Therapeutic Agents That Inhibit Angiogenesis

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39.1 Introduction

Angiogenesis is a complex multistep process leading to the formation of new blood vessels from the existing vascular network, tightly regulated by pro-angiogenic and antiangiogenic growth factors. Several potential pathways for tumor-induced angiogenesis have been proposed: (1) secretion by tumor cells of endothelial growth factors which may stimulate tyrosine kinase activities in endothelial cells (ECs), being vascular endothelial growth factor (VEGF) the most specific and potent pro-angiogenic agent; (2) downregulation of naturally occurring inhibitors of angiogenesis; (3) circulating CD34-positive EC precursors may contribute to vasculogenesis, by presently unknown mechanisms mainly involving genetic regulations.

Targeting the molecular pathways involved in tumor progression by biologically designed treatments is a new therapeutic paradigm aimed to reach cancer growth control. Inhibition of angiogenesis presents certain advantages on conventional therapies, such as the direct accessibility from the circulation, the potential low rate of drug resistance and the favorable toxicologic profile.

A recently proposed classification of antiangiogenic agents is based on their mechanisms of action, and include three categories: (1) direct antiangiogenic drugs acting by targeting the endothelial cells and their functions involved in angiogenesis (proliferation, migration, formation of new vessels); (2) indirect antiangiogenic drugs that inhibit the production of angiogenic factors by tumor and microenvironment cells, and/or interfere with extracellular processes; (3) mixed antiangiogenic drugs that may be able to interfere with both endothelial and tumor cells [1].

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A functional classification, based on the potential targets for inhibition of angiogenesis, includes drugs that are directed towards activated endothelial cells, pericytes, hypoxia pathways, and nitric oxide [2].

- Endogenous antiangiogenic agents such as angiostatin, endostatin, caplostatin, and thrombospondin-1 (TSP-1) selectively block the proliferation and migration of intratumoral vascular endothelium and induce prolonged tumor dormancy in several experimental models [2]. In 2005, rh-endostatin was approved in China for the treatment of advanced non-small-cell lung cancer (NSCLC) based on the positive results of the phase III trial by Sun et al. [3]. Moreover, the recent identification and characterization of the endothelial progenitor cell (EPC) and its capability to migrate from bone marrow into circulation and then into other tissues where it stimulates angiogenesis may also open new selective anti-EPC therapeutic strategies [4–6].
- Pericytes are mural cells differentiated from pools of c-kit+sca-1+VEGFR-1+ perivascular progenitor cells mobilized from bone marrow in response to the platelet derived growth factor (PDGF)-BB. When PDGF is overexpressed, tumor microvasculature is covered by a high number of mural cells and tumor growth is accelerated. Drugs targeting PDGFR-β inhibit the recruitment of pericytes, induce dilation of tumor vessels and stimulate endothelial cells to enter apoptosis. The combined block of both VEGFR and PDGFR-β by multitarget tyrosine kinase inhibitors, such as sunitinib, increases the antiangiogenic effect, even in late stage solid tumors [7–10].
- Among angiogenesis promoting molecules identified and purified up to now, VEGF is a key regulator of angiogenesis, overexpressed in the majority of human tumor types. The most advanced in the clinic, among the anti-VEGF inhibitors, are the humanized monoclonal antibody bevacizumab and a number of VEGFRs selective tyrosine kinase inhibitors (Table 39.1). Additional compounds targeting VEGF in clinical development include a VEGF trap and antibodies against VEGFR-2,

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Table 39.1 Angiogenesis inhibitors approved for clinical use

Date approved	Drug	Place	Disease
May 2003	Bortezomib	USA (FDA)	Multiple myeloma
December 2003	Thalidomide	Australia	Multiple myeloma
February 2004	Bevacizumab[a]	USA (FDA)	Colorectal cancer
November 2004	Erlotinib	USA (FDA)	Lung cancer
December 2004	Bevacizumab[a]	Switzerland	Colorectal cancer
December 2004	Macugen[a]	USA (FDA)	Macular degeneration
January 2005	Bevacizumab[a]	EMEA	Colorectal cancer
September 2005	Endostatin[a]	China	Lung cancer
December 2005	Sorafenib[a]	USA (FDA)	Kidney cancer
December 2005	Revlimid	USA (FDA)	Myelodysplastic syndrome
January 2006	Sunitinib[a]	USA (FDA)	Gastric (GIST), kidney cancer
June 2006	Lucentis	USA (FDA)	Macular degeneration
October 2006	Bevacizumab[a]	USA (FDA)	Lung cancer

[a]"Pure" antiangiogenic agents

VEGFR-1. Interestingly, anti-VEGF agents not only arrest proliferation and migration of endothelial cells, but also induce regression of existing vessels by inducing apoptosis and by suppressing the mobilization of EPCs from bone marrow [11–13].

- Oxygen limitation is central in regulating angiogenesis, glucose metabolism, survival, and tumor growth [2]. A major key factor is the hypoxia-inducible factor (HIF) which is required transcriptional factor in nutrient stress signaling. HIF is a pleiotropic factor that controls the expression of VEGF-A and angiopoietin-2 [14]. In the nucleus, HIF links the hypoxia-response-elements that control oxygen tension via oxidizing enzymes and hydroxylases. Moreover, HIF also transcriptionally regulates the expression of MET promoter that is a key regulator of invasive growth by driving cell motility and metastasis [2].

Nitric oxide (NO) is a multifunctional gaseous molecule and a highly reactive free radical that regulates several vascular functions, being capable to mediate VEGF and angiopoietin-1-induced angiogenesis in vivo [15]. Inhibition of NO signaling is another potential antiangiogenic therapeutic strategy. A number of compounds interfere with NO production, such as cavtratin, a caveolin-1 derived peptide, L-NNa, and NO-donating nonsteroidal anti-inflammatory drugs (NSAIDs) [16–18].

More than 50 antiangiogenic agents with different mechanisms of action have been discovered up to now. They include tyrosine kinase inhibitors (TKIs), monoclonal antibodies, small molecule inhibitors and transcription inhibitors. Recently, nanobodies have been introduced into the antiangiogenic armamentarium.

39.2 Anti-VEGF Targeted Therapies

39.2.1 Bevacizumab

Bevacizumab is a humanized monoclonal antibody against VEGF. In February 2004, bevacizumab received the Food and Drug Authorization (FDA) approval for first-line therapy of colorectal cancer (CRC) in combination with an IFL-based (irinotecan, fluorouracil and leucovorin) regimen. In CRC, a randomized multicenter study evaluating the clinical benefits of bevacizumab combined with a bolus IFL in front-line treatment of CRC showed an advantage in median duration of overall survival, median duration of progression-free survival (PFS), response rate (RR) and median duration of response [19]. The most significant clinical toxicities reported in bevacizumab-treated patients were thrombosis, hypertension, proteinuria, and bleeding. In addition, gastrointestinal perforation (1.5 %) as well as an increase in diarrhea, leukopenia, and hypertension was also described. Results from the Three Regimens of Eloxatin Evaluation (TREE)-2 trial study in patients with metastatic CRC showed that the addition of bevacizumab to an oxaliplatin/fluorouracil regimen in first-line therapy improves RR and time to progression (TTP) with acceptable tolerability, and no unexpected toxicity [20]. Several other trials studying the clinical benefits of bevacizumab combined with different anti-EGFR agents, such as erlotinib, panitumumab, or cetuximab are ongoing.

The clinical utility of bevacizumab in metastatic RCC was investigated in a randomized phase II trial in which 116 patients with metastatic clear-cell RCC refractory to immunotherapy were randomized to receive placebo, low-dose (3 mg/kg) bevacizumab, or high-dose (10 mg/kg) bevacizumab given intravenously every 2 weeks. Patients treated with bevacizumab experienced a significant prolongation of TTP compared with the placebo group [21]. There were four partial responses (10 % ORR), all in the high-dose bevacizumab arm. There were no life threatening toxicities or deaths attributable to bevacizumab. Common toxicity included hypertension and proteinuria, more commonly seen in the high-dose bevacizumab arm. All side effects were reversible with cessation of therapy. Grade 1 or 2 hemoptysis was observed in two patients receiving bevacizumab and two patients receiving placebo. No thromboembolic events were reported in any arm.

In a double-blind phase II trial comparing bevacizumab plus erlotinib versus bevacizumab plus placebo, data showed that the combination was safe and well tolerated, although adding erlotinib did not improve efficacy [22]. However, PFS of 8.5 months observed in patients treated with bevacizumab [23] appears to be more favorable than what is reported with the use of IFN-α, suggesting a potential clinical benefit of bevacizumab in RCC. Given the promising activity of bevacizumab, several clinical trials investigating

the clinical advantages of bevacizumab with IFN-α2b therapy (CALGB 90206; $n=600$) (Rini = 72) or in addition to erlotinib plus imatinib are currently in progress [24].

The addiction of bevacizumab to carboplatin/paclitaxel regimen caused a higher RR (31.5 % in the high-dose arm versus 18.8 %) and longer median TTP (7.4 months in the high-dose arm versus 4.2 months) than chemotherapy alone in NSCLC patients [25]. Bevacizumab was generally well tolerated. However, severe haemoptysis episodes among patients with squamous cell histology and central tumor mass were observed. Another study evaluated the clinical benefits of bevacizumab with the carboplatin/paclitaxel regimen as first-line therapy in 842 patients with NSCLC. This US cooperative group phase III trial (E4599) reported a higher RR, longer PFS and increased survival in the bevacizumab/chemotherapy arm compared with the chemotherapy-alone arm [26]. In a trial combining bevacizumab with erlotinib in patients with recurrent NSCLC, preliminary data showed a promising antitumor activity, with partial response achieved in 20 % of patients and stable disease in 65 % [27]. The most common adverse events reported ranged from mild-to-moderate and included rash, diarrhea and proteinuria. Bevacizumab in combination with erlotinib is still under investigation in recurrent or refractory NSCLC [28].

A phase II study assessed the combination of bevacizumab plus gemcitabine in 52 patients with stage IV pancreatic cancer [29]. The objective RR was 21 %, the median PFS 5.4 months and the 6-month OS of 77 %. Adverse events included hypertension, thrombosis, and bleeding episodes. The promising efficacy prompted two ongoing trials: the European phase III trial (BO17706) and the US Cooperative Group phase III trial (CALGB 80303), investigating both the therapeutic benefits of bevacizumab when added to gemcitabine alone or with erlotinib.

Zhu et al. reported encouraging results from a phase II study investigating the therapeutic benefits of bevacizumab combined with a gemcitabine and oxaliplatin regimen in patients with advanced hepatocellular carcinoma [30]. Data suggested that this combination was generally well tolerated, with the most common grade 3–4 adverse events being fatigue, transient elevation of transaminases, nausea/vomiting and hypertension. Although no patients achieved a complete response, the overall response rate was 20 % and the rate of PFS at 6 months 48 %.

In a phase III metastatic breast cancer (mBC) trial, the addition of bevacizumab to capecitabine in a cohort of taxane-refractory and-anthracycline-refractory patients failed to show an improvement in PFS and OS [31]. The addition of bevacizumab to a carboplatin/albumin-bound form of paclitaxel and trastuzumab regimen showed promising antitumor activity among patients with HER2-positive mBC [32]. Of nine evaluable patients, eight achieved a major clinical response. The clinical activity of bevacizumab alone or in combination is being examined in ovarian cancer, recurrent cervical cancer, hormone-refractory prostate cancer, refractory or relapsed AML and malignant melanoma, as well as head and neck cancer. Bevacizumab is also being evaluated as adjuvant treatment of CRC in combination with different regimens including FOLFOX4, FOLFOX6, or XELOX (capecitabine/oxaliplatin) in the National Surgical Adjuvant Breast and Bowel Project (NSABP) C08 trial, the AVANT (AVastin adjuvANT) trial, and the Intergroup Rectal Adjuvant trial [33].

39.2.2 HuMV833

HuMV833 is a humanized monoclonal antibody that recognizes VEGF121 and VEGF165 isoforms. Results from a phase I trial showed that HuMV833 was safe and well tolerated in patients with solid tumors [34]. Infusions were well tolerated without any grade 3 or 4 toxicities. The most common adverse events included fatigue/asthenia, nausea, vomiting, gastrointestinal symptoms, and rash. Recently, phase II/III clinical trials of HuMV833 have begun in Europe.

39.2.3 VEGF-Trap

VEGF-Trap is a novel, high-affinity molecule to VEGF molecule, generated as a fusion molecule of the VEGF receptor extracellular domain and the Fc portion of immunoglobulin G1. Data from phase I studies showed the clinical feasibility of these agents, which are currently being investigated in phase II/III trials [35, 36]. In particular, VEGF-Trap R1R2 is a derivative of the soluble form of VEGFR-1, which irreversibly binds to VEGF. This VEGF blocker is a chimeric fusion molecule composed of the second immunoglobulin domain of VEGFR-1 and combined with the third immunoglobulin domain of VEGFR-2 [37]. VEGF-Trap R1R2 was engineered to minimize interactions with the extracellular matrix, maintaining its potent affinity for VEGFR-2. Preclinical studies showed tumor growth inhibition in various models [38, 39]. In a phase I study, VEGF-Trap administered to patients with solid tumors did not induce antibody response with evidence of biological activity [40]. At present, a phase I clinical is investigating the side effects of VEGF-Trap R1R2 in patients with relapsed or refractory advanced solid tumors or non-Hodgkin's lymphoma.

39.3 Anti-VEGF Receptors Targeted Therapies

39.3.1 IMC-1C11

Among VEGF family receptor, the VEGFR 2/kinase-insert-domain-containing receptor (KDR) appear to be significantly upregulated during tumorigenesis. IMC-1C11, a chimeric

monoclonal anti-KDR antibody, blocks the binding of the ligand to the receptor and subsequently inhibits downstream events such as VEGFR and MAPK activation, inhibiting VEGFR-induced endothelial cell proliferation [41]. This agent is presently being examined in a phase I trial in patients with liver metastases from CRC carcinoma. IMC-1C11 appears to be safe and well tolerated, although 7 out of 14 patients experienced detectable levels of antibodies against IMC-1C11 [42]. A fully human anti-VEGFR-2 has been produced as a second-generation agent to be less immunogenic for chronic administration as monotherapy or in combination with chemotherapy or radiotherapy [43].

39.3.2 Sunitinib Malate

Sunitinib is an oral inhibitor of VEGFR-1, VEGFR-2, stem-cell factor receptor (c-KIT), PDGFRα, PDGFRβ, and fetal liver tyrosine kinase receptor 3 (FLT3) [44–46]. A phase I study investigating the biological activity of sunitinib in patients with AML showed that the phosphorylation of FLT-3 was inhibited in 50 % of patients with wild-type FLT-3 and in 100 % of patients with mutated FLT-3. The majority of gastrointestinal stromal tumors (GISTs) harbor mutations in the receptor tyrosine kinase KIT or PDGFR-A and are responsive to imatinib. Unfortunately, tumors develop resistance due to amino acid mutations in the kinase domain of the targeted receptor, thus preventing or weakening the interaction with the inhibitor. In vitro studies have demonstrated that sunitinib potently inhibited various imatinib-resistant KIT variants [47, 48]. In phase I studies sunitinib showed a manageable toxicity and the most common side effects reported were fatigue, hypertension, sore mouth, skin, gastrointestinal and hematological toxicity [49, 50]. Among side effects, an increased incidence of hypothyroidism was reported, related to a reduced drug-induced vascularity of thyroid gland [51]. Sunitinib demonstrated activity in patients affected by NSCLC, neuroendocrine and other tumor types, although most data are available for patients affected by GIST and RCC [52, 53].

The therapeutic advantages of sunitinib were examined as second-line therapy in patients with RCC. RCC is one of the most resistant tumor types, and, cytokines were the only moderately active agents. Two phase II clinical trials evaluated the activity of sunitinib (50 mg daily administered for 4 weeks followed by 2 weeks off) in patients with RCC after failure of previous immunotherapy [54, 55]. In the first study of Motzer et al. ($n=63$) 40 % of partial responses were reported and TTP was 8.7 months. In addition, 27 % of patients demonstrated stable disease lasting more than 3 months. The most common reported adverse event was fatigue (grade 3 in 11 % of patients). Therapeutic activity was confirmed by a further open-label study single-arm clinical trial as second-line treatment of mRCC that had progressed despite previous cytokine therapy. A partial response was observed in 36 of 106 patients (34 %) and the median progression-free survival was 8.3 months. Recently, Motzer et al. compared IFN-α to sunitinib as first line therapy in metastatic RCC in a phase III randomized clinical trial. Sutininib showed a statistically significant higher response rate (31 versus 6 %) and PFS (11 versus 5 months) as compared to IFN-α (HR 0.42; $p<0.001$) [9].

This agent was also investigated in a phase III trial in 312 patients affected by GISTs who progressive after imatinib therapy. The trial was unblinded because a planned interim analysis showed significant better outcome in the sunitinib arm [56]. Median time to tumor progression was significantly longer in the sunitinib group compared to placebo (27.3 versus 6.4 weeks, respectively; HR: 0.33; $p<0.0001$). The confirmed objective response rate was 7 % in the sunitinib group versus 0 % in the placebo group ($p=0.006$). The survival benefit could be underestimated as a result of the cross-over of patients receiving placebo.

Clinical activity of sunitinib is being investigated in NSCLC and preliminary data have shown that stable disease and partial response have been achieved [52]. Adverse events were generally mild-to-moderate and included fatigue, diarrhea and nausea. On the basis of these clinical results, in January 2006, sunitinib was approved by FDA for the treatment of advanced RCC refractory to cytokine therapy and for imatinib-resistant or imatinib-intolerant GIST.

39.3.3 Sorafenib

Sorafenib is an oral inhibitor targeting multiple kinases including VEGFR-2, VEGFR-3, PDGFR, FLT-3, c-KIT, c-Raf1, and B-Raf [57]. In a phase I study sorafenib showed activity in various tumor types and a manageable toxicity profile, both in monotherapy and in association with chemotherapy [58, 59]. Dose limiting toxicity was associated with diarrhea, fatigue, hypertension, skin rash, and hematological toxicity. The dose of 400 mg twice daily was recommended for phase II evaluation, showing interesting antitumoral activity in patients with various types of solid tumors, especially renal cell carcinoma (RCC) [60–63]. Data from a large phase II randomized discontinuation trial of sorafenib in patients with mRCC demonstrated significant disease-stabilizing activity in the arm receiving the active drug compared with placebo group. During the run-in period, 73 out of 202 patients had tumor shrinkage of >25 % and median overall PFS was 29 weeks for the overall population [64]. The most common drug-related adverse effects included rash, hand–foot syndrome, and fatigue. Grade 3/4 drug-related events occurred in 47 % of patients, with the most common being hypertension (24 %), hand–foot syndrome

(13 %), and fatigue (5 %) No patient died from sorafenib-induced toxicity. A subsequent phase III study including 769 patients with advanced or metastatic cytokine-refractory RCC, demonstrated antitumoral activity of sorafenib in this population and significant clinical benefit [65]. Median PFS was indeed 24 weeks in the sorafenib arm versus 12 weeks in the placebo group (HR = 0.44; $p < 0.00001$). Common side effects were diarrhea (33 %), rash (34 %), hand–foot skin reactions (27 %), fatigue (26 %), and hypertension (11 %). Updated results recently reported demonstrated a survival advantages as compared to placebo (median OS of 19.3 versus 15.9 months, respectively. HR = 0.77; $p = 0.015$) [66]. Based of these results sorafenib was approved by FDA for the treatment of advanced or metastatic RCC refractory to cytokine therapy. Feasibility of combination therapy with Interferon-α (IFNα) is under evaluation in phase II trials, as well as direct comparison between these two agents in patients with RCC [67–69]. Additional investigations are underway to better define role of sorafenib in combination with other targeted agents or cytokines in RCC and as adjuvant therapy following a nephrectomy. Ongoing phase III studies are evaluating the activity of sorafenib for treatment of HCC, melanoma, and NSCLC.

39.3.4 PTK-787/ZK-222584

Vatalanib (PTK787/ZK 222584) is a small molecule orally active TKI of all known VEGFRs, PDGFR tyrosine kinases and the c-kit protein tyrosine kinase. In phase I studies the most common side effects reported were fatigue, hypertension, nausea, vomiting, dizziness, and transaminases elevation [70–72]. Dose identified for further examination was 1250 mg daily. In phase II studies vatalanib showed clinical activity in several solid tumors [73–75]. Phase III trials did not show conclusive results in patients affected by mCRC. In fact CONFIRM-1 study, including 1168 patients with mCRC, demonstrated no beneficial effect of adding vatalanib (1250 mg once a day) to chemotherapy (FOLFOX-4) in first line treatment [76]. Adverse events attributable to PTK/ZK were generally reversible and similar to other antiangiogenic agents. In the phase III placebo-controlled CONFIRM-2 study, enrolling 855 patients with mCRC, adding valatinib to the same chemotherapy regimen (FOLFOX-4) in second line of treatment, demonstrated a longer PFS versus chemotherapy alone (5.5 months versus 4.1 months; HR: 0.83; $p = 0.026$). Response rate was similar in the two arms (18.5 % in the PTK/ZK arm versus 17.5 % in the placebo arm) and survival advantage was not reported (OS was 12.1 months in the PTK/ZK arm and 11.8 months in the placebo arm. HR: 0.94; $p = 0.511$) [77]. Recently, a meta-analysis of these two studies was performed, based on the preliminary results from CONFIRM trials that showed a greater clinical benefit and improvement of PFS in patients with high LDH levels. This analysis confirm that the effect of vatalanib in improvement of PFS is strong in high LDH population (HR 0.65, $p < 0.001$), as compared to overall population (HR 0.85, $p = 0.005$) [78].

A report of preliminary results from a phase II clinical trial investigating the therapeutic benefits of PTK-787/ZK-222584 as a single second-line agent for NSCLC showed that disease control was achieved in 58 % of patients [75]. Other clinical trials investigating PTK-787/ZK-222584 in metastatic neuroendocrine tumors and imatinib mesylate-resistant metastatic GIST are currently ongoing [74, 79].

Our group designed a dose finding phase I study with PTK787/ZK in combination with chemotherapy and trastuzumab, involving patients affected by HER-2/*neu* positive mBC who progressed after trastuzumab and anthracycline-based and/or taxane-based chemotherapy for metastatic disease. The schedule of study treatment includes vinorelbine (day 1-8-15, q 28 days, dose ranging from 25 to 30 mg/mq), weekly trastuzumab and daily PTK787/ZK (dose ranging from 500 to 1250 mg). Four dose levels are planned: the primary objective of the present study is to determine the maximum tolerated dose (MTD) and the pharmacokinetics interactions of PTK787/ZK with the cytotoxic agents. The secondary objective of the study is to evaluate the tolerability and the optimal schedule of the schedule.

39.3.5 AMG-706

AMG 706 is an oral small molecule multi-kinase inhibitor with both antiangiogenic and direct antitumor activity that selectively targets VEGF, PDGF, and Kit receptors [80]. It has demonstrated antiangiogenic and antitumor activity. Data from a phase I study presented at the 2005 American Society of Clinical Oncology (ASCO) meeting showed that AMG-706 in patients with advanced solid tumors was safe at doses up to 125 mg/day [81, 82]. Preliminary data were promising, as they indicated vascular changes and stable disease in the majority of patients. Most frequently reported adverse events were hypertension, fatigue, diarrhea, headache, and nausea. AMG-706 is currently undergoing several phase I/II clinical trials alone or in combination with chemotherapy in CRC, GIST, NSCLC and thyroid cancer. Among these studies, a phase I clinical trial evaluating the safety and clinical activity of AMG-706 in thyroid cancer showed that this agent was well tolerated and objective response was achieved in 43 % of patients [83]. AMG-706 is also being investigated in combination with panitumumab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody, in NSCLC, and preliminary data revealed that this regimen was safe and exhibited clinical activity [84].

39.3.6 ZD-6474 (Zactima, Vandetanib)

ZD6474 is a dual-kinase inhibitor that inhibits VEGFR-2, but also has moderate anti-EGFR activity and RET receptor. In phase I evaluation, ZD6474 was well tolerated at daily dose of 100–300 mg/day and adverse events commonly reported were diarrhea, rash, fatigue and asymptomatic QT-prolongation [85]. Preclinical studies have yielded data consistent with a potent inhibition of the VEGF signaling pathway, suggesting potential use in a broad range of tumors including colon, lung, prostate, breast, and ovarian cancers [86]. However, most clinical data in phase II studies are available in NSCLC. Adding of ZD6474 to carboplatin/paclitaxel in first line chemotherapy is safe and did not significantly increase treatment toxicity. In a phase II randomized study performed by Heymach et al., patients with locally advanced or metastatic NSCLC after failure of first line platinum-based chemotherapy were randomized to receive docetaxel plus ZD6474, either at dose of 100 mg 300 mg, or docetaxel alone. Median PFS was higher in patients receiving the combined therapy (19 versus 17 versus 12 weeks, respectively) [87]. Recently, a double-blind phase II randomized trial compared ZD6474 300 mg with gefitinib 250 mg in 168 advanced previously treated NSCLC patients, with the option of crossover at the time of progression. Preliminary data showed a response rate of 8 % in the ZD6474 arm and 1 % in the gefitinib arm and a statistically significant longer PFS with ZD6474 was reported (11.9 versus 8.1 weeks, respectively; $p = 0.011$) [88]. These positive results were not confirmed in BC: in 44 patients refractory to anthracycline/taxane, ZD6474 either at dose of 100 mg or 300 mg did not show clinical activity [89]. ZD6474 displayed also promising evidence of activity in patients with hereditary medullary thyroid carcinoma; in a phase II trial in 15 evaluable patients, three had partial response and ten stable disease [90].

39.3.7 Axitinib (AG-013736)

Axitinib is an oral multitargeted TKI with inhibitory effects against VEGFR-2, VEGFR-3, and PDGFR-β, evaluated in a phase I clinical study among patients with advanced solid tumors, AG-013736 demonstrated clinical activity in RCC, adenoid cystic cancer and NSCLC [91]. Dose-limiting toxicities included hypertension, hemoptysis, and stomatitis [92], manageable with appropriate medication or dose reduction. A phase I study identified maximum tolerated dose of 5 mg twice daily. Rini and colleagues evaluated axitinib in a phase II trial in 52 patients with advanced RCC refractory to one prior cytokine-based therapy. Partial response was reported in 24 patients (46 %) and stable disease in a further 40 %. Median TTP has not been reached after 12- to 18-month follow-up in all patients. Most common serious adverse events (grade 3–4) were diarrhea (8 %), hypertension (15 %), and fatigue (8 %) [93]. Axitinib has been also evaluated in 32 patients with advanced thyroid cancer, refractory to or not suitable candidates for Iodine therapy. Although response assessments are still ongoing, partial response was achieved in three patients [94].

39.3.8 Other Multitargeted TKIs in Clinical Development

GW-786034 is another oral TKI with activity against VEGFR that has demonstrated antitumor and antiangiogenic activity in vitro and in vivo [95]. It is currently undergoing phase II clinical trials on refractory multiple myeloma, soft tissue sarcoma, ovarian cancer, and RCC.

CP-547,632 has shown antitumor and antiangiogenic activity against VEGFR-2 in several preclinical models [96]. This agent is currently in a phase II clinical trial for recurrent or persistent small-volume ovarian cancer.

The agent AEE-788 is a dual family EGFR/ErbB2 and VEGFR TKI [97], showing interesting antitumor activity in various models [98, 99]. In phase I studies AEE788 was generally well tolerated and most of the adverse events were generally mild or moderate diarrhea, asthenia, anorexia, rash, nausea, and vomiting [100–102]. Dose-limiting toxicities were observed at the 500–550 mg dose levels. Enrollment in clinical trials is ongoing.

XL647 is an orally bioavailable small molecule with inhibitory effect on EGFR, HER-2, VEGFR2/KDR and EphB4. In a phase I study XL647 showed a satisfactory toxicity profile. The maximum tolerated dose was established in 4.68 mg/kg orally administered for 5 consecutive days every 2 weeks [103].

CEP-7055 is a novel orally active inhibitor of all three VEGFR kinases with broad preclinical antitumor and antiangiogenic activity [104], now entering a phase I clinical study.

39.3.9 RPI-4610

RPI-4610 belongs to a new class of drug termed chemically stabilized ribozymes, synthesized to target and cleave a specific mRNA sequence. RPI-4610, administered intravenously, targets the VEGFR-1 mRNA [105]. A phase I clinical study in patients with refractory solid tumors showed grade 1/2 infusion reactions as the most common toxicities [106]. A phase II clinical trial is currently investigating the effectiveness of RPI-4610 in patients with metastatic RCC.

39.4 Targeted Therapies Interfering with Metalloproteinases

The involvement of proteolytic activity of metalloproteinases (MMPs) in angiogenesis is well established. The extracellular matrix (ECM) surrounding endothelial cells must be broken down to allow cell migration and proliferation. MMPs belong to a family of proteolytic enzymes that degrade ECM components and contribute through this mechanism to the angiogenic process. Aberrant MMP expression contributes to the invasive growth and spread of a variety of solid malignancies [11, 107, 108]. It has been suggested that the control of MMPs activity through inhibitors has been considered a potential target for anticancer therapy, but clinical trials yielded disappointing results. Incyclinide, an oral MMP inhibitor (MMPI), showed biological activity in AIDS-related Kaposi's sarcoma [109]. The most common adverse events were photosensitivity and rash. BMS-275291, a broad spectrum MMPI, failed to achieve partial or complete tumor responses in patients with advanced or metastatic cancer [110]. The most frequently reported adverse events were joint toxicity, rash, fatigue, headache and nausea. In a recent study evaluating two different doses, this agent demonstrated limited clinical activity in hormone-refractory prostate cancer with bone metastases [111]. Other MMPIs, such as BAY-129566 or BB-2516, have failed to show therapeutic activity in human malignancies despite preclinical antimetastatic and antiangiogenic activity. The reasons for the disappointing results observed with MMPIs in cancer therapy remain unclear. The negative results of MMPIs reported in several studies have definitely raised serious concerns about the opportunity to continue the evaluation of MMPIs as a therapeutic anticancer strategy.

39.5 Targeted Therapies Directly Interfering with Endothelial Cells

Thrombospondin-1 (TSP-1) is a naturally occurring inhibitor of angiogenesis that limits vessel density in normal tissues and curtails tumor growth. ABT-510, a promising new agent, is a TSP-1 analogue. A phase I study in patients with advanced solid malignancies showed a favorable toxicity profile, with the most common toxicities observed being injection-site reactions and fatigue. Stability of disease was observed in a significant number of patients, warranting further clinical trials [112]. A phase II trial testing the clinical benefits of ABT-510 in head and neck cancer is currently underway. An additional avenue being explored is integrin protein, which plays an essential role in cell–cell and cell–matrix adhesion. Integrins are cell surface adhesion molecules coupling the extracellular environment to the cytoskeleton, as well as receptors for transmitting signals important for cell migration, invasion, proliferation and survival. Deregulation of adhesion can lead to pathological processes, including tumor metastasis, either by disrupting the normal anchorage, thereby altering cell movement and regulatory signaling, or by promoting inappropriate adhesion. One member of the integrin family, αvβ3-integrin is overexpressed in tumor cells Supporting evidence for the integrin involvement in tumor angiogenesis was recently reported by Nikolopoulos and colleagues who demonstrated that the subunit of integrin promotes endothelial migration and invasion [113]. Therefore, agents targeting integrins are currently being evaluated as potential therapeutic options to treat tumors. Such agents include abegrin, a monoclonal antibody, and cilengitide (EMD-121974), a cyclic peptide. Both interfere with the αvβ3-integrin and are under investigation in phase I/II studies.

Another antiangiogenic drug tested in human malignancies is thalidomide, although its exact mechanism of action is still unclear. Thalidomide was once notorious for producing severe deformities in the arms and legs of newborn babies whose mothers were given the drug during pregnancy. Off the market for decades, it has recently emerged as a somewhat effective treatment for several cancers. Thalidomide and its immunomodulatory analogues are being investigated in several phase II/III trials for treating various tumors including multiple myeloma, RCC, prostate cancer, and hepatocellular cancer [114]. Finally, endostatin, an endogenous angiogenesis inhibitor, represents an additional target for cancer therapy [115]. Clinical trials evaluating the safety of r-hu endostatin in patients with advanced solid tumors showed that this agent has a good safety profile, but modest antitumor activity [116], with positive results only in NSCLC.

39.6 New Strategies for Antiangiogenic Therapy Combinations

39.6.1 Broad-Spectrum Targeting

Recently Folkman proposed the paradigm to block tumor growth indefinitely by the use of a broad-spectrum single multitarget agent capable simultaneously to interfere with several angiogenesis pathways or, alternatively, by combinations of different highly selective drugs. Two examples of the first strategy are the multitarget tyrosine kinase inhibitors sunitinib and sorafenib.

Examples of the second strategy include the combinations of bevacizumab and anti-EGFR agents taking into account that angiogenesis is linked to other tumor molecular pathways. Preclinical studies demonstrated that interactions between EGFR and VEGF signaling pathways sustained tumor growth and progression. They exert effects

both directly and indirectly on tumor cells, and combining drugs targeting both the targets confer additional clinical benefit. EGFR has been detected in the endothelial cells of tumor vasculature preclinically [117]. Co-expression of EGFR and TGFα has been correlated with increased vascularity in invasive BC [118]. VEGF is also downregulated by EGFR inhibition [119, 120] and a recent study suggests that blockade of VEGF may also inhibit the EGFR autocrine signaling [121].

A number of preclinical studies investigating the antitumor activity of combined anti-EGFR and anti-VEGF agents suggest promising activity [122–126].

Furthermore, VEGF blockade is critical in preventing resistance to EGFR inhibition. The use of agents such as erlotinib and bevacizumab targeting different signaling pathways and affecting different cell types (tumor cell and endothelial cell) may abide by different rules than standard cytotoxic chemotherapy because of the significant cross talk between the pathways in numerous cell types. These encouraging data have led to the initiation of a number of clinical studies evaluating the combination of erlotinib with bevacizumab in a number of tumor types, including phase II trials in RCC [127] and mBC [128] and a phase I study in patients with HNSCC [129].

Herbst et al. [130] evaluated the combination of bevacizumab and erlotinib in 40 patients (34 patients at phase II dose) with previously treated NSCLC. Eight patients (20.0 %) had partial response and 26 (65.0 %) had stable disease as their best response. The median OS for the 34 patients treated at phase II dose was 12.6 months, with a PFS of 6.2 months. The most common adverse events were mild to moderate rash, diarrhea and proteinuria.

In another multicenter phase II trial, 63 patients with metastatic RCC were treated with bevacizumab 10 mg/kg intravenously every 2 weeks and erlotinib 150 mg orally daily. Fifteen (25 %) of 59 assessable patients had objective response and an additional 36 patients (61 %) had stable disease after 8 weeks of treatment. The median and 1-year PFS was 11 months and 43 % respectively. After a median follow-up of 15 months, median OS has not been reached: survival at 18 months was 60 %. Treatment was generally well-tolerated: only two patients discontinued treatment because of toxicity (skin rash); grade 1/2 skin rash and diarrhea were the most frequent treatment-related toxicities [131].

Encouraging results have been reported with the combination of two different monoclonal antibodies with or without chemotherapy. The BOND II Trial evaluated the combination of cetuximab/bevacizumab +/– irinotecan in mCRC patients after irinotecan failure. Preliminary data showed a better efficacy in the experimental arm in terms of response rate (37 % versus 20 %) and median TTP (7.9 months versus 5.6 months) [132].

Based on preclinical results showing co-upregulation of VEGF and HER-2/neu in breast cancer, a recent phase I/II trial explored the combination of trastuzumab and bevacizumab in relapsed mBC HER-2 positive [133].

39.6.2 Metronomic Chemotherapy

Another therapeutic strategy is based on the use of antiangiogenic schedules of cytotoxic agents (i.e., metronomic chemotherapy) aimed to block endothelial cells proliferation. The most studied cytotoxic agent is oral cyclophosphamide, which is more active combined with selective antiangiogenic agents such as the TSP-1 peptide ABT-510, thalidomide and celecoxib, or anti-VEGF agents [134].

A recent phase II study in pediatric cancer patients with recurrent or progressive poor prognosis tumors for which no curative therapy remained, Kieran et al. [135] tested the continuous administration of oral thalidomide, celecoxib with alternating etoposide and cyclophosphamide. Such a therapy was well-tolerated and 40 % of patients had clinical benefit with 25 % of all cases continuing to be progression-free for more than 123 weeks. Elevated circulatory levels of TSP-1 correlated with prolonged response.

39.6.3 Bidirectional Block of Angiogenic Balance

At a certain time the angiogenic activity of a tumor is the result of the net balance between pro- and contra-angiogenic molecules. It is reasonable to hypothesize that the concurrent use of an anti-pro-angiogenic molecule (i.e., anti-VEGF) and a naturally occurring angiogenesis inhibitor (i.e., endostatin) should be more effective than the use of each single class of agents alone. Such a strategy is now feasible in the clinic due to the approval in China of therapy for NSCLC with rh-endostatin, a tumor type for which also bevacizumab is active and approved [3].

39.6.4 Nanobodies

A new attractive category of targeted agents is that of nanobodies, consisting of the smallest functional fragment of a single-chain antibody, with interesting pharmacodynamic and pharmacokinetic characteristics such as: high stability, alternative routes of administration (intranasal, pulmonary, oral, transdermal, etc.), high tissue penetration (properties more similar to small molecules) and high affinity/potency, to the target, and low inherent toxicity. In addition, nanobodies are easier to of manufacture in bacteria and yeasts, have low

immunogenicity and a wider range of epitopes including cavities. The BIV nanobody (Ablynx) has shown a high affinity against ~100 targets, one of them being VEGF-A; it has been demonstrated his activity as a potent antagonist in vitro against ~20 targets. In vivo his efficacy has been shown in over ten animal models [136]. Preclinical development is ongoing at the moment and phase I/II clinical trials are planned to be started in 2007.

39.7 Conclusions

From 2004 antiangiogenic agents improved the antitumor therapeutic armamentarium. Up to now four inhibitors of angiogenesis has been approved by FDA. Bevacizumab significantly improved clinical outcome of patients with advanced CRC, NSCLC, and BC as compared with standard chemotherapy. Two multitargeted TKI compounds, namely sunitinib and sorafenib, changed the natural history of advanced RCC, a tumor type characterized by its poor responsiveness to cytotoxic and immunomodulatory agents.

Taking into account that tumor angiogenesis is a multistep process very complex, novel strategies based on the use of broad-spectrum antiangiogenic compounds, schedules of multitargeted agents (i.e., anti-VEGF + anti-EGFR or antimTOR) as well as the development of new highly active and selective drugs (i.e., nanobodies) are expected to further ameliorate the efficacy of antiangiogenic therapy in future years.

With the promising results obtained in patients with advanced, metastatic disease it is likely to be reproduced and enhanced in the adjuvant setting. Because the angiogenic switch is an early event in tumor growth, the capability to develop orally active and safe angiogenic inhibitors may open the opportunity to develop novel strategies of chemoprevention for high-risk subjects.

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