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APPLICATION NUMBER:

761099Orig1s000

NON-CLINICAL REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: BLA 761099
Supporting document/s: 003
Applicant's letter date: June 29, 2018
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Product: PF-06439535
Indication: Metastatic colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, metastatic renal cell carcinoma, and cervical cancer
Applicant: Pfizer, Inc.
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1 Executive Summary

1.1 Introduction

Pfizer has submitted biologics license application (BLA) 761099 under section 351(k) of the Public Health Service Act (PHS Act) for PF-06439535, a proposed biosimilar for US-licensed Avastin (bevacizumab). PF-06439535 is a recombinant humanized IgG1 monoclonal antibody directed against vascular endothelial growth factor (VEGF). The established mechanism of action of bevacizumab is to bind VEGF and prevent its interaction with the VEGF receptors (VEGFR-1 and VEGFR-2) on the surface of endothelial cells, thus inhibiting vascular endothelial cell proliferation and angiogenesis.

1.2 Brief Discussion of Nonclinical Findings

The Applicant conducted in vitro pharmacology studies comparing the functional and binding properties of PF-06439535, US-licensed Avastin, and EU-approved bevacizumab. These studies were reviewed in detail by the CMC team as part of the analytical similarity assessment. From a nonclinical perspective, PF-06439535, US-licensed Avastin, and EU-approved bevacizumab exhibited relatively similar in vitro inhibition of VEGF-induced human umbilical vein endothelial cell (HUVEC) proliferation; binding to human VEGF₁₆₅/VEGF₁₂₁/VEGF₁₈₉/VEGF₂₀₆, C1q, FcγR receptors, and FcRn. PF-06439535 also exhibited a lack of antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activity, as expected based on a similar lack of activity with the innovator product. Based on the results from these in vitro comparative pharmacology studies, the Applicant did not conduct any in vivo pharmacology studies with PF-06439535.

The Applicant compared the nonclinical toxicity and toxicokinetics of PF-06439535 and EU-approved bevacizumab in a GLP-compliant 1-month repeat-dose toxicology study in sexually and skeletally immature male cynomolgus monkeys. Twice weekly intravenous (IV) administration of 10 mg/kg PF-06439535 or EU-approved bevacizumab resulted in minimal to moderate physeal dysplasia in the growth plate of the distal femur, an expected pharmacologic response. The incidence and severity of physeal dysplasia was relatively similar between PF-06439535 and EU-approved bevacizumab. Overall, there were no biologically significant differences in toxicity, toxicokinetics, or immunogenicity between PF-06439535 and EU-approved bevacizumab. Anti-drug antibodies (ADAs) were not detected in monkeys dosed with PF-06439535 or EU-approved bevacizumab. From a nonclinical perspective, the Applicant provided an acceptable scientific bridge based on comparative analytical similarity data between PF-06439535, US-licensed Avastin, and EU-approved bevacizumab to justify the relevance of the results from the 1-month toxicology study using a non-US-licensed comparator (EU-approved bevacizumab).

Although rats are not a pharmacologically relevant species for toxicity assessment of bevacizumab (reviewed under BLA 125085), (b) (4) the Applicant also conducted a GLP-compliant 2-week repeat-dose non-

comparative toxicology study in Sprague-Dawley rats investigating twice weekly IV administration of 0, 15, or 150 mg/kg PF-06439535. There were no mortalities in this study and the only evidence of potential non-target-mediated toxicity was increased mean liver weight at the high dose level that correlated histologically with sinusoidal cell hyperplasia. Both animal studies also employed the intended clinical formulation of PF-06439534, which includes compendial excipients not included in US-licensed or EU-approved bevacizumab including a high level of succinic acid. The level of succinic acid delivered to rats in the PF-06439535 drug product exceeded the level of succinic acid that patients will receive clinically with PF-06439535 providing reasonable toxicological coverage for this concentration considering that succinic acid is an endogenous substance that is a component of the Krebs cycle, and it occurs widely as a natural constituent of plants and animals consumed orally at high levels as food by humans.

Overall, the submitted nonclinical data suggest that PF-06439535 had similar in vitro activity and in vivo toxicity, pharmacokinetics/pharmacodynamics (PK/PD), and immunogenicity as bevacizumab. Thus, the submitted nonclinical data support a demonstration of PK/PD similarity.

1.3 Recommendations

1.3.1 Approvability

The submitted nonclinical studies support the similarity of PF-06439535 to US-licensed Avastin. From a pharmacology/toxicology perspective, there are no residual uncertainties regarding PK/PD and safety as it pertains to a demonstration of biosimilarity between PF-06439535 and US-licensed Avastin, and approval of PF-06439535 is recommended.

1.3.2 Additional Non-Clinical Recommendations

None.

1.3.3 Labeling

Since PF-06439535 is a proposed biosimilar to US-licensed Avastin, sections of the labeling relevant to nonclinical studies are identical to those in the FDA-approved labeling for US-licensed Avastin with the exception of the addition of biosimilar labeling terminology.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number: 216974-75-3

2.1.2 Proper Name: ZIRABEV

2.1.3 Code Name: PF-06439535; bevacizumab-Pfizer

2.1.4 Chemical Name: Recombinant humanized IgG1 kappa monoclonal antibody directed against vascular endothelial growth factor (VEGF)

2.1.5 Molecular Formula/Molecular Weight: Approximately 149 kDa. PF-06439535 has the same primary amino acid sequence as bevacizumab (US-licensed Avastin).

2.1.6 Structure: IgG1 kappa monoclonal antibody composed of two identical heavy chains and two identical light chains covalently linked with four inter-chain disulfide bonds. PF-06439535 is produced using a recombinant Chinese hamster ovary (CHO) cell line that contains the DNA encoding the sequence for bevacizumab.

2.1.7 Pharmacologic Class:

Vascular endothelial growth factor (VEGF) directed antibody

2.2 Relevant IND/s:

IND 117038 for PF-06439535 and BLA 125085 for bevacizumab (US-licensed Avastin)

2.3 Drug Formulation

PF-06439535 is supplied as a 25 mg/mL liquid concentrate for solution for infusion in (b) (4) clear glass vials containing either 100 mg/4 mL or 400 mg/16 mL.

Table 1: Composition of 100 mg/4 mL PF-06439535 Drug Product

Name of Ingredients	Reference to Standard	Function	Unit Formula (mg/mL)	Unit Formula (mg/vial)
PF-06439535	In-house specification	Active ingredient	25	100
Succinic Acid	NF, JPE	(b) (4)	2.36	9.44
Sucrose	NF, Ph. Eur., JP		85	340
Edetate Disodium Dihydrate (EDTA)	USP, Ph. Eur., JP		0.05	0.2
Polysorbate 80	NF, Ph. Eur., JP		0.2	0.8
Water for injection	USP, Ph. Eur., JP		q.s. to 1.0 mL	q.s. to 4.0 mL

400 mg/16 mL presentation contains 37.76 mg succinic acid, 1360 mg sucrose, 0.8 mg EDTA, and 3.2 mg polysorbate 80; (Table excerpted from Applicant's submission)

2.4 Comments on Novel Excipients

There are no novel excipients or any excipients of human or animal origin in PF-06439535, but all of the excipients in PF-06439535 are different than those in US-licensed Avastin. The levels of sucrose, EDTA, and polysorbate 80 included in the proposed formulation of PF-06439535 are adequately supported from a safety perspective because they are within the range of previously approved IV products; however, the amount of succinic acid in PF-06439535 (2.36 mg/mL) appears to be higher than has been found in previously approved IV products. The amount of succinic acid administered to humans at the recommended PF-06439535 doses of 15 mg/kg once every 3 weeks and 10 mg/kg once every 2 weeks are ~85 mg once every 3 weeks and ~57 mg once every 2 weeks, respectively, compared to (b) (4) mg/week (b) (4) mg over 3 weeks) and (b) (4) mg once every 3 weeks for the approved products PARSABIV and KADCYLA. In addition, the IV radiopharmaceutical TechnScan MAA contains (b) (4) g

of succinic acid per vial and is usually reconstituted with 5-10 mL of solution, resulting in a concentration of (b) (4) mg/mL, which would cover the concentration of succinic acid in PF-06439535, although it is unclear how much succinic acid patients receive per dose. The nonclinical data also provides support for the safety of succinic acid, as succinic acid was present at the same concentration as that in the intended clinical formulation in the formulations given to animals in both the rat and monkey studies included in the nonclinical package for PF-06439535. Monkeys received up to 10 mg/kg PF-06439535 twice weekly (b) (4) in the 1-month toxicology study without significant toxicity and rats received up to 150 mg/kg (b) (4) once every 3-4 days for 2 weeks without significant toxicity. In addition, there are minimal safety concerns related to the systemic toxicity of the compendial excipient succinic acid because it is an endogenous substance that is a component of the Krebs cycle, and it occurs widely as a natural constituent of plants and animals consumed orally at high levels as food by humans. Overall, based on the totality of nonclinical and clinical data, there are no nonclinical safety concerns with the proposed composition of the drug product.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

US-licensed Avastin has been approved for the treatment of metastatic colorectal cancer; first-line unresectable, locally advanced, recurrent, or metastatic non-squamous non-small cell lung cancer; recurrent glioblastoma; metastatic renal cell carcinoma; persistent, recurrent, or metastatic cervical cancer; and epithelial ovarian, fallopian tube, or primary peritoneal cancer. Since PF-06439535 is a proposed biosimilar to US-licensed Avastin, the proposed indications and dosing regimens for PF-06439535 are the same as those in the FDA-approved labeling for US-licensed Avastin, with the exception of the epithelial ovarian, fallopian tube, and primary peritoneal cancer indication, which is protected under orphan drug exclusivity.

2.7 Regulatory Background

US-licensed Avastin was originally approved on February 26, 2004 under BLA 125085 for the first-line treatment of metastatic colorectal cancer in combination with 5-fluorouracil based chemotherapy. Key FDA regulatory interactions for PF-06439535 held under IND 117038 that are relevant to the nonclinical discipline are listed below:

- A Biosimilar Initial Advisory meeting was held on May 22, 2013 to obtain preliminary feedback on the proposed biosimilar development program. The nonclinical team agreed that the design of the proposed 4-week toxicology study in cynomolgus monkeys appeared reasonable to support initiation of clinical studies with PF-06439535.
- Pfizer submitted IND 117038 to the Division of Oncology Products 2 (DOP2) on December 6, 2013, and it was safe to proceed as of January 3, 2014.
- A BPD Type 3 meeting was held on April 15, 2015. The nonclinical team agreed that the in vivo nonclinical program for PF-06439535, together with the analytical data and PK similarity results, would be sufficient to support filing a 351(k) BLA

from a nonclinical perspective provided that Pfizer has established an acceptable scientific bridge to justify the relevance of data obtained using EU-approved bevacizumab.

- A BPD Type 4 meeting was held on September 12, 2017 to discuss and obtain concurrence on the overall content and format of the planned 351(k) BLA submission. The nonclinical team agreed that the proposed structure and format of the nonclinical pharmacology/toxicology sections generally appeared adequate to support the filing of a 351(k) BLA.
- BLA 761099 was initially submitted to DOP2 on January 26, 2018. The application was fileable from a nonclinical perspective; however, other disciplines had filing issues. Pfizer subsequently withdrew the BLA on March 9, 2018.
- BLA 761099 was re-submitted to DOP2 on June 29, 2018.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Study #	Study title
N/A (EDR 4.2.1.1)	Summary of In Vitro Primary Pharmacodynamics
LAB-22054 (EDR 3.2.R)	Determination of the Biological Activity of Bevacizumab (PF-06439535) by Inhibition of Cell Growth Assay
N/A (EDR 3.2.R.3.2.2)	Biological Activity – Functional Characterization of the Fab Domain
N/A (EDR 3.2.R.3.2.2)	Biological Activity – Functional Characterization of the Fc Domain

Pharmacokinetics

Study #	Study title
15-1323	Validation for Determination of Bevacizumab-EU and PF-06439535 in Cynomolgus Monkey Serum by ELISA
151325	Validation of an ECL Assay for the Detection of Anti-Drug Antibodies (ADA) Directed Against Bevacizumab in Cynomolgus Monkey Serum
151326	Validation of an ECL Assay for the Detection of Anti-Drug Antibodies (ADA) Directed Against PF-06439535 in Cynomolgus Monkey Serum
091847	Validation of a Ligand Binding Assay for the Quantitation of PF-06439535 in Sprague Dawley Rat Serum
092141	Validation of a Ligand Binding Assay for the Detection of Anti-Drug Antibodies to PF-06439535 in Sprague Dawley Rat Serum

General Toxicology/Toxicokinetics

Study #	Study title
14MA078/8305590	2-Week Intravenous Bolus Toxicity and Toxicokinetic Study with PF-06439535 in Rats
13GR179	1-month Intravenous Bolus Toxicity Study of PF-06439535 In Cynomolgus Monkeys

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Pharmacology/toxicology review of IND 117038 by Dr. Emily F. Wearne (formerly Emily M. Fox)

Toxicology review of BLA 125085 by Dr. Barbara J. Wilcox.

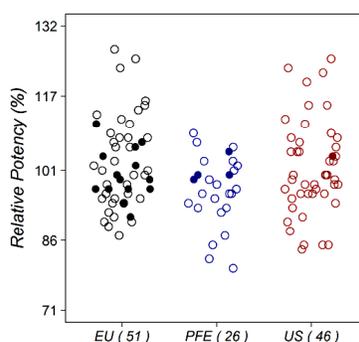
4 Pharmacology

4.1 Primary Pharmacology

The Applicant conducted in vitro pharmacology studies comparing the functional and binding properties of PF-06439535, US-licensed Avastin, and EU-approved bevacizumab. These studies were reviewed in detail by the CMC team as part of the analytical similarity assessment but are summarized herein from a nonclinical perspective. The Applicant submitted a summary Primary Pharmacodynamics report to Module 4.2.1.1 of the EDR containing tables with links to the methods and/or results for each pharmacology assay, but the primary data is in Module 3.2.R of the EDR.

The Applicant compared the ability of PF-06439535, US-licensed Avastin, and EU-approved bevacizumab to inhibit human VEGF₁₆₅-induced proliferation of human umbilical vein endothelial cells (HUVEC). HUVEC cells in 96-well plates were treated with serial dilutions (0.007813-4 µg/mL) of PF-06439535, US-licensed Avastin, or EU-approved bevacizumab and incubated with diluted human VEGF₁₆₅ for two days. Cell proliferation was measured using the CellTiter Glo® assay system whereby inhibition of cell growth was inversely proportional to the amount of ATP in each well. As shown in Figure 1, PF-06439535, US-licensed Avastin, and EU-approved bevacizumab exhibited similar dose-dependent inhibition of VEGF₁₆₅-induced HUVEC cell proliferation.

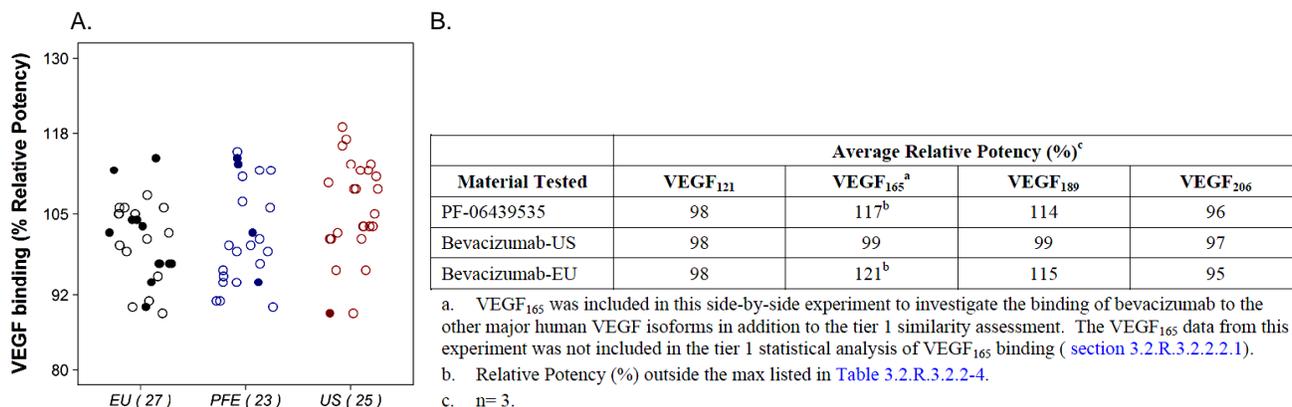
Figure 1: Effect of PF-06439535, US-licensed Avastin, and EU-approved Bevacizumab on In Vitro VEGF₁₆₅-induced HUVEC proliferation



PFE = PF-06439535. Numbers in parentheses indicate the number of lots assessed. Solid circles indicate lots that have been used in clinical studies. (Applicant figure excerpted from report entitled "Biological Activity-Functional Characterization of the Fab Domain")

As assessed by binding enzyme-linked immunosorbent (ELISA) assays, PF-06439535, US-licensed Avastin, and EU-approved bevacizumab exhibited similar dose-dependent binding to human VEGF₁₆₅, VEGF₁₂₁, VEGF₁₈₉, and VEGF₂₀₆ (see Figure 2).

Figure 2: Effect of PF-06439535, US-licensed Avastin, and EU-approved Bevacizumab on In Vitro Binding to Soluble Human VEGF Isoforms



Panel A: VEGF₁₆₅ only. Numbers in parentheses indicate the number of lots assessed. Solid circles indicate lots that have been used in clinical studies. PFE = PF-06439535. Panel B: All four VEGF isoforms were assessed. (Applicant figure excerpted from report entitled “Biological Activity-Functional Characterization of the Fab Domain”)

Since PF-06439535 is an IgG1 antibody, the Applicant evaluated in vitro binding to Fc receptors and Fc effector function. From a nonclinical perspective, PF-06439535, US-licensed Avastin, and EU-approved bevacizumab exhibited relatively similar binding to C1q, FcγRIIIa, FcγRI, FcγRIIIa, FcγRIIb, and FcγRIIIb (see Table 2). Based on percent K_D values, PF-06439535 appeared to exhibit slightly higher binding affinity for FcγRIIIa 158F compared to US-licensed Avastin and EU-approved bevacizumab; however, only one lot of each antibody was assessed. Further, consistent with literature reports regarding Fc-mediated effector function of bevacizumab¹, PF-06439535, US-licensed Avastin, or EU-approved bevacizumab did not induce in vitro antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) activity against VEGF-expressing DLD-1 adenocarcinoma cells (see Figure 3). In contrast, the positive control anti-HER2 antibody trastuzumab induced dose-dependent ADCC activity against HER2-positive SKBR3 cells, and the positive control anti-CD20 antibody rituximab induced dose-dependent CDC activity against CD20-positive Ramos cells (data not shown). In the ADCC assay, peripheral blood mononuclear cells (PBMCs) were used as effector cells and cell lysis was measured using the ToxiLight reagent, which detects the release of adenylate kinase from damaged cells. In the CDC assay, human complement was used to assess complement-mediated cell lysis as measured by ToxiLight reagent.

Table 2: Effect of PF-06439535, US-licensed Avastin, and EU-approved Bevacizumab on In Vitro Binding to Fcγ Receptors and C1q

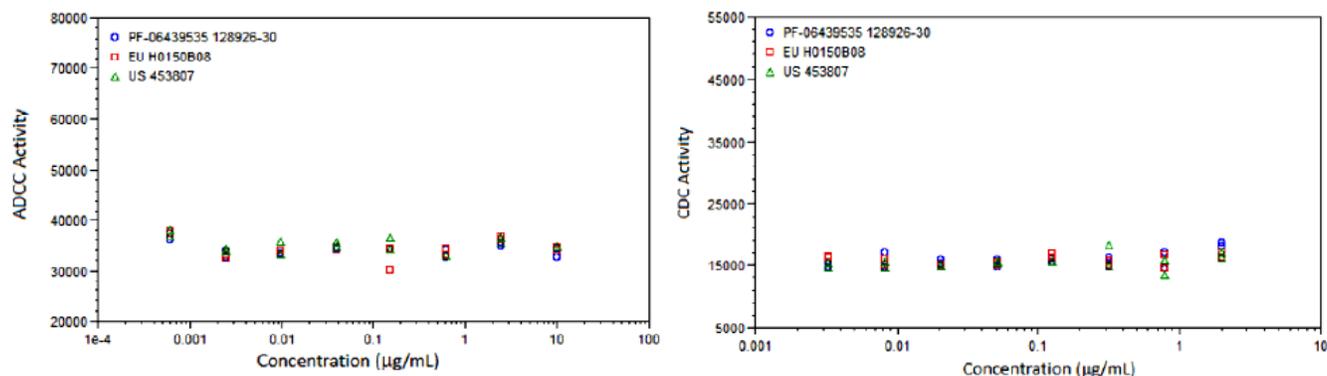
	Method	Parameter	PF-06439535 RM ^a	PF-06439535 IC ^b	US-licensed Avastin	EU-approved Bevacizumab
C1q ^c	Immunoassay	% Relative Binding	100	N/A	98	101

¹ Wang Y, D Fei, M Vanderlaan, A Song, 2004, Biological activity of bevacizumab, a humanized anti-VEGF antibody in vitro, *Angiogenesis*, 7(4):335-345.

	Method	Parameter	PF-06439535 RM ^a	PF-06439535 IC ^b	US-licensed Avastin	EU-approved Bevacizumab
Fc γ RIIIa 158F ^c	SPR	K _D , M	5.24E-07	5.70E-07	7.07E-07	6.30E-07
		% K _D	100	92	75	83
Fc γ RIIIa 158V ^c	SPR	K _D , M	1.03E-07	1.06E-07	9.81E-08	1.04E-07
		% K _D	100	97	105	100
Fc γ RI ^c	SPR	K _D , M	9.66E-09	1.04E-08	9.93E-09	9.52E-09
		% K _D	100	94	97	100
Fc γ RIIIa 131H ^c	SPR	K _D , M	3.00E-06	2.80E-06	2.61E-06	2.54E-06
		% K _D	100	107	115	118
Fc γ RIIIa 131R ^c	SPR	K _D , M	4.13E-06	3.75E-06	3.80E-06	3.76E-06
		% K _D	100	110	110	109
Fc γ RIIb ^{d,e}	SPR	K _D , M	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05
Fc γ RIIIb ^{d,e}	SPR	K _D , M	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05

Reviewer generated table based on results from report entitled “Biological Activity-Functional Characterization of the Fc Domain.” SPR: Surface plasmon resonance; N/A: Not applicable; ^a: Reference material PF-06439535 (lot# 128926-30) was set as 100%; ^b: PF-06439535 (lot# 128926-30) was tested against itself as an internal control; ^c: n=3; ^d: Curves did not achieve saturation at highest concentration (10 μ M) due to low affinity interaction with captured ligand; ^e: n=2

Figure 3: Effect of PF-06439535, US-licensed Avastin, and EU-approved Bevacizumab on In Vitro ADCC and CDC

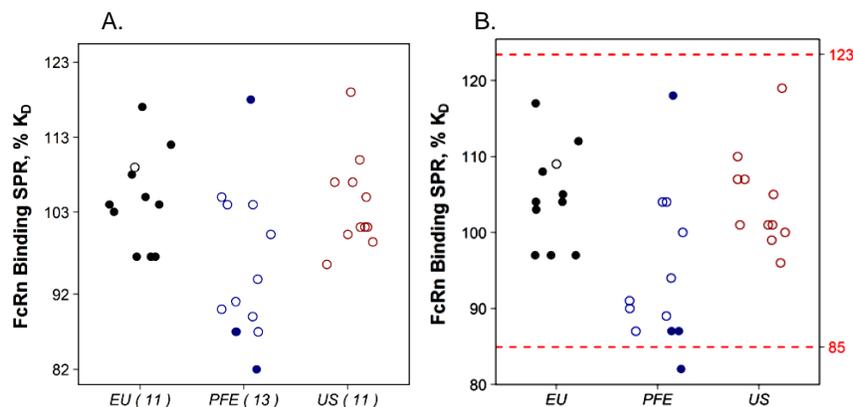


(Applicant figures excerpted from report entitled “Biological Activity-Functional Characterization of the Fc Domain”)

Notably, only one lot each of PF-06439535, US-licensed Avastin, and EU-approved bevacizumab were assessed in the VEGF₁₂₁, VEGF₁₈₉, VEGF₂₀₆, ADCC, CDC, and Fc γ R assays.

In addition, SPR analysis demonstrated that PF-06439535, US-licensed Avastin, and EU-approved bevacizumab exhibited relatively similar binding to FcRn (see Figure 4). Although there was a trend towards a modestly lower affinity for the receptor by PF-06439535, these decreases were generally still within the range of upper and lower limits for comparability.

Figure 4: Effect of PF-06439535, US-licensed Avastin, and EU-approved Bevacizumab on In Vitro FcRn Binding



PFE = PF-06439535. Solid circles indicate lots that have been used in clinical studies. Panel A: Numbers in parentheses indicate the number of lots assessed. Panel B: Red dashed lines represent the upper and lower limits of the statistical quality range. (Applicant figures excerpted from report entitled “Biological Activity-Functional Characterization of the Fc Domain”)

Overall, from a nonclinical perspective, PF-06439535, US-licensed Avastin, and EU-approved bevacizumab exhibited relatively similar in vitro inhibition of VEGF-induced HUVEC cell proliferation; binding to human VEGF₁₆₅/VEGF₁₂₁/VEGF₁₈₉/VEGF₂₀₆, C1q, Fc γ R receptors, and FcRn; and lack of ADCC and CDC activity. Based on the results from these in vitro comparative pharmacology studies, the Applicant did not conduct any in vivo pharmacology studies with PF-06439535.

4.2 Secondary Pharmacology

Secondary pharmacology studies were not conducted.

4.3 Safety Pharmacology

Stand-alone safety pharmacology studies were not conducted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

PK/ADME studies were not conducted.

The Applicant submitted validation reports for assays: 1) quantifying PF-06439535 (rat and monkey) and EU-approved bevacizumab (monkey) in serum and 2) detecting the presence of ADAs in rat and monkey serum samples. Findings are summarized in Table 3. All five assays were validated.

Table 3: Summary of Nonclinical Pharmacokinetic Validation Reports

Study #	Species	Analyte	Method	Quantitation Range ($\mu\text{g/mL}$)	Stability	Drug Tolerance
15-1323	Monkey	PF-06439535, EU-approved bevacizumab	ELISA	10-400	24 hrs at RT; 5 freeze/thaw cycles;	N/A

Study #	Species	Analyte	Method	Quantitation Range (µg/mL)	Stability	Drug Tolerance
					56 days at -70°C	
15-1325	Monkey	Anti-EU-approved bevacizumab antibodies	ECL	N/A	Positive control: 24 hrs at RT; 6 cycles at -70	HPC and LPC can tolerate at least 200 µg/mL and 50 µg/mL free bevacizumab in serum, respectively.
151326	Monkey	Anti-PF-06439535 antibodies	ECL	N/A	Positive control: 24 hrs at RT; 6 cycles at -70	HPC and LPC can tolerate at least 200 µg/mL and 25 µg/mL free PF-06439535 in serum, respectively.
091847	Rat	PF-06439535	Ligand binding	10-400	24 hrs at RT; 5 freeze/thaw cycles; 42 days at -70°C	N/A
092141	Rat	Anti-PF-06439535 Antibodies	Ligand Binding	N/A	Positive control: 24 hrs at RT; 5 freeze-thaw cycles	HPC and LPC can tolerate at least 200 µg/mL and 12.5 µg/mL free PF-06439535 in serum, respectively.

RT = Room temperature; HPC = high positive control (6000 ng/mL); LPC = low positive control (200 ng/mL); ECL = Electrochemiluminescent; N/A = Not Applicable

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose studies were not conducted.

6.2 Repeat-Dose Toxicity

Study title: 2-Week Intravenous Bolus Toxicity and Toxicokinetic Study with PF-06439535 in Rats

Study no.: 14MA078/8305590
 Study report location: EDR 4.2.3.2
 Conducting laboratory and location: (b) (4)
 Date of study initiation: August 28, 2014
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PF-06439535, 14J138K002 (J33002), 98.4%

Key Study Findings

- There were no mortalities
- There was a statistically significant increase in mean liver weight in male rats dosed with 150 mg/kg PF-06439535 compared to controls, which correlated histologically with minimal sinusoidal cell hyperplasia. Additional histologic findings in the liver included hepatocyte degeneration/necrosis, inflammation/mineralization, and tension lipidosis.
- One high dose rat exhibited slight lobular atrophy in the pancreas
- Anti-drug antibodies (ADAs) were not detected in rats dosed with PF-06439535

Methods

Doses: Group 1: 0 mg/kg PF-06439535
 Group 2: 15 mg/kg PF-06439535
 Group 3: 150 mg/kg PF-06439535
 Frequency of dosing: Twice weekly on Days 1, 4, 8, 11, and 15
 Route of administration: IV injection into tail vein
 Dose volume: 10 mL/kg
 Formulation/Vehicle: (b) (4), 85 mg/mL sucrose, 0.05 mg/mL EDTA, 0.2 mg/mL polysorbate 80, pH 5.5
 Species/Strain: Sprague Dawley rats/Crl:CD[SD]
 Number/Sex/Group: 10/sex/group
 Age: 7-8 weeks old
 Weight: 226-302 g (M); 187-228 g (F)
 Satellite groups: 3/sex in Group 1 and 4/sex in Groups 2-3 for toxicokinetics
 Unique study design: Immunogenicity
 Deviation from study protocol: None that impacted study interpretation

Reviewer's Note: (b) (4) to assess the potential for non-target-mediated toxicity of PF-06439535.

Observations and Results

Mortality: Checked twice daily

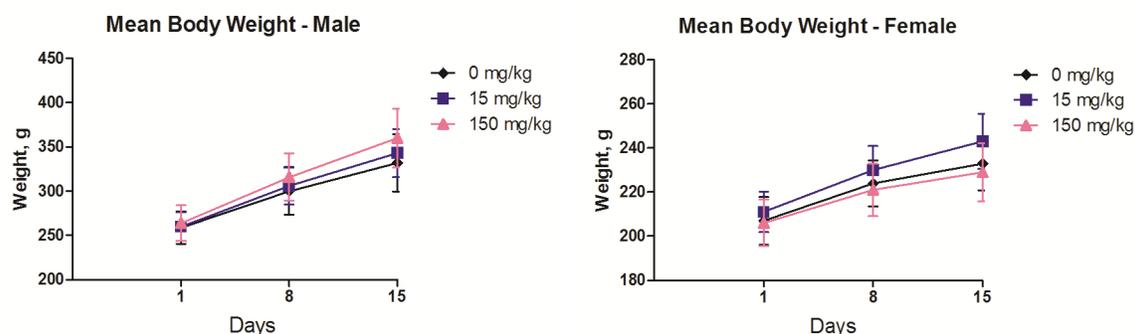
None.

Clinical Signs: Observed once daily; detailed observations were conducted pre-dosing, weekly during the dosing phase, and on days of scheduled euthanasia

Unremarkable.

Body Weights: Recorded weekly

Unremarkable.

Figure 5: Mean Body Weight Values in Male and Female Rats**Food Consumption: Recorded weekly**

Unremarkable.

Ophthalmoscopy

Ophthalmologic examinations were performed once pre-dosing in all rats and on Day 15 in control and high dose rats. Findings were unremarkable.

ECG: Not applicable**Hematology**

Blood samples were collected from fasted rats on Day 16. Findings were unremarkable.

Clinical Chemistry

Blood samples were collected from fasted rats on Day 16. Comparison of serum globulin concentrations to the plasma concentration of the test article suggested that higher serum globulin concentrations and total protein observed in high dose rats were likely due to the physical presence of the test article and not a toxicologically significant effect.

Table 4: Changes in Clinical Chemistry Compared to Controls in Rats

Dose (mg/kg/day)	15		150	
	M	F	M	F
Total protein			7%↑*	
Globulin			24%↑*	14%↑*
Albumin:globulin			18%↓*	8%↓*

% relative to controls on Day 16; *, P ≤ 0.05

Urinalysis

Urine was collected from fasted rats on the day of necropsy. Findings were unremarkable.

Gross Pathology

Ten rats/sex/group were necropsied on Day 16.

Table 5: Gross Pathology Findings in Rats after 2 Weeks of Treatment

Dose (mg/kg/day)		0		15		150	
Sex		M	F	M	F	M	F
# of rats examined		10	10	10	10	10	10
Liver	Raised area						1
Lymph node, other	Large			1	1	1	
Stomach	Discolored			2	1	1	

Organ Weights

There was a statistically significant increase in mean liver weight (absolute and relative to body and brain weight) in male rats dosed with 150 mg/kg PF-06439535 compared to controls, which correlated histologically with minimal sinusoidal cell hyperplasia.

Table 6: Organ Weight Changes in Rats after 2 Weeks of Treatment

Dose (mg/kg)		15		150	
Sex		M	F	M	F
Brain	Relative to Body Weight	8%↓*		13%↓*	
Liver	Absolute		17%↑*	25%↑*	5%↑
	Relative to Body Weight		11%↑*	14%↑*	6%↑
	Rel to Brain Weight		15%↑*	31%↑*	4%↑
Thymus	Absolute	29%↑*		28%↑*	
	Relative to Body Weight	24%↑*		16%↑	
	Relative to Brain Weight	36%↑*		34%↑*	

% relative to controls; *, $P \leq 0.05$

Histopathology

Adequate Battery: Yes. All tissues were examined from control and high dose rats, and macroscopic lesions and the liver were also examined in low dose rats.

Peer Review: Yes

Table 7: Histological Findings in Rats after 2 Weeks of Treatment

Dose (mg/kg/day)		0		15		150	
Sex		M	F	M	F	M	F
# of rats examined		10	10	0	0	10	10
Brain	Mononuclear cell infiltrate, perivascular, Min						1
Duodenum	Eosinophil infiltrate, Min						1
Eye	Inflammation/mineralization Min					1	
Injection site	Erosion/ulcer Min	2				2	1
	Slight					1	
	Foreign material	2				3	
	Inflammation, chronic, perivascular, Slight						1
	Inflammation, mixed cell, dermis, Min	1				2	1
Liver	# of rats examined	10	10	10	10	10	10
	Degeneration/necrosis hepatocytes, Min						1

Dose (mg/kg/day)		0		15		150	
Sex		M	F	M	F	M	F
# of rats examined		10	10	0	0	10	10
	Hyperplasia, sinusoidal cell Min					8	4
	Mixed cell infiltrate, Min					1	
	Mixed cell inflammation, Min						1
	Inflammation/mineralization Slight						1
	Lipidosis, tension, Min						1
Lung	Hemorrhage, Min	1	1			2	1
	Inflammation, mixed cell Min					1	
	Slight	1				1	
Lymph node, other	# of rats examined	0	0	1	1	1	0
	Increased lymphocytes Min			1			
	Slight				1	1	
Pancreas	Atrophy, lobular, Slight						1
Prostate	Mononuclear cell infiltrate Min	2				1	
	Moderate						
Stomach, glandular	Eosinophil infiltrate, Min					1	
Stomach, non-glandular	# of rats examined	10	10	2	1	10	10
	Eosinophil infiltrate, Min					1	
Testis	Degeneration/atrophy, seminiferous epithelium Min	1				2	
Thyroid	Thymus, ectopic						1

Special Evaluation:

Immunogenicity

Anti-drug antibody (ADA) Analysis:

Blood was collected prior to dosing on Days 1 and 15. ADAs were not detected in any rats dosed with vehicle or PF-06439535; however, ADA production may have been masked by high PF-06439535 concentrations.

Toxicokinetics

- Blood samples were collected pre-dosing and 1, 8, 24, and 72 hours post-dose on Days 1 and 11, as well as pre-dosing and 24 hours post-dose on Day 15
- PF-06439535 exposures increased slightly less than dose proportionally on Days 1 and 11
- There were no significant sex-related differences in exposure
- There was evidence of accumulation based on C_{max} (≤ 2.4 -fold) and AUC_{72} (≤ 3 -fold) on Day 11 compared to Day 1

Table 8: Summary of Mean PF-06439535 Toxicokinetic Data in Rats

Dose (mg/kg/dose) ^a	Study Day	Sex ^b	C _{max} (µg/mL)	AUC ₇₂ (µg•h/mL)	AUC ₇₂ /Dose (µg•h/mL/(mg/kg))
15	1	Male	308 ± 34.2	12200 ± 904	813 ± 60.3
		Female	308 ± 8.54	12000 ± 624	800 ± 41.6
	11	Male	659 ± 53.9	31600 ± 2360	2110 ± 157
		Female	720 ± 56.3	35800 ± 2720	2390 ± 181
150	1	Male	2740 ± 341	110000 ± 7760	733 ± 51.7
		Female	2780 ± 1170	96800 ± 20000	645 ± 133
	11	Male	6260 ± 394	250000 ± 34100	1670 ± 227
		Female	6660 ± 400	258000 ± 12400	1720 ± 82.7

Notes: Group mean and individual TK data are presented in Supportive Tables 7.1, and 7.2.
AUC₇₂ = Area under the concentration-time curve from time 0 to 72 hours; C_{max} = Maximum observed concentration; h = hour.

a. Animals were dosed on Study Days 1, 4, 8, 11 and 15.

b. 4 animals/sex/dose group.

(Table excerpted from Applicant's submission)

Dosing Solution Analysis

Met acceptance criteria.

Study title: 1-month Intravenous Bolus Toxicity Study of PF-06439535 In Cynomolgus Monkeys

Study no.:	13GR179
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	Pfizer Worldwide Research & Development Drug Safety Research & Development Eastern Point Road Groton, CT 06340
Date of study initiation:	July 2, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-06439535, 13J138K003 (G27629; PFX000161), 99%; EU-approved bevacizumab, H0150B08 (Days 1, 4, 8, 11, 15, 18), 98.1% and B7100B06 (Days 22, 25, 29), 98%

Key Study Findings

- There were no mortalities
- Minimal to moderate physal dysplasia in the growth plate of the distal femur was observed in all monkeys treated with PF-06439535 or EU-approved bevacizumab, and the incidence and severity were relatively similar between treatment groups
- There were no biologically significant differences in toxicity or toxicokinetics between PF-06439535 and EU-approved bevacizumab
- ADAs were not detected in monkeys dosed with PF-06439535 or EU-approved bevacizumab

Methods

Doses: Group 1: 0 mg/kg (vehicle for PF-06439535)
 Group 2: 10 mg/kg PF-06439535
 Group 3: 0 mg/kg (vehicle for EU-approved bevacizumab)
 Group 4: 10 mg/kg EU-approved bevacizumab
Frequency of dosing: Twice weekly for 1 month (Days 1, 4, 8, 11, 15, 18, 22, 25, and 29)
Route of administration: IV bolus injection
Dose volume: 0.4 mL/kg
Formulation/Vehicle: **PF-06439535:** (b) (4), 85 mg/mL sucrose, 0.05 mg/mL EDTA, 0.2 mg/mL polysorbate-80, pH 5.5
EU-approved bevacizumab: (b) (4) trehalose, (b) (4) sodium phosphate (b) (4)
 polysorbate-20, pH (b) (4)
Species/Strain: Cynomolgus monkey
Number/Sex/Group: 4 males/group
Age: Sexually and skeletally immature monkeys, ages 2 years and 5 months to 3 years and 3 months
Weight: 2.6-4.2 kg
Satellite groups: None
Unique study design: Immunogenicity
Deviation from study protocol: None that impacted study interpretation

Dose/animal Justification: According to the Applicant, the dosing regimen was selected based on the 4-week toxicology study with bevacizumab, in which the key finding was physeal dysplasia in male cynomolgus monkeys dosed with 10 mg/kg and 50 mg/kg bevacizumab twice weekly by IV infusion. The Applicant chose male cynomolgus monkeys less than approximately four years old because younger monkeys are more likely to have open growth plates in long bones. All monkeys in the study were sexually immature based on the microscopic appearance of the male reproductive tract tissues, and skeletally immature based on the presence of active (open) growth plates observed microscopically in the distal femur.

Observations and Results

Mortality: Checked twice daily

None

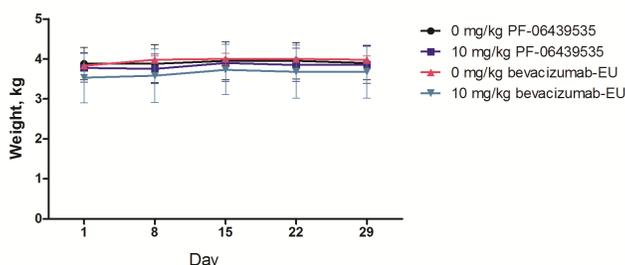
Clinical Signs: Observed pre-dose, approximately 1-4 hours after the last animal was dosed, and at the end of the workday on dosing days; observed twice daily on non-dosing days

Unremarkable; no apparent differences were noted between PF-06439535 and EU-approved bevacizumab.

Body Weights: Recorded twice a week

Unremarkable; no apparent differences were noted between PF-06439535 and EU-approved bevacizumab.

Figure 6: Mean Body Weight Values in Male Monkeys



Food Consumption: Recorded daily

Feed consumption was qualitatively assessed, and the data were not reported. According to the Applicant, there were no changes in food consumption related to PF-06439535 or EU-approved bevacizumab.

Ophthalmoscopy

Ophthalmologic examinations were performed once pre-dosing and on Day 30. Findings were unremarkable and there were no apparent differences between PF-06439535 and EU-approved bevacizumab.

ECG

Electrocardiograms and respiratory rates were obtained once pre-dosing (Day -7), as well as pre-dose and approximately 1-2 hours post-dose on Day 29. Findings were unremarkable and there were no apparent differences between PF-06439535 and EU-approved bevacizumab in ECG parameters, waveform morphologies, or respiratory rates.

Hematology

Blood samples were collected from fasted monkeys pre-dosing and on Day 30. There were no toxicologically meaningful differences between PF-06439535 and EU-approved bevacizumab.

Table 9: Changes in Hematology Compared to Controls in Male Monkeys

Males	PF-06439535	EU-approved bevacizumab
Dose (mg/kg)	10	10
Reticulocytes	21%↑	13%↓
Neutrophils	63%↑	130%↑
White blood cells	8%↑	24%↑
Eosinophils	36%↑	21%↓
APTT	4%↓	6%↓*

% relative to controls on Day 30; *, $P \leq 0.05$

Clinical Chemistry

Blood samples were collected from fasted monkeys pre-dosing and on Day 30. There were no toxicologically meaningful differences between PF-06439535 and EU-approved bevacizumab.

Table 10: Changes in Clinical Chemistry Compared to Controls in Male Monkeys

Males	PF-06439535	EU-approved bevacizumab
Dose (mg/kg)	10	10
GGT	29%↓	26%↓
Albumin	4%↓*	3%↑
Globulin	0.6%↑	13%↑**
Total Protein	2%↓	7%↑**
Blood urea nitrogen	24%↑*	8%↑
Creatinine	19%↑*	7%↑

% relative to controls on Day 30; *, $P \leq 0.05$; **, $P \leq 0.01$

Urinalysis

Urine was collected from fasted monkeys on Day 32. Findings were unremarkable and there were no apparent differences between PF-06439535 and EU-approved bevacizumab.

Gross Pathology

Four monkeys/group were necropsied on Day 32, three days after the last dosing day. Findings were unremarkable and there were no apparent differences between PF-06439535 and EU-approved bevacizumab.

Organ Weights

There were no toxicologically meaningful differences between PF-06439535 and EU-approved bevacizumab.

Table 11: Changes in Organ Weights in Male Monkeys

Males	PF-06439535	EU-approved bevacizumab
Dose (mg/kg)	10	10
Heart	Absolute	12%↓*
	Relative to body weight	11%↓
	Relative to brain weight	0%↓
		9%↓
		0%
		14%↓

% relative to controls on Day 32; *, $P \leq 0.05$

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

The incidence and severity of physeal dysplasia in the growth plate of the distal femur was relatively similar between PF-06439535 and EU-approved bevacizumab. One monkey dosed with PF-06439535 exhibited minimal multifocal mineral deposition in the brain. According to the toxicology review for BLA 125085 (US-licensed Avastin), mineralization was observed in the brain of one control monkey and two monkeys dosed with 50 mg/kg bevacizumab in the 4-week toxicology study. Dr. Barbara J. Wilcox concluded that these findings did not appear to be related to study drug administration. Thus, histologic mineral deposition in the brain is unlikely to be toxicologically relevant. Overall, there were no toxicologically significant differences in histopathological findings between PF-06439535 and EU-approved bevacizumab.

Table 12: Histological Findings in Male Monkeys

		Males	PF-06439535		EU-approved bevacizumab	
		Dose (mg/kg)	0	10	0	10
		# of monkeys examined	4	4	4	4
Bone, Femur	Dysplasia: physeal					
	Min			1		
	Mild			1		2
	Moderate			2		2
Colon	Infiltration, mononuclear cell				1	
	Min					
	Mild			1		
Lung	Fibrosis interstitial, Min					1
	Chronic inflammation, pleura					
	Min			1		
Heart	Infiltration, myocardium					
	mononuclear cell					
	Min	2		1	1	1
	Mild			1	1	
Thyroid	Cyst			1	1	4
Parathyroid	Cyst			1	1	2
Salivary Gland	Infiltration, mononuclear cell					
	Min			2		1
Tongue	Infiltration, mononuclear cell					
	Min				1	2
Brain	Deposition: mineral, multifocal, Min			1		
Epididymis	Oligospermia					
	Min	2			2	
	Moderate			1		
Injection Site	Inflammation					
	Min	1		2	1	3
	Mild	3		1	1	1
	Moderate			1	2	

Special Evaluation

Immunogenicity: Anti-Drug Antibody (ADA) Assessment

Blood was collected prior to dosing on Days 1, 15, and 25. ADA samples from the control groups were not analyzed. ADAs were not detected in any monkeys dosed with PF-06439535 or EU-approved bevacizumab; however, ADA production may have been masked by high PF-06439535 or EU-approved bevacizumab concentrations.

Toxicokinetics

- Blood samples were collected pre-dosing and 1, 8, 24, and 72 hours post-dose on Days 1 and 25; pre-dosing, 1, and 8 hours post-dose on Days 11 and 15; and on Day 30
- T_{max} was 1 hour for PF-06439535 and EU-approved bevacizumab
- Exposure levels (C_{max} and $AUC_{(0-72)}$) to PF-06439535 and EU-approved bevacizumab were relatively similar, although the C_{max} and $AUC_{(0-72)}$ of PF-06439535 were 81% and 82.3% of those for EU-approved bevacizumab on Day 1, respectively
- There was evidence of accumulation on Day 25 compared to Day 1 for both PF-06439535 (≤ 3.8 -fold) and EU-approved bevacizumab (≤ 3 -fold)
- Overall, a similar toxicokinetic profile was observed for PF-06439535 and EU-approved bevacizumab

Table 13: Summary of Mean Toxicokinetic Data in Male Cynomolgus Monkeys

Dose (mg/kg)	Day	Parameter	Test (PF-06439535)	Reference (Bevacizumab-EU)	Ratio (Test/Reference)
10	1	C_{max} ($\mu\text{g/mL}$)	241 ± 37.2	298 ± 29.6	0.809
		$AUC_{(0-72)}$ ($\mu\text{g}\cdot\text{h/mL}$)	12100 ± 876	14700 ± 2260	0.823
	25	C_{max} ($\mu\text{g/mL}$)	789 ± 54.7	853 ± 91.5	0.925
		$AUC_{(0-72)}$ ($\mu\text{g}\cdot\text{h/mL}$)	45500 ± 5420	45100 ± 3670	1.01

(Table excerpted from Applicant's submission)

Table 14: Mean C_{max} and AUC Ratios for PF-06439535 Relative to EU-approved Bevacizumab

Dose (mg/kg)	Test Article	Day	C_{max} ($\mu\text{g/mL}$)			T_{max} (h)			$AUC_{(0-72)}$ ($\mu\text{g}\cdot\text{h/mL}$)		
			Mean	SD	n	Mean	SD	n	Mean	SD	n
10	PF-06439535	1	241	37.2	4	1.0	0.0	4	12100	876	4
		25	789	54.7	4	1.0	0.0	4	45500	5420	4
	Bevacizumab-EU	1	298	29.6	4	1.0	0.0	4	14700	2260	4
		25	853	91.5	4	1.0	0.0	4	45100	3670	4

$AUC_{(0-72)}$ = Area under the serum drug concentration-time curve for 0-72h; C_{max} = Highest drug concentration observed in serum; h = hour; n = number; SD = Standard Deviation; T_{max} = Time at which C_{max} was first observed.

(Table excerpted from Applicant's submission)

Dosing Solution Analysis

Met pre-specified acceptance criteria.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

EMILY F WEARNE
01/14/2019 11:20:59 AM

WHITNEY S HELMS
01/16/2019 11:23:31 AM

I concur with Dr. Wearne's conclusions that the pharmacology/toxicology data is generally supportive of the similarity of Zirabev to the innovator product. There are no outstanding issues from a pharmacology/toxicology perspective that would prevent approval of the biosimilar product.