Clinical Applications of VEGF-Trap (Aflibercept) in Cancer Treatment

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Angiogenesis is one of the key acquired characteristics or “hallmarks” essential for the growth and development of all solid tumor types. The antiangiogenic agent vascular endothelial growth factor-Trap (VEGF-Trap) (aflibercept), which is a composite decoy receptor based on VEGF receptor-1 and VEGF receptor-2 fused to an Fc segment of immunoglobulin G1 that binds specifically to VEGF, has demonstrated preclinical efficacy in a range of different tumor types. VEGF-Trap exerts its antiangiogenic effects through regression of tumor vasculature, remodeling or normalization of surviving vasculature, and inhibition of new tumor vessel growth. Preclinical and clinical studies have reported that VEGF-Trap can be combined effectively with both chemotherapy and radiotherapy. This review examines the main effects of VEGF-Trap on tumor vasculature and on different types of solid tumors, and explores the preclinical and clinical benefits of incorporating VEGF-Trap into anticancer treatment strategies. [J Chin Med Assoc 2010;73(9):449–456]

Key Words: angiogenesis, antiangiogenic agent, clinical application, VEGF-Trap

Introduction

Angiogenesis is the process of new blood vessel formation. It is an important process in the growth of malignant tumors as tumors need to establish their own blood supply to grow beyond 1–2 mm in diameter. The predominant regulator of tumor angiogenesis is vascular endothelial growth factor (VEGF),1,2 which is the only angiogenic factor known to be present throughout the entire tumor lifecycle.3 This continuous expression, along with the proposed genetic stability of VEGF and endothelial cells,2,4 makes direct and continuous targeting of VEGF an important antitumor strategy.3

VEGF-Trap (aflibercept), an engineered protein which contains the extracellular domain 2 of VEGF receptor-1 (VEGFR-1, Flt-1) and extracellular domain 3 of VEGFR-2 (Flk-1, KDR) fused to the Fc portion of human immunoglobulin G1 (Figure 1), binds to all isoforms of VEGF and to placental growth factor.5 VEGF-Trap exerts its antiangiogenic effects through regression of tumor vasculature,6,7 remodeling or normalization of surviving vasculature,8,9 and inhibition of new tumor vessel growth.9 Given the central role of angiogenesis in tumor development, antiangiogenic agents should be considered a pillar of anticancer therapy, alongside surgery, radiotherapy, chemotherapy, and hormonal therapy.

This review examines the effects of VEGF-Trap on tumor vasculature, and how these translate into preclinical and clinical efficacy. This review also examines the preclinical and clinical benefits of incorporating VEGF-Trap into anticancer treatment strategies, including which agents can be combined with VEGF-Trap.

Effect of VEGF-Trap on Tumor Vasculature

VEGF-Trap is a composite decoy receptor based on VEGFR-1 and VEGFR-2 fused to an Fc segment of immunoglobulin G1 that binds specifically to VEGF,
preventing activation of the VEGF receptor. VEGF-Trap inhibits VEGF extracellularly and may therefore inhibit angiogenesis without disrupting targets outside the VEGF pathway. Based on preclinical models, it has been proposed that VEGF-Trap exerts antivascular effects by rapid regression of existing tumor vessels, as shown by significant reductions in microvascular density, remodeling or normalization of surviving mature vasculature, and ongoing inhibition of new tumor vessel growth. This results in maintenance of a more functional and normal vasculature, potentially improved capacity for drug delivery, and inhibition of tumor growth and metastasis.

Preclinical models have indicated that VEGF-Trap can cause regression of existing tumor vasculature. Significant reductions in tumor vascular volume and density following a single infusion of VEGF-Trap alone have been reported. In a preclinical model using human neuroblastoma xenografts, high doses of VEGF-Trap can lead to regression of co-opted vascular structures. Huang et al. reported that VEGF-Trap abolishes mature, preexisting vasculature in established xenografts. Fukasawa and Korc. found that VEGF-Trap, initiated 2 days after tumor cell inoculation, markedly decreases tumor microvessel density in the subcutaneous growth of 4 pancreatic cancer cell lines. Kadenhe-Chiweshe et al. found that treatment with VEGF-Trap in late-stage hepatoblastoma xenografts results in the initial collapse of the vasculature. Frischer et al. reported that VEGF-Trap induces regression of the vessels of established Wilms’ tumor xenografts. Treatment with VEGF-Trap nearly eradicates tumor vasculature. Rare persisting vessels are characterized by a large caliber, quiescence, and arterialization (both phenotypic and molecular), resulting in nearly avascular tumors. Persistent vessels in tumors treated with VEGF-Trap display specific morphologic and molecular features, suggestive of arterialization. Huang et al. confirmed this finding.

Similar to Avastin, which results in the inhibition of new vessel growth in preclinical models, VEGF-Trap has a marked effect on preexisting or newly formed vessels, which are required for tumor growth and metastasis. In preclinical models, VEGF-Trap has been shown to remodel or normalize the surviving mature vasculature of tumors.

Inai et al. reported that treatment with VEGF-Trap causes robust and early changes in endothelial cells, pericytes, and the basement membrane of vessels in spontaneous islet-cell tumors of RIP-Tag2 transgenic mice and in subcutaneously implanted Lewis lung carcinomas. They found that within 24 hours, endothelial fenestrations in RIP-Tag2 tumors disappeared, vascular sprouting was suppressed, and patency and blood flow ceased in some vessels. By 7 days, vascular density decreased more than 70%. In both tumors, pericytes did not degenerate to the same extent as endothelial cells, and those on surviving tumor vessels acquired a more normal phenotype.
remodeling of the blood vessels in disseminated ovarian carcinoma. Baffert et al.17 identified the sequence of events and the fate of endothelial cells, pericytes, and the vascular basement membrane during capillary regression in mouse tracheas after VEGF signaling was blocked with VEGF-Trap. Within 1 day, patency was lost and fibrin accumulated in some tracheal capillaries. Apoptotic endothelial cells marked by activated caspase-3 were present in capillaries without blood flow. VEGF inhibition was accompanied by a 19% decrease in tracheal capillaries over 7 days and 30% over 21 days. During this period, pericytes moved away from regressing capillaries onto surviving vessels. Empty sleeves of basement membrane were left behind by regressing endothelial cells, persisted for approximately 2 weeks, and served as a scaffold for vascular regrowth after treatment ended. The amount of regrowth was limited by the number of surviving basement membrane sleeves. Baffert et al.’s17 findings demonstrate that after treatment with VEGF-Trap, some normal capillaries regress in a systematic sequence of events initiated by a cessation of blood flow, followed by apoptosis of endothelial cells, migration of pericytes away from regressing vessels, and formation of empty basement membrane sleeves that can facilitate capillary regrowth.

These effects of VEGF-Trap on tumor vascular may help make tumor cells more sensitive to cytotoxic chemotherapy.19–21 In terms of clinical significance, normalization of tumor vasculature by VEGF-Trap can maximize efficacy of concomitant therapy. The unregulated nature of tumor angiogenesis leads to the production of structurally and functionally abnormal vasculature, characterized by a number of different features as follows: increased vessel density, diameter, length and tortuosity, abnormally high interstitial fluid pressure, and increased vascular permeability. These abnormalities prevent the effective delivery of therapy to the tumor. The entry of large molecules, such as chemotherapeutic agents, into the tumor is impeded. Hypoxia results from inconsistent oxygen supply within the tumor, producing regions that are resistant to radiotherapy and some cytotoxic agents.19,20 Antiangiogenic therapy, such as VEGF-Trap, normalizes the tumor vasculature, pruning excess vessels and reducing interstitial fluid pressure and vessel permeability. This increases both the delivery of anticancer agents to the tumor and the sensitivity of tumor cells to those agents.

Effect of VEGF-Trap on Solid Tumors

Solid tumors are unable to grow beyond a size of 1–2 mm, or undergo further growth and metastasis, without the ability to establish their own blood supply through angiogenesis.2,4,22 An anticancer agent such as VEGF-Trap, which inhibits this process, should therefore have activity against all types of solid tumors. Preclinical studies of VEGF-Trap in different tumor types have demonstrated that VEGF inhibition produces preclinical benefits for the xenograft models (Table 1).6–8,10–12,23–26 Holash et al7 found that VEGF-Trap effectively suppresses tumor growth and vascularization in vivo, resulting in stunted and almost completely avascular tumors. In a preclinical model using human neuroblastoma xenografts, high-dose VEGF-Trap causes the greatest inhibition of tumor growth (81% compared with controls).6 Huang et al10 reported that VEGF-Trap causes marked tumor regression, including
regression of lung micrometastases. Byrne et al found that systemic administration of VEGF-Trap inhibits the growth of disseminated cancer. Fukasawa and Korc found that VEGF-Trap, initiated 2 days after tumor cell inoculation, suppresses the subcutaneous growth of 4 pancreatic cancer cell lines and also attenuates intrapancreatic tumor growth and metastasis in an orthotopic model using PANC-1 cells when initiated 3 weeks after tumor implantation. Therefore, VEGF-Trap may represent an exceedingly useful therapeutic modality for pancreatic ductal adenocarcinoma. Frischer et al reported that VEGF-Trap delays subcutaneous growth in the 6 Ewing’s sarcoma family of tumor cell line xenograft models. Kadenhe-Chiweshe et al found that treatment with VEGF-Trap in late-stage hepatoblastoma xenografts results in significant tumor regression. Verheul et al tested the activity of VEGF-Trap in an orthotopic murine model of renal cancer with spontaneous lung metastases. In the prevention model, VEGF-Trap inhibited tumor growth by 87 ± 14% compared with controls (p = 0.007) and significantly prolonged survival. In the intervention model, VEGF-Trap inhibited tumor growth by 74 ± 9% (p < 0.001), and the formation of lung metastases was inhibited by 98% (p < 0.004). The investigators concluded that VEGF-Trap is a potent inhibitor of renal cell carcinoma growth and metastasis formation. These results support further clinical development of VEGF-Trap for renal cell cancer and other cancer types. Gomez-Manzano et al tested VEGF-Trap in an intracranial glioma model. They found that VEGF-Trap treatment was efficacious in both initial and advanced phases of tumor development by significantly increasing overall survival. Furthermore, this effect was enhanced in animals treated with more prolonged regimens. These results suggest that VEGF-Trap could be effective in treating both patients with recurrent or progressive resectable glioblastoma and patients who have undergone extensive initial surgery, and indicate that the clinical success of VEGF-Trap may depend on a prolonged treatment in combined therapy aiming to simultaneously inhibit angiogenesis and tumor invasion. Le et al found that VEGF-Trap as a single treatment inhibited tumor microvessel density, tumor vasculature, cell proliferation, and tumor growth of BT474 xenografts in a dose-dependent manner from 2.5 mg/kg to 25 mg/kg. They also found that VEGF-Trap decreased levels of both human VEGF and placental growth factor protein in vivo. Lu et al reported that treatment with VEGF-Trap caused 40–50% reductions in the growth of A549 non-small cell lung cancer and PANC-1 pancreatic cancer xenografts and reduced microvessel density in the tumors.

**Preclinical and Clinical Benefits of Incorporating VEGF-Trap into Anticancer Treatment Strategies**

The precise nature of VEGF inhibition by VEGF-Trap makes this agent an ideal partner for combination with approaches aimed at other mechanisms of tumor proliferation. Additional benefit may be gained through normalization of tumor vasculature by VEGF-Trap, which reduces interstitial pressure and increases the delivery of other agents to the tumor. The ability to apply direct and continuous VEGF inhibition with VEGF-Trap in combination with other treatment modalities may increase the available therapeutic options in a number of tumor types. The specific chemotherapy, radiotherapy, and targeted therapy agents used in clinical practice vary considerably between tumor types. Preclinical and clinical studies have investigated VEGF-Trap in combination with the most frequently used regimens in each indication (Table 2).

Hu et al examined combination treatment with VEGF-Trap and conventional cytotoxic chemotherapy. Their study assessed the efficacy of VEGF-Trap combined with paclitaxel in a mouse model of human ovarian cancer, and they showed that tumor burden after VEGF-Trap plus paclitaxel was reduced by approximately 98% versus controls. Diaphragmatic and hepatic tumors were not found in the VEGF-Trap plus paclitaxel group in contrast to controls, indicating a lack of metastasis. The investigators concluded that combination therapy with VEGF-Trap plus paclitaxel may provide a novel, long-lasting therapeutic strategy for treatment of patients with ovarian cancer. Le et al found that dual targeting of VEGF (with VEGF-Trap) and human epidermal growth factor receptor-2 (HER2) (with trastuzumab) pathways results in greater growth inhibition of HER2-overexpressing breast cancer xenografts than either agent alone. They found that treatment with a combination of VEGF-Trap (2.5–10 mg/kg) and trastuzumab (1 mg/kg) produced a significantly greater inhibition of BT474 tumor growth than that of either individual agent, and was associated with a greater inhibition of tumor microvessel density and tumor cell proliferation. Thus, VEGF-Trap in combination with trastuzumab produces superior
growth inhibition of tumor xenografts that overexpress HER2, which may result from inhibition of both tumor angiogenesis and proliferation. This finding indicates that similar mechanisms may contribute to the clinical antitumor activity of trastuzumab in combination with inhibitors of the VEGF signaling pathway in women with breast cancer that overexpress HER2.26 Lu et al27 reported that the combination of

### Table 2. Preclinical and clinical studies of VEGF-Trap (aflibercept) alone and in combination with anticancer treatment strategies

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<td><strong>BT474 breast cancer</strong></td>
<td>(1) VEGF-Trap (2.5 mg/kg) alone, twice weekly for 3 wk; (2) VEGF-Trap (2.5 mg/kg) + trastuzumab (1 mg/kg), twice weekly for 3 wk</td>
<td>VEGF-Trap + trastuzumab has greater inhibition effect than either alone, especially in tumors overexpressing HER2</td>
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<td><strong>OVCAR-3 ovarian cancer</strong></td>
<td>(1) VEGF-Trap (10 mg/kg) alone, thrice weekly for 4 wk; (2) VEGF-Trap (10 mg/kg) + paclitaxel (10 mg/kg), thrice weekly for 4 wk</td>
<td>VEGF-Trap + paclitaxel is a novel strategy for treatment of ovarian cancer</td>
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<td><strong>U87 glioblastoma</strong></td>
<td>(1) VEGF-Trap (2.5–25 mg/kg) alone, every 3 d for 3 wk; (2) VEGF-Trap (2.5–25 mg/kg), every 3 d for 3 wk + a single dose of 10 Gy or fractionated RT (3 × 5 Gy) in 3 scheduling protocols</td>
<td>VEGF-Trap + radiation is a better strategy than radiation alone for treatment of glioblastoma</td>
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<td>47</td>
<td>VEGF-Trap (0.3–7.0 mg/kg) alone i.v. every 2 wk</td>
<td>Well tolerated</td>
<td>30</td>
<td></td>
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<td>6</td>
<td>VEGF-Trap (2.0 mg/kg, 4.0 mg/kg) + FOLFOX-4 i.v. every 2 wk</td>
<td>Safe</td>
<td>31</td>
<td></td>
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<td>32</td>
<td>VEGF-Trap (2.0 mg/kg, 4.0 mg/kg, 5.0 mg/kg) + FOLFOX-4 i.v. every 2 wk</td>
<td>Safe</td>
<td>32</td>
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<td>10</td>
<td>VEGF-Trap (2.0 mg/kg, 4.0 mg/kg) + I-LV5FU2 i.v. every 2 wk</td>
<td>Safe</td>
<td>33</td>
<td></td>
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<td>27</td>
<td>VEGF-Trap (4.0 mg/kg) + I-LV5FU2 (standard dose) i.v. every 2 wk</td>
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<td>VEGF-Trap (2.0–6.0 mg/kg) + I-LV5FU2 (fixed standard dose) i.v. every 2 wk</td>
<td>Safe</td>
<td>35</td>
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<td>54</td>
<td>VEGF-Trap (2.0–9.0 mg/kg) + docetaxel (fixed dose 75 mg/m²) i.v. every 3 wk</td>
<td>Safe</td>
<td>36</td>
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<td>16</td>
<td>VEGF-Trap (4.0, 5.0, 6.0 mg/kg) + docetaxel (fixed dose 75 mg/m²) &amp; cisplatin (fixed dose 75 mg/m²) i.v. every 3 wk</td>
<td>Safe</td>
<td>37</td>
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<td>32</td>
<td>VEGF-Trap (4.0, 6.0 mg/kg) i.v. every 2 wk followed by standard weekly i.v. gemcitabine (7 wk on/1 wk off, then 3 wk on/1 wk off every 4 wk)</td>
<td>Safe</td>
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<td>2 or 4 mg/kg, i.v. every 2 wk</td>
<td>With antitumor effect on EOC population</td>
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<td>12</td>
<td>4 mg/kg, i.v. every 2 wk</td>
<td>With activity in prolonging time to repeat paracentesis</td>
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<td>51</td>
<td>4 mg/kg, i.v. every 2 wk</td>
<td>Well tolerated</td>
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<td>94</td>
<td>4 mg/kg, i.v. every 2 wk</td>
<td>Well tolerated</td>
<td>42</td>
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<tr>
<td>38</td>
<td>4 mg/kg, i.v. every 2 wk</td>
<td>Well tolerated</td>
<td>43</td>
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<td>22</td>
<td>4 mg/kg, i.v. every 2 wk</td>
<td>Well tolerated</td>
<td>44</td>
<td></td>
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<tr>
<td>27</td>
<td>4 mg/kg, i.v. every 2 wk</td>
<td>Safe</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>4 mg/kg, i.v. every 2 wk</td>
<td>Well tolerated</td>
<td>46</td>
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VEGF = vascular endothelial growth factor; HER2 = human epidermal growth factor receptor-2; RT = radiation treatment; i.v. = intravenously; FOLFOX-4 = oxaliplatin/5-fluorouracil/leucovorin; I-LV5FU2 = irinotecan, 5-fluorouracil and leucovorin; EOC = epithelial ovarian cancer.
6-(2-aminoethyl)amino-5-chlorouracil, a potent and specific small-molecule inhibitor of the catalytic activity of thymidine phosphorylase, and VEGF-Trap produced additive antitumor activity that was significantly greater than VEGF-Trap alone in A549 non-small cell lung cancer and Panc-1 pancreatic cancer xenografts. These studies suggest that inhibitors of thymidine phosphorylase could be used to augment the clinical efficacy of VEGF-Trap. Wachsberger et al reported that VEGF-Trap plus radiation was clearly better than radiation alone in a U87 subcutaneous xenograft model. Although high-dose VEGF-Trap (10 mg/kg or 25 mg/kg) significantly reduces tumor growth over that of radiation treatment alone, there is no additional benefit to combining high-dose VEGF-Trap with radiation treatment, and it is unclear whether such high doses can be used clinically without incurring normal tissue toxicities. Thus, information on lower doses of VEGF-Trap and ionizing radiation is of clinical relevance.

The safety, pharmacokinetics, and pharmacodynamics of VEGF-Trap administered intravenously (IV) every 2 weeks were evaluated in a phase I clinical trial. The study enrolled 47 patients with refractory solid tumors or non-Hodgkin’s lymphoma with adequate organ function at doses ranging from 0.3 to 7.0 mg/kg IV every 2 weeks. Dose-limiting toxicities were rectal ulceration and proteinuria at the 7.0 mg/kg dose. Other mechanism-specific toxicities included hypertension. On the basis of these observations and pharmacokinetics, the recommended phase II dose of VEGF-Trap as a single agent is 4 mg/kg every 2 weeks. It was concluded that IV VEGF-Trap is well tolerated at the dose levels tested.

In phase I trials, large safety studies have explored the combination of VEGF-Trap with a variety of chemotherapy agents and regimens such as FOLFOX-4, I-LV5FU2, docetaxel, docetaxel and cisplatin, and gemcitabine in advanced solid tumors. Phase II trials in several diseases are ongoing, including ovarian cancer, metastatic colorectal cancer, platinum- and erlotinib-resistant non-small cell lung adenocarcinoma, recurrent or metastatic gynecologic soft-tissue sarcomas, recurrent or metastatic transitional cell carcinoma, recurrent inoperable stage III or stage IV melanoma, and recurrent temozolomide-resistant glioblastoma.

In conclusion, angiogenesis is a hallmark of cancer throughout the lifecycle of the tumor. The predominant proangiogenic factor at the centre of the angiogenic pathway is VEGF. The continuous expression of VEGF by the tumor makes it a rational target for cancer therapy. Direct inhibition of VEGF by VEGF-Trap allows for greater precision than other means of targeting the VEGF pathway, thereby avoiding unwanted inhibitory effects on non-VEGF-mediated functions. The key role of angiogenesis in tumor growth and development suggests that the angiogenesis inhibitor VEGF-Trap is likely to have potential benefits in the treatment of multiple tumor types. Numerous preclinical studies have demonstrated the efficacy of VEGF-Trap in ovarian cancer, breast cancer, non-small cell lung cancer, pancreatic cancer, neuroblastoma, glioblastoma, glioma, hepatoblastoma, Ewing’s sarcoma family of tumors, Wilms’ tumor, and renal cell cancer xenograft models. In preclinical studies, VEGF-Trap has proven its efficacy in combination with a broad range of conventional anticancer therapies, including cytotoxic chemotherapy, radiotherapy, and targeted therapy.

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