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Critical Role of Anti-Angiogenesis and VEGF Inhibition in Colorectal Cancer

Guest Editors
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16 figures and 20 tables, 2005

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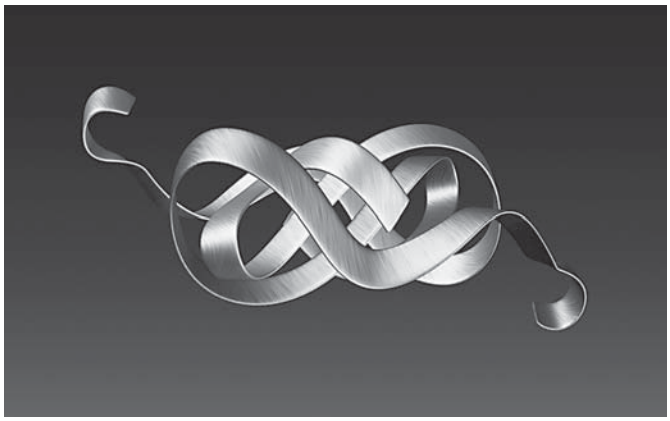

Fig. 1. VEGF structure.

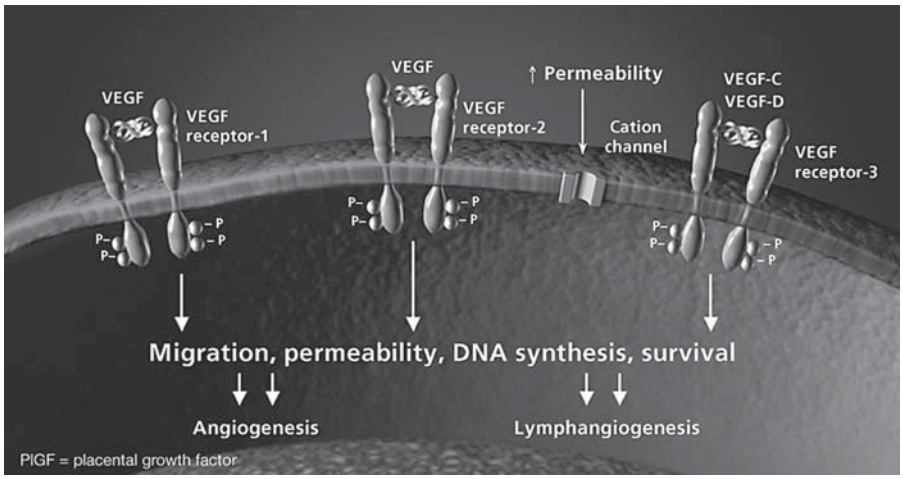

Fig. 2. The VEGF family and its receptors. Reproduced with permission from Ferrara et al., Copyright Nature Medicine 2003 [3].

and is more freely diffusible [4]. The heparin proteoglycan forms of VEGF can be released from cellular surfaces and extracellular matrices by heparinases, plasmin and matrix metalloproteinases (MMP), proteases that are proteolytically activated during tissue remodeling [5]. VEGF itself activates proteinase cascades leading to plasmin and MMP generation, thereby creating a positive feedback loop for VEGF activity.

VEGF gene expression is up-regulated by a variety of factors. A number of growth factors have been demonstrated to induce VEGF gene expression, including PDGF, fibroblast growth factor (FGF), epidermal growth factor (EGF), tumor necrosis factor (TNF), transforming growth factor-β and interleukin-1 (IL-1) [6]. Another inducer of VEGF is hypoxia. Hypoxic induction of VEGF appears to be a ubiquitous response, since a wide range of cultured cells have been observed to increase VEGF mRNA levels by approximately 10- to 50-fold in response to lowering oxygen levels from ambient 21% to 0–3% [7]. Similar induction of VEGF is seen in response to hypoxia in vivo; occlusion of coronary arteries induces ischemia and rapid induction of VEGF mRNA expression in porcine hearts [8]. Acidosis, like hypoxia, is a consequence of inadequate perfusion and an effective inducer of VEGF [9].

The physiological effects of VEGF are mediated through binding to two homologous VEGF receptors, VEGF receptor-1 (Flt-1) and VEGF receptor-2 (KDR), which are expressed on vascular endothelial cells [reviewed in Thomas, 1996, 10]. Like other growth factor transmembrane tyrosine kinase receptors, VEGF receptors undergo ligand-induced dimerization. This triggers signal transduction by promoting receptor phosphorylation and subsequent recruitment of specific downstream signal transduction mediators. A third receptor, VEGF receptor-3 (Flt-4) has been shown to be involved in VEGF-C- and -D-mediated lymphangiogenesis [6] (fig. 2).

VEGF binding to VEGF receptor-2 elicits an efficient endothelial cell response [11]. Although VEGF receptor-1 binds VEGF with high affinity, it is believed to act primarily by modulating the availability of VEGF for binding to VEGF receptor-2. A further level of regulatory control is afforded by the existence of a soluble form of VEGF receptor-1 (sVEGF receptor-1), which lacks intracellular kinase domains [12]. sVEGF receptor-1 retains its specific high affinity for VEGF and inhibits VEGF-induced mitogenesis, presumably by sequestering VEGF. In addition to sequestering VEGF, sVEGF receptor-1 may further inhibit VEGF-mediated signaling by heterodimerization with VEGF receptor-2; heterodimerization with sVEGF receptor-1 would in effect ablate the signal transduction properties of VEGF receptor-2, because sVEGF receptor-1 lacks intracellular tyrosine kinase domains [10]. However, the roles and interactions of VEGF receptor-1 and VEGF receptor-2 remain to be fully elucidated.

The Role of VEGF

VEGF induces angiogenesis via a direct effect on endothelial cells [13]. In in vitro experiments using microvascular endothelial cells grown on the surface of three-dimensional collagen gels, VEGF was shown to induce the cells to invade the underlying matrix and to form capillary-like tubules.

VEGF also elicits non-mitogenic responses by vascular endothelial cells. Endothelial cells in newly formed vasculature undergo apoptosis in the absence of survival signals. By inducing anti-apoptotic signals, VEGF is instrumental in maintaining the viability of immature vasculature [14]. In addition, there is in vivo evidence that VEGF can rapidly increase vascular permeability, allowing leakage of plasma proteins and development of an extravascular matrix, which further enhances the environment for subsequent endothelial cell growth [10]. Such increased vascular permeability also has the effect of increasing interstitial pressure. Finally, VEGF induces chemotaxis [15], and the expression of plasminogen activators [16] and collagenases [17] in endothelial cells. VEGF is therefore a key mediator of angiogenesis as it facilitates blood vessel growth and remodelling processes, as well as providing mitogenic and survival stimuli for endothelial cells.

VEGF also influences the immune system in a number of different ways. Murine studies have demonstrated that VEGF directly interferes with T cell development from early hematopoietic progenitor cells, and results in defective dendritic cell function [18]. Additionally, VEGF has been shown to have a mobilizing effect on circulating endothelial precursor cells and hematopoietic stem cells, as well as mediating monocyte migration [6].

Given its key role in angiogenesis, it is not surprising that VEGF is central to the processes of embryonic vasculogenesis [19, 20]. Deletion of a single VEGF allele results in abnormal blood vessel development and lethality in murine embryos. VEGF and angiogenesis are also required for endochondral bone formation [21]. Although VEGF mRNA remains detectable in several organ types in adults, angiogenesis is minimal in adult men and is largely restricted to neovascularization processes in the estrus cycle in females [22]. However, a role for VEGF in wound healing is established [23], with animal experiments demonstrating induction of VEGF mRNA following injury.

Given the role of VEGF as a key mediator of normal physiological angiogenesis, abnormalities of VEGF expression have the potential to be important in disease processes. A large body of evidence exists to support a role for VEGF in the pathogenesis of diseases characterized by neovascularization, including ocular diseases and inflammatory conditions. Additionally, biopsies representing a large number of human tumor types have been shown to exhibit enhanced expression of VEGF, and VEGF has been recognized to be fundamental to tumorigenesis and disease progression in a wide range of human cancers [24].

Why VEGF-Mediated Angiogenesis Is Essential for Cancer Growth

Without an adequate vascular supply, solid tumors can grow only to a critical size of 1–2 mm (or about 10^6 cells), primarily due to lack of oxygen and nutrients [25, 26]. Folkman (1971) [27] hypothesized that tumor blood vessel formation was dependent on a tumor angiogenic factor (TAF), and that its blockade during the period when a tumor is most vulnerable (i.e. prior to angiogenesis) may restrict tumor growth. VEGF was later identified as one of the most potent TAF molecules [28].

Tumors may remain dormant, in an avascular phase, maintaining a steady state between cell proliferation and apoptosis before converting to an angiogenic phenotype. This conversion, which is known as the 'angiogenic switch', is due to an alteration in the balance of inhibitory and stimulatory factors such that growth stimulation

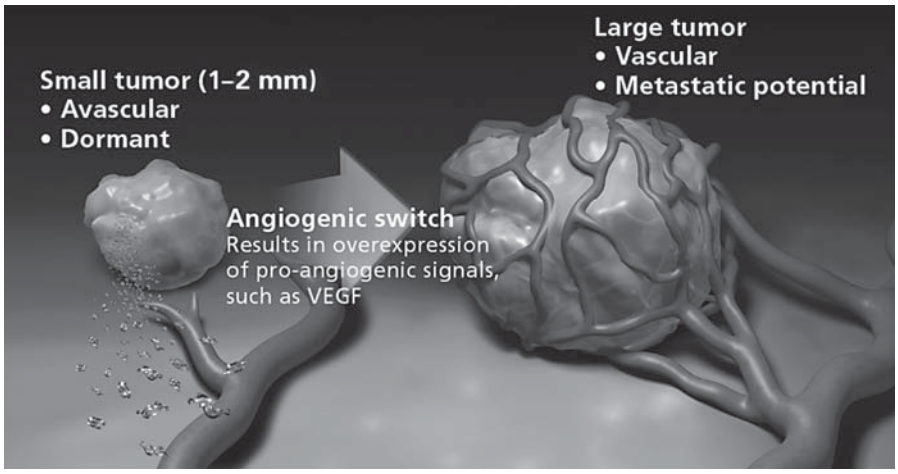

Fig. 3. VEGF and other signals promote the angiogenic switch in tumors.

is favored [26] (fig. 3). VEGF acts as the central mediator of tumor angiogenesis, stimulating the growth of new blood vessels from nearby capillaries and allowing tumors to access the oxygen and nutrients they need to grow [26].

As solid tumors grow in size, the cells within the expanding mass frequently become hypoxic because of increasing distance from the nearest blood vessels. For instance, in human glioblastoma multiforme tumors, expression of VEGF mRNA was maximal in regions characterized by necrosis and a lack of vasculature, and therefore hypoxic [7]. Such a distribution pattern for VEGF expression is consistent with the hypothesis that tumor angiogenesis may be driven, at least in part, by hypoxia [1]. Further evidence for the role of hypoxia in the control of VEGF production is provided by the example of the von Hippel-Lindau (VHL) protein. VHL is ubiquitinized under normoxic conditions, and in this state can degrade hypoxia inducible factor-1α (HIF-1α) [29, 30]. Degradation prevents HIF-1α dimerization and binding to a promoter on the VEGF gene. This in turn suppresses VEGF gene transcription and VEGF protein production. Under hypoxic conditions, VHL is not ubiquitinized and does not degrade HIF-1α. This allows HIF-1α dimerization and binding to the VEGF gene promoter, stimulating VEGF production and angiogenesis. Mutation of VHL so that it is dysfunctional results in VHL disease, which is characterized by high levels of VEGF and results in highly vascular renal cell tumors [30].

The expression of VEGF by tumor cells is also potentiated by common genetic events that lead to malignant transformation, such as those that cause aberrant mitogenesis and resistance to apoptosis. Experimental evidence suggests that the loss of tumor suppressor genes such as p53, and activation of oncogenes such as Kras, Hras, v-src, human epidermal growth factor (HER) 2, HER1/EGF receptor (EGFR), FOS, trkB, V-p3K, PTTG1 and Bcl-2 is associated with increased VEGF expression [31]. For example, in a series of head and neck tumor biopsies, VEGF was significantly correlated with expression of both EGFR and HER2 [32].

PDGF, FGF, TNF and IL-1 are some of a number of growth factors that have been demonstrated to up-regulate VEGF gene expression [6]. In this way, tumor-derived growth factors promote tumor angiogenesis. Furthermore, recent evidence suggests that some tumor cell lines may express VEGF and VEGF receptors, so that VEGF can act as both an autocrine and paracrine factor, leading to a positive feedback loop for a direct effect on tumor cells [33]. Notably, tumor cell lines of non-endothelial origin expressing both VEGF and VEGF receptor-1 are wide-ranging and include melanoma, ovarian, pancreatic and prostate carcinomas [33].

VEGF also plays an integral part in tumor growth by protecting the neovasculature of tumors from apoptosis, through induction of the anti-apoptotic factors Bcl-2 [34] and survivin [35]. Additionally, VEGF mediates the secretion and activation of enzymes involved in degrading the extracellular matrix, such as plasminogen activator [16] and the MMP interstitial collagenase, allowing unhindered development of further blood vessels [17].

The activities of VEGF described show that, in addition to being key to the angiogenic switch and vascularization of the tumor, VEGF mediates the secretion and activity of enzymes that degrade the extracellular matrix and also encourages tumor growth by protecting neovas-

culature from apoptosis. Thus, because angiogenesis and maintenance of the tumor vasculature are essential for cancer growth and VEGF is essential for tumor angiogenesis, VEGF is a critical factor in tumor development.

The Impact of VEGF on Tumor Blood Vessels

A number of studies have been undertaken to evaluate tumor vasculature and have demonstrated significant differences between the vasculature of tumors and that of normal tissue [36–40]. Of note, tumor vasculature has been shown to have increased variability in pore size compared with the vasculature of normal tissue, and studies of permeability using various dye reagents show increased permeability in tumor vessels compared with normal vessels [37]. The increased concentrations of VEGF in tumor also result in blood vessels that are structurally different from normal blood vessels. In contrast to the architecture of normal vasculature, tumor vasculature is irregularly shaped, dilated and tortuous, with numerous blind ends [26, 39, 41, 42] (fig. 4). Additionally, the vessels are not organized into a hierarchy of definitive venules, arterioles and capillaries like normal blood vessels, but instead have chaotic versions of all of them [41]. Tumor vasculature is also functionally abnormal, demonstrating increased leakage and hemorrhage compared with normal vessels, and, as a consequence of the increased permeability of tumor vessels, interstitial pressure is increased [26, 43] (fig. 5).

As a result of the disordered architecture of tumor vasculature, tumor blood flow is often suboptimal, with areas of stagnation due to dead-end vessels and disordered blood flow due to abnormal connections between vessels [26]. This in turn predisposes to areas of hypoxia, further stimulating VEGF release and creating further disorganized vasculature. In situ analysis of tumor specimens undergoing neovascularization reveals clustering of capillaries alongside VEGF-producing cells in close proximity to areas of necrosis [7].

It has been proposed that inhibiting VEGF may result in the remodeling of the tumor vasculature, leading to decreases in tumor perfusion, microvascular density, vascular volume and interstitial fluid pressure in patients

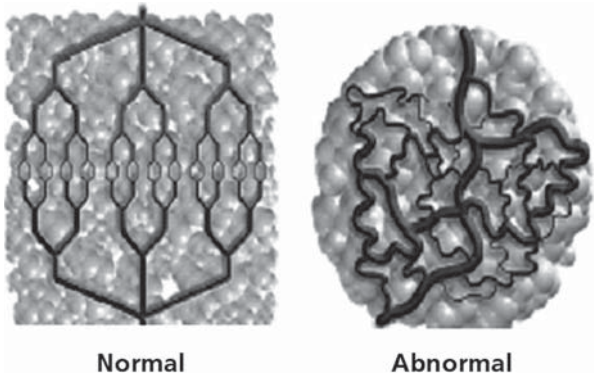

Fig. 4. Blood vessels under the influence of VEGF are physically abnormal and inefficient. Reproduced with permission from Jain, Copyright Nature Medicine 2001 [43].

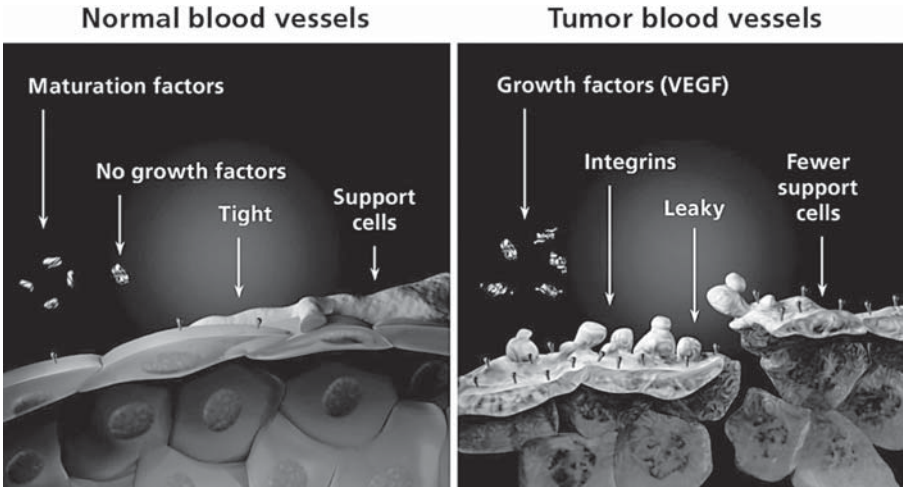

Fig. 5. Tumor vasculature is structurally abnormal.

with colorectal cancer [44]. The resulting vasculature will be more efficient, allowing the effective delivery of chemotherapy to the tumor. Preclinical studies have shown that anti-VEGF therapy is synergistic with chemotherapy, resulting in effective tumor growth inhibition [45]. Anti-VEGF therapy will potentially have broad applicability, because progression of all solid tumor types (as well as hematological malignancies) is dependent on VEGF, making VEGF an important therapeutic target in the treatment of cancer.

In summary, tumor blood vessels produced due to VEGF stimulation are structurally and functionally irregular, with dead ends, disordered blood flow and increased permeability. These irregularities in blood flow lead to further tumor hypoxia and subsequent increases in VEGF production. Thus, a positive feedback loop is established by which the tumor keeps producing VEGF. This ensures that the immature tumor vasculature is maintained, but also means that tumor vasculature is consistently abnormal.

Discussion and Conclusions

VEGF, a member of the PDGF family of mitogens, effects its biological activity via transmembrane tyrosine kinase receptors on endothelial cells. VEGF is required for angiogenesis in embryonic development, but in adults its role is largely restricted to the angiogenic processes of the female estrus cycle and wound healing. However, VEGF plays a pivotal role in the pathogenesis of diseases characterized by neovascularization, including a wide range of human cancers. In order to grow beyond 1–2 mm diameter, human tumors must trigger the angiogenic switch, thereby developing vasculature for effective transfer of oxygen and nutrients. Up-regulation of VEGF in malignant transformation may result from a number of different factors including oncogene activation, inhibition of tumor suppression molecules and release of growth factors as well as tumor hypoxia and necrosis. In addition to acting as a mitogenic signal for vascular endothelial cells, VEGF also protects tumor vasculature from apoptosis through induction of anti-apoptotic factors. Furthermore, VEGF mediates the secretion and activation of enzymes involved in degrading the extracellular matrix thereby further facilitating tumor angiogenesis. The tumor vasculature that results from the activity of VEGF is irregularly shaped, dilated and tortuous, with numerous blind ends, and is functionally abnormal, demonstrating increased leakage and hemorrhage compared with normal vessels. The disordered architecture of tumor vasculature leads to poor tumor perfusion and areas of hypoxia, which, in turn, further stimulate VEGF release and create an additional positive feedback loop for tumor angiogenesis. The evidence demonstrates the pivotal role of VEGF in tumor angiogenesis and consequently, the pathogenesis of a wide range of human cancers. The evidence also suggests that VEGF has many of the attributes of a potential therapeutic target whose inhibition will have specific anticancer effects.

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Introduction

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Cancer is the second leading cause of death in western countries [1], preceded only by cardiovascular disease. Colorectal cancer (CRC) is the third most common cancer, with over 1 million new cases diagnosed worldwide each year and around half of all patients dying due to the disease [2].

Although surgery is the principal treatment for primary CRC, chemotherapy alone or in combination with radiotherapy is often administered before surgery to make the cancer resectable, and after surgery to prevent recurrence. In patients with recurrent metastatic disease, chemotherapy has been the treatment of choice for many years. A combination of systemic chemotherapies and radiotherapy has provided improvements in clinical outcomes, but often at the expense of increased toxicity. Our enhanced knowledge of cancer pathogenesis, together with advances in pharmaceutical biotechnology, has led to the development of novel, targeted agents with potentially increased efficacy and limited toxicity compared with conventional chemotherapies.

The components of a number of biological processes that are essential for tumor growth and progression have been targeted, including the cell cycle, cellular adhesion, cellular migration, growth factor secretion and angiogenesis. Angiogenesis is the growth of new blood vessels from existing vessels and plays a major role in malignant progression [3]. The role of angiogenesis in tumor growth and metastasis is apparent from the high density of vasculature seen in large, established tumors. Angiogenesis is regulated by pro-angiogenic and anti-angiogenic factors, with vascular endothelial growth factor (VEGF) being the key mediator. VEGF expression has been shown to be upregulated in many tumor types and has been shown to be of a prognostic value [4]. Furthermore, VEGF levels are linked to the degree of tumor angiogenesis [5]. These observations support the role of VEGF as the key mediator of tumor angiogenesis and make it an important therapeutic target.

The role of VEGF in tumor progression has been successfully evaluated using a number of molecular techniques, including antisense oligonucleotides directed against VEGF mRNA [6], mutant dominant-negative VEGF receptors [7] and receptor tyrosine kinase inhibitors [8]. The most successful strategy to date has been the use of monoclonal antibodies [9] that bind VEGF and prevent it from binding and activating its receptors VEGF receptor-1 and -2 (also known as Flt-1 and KDR).

Preclinical studies have demonstrated that anti-VEGF antibodies block tumor angiogenesis [10], inhibit tumor growth by up to 90% [11] and reduce the number of metastases [12, 13]. These observations suggest that targeting VEGF is likely to be an effective anticancer therapy.

Bevacizumab (Avastin®) is a humanized monoclonal antibody developed from A4.6.1, a murine antibody that recognizes all isoforms of VEGF [14]. Recent studies have shown that bevacizumab may not only inhibit angiogenesis but may also improve the delivery of chemotherapy to the tumor. The administration of A4.6.1 increased intratumoral concentrations of chemotherapy (irinotecan) in a mouse model [15]. Another study showed

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that treatment with bevacizumab led to a decrease in microvessel density, tumor blood perfusion and interstitial fluid pressure, thus potentially improving access for chemotherapy in patients with colorectal cancer [16].

It has been hypothesized that in addition to supporting tumor growth by inducing angiogenesis, VEGF may also act directly through VEGF receptors expressed by tumor cells [17]. Since many tumors express or have the capacity to express VEGF, expression of VEGF receptors by tumor cells implicates a potential role for autocrine and paracrine activity in regulating tumor growth. Recent studies have shown that disruption of the VEGF/VEGF receptor autocrine loop leads to tumor cell growth arrest and apoptosis, indicating that VEGF and its receptors may directly regulate tumor growth [18, 19]. Therefore, blockade of VEGF is a rationally developed therapeutic approach.

Bevacizumab is the first anti-VEGF and anti-angiogenic agent to show clinical benefit when combined with standard chemotherapy in patients with metastatic CRC. In February 2004, the US Food and Drug Administration (FDA) approved the use of bevacizumab in combination with 5-fluorouracil (5-FU)-based chemotherapy for the first-line treatment of metastatic colorectal cancer. The European Commission (EC) recently approved bevacizumab as first-line therapy for metastatic CRC in combination with intravenous 5-FU/leucovorin with or without irinotecan.

The articles herein present a detailed discussion of VEGF and its role in angiogenesis and tumor development, together with clinical data on bevacizumab and information regarding its future development. The information presented will provide:

- an overview of VEGF, its biological activity in angiogenesis and its essential role in tumor development
- information on why VEGF is an attractive therapeutic target and an overview of the approaches used to inhibit VEGF
- an overview of the development of bevacizumab and preclinical data regarding its efficacy and safety
- detailed clinical data from phase II/III trials of bevacizumab in combination with standard chemotherapy regimens for metastatic CRC
- data on the safety profile of bevacizumab and recommendations for the management of patients treated with bevacizumab
- a review of the ongoing development of bevacizumab in both primary and metastatic CRC
- details of trials that have investigated the potential of adding bevacizumab to standard therapies in other indications, together with information regarding the ongoing development program in these indications.

In this way, readers should obtain a full picture of the rationale for anti-VEGF therapy with bevacizumab, the significant efficacy benefits that are obtained by combining bevacizumab with standard chemotherapy in metastatic CRC, the safety profile of bevacizumab and how to manage associated side effects, and the future development of bevacizumab in colorectal cancer and other malignancies.

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fied (table 1). Most of these molecules, such as basic fibroblast growth factor and matrix metalloproteinases, have a relatively broad range of action, with effects on other systems in addition to their potential role in angiogenesis.

Vascular endothelial growth factor (VEGF) has been identified as the central mediator of angiogenesis [3, 4; reviewed by Ferrara et al.; 5, 6]. The role of VEGF as a key mediator of angiogenesis is discussed earlier in this issue [7].

VEGF expression is elevated in many cancers including colorectal cancer (CRC), breast, lung and other tumors [8–10]. The level of VEGF expression also correlates with microvessel density and metastatic spread in some tumor types, including colorectal, breast and cervical cancer and melanoma. Given the central role of VEGF in tumor angiogenesis and the correlation with tumor growth, VEGF has emerged as the most promising therapeutic target for angiogenesis inhibition.

Targeting VEGF as a Therapeutic Strategy

The most powerful rationale for targeting VEGF is its central role in tumor angiogenesis and its expression by many tumors. Several other characteristics also make VEGF an attractive target. Because VEGF circulates in the blood, and acts directly on endothelial cells, it is not necessary to penetrate tumor tissue to inhibit tumor angiogenesis through VEGF. While VEGF is a potent mitogen for endothelial cells, it has little effect on other cell types, and so should not affect other physiological processes. Angiogenesis has limited importance in normal physiology except wound healing and female reproduction and therefore inhibition of VEGF would not be expected to cause the range of side effects that can occur with other cancer treatments, particularly chemotherapy.

In addition, VEGF acts on endothelial cells, which are relatively stable, quiescent in adults and have a lifespan of many years. This stability means that the cells are less likely to mutate to a treatment-resistant phenotype than genetically unstable tumor cells, making them a more attractive target for long-term therapy.

Actions of VEGF on Tumor Vasculature

In physiological angiogenesis, VEGF stimulates the formation of new blood vessels and maintains immature vessels in coordination with other factors to ensure blood vessels have a normal structure and function. This coordination is lost in tumor blood vessels stimulated by VEGF, leading to prolific growth of disordered vessels with blind ends. VEGF also increases the permeability of blood vessels, resulting in poorly perfused tumors, with subsequent hypoxia stimulating further VEGF production. In addition, the leaky blood vessels result in high tumor interstitial pressure. These effects make it difficult for chemotherapy to access to tumor tissue. Inhibition of VEGF results in normalization of permeability and reduced interstitial pressure, improving accessibility for treatments such as chemotherapy.

Regulators of VEGF Production

The most significant regulator of VEGF production is hypoxia. As a tumor increases in mass and becomes hypoxic, VEGF is induced and stimulates growth of new vessels. Transcription of VEGF mRNA is up-regulated in hypoxia through transcription factors known as hypoxia-inducible factors (HIFs) that bind to the VEGF promoter [11, 12]. The up-regulation of VEGF in the hypoxic state is highly specific: HIFs do not increase expression of other members of the VEGF gene superfamily [13]. Under normal oxygen tension, VEGF is suppressed by the product of a tumor suppressor gene known as the von Hippel Lindau (VHL) gene, which is involved in the degradation of VEGF protein. Patients with a defective VHL gene suffer from VHL disease, a condition in which there is excessive blood vessel formation resulting in angiomas in the retina and cerebellum as well as other areas. Notably, patients with VHL disease also have a markedly increased susceptibility to renal and brain cancers, suggesting an important role for VEGF in the pathogenesis of these tumors [14].

VEGF production is up-regulated by several major growth factors which are frequently expressed by tumors, including epidermal growth factor, transforming growth factor-α and -β, fibroblast growth factor and platelet-derived growth factor [6]. Hormones such as estrogen and thyroid-stimulating hormone [14] and inflammatory cytokines including interleukin-1 and interleukin-6 also induce VEGF expression in many cell types [13]. Mutations in tumor suppressor genes including p53 [15] and oncogenes such as *ras* [16] have also been shown to up-regulate VEGF. Induction of VEGF expression appears to be characteristic of many tumor types and it is likely that inhibition of VEGF would inhibit the angiogenic activity of these tumors.

Other Activities of VEGF

The effects of VEGF on endothelial cells and vasculature are well documented. However, VEGF has other roles that influence tumor growth and progression, including inhibiting functional maturation of dendritic cells [17] and enhanced adhesion of natural killer cells to microvessels [18]. The importance of these effects in human tumors is not fully established, but data suggest that induction of VEGF may contribute to the evasion of host immune responses by growing tumors.

Direct effects of VEGF on apoptosis in tumor cells have also been described. In murine and human tumor cell lines, addition of VEGF up-regulated expression of the anti-apoptotic gene *bcl-2* and addition of anti-VEGF antibodies induced apoptosis [19]. Similarly, in human breast cancer cell lines, induction of VEGF expression by hypoxia provided an autocrine survival signal, while addition of anti-VEGF expression induced apoptosis [20]. These data suggest that VEGF expression may have direct, autocrine effects in promoting tumor cell growth and survival, in addition to promoting angiogenesis.

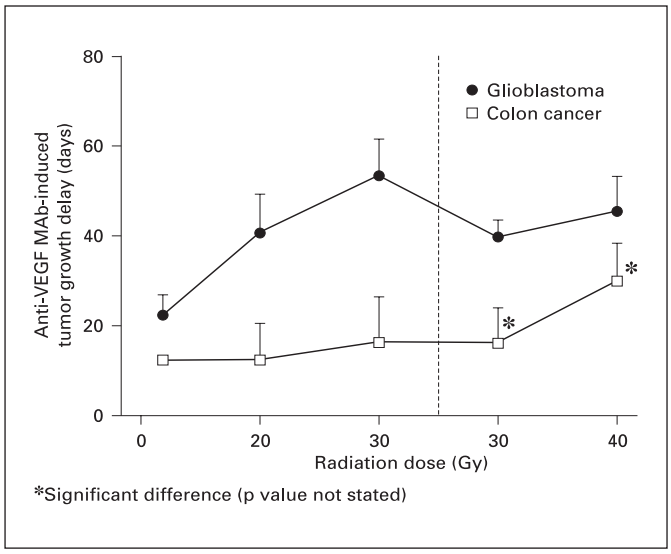

Fig. 1. Tumor growth delay in mice treated with A4.6.1 in combination with radiotherapy. Reproduced with permission from Lee et al., Copyright American Association for Cancer Research 2000 [37].

Prognostic Significance of VEGF Expression

Increased neovascular formation and intratumoral microvessel density in human tumors are associated with poorer prognosis [10]. These findings appear to be correlated with the degree of VEGF expression, as VEGF expression was also found to be a powerful prognostic indicator in a range of solid tumors [10]. The prognostic significance of VEGF expression has subsequently been confirmed in a variety of different solid tumors and hematological malignancies [10, 21–24].

Approaches to Targeting VEGF

VEGF is an ideal therapeutic target; it is crucial for tumor growth and progression with limited applications in normal physiology. Several approaches have been investigated, including agents that target either VEGF or its cell surface receptors. Receptor-targeted molecules include monoclonal antibodies and inhibitors of VEGF-receptor tyrosine kinases. Molecules targeting VEGF include monoclonal antibodies and soluble receptor constructs. A disadvantage of receptor-targeted approaches is that the VEGF receptors (VEGF receptors 1 and 2) may also bind different members of the VEGF superfamily and affect systems other than angiogenesis [25, 26]. The same argument applies to soluble receptor constructs, which may also bind to factors other than VEGF. Therefore, one of the major advantages in targeting VEGF, the fact that effects on normal physiological processes are minimized, may be reduced by approaches that do not target the VEGF molecule with high specificity.

The best-studied and most advanced approach to VEGF inhibition is the humanized monoclonal antibody bevacizumab (Avastin®), which is the only anti-angiogenic agent approved for treatment of cancer. In a large randomized controlled trial, the addition of bevacizumab to standard chemotherapy for patients with previously untreated metastatic CRC resulted in a 30% increase in median survival (Hurwitz et al. 2004 [27], described in more detail later in this issue). Bevacizumab was developed from a murine antibody to human VEGF by recombinant DNA technology [28] and was selected for clinical development based on preclinical evidence showing high antiangiogenic and antitumor activity.

Preclinical Evidence for Bevacizumab Activity

The murine parent antibody of bevacizumab, muMAb A.4.6.1, was first evaluated in mouse models where it completely suppressed neovascularization of rhabdo-

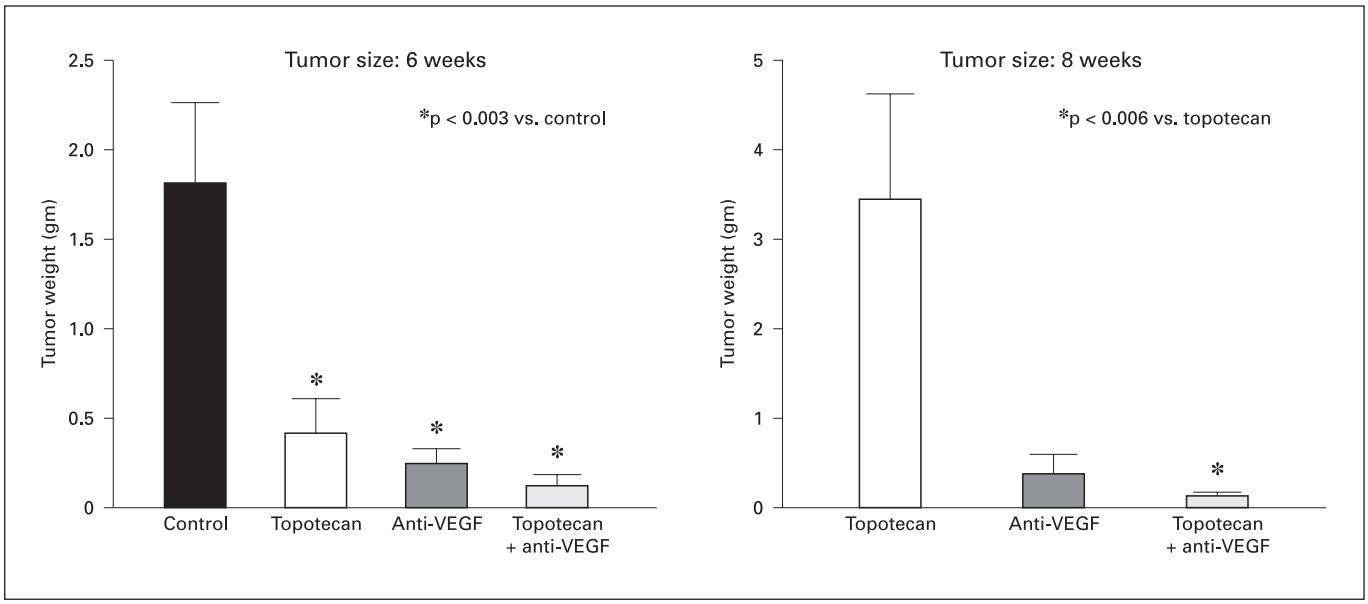

Fig. 2. A4.6.1 plus topotecan in Wilms tumor. Reproduced with permission. Copyright Elsevier 2001 [35].

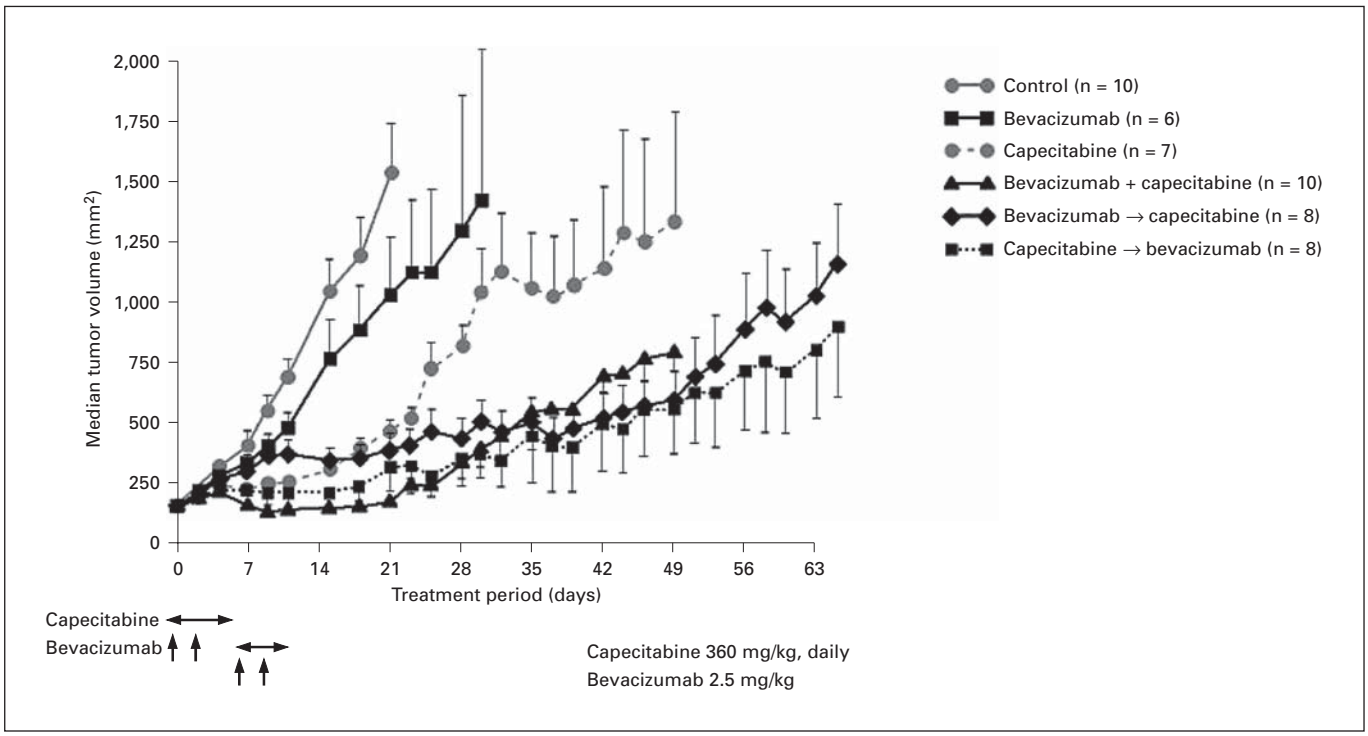

Fig. 3. Synergistic effect of Avastin® and capecitabine in a preclinical CRC model [36].

myosarcoma [29] and reduced vascular permeability, vessel diameter and tortuosity within tumors [30]. At doses of ≥2.5 mg/kg, muMAb A.4.6.1 was able to suppress tumor growth [31]. Subsequently, the humanized antibody bevacizumab was found to have antitumor effects and to inhibit VEGF-induced growth of endothelial cells in vitro in a similar fashion to the murine parent [28].

In animal xenograft models, bevacizumab was shown to have profound effects on tumor vasculature. Tumor vascular density was markedly lowered and interstitial pressure decreased by 75% in colon xenografts [9], while vascular permeability was decreased in breast tumor xenografts in athymic rats [32]. Bevacizumab has demonstrated synergy in combination with chemotherapy or radiotherapy in vitro, where bevacizumab overcomes VEGF-induced protection of endothelial cells against docetaxel treatment [33], and in vivo, where bevacizumab enhances tumor suppression in animals when added to cisplatin (Platinol®) [34], topotecan (Hycamtin®) [35], capecitabine (Xeloda®) [36] or radiation [37] (fig. 1–3).

The safety and pharmacokinetics of bevacizumab were evaluated in young adult cynomolgus monkeys. Following twice-weekly administration of bevacizumab at doses up to 50 mg/kg, the only side effects seen were physeal dysplasia and reduction in ovarian and uterine weight. Both of these effects were reversible on cessation of treatment and no other treatment-related effects were observed [38]. The pharmacokinetics of bevacizumab were predictable, with a terminal half-life of 1–2 weeks, clearance of 5 ml/day/kg and 100% bioavailability [39]. The encouraging data from these preclinical studies formed the foundation for the extensive clinical trials program, which led to the approval of bevacizumab in combination with chemotherapy for treatment of metastatic CRC and the ongoing evaluation of the antibody in a wide variety of other solid tumors and hematological malignancies.

Conclusions

Angiogenesis, mediated by VEGF, is crucial for tumor growth and normal development, but has limited applications in adults. Anti-angiogenic therapy has therefore been the subject of intensive research, with VEGF representing the best therapeutic target.

Targeting VEGF in cancer therapy has a number of advantages. Because VEGF is a circulating molecule, therapy does not need to penetrate the tumor, and inhibition of circulating VEGF reduces vascular permeability and thus tumoral interstitial pressure, permitting easier penetration of the tumor by conventional chemotherapeutic targets.

The clinical development of anti-angiogenic therapy is now at an advanced stage in a variety of tumors, but the only agent to have demonstrated a significant anticancer benefit is the humanized monoclonal antibody bevacizumab, which targets VEGF directly. Preclinical data demonstrated that bevacizumab has high antitumor activity with a favorable and predictable safety profile, and a randomized trial has shown a significant survival advantage for the addition of bevacizumab to chemotherapy in CRC. Trials in other settings are ongoing.

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platin (Eloxatin®) and capecitabine (Xeloda®). The survival of patients with metastatic disease has improved with the use of first-line combinations of 5-FU/LV with these new agents. The addition of irinotecan to bolus 5-FU/LV significantly improved median OS from 12.6 to 14.8 months (p = 0.042) [3]. A greater improvement in OS was seen when irinotecan was added to infusional 5-FU/LV (14.1 vs. 17.4 months, p = 0.032) [2]. Irinotecan was approved for the treatment of metastatic CRC in combination with bolus 5-FU/LV in 1996 and this combination, known as IFL, became the new first-line standard of care in the US for patients able to tolerate intensive chemotherapy [2, 3]. This was the standard regimen for the treatment of metastatic CRC at the time that phase III trials of bevacizumab were initiated.

Oxaliplatin is a third-generation platinum analog, which was approved in the US in 2002 as second-line therapy for CRC refractory to first-line IFL. The use of first-line oxaliplatin with infusional 5-FU/LV (FOLFOX) resulted in an OS of 16.2 months compared with 14.7 months in patients treated with 5-FU/LV alone [4]. Although the addition of oxaliplatin to 5-FU/LV improved OS, the outcome was not statistically significant (p = 0.12). In another study by Giacchetti et al., the addition of oxaliplatin to first-line 5-FU/LV also failed to significantly improve OS (19.4 months in the combination arm and 19.9 in the 5-FU/LV arm) [5]. A more recent study by Goldberg et al. (2004) demonstrated a survival benefit in patients treated with first-line FOLFOX compared with patients treated with IFL (19.5 vs. 15.0 months, respectively) [6]. Improvements in progression-free survival (PFS) and response rate were also seen (8.7 vs. 6.9 months and 45% vs. 31%, respectively). Based on these data, the US Food and Drug Administration (FDA) recently approved the FOLFOX regimen as first-line therapy for metastatic CRC.

Finally, capecitabine is an oral fluoropyrimidine indicated for first-line treatment of patients with metastatic CRC. Two large, randomized phase III trials compared capecitabine (1,250 mg/m^2 twice daily for the first 2 weeks of a 3-week cycle) (n = 603) with bolus 5-FU/LV (Mayo Clinic regimen) (n = 604) in patients with previously untreated metastatic CRC. While time-to-progression (TTP) and OS were similar in both treatment groups (4.6 vs. 4.7 months and 12.9 vs. 12.8 months, respectively), response rate was significantly higher in patients treated with capecitabine [7]. Safety analysis showed that capecitabine has a safety profile superior to that of 5-FU/LV, with a significantly lower incidence of adverse events and fewer hospitalizations [8].

While these new drugs have improved clinical outcomes for patients with CRC, the poor prognosis of this disease means that new therapies are still needed. Ideally, these would improve survival without increasing toxicity. A number of novel targeted therapies have been developed with this aim. Therapies targeting components of signaling pathways known to drive tumor progression have produced improvements in clinical outcomes in a number of indications; for example, trastuzumab (Herceptin®) in breast cancer [9] and erlotinib (Tarceva®) in non-small cell lung cancer [10].

Studies have shown that tumor progression and metastasis are dependent on angiogenesis (the formation of new blood vessels from existing vasculature), which provides the tumor with essential nutrients and oxygen [11]. These observations led to the hypothesis that blockade of anti-angiogenic signals combined with cytotoxic agents may result in effective tumor regression.

Bevacizumab is a humanized monoclonal antibody designed to inhibit vascular endothelial growth factor (VEGF), the key mediator of tumor angiogenesis [12]. Bevacizumab is currently approved in the USA and EU for the treatment of metastatic CRC in combination with first-line 5-FU-based chemotherapy, and is being evaluated in combination with standard therapies for a range of indications, including CRC.

The aim of this review is to provide an overview of the current clinical data for bevacizumab in combination with standard therapy in metastatic CRC.

Phase I Data with Bevacizumab

Early, phase I studies of bevacizumab focussed on the pharmacokinetics and safety of the drug. In the first of two trials, 25 patients with refractory solid tumors were treated with bevacizumab monotherapy at doses ranging from 0.1 to 10 mg/kg administered as a 90-min intravenous (i.v.) infusion on days 0, 28, 35 and 42 [13]. Pharmacokinetic assessments revealed that bevacizumab has a half-life of around 21 days in humans, which is comparable to the half-life of similar Fc fragment-based antibodies.

Infusions of bevacizumab were well tolerated, with no reported grade 3 or 4 infusion-related toxicities. The most common adverse events related to bevacizumab therapy included tumor-related bleeding, hypertension, headache, nausea and grade 1/2 asthenia. Of the 25 patients, two (8%) had a minor response and 12 (48%) had stable disease. Interestingly, five of the 12 patients who had sta-

ble disease had renal cell cancer, which is characterized by elevated levels of VEGF due to a mutation of the von Hippel-Lindau (VHL) gene that results in its inactivation and upregulation of hypoxia inducible factor 1 alpha (Hif1α), which in turn upregulates many angiogenic factors, including VEGF [14].

In a second phase I trial, bevacizumab was evaluated in combination with chemotherapy in 12 patients with previously treated metastatic cancer [15]. Bevacizumab 3 mg/kg weekly was administered in combination with chemotherapy: doxorubicin 50 mg/m^2 every 4 weeks (n = 4); carboplatin at area under the curve of 6 plus paclitaxel (Taxol®) 175 mg/m^2 every 4 weeks (n = 4); or 5-FU 500 mg/m^2 plus LV 20 mg/m^2 weekly for the first 6 weeks of an 8-week cycle (n = 4). Treatment was well tolerated without apparent synergistic toxicity. Three patients (one in each treatment arm) responded to treatment and continued receiving bevacizumab for more than 9 months.

The effects of long-term bevacizumab therapy were evaluated in an extension study involving patients from these trials and the initial phase II trial program [16]. The study evaluated 35 patients with advanced solid tumors who had received bevacizumab for at least 1 year. Six patients were treated for over 2 years. Of the 35 patients, 23 patients received bevacizumab in combination with chemotherapy. All patients underwent an observation period of 6 months, during which treatment was ceased. Therapy was re-introduced on disease progression and continued for a mean duration of 14 months, excluding the observation period. A complete response was seen in three patients, partial response in 15 patients and stable disease in 14 patients. Median survival had not been reached, but at the time of the report was >27.5 months, with 71% of patients alive at 2 years. In general, long-term therapy with bevacizumab was well tolerated; the overall rate of adverse events per patient-year of exposure to bevacizumab was lower in patients treated for longer than 1 year than in the overall trial population.

Overall, these early studies demonstrated that bevacizumab has the potential to be combined with many standard chemotherapy regimens and to be used in many of the major cancer types, including colorectal, non-small cell lung, breast and renal cell cancers. The development of bevacizumab is most advanced in metastatic CRC, which is the topic of the remainder of this review.

Phase II and III Trials of Bevacizumab for the Treatment of Metastatic CRC

Bevacizumab plus IFL

The first reported phase III trial of bevacizumab in metastatic CRC evaluated bevacizumab in combination with first-line irinotecan-based chemotherapy [17]. AVF2107 was a blinded, placebo-controlled, randomized phase III study designed to determine whether bevacizumab (5 mg/kg i.v. every 2 weeks) in combination with irinotecan (125 mg/m^2 by i.v. infusion), 5-FU (500 mg/m^2 by i.v. bolus) and LV (20 mg/m^2 by i.v. bolus) (IFL) weekly for 4 of 6 weeks prolongs OS in patients with previously untreated metastatic CRC. As previously discussed, when the trial was initiated in September 2000, IFL was the standard therapy for metastatic CRC in the US.

Patients were randomly assigned to one of three arms: IFL/placebo (n = 411); IFL/bevacizumab 5 mg/kg (n = 402); and 5-FU/LV (Roswell Park regimen)/bevacizumab (n = 110) in which LV 500 mg/m^2 was administered over 2 hours by i.v. infusion, and 5-FU 500 mg/m^2 was administered by i.v. bolus weekly for 6 weeks of an 8-week cycle [17]. The latter arm was used as a comparator to ensure that safety in the IFL arms was acceptable because no data were available to indicate the safety profile of bevacizumab when combined with irinotecan. The 5-FU/LV arm closed after an independent data monitoring committee reviewed safety data when approximately 100 patients had been enrolled to each arm (n = 313) and concluded that safety in the IFL arms was comparable to that in the 5-FU/LV arm. Patients were treated until disease progression, after which patients in the IFL/bevacizumab and 5-FU/LV/bevacizumab arms could continue bevacizumab therapy. No crossover to bevacizumab was allowed in the IFL-alone arm, meaning that any improvement in survival could be attributed to bevacizumab therapy if second-line therapies were balanced between the arms.

The primary endpoint was OS, which was significantly increased by 30%, from 15.6 to 20.3 months ($p < 0.001$), on the addition of bevacizumab (fig. 1) [17]. Second-line therapies were balanced between the arms, indicating that the improvement in survival was attributable to bevacizumab therapy. In addition, PFS was significantly increased by 71%, from 6.2 to 10.6 months ($p < 0.001$). The overall response rate was 44.8% and 34.8% for the IFL/bevacizumab and IFL/placebo arms, respectively ($p = 0.004$; table 1).

Following adjustment of factors that are prognostic for OS, PFS and objective response, the clinical benefit of adding bevacizumab to chemotherapy was shown to ex-

tend to all patient subgroups, including those defined by age, sex, performance status, location of primary tumor, number of organs involved and duration of metastatic disease [18]. These observations suggest that patient and disease characteristics should not be used to select patients to receive bevacizumab therapy.

A further subgroup analysis showed that patients who were given first-line IFL/bevacizumab followed by oxaliplatin-based post-progression therapy had a median OS of 25.1 compared to 19.6 months for patients in this arm who did not receive oxaliplatin post progression [19]. These data are consistent with the notion that there may be a subset of patients who may still benefit from treatment past the amount of progression permissible in most protocols; i.e. in settings where the clinician believes there has been a marked effect on the rate of tumor progression. To date, however, no prospective clinical trial data are available to address what value, if any, is conferred by continuation of bevacizumab past progression.

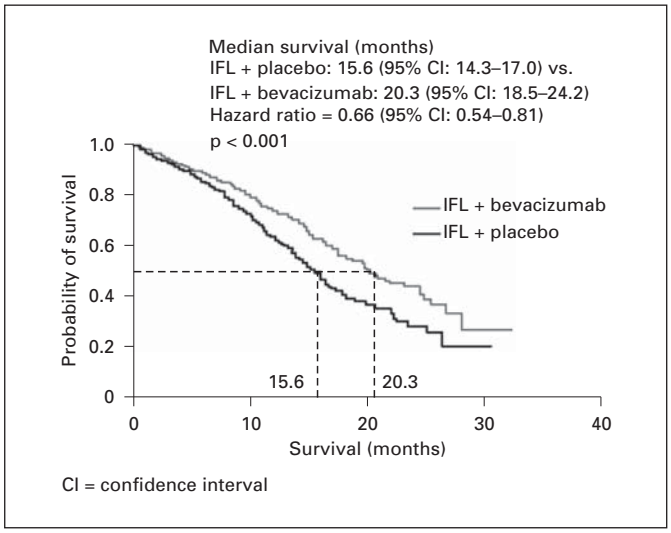

Fig. 1. Kaplan-Meier estimate of overall survival. Reproduced with permission. Copyright 2004 Massachusetts Medical Society. All rights reserved [17].

The addition of bevacizumab to chemotherapy was generally well tolerated and did not exacerbate chemotherapy-related adverse events (table 2). Safety results were not adjusted for treatment duration, which was 40.4 weeks in the IFL/bevacizumab arm and 27.6 weeks in the IFL/placebo arm [17].

Potential safety concerns identified in phase II trials of bevacizumab included hypertension, proteinuria, epistaxis, thrombosis, gastrointestinal (GI) bleeding, fever, rash and headache [20]. Of these, the most common side effect attributable to bevacizumab therapy in the phase III trial was hypertension (IFL plus bevacizumab, 22.4% vs. IFL plus placebo, 8.3%), which was easily manageable using oral antihypertensives without any lasting clinical consequences. Thromboembolic events occurred at a similar incidence in the IFL/bevacizumab and IFL/placebo arms (19.4 vs. 16.2%), but further analysis revealed that arterial thromboembolic events were more frequent in the bevacizumab arm [17]. A subsequent pooled analysis of five randomized trials in CRC and other tumors has indicated that the incidence of arterial thromboembolic events associated with bevacizumab is 3.8 vs. 1.7% with chemotherapy alone [21].

From a clinical perspective, the most significant side effect was GI perforation. Although infrequent, occurring in <2% of patients treated with bevacizumab, one patient died as a direct result of this complication [17]. In a US surveillance registry opened to enrol 2,000 patients with metastatic CRC receiving bevacizumab with first-line chemotherapy, the incidence of GI perforation was 1.6% in the 1,367 patients enrolled to date [22].

Table 1. Analysis of efficacy [17, 20, 23]

	AVF2107			AVF0780			AVF2192		
	IFL + placebo (n = 411)	IFL + bevacizumab 5 mg/kg (n = 402)	p value	5-FU/LV (n = 36)	5-FU/LV + bevacizumab 5 mg/kg (n = 35)	p value	5-FU/LV + placebo (n = 105)	5-FU/LV + bevacizumab 5 mg/kg (n = 104)	p value
Median OS, months	15.6	20.3	<0.001	13.8	21.5	0.137	12.9	16.6	0.1597
PFS, months	6.2	10.6	<0.001	5.2	9.0	0.005	5.5	9.2	0.0002
Overall response rate, %	34.8	44.8	0.004	17	40	0.029	15.0	26.0	0.0552
Duration of response, months	7.1	10.4	0.001	–	–	–	6.8	9.2	0.1184

Bevacizumab plus 5-FU-Based Chemotherapy

The initial trial (AVF0780) of this combination was a randomized, open-label, double-blind, phase II trial in 104 patients with untreated metastatic CRC [20]. Patients were randomized to one of three treatment arms: control (5-FU 500 mg/m^2 and LV 500 mg/m^2 [Roswell Park regimen]) (n = 36); bevacizumab 5 mg/kg every 2 weeks plus 5-FU/LV (n = 35); and bevacizumab 10 mg/kg every 2 weeks plus 5-FU/LV (n = 33). 5-FU/LV was given weekly for the first 6 weeks of each 8-week cycle. Patients were treated until disease progression, after which they could receive bevacizumab 10 mg/kg monotherapy every 2 weeks. Patients in the 5-FU/LV arm were allowed to crossover to receive bevacizumab on disease progression, which biases against observing a survival advantage for bevacizumab therapy.

The addition of bevacizumab 5 mg/kg to 5-FU/LV significantly improved the primary endpoint of TTP by 73%, from 5.2 to 9.0 months (table 1) [20]. Tumor response rate was increased from 17 to 40% and there was also an increase in median OS from 13.8 to 21.5 months.

Bevacizumab 5 mg/kg was generally well tolerated and did not increase chemotherapy-related toxicity. Bevacizumab therapy was associated with mild-to-moderate fever, headache, rash and chills. Bleeding (mainly epistaxis), hypertension, thrombosis and proteinuria, all of which were increased in patients receiving bevacizumab plus chemotherapy, are being monitored in all trials of bevacizumab.

In a second randomized, double-blind, controlled, phase II trial (AVF2192), bevacizumab was evaluated in combination with 5-FU/LV in patients with untreated metastatic CRC who were not optimal candidates to receive first-line irinotecan [23]. A total of 209 patients were randomized to either placebo plus 5-FU/LV (600 mg/m^2 and 500 mg/m^2, respectively) (n = 105) (Roswell Park regimen) or bevacizumab 5 mg/kg every 2 weeks plus 5-FU/LV (n = 104). The chemotherapy regimen was administered weekly for 6 weeks of an 8-week cycle.

The addition of bevacizumab to 5-FU/LV significantly prolonged the TTP by 67%, from 5.5 to 9.2 months (p = 0.0002) (table 1) [23]. The primary endpoint of survival was also improved by 29%, from 12.9 to 16.6 months, but was not statistically significant (p = 0.1597). This lack of significance in survival may be explained by the small number of patients in the trial and the increased availability of second-line therapies compared to previous trials.

The safety profile was similar to that reported for the phase III trial. Grade 3 hypertension, which was managed with oral medications, and asymptomatic proteinuria were the most common bevacizumab-related events. Bevacizumab therapy was also associated with GI perforation, which was reported in two (2%) patients receiving bevacizumab; there were no reported incidences of GI perforation in the control arm.

A combined analysis of the two phase II trials and the third arm of the pivotal phase III trial showed that adding bevacizumab to 5-FU/LV improves OS and PFS (table 3) [24]. The analysis included a control arm of patients with metastatic CRC treated with either 5-FU/LV or IFL. In the phase III trial, only patients from the IFL arm who were concomitantly enrolled with those receiving 5-FU/

Table 2. Bevacizumab does not increase chemotherapy-related toxicities

Adverse event	Study 2192 [23]*		Study 2107 [17]*	
	5-FU/LV + placebo (n = 104)	5-FU/LV + bevacizumab 5 mg/kg (n = 100)	IFL + placebo (n = 397)	IFL + bevacizumab 5 mg/kg (n = 393)
Diarrhea, grade 3 or 4, %	40	39	24.7	32.4
Leucopenia, grade 3 or 4, %	7	5	31.1	37
Vomiting, grade 3 or 4, %	10	7	10.4	7.7
All-cause mortality at 60 days, %	13.5	5.0	4.9	3.0
Adverse event leading to death, %	7	4	2.8	2.6
Adverse event leading to study discontinuation, %	12	10	7.1	8.4

NB = Results not adjusted for different time on therapy.
* Roche data on file.

Table 3. Combined analysis: efficacy [24]

Assessment	5-FU/LV or IFL[a] (n = 241)	5-FU/LV + bevacizumab 5 mg/kg (n = 249)
Patients who died	165	151
Median duration of survival[b], months	14.6	17.9
Hazard ratio[c] stratified analysis	–	0.74
p value (log rank)	–	0.0081
Median progression free survival, months	5.6	8.8
Hazard ratio[c] stratified analysis	–	0.63
p value, log rank	–	0.0001

[a] Patients in control arms of phase II/III bevacizumab studies.
[b] Summary statistics are shown from Kaplan-Meier analysis.
[c] Relative to 5-FU/LV or IFL arm; estimated by Cox regression.

LV plus bevacizumab were included as part of the control group. This group of patients was compared with patients from these studies who were treated with 5-FU/LV plus bevacizumab 5 mg/kg every 2 weeks. The addition of bevacizumab to 5-FU/LV increased OS and PFS from 14.6 to 17.9 months (p = 0.0081) and 5.6 to 8.8 months (p = 0.0001), respectively. This improvement in OS and PFS is similar to or better than that observed when irinotecan or oxaliplatin is added to 5-FU/LV [3, 4].

Bevacizumab plus Capecitabine

The effect of bevacizumab and capecitabine on the in vivo growth of CRC xenografts in nude mice has been studied and suggests that these agents have at least additive effects independent of treatment sequence [25]. This study provides evidence to support the use of bevacizumab combined with capecitabine to treat CRC.

To date, only one trial of bevacizumab plus capecitabine alone has been reported and this was in patients with metastatic breast cancer. Enrolled patients had previously been treated with both an anthracycline and a taxane. The trial failed to achieve its primary endpoint (PFS), but the addition of bevacizumab to capecitabine produced a significant increase in the objective response rate in patients treated with capecitabine plus bevacizumab compared with capecitabine alone (19.8% vs. 9.1%, p = 0.001; assessed by an independent review facility) [26]. The failure of this trial to show an improvement in PFS may be due to the fact that the patients in this trial had failed previous therapy and that the cancer may be at a later stage when there is less influence of VEGF [27]. Side effects were consistent with those reported in other trials of bevacizumab and included hypertension, proteinuria and epistaxis in the bevacizumab arm, with hypertension being the most common side effect (17.9% in the bevacizumab plus capecitabine arm vs. 0.5% capecitabine-alone arm). This trial provides an indication that administering bevacizumab with capecitabine is feasible and has biological activity.

While capecitabine plus bevacizumab has not yet been evaluated in metastatic CRC, the above data together with the similarity of outcomes for capecitabine and 5-FU/LV suggest that this combination will have similar efficacy to 5-FU/LV plus bevacizumab, while the safety profile may be improved.

Bevacizumab plus Oxaliplatin-Based Chemotherapy

Several phase II and III trials have been designed to investigate bevacizumab in combination with oxaliplatin-based regimens. NO16996 and TREE-2 are ongoing trials evaluating the addition of bevacizumab to oxaliplatin-based regimens as first-line therapy in patients with metastatic CRC. In the phase II trial TREE-2, bevacizumab significantly improved overall response rates when combined with either modified FOLFOX6, bolus 5-FU/LV plus oxaliplatin (bFOL) or capecitabine plus oxaliplatin (XELOX) (p = 0.004, pooled logistical regression analysis) [28].

Safety data show that grade 3/4 hypertension is increased when bevacizumab is added to any of the chemotherapy regimens. To date, the most serious side effect has been GI perforation. Although rare, it is potentially life-threatening. A detailed discussion on the safety profile of bevacizumab is provided in the accompanying article authored by Gordon and Cunnningham.

The recently completed E3200 trial evaluated bevacizumab 10 mg/kg every 2 weeks plus FOLFOX4 versus FOLFOX4 alone and bevacizumab alone in 829 previously treated patients with metastatic CRC [29]. Treat-

ment with this combination regimen significantly improved median OS from 10.8 months with FOLFOX4 alone, to 12.9 months (p = 0.0018). Patients treated with bevacizumab plus FOLFOX had an increased incidence of grade 3/4 hypertension, bleeding, sensory neuropathy and vomiting events compared with patients treated with FOLFOX4 alone.

Furthermore, preliminary data from a phase II study of bevacizumab combined with XELOX in first-line treatment of 30 patients with metastatic CRC have shown this regimen to be highly active [30]. Median TTP was approximately 12 months and the response rate was 57%. The regimen was generally well tolerated.

Together, the safety data indicate that combining bevacizumab with oxaliplatin-containing and infusional 5-FU regimens is well tolerated and does not substantially alter the toxicity profiles of these regimens.

Conclusions

Bevacizumab is the first anti-angiogenic agent with demonstrated anticancer benefit. The addition of bevacizumab to first-line chemotherapy provides unprecedented improvement in PFS and OS in patients with metastatic CRC when combined with IFL. There is also significant survival benefit for the combination of bevacizumab with 5-FU/LV. In second-line refractory metastatic CRC, the combination of bevacizumab with FOLFOX also confers a clinically significant survival advantage. Based on the unique mechanism of action of bevacizumab, which is different to that of other therapies used to treat CRC, and the consistent survival benefit seen with all regimens tested to date, it is expected that bevacizumab will be an effective partner for all other currently used regimens, hopefully increasing survival and improving patients' lives without causing additional toxicity. Bevacizumab is currently being investigated with other chemotherapy regimens (capecitabine monotherapy, FOLFOX, 5-FU/LV plus irinotecan [FOLFIRI], XELOX and capecitabine plus irinotecan [XELIRI]).

Based on these data for bevacizumab in combination with 5-FU-based chemotherapy, the US FDA awarded a licence for the use of bevacizumab in combination with first-line 5-FU-based regimens for the treatment of metastatic CRC. A European license was granted in January 2005 for the first-line use of bevacizumab in combination with i.v. 5-FU/LV with or without irinotecan.

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cer therapy. Approval of bevacizumab was based on the results of several large phase II and III studies in patients with previously untreated metastatic CRC [5–8]. A randomized phase III trial in patients with previously untreated metastatic CRC showed a 30% increase in median overall survival (OS) and a 71% increase in progression-free survival (PFS) for patients treated with bevacizumab plus irinotecan/5-fluorouracil (5-FU)/leucovorin (LV) (IFL) compared with those treated with IFL alone [5]. Two phase II studies have shown that adding bevacizumab to first-line 5-FU/LV in patients with metastatic CRC significantly improves PFS from 5.2 to 9.0 months and from 5.5 to 9.2 months, respectively (an approximately 70% increase) [6, 7]. Combining data from these three studies has demonstrated that adding bevacizumab to first-line 5-FU/LV-based chemotherapy significantly improves OS [8]. Thus, major studies of bevacizumab as first-line therapy for metastatic CRC demonstrate that it has clinically significant benefit when added to intravenous 5-FU with or without irinotecan.

Angiogenesis is a critical process in tumor development, but has limited roles in normal adult physiology [9]. Furthermore, the pro-angiogenic factor VEGF is required for the maintenance of immature blood vessels, such as those that are found in tumors, but not of the mature vasculature typical of adult organs and tissues [10]. Based on these features, it would be expected that bevacizumab would have limited toxicity and would not be associated with the gastrointestinal (GI), myelosuppressive and other effects typical of chemotherapy.

In clinical trials of bevacizumab monotherapy in renal cell cancer [11], breast cancer [12] and other tumors [13], few grade 3 or 4 adverse events and no dose-limiting or infusion-related toxicities have been observed. The most commonly observed adverse events were hypertension, proteinuria and bleeding, which were generally mild to moderate and manageable, and thrombosis. A similar side-effect profile for bevacizumab was observed in early trials in combination with chemotherapy [14]. No exacerbation of chemotherapy-related toxicity was seen in these trials, and this observation has been confirmed in the phase II and III trials in CRC [5–7].

Based on these data, the proven efficacy of bevacizumab in a variety of tumor types, and the known role of VEGF in the progression of many tumors [15], bevacizumab is being investigated in a wide range of tumor types and in combination with a variety of different chemotherapeutic and other agents [reviewed by Chen et al., 2004, 16]. To date, the side-effect profile of bevacizumab has been consistent and generally manageable. The side effects of bevacizumab are discussed below, together with management recommendations.

Table 1. Safety in the randomized study of bevacizumab plus IFL versus IFL alone in patients with previously untreated metastatic CRC. Reproduced with permission. Copyright 2004 Massachusetts Medical Society. All rights reserved [5]

Adverse event	IFL % patients n = 397	IFL + bevacizumab % patients n = 393
Any grade 3/4 adverse event	74.0	84.9*
Hospitalization due to adverse event	39.6	44.9
Discontinuation due to adverse event	7.1	8.4
Death from adverse event	2.8	2.6
Death within 60 days of treatment	4.9	3.0
Grade 3/4 leucopenia	31.1	37.0
Grade 3/4 diarrhea	24.7	32.4
Hypertension (all grades)	8.3	22.4*
Grade 3/4 hypertension	2.3	11.0*
Any thrombotic event	16.2	19.4
Deep thrombophlebitis	6.3	8.9
Pulmonary embolus	5.1	3.6
Grade 3/4 bleeding	2.5	3.1
Proteinuria all grades	21.7	26.5
Proteinuria grade 3	5.8	3.1
Proteinuria grade 4	0.8	0.8
GI perforation	0	1.5

Data were not adjusted for differences in the median duration of therapy between the group given IFL plus placebo and the group given IFL plus bevacizumab (27.6 vs. 40.4 weeks).

* $p < 0.01$.

Safety of Bevacizumab in Combination with Chemotherapy

In early phase I trials, bevacizumab alone and in combination with carboplatin (Paraplatin®) plus paclitaxel (Taxol®), doxorubicin (Adriamycin®) or 5-FU was well tolerated at doses of bevacizumab associated with VEGF blockade, with no evidence of exacerbation of chemotherapy-related toxicities and no cumulative or late toxicities [13, 14]. In subsequent phase II and III trials of bevacizumab [5–7, 11, 12, 17–19], the side effects associated with bevacizumab therapy were: hypertension (the most common bevacizumab-related event); generally asymptomatic proteinuria; arterial thrombosis; effects on wound healing; bleeding events, which were usually minor mucocutaneous events; and rare but potentially life-

Table 2. Hypertension (%) in clinical trials of bevacizumab in metastatic CRC [5–7]

Trial	Regimen	All grades	Grade 3/4	Grade 3	Grade 4
AVF0780	5-FU/LV	3	0	NR	NR
	5-FU/LV + bevacizumab 5 mg/kg	11	8.6	NR	NR
	5-FU/LV + bevacizumab 10 mg/kg	28	25	NR	NR
AVF2107	IFL	8.3	NR	2.3	0
	IFL + bevacizumab 5 mg/kg	22.4	NR	11	0
AVF2192	5-FU/LV	4.8	NR	2.9	0
	5-FU/LV + bevacizumab 5 mg/kg	32.0	NR	16.0	0

NCI CTC definitions of hypertension are: grade 1, asymptomatic, transient (<24 h) increase by >20 mm Hg (diastolic) or to >150/100 mm Hg if previously within normal limits (WNL) (intervention not indicated); grade 2, recurrent or persistent (≥24 h) or symptomatic increase by >20 mm Hg (diastolic) or to >150/100 mm Hg if previously WNL (monotherapy may be indicated); grade 3, requiring more than one drug or more intensive therapy than previously; grade 4, life-threatening consequences (e.g. hypertensive crisis).

NR = Not reported.

threatening occurrences of GI perforation. In the phase III trial of bevacizumab plus IFL versus IFL alone in 813 patients with metastatic CRC, there was a 10% absolute increase in the incidence of grade 3/4 adverse events, primarily due to the increase in grade 3 hypertension [5] (table 1). It is interesting to note that in this trial bevacizumab did not increase, and actually appeared to reduce, 60-day mortality, a combined measure of treatment-related death and treatment efficacy. Similar results were observed in the phase II trial in which patients unsuitable for first-line irinotecan therapy were randomized to 5-FU/LV with or without bevacizumab [7].

Trials to date indicate that the side effects associated with bevacizumab therapy are generally mild to moderate in severity and manageable, although there are specific, uncommon events that are more severe and can be life-threatening. The side-effect profile of bevacizumab is considered in detail below.

Hypertension

Hypertension is the most common side effect seen in trials of bevacizumab plus chemotherapy, with an overall incidence of 22–32% and grade 3/4 events in 11–16% of patients with metastatic CRC [5–7] (table 2). In a retrospective study of medical records from 40 CRC patients treated with bevacizumab, 30% had hypertension of any degree that was new or worsened [20]. In the randomized phase II trial of bevacizumab plus 5-FU/LV versus 5-FU/LV alone in patients with previously untreated metastatic CRC, grade 3/4 hypertension was not seen in patients treated with 5-FU/LV alone but occurred in 3/35 patients treated with bevacizumab at the 5 mg/kg dose level and in 8/32 patients treated at the 10 mg/kg dose level [6]. These data suggest a possible dose relationship. In the randomized phase III trial of bevacizumab 5 mg/kg every 2 weeks plus IFL versus IFL alone, grade 3 hypertension occurred in 11.0% of patients in the bevacizumab arm compared with 2.3% in the control arm [5]; similarly, in the phase II trial of first-line 5-FU/LV with or without bevacizumab in patients unsuitable for irinotecan therapy, the incidence was 16.0%, compared to 3% in the control arm [7]. These data suggest that at the recommended bevacizumab dose, the incidence of grade 3/4 hypertension is approximately 10–15%.

Most of these events are grade 3; few grade 4 events have been observed and only 0.7% of all bevacizumab-treated patients have discontinued therapy due to hypertension [21]. In the Common Toxicity Criteria version 2.0 used in trials of bevacizumab to date, grade 3 hypertension is defined as an increase in blood pressure that requires the initiation of oral antihypertensive therapy or a change in existing antihypertensive therapy to control blood pressure. Based on this, management of hypertension in patients treated with bevacizumab generally requires the administration of standard oral antihypertensive medication. Drugs used successfully in trials of bevacizumab include angiotensin-converting enzyme inhibitors and calcium channel blockers. In the phase III randomized study [5], all instances of hypertension were manageable in this way and no long-term complications of hypertension were

Table 3. Incidence (%) of proteinuria in trials in metastatic CRC [5–7]

Trial	Regimen	All grades	Grade 3/4	Grade 3	Grade 4
AVF0780	5-FU/LV	11.4	NR	NR	NR
	5-FU/LV + bevacizumab 5 mg/kg	22.8	NR	NR	NR
	5-FU/LV + bevacizumab 10 mg/kg	28.1	NR	NR	NR
AVF2107	IFL	21.7	NR	0.8	0
	IFL + bevacizumab 5 mg/kg	26.5	NR	0.8	0
AVF2192	5-FU/LV	19.2	NR	0	0
	5-FU/LV + bevacizumab 5 mg/kg	38.0	NR	1.0	0

evident: no patient required discontinuation of bevacizumab due to hypertension and there were no deaths or hospitalizations due to hypertension. In the earlier phase II study [6], one patient stopped bevacizumab treatment because of angina and hypertension.

In clinical trials, the onset of hypertension in bevacizumab-treated patients occurred at any time during therapy and did not appear to differ from that in patients treated with chemotherapy alone (Roche data on file). Langmuir et al., 2002 [22] have reported that in a group of 35 patients treated with bevacizumab for longer than a year, the incidence of hypertension is lower than in the overall population of patients. This suggests that hypertension tends to develop during the first year of therapy if it is going to develop. However, because of the limited sample size, further research is needed to fully clarify when hypertension is most likely to occur.

The mechanism of bevacizumab-related hypertension is not clearly understood. However, it seems likely that it may be related to inhibition of VEGF by bevacizumab, which is known to decrease the production of nitric oxide [23]. Nitric oxide is a known vasodilator, and therefore decreased production due to VEGF inhibition would result in vasoconstriction and increased blood pressure. This specific mechanism for bevacizumab-related hypertension fits with the fact that hypertension resolves in most patients once bevacizumab therapy is withdrawn. It is also interesting to note that hypertension appears to be a class effect of agents targeting the VEGF pathway and has been observed with a number of such agents [24–26].

In summary, patients who develop hypertension while receiving bevacizumab can be managed using standard oral antihypertensive therapy. To ensure that hypertension is identified and treated as early as possible, it is recommended that patients receiving bevacizumab have their blood pressure monitored at least every 2–3 weeks. Bevacizumab should not be initiated in patients with uncontrolled hypertension. Furthermore, if hypertension cannot be managed using standard oral antihypertensive therapy then bevacizumab therapy should be stopped. Similarly, bevacizumab should be permanently discontinued in any patient who experiences a hypertensive crisis.

Proteinuria

The incidence of proteinuria in trials of bevacizumab in metastatic CRC has been reported as 22.8–38% [5–7]. This compares to an incidence of 11.4–21.7% in patients treated with chemotherapy alone in these trials. In a retrospective study of medical records from 40 CRC patients treated with bevacizumab, 22.5% had proteinuria [20]. In the full safety population of 1,132 patients with different types of cancer treated in clinical trials of bevacizumab, the incidence was 23% [27].

Bevacizumab-related proteinuria is predominantly an asymptomatic event detectable only through laboratory analysis, as it is in patients treated with chemotherapy alone. In the phase II and phase III randomized trials of bevacizumab in metastatic CRC, in which patients with significant baseline proteinuria were excluded, there was no increase in the incidence of symptomatic, grade 3/4 proteinuria in bevacizumab-treated patients compared to those treated with chemotherapy alone, and grade 4 events were not observed [5–7] (table 3). Nephrotic syndrome has been seen only rarely in trials of bevacizumab.

Monitoring of patients treated with bevacizumab for proteinuria is recommended. This can be done using regular dipstick urinalyses. Those with a dipstick reading $\geq$2+ should be monitored further by 24-hour urine collection for total protein. It is recommended that bevacizumab therapy be interrupted in patients with proteinuria $\geq$2 g/24 h and that therapy be discontinued if a patient develops nephrotic syndrome, which is defined as persistent marked proteinuria resulting in hyperalbuminemia, hypercholesterolemia and edema.

Thrombosis

An apparently increased risk of thromboembolic events in bevacizumab-treated patients was detected in the phase II study in metastatic CRC in which patients received bevacizumab 5 or 10 mg/kg every 2 weeks [6]. The background incidence of thromboembolic events is high in patients with metastatic CRC and associated with both the disease and chemotherapy [28], but this trial suggested that these events were increased with bevacizumab therapy. In the light of these findings, thromboembolic events were analyzed in detail in subsequent studies in metastatic CRC [5, 7]. These analyses revealed that bevacizumab does not increase the overall risk of thromboembolism (incidence 18.0–19.4 vs. 16.2–18.3%) or the risk of venous thromboembolic events. However, an increased risk of arterial thromboembolism was noted, although such events were uncommon [29]. Both of these trials suggested that the risk of arterial thromboembolic events is increased two-fold in bevacizumab-treated patients, prompting a retrospective analysis of five trials to try to identify the true risk [30].

This analysis examined five completed clinical trials of bevacizumab: the phase III trial in first-line metastatic CRC (n = 925) [5]; the phase II trial of two bevacizumab doses in first-line metastatic CRC (n = 104) [6]; the phase II trial in patients with metastatic CRC not suitable to receive irinotecan (n = 209) [7]; a phase III trial in relapsed metastatic breast cancer (n = 462) in which patients were randomized to capecitabine (Xeloda®) plus placebo (n = 230) or capecitabine plus bevacizumab (n = 232) [31]; and a phase II trial in advanced or recurrent non-small cell lung cancer (NSCLC) in which patients were randomized to carboplatin/paclitaxel plus placebo or one of two bevacizumab doses (n = 98) [19]. The analysis revealed that the incidence of arterial thromboembolic events was 3.8% in patients receiving bevacizumab plus chemotherapy compared with 1.7% in patients receiving chemotherapy alone [30]. Mortality due to arterial thromboembolic events was 0.8% compared with 0.4%. The events that occurred included cardiovascular accident (stroke), transient ischemic attack, subarachnoid hemorrhage, myocardial infarction and angina.

Based on this analysis, in August 2004 the US Food and Drug Administration (FDA) required that a Dear Doctor letter be sent to all US oncologists describing the increased risk of developing arterial thromboembolic events in patients treated with bevacizumab. The letter included the information that patients with a history of arterial thromboembolic events and those aged >65 years have an increased risk of developing arterial thromboembolic events during bevacizumab therapy.

Table 4. Patients receiving warfarin and incidence of bleeding in the phase III trial of IFL with or without bevacizumab [32]

Therapy	Patients receiving full-dose warfarin	Bleeding incidence
IFL + placebo, n = 396	30 (8%)	2 (6.6%)
IFL + bevacizumab, n = 392	53 (14%)	2 (3.8%)

Information from the phase III trial of bevacizumab in metastatic CRC suggests that patients at risk of arterial thromboembolic events who receive bevacizumab can benefit from therapy [30]. Patients who developed venous thromboembolism were managed using full-dose anticoagulation, as described below [32]. A subgroup analysis of the phase III trial of IFL with or without bevacizumab demonstrated that patients aged >65 years who are treated with bevacizumab obtain survival benefit (24.2 vs. 14.9 months; hazard ratio = 0.61) despite the increased risk of arterial thromboembolism [33]. Furthermore, Hambleton et al. [32] analyzed hemorrhagic complications in patients in the phase III study who received anticoagulant therapy (full-dose warfarin) for thrombotic events, and found no evidence for any increase in these complications for patients in the bevacizumab arm (table 4).

In summary, patients treated with bevacizumab are at an increased risk of arterial thromboembolic events, particularly those aged >65 years or with a history of such events. Data indicate that patients aged >65 years benefit from bevacizumab therapy despite being at increased risk, and that patients can receive full-dose anticoagulation without any increase in the risk of bleeding. However, patients experiencing an arterial thromboembolic event while receiving bevacizumab should discontinue therapy immediately.

Wound Healing Complications

Angiogenesis is a critical process in wound healing and therefore bevacizumab inhibition of VEGF blockade would be expected to interfere with the wound healing process. The effects of bevacizumab on wound healing have been analyzed in most detail in a phase III trial [34]. The investigators examined the incidence of wound healing complications for patients who underwent surgery at

Table 5. Incidence of wound-healing complications and bleeding events in patients who underwent surgery 28–60 days prior to or during bevacizumab therapy [34]

	IFL + bevacizumab	5-FU/LV + bevacizumab	IFL
Surgery 28–60 days before treatment			
Underwent surgery/total no. of patients (%)	173/402 (43.0)	45/110 (40.1)	180/411 (43.8)
Incidence of bleeding events, %	1.7	2.2	2.8
Incidence of wound healing events, %	1.7	0	0
Surgery during treatment			
Underwent surgery/total no. of patients (%)	40/402 (9.9)	15/110 (13.6)	25/411 (6.1)
Incidence of events, %	10.0	6.7	0

28–60 days prior to starting bevacizumab therapy and for those patients who underwent surgery during bevacizumab therapy. Their analysis demonstrated that there was no increase in the risk of wound healing complications in patients who underwent surgery prior to bevacizumab therapy (incidence 3.4 vs. 2.2 vs. 2.8% in the IFL plus bevacizumab, 5-FU/LV plus bevacizumab and IFL alone arms, respectively); however, patients undergoing surgery during bevacizumab therapy are at an increased risk of wound healing complications (incidence 10.0 vs. 6.7 vs. 0%) (table 5) [34].

Based on this analysis, it is currently recommended that bevacizumab should be discontinued in patients who develop wound dehiscence requiring medical intervention and that, where possible, bevacizumab therapy should be discontinued prior to elective surgery. Based on clinical experience and the half-life of bevacizumab (21 days) [21], the gap between bevacizumab discontinuation and surgery should be at least 30 days. Emergency surgery during bevacizumab therapy can be undertaken following a careful risk:benefit analysis.

Bleeding

In patients with metastatic CRC treated with bevacizumab 5 mg/kg every 2 weeks in combination with chemotherapy, the incidence of grade 3/4 bleeding events was 3.1–5.1% [5–7] (table 6). This is considered to be not different to the incidence in patients treated with chemotherapy alone (2.5–2.9%). Similarly, in the full safety population of 1,132 patients with different tumor types, the incidence of grade 3 and 4 bleeding events was 4.0% [27].

Serious hemorrhagic complications have been observed in patients with NSCLC treated with bevacizumab [19]. Six of 66 patients (9%) experienced life-threatening pulmonary hemorrhage and/or hemoptysis, with four of these episodes being fatal. Based on a multivariate analysis, patients with squamous cell tumors were most at risk of hemorrhage, and these patients have been excluded from subsequent trials of bevacizumab in NSCLC. The incidence of pulmonary hemorrhage in lung tumors may also be related to the efficacy of bevacizumab, with many of the tumors involved being necrotic or having central cavitation, and the proximity of the tumors to major blood vessels. However, further research is warranted.

In other settings, the hemorrhagic complications of bevacizumab have been largely limited to minor epistaxis. These events are most commonly grade 1, last less than 5 min and resolve without medical intervention. They occur in 20–40% of bevacizumab-treated patients. Gingival and vaginal bleeding have also been observed, but are less common.

It is important to note that the risk of central nervous system (CNS) hemorrhage in patients with CNS metastases receiving bevacizumab has not been fully evaluated because these patients were excluded from clinical trials. Furthermore, no information is available on the safety profile of bevacizumab in patients with congenital bleeding diathesis, acquired coagulopathy or in patients receiving full dose of anticoagulants for the treatment of thromboembolism prior to starting bevacizumab treatment. However, as noted above, patients who developed thrombosis while receiving bevacizumab did not appear to be at increased risk of serious bleeding when treated with full-dose warfarin and bevacizumab concomitantly [32]. Similarly, no increased bleeding risk was reported with concomitant use of low-dose aspirin and bevacizumab [35].

Recommendations for the management of bleeding events in patients receiving bevacizumab include: epistaxis can be managed using standard first-aid tech-

Table 6. Incidence (%) of bleeding events in trials of bevacizumab in metastatic CRC [5–7]

Trial	Regimen	All grades	Grade 3/4	Grade 3	Grade 4
AVF0780	5-FU/LV	11*	0*	NR	NR
	5-FU/LV + bevacizumab 5 mg/kg	52*	0*	NR	NR
	5-FU/LV + bevacizumab 10 mg/kg	69*	9.4*	NR	NR
AVF2107	IFL	NR	2.5	NR	NR
	IFL + bevacizumab 5 mg/kg	NR	3.1	NR	NR
AVF2192	5-FU/LV	NR	2.9	1.9	1.0
	5-FU/LV + bevacizumab 5 mg/kg	NR	5.1	3.0	2.0

* Epistaxis + GI hemorrhage.

niques, including local pressure; patients with congenital bleeding diathesis, acquired coagulopathy and those receiving full-dose anticoagulation for the treatment of thromboembolism prior to starting bevacizumab treatment should be treated with bevacizumab with caution; bevacizumab should not be used in patients with CNS metastases; and patients requiring medical intervention for the management of bleeding should discontinue bevacizumab.

Gastrointestinal Perforation

GI perforation is an infrequent (incidence approximately 1.5%) but potentially life-threatening event that has occurred in a small percentage of patients with CRC treated with bevacizumab (table 7). In the phase III study, six patients (1.5%) receiving bevacizumab experienced GI perforation, several of whom died as a result [5]. These events included:

- sub-diaphragmatic air on plain abdominal radiograph not requiring surgery; the patient recovered and remained on study
- a perforated stomach ulcer; the patient recovered and remained on study
- colonic perforation associated with carcinomatosis; the patient recovered and remained on study
- colonic perforation with abdominal abscess; the patient died
- small bowel obstruction, abscess and perforated transverse colon; the patient recovered but was withdrawn from the study
- bowel obstruction, ileal necrosis and perforation; the patient recovered but was withdrawn from the study.

In the phase II trial in patients unsuitable for irinotecan therapy, GI perforations included:

Table 7. Incidence (%) of GI perforations in trials in CRC to date [5–7]

Regimen	Incidence of GI perforation
AVF2107	
IFL + placebo (n = 397)	0
IFL + bevacizumab (n = 393)	1.5
5-FU/LV + bevacizumab (n = 110)	0.9
AVF2192	
5-FU/LV + bevacizumab (n = 104)	0
5-FU/LV + bevacizumab (n = 100)	2.0

Note that GI perforation was not observed in the phase II trial of 5-FU/LV with or without two doses of bevacizumab [6].

- perforated diverticulum in sigmoid colon; the patient died
- perforated diverticulum in sigmoid colon; the patient recovered and remained on study.

The common feature of these GI perforations was intra-abdominal inflammation, either due to gastric ulcer disease, tumor necrosis or diverticulitis, or chemotherapy-associated colitis [19]. Preliminary data from a safety registry of bevacizumab with first-line chemotherapy in 1,367 patients with metastatic CRC showed that the incidence of GI perforation was 1.6%. More than half of patients experiencing GI perforation had one or more identified risk factors, such as acute diverticulitis, obstruction, tumor at site of perforation, abdominal carcinomatosis or a history of abdominal radiation [36].

Therefore, some patients with metastatic CRC and an intra-abdominal inflammatory process may be at increased risk for the development of GI perforation when

treated with bevacizumab and chemotherapy. These patients should therefore be treated with caution. Bevacizumab should be permanently discontinued in patients who develop GI perforation. Furthermore, early detection of GI perforation is essential and patients receiving bevacizumab need to be monitored carefully for signs of GI perforation. These include abdominal pain associated with constipation and/or vomiting.

Long-Term Safety of Bevacizumab Therapy

A long-term extension study has evaluated bevacizumab-related side effects in 35 patients with advanced solid tumors treated with bevacizumab with or without chemotherapy for more than 1 year [22]. The profile of events was similar to that seen in other trials of bevacizumab (predominantly mild and manageable hypertension, bleeding, proteinuria and/or thrombosis) and no unexpected events or deaths occurred with long-term bevacizumab treatment. Only one patient discontinued treatment due to an adverse event. Overall the rate of adverse events per patient-year was lower, primarily due to a lower risk of hypertension over time. In addition to these data, Yang, 2004 [37] has reported on four patients with renal cell cancer who have been treated with bevacizumab for up to 5 years. The only reported long-term side effect in these patients was proteinuria, which was substantial and affected three patients. However, renal function remained normal. Together, these data indicate that single-agent bevacizumab is not associated with cumulative or late toxicity and can therefore be used long term in patients with cancer who continue to respond to therapy.

Conclusions

Bevacizumab is the first anti-angiogenic agent to be approved for the treatment of cancer, and prolongs survival when added to standard chemotherapy for treatment of CRC [5, 8]. Bevacizumab has a favorable safety profile for combination with chemotherapy: it does not exacerbate the toxicity associated with chemotherapy; and the side-effect profile of bevacizumab is well defined and does not overlap with those of chemotherapeutic agents. The most common side effect of bevacizumab, hypertension, is generally easily manageable with standard oral antihypertensive medication and does not usually require interruption of treatment. Minor mucocutaneous bleeding events such as epistaxis are also more common with bevacizumab, but are easily manageable using standard first aid techniques; the incidence of asymptomatic proteinuria is also increased, but symptomatic events are uncommon and occur at a similar incidence with chemotherapy and chemotherapy plus bevacizumab.

Serious adverse events are uncommon with bevacizumab combination therapy, but GI perforation and arterial thrombosis have been identified as potentially life-threatening events that require careful monitoring. Patients presenting with abdominal pain on bevacizumab therapy should be monitored for signs of GI perforation and bevacizumab should be stopped if GI perforation is suspected or the patient has wound healing complications.

Overall, the addition of bevacizumab to standard chemotherapy is well tolerated with a generally favorable and manageable safety profile. Bevacizumab does not increase the incidence or severity of the side effects of chemotherapy, making it suitable for use in combination with standard chemotherapy.

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KARGER

Chemotherapy Options for Metastatic CRC

The development of new chemotherapies has improved survival in metastatic CRC, but the optimal treatment strategy remains to be defined, particularly the best sequence of drug administration [6]. A lack of head-to-head comparative efficacy trials for the first- and second-line treatment of metastatic CRC has resulted in wide variation in clinical practice between countries, regions and even hospitals [7, 8]. Choice of treatment is often dependent on factors such as physician and patient preference and convenience.

Currently, combinations usually incorporate a fluoropyrimidine (5-FU or capecitabine [Xeloda®]) and either oxaliplatin (Eloxatin®) or irinotecan (Camptosar®). Although 5-FU-containing regimens are most often used in the management of metastatic CRC, no consensus exists for the optimal treatment schedule for 5-FU. 5-FU is administered using various dosing schedules and approaches, including both bolus and short-term and protracted infusions, all with the aim of improving survival while limiting tolerability. 5-FU is usually combined with leucovorin (LV), as this combination produces higher response rates than 5-FU alone [9–11].

5-FU in Metastatic CRC

Administration of 5-FU as a continuous infusion is more effective than bolus 5-FU [12]. Two studies demonstrated that infusional 5-FU/LV produced higher response rates and prolonged progression-free survival (PFS) compared with the bolus Mayo Clinic regimen [10, 13]. However, both of these trials showed that there was no significant difference in overall survival (OS). Compared with patients given a bolus injection, patients given infusional 5-FU have a lower incidence of gastrointestinal (GI) and hematologic toxicity. However, a skin toxicity, hand-and-foot syndrome, is increased in patients receiving infusional 5-FU [10, 13]. Based on these and similar data, infusional 5-FU is widely used in Europe, both as monotherapy and in combination with irinotecan and oxaliplatin.

5-FU-Based Combinations in Metastatic CRC

To improve survival, 5-FU must be used in combination with other chemotherapeutic agents [9, 10, 12]. Typical chemotherapy combination regimens are shown in table 1. Combination of irinotecan with 5-FU produces significant improvements in survival compared with 5-FU/LV alone [14–16] (table 2). Addition of oxaliplatin to 5-FU/LV has also shown greater therapeutic benefit than 5-FU/LV alone, with phase III trials showing improvements in PFS [17–19] (table 3). Regimens such as FOLFOX (5-FU/LV plus oxaliplatin) and FOLFIRI (5-FU/LV plus irinotecan) (defined in tables 2 and 3) can prolong median OS in advanced CRC to 18+ months. Review of the irinotecan and oxaliplatin combination trials (tables 2 and 3) shows greater benefit in later trials, perhaps due to increased access to all active drugs (irinotecan, oxaliplatin and 5-FU).

Although combinations including 5-FU/LV with irinotecan or oxaliplatin have all shown efficacy in the treatment of advanced CRC, current data do not definitively show which is the best regimen or the best sequence of treatment. Comparison of first-line treatment with IFL or FOLFOX4 showed that IFL was a more toxic regimen than FOLFOX4. This greater toxicity is likely to be due to the use of bolus 5-FU in the IFL regimen rather than infusional 5-FU used in FOLFOX4. In addition, only data for high-dose irinotecan were reported, even though this dose was reduced during the trial due to toxicity. Compared to IFL (5-FU/LV/irinotecan), FOLFOX showed better efficacy. Median time to disease progression (TTP) differed significantly between patients receiving IFL (6.9 months) and those receiving FOLFOX (8.7 months) [20]. FOLFOX also produced longer OS: 19.5 months compared with 15.0 months for IFL. However, it should be noted that the design of this study may have impacted on the results. Most importantly, imbalances in second-line therapy between the IFL and FOLFOX arms, which led to more FOLFOX-treated patients receiving second-line irinotecan than IFL-treated patients receiving second-line oxaliplatin, may have biased OS in favor of the FOLFOX arm.

Recent studies comparing oxaliplatin- and irinotecan-based regimens have produced inconsistent results. In a phase II trial (n = 225), when combined with bolus 5-FU, both irinotecan and oxaliplatin were equally effective when given first line to patients with metastatic CRC (response rate 33 vs. 32%; TTP 8.9 vs. 7.6 months; OS 17.6 vs. 17.4 months) [21]. The authors state that irinotecan-based therapy may be preferable to avoid any neurotoxicity associated with the oxaliplatin regimen. Similarly, in a phase III trial involving 2,135 patients, OS was not significantly different for patients treated with irinotecan or oxaliplatin combined with the modified de Gramont regimen first or second line (OS 16.3 vs. 15.2 months, p = 0.165) [22]. In contrast, in a recent phase III trial of irinotecan plus high-dose folinic acid/5-FU (IFIFAFU) versus oxaliplatin plus high-dose folinic acid/5-FU (OXAFAFU) (n = 274), the OXAFAFU regimen was more ac-

Table 1. Commonly used regimens in CRC

Regimen	Drug	Dose	Day	Schedule*
Mayo Clinic	dl-LV	20 mg/m^2 bolus	1–5	Every 4 weeks (5-week cycle)
	5-FU	425 mg/m^2 bolus	1–5	Every 4 weeks (5-week cycle)
Roswell Park	dl-LV	500 mg/m^2 over 2 h	1	Once weekly for 6 weeks (8-week cycle)
	5-FU	500 mg/m^2 by slow push	1	Once weekly for 6 weeks (8-week cycle)
De Gramont (LVFU2)	dl-LV	200 mg/m^2 over 2 h	1 and 2	Every 2 weeks
	5-FU	400 mg/m^2 bolus	1 and 2	Every 2 weeks
	5-FU	600 mg/m^2 over 22 h	1 and 2	Every 2 weeks
AIO	dl-LV	500 mg/m^2 over 2 h	1	Once weekly for 6 weeks (8-week cycle)
	5-FU	2.3–2.6 g/m^2 over 24 h	1	Once weekly for 6 weeks (8-week cycle)
FOLFOX4	Oxali	85 mg/m^2	1	Every 2 weeks
	dl-LV	200 mg/m^2 followed by	1 and 2	Every 2 weeks
	5-FU	400 mg/m^2 bolus	1 and 2	Every 2 weeks
	5-FU	600 mg/m^2 over 22 h	1 and 2	Every 2 weeks
FOLFOX6	Oxali	100 mg/m^2 over 2 h	1	Every 2 weeks
	dl-LV	400 mg/m^2 over 2 h followed by	1	Every 2 weeks
	5-FU	400 mg/m^2 bolus	1	Every 2 weeks
	5-FU	2.4–3 g/m^2 over 46 h	1	Every 2 weeks
FUFOX	Oxali	50 mg/m^2 over 2 h	1	Once weekly for 4 weeks (5-week cycle)
	dl-LV	500 mg/m^2 over 2 h	1	Once weekly for 4 weeks (5-week cycle)
	5-FU	2.0 g/m^2 over 24 h	1	Once weekly for 4 weeks (5-week cycle)
Saltz	Irino	125 (reduced to 100) mg/m^2 over 1.5 h	1	Once weekly for 4 weeks (6-week cycle)
	dl-LV	20 mg/m^2 slow push (15 mins)	1	Once weekly for 4 weeks (6-week cycle)
	5-FU	500 (reduced to 400) mg/m^2 bolus	1	Once weekly for 4 weeks (6-week cycle)
Douillard	Irino	180 mg/m^2 over 1.5–2 h	1	Every 2 weeks
	dl-LV	200 mg/m^2 followed by	1 and 2	Every 2 weeks
	5-FU	400 mg/m^2 bolus	1 and 2	Every 2 weeks
	5-FU	600 mg/m^2 over 22 h	1 and 2	Every 2 weeks
FOLFIRI	Irino	180 mg/m^2 over 1.5–2 h	1	Every 2 weeks
	dl-LV	400 mg/m^2 over 2h followed by	1	Every 2 weeks
	5-FU	400 mg/m^2 bolus	1	Every 2 weeks
	5-FU	2.4–3 g/m^2 over 46 h	1	Every 2 weeks
XELOX	Oxali	130 mg/m^2	1	Every 3 weeks
	Cape	850–1,000 mg/m^2 b.d.	1–14	Every 3 weeks
XELIRI	Irino	250 mg/m^2 i.v.	1	Every 3 weeks
	Cape	800–1,000 mg/m^2 b.d.	1–14	Every 3 weeks

Oxali = Oxaliplatin; Irino = irinotecan; Cape = capecitabine.
* All regimens are given until progression or unacceptable toxicity.

tive (OS 18.9 vs. 15.6 months; p = 0.032) and showed less toxicity than IRIFAFU in patients with advanced CRC [23].

No comparisons of the therapeutic benefit of FOLFIRI and FOLFOX, which are both infusional 5-FU-based regimens and any comparison of which would illustrate the relative benefit of irinotecan and oxaliplatin, have been reported. To evaluate whether FOLFIRI or FOLFOX is preferable as first-line therapy, sequencing of these drugs was compared in a phase III trial (FOLFIRI → FOLFOX6 vs. FOLFOX6 → FOLFIRI) [24]. Both treatment sequences showed similar OS and PFS, and the toxicity

Table 2. Phase III trials showing the benefit of adding irinotecan to 5-FU/LV for the first-line treatment of patients with metastatic CRC

Regimen	Median OS months	Median PFS months
5-FU/LV (Mayo)	12.6	4.3
5-FU/LV + irinotecan [15]	14.8 ($p < 0.05$)	7.0 ($p < 0.01$)
LV5FU2	14.1	4.4
5-FU/LV + irinotecan [14]	17.4	6.7 ($p < 0.001$)
5-FU/LV (AIO)	16.9	6.4
5-FU/LV infusion + irinotecan [16]	20.1	8.5 ($p < 0.001$)

AIO = Arbeitsgemeinschaft Internische Onkologie.

Table 3. Phase III trials showing the benefit of adding oxaliplatin to 5-FU/LV in the first-line treatment of patients with metastatic CRC

Regimen	Median OS months	Median PFS months
5-FU/LV CMI	19.9	6.1
5-FU/LV + oxaliplatin [17]	19.4	8.7 ($p < 0.05$)
5-FU/LV	14.7	6.2
5-FU/LV + oxaliplatin [18]	16.2	9.0 ($p < 0.001$)
Bolus 5-FU/LV	16.1	5.3
5-FU/LV infusion + oxaliplatin [19]	20.4	7.8 ($p < 0.001$)

profiles were as expected: grade 3/4 GI toxicities, mucositis and grade 2 alopecia were more frequently seen with FOLFIRI, and grade 3/4 neutropenia and neurotoxicity were more frequent with FOLFOX6. The results of this study, which show OS is similar regardless of treatment sequence, were confirmed by analysis of data from seven phase III trials in advanced CRC [25]. OS was strongly associated with the use of three active drugs (5-FU/LV, irinotecan and oxaliplatin), irrespective of the order in which patients received the drugs in the course of disease. Therefore, to maximize OS, it is important to make all drugs that have well-demonstrated clinical activity available to all patients with advanced CRC. Grothey et al. [25] also state that PFS is a better measure of drug efficacy because the effect of second-line therapy is excluded.

Capecitabine in Metastatic CRC

Another drug that has shown clinical benefit in metastatic CRC is capecitabine [26]. Capecitabine is a tumor-selective oral fluoropyrimidine generating 5-FU preferentially in tumor tissue via the activity of thymidine phosphorylase. In patients with metastatic CRC, capecitabine produces improved objective tumor responses and has greater tolerability than 5-FU/LV (Mayo Clinic regimen) [27].

As well as its use as monotherapy, capecitabine can be administered orally in combination with oxaliplatin (XELOX) or irinotecan (XELIRI), with similar efficacies in both regimens. In a trial of 161 patients with metastatic CRC, the response rate in patients receiving capecitabine plus irinotecan (41%) was lower than that in patients receiving capecitabine plus oxaliplatin (51%) [28]. There was no significant difference in median OS (17 months in both groups) or PFS (7.1 months in the irinotecan group and 7.2 in the oxaliplatin group). Overall, this study showed that XELOX and XELIRI are highly effective and well-tolerated first-line treatments for metastatic CRC.

Combining Bevacizumab with 5-FU-Based Regimens – Current Status

As studies have shown that none of the currently used regimens have demonstrated clear superiority over other regimens [25], it is likely that bevacizumab will be combined with many different chemotherapy regimens. Initial studies have examined the benefits of combining bevacizumab with 5-FU and IFL, and ongoing studies are examining combinations with oxaliplatin and capecitabine.

Bevacizumab, the first approved therapy designed to inhibit angiogenesis, has considerable clinical benefits as first-line therapy for metastatic CRC. Studies have shown that bevacizumab combined with 5-FU/LV (Roswell Park regimen) is associated with clinical benefit [4, 29, 30]. Combined analysis of the data from three trials, in which patients treated with 5-FU/LV and bevacizumab 5 mg/kg (excluding patients from the third arm of the phase III trial) were compared with those treated with 5-FU/LV of IFL, demonstrated that addition of bevacizumab to 5-FU/LV significantly improved OS (17.9 vs. 14.6 months; $p < 0.0081$) and PFS (8.8 vs. 5.6 months $p < 0.0001$), compared with 5-FU/LV alone [31].

In a phase III trial involving 813 patients with previously untreated metastatic CRC who were treated with IFL with or without bevacizumab (5 mg/kg every 2 weeks), IFL plus bevacizumab produced significant increases in OS compared with IFL plus placebo (table 4) [5]. This trial also showed that response rates and PFS were significantly improved in patients receiving IFL plus beva-

Table 4. Summary of the therapeutic benefit demonstrated in trials with bevacizumab in combination with chemotherapy in patients with advanced CRC

Trial	Regimen	Median OS months	Median PFS months	Response rate %
AVF0780g [4]	Bevacizumab/5-FU/LV	21.5 and 16.1*	7.4**	32**
	vs.			
	placebo/5-FU/LV	13.8, $p > 0.05$	5.2, $p = 0.013$	17, $p = 0.086$
AVF2192g [29]	Bevacizumab/5-FU/LV	16.6	5.5	26
	vs.			
	placebo/5-FU/LV	12.9, $p = 0.160$	9.2, $p < 0.001$	15, $p = 0.055$
AVF2107g [5]	Bevacizumab/IFL	20.3	10.6	45
	vs.			
	placebo/IFL	15.6, $p < 0.0001$	6.2, $p < 0.001$	35, $p = 0.004$

* Results shown for bevacizumab 5 and 10 mg/kg respectively; ** pooled data (bevacizumab 5 and 10 mg/kg)

cizumab compared to those given IFL plus placebo. Retrospective analysis demonstrated that patients treated with IFL plus bevacizumab first line who then received oxaliplatin following progression had extended survival compared with patients treated with IFL plus placebo followed by oxaliplatin [5, 31]. The subgroup of 97 patients who progressed after treatment with IFL plus bevacizumab and then received second-line oxaliplatin had median OS of 25.1 months, compared with 22.2 months in the 109 patients who received IFL plus placebo followed by second-line treatment with oxaliplatin [32].

The significant survival benefits seen when bevacizumab is combined with 5-FU/LV and IFL in patients with metastatic CRC have led to its approval for the first-line treatment of metastatic CRC in combination with 5-FU-based regimens. Furthermore, the consistent effect of bevacizumab on survival (table 4) and the use of different chemotherapy regimens depending on local practice and patient preference have led to the investigation of the role of bevacizumab in other combinations and settings in CRC. In patients with metastatic CRC, treatment with bevacizumab plus chemotherapy has consistently extended median OS by approximately 4–5 months compared with chemotherapy alone. This evidence clearly suggests that adding bevacizumab to oxaliplatin-based combinations will further improve outcomes.

Survival data for bevacizumab in combination with first-line oxaliplatin or infusional 5-FU regimens have not been reported. Similarly, efficacy data for bevacizumab plus capecitabine alone are not yet available, although this combination has been shown to have biological activity and to be well tolerated in metastatic breast cancer [33]. However, several studies have reported preliminary data with these regimens [34–36]. Results from a phase II trial have demonstrated that the combination of XELOX and bevacizumab is an active regimen in the first-line treatment of metastatic CRC [34]. Patients received oxaliplatin (85 mg/m^2 on day 1), capecitabine (1,000 mg/m^2 bid on days 1–5 and days 8–12) and bevacizumab (10 mg/kg on day 1) administered on a 2-week cycle. In the 30 evaluable patients, 57% had a complete or partial response. Although hand-foot syndrome and diarrhea resulted in most patients requiring capecitabine dose modification, following modification the regimen was well tolerated. This study is continuing enrolment using a capecitabine dose of 850 mg/m^2 bid.

Combining Bevacizumab with Oxaliplatin-Based Regimens – Current Status

Three large studies are investigating the combination of bevacizumab with oxaliplatin-based chemotherapy regimens for the first-line (studies TREE-2 and NO16966) and second-line therapy (study E3200) of metastatic CRC.

TREE-2 is a randomized clinical study examining the benefit of three oxaliplatin-based infusional 5-FU regimens plus bevacizumab in 223 patients with metastatic CRC [35]. Patients receive bFOL (bolus 5-FU/LV plus oxaliplatin) plus bevacizumab (5 mg/kg every 2 weeks), XELOX plus bevacizumab (7.5 mg/kg every 3 weeks), or a modified FOLFOX6 regimen (mFOLFOX6) plus bevacizumab (5 mg/kg every 2 weeks). The trial is now fully recruited and preliminary efficacy data are available. An analysis of response showed a best response rate of 63%

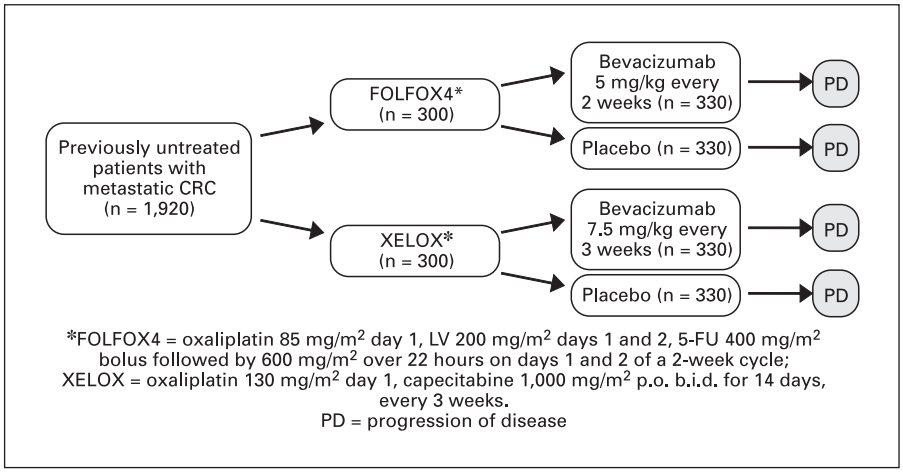

Fig. 1. Schematic diagram illustrating the design of the phase III trial NO16966C examining bevacizumab combined with oxaliplatin-based chemotherapy for the treatment of patients with metastatic CRC (q2w = every 2 weeks).

with the mFOLFOX6 plus bevacizumab regimen, 43% with the bFOL combination regimen and 57% with the XELOX combination regimen [37]. This was a significant improvement over the response rates seen with these regimens alone in TREE-1. Preliminary safety data indicate that all three study regimens are well tolerated, with a similar number of grade 3/4 events during the first 12 weeks of exposure and overall [35, 37]. Importantly, when the toxicity results are compared with those of the TREE-1 study, no increase in the incidence of adverse events associated with chemotherapy has been seen [35].

Study NO16966 is a randomized study with a 2×2 factorial design investigating the efficacy of XELOX with or without bevacizumab (7.5 mg/kg every 3 weeks) versus FOLFOX4 with or without bevacizumab (5 mg/kg every 3 weeks) as first-line treatment for patients with metastatic CRC (fig. 1). It is planned that a total of 1,960 patients will be recruited. The primary objectives of the study are to demonstrate at least equivalent TTP with XELOX (with/without bevacizumab) versus FOLFOX4 (with/without bevacizumab) and to show superior TTP with bevacizumab plus XELOX/FOLFOX versus XELOX/FOLFOX alone. No data are available yet from this study.

E3200 evaluated a higher dose of bevacizumab (10 mg/kg every 2 weeks) in combination with FOLFOX4 as second-line therapy for metastatic CRC [36]. Patients previously treated with a fluoropyrimidine and irinotecan were randomized to one of three arms (FOLFOX4 plus bevacizumab or FOLFOX4 alone or bevacizumab alone). Objectives of the study were to compare response, TTP and OS and examine toxicity. It is important to note that second and later lines of therapy may not be the optimal setting for bevacizumab due to the decreased influence of vascular endothelial growth factor (VEGF) [38] and as shown by Chen et al. [39]. However, recent data from E3200 demonstrate a significant improvement in OS with FOLFOX4 plus bevacizumab versus FOLFOX4 alone (12.9 vs. 10.8 months, respectively; hazard ratio [HR] = 0.76; p = 0.0018) [36]. Interim safety results suggest that the addition of bevacizumab to FOLFOX4 does not substantially alter these regimens' toxicity profiles. As with previous studies, hypertension was associated with use of bevacizumab [36]. Fistula and bowel perforation may also be related to the use of bevacizumab, but occur infrequently. However, no increased risk of thromboembolism was evident. Oxaliplatin-associated events were as expected, although the incidence of neurotoxicity was increased in the bevacizumab arm [36]; this is likely to be due to an increased duration of oxaliplatin therapy in the bevacizumab arm, although toxicity data over time have not been reported. Overall, the results illustrate that bevacizumab, even at a dose of 10 mg/kg every 2 weeks, can be safely administered with FOLFOX4, that adding bevacizumab to FOLFOX4 does not substantially alter the toxicity profile of FOLFOX4 and that bevacizumab is effective in combination with FOLFOX4 [36].

Initial data from the TREE-2 and E3200 studies suggest that bevacizumab is an effective agent when combined with infusional 5-FU-containing, capecitabine-containing and oxaliplatin-containing regimens in the first- and second-line setting in patients with metastatic CRC. The combinations are well tolerated and bevacizumab does not exacerbate the safety profile of the che-

motherapy regimen. The studies conducted to date have validated the hypothesis that inhibition of angiogenesis by bevacizumab is a valid approach in metastatic CRC. The future clinical role of bevacizumab in CRC will be driven by clinical data. Consequently, in addition to the ongoing studies described above, a number of other trials are ongoing.

Clinical Development Plans for Bevacizumab in CRC

Preclinical studies suggest that anti-angiogenic therapy should be used early in disease (either as adjuvant therapy or early in metastatic disease) to optimize its benefits [38]. VEGF plays an essential role in tumor angiogenesis by stimulating the growth of blood vessels that are essential for tumor growth. In addition, a positive correlation has been repeatedly demonstrated between circulating levels of VEGF and either tumor progression or patient survival [40–42]. At early stages in the disease, tumors are more reliant on VEGF than later in development, when other growth factors increasingly play a role [38]. In this regard, anti-VEGF treatment is likely to be most effective early in disease.

Trials in Metastatic CRC

In phase II and III trials to date, bevacizumab given as first-line treatment combined with chemotherapy has significantly improved response rates, TTP and OS in patients with metastatic CRC [4, 5]. Furthermore, bevacizumab has demonstrated a significant survival benefit when combined with chemotherapy in patients with metatstatic CRC who have failed previous chemotherapy [36]. Given our understanding of angiogenesis the focus of trials of bevacizumab in metastatic CRC remains on first-line treatment.

Chemotherapy Re-Introduction Strategy

The optimal use of chemotherapy to treat metastatic CRC remains the subject of debate. One area that is being examined is strategies to reduce the toxicity of chemotherapy regimens. The OPTIMOX or 'stop-and-go' approach has been used to reduce the cumulative sensory neurotoxicity of oxaliplatin, and involves administering a fixed number of cycles of FOLFOX, followed by a fixed number of cycles of 5-FU/LV with subsequent reintroduction of FOLFOX [43]. This approach maintains the efficacy of FOLFOX. Given that bevacizumab increases PFS and treatment duration, cumulative neurotoxicity could be an issue when it is administered in combination with oxaliplatin-containing regimens. Therefore, the OPTIMOX approach is being examined in clinical trials to try to limit this while maintaining efficacy.

The 'stop and go' strategy will be used in the DREAM trial, which is a randomized, international, phase III study with a 2×2 factorial design being conducted with the French clinical trials group, GERCOR. In this trial, patients will initially be randomized to bevacizumab with modified FOLFOX7 (mFOLFOX7) or XELOX2 for six cycles, with bevacizumab continuing until tumors reattain their original size or new tumors appear, when FOLFOX7 or XELOX2 will be re-introduced. There will be a second randomization to erlotinib (Tarceva®), an epidermal growth factor receptor-specific small molecule tyrosine kinase inhibitor, or placebo. A total of 640 patients will be recruited with the aim of showing an improvement in PFS with erlotinib.

CONcePT is another placebo-controlled trial with a 2×2 factorial design that will recruit 532 patients with metastatic CRC (fig. 2). This trial is examining the time-to-treatment failure of four treatment strategies aimed at reducing the neurotoxicity associated with mFOLFOX7 plus bevacizumab. All patients receive mFOLFOX plus bevacizumab. Patients are initially randomized to receive either placebo or calcium and magnesium infusions. This is followed by a second randomization to receive mFOLFOX either as continuous treatment until progression or using a 'stop and go' approach. Under the 'stop and go' strategy patients would 'stop' treatment after a predetermined cumulative oxaliplatin dose or when early sensory neurotoxicity is detected. Therapy would then be re-initiated after a predetermined interval when the sensory neurotoxicity has resolved or when oxaliplatin treatment is required to stop tumor progression.

These and other trials should provide insight into how best to manage patients who develop neurotoxicity while receiving oxaliplatin-containing chemotherapy in combination with bevacizumab.

Other Trials in Metastatic CRC

As a result of the many different chemotherapeutic combinations available to treat metastatic CRC, many chemotherapy regimens are being considered for combination with bevacizumab. Therefore, to examine the therapeutic benefit of bevacizumab in combination with all currently administered chemotherapies, various other phase II trials are planned or ongoing.

Several phase II trials are investigating bevacizumab in combination with capecitabine regimens. When used

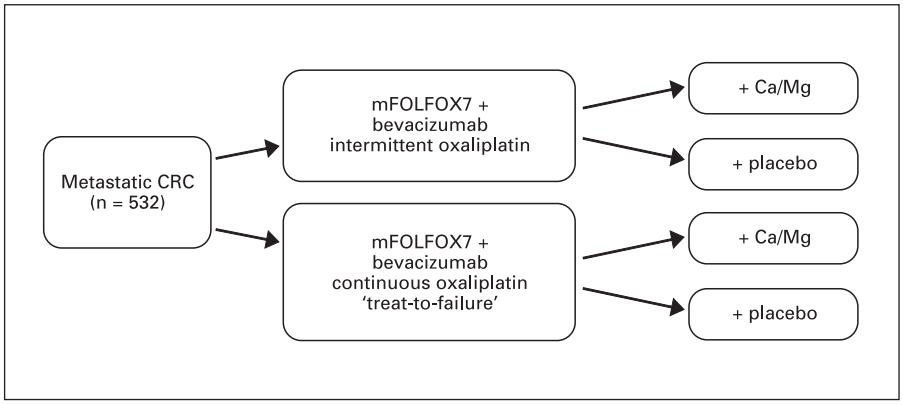

Fig. 2. Schematic diagram illustrating the 2 × 2 randomization of the phase III CONcePT trial for the prevention of neurotoxicity during bevacizumab + oxaliplatin-based chemotherapy.

as a single agent, capecitabine has demonstrated at least equivalent efficacy and better tolerability than intravenous 5-FU/LV in the metastatic CRC and adjuvant settings [24, 44].

Bevacizumab is also being evaluated in combination with non-toxic lower-dose chemotherapy (metronomic therapy). This method of administration has the potential to reduce the tumor vasculature and not just target the tumor cells. Metronomic chemotherapy combined with anti-VEGF therapy could make the vasculature more susceptible [45].

Clinical trials are also investigating bevacizumab in combination with other biological therapies, including cetuximab (Erbitux®), which is approved for use in patients with metastatic CRC who have failed prior irinotecan-containing chemotherapy. Phase II trials of bevacizumab in combination with cetuximab and irinotecan in bevacizumab-naïve (trial BOND-2) and bevacizumab-refractory (trial BOND-3), pretreated patients are currently underway [46]. BOND-2 has reported promising data indicating that bevacizumab improves response rates and PFS [46]. Finally, trials to examine the feasibility of bevacizumab therapy in patients whose liver metastases can be rendered resectable are planned.

Trials in Adjuvant CRC

As previously described, studies have shown that VEGF is essential for the progression and metastasis of CRC and VEGF is a prognostic indicator of OS, overall prognosis, time to recurrence and metastasis [40–42]. Furthermore, following surgery, VEGF is involved in the angiogenic switch to vascular malignant growth of micrometastases, which can cause tumor relapses. Consequently, anti-VEGF therapy has the potential to be used as an adjuvant treatment in CRC. Treating a liver metastasis mouse model with the anti-VEGF monoclonal antibody A4.6.1, the murine parent antibody of bevacizumab, not only inhibited the growth of xenografts but also markedly reduced both the number and size of liver metastases [47]. This, together with the significant survival improvements with bevacizumab as first-line therapy and its good tolerability in patients with metastatic CRC, suggests that bevacizumab may offer a useful option for adjuvant treatment of patients with CRC [4, 5]. As a result, several trials will examine the use of bevacizumab as adjuvant treatment.

In the adjuvant setting, it is especially important to address the tolerability of bevacizumab. In patients receiving bevacizumab, an increased risk of arterial thromboembolic events is seen. As an anti-angiogenic agent, bevacizumab also has the potential to interfere with wound healing following surgery, which could compromise its use in the adjuvant treatment setting. Administration of bevacizumab to patients with metastatic CRC has also resulted in GI perforations.

However, analysis of wound healing and bleeding complications in patients who underwent surgery 28–60 days prior to starting bevacizumab therapy revealed no increase in adverse events [48]. Therefore, surgery before the initial use of bevacizumab in the primary disease setting should not lead to a significant increase in the incidence of wound healing or bleeding complications. These results are encouraging for the safe use of bevacizumab in the adjuvant setting. Planned studies of bevacizumab in the adjuvant setting will carefully monitor safety data on an ongoing basis.

Until recently, the standard adjuvant therapy has been 5-FU/LV. However, the 3-year results of the MOSAIC

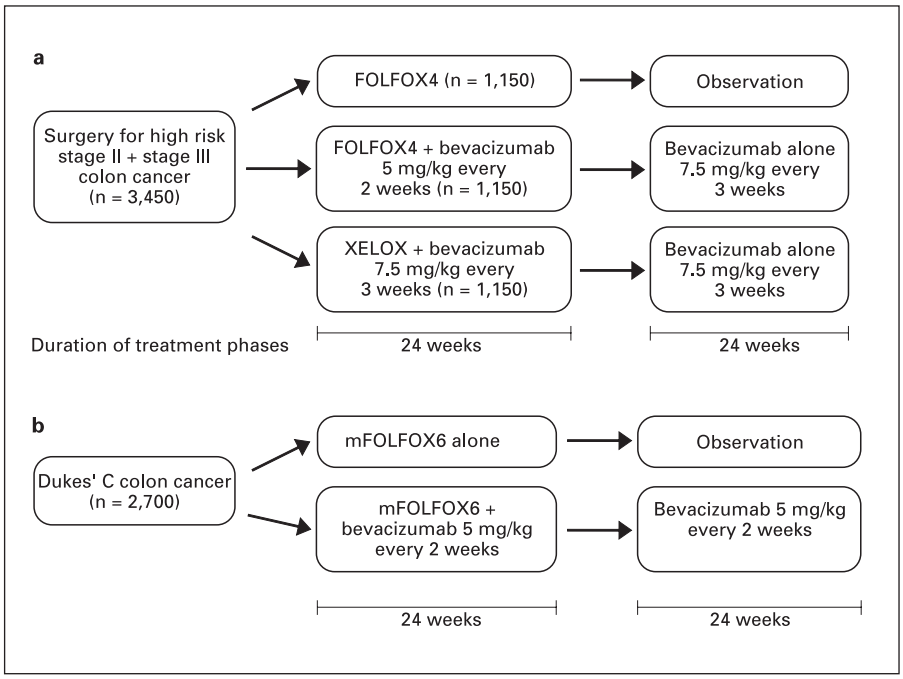

Fig. 3. Schematic diagram illustrating the design of two phase III trials of bevacizumab combined with oxaliplatin-based chemotherapy for the adjuvant treatment of patients with stage II or III CRC: AVANT (**a**) and NSABP C-08 (**b**) (q2w = every 2 weeks; q3w = every 3 weeks).

trial have shown that oxaliplatin combined with 5-FU/LV is a superior regimen to 5-FU/LV in this setting [49]. Disease-free survival (DFS) was significantly improved with FOLFOX4 compared with 5-FU/LV alone (77.8% vs. 72.9%, respectively; HR = 0.77 $p < 0.01$). In addition, the X-ACT trial has shown that capecitabine is at least equivalent to the 5-FU/LV [44]. Three-year multivariate analysis shows that, compared with 5-FU/LV, treatment with capecitabine has a significant effect on DFS, relapse-free survival and OS. FOLFOX or XELOX is thus likely to become the standard regimen for the adjuvant treatment of CRC. Consequently, the clinical development program for bevacizumab in adjuvant CRC is focused on assessing the clinical benefit gained by adding bevacizumab to oxaliplatin-containing regimens.

Combining Bevacizumab with FOLFOX in the Adjuvant Setting

Up to 350 centers in 36 different countries are participating in AVANT (BO17920), which is an open-label, three-arm phase III study in patients with stage II or III colon cancer (fig. 3a). Following surgery, 3,450 patients will be randomized to receive FOLFOX4 plus bevacizumab (5 mg/kg every 3 weeks) or XELOX plus bevacizumab (7.5 mg/kg every 3 weeks) or FOLFOX4 alone for 24 weeks. Patients in the bevacizumab arms will then continue to receive bevacizumab (7.5 mg/kg every 3 weeks) for a further 24 weeks, whereas patients in the FOLFOX arm will be observed. The primary endpoint of AVANT is DFS and the secondary endpoints are safety and OS.

NSABP (The National Surgical Adjuvant Breast and Bowel Project) C-08 is a US Cooperative Group phase III study that is examining the benefit of adding bevacizumab to FOLFOX in 2,600 patients (fig. 3b). Patients with resected stage II or III colon cancer will be stratified depending on the number of nodes involved. These patients will then be randomized to receive modified FOLFOX6 (every 2 weeks for 12 cycles) alone or in combination with bevacizumab (5 mg/kg every 2 weeks for 1 year). The primary aim of this study is to establish whether DFS can be improved by the addition of bevacizumab to modified FOLFOX6. Other goals are to compare the relative efficacy of the two treatment regimens and to assess adverse events related to bevacizumab.

E5202 is a phase III adjuvant trial evaluating bevacizumab plus mFOLFOX6 versus FOLFOX6 alone in 3,000 patients with stage II colon cancer. Molecular markers (18q loss of heterozygosity) will define if a patient is designated as high risk and randomized to receive adjuvant therapy (mFOLFOX6 plus bevacizumab or mFOLFOX6 alone), or low risk, and followed for observation only.

Table 5. Summary of phase III trials with bevacizumab-based combinations in patients with CRC

Name	Investigating bevacizumab in combination with	Patients	Status
Metastatic CRC, first-line			
Hurwitz et al. [5]	IFL	923	Completed
NO16966C	XELOX or FOLFOX	1,920	Accrual complete
TREE-2 [35, 37]	mFOLFOX6	223	Accrual complete
CONcePT	mFOLFOX6	532	Planned
Metastatic CRC, second-line			
E3200 [36]	FOLFOX4	829	Completed
Stage II or III CRC, adjuvant treatment			
AVANT	FOLFOX4 or XELOX	3,450	Ongoing
NSABP C-08	mFOLFOX6	2,600	Ongoing

Bevacizumab in the Neoadjuvant Setting

Unlike colon cancers, the optimal method of management for rectal cancers is dependent on tumor stage at the time of diagnosis. Although surgical resection is the standard treatment for rectal cancers, many patients present with locally advanced (stage T4–T4), unresectable disease. Neoadjuvant chemoradiotherapy comprising radiotherapy and 5-FU/LV is often used to down-stage the cancer to render it resectable. Bevacizumab in combination with chemoradiotherapy has been evaluated in patients with stage T3 or T4 rectal cancer [50]. In this study, nine patients with primary and locally advanced adenocarcinoma of the rectum received neoadjuvant bevacizumab 5 mg/kg (6 patients) or 10 mg/kg (3 patients) followed 2 weeks later by three 2-week cycles of bevacizumab, 5-FU and radiotherapy. Six patients in the 5 mg/kg arm completed therapy without any dose-limiting toxicities, and underwent surgical resection without any complications. None of the three patients treated with bevacizumab 10 mg/kg had dose-limiting toxicities and one patient underwent resection without complication. At the time of the report, the remaining two patients were scheduled for surgery. These early data suggest that the combination of bevacizumab with chemoradiotherapy may be feasible for the neoadjuvant treatment of rectal cancers.

The liver is a common site for metastases originating from cancer of the colon or rectum and is often unresectable. Neoadjuvant therapy aims to downsize unresectable metastases so that they can be surgically removed. Preclinical studies have demonstrated that anti-VEGF therapy reduces the number and size of liver metastases in a CRC xenograft mouse model [47]. Furthermore, VEGF receptors (VEGF receptor-1 and -2) and ribosomal RNA for these receptors are highly expressed in human liver metastases from primary colorectal carcinomas [47]. Together, these studies support the use of bevacizumab for the management of liver metastases. Clinical trials are investigating the feasibility of liver resection following treatment with bevacizumab combined with chemotherapy. In the pivotal phase III trial [5], six out of 40 patients who underwent surgery during bevacizumab treatment had liver metastasectomy. No patients experienced any complications, and two of the six patients resumed bevacizumab treatment after surgery. Further trials of neoadjuvant Avastin are planned, including a study of the effect of bevacizumab, cetuximab and FOLFOX on resectable liver metastases.

Conclusions

Phase II and III trials have shown that, when combined with standard chemotherapy regimens, bevacizumab can significantly improve the outcomes of patients with CRC [4, 5]. Adding bevacizumab to chemotherapy has shown consistent efficacy with a good tolerability profile. Furthermore, results to date suggest that the incremental increases in therapeutic benefit seen upon addition of bevacizumab to standard chemotherapy regimens can be translated to other regimens. The current clinical program will investigate this, examining bevacizumab in combination with commonly used chemotherapy regimens given to patients with metastatic CRC (table 5). The program will also determine the activity of bevacizumab in combination with FOLFOX and XELOX in the adjuvant setting (table 5). Bevacizumab has the potential to further improve the outcome of patients with CRC, with improved efficacy in both the first-line metastatic and adjuvant settings when combined with all standard chemotherapy regimens.

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KARGER

Introduction

In phase II and III clinical trials, bevacizumab (Avastin®), a monoclonal antibody targeted against vascular endothelial growth factor (VEGF), has been shown to have unprecedented survival benefit in patients with metastatic colorectal cancer (CRC) [1, 2]. These data led to its approval by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of patients with previously untreated metastatic cancer of the colon or rectum, in combination with intravenous 5-fluorouracil (5-FU)-based chemotherapy.

Bevacizumab also has the potential to increase survival in malignancies other than CRC. As solid tumors cannot grow without the nutritional support provided by a blood supply, angiogenesis and VEGF play a central role in tumor progression [3]. VEGF overexpression correlates with poor prognosis and survival rates in patients with these cancers. Bevacizumab binds to VEGF, inhibiting angiogenesis and thereby preventing tumor growth and metastasis [4]. Consequently, the therapeutic benefit of bevacizumab is also being investigated in other tumors.

Trials are in progress investigating the potential of bevacizumab in indications with a high unmet need, including metastatic renal cell cancer (RCC), non-small cell lung cancer (NSCLC), pancreatic cancer and breast cancer. These cancers have high prevalence and mortality rates. In addition, multiple phase II non-randomized trials in other indications, such as melanoma, head and neck cancer, hepatocellular carcinoma and hematological malignancies, are ongoing in the US. By providing new treatment options in these tumor types, bevacizumab offers the potential to increase survival without substantially adding to the toxicity of standard therapy.

Metastatic RCC

RCC usually occurs in adults between the ages of 50 and 70 and is the most common cancer of the kidney, accounting for 3% of all human cancers and over 90% of malignant kidney tumors. Between 25 and 30% of patients have metastases at the time of diagnosis and RCC is responsible for approximately 95,000 deaths per year worldwide [5]. RCC is classified into five types but most patients (between 70 and 80%) with RCC have the clear cell type.

The treatment of RCC depends on the stage and the patient's overall physical health. Surgery is typically performed in lower stage disease, with systemic therapy reserved for when there has been spread of the cancer. Unfortunately, RCC tends to be very resistant to chemotherapy with generally limited responses in patients with metastatic RCC. Consequently, various types of immunotherapy are currently preferred [6]. Immunotherapy with cytokines, such as interferon-α and interleukin-2 (IL-2), can result in a modest improvement in median survival and in selected patients has led to long-term survival [7]. However, in the majority of patients with RCC, therapeutic benefit remains limited and new treatment options are therefore needed.

Most patients with clear cell RCC have loss of function of the von Hippel-Lindau (VHL) tumor suppressor gene, which under normal conditions has a role in the regulation of pro-angiogenic factors in response to hypoxia [8]. In RCC, mutations in the VHL gene lead to the overexpression of factors normally controlled by VHL, including VEGF [9]. Furthermore, increasing VEGF levels correlate with reduced survival [10]. Therefore, inhibition of VEGF with an anti-VEGF drug such as bevacizumab could be a viable therapeutic strategy in clear-cell RCC.

Biological activity with bevacizumab has already been shown in patients with metastatic RCC refractory to immunotherapy [11]. In a randomized, double-blind phase II trial of 116 patients, bevacizumab 3 or 10 mg/kg every 2 weeks was compared with placebo. Crossover from placebo to bevacizumab 3 mg/kg with or without thalidomide was permitted, so overall survival (OS) was only a secondary endpoint. The two primary endpoints were time to progression (TTP) and response. This trial was closed early because progression-free survival (PFS) was significantly longer in patients treated with bevacizumab 10 mg/kg than in those receiving placebo (hazard ratio [HR] = 2.55, $p < 0.001$). Albeit smaller and of borderline significance, an effect on PFS was also seen with bevacizumab 3 mg/kg (HR = 1.26, $p = 0.053$). Survival benefit was not observed, possibly because patients in the placebo arm were allowed to cross over to receive bevacizumab alone or with thalidomide at progression, or due to the small numbers of patients enrolled. There were no life-threatening toxicities or deaths related to bevacizumab therapy. Side effects were minimal, with the most common adverse events being hypertension and asymptomatic proteinuria. Four patients have been undergoing long-term bevacizumab therapy without tumor progression for 3 to 5 years; even though three have substantial proteinuria, these patients have normal renal function [12]. Patients in the placebo arm who showed tumor growth were allowed to cross over to a small pilot study

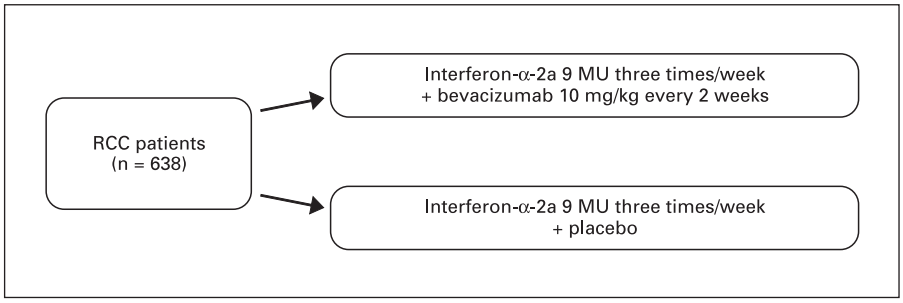

Fig. 1. Study design of BO17705 – a Roche-sponsored phase III trial of interferon-α-2a with and without bevacizumab in patients with advanced renal cell cancer.

of bevacizumab combined with thalidomide and were followed for toxicity. The authors concluded that the combination showed no unexpected toxic effects but thalidomide did not add to the efficacy of bevacizumab in these patients [12, 13].

Examination of the total tumor burden in bevacizumab-treated patients in this trial showed that those who progressed after receiving bevacizumab 10 mg/kg had a lower tumor burden than at baseline; those who progressed while receiving bevacizumab 3 mg/kg had net stability of tumor volume, which is rarely observed in patients receiving placebo [12]. Based on their observations, the authors noted that investigating the effect of bevacizumab on survival in patients allowed to continue on bevacizumab despite tumor progression would be valuable. They also concluded that combining bevacizumab with other agents could lead to even greater benefit in patients with RCC.

Like VEGF, epidermal growth factor receptor (EGFR) is another biological target being studied in RCC. EGFR is overexpressed in RCC, and its blockade decreases the proliferation of RCC cells. As a result, trials are examining bevacizumab plus erlotinib (Tarceva®), an inhibitor of EGFR signalling. Initial results from a phase II trial examining this combination have been reported for 59 patients with metastatic RCC [14]. Bevacizumab (10 mg/kg every 2 weeks) and erlotinib (150 mg daily) has proved to be one of the most active combinations examined in metastatic RCC to date (table 1). The median OS was almost 23 months and the median TTP was just over 11 months. The median TTP compares favorably to bevacizumab alone (approximately 5 months) and immunochemotherapy regimens (interferon-α-2a plus vinblastine (Velbe®), 5 months; interferon-α-2a + IL-2 + 5-FU, 6 months [15]). The regimen was well tolerated with fewer than half of the patients having grade 3/4 toxicity. The most common side effects were rash and diarrhea related to erlotinib therapy (table 4). The substantial clinical activity of the combination of bevacizumab and erlotinib in RCC, which is superior to the activity of either agent when used as monotherapy, illustrates that targeting different pathways involved in tumor development is a feasible and active approach and that further studies are warranted. Triple combination therapy with bevacizumab in RCC is also being investigated in a phase I/II trial that combines bevacizumab (10 mg/kg every 2 weeks), erlotinib (150 mg daily) and imatinib (Glivec®), a tyrosine kinase inhibitor (300, 400 to 600 mg/day dose escalation) in advanced RCC [16].

Table 1. Responses to bevacizumab and erlotinib in patients with metastatic RCC (59 evaluable patients) [14]

Response	Patients (%)
Partial response	13 (22)
Minor response	13 (22)
Stable disease	23 (39)
Continued progression	8 (14)

The main focus of bevacizumab development in RCC is in combination with standard therapy, as it is in other indications. Thus, the combination of bevacizumab and immunotherapy is being investigated in patients with metastatic RCC. A phase III trial evaluating the survival benefit of adding bevacizumab to interferon-α-2a will recruit up to 638 patients (BO17705) (fig. 1). Patients will receive interferon-α-2a (9 MU 3 times per week for a maximum of 52 weeks) with or without bevacizumab 10 mg/kg every 2 weeks until progression. In addition, a US Cooperative Group trial is investigating the survival benefit of bevacizumab in combination with interferon-α-2b (CALGB 90206; 600 patients). These trials should build on the existing evidence that bevacizumab is an active drug in RCC and help to establish the role of bevacizumab in this tumor type.

Metastatic NSCLC

NSCLC is the most common cancer, affecting over 800,000 people worldwide. The majority of people diagnosed with NSCLC are unsuitable for surgery. Several agents, including gemcitabine (Gemzar®), paclitaxel (Taxol®), docetaxel (Taxotere®), topotecan (Hycamtin®), irinotecan (Camptosar®) and vinorelbine have been shown to be active in the treatment of advanced NSCLC [17]. These chemotherapeutic agents are usually combined, typically with either cisplatin (Platinol®) or carboplatin (Paraplatin®) (e.g. gemcitabine/cisplatin) [18]. Standard therapy in the US is paclitaxel/carboplatin and in Europe, gemcitabine/cisplatin. Systemic chemotherapy can produce responses and palliation of symptoms for short durations in patients with advanced disease. However, chemotherapy offers only modest improvements in median survival and OS is poor. Unfortunately, the outcome of standard treatment is poor in all but the most localized cancers, and only a small minority of patients are cured.

The efficacy of gemcitabine/cisplatin has been demonstrated in phase III clinical trials. The combination of gemcitabine and cisplatin has shown improvements in response rate (30 vs. 11%), median TTP (5.6 vs. 3.7 months) and OS (9.1 vs. 7.6 months) compared with cisplatin alone [19]. However, these improvements are small and new treatments that improve outcomes are needed. Furthermore, because treatment is unsatisfactory for almost all patients with NSCLC, most eligible patients should be considered for clinical trials.

Clinical trials are now investigating the use of targeted therapies such as monoclonal antibodies, vaccines and gene therapy. Angiogenesis inhibitors are a promising area of investigation in NSCLC. The malignancy is a rational target for angiogenesis inhibitors, particularly anti-VEGF therapies, as a number of studies have illustrated that VEGF overexpression is linked to a worse prognosis [20] and patients with increased VEGF expression have a shorter OS. Studies are therefore in progress to examine the therapeutic benefit of inhibiting the VEGF pathway.

A phase II trial of 99 patients with metastatic NSCLC demonstrated that adding bevacizumab to chemotherapy increased therapeutic benefit compared to chemotherapy alone [21]. Patients in this trial received carboplatin/paclitaxel alone or chemotherapy plus either bevacizumab 7.5 or 15 mg/kg every 3 weeks. Patients receiving chemotherapy plus bevacizumab 15 mg/kg had a higher response rate, longer median TTP and a modest increase in survival compared with patients receiving chemotherapy alone (table 2). Although patients given chemotherapy plus bevacizumab 7.5 mg/kg showed similar outcomes to the control group (table 2), it should be noted that patients in the control arm had a particularly long median survival (14.9 months compared to 8 months in similar patient populations [17, 22]), which may be due in part to 19 of the 32 control patients crossing over to receive bevacizumab following disease progression. In addition, 10 patients in the bevacizumab 7.5 mg/kg arm had squamous NSCLC, which is associated with an increased risk of severe hemoptysis in this indication. This may have further biased against observing a benefit for bevacizumab therapy.

Table 2. Responses to bevacizumab plus chemotherapy in patients with metastatic NSCLC (99 evaluable patients) [21]

	Chemotherapy alone (n = 32)	Chemotherapy + bevacizumab (7.5 mg/kg) (n = 32)	Chemotherapy + bevacizumab (15 mg/kg) (n = 34)
Response rate, %	18.8	28.1	31.5
TTP, months	4.2	4.3	7.4
OS, months	14.9	11.6	17.7

In general, adverse events (e.g. leukopenia, hypertension) were mild and easily managed. However, six patients receiving bevacizumab developed severe hemoptysis (five receiving bevacizumab 7.5 mg/kg and one receiving the 15 mg/kg dose). Analysis of risk factors suggested that patients with squamous cell histology and/or central, cavitary or necrotic tumors may be at greatest risk of this complication. Consequently, entry criteria for future NSCLC trials have been adjusted to exclude patients with squamous cell histology (around 30% of NSCLC patients).

Bevacizumab combined with carboplatin/paclitaxel has been further examined in a US Cooperative Group phase III randomized trial (E4599) that accrued 878 patients with advanced non-squamous cell NSCLC [23]. Patients were randomized to receive paclitaxel plus carboplatin with or without bevacizumab (15 mg/kg every 3 weeks). Patients given chemotherapy plus bevacizumab 15 mg/kg had a higher response rate, longer PFS and an increase in survival compared with patients on chemotherapy alone (table 3). Both regimens were generally well tolerated (table 4). Selected toxicities (chemotherapy alone vs. chemotherapy plus bevacizumab) include: neutropenia (16.4 vs. 24%); thrombocytopenia (0 vs. 1.4%); hemorrhage (0.7 vs. 4.5%); hypertension (0.7 vs. 6.0%).

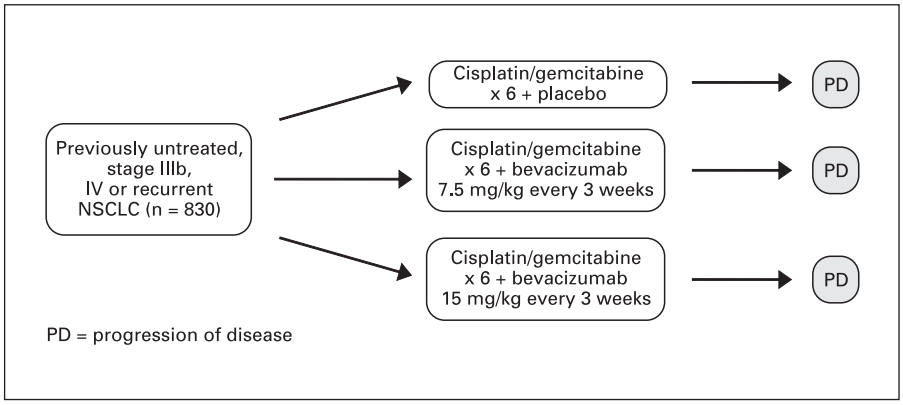

Fig. 2. Study design of BO17704 – phase III trial of gemcitabine/cisplatin with and without bevacizumab in patients with locally advanced or metastatic non-small cell lung cancer.

Table 3. Response to bevacizumab plus carboplatin/paclitaxel in patients with advanced NSCLC in Study E4599 (707 evaluable patients) [23]

	Carboplatin/ paclitaxel alone (n = 350)	Carboplatin/paclitaxel + bevacizumab (15 mg/kg) (n = 357)	p value
Response rate, %	10.0	27.2	<0.0001
PFS, months	4.5	6.4	<0.0001
OS, months	10.2	12.5	0.0075

The incidence of hemoptysis was decreased compared with the NSCLC phase II trial, but there was a 1.8% incidence (8 cases) in the bevacizumab plus chemotherapy group, five of which were fatal.

In Europe, the main focus will be on the addition of bevacizumab to cisplatin/gemcitabine. A phase III trial (BO17704) will recruit 830 patients with histologically confirmed, locally advanced (stage IIIb, IV or recurrent) NSCLC (fig. 2). The study has a two-stage design. Initially, 210 patients will be randomized in a 1:1:1 ratio to one of three arms (cisplatin 80 mg/m^2 plus gemcitabine 1,250 mg/m^2 alone on days 1 and 8 of a 3-week cycle [arm A] or with bevacizumab at a dose of either 7.5 mg/kg [arm B] or 15 mg/kg [arm C] every 3 weeks). Following assessment based on response and safety, randomization will then continue to the control arm (cisplatin/gemcitabine) and one of the bevacizumab arms (in a 1:1 ratio); a further 840 patients will be recruited. As OS is the primary endpoint, no crossover will be allowed. Secondary endpoints include PFS, response rate and toxicity. A further phase II trial is investigating cisplatin/gemcitabine plus bevacizumab in patients with malignant mesothelioma [24].

In addition to these trials, bevacizumab is being examined with anti-EGFR therapy. The EGFR inhibitor, erlotinib, has previously shown single-agent activity in NSCLC [25]. Data from a randomized trial of 731 patients with advanced NSCLC following failure of first- or second-line therapy demonstrate that erlotinib prolongs survival (6.7 months with erlotinib vs. 4.7 months with placebo) [25]. Consequently, studies are investigating the combination of the targeted agents bevacizumab and erlotinib. In one trial, patients with stage IIIB/IV locally advanced or metastatic non-squamous NSCLC were recruited [26]. Patients had to have received at least one prior chemotherapy regimen. Erlotinib was given once daily, with bevacizumab administered on day 1 of each 21-day cycle. Patients received bevacizumab plus erlotinib at one of three dose levels: erlotinib 100 mg/day plus bevacizumab 7.5 mg/kg; erlotinib 100 mg/day plus bevacizumab 15 mg/kg; or erlotinib 150 mg/day plus bevacizumab 15 mg/kg. The primary endpoints were to establish the maximum tolerated dose (MTD) and to examine the toxicity profile of the combination (phase I), as well as to assess response rate and tolerability (phase II). The regimen was well tolerated with promising activity, with a response rate of 20% and a median OS of 12.6 months [26]. Further phase II trials of this combination are planned in patients with NSCLC.

Metastatic Pancreatic Cancer

Pancreatic cancer is a difficult condition to treat. Symptoms are minor until the disease has significantly progressed, and following diagnosis the tumor is often resistant to standard anticancer therapies. As a result, pancreatic cancer is associated with high mortality, with

Table 4. The most common grade 3/4 adverse events experienced with bevacizumab-based therapy for RCC, NSCLC, pancreatic cancer and metastatic breast cancer

Grade 3/4 adverse events, %	RCC		NSCLC		Pancreatic			Breast			
	AT [14]	ATI [16]	ACP [23]	AT [55]	AXR [34	AG [30]	A [38]	AP [42]	AN [40]	AT [56]	AX [57]
Bleeding	8	2	–	–	10	2	0	1	–	–	0.4
Diarrhea	13	29	–	0	–	–	–	–	–	4	–
Hematological events*	–	–	29	–	–	–	–	5	76	–	–
Hemorrhage	–	–	5	–	–	–	–	–	0	–	–
Hypertension	10	2	6	–	2	4	17	13	0	8	18
Nausea/vomiting	10	13	–	–	–	–	–	–	7	4/4	3
Neuropathy	3	0	–	–	–	–	–	21	2	–	–
Proteinuria	8	2	–	–	–	2	2	2	2	–	1
Rash	13	27	–	6	–	–	–	–	–	–	–
Thromboembolic events**	–	–	6	–	6	13	5	1	2	4	6

A = Bevacizumab; T = erlotinib; I = imatinib; C = carboplatin; P = paclitaxel; X = capecitabine; R = radiotherapy; G = gemcitabine; N = vinorelbine.

* Including neutropenia, febrile neutropenia, thrombocytopenia, anemia.

** Including thrombosis, pulmonary embolism, phlebitis.

only 20% of patients surviving to 1 year [27]. In patients with advanced pancreatic cancer, 1-year survival drops to approximately 10%. The current standard treatment is gemcitabine monotherapy, but researchers are examining combinations of gemcitabine with other chemotherapeutic agents, such as oxaliplatin, and with vaccines, EGFR inhibitors and anti-angiogenic therapy.

VEGF signalling, through its receptors, plays a critical role in angiogenesis in patients with pancreatic cancer. High VEGF expression correlates with advanced-stage, post-operative recurrence, lymph node and distant metastases [28]. Patients with pancreatic cancer with high VEGF expression also have decreased survival compared with patients with pancreatic cancer with low VEGF expression [29]. These findings suggest that VEGF could be a target for anticancer therapy in patients with metastatic pancreatic cancer.

In a phase II study of 52 patients with stage IV pancreatic cancer (AVF2355s), adding bevacizumab 10 mg/kg every 2 weeks to the current standard of care, gemcitabine, showed promising efficacy [30]. Partial responses were seen in 19% of patients, with a further 48% having stable disease. Median OS was 8.7 months and the median TTP was 5.8 months. OS at 1 year was estimated at 29%. Although in general the combination was well tolerated (table 4), one patient with a tumor extending into the duodenum developed a fatal gastrointestinal (GI) bleed. Two patients also developed bowel perforations (one grade IV and one fatal). The promising efficacy has led to the initiation of two first-line phase III studies.

A European phase III trial (BO17706) will compare gemcitabine (1,000 mg/m^2 for 7 of the first 8 weeks and then for 3 weeks of every 4-week cycle) plus erlotinib (100 mg/day) with gemcitabine plus erlotinib plus bevacizumab 5 mg/kg every 2 weeks (fig. 3). Several lines of evidence suggest that combining bevacizumab and erlotinib in this trial may be beneficial. First, it has recently been reported that adding erlotinib to gemcitabine significantly improves OS in patients with pancreatic cancer (HR = 0.81; p = 0.025) [31]. Side effects were as expected based on the safety profiles of these agents. Furthermore, combining erlotinib with bevacizumab has demonstrated activity in several tumor types, including NSCLC and RCC [14, 26]. Finally, data indicate that the EGFR and VEGF pathways are interlinked and that VEGF upregulation contributes to resistance to anti-EGFR therapy [32, 33].

In the US, a Cooperative Group phase III trial of first-line gemcitabine with or without bevacizumab 10 mg/kg every 2 weeks is being run by the Cancer and Leukemia Group B (CALGB) (CALGB 80303). The trial design is shown in fig. 4. The primary endpoint is OS (with 90% power to detect a 35% increase in survival from 6 to 8.1 months).

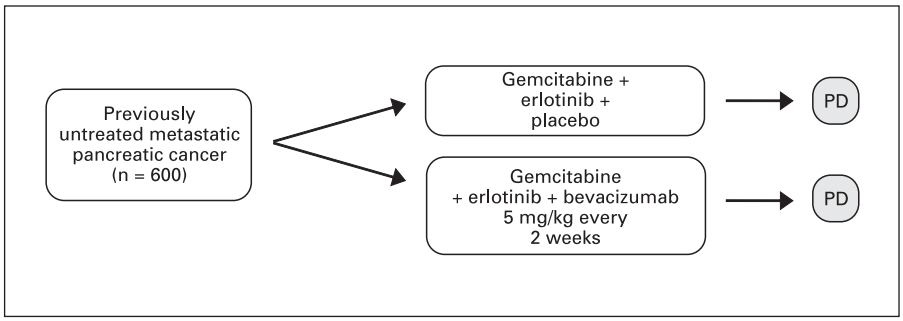

Fig. 3. Study design of BO17706 – a Roche-sponsored phase III trial of gemcitabine and erlotinib with and without bevacizumab in patients with advanced pancreatic cancer.

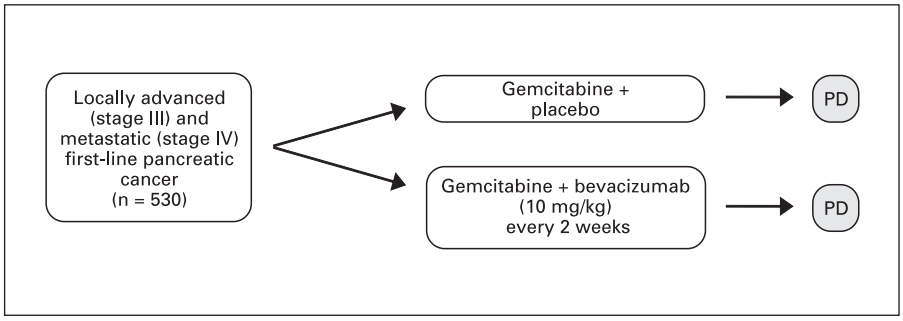

Fig. 4. Study design of CALGB 80303 – a US phase III trial of gemcitabine with and without bevacizumab in patients with advanced pancreatic cancer.

A number of ongoing clinical trials are also studying bevacizumab combined with various other chemotherapy regimens as well as with radiotherapy. Preliminary analysis of one ongoing phase I trial to determine the MTD of bevacizumab (five dosing groups: 2.5–10 mg/kg) in combination with capecitabine and radiotherapy has shown evidence of activity [34]. Of 47 patients with advanced pancreatic cancer, 19% had a partial response to this combination therapy and 57% had stable disease. The combination was well tolerated, although three patients had grade 3 ulceration with bleeding and one had grade 3 GI perforation.

Metastatic Breast Cancer

Breast cancer is the most common cancer in women and is the leading cause of death for women aged 40–55 years. In Europe, around 250,000 women develop the disease each year, and 50,000 will die from it. Advances have been made in the treatment of metastatic breast cancer with the introduction of novel chemotherapeutic agents, endocrine therapies and biological treatments.

Targeted therapies have already been shown to be effective in the treatment of metastatic breast cancer. Trastuzumab (Herceptin®), a humanized monoclonal antibody, blocks the function of human epidermal growth factor receptor-2 (HER2) protein [35]. Trastuzumab has proved effective in clinical trials both as a single agent and in combination with chemotherapy [36, 37]. Trastuzumab is approved for the treatment of HER2-positive metastatic breast cancer, first line in combination with paclitaxel or docetaxel or as monotherapy in later disease. Thus, there is significant support for biological approaches in this indication.

As VEGF levels are increased in approximately 30–60% of breast cancers and many studies show a link between VEGF and prognosis, targeted anti-VEGF therapy may also be of benefit. A phase II dose-escalation study in patients who had been previously treated for metastatic breast cancer has been reported [38]. Patients were treated with bevacizumab 3, 10 or 20 mg/kg every 2 weeks until disease progression. Comparable to response rates with other drugs in similar patient populations, 16% of patients had stable disease or better after 5 months of bevacizumab therapy. Bevacizumab treatment was well tolerated (table 4).

Based on these results, a phase III trial examining the therapeutic benefit of bevacizumab 15 mg/kg every 3 weeks plus capecitabine 2,500 mg/m^2 daily compared to capecitabine alone in 462 patients with metastatic breast cancer resistant to taxanes and anthracyclines was conducted [39]. The objective response rate was significant-

ly higher with the combination than with capecitabine alone (independently assessed response rates 19.8 vs. 9.1% respectively, p = 0.001; investigator-assessed response rates 30.2 vs. 19.1% respectively, p = 0.006). However, the trial did not meet its primary endpoint of PFS, which was similar with both regimens (4.86 vs. 4.17 months, p = 0.857). The combination was well tolerated and the incidence and severity of capecitabine-related toxicities were not affected by the addition of bevacizumab. Furthermore, bevacizumab-related side effects were similar to those in the phase II trial described above.

The trial showed that bevacizumab is active in combination with capecitabine. Although responses last for a short time, there is a biological effect, as shown by the significant increase in response rate. In addition, the data indicate that capecitabine and bevacizumab can be safely combined. In contrast, a subsequent phase II trial has suggested that combining bevacizumab with vinorelbine in heavily pretreated patients with metastatic breast cancer may extend time on study, but not increase response rates [40]. However, the mechanism of action of bevacizumab, together with the decrease in the relative influence of VEGF as breast cancer progresses, suggest that bevacizumab may be more effective when used earlier in the disease and in patients with a smaller tumor burden [41]. Further trials of bevacizumab in metastatic breast cancer are ongoing.

The US Eastern Cooperative Oncology Group is conducting trial AVF2293s/E2100, a randomized, open-label phase III trial of patients with metastatic breast cancer who receive first-line paclitaxel 90 mg/m^2 for 3 weeks of a 4-week cycle with or without bevacizumab 10 mg/kg every 2 weeks [42]. The primary objective of this trial is PFS. The patients recruited to this trial are less heavily pretreated than those who were recruited to the previous phase III trial of bevacizumab plus capecitabine in metastatic breast cancer. A total of 715 patients have been randomized to the two treatment arms. A significant, 2-fold increase in response rate was observed in patients receiving bevacizumab plus paclitaxel compared with paclitaxel alone in metastatic breast cancer (28.2 vs. 14.2%, $p < 0.0001$). There was also a significant increase ($p < 0.001$) in median PFS in patients receiving bevacizumab plus paclitaxel compared with paclitaxel alone (10.97 months vs. 6.11 months, respectively).

Preliminary safety data have been reported and side effects are as expected based on data from previous trials of bevacizumab. Increases in the incidence of hypertension, bleeding and proteinuria were seen in the bevacizumab plus paclitaxel treatment arm (table 4). Bevacizumab is also being investigated in metastatic breast cancer in combination with targeted therapies such as trastuzumab and erlotinib.

Other Indications

VEGF is active in a number of other malignancies including prostate cancer, ovarian cancer and hematological malignancies. For example, VEGF levels have been linked with poor prognosis in leukemia and non-Hodgkin's lymphoma [43]. As a result, studies are also investigating the role of bevacizumab in these diseases. In a phase II trial of bevacizumab (10 mg/kg) alone in 43 patients with relapsed, aggressive non-Hodgkin's lymphoma (S0108), response rate and 6-month PFS were similar to those seen in other tumor types treated with single anti-angiogenic agents. Bevacizumab was also well tolerated in these patients [44].

Several phase II trials are investigating the clinical benefit of bevacizumab, either alone or in combination with commonly used chemotherapy regimens, in patients with ovarian cancer. Preliminary results from a phase II trial of bevacizumab (15 mg/kg) in persistent or recurrent epithelial ovarian cancer showed efficacy responses as well as good tolerability [45].

Study CALGB 0006 has investigated the benefit of bevacizumab in prostate cancer [46]. Adding bevacizumab to docetaxel plus estramustine (Emcyt®) in patients with hormone-refractory prostate cancer resulted in 17 of the 32 patients with measurable disease having a partial response. The safety profile in this study was similar to that seen with docetaxel and estramustine alone. However, one patient died of mesenteric vein thrombosis considered to be related to thrombotic complications due to bevacizumab and estramustine. The high response rates in this trial warrant further study. Other studies with bevacizumab, either alone or in combination with chemotherapy regimens, have reported preliminary data in patients with gastric cancer [47, 48] and hepatocellular carcinoma [49–51].

Phase II studies are also investigating the use of bevacizumab, combined with other treatments, in patients with a number of hematological malignancies including diffuse large B cell lymphoma, relapsed aggressive non-Hodgkin's lymphoma, multiple myeloma, acute myeloid leukemia and chronic myelogenous leukemia. Preliminary data from 48 patients with acute myeloid leukemia have shown that cytotoxic chemotherapy fol-

lowed by bevacizumab had a favorable overall response rate (48%) [39]. Median disease-free and OS were 7 and 16 months, respectively. The authors concluded that VEGF neutralization with bevacizumab might directly result in leukemic cell death and the use of bevacizumab in acute myeloid leukemia warrants further study. Other studies are investigating the use of bevacizumab alone or in combination for other tumors, such as head and neck squamous cell carcinoma [52], metastatic carcinoma of unknown primary site [53] and advanced carcinoid [54].

Conclusions

Cancer therapies with a different mechanism of action to traditional chemotherapies are urgently needed. Ideally, new treatments, which will probably be used in combination with existing therapy, should improve survival while adding limited toxicity. Currently available data indicate that bevacizumab can improve efficacy in patients with metastatic RCC, NSCLC, pancreatic cancer and breast cancer when used first line in combination with standard regimens. Furthermore, the side-effect profile in all of these indications is consistent with that observed in CRC; hypertension, proteinuria and minor bleeding are the most commonly observed side effects (table 4).

The significant advances in the field of angiogenesis provide hope for the future development of therapeutic strategies to inhibit the growth of a wide range of tumor types. Further clinical trials will lead to the optimization and refinement of the use of bevacizumab to further improve the clinical benefit and safety for many patients with VEGF-driven cancers.

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