REVIEW

Targeting vessels to treat hepatocellular carcinoma

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ABSTRACT

The process of blood vessel proliferation, known as angiogenesis, is essential during embryonic development and organogenesis. In adult life, it participates in normal tissue repair, wound healing, and cyclical growth of the corpus luteum and the endometrium. Crucial as it is, angiogenesis can become pathological, and abnormal angiogenesis contributes to the pathogenesis of inflammatory and neoplastic diseases. The present review highlights the evidence for the role of angiogenesis in HCC (hepatocellular carcinoma) and discusses the increasing importance of inhibitors of angiogenesis in HCC therapy.

NORMAL ANGIOGENESIS AND THE CONTROL OF NEW VESSEL FORMATION

Blood vessels arise from endothelial precursors, which share a common origin with haemopoietic progenitors. These progenitors assemble into an early vascular labyrinth of small capillaries. At this stage, these cells already exhibit arterial or venous features, indicating that vascular cell specification is determined not only by haemodynamic factors, but also by genetic factors [1]. During angiogenesis, a vascular plexus expands and remodels into a vascular network of larger vessels branching into smaller ones [2]. This occurs essentially through two different processes either by sprouting, a linear expansion of the blind tip of capillaries, or by intussusception, the splitting of existing capillaries [3]. After forming vessels, endothelial cells are covered by pericytes and smooth muscle cells, which provide wall integrity and allow regulation of vessel perfusion [4,5].

The maturation of new vessel networks is regulated by physical forces as well as by molecular influences. In solid tumours, the structure and organization of the vessels are abnormal, resulting in uneven perfusion with hypoperfused zones. Tumoral vessels are more porous than normal vessels and, in tumours, the interstitial pressure is typically increased, a phenomenon which impairs the delivery of drugs [6].

Numerous factors and environmental states, such as hypoxia, injury and glycaemic state, regulate microvascular tissue density. Some of the principal activators of angiogenesis are VEGF (vascular endothelial growth factor), bFGF [basic FGF (fibroblast growth factor)] and TGFs (transforming growth factors), whereas inhibitors of angiogenesis include, among many others, angiotatin, endostatin and thrombospondin-1 [7,8].

In order to grow, tumours require new vessels, and the point at which they acquire the ability to promote the formation of their own vasculature, known

Key words: angiogenesis, growth factor, hepatocellular carcinoma (HCC), inflammation, liver, vascular endothelial growth factor (VEGF).

Abbreviations: Ang, angiopoietin; (b)FGF, (basic) fibroblast growth factor; COX-2, cyclo-oxygenase-2; ERK, extracellular-signal-regulated kinase; FKBPs, FK506-binding protein; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HGF, hepatocyte growth factor; HIF-1, hypoxia-inducible factor-1; IL, interleukin; iκB, inhibitory κB; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κB; NK4, natural killer cell transcript 4; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PG, prostaglandin; PI3K, phosphoinositide 3-kinase; STAT, signal transducer and activator of transcription; TACE, transarterial chemoemobolization; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

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as the angiogenic switch, is the critical oncogenic step [9,10].

**VEGFs AND THEIR RECEPTORS**

The VEGF family comprises seven secreted glycoproteins that are designated VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, PIGF (placental growth factor) and VEGF-F [11–13]. VEGF-A is the best characterized and most studied of the family members. Its gene is localized on the short arm of chromosome 6, and mRNA splicing and proteolytic processing yields different isoforms, the predominant ones are VEGF165, VEGF189, VEGF211 and VEGF225 [14]. VEGF-A binds and activates two tyrosine kinase receptors: VEGFR1 (VEGF receptor 1; also known as Flt-1) and VEGFR2 (VEGF receptor 2; also known as KDR/Flk-1). VEGFR1 plays a dual role in angiogenesis, as an inhibitor in the embryo and as a stimulator in adulthood, when it promotes tumour growth, metastasis and inflammation. VEGFR2 is a signal transducer for pathological angiogenesis, including cancer and diabetic retinopathy [15].

Several independent pathways have been described to regulate VEGF-A gene expression. Among these, hypoxia through HIF-1 (hypoxia-inducible factor-1) [16] plays a major role and, additionally, numerous cytokines and growth factors, including endothelial growth factor, TGF-α, TGF-β, HGF (hepatocyte growth factor), IGF-1 (insulin-like growth factor-1), FGF, PDGF (platelet-derived growth factor), IL (interleukin)-1 and IL-6 can regulate VEGF-A expression in different cell types [17]. It was described a few years ago that LXR (liver X receptor) could also regulate VEGF expression, specially under inflammatory conditions [18].

Upon ligand binding, tyrosine residues of VEGFRs are phosphorylated, resulting in the activation of a number of downstream signalling molecules. VEGFR phosphorylation activates the MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase]/ERK cascade via Ras/Raf stimulation, PKC (protein kinase C) and calcium signals, via activation of PLCγ (phospholipase Cγ), and Akt, via stimulation of PI3K (phosphoinositide 3-kinase). Another kinase positively controlled by Akt is mTOR (mammalian target of rapamycin), which regulates the synthesis of several proteins, such as HIF. Coordinated activation of these pathways results in cell proliferation, cell survival and angiogenesis (Figure 1) [11]. The effects of VEGF on tumour growth include stimulation of neovascularization, increased vessel permeability, increased intra-tumoral pressure and inhibition of the maturation of dendritic cells from haemopoietic progenitors, altering the host immune response [19]. Other growth factors are also able to stimulate angiogenesis. HGF binds to its tyrosine kinase receptor (met) and stimulates the same signalling cascades as VEGF. Moreover, HGF induces angiogenesis by stimulating the production of VEGF and inhibiting thrombospondin-1 expression [20]. These receptors and their downstream signalling pathways are at play in tumour cells as well as in endothelial cells and, depending on the pharmacological intervention, they may affect both cell types.

**HCC (HEPATOCELLULAR CARCINOMA)**

HCC is the fifth most common cancer in the world and the third most common cause of cancer-related death, with more than 500 000 new cases a year [21]. HCC accounts for more than 80% of primary liver cancers. A total of 80% of HCC cases occur in Africa and Asia, and China alone accounts for more than 50% of the cases worldwide [22]. Over the last few years, evidence indicates that the incidence of HCC is rising worldwide because of the increased incidence of HCV (hepatitis C virus) infection and NASH (non-alcoholic steatohepatitis).

Therapeutic options for HCC depend entirely on the stage of the tumour. Only patients with small HCCs are amenable to curative treatments, such as surgical resection, liver transplantation or percutaneous ablation [23]. These therapies provide a high rate of complete responses and improve survival, resulting in 5-year survival of over 50% [24]. Unfortunately, the diagnosis of HCC is, in the majority of patients, made at an advanced stage thus precluding curative treatments [25]. Patients with unresectable tumours or metastatic disease have poor survival, despite non-surgical therapies, according to an analysis of 16 randomized control trials [26]: ten trials with loco-regional therapies [percutaneous ethanol injection, percutaneous acetic acid injection, radiofrequency ablation and TACE (transarterial chemoembolization)] and six trials with systemic therapies (four of them including tamoxifen). Four of these trials demonstrated a better local HCC control in tumours larger than 2 cm treated by radiofrequency ablation compared with ethanol injection, whereas no survival advantages were obtained from systemic treatments in patients with advanced HCC [26].

Several groups have proposed algorithms assessing HCC prognosis and assisting in selecting therapy, such as BCLC (Barcelona–Clinic Liver Cancer), CLIP (Cancer of the Liver Italian Program) and CUPI (Chinese University Prognostic Index) among others [27,28]. There is an urgent need not only to conduct randomized clinical trials in HCC with new molecular-targeted inhibitors, but also to design tools identifying the most adequate drug for each patient. It is clearly becoming evident that the molecular classification of HCCs based on the pathways implicated will permit individualized treatments, among them anti-angiogenic drugs.
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Figure 1 An overview of the VEGF signalling pathway
cPLA, cytosolic phospholipase A; DAG, diacylglycerol; Fak, focal adhesion kinase; IP3, Ins(1,4,5)P3; MEK, MAPK/ERK kinase; NFAT, nuclear factor of activated T-cells; PIP2, PtdIns(4,5)P2; PLC-γ, phospholipase C-γ; PKC, protein kinase C; PP2B, protein phosphatase 2B; SHP-1/SHP-2, Src homology 2 domain-containing protein tyrosine phosphatase-1/-2.

ANGIOGENESIS IN HCC

Small tumour cells can survive by obtaining nutrients and oxygen by simple diffusion, but to be able to grow and invade neighbouring tissues a solid tumour must develop its own blood vessel network [10]. Several cancer therapies are now directed against the tumour vasculature. HCC is a highly vascular tumour characterized by neovascularization and arterial perfusion. These characteristics permit the radiological diagnosis of HCC after injection of a contrast agent without histological confirmation [28]. Numerous factors regulate hepatocellular angiogenesis either positively or negatively [29]. Liver angiogenesis is particular. In the liver, large vessels lined by a continuous endothelium and sinusoids lined by a fenestrated endothelium coexist [30]. Liver-specific secreted factors, i.e. ANGPTL3 [Ang (angiopoietin)-like factor 3], have a role in the regulation of hepatic angiogenesis [31], and this process is under the influence of HSCs (hepatic stellate cells). HSCs are liver-specific pericytes which express VEGFR1, VEGFR2 and Tie-1 constitutively and migrate in response to VEGF and Ang-1 [32].

In HCC, serum VEGF levels are correlated with the absence of a tumour capsule, the presence of intra-hepatic metastasis, the presence of microscopic venous invasion and an advanced stage [33–36]. Serum levels correlate with VEGF tumour expression, suggesting that serum VEGF levels may be useful as a predictive marker of microvascular density and vascular invasiveness in HCC, one of the main negative prognostic indicators of HCC [37–40]. High serum VEGF levels prior to liver resection are a predictor of microscopic venous invasion in HCC [38,41], whereas high levels before radiofrequency ablative treatment predict a poor post-treatment prognosis [42]. Polymorphisms of the VEGF gene may be of prognostic significance for patients with HCC, although no relationship between any VEGF polymorphism and serum VEGF levels has been found as yet [43].

The Ang/Tie-2 system plays an important role in HCC angiogenesis [44]. An increased tumoral expression of Ang-2 in comparison with Ang-1 has been associated with tumour diameter, microvessel density and portal vein invasion [45–47]. Ang-2 appears to exert its angiogenic action in HCC synergistically with VEGF [48].

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Expression of Ang-2 is increased specifically in hypervascular HCC, and in vivo gene transference of a soluble Tie-2 receptor for Ang-2 had antitumoral and anti-angiogenic effects in experimental HCC [9].

The HGF pathway has been highlighted as a potential target. HGF is overexpressed in HCC [49], and HGF enhances VEGF production and promotes angiogenesis [50]. NK4 (natural killer cell transcript 4) is a proteolytic digestion product of HGF, which competitively antagonizes HGF-induced phosphorylation of Met, an inhibition that can result in a complete interruption of biological events driven by HGF/Met receptor signalling. NK4 has inhibitory effects on angiogenesis, and its adenovirus-mediated administration resulted in tumour growth suppression in an experimental model of HCC [51].

An additional factor that influences tumorigenesis in general and the development of new vessels in particular is chronic inflammation. The relationship between inflammation and angiogenesis was thought to be that both share some common pathways, but it is becoming clearer that reciprocal modulation occurs between these two crucial processes [52]. In HCC, one of the main risk factors is related to inflammation due to viral infection, and viral proteins, such as HBx (hepatitis B virus X protein) and HCV structural core protein, can activate NF-κB (nuclear factor κB) [53]. NF-κB is one of the principal factors implicated in the regulation of inflammation, tumour suppression, control of proliferation, fibrosis and cirrhosis in a continuum process that Elsharkawy and Mann [54] have named the hepatic inflammation–fibrosis–cancer axis. The NF-κB pathway is activated by multiple stimuli, including TNF-α (tumour necrosis factor-α), LPS (lipopolysaccharide) and ROS (reactive oxygen species). IκB (inhibitory κB) is a protein which normally sequesters NF-κB in the cytoplasm. When the pathway is activated, IκB is ubiquitinated and degraded by the proteasome. NF-κB is then free to translocate to the nucleus and to regulate the expression of numerous genes [55]. The activation of NF-κB can increase the release of IL-6 and consequently the activation of STAT-3 (signal transducer and activator of transcription-3), leading to the secretion of VEGF [56]. VEGF is linked to the inflammatory pathway at, at least, two critical points: (i) VEGF stimulates activation of STAT-3, creating a first short loop of autoregulation that would eventually amplify its own secretion [57–59]; and (ii) VEGF also activates NF-κB through the Akt pathway, again feeding the feedback loop for its secretion through the IL-6/STAT-3 pathway [60] (Figure 2).

The VEGF gene has binding sites for factors that are involved in the inflammatory and angiogenic response (AP-1, AP-2, Egr-1, Smad and NF-1) [17]. In chronic inflammation, hepatocytes undergo to a state where ECM (extracellular matrix) deposition and fibrosis is favoured, which is related to TGF-β signalling, Smad3 activation and stimulation of the JNK (c-Jun N-terminal kinase) pathway (which would be amplified by inflammatory signals), increasing the risk of cancer [61]. An additional system involved in angiogenesis controlled by inflammatory signals is the Ang-1/Ang-2 system, where Ang-1 binding to Tie-2 stabilizes the new vessels. Ang-2 antagonizes this effect, sensitizing endothelial cells to inflammatory stimuli, especially at the step of cell adherence to vessels [62]. HIF-1α stimulates angiogenesis by activating transcription of the gene encoding VEGF, a process that, besides the regulation of hypoxia, is also regulated by IL-1β, TNF-α and NO [63,64]. The tumour suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10) could also participate in this network, as it down-regulates the Akt pathway, HIF-1α expression and HIF-mediated transcription, which includes VEGF [65] (Figure 2).

COX-2 (cyclo-oxygenase-2) expression is undetectable in most normal tissues and it is induced after exposure to pro-inflammatory cytokines, mitogens, tumour promoters and growth factors. It is well-established that COX-2 is overexpressed in many premalignant and malignant lesions, including HCC [66,67]. The overexpression of COX-2 leads to an increase in PG (prostaglandin) levels, which affect several mechanisms involved in carcinogenesis, such as angiogenesis, inhibition of apoptosis and stimulation of cell growth [68,69]. Experimental studies on liver cancer have shown that NSAIDs (non-steroidal anti-inflammatory drugs), including both selective and non-selective COX-2 inhibitors, exert chemopreventive as well as therapeutic effects [70]. The COX-2 pathway may be involved in the regulation of angiogenesis in HCC. Inhibition of COX-2 expression results in decreased proliferation, migration and differentiation in vitro and neovascularization in vivo. These effects were partially reversed by the addition of exogenous PGE2. COX-2 inhibition with SC-58635 resulted in PGE2 reduction, down-regulation of four PGE2 receptor subtypes and downexpression of pro-angiogenic factors, such as VEGF, HGF and FGF [71].

The small Rho GTPase Rac has been suggested to participate in the control of angiogenesis. HCC microarray evaluation of the expression of Rac found that its expression correlates with VEGF [72]. Rac induces the transcriptional activation of VEGF by direct interaction with HIF-1α. In hypoxic conditions, Rac promotes angiogenesis through stabilization of HIF-1α [72]. The main downstream effector of Rac, Pak1 (p21-activated protein kinase), is overexpressed in human HCC [73]. FTY720, an inhibitor of Rac, had antitumoral and anti-angiogenic effects in an experimental model of HCC [74].

Tumour vessels exhibit an abnormal association with adventitial vascular cells, such as pericytes, which regulate vessel stability. Angiogenesis and vascular remodelling involve pericyte recruitment (through PDGF, TGF-β,
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**Figure 2** Central role of the VEGF and NF-κB pathway in the control of inflammation-mediated angiogenesis

HBx, hepatitis B virus X protein; JNK, c-Jun N-terminal kinase; PTEN, phosphatase and tensin homologue deleted on chromosome 1.

**Figure 3** Site of action of some of the anti-angiogenic drugs that are currently in clinical trials for treatment of HCC

Ab, antibody; IFN-γ, interferon γ; NK cells, natural killer cells; TK, tyrosine kinase.
Angs and NO, among others) and secretion of angiogenic factors by pericytes to attract endothelial cells [75]. The presence of PDGF-B and its appropriate mode and timing of action appears critical for correct vessel maturation [75]. PDGFR (PDGF receptor) is expressed on endothelial cells of metastatic HCC, and administration of imatinib, a tyrosine kinase inhibitor of PDGFR, in combination with interferon was able to inhibit tumour growth in a xenograft model [77]. Sunitinib is another potent inhibitor of PDGFR and VEGFR, which has proved to efficiently treat solid tumours in vitro and in animal models, but has not yet been tested in HCC.

An interesting alternative target in the pathway of angiogenesis is mTOR. mTOR is a serine/threonine kinase member of PI3K-related kinase family, which adjusts cell growth and proliferation to the availability of nutrients and stimulation by growth factors [78]. Over-activation of this pathway uncouples proliferation via cues associated with resource limitation and is frequently active in oncogenesis [79]. The PI3K/Akt/mTOR pathway is active in approx. 40% of HCC [80,81]. Rapamycins are highly specific inhibitors of mTOR with immunosuppressant, antifungal and antitumour properties. Rapamycins bind the cytosolic immunophilin FKBP (FK506-binding protein)-12. The FKBP–rapamycin complex binds with the FRB (FKBP–rapamycin-binding) domain of mTOR inhibiting its activity. Inhibition of mTOR induces arrest of the cell cycle [82] and interrupts downstream propagation of PI3K/Akt-mediated proliferative signals [83]. Guba et al. [84] were the first to recognize that rapamycin has anti-angiogenic properties by inhibiting the production of VEGF. In an in vivo orthotopic syngeneic model of HCC, rapamycin slowed the progression of HCC and improved survival [85]. Inhibition of mTOR primarily affected proliferation of endothelial cells, rather than proliferation of malignant cells, and blocked angiogenesis by spouting, leaving tumours to grow via the less efficient intussusception mechanism [85] (Figure 3).

One of the most important tumour suppressors in human oncology is p53. This is particularly relevant in HCC, where multiple factors favouring the development of HCC affect p53 [86]. Recent results have shown that, besides its role as a guardian of the genome, p53 also inhibits tumour angiogenesis [87]. p53 inhibits angiogenesis by regulating the expression of the enzyme prolyl-4-hydroxylase, which cleaves endogenous angiogenesis inhibitors endostatin and tumstatin from collagen 18 and 4 respectively [88].

**ANTI-ANGIOGENIC THERAPY IN HCC**

Anti-angiogenic therapy destroys the tumour vasculature, thus depriving the tumour of oxygen and nutrients. An alternative hypothesis indicates that anti-angiogenic drugs normalize the structure and function of the tumour vasculature, decreasing intra-tumour pressure and improving delivery of conventional chemotherapy [89]. A better knowledge of the molecular and cellular pathways involved in these processes will lead to more effective therapies. One pathway distinguishes anti-angiogenic drugs that inhibit the formation of new vessels and antivascular drugs designed to selectively destruct pre-existing tumour blood vessels. Both types of agents have been investigated in preclinical studies. There is a wide number of drugs available, some of them target endothelial cells directly, whereas others target molecule

### Table 1 Agents with anti-angiogenic activity that are being investigated in clinical trials according to ClinicalTrials.gov

<table>
<thead>
<tr>
<th>Mechanism of action</th>
<th>Drug name</th>
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<td>Drugs that inhibit endothelial cells directly</td>
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<td></td>
<td>CNGRC peptide TNF-α conjugate (NGR-TNF)</td>
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<td></td>
<td>Cyclophosphamide (cytoxan)</td>
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<td></td>
<td>Combretastatin A4 phosphate</td>
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<td></td>
<td>Dimethylxanthenone acetic acid docetaxel (taxotere)</td>
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<td></td>
<td>Lenalidomide</td>
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<td></td>
<td>LY317615 (enzastaurin)</td>
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<tr>
<td></td>
<td>Paclitaxel</td>
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<td></td>
<td>Paclitaxel albumin-stabilized nanoparticle formulation (abraxane)</td>
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<tr>
<td></td>
<td>Soya bean isoflavone (genistein; soya bean protein isolate)</td>
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<td></td>
<td>Tamoxifen citrate</td>
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<td></td>
<td>Thalidomide</td>
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<tr>
<td>Drugs that block activators of angiogenesis</td>
<td>ADH-1 (exeherin™)</td>
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<td>AG-013736</td>
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<td></td>
<td>AMG-706</td>
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<tr>
<td></td>
<td>Anti-VEGF antibody (bevacizumab; avastin™)</td>
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<td></td>
<td>AZD2171</td>
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<td></td>
<td>Bay 43-9006 (sorafenib tosylate)</td>
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<td>BMS-582664</td>
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<td>CHIR-265</td>
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<td></td>
<td>GW786034 (pazopanib)</td>
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<td>PI-88</td>
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<td>PTK787/ZK 222584 (vatalanib)</td>
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<td></td>
<td>RAD001 (everolimus)</td>
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<td>Suramin</td>
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<td>SU11248 (sunitinib malate)</td>
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<td>XL184</td>
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<td></td>
<td>ZD6474</td>
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<tr>
<td>Drugs that inhibit endothelial-specific integrin/survival signalling</td>
<td>ATN-161</td>
</tr>
<tr>
<td>Drugs that block matrix breakdown</td>
<td>EMD 121974 (silengitide)</td>
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More information about the trials is available at the ClinicalTrials.gov website (www.clinicaltrials.gov).
activators of angiogenesis. Table 1 lists the drugs presently tested in clinical trials in patients with HCC.

Drugs targeting VEGF or VEGFR include neutralizing antibodies to the VEGF ligand or VEGFR, soluble VEGFR hybrids and VEGFR tyrosine kinase inhibitors. VEGF ligand monoclonal antibodies are highly specific, eliminating free circulating VEGF and preventing it from binding to its receptors (Figure 3). Bevacizumab is a humanized monoclonal antibody that complexes with VEGF. In a phase II study, Schwartz et al. [90] administered bevacizumab to 30 patients with HCC. They observed a partial response in 10% of the patients and disease stabilization in 70%. The median time to progression was 6.5 months. The combination of bevacizumab with gemcitabine and oxaliplatin was tested in 33 patients with unresectable HCC. Of these patients, 18% had a partial response and 24% had disease stabilization [91]. Several trials are now testing bevacizumab in combination for HCC (Table 2).

Thalidomide has been revived as an anti-angiogenic agent. It has been found to affect HCC perfusion as assessed by microbubble sonography [92]. Thalidomide and its derivatives act through blocking VEGF and bFGF. However, clinical trials with thalidomide in HCC gave variable results [93]. A 30% transient stabilization of HCC was reported with high-dose thalidomide, but this regimen was associated with significant neurological toxicity [94]. Gradual dose escalation was tolerated better in a trial that enrolled 27 patients, but only a modest response could be achieved (two stable diseases) [95]. Neither complete nor partial responses were observed in 19 patients treated with thalidomide in combination with epirubicin [96] (Figure 3).

Over the last few years, several tyrosine kinase inhibitors have reached the clinic [97]. As tyrosine kinase domains have high homology, these drugs frequently inhibit more than one receptor and have different profiles of inhibition. In particular, one tyrosine kinase inhibitor has been tested in patients with HCC, namely sorafenib. Sorafenib is a potent inhibitor of Raf-1, a kinase that links growth receptor activation to the MAPK pathway. Sorafenib also has significant activity against several receptor tyrosine kinases involved in neovascularization and tumour progression, including VEGFRs and PDGFRs [98] (Figure 3). In a phase II study, 137 patients, with compensated cirrhosis and inoperable HCC with no prior systemic treatment, took sorafenib (400 mg, twice a day) in 4-week cycles; three (2.2%) patients achieved a partial response, eight (5.8%) had a minor response, and 46 (33.6%) had stable disease for at least 16 weeks [99]. The drug was particularly effective in patients with overactive Raf kinase, as demonstrated by immunohistochemistry for phosphorylated ERK, a downstream target [99]. A landmark phase III trial confirmed these results. A total of 600 patients were randomized to sorafenib or placebo. The second interim analysis of this trial found an effect which led to its interruption. The median survival was 7.9 months in the placebo group and 10.7 months in the sorafenib group (P < 0.0006). The median time to symptomatic progression was, however, not significant between the two groups. Several trials are actively enrolling patients combining sorafenib with conventional chemotherapy [taglura/uracil (ClinicalTrials.gov accession number NCT00494299; see http://www.clinicaltrials.gov/)], antiangiogenic drugs [bevacizumab (NCT00598952) and sirolimus (NCT00509613)] or other signal transduction kinase inhibitors [perifosine (NCT00398814)]. One particularly interesting approach is the combination of sorafenib with TACE, as TACE has been shown to improve the survival of patients with HCC and this procedure

<table>
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<th>Table 2</th>
<th>Trials of anti-angiogenic therapies for HCC that are currently active according to ClinicalTrials.gov</th>
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<tr>
<td>Trial</td>
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<td>NCT00049322</td>
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<td>in patients with unresectable HCC receiving chemoembolization</td>
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<tr>
<td>Efficacy and safety study of bevacizumab and erlotinib to treat primary liver cancer that can not be removed by surgery</td>
<td>NCT00242502</td>
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<tr>
<td>TACE plus bevacizumab for treatment of HCC</td>
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<td>Bevacizumab and erlotinib in inoperable and metastatic HCC</td>
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<td>Phase II study of bevacizumab and TACE</td>
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<tr>
<td>in patients with unresectable HCC</td>
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<td>Phase II study of bevacizumab and erlotinib hydrochloride in patients with advanced HCC</td>
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<td>Bevacizumab in advanced HCC</td>
<td>NCT00162669</td>
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<tr>
<td>Phase II study of hepatic arterial infusion</td>
<td>NCT00492999</td>
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<tr>
<td>comprising fluoruridine and dexamethasone in combination with systemic bevacizumab in patients with unresectable primary hepatic malignancy</td>
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<tr>
<td>Phase I randomized study of sorafenib and bevacizumab in patients with refractory, metastatic or unresectable solid tumours</td>
<td>NCT00098592</td>
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<tr>
<td>Proton therapy and bevacizumab for primary liver tumours</td>
<td>NCT00426829</td>
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<tr>
<td>Phase I study of sirolimus and bevacizumab in patients with unresectable HCC</td>
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stimulates the production of angiogenic factors in the liver [100]. A trial (NCT00478374) is actively enrolling patients to investigate the combination of sorafenib with TACE.

SIDES EFFECTS OF ANTI-ANGIOGENIC TREATMENTS

As angiogenesis, even in adult life, is a normal process that is active under physiological conditions, blocking VEGF pathways is not free from side effects. Evidently, tissue repair is affected, and anti-angiogenic drugs should be stopped before elective surgery [101]. Effects on vessels may sometimes lead to arterial hypertension or aggravation of this condition. Both cirrhosis and portal hypertension may be altered by these therapies [102,103]. Zhu et al. [91] reported three oesophageal bleeds in 33 patients with unresectable HCC treated with bevacizumab in combination with gemcitabine and oxaliplatin. Schwartz et al. [90] observed the same rate of oesophageal bleeds with bevacizumab monotherapy. Therefore it might be wise to endoscopically eradicate varices before contemplating such a treatment. In animal models, however, it has been reported that anti-angiogenic therapy decreases hyperdynamic splanchnic circulation and the formation of portal-systemic collateral vessels, suggesting that anti-angiogenic therapy is an option for the treatment of portal hypertension [104,105].

Hand–foot syndrome, which is a painful drug-induced exfoliative dermatitis, is a frequent and limiting side effect of these drugs and requires early administration of ointment to avoid the decrease in the anti-angiogenic therapy. However, in comparison with conventional chemotherapies, anti-angiogenic drugs are generally safe and well tolerated. Other side effects are abdominal pain, diarrhoea and nausea. Infrequent serious adverse events include gastrointestinal perforation, haemorrhage, arterial thromboembolic events, hypertensive crisis, wound healing complications, neutropenia, nephrotic syndrome, reversible posterior leukoencephalopathy syndrome and congestive heart failure [106].

FUTURE PERSPECTIVES

HCC is serial killer which has been neglected for too long. It is one of the most frequent solid tumours and one of the primary causes of cancer-related death worldwide. The diagnosis is still too frequently made at advanced stages which excludes curative options. Several chemotherapeutic protocols have been investigated to offer to these patients effective non-surgical treatment, but the results of these have not been encouraging. This is changing with the availability of drugs that interfere with the tumour vasculature, although the only systemic treatment that had a significant effect on survival is the anti-angiogenic sorafenib.

One of the critical factors involved in HCC pathogenesis is the imbalance in the control of angiogenesis, which leads to hypervascularity, vascular invasion and poor prognosis. Anti-angiogenic drugs with specific targets and an acceptable profile of side effects will find their place in combination with conventional chemotherapy or as adjuvant therapy in the treatment of HCC. Appropriate selection of patients who benefit from targeted therapies will be a critical point in the design of forthcoming trials. Despite the link between VEGF levels with tumour progression and poor prognosis, pre-treatment VEGF levels are not predictive of a response to anti-angiogenic therapy [107]. One of the more critical challenges ahead is the development of distinctive markers identifying which therapeutic targets will work best in patients with different tumour types.

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