

SYSTEMIC PHARMACOKINETICS AND PHARMACODYNAMICS OF INTRAVITREAL AFLIBERCEPT, BEVACIZUMAB, AND RANIBIZUMAB

ROBERT L. AVERY, MD,* ALESSANDRO A. CASTELLARIN, MD,* NATHAN C. STEINLE, MD,* DILSHER S. DHOOT, MD,* DANTE J. PIERAMICI, MD,* ROBERT SEE, MD,* STEPHEN COUVILLION, MD,* MA'AN A. NASIR, MD,* MELVIN D. RABENA, BS,* MAURICIO MAIA, PhD,† SHERRI VAN EVEREN, PHARM D,† KHA LE, PhD,† WILLIAM D. HANLEY, PhD†

Purpose: To evaluate the systemic pharmacokinetics (PKs) of aflibercept, bevacizumab, and ranibizumab in patients with neovascular age-related macular degeneration (AMD), diabetic macular edema (DME), or retinal vein occlusion (RVO).

Methods: Prospective, open-label, nonrandomized clinical trial of patients with AMD, DME, or RVO who were anti-vascular endothelial growth factor (VEGF) naïve or had not received anti-VEGF for ≥ 4 months. Patients received 3 monthly intravitreal injections of aflibercept 2.0 mg, bevacizumab 1.25 mg, or ranibizumab (0.5 mg for AMD/RVO, 0.3 mg for DME). The main outcome measures were serum PKs and plasma free-VEGF concentrations after the first and third injections.

Results: A total of 151 patients were included. In AMD/DME/RVO, systemic exposure to each drug was highest with bevacizumab, then aflibercept, and lowest with ranibizumab. Ranibizumab cleared from the bloodstream more quickly than bevacizumab or aflibercept. Aflibercept treatment resulted in the greatest reductions in plasma free-VEGF relative to baseline levels, whereas ranibizumab treatment resulted in the smallest decreases in plasma free-VEGF.

Conclusion: The three anti-VEGF treatments examined in this analysis demonstrated notable differences in systemic PKs. Generally, the reduction in plasma free-VEGF levels correlated with elevated levels of circulating anti-VEGF agents, with the reduction in free-VEGF levels greatest with aflibercept and least with ranibizumab.

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Vascular endothelial growth factor (VEGF) is a critical mediator of physiological angiogenesis and pathological angiogenesis.¹ Several ophthalmic diseases, including neovascular (wet) age-related macular degeneration (AMD), diabetic macular edema (DME), and macular edema after retinal vein occlusion (RVO), are characterized by abnormal angiogenesis and increased vascular permeability in the retina, which ultimately can lead to vision loss if left untreated.² The three most commonly prescribed anti-VEGF therapy options available in clinical practice for the treatment of AMD, DME, and RVO are aflibercept, bevacizumab, and ranibizumab.^{3–12}

These 3 anti-VEGF treatments differ in their molecular structure and properties. Aflibercept is a fusion protein (115 kDa) comprising the second Ig

domain of human VEGFR1, the third Ig domain of human VEGFR2, and the Fc region of a human IgG1.¹³ Aflibercept binds multiple isoforms of human VEGF-A, VEGF-B, and placental growth factor.¹¹ The reported estimated terminal elimination half-life of free aflibercept in plasma is 5 days to 6 days after intravenous administration of doses ranging from 2 mg/kg to 4 mg/kg.¹² The vitreous half-life of aflibercept in rabbits is 3.63 days.¹⁴ At the time of this publication, there are no data available on the half-life of aflibercept in the human eye. Ranibizumab and bevacizumab bind to all human VEGF-A isoforms.^{15–17} Bevacizumab is a full-length monoclonal antibody (149 kDa) that was developed for cancer therapy.¹⁶ In human nonvitrectomized eyes, the aqueous half-life of bevacizumab was 9.82 days.¹⁸

Ranibizumab is an anti-VEGF-A affinity-matured monovalent monoclonal antibody fragment (48 kDa) that was designed for ocular use.¹⁵ It does not contain the Fc antibody region, and hence, it is cleared from the bloodstream more rapidly and has a short systemic elimination half-life of ~2 hours.¹⁹ Using a population pharmacokinetic (PK) analysis from 674 patients with

From the *California Retina Consultants, Santa Barbara, California; and †Genentech, Inc, South San Francisco, California.

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Reprint requests: Robert L. Avery, MD, California Retina Consultants, 525 East Micheltorena Street, Suite A, Santa Barbara, CA 93103; e-mail: bobave@gmail.com

wet AMD, Xu et al¹⁹ estimated the vitreous half-life of ranibizumab to be ~9 days. This approach, however, is not applicable to bevacizumab and aflibercept because upon entering the systemic circulation they undergo recycling via FcRn receptor.¹⁹ In primates, the vitreous elimination half-life of ranibizumab is reported to be ~3 days.²⁰ The aqueous half-life of intravitreal ranibizumab 0.5 mg is 7.19 days in human nonvitrectomized eyes.²¹ The systemic PK values of ranibizumab in patients with RVO and DME were shown to be similar to those in patients with AMD; thus, systemic exposure of ranibizumab is not considered dependent on disease state.²²

Concerns have been raised regarding potential adverse effects resulting from the systemic suppression of VEGF from intraocular anti-VEGF treatments.²³ From clinical experience in oncology, there are known adverse effects related to blocking VEGF in the systemic circulation.²⁴ These adverse effects include cardiovascular and arterial thromboembolic effects (ATE), renal and gastrointestinal (GI) effects, and wound-healing complications. The majority of comparative safety data for intravitreal use of anti-VEGF therapeutics come from large clinical trials in neovascular AMD patients.^{25–30} In patients with AMD, greater decreases in serum and plasma free-VEGF have been observed with bevacizumab and aflibercept compared with ranibizumab.^{31–35} Additionally, several studies have explored the impact of anti-VEGF therapeutics on serum³⁶ and plasma^{37,38} VEGF levels in patients with AMD. However, studies comparing the systemic levels of aflibercept, bevacizumab, and ranibizumab and their relative effects on circulating VEGF in patients with DME and RVO are lacking. Because systemic safety concerns have recently been raised in the non-AMD populations,³⁹ it is important to assess systemic PK of intravitreal anti-VEGF agents in these groups as well.

The objective of the current prospective study was to evaluate and compare serum drug concentrations and plasma free-VEGF concentrations in patients with AMD, DME, or RVO receiving intravitreal injections of aflibercept, bevacizumab, or ranibizumab. Additionally, values were compared across indications to identify if disease impacts any differences observed.

Methods

Study Design

The study protocol was Institutional Review Board approved and was conducted in accordance with U.S. Food and Drug Administration Good Clinical Practice guidelines. Written informed consent was provided by all patients for study participation. This study was

Health Insurance Portability and Accountability Act compliant and adhered to the tenets of the declaration of Helsinki. This study is registered at clinicaltrials.gov (NCT02118831).

This prospective study enrolled patients from several offices of a single private practice (California Retina Consultants, Santa Barbara, CA) who were naive to anti-VEGF therapy or had not received treatment with anti-VEGF for at least 4 months. Patients were sorted into 3 groups based on eye disease (neovascular AMD, DME, or RVO), with at least 15 patients per disease state for each drug group. Patients received 3 monthly intravitreal injections (re-treatment could be given within 21–35 days from previous injection) of aflibercept 2.0 mg, bevacizumab 1.25 mg, or ranibizumab (0.5 mg for AMD and RVO patients and 0.3 mg for DME patients). Patients were excluded from the study if they were unwilling to receive three monthly injections of an anti-VEGF agent, had been treated with a systemic anti-VEGF agent for cancer in the past year, were currently undergoing dialysis, or if they had received a vitrectomy in the treated eye.

Sample Collection and Bioanalytical Methods

Blood samples were collected at screening, 3 hours after injection, and at Days 1, 3, 7, and 28 after Dose 1 and Dose 3 for PK and systemic VEGF analyses. Analyses of serum drug levels and plasma concentrations of free-VEGF have been described in detail previously.³² CTAD (citrate, theophylline, adenosine, and dipyridamole) tubes were used for the collection of plasma samples because of their ability to preserve platelets and prevent activation.^{40,41} VEGF was measured in plasma samples, as opposed to serum, to prevent or minimize release of VEGF from platelets.⁴² Serum levels of aflibercept, bevacizumab, and ranibizumab were analyzed using solution phase enzyme-linked immunosorbent assays (ELISA). The lower limits of quantitation (LLOQ) for aflibercept, bevacizumab, and ranibizumab were 1,000 pg/mL, 313 pg/mL, and 15.0 pg/mL, respectively. Plasma concentrations of free-VEGF were determined using the Quantikine ELISA kit, with an LLOQ of 10 pg/mL (R&D Systems, Minneapolis, MN). As noted by the manufacturers of this kit, this assay is not suited to measure total VEGF in the presence of anti-VEGFs, because of the binding properties of the capture and detection reagents used in the assay. However, this assay is commonly used for measuring free-VEGF levels in samples from patients treated with anti-VEGF therapeutics. Target-therapeutic complexes are in a state of binding equilibrium, which we expect to be minimally affected by the 1:2 sample dilution performed in this assay.

PK Analyses

PK parameters were analyzed using noncompartmental analysis, and descriptive statistics were used to summarize the results. Because this was a PK study, a formal sample size calculation was not done. Non-compartmental PK analysis was used to evaluate drug concentration data and determine area under the curve (AUC) over 28 days (AUC_{0-28} [Dose 1], AUC_{60-88} [Dose 3]), peak concentration (C_{max}), trough concentration (C_{min}), and serum half-life for each anti-VEGF. To better estimate the serum half-lives of bevacizumab and aflibercept (which are estimated to have longer systemic half-lives because of their Fc portion), a protocol amendment was implemented in the middle of the study to collect PK data at later time points after the third dose if the patient had not received another injection the next month for his or her eye disease. As a result, only a small fraction of patient data was available for an accurate half-life estimation, and the serum half-life data were pooled over all three indications. Serum drug concentrations of aflibercept, bevacizumab, and ranibizumab and plasma free-VEGF levels that measured lower than the LLOQ were assigned a value equal to 50% of the LLOQ (rather than a value of 0).

Results

Patients

A total of 151 patients were enrolled in this study, 57 in the AMD group, 46 in the DME group, and 48 in the RVO group (Table 1).

Systemic Exposure of Aflibercept, Bevacizumab, and Ranibizumab in Patients With AMD, RVO, and DME

PK data for patients with AMD have been previously published.³² Mean systemic exposure data for the AMD, DME, and RVO groups are summarized in Tables 2–4. Subsequent to the initial injection (Day 0), C_{max} was reached at median time to C_{max} (t_{max}) of 1, 7, and 1 day for aflibercept, bevacizumab, and ranibizumab, respectively, for patients with AMD, DME, and RVO.

Based on the Dose 3/Dose 1 geometric mean ratios of C_{min} , C_{max} , and AUC_{0-28} , ranibizumab did not demonstrate systemic accumulation between Dose 1 and Dose 3 as evidenced by accumulation ratios close to 1 (Table 5); however, bevacizumab and aflibercept seemed to exhibit systemic drug accumulation. Mean $\pm$ SD serum half-lives after intravitreal administration for aflibercept, bevacizumab, and ranibizumab were

Table 1. Patient Demographics and Baseline Characteristics

Characteristic	AMD (n = 57)	DME (n = 46)	RVO (n = 48)
Age, mean, years	76.9	59.7	67.3
Male, n (%)	28 (50.0)	24 (51.1)	27 (57.4)
Treatment group, n			
Aflibercept	22	15	15
Bevacizumab	15	15	15
Ranibizumab	20	16	18
Baseline free-VEGF level, mean, pg/mL			
Aflibercept	19.2	20.2	18.9
Becavizumab	22.5	22.8	23.5
Ranibizumab	17.0	20.9	22.6

11.4 ± 4.8 days (n = 39), 18.7 ± 5.8 days (n = 7), and 5.8 ± 1.8 days (n = 43), respectively.

Systemic exposure for each anti-VEGF therapeutic did not seem to differ by indication and was consistently highest with bevacizumab, followed by aflibercept, and lowest with ranibizumab (Figure 1). After Dose 3, systemic exposure to aflibercept ranged 43- to 107-fold, 7- to 13-fold, and 13- to 19-fold higher than systemic exposure to ranibizumab, whereas that of bevacizumab was 399- to 788-fold, 21- to 30-fold, and 67- to 91-fold higher than that of ranibizumab based on C_{min} , C_{max} , and AUC, respectively, over the 3 indications. Mean serum concentrations of aflibercept were higher than its reported half-maximal inhibitory concentration (IC_{50}) for VEGF inhibition (0.068 nM)⁴³ at most time points after Dose 1 and Dose 3 (Figure 1). Mean serum concentrations of bevacizumab were also above its reported IC_{50} (0.668 nM)⁴³ at most time points after Dose 3 for all indications (Figure 1). For Dose 1 and Dose 3, mean serum concentrations of ranibizumab met or exceeded its reported IC_{50} (0.060 nM)⁴³ at the 3-hour and 24-hour time points postinjection, but fell below the IC_{50} for the remaining time points.

Systemic Plasma Free-VEGF Levels

Mean free-VEGF levels in plasma were balanced at baseline for each indication and were comparable with values reported previously.^{35,44} Mean baseline VEGF levels are summarized in Table 1. It is important to note that individual patient data for plasma free-VEGF levels at baseline ranged widely, from below the assay's LLOQ (10 pg/mL) to 264 pg/mL in patients with AMD, from <10 pg/mL to 558 pg/mL in patients with DME, and from <10 pg/mL to 615 pg/mL in patients with RVO (Figure 2).

Mean plasma free-VEGF profiles over time after intravitreal administration of aflibercept, bevacizumab, and ranibizumab in the AMD, DME, and RVO groups are shown in Figure 3. For Dose 1 and Dose 3, the greatest decreases in plasma free-VEGF levels

were observed with aflibercept for all 3 indications (Figure 3). Mean plasma VEGF levels in patients who received aflibercept fell below the LLOQ 3 hours postinjection and remained below the LLOQ at the Day 1, 3, and 7 time points for all 3 indications. Patients who received bevacizumab had notable decreases from baseline in free-VEGF levels; however, in patients with DME and RVO, mean free-VEGF levels remained above the LLOQ at all time points. In patients with AMD, free-VEGF levels were below the LLOQ after Dose 3 at the Day 1, 3, and 7 time points. Patients who received ranibizumab experienced the least amount of change in mean free-VEGF levels. Overall, there were no notable changes in mean and median free-VEGF levels from baseline for ranibizumab (Figures 2 and 3) over all the 3 indications.

Discussion

This study provides evidence that aflibercept, bevacizumab, and ranibizumab exhibit different systemic exposures and effects on systemic VEGF after intravitreal injection. Systemic exposure of each anti-VEGF drug did not seem to differ by indication and was consistently highest with bevacizumab and lowest with ranibizumab. Systemic ranibizumab concentrations remained below its IC_{50} (0.06 nM)⁴³ at most observed time points for all 3 indications. However, after the third doses, systemic levels of aflibercept and bevacizumab exceeded their respective IC_{50} for VEGF inhibition for all 3 indications. Ranibizumab demonstrated no systemic accumulation between Doses 1 and 3, whereas bevacizumab showed substantive drug accumulation after Dose 3 compared with Dose 1, which ranged from 30% to 95% accumulation based on C_{min} , C_{max} , or AUC across indications (Table 5). The only exception was when the accumulation ratio was calculated using C_{min} in patients with DME, which showed no accumulation for any of the three

Table 2. Mean Systemic Exposures of Aflibercept, Bevacizumab, and Ranibizumab in Patients With AMD³²

Parameter	Aflibercept	Bevacizumab	Ranibizumab	Geometric Mean Ratio (AFL/RBZ) [95% CI]	Geometric Mean Ratio (BEV/RBZ) [95% CI]
First dose					
C_{max} , nM, mean (SD)	0.45 (0.29), n = 21	0.76 (0.31), n = 15	0.11 (0.13), n = 20	4.65 [3.07–7.05]	8.80 [5.59–13.87]
C_{min} , nM, mean (SD)	0.05 (0.02), n = 20	0.45 (0.16), n = 15	0.002 (0.002), n = 19	37.28 [23.72–58.6]	321.61 [197.5–523.7]
AUC _{0–28} , nM·h, mean (SD)	4.32 (1.77), n = 20	16.10 (5.75), n = 15	0.46 (0.24), n = 19	9.49 [7.37–12.22]	35.73 [27.2–46.94]
Third dose					
C_{max} , nM, mean (SD)	0.58 (0.52), n = 21	1.47 (0.55), n = 15	0.08 (0.06), n = 18	6.74 [4.46–10.18]	20.97 [13.37–32.89]
C_{min} , nM, mean (SD)	0.07 (0.03), n = 21	0.70 (0.29), n = 14	0.002 (0.002), n = 18	52.92 [33.83–82.8]	500.31 [304.51–822.03]
AUC _{60–88} , nM·h, mean (SD)	5.38 (1.77), n = 21	29.12 (10.35), n = 14	0.52 (0.59), n = 18	12.58 [9.33–16.96]	67.24 [48.26–93.68]

AFL, aflibercept; BEV, bevacizumab; RBZ, ranibizumab.

Table 3. Mean Systemic Exposures of Aflibercept, Bevacizumab, and Ranibizumab in Patients With DME

Parameter	Aflibercept	Bevacizumab	Ranibizumab	Geometric Mean Ratio (AFL/RBZ) [95% CI]	Geometric Mean Ratio (BEV/RBZ) [95% CI]
First dose					
C_{max} , nM, mean (SD)	0.53 (0.39), n = 15	0.75 (0.24), n = 15	0.12 (0.19), n = 15	7.32 [4–13.4]	12.01 [6.57–21.97]
C_{min} , nM, mean (SD)	0.04 (0.02), n = 15	0.35 (0.18), n = 15	0.0006 (0.0008), n = 15	79.75 [44.99–141.36]	844.04 [476.18–1496.1]
AUC _{0–28} , nM·h, mean (SD)	3.41 (1.32), n = 15	14.26 (4.67), n = 15	0.24 (0.08), n = 15	13.96 [10.44–18.67]	59.45 [44.47–79.47]
Third dose					
C_{max} , nM, mean (SD)	0.57 (0.33), n = 15	1.18 (0.45), n = 14	0.04 (0.03), n = 15	13.59 [9.63–19.18]	30.29 [21.34–43.01]
C_{min} , nM, mean (SD)	0.045 (0.024), n = 12	0.42 (0.25), n = 14	0.0007 (0.0009), n = 15	107 [52.27–219.03]	787.55 [390.1–1589.93]
AUC _{60–88} , nM·h, mean (SD)	4.35 (1.36), n = 14	20.39 (6.83), n = 14	0.23 (0.08), n = 15	19.44 [14.66–25.76]	90.52 [68.3–119.97]

AFL, aflibercept; BEV, bevacizumab; RBZ, ranibizumab.

Table 4. Mean Systemic Exposures of Aflibercept, Bevacizumab, and Ranibizumab in Patients With RVO

Parameter	Aflibercept	Bevacizumab	Ranibizumab	Geometric Mean Ratio (AFL/RBZ) [95% CI]	Geometric Mean Ratio (BEV/RBZ) [95% CI]
First dose					
C_{max} , nM, mean (SD)	0.76 (0.96), n = 15	0.77 (0.33), n = 15	0.08 (0.10), n = 18	7.52 [4.14–13.63]	13.45 [7.42–24.4]
C_{min} , nM, mean (SD)	0.04 (0.03), n = 15	0.37 (0.11), n = 15	0.0015 (0.0011), n = 18	31.07 [18.09–53.36]	335.46 [195.33–576.11]
AUC_{0-28} , nM·h, mean (SD)	4.26 (1.95), n = 15	14.76 (3.25), n = 15	0.33 (0.13), n = 18	12.45 [9.45–16.39]	47.05 [35.73–61.95]
Third dose					
C_{max} , nM, mean (SD)	0.59 (0.28), n = 14	1.20 (0.27), n = 15	0.067 (0.049), n = 18	9.84 [6.74–14.38]	22.07 [15.21–32.01]
C_{min} , nM, mean (SD)	0.05 (0.02), n = 14	0.48 (0.13), n = 15	0.0019 (0.0017), n = 18	43.38 [25.04–75.16]	398.52 [232.42–683.32]
AUC_{60-88} , nM·h, mean (SD)	4.91 (1.62), n = 14	22.49 (4.04), n = 15	0.33 (0.12), n = 18	14.83 [11.82–18.6]	70.68 [56.58–88.3]

AFL, aflibercept; BEV, bevacizumab; RBZ, ranibizumab.

therapeutics. There seemed to be a trend of systemic accumulation after Dose 3 compared with Dose 1 for aflibercept, based on the consistently >1 geometric mean ratios between Dose 3 and Dose 1 using C_{min} , C_{max} , or AUC (ranging from 19 to 51%), although the 95% confidence intervals (CIs) spanned one in most indications (Table 5). Because bevacizumab and aflibercept contain Fc regions, their higher systemic exposures compared with ranibizumab may result from a decreased rate of systemic clearance by FcRn-mediated recycling within endothelial cells (a mechanism protecting Fc-containing molecules from intracellular degradation within endosomes, thus decreasing their systemic clearance).⁴⁵ In contrast, ranibizumab is a Fab fragment, and thus is cleared from the systemic circulation more rapidly.

Concerning systemic VEGF levels, aflibercept resulted in the greatest suppression of plasma free-VEGF, with most samples falling below the LLOQ for all three indications. Mean plasma free-VEGF levels in patients who received ranibizumab remained >10 pg/mL at every time point. In patients with RVO and DME who received bevacizumab, VEGF levels remained above the LLOQ after the first and third doses. There were no differences between plasma free-VEGF levels with ranibizumab 0.3 mg (patients with DME) compared with 0.5 mg (patients with AMD and RVO) (Figure 2). In patients with AMD, plasma free-VEGF levels remained above the assay's LLOQ after the first bevacizumab dose only. In this study, of the three anti-VEGF therapeutics examined, ranibizumab demonstrated the smallest effect on plasma free-VEGF levels. The measurement of free-VEGF in the presence of anti-VEGF mAb may be confounded by the dissociation of the complex these two molecules form in vivo. Anti-VEGF/VEGF complexes in samples may undergo dissociation upon dilution. However, based on published binding affinity properties of these three therapeutics, it can be concluded that the observations made in this study were not likely affected by complex dissociation incurred by sample dilution performed during sample testing.

The results described herein are consistent with other published studies in AMD.^{33–38} Several recent studies have shown that mean systemic VEGF levels are markedly reduced from baseline in patients with AMD receiving aflibercept injection, and these reductions have lasted 1 month or longer, whereas the VEGF levels in patients who received ranibizumab injection did not change significantly.^{34,38} A prospective, interventional case series by Wang et al reported that, in patients with AMD, 0.5-mg ranibizumab did not have a significant impact on plasma VEGF concentrations over 2 months.³⁵ However, patients treated

Table 5. Accumulation Ratios for the Dose 3/Dose 1 Geometric Mean Ratios of C_{min} , C_{max} , and AUC_{0-28}

Accumulation Ratio (95% CI)	AMD	DME	RVO
Aflibercept			
C_{min}	1.37 (1.03–1.83)	1.42 (0.89–2.26)	1.51 (0.92–2.48)
C_{max}	1.19 (0.77–1.84)	1.18 (0.75–1.85)	1.29 (0.65–2.57)
AUC_{28}	1.27 (1.01–1.59)	1.31 (0.98–1.76)	1.22 (0.87–1.70)
Bevacizumab			
C_{min}	1.51 (1.11–2.04)	0.99 (0.51–1.90)	1.29 (1.03–1.60)
C_{max}	1.95 (1.43–2.68)	1.60 (1.22–2.11)	1.62 (1.30–2.01)
AUC_{28}	1.80 (1.34–2.41)	1.43 (1.08–1.89)	1.54 (1.32–1.79)
Ranibizumab			
C_{min}	0.97 (0.49–1.93)	1.06 (0.49–2.29)	1.08 (0.53–2.23)
C_{max}	0.82 (0.50–1.34)	0.64 (0.32–1.26)	0.99 (0.59–1.66)
AUC_{28}	0.96 (0.67–1.36)	0.94 (0.70–1.27)	1.02 (0.80–1.32)

with aflibercept 2.0 mg showed dramatic reductions from baseline in VEGF levels at 1 week and 1 month postinjection, and VEGF levels did not return to near-baseline values until 2 months postinjection.³⁵ Similarly, a prospective randomized controlled trial in patients with AMD reported that plasma VEGF levels were reduced from baseline levels below the minimum detectable dose (MDD) in 89.5% of patients 1 week after injection of aflibercept and remained below the MDD at 1 month, but ranibizumab did not produce significant changes in plasma VEGF levels at either time point.³⁴

Limited data are available on the impact of anti-VEGF treatment on plasma VEGF levels in patients with DME and RVO. The results of the present study for patients with DME were similar to those of a smaller study by Zehetner et al,⁴⁶ which reported significant reductions in plasma VEGF levels from baseline after 1 week ($P = 0.008$) and levels remained reduced at 4 weeks ($P = 0.012$) in patients with DME treated with bevacizumab ($n = 10$). Conversely, plasma VEGF levels in patients with DME treated with ranibizumab ($n = 10$) were not significantly affected.⁴⁶

The clinical implications of suppression of systemic VEGF after intravitreal anti-VEGF therapeutic administration are not known. From clinical experience in oncology, VEGF suppression after systemic administration of anti-VEGF agents has been associated with cardiovascular, arterial thromboembolic, renal, and GI adverse effects and wound-healing complications.²⁴

Few head-to-head studies have been conducted between ranibizumab and aflibercept. In the VIEW I and II clinical trials, the ocular and systemic safety profiles of ranibizumab and aflibercept in patients with AMD were found to be similar.^{26,27} Additionally, in a study comparing ranibizumab, aflibercept, and bevacizumab in patients with DME, there were no significant differences in systemic safety including the rates of serious adverse events (SAEs), hospitalization, death, or major cardiovascular events at 1 year.⁸ At

2 years, more Antiplatelet Trialists Collaboration (APTC) events were noted in the ranibizumab arm ($P = 0.047$), although there was a baseline imbalance with more ranibizumab patients with a pretreatment cardiovascular history (coronary artery disease, myocardial infarction, transient ischemic attack, and cerebrovascular accident) than aflibercept patients (70 vs. 49), and when adjusting for multiple baseline characteristics, the differences were no longer statistically significant (overall $P = 0.09$).⁴⁷ Furthermore, the authors noted that this increased incidence of ATEs was not observed in previous trials with ranibizumab, and the implications of the increased rate of APTC events with ranibizumab found in that study were uncertain because of this inconsistency.⁴⁷

The efficacy and safety profiles of bevacizumab and ranibizumab have been compared in several clinical trials.^{25,28,30} Although the efficacy of both treatments has been comparable, differences in their respective safety profiles have been reported. In the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT), a higher percentage of bevacizumab patients had ≥ 1 systemic SAEs compared with ranibizumab patients (39.9 vs. 31.7%; adjusted risk ratio [RR], 1.30; 95% CI, 1.07–1.57; $P = 0.009$).⁵ In the alternative treatments to Inhibit VEGF in Age-related choroidal Neovascularization (IVAN) randomized trial of bevacizumab and ranibizumab, serum VEGF levels were significantly lower after bevacizumab administration compared with ranibizumab (geometric mean ratio, 0.47; 95% CI, 0.41–0.54, $P < 0.001$).³¹ Median serum VEGF levels in patients receiving bevacizumab decreased from 203 pg/mL at baseline to 83 pg/mL at 1 year, whereas median serum VEGF concentrations in patients receiving ranibizumab decreased from 173 pg/mL at baseline to 151 pg/mL at 1 year. The incidence of systemic SAEs in IVAN did not differ between ranibizumab and bevacizumab (9.6 vs. 12.5%; $P = 0.25$);

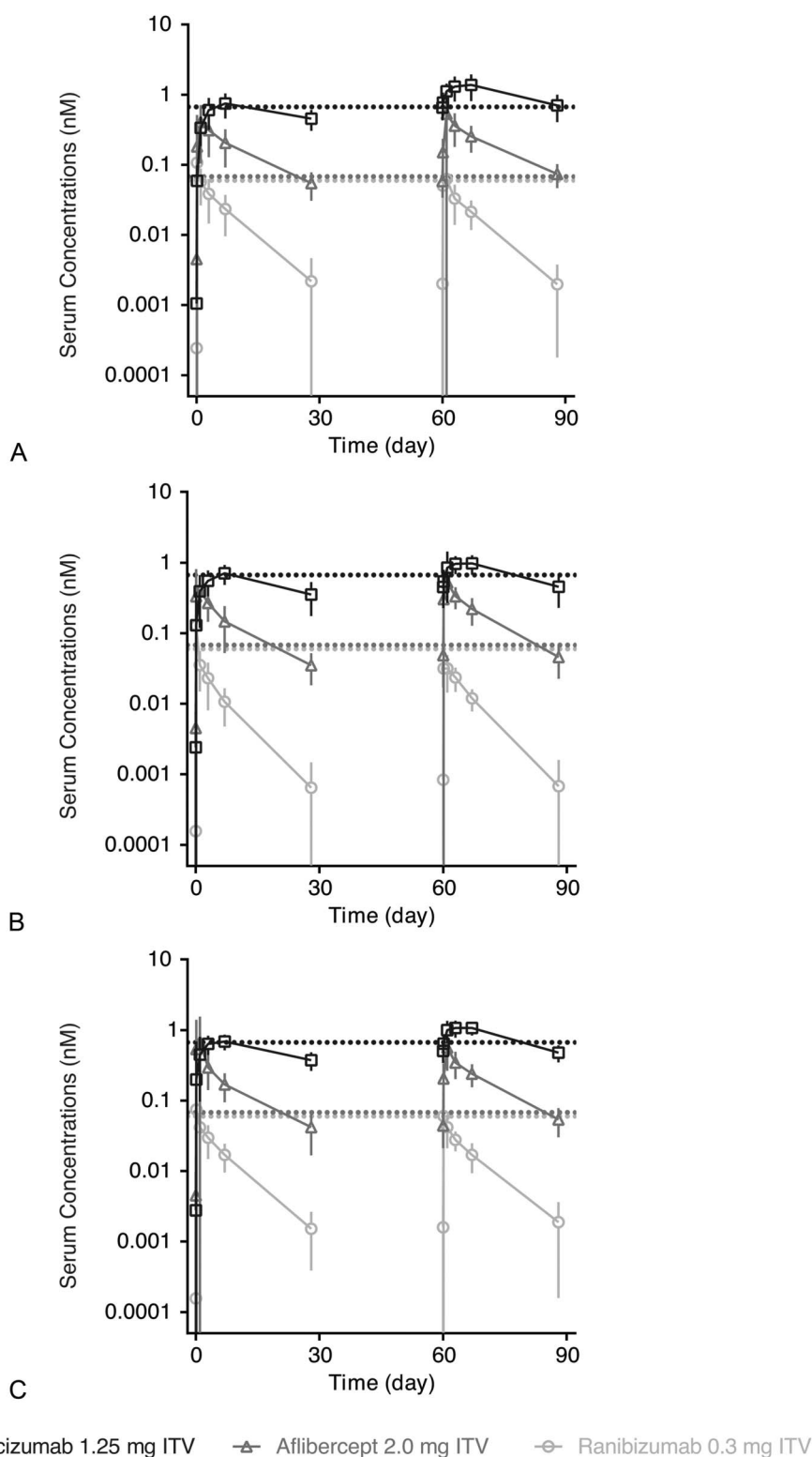


Fig. 1. Mean (SD) serum concentration–time profiles of each drug after intravitreal injection with aflibercept, bevacizumab, or ranibizumab in (A) patients with AMD, (B) patients with DME, and (C) patients with RVO. The black, gray, and light gray lines represent the IC₅₀ for bevacizumab, aflibercept, and ranibizumab, respectively. ITV, intravitreal. Figure 1A was reproduced, with permission, from Avery RL, Castellarin AA, Steinle NC, et al. Systemic pharmacokinetics following intravitreal injections of ranibizumab, bevacizumab or aflibercept in patients with neovascular AMD. *Br J Ophthalmol* 2014;98:1636–1641. <http://bj.o.bmj.com/content/98/12/1636>; licensed under CC BY-NC 3.0.

however, more patients who received ranibizumab experienced an ATE or heart failure than patients who received bevacizumab ($P = 0.03$). An analysis of 506 patients from IVAN found that SAEs (exclud-

ing cardiovascular-related SAEs) did not seem to be associated with the change in the levels of serum VEGF using samples taken at 0, 1, 11, 12, 23, and 24 months ($P = 0.92$).⁴⁸

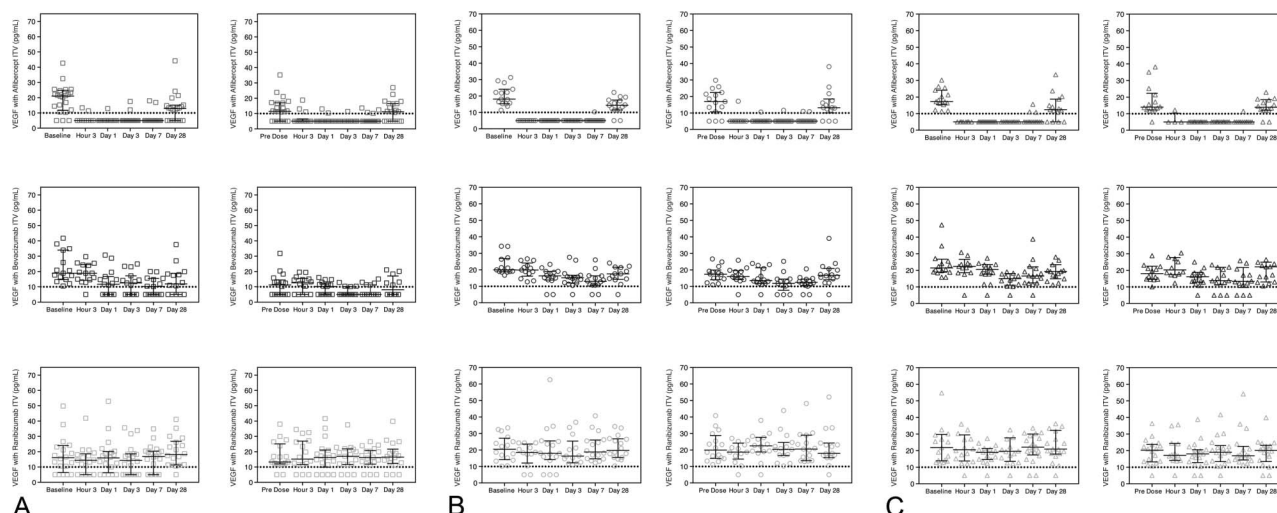


Fig. 2. Plasma levels of free-VEGF in (A) patients with AMD, (B) patients with DME, and (C) patients with RVO. Lines represent median and interquartile range. Dotted line represents the LLOQ. Outliers (>75 pg/mL) are not illustrated for better visualization of the data, but are included in the median and interquartile range (AMD: 1 level in patient treated with ranibizumab; DME: 2 levels in patients treated with bevacizumab, 5 levels in patients treated with ranibizumab; RVO: 13 levels in patients treated with bevacizumab, 6 levels in patients treated with ranibizumab). Plasma VEGF levels that measured lower than the LLOQ were assigned a value of 50% of the LLOQ. ITV, intravitreal. Figure 2A was reproduced, with permission, from Avery RL, Castellano AA, Steinle NC, et al. Systemic pharmacokinetics following intravitreal injections of ranibizumab, bevacizumab or aflibercept in patients with neovascular AMD. *Br J Ophthalmol* 2014;98:1636–1641. <http://bjoo.bmj.com/content/98/12/1636>; licensed under CC BY-NC 3.0.

Meta-analyses of clinical trials have also reported potential safety differences between bevacizumab and ranibizumab. Zhang et al⁴⁹ performed a meta-analysis of 4 clinical trials and 11 retrospective case series to examine the efficacy and safety of bevacizumab and ranibizumab in patients with AMD. Ranibizumab was found to be associated with a lower incidence of venous thrombotic events (RR, 0.27; 95% CI, 0.08–0.89; $P = 0.03$), but the risk of ATEs did not differ. A recent meta-analysis of patients with AMD, RVO, or DME treated with either ranibizumab or bevacizumab reported no increased risk of major cardiovascular events or nonocular hemorrhagic events.⁵⁰ However, for the trials that compared bevacizumab and ranibizumab directly, bevacizumab was found to have increased risk of venous thromboembolic events (odds ratio, 3.45; 95% CI, 1.25–9.54; $P = 0.02$).⁵⁰

Three meta-analyses of AMD trials have identified an increased risk of GI adverse events with bevacizumab versus ranibizumab.^{51–53} In a meta-analysis of data from the CATT, IVAN, GEFAL, MANTA, and LUCAS trials, a higher percentage of patients randomized to bevacizumab experienced a GI SAE compared with patients randomized to ranibizumab (3.1 vs. 1.6%) and the risk of a GI SAE with bevacizumab was significantly higher versus ranibizumab (RR, 1.94; 95% CI, 1.20–3.14, $P = 0.007$).⁵¹ Two Cochrane reports published in 2014 found a higher risk of GI adverse events with bevacizumab versus ranibizumab (RR, 1.82; 95% CI, 1.04–3.19, $P = 0.04$, in one, and RR, 2.24; 95% CI, 1.10–4.55, in the other).^{52,53}

One of these Cochrane meta-analyses found a significant increase in systemic SAEs with bevacizumab compared with ranibizumab (RR, 1.27; 95% CI, 1.06–1.52).⁵² The second Cochrane meta-analysis included unpublished trials including LUCAS and found the risk to no longer be statistically significant (RR, 1.08; 95% CI, 0.90–1.31, $P = 0.42$).⁵³ However, excluding the LUCAS trial, they found the results to be significant with RR, 1.19; 95% CI, 1.06–1.34, $P = 0.0038$.⁵³ The LUCAS authors noted a statistically significant imbalance at baseline with more patients with previous MIs in the ranibizumab arm ($P = 0.02$). The increase in systemic SAEs was driven primarily by an increase in cardiovascular events in the ranibizumab arm, which was also statistically significant at year 1, but not at year 2.^{54,55} Hence, it is possible that the baseline imbalance in LUCAS may be responsible for the finding of no statistically significant difference in systemic SAEs in this second Cochrane meta-analysis.⁵⁶

Intravitreal anti-VEGF therapy seems safe in the general population enrolled into numerous clinical trials, but there may be select at-risk populations, such as patients with diabetes or a history of recent MI or cerebrovascular accident, in whom there may be increased systemic risk to sustained suppression of systemic VEGF levels.^{23,56} In fact, a recent, focused meta-analysis described a possibly increased risk of death in diabetic patients receiving monthly anti-VEGF agents for 2 years.³⁹

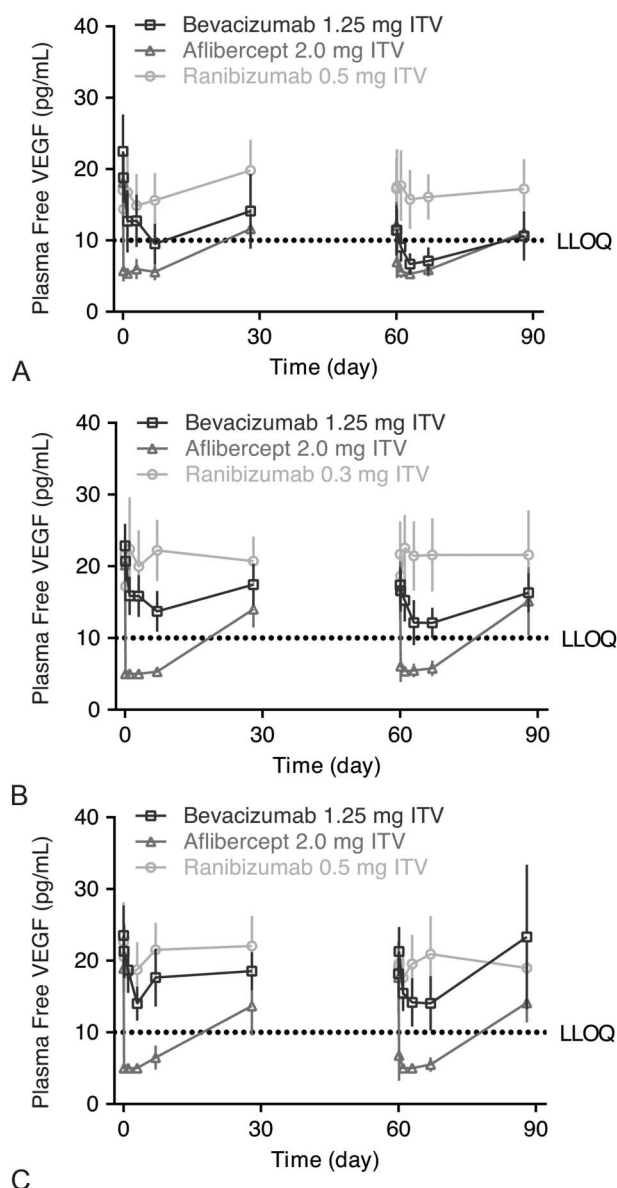


Fig. 3. Mean (95% CI) plasma free-VEGF with bevacizumab, ranibizumab, and aflibercept in (A) patients with AMD, (B) patients with DME, and (C) patients with RVO. ITV, intravitreal. Figure 3A was reproduced, with permission, from Avery RL, Castellarin AA, Steinle NC, et al. Systemic pharmacokinetics following intravitreal injections of ranibizumab, bevacizumab or aflibercept in patients with neovascular AMD. *Br J Ophthalmol* 2014;98:1636–1641. <http://bjo.bmj.com/content/98/12/1636>; licensed under CC BY-NC 3.0.

Another at-risk population is retinopathy of prematurity (ROP) infants, because VEGF has a role in organogenesis and neurodevelopment. Two recent studies have raised concerns about a potential increased risk of neurodevelopmental abnormalities in ROP infants treated with bevacizumab relative to laser alone.^{57,58} Because of the ROP baby's small size, the total blood volume is ~20-fold less than that of adults, but the dose of bevacizumab typically admin-

istered is one half of the adult dose. A recent PK study in ROP infants found a serum bevacizumab C_{max} of 1,002 ng/mL at 2 weeks, ~9-fold higher than the C_{max} at 1 week after the first dose in the present study.⁵⁹ Bevacizumab was detected and free-VEGF was reduced in the serum out to 8 weeks in this ROP study, which did not evaluate ranibizumab.⁵⁹ Because the present study found much lower systemic exposure from ranibizumab compared with bevacizumab across three disease states, if these findings can be extrapolated to ROP, it would support the contention that ranibizumab may be safer than bevacizumab in infants.⁶⁰ Ongoing trials are evaluating lower doses of both bevacizumab and ranibizumab in ROP.

The present study provides important insight into the differences in systemic distribution of these anti-VEGF agents and circulating levels of VEGF in patients with AMD, DME, and RVO. Despite its relatively small sample size and lack of formal statistical analysis, the present study prospectively evaluated head-to-head all the commonly used intravitreal anti-VEGF therapeutics across multiple disease states over several time points. Nearly 2,000 blood samples were each analyzed for free-VEGF and drug concentrations, making it the largest reported comparative PK/pharmacodynamic study of these 3 agents. An additional strength of this study is the use of CTAD collection tubes, which minimize the impact of platelet rupture and/or activation that can lead to release of VEGF from platelets, which could erroneously skew the data toward higher or more variable VEGF levels.³² Furthermore, unlike most previous studies that did not measure drug levels, comparisons of systemic drug levels and VEGF levels were analyzed and demonstrated that higher systemic concentrations of the drug correlated with more suppression of plasma free-VEGF levels.

Conclusions

In patients with AMD, DME, and RVO, the three anti-VEGF treatments examined in this analysis demonstrated notable differences in systemic PKs and plasma free-VEGF levels. Although clinically meaningful differences in the systemic safety profiles have yet to be elucidated, these differences could provide a plausible biologic explanation for potential differences in systemic safety risks among aflibercept, bevacizumab, and ranibizumab.

Key words: age-related macular degeneration, anti-VEGF, diabetic macular edema, pharmacokinetics, retinal vein occlusion.

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References

- Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003;9:669–676.
- Stewart MW. The expanding role of vascular endothelial growth factor inhibitors in ophthalmology. *Mayo Clin Proc* 2012;87:77–88.
- Lucentis (Ranibizumab Injection) Intravitreal Injection Prescribing Information. 2014. Available at: www.gene.com/download/pdf/lucentis_prescribing.pdf. Accessed June 12, 2014.
- Korobelnik JF, Do DV, Schmidt-Erfurth U, et al. Intravitreal aflibercept for diabetic macular edema. *Ophthalmology* 2014;121:2247–2254.
- Martin DF, Maguire MG, Ying GS, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med* 2011;364:1897–1908.
- Nepomuceno AB, Takaki E, Paes de Almeida FP, et al. A prospective randomized trial of intravitreal bevacizumab versus ranibizumab for the management of diabetic macular edema. *Am J Ophthalmol* 2013;156:502–510.e502.
- Rajendram R, Fraser-Bell S, Kaines A, et al. A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. *Arch Ophthalmol* 2012;130:972–979.
- Wells JA, Glassman AR, Ayala AR, et al. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *N Engl J Med* 2015;372:1193–1203.
- Hirose M, Matsumiya W, Honda S, Nakamura M. Efficacy and visual prognostic factors of intravitreal bevacizumab as needed for macular edema secondary to central retinal vein occlusion. *Clin Ophthalmol* 2014;8:2301–2305.
- Narayanan R, Panchal B, Das T, et al. A randomised, double-masked, controlled study of the efficacy and safety of intravitreal bevacizumab versus ranibizumab in the treatment of macular oedema due to branch retinal vein occlusion: MARVEL Report No. 1. *Br J Ophthalmol* 2015;99:954–959.
- Heier JS, Clark WL, Boyer DS, et al. Intravitreal aflibercept injection for macular edema due to central retinal vein occlusion: two-year results from the COPERNICUS study. *Ophthalmology* 2014;121:1414–1420.
- EYLEA (Aflibercept Injection) Intravitreal Injection Prescribing Information. Regeneron Pharmaceuticals, Inc. Tarrytown, NY. 2016. Available at: <https://www.regeneron.com/Eylea/eylea-fpi.pdf>. Accessed June 22, 2016.
- Papadopoulos N, Martin J, Ruan Q, et al. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. *Angiogenesis* 2012;15:171–185.
- Park SJ, Oh J, Kim YK, et al. Intraocular pharmacokinetics of intravitreal vascular endothelial growth factor-Trap in a rabbit model. *Eye (Lond)* 2015;29:561–568.
- Ferrara N, Damico L, Shams N, et al. Development of ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. *Retina* 2006;26:859–870.
- Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov* 2004;3:391–400.
- Lowe J, Araujo J, Yang J, et al. Ranibizumab inhibits multiple forms of biologically active vascular endothelial growth factor in vitro and in vivo. *Exp Eye Res* 2007;85:425–430.
- Krohne TU, Eter N, Holz FG, Meyer CH. Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. *Am J Ophthalmol* 2008;146:508–512.
- Xu L, Lu T, Tuomi L, et al. Pharmacokinetics of ranibizumab in patients with neovascular age-related macular degeneration: a population approach. *Invest Ophthalmol Vis Sci* 2013;54:1616–1624.
- Gaudreault J, Fei D, Rusit J, et al. Preclinical pharmacokinetics of Ranibizumab (rhuFabV2) after a single intravitreal administration. *Invest Ophthalmol Vis Sci* 2005;46:726–733.
- Krohne TU, Liu Z, Holz FG, Meyer CH. Intraocular pharmacokinetics of ranibizumab following a single intravitreal injection in humans. *Am J Ophthalmol* 2012;154:682–686.e682.
- Zhang Y, Yao Z, Kaila N, et al. Pharmacokinetics of ranibizumab after intravitreal administration in patients with retinal vein occlusion or diabetic macular edema. *Ophthalmology* 2014;121:2237–2246.
- Avery RL. What is the evidence for systemic effects of intravitreal anti-VEGF agents, and should we be concerned? *Br J Ophthalmol* 2014;98:i7–i10.
- Chen HX, Cleck JN. Adverse effects of anticancer agents that target the VEGF pathway. *Nat Rev Clin Oncol* 2009;6:465–477.
- Chakravarthy U, Harding SP, Rogers CA, et al. Alternative treatments to inhibit VEGF in age-related choroidal neovascularisation: 2-year findings of the IVAN randomised controlled trial. *Lancet* 2013;382:1258–1267.
- Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. *Ophthalmology* 2012;119:2537–2548.
- Schmidt-Erfurth U, Kaiser PK, Korobelnik JF, et al. Intravitreal aflibercept injection for neovascular age-related macular degeneration: ninety-six-week results of the VIEW studies. *Ophthalmology* 2014;121:193–201.
- Kodjikian L, Souied EH, Mimoun G, et al. Ranibizumab versus bevacizumab for neovascular age-related macular degeneration: results from the GEFAL noninferiority randomized trial. *Ophthalmology* 2013;120:2300–2309.
- Krebs I, Schmetterer L, Boltz A, et al. A randomised double-masked trial comparing the visual outcome after treatment with ranibizumab or bevacizumab in patients with neovascular age-related macular degeneration. *Br J Ophthalmol* 2013;97:266–271.
- Martin DF, Maguire MG, Fine SL, et al. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology* 2012;119:1388–1398.
- Chakravarthy U, Harding SP, Rogers CA, et al. Ranibizumab versus bevacizumab to treat neovascular age-related macular degeneration: one-year findings from the IVAN randomized trial. *Ophthalmology* 2012;119:1399–1411.
- Avery RL, Castellarin AA, Steinle NC, et al. Systemic pharmacokinetics following intravitreal injections of ranibizumab, bevacizumab or aflibercept in patients with neovascular AMD. *Br J Ophthalmol* 2014;98:1636–1641.
- Carneiro AM, Costa R, Falcao MS, et al. Vascular endothelial growth factor plasma levels before and after treatment of neovascular age-related macular degeneration with bevacizumab or ranibizumab. *Acta Ophthalmol* 2012;90:e25–e30.

34. Zehetner C, Kralinger MT, Modi YS, et al. Systemic levels of vascular endothelial growth factor before and after intravitreal injection of aflibercept or ranibizumab in patients with age-related macular degeneration: a randomised, prospective trial. *Acta Ophthalmol* 2015;93:e154–e159.
35. Wang X, Sawada T, Sawada O, et al. Serum and plasma vascular endothelial growth factor concentrations before and after intravitreal injection of aflibercept or ranibizumab for age-related macular degeneration. *Am J Ophthalmol* 2014;158:738–744.e731.
36. Gu X, Yu X, Dai H. Intravitreal injection of ranibizumab for treatment of age-related macular degeneration: effects on serum VEGF concentration. *Curr Eye Res* 2014;39:518–521.
37. Enders P, Muether PS, Hermann M, et al. Long-term alterations of systemic vascular endothelial growth factor levels in patients treated with ranibizumab for age-related macular degeneration. *Retina* 2015;35:454–458.
38. Yoshida I, Shiba T, Taniguchi H, et al. Evaluation of plasma vascular endothelial growth factor levels after intravitreal injection of ranibizumab and aflibercept for exudative age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 2014;252:1483–1489.
39. Avery RL, Gordon GM. Systemic safety of prolonged monthly anti-vascular endothelial growth factor therapy for diabetic macular edema: a systematic review and meta-analysis. *JAMA Ophthalmol* 2016;134:21–29.
40. Aguilar-Mahecha A, Kuzyk MA, Domanski D, et al. The effect of pre-analytical variability on the measurement of MRM-MS-based mid- to high-abundance plasma protein biomarkers and a panel of cytokines. *PLoS One* 2012;7:e38290.
41. Mody M, Lazarus AH, Semple JW, Freedman J. Preanalytical requirements for flow cytometric evaluation of platelet activation: choice of anticoagulant. *Transfus Med* 1999;9:147–154.
42. Gunsilius E, Petzer A, Stockhammer G, et al. Thrombocytes are the major source for soluble vascular endothelial growth factor in peripheral blood. *Oncology* 2000;58:169–174.
43. Yu L, Liang XH, Ferrara N. Comparing protein VEGF inhibitors: in vitro biological studies. *Biochem Biophys Res Commun* 2011;408:276–281.
44. Stewart MW, Rosenfeld PJ. Predicted biological activity of intravitreal VEGF Trap. *Br J Ophthalmol* 2008;92:667–668.
45. Ternant D, Paintaud G. Pharmacokinetics and concentration-effect relationships of therapeutic monoclonal antibodies and fusion proteins. *Expert Opin Biol Ther* 2005;5(suppl 1):S37–S47.
46. Zehetner C, Kirchmair R, Huber S, et al. Plasma levels of vascular endothelial growth factor before and after intravitreal injection of bevacizumab, ranibizumab and pegaptanib in patients with age-related macular degeneration, and in patients with diabetic macular oedema. *Br J Ophthalmol* 2013;97:454–459.
47. Wells JA, Glassman AR, Ayala AR, et al. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema: two-year results from a comparative effectiveness randomized clinical trial. *Ophthalmology* 2016;123:1351–1359.
48. Chakravarthy U, Rogers C, Reeves B. Alterations in serum vascular endothelial growth factor after intraocular administration of anti-VEGF drugs, is there an association with safety outcomes? *Invest Ophthalmol Vis Sci* 2014;55:871.
49. Zhang XY, Guo XF, Zhang SD, et al. Comparison of bevacizumab and ranibizumab in age-related macular degeneration: a systematic review and meta-analysis. *Int J Ophthalmol* 2014;7:355–364.
50. Thulliez M, Angoulvant D, Le Lez ML, et al. Cardiovascular events and bleeding risk associated with intravitreal anti-vascular endothelial growth factor monoclonal antibodies: systematic review and meta-analysis. *JAMA Ophthalmol* 2014;132:1317–1326.
51. Scott L, Chakravarthy U, Nash R. Gastrointestinal serious adverse events in patients treated with intraocular ranibizumab or bevacizumab for age-related choroidal neovascularisation, what do the recent trials tell us? *Invest Ophthalmol Vis Sci* 2014;55:1648.
52. Solomon SD, Lindsley K, Vedula SS, et al. Anti-vascular endothelial growth factor for neovascular age-related macular degeneration. *Cochrane Database Syst Rev* 2014:CD005139.
53. Moja L, Lucenteforte E, Kwag KH, et al. Systemic safety of bevacizumab versus ranibizumab for neovascular age-related macular degeneration. *Cochrane Database Syst Rev* 2014:CD011230.
54. Berg K, Pedersen TR, Sandvik L, Bragadottir R. Comparison of ranibizumab and bevacizumab for neovascular age-related macular degeneration according to LUCAS treat-and-extend protocol. *Ophthalmology* 2015;122:146–152.
55. Berg K, Hadzalic E, Gjertsen I, et al. Ranibizumab or bevacizumab for neovascular age-related macular degeneration according to the Lucentis Compared to Avastin Study Treat-and-Extend Protocol: two-year results. *Ophthalmology* 2016;123:51–59.
56. Avery RL. Re: Berg et al.: comparison of ranibizumab and bevacizumab for neovascular age-related macular degeneration according to LUCAS treat-and-extend protocol. *Ophthalmology* 2016;123:e14–e16.
57. Lien R, Yu MH, Hsu KH, et al. Neurodevelopmental outcomes in infants with retinopathy of prematurity and bevacizumab treatment. *PLoS One* 2016;11:e0148019.
58. Morin J, Luu TM, Superstein R, et al. Neurodevelopmental outcomes following bevacizumab injections for retinopathy of prematurity. *Pediatrics* 2016;137:1–10.
59. Kong L, Bhatt AR, Demny AB, et al. Pharmacokinetics of bevacizumab and its effects on serum VEGF and IGF-1 in infants with retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 2015;56:956–961.
60. Avery RL. Bevacizumab (Avastin) for retinopathy of prematurity: wrong dose, wrong drug, or both? *J AAPOS* 2012;16:2–4.