Advances in Combination of Antiangiogenic Agents Targeting VEGF-binding and Conventional Chemotherapy and Radiation for Cancer Treatment

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Despite great efforts and resources being devoted to treatment, the incidence and mortality of numerous cancers have not decreased in recent decades. This is a result of the resistance of cancer cells to chemotherapeutic agents and radiotherapy. The development of antiangiogenic agents that target vascular endothelial growth factor (VEGF) provides a new option for treatment of cancer. Major advances have been achieved with cancer therapy based on antiangiogenic VEGF-targeted agents in the past few years, and some of the recently approved therapies are now being used in daily clinical practice. A further challenge is finding a more efficacious combination of antiangiogenic VEGF-targeted therapies and conventional radio- and chemotherapies. This review outlines the current preclinical and clinical cancer treatments using optimized combinations of antiangiogenic VEGF-targeted agents and conventional radiochemotherapy and highlights that better scheduling for the combination of radiochemotherapy and antiangiogenic VEGF-targeted agents should be developed to achieve better treatment outcomes. [*J Chin Med Assoc* 2010;73(6):281–288]

Key Words: antiangiogenic agents, bevacizumab, VEGF-targeted agents, VEGF-Trap

Introduction

Vascular endothelial growth factor (VEGF) stimulates proliferation and migration of endothelial cells and plays a pivotal role in vasculogenesis, angiogenesis, and endothelial integrity and survival.[1] VEGF is a crucial promoter of blood vessel growth during embryonic development and tumorigenesis.[2] To grow beyond a few millimeters in size, solid tumors must develop an angiogenic phenotype that promotes the establishment of an expanding vascular network for delivery of oxygen and other nutrients.[3] VEGF is well established as a central mediator in this process.[4,5] VEGF promotes endothelial cell proliferation, migration and survival, as well as mobilization of bone-marrow-derived endothelial precursors, in support of tumor angiogenesis. In addition, VEGF is a potent stimulator of vessel permeability, having originally been recognized for its function as a vascular permeability factor.[6] As a result of its fundamental role in tumor angiogenesis, VEGF serves as a logical target for antiangiogenic cancer therapy.

A number of antiangiogenic agents that target VEGF, including bevacizumab (Avastin; Roche, Nutley, NJ, USA), VEGF-Trap (Regeneron Pharmaceuticals, Tarrytown, NY, USA), and KH902/903 (Kanghong Biotech, Chengdu, Sichuan Province, China), have now been described and are currently in clinical trials, or are pending approval for clinical use in the treatment of cancer and other angiogenesis-dependent diseases.[7]

Dysfunctional tumor vessels can be a significant barrier to effective cancer therapy. However, increasing evidence[8,9] suggests that VEGF inhibitors can effect transient "normalization" of the tumor vasculature, thereby improving tumor perfusion and, consequently, delivery of systemic chemotherapy. This transient vessel normalization mechanism in tumor vessels treated

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with antiangiogenic agents that target VEGF remodels tumor vessels and partially overcomes the physiological barriers to drug and oxygen delivery within tumors through improvement in their functional efficiency, thus enhancing the delivery and antitumor activity of chemotherapy and radiation.

A further challenge is how to optimize the combination of antiangiogenic VEGF-targeted therapies and conventional radio- and chemotherapies. This review intends to integrate recent research results of antiangiogenic agents that target VEGF, e.g. bevacizumab (Avastin), VEGF-Trap, and the results of these agents in combination with radio- and chemotherapies, into basic information for developing a rational modality for cancer treatment.

Antiangiogenic VEGF-targeted Agents

Antiangiogenic VEGF-targeted agents, e.g. bevacizumab, and anti-VEGF immunoglobulin G (IgG) Fc-cytokine receptor molecules (VEGF-Trap, KH902/903) bind and neutralize VEGF.[10,11]

Bevacizumab is a humanized, monoclonal anti-VEGF antibody that neutralizes all isoforms of human VEGF.[12] It is the first antiangiogenic VEGF-targeted agent for cancer therapy approved by the United States Food and Drug Administration. *In vitro* studies have shown that bevacizumab causes decreased survival of human umbilical vascular endothelial cells and decreases VEGF-induced human umbilical vascular endothelial cell permeability.[13] This humanized antibody has been shown to inhibit bovine capillary endothelial cell proliferation in response to VEGF and has shown antitumor effects in many cancer cell lines.[14] In addition, preclinical studies have shown that bevacizumab has activity against metastases.[12,15]

VEGF-Trap is an engineered protein that contains extracellular domain 2 of VEGF receptor 1 (VEGFR1, Flt-1) and extracellular domain 3 of VEGFR2 (Flk-1/KDR) fused to the Fc portion of human IgG1,[16] and binds to all isoforms of VEGF and placental growth factor. The antitumor efficacy of VEGF-Trap has been investigated in several tumor xenograft models.[11,17,18]

KH902 is an engineered protein that contains extracellular domain 2 of VEGFR1 and extracellular domains 3 and 4 of VEGFR2 fused to the Fc portion of human IgG1. Previous results have indicated that it can efficiently bind VEGF.[19] It has also been suggested that KH902 has promise as a local antiangiogenic treatment of human choroidal neovascularization-related age-related macular degeneration.[19,20] Now, KH902 has been approved by the State Food and Drug Administration of China, and a phase II trial for human choroidal neovascularization-related age-related macular degeneration as well as a phase I trial for several solid tumors are ongoing.

Although continued VEGF inhibition is thought to maintain important antiangiogenic effects that keep tumor cells from growing and spreading, cessation of VEGF suppression might diminish those effects. In preclinical models, withdrawal of an anti-VEGF agent has been shown to result in regrowth of tumor vessels. In particular, both the rate and the amount of vascular regrowth observed following withdrawal of VEGF inhibition has been consistent with normal tumor development.[21,22] The current understanding of tumor biology, based primarily on preclinical observations, suggests that antitumor strategies must be made versatile over time to remain effective. As observed in preclinical models, the ability to maintain direct VEGF inhibition as part of an overall antitumor strategy might be a function of the specificity of direct VEGF inhibitors. This specificity might facilitate combination with approaches that target other mechanisms of tumor proliferation.[23,24]

In addition to antitumor activity demonstrated in single-agent experiments, direct VEGF inhibition has been shown to be active in combination with a range of modalities that target other mechanisms of tumor proliferation.[12] This ability to apply direct and continuous VEGF inhibition, either alone or with other modalities, could add versatility to an overall antitumor approach.

Antiangiogenic VEGF-targeted Agents in Combination With Conventional Radiation

One should be able to improve radiation response to inhibit tumor progression. Combination with antiangiogenic VEGF-targeted agents and radiation is a logical step. Preclinical and clinical studies have investigated the potential of combining antiangiogenic VEGF-targeted agents and radiation; these studies are summarized in Table 1.[25–30] The radiation treatments have involved single and fractionated schedules. For single radiation treatments, there are clear differences in the total doses given, whereas in the fractionated studies, not only do the total doses vary considerably, but there are also large differences in the number of fractions given and the time over which the doses are delivered. The lack of standardization in combination with antiangiogenic VEGF-targeted agents and radiation is obvious. It is true not only for the drug doses and treatment times used, but also for the different combination schedules applied with radiation. The schedules could

Table 1. Preclinical and clinical studies of bevacizumab (Avastin) in combination with radiation

Tumor type	Radiation schedule	Avastin schedule	Ref.
Preclinical			
Lewis lung carcinoma	2 × 20 Gy, d 0 + 1	10 μg/d, IP, d 0 + 1	25
SQ-20B squamous cell carcinoma	4 × 10 Gy, d 0–3	10 μg/d, IP, d 0–3	25
Seg-1 esophageal adenocarcinoma	4 × 5 Gy, d 0–3	10 μg/d, IP, d 0–3	25
U87 glioblastoma	8 × 5 Gy, d 0, 1, 4, 5, 7, 8, 11, and 12	10 μg/d, IP, d 0, 1, 4, 5, 7, 8, 11, and 12	25
U87 glioblastoma	8 × 5 Gy, d 0–3, and 7–10	5 or 25 μg/kg/d, IP, d 0–3	26
Seg-1 esophageal adenocarcinoma	4 × 5 Gy, d 0–3	5 or 25 μg/kg/d, IP, d 0–3	26
U87 glioblastoma	1 × 20–30 Gy, d 11	100 μg/d, IP, d 0, 2, 4, 6, 8, 10, and 12	27
LS1747 colon adenocarcinoma	1 × 20–30 Gy, d 11	100 μg/d, IP, d 0, 2, 4, 6, 8, 10, and 12	27
B16F10 murine melanoma cells	5 Gy at a dose rate of 1.4 Gy/min	10 mg/kg IV in a single injection	28
SCK murine mammary carcinoma	5 Gy at a dose rate of 1.4 Gy/min	10 mg/kg IV in a single injection	28
MA148 human ovarian carcinoma	5 Gy at a dose rate of 1.4 Gy/min	10 mg/kg IV in a single injection	28
Clinical			
Glioblastoma	60.0 Gy in 30 fractions started within 3–5 wk after surgery	10 mg/kg every 2 wk. Concurrently, temozolomide was given daily at 75 mg/m^2 for 42 d during radiation	29
Rectal cancer	50.4 Gy of external beam radiation therapy to the tumor in 28 fractions	15 mg/kg d 1 + 10 mg/kg d 8 and 22. Capecitabine (625 mg/m^2 bid) and oxaliplatin (50 mg/m^2/wk) were administered concurrently with radiation	30

IP = intraperitoneal; IV = intravenous; bid = twice daily.

include administering the VEGF-targeted agents during the radiation treatment,[25,26] before starting the radiation,[27] after completing the radiation, or in a combination of before, during and after radiation. Whether or not the combination of VEGF-targeted agents and radiation is superior to either treatment alone should be explored. In one preclinical study, it was reported that the transient modulation of tumor physiology caused by antiangiogenic VEGF-targeted therapy improved the effect of radiation treatment.[28] In that study, tumor growth delay was enhanced when single dose or fractionated radiotherapy was initiated within the tumor oxygenation window, as compared with other treatment schedules. Mechanistically, antiangiogenic VEGF-targeted agents are unable to sensitize tumor cells to radiation directly but they do exhibit varying levels of radiosensitization of endothelial cells, which leads to an improved radiation response. The study indicates that tumor endothelial cell sensitization and increased tumor tissue oxygenation *in vivo* are integral to the mechanism of action at both the cellular and physiological levels. The results are of immediate translational importance because the clinical benefits of bevacizumab therapy might be increased by more precise scheduling to ensure that radiation is given during periods of peak radiosensitivity. Ou et al have reported that the imaging of hypoxia-inducible factor-1 activity is useful in determining the oxygenation window. Their results suggest that an optimal window exists for combining bevacizumab with radiotherapy, which determines whether or not the combination will be beneficial.[31] One study has shown that VEGF-Trap plus radiation is clearly better than radiation alone in a U87 subcutaneous xenograft model, although high doses of VEGF-Trap alone are highly efficacious.[32]

Antiangiogenic VEGF-targeted Agents in Combination With Conventional Chemotherapy

Numerous studies have investigated the potential combination of VEGF-targeted agents with chemotherapeutic drugs, and these are summarized in Table 2.[33–53] As can be seen, the schedules used are highly variable. What is clear is that the majority of studies have reported an increased benefit of the combination approach, although in a few examples, no additional

Table 2. Preclinical and clinical studies of bevacizumab (Avastin) in combination with chemotherapy

Tumor type	Chemotherapy schedule	Avastin schedule	Ref.
Preclinical			
MCF-7 spheroids	Doxorubicin, 5 mg/kg, IV, 1×/wk	200 μg, IP, 2×/wk	33
SK-NEP-1 Wilms' tumor	Topotecan, 0.36 mg/kg, IP, d 7–11, 14–18, 28–32, and 3–39	100 μg, IP, 2×/wk for 5 wk	34
CWR22R prostate	Paclitaxel, 6.25 mg/kg, SC, 5×/wk for 3 wk	5 mg/kg, IP, 2×/wk for 4 wk	35
OVCAR3 tumors	Paclitaxel, 20 μg/g, IP, 2×/wk to 3×/wk for 6 wk	5 μg/g, IP, 2×/wk for 6 wk	36
HT29 colon cancer	CPT-11, 100 mg/kg, IP, d 7	200 μg, IP, d 0 + 4	37
MA148 human epithelial ovarian carcinoma	Carboplatin, 32.5 mg/kg, IP, every 3 d for 5 treatments starting d 10	2 mg, IP, every 3 d for 10 treatments starting d 10	38
NB-1691 human neuroblastoma	Topotecan 1 mg/kg	200 μg in a single injection	39
SK-N-AS human neuroblastoma	Topotecan 1 mg/kg	200 μg in a single injection	39
Clinical			
Breast cancer	Vinorelbine, 25 mg/m^2/wk IV	10 mg/kg IV every 2 wk	40
Breast cancer	Docetaxel, 35 mg/m^2/wk IV for 3 wk of a 4-wk cycle	10 mg/kg IV every 2 wk	41
Breast cancer	Docetaxel, 75 mg/m^2 IV every 3 wk	15 mg/kg IV every 3 wk	42
Breast cancer	Xeloda, 2,500 mg/m^2 orally daily for 2 wk of a 3-wk cycle	15 mg/kg IV every 3 wk	43
Breast cancer	Paclitaxel	10 mg/kg every 2 wk	44
Previously untreated mCRC	5-FU/LV, IV 5-FU 500 mg/m^2 and LV 500 mg/m^2/wk for the first 6 wk of an 8-wk cycle (Roswell Park regimen)	5 or 10 mg/kg every 2 wk	45
Previously untreated mCRC	5-FU/LV	5 mg/kg every 2 wk	46
Previously untreated NSCLC	Paclitaxel (200 mg/m^2) and carboplatin (6 mg/mL/min) on d 1, every 3 wk for 6 cycles	15 mg/kg, on d 1, every 3 wk	47
Previously untreated NSCLC	Paclitaxel (200 mg/m^2) and carboplatin (6 mg/mL/min) every 3 wk for 6 cycles	7.5 mg/kg or 15 mg/kg	48
Unresectable PC	Gemcitabine, 1,000 mg/m^2 IV over 30 min on d 1, 8, and 15 every 28 d	10 mg/kg after gemcitabine on d 1 and 15	49
Previously untreated mCRC	IFL, 5-FU 500 mg/m^2 + LV 20 mg/m^2 + irinotecan 125 mg/m^2 given 4/6 wk	5 mg/kg every 2 wk	50
Previously untreated mCRC	5-FU/LV, bolus 5-FU 500 mg/m^2 + LV 500 mg/m^2 given 6/8 wk	5 mg/kg every 2 wk	50
Previously untreated mCRC	FOLFOX-4, oxaliplatin 85 mg/m^2, LV 200 mg/m^2, bolus 5-FU 400 mg/m^2 and 600 mg/m^2	5 mg/kg every 2 wk	51
Previously untreated mCRC	FOLFOX-4, oxaliplatin 85 mg/m^2, LV 200 mg/m^2, bolus 5-FU 400 mg/m^2 and 600 mg/m^2	5 mg/kg every 2 wk	52
Previously untreated mCRC	XELOX, oxaliplatin 130 mg/m^2 IV, capecitabine 1,000 mg/m^2 bid oral d 1–14, q3w, 2 × 2 factorial design	7.5 mg/kg every 3 wk	52
Previously untreated mCRC	FOLFOX, oxaliplatin 85 mg/m^2, LV 350 mg, 5-FU bolus 400 mg/m^2 and 2,400 mg/m^2, CIV over 46 hr	5 mg/kg q14d or 7.5 mg/kg q21d	53
Previously untreated mCRC	bFOL, oxaliplatin 85 mg/m^2 d (d) 1&15, LV 20 mg/m^2 and bolus 5-FU 500 mg/m^2 d 1, 8, 15 q4w	5 mg/kg q14d or 7.5 mg/kg q21d	53
Previously untreated mCRC	CapeOx, oxaliplatin 130 mg/m^2 d 1, capecitabine 1,000–850 mg/m^2 bid for 14 d	5 mg/kg q14d or 7.5 mg/kg q21d	53

IV = intravenous; IP = intraperitoneal; SC = subcutaneous; mCRC = metastatic colorectal cancer; 5-FU = 5-fluorouracil; LV = leucovorin; NSCLC = non-small-cell lung cancer; PC = pancreatic cancer; IFL = irinotecan/5-FU/LV; FOLFOX-4 = oxaliplatin/5-FU/LV; XELOX = xeloda + oxaliplatin; bid = twice daily; FOLFOX = 5-FU/LV + oxaliplatin; CIV = common iliac vein; bFOL = oxaliplatin weekly + 5-FU + low-dose LV; CapeOx = capecitabine + oxaliplatin.

benefit was found. In clinical trials to date, the addition of bevacizumab to conventional chemotherapy has generally improved survival and response rate by 10–15% and has been shown to cause clinically evaluable changes in tumor physiology.[12,50] To date, the most convincing clinical study showing the potential benefit of combining VEGF-targeted agents and chemotherapy drugs comes from a phase III trial that has combined bevacizumab with irinotecan, fluorouracil and leucovorin (IFL) in previously untreated metastatic colorectal cancer.[50] In that study, patients were randomized to receive IFL plus bevacizumab or IFL and placebo, and the results showed that the addition of bevacizumab to the chemotherapy regimen significantly improved survival. However, not every combination study with bevacizumab has shown improved efficacy. Patients with metastatic breast cancer in a phase III trial did not benefit from the addition of bevacizumab to capecitabine.[43]

Other investigators have examined combination treatment with VEGF-Trap and conventional cytotoxic chemotherapy. One study has assessed the efficacy of VEGF-Trap combined with paclitaxel in a mouse model of human ovarian cancer, and has shown that tumor burden after VEGF-Trap plus paclitaxel was reduced by approximately 98% versus controls. Morphological analysis showed that most residual tumors had degenerative changes. Diaphragmatic and hepatic tumors were not found in the VEGF-Trap plus paclitaxel group in contrast to controls, which indicated a lack of metastasis. *In vivo* fluorescein-isothiocyanate-labeled lectin tumor vessel imaging showed sparse, short, straight vessels in treated mice compared with controls, in which vessels were numerous, irregular, tortuous, and leaky. It has been concluded that combination therapy with VEGF-Trap plus paclitaxel might provide a novel, long-lasting therapeutic strategy for treatment of patients with ovarian cancer associated with ascites. Correlative work has shown a significant decrease in tumor vasculature in tumors treated with VEGF-Trap and paclitaxel. Treatment with VEGF-Trap or paclitaxel alone has resulted in only modest rates of apoptosis (10% and 40%, respectively), whereas the combination of VEGF-Trap and paclitaxel has led to apoptosis in more than 90% of tumor cells.[54]

Adverse Effects of Antiangiogenic VEGF-targeted Agents in Combination With Conventional Radio- and Chemotherapy

As mentioned above, VEGF-targeted antiangiogenesis is an important strategy in the treatment of different cancers, alone or in combination with conventional radio- and chemotherapy. However, it is important to analyze the adverse effects of these agents in cancer as well as in normal tissues. The known adverse effects of bevacizumab in combination with chemotherapy are listed in Table 3.[40–46,50] Venous thromboembolism was the most significant adverse event, together with hypertension, proteinuria, and epistaxis in a randomized phase II study that evaluated the efficacy and safety of bevacizumab in combination with 5-fluorouracil plus leucovorin in patients with previously untreated advanced colorectal cancer.[45] Antiangiogenic VEGF-targeted agents could also cause decreased matrix deposition in the supporting layers of vessels.[55] Therefore, the final picture of antiangiogenic VEGF-targeted therapy might consist of not only a tendency to bleed, but also an increased frequency of thrombotic events. Bevacizumab combined with IFL produced a higher risk for hypertension and epistaxis. In 2% of patients, there were wound-healing problems and gastrointestinal perforations.[56] Furthermore, in a phase III trial of the paclitaxel–carboplatin combination versus paclitaxel, carboplatin and bevacizumab as first-line treatment in 878 patients with advanced non-squamous non-small-cell lung cancer, fatal pulmonary bleeding was observed in 1.2% of the patients.[47] Preliminary toxicity data of VEGF-Trap are consistent with inhibition of the VEGF pathway. The most common grade 3/4 toxicities are proteinuria, hypertension, venous thromboembolic disease, and leukopenia.[57]

In conclusion, antiangiogenic agents that target VEGF have an additive or synergistic effect when used in combination with conventional chemotherapy or radiotherapy, as mentioned above. One potential explanation hypothesized for this synergy is that, as antiangiogenic agents begin to restore a balance between pro- and antiangiogenic cytokines, tumor vessels, at least transiently, display a structural and functional phenotype more reflective of normal blood vessels.[58] This process, termed vascular normalization, remodels tumor vessels, and enhances delivery and perfusion of conventional chemotherapies. This process of vascular normalization seems to be transient, however, with a relatively narrow window during which synergy is likely to be achieved, and after which, the tumor vasculature is destroyed. On this basis, better scheduling for combination of radiochemotherapy and antiangiogenic VEGF-targeted agents should be developed to achieve a better treatment outcome for cancer.

This review has partially outlined the current preclinical and clinical treatments of cancer with combination of antiangiogenic VEGF-targeted agents and

Table 3. Adverse effects of bevacizumab (Avastin) in combination with chemotherapy

Adverse effect	Incidence of chemotherapy (total patients)	Incidence of bevacizumab in combination with chemotherapy (total patients)	Ref.
Hypertension	8.3% (397)	22.4% (393)	50
	0.5% (215)	17.9% (229)	43
	Data not shown	25.5% (55)	40
	Data not shown	18.5% (27)	41
	2.0% (332)	<16.0% (350)	44
	3% (35)	11% (35, 5 mg/kg); 28% (32, 10 mg/kg)	45
	2.9% (104)	16.0% (100)	46
Proteinuria	21.7% (397)	26.5% (393)	50
	0% (215)	0.9% (229)	43
	Data not shown	25.5% (55)	40
	Data not shown	40.7% (27)	41
	0% (332)	2.0% (350)	44
	11% (35)	23% (35, 5 mg/kg); 28% (32, 10 mg/kg)	45
	0% (104)	1.0% (100)	46
Thrombotic event	16.2% (397)	19.4% (393)	50
	3.7% (215)	5.6% (229)	43
	Data not shown	3.6% (55)	40
	Data not shown	7.4% (27)	41
	4.0% (332)	2.0% (350)	42
	9% (35)	26% (35, 5 mg/kg); 13% (32, 10 mg/kg)	45
	18.3% (104)	18.0% (100)	46
Bleeding	2.5% (397)	3.1% (393)	50
	0.5% (215)	0.4% (229)	43
	0% (332)	<3.0% (350)	44
	2.9% (104)	5.0% (100)	46
GI bleeding	0% (35)	6% (35, 5 mg/kg); 16% (32, 10 mg/kg)	45
Vomiting	1.9% (215)	2.6% (229)	43
	Data not shown	40.0% (55)	40
Fatigue	Data not shown	85.1% (27)	41
Neurosensory	Data not shown	50.9% (55)	40
Neuropathy	Data not shown	18.5% (27)	41
Neutropenia	Data not shown	25.9% (27)	41
Epistaxis	Data not shown	20.0% (55)	40
	Data not shown	3.7% (27)	41
	11% (35)	46% (35, 5 mg/kg); 53% (32, 10 mg/kg)	45
Pericardial effusion	Data not shown	0.02% (55)	40
CHF/cardiomyopathy	1% (215)	3.0% (229)	43
Leukopenia	3% (35)	11% (35, 5 mg/kg); 3% (32, 10 mg/kg)	45
	Data not shown	37.0% (27)	41
Stomatitis	Data not shown	40.7% (27)	41
Infection	Data not shown	18.5% (27)	41
Eye tearing	Data not shown	55.6% (27)	41
Hand-foot syndrome	24.2% (215)	27.5% (229)	43
Dyspnea	Data not shown	70.4% (27)	41
Diarrhea	83% (35)	91% (35, 5 mg/kg); 75% (32, 10 mg/kg)	45

GI = gastrointestinal; CHF = congestive heart failure.

conventional radiochemotherapy, to optimize this kind of combination.

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