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Vascular Endothelial Growth Factor (VEGF) Pathway as a Therapeutic Target in Gynecologic Malignancies

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Abstract

Vascular endothelial growth factor (VEGF) plays a critical role in angiogenesis, which is required for tumor growth and metastasis. In this article, a review of the functional and biological roles of the VEGF pathway in driving angiogenesis and growth of gynecologic malignancies was performed. Based on the biological functions of VEGF, multiple approaches for targeting the VEGF/VEGF-receptor complex have been developed and many of these have demonstrated substantial activity in preclinical models. These promising data have led to rapid clinical development of VEGF-targeted agents. Therefore, we also assessed the status of VEGF-targeted therapies and associated toxicities in gynecologic malignancies. However, many questions remain related to optimal dosing, sequencing of therapies, management of toxicities, appropriate patient selection, and assessment of response, which will require further studies. Nevertheless, VEGF-targeted therapies offer hope for improving the outcome of cancer patients.

Keywords

Vascular endothelial growth factor (VEGF); angiogenesis; gynecologic cancer

Angiogenesis Pathways

Angiogenesis is an important physiological process during fetal development and growth as well as in mature tissue remodeling and repair. For cancer expansion and dissemination, both primary lesions and metastatic tumors must develop a new vascular supply in order to survive. Without angiogenesis, it is thought that tumor implants would be unable to grow beyond 1 mm in size [1]. Angiogenesis is important for supplying a variety of substances including oxygen, nutrients, hormones, and growth factors. Angiogenesis is tightly regulated by balancing pro- and anti-angiogenic factors. The early initiation of angiogenesis, or the “angiogenic switch”, is essential for cancer survival and occurs when stimulatory factors overcome inhibitory factors

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Précis

This review comprehensively discusses the VEGF pathway and VEGF-targeted therapies in gynecologic malignancies

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promoting the formation of new blood vessels [2]. This favorable balance for development of neovasculature allows for relentless tumor growth.

Research over the last several decades has demonstrated that tumor neovascularization is highly complex and likely to involve multiple pathways. Angiogenesis can occur by sprouting or nonsprouting processes [3]. Sprouting angiogenesis occurs by branching of new capillaries from preexisting blood vessels whereas nonsprouting angiogenesis results from enlargement, splitting, and fusion of preexisting vessels produced by proliferation of endothelial cells within the vessel wall. Another mechanism called vessel cooption is likely to play a role in the spread of many solid tumors whereby tumor cells initially attach to the preexisting host vasculature [4]. This cooption is followed by destabilization of the host vasculature, central necrosis, increased production of angiogenic cytokines and finally, recruitment of new blood vessels at the periphery. An additional mechanism involves vasculogenic mimicry, which reflects the ability of aggressive tumor cells to express vascular-associated genes and form vasculogenic-like networks [5,6]. Thus, to develop effective anti-angiogenic approaches, it is important to understand the various mechanisms involved in tumor neovascularization.

Research investigating the molecular basis of angiogenesis has identified multiple pathways that contribute to tumor angiogenesis. These include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF). Based on the central role of VEGF in tumor angiogenesis and growth, it has emerged as the most promising therapeutic target for angiogenesis inhibition. In this review, we will focus on the role of the VEGF-signaling pathway in tumor angiogenesis and its relevance as a therapeutic target.

Vascular Endothelial Growth Factor Ligand (VEGF)

VEGF, a 35- to 45-kD dimeric polypeptide, plays a critical role in normal and pathologic angiogenesis. It was initially described by Dvorak and associates as vascular permeability factor (VPF) [7]. Subsequently, Ferrara and colleagues isolated and cloned VEGF-A as an endothelial specific mitogen [8]. Other members of the VEGF family include VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factors (PlGF) 1 and 2 (Figure 1).

The VEGF-A gene, *via* alternative splicing, yields several isoforms of 121, 165, 189, and 206 amino acids. Of these, VEGF-165 is the predominant isoform and plays a critical role in tumor angiogenesis. VEGF-A is expressed in macrophages, T cells, smooth muscle cells, mesangial cells, kidney cells, osteoblasts, astrocytes, and malignant tumor cells [9]. Tumor cells secrete VEGF in response to many stimuli including hypoxia, low pH, or cellular stress, which are prevalent in most solid tumors. We have recently demonstrated that chronic behavioral stress can also promote VEGF production *via* stimulation of the β nergic receptor (encoded by *ADRB2*) on ovarian cancer cells [10]. The activation of the VEGF receptors by VEGF promotes intracellular signaling pathways that promote vascular permeability, endothelial cell proliferation and migration, and stabilization of new blood vessels [11]. In addition, VEGF promotes mobilization of endothelial progenitor cells from the bone marrow that likely play a significant role in tumor neovascularization.

Among the many functions of VEGF, it plays a pivotal role in vessel permeability and contributes to the development of pleural effusion and ascites [12,13]. By increasing vascular permeability, VEGF allows leakage of multiple plasma proteins. Some proteins, such as plasminogen activator, matrix metalloproteinases, interstitial collagenases, and gelatinase A, degrade the extracellular matrix to allow space for new cell growth [14]. Another plasma protein, fibrinogen, also leaks into the extravascular environment and serves as a foundation for microvessel growth [15]. VEGF also serves as a potent mitogen for vascular endothelial cells, which proliferate and migrate to the area to begin formation of new blood vessels [11].

Finally, once new vessels are formed, VEGF functions as a survival factor and inhibits apoptosis of the poorly formed vasculature [16]. Through this mechanism, tumor cells are able to sustain growth by securing continued blood flow and nutrition.

The role of VEGF-B and PlGF in tumor angiogenesis is not fully understood. It is possible that their function may be somewhat redundant as mice null for these proteins have no overt developmental abnormalities [17]. VEGF-C and VEGF-D are essential for embryonic and postnatal lymphangiogenesis. For example, VEGF-C homozygous deletion in mice is embryonically lethal and heterozygous deletion results in abnormalities associated with defective lymphatic development [18].

Vascular Endothelial Growth Factor Receptors (VEGFR)

VEGF exerts its biologic effect through interaction with receptors present on the cell surface. These transmembrane tyrosine kinase receptors include VEGF receptor-1 (VEGFR-1, Flt-1) and VEGFR-2 (kinase insert domain-containing receptor, Flk-1), which are predominantly present on vascular endothelial cells. Both VEGFR-1 and VEGFR-2 have seven immunoglobulin-like domains in the extracellular domain, a single transmembrane region, and a consensus tyrosine kinase sequence that is interrupted by a kinase-insert domain [19,20]. In addition, VEGFR-3 (Flt-4) is expressed on vascular and lymphatic endothelium while the neuropilin receptor is expressed on vascular endothelium and neurons [13]. VEGF binding to its receptor causes dimerization and phosphorylation of the intracellular receptor kinases, which in turn activates a cascade of downstream proteins. VEGFR-1 is critical for physiological and developmental angiogenesis; however, its role in tumor angiogenesis remains unclear. In addition to VEGF-A, VEGFR-1 also binds VEGF-B and PlGF. Gene targeting studies have demonstrated that VEGFR-1 null mice die *in utero* between days 8.5 and 9.5 [21,22]. In these embryos, endothelial cells develop, but do not organize into vascular channels. VEGFR-2 appears to be the main receptor responsible for mediating the proangiogenic effects of VEGF. Both homozygous and heterozygous VEGFR-2 knock out mice die *in utero* due to defective vascular development [23]. Specifically, there is lack of vasculogenesis, and failure to develop blood islands and organized blood vessels in VEGFR-2 null mice, resulting in early embryonic death. VEGF binding to the extracellular domain of the receptor results in dimerization and autophosphorylation of the intracellular tyrosine kinases. This activates multiple downstream proteins which play functional roles in cell survival and proliferation. For example, VEGF induces endothelial cell proliferation by activating the protein kinase C-Raf-Mek-Erk pathway [24]. The prosurvival effects of VEGF/VEGFR-2 are mediated by the PI3 kinase-Akt pathway [25]. Recent studies indicate that VEGF receptors are also expressed by some tumor cells and may represent an additional target [6,26–28].

VEGF and Gynecologic Malignancies

VEGF and Ovarian Cancer

The biological roles of VEGF predict that its expression should be related to clinical outcome in cancer patients. Indeed, VEGF expression has been correlated with both disease-free and overall survival in a variety of gynecologic cancers. VEGF expression has been evaluated in women with ovarian carcinoma in several studies. Using enzyme immunoassay and Western blot analysis, Kassim and colleagues [29] found some degree of VEGF expression in all ovarian cancer specimens examined and the level of VEGF expression was significantly higher in tumor specimens compared to benign ovarian tissue. In addition, rising titers of VEGF in cytosolic fractions from tumor specimens correlated with increasing stage and decreased survival. Paley and colleagues [30] used *in situ* hybridization in early stage ovarian cancers and also found that increased VEGF expression correlated with worse disease-free and overall survival. Higher serum VEGF levels have also been associated with ovarian cancer when

compared to benign adnexal masses [31]. Furthermore, in women with ovarian cancer, high serum levels of VEGF are an independent risk factor for ascites, advanced stage disease, undifferentiated histology, more metastasis, and decreased survival [31–33]. Collectively, these studies provide relatively consistent evidence that higher VEGF levels are associated with aggressive clinical behavior in ovarian carcinoma.

VEGF and Endometrial Cancer

There are limited data regarding VEGF expression in endometrial cancer specimens. Holland and associates [34] used *in situ* hybridization to document VEGF expression in 100% of the endometrial cancer specimens examined. In addition, they found no expression of VEGF in benign endometrial tissue and only 20% of tissue samples with atypical hyperplasia expressed VEGF. Immunohistochemical expression of VEGF in tumor specimens has been correlated with higher histologic grade [35] as well as greater depth of myometrial invasion, lymphovascular space invasion, lymph node metastasis, and shorter disease-free survival [36]. The functional significance of the VEGF/VEGFR axis in endometrial cancer remains to be demonstrated.

VEGF in Cervical Cancer

In tissue specimens from patients with cervical cancer, VEGF expression has been noted immunohistochemically in 94% of the samples [37]. Intratumoral protein levels of VEGF are increased in patients with cervical cancer when compared to normal cervical tissue [38]. For these women with cervical cancer, increasing intratumoral levels of VEGF also correlated with higher stage, increased risk of lymphovascular space invasion, greater likelihood of parametrial spread and lymph node metastasis [38]. In addition, higher levels of intratumoral cytosolic VEGF concentration predicted worse disease-free and overall survival [39]. When cervical cancer specimens were examined for VEGF expression using immunohistochemistry, Lancaster and colleagues [37] found that higher VEGF expression was an independent prognostic factor for poor disease-free and overall survival. Other authors, however, were not able to corroborate this finding [40]. Increased VEGF expression seen peritumorally predicted increased lymphatic vessel density and lymphovascular space invasion and, in turn, increased lymph node metastasis [41]. Lebrecht and associates [42] compared serum VEGF levels in women with cervical cancer, cervical dysplasia, and healthy controls. They found increased levels of VEGF in women with dysplasia compared to controls and higher levels of serum VEGF in women with cancer when compared to those with dysplasia. In addition, higher levels of serum VEGF correlated with advanced stage in women with cervical cancer. Surprisingly, however, there was no correlation with serum VEGF and overall survival.

VEGF-Targeted Therapies

Therapies Targeting VEGF

The biological and clinical significance of the VEGF pathway in tumor angiogenesis suggests its value as a therapeutic target in gynecologic malignancies. There are many potential strategies to inhibit the VEGF pathway for therapeutic applications (Figure 2). Bevacizumab (Avastin, Genentech) is a 149-kD recombinant humanized monoclonal IgG1 antibody directed against human VEGF. It has been approved by the Food and Drug Administration (FDA) for the upfront treatment of patients with advanced colorectal cancers in conjunction with intravenous 5-fluorouracil-based chemotherapy [43]. This approval was based on significant improvement in both progression-free and overall survival. Although side effects of bevacizumab are typically mild or moderate, severe, life threatening morbidities have been associated with this drug. These include bowel perforations, thromboembolic events, hemorrhage, and sequelae from grade 3 or 4 hypertension.

In pre-clinical models of ovarian cancer, a VEGF-targeted antibody alone had minimal effect on tumor burden, but markedly decreased ascites [44]. However, in combination with paclitaxel, tumor burden, along with ascites, was significantly decreased [45]. Prospective and retrospective trials with bevacizumab as both single agent and in combination therapies have been reported in patients with ovarian and cervical cancers.

Two phase II trials using bevacizumab in high-grade carcinoma of the ovary were presented at the American Society of Clinical Oncology meetings in 2005 and 2006 (Table 1). The Gynecologic Oncology Group administered single-agent bevacizumab at 15 mg/kg every three weeks to 62 women with ovarian carcinoma (GOG 170-D) [46]. Eligible patients were restricted to 2 prior chemotherapy regimens and 42% of women enrolled had platinum-sensitive disease. Three patients (5%) had a complete response, and 4 patients (13%) had a partial response for an overall response rate of 18% with a median response duration of 10.25 months. An additional 34 patients (55%) had stabilization of disease. In the entire cohort of 62 women, 24 (39%) had a progression-free survival of greater than 6 months. Bevacizumab is the first anti-angiogenic agent to demonstrate such encouraging results as a single agent in ovarian cancer. When compared to historic controls from prior GOG studies, patients who received bevacizumab had a significantly longer disease-free interval than other second line agents from previous trials. There were no reports of bowel perforation in this study, however, 4 patients had grade 3 hypertension and another 3 patients reported grade 3 pain.

In 2006, Cannistra and colleagues reported the results of 44 patients with platinum-resistant ovarian cancer who received bevacizumab at 15 mg/kg every three weeks [47]. Eligible patients had received and failed a platinum-based therapy either as initial therapy or at time of first recurrence. In addition, these patients had tumor progression on topotecan and/or liposomal doxorubicin prior to initiating bevacizumab. Patients could not have received more than 3 prior chemotherapy regimens. There were no complete responders and 7 partial responders for an overall response rate of 16% in this cohort. The median duration of response was only 4.2 months, much lower than the 10.25 months reported by Burger and associates in 2005. Six patients had a grade 3 or worse hypertensive episode including 1 patient with a hypertensive encephalopathy who ultimately died of this side effect. Another 5 patients (11%) had a bowel perforation with 1 fatality. None of the women with intestinal perforations were among those who responded. The unexpected high rate of bowel perforations led the investigators to close the study ahead of the targeted accrual of 53 patients.

The two single-agent phase II studies described above enrolled women with recurrent ovarian cancer who had received 3 or fewer chemotherapy regimens. Retrospective studies with bevacizumab as palliative therapy have also been published. Numnum and colleagues [48] administered bevacizumab (15 mg/kg every three weeks) to highly pretreated women (median 7 prior regimens) in an attempt to decrease symptomatic ascites and reduce the requirement for paracentesis. All had resolution of their ascites within nine weeks (median 4.5 weeks) and all had stabilization of disease. There were no grade 3 or 4 toxicities reported in these 4 patients. Monk and coworkers [49] also administered bevacizumab to 32 heavily pretreated patients (median 5.3 prior regimens) with recurrent, platinum-resistant ovarian cancer. Among these patients, 23 received single agent bevacizumab 15 mg/kg given every three weeks. Seven patients who originally responded to single agent bevacizumab had a traditional cytotoxic agent added when they had tumor progression on the single agent regimen. Two patients were initiated on combination bevacizumab and traditional chemotherapy. One patient had a complete response and 4 patients had partial response. All five of these patients had received single agent bevacizumab. The overall response rate of 16% was similar to the other single agent studies. In addition, 20 patients (63%) had stable disease. Two of the women who originally achieved partial response with single agent bevacizumab eventually progressed but attained stabilization of their disease when a cytotoxic agent was added to bevacizumab. The

median overall survival was 6.9 months and the median progression-free survival was 5.5 months. Three grade 3 toxicities occurred: 1 patient developed a hypertensive emergency, 1 patient had proteinuria, and 1 patient who had undergone 7 prior debulking surgeries developed an enterocutaneous fistula. There were no reported bowel perforations.

Bidus and coworkers [50] reported on 3 women with low-grade ovarian cancer who received single agent bevacizumab at 15 mg/m² every three weeks (*note*: bevacizumab can be administered on a per kilogram or BSA dosing schedule). Two women had low-grade serous lesions while the other had a grade 1 mixed serous and endometrioid histology. All three achieved durable response and remained without tumor progression at the time of publication. Two had complete responses and were without evidence of disease at 15 and 22 months. The third had a partial response without progression of disease at 15 months after bevacizumab initiation. All three patients reported fatigue and myalgia and the 2 patients with osteoarthritis reported worsening of those symptoms. There were no reports of bowel perforation in any of the 3 women.

The above studies of single agent bevacizumab in women with high-grade ovarian carcinoma resulted in response rates of 16–18%, with an additional 55–63% of women achieving stable disease. These results are as good or better than typical rates from traditional second-line chemotherapeutic agents in this group of patients. Cytotoxic and anti-angiogenic agents can be used in combination for enhanced activity. Teicher and colleagues [51] were the first to show synergistic activity between these agents for treatment of tumors in mice. Similarly, in multiple studies, we and others have noted enhanced anti-tumor activity when a cytotoxic agent is combined with an anti-angiogenic agent [28,52–55]. Based on these preclinical data and clinical data from other cancer sites such as colorectal cancer [43] demonstrating improved response rates of antiangiogenic agents with a traditional cytotoxic agent, such combinations are also being evaluated in gynecologic malignancies. In 2005, Garcia and coworkers [56] presented the interim findings from a National Cancer Institute trial (NCI 5789) of 29 patients with recurrent ovarian cancer. These patients had received a median of 2 prior chemotherapeutic regimens (range, 1–3) and 42% had platinum-sensitive disease. Eligible patients were given bevacizumab at 10 mg/kg biweekly with metronomic oral cyclophosphamide at 50 mg daily. The investigators found an overall response rate of 28% with no complete responders and 8 partial responders. The median duration of response was 4.9 months. In addition, 18 women (62%) achieved stable disease with 57% of enrollees attaining a progression-free interval of greater than 6 months. The median time to progression was 7.5 months and overall survival was 13 months. Toxicities included 3 patients with grade 3 hypertension, 1 with grade 3 proteinuria, and 1 patient with bowel perforation. This study will go on to enroll a total 55 patients and we await the complete findings.

In another combination study, 10 patients were treated with bevacizumab on a biweekly basis with a taxane given weekly. [57]. This retrospective study reviewed varying doses of the combination given as palliative therapy to heavily pretreated women with recurrent, platinum-resistant ovarian cancer who had received a median of 4 prior cycles (range, 2–8). One patient was taken off therapy 20 days after initiating bevacizumab and taxane due to worsening of her performance status. The remaining nine patients completed at least 1 cycle of the two drugs. All nine patients had decreased CA125 and improvement of their symptoms. The median number of cycles received was 4 with some patients continuing on therapy at time of publication. Unfortunately, objective response rates using radiographic criteria were not monitored and therefore not reported. There were no grade 3 or 4 toxicities reported in any of the ten patients.

Wright and colleagues [58] retrospectively reported on 23 patients with recurrent, platinum-resistant ovarian cancer who received a variety of different combination regimens of

bevacizumab and cytotoxic chemotherapy. These women had received a median of 7 prior regimens (range, 2–15). Sixty-five percent were treated with bevacizumab and cyclophosphamide while another 26% received bevacizumab and 5-fluorouracil. In their series, 8 patients (35%) had a partial response and another 10 patients (44%) had stable disease. There were no complete responses noted. The median time to progression was 5.6 months in those women who had a partial response and 2.3 months in those who had stable disease. Three women (13%) achieved a progression-free interval of greater than 6 months. Toxicities included 1 patient with grade 4 neutropenia and 1 with grade 4 hypertension and resultant encephalopathy. Two patients developed chylous ascites and another 2 (9%) had bowel perforation. One woman had a small bowel perforation and died of this complication while the other had a large bowel perforation requiring an exploratory laparotomy and diversion. She succumbed to her disease 10 weeks after the incident.

Based on the encouraging phase II trial data, bevacizumab has been rapidly incorporated into phase III trials. The Gynecologic Oncology Group (GOG) trial 218 will evaluate bevacizumab in adjuvant and consolidation settings in women with ovarian cancer after a suboptimal tumor reductive surgery. Eligible patients are randomized to one of three arms: 1) current standard of care therapy with carboplatin and paclitaxel (and placebo) followed by observation (placebo) for 15 months; 2) carboplatin, paclitaxel and bevacizumab followed by observation (placebo) for 15 months; 3) carboplatin, paclitaxel and bevacizumab followed by bevacizumab maintenance for 15 months. ICON 7 is another upfront randomized, two-arm, multicenter Gynaecologic Cancer Inter Group (GCIG) trial designed to evaluate the safety and efficacy of adding bevacizumab to standard chemotherapy (paclitaxel and carboplatin) in patients with advanced epithelial ovarian or primary peritoneal cancer. With regard to relapsed ovarian cancer, GOG 213 will examine the role of surgery and biologic agents in women with recurrent, platinum-sensitive ovarian cancer. However, the exact design of this trial has not been finalized.

Bevacizumab has also been given to women with cervical carcinoma. Wright and associates [59] gave combination bevacizumab and 5-fluorouracil or capecitabine to women with recurrent cervical cancer (4 squamous, 1 adenocarcinoma, and 1 undifferentiated carcinoma). All had received chemoradiation and at least 1 other chemotherapy regimen prior to combination therapy with bevacizumab. In this small, retrospective group of 6 women with cervical cancer, 1 patient achieved a complete response, 1 a partial response and 2 had disease stabilization. Unfortunately, all 4 eventually had disease progression at a median time of 4.3 months and none had a progression-free interval of greater than 6 months. The regimen was seemingly well-tolerated with no bowel perforations or grade 3 or 4 hypertension. One patient, however, did have grade 4 neutropenic sepsis.

Other agents targeting the VEGF pathway are also being rapidly developed. VEGF-Trap (Sanofi-Aventis) inactivates VEGF by acting as a decoy receptor for VEGF, preventing binding of the ligand to its natural receptors. This construct incorporates domains of both VEGFR-1 and VEGFR-2 and binds VEGF with significantly higher affinity than previously reported VEGF antagonists [60]. This inhibitor appears to have prolonged *in vivo* half-life and can effectively suppress the growth and neovascularization of a number of tumors *in vivo* [60, 61]. In preclinical studies of ovarian carcinoma, systemic administration of VEGF-Trap prevented ascites accumulation and also inhibited the growth of disseminated cancer [62]. Single agent VEGF-Trap or paclitaxel reduced tumor burden by 55% when compared to control mice. In combination with paclitaxel, VEGF-Trap reduced tumor burden by 98% when compared to controls [52]. Clinically, Dupont and colleagues [63] performed a phase I study of the VEGF-Trap in patients with advanced solid malignancies. At each dose level, 3–6 patients were treated with VEGF-Trap and despite doses of 800 mcg/kg subcutaneously biweekly, maximum tolerated dose (MTD) was not reached. Grade 3 hypertension was observed in 6% of patients. No other dose related patterns of adverse events were observed.

The GOG is planning to open phase II studies with VEGF-trap in multiple gynecological malignancies.

Therapies Targeting VEGF-R

Currently there are a number of small molecule tyrosine kinase inhibitors in clinical trials. All of these agents are oral compounds with seemingly low side effect profiles. PTK787/ZK222584 (Vatalanib, Novartis) inhibits the adenosine triphosphate binding site targeting VEGFR-1, VEGFR-2, and PDGFR, with highest activity against VEGFR-2. Single agent PTK787 reduced ascites and increased survival in an ovarian cancer mouse model [64].

AG-013736 (Pfizer) is a direct inhibitor of VEGFR-1, VEGFR-2, and PDGF- β receptor [65]. Sunitinib (Sutent, Pfizer) works by targeting multiple tyrosine kinase receptors including VEGFR-2 and PDGFR. In addition, it also inhibits c-kit and FLT-3 [66]. This medication has been approved by the FDA for patients with gastrointestinal stromal tumors (GIST) and advanced kidney cancers. ZD7474 (Zactima, Astra Zeneca) and AEE788 (Novartis) are dual specific inhibitors of VEGFR and EGFR. Both drugs are in clinical trials and neither has received FDA approval to date. In an ovarian cancer nude mice model, single agent ZD6474 had a significant anti-tumor effect [67]. Single agent AEE788 or paclitaxel had an intermediate effect when compared to controls and combination therapy [28]. Combination therapy with AEE788 and paclitaxel greatly reduced tumor burden when compared to control mice. The greater efficacy occurred, in part, due to significantly higher apoptosis of tumor-associated endothelial cells in the combination group compared to the single agent or control groups [28]. These studies show promise for targeted therapies against ovarian cancer, especially in combination with traditional cytotoxic agents.

Another small molecule kinase inhibitor, Sorafenib (Nexavar, Bayer) targets the RAF/MEK/ERK signaling pathway [68]. This, in turn, inhibits cell proliferation and the VEGFR-2/PDGFR pathway. It has also been shown to inhibit several receptor kinases including VEGFR. This drug has been FDA-approved for advanced renal cell carcinoma [69]. Other than bevacizumab, Sorafenib is the only VEGF-targeted agent with any clinical data published in ovarian cancer patients. Siu and colleagues [70] performed a multicenter phase I trial of combination sorafenib and gemcitabine. Of the 42 patients enrolled, 6 women had ovarian cancer. Of these 6 women, 3 had a partial response and 1 had stabilization of disease. Phase I and phase II trials with Sorafenib and other small molecule tyrosine kinase inhibitors are currently ongoing in patients with gynecologic malignancies.

2C3 (Peregrine Pharmaceuticals, Inc) a murine monoclonal antibody that selectively inhibits VEGF binding to VEGFR-2, but not VEGFR-1. In theory, this could reduce tumor angiogenesis as mediated by the VEGFR-2 receptor without reducing the normal physiologic effects of VEGF through its binding to VEGFR-1. Preclinical studies with an orthotopic breast cancer model in nude mice showed inhibition of primary tumor growth and reduced implantation and growth of simulated metastasis [71].

Another drug, Angiozyme (Ciron Corp.) is a hammerhead ribozyme that recognizes and cleaves the mRNA that encodes for VEGFR-1. This compound eliminates the effects of VEGF by destroying its receptor before protein synthesis. We have recently developed a method for highly efficient delivery of short interfering RNA (siRNA) that may be useful for targeting the VEGF/VEGFR pathway [53].

Complications of Anti-Angiogenic Therapy

Treatment related side effects from anti-angiogenic therapies are generally manageable. When these medications were in their developmental stage, many thought these therapies would have

minimal toxicity as they would be directed at the active site of angiogenesis in malignant tissues. However, targeting the VEGF/VEGFR axis may affect the survival of both proliferating and quiescent endothelial cells. Therefore, some of the toxicities realized in the clinical studies of anti-angiogenic therapies may be related to the disruption of normal vasculature [72]. The increased arterial thromboembolic events, including cerebral infarction, transient ischemic attacks (TIAs), myocardial infarction and angina may be related to this phenomenon. In reviewing the clinical trials that won bevacizumab approval for the first-line treatment for metastatic colon and rectal cancer, investigators found the overall incidence of arterial thromboembolic events to be 4.4% in those who received 5-FU and bevacizumab *versus* 1.9% for those who received 5-FU only. These data led the FDA to issue a drug warning in August 2004 regarding this increased risk, but concluded that the benefits of bevacizumab likely outweigh this risk [73].

Hypertension is one of the most common side effects of bevacizumab therapy with an overall incidence of 22–32% [74]. Although the mechanism behind bevacizumab-related hypertension is not fully understood, it is thought to be related to decreased production of nitric oxide as a result of VEGF inhibition [75]. Elevated blood pressure may be seen with any of the anti-VEGF/VEGFR medications as the hypertension appears to be related to inhibition of the VEGF pathway rather than to individual drugs [63]. Most patients with hypertension can be managed with oral anti-hypertensive agents such as angiotensin-converting enzyme (ACE) inhibitors and calcium channel blockers [43].

Another side effect of bevacizumab therapy, proteinuria, has been reported in as many as 38% of patients [74]. Most often, this proteinuria is asymptomatic but routine monitoring should be employed for all patients on anti-VEGF therapy. It is recommended that anti-VEGF therapy should be interrupted if 24 hour collection of protein is > 2 g and discontinued altogether if nephrotic syndrome develops.

Bleeding disorders have been reported in patients receiving bevacizumab. Apart from pulmonary bleeding in patients with lung cancer, most bleeding complications are relatively minor with epistaxis being reported most often [74]. Gingival and vaginal bleeding have also been noted. Most instances are easily controlled with direct pressure and bevacizumab therapy should be discontinued if more invasive treatments are needed to stop bleeding. For patients on therapeutic anticoagulation for DVT, pulmonary embolus, or atrial fibrillation, anti-angiogenic therapy should be undertaken with great care.

VEGF is known to play a critical role in the physiologic angiogenesis required for wound healing. This could be of particular importance when considering anti-angiogenic therapy as front-line adjuvant treatment of ovarian cancer after cytoreductive surgery. Scappaticci and colleagues [76] reviewed the pooled data from two randomized trials with bevacizumab for the treatment of colorectal cancer. Based on concerns with potential wound complications for patients on anti-angiogenic therapy, those two trials were designed so that patients would receive adjuvant chemotherapy > 28 days postoperatively. For these patients, there was no significant increase in risk of wound complications in the patients who received bevacizumab (1.3%) *versus* controls (0.5%). However, a subgroup of patients required surgical intervention while enrolled on protocol and actively receiving bevacizumab. In these patients, there was a statistically significant increased risk of wound complication in the bevacizumab group (13%) when compared to the controls (3.4%). Because of these concerns for wound healing in postoperative patients, women enrolling in GOG 218 will start bevacizumab/placebo therapy with cycle 2, which will be >28 days after surgery.

Among the most well-publicized and feared complications of bevacizumab is intestinal perforation. In the studies with colorectal cancer patients, the rate of bowel perforation was

1.5% [74]. In the seven studies with bevacizumab in recurrent ovarian cancer described above, the overall rate of bowel perforation was 4% (Table 2). Identified risk factors for perforation in patients with colorectal cancer included acute diverticulitis, history of abdominal radiation, tumor at the site of perforation, abdominal carcinomatosis, and obstruction [74]. These risk factors are likely minimal in patients with colorectal cancer receiving anti-angiogenic therapy as part of their front-line chemotherapy. In women with recurrent ovarian cancer, in contrast, the risk factors of tumor at the site of perforation, abdominal carcinomatosis, and obstruction are essentially ubiquitous. Therefore, it is not surprising to see a much higher rate of bowel perforation in these patients. If an intestinal perforation occurs, clinical presentation will dictate whether surgical *versus* conservative medical management should be undertaken. Nevertheless, anti-VEGF therapy should be discontinued after such an event.

Challenges and Future Directions

Although VEGF-targeted therapies appear promising in treating gynecologic malignancies, many questions remain unanswered. The clinical application of VEGF-targeted therapy is more complex than previously appreciated. Many potential mechanisms of action have been proposed including blockade of vessel formation, pruning of existing vessels, normalization of tumor vasculature and microenvironment, and reduction of interstitial fluid pressure (reviewed in detail by R. Jain) [77]. Although there is clinical evidence for some of these mechanisms, others remain to be proven in clinical settings [78]. To the extent that some anti-angiogenic agents are showing promise even as single agents, it is important to understand the underlying mechanisms so that more rational combinations with chemotherapy and sequence of administration can be addressed in future clinical trials.

Another challenge with biological therapies lies in the selection of appropriate patients. Most trials to date have not required that either VEGF ligand or VEGF receptor levels be checked prior to patient enrollment. Essentially, some of the “targeted” therapies have been administered without any knowledge of whether the “target” was present. Ideally, target validation would be part of future prospective protocols involving any biological agent. To date, clinical development of most biological agents has followed the “maximum tolerated dose” schemas. However, with some biological therapies, MTD may not be reached and the “optimal biologic dose” may actually be well below the MTD. For example, in a phase II trial of bevacizumab in colorectal cancer, the 5 mg/kg dose seemed to be more efficacious than the 10 mg/kg dose, but the toxicity was dose dependent [79]. For some agents, treatment at close to MTD may not be needed or desirable. Therefore, reproducible and reliable methods for determining the “optimal biological dose” or “biologically effective dose” are needed.

It is currently unknown whether traditional markers of response are appropriate or adequate for patients being treated with biological agents such as anti-VEGF therapy. It is unlikely that most biological agents will result in substantial responses when given as monotherapy, and maximal responses typically occur in combination with traditional cytotoxic agents. Thus, traditional modalities including imaging (CT, MRI) or circulating tumor markers (e.g. CA125) may not be useful in such trials. In addition, repetitive biopsies are neither practical nor desirable in most patients undergoing therapy with these agents. Even in the setting of “objective responses”, additional biomarkers are needed that will help determine the mechanism of effect. For example, in the bevacizumab phase II GOG trial, an 18% response rate was noted. However, an unanswered question is whether this “response” reflected destruction of tumor cells or simply resolution of edema. New biomarkers may also be useful for selection of appropriate patients. We and others are exploring the utility of several candidate biomarkers including circulating growth factors, soluble receptors, circulating endothelial cells, and circulating cell-free nucleic acids. Other alternatives to invasive strategies include imaging of tumor blood flow and permeability by functional imaging. For example, Willett

and colleagues [80] examined patients undergoing treatment with bevacizumab and combined modality therapy for locally advanced rectal cancer with functional CT imaging. There was a significant decrease in tumor blood perfusion and blood volume with a decrease in tumor MVD suggesting a correlation between invasive and non-invasive strategies. However, the circulating biomarkers and imaging modalities have not been validated as surrogate markers for response to anti-angiogenic therapy.

Anti-angiogenic therapies primarily target the endothelial cells, which are thought to be genetically stable. However, it is becoming apparent from recent trials that the median survival benefit from addition of bevacizumab to chemotherapy is subsequently lost with acquisition of resistance and disease progression in metastatic colorectal cancer [43]. The mechanisms of resistance are not fully understood, but may be related to upregulation of other angiogenic factors and maturation of the tumor vasculature may allow these vessels to become resistant to VEGF-targeted therapies [77,81]. These mechanisms will be important to decipher for additional gains in anti-angiogenic therapeutic approaches.

Conclusions

Over three decades of research in tumor angiogenesis has yielded substantial advances in our knowledge about the biological processes involved in tumor growth and metastasis. Among the many factors involved in angiogenesis, VEGF is the most potent. Based on its high expression in ovarian, endometrial, and cervical cancers, VEGF represents a promising therapeutic target. Recent successes with anti-angiogenic agents in clinical trials further support the value of these approaches. Most of the clinical studies thus far have used bevacizumab either as monotherapy or in combination with traditional cytotoxic agents in women with recurrent ovarian cancer. These studies have reported promising response rates of 16–18% as a single agent and up to 35% in combination with cytotoxic chemotherapy. Clearly a better understanding of the process and mechanisms of angiogenesis is needed to make additional advances in anti-angiogenic therapy. It is likely that multiple angiogenic markers will have to be targeted to make further gains in therapeutic efficacy. However, the exciting developments thus far hold promise for improved cure rates with lower overall toxicity in patients with gynecologic and other malignancies.

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Figure 1.
The VEGF ligands and receptors.

Figure 2.
VEGF/VEGFR-targeted therapies.

Table 1

Summary of bevacizumab use in patients with recurrent ovarian cancer

Author	Year	n	Regimen	CR (%)	PR (%)	SD (%)	PFI (months)
Burger [46]	2005	62	Bevacizumab 15 mg/kg every 3 weeks	5	13	55	10.3
Cannistra [47]	2006	44	Bevacizumab 15 mg/kg every 3 weeks	0	16	NR	4
Numnum [48]	2006	4	Bevacizumab 15 mg/kg every 3 weeks	0	0	100	5.5+
Monk [49]	2006	23	Bevacizumab 15 mg/kg every 3 weeks	4	18	78	5.5
		7	Bevacizumab then chemo added	0	0	14	
		2	Bevacizumab + chemo	0	0	0	
Garcia [56]	2005	29	Bevacizumab 10 mg/kg every 2 weeks + Cyclophosphamide 50 mg daily	0	28	62	7.5
Wright [58]	2006	23	Bevacizumab at varying doses + Multiple cytotoxic agents	0	35	44	5.6 for PR 2.3 for SD

CR – complete response; PR – partial response; SD – stable disease; PFI – progression-free interval; NR – not reported

Table 2

Number and percent of bowel perforations in published studies using bevacizumab in women with recurrent ovarian cancer

Author	Year	n	Number of bowel perforations	% of bowel perforations
Burger [46]	2005	62	0	0
Cannistra [47]	2006	44	5	11
Numnum [48]	2006	4	0	0
Monk [49]	2006	32	0	0
Garcia [56]	2005	29	1	3
Cohn [57]	2006	10	0	0
Wright [58]	2006	23	2	9
Total		204	8	4