Clinical Pharmacology: Concise Drug Reviews

Bevacizumab

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Evaluate the clinical use of bevacizumab, both for cancer and for non-oncologic diseases, and discuss approved and investigational combination chemotherapies that include bevacizumab.
2. Describe the pharmacology of bevacizumab and its mechanism of action in order to predict degrees of patient response.

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INTRODUCTION

Vascular endothelial growth factor A (VEGF) is a potent proangiogenic growth factor that stimulates the proliferation, migration, and survival of endothelial cells. As one of the more important proteins also expressed by tumor cells, VEGF is an important target of anticancer therapy [1].

Bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA) is a humanized anti-VEGF monoclonal IgG₁ antibody (molecular weight, 149 kDa) [2, 3]. In com-

bination with chemotherapy, it is approved for the treatment of advanced colorectal cancer (CRC), advanced non-small cell lung cancer (NSCLC), metastatic breast cancer (MBC) [2, 3], and advanced renal cell cancer [3] (Table 1) [4–12]. As a single agent, it has been approved by the U.S. Food and Drug Administration (FDA) for second-line treatment of advanced glioblastoma multiforme. Further studies are being conducted in other solid tumors as well, indicative of the potential therapeutic benefit of bevacizumab in combination anticancer therapy [13–16].

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Table 1. Approved indications of bevacizumab and main results of pivotal studies

Cancer type	Combination therapy	Dosage	Median PFS without bevacizumab ^a	Median PFS with bevacizumab ^a	HR (95% CI)	p-value	Median OS without bevacizumab ^a	Median OS with bevacizumab ^a	HR (95% CI)	p-value
CRC (first-line)	Bolis IFL [4] 5-FU and LV [5]	5 mg/kg every 2 wks [2, 3]	6.2	10.6	0.54 (0.45–0.66)	<.001	15.6	20.3	0.66 (0.54–0.81)	<.001
		5 mg/kg every 2 wks [2, 3]	5.2	9.0	0.46 (0.34–0.73)	.005	13.6	17.7	0.52	.073
		10 mg/kg every 2 wks [2, 3]	5.2	7.2	0.66 (0.54–0.81)	.22	13.6	15.2	1.01	.98
	FOLFOX4/XELOX [6]	5 mg/kg every 2 wks/7.5 mg/kg every 3 wks [3]	8.0	9.4	0.83 (0.72–0.95)	.002	19.9	21.3	0.89 (0.76–1.03)	.08
CRC (second line)	FOLFOX4 [7]	10 mg/kg every 2 wks [2, 3]	4.7	7.3	0.52 (0.42–0.65)	<.0001	10.8	12.9	0.75 (0.63–0.89)	.001
NSCLC (first line)	Cisplatin and gemcitabine [8]	7.5 mg/kg every 3 wks [3]	6.1	6.7	0.75 (0.62–0.91)	.003	NA ^b	NA	NA	NA
		15 mg/kg every 3 wks [3]	6.1	6.5	0.82 (0.68–0.98)	.03	NA ^b	NA	NA	NA
	Carboplatin and paclitaxel [9]	15 mg/kg every 3 wks [2, 3]	4.5	6.2	0.66 (0.56–0.76)	<.0001	10.3	12.3	0.79 (0.67–0.92)	.003
MBC (first line)	Paclitaxel [10]	10 mg/kg every 2 wks [2, 3]	5.8	11.4	0.42 (0.34–0.52)	<.0001	24.8	26.5	0.87 (0.72–1.05)	.14
		7.5 mg/kg every 3 wks	8.0	8.7	0.69 (0.54–0.89)	.003	NA	NA	NA	NA
	Docetaxel [11]	10 mg/kg every 3 wks	8.0	8.8	0.61 (0.48–0.78)	.0001	NA	NA	NA	NA
RCC (first line)	Interferon- α 2a [12]	10 mg/kg every 2 wks [3]	5.4	10.2	0.63 (0.52–0.75)	.0001	NR	NR	NR	NR

^aValues in months.

^bDuration of follow-up was not sufficient for overall survival analysis.

Abbreviations: 5-FU, 5-fluorouracil; CI, confidence interval; CRC, metastatic colorectal cancer; FOLFOX-4, 5-FU, LV, and oxaliplatin; HR, hazard ratio; IFL, irinotecan, 5-FU, and LV; LV, leucovorin; MBC, metastatic breast cancer; NA, not applicable; NR, not reached; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; RCC, renal cell carcinoma; XELOX, capecitabine and oxaliplatin.

CLINICAL USE

Bevacizumab is a clear, colorless solution administered i.v. [2, 3] in combination with chemotherapy in doses of 5, 7.5, 10, or 15 mg/kg as shown in Table 1.

Apart from these regimens, bevacizumab is also being tested off label in other malignancies. In ovarian cancer, the drug is being tested at a dose of 10 mg/kg every 2 weeks in combination with cyclophosphamide [13] and at a dose of 15 mg/kg every 3 weeks in combination with carboplatin and paclitaxel [16]. Promising results are being achieved in patients with chronic diffuse diabetic macular edema and age-related macular degeneration (AMD), for which bevacizumab is being injected intravitreally at a dose of 1.25 mg [15]. Ranibizumab (Lucentis®; Genentech, Inc., South San Francisco, CA), the Fab fragment derived from the same parent molecule as bevacizumab, has been FDA approved for AMD. It is given by monthly intravitreal injections for 1 year.

The maximum-tolerated dose of bevacizumab is 20 mg/kg, at which 25% of patients suffer from grade ≥ 3 toxicity (on a scale of 1–5, according to the Common Toxicity Criteria) including headache, nausea, and vomiting [17].

MECHANISM OF ACTION

Cancer cells and tissues often have high metabolic rates; this may result in a demand for oxygen and nutrients exceeding the supply. As a result, these tissues are characterized by the presence of hypoxia, which is also the

primary factor controlling angiogenesis. Under hypoxic conditions, hypoxia inducible factor (HIF) binds to the hypoxia response element present in the *VEGF* gene, thus inducing the transcription of VEGF protein [18]. Circulating VEGF binds to VEGF receptor (VEGFR)-1 and VEGFR-2 and to its coreceptors neuropilin (NRP)-1 and NRP-2 with high binding affinity [19]. These receptors are expressed on the surface of endothelial cells, and they play a critical role in the development of angiogenesis by stimulating the recruitment and proliferation of endothelial cells [1].

Bevacizumab acts by selectively binding circulating VEGF, thereby inhibiting the binding of VEGF to its cell surface receptors. This inhibition leads to a reduction in microvascular growth of tumor blood vessels and thus limits the blood supply to tumor tissues. These effects also lower tissue interstitial pressure, increase vascular permeability, may increase delivery of chemotherapeutic agents, and favor apoptosis of tumor endothelial cells [20].

An in vivo study on vascular regrowth in mice showed that, upon interruption of anti-VEGF therapy, the tumor vasculature resumed multiplication and reached the baseline growth rate within 7 days. The regrowth of the tumor vessels occurred from the empty sleeves and pericytes of the vascular basement membrane. When the anti-VEGF therapy was continued, the tumor vasculature became sensitive once more as under baseline conditions [1].

OTHER INVESTIGATIONAL COMBINATION THERAPIES

In addition to the approved combination therapies (Table 1), bevacizumab is being investigated with other drug combinations as well.

In NSCLC, in combination with erlotinib, an epidermal growth factor receptor inhibitor, a randomized phase II trial with this combination suggested a better safety profile than with the combination of bevacizumab (15 mg/kg every 3 weeks) plus docetaxel or pemetrexed. In that trial, 28% of patients discontinued treatment in the bevacizumab–chemotherapy arm as a result of adverse effects, versus 24% of patients treated with only chemotherapy. The progression-free survival (PFS) intervals were similar in the bevacizumab–erlotinib and bevacizumab–chemotherapy arms (4.4 months versus 4.8 months) [21].

In MBC, a single-arm, phase II study of bevacizumab in combination with docetaxel and trastuzumab showed a PFS interval of 7.5 months (95% confidence interval [CI], 6.2–8.3 months) [22]. In other preliminary reports, the combination of bevacizumab (10 mg/kg every 2 weeks) with trastuzumab in human epidermal growth factor receptor 2–positive MBC patients appeared to be well tolerated, with few grade 3 or 4 side effects [23].

In pancreatic cancer, bevacizumab (10 mg/kg every 2 weeks) was tested in combination with gemcitabine in a randomized, double-blind, placebo-controlled, phase III study. However, this combination did not result in a significant improvement in any of the clinical endpoints when compared with gemcitabine alone [14]. In another preliminary report from a phase III study, bevacizumab was added to the combination of gemcitabine and erlotinib in first-line metastatic pancreatic cancer, resulting in a marginal, albeit statistically significant, longer PFS time (3.6 months versus 4.6 months; hazard ratio [HR], 0.73; 95% CI, 0.61–0.86; $p = .0002$) [24].

PHARMACOKINETICS

Serum concentrations of bevacizumab can be analyzed using enzyme-linked immunosorbent assays (ELISAs). In a study analyzing 491 patients receiving 1–20 mg/kg of bevacizumab every 1, 2, or 3 weeks, the estimated half-life was 19.9 days (range, 11–50 days) and the predicted time to reach steady-state was approximately 100 days [25].

Protein Binding

A recent study reported that bevacizumab binds >97% of serum VEGF. Serum VEGF is predominantly derived from platelets, which have been shown to take up bevacizumab [17]. Platelets may release bevacizumab at sites of endothelial damage and thus deliver it to procoagulatory angiogenic

tumor sites at relatively high concentrations, targeting the tumor cell VEGF [17].

However, blockade of platelet VEGF appears to play an important role in the development of serious side effects related to bevacizumab therapy, including: hypertension, impaired wound healing, bleeding, and gastrointestinal perforations [17].

Distribution

A two-compartment model with first-order elimination estimated that the volume of distribution of bevacizumab was 2.39 l for a typical female and 3.29 l for a typical male, which is about the expected normal plasma volume [25]. Studies in Cynomolgus monkeys revealed that the weekly administration of 2–3 mg/kg bevacizumab resulted in sustained serum levels of 10–30 $\mu\text{g/ml}$, which appears to be enough to suppress VEGF activity. The distribution of bevacizumab was limited to the tumor vasculature with minimal extravascular distribution [26]. In addition, scintigraphic visualization of VEGF expression in mouse models showed the accumulation of radiolabeled bevacizumab to be higher in tumor tissues than in normal tissues, and that the uptake in normal tissues decreased over time [27].

Elimination

The neonatal Fc Receptor (FcRn) plays a major role in the clearance of bevacizumab. The antibody is taken up by pinocytosis into endosomes of catabolic cells where it binds to FcRn. This binding delays the degradation of the antibody and protects it from systemic elimination, resulting in a longer half-life [28].

The estimated clearance of bevacizumab is 0.207 l/day (95% CI, 0.188–0.226 l/day). Elimination of bevacizumab is correlated with body weight, gender, serum albumin, alkaline phosphatase (ALP), and serum aspartate aminotransferase (AST). In cases of extreme body weight, the clearance of bevacizumab can be up to 30% lower (body weight < 49 kg) or up to 30% higher (body weight > 114 kg) than the mean value. In addition, the clearance of bevacizumab is, on average, 26% higher in males than in females. Patients with low serum albumin (<29 g/l) have, on average, a 29% higher clearance rate, whereas patients with higher ALP (>483 IU/L) levels have a 23% higher clearance rate. Patients with elevated AST have an approximately 10% lower bevacizumab clearance rate [25]. The clearance of bevacizumab also depends on the tumor burden. Patients with a higher tumor burden have a higher clearance rate than patients with a tumor burden below the median (0.249 l/day versus 0.199 l/day) [29]. The potential

implications of variability in elimination for the activity and safety of bevacizumab are poorly understood.

Special Populations

Demographic studies indicate that dose adjustments are not necessary for differences in age or gender. No studies have been conducted to explore the pharmacokinetics of bevacizumab in patients with renal or hepatic dysfunction [2, 3].

In recent phase I studies, the use of bevacizumab in pediatric patients was analyzed. Dose levels of 5, 10, and 15 mg/kg of bevacizumab were tolerated in this group of patients [30]. In geriatric patients, a $\geq 2\%$ higher incidence of adverse effects was observed than in patients aged < 65 years [2, 3]. Nevertheless, the analysis of data pooled from two placebo-controlled studies, including 43% of patients aged ≥ 65 years and 27% aged > 70 years with metastatic CRC, indicated that adding bevacizumab to fluorouracil-based chemotherapy resulted in a longer PFS interval (9.2 months with bevacizumab versus 6.2 months with placebo; HR, 0.52; 95% CI, 0.40–0.67; $p < .0001$). Bevacizumab-associated adverse effects were similar to those reported in overall population studies [31].

PHARMACOGENETICS

Genetic variations in *VEGF* or VEGF receptors can be possible markers for the evaluation of therapy response. There are five functional single nucleotide polymorphisms (SNPs) identified in the 5' and 3' regions of *VEGF*. These SNPs result in decreased VEGF production or increased promoter activity. In addition, there are several nonsynonymous SNPs in the coding region of *KDR* [32] and *HIF-1 α* [18], which are responsible for their greater expression.

The prolonged plasma half-life of bevacizumab can be explained by different alleles of *FcRn*. There are variable numbers of tandem repeats (VNTRS) within the promoter of the *FCGRT* gene (which codes Fc), consisting of five different alleles. The *VNTR3* allele is associated with higher FcRn expression and it is possible that individuals carrying this allele have a longer plasma half-life of bevacizumab [33].

There is also evidence for inheritance of conserved *VEGF* haplotypes. The haplotype with a polymorphism at $-460/+405$ was found to be associated with altered VEGF production in vitro. Carriage of this type of polymorphism significantly alters VEGF promoter activity and responsiveness [34].

Because these gene differences may contribute to variation in VEGF production, individual patients carrying one or more of these SNPs may have different efficacy or toxicity responses to anti-VEGF therapy. However, further studies are needed.

SIDE EFFECTS

Common side effects of bevacizumab are hypertension, asymptomatic proteinuria, thromboembolic events, gastrointestinal perforation, and wound healing complications. Some of the adverse effects, like thromboembolism, are more common at higher doses of bevacizumab (10–15 mg/kg). A meta-analysis showed that 11.9% (95% CI, 6.8%–19.9%) of patients, with different malignant diseases, developed thromboembolism (relative risk [RR], 1.33; 95% CI, 1.13–1.56; $p < .001$) when treated with bevacizumab at higher doses, compared with controls. The risk was similarly higher for bevacizumab at 2.5 mg/kg per week (RR, 1.31; 95% CI, 1.08–1.60; $p = .007$) and 5 mg/kg per week (RR, 1.31; 95% CI, 1.02–1.68; $p = .04$) [35]. In another study, in the first-line of treatment of NSCLC patients, fatal pulmonary hemorrhage was observed at both dose levels. The current label contraindicates use in squamous cell NSCLC. Combination therapy with bevacizumab was associated with a higher risk for treatment-related deaths than with chemotherapy alone (15 patients versus 2 patients; $p = .001$) [9]. A reduction in left ventricular ejection fraction was also suggested as a possible side effect during the long-term use of bevacizumab; however, it cannot be excluded that this could have been caused by the chemotherapy as well [36].

Depending on the severity of adverse effects, the administration of bevacizumab should be discontinued temporarily or permanently. In cases with moderate to severe proteinuria or uncontrolled hypertension, bevacizumab therapy should be temporarily discontinued until stabilization of the patient's condition. Therapy should be permanently discontinued in cases of gastrointestinal perforation, fistula formation, serious bleeding, arterial thromboembolic events, nephrotic syndrome, hypertensive crisis, or hypertensive encephalopathy [2, 3].

Bevacizumab in combination with sunitinib causes microangiopathic hemolytic anemia; this combination is not recommended for use [37]. In combination with carboplatin and paclitaxel, bevacizumab reduces incidentally, but substantially, the mean exposure to paclitaxel [2].

DRUG RESISTANCE

Mechanisms of resistance to antiangiogenic therapy include the existence of or development of alternative angiogenic pathways. Anti-VEGF drug resistance might also occur by increasing levels of growth factor or the VEGFRs, which might lead to specific angiogenesis inhibitor-related toxicities, as mentioned above [38]. The greater levels of receptors might lead to more problems when the anti-VEGF drug is discontinued, and to faster regrowth of the tumor vasculature [1].

Thus, the combination of bevacizumab with chemotherapy that acts against different targets or pathways might help in the prevention of anti-VEGF drug resistance.

PATIENT SELECTION

Elevated levels of circulating VEGF have been associated with a poor prognosis and greater risk for the development of metastases in patients with different cancer types [39]. There are many techniques used to evaluate VEGF expression in human tumors, including immunohistochemistry, ELISA, chemiluminescence immunosorbent assay, Western blotting, and in situ hybridization [40].

The identification and validation of a biomarker assisting in patient selection are important future developments, and circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPs) possibly may serve this purpose. CECs and CEPs contribute to angiogenesis by their incorporation into growing blood vessels and differentiation into endothelial cells [41, 42]. Studies also suggest that measurement of CECs and CEPs could be used for evaluation of the response to antiangiogenic therapy. After the administration of 5 or 10 mg/kg of bevacizumab in locally advanced rectal carcinoma patients, CEC levels were lower on days 3 and 12 than at baseline ($p < .01$) [42]. In breast cancer patients, treatment with bevacizumab and combination chemotherapy resulted in a lower total number of CECs ($p = .07$) and CEPs ($p = .02$) than at baseline [43].

However, individualized therapy with bevacizumab is hampered by the lack of a validated biomarker to guide patient selection, dosing, and monitoring of bevacizumab therapy.

DURATION OF TREATMENT

Inhibition of angiogenesis by an anti-VEGF drug is not irreversible. Thus, the ~20% of the tumor vasculature that remains undestroyed can regrow again after drug interruption, and revascularize the tumor [1]. This suggests that the duration of bevacizumab therapy is of major importance.

In clinical studies, bevacizumab therapy is usually used until progressive disease (PD). Discontinuation before

reaching PD might result in the regrowth of tumor vasculature [1]; however, clinical proof of this hypothesis is lacking. There is, at best, a suggestion of such an effect, as seen in a study with 5-fluorouracil, leucovorin, and oxaliplatin and capecitabine and oxaliplatin (XELOX) combination. In that large, phase III study, the bevacizumab combination with XELOX was discontinued as a result of oxaliplatin adverse effects. This may have affected the study outcome, because in these patients bevacizumab was discontinued before PD had developed [6].

Another study explored the use of bevacizumab therapy beyond PD in patients with metastatic CRC. The median OS time was 31.8 months (95% CI, 27.9 months to not reached) in patients treated with bevacizumab, versus 19.9 months (95% CI, 18.0–22.0 months) in those treated with chemotherapy only. However, the study was not randomized and the group of patients who received bevacizumab beyond progression had less aggressive tumor biology [44].

Bevacizumab monotherapy was analyzed in a small study in patients with MBC after complete response was achieved with a chemotherapy and bevacizumab regimen [45]. Preliminary results suggested that a group of patients might benefit from bevacizumab monotherapy, but more studies are needed for further evaluation.

The use of bevacizumab therapy is increasing beyond its indication in several other types of malignancies as well [13–16]. However, one of the major issues is the cost-effectiveness of the therapy. In a number of European countries, the estimated cost per life-year gained in the treatment of metastatic CRC with bevacizumab is about €80,000. The combination of bevacizumab with irinotecan, 5-fluorouracil, and leucovorin (the IFL regimen) was considered a non-cost-effective treatment by the English National Institute for Health and Clinical Excellence [46, 47].

AUTHOR CONTRIBUTIONS

Conception/Design: Jan H. M. Schellens

Provision of study material or patients: Jan H. M. Schellens

Collection and/or assembly of data: Filis Kazazi-Hyseni

Data analysis and interpretation: Jan H. M. Schellens, Filis Kazazi-Hyseni

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