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Liver cancer: Targeted future options

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Abstract

Hepatocellular carcinoma (HCC) has a poor prognosis and systemic chemotherapies have disappointing results. The increasing knowledge of the molecular biology of HCC has resulted in novel targets, with the vascular endothelial growth factor and epidermal growth factor receptor (EGFR)-related pathways being of special interest. New blood vessel formation (angiogenesis) is essential for the growth of solid tumors. Anti-angiogenic strategies have become an important therapeutic modality for solid tumors. Several agents targeting angiogenesis-related pathways have entered clinical trials or have been already approved for the treatment of solid tumors. These include monoclonal antibodies, receptor tyrosine kinase inhibitors and immunomodulatory drugs. HCC is a highly vascular tumor, and angiogenesis is believed to play an important role in its development and progression. This review summarizes recent advances in the basic understanding of the role of angiogenesis in HCC as well as clinical trials with novel therapeutic approaches targeting angiogenesis and EGFR-related pathways.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and is the seventh most frequent cause of cancer related death in Europe[1]. It is the fifth most common cancer in men and eighth most common cancer in women worldwide, resulting in at least 500 000 deaths per year[2]. HCC accounts for 90% of all liver cancers. Its crude incidence in the European Union is 8.29/100 000. Areas such as Asia and sub-Saharan Africa with high rates of infectious hepatitis have incidences as high as 120 cases per 100 000. HCC is four to eight times more common in men and usually associated with chronic liver injury such as hepatitis B HBV, hepatitis C HCV and alcoholic cirrhosis. Most HCCs arise from chronic liver disease and cirrhosis, caused mainly by viral infections, fatty liver disease or alcohol induced cirrhosis[3].

The management of HCC patients is multidisciplinary and treatment is influenced by the stage of the disease, by the liver function (underlying liver cirrhosis) and by the patient's performance status. Potential curative therapy options such as liver transplantation, liver resection and

local liver ablation are only considered for patients with early stage HCC and with preserved liver function[4]. Most HCC patients are at an intermediate or late disease stage and the therapeutic options are limited to transarterial chemoembolization (TACE) or systemic chemotherapy. However, many patients are not suitable for TACE and the efficacy of conventional systemic cytotoxic chemotherapy is modest with limited benefit. Although a few randomized trials have been conducted, no single cytotoxic regimen has emerged as superior to any other, and no drug or regimen has been shown to improve survival. Therefore new therapeutic options targeting specific pathways and new drugs are of urgently needed. New insights into the biology of hepatocarcinogenesis have been identified new therapeutic approaches like including antiangiogenesis or and inhibition of specific growth factors like such as the Epidermal growth factor receptor (EGFR) or the Insulin like growth factor receptor 1 (IGF1R)[5].

New blood vessel formation (angiogenesis) is fundamental to tumor growth and spread. In adults, physiological angiogenesis is limited to a small number of specific processes, such as wound healing, tissue repair and the female reproductive cycle[6]. Following the pioneering work of Judah Folkman it was recognized that angiogenesis plays an important role in tumor development, progression, and metastasis[7]. Tumors require nutrients and oxygen in order to grow, and new blood vessels, formed by the process of angiogenesis, provide these substrates. Tumor blood vessels are generated by various mechanisms, such as co-option of the existing vascular network, expansion of the host vascular network by budding of endothelial sprouts (sprouting angiogenesis), remodeling and expansion of vessels by the insertion of interstitial tissue columns into the lumen of pre-existing vessels (intussusceptive angiogenesis) and homing of endothelial cell precursors (EPC; CEP) from the bone marrow or peripheral blood to the endothelial lining of neovessels (vasculogenesis)[8]. Bone marrow derived progenitor cells contribute significantly to neovascularization in a variety of tumors[9-12].

The key mediator of angiogenesis is the vascular endothelial growth factor (VEGF). Therefore, VEGF and its receptors are interesting targets for anticancer therapies. VEGF signaling inhibition has been shown to result in significant tumor growth delay in a wide range of animal models[13]. Even a single VEGF allele knock-out has been shown to lead to embryonic lethality in mice[14]. The clinical benefit of this approach has also been confirmed and concentrated efforts in recent years have resulted in a number of novel anti-angiogenic agents. The humanized monoclonal anti-VEGF antibody bevacizumab is the first VEGF-targeting drug, which is officially approved as first-line therapy in patients with metastatic colorectal cancer[15].

Tight control of angiogenesis is maintained by a balance of endogenous anti-angiogenic and proangiogenic factors. VEGF has a key, rate-limiting role in promoting tumor angiogenesis and exerts its effects by binding to one of three tyrosine kinase receptors: VEGFR-1, VEGFR-2 and VEGFR-3. VEGFR-1 [ligands include VEGF-A, VEGF-B and placental growth factor (PIGF)] and VEGFR-2 (ligands include VEGF-A, VEGF-C and VEGF-D) are predominantly expressed on vascular endothelial cells, and activation of VEGFR-2 appears to be both, necessary and sufficient, to mediate VEGF-dependent angiogenesis and induction of vascular permeability[16]. VEGF-A binds to VEGFR-1 and VEGFR-2, whereas VEGF-B and PlGF only bind to VEGFR-1. Both receptor tyrosine kinases are expressed in all adult endothelial cells except for endothelial cells in the brain. VEGFR-1 is also expressed on hematopoietic stem cells (HSC), vascular smooth muscle cells, monocytes, and leukemic cells[17]. VEGFR-2 is also expressed on endothelial progenitor cells and megakaryocytes[18,19]. Although the exact contribution of VEGFR-1 signaling to angiogenesis is unclear, it has been shown to co-operate directly with VEGFR-2 *via* heterodimerization, as well as to bind two additional VEGF homologues, VEGF-B and PIGF[20]. VEGFR-3, which is largely restricted to lymphatic endothelial cells, binds the VEGF homologues VEGF-C and VEGF-D and may play an important role in the regulation of lymphangiogenesis.

HCC is one of the most vascular solid tumors and is characterized by an abnormal vascular structure. Therefore, proangiogenic factors such as VEGF and plateletderived growth factor (PDGF) are of major importance and are upregulated in hepatocarcinogenesis. Sorafenib, a multikinase inhibitor blocking VEGFR signaling was the first targeted agent showing an overall survival benefit in HCC patients[21] and provided a breakthrough in modern HCC therapy.

New insides into the pathological and molecular mechanisms of HCC have led to the development of numerous targeting agents. This review summarizes a selection of these new drugs.

ANTI-ANGIOGENIC THERAPIES

As already mentioned above, HCC is a highly vascular tumor and so antiangiogenic therapies are of major interest[22-23]. In a variety of solid tumors, anti-angiogenic therapies like bevacizumab or sunitinib have already proven clinical efficacy. Most of these compounds can be broadly classified into two main categories: small-molecule kinase inhibitors and monoclonal antibodies.

Sorafenib

Sorafenib is an oral tyrosine kinase inhibitor (RTKI) blocking several receptors including VEGFR1-3, PDGFR β, c-KIT and FLT-3. Having shown anti HCC activity in several preclinical models Sorafenib[24-26], progressed to clinical studies. The results of four phase I studies were summarized in a review describing the tolerability and the pharmacokinetics of sorafenib in pre-treated patients[27]. Sorafenib was well tolerated and the maximal tolerated dose (MTD) was 400 mg twice daily. The most common adverse events were fatigue, diarrhea, rash and hand-foot

skin reaction. Based on these results, phase Ⅱ and Ⅲ studies with 400 mg twice a day were started. The phase Ⅱ trials confirmed the antitumor efficacy and tolerability of the drug[28].

In two large randomized, multicenter, controlled clinical phase Ⅲ trials sorafenib given as first systemic agent showed an overall survival benefit for patients with unresectable HCC[21]. In the sorafenib HCC assessment randomized protocol (SHARP) patients not eligible for locoregional therapy were randomly assigned to sorafenib 400 mg twice daily or placebo. The study included 602 primarily European patients (sorafenib $n = 299$, placebo $n = 303$) and inclusion criteria were ECOG performance status ≤ 2, Child Pugh liver function class A as well as no prior systemic therapy. The results of the study showed a significant prolongation of the time to progression (TTP) from 2.8 to 5.5 mo [HR = 0.58, 95% confidence interval (CI) = 0.45 - 0.74, $P < 0.0001$] and an improvement of survival from 7.9 to 10.7 mo (HR = 0.69, 95% CI = 0.55 - 0.87, $P < 0.0001$) in the sorafenib treatment arm.

A similar study was performed in mainly Asian patients where 271 patients were allocated to sorafenib or placebo[29], randomized in a 2:1 ratio. The outcome of this study showed a median overall survival of 6.5 mo with sorafenib treatment compared to 4.2 mo in the control group. The discordance in the OS benefit between the SHARP and Asian could be related to a divergence in selection of patients. In the Asian population unfavorable prognostic factors including the rate of Hepatitis B virus infections, the stage of disease (Asian population showed more level C Barcelona clinic liver criteria), age (Asians were younger) and performance status (Asian included more ECOG 2) were more often observed. The adverse event profile of the two large phase Ⅲ trails was similar, with hand-foot syndrome (8% Europe, 11% Asian), fatigue (8%-10%), and diarrhea (9%) the most common.

Sunitinib
Sunitinib is an oral RTKI, targeting the VEGFR1-3, the platelet derived growth factor receptor PDGFR α and β and the stem cell factor receptor (KIT). In several preclinical studies sunitinib showed anti HCC activity[30]. To date, Sunitinib is approved for the treatment of advanced renal cell carcinoma (RCC) and gastrointestinal stroma tumors (GIST) after disease progression or intolerance to imatinib mesylate[31]. In RCC and GIST sunitinib is administered at a dose of 50 mg/d for 4 wk followed by 2 wk of no treatment. The optimal treatment dose assessed in preclinical studies was used in phase Ⅰ studies and was well tolerated. Described adverse events of fatigue, hypertension and skin toxicity with sunitinib are typical for VEGFR tyrosine kinase inhibitors[32].

Suntinib was evaluated in HCC in two phase Ⅱ trials using different doses of the drug. Zhu *et al* analysed 34 patients and reported a 2.9% response rate, a median progression free survival (PFS) of 3.9 mo, and a median overall survival (OS) of 9.8 mo. The administered dose of sunitinib was 37.5 mg daily for 4 wk (d1-d28) at time intervals of 6 wk[33]. In another study by Faivre *et al*[34]

where 37 patients were included, 50 mg sunitinib was given daily for 4 wk followed by two weeks off-treatment in 6 wk cycles. The authors reported similar results with a response rate of 2.7%, PFS of 5.2 mo and an OS of 11.2 mo. A higher dose of suntinib used in the study by Zhu revealed a high toxicity rate and 10% of deaths were treatment related. Therefore the authors concluded that 50 mg/d sunitinib is not appropriate and that the dose should be reduced to 37.5 mg without the 2 wk wash-out phase[32].

A direct comparison of sunitinib and sorafenib in a randomized phase Ⅲ study in advanced HCC was discontinued in April 2010 after the first review by an independent data monitoring committee. The study was terminated based on higher incidence rates of serious adverse events in the sunitinib treatment arm compared to the sorafenib arm and because the preliminary data did not meet the primary study endpoints (sunitinib did not improve survival compared to sorafenib) (Pfizer press release April 22, 2010).

Cediranib
Cediranib (AZD2171, Recentin®) is a potent inhibitor of both VEGFR-1 and VEGFR-2. It also has activity against c-kit, PDGFR-β, and FLT4 at nanomolar concentrations[35]. Cediranib has been shown to inhibit VEGF signaling. In our study, cediranib was well tolerated up to 45 mg/d in patients with a broad range of solid tumors[36]. The most common toxicities include diarrhea, dysphonia, and hypertension. In a phase Ⅱ study with cediranib in 28 patients with advanced HCC, 19 patients were evaluable for toxicity[37]. The main adverse events were fatigue, hypertension and anorexia.

Vatalanib
Vatalanib (formerly PTK787/ZK 222584) is an oral angiogenesis inhibitor that is active against VEGFR and PDGFR tyrosine kinases, thereby offering a novel approach to inhibiting tumor growth[38]. This drug interferes with the ATP binding sites of VEGF receptors. In a phase Ⅰ study by us, vatalanib was well tolerated and showed clinical activity in a variety of solid tumors[39]. Preclinical studies suggested anti-angiogenic and angiogenesis-independent effects on HCC growth arrest[40]. In a phase Ⅰ study of vatalanib in 18 patients with unresectable HCC, nine patients had a best response of stable disease (SD), and nine patients had progressive disease (PD)[41].

Bevacizumab
Bevacizumab (Avastin®) is a humanized monoclonal antibody IgG1. It was created from a murine anti-human VEGF monoclonal antibody that blocks the binding of human VEGF to its receptors, thereby disrupting autocrine and paracrine survival mechanisms mediated by VEGFR-1 and VEGFR-2[42]. Bevacizumab is the first VEGF targeting drug, which is officially approved for cancer therapy. Initially, Bevacizumab demonstrated survival benefits in patients with metastatic colon cancer when combined with conventional chemotherapy[15].

Since then, it has been tested in several other cancer types. In patients with HCC, bevacizumab was examined as monotherapy or in combination therapies. In a phase Ⅱ study, monotherapy with bevacizumab was examined in 46 patients with advanced HCC[43]. Six patients had objective responses (13%) and 65% were progressive after 6 mo. Median progression-free survival was 6.9 mo and overall survival rate was 53% at 1 year. The main adverse events were hypertension, thrombosis. Bevacizumab was associated with significant reductions in tumor enhancement by dynamic contrast-enhanced magnetic resonance imaging and reductions in circulating VEGF-A and stromal-derived factor-1 levels. In a phase Ⅱ study, bevacizumab was studied in combination with gemcitabine and oxaliplatin in patients with advanced HCC[44]. The overall response rate was 20% in evaluable patients. An additional 27% of patients had SD with a median duration of 9 mo. The median overall survival was 9.6 mo and the median progression-free survival was 5.3 mo. Main bevacizumab-related side effects were hypertension, bleeding, and proteinuria.

TARGETING THE EPIDERMAL GROWTH FACTOR RECEPTOR PATHWAY

The epidermal growth factor receptor is upregulated in HCC and plays an important role in tumor progression[45,46]. The ligands of the EGFR EGF and TGF-α have been identified as key stimuli for HCC cell proliferation. The inhibition of EGFR signalling can either be by extracellular neutralizing antibodies such as cetuximab and panitumumab or by receptor tyrosine kinase inhibitors such as gefitinib, erlotinib and lapatinib. To date these targeted agents are being assessed in clinical trials.

Gefitinib

Gefitinib is an oral EGFR tyrosine kinase inhibitor approved for the treatment of non small cell lung cancer patients having an activating mutation in the EGFR gene[47]. The beneficial effect of Gefitinib is well analysed in many solid tumors including lung cancer, colorectal cancer, breast cancer. However, only a few studies have evaluated the effect of Gefitinib in HCC. The only reported study is a phase Ⅱ study with 31 advanced HCC patients presented at the 2006 ASCO conference in which Gefitinib induced 3% of objective responses and 22.6% of stable disease. Median PFS and OS were 2.8 and 6.8 mo, respectively[48]. The final outcome of this study is not yet published in MedLine. At the 2010 ASCO conference, a pilot study, analyzing the feasibility of gefitinib in adjuvant treatment of HCC patients was presented[49]. The study protocol includes a large biomarker program to identify prognostic as well as predictive markers and first results are awaited.

Erlotinib

Erlotinib is another oral EGFR RTKI that has showed clinical efficacy in the therapy of HCC. In two phase Ⅱ studies reported by Philip *et al*[50] and by Thomas *et al*[51] erlotinib showed antitumor activity and a PFS of 3.2/3.1 mo respectively and an OS 13/10.75 mo respectively. These studies included 38/40 patients respectively, with advanced nonresectable HCC. The side effects reported in these studies of erlotinib were rash, diarrhea or other skin events (acne, dry skin, pruritus).

In a recently published phase Ⅱ study in 40 HCC patients, a combination therapy of erlotinib with bevacizumab was assessed[52]: The rationale of this combination is based on preclinical models where a dual inhibition of VEGFR and EGFR showed additive effects. The combination therapy showed antitumor activity and the 16 wk PFS was 62.5% (primary end point), with 10 patients achieving a partial response, giving a confirmed overall response rate of 25%. The median PFS was 39 wk (95% CI, 26 to 45 wk; 9.0 mo), and the median overall survival was 68 wk (95% CI, 48 to 78 wk; 15.65 mo). Compared these results with the sorafenib studies (phase Ⅱ and SHARP trials) the authors identified more favourable results from combination therapy compared to monotherapy.

These retrospective comparisons are of minor scientific relevance and should be tested in prospective studies. Several new studies comparing combination therapies of EGFR and VEGFR TKIs with the standard VEGFR TKI sorafenib (bevacizumab + erlotinib versus sorafenib; erlotinib and sorafenib versus sorafenib) will identify the most effective treatment with the best tolerability.

Further anti-EGFR-based approaches include cetuximab, a chimeric monoclonal antibody against EGFR, and lapatinib, a selective dual inhibitor of both EGFR and ErbB2 tyrosine kinases. Both agents are currently being evaluated in clinical trials for patients with HCC.

EVALUATION OF BIOMARKERS

A major focus of research on targeted therapies should be the definition of predictive biomarkers which will allow identification of potential responders. To date there is no direct evidence on which HCC patients respond to targeted therapies. Various possible biomarkers have been postulated, but adequate valuation in prospective studies is still lacking. Possible candidates that could be considered are clinical parameters like blood pressure increase, various proteins assessed by biochemical methods [e.g. phosphorylated extracellular signal regulated kinase (pERK)] or levels of circulating endothelial cells and progenitor cells (CEC, CEPs). In addition, angiogenic factors and new imaging strategies as DCE-MRI[53] are currently being evaluated as possible biomarkers.

About Alfa *et al*[28] showed that standard RECIST criteria for the evaluation of response to sorafenib therapy in HCC patients are not ideal, because sorafenib-treated tumors do not decrease in size, although the necrotic index increases. In the same study, better responses to sorafenib were observed in patients with high mitogen

activated protein kinase (MAPK) activity and resulted in a prolonged time to progression. An increased activity of MAPK was defined by elevated tumor cell pERK immunohistochemical staining intensity (2 - 4 +) compared to normal (0 - 1 +) at baseline assessment before treatment. Greater activation of the Ras signalling pathway could be due to loss of sprouty and spreads[54,55]. Sprouty and spreads downregulation may reduce the threshold for cells to acquire malignant features. Lung cancer patients with upregulated Ras activity (battle trial) also show good response rates to sirafenib.

Hypertension is a common side effect of antiangiogenic therapies. In renal cell cancer, studies identified a correlation between blood pressure elevation and better overall survival benefit on axitinib therapy[56]. Kim *et al*[57] assessing the predictive effect of hypertension in patients taking sorafenib for advanced HCC, identified elevated blood pressure levels as a positive predictive marker for sorafenib therapy.

A recently published study proposed early alpha- fetoprotein (AFP) as a predictive marker for therapeutic effects in HCC patients treated with antiangiogenic therapies[58].

One of the largest biomarker programs integrated in a phase Ⅱ trial by Zhu *et al*[33] included the assessment of angiogenic serum markers, CEPs and CECs, DCE- MRI and immunohistochemical analyses of tumor samples. In HCC patients, the authors found that rapid changes in vascular permeability and in circulating inflammatory biomarkers reflected response or resistance to sunitinib therapy. Thus, these candidate biomarkers should continue to be actively explored in trials of antiangiogenic agents in patients, with the goal of improving and individualizing cancer therapy[53].

CONCLUSION

Molecular targets are of relevance in the treatment of HCC. Angiogenesis is upregulated in HCC and provides a target for novel agents. Therefore, VEGF and its receptors comprise the most important pathway in regulating neoangiogenesis, vasculogenesis and recruitment of endothelial progenitor cells. Further, VEGF stimulates proliferation, migration and survival of HCC cells directly and is of prognostic relevance. Further targets such as EGFR provide the possibility of combination therapies in order to enhance treatment efficacy. A number of clinical trials are underway to examine these novel agents in the hope of improving treatment modalities in advanced HCC.

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