Aflibercept in epithelial ovarian carcinoma

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Angiogenesis is a hallmark of malignant transformation. With improved understanding of angiogenic signaling in both the normal and malignant state, there have been a number of agents developed that target VEGF signaling. These targeted agents can affect downstream VEGF signal transduction via unique mechanisms at different cellular and extracellular locations. The aflibercept, or VEGF-Trap, molecule is the subject of this article. Its molecular structure, pharmacokinetic and pharmacodynamic profile, and preclinical and early clinical data in epithelial ovarian carcinoma is reviewed. For comparison, other anti-angiogenic agents that have been or are currently being studied in epithelial ovarian carcinoma are also summarized. Finally, the anticipated role of aflibercept in the treatment of epithelial ovarian carcinoma is also discussed.

Epithelial ovarian cancer (EOC) is a malignancy that afflicts one in 70 women. It occurs most frequently at approximately 60 years of age, and is more common in Caucasian women (45%) than in women of other races (Black: 38%; all others: 17%). In the USA, an estimated 22,000 women are diagnosed with EOC annually [1]. In Europe, the incidence is substantially higher, with an estimated 44,000 cases identified per year. Most women who present with EOC are found with advanced disease; approximately 75% of patients are stage III–IV at diagnosis. Surgical cytoreduction and cytotoxic chemotherapy are the cornerstones of primary therapy for advanced disease, and most patients achieve a complete clinical remission in response to primary therapy. Unfortunately, recurrence is both common and lethal; 5-year survival for stage IIIC and IV patients is 29 and 13%, respectively [2].

Like most other solid tumors, EOCs exhibit a large number of molecular abnormalities that are thought to be central to carcinogenesis and metastatic spread. Among these, increased VEGF signal transduction, an important step in tumor-associated angiogenesis, appears to be one of the primary means by which malignant ovarian cells grow and disseminate.

Targeted inhibition of VEGF signaling has been shown to be an effective way to treat many different solid tumors, including EOC [3]. Since the introduction of monoclonal antibodies such as bevacizumab (Avastin™, Genentech, CA, USA), significant emphasis has been placed on the development of other VEGF pathway inhibitors that have the potential to provide improved efficacy in the treatment of malignancy. One of these, the subject of this review, is the drug aflibercept, also known as VEGF-Trap.

Contemporary adjuvant chemotherapy for EOC in the primary treatment setting is platinum and taxane based. Efforts to improve response rate and progression-free survival by adding cytotoxic agents to platinum and taxane therapy have not been successful [4]. In general, EOC is a relatively chemosensitive disease, as up to 80% of patients achieve a complete clinical remission. Unfortunately, the recurrence rate for EOC in patients who achieve this status is approximately 80% [5]. Treatment in the recurrent setting may include surgery, radiotherapy and chemotherapy, or any combination thereof. In nearly all cases, the approach is considered palliative. As such, there is no clear management algorithm and treatment options are, in large part, individualized. The manner in which they are individualized depends upon a number of factors, such as the location and estimated number of recurrent implants, the patient's previous response to platinum- and taxane-based chemotherapy and functional status. Figure 1 illustrates the typical treatment and survival timeline for patients with EOC. Table 1 lists agents offered by the National Comprehensive Cancer Network (NCCN) as acceptable for use in patients with recurrent EOC [6]. Of note, only one targeted biologic agent is listed, bevacizumab. This agent has become more widely used in the past few years after multiple Phase II studies have demonstrated its activity in EOC [7–9].

The VEGF family is composed of five glycoproteins referred to as VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF. Expression

Drug Evaluation
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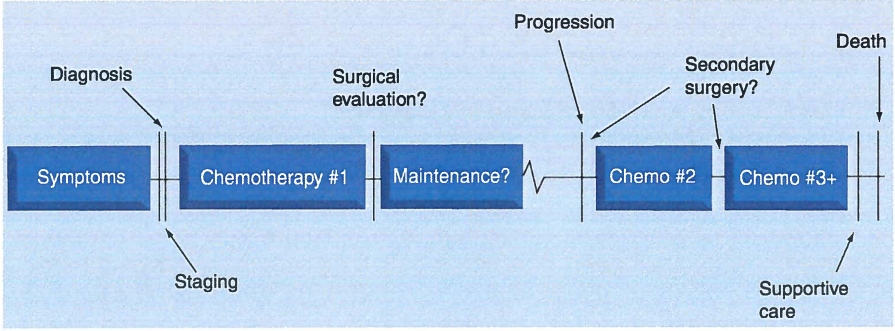

Figure 1. Epithelial ovarian cancer: natural history.

of the different VEGF ligands are associated, somewhat uniquely, with key events in physiologic vasculogenesis and angiogenesis from embryonic life forward [10]. The most well described of these ligands is VEGF-A (VEGF). In mice, homozygous deletion of the VEGF is embryologically lethal, resulting in defects in vasculogenesis and cardiovascular abnormalities. VEGF-A is also important to a number of postnatal angiogenic processes, including wound healing, ovulation, menstruation, maintenance of blood pressure and pregnancy. PlGF and VEGF-B, in contrast to VEGF-A, do not appear to be essential to organogenesis or early development in mice. PlGF knockout mice, however, show a reduced ability to respond to ischemic damage through angiogenesis and adaptive arteriogenesis, suggesting a role for PlGF in pathologic states in the adult [10]. VEGF is alternatively spliced after translation, forming numerous isoforms of varying lengths (e.g., 121-, 145-, 165- and 206-amino acid proteins). Of these isoforms, $VEGF_{165}$ is predominant, and is overexpressed in a number of solid tumors. The predominant isoform of PlGF (PlGF-1) has close structural similarity to VEGF-A [11].

VEGF ligands as well as PlGF bind to and activate three closely related transmembrane receptor tyrosine kinases, referred to as VEGF receptor (VEGFR)1 (FLT1), VEGFR2 (KDR) and VEGFR3 (FLT4). The multiple different VEGF ligands have unique binding specificities for each VEGFR tyrosine kinase receptor, contributing to their diversity of function. After ligand binding to VEGF receptors, each tyrosine kinase activates a complex network of unique

Table 1. National Comprehensive Cancer Network acceptable drugs in recurrent epithelial ovarian carcinoma.

	Cytotoxic therapy	Hormonal therapy	Targeted therapy
Preferred agents	Cisplatin (if platinum-sensitive) Carboplatin (if platinum-sensitive) Gemcitabine Carboplatin/paclitaxel (category 1) (if platinum-sensitive) Gemcitabine/carboplatin Liposomal doxorubicin Topotecan	–	–
Other potentially active agents	Altretamine Capecitabine Cyclophosphamide Docetaxel Etoposide, oral Ifosfamide Irinotecan Melphalan Oxaliplatin Paclitaxel Vinorelbine	Anastrozole Letrozole Tamoxifen	Bevacizumab

Data taken from [6].

downstream signaling pathways. VEGFR2 is expressed primarily within the endothelium, and is considered the primary mediator of VEGF-induced angiogenesis [12]. VEGFR1 is expressed in multiple tissues, including vascular endothelium; however, its exact role in signal transduction-mediated angiogenesis is unclear. It has a tenfold higher binding affinity to VEGF than VEGFR2, but VEGFR1 is much less involved with subsequent signal transduction activity than VEGFR2 [12]. VEGFR3 preferentially binds VEGF-C and VEGF-D, and is found primarily with lymphatic endothelial cells. Recent data has demonstrated that VEGFR3 is also located within the vascular endothelium. Targeting VEGFR3 leads to tumor regression in some mouse xenograft studies [13].

Neuropilins are a group of VEGFRs that were described subsequent to initial characterization of the VEGF receptor family. They were originally named based on their role in axonal guidance in the developing nervous system, and were then found expressed on endothelial cells as well as overexpressed on tumor cells. Neuropilins have since been found to be important co-receptors for VEGFR signaling, and also possess intrinsic pro-angiogenic signaling capability. They are promising targets for anti-angiogenic therapy, particularly in combination with cytotoxics and/or other vascular-focused therapy. Development of neuropilin inhibitors is currently underway and awaits clinical investigation [12].

Overview of the market

Despite the promise of anti-angiogenic agents in the treatment of EOC, there are relatively few clinically available drugs available at the current time. There are, however, multiple drugs under study (see below). Each of these agents can be categorized mechanistically. A brief description of each follows (Table 2).

Monoclonal antibodies targeting the VEGF pathway

VEGF monoclonal antibodies (Mabs) act by antagonizing the VEGF receptors (primarily KDR and FLT1) or their respective ligands. Currently, the most widely used VEGF Mab is bevacizumab, which is a chimeric murine/human antibody targeting the VEGF ligand. It currently has US FDA approval in combination with 5-fluorouracil-based therapy in metastatic colorectal carcinoma, metastatic breast carcinoma in combination with paclitaxel and in nonsquamous, non-small-cell lung cancer patients in combination with carboplatin and paclitaxel [3].

Although not formally approved for EOC, bevacizumab has been widely studied in multiple Phase II trials as both a single agent and in combinations with both cytotoxic and other targeted therapies (Table 3) [7–9]. Several EOC Phase III trials in both the primary and recurrent settings are underway (Table 4).

Tyrosine kinase inhibitors targeting the VEGF pathway

VEGF tyrosine kinase inhibitors (TKIs) are small molecules that bind specific intracellular domains of VEGFR receptor tyrosine kinases (RTKs), inhibiting phosphorylation and subsequent signal transduction. Most RTK inhibitors are relatively selective, however, not specific for a unique RTK domain. As a result, they function, to varying degrees, as multikinase inhibitors. Commonly, other RTK systems affected are within the EGF and PDGF receptor families. In addition, small-molecule TKIs frequently act on downstream signaling effectors such as Raf, Src and Met [10].

There are no VEGF TKIs currently approved in EOC; however, several have been studied in the Phase II setting and have shown some activity in combination with other agents in heavily pretreated patients [14–23] (Table 5).

Table 2. Phase III VEGF-inhibitor trials in epithelial ovarian carcinoma.

Trial name	Combination	Treatment setting	Anticipated accrual
GOG-218	Carboplatin, paclitaxel, bevacizumab	Primary	2000
ICON-7	Carboplatin, paclitaxel, bevacizumab	Primary	1444
ICON-6	Carboplatin, paclitaxel, cediranib‡	Recurrent	2000
GOG-213	Carboplatin, paclitaxel, bevacizumab	Recurrent*	660
OCEANS	Carboplatin, paclitaxel, bevacizumab	Recurrent*	440

*Platinum sensitive.
‡VEGFR-2 tyrosine kinase inhibitor.
GOG: Gynecologic Oncology Group; ICON: Internationational Collaborative Ovarian Neoplasm.

Table 3. Bevacizumab in epithelial ovarian carcinoma (Phase II).

Variable	Cannistra (n = 44) (%)	Garcia* (n = 70) (%)	Burger (n = 62) (%)
Previous regimens			
1		100	34
2	52		
3	48		
Response rate			
CR	0	0	3
PR	16	24	18
GI perforations	11	6	0
Arterial thrombosis	7	4	0
Bevacizumab-related deaths	7	4	0
Ref.	[8]	[9]	[7]

*Combined with oral cyclophosphamide.
CR: Complete response; GI: Gastrointestinal; PR: Partial response.

Fusion proteins

Both monoclonal antibodies and TKIs act via direct interaction with binding domains on either soluble VEGF ligands or membrane-bound receptors. Another, unique method to inhibit VEGF-mediated angiogenic signaling involves the use of a soluble fusion protein comprised of truncated VEGFR1 and VEGFR2 binding domains combined with the Fc portion of IgG1. This molecule, via a mechanism similar to that of VEGF Mabs, serves to function as a decoy receptor, binding with high affinity to the VEGF-A ligand and thus preventing VEGFR1 and VEGFR2 binding and subsequent stimulation [24]. Despite the mechanistic similarity between Mabs and fusion proteins, there are clear structural and pharmacokinetic differences between them, the functional significance of which are incompletely understood. The only VEGF fusion protein currently in clinical use is aflibercept.

Introduction to the compound

The aflibercept molecule (parental VEGF -Trap) was originally synthesized as a fusion protein combining the constant region (Fc) of IgG1 with the first three domains of VEGFR1 ($VEGF_{\delta B1}$). It was found to have impressive, picomolar binding affinity to VEGF ligand and promising anti-tumor activity in transformed cancer cell lines. Unfortunately, it was also found in its parental form to have a significant positive charge, and as a result, to bind nonspecifically to negatively charged extracellular matrix proteins, causing its systemic half-life to be poor. It was then modified to include the Fc region of IgG1 fused with domain two of VEGFR1 and domain three of VEGFR2 ($VEGF_{\delta R1R2}$) (Figure 2). This modification served to maintain its high VEGF-A ligand affinity and relative specificity, and to significantly prolong its *in vivo* half-life, making it clinically useful. It also has strong, picomolar binding affinity for PlGF. All of the aflibercept variants were produced and purified from Chinese hamster ovary cells [24].

Aflibercept has undergone extensive preclinical testing to establish it as an effective inhibitor of angiogenesis and tumor growth in animal models. Subcutaneous mouse tumor xenograft studies using cancer cell lines from multiple species (murine melanoma, human rhabdomyosarcoma and rat glioma) were

Table 4. Aflibercept-related adverse effects.

Year	Publication form	Author	AF doses (mg/kg)	Combination agent(s)	G2 events (%)	G3–G4 events (%)	Ref.
Phase I							
2008	Abstract	Rixe	2–6	Irinotecan, 5-FU, leucovorin	Dysphonia (24), epistaxis (18)	Hypertension (32), proteinuria (11)	[31]
2008	Abstract	Freyer	4, 6	Docetaxel, cisplatin	Epistaxis (19), proteinuria (17) dysphonia (13)	Hypertension (13)	[36]
2008	Absract	Limentani	2, 3, 4, 5	Oxaliplatin, 5-FU, leucovorin (FOLFOX-4)	Not reported	Hypertension (13), proteinuria (13), hemorrhagic events (3), deep vein thrombosis (3)	[35]
2008	Abstract	Coleman	2–6	Docetaxel 75 mg/kg	Fatigue (33), mucositis (11)	Hypertension (22), neutropenia (89)	[37]
Phase II							
2007	Abstract	Tew	2, 4	None	Not reported	Hypertension (9), proteinuria (4), encephalopathy (2), renal failure (2)	[32]
2008	Unpublished	Coleman	6	Docetaxel 75 mg/kg	Study in progress	Study in progress	

5-FU: 5-fluorouracil; AF: Aflibercept.

Table 5. Tyrosine kinase inhibitors in epithelial ovarian carcinoma.

Drug	Generic name	Target	Current trials in ovarian carcinoma	US FDA approval (indication)	Ref.
SU11248	Sutent	VEGFR, EGFR, PDGFR, c-KIT	Phase II/III	Yes (renal)	[14]
ZD6474	Vandetanib	VEGFR, EGFR	Phase II	Yes	[21]
Sorafenib	Sorafenib	RAF-1, VEGFR-2, EGFR-3 PDGFR-β, FLT-3, c-KIT	Phase I/II/III	Yes (renal)	[16]
AZD2171	Cediranib	VEGFR-1-3, PDGFR, c-KIT	Phase I/II/III	No	[22]
GW786034	Pazopanib	VEGFR1-3, PDGFR-α/β, c-KIT	Phase II	No	[15]
PTK787	Vatalanib	VEGFR-1-3, PDGFR, c-KIT	Phase I	No	[20]
BIBF 1120	Vargatef	VEGFR, PDGFR, FGFR	Phase I	No	[17]
BMS-582664	Brivanib	VEGFR-2, FGFR	Phase I	No	[18]

performed. Aflibercept at high (25 mg/kg) and low (2.5 mg/kg) doses was administered subcutaneously twice-weekly. Measurement of tumor volumes at multiple time points from initiation of therapy demonstrated significant regression in tumor in two of the three models. Examination of tumor sections from sacrificed treatment and control mice demonstrated substantial decreases in tumor vascularity in treated mice, particularly those who received high doses [24].

With the knowledge that VEGF plays an important role in the development of malignant ascites, a mouse model of ovarian cancer using VEGF overexpressing SKOV-3 cells was created. Mice were administered aflibercept 25 mg/kg twice-weekly or placebo. Aflibercept treated mice developed minimal to no ascites in comparison to placebo-treated mice. In a similar experiment using OVCAR-3 cells, both inhibition of ascites as well as tumor growth were significantly improved with aflibercept relative to placebo [25].

Initial anti-angiogenic strategies targeting VEGF were tested in preclinical models and in humans who were known to have minimal disease (e.g., early stage or immediately following surgical cytoreduction). It has been postulated that pro-angiogenic growth factors secreted by pericytes found in established or bulky tumors would make them refractory to VEGF antagonism. To test this in the preclinical setting, mice with bulky, orthotopic, SK-NEP-1 (Wilms tumor) xenografts were injected subcutaneously with aflibercept 500 µg or an equivalent placebo volume twice-weekly. Mice were then sacrificed at multiple time points and their tumors were weighed. Significant differences between treated mice and controls were observed at multiple time points, with a 79.3% decrease noted in VEGF treated mice at the longest tested treatment duration (36 days). Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) and platelet endothelial cell adhesion molecule-1 (PECAM-1) staining strongly indicated that apoptosis was the mechanism responsible for the loss of tumor cells [26].

Aflibercept was also evaluated in an orthotopic murine renal cancer cell model. Aflibercept's ability to prevent tumor formation after orthotopic injection of luciferase-tagged renal cancer cells, as well as its effect on measurable tumor burden, was assessed. In the prevention assay, twice-weekly injections of aflibercept 10 mg/kg versus control were performed starting day 3 or 4 after injection of cancer cells. Luciferase assay at 30 days demonstrated an 87% decrease in tumor

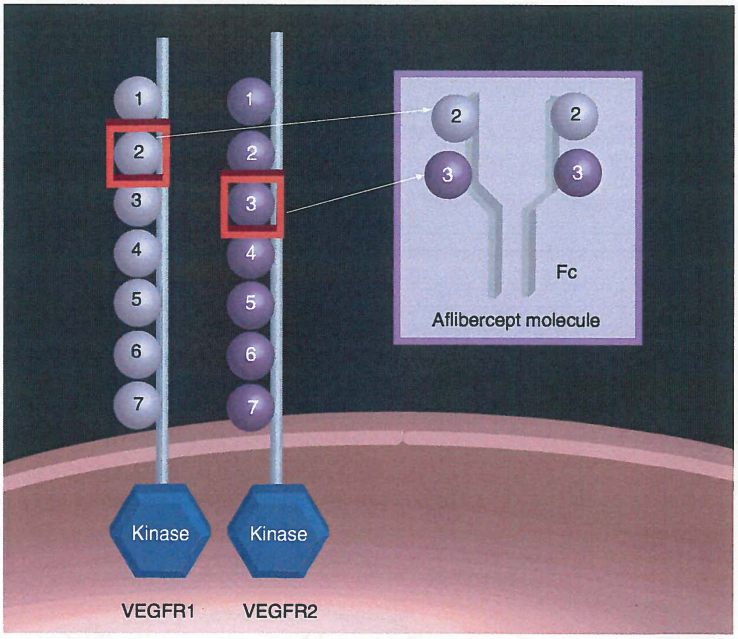

Figure 2. Aflibercept/VEGF$_{R1R2}$ structure. Domain 2 of VEGFR1 and domain 3 of VEGFR2 complexed with the Fc portion of human IgG1.

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