial response (2 unconfirmed), with a disease-control rate of 60%. First-line treatment with brivanib alaninate was also associated with a median time to progression (TTP) of 2.8 months and a median overall survival of 10 months [78]. In cohort B, 1 of 37 evaluable patients had a partial response and 16 had stable disease following second-line treatment with brivanib alaninate; the disease-control rate was 46%. Second-line treatment with brivanib alaninate was also associated with a median investigator-assessed TTP of 2.7 months and a median overall survival of 9.8 months. The most frequently reported grade 3/4 adverse events across the two cohorts were fatigue, hypertension and diarrhea. Serious adverse events included grade 3 encephalopathy; grade 3/4 abdominal pain; grade 3 diarrhea; grade 3 vomiting, asthenia [78]. Based on these encouraging results, a phase III randomized study of brivanib versus sorafenib in the front-line setting is planned to explore the potential additional clinical efficacy of brivanib provided through inhibition of FGFR (NCT00858871). Another randomized, double-blind, multicenter phase III study of brivanib versus placebo as adjuvant therapy to transarterial chemoembolization (TACE) in patients with unresectable HCC is to assess the benefits of using brivanib systemic therapy in combination with localized TACE therapy compared with TACE plus placebo (NCT00908752).

Dovitinib lactate (TKI258; formerly CHIR-258) is the orally bioavailable lactate salt of a benzimidazole-quinolinone compound with potential antineoplastic activity. Dovitinib targets VEGFR1-3; PDGFR-β; FGFR1-3; FLT-3; and cKIT, Ret, TrkA, and csf-1 RTKs [79]. Dovitinib showed a dose- and exposure-dependent inhibition of RTKs expressed in tumor xenografts and stromal components in several preclinical models [80]. Dovitinib inhibited PDGFR-β and ERK phosphorylation within 2 h following dosing, and this was sustained for 24 h [79]. In leukemia xenograft models, it induced tumor regression and eradication of AML cells from the bone marrow and decreased cellular proliferation [80]. In human colon xenograft models, dovitinib inhibited VEGFR-1 and -2, FGFR-1 and -3 and PDGFR-β and had antitumor and antiangiogenic activity [79]. A phase dose finding study of TKI258 has shown that TKI258 is safe and well tolerated at dose below 500 mg/day. The most frequent drug-related adverse events were grade fatigue, diarrhea, and dehydration [81]. A phase II open-label study to delineate the safety and efficacy of dovitinib in FGFR-1 amplified and non-amplified metastatic HER2 negative breast cancer is ongoing (NCT00958971). Another phase II study of dovitinib in advanced urothelial carcinoma aims to evaluate the efficacy of dovitinib in patients with advanced urothelial cancer (NCT00790426). Current there are no clinical trials to evaluate the efficacy of dovitinib in HCC yet. Because FGF, PDGF and VEGF are required for survival, angiogenesis, cell migration, cell division, suppression of the VEGFR/FGFR/PDGFR by dovitinib may represent a novel approach for treatment of HCC. Preclinical studies are under way to study this possibility.

2.2. Targeting EGFR pathway

EGFR is expressed in a high proportion of HCCs and proliferation of HCC cells depends on stimulation of EGFR by TGF-α or EGF [82–84]. Therefore EGFR represents a molecular target for therapy of HCC. EGFR inhibitors, such as neutralizing monoclonal antibodies (cetuximab or panitumumab) or tyrosine kinase inhibitors gefitinib, erlotinib, and lapatinib have shown to inhibit HCC growth and metastasis formation *in vitro* and *in vivo* [85–89]. In a phase II study 30 patients with advanced or metastatic HCC received cetuximab, no clinical responses were seen and disease stabilization was observed in 17% of patients. Median PFS and OS were 1.4 and 9.6 months, respectively [90]. In another similar study, 27 advanced HCC patients were administered cetuximab. The disease stabilization rate was 44.4% (no objective responses) and median PFS – 1.8 months [91]. A phase II multicentre study of cetuximab in patients with EGFR-undetectable refractory metastatic CRC has shown that there were four cetuximab-related adverse events greater than grade 3: infusion related reaction, hypomagnesaemia, dyspnea and skin rash [92]. Currently, anti-EGFR-based approaches are being tested in clinical trials, especially in combination with either conventional cytostatics or with other targeted agents. The clinical efficacy of cetuximab combined with standard chemotherapy was analyzed in a multicenter phase II study, which included 43 treatment-naive advanced HCC patients. Patients were treated with cetuximab (standard regimen) and chemotherapy repeated every 2 weeks (gemcitabine + oxaliplatin). The objective response rate was 23% and disease stabilization was observed in 65% of patients [93]. Lapatinib was evaluated in a cohort of 40 patients with advanced HCC in a phase II study [94]. The most frequently reported lapatinib-related adverse events were diarrhea and rash [95]. The objective response rate was 5%. Median PFS and OS were 2.3 and 6.2 months, respectively. In a similar study in a group of 31 patients, gefitinib induced 3% of objective responses and 22.6% of stable disease. Median PFS and OS were 2.8 and 6.8 months, respectively [96]. A phase II clinical trial with erlotinib for advanced HCC showed good response in approximate one third of the patients and a prolonged survival with mild and tolerate side effects [97]. Dose-limiting toxicities consist of grade 3/4 skin toxicity or diarrhea [97]. Another phase II trial conducted in patients with advanced HCC evaluated the efficacy of a combination of erlotinib and bevacizumab [39]. Ten patients achieved a partial response for a confirmed overall response rate of 25%. The median PFS was 39 weeks and the median overall survival was 68 weeks. Based on the above encouraging results, a randomized phase II study of the combination of bevacizumab and erlotinib compared to sorafenib in the first-line treatment of patients with advanced HCC is conducted (NCT003653391 and NCT00881751).

2.3. Targeting the IGFR pathway

There is evidence that IGF-I and IGF-II and their receptor IGF-1R are involved in the development and progression of HCC. Aberrant activation of IGF signaling pathway resulting from upregulation and activation of IGF-1R were detected in 20% of HCC [98]. Additionally, IGF-II expression was increased in 12–44% of HCC samples [98]. In addition to the increased expression of IGF-II, a simultaneous reduction of IGF binding protein 3 and enhanced proteolytic cleavage of IGFBPs often occurred [99]. Both mechanisms lead to an excessive increase in the amount of bioactive IGF, which further enhances the mito-oncogenic effects of IGFR-signaling in HCC. Upon binding of IGF-I or IGF-II to IGF-1R, downstream cell signaling occurs, with resultant activation of several pathways also common to EGFR, including Ras/Raf/MEK/ERK, PI-3K/Akt/mTOR, and Jak/Stat. Thus, a promising approach of innovative HCC treatment may be blockade of the IGF/IGFR, but also the Raf/MEK/ERK, Jak/Stat, and mTOR signaling systems, which are functionally upregulated in HCC cells [100–102] and *in vivo* [103]. In a xenograft HCC model, monoclonal antibody targeting IGF-1R delayed tumor growth and improved survival of

treated animals [104]. In a phase I clinical study one patient with heavily pretreated HCC experienced stable disease exceeding 9 months following administration of IMC-A12 monoclonal antibody [105]. So far IGF-1R inhibition appears to be well tolerated in the preliminary clinical studies conducted [106–108]. A phase II study of IMC-A12 in patients with metastatic castration-resistant prostate cancer has shown that the most common adverse events at least possible related to IMC-A12 were fatigue, hyperglycemia, thrombocytopenia, muscle spasms, leukoencephalopathy [109]. Recently, BMS-754807, a potent and reversible inhibitor of the IGF-1R/insulin receptor (IR) family kinases, is developed. BMS-754807 was active *in vivo* in multiple xenograft tumor models and showed *in vitro* synergies when combined with cytotoxic, hormonal, and targeted agents [110]. BMS-754807 is currently in phase I development for the treatment of a variety of human cancers including HCC. Since crosstalk between the IGFR and other RTKs exist [111], IGF therapy has to be combined with other RTK inhibitors to enhance the antiproliferative effect. Support to this concept, it has been shown that concomitant inhibition of IGFR and EGFR enhanced the antineoplastic effect of respective monotherapies [88,112,113].

2.4. Targeting the c-Met pathway

Numerous experimental and clinical data indicate a particular role of HGF and c-Met in growth, survival and invasion by tumor cells. Like EGFR, HGF and c-Met have been implicated in HCC [114,115]. Point mutations in *MET* have been identified in HCC [116,117]. A study conducted on 18 resected HCC patients found a correlation between overexpression of c-Met mRNA and early stage of the disease but no association with survival [115]. Recent biomarker data from a phase III sorafenib study identified elevated HGF levels as a potential predictor of poor prognosis in HCC [118]. Although the clinical efficacy of targeting c-Met has yet to be determined, several studies with receptor- and ligand-specific antibodies and TKIs are ongoing or in development [119]. Foretinib (GSK1363089; XL-880) is an orally bioavailable small molecule with potential antineoplastic activity. This compound binds to and selectively inhibits c-Met and VEGFR-2, which may result in the inhibition of tumor angiogenesis, tumor cell proliferation and metastasis [120]. A phase I dose escalation study of foretinib has shown that the MTD of foretinib was determined to be 80 mg. Dose-limiting toxicity were hypertension and dehydration at 120 mg/day and diarrhea at 100 mg/day. Frequent adverse events associated with foretinib were hypertension, fatigue, nausea, diarrhea, proteinurea, and increased lactate dehydrogenase [121]. A phase I/II, open-label, multicenter study of foretinib in adult subjects with HCC is ongoing (NCT00920192). The purpose of this study is to assess the safety and tolerability of foretinib when used in the treatment of patients with advanced HCC.

Apart from the above-mentioned drugs, various small molecule inhibitors with various mechanisms of action have been developed for the treatment of HCC and various cancers (Table 1). They include cediranib (AZD2171, Recentin), TSU-68, ABT-869, axitinib (also known as AG013736), BIBF 1120, pazopanib (GW-786034), vandetanib, XL-184, and regorafenib. The future landscape for randomized phase II and III studies of these molecules will be based on head-to-head comparisons with sorafenib.

3. Targeting the intracellular kinases for the treatment of HCC

The biological effects of receptor tyrosine kinase activation are mediated by a complex cascade of intracellular signaling molecules that are potential targets for therapy, including the PI3K/Akt/mTOR, Raf/MEK/ERK, and Jak/Stat pathways.

3.1. Targeting the Raf/MEK/ERK pathway

The Raf/MEK/ERK pathway appears to be one of the most significant cellular signaling sequences in the development and maintenance of HCC [122]. This pathway transduces extracellular signals from ligand-bound RTKs, such as EGFR, IGFR, VEGFR, c-Met and PDGFR to the nucleus. In addition, HCV core protein can directly activate the Raf/MEK/ERK cascade [123]. Overexpression of Raf-1 was shown in 50% of HCC biopsies, while increased activation of Raf-1 protein was found in 100% of the evaluated HCC biopsies [124]. Loss of Raf kinase inhibitor protein was demonstrated to stimulate HCC proliferation and migration [125]. Upregulated activity of the MEK/ERK has been well documented in HCC cell lines, *in vivo* HCC models and in human HCC specimens [126–128]. Activated ERK regulates cell cycle progression, apoptosis resistance, extracellular matrix remodeling, cellular motility, angiogenesis, and drug resistance. Inhibition of MEK kinase was shown to prevent development of HCC and increase apoptosis in existing HCC tumors in mice [129]. In another study administration of MEK inhibitor resulted in dose-dependent growth inhibition of HCC xenografts [130]. Recent study showed that MEK inhibitor AZD6244 plus rapamycin treatment had antitumor and antiangiogenic effects in preclinical models of human HCC [131]. Sorafenib, a multi tyrosine kinase inhibitor, inhibits Raf-1 [49]. In preclinical study of HCC, sorafenib treatment resulted in suppression of tumor growth, reduction in cell proliferation and induction of apoptosis but upregulation of phospho-c-Raf Ser338 and phospho-ERK Thr202/Tyr204 [57]. Pharmacological inhibition of the MEK/ERK pathway by the MEK inhibitor AZD6244 enhanced the antitumor effect of sorafenib in both orthotopic and ectopic models of HCC [57]. Given the urgent need for effective therapies in HCC, clinical evaluating sorafenib/AZD6244 and AZD6244/RAPA combinations seem warranted. A phase I/II study of AZD6244 in combination with sorafenib in advanced HCC is planned (NCT01029418). Another phase I/II study of the combination of MEK inhibitor RDEA119 and sorafenib in patients with advanced cancer are ongoing (NCT00785226).

3.2. Targeting the PI3K/Akt/mTOR pathway

The PI3K/Akt/mTOR protein cascade is one of major signaling pathways associated with RTKs, which have been identified in cancer cells [132]. In HCC, activation of PI3K/Akt/mTOR pathway correlates with poor prognosis [133]. Patients with mutations in the catalytic domain of the *phosphoinositide-3 kinase (PIK3CA)* gene often have elevation of Akt expression and subsequently activation of mTOR [134]. mTOR regulates the phosphorylation of p70S6 serine-threonine kinase (S6K1) and the translational repressor protein PHAS-1/4E-BP, which in turn regulate the expression of VEGF and the translation of proliferation- and angiogenesis-relevant proteins such as c-myc, cyclin-D1, ornithine decarboxylase, and hypoxia-induced factor 1-α. Phosphorylation of mTOR and S6K1 were detected in human HCC [38,135]. In nontransformed cells the PI3K/AKT/mTOR pathway is controlled by PTEN, a tumor suppressor, which inhibits this pathway by reversing the PI3K reaction and blocking Akt activation. PTEN expression was reduced or absent in almost half of the studied HCCs, and hepatocyte-specific abrogation of PTEN expression in mice results in the development of HCC [136]. These indicate that targeting the PI3K/Akt/mTOR pathway may represent an approach for HCC therapy.

PI3K/AKT/mTOR pathway may be inhibited at various levels. PI3K inhibitors such as wortmannin, LY294002 and FTY720 have demonstrated some antitumor activity in preclinical HCC models [137,138]. However, the most promising target in this pathway is represented by mTOR. Rapamycin was shown to inhibit prolifera-

tion of HCC cell line *in vitro* and growth of HCC tumors in animal models [139]. The effectiveness of mTOR blockade with rapamycin analog everolimus (RAD001) has been tested in preclinical HCC models [140]. The preclinical data conducted so far showed that mTOR inhibitors, including rapamycin and RAD001, are promising agents for future HCC therapy. Recently, three analogs of rapamycin with superior pharmacokinetic and biological properties, CCI-779, AP23573, and WYE-125132 have been synthesized and tested in clinical trials for different malignancies [141,142]. A phase I clinical evaluating the efficacy of sirolimus in 21 advanced HCC patients was conducted [143]. One patient experienced partial response and 5 remained progression-free at 3 months. Median overall survival was 6.5 months. Another phase I/II trial evaluating everolimus for advanced HCC is currently starting to recruit patients [144] (NCT00390195). A phase I study of everolimus has shown that everolimus is well tolerated at the dose of 5 mg/m^2. Dose-limiting toxicities consist of diarrhea, mucositis, and elevation of ALT at 6.5 mg/m^2 [145]. The use of rapamycin analogs has also been demonstrated to enhance the antineoplastic activity of conventional cytotoxic drugs such as doxorubicin or vinblastine [139,146,147]. Our recent studies showed that rapamycin plus sorafenib [57], rapamycin plus MEK inhibitor AZD6244 [131], or rapamycin plus bevacizumab [38] treatments inhibited HCC growth to a greater degree than monotherapies. These data provide a strong rationale for clinical investigation of these combinations in patients with HCC.

3.3. Targeting the Jak/Stat pathway

The Jak/Stat pathway is activated by more than 40 cytokines and growth factors and is involved in multiple cell functions such as differentiation, proliferation, and apoptosis [148,149]. In this pathway, the cytokines induce phosphorylation of the Janus tyrosine kinases (Jak1, 2 and 3, Tyk2), followed by activation of Stat1-6 [148,149]. Both hepatitis B and C viruses are able to induce Ras and Jak/Stat pathways [150–152]. In HCC, phosphorylation of Jak1, Jak2, and Tyk2 tyrosine kinases was not detected in normal livers but increased significantly from surrounding non-neoplastic livers to HCCs [153]. Activation of Stat1, Stat3, and Stat5 was statistically higher in tumors than in respective surrounding livers, with pStat3 being higher in HCC with poor prognosis than HCC with better prognosis [153]. The levels of Jak/Stat targets, including Bcl-xl, Mcl-1, cyclin D1, and c-Myc were markedly increased in the majority of HCCs [153]. Furthermore, treatment of hepatoma cells with Jak/Stat inhibitor in combination with demethylating agents induces apoptosis [153]. These data demonstrate the importance of Jak/Stat pathway in HCC development and suggest the use of Jak/Stat inhibitors such as AZD1480 for the treatment of HCC. A phase I/II, open-label multicenter study to assess the safety, tolerability, pharmacokinetics and preliminary efficacy of the JAK2 inhibitor AZD1480 administered orally to patients with primary myelofibrosis and post-polycythaemia vera/essential thrombocythaemia myelofibrosis is planned (NCT00910728). It is interesting to determine if AZD1480 has any antitumor activity in HCC.

4. Discussion

Hepatocarcinogenesis is a complex process involving various, diverse signaling pathways. Clinical trials indicate that growth factors such as bFGF, IGFs, VEGF, EGF, HGF and PDGF and its receptors play important roles in HCC cancer etiology and progression, thus providing rational targets for innovative HCC therapy. Antiangiogenic strategies are likely to continue to be important in HCC treatment. Preclinical studies have identified four potential mechanisms of resistance to angiogenesis inhibitors: (1) activation and/or upregulation of alternative angiogenic signaling pathways by tumor cells; (2) recruitment of bone marrow-derived cells that up-regulate angiogenic pathways; (3) increased pericyte coverage leading to VEGF-independent tumor vasculature; and (4) activation of invasion and/or metastasis mechanisms to supply tumor cells via established vasculature rather than stimulation of new blood vessels. During progression of malignant disease, these mechanisms may play a role in resistance to antiangiogenic therapies. The tumor cells can also become resistant to TKIs, mostly due to new mutations in the tyrosine kinase, drug efflux mechanisms, receptor down-regulation, and loss of tyrosine kinase inhibitor pathways. Studies on determination of molecular pathways responsible for HCC development and progression indicate existence of crosstalk between various levels of different signal transducing cascades and redundant signaling pathways. Understanding these mechanisms is expected to help to identify novel mechanism of resistance to targeted agents and determine molecular targets in complementary pathways, which must be blocked simultaneously. Unfortunately, there is currently no single known inhibitor of all relevant tyrosine kinases in HCC. Therefore, a combination of different agents or a single "unspecific" inhibitor of several pathways may offer advantages over inhibition of a single pathway. The heat shock protein 90 (Hsp90) is an essential chaperone for function and integrity of a wide range of oncogenic client proteins including ErbB2, c-Raf, cdk-4/6, Stat-3, hTERT, IGF-1R, Src, Brc/Abl, Akt, HIF-1 alpha [154,155]. Hsp90 is overexpressed in HCC and its expression is associated with a poor prognosis [156]. Furthermore, Hsp90 inhibition improved the antiangiogenic effects of rapamycin by reducing the expression of PDGFR-β on vascular smooth muscle cells, and diminishing VEGFR-2 expression on endothelial cells [157]. Based on the above information, it appears that targeting Hsp90 may be an attractive strategy in HCC therapy. Several Hsp90 inhibitors such as IPI-504 [158,159], PU-H71 [160], and KOS-953 [161] are undergoing clinical development in patients with various tumors.

Sorafenib is the first medication that is now approved for the treatment of patients with advanced HCC. Although sorafenib has shown a modest survival benefit for patients with early disease, more novel drugs with better efficacy are needed for advanced and sorafenib-resistant HCC patients for whom no other treatment choices exist. Clinical benefits such as seen with brivanib in the first-line setting and after sorafenib highlight the potential to improve the clinical course of patients with advanced HCC. Most if not all TKIs (Table 1) lack substantial benefit when given as monotherapy but create a chronic disease state, which is no longer immediately life threatening. In general, side effects of these newly developed agents are minimal when compared to conventional chemotherapeutic agents. Preclinical evidence of synergistic antitumor achievable by combining targeted agents that block multiple signaling pathways has recently emerged [51,57,131,162–166]. Such combinations could lower the effective dose of each agent but retain comparable or enhanced activity; thus reducing the likelihood of drug toxicity and the selection pressure for drug resistant mutations. Combining multiple small molecule TKIs (erlotinib plus bevacizumab, bevacizumab plus rapamycin, sorafenib plus MEK inhibitor AZD6244), or combining a TKI with conventional chemotherapy (sorafenib plus doxorubicin) and/or radiotherapy are more likely to improve the clinical response in patients with advanced HCC with tolerable and manageable side effects. Our data support the concept that multitargeting HCC cells for advanced treatment efficacy and show that sorafenib plus mTOR or MEK inhibition appear to be a promising combination, warranting further elucidation in clinical trials [51,57]. Furthermore, the use of targeted therapies such as sorafenib might also improve HCC recurrence in patients who undergo resection, local ablation and transarterial chemoembolization, which is currently being tested in several studies. Since,

regrowth of HCC tumors following surgery or chemoembolization is associated with rapid angiogenesis, antiangiogenic strategies may prove effective in reducing the relapse rate. If sorafenib in combination with other agents proves to have survival benefits in advanced HCC, future trials should assess whether neoadjuvant treatment with the combination, administered before or after successful ablation or tumor resection or transarterial chemoembolization, may help further to increase survival time and time to tumor recurrence in patients with HCC.

5. Conclusion

Several promising multitargeted molecules are developed and currently under investigation for the treatment of HCC. Unfortunately, HCC patients' tumors are refractory to many targeted therapies. Most of tumor cells harbor multiple genetic defects, and inhibiting one tyrosine kinase may not be sufficient. Even though blocking the angiogenesis has been shown to prevent tumor progression in the short-term, eventual progression of disease in the presence of angiogenic therapies has been observed in clinical studies [40–42]. Therefore, resistance to treatment remains the major challenge to targeted cancer therapy. In case of tyrosine kinase inhibitors having tested or used for treatment of HCC and other malignancies, validated biomarkers are still missing. In the long-term, a useful tool for determining an appropriate treatment strategy for HCC may be the identification of appropriate biomarkers, which may help to establish the extent of disease and predict the effectiveness of treatment in a specific patient. In addition, development of therapeutic approaches based on molecularly targeted drugs must take into account that these agents need be low toxic and attention will have to be paid to the emergence and management of toxicity related to the usage of new agents. Because of the high prevalence of liver cirrhosis in HCC patients, to achieve long-term survival of the majority of patients, targeted anti-cancer therapies will need to be coupled with strategies aimed at reversing the progression of chronic liver disease.

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