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Review Article

It takes two to tango: Combinations of conventional cytotoxics with compounds targeting the vascular endothelial growth factor–vascular endothelial growth factor receptor pathway in patients with solid malignancies

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Through advances in molecular biology, insight into the mechanisms driving malignancies has improved immensely and as a result, various factors playing an essential role in the biology of numerous tumor types have been revealed. By using compounds that specifically block the function of a single factor being crucial for tumor pathogenesis, it was hoped to exert antitumor activity while avoiding toxicities characteristic for conventional chemotherapy. One of the processes of crucial importance in the development of cancer, and consequently an attractive target, is angiogenesis. In recent years, several key factors for angiogenesis have been identified, including ligands, receptors, and transduction signaling factors. Of these, the vascular endothelial growth factor (VEGF) pathway has been found to be activated in numerous tumor types and considered one of the main drivers of angiogenesis. Roughly, VEGF-mediated angiogenesis can be inhibited by two approaches: either by monoclonal antibodies directed towards VEGF or its corresponding receptors, or by kinase inhibitors targeting the signal transduction of the VEGF receptors. As monotherapy, several kinase inhibitors exert antitumor activity in tumor types such as renal cell carcinoma. However, in most tumor types, the antitumor activity of compounds targeting the VEGF pathway is limited. In recent years, evidence is mounting that the paradigm of one single factor that drives malignant behavior applies rarely and is an oversimplification for most tumors in which there are multiple driving pathways. Consequently, multitargeting rather than single-targeting approaches are required. One of the means is by combining targeted agents with conventional cytotoxics. As the VEGF pathway also affects the sensitivity of tumor cells to chemotherapeutics, combinations of compounds targeting this pathway and conventional cytotoxics have been explored. This review addresses such combinations. (*Cancer Sci* 2010; 101: 7–15)

Recently, anticancer therapy has focused on cancer cell-specific therapy, often referred to as targeted therapy. Mainly through improved molecular techniques, numerous factors involved in tumor pathogenesis have been identified. Such factors are frequently expressed both in tumor cells, and in adjacent normal cells, supporting tumor growth. Examples of tumor-driving factors include ligands (e.g. VEGF, and hepatocyte growth factor), receptors (e.g. c-KIT, VEGFR, EGFR, and human EGFR-2), and factors involved in signal transduction pathways. Initially, it was hoped that one or only a few factors would drive malignant behavior of solid tumors, and that inhibiting these factors would exert antitumor activity.

Indeed, the concept of a single pathway driving malignant behavior is illustrated by the example of GIST. GIST is driven by activating mutations in the *c-KIT* gene.[1] The introduction of imatinib, a TKI targeting c-KIT, dramatically improved the outcomes of advanced GIST patients.[2,3] However, in contrast to GIST, in most tumors multiple pathways are active in parallel, therefore targeting one or a few pathways will frequently not yield significant antitumor activity. Multiple driving pathways require multitargeting approaches, which can be achieved by several means; cancer cell-specific drugs are designed to have a broader range of activity, cancer cell-specific drugs can be combined, and targeted therapy may be combined with conventional chemotherapy. The present review addresses the rationale and currently available data on combinations of conventional chemotherapy and cancer cell-specific therapies directed towards the VEGF pathway.

Vascular endothelial growth factor-driven angiogenesis as a target for therapy in solid tumors

Angiogenesis is crucial for tumor growth and dissemination and therefore forms an attractive pathway to target. In this process, the VEGF family plays a central role. VEGF-A is the major pro-angiogeneic factor, usually referred to as VEGF. Other family members include VEGF-B, VEGF-C, VEGF-D, and placental growth factor. In addition to tumor cells, VEGF is produced by a number of cells, such as platelets, stromal, and muscle cells. Although VEGFR is sometimes expressed by tumor cells, VEGF's predominant site of action is at endothelial cells. Binding of VEGF to VEGFR-1 and VEGFR-2 initiates a cascade of downstream intracellular signal transduction pathways resulting in endothelial cell proliferation and migration, vascular permeability, and subsequently to the formation of new blood vessels.[4]

VEGF is overexpressed in many solid tumor types as a consequence of several underlying mechanisms. VEGF can be induced by a number of genetic and epigenetic alterations, by cytokines, growth factors, hormones, and hypoxia. One of the best examples elucidated thus far is in clear-cell RCC, in which the activity of the *VHL* gene is disrupted due to mutations or

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methylation. Normally, VHL binds to and inactivates the transcription factor hypoxia inducible factor 1-α. Due to the disrupted VHL function in RCC, however, hypoxia inducible factor 1-α levels are elevated, inducing transcription of many factors including VEGF.[5] In many cancer types, increased VEGF expression is associated with poor outcome, irrespective of tumor stage or grade.[6] A higher potency to disseminate and chemoresistance account for this, thus rendering the VEGF pathway one of the most attractive targets for anticancer therapy.

Clinical studies on single agents targeting the VEGF pathway

Currently, the VEGF pathway can be blocked by mAb or kinase inhibitors. Concerning mAb, only bevacizumab has been extensively explored in the clinic. Bevacizumab is directed towards VEGF, thereby hindering its attachment to receptors. As bevacizumab does not bind factors other than VEGF, bevacizumab is regarded as a truly single factor-targeting treatment. In contrast, kinase inhibitors targeting VEGFR often abrogate the function of other factors as well, therefore being less specific. Recently, compounds targeting the VEGF pathway have been widely explored. One of the first issues that had to be solved in the context of these studies was how to reliably assess their activity in clinical studies. Historically, the RR was used for this purpose, but data are accumulating that for many antitumor agents, in particular those targeting the VEGF pathway, antitumor activity is not adequately reflected by changes in size but more relevantly by parameters reflecting tumor stabilization, such as the ratio of tumor progression before and after starting the agent of interest, the PFS, and progression-free rate at a certain time point.

Bevacizumab. The first proof of the efficacy of an anti-angiogenesis treatment in human malignancy was established in advanced RCC. Monotherapy bevacizumab induced a low RR (10%), but PFS almost doubled compared to placebo.[7] However, apart from RCC, monotherapy bevacizumab has been explored only in a few other tumor types and only in non-randomized settings. In cervical cancer and castrate refractory prostate cancer, no antitumor activity was observed (Table 1). In HCC, bevacizumab induced a 6-months progression-free rate of 65% compared with 40% in historical controls, although fair comparison remains difficult without a randomized control arm.[8] For ovarian cancer, two non-randomized phase II studies in heavily pretreated patients have been published, both showing interesting PFS and OS[9,10] compared to historical controls.[11] It was concluded that bevacizumab has activity against ovarian cancer, albeit this conclusion is based on non-randomized studies. Furthermore, bevacizumab has recently been Food and Drug Administration (FDA)-approved based on data of two non-randomized studies in patients with previously treated glioblastoma (AVF3708g and NCI 06-C-0064E), both not published as full papers yet. Another non-randomized phase II study confirmed bevacizumab's activity in pretreated glioblastoma.[12]

Tyrosine kinase inhibitors. The first two TKI targeting VEGFR that were widely explored in solid tumors are sunitinib and sorafenib. In addition to VEGFR-2, sunitinib also inhibits c-KIT, FMS-like tyrosine kinase 3, PDGF-α, and PDGF-β. Sorafenib is a potent Raf kinase inhibitor that directly suppresses tumor cell proliferation, and also targets VEGFR-2, VEGFR-3, and PDGFR-β. Sunitinib improves PFS and RR compared with IFN-α as first-line therapy for advanced clear-cell RCC. PFS and RR were 11 months and 47% in the sunitinib group, compared with 5 months and 12% in the IFN-α group respectively.[13] A trend to better OS in the sunitinib group was observed (26.4 *vs* 21.8 months [$P = 0.051$]). Within the IFN-α group, however, the majority of patients received active post-study antitumor treatment, obscuring the true impact of sunitinib on OS. In the subgroups not receiving post-study therapy, sunitinib doubled the OS compared with IFN-α (28.1 *vs* 14.1 months, respectively),[14] strongly supporting sunitinib's activity in RCC. Furthermore, sunitinib is active in patients with advanced GIST failing to imatinib.[15]

Sorafenib improved PFS in patients with advanced clear-cell RCC pretreated with cytokine-containing therapy in a placebo-controlled phase III trial (median PFS 5.5 *vs* 2.8 months). Subsequently, the trial was stopped early and patients receiving placebo were allowed to cross over to sorafenib.[16] Although an intent-to-treat analysis demonstrated no OS benefit (17.8 *vs* 15.2 months, respectively), censoring placebo patients indicated

Table 1. Trials with monotherapy bevacizumab

Indication	Study phase	Patients (*n*)	Agent	End points	Reference
Metastatic clear-cell RCC 2nd line	II	116	Bevacizumab 3 and 10 mg/kg q2w or placebo	PFS bevacizumab 10 mg/kg 4.8 m; 3 mg/kg 3.0 m; placebo 2.5 m OS ns RR 10% bevacizumab 10 mg/kg	(7)
Metastatic castrate refractory prostate carcinoma	II	15	Bevacizumab 10 mg/kg q2w non-randomized	No objective response	(73)
Platinum resistant epithelial ovarian/peritoneal serous cancer 3rd/4th line	II	44	Bevacizumab 15 mg/kg q3w non-randomized	PFS 4.4, OS 10.7 m, PR 15.9%, perforation 11%, 3 deaths (historical PFS 2.3–3.4 m, OS 8–10.3 m)	(10)
Epithelial ovarian/primary peritoneal cancer 2nd/3rd line	II	62	Bevacizumab 15 mg/kg q3w non-randomized	PFS 4.7 m, OS 17 m RR 21%	(9)
Recurrent cervical cancer 2nd/3rd line	II	46	Bevacizumab 15 mg/kg q3w non-randomized	PFS 3.4 m, OS 7.3 m, PR 10.9% (historically OS 4–6.6 m)	(74)
Non-metastatic unresectable HCC	II	46	Bevacizumab 5 or 10 mg/kg q2w non-randomized	PFS 6.9 m, PFS rate 65% at 6 m, OS not available	(8)
Recurrent glioblastoma	II	48	Bevacizumab 10 mg/kq q2w non-randomized	PFS 16 w, OS 31 w, RR 35% (MacDonald criteria)/71% (Levin criteria)	(12)

HCC, hepatocellular carcinoma; OS, overall survival; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; RR, response rate; w, weeks; m, months.

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Table 2. Trials with monotherapy tyrosine kinase inhibitors

Indication	Study phase	Patients (*n*)	Agent	End points	Reference
Metastatic clear-cell RCC 1st line	III	750	Sunitinib 50 mg 4 weeks q6w, or IFNα 9 MU 3/week	PFS sunitinib 11 m *vs* IFNα 5 m OS 26.4 *vs* 21.8 m, RR 42% *vs* 12%	(14)
Metastatic clear-cell RCC 1st line	II	189	Sorafenib 400 mg bid or IFNα 9 MU 3/week. Cross-over to sorafenib 600 mg bid or sorafenib 400 mg bid	PFS sorafenib 5.7 m *vs* IFNα 5.6 m, PR 5 *vs* 7% Cross-over: PFS sorafenib 600 mg 3.6 m; 400 mg 5.3 m	(18)
Metastatic clear-cell RCC 2nd line	III	903	Sorafenib 400 mg bid or placebo	Interim analysis: PFS sorafenib 5.5 m *vs* placebo 2.8 m. Cross-over: OS sorafenib 17.8 m *vs* placebo 15.2 m (ns) OS (placebo censored) sorafenib 17.8 m *vs* placebo 14.3 m	(16,17)
Metastatic clear-cell RCC 1st/2nd line	III	434	Pazopanib 800 mg or placebo	PFS pazopanib 9.2 m *vs* placebo 4.2 m, OS awaited, RR 30% *vs* 3%	(20)
Metastatic castrate refractory prostate cancer 1st line	II	55	Sorafenib 400 mg bid non-randomized	PFS 8 w, PFS rate 1 y 13%, OS not reached	(75)
Metastatic soft tissue sarcoma 2nd line	II	142	Pazopanib 800 mg non-randomized	PFS rate 12 w 36–49% (leiomyosarcoma, synovial); PFS rate 1 y 14%, OS rate 1 y 34%	(76)
Advanced HCC 1st/2nd line	II	34	Sunitinib 37.5 mg/d 4w q6w non-randomized	PFS 3.9 m, OS 9.8 m RR 2.9%, 50% stable disease	(28)
Advanced HCC 1st line	III	271	Sorafenib 400 mg bid or placebo	PFS sorafenib 2.8 m *vs* placebo 1.4 m, OS 6.5 m *vs* 4.2 m	(77)
Advanced HCC 1st line	III	602	Sorafenib 400 mg bid or placebo	PFS sorafenib 5.5 m *vs* placebo 2.8 m, OS 10.7 m *vs* 7.9 m	(19)
Glioblastoma 1st/2nd line	II	16	Cediranib 45 mg/d non-randomized	PFS 3.7 m, OS 7 m	(29)

HCC, hepatocellular carcinoma; OS, overall survival; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; RR, response rate; w, weeks; m, months; y, years; ns, non-significant.

a better OS for those receiving sorafenib (17.8 *vs* 14.3 months), suggesting an important cross-over effect.[17] Surprisingly, given the effects of sorafenib in second-line treatment, sorafenib and IFN-α had equivalent activity as first-line treatment of metastatic RCC.[18] In HCC, sorafenib yielded a 2% RR, but significantly improved PFS and OS over placebo. PFS was 5.5 *versus* 2.8 months, and OS was 10.7 *versus* 7.9 months, respectively.[19] This study clearly shows that antitumor activity of VEGF-targeting agents is frequently not appropriately reflected in RR. Furthermore, sunitinib and sorafenib have been studied in a wide range of other tumor types (Table 2).

In addition to sunitinib and sorafenib, the number of TKI targeting the VEGFR is rapidly increasing, as is the number of tumor types in which they are assessed. Recently, the results of a randomized placebo-controlled phase III trial of pazopanib as first- or second-line treatment in clear-cell RCC were presented. Compared to placebo, pazopanib improved RR and PFS.[20] Although the outcomes of many of the studies exploring pazopanib and other novel VEGFR TKI are promising, the lack of results from randomized studies is insufficient to give an exact definition of their role in this process. However, it is not unrealistic that besides a few exceptions, the activity of these agents as monotherapy is at best modest for most tumor types.

Rationale to combine compounds inhibiting the VEGF-pathway with conventional chemotherapy

There are several potential reasons rendering VEGF pathway-inhibiting drugs attractive to combine with conventional chemotherapeutic drugs. Besides promoting angiogenesis, VEGF expression can confer resistance against chemotherapy, potentially contributing to the generally worse outcome for patients with VEGF-overexpressing tumors. In xenografts, VEGF-producing tumor cell lines formed highly vascular tumors with accelerated growth compared to the parental cell lines, and exhibited less sensitivity to doxorubicin. Adding an anti-VEGF mAb reinforced the antitumor effects of doxorubicin.[21] Several mechanisms explaining how VEGF may confer chemoresistance, and why combinations of conventional chemotherapy with VEGF pathway-inhibiting agents may yield synergistic interaction have been revealed.

Increased VEGF expression can protect tumor endothelial cells from apoptosis, through increased levels of Bcl-2 and survivin, two anti-apoptotic factors.[22,23] Furthermore, VEGF overexpression may account for chemoresistance through increased IFP in tumors. Tumor vasculature is more fragile and leaky than normal vasculature, leading to elevated IFP, which hinders the delivery of drugs from the circulation into tumors.[24] Abnormal tumor vasculature also leads to reduced blood flow and perfusion, thereby further impairing delivery of anticancer drugs. Normalization of tumor vasculature by anti-angiogenic drugs can transiently reverse these abnormalities, and enhance the effects of chemotherapy (or radiotherapy), provided that it is administered during the ''normalization window''.[25,26] In a small series of six patients with locally advanced CRC, bevacizumab decreased tumor perfusion, vascular volume, microvascular density, and IFP, all being signs of tumor vasculature normalization.[27] Furthermore, there was no change in

FDG-PET uptake, despite less blood flow, indicating increased efficiency of blood vessels.[27] Consistently, in HCC patients treated with sunitinib, and in glioblastoma patients treated with cediranib, signs of reduced vascular permeability corresponding with vascular normalization were seen.[28,29] A third VEGF-mediated mechanism that may contribute to tumor growth and tumor cell repopulation after chemotherapy is the VEGF-mediated mobilization of circulating EPC after cytotoxic therapy. It is hypothesized that EPC are mobilized from the bone marrow and transported through the circulation to become incorporated into the walls of growing blood vessels,[30–32] thereby contributing to blood vessel formation and tumor regrowth after chemotherapy-induced cytotoxic effects. Both clinical and preclinical data showed substantial increases in viability and mobilization of EPC post-chemotherapy.[33–35] Notably, EPC induction by cytotoxic drugs seems to be drug-dependent. Paclitaxel, 5-fluorouracil (5-FU), and docetaxel cause acute elevations in viable EPC levels, unlike other cytotoxic agents (e.g. gemcitabine, cisplatin, and doxorubicin).[34] VEGF's role has been demonstrated as the rapid induction of EPC was blocked when an anti-VEGFR mAb was added prior to paclitaxel-containing chemotherapy. Furthermore, combining the anti-VEGFR mAb with paclitaxel yielded synergistic antitumor effects that could not be observed with gemcitabine.[34] However, debate is still ongoing concerning the identity and relative contribution to tumor angiogenesis of EPC, as extreme variability in the contribution of EPC to tumor vasculature were reported. Altogether, several ways may yield synergy between conventional chemotherapy and VEGF pathway-targeting drugs.

Combinations of bevacizumab and chemotherapy

Bevacizumab has been combined with various chemotherapeutic regimens in a wide range of tumor types. In general, combining bevacizumab with chemotherapy is safe, although exceptions exist; combining bevacizumab with doxorubicin in soft tissue sarcoma yielded a greater than expected cardiotoxicity.[36] Given the great number of studies on bevacizumab-containing regimens, only those combinations for which randomized data are available, and which have been published as full papers, will be addressed here.

Colorectal cancer. The first hint of bevacizumab's activity in metastatic CRC was observed in a phase III trial, comparing irinotecan, 5-FU, and leucovorin with or without bevacizumab. The addition of bevacizumab improved OS and PFS significantly. OS was 20.3 months in the combination arm, compared with 15.6 months for irinotecan, 5-FU, and leucovorin, whereas PFS was 10.6 and 6.2 months respectively. Furthermore, RR increased from 34.8% to 44.8%.[37] More recently, bevacizumab was explored in combination with two nowadays more widely used first-line treatment schedules for metastatic CRC; capecitabine plus oxaliplatin and FOLFOX-4. Again, PFS was improved in the combination arm, but only slightly (9.4 *vs* 8.0 months), while OS and RR were comparable.[38] In addition to combination regimens such as capecitabine plus oxaliplatin, monotherapy 5-FU or capecitabine is frequently used in patients considered unfit for combinations. The added value of bevacizumab to capecitabine, or capecitabine plus mitomycin C was investigated as first-line therapy for metastatic CRC. RR and PFS were significantly improved in the bevacizumab-containing regimens, but OS was unchanged.[39] Recently, the value of bevacizumab was assessed in stage II and III CRC, in which patients were treated with adjuvant FOLFOX-6 with or without bevacizumab. After a median follow up of 36 months, disease-free survival was comparable in both treatment arms.[40] In contrast to the findings in first-line and adjuvant settings, bevacizumab added to FOLFOX-4 significantly improved RR, PFS, and OS when given as second-line treatment for metastatic CRC.[41] In conclusion, bevacizumab added to conventional chemotherapy in CRC may enhance antitumor effects, but the extent to which this occurs is not fully elucidated, and seems to be dependent on regimen and setting.

Breast cancer. The first randomized study in MBC compared capecitabine with capecitabine plus bevacizumab as second- and third-line treatment of MBC. The combination regimen improved RR, but there was no PFS or OS benefit.[42] However, bevacizumab added to paclitaxel as first-line treatment of MBC did show a benefit in PFS. In this trial, bevacizumab combined with weekly paclitaxel significantly improved the RR from 21.2% to 36.9% and PFS from 5.9 to 11.8 months.[43] Although less striking, preliminary data showed that bevacizumab improves RR and PFS when added to docetaxel.[44] So also in MBC, the effects of bevacizumab seem regimen dependent.

Non-small cell lung cancer. Two randomized phase III studies explored bevacizumab with first-line chemotherapy in NSCLC. Bevacizumab with carboplatin and paclitaxel significantly improved both OS and PFS with 8 and 6 weeks respectively; however, this was at the cost of significant side effects in terms of bleeding, hypertension, and grade 4 neutropenia. Even though patients with squamous cell tumors were excluded, lethal pulmonary hemorrhage occurred in 1.2%.[45] In the second study (AVAiL), two dose levels of bevacizumab were combined with gemcitabine and cisplatin in advanced NSCLC. PFS was 6.1 months in the chemotherapy alone arm, compared with 6.7 and 6.5 months for the bevacizumab low and high dose, respectively. RR was 20.1% in the chemotherapy alone group, compared with 34.1% and 30.4% in the chemotherapy plus bevacizumab low and high dose groups, respectively. The incidence of serious adverse events and pulmonary hemorrhages were comparable in all groups.[46] So, bevacizumab modestly enhanced the outcomes of platinum-based chemotherapy, but at the expense of increased toxicity. Particularly in older patients, toxicity seems to outbalance antitumor activity[47] and as most NSCLC patients are of older age with comorbidity, only a minority of patients may benefit from bevacizumab added to chemotherapy.

Pancreatic cancer. Many approaches to improve the outcomes of the current standard in advanced pancreatic cancer (gemcitabine) have failed, including the addition of bevacizumab. In a large phase III trial, adding bevacizumab to gemcitabine failed to improve RR, PFS, and OS.[48] Data of bevacizumab added to gemcitabine/erlotinib were recently published. Median OS was equivalent in both groups, while adding bevacizumab significantly improved PFS (4.6 *vs* 3.6 months).[49]

Combinations of receptor TKI and chemotherapy

As previously mentioned, TKI harbor a broader range of activity than mAb. Consequently, TKI may be more effective than antibodies, but at the cost of more toxic effects. Accordingly, combinations of chemotherapy with VEGFR TKI seem less feasible than combinations of chemotherapy with bevacizumab. Unfortunately, data of randomized trials on VEGFR-targeting TKI-containing regimens are currently scarce. In addition to the few randomized trials, combinations explored in phase I, including toxicity and interaction issues that arise from these studies, are discussed. As the outcomes of single-arm efficacy studies on combinations without a control arm are hard to interpret, these will not be addressed.

Combinations of Sorafenib and Chemotherapy

Phase I on sorafenib-containing combinations. Numerous chemotherapeutic drugs have been combined with sorafenib and evaluated for toxicity, and pharmacokinetic interactions (Table 3). In the majority of these trials the toxicity profiles

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Table 3. Phase I trials combining sorafenib with chemotherapy

Study population	Patients (*n*)	Agent	End points	Reference
Refractory solid tumors (*n* = 15) and metastatic melanoma (*n* = 24)	39	Sorafenib (100, 200, 400 mg bid) Carboplatin (AUC6) and paclitaxel (225 mg/m^2)	DLT: 6 rash, 1 hypertension Melanoma: 1 CR, 9 PR, PFS 10.2 m, RR 26%	(54)
Refractory solid tumors (*n* = 27) and CRC (*n* = 10)	37	Sorafenib (200, 400 mg bid) Oxaliplatin (130 mg/m^2)	MTD not reached DLT: 2 diarrhea gr 3. 2 PR (6%), SD >10 w 43% solid tumors, 78% CRC	(53)
Refractory solid tumors (*n* = 19) and pancreatic cancer (*n* = 23)	42	Sorafenib (100, 200, 400 mg bid) Gemcitabine (1000 mg/m^2)	MTD not reached DLT: 1 fatigue gr 3 2 PR (11%), 25 SD	(52)
Refractory solid tumors (*n* = 34) and advanced HCC (*n* = 18)	52	Sorafenib (100, 200, 400 mg bid) Doxorubicin (60 mg/m^2)	MTD not reached DLT: 7 HFSR 1 gr 3 diarrhea Solid tumor: 1 PR, 15 SD (48%) HCC: 1 PR (6%), 10 SD (63%)	(50,51)
Refractory solid tumors (*n* = 20) and CRC (*n* = 14)	34	Sorafenib (100, 200, 400 mg bid) Irinotecan (125 mg/m^2 or 140 mg)	MTD not reached DLT: 1 hemorrhage, 2 HFSR 22 SD (67%), 1 PR	(55)

CR, complete response; CRC, colorectal carcinoma; DLT, dose-limiting toxicity; HCC, hepatocellular carcinoma; HFRS, hand–foot skin reaction; MTD, maximum tolerated dose; PFS, progression-free survival; PR, partial response; RR, response rate; SD, stable disease.

encountered were deemed acceptable and similar to the expected toxicity from each agent when given as monotherapy. Sorafenib (from day 4 at 100, 200, or 400 mg twice a day) in combination with doxorubicin (60 mg/m^2, every 3 weeks) has been explored in a dose escalation (n = 34), and an extension part (n = 18), the latter enrolling only advanced HCC patients.[50,51] The most frequent grade 3–4 drug-related adverse events were neutropenia (56%), lymphopenia (18%), fatigue (12%), and HFSR (12%). The frequency of cardiotoxicity was not higher than expected from monotherapy doxorubicin. In HCC patients, a high incidence of hepatic toxicity (change >2 grades from baseline) was observed: bilirubin (62%), albumin (24%), and alkaline phosphatase (17%). Furthermore, grade 3 diarrhea was observed (18%), and two patients withdrew from treatment due to adverse events (renal failure grade 4 and hepatic encephalopathy). DLT were experienced by eight patients (20%), mainly HFSR and diarrhea. The MTD was not reached. Sorafenib increased doxorubicin exposure, with an increase in AUC of 21% and C_{max} of 33%. The pharmacokinetics of sorafenib and one of doxorubicin's active metabolites, doxorubicinol, were not affected.[51]

Sorafenib continuously (100, 200, or 400 mg bid) has been combined with gemcitabine (1000 mg/m^2; day 1, 8, 15; every 4 weeks).[52] The most frequent adverse events were constitutional (fatigue 78.6%), gastrointestinal symptoms, dermatological, and bone marrow toxicities. Common grade 3–4 adverse events were thrombocytopenia (28.6%), lymphopenia (21.4%), lipase elevation (19%), neutropenia (16.7%), fatigue (14.3%), thrombosis (11.9%), and hypertension (7.1%). Grade 3–4 elevations in hepatic transaminases or bilirubin occurred in 5–10%. One DLT, grade 3 fatigue, was observed in the cohort with 400 mg bid sorafenib. Therapeutic dosages of gemcitabine and sorafenib (400 mg bid) could be administrated without reaching the MTD. No clinically relevant pharmacokinetic interaction between sorafenib and gemcitabine was observed.[52]

Sorafenib (200 or 400 mg continuously from day 4) was combined with oxaliplatin (130 mg/m^2) in 27 patients with refractory solid tumors, and 10 patients with refractory CRC in the extension part. Adverse events were generally mild to moderate. Common adverse events were diarrhea (43%), neuropathy (37%), and dermatological toxicities (51%). Two DLT were reported (grade 3 diarrhea), and the MTD was not reached. No pharmacokinetic interaction between sorafenib and oxaliplatin was found.[53]

The combination of sorafenib (100, 200, or 400 mg bid days 2–19) combined with paclitaxel (225 mg/m^2 every 3 weeks) and carboplatin (AUC6) showed promising results in 39 patients with advanced cancer, of which 24 were melanoma.[54] All patients experienced treatment-related adverse events, mostly hematological (95%), dermatological (85%), fatigue (59%), sensory neuropathy (59%), nausea (56%), and arthralgia (26%). Grade 4 neutropenia occurred in 62%, and HFSR grade 3 was reported in 23%. Seven patients experienced a DLT, grade 3 rash/HFSR in six patients, and hypertension in one patient. There was no clear dose-dependent relationship in treatment-related adverse events. The recommended phase II dose was sorafenib 400 mg bid, carboplatin AUC6, and paclitaxel 225 mg/m^2. Although clearance of paclitaxel is dependent on the cytochrome P450 enzymes, sorafenib had no apparent effect on the pharmacokinetics of paclitaxel. One complete response and nine partial responses were observed, all among patients with melanoma.[54]

Sorafenib (100, 200, and 400 mg bid) was combined with irinotecan (125 mg/m^2 on days 1, 8, 15, and 22 of each 6-week cycle) in patients with advanced solid tumors, and in an extended cohort in CRC patients, receiving fixed-dose irinotecan (140 mg weekly). Three DLT related to sorafenib were found, with sorafenib 400 mg bid, one cerebellar hemorrhage, and two HFSR. Frequent drug-related toxicities were gastrointestinal, dermatological, constitutional, and metabolic, mostly grade 1–2. Grade 3–4 adverse events were diarrhea (40%), infection/neutropenic fever (35%), leukopenia (10%), and neutropenia (5%). The MTD was not reached. Irinotecan had no impact on sorafenib's pharmacokinetics. In contrast, sorafenib doses higher than 200 mg bid significantly increased irinotecan and SN38 exposure; however, this was not associated with increased toxicity.[55]

Randomized trials on sorafenib-containing combinations. *Melanoma.* The promising results of sorafenib combined with paclitaxel and carboplatin in the abovementioned phase I trial prompted further studies in melanoma patients. Recently, a phase III study was published in which 270 patients with advanced melanoma received second-line therapy with carboplatin (AUC6) and paclitaxel (225 mg/m^2, every 3 weeks) with sorafenib (400 mg bid) or placebo. Disappointingly, no difference was observed in any of the end points of the study. Dermatological events (91% *vs* 73%), and fatigue (75% *vs* 57%) were more common in patients treated with chemotherapy plus

sorafenib.[56] Another study with a comparable design is expected to complete accrual in 2010.

Based on a phase I study, published as an abstract, it was shown that sorafenib (400 mg bid) can be safely combined with dacarbazine (1000 mg/m^2, every 3 weeks). This combination was compared with dacarbazine alone as first-line treatment in advanced melanoma patients (n = 101). A trend for improved PFS was observed for the sorafenib group (21.1 *vs* 11.7 months) without any difference in OS. The combination of sorafenib with dacarbazine in therapeutic dosage was well tolerated and had a manageable toxicity profile. Grade 3–4 adverse events were reported in 50% of patients in the control arm, and in 69% of patients in the sorafenib plus dacarbazine arm, 51% of the sorafenib-treated patients had grade 3–4 hematological toxicity.[57]

Combinations of Sunitinib and Chemotherapy

Phase I on sunitinib-containing combinations. Currently, several combinations of conventional chemotherapy and sunitinib are being studied in phase I/II settings, including combinations with ifosfamide, capecitabine, carboplatin plus paclitaxel, gemcitabine, irinotecan, gemcitabine plus cisplatin, and 5-FU plus irinotecan. Most combinations of sunitinib plus conventional chemotherapy seem feasible, but at the expense of increased hematological toxicity. The severity and frequency of neutropenia is probably determined by the dose and schedule of sunitinib, and on the toxicity profile of the cytotoxic agent used. For example, sunitinib combined with capecitabine resulted in grade 4 neutropenia in <10% of patients, whereas sunitinib in combination with irinotecan or carboplatin/paclitaxel resulted in grade 3–4 neutropenia in 30–60% of patients. Furthermore, sunitinib in combination with ifosfamide was not feasible without growth factor support.[58] However, none of these studies have thus far been published as full papers, and therefore will not be discussed in further detail. The same applies to randomized trials exploring sunitinib-containing combinations. Many such trials are ongoing but have not been published yet.

Other TKI

Phase I trials on vandetanib-containing combinations. Vandetanib is an orally administered TKI of VEGFR2, VEGFR3, RET, and EGFR. As monotherapy, it is well tolerated dosed at 300 mg per day.[59] In a phase I study, 21 patients with advanced NSCLC received vandetanib (100 or 300 mg) with pemetrexed (500 mg/m^2, every 3 weeks) as second-line therapy. Both dose levels were well tolerated. Two DLT were reported, asymptomatic QTc prolongation and interstitial lung disease, which resolved after steroid therapy. Most common adverse events were rash, anorexia, fatigue, and diarrhea (all approximately 50%), and most were grade 1–2. No pharmacokinetic interactions were found.[60]

The safety and tolerance of vandetanib plus FOLFOX-6 was recently investigated in patients with advanced CRC as first- or second-line chemotherapy. Seventeen patients received 14-day treatment cycles of mFOLFOX-6 plus vandetanib (100 or 300 mg). Both dose levels were tolerable, but a DLT (diarrhea) occurred in each cohort. Overall, the most common adverse events were diarrhea, nausea, lethargy (all 65%), neutropenia, and neuropathy (both 59%). There was no pharmacokinetic interaction. At steady-state exposure to vandetanib, there was an increase in exposure to oxaliplatin, but time-dependent increases have also been observed previously with oxaliplatin as monotherapy.[61]

Phase II randomized trials on vandetanib-containing combinations. Vandetanib has been studied with docetaxel in advanced NSCLC patients as second-line treatment in a randomized phase II study. Advanced NSCLC patients (n = 127) were treated with docetaxel (75 mg/m^2, every 3 weeks) combined with either placebo or vandetanib (100 or 300 mg). Diarrhea and rash were most frequent and severe in patients receiving vandetanib 300 mg. Patients in both vandetanib groups showed a modest increase in blood pressure at 6 weeks. Asymptomatic QTc prolongation was only observed in the vandetanib-treated patients. Though not adequately powered to detect small differences, RR and PFS were significantly improved in the vandetanib 100 mg group, compared with the other two groups. Combined use did not cause detectable changes in the pharmacokinetic profile of either drug.[62] Currently, a randomized phase III trial of docetaxel with vandetanib or placebo as second-line therapy for advanced NSCLC is ongoing.

In another randomized phase II study, the combination carboplatin/paclitaxel was compared with vandetanib monotherapy, and with carboplatin/paclitaxel combined with vandetanib in advanced NSCLC patients as first-line therapy. The vandetanib monotherapy arm was stopped early after an interim analysis. Treatment was tolerable in all three groups, but more patients receiving vandetanib plus carboplatin/paclitaxel experienced insomnia, anorexia, depression, grade 3–4 diarrhea, asymptomatic QTc interval prolongation, skin disorders, and hypertension. Neutropenia was the most frequently reported grade 3 adverse event, equally distributed among the chemotherapy-containing arms. A statistically significant improvement in PFS of only 1 week was observed in the group treated with vandetanib and chemotherapy, compared with chemotherapy alone. OS and RR were not significantly different. No detectable changes in pharmacokinetic exposure to vandetanib with the addition of carboplatin/paclitaxel were observed.[63]

Phase I trial on cediranib-containing combinations. The TKI cediranib targets VEGFR, PDGFRβ, and c-kit. In a phase I study, cediranib (daily 30 or 45 mg) was combined with carboplatin (AUC6) and paclitaxel (200 mg/m^2, every 3 weeks) in patients with advanced NSCLC as first-line therapy. Toxicity was manageable, and common side effects were fatigue, diarrhea, anorexia, and neutropenia. No DLT were reported. Steady-state levels of cediranib were comparable to those seen in single-agent therapy. Carboplatin clearance was unchanged, but paclitaxel clearance was decreased in cycle 2, which was reflected in the nadir of the platelet counts.[64]

In another phase I trial, cediranib (30 or 45 mg daily) was added to mFOLFOX-6 (every 14 days) in 16 metastatic CRC patients. One DLT, grade 3 diarrhea, was observed. Common grade 3 cediranib-related toxicities included hypertension, diarrhea, fatigue, and anorexia. There were no pharmacokinetic interactions between cediranib and 5-FU or free plasma oxaliplatin.[65] Currently, a phase III trial has been initiated, in which FOLFOX plus bevacizumab is compared with FOLFOX plus cediranib as first-line treatment of metastatic CRC.[66]

Combine targeted therapy and chemotherapy with caution, more is not always better

To further improve the outcomes of combinations of agents targeting the VEGF pathway and chemotherapy, recently two large studies have been published in which an EGFR-targeting drug was added in patients with metastatic CRC as first-line therapy. Unexpectedly, adding panitumumab or cetuximab, resulted in worse outcome and increased toxicity.[67,68] Panitumumab was added to bevacizumab and oxaliplatin- and irinotecan-based chemotherapy. PFS was 10.0 and 11.4 months, and OS was 19.4 and 24.5 months for the groups with or without panitumumab respectively.[67] Similarly, cetuximab, added to capecitabine, oxaliplatin, and bevacizumab, resulted in significantly shorter PFS, 9.4 compared with 10.7 months for patients with or without cetuximab respectively. OS and RR were comparable.[68] Although a combination of agents targeting multiple

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signal-transduction pathways appears reasonable, the results from these studies show that theory may differ from practice. The underlying mechanisms for these results are unclear. There are no available data of a possible pharmacokinetic interaction. A possible pharmacodynamic interaction induced by EGFR inhibition could have led to diminished therapeutic effects of bevacizumab and/or chemotherapy, perhaps through EGFR-mediated alterations of downstream targets required for the activity of bevacizumab, but this is speculative.

Conclusions and future perspectives

Though combining VEGF pathway inhibitors with conventional chemotherapy is theoretically attractive, this has currently only been proven for a few indications. Bevacizumab can improve the outcomes of conventional chemotherapy, but this is highly dependent on tumor type, stage, and chemotherapeutic regimen. With respect to kinase inhibitors, which in general are more difficult to combine with chemotherapy, randomized studies evaluating their added value are ongoing. Obviously, the availability of biomarkers enabling the identification of patients likely to benefit from combined regimens will augment the risk–benefit ratio of this approach. Biomarkers currently assessed include radiological tests to determine parameters such as vascular density, permeability, and volume.[27,29] With respect to soluble markers, baseline VEGF levels and outcome to antiangiogenic therapy as monotherapy have shown conflicting results. The predictive value in combination regimens remains to be established.[69,70] Increased levels of placental growth factor were associated with better outcome in CRC patients treated with bevacizumab and chemoradiation.[71] Furthermore, inflammatory proteins may be potential biomarkers; increased interleukin-6 levels were associated with worse outcome in patients with CRC and HCC, treated with bevacizumab and sunitinib respectively.[28,71] Also, circulating endothelial cells and EPC may emerge as useful biomarkers. Polymorphisms in the *VEGF* gene are another promising predictive factor. The *VEGF-2578 AA* genotype was associated with better OS in patients with MBC treated with bevacizumab and paclitaxel.[72] Whether or not this holds true for other combination regimens and other polymorphisms remains to be established. Clearly, the need for markers predictive for outcome to combinations of conventional chemotherapy and VEGF pathway-targeting drugs is high. In particular through the introduction of such predictive markers and thereby improved treatment individualization, combinations of conventional chemotherapy and VEGF pathway-targeting drugs are likely to redeem their great promise.

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Disclosure Statement

None.

Abbreviations

AUC	area under the curve
CRC	colorectal cancer
DLT	dose-limiting toxicity
EGFR	epidermal growth factor receptor
EPC	endothelial progenitor cell
FOLFOX	folinic acid/fluorouracil oxaliplatin
GIST	gastrointestinal stromal tumor
HCC	hepatocellular carcinoma
HFSR	hand–foot skin reaction
IFN	interferon
IFP	interstitial fluid pressure
MBC	metastatic breast cancer
MTD	maximum tolerated dose
NSCLC	non-small cell lung cancer
OS	overall survival
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PFS	progression-free survival
RCC	renal cell cancer
RR	response rate
TKI	tyrosine kinase inhibitor
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VHL	von hippel Lindau
5-FU	5-fluorouracil

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