

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761099Orig1s000

PRODUCT QUALITY REVIEW(S)



Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Biotechnology Products

LABELS AND LABELING ASSESSMENT

Date of review:	June 10, 2019
Reviewer:	Vicky Borders-Hemphill, PharmD Labeling Assessor Office of Biotechnology Products (OBP)
Through:	Jee Chung, PhD, Product Quality Assessor OBP/Division of Biotechnology Review and Research IV
Application:	BLA 761099
Applicant:	Pfizer Inc.
Submission Date:	June 29, 2018
Product:	Zirabev (bevacizumab-bvzr)
Dosage form(s):	injection
Strength and Container-Closure:	100 mg/4 mL (25 mg/mL) or 400 mg/16 mL (25 mg/mL) in a single-dose vial
Purpose of review:	The Applicant submitted a biologics license application for Agency review
Recommendations:	The container labels (submitted on December 14, 2018), carton labeling (submitted on February 21, 2019), and prescribing information (submitted on May 29, 2019) were assessed and found to be acceptable (see Appendix C) from an OBP labeling perspective.

Materials Considered for this Label and Labeling Assessment	
Materials Assessed	Appendix Section
Proposed Labels and Labeling	A
Evaluation Tables	B
Acceptable Labels and Labeling	C

n/a = not applicable for this assessment

DISCUSSION

We evaluated the proposed labels and labeling for compliance with applicable requirements in the Code of Federal Regulations (see Appendix B).

CONCLUSION

The container labels (submitted on December 14, 2018), carton labeling (submitted on February 21, 2019), and prescribing information (submitted on May 29, 2019) were reviewed and found to be acceptable (see Appendix C) from an OBP labeling perspective.

APPENDICES

Appendix A: Proposed Labeling

- Prescribing Information (submitted on October 2, 2018
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Container Labels (submitted on June 29 ,2018)



Appendix B: Evaluation Tables

Evaluation Tables: Label^{1,2} and Labeling³ Standards

Container⁴ Label Evaluation

Proper Name <i>(for container of a product capable of bearing a full label)</i>	Acceptable
21 CFR 610.60, 21 CFR 201.50, 21 CFR 201.10	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: <i>For the 400 mg/16 mL vial: acceptable provided the DMEPA-approved suffix is applied to the proper name</i>	
Recommended labeling practices (placement of dosage form)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: For the 400 mg/16 mL vial: If space permits, consider adding the dosage form "Injection" to appear underneath the proper name. <i>The Applicant revised as requested</i>	
Manufacturer name, address, and license number <i>(for container of a product capable of bearing a full label)</i>	Acceptable
21 CFR 610.60 (a)(2), 21 CFR 201, 21 CFR 201.1(a), 21 CFR 201.1(h)(5), 21 CFR 201.1(h)(6), 21 CFR 201.100(e)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (using the following qualifying statement "Manufactured by:")</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: If space permits, relocate the US License number to appear underneath the Manufacturer name and address. <i>The Applicant revised as requested</i>	
Lot number or other lot identification <i>(container capable of bearing a full label shall bear)</i>	Acceptable
21 CFR 610.60, 21 CFR 201.18, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Expiration date <i>(container capable of bearing a full label shall bear)</i>	Acceptable

¹ Per 21 CFR 1.3(b) *Label* means any display of written, printed, or graphic matter on the immediate container of any article, or any such matter affixed to any consumer commodity or affixed to or appearing upon a package containing any consumer commodity.

² Per CFR 600.3(dd) *Label* means any written, printed, or graphic matter on the container or package or any such matter clearly visible through the immediate carton, receptacle, or wrapper.

³ Per 21 CFR 1.3(a) *Labeling* includes all written, printed, or graphic matter accompanying an article at any time while such article is in interstate commerce or held for sale after shipment or delivery in interstate commerce.

⁴ Per 21 CFR 600.3(bb) *Container* (referred to also as "final container") is the immediate unit, bottle, vial, ampule, tube, or other receptacle containing the product as distributed for sale, barter, or exchange.

21 CFR 610.60, 21 CFR 201.17	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Product Strength	Acceptable
21 CFR 201.10(d)(1), 21 CFR 201.100(b)(4)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (expression of strength for injectable drugs): Reference: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 line 176 USP General Chapters: <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Multiple dose containers (recommended individual dose)	Acceptable
21 CFR 610.60, 21 CFR 201.55	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Statement: "Rx only"	Acceptable
21 CFR 610.60, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Medication Guide	Acceptable
21 CFR 610.60, 21 CFR 208.24	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
No Package for container	Acceptable
21 CFR 610.60	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Proper Name (for container bearing a partial label)	Acceptable
21 CFR 610.60, 21 CFR 201.10	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: <i>For the 100 mg/4 mL vial: acceptable provided the DMEPA-approved suffix is applied to the proper name</i>	
Manufacturer name, address, and license number (for container bearing a partial label)	Acceptable
21 CFR 610.60, 21 CFR 201.10 <i>For the 100 mg/4 mL vial</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (U.S license number for container bearing a partial label): For the 100 mg/4 mL vial</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Lot number or other lot identification <i>(for container bearing a partial label)</i>	Acceptable
21 CFR 610.60, 21 CFR 201.10 <i>For the 100 mg/4 mL vial</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Expiration date <i>(for container bearing a partial label)</i>	Acceptable
21 CFR 201.17 <i>For the 100 mg/4 mL vial</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
No container label	Acceptable
21 CFR 610.60	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Ferrule and cap overseal <i>(for vials only)</i>	Acceptable
Recommended labeling practices: United States Pharmacopeia (USP), General Chapters: <7> Labeling (Ferrules and Cap Overseals) Comment/Recommendation: <i>Confirm there is no text on the ferrule and cap overseal of the vials.</i> Pfizer confirms that no text or other markings are present on the ferrule and cap overseal of the vials.	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Visual inspection <i>(for vials only)</i>	Acceptable
21 CFR 610.60	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation: Confirm there is sufficient area on the container to allow for visual inspection when the label is affixed to the vial and indicate where the visual area of inspection is located per 21 CFR 610.60(e).

Pfizer confirms there is sufficient area on the container to allow for visual inspection when the label is affixed to the vial. The open space between the ends of the applied label is approximately 2 mm wide for 5 mL vial and approximately 5 mm wide for 20 mL vial.



<u>NDC numbers</u>	Acceptable
21 CFR 201.2, 21 CFR 207.35	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<u>Route of administration</u>	Acceptable
21 CFR 201.5, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (route of administration statement to appear after the strength statement on the principal display panel)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<p>Comment/Recommendation: Revise the route of administration statement from "(b) (4)" to read as follows: "For Intravenous Infusion After Dilution" <i>The Applicant revised as requested</i></p>	
<u>Preparation instructions</u>	Acceptable
21 CFR 201.5	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<u>Package type term</u>	Acceptable

<i>Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use</i> <i>USP chapter <659> Packaging and Storage Requirements</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Misleading statements	Acceptable
21 CFR 201.6	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Prominence of required label statements	Acceptable
21 CFR 201.15	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Spanish-language (Drugs)	Acceptable
21 CFR 201.16	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
FD&C Yellow No. 5 and/or FD&C Yellow No. 6	Acceptable
21 CFR 201.20	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Phenylalanine as a component of aspartame	Acceptable
21 CFR 201.21	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Sulfites; required warning statements	Acceptable
21 CFR 201.22	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Bar code label requirements	Acceptable
21 CFR 201.25, 21 CFR 610.67	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products)	Acceptable
21 CFR 610.68, 21 CFR 201.26	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Net quantity	Acceptable
21 CFR 201.51	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Usual dosage statement	Acceptable
21 CFR 201.55, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Inactive ingredients	Acceptable
21 CFR 201.100	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Storage requirements	Acceptable
Recommended labeling practices: USP General Chapters <7> Labeling USP General Chapters <659> Packaging and Storage Requirements	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: Revise the storage statement to read as follows for clarity: "Store refrigerated at 2°C to 8°C (36°F to 46°F)." <i>The Applicant revised as requested</i>	
Dispensing container	Acceptable
21 CFR 201.100	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Package Label⁵ Evaluation

Proper name	Acceptable
21 CFR 610.61, 21 CFR 201.50, 21 CFR 201.10	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: <i>acceptable provided the DMEPA-approved suffix is applied to the proper name</i>	
Manufacturer name, address, and license number	Acceptable
21 CFR 610.61, 21 CFR 201.1(a), 21 CFR 201.1(h)(5), 21 CFR 201.1(h)(6), 21 CFR 201.100(e)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: Relocate the placeholder for the US license number to appear beneath the manufacturer name and address and before the distributor's name and address. <i>The Applicant revised as requested</i>	
Lot number or other lot identification	Acceptable
21 CFR 610.61	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

⁵ Per 21 CFR 600.3(cc) *Package* means the immediate carton, receptacle, or wrapper, including all labeling matter therein and thereon, and the contents of the one or more enclosed containers. If no package, as defined in the preceding sentence, is used, the container shall be deemed to be the package. Thus, this includes the carton, prescribing information, and patient labeling.

	<input type="checkbox"/> N/A
Expiration date	Acceptable
21 CFR 610.61, 21 CFR 201.17	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Preservative	Acceptable
21 CFR 610.61	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Number of containers	Acceptable
21 CFR 610.61	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Strength/volume	Acceptable
21 CFR 610.61, 21 CFR 201.10, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (expression of strength for injectable drugs): Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 line 176 USP General Chapters: <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Storage temperature/requirements	Acceptable
21 CFR 610.61	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: Revise the storage statement to read as follows for clarity: "Store refrigerated at 2°C to 8°C (36°F to 46°F)." <i>The Applicant revised as requested</i>	
Handling: "Do Not Shake", "Do not Freeze" or equivalent	Acceptable
21 CFR 610.61	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Multiple dose containers (recommended individual dose)	Acceptable
21 CFR 610.61	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Route of administration	Acceptable
21 CFR 610.61, 21 CFR 201.5, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Recommended labeling practices:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

<p>Comment/Recommendation: Revise the route of administration statement from " (b) (4) to read as follows: "For Intravenous Infusion After Dilution" <i>The Applicant revised as requested</i></p>	<input type="checkbox"/> N/A
<p>Known sensitizing substances</p>	<p>Acceptable</p>
<p>21 CFR 610.61</p>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
<p>Inactive ingredients</p>	<p>Acceptable</p>
<p>21 CFR 610.61, 21 CFR 201.100</p>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<p>Recommended labeling practices: USP General Chapters <1091> Labeling of Inactive Ingredients USP General Chapters <7> Labeling</p>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<p>Comment/Recommendation: (b) (4) (b) (4)</p> <p>Revise to appear as follows: For consistency with the prescribing information, revise each presentation as follows: "Each carton contains ...Zirabev. Each mL of solution contains 25 mg bevacizumab-bvzr, edetate disodium dihydrate (0.05 mg), polysorbate 80 (0.2 mg), succinic acid (2.36 mg), sucrose (85 mg), and Water for Injection, USP." <i>The Applicant revised as requested</i></p>	
<p>Source of the product</p>	<p>Acceptable</p>
<p>21 CFR 610.61</p>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
<p>Minimum potency of product</p>	<p>Acceptable</p>
<p>21 CFR 610.61</p>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<p>Rx only</p>	<p>Acceptable</p>
<p>21CFR 610.61, 21 CFR 201.100</p>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<p>Divided manufacturing</p>	<p>Acceptable</p>
<p>21 CFR 610.63 (Divided manufacturing responsibility to be shown)</p>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
<p>Distributor</p>	<p>Acceptable</p>
<p>21 CFR 610.64 (Name and address of distributor)</p>	<input checked="" type="checkbox"/> Yes

	<input type="checkbox"/> No <input type="checkbox"/> N/A
Bar code	Acceptable
21 CFR 610.67, 21 CFR 201.25	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products)	Acceptable
21 CFR 610.68, 21 CFR 201.26	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
NDC numbers	Acceptable
21 CFR 201.2, 21 CFR 207.35	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Preparation instructions	Acceptable
21 CFR 201.5	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Package type term	Acceptable
<i>Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use</i> <i>USP chapter <659> Packaging and Storage Requirements</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: Relocate the "Discard Unused Portion" statement to appear underneath the package type term statement "One Single-Dose Vial" <i>The Applicant revised as requested</i>	
Misleading statements	Acceptable
21 CFR 201.6	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Prominence of required label statements	Acceptable
21 CFR 201.15	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Spanish-language (Drugs)	Acceptable
21 CFR 201.16	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
FD&C Yellow No. 5 and/or FD&C Yellow No. 6	Acceptable

21 CFR 201.20	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Phenylalanine as a component of aspartame	Acceptable
21 CFR 201.21	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Sulfites; required warning statements	Acceptable
21 CFR 201.22	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Net quantity	Acceptable
21 CFR 201.51	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Usual dosage statement	Acceptable
21 CFR 201.55, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Dispensing container	Acceptable
21 CFR 201.100	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
Medication Guide	Acceptable
21 CFR 610.60, 21 CFR 208.24	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Prescribing Information Evaluation

PRESCRIBING INFORMATION

Highlights of Prescribing Information	
PRODUCT TITLE	Acceptable
21 CFR 201.57(a)(2)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices reference: Draft Guidance for Industry on Product Title and Initial U.S. Approval in the Highlights of Prescribing Information for Human Prescription Drug and Biological Products - Content and Format (January 2018), which, when finalized, will represent FDA's current thinking on topic</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

DOSAGE AND ADMINISTRATION	Acceptable
<i>Recommended labeling practices reference: USP nomenclature for diluents and intravenous solutions</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
DOSAGE FORMS AND STRENGTHS	Acceptable
21 CFR 201.57(a)(8), 21 CFR 201.10, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018)</i> <i>USP chapter <659> Packaging and Storage Requirements</i> <i>USP General Chapters: <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Full Prescribing Information	
2 DOSAGE AND ADMINISTRATION	Acceptable
21 CFR 201.57(c)(3)(iv)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices reference: USP nomenclature for diluents and intravenous solutions</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
3 DOSAGE FORMS AND STRENGTHS	Acceptable
21 CFR 201.57(c)(4)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018)</i> <i>USP chapter <659> Packaging and Storage Requirements</i> <i>USP General Chapters: <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
11 DESCRIPTION	Acceptable
21 CFR 201.57(c)(12), 21 CFR 610.61 (m), 21 CFR 610.61(o), 21 CFR 610.61 (p), 21 CFR 610.61 (q)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: USP General Chapters <1091>, USP General Chapters <7></i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Comment/Recommendation: We deleted the proprietary name and only included the proper name-suffix since this 1st paragraph discuss the drug substance. <i>The applicant revised as requested</i>	

The applicant revised as requested

We condensed the inactive ingredient information for both formulations into one paragraph and revised the quantitative information for each inactive ingredient to the amount per mL of solution. We relocated the quantitative amounts to appear in parenthesis after the inactive ingredient name to help distinguish the active ingredient from the inactive ingredients in this list. Ensure that the carton labeling expresses the amount of ingredients per mL and in the same format.

The applicant revised as requested

<u>16 HOW SUPPLIED/ STORAGE AND HANDLING</u>	<u>Acceptable</u>
21 CFR 201.57(c)(17)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<u>MANUFACTURER INFORMATION</u>	<u>Acceptable</u>
21 CFR 201.100(e), 21 CFR 201.1	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Recommended labeling practices references: 21 CFR 610.61(b) (add the US license number for consistency with the carton labeling), and 21 CFR 610.64 (Name and address of distributor may appear and use a qualifying phrase for consistency with the carton labeling, when applicable)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A



Vicky
Borders-Hemphill

Digitally signed by Vicky Borders-Hemphill
Date: 6/10/2019 07:49:47AM
GUID: 50814c7000007a3d59329f660d8ddf02



Jee
Chung

Digitally signed by Jee Chung
Date: 6/13/2019 10:26:01AM
GUID: 508da6da000265c6b990d788ce889c0b

Recommendation: Approval

BLA Number: **761099**

Review Number: **1**

Review Date: 4/4/19

Drug Name/Dosage Form	ZIRABEV (Bevacizumab-bvzr) Solution for intravenous infusion
Strength/Potency	25 mg/ml
Route of Administration	Intravenous infusion
Rx/OTC dispensed	Rx
Indication	Treatment of metastatic colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, metastatic renal cell carcinoma, cervical cancer.
Applicant/Sponsor	Pfizer
US agent, if applicable	Riddhi Dedhia

Product Overview

Bevacizumab-bvzr is a recombinant humanized IgG1 monoclonal antibody developed as a biosimilar to US-licensed Avastin. Bevacizumab-bvzr binds to VEGFA isoforms that have been identified as regulators of developmental, physiological and pathological neovascularization, which support tumor growth and metastasis. Bevacizumab-bvzr binding to VEGFA blocks the ability of VEGFA to bind its receptors on the cell surface, resulting in inhibition of receptor-associated kinase activity and associated downstream effects. The Fc portion of the antibody contains the typical immunoglobulin N-linked glycans that can impact in vivo antibody half-life and effector functionality. Although the Fc portion of an IgG1 molecule can induce effector functions such as antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC), the levels of these activities are very low due to a mainly soluble VEGFA target, and therefore effector function is not thought to contribute to the mechanism of action of bevacizumab-bvzr. Bevacizumab-bvzr is produced in a CHO cell line. Drug product is manufactured to the same concentration and presentation as US licensed Avastin, i.e. as a 25 mg/mL solution of bevacizumab-bvzr in 100 mg/4 mL and 400 mg/16 mL preservative-free, single-dose vials. However, the formulation consists of different excipients as compared to US-licensed Avastin.

Quality Review Team

Discipline	Reviewer	Branch/Division
RBPM	Anh-Thy Ly	OPQ/OPRO/DRBPMI/RBPMBI
Drug Substance	Jee Chung	OPQ/OBP/DBRRIV
Drug Product	Jee Chung	OPQ/OBP/DBRRIV
Immunogenicity	Jee Chung	OPQ/OBP/DBRRIV
Labeling - OBP	Vicky Borders-Hemphill	OPQ/OBP
Facility	Michael Shanks	OPQ/OPF/DIA/IAB1
Facility	Zhihao Peter Qiu	OPQ/OPF/DIA/IAB1
Microbiology- DS	Aimee Cunningham	OPQ/OPF/DMA/MABIV
Microbiology- DP	Jessica Hankins	OPQ/OPF/DMA/MABIV
Team Lead - OBP	Chana Fuchs	OPQ/OBP/DBRRIV
Team Lead - DMA	Reyes Candau-Chacon	OPF/DMA/BIV
Team Lead – DIA	Zhihao Peter Qiu	OPQ/OPF/DIA/IAB1
Application Team Lead	Chana Fuchs	OPQ/OBP/DBRRIV

Core Multidisciplinary Review Team:

Discipline	Reviewer	Office/Division
RPM	Gina Mehta	OND/OHOP/DOP2
Cross-disciplinary Team Lead	Martha Donoghue	OND/OHOP/DOP2
Medical Officer	Sandra Casak	OND/OHOP/DOP2
Pharm/Tox	Emily Wearne	OND/OHOP/DHOT
Clinical Pharmacology	Theingi Thway	OTS/OCP
Statistics	Tianjao Dai (CMC) Haiyan Chen (OND)	OTS/OB/DBVI OTS/OB/DBV

1. Names:

- a. Proprietary Name: Zirabev
- b. Trade Name: Zirabev
- c. Non-Proprietary Name/USAN: bevacizumab-bvzr
- d. CAS Name: 216974-75-3
- e. Common Name: PF-06439535
- f. INN Name: bevacizumab
- g. Compendial Name: not applicable
- h. OBP systematic name: MAB HUMANIZED (IgG1) Anti-P15692 (VEGFA_Human)
[PF06439535]

Submissions Reviewed:

Submission(s) Reviewed	Document Date
0003 – BLA resubmission	06/29/2018
0004	07/25/2018
0006	08/31/2018
0010	10/02/2018
0014	10/31/2018
0016	11/16/2018
0017	12/03/2018
0018	12/14/2018
0019	12/14/2018
0020	12/20/2018
0021	12/21/2018
0022	1/09/2019
0024	01/15/2019
0025	01/18/2019
0026	1/22/19
0027	1/3/19
0028	02/06/2019
0029	02/08/2019
0030	2/14/19
0031	2/15/19
0032	2/21/19
0034	3/12/19
0035	3/26/19

Quality Review Data Sheet

1. Legal Basis for Submission: 351(k)

2. Related/Supporting Documents:

A. DMFs:

DMF #	DMF Type	DMF Holder	Item referenced	Code ¹	Status ²	Date Review Completed	Comments
(b) (4)	V	(b) (4)	(b) (4)	3	N/A		
	V			1	Adequate	07/12/2018	
	II			1	adequate		DMF was looked at for purpose of this BLA. No specific DMF review was written. Information is incorporated into the BLA review.
	III			3	N/A		Not reviewed. Sufficient Leachables and Extractables data was in the BLA, supported by product quality and stability data.
	III			3	N/A		Not reviewed. Sufficient Leachables and Extractables data and primary stability program in BLA.
	III			3	N/A		Not reviewed. Sufficient Leachables and Extractables data and primary stability program in BLA.
	III			3	N/A		Not reviewed. Sufficient Leachables and Extractables data and primary stability program in BLA.

1. Action codes for DMF Table:

1- DMF Reviewed; Other codes indicate why the DMF was not reviewed, as follows: 2- Reviewed previously and no revision since last review; 3- Sufficient information in application; 4- Authority to reference not granted; 5- DMF not available; 6- Other (explain under "comments")

2. Adequate, Adequate with Information Request, Deficient, or N/A (There is enough data in the application; therefore, the DMF did not need to be reviewed.)

B. Other documents: IND, Referenced Listed Drug (RLD), or sister application: NONE

3. Consults: NONE

Executive Summary

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

Recommendation:

The Office of Biotechnology Products, OPQ, CDER, recommends approval of BLA 761099 for Zirabev (bevacizumab-bvzr) manufactured by Pfizer Inc. The data submitted in this application, including the analytical similarity assessment, are adequate to support the conclusion that:

- The biological product, bevacizumab-bvzr, is highly similar to US-licensed Avastin notwithstanding minor differences in clinically inactive components.
- A sufficient scientific bridge was established to support the use of EU-approved bevacizumab as a comparator in clinical trials supporting this application.
- The manufacture of bevacizumab-bvzr is well-controlled and yields a consistently high quality product that is pure and potent. The conditions used in manufacturing have been sufficiently validated, and a consistent product is produced from the multiple production runs presented.

As summarized in the following sections of this review, OBP, DMA and DIA reviewers have all concluded that this BLA should be approved. Therefore, I recommend that this product be approved for human use under conditions specified in the package insert.

B. Approval Action Letter Language:

- Manufacturing locations:
 - Drug Substance: Wyeth BioPharma. One Burt Road, Andover, MA (FEI: 1222181)
 - Drug Product: **Manufacturing:** Pharmacia and Upjohn Company LLC. 7000 Portage Road, Kalamazoo, MI (FEI: 1810189)
Release testing: Wyeth BioPharma. One Burt Road, Andover, MA (FEI: 1222181) AND Pharmacia and Upjohn Company LLC. 7000 Portage Road, Kalamazoo, MI (FEI: 1810189)
Labeling and Packaging: Pharmacia and Upjohn Company LLC. 7000 Portage Road, Kalamazoo, MI (FEI: 1810189)
Stability testing: Wyeth BioPharma. One Burt Road, Andover, MA (FEI: 1222181) AND Pharmacia and Upjohn Company LLC. 7000 Portage Road, Kalamazoo, MI (FEI: 1810189) AND (b) (4)

“Under this license, you are approved to manufacture bevacizumab-bvzr drug substance at Wyeth BioPharma, Andover, MA. The final formulated product will be manufactured, filled, labeled and packaged at Pharmacia and Upjohn Company LLC, Kalamazoo, MI and tested for release and stability at Wyeth BioPharma, Andover, MA.”

- Fill size and dosage form
 - 100 mg/4 mL single-dose vial, Injection, for intravenous use
 - 400 mg/16 mL single-dose vial, Injection, for intravenous use

- Dating period:
 - Drug Product:
 - 100mg vials - 42 months at 2-8 °C
 - 400 mg vials – 42 months at 2-8 °C
 - Drug Substance: (b) (4) months: (b) (4) °C
 - For packaged products: “Not packaged”
 - Stability Option (select one below):
 - We have approved the stability protocol(s) in your license application for the purpose of extending the expiration dating of your drug substance and drug product under 21 CFR 601.12.
- Exempt from lot release
 - Yes. ZIRABEV is a specified product exempt from lot release in accordance with 21 CFR 601.2a.

C. Benefit/Risk Considerations:

ZIRABEV (bevacizumab-bvzr) is a biosimilar to US-licensed Avastin, and is intended to deliver bevacizumab by the same route of intravenous administration for the same indications as the US-licensed Avastin reference product, other than those indications protected under the orphan exclusivity. The analytical similarity of bevacizumab-bvzr to US-licensed Avastin was evaluated using methods to assess physicochemical and functional properties of the products. The quality attributes evaluated covered primary and higher order structure, glycosylation, antibody Fab and Fc mediated biological activities, product related species, drug product attributes, and the stability profile of the products. For some attributes, multiple orthogonal methods were used. The formulation buffer for bevacizumab-bvzr is different from US- licensed Avastin and EU-approved bevacizumab. The methods were evaluated for impact due to the different formulations, and for some higher order structure methods all products were dialyzed into a common buffer.

To establish the analytical portion of the scientific bridge to justify the use of clinical data derived from European Union (EU)-approved bevacizumab in support of this BLA, the applicant provided pairwise analytical comparisons between bevacizumab-bvzr and EU-approved bevacizumab and between US-licensed Avastin and EU-approved bevacizumab. Forty-six US-licensed Avastin and 51 EU-approved bevacizumab lots were used in the analytical similarity study compared to 12 independent bevacizumab-bvzr drug substance lots sourced as 8 DP lots from independent DS lots and 4 additional DS lots. Both 100 mg and 400 mg DP presentations were used in the analytical similarity study.

Review of manufacturing has identified that the methodologies used for drug substance and drug product manufacturing, release and stability testing are robust and sufficiently controlled to result in a consistent and safe product. The drug substance manufacturing process is robust for inactivation and removal of adventitious agents. The BLA is recommended for approval from a sterility assurance and microbiology product quality perspective. The DIA review recommends approval of the commercial manufacture of bevacizumab-bvzr DS at Wyeth BioPharma and of Zirabev DP at Pharmacia and Upjohn Company LLC. OBP product quality and immunogenicity assay, DMA microbiological drug substance and drug product, DIA facility, and OBP labeling technical assessments are located as separate documents in Panorama.

D. Recommendation on Phase 4 (Post-Marketing) Commitments, Requirements, Agreements, and/or Risk Management Steps, if approvable: None

II. Summary of Quality Assessments:

A. Analytical Similarity Assessment:

The reference product, US-licensed Avastin (bevacizumab) was approved in the U.S. on February 26, 2004. Bevacizumab-bvzr, US-licensed Avastin, and EU-approved bevacizumab are available in two strengths, 100 mg/4 mL and 400 mg/16mL per vial, as a preservative-free, single-dose product for intravenous infusion. The formulation buffer for bevacizumab-bvzr is different from US- licensed Avastin and EU-approved bevacizumab. The totality of the analytical data provided is sufficient to support a demonstration that bevacizumab-bvzr is “highly similar” to US-licensed Avastin.

Clinical studies supporting this application used a non-US-licensed comparator product, EU-approved bevacizumab. To justify the use of these comparative clinical data to support a demonstration of biosimilarity of bevacizumab-bvzr to US-licensed Avastin, the applicant performed an analytical study as well as a pharmacokinetic study (described below) to establish an adequate scientific bridge for the products.

A 3-way evaluation of analytical similarity of bevacizumab-bvzr, US-licensed Avastin and EU-approved bevacizumab was demonstrated using 8 bevacizumab-bvzr drug product and 4 additional bevacizumab-bvzr drug substance lots, 46 US-licensed Avastin lots, and 51 EU-approved bevacizumab lots. The 8 bevacizumab-bvzr drug product lots were derived from independent drug substance lots and included lots used in the comparative PK and comparative clinical studies, and process validation lots. US-licensed Avastin and EU-approved bevacizumab DP lots were collected over a period of four years and covered a remaining shelf-life span of <1 months to 21 months from expiry. Results that underwent statistical analysis focused on independent lots and did not include DP lots that originated from the same lot of drug substance. The number of lots analyzed for each quality attribute was justified by the Applicant. Both drug product strengths for which the Applicant is requesting approval were represented in the analytical similarity assessment.

A single-dose pharmacokinetic similarity study in healthy male subjects provided a 3-way comparison of bevacizumab-bvzr, US-licensed Avastin and EU-approved bevacizumab with the goal of addressing residual uncertainties remaining from the analytical similarity studies and supporting PK similarity. The study was also used to provide the PK portion of the scientific bridge to support the relevance of comparative clinical data generated using EU-approved bevacizumab.

The strength of U.S.-licensed Avastin is labeled in mass per unit volume (mg/mL). U.S.-licensed Avastin is filled into single-use vial with a volume of 100mg/4 mL and 400mg/16 mL¹. Pfizer is seeking approval for bevacizumab-bvzr for the same strength as U.S.-licensed Avastin. Comparative in vitro potency, protein concentration (mg/mL) data reviewed as part of the analytical similarity assessment, and validation of extractable (deliverable) volume data reviewed as part of the drug product manufacturing process control were used to inform the assessment of whether the proposed presentation of bevacizumab-bvzr has the same strength

¹ U.S. Prescribing Information, U.S.-licensed Avastin. Accessed 4/2 /2019 from https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/125085s323lbl.pdf

as the presentation of U.S.-licensed Avastin. Based on these comparative data, the 100 mg/4 mL and 400 mg/16 mL single use vials have the same total content of drug substance in units of mass in a container and the same concentration of drug substance in units of mass per unit volume as the respective presentations of U.S.-licensed Avastin. These presentations meet the statutory “same strength” requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.

The analytical similarity assessment of bevacizumab-bvzr, US-licensed Avastin and EU-approved bevacizumab used a comprehensive set of validated or qualified assays, as listed in Table 1 below:

Table 1: Summary of Similarity Assessment

Category	Assays and Attributes by Analytical Techniques	Risk Category
Primary Structure and Post-translational Modifications	• Sequence - Amino Acid Analysis by LC-MS/MS peptide mapping and Edman degradation	3
	• Molecular mass	
	○ Intact Mass: nano-ESI MS;	3
	○ LC/MS subunit analysis (Ide digestion);	3
	○ LC/MS Peptide Mapping (reduced and non-reduced)	3
	○ Non-Reduced LC/MS Peptide Map Profile	3
Higher Order Structure	• N-linked Glycans by HILIC/MS	
	○ % High Mannose	2
	○ % Afucosylation	2
	○ % Galactosylation	2
	○ % Sialylation	3
Biological Activity – Fab mediated	• Secondary Structure: Alpha helix and Beta sheets	
	○ FTIR	3
	○ Far UV-CD	3
	• Tertiary Structure:	
	○ Near UV-CD	3
	○ Intrinsic Fluorescence Spectroscopy	3
	• Melting Temperature	
	○ DSC	3
	• Potency by proliferation inhibition bioassay in HUVEC	1
	• VEGF-A ₁₆₅ binding by ELISA	1
	• VEGF-A isoforms 121, 189, and 206 binding by ELISA	3
	• VEGFA family specificity Binding to VEGFC and VEGFD by ELISA	3

Biological Activity – Fc mediated	<ul style="list-style-type: none"> • FcRn binding by SPR • FcγRIIIa (158V) %KD by SPR • FcγRIIIa (158F) by SPR • FcγRIa binding by SPR • FcγRIIa (131H and 131R) binding by SPR • FcγRIIb binding by SPR • FcγRIIIb binding by SPR • C1q binding by ELISA • ADCC activity in DLD-1 (VEGFA secreting) • ADCC activity in SKOV-3 (VEGFA membrane bound) • CDC activity in DLD-1 (VEGFA secreting) • CDC activity in SKOV-3 (VEGFA membrane bound) 	<p>2</p> <p>2</p> <p>2</p> <p>3</p> <p>3</p> <p>3</p> <p>3</p> <p>3</p> <p>3</p> <p>3</p> <p>3</p> <p>3</p>
Product-related Substances and Impurities	<ul style="list-style-type: none"> • Charge variants by iCE - acidic, main and basic peaks, profile. • Size variants by SE-HPLC - % Monomer, % HMMS • Size variants by cGE reduced - % fragments, % HC+LC • Size variants by cGE nonreduced - % intact IgG 	<p>2</p> <p>2</p> <p>3</p> <p>3</p>
Protein Concentration	<ul style="list-style-type: none"> • UV 280 	<p>2</p>
Disulfide bonds	<ul style="list-style-type: none"> • LC/MS-non-reducing peptide mapping with Lys-C 	<p>NC</p>
Comparative Forced Degradation	<ul style="list-style-type: none"> • Forced degradation at 40°C - analysis by SE-HPLC, iCE, CGE reducing, potency and subvisible particles • Forced degradation by light (51 klux) and 25°C - analysis by SE-HPLC, iCE, CGE reducing, potency, peptide mapping, and subvisible particles • Forced degradation in Phosphate Buffer pH 7.5; 14 days at 40°C - analysis by SE-HPLC, iCE, CGE reducing, potency, peptide mapping, and subvisible particles • Forced degradation by peracetic acid: analysis by FcRn binding ELISA, potency, and peptide mapping. 	<p>3</p> <p>3</p> <p>3</p> <p>3</p>

NC = not categorized

Each of the attributes described in Table 1 above met the pre-determined criteria for the pairwise comparison of bevacizumab-bvzr to US-licensed Avastin and to EU-approved bevacizumab with the exceptions described below. In each of these cases, the impact of the differences in the attributes and resulting residual uncertainty was mitigated by additional information and analysis provided by the applicant.

- Differences in levels of glycosylation were observed between bevacizumab-bvzr, US-licensed Avastin, and EU-approved bevacizumab. The data provided showed that bevacizumab-bvzr lots had higher levels of afucosylation, terminal galactosylation, and high mannose content.
 - Afucosylation: The bevacizumab-bvzr lots tested fail to meet the afucosylation ranges of the US-licensed Avastin lots used in this analysis. Higher levels of afucosylation were detected for all bevacizumab-bvzr lots by HILIC/MS, with levels above what was detected in US- licensed Avastin lots. Afucosylation levels in the EU-approved bevacizumab lots tested spanned the range of the US-licensed Avastin lots and overlapped with some of the bevacizumab-bvzr lots. The level of antibody fucosylation

is relevant for effector activities of the antibody. No ADCC activity was identified for all three compared products using the assays and cell lines identified in the table above. Additional comparative assessment of the biological activity of bevacizumab-bvzr, US-licensed Avastin and EU-approved bevacizumab for FcγRIIIa binding showed that there are no differences observed in these assays.

- High mannose: Differences between bevacizumab-bvzr, US-licensed Avastin, and EU-approved bevacizumab were observed in the level of high mannose N-linked glycans. Bevacizumab-bvzr, contains higher levels of high mannose content compared to US-licensed Avastin and EU-approved bevacizumab, with levels that do not overlap in their ranges. Higher levels of high mannose glycans can result in faster clearance of product in vivo and therefore affect the PK of the product. The amounts and difference in high mannose levels were within ranges identified in the literature as not having an impact on PK of various monoclonal antibodies studied. A lack of impact on clearance was verified in the PK and clinical comparative studies.
- Galactosylation: Galactose levels in bevacizumab-bvzr were overlapping with the levels seen for the differences in galactose levels were noted for US-licensed Avastin, however, bevacizumab-bvzr levels ranged to the upper end and exceeded the levels of terminal galactose in US-licensed Avastin. Galactose levels can impact CDC activity. Relevance of any differences noted were assessed by comparative C1q binding studies and comparative analysis of CDC activity using in DLD-1 and SKOV3 cell lines. The results of the C1q binding indicate that all three products have similar C1q binding properties. The CDC study results showed that all three products equally failed to induce CDC activity as compared to the positive control. Lots of bevacizumab-bvzr that had the higher range of galactosylation above the US-licensed Avastin range were associated with bioreactor sparger clogging resulting in inability to maintain adequate dissolved oxygen levels. Resolution of this issue brought back the galactosylation to within the galactose range identified for the US-licensed Avastin.
- Similar binding affinities by all three products (bevacizumab-bvzr, US-licensed Avastin, and EU-approved bevacizumab) were identified for the VEGFA isoforms tested except for VEGFA 206 that showed a higher binding affinity by bevacizumab-bvzr. The specific relevance of VEGFA 206 is that this isoform is associated with the cell membrane and therefore has the potential to result in effector function activity. This concern is mitigated by results from the functional ADCC and CDC assays using the SKOV-3 cells in which VEGFA is membrane bound. The results showed an absence of effector functions for bevacizumab-bvzr, US-licensed Avastin, and EU-approved bevacizumab. Thus, the higher binding by bevacizumab-bvzr to the VEGFA 206 isoform would not preclude the conclusion that bevacizumab-bvzr is highly similar to US-Avastin
- Differences in charge were noted in iCE assay data:
 - bevacizumab-bvzr was noted to have a higher level of total basic peaks when separated based on charge, with an additional basic peak in bevacizumab-bvzr as compared to US-licensed Avastin and EU-approved bevacizumab lots. Differences in the level of main peak were the result of the differences in basic peak. To elucidate the differences observed in the basic peaks, a lot of each of the three products, bevacizumab-bvzr, US-licensed Avastin and EU-approved bevacizumab, were fractionated using preparative CEX-HPLC for analysis by LC/MS. Charge variants separated by CEX-HPLC showed the same charge species in all three products with the exception of two basic peaks (B3 and

B4). LC/MS identified that the majority of the variants identified in bevacizumab-bvzr were also present in US-licensed Avastin and in EU-approved bevacizumab, with the exception of a H chain VHS signal peptide extension. The amount of this signal peptide variant as part of the basic peaks is controlled by the release testing acceptance criteria.

- Samples were also digested with carboxypeptidase B prior to iCE analysis. The carboxypeptidase B treatment removes C-terminal lysine. Results showed that the higher levels of basic peaks in bevacizumab-bvzr are due to the presence of C-terminal lysine variant and resulted in similar peak profiles of the three products in the carboxypeptidase B treated samples. Based on current knowledge and literature reports (Liu, H., et. al. *Mabs* 2015 Sept-Oct; 6(5): 1145-1154), differences in the levels of C-terminal lysine residues of monoclonal antibodies administered by the intravenous route are not expected to impact product performance as it is typically removed in vivo shortly after administration.
- The results of the percent of fragments, HC+LC, HMMS, and intact IgG showed that bevacizumab-bvzr lots contained fewer fragments and HMMS, and higher levels of HC+LC and intact IgG from what was observed in the US-licensed Avastin and the EU-approved bevacizumab lots. Based on data provided, the results correlate with the estimated age of the materials tested. Therefore, these differences are attributed to the relative age of each product and are not due to clinically relevant differences between bevacizumab-bvzr, US-licensed Avastin, and EU-approved bevacizumab.
- Protein concentration: Based on the three methods used to determine the extinction coefficient, Pfizer selected 1.65 mL mg⁻¹cm⁻¹ for evaluation of the protein concentration. The mean values for protein concentration for bevacizumab-bvzr, US-licensed Avastin, and EU-approved bevacizumab were provided. The data show that approximately a third of bevacizumab-bvzr lots were outside of the EU-approved bevacizumab lots tested quality range of 24.4-26.4 mg/mL. The small difference in protein content is not likely to have impact on the results from the clinical studies based on the fact that the potency of the products was analytically similar, the differences in protein concentration is very small, and based on the fact that the product is dosed to saturation. This conclusion is also supported by the comparative PK study results.

The totality of the analytical data supports a demonstration that bevacizumab-bvzr, US-licensed Avastin, and to EU-approved bevacizumab are highly similar in terms of protein sequence, protein structure, and biological functions linked to the mechanisms of action and met expectations for analytical similarity.

The immunogenicity assays used to evaluate antidrug antibodies (ADA) are adequately validated. Patients developed similarly low binding and neutralizing responses to bevacizumab-bvzr and US-licensed Avastin

B. CQA Identification, Risk and Lifecycle Knowledge Management

Tables 2 and 3, below, summarize the critical quality attributes and their control strategy that are relevant specifically to the API and Drug Substance. For additional information, see the OBP quality technical assessment and the Drug Substance Microbiology technical assessment in Panorama.

Table 2: Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

CQA (type)	Risk	Origin	Control Strategy	Other
Binding to VEGFA and neutralization (Potency by HUVEC Proliferation Inhibition Activity)	Potency, Efficacy and safety	Intrinsic to the molecule. Impacted by in process and stability, tryptophan oxidation in CDR. Fragmentation in CDR3, Aggregation	(b) (4)	Additionally, controlled by other CQAs that impact potency
Glycosylation - high mannose (product-related impurities)	PK/Efficacy	Intrinsic to molecule and cell culture. (b) (4) Does not change during storage.		
Glycosylation - sialic acid (product-related impurities)	PK/Efficacy	Intrinsic to molecule and cell culture. (b) (4) Does not change during storage.		
High Molecular Mass (HMMS)/Aggregates (product-related impurities)	Efficacy, PK and immunogenicity	Manufacturing process and storage conditions Minimal increase is expected on DS stability under controlled conditions.		Aggregates are increased upon exposure to light, and high pH stress.
Fragmentation (product-related impurities)	Efficacy/potency	Manufacturing process and storage conditions. Minimal change is expected on DS stability under controlled conditions		Increased upon exposure to heat, high pH stress, and light stress.
HC Trp50/108 oxidation (product-related impurities)	Efficacy (impacts VEGFA binding)	Manufacturing process and exposure to light		These non-exposed amino acids are not easy to oxidize
HC Met 258 oxidation (product-related impurities)	PK (impacts FcRn binding)	Manufacturing process and inappropriate storage (high temperature, exposure to light)		This quality attribute was not monitored on every lot. Pfizer committed to implement routine monitoring in the BLA.
FcRn binding	Efficacy Pharmacokinetics	(b) (4)		

Charge Variant Profile	Efficacy, PK	(b) (4) storage	(b) (4)
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C. Drug Substance bevacizumab-bvzr Quality Summary

CQA Identification, Risk, and Lifecycle Knowledge Management

Table 3: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management

CQA (type)	Risk	Origin	Control Strategy	Other
Bioburden	Safety, purity, and efficacy (degradation or modification of the product by microbial contamination)	Raw materials or manufacturing process	(b) (4)	
Endotoxin	Safety and purity	Raw materials or manufacturing process		
Host Cell Proteins (Process-related impurity)	Safety and Immunogenicity	Process-related impurity from the production cell line, and cell culture/harvest process		(b) (4)
Host Cell DNA (Process-related impurity)	Safety	Process-related impurity from the production cell line and the cell culture/harvest process		(b) (4)
(b) (4) (Process-related impurity)	Safety and Immunogenicity	Process-related impurity (b) (4)		(b) (4)
(b) (4) (Process-related impurity)	Safety	Cell culture media		
(b) (4)	Safety	Cell culture media		

(Process-related impurity)			(b) (4)
(b) (4) (Process-related impurity)	Safety	Cell culture media	
(b) (4) (Process-related impurity)	Safety	Cell culture media	
(b) (4) (Process-related impurity)	Safety	Cell culture media	
(b) (4) (Process-related impurity)	Safety	Cell culture media	
Viruses (Contaminant)	Safety	Contamination during manufacture.	(b) (4)
Mycoplasma (Contaminant)	Safety	Mycoplasma introduced during cell culture operations	(b) (4)
Leachables (Process-related impurity)	Safety	(b) (4)	
Visual appearance (Color and opalescence) (general)	Safety	Manufacturing process and formulation	

Protein content (general)	Impact on efficacy	DS manufacture (b) (4)	(b) (4)
pH	Safety, efficacy	Manufacturing process (formulation)	
Identity	Safety and Efficacy	NA	

- Description:**
 Bevacizumab-bvzr is a recombinant humanized IgG1 kappa monoclonal antibody produced in CHO cells consisting of two heavy chains (453 amino acids each) and two light chains (214 amino acids each). The antibody targets human vascular endothelial growth factor A (VEGFA). The bevacizumab-bvzr DNA sequence was derived from literature and the sequence information was used as a template to synthesize the corresponding DNA sequences for the HC and LC variable regions. The DNA sequence derived from literature was verified by peptide mapping using US-licensed Avastin. Bevacizumab-bvzr has an average molecular weight of 149.2 kDa and a pI of ~8.2 (main species). It has the typical structure of an IgG1 antibody with 4 interchain disulfide bonds and 12 intrachain disulfide bonds and typical N-linked glycan structures in the heavy chain at Asn 303 that are predominantly core fucosylated, complex-type, bi-antennary structures, G0F/G0F (major species), and G0F/G1F, G1F/G1F, and G0F/G0F + Lys (minor species).
- Mechanism of Action (MoA):**
 Bevacizumab-bvzr binds specifically to VEGFA and prevents the interaction of VEGFA with its receptors, thereby inhibiting the known functional activities of VEGFA. According to the Avastin USPI, preventing the normal biological activity of VEGFA regresses existing vascularization of tumors, inhibits formation of new tumor vasculature and normalizing remaining tumor vasculature, thereby inhibiting tumor growth. The same MoA of bevacizumab, i.e. inhibition of VEGFA-induced angiogenesis and vascular permeability, is identified for each of the approved indications.

VEGFA, a member of the cysteine knot growth factors family of proteins, is responsible for regulating vasculogenesis and angiogenesis under both normal (e.g. developmental and wound repair functions) and pathophysiological (e.g. tumor growth) conditions. VEGFA provides several functions that are important for angiogenesis and include induction of endothelial cell proliferation and survival, increase in vascular permeability, and chemotaxis and homing of bone marrow cells for hematopoiesis. The main identified receptors that bind VEGFA and mediate vasculogenesis/ angiogenesis and chemotaxis/hematopoiesis are VEGF receptor 2 (VEGFR2/ KDR) and receptor 1 (VEGFR1/Flt-1), respectively. VEGFA can exist in several isoforms due to differential splicing that exert local as well as distal signaling events. Most splice isoforms larger than VEGFA148 contain a significant part of the heparin binding site sequence and thus are anticipated to have some association with the heparin on the cell surface. VEGFA splice isoforms 165 and larger isoforms also contain a binding site for the neuropilin co-

receptors that enhances VEGF signaling. The VEGFA epitope recognized by US-licensed Avastin and bevacizumab-bvzr is conserved in all splice isoforms.

- **Potency Assay:**

The potency assay used to measure bevacizumab-bvzr activity is a cell-based anti-proliferation assay using human umbilical vein endothelial cells (HUVEC). HUVECs are known to express VEGFA receptors, VEGFR1 and VEGFR2, as well as VEGFA neuropilin co-receptors and proliferate in the presence of exogenously added VEGFA. The potency assay entails the introduction of bevacizumab-bvzr along with VEGFA 165 isoform to HUVECs at the same time followed by incubation for 48 hours. At the end of the incubation period, HUVEC proliferation is measured and the percent inhibition of proliferation by bevacizumab-bvzr is determined and compared to the bevacizumab-bvzr reference standard.

- **Reference Materials:**

A two-tier reference standard system is in place, which is consistent with ICH Q6B. (b) (4)

The PRM was tested by release and additional characterization assays and is adequately qualified. (b) (4)

Following qualification, the PRM potency is assigned a value of (b) (4)%. Qualification parameters for future reference standards are not included in the BLA. A description of the reference standard stability program is included in the BLA.

- **Critical starting materials or intermediates:**

(b) (4)

- **Manufacturing process summary:**

(b) (4)
(b) (4)

[Redacted] (b) (4)

- Container closure: The DS container closure system is a [Redacted] (b) (4)

- Dating period and storage conditions: [Redacted] (b) (4)

D. Drug Product Zirabev Quality Summary:

Table 4, below, provides a summary of the identification, risk, and lifecycle knowledge management for drug product CQAs that derive from the drug product manufacturing process and general drug product attributes. For additional information, see the OBP quality technical assessment and the Drug Product Microbiology technical assessment in Panorama.

Table 4: Drug Product CQA Identification, Risk, and Lifecycle Management

CQA (type)	Risk	Origin	Control Strategy	Other
Sterility (contaminant)	Safety (infection), purity, and efficacy via degradation or modification of products by microbial contamination	Manufacturing process or failure of container closure integrity	[Redacted] (b) (4)	
Endotoxin	Safety (pyrogenic fever, increased immunogenicity risk) and purity	Raw materials, manufacturing process or failure of container closure integrity	[Redacted] (b) (4)	

Container closure integrity	Safety (maintenance of sterility during shelf life)	Storage conditions	(b) (4)
Osmolality (general)	Safety	Formulation (Osmolality is a surrogate test for excipients levels)	
Appearance (General) Visible Particulate Matter (product or process related impurities)	Safety and immunogenicity	DS and DP Manufacturing processes, formulation, interaction with the container closure system	
Subvisible Particulate Matter (product or process related impurities)	Safety and Immunogenicity	Manufacturing process and CCS. Could be product or foreign particles.	
Color of solution (general)	Safety and Efficacy	Formulation, contamination or degradation	
Polysorbate 80 (general/critical excipient)	Safety and Efficacy (impacts aggregate)	Formulation	
pH (general)	Safety	Formulation	
Turbidity (general and process or product related impurities)	Safety	Manufacturing process and formulation	
Extractable Volume (general)	Efficacy/Dosing	Manufacturing process	
Protein Concentration (general)	Efficacy and Safety	Formulation	
Identity (general)	Safety and Efficacy	Intrinsic to molecule	
Leachables (process-related impurities)	Safety	Manufacturing equipment and CCS	

- **Potency:**

The potency of bevacizumab-bvzr is defined as the percent inhibition of proliferation relative to bevacizumab-bvzr reference material. The potency assays are the same as those described in the Drug Substance section of this review.

Strength: 25 mg/mL

The bevacizumab-bvzr drug product (DP) is supplied in two presentations for intravenous infusion. Both presentations contain 25 mg/mL of bevacizumab-bvzr.

- a 100 mg/4 mL sterile, preservative-free solutions in single dose vials to deliver 4 mL
- a 400mg /16 mL sterile, preservative-free solutions in single dose vials to deliver 16 mL

Comparative data for protein concentration demonstrate that bevacizumab-bvzr has the same total content of therapeutic antibody as US-licensed Avastin. Bevacizumab-bvzr meets the statutory requirement to have the same strength as the reference product described under section 351(k)(2)(A)(i)(IV) of the PHS Act.

- **Summary of Product Design:**

Bevacizumab-bvzr is a sterile 25 mg/mL bevacizumab-bvzr liquid filled into (b) (4) glass vials. The drug product is supplied as a 100 mg or 400 mg, sterile, single-dose, preservative-free solution in a vial to deliver 4 mL or 16 mL of bevacizumab-bvzr for intravenous infusion. The container closure system consists of a 5 mL (100 mg) or 20 mL (400 mg) (b) (4) glass vial, elastomeric stopper, and aluminum crimp seal with flip off cap.

- **List of Excipients:**

There are no further changes in excipients from DS. Each vial of bevacizumab-bvzr contains 25 mg/mL of bevacizumab-bvzr, 85 mg/mL sucrose, 0.05 mg/mL edetate disodium dehydrate (EDTA), 0.2 mg/mL polysorbate 80, (b) (4) at pH 5.5. Additional excipient information is located in the Product Quality primary technical assessment in Panorama.

- **Reference Materials:**

The drug product reference standard is the same as was described for bevacizumab-bvzr drug substance. There is no formal drug product reference standard. Bevacizumab-bvzr RS information is located in the drug substance section of the OBP Product Quality primary technical review in panorama.

- **Manufacturing process summary:**

Drug Product manufacturing occurs at Pharmacia and Upjohn Company, LLC in Kalamazoo, MI. The manufacturing process involves the following steps:

(b) (4)

(b) (4)

(b) (4)

- **Container closure:**

The 100 mg/4 mL bevacizumab-bvzr drug product presentation is supplied in 5 mL (b) (4) glass vial with a 13 mm (b) (4) elastomer stopper (b) (4), a 13mm aluminum seal and a flip-off cap; The 400 mg/16 mL bevacizumab-bvzr drug product presentation is supplied in a 20 mL (b) (4) (b) (4) glass vial with a 20 mm (b) (4) elastomer stopper (b) (4), a 20 mm aluminum seal, and a flip-off cap.

- **Dating period:**

Drug product stability data were provided for both the 100mg and 400 mg vial presentations. The expiration dating for both presentations on approval will be 42 months when stored at 2-8 °C. (b) (4)

(b) (4). The BLA contains an annual stability protocol for each presentation of the drug product.

- E. Biopharmaceutics Considerations: N/A
- F. Novel Approaches/Precedents: None
- G. Any Special Product Quality Labeling Recommendations:
Single dose vial, do not reuse. Store at 2-8°C. Protect from light.
- H. Establishment Information:

Overall Recommendation: Approve					
DRUG SUBSTANCE					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Cell bank manufacture and storage; drug substance manufacture; drug substance in-process control testing; drug substance storage; drug substance release and stability testing	Wyeth BioPharma, One Burt Road Andover, MA	1222181	PLI needed	One Item 483 on lack of adequate control of critical material and reagents	Approve
Drug substance storage	Pharmacia and Upjohn Company LLC Kalamazoo, MI	1810189	PLI needed	Three Items	Approve
Drug substance in-process testing	(b) (4)	(b) (4)	Facility Adequate	N/A	Approve

Cell bank storage	Pfizer Ireland Pharmaceuticals, Dublin, Ireland	3004145594	Facility Adequate	N/A	Approve
DRUG PRODUCT					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
DP release and stability testing	Wyeth BioPharma, Andover, MA	1222181	PLI needed	One Item	Approve
DP manufacture, DP in-process control testing, primary packaging, DP release testing, DP stability testing, batch release, and secondary packaging and labeling	Pharmacia and Upjohn Company LLC Kalamazoo, MI	1810189	PLI needed	Three Items	Approve
Drug product stability testing	(b) (4)		Facility Adequate	N/A	Approve

I. Facilities:

Wyeth BioPharma (FEI 1222181)

A pre-license inspection (PLI) of this drug substance manufacturing facility was conducted for BLA 761099 on 01/14/2019 – 01/18/2019, and a 1-item FDA Form 483 was issued at the conclusion of the inspection on lack of adequate control of critical material and reagents. Comprehensive reviews of manufacturing areas, utilities, equipment, processes and procedures were conducted to evaluate product quality, compliance to commitments in the BLAs and compliance to CGMPs.

The CDER review for this PLI under CMS work activity 254288 was performed by OPF/DIA and was found acceptable. The inspection was classified as VAI. The FDA 483 observation, EIR Narratives, exhibits, firm’s written 483 responses, and associated attachments were reviewed and a recommendation of approve with an adequate compliance status was made by CDER/OPQ/OPF.

Pharmacia and Upjohn Company LLC (FEI 1810189)

A cGMP surveillance and pre-license inspection of Pharmacia and Upjohn Company LLC, Kalamazoo, MI, for Drug Product manufacturing were completed in support of BLA 761099. The inspection occurred from 09/10-21/2018 and was conducted in accordance with (b) (4)

[Redacted]

The pre-license component of this inspection was for this BLA, BLA 761099/0, and included coverage of the Quality, Facilities & Equipment, Materials, Production, Packaging & Labeling, and Laboratory systems for the profile classes: (b) (4). A 3-item FDA 483 was issued at

the end of the inspection, citing (1) Procedures designed to prevent microbiological contamination of drug products purporting to be sterile are not followed. (2) Employees are not given training in the particular operations they perform as part of their function. (3) Deviations from written production and process control procedures are not recorded. The inspection was classified as VAI; and approval of BLA 761099/0 was recommended.

J. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols approved:

1. Annual stability protocol for drug substance
2. Stability Protocol for primary and working reference material
3. [REDACTED] (b) (4)
validation protocols
4. [REDACTED] (b) (4)
validation protocols
5. [REDACTED] (b) (4) validation protocols
6. Cell bank stability protocol
7. [REDACTED] (b) (4) qualification protocol
8. [REDACTED] (b) (4) protocol
9. [REDACTED] (b) (4) qualification protocol

ii. Outstanding review issues/residual risk: None

iii. Future inspection points to consider: None

b. Drug Product

i. Protocols approved:

1. Annual stability protocol for drug product
2. Stability protocol for the extension of drug product shelf-life

ii. Outstanding review issues/residual risk: None

iii. Future inspection points to consider: none

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

#	Checklist	Yes	No	N/A
Product Type				
1.	Recombinant Product	X		
2.	Naturally Derived Product		X	
3.	Botanical		X	
4.	Human Cell Substrate/source material		X	
5.	Non-Human Primate Cell Substrate/Source Material		X	
6.	Non-Primate Mammalian Cell Substrate/source material	X		
7.	Non-Mammalian Cell Substrate/Source Material		X	
8.	Transgenic Animal source		X	
9.	Transgenic Plant source		X	
10.	New Molecular Entity		X	
11.	PEPFAR drug		X	
12.	PET drug		X	
13.	Sterile Drug Product	X		
14.	Other: [fill in information]			
Regulatory Considerations				
15.	Citizen Petition and/or Controlled Correspondence Linked to the Application [fill in number]		X	
16.	Comparability Protocol(s)		X	
17.	End of Phase II/Pre-NDA Agreements		X	
18.	SPOTS (special products on-line tracking system)		X	
19.	USAN assigned name	X		
20.	Other [fill in]			
Quality Considerations				
21.	Drug Substance Overage		X	
22.	Design Space	Formulation		X
23.		Process		X
24.		Analytical Methods		X
25.		Other		X
26.	Other QbD Elements		X	
27.	Real Time release testing (RTRT)		X	
28.	Parametric release in lieu of Sterility testing		X	
29.	Alternative Microbiological test methods		X	
30.	Process Analytical Technology in Commercial Production		X	
31.	Non-compendial analytical procedures	Drug Product	X	
32.		Excipients		X
33.		Drug Substance	X	
34.	Excipients	Human or Animal Origin		X
35.		Novel		X
36.	Nanomaterials		X	
37.	Genotoxic Impurities or Structural Alerts		X	
38.	Continuous Manufacturing		X	
39.	Use of Models for Release		X	
40.	Other {fill-in}			



Joel
Welch

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Chana
Fuchs

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Zihao Peter
Qiu

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Reyes
Candau-Chacon

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BLA STN 761099

Zirabev (Bevacizumab-bvzr)

Pfizer, Inc.

Jee Chung, Ph.D. Product Quality Reviewer
Chana Fuchs Ph.D., Team Leader

Office of Biotechnology Products
Division of Biotechnology Review and Research IV

OBP CMC Review Data Sheet

1. BLA#: 761099

2. Review Date: 3/26/19

3. Primary Review Team:

- a. Medical Officer: Sandra Casak
- b. Pharm/Tox: Emily Wearne
- c. Product Quality Team: Jee Chung
- d. Facilities: Aimee Cunningham, Jessica Hankins, Michael Shanks
- e. Clinical Pharmacology: Theingi Thway
- f. Statistics: Haiyan Chen (Biometrics) and Tianjiao Dai (CMC Statistics)
- g. OBP Labeling: Vicky Borders-Hemphill
- h. RBPM: Gina Mehta (OND) and Anh-Thy Ly (OPQ)

4. Major GRMP Deadlines:

- a. Filing Meeting: 8/14/2018
- b. Mid-cycle meeting: 11/26/18
- c. Wrap-up meeting: 5/9/2019
- d. Primary review due: 3/28/19
- e. Secondary review due: 4/7/19
- f. PDUFA action date: 6/26/19

5. Communications with Sponsor and OND:

Communication/Document:	Date:
74-day Letter	9/11/18
Information Request-2 (IR2)	11/15/18
IR3 (midcycle communication)	12/3/18
IR4	12/20/18
IR5 (Analytical Similarity only)	1/29/19
IR6 (DP manufacturing and others)	1/29/19
IR 7 (Immunogenicity)	2/7/19
IR8 (DS and DP)	3/4/19
IR10	3/21/19

6. Submission Reviewed:

Submission:	Date Received:	Review Completed (yes or no)
761099/0010: 74-day Response-1	10/2/18	Yes
761099/0014: 74-day Response-2	10/31/18	Yes
761099/0018: 74-day Response-3	12/14/18	Yes (ADCC and CDC)
761099/0017: IR2 Response-1	12/3/18	Yes
761099/0018: IR2 Response-2	12/14/18	Yes
761099/0021: IR3 Response-1	12/21/18	Yes
761099/0025: IR3 Response-2	1/18/19	Yes

761099/0022: IR4	1/9/119	Yes
761099/0026: IR4 Response 6	1/22/19	Yes
761099/0028: IR5 (C1q binding and expired lots)	2/6/19	Yes
761099/0029: IR6	2/8/19	Yes
761099/0030: IR7 (Immunogenicity)	2/14/19	Yes
761099/0031: IR5 (VEGFA membrane and FcgR additional lots)	2/15/19	Yes
761099/0034: IR8	3/11/19	Yes
761099/0035:IR10	3/26/19	Yes

7. Drug Product Name/Code/Type:

- a. Proprietary Name: Zirabev
- b. Trade Name: Zirabev
- c. Non-Proprietary Name/USAN: bevacizumab-bvzr
- d. CAS Name: 216974-75-3
- e. Common Name: PF-06439535
- f. INN Name: bevacizumab-bvzr
- g. Compendial Name: not applicable
- h. OBP systematic name (refer to OPQ-SOP-OBP-3006):
MAB HUMANIZED (IgG1) Anti-P15692 (VEGFA_Human) [PF06439535]

8. Pharmacological Category: Therapeutic recombinant human monoclonal antibody to VEGFA

9. Dosage Form: Injection

10. Strength/Potency:

- (i): 100 mg/4mL and 400 mg/16 mL
- (ii): Cell growth inhibition assay

11. Route of Administration: Intravenous

12. Referenced Drug Master Files (DMF):

DMF#	DMF Holder	Item Referenced	Letter of Cross-Reference	Comments (status)
(b) (4)			Yes	Sufficient manufacturing process information (adequate)
			Yes	Sufficient leachables and extractables and primary stability data in the BLA (adequate)
			Yes	Sufficient leachables and extractables and primary stability data in the BLA (adequate)

(b) (4)	Yes	Sufficient leachables and extractables and primary stability data in the BLA (adequate)
	Yes	Sufficient leachables and extractables and primary stability data in the BLA (adequate)

13. Inspectional Activities:

The pre-license inspection of the drug substance manufacturing facility at Pfizer Inc., Andover, MA was conducted from January 9th, 2019 to January 18th, 2019. The first part of the inspection from January 9th to 11th, and 16th-17th covered the analytical similarity testing and storage areas for US-licensed Avastin and EU-approved bevacizumab and was led by Sean Marcsisin (ORA), with Jee Chung (CDER/OPQ/OBP/DBRRIV) and Rukman De Silva (CDER/OPQ/OBP/DBRRIV). The second half of the inspection was led by Maxwell Van Tassell (CDER/OPQ/OPF/DMA), with Aimee Cunningham (CDER/OPQ/OPF/DMA) and Jee Chung and covered the PF-06439535 drug substance manufacturing areas, QC laboratories, warehouse, and cell bank storage.

The inspection was system-based and covered Quality, Facilities and Equipment, Production, Materials, and Laboratories. The inspection was limited to the manufacturing of PF-06439535 DS and the analytical similarity data and components.

Two Form FDA 483 were issued, first Form FDA 483 contained two-item deficiencies and the second Form FDA 483 contained a single item deficiency. The deficiencies were laboratory control mechanisms to trend and investigate multiple assay failures, the firm did not follow established procedures to document all instrument checks and associated maintenance for (b) (4) instrument used to determine protein concentration, and insufficient control over critical material and reagent used for release and stability testing. Pfizer addressed these issues in their response to the observations.

A final compliance status of voluntary action indicated (VAI) was given to the facility.

14. Consults Requested by OBP:

None

15. Quality by Design Elements:

The following was submitted in the identification of QbD elements (check any that apply):

	Design Space
X	Design of Experiments
	Formal Risk Assessment/Risk Management
	Multivariate Statistical Process Control
	Process Analytical Technology
	Expanded Change Protocol

16. Precedents:

None

17. Administrative:

A. Signature Block

Name and Title	Signature and Date
Jee Chung, Ph.D. Product Quality Reviewer Division of Biotechnology Review and Research IV Office of Biotechnology Products Office of Pharmaceutical Quality, FDA	See electronic signature in Panorama
Chana Fuchs, Ph.D. Product Quality Team Lead Division of Biotechnology Review and Research IV Office of Biotechnology Products Office of Pharmaceutical Quality, FDA	See electronic signature in Panorama
Joel Welch, Ph.D. Acting Review Chief Division of Biotechnology Review and Research IV Office of Biotechnology Products Office of Pharmaceutical Quality, FDA	See electronic signature in Panorama

B. CC Block

Recipient	Date
Clinical Division BLA RPM	
Division of Biotechnology Review and Research IV File/BLA STN 761028	

Summary of Quality Assessments

I. Primary Reviewer Summary Recommendation

We recommend approval of STN 761099 for Zirabev (bevacizumab-bvzr) manufactured by Pfizer, Inc.. The data submitted in this application are adequate to support the conclusion that the manufacture of Zirabev (bevacizumab-bvzr) is well controlled and leads to a product that is pure and potent. The product is free of endogenous and adventitious infectious agents sufficient to meet the parameters recommended by FDA. The conditions used in manufacturing have been sufficiently validated and a consistent product has been manufactured from multiple production runs. It is recommended that this product be approved for human use under the conditions specified in the package insert.

We recommend an expiration dating period of (b) (4) months for PF-06439535 drug substance when stored at (b) (4) °C

We recommend an expiration dating period of 36 months for PF-06439535 drug product when stored at 2-8°C protected from light.

II. List of Deficiencies to be Communicated

There are no product quality deficiencies precluding approval of this BLA.

III. List of Post-Marketing Commitments/Requirements- None

IV. Review of Common Technical Document- Quality Module 1

A. Environmental Assessment of Claim of Categorical Exclusion

A categorical exclusion is claimed for the requirement to prepare an environmental assessment in accordance with 21 CFR 25.31(c).

The claim of categorical exemption is accepted.

V. Primary Container Labeling Review

A separate primary container labeling review was performed by Vicky Borders-Hemphill, OBP.

VI. Review of Common Technical Document- Quality Module 3.2

The review of module 3.2 is provided below.

VII. Review of Immunogenicity Assays- Module 5.3.1.4

A review of the product immunogenicity assays is included at the end of the primary review document.

3.2.R.3 Analytical Similarity Assessments (Comparative Physicochemical and Functional Assessment)

The analytical similarity development program for Pfizer's PF-06439535 spanned over 5 years starting with reference product analysis in 2011 to execution of process validation studies in 2016. The analytical similarity assessment included a comparison of three products, PF-06439535, US-Avastin,

and EU-Avastin. The comparison between US-Avastin and EU-Avastin was conducted to provide a scientific bridge to support the use of EU-Avastin as a comparator in the clinical studies.

Terminology

Pfizer’s proposed biosimilar product is referred to as PF-06439535 in the submission as well as in the BLA review. The reference product (RP) or the US-licensed bevacizumab used in the similarity studies is referred to in the submission as bevacizumab-US by Pfizer. The EU-approved bevacizumab is referred to as bevacizumab-EU in the submission. Therefore, in figures and tables that are copied from the submission will refer to US-licensed bevacizumab and EU-approved bevacizumab as bevacizumab-US and bevacizumab-EU, respectively.

***Reviewer Comment:** In the text of this review, US-licensed bevacizumab and EU-approved bevacizumab are referred to as US-Avastin and EU-Avastin for brevity.*

3.2.R.3.1.1 Overall Strategy-Criteria for Assessing Similarity

Pfizer took a stepwise approach to assessing analytical similarity between PF-06439535, US-Avastin, and EU-Avastin, that are stated to be aligned with the recommendations provided to Pfizer from published FDA and ICH guidance documents, as well as formal Biological Product Development (BPD) meetings type 3 and 2 held April 15, 2015 and February 13, 2017. The first step in Pfizer’s stepwise approach was a determination of the RP quality attributes (QA) and sequence identification. The second step consisted of risk assessments on the QAs for their ability to impact safety, efficacy, pharmacokinetic/pharmacodynamics (PK/PD), and immunogenicity and ranking the QAs into high, moderate, and low risks. The third step of the stepwise approach was to take the QAs and place them into one of three statistical tiers with consideration for method capabilities as shown below:

- Tier 1: Equivalence testing. Highest level of risk and significance to similarity, such as attributes likely to impact clinically relevant mechanism(s) of action (MoA) of the product for each indication for which approval is sought.
- Tier 2: Quality ranges. QAs with moderate level of risk and significance to similarity
- Tier 3: Tabular/graphical raw data. Generally QA with lowest level of risk and significance to similarity or reflective of method capabilities not amenable to Tier 1 or Tier 2 statistical analysis.

The following tables provided in the submission list the QAs, their respective tiers, and the rationale for assignments.

Table 3.2.R.3.1.1-1. Statistical Tier Rankings for Bevacizumab Potency/Biological Activity Assays

QA/Methodology	Tier	Rationale
VEGF Binding to Fab Domain		
Inhibition of Cell Growth Assay	1	The ability of bevacizumab to bind VEGF and inhibit tumor cell proliferation is a known MoA. It was assessed in an inhibition of cell growth assay using HUVEC that express VEGFR1 and VEGFR2. The assay measures binding to the target antigen VEGF, and inhibition of VEGF receptor-mediated proliferation. Equivalence of PF-06439535 and licensed bevacizumab was demonstrated based on the Tier 1 assessment of the results from multiple reference product and PF-06439535 lots.
Binding to VEGF ₁₆₅ Target Antigen by ELISA	1	The ability of bevacizumab to bind to the target antigen VEGF ₁₆₅ is a known MoA. Binding to VEGF ₁₆₅ , as assessed by an ELISA, is a direct and sensitive measure of target binding. Equivalence of PF-06439535 and licensed bevacizumab was demonstrated based on the Tier 1 assessment of the results from multiple reference product and PF-06439535 lots.
Binding to other VEGF isoforms (VEGF ₁₂₁ , VEGF ₁₈₉ , VEGF ₂₀₆) by ELISA	3	The ability of bevacizumab to bind to all VEGF isoforms is a known attribute (Muller et al., 1998). Binding to VEGF ₁₂₁ , VEGF ₁₈₉ , and VEGF ₂₀₆ , as assessed by an ELISA, is a direct and sensitive measure of target binding.

Table 3.2.R.3.1.1-1. Statistical Tier Rankings for Bevacizumab Potency/Biological Activity Assays

QA/Methodology	Tier	Rationale
Effector Function via Fc Domain		
Peripheral Blood Mononuclear Cell (PBMC) Antibody-Dependent Cellular Cytotoxicity (ADCC) Assay	3	ADCC is not relevant to the MoA (Wang et al., 2004). Therefore, confirmation of lack of ADCC response was demonstrated using representative reference product and PF-06439535 lots.
Complement-Dependent Cytotoxicity (CDC) Assay	3	CDC is not relevant to the MoA (Wang et al., 2004). Therefore, confirmation of lack of CDC response was demonstrated using representative reference product and PF-06439535 lots.
Binding to C1q by Immunoassay	3	The binding of the Fc domain to the complement protein, C1q is a known prerequisite to CDC activity. However, CDC function does not play a role in the in vitro MoA of bevacizumab (Wang et al., 2004). The C1q ELISA assay was used to monitor Fc domain-mediated binding to C1q in representative reference product and PF-06439535 lots.
Binding to Fc γ RI, Fc γ RIIb, Fc γ RIIa (131H and 131R), Fc γ RIIIa (158F and 158V) and Fc γ RIIIb by Surface Plasmon Resonance (SPR)	3	Binding to Fc γ receptors such as Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIIa and Fc γ RIIIb is involved in Fc-mediated functions. However, ADCC and other Fc-mediated functions do not play a role in the bevacizumab MoA. Therefore, similarity of PF-06439535 and bevacizumab reference product is demonstrated based on raw data from representative lots.
Binding to FcRn by Surface Plasmon Resonance (SPR)	2	Binding of monoclonal antibodies to FcRn is reported to mediate in vivo clearance (Roopenian & Akilesh, 2007). Similarity in binding to FcRn is demonstrated based on Tier 2 assessment of multiple reference product and PF-06439535 lots.

Table 3.2.R.3.1.1-2. Evaluation of Bevacizumab Physicochemical QAs for Statistical Tier Ranking

Quality Attribute	Methodology	Tier	Rationale and Justification
Primary Structure and Molecular Mass			
Primary Structure	Mass Spectrometry (LC/MS, LC/MSMS), Edman Sequencing	3	Amino acid sequence defines an antibody's function and properties. The cell line used to produce PF-06439535 was confirmed to produce a product with the identical primary sequence to that of bevacizumab-US and bevacizumab-EU, as determined by numerous comparative heightened characterization methods including Edman degradation and several chromatographic and mass spectrometric methods. Comparative confirmation of primary structure involved analytical methodologies that result in data that are qualitative in nature and make it appropriate for Tier 3. While these methods also provide information about the product isoform profile, attribute-specific tests that are more appropriate for statistical analysis are discussed below.
Protein Concentration			
Protein Concentration	UV 280 nm absorbance	2	At current dosing, bevacizumab will produce complete suppression of free serum and likely at or above saturation at target tumor site as well (Gordon et al, 2001 and Bai et al, 2014). Therefore similarity of multiple reference product and PF-06439535 is demonstrated based on Tier 2 analysis.
Posttranslational Modifications			
N-linked Glycan Profile	Hydrophilic Interaction Liquid Chromatography (HILIC)	3	The N-linked glycan species observed in bevacizumab are present on endogenous human immunoglobulins; therefore these structures are not expected to impact immunogenicity. PK of the commonly occurring mAb glycoforms does not appear to differ significantly (Putnam et al, 2010). N-linked glycans on the IgG Fc do not play a role in efficacy unless the MoA involves ADCC and/or CDC. ADCC and CDC are not implicated as part of the MoA for bevacizumab (Wang et al, 2004). Therefore, comparison of the overall N-linked glycan profile is appropriate for demonstration of similarity and ensures all the major N-linked glycan species are taken into account.
Charge Heterogeneity			
Basic species	iCE	2	The C-terminal lysine residue of a mAb is not involved in biological activity, neither target antigen binding nor Fc domain-functions. Basic species are not thought to impact PK (Schiestl et al., 2011). However, it has been reported that basic species are associated with a decrease in activity for bevacizumab (EPAR, 2005). Although no discernible effect on biological activity has been observed for PF-06439535 enriched in basic species, a demonstration of similarity based on Tier 2 analysis of multiple reference product and PF-06439535 lots was considered appropriate due to the uncertainty created by published literature.
Acidic species	iCE	2	Acidic species in mAbs have no known impact on safety or efficacy and are not thought to impact PK (Khawli et al, 2010; Putnam et al, 2010). The presence of low levels of sialylated N-linked glycans in the Fc in acidic species is unlikely to impact PK or biological activity (Liu, 2015). However, the demonstration of similarity based on Tier 2 analysis of multiple reference product and PF-06439535 lots remains an FDA expectation for acidic species.

Table 3.2.R.3.1.1-2. Evaluation of Bevacizumab Physicochemical QAs for Statistical Tier Ranking

Quality Attribute	Methodology	Tier	Rationale and Justification
Purity			
Monomer	SE-HPLC	2	Monomer is the primary active species. A decrease of monomer would be potentially relevant to efficacy due to the expected loss of biological activity and have an impact on PK/PD and increased risk of immunogenicity. Therefore, similarity of multiple reference product and PF-06439535 lots is demonstrated based on Tier 2 analysis.
HMMS	SE-HPLC	2	The levels of HMMS may impact immunogenicity. Therefore, similarity of multiple reference product and PF-06439535 lots is demonstrated based on Tier 2 analysis.
Fragments	CGE (Reducing)	3	IgG fragmentation may theoretically expose a new epitope, causing increased immunogenicity. The levels of fragments in reference product and PF-06439535 are low (<3.5%) and therefore, are not expected to impact immunogenicity. A demonstration of similarity based on raw data from representative reference product and PF-06439535 lots is considered appropriate.
Heavy and Light Chain (HC + LC)	CGE (Reducing)	3	Heavy chain and light chain (assessed following reduction) is closely related to fragment levels. A decrease in heavy chain and light chain would be, potentially, relevant to immunogenicity. However, the levels of heavy and light chain in reference product are high (>96%) and therefore, not expected to impact immunogenicity. A demonstration of similarity based on raw data from representative reference product and PF-06439535 lots is considered appropriate.
Higher Order Structure (HOS)			
Secondary structure	Far-UV CD, FTIR Spectroscopy	3	Secondary structure contributes to antibody folding, function and properties including stability. Far-UV circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR) spectra are interpreted qualitatively and the similarity assessment is based on profile overlay. The methods are qualitative in nature and demonstration of similarity based on raw data from representative reference product and PF-06439535 lots was considered appropriate.
Tertiary structure	Near-UV CD, Fluorescence Spectroscopy	3	Tertiary structure contributes to antibody function and properties including stability. Near-UV CD and fluorescence spectra are interpreted qualitatively and the similarity assessment is based on profile overlay. The methods are qualitative in nature and demonstration of similarity based on raw data from representative reference product and PF-06439535 lots was considered appropriate.
Thermal stability of HOS	DSC	3	Differential Scanning Calorimetry (DSC) characterizes thermal unfolding of protein domains, which is indicative of overall integrity of HOS. DSC unfolding thermograms are interpreted qualitatively and the similarity assessment is based on profile overlay. The methods are qualitative in nature and demonstration of similarity based on raw data from representative reference product and PF-06439535 lots was considered appropriate.

Reviewer Comments:

- *The stepwise approach taken for QAs and statistical tiers is consistent with advice previously provided to Pfizer during the BPD type 2 meeting held 20140716. However, as described below, the risk ranking of the following QAs is not justified and evaluation by quality range, rather than graphical comparison, is recommended. In addition, several QAs that were previously recommended to be assessed were not evaluated as indicated below.*
 - *ADCC Activity: Pfizer did not evaluate the ability to induce ADCC using cells that express membrane bound form of VEGFA. Because VEGFA is also known to exist associated to the extracellular matrix of cells and not only in a soluble state, Pfizer should evaluate the ability for their product to bind to cell-surface associated VEGFA and induce ADCC. IR was sent to request the ADCC data (see IR response below).*
 - *CDC Activity: For the same reason stated above for ADCC activity evaluation, IR was sent to request CDC data using cells that express membrane bound form of VEGFA and sponsor stated that requested data will be provided by 12/14/18. (see response in section 3.2.R3.2.2 Biological Activity)*
 - *FcγRIIIa (158F and 158V variants): The justification provided for placement of the binding activity evaluations for these two Fcγ receptors is not acceptable because the risk of binding to these receptors is not strictly associated with their linkage or lack thereof to the MoAs, but with safety as related to unwanted side-effects, especially given that VEGFA is not only soluble, but also exists as cell-surface associated VEGFA. IR was sent to include binding affinity assessment to FcγRIIIa (158F and 158V variants) to be in a higher risk category with applicable data analysis (see response in section 3.2.R3.2.2 Biological Activity Functional Characterization of the Fc domain).*
 - *N-glycan profile: The justifications provided by Pfizer for the evaluation of the N-linked glycan profile by a Tier 3 data analysis are not acceptable because the risk of this QA to affect PK and safety via unwanted Fc effector function should be considered and not just that the QA attribute is unlikely to affect PK based on literature of “commonly occurring mAb glycoforms” and the MoA. IR was sent to include a quantitative glycan analysis in a higher risk category with applicable data analysis (see response in section 3.2.R.3.2.3 N-Linked glycan Structure).*
- *There are two QAs that were not assessed as part of the similarity exercise. The QAs are binding to a short VEGFA isoform as part of the binding assessment evaluated by and binding specificity to VEGFA versus other VEGF family members. During the 2013 BIA meeting, the FDA recommended that binding specificity of the products to VEGFA to be included in the analytical similarity assessment. IR was sent on September 11, 2018 to request binding specificity data.*

IR Response 10/31/18: Sponsor provided ELISA binding results of all three products, PF-06439535 (reference standard lot 128926-30), US-Avastin (453807), and EU-Avastin (H0150B08) tested side-by-side to VEGF family members VEGFC and VEGFD. Briefly, the ELISA uses VEGF ligands, i.e. VEGFA, VEGFC, and VEGFD to capture either the products under investigation, PF-06439535, US-Avastin, and EU-Avastin or VEGFR2, which is known to bind to all three VEGF ligands as a positive control. The results showed that PF-06439535 as well as US-Avastin, EU-Avastin did not bind to VEGFC and VEGFD isoforms. Hence, the binding of PF06439535 to VEGF ligands was demonstrated to be specific for VEGFA.

Reviewer Comment: *The response is acceptable.*

- *Regarding binding analysis, the Agency recommended that Pfizer assessment of binding to VEGF-A by ELISA include at least two isoforms, a long and a short isoform such as VEGFA-165 and VEGFA-121, respectively, and the data to be evaluated by equivalence test. In this case, Pfizer did not include a short isoform for evaluation by equivalence test. However Pfizer included VEGFA-121, among other isoforms, as part of the analytical similarity assessment and evaluated the data by graphical comparison. , Specifically for the ELISA assay conducted with VEGFA-121, Pfizer used 6 lots each of PF-06439535, US-Avastin, and EU- Avastin, which provide adequate confidence in the results of the assay.*

Pfizer provided a description of both equivalence test and quality range method. Quality ranges (QR) for Tier 2 attributes were derived from the mean plus/minus X times the standard deviation (\pm XSD) of the RP. The value X used for all QRs is 3. The justification for the use of the multiplier 3 is stated to be provided in the respective QA analysis sections. For similarity conclusion, attributes evaluated by the equivalence test must meet the equivalence criteria and for attributes evaluated by quality ranges, 90% of the lots tested must be within the QRs. For QA evaluated by graphical comparison, the following table lists the acceptance criteria.

Table 3.2.R.3.1.1-3. Criteria for Similarity Used to Assess PF-06439535, bevacizumab-US, and bevacizumab-EU

Section or Quality Attribute	Section Number	Criteria for Similarity ^a
Primary Structure and Posttranslational Modifications	3.2.R.3.2.1	Identical amino acid sequence.
		Similar molecular mass and size.
		Similar posttranslational modifications.
VEGF binding to Fab Domain	3.2.R.3.2.2	Similar range of inhibition of VEGF response using the inhibition of cell growth assay.
		Similar range of binding to VEGF using VEGF ₁₆₅ binding ELISA.
		Similar binding to VEGF using VEGF ₁₂₁ , VEGF ₁₈₉ and VEGF ₂₀₆ binding ELISAs.
Effector Function via Fc Domain	3.2.R.3.2.2	Confirms lack of response in ADCC assay.
		Confirms lack of response in CDC assay.
		Similar range of binding to FcRn by SPR.
		Similar dose-dependent response curve in binding to C1q by immunoassay.
N-linked Glycan Profile	3.2.R.3.2.3	Similar N-linked glycan profile, structure, composition, sialic acid levels, and glycosidic linkages.
Charge Heterogeneity	3.2.R.3.2.4	Similar range for levels of acidic, main, and basic isoforms .
		Similar identity of major and minor charge isoforms.
Product Purity	3.2.R.3.2.5	Similar range for levels of monomer, HC + LC, Intact IgG, HMMS and fragment content.
		Similar banding pattern for western blot and SDS-PAGE.
Disulfide Bonds	3.2.R.3.2.6	Similar state of cysteines and disulfide bonds.
Higher Order Structure	3.2.R.3.2.7	Similar secondary, tertiary structure and thermal stability.
Protein Concentration	3.2.R.3.2.8	Similar range for protein concentration.
Forced Degradation	3.2.R.3.3	Similar degradation profiles under forced degradation conditions (elevated temperature, light exposure, and forced deamidation) and demonstrate there are no new degradation products

a. Quality attributes assigned to tier 1 or tier 2 in Table 3.2.R.3.1.1-1 and Table 3.2.R.3.1.1-2 will also be assessed against the statistical acceptance criteria.

Reviewer Comments:

- *The equivalence testing methodology will be deferred to CMC stats reviewer.*
- *The acceptance criteria for graphical comparison analysis appear reasonable for the QA that are appropriately categorized for this type of analysis.*
- *The justification for setting 3 as the multiplier for all the QAs evaluated by QR is stated to be based on lot-to-lot variability and method capability. The justification provided by Pfizer is adequate.*

3.2.R.3.1.2 Lots Enrolled in Similarity

Drug Product Information:

PF-06439535, US-Avastin, and EU-Avastin are available in two strengths, 100 mg and 400 mg per vial, as a preservative-free, single-use product for intravenous infusion. Each vial contains either 4 mL (100 mg) or 16 mL (400 mg) of solution containing the active ingredient. The formulation buffer for PF-06439535 is different than US-Avastin and EU-Avastin products and consists of succinic acid, sucrose, edetate disodium dehydrate (EDTA), polysorbate 80, (b) (4) pH 5.5. The container closure system (CCS) is similar to US-Avastin and EU-Avastin CCS and consists of 5 mL or 20 mL (b) (4) glass vials with elastomeric stopper and aluminum seal with (b) (4) flip-off cap. Both 100 mg and 400 mg presentations were used in the similarity study.

Lots Tested in the Analytical Similarity Assessment

A total of 12 PF-06439535 DS lots were used in the analytical similarity exercise. The majority of the samples were supplied as DP for the analytical similarity testing, i.e. 8 DP lots from 8 independent DS lots and 4 DS lots, which were never processed into DP. The PF-064395435 DS and DP lot numbers used in the similarity studies are shown below.

Table 3.2.R.3.1.2-1. Lot Genealogy

Drug Substance Batch	Drug Product Presentation (mg)	Drug Product Lot	Additional Batch/Lot Details
• 12P138K601	N/A	N/A	Refer to Section 3.2.S.4.4 Batch Analyses and Section 3.2.P.5.4 Batch Analyses for date of manufacture, manufacturing site, and manufacturing process
• 12P138K603	N/A	N/A	
• 13J138K002(G27608)	N/A	N/A	
13J138K003(G27629)	100	J12512-W	
	400	• H49500 ^a	
13J138K004(G27631)	100	• J12513-W	
	400	H90818-W	
14J138K001(J32980)	100	• J76233-W	
	400	J76228-W	
14J138K002(J33002)	100	J76232-W	
	400	• J61289-W ^b	
• 14J138K003(J33003)	N/A	N/A	
14J138K004(J33004)	100	J76234-W	
	400	• J76229-W ^b	
16J138K001(R12923)	100	• R64866-W	
	400	R59890-W	
16J138K002(R32728)	100	R64865-W	
	400	• R59889-W ^b	
16J138K003(R32744)	100	• R64867-W	
	400	R59891-W	

a. Used in the comparative PK study

b. Used in the comparative safety and efficacy study

• Bold, bulleted DS batches and DP lots were selected as independent lots for statistical analyses.

N/A = No drug product made from the drug substance

Reviewer Comment: The DS lot 12P138K603 shown in the table above is the source of the “clinical reference material”. This reference material was used to release all lots manufactured by the clinical process, including the lots used in the clinical studies, as well as the PPQ lots (beginning with 16J...).

US-Avastin and EU-Avastin DP lots were collected over a period of 4 years (2013-2017) and covered remaining shelf-life span of <1 month to 21 months till expiry. A total of 46 US-Avastin and 51 EU-Avastin lots were used in the analytical similarity study. Both 100 mg and 400 mg DP presentations were used in the analytical similarity exercise. Lot information with testing dates and labeled expiry dates was provided in Table 3.2.R.3.5-1 in section 3.2.R.3.5 Appendix-Raw Data (table not shown in the review). All lots used in the clinical studies were included in the analytical similarity assessment acquired in the open market at regular intervals and with no pre-specified criteria.

The sponsor stated that the US-Avastin and EU-Avastin lots were aliquoted and stored frozen between -60 to -90°C until needed for testing in the analytical similarity to prevent changes to the QAs until all the testing were completed. The samples were aliquoted for single use, thawed once, and used prior to expiry. Pfizer provided data to demonstrate that the single freeze-thaw cycle did not itself produce changes to the product quality by providing data up to 3 cycles. Product QAs evaluated included protein concentration (UV 280), charge heterogeneity (iCE), aggregation (SE-HPLC), fragmentation (CGE reduced and non-reduced (nr)), glycan profile (HILIC), potency (cell-based), primary structure (LC/MS-subunit analysis), and secondary structure changes (near-UV-CD). Pfizer also evaluated impact of long term storage of the aliquoted samples at -60 to -90°C (up to 44 months). Both US-Avastin and EU-Avastin lots were used in the sample handling studies.

The results from the sampling handling studies showed a slight increasing trend for aggregates (HMMS) after the third freeze/thaw cycle and an increasing trend for fragments (CGE reduced) after 44 months of storage (see data below). At all other testing time intervals for both the freeze/thaw study and the long-term storage study, the product QA remained stable with no apparent trends with the exceptions already noted for HMMS and fragments. However, no changes to product quality were observed after a single freeze/thaw cycle and storage at least up to 27 months.

Table 3.2.R.3.6-5. Multiple Freeze Thaw Study Results: Monomer, HMMS and LMMS

Lot Number	Quality Attribute (%)	Fresh	1X F/T	2X F/T	3X F/T
Bevacizumab-EU B8507H18	Monomer	97.6	97.6	97.4	97.4
	HMMS	2.2	2.3	2.4	2.4
	LMMS ^a	<0.4	<0.4	<0.4	<0.4
Bevacizumab-US 3022680	Monomer	97.1	97.0	96.7	96.9
	HMMS	2.6	2.8	3.0	2.8
	LMMS	<0.4	<0.4	<0.4	<0.4
Bevacizumab-US 3115915	Monomer	97.6	97.7	97.4	97.5
	HMMS	2.2	2.2	2.4	2.4
	LMMS	<0.4	<0.4	<0.4	<0.4

a. Quantitation (QL) = 0.4%

Table 3.2.R.3.6-17. Reference Product Stored at -60 to -90 °C: HC+LC (%) by CGE (reducing)

Lot Number	Quality Attribute	Mar-13 (Initial)	Jun-14 (15 mon)	Jun-15 (27 mon)	Nov-16 (44 mon)
Bevacizumab-US 453807 ^a	HC + LC	97.3	96.9	97.0	95.8
	Fragments	2.4	2.7	2.5	3.6
Bevacizumab-EU H0150B08 ^b	HC + LC	96.9	97.4	97.4	96.6
	Fragments	2.5	2.3	2.2	3.0

a. Expired 30Nov13

b. Expired 30Apr14

Reviewer Comment: The sample storage and handling data support the intended single freeze/thaw of samples prior to testing in the analytical similarity assessment. The lot and sample handling information are acceptable.

3.2.R.3.1.3 Description of Characterization Methods

The sponsor provided general descriptions of the characterization methods used in the analytical similarity studies listed below:

- primary structure (LC/MS/MS, peptide mapping/Edman degradation, nano-electrospray ionization MS, LC/MS)
- biological activity (binding by ELISA, ADCC, CDC, C1q binding by MSD (Meso Scale Discovery), binding to Fcγ receptors and FcRn by SPR),
- N-glycan mapping by HILIC/MS,
- charge heterogeneity by CEX-HPLC,
- organic-SEC/ESI MS,

- amino acid determination by LC/MS/MS-peptide mapping using trypsin and Lys-C digestion,
- SDS-PAGE/Western blotting,
- free sulfhydryl analysis by VIS spectrometry using Ellman's Reagent,
- disulfide bond determination by reducing and non-reducing peptide mapping followed by LC/MS,
- higher order structure (Far-UV Circular dichroism (CD) spectroscopy, near-UV CD, Fourier transform infrared (FTIR), intrinsic fluorescence emission spectroscopy, and differential scanning calorimetry (DSC),
- HIAC during forced degradation studies.

The sponsor stated due to the difference in product formulation, the methods were evaluated during method characterization studies for the impact of the different formulation. The methods are stated to be evaluated for mass accuracy (for MS), sensitivity, and resolution (MS and HPLC methods). Majority of the method descriptions end with the statement, "The comprehensive assessment of instrument performance and results in terms of mass accuracy, sensitivity, and resolution ensures that this method is suitable for assessing similarity between PF-06439535, and bevacizumab licensed products." Data associated with the "comprehensive assessment" were not provided to support method suitability. Only two method qualification technical reports for VEGFA binding by ELISA and FcRn binding by surface plasmon resonance were provided.

Reviewer Comments:

- *Technical reports for only VEGFA binding by ELISA and FcRn by SPR were provided and were adequately qualified for precision, accuracy, and specificity.*
- *IR was sent to request method qualification data for all other assays used in the analytical similarity study, e.g. glycan analysis using mass spectrometry, ADCC, CDC, C1q binding, etc. in order to determine method suitability.*

IR Response 10/2/18 Query #5: Sponsor provided method qualification reports for the assays used in the similarity studies.

- *Also note that BPD type 4 meeting requested SOPs and sponsor stated that they will provide SOPs for release and stability methods, but for analytical similarity either SOPs or detailed description of methods. The method descriptions are general and do not have data for majority of the methods described, e.g. MS. IR was sent to request SOPs in addition to the method qualification data.*

IR Response 10/2/18 Query #5: Sponsor provided method qualification reports for the assays used in the similarity studies.

- *The higher order structure methods such as FTIR, Far-UV CD, Near-UV CD, intrinsic fluorescence emission spectroscopy, and DSC all state that samples were dialyzed into a common buffer. It is likely because the formulation is different, but sponsor stated that their characterization studies indicated no impact of the methods because of the formulation differences. Therefore, it is not clear why samples were dialyzed. Sponsor should provide a justification and whether the dialyzation step itself did not alter product quality that may hamper interpretation of the results. IR was sent for additional information and data about the dialysis process and whether these methods required dialyzation.*

IR Response 10/2/18:

Sponsor states that all samples including PF-06439535 were dialyzed side-by-side for the same amount of time in PF-06439535 formulation buffer. Sponsor states that dialyzation process has little impact on product quality based on platform knowledge. However, no data were provided.

Reviewer Comment: *Ideally, data from side-by-side dialyzation to Avastin formulation buffer for all three products would have addressed the issue regarding product impact due to the dialyzation process. However, given the additional information that the dialyzation process occurred side-by-side and the higher order spectral data from chemically or thermally denatured PF-06439535 for comparison supports Pfizer's claim that the dialyzation process did not affect product quality, and by my assessment side-by-side dialization would have low risk in impacting only one of the three products and thereby the analytical similarity results. Therefore, the response is acceptable.*

- *Based on the review of the method qualification reports submitted in response to the IR, the methods are considered adequately qualified.*

Review of the Three-Way Analytical Similarity Study Results:

The following table summarizes the three-way analytical similarity study results. Brief reviewer comments and conclusions are shown in the last column of the summary table and more detailed descriptions and discussions of the analytical similarity assessment follow the summary table. A number of comments refer to age of product. For information about impact of age of products to product purity attributes, see review in section 3.2.R.3.2.5

BLA 761099 PF-06439535 Similarity Summary Table

Category	Test		Tier	Number of Lots (US-Avastin: PF-06439535: EU-Avastin)	US-Avastin Range	PF-06439535 Range	EU- Avastin Range	PF-06439535:US-Avastin/ PF-06439535:EU-Avastin/ EU-Avastin:US-Avastin
Primary Structure and Post-translational Modifications	Sequence	Amino Acid Analysis by LC-MS/MS peptide mapping and Edman degradation	3	1:1:1	Visually similar	Visually similar	Visually similar	Pass/Pass/Pass <i>100% sequence coverage</i>
	Molecular Mass	Intact Mass: nano-ESI MS	3	1:1:1	Visually Similar and similar intact mass	Visually Similar and similar intact mass	Visually Similar and similar intact mass	Pass/Pass/Pass <i>Note that a difference in peak height for mannose is observed; see glycan analysis</i>
		LC/MS subunit analysis (Ide digestion)	3	1:1:1 quantified 13:4:13 profile only	Visually Similar	Visually Similar	Visually Similar	Pass/Pass/Pass <i>Differences in peak heights noted for isoforms containing C-terminal lys as well as glycan differences. However no new peaks are observed.</i>
		LC/MS Peptide Mapping (reduced and non-reduced)	3	1:1:1	Visually Similar	Visually Similar	Visually Similar	Pass/Pass/Pass <i>No new peptides observed compared to US-licensed Avastin; however,</i>

								<i>sequence coverage is 96.7% for HC and 97.7% for LC due to small undetected peptides in all three products</i>
		Non-Reduced LC/MS Peptide Map Profile	3	1:1:1	Visually Similar	Visually Similar	Visually Similar	Pass/Pass/Pass <i>All 16 disulfide bonds with correct linkages detected.</i>
	N-linked Glycans by HILIC/MS	% High Mannose	2	46:12:51	0.5-1.1	1.3-3.1	0.5-1.2	Fail/Fail/Pass <i>IR was sent for pairwise and response is incorporated above. Difference not likely to have a clinical impact (see discussion in text)</i>
		% Afucosylation	2	46:12:51	2.4-3.3	2.3-4.6	2.2-4.3	Fail/Pass/Fail <i>IR was sent for pairwise and response is incorporated above. Difference not likely to impact the scientific bridge conclusion or have a clinical impact (see discussion in text)</i>
		% Galactosylation	2	46:12:51	6.5-18.9	13.0-22.1	6.0-21.7	Fail/Pass/Fail <i>IR was sent for pairwise and response is incorporated above.</i>

								<i>Difference not likely to have a clinical impact (see discussion in text)</i>
		% Sialylation	3	46:10:51	< QL	0.4-0.7	<QL	Fail/Fail/Pass <i>Similarly low</i>
Higher Order Structure	Secondary Structure: α helix and β sheets	FTIR	3	4:4:1	Visually Similar	Visually Similar	Visually Similar	Pass/Pass/Pass
		Far UV-CD	3	5:4:15	Visually Similar	Visually Similar	Visually Similar	Pass/Pass/Pass
	Tertiary Structure	Near UV CD	3	5:4:15	Visually Similar	Visually Similar	Visually Similar	Pass/Pass/Pass
		Intrinsic Fluorescence Spectroscopy	3	4:4:1	Visually Similar	Visually Similar	Visually Similar	Pass/Pass/Pass
	Melting Temperature	Differential Scanning Calorimetry	3	5:4:15	Visually Similar	Visually Similar	Visually Similar	Pass/Pass/Pass
Biological Activity	Fab	HUVEC Bioassay	1	46:10:43	84-125	85-106	87-127	Pass/Pass/Pass
		VEGFA165 Binding by ELISA	1	25:10:27	89-119	90-115	89-114	Pass/Pass/Pass
		VEGFA isoform 121, 189, and 206 binding by ELISA	3	6:6:6	% Binding Rel to RM from orig dataset n=1 121: 98 189: 99 206: 97 EC50 ng/mL from orig dataset 121: 31 189: 28 206: 24 Min/Max 121: 87/114 189: 93/118 206: 81/103	% Binding Rel to RM from orig dataset n=1 121: 98 189: 114 206: 96 EC50 ng/mL from orig dataset 121: 31 189: 25 206: 24 Min/Max 121: 98/107 189: 106/114 206: 96/121	% Binding Rel to RM from orig dataset n=1 121: 98 189: 115 206: 95 EC50 ng/mL from orig dataset 121: 29 189: 22 206: 23 Min/Max 121: 88/110 189: 99/115 206: 95/107	Similar IR Response 1/18/19 provided data from 5 additional lots. The results are similar for all three products for VEGFA 121 and 189. PF-06439535 binds with higher affinity to VEGFA 206, but based on no detectable effector functions, the difference is acceptable.

		VEGFA family specificity Binding to VEGFC and VEGFD ELISA	3	1:1:1	Visually Similar	Visually Similar	Visually Similar	Specific for VEGFA no binding to VEGFC or VEGFD
	Fc	FcRn by SPR	2	11:12:11	96-119	82-118	97-117	Pass/Pass/Pass <i>IR was sent for pairwise and response is incorporated above.</i>
		FcgRIIIa 158V %KD SPR	2	10:10:10	83-109	85-113	92-109	Pass/Pass/Pass <i>IR was sent for pairwise comparisons and results are incorporated above from 12/21/18 response.</i>
		FcgRIIIa 158F SPR	2	10:10:10	97-121	87-120	91-137	Pass/Pass/Pass <i>IR was sent for pairwise comparisons and results are incorporated above from 12/21/18 response.</i>
		FcgRI, RIIa (131H and 131R), RIIb, RIIIb by SPR	3	1:1:1	Visually Similar	Visually Similar	Visually Similar	Similar <i>IR was sent to request data from additional lots. See</i>

								<i>IR response in section 3.2.S.3.2.2</i>	
		C1q binding by ELISA	3	5:5:5	99-113%	99-113%r	103-112%	Similar	
		ADCC	DLD-1 (VEGF A secreting)	3	1:1:1	Visually Similar	Visually Similar	Visually Similar	Similar <i>No ADCC activity</i>
			SKV03 (VEGF A membrane bound)	3	1:1:1				<i>Data provided on 12/14/18, but sponsor used expired lots. IR for use of expired lots and SKOV-3 expression of membrane bound VEGFA was sent. See discussion in section 3.2.R.3.2.2</i>
		CDC	DLD-1 (VEGF A secreting)	3	1:1:1	Visually Similar	Visually Similar	Visually Similar	Similar <i>No CDC activity</i>
			SKOV-3 (VEGF A membrane bound)	3	1:1:1				<i>Data provided on 12/14/18, but sponsor used expired lots. See IR response in section 3.2.R.3.2.2</i>
Charge Heterogeneity	iCE	% Acidic peaks	2	46:12:44	25.4-32.6	22.0-28.0	24.0-33.6	Pass/Pass/Pass <i>IR was sent for pairwise comparisons and results are incorporated above from 12/21/18 response.</i>	

								<p><i>Note that EU data stated in this table are from 43 lots because charge variants were not assessed for EU lot B7227B08 according to raw data table in the Appendix. Also during verification of data I deleted one more lot that was overlooked by sponsor. Therefore, only 42 EU lots can be considered independent for charge variant analysis. The results do not change.</i></p>
		%Basic peaks	2	46:12:44	3.6-6.5	6.0-17.4	3.7-7.0	<p>Fail/Fail/Pass IR was sent for pairwise comparisons and results are incorporated above from 12/21/18 response. Note that basic fails due to higher C-terminal lysine residue and has no impact to safety and efficacy because the Lys residue is typically cleaved off in circulation once</p>

								<i>administered to patients.</i>
Product Purity	SE-HPLC	% Monomer	2	46:12:44	96.2-98.0	98.3-99.4	96.1-97.8	Fail/Fail/Pass <i>IR was sent for pairwise comparisons and results are incorporated above from 12/21/18 response. The difference in monomer is due to lot age and is considered acceptable</i>
		% HMMS	2	46:12:44	1.9-3.2	0.6-1.6	2.1-3.4	Fail/Fail/Pass <i>IR was sent for pairwise comparisons and results are incorporated above from 12/21/18 response. The difference in monomer was shown to be due to lot age and is considered acceptable</i>
	CGE reduced	% fragments	3	46:28:51	1.7-3.6	0.7-1.0	1.9-4.2	<i>PF-06439535 has less fragments due to difference in age of the products</i>
		% HC + LC	3	46:28:51	95.7-97.9	97.7-98.9	95.3-97.8	<i>PF-06439535 has less fragments due to age of the products</i>
	CGE nonreduced	% Intact IgG	3	46:28:51	95.7-97.7	96.6-98.0	94.8-97.4	<i>PF-06439535 has higher level of intact IgG due to</i>

								<i>the difference in age of the products</i>
Protein Concentration	UV 280		2	46:16:51	24.3-26.3	23.6-25.0	24.7-26.1	Pass/Fail/Pass IR was sent for pairwise comparisons and results are incorporated above from 12/21/18 response. See discussion for difference of PF compared to EU QR
Disulfide Bonds	LC/MS-non-reducing peptide mapping with Lys-C		Not tiered	1:1:1				Similar profiles and all 16 disulfide bonds were correctly linked
Comparative Forced Degradation	Temp (40°C) 1 month	iCE, SE-HPLC, CGE (reducing), Subvisible and potency	3	6:12:6 12 PF lots are from 5 independent lots				Similar Trends Decreasing trend in potency for US and EU; not so apparent potency decrease in PF; increasing trend in acidic peaks, HMMS, and fragments for all three products; note that the trends are not as dramatic for PF for HMMS and fragments
	Light (5.1 klux) and 25°C for 14 days	iCE, SE-HPLC, CGE (reducing), Subvisible potency, and peptide mapping	3	1:2:1 2 DP lots for PF represented 100 and 400 mg/vial and US=400 mg/mL EU=100 mg/mL				Similar Trends Similar degradation pathway as elevated temperature with the addition of met-oxidation in peptide H16ox (255-280),

								<i>H26 (347-366), and H31 (421- 445)</i>
	Phosphate Buffer pH 7.5; 14 days at 40°C	iCE, SE-HPLC, CGE (reducing), Subvisible particles, potency, and peptide mapping	3	1:2:1 2 DP lots for PF represented 100 and 400 mg/vial and US Avastin=400 mg/mL EU Avastin=100 mg/mL				<i>Similar Trends Similar degradation pathway as elevated temp except the degree of degradation was also similar for PF. Increase in deamidation peptide H2 (44-55), H4 (77-98), H8H9 (154-216), and H28 (377-398) and increase in glutamic acid cyclization H1(1-43)</i>
	Peracetic Acid	FcRn binding, potency, peptide mapping	3	1:1:1				<i>Similar Trends Predominantly increase in met-ox (H16, H26, and H31 (met 258, 364, and 434), which decreased FcRn binding. Potency was unaffected.</i>

3.2.R.3.2.1 Primary Structure and Posttranslational Modifications

The sponsor used several methods to determine the primary structure and post-translational modifications of PF-06439535, US-Avastin, and EU-Avastin. Single lots of each product were used to determine the complete amino acid sequence, PF-06439535 lot 128926-30, US-Avastin lot 453807, and EU-Avastin lot H0150. Intact mass using nanoelectrospray ionization mass spectrometry (MS) (nano-ESI MS) and LC/MS peptide mapping using Lys-C (reduced and unreduced) were determined using the same single lots shown above for amino acid determination. For subunit analysis, four lots of PF-06439535 and US-Avastin were used, whereas up to 13 lots of EU-Avastin were used (see Table 3.2.R.3.7-10 provided in section 3.2.R.3.7).

Amino Acid Analysis

Pfizer took a stepwise approach to determine the primary structure, beginning with literature and patent information searches for bevacizumab (patent number 2002/0032315 A1 (Baca et al., 2002)). The sequence obtained in this manner was then confirmed experimentally using LC-MS/MS-peptide mapping with multiple proteases (trypsin with/without PNGase, chymotrypsin, Lys-C, Asp-N, and V8) and automated Edman degradation. To differentiate between lys and gln residues that are both 128 Da, the peptide maps containing lysine residues were guanidinated to create a homoarginine side chain that would add +42 Da onto the peptide with lys residue and isomeric leucine and isoleucine differentiation was performed using a proprietary software algorithm that incorporates information from local sequence environment, extensive antibody database, and yields the probability of occurrence for each residue. Edman degradation sequencing provided 100% sequence coverage for all three products (amino acid sequences for the light and heavy chain were provided but not shown in the review).

Intact Molecular Mass

Intact molecular masses of the three products were determined using nanoelectrospray ionization MS (nano-ESI MS) with a high-resolution, hybrid quadrupole time-of-flight MS with a mass accuracy of ± 50 ppm. The results showed three major isoforms that corresponded to different N-linked glycan species, G0F/G0F, G0F/G1F, and G1F/G1F. Differences in the levels of C-terminal lys residue linked isoform and Man5/Man5 were observed between PF-06439535 and the Avastin lots (US-Avastin and EU-Avastin), in which PF-06439535 showed higher levels of the isoforms (see figure and table below; peaks 1 and 2 representing Man5 and C-terminal lys are highlighted in yellow).

Figure 3.2.R.3.2.1-4. Analysis of Intact PF-06439535, Bevacizumab-US, and Bevacizumab-EU by nanoESI MS

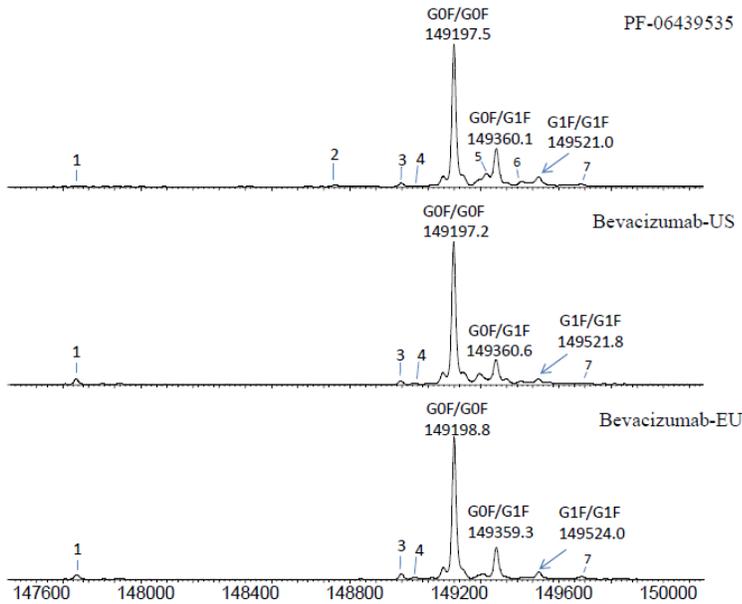


Figure 3.2.R.3.2.1-4 Deconvoluted, zero-charge mass spectra for intact PF-06439535, bevacizumab -US, and bevacizumab -EU.

Table 3.2.R.3.2.1-3. Comparison of Intact Isoforms Observed in PF-06439535, Bevacizumab-US, and Bevacizumab-EU

Isoform ^a	Relative Abundance ^b			Results
	PF-06439535	Bevacizumab-US	Bevacizumab-EU	
G0F/G0F	Major (63%)	Major (76%)	Major (72%)	Major glycoform for all three products
G0F/G1F	Minor (16%)	Minor (13%)	Minor (16%)	Minor glycoform for all three products
G1F/G1F	Trace (4%)	Trace (4%)	Trace (4%)	Trace glycoform for all three products
1-G0F/unoccupied	Trace (1%)	Trace (3%)	Trace (2%)	Trace glycoform for all three products
2-Man5/Man5	Trace (1%)	ND ^c	ND	Trace glycoform in PF-06439535; not detected in bevacizumab-US, and bevacizumab-EU
3-G0F/G0F-GlcNAc	Trace (2%)	Trace (2%)	Trace (3%)	Trace glycoform for all three products
4-G0/G0F	Trace (1%)	Trace (1%)	Trace (1%)	Trace glycoform for all three products
5-G0F/G0F + 1K	Minor (9%)	ND	ND	Minor glycoform in PF-06439535; not detected in bevacizumab-US, and bevacizumab-EU

6- G0F/G0F + 2K	Trace (3%)	ND	ND	Trace glycoform in PF-06439535; not detected in bevacizumab-US, and bevacizumab-EU
7-G1F/G2F	Trace (1%)	Trace (1%)	Trace (2%)	Trace glycoform for all three products

b. All glycoforms contain two L chains, two H chains, and 16 disulfide bonds, with or without C-terminal lysine in the H chain as noted (+ 1 Lys and +2 Lys). All glycoforms are assessed relative to the three major N-linked glycoforms: G0F/G0F, G0F/G1F and G1F/G1F.

c. Abundance categories based on relative % abundance and assay capability. Major: >20%, Minor: 5 to 20%, Trace: <5%. Relative % abundance was calculated by normalizing the peak heights of each intact isoform on a scale between 0-100%: Relative % abundance for each intact isoform = peak height of the isoform / sum of peak heights for all isoforms. The relative % abundance values for all intact isoforms total 100%.

ND=Not detected

LC/MS-Subunit Analysis for Post-translational Modifications

Post-translational modifications and heterogeneity at the subunit level were determined using LC/MS method. The products were digested using IdeS (immunoglobulin-degrading enzyme of *Streptococcus pyogenes*) that cleaves monoclonal antibodies just below the hinge region at a specific G-G sequence motif to yield one Fab2 and two single chain Fc (scFc) fragments (see figure below). The resulting subunits were then reduced to generate light (L) chain (1-214), Fd' domain (1-242), and heavy (H) scFc fragments (243-452). After IdeS digestion and reduction, the samples are analyzed using RP-UHPLC coupled to ESI-QTOF MS.

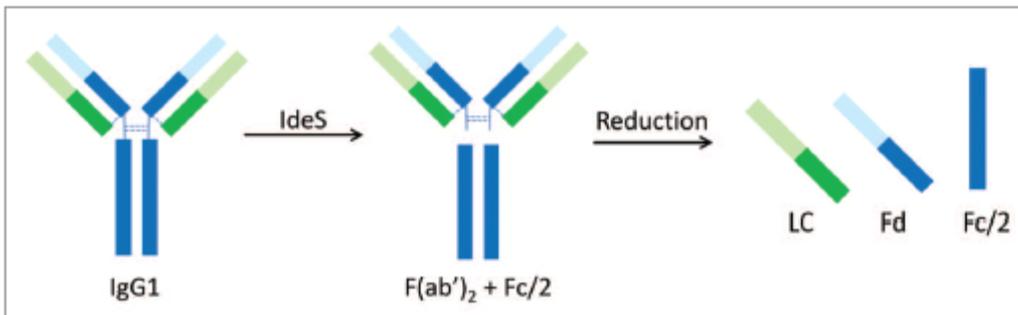


Figure 1. Limited proteolysis of IgG1 by IdeS.

Figure of IdeS digestion of IgG1 is taken from 2014 mAbs 6:4, 879-893

The results of LC/MS-subunit analysis confirmed similar molecular masses to the theoretical masses for the three different subunits, 25220.463 Da for scFc (G0F), 23436.434 Da for L chain, and 25929.659 Da for Fd'. The results of the measured masses were within ± 2 ppm (MS limitation) of each other, e.g. 25220.502 Da, 25220.503 Da, and 25220.509 Da for PF-06439535, US-Avastin, EU-Avatin, respectively for scFc (G0F) subunit. Small peak corresponding to glycation of the L chain and H chain were detected on the chromatograms (see figures below). In addition, the subunit analysis identified the higher levels of Man5 and C-terminal lys residue in PF-06439535 that were also observed during intact mass analysis by nanoESI method (see figure below).

Figure 3.2.R.3.2.1-5. LC/MS of scFc Domain of PF-06439535, Bevacizumab-US, and Bevacizumab-EU

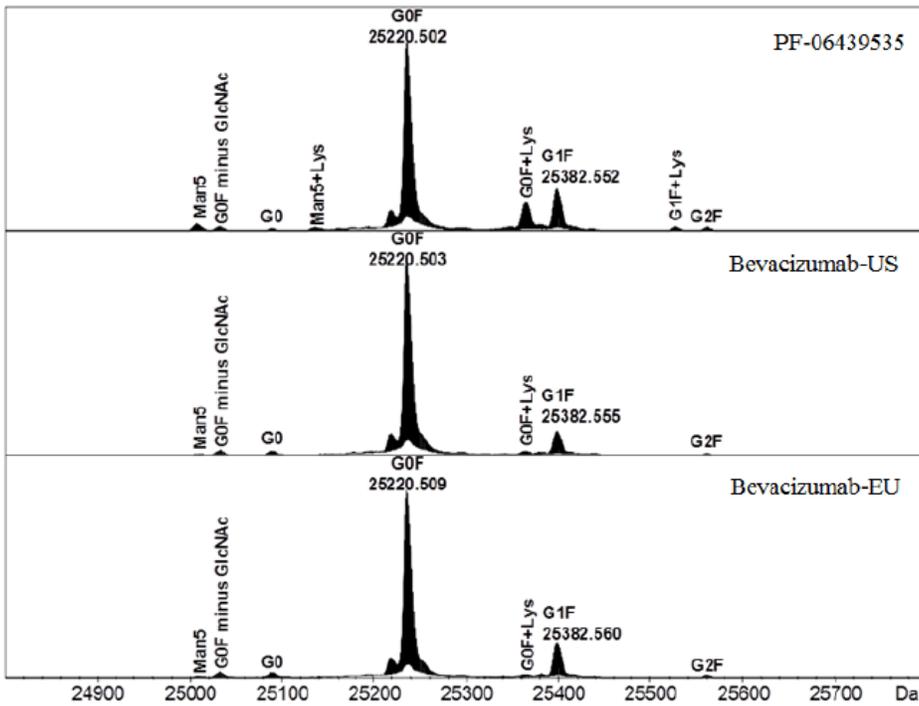


Figure 3.2.R.3.2.1-5 Zero-charge mass spectra of the H chain scFc domains for PF-06439535, bevacizumab-US, and bevacizumab-EU.

Figure 3.2.R.3.2.1-6. LC/MS of Fd' Domain of PF-06439535, Bevacizumab-US, and Bevacizumab-EU

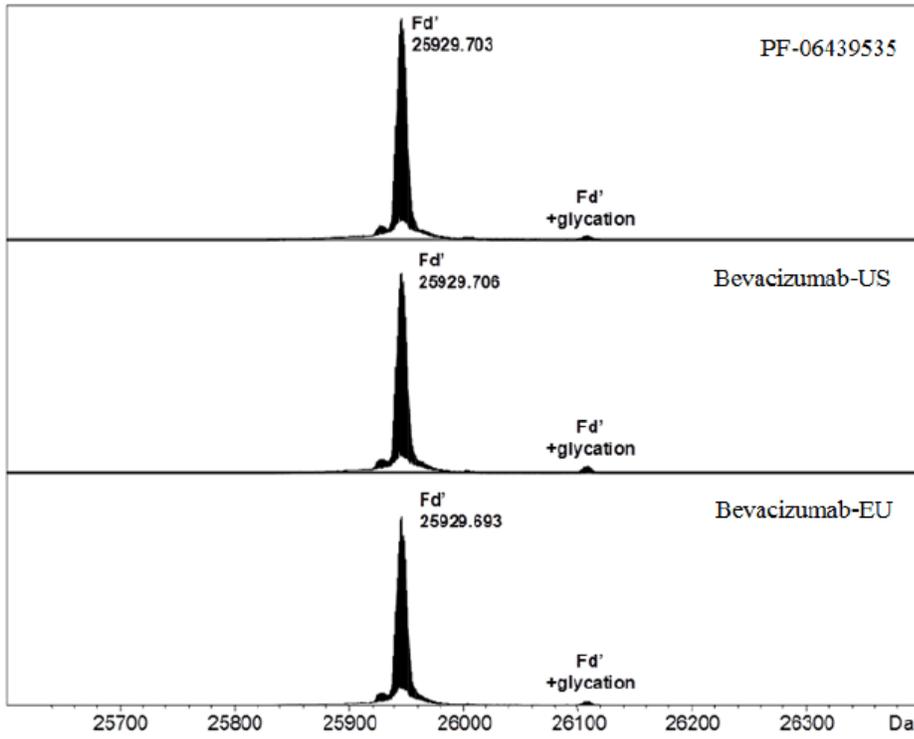


Figure 3.2.R.3.2.1-6 Zero-charge mass spectra the H chain Fd' domains for PF-06439535, bevacizumab-US, and bevacizumab-EU.

Figure 3.2.R.3.2.1-7. LC/MS of L Chain Subunit of PF-06439535, Bevacizumab-US, and Bevacizumab-EU

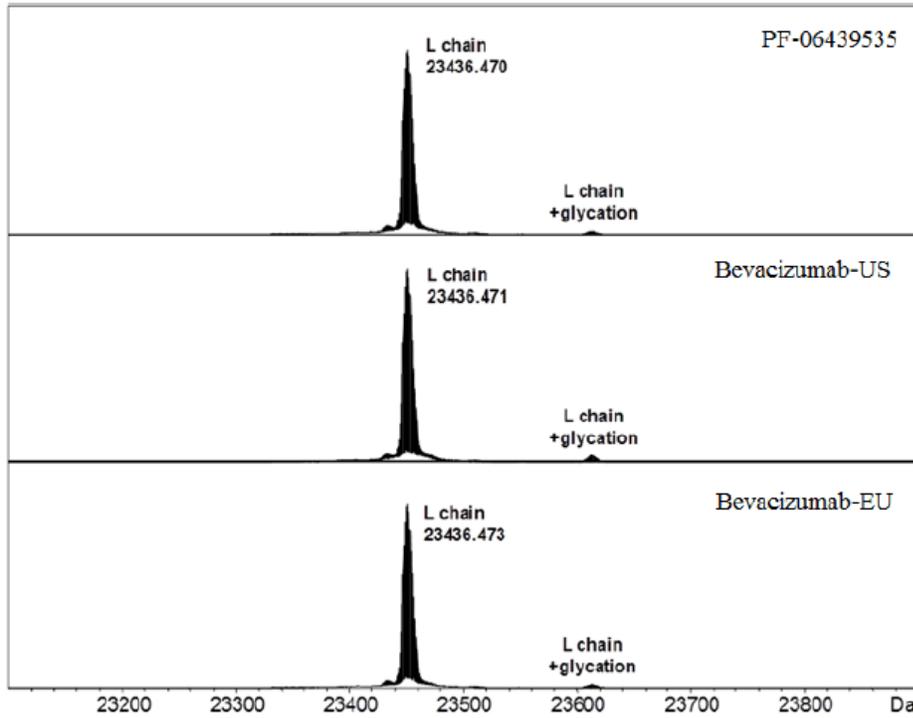


Figure 3.2.R.3.2.1-7 Zero-charge mass spectra of L chain subunits for PF-06439535, bevacizumab-US, and bevacizumab-EU.

The amounts of subunit isoforms are quantified below in Table 3.2.R.3.2.1-5 and show that PF-06439535 major form, G0F is 13% below US-Avastin and 8% below EU-Avastin. The difference is largely due to C-terminal lys attached G0F isoform present in higher amounts in PF-06439535.

Table 3.2.R.3.2.1-5. Comparison of Isoforms Observed in PF-06439535, Bevacizumab-US, and Bevacizumab-EU by LC/MS –Subunit Analysis

Subunit	Isoform	Relative Abundance ^a			Results
		PF-06439535	Bevacizumab-US	Bevacizumab-EU	
scFc	G0F	Major (67%)	Major (80%)	Major (75%)	Most abundant N-linked glycoform for all products
	G1F	Minor (15%)	Minor (10%)	Minor (14%)	Minor level N-linked glycoform in all products
	G2F	Trace (1%)	Trace (1%)	Trace (1%)	Trace level N-linked glycoform in all products
	Man5	Trace (2%)	Trace (<1%)	Trace (<1%)	Trace-level in all products
	G0F minus GlcNAc ^c	Trace (1%)	Trace (2%)	Trace (3%)	Trace-level in all products
	G0	Trace (1%)	Trace (2%)	Trace (2%)	Trace-level in all products
	Aglycosylated ^b	Trace (<1%)	Trace (3%)	Trace (3%)	Trace-level in all products
scFc+Lys	G0F	Minor (10%)	Trace (2%)	Trace (2%)	Minor level N-linked glycoform in PF-06439535 and trace level in bevacizumab-US and bevacizumab-EU
	G1F	Trace (1%)	Trace (<1%)	Trace (<1%)	Trace-level in all products
	Man5	Trace (1%)	Trace (<1%)	Trace (<1%)	Trace-level in all products
Fd ^d	Unmodified	Major (98%)	Major (96%)	Major (97%)	Most abundant isoform for all products
	Glycation	Trace (2%)	Trace (4%)	Trace (3%)	Trace-level in all products
L Chain	Unmodified	Major (97%)	Major (96%)	Major (97%)	Most abundant isoform for all products
	Glycation	Trace (<1%)	Trace (3%)	Trace (2%)	Trace-level in all products

a. Abundance categories based on relative % abundance and assay capability. Major: >20%, Minor: 5 to 20%, Trace: <5%. Relative % abundance was calculated by normalizing the peak heights of each subunit isoform on a scale between 0-100%: Relative % abundance for each subunit isoform = peak height of the isoform / sum of peak heights for all isoforms. The relative % abundance values for all subunit isoforms total 100%.

b. Aglycosylated scFc elutes as a separate peak from the N-linked glycosylated scFc species

LC/MS-Peptide Mapping (reduced and non-reduced) for Orthogonal Primary Structure and Post-Translational Modifications

The three products were reduced, alkylated, and digested using endoproteinase Lys-C to generate peptides for sequence and post-translational modifications. The resulting peptides provided 97.7% and 96.7% sequence coverage for L and H chains, respectively for all three products. In addition, the N-linked glycosylation site was confirmed to occur on Asn residue 303. The results showed low levels of methionine oxidation, less than equal to 3% in all three products (1-3% in PF-06439535, 1-2% in US-Avastin, and 1-2% in EU-Avastin), and succinimide intermediate, less than equal to 5% in all three products (2-3% in PF-06439535, 2-4% in US-Avastin, and 1-5% in EU-Avastin) were detected in all three products. Deamidation was also detected at higher amounts on peptide H28, 16%, 13%, and 12% in PF-06439535, US-Avastin, and EU-Avastin, respectively, whereas the other four peptides (H4, H18H19, H27, and H31) were less than equal to 2-7%. Pfizer did note that higher deamidation was observed due to the digestion conditions of high pH (8.2) over prolonged time period (18 h).

Reviewer Comments:

- *EU-Avastin lots stated in several tables, e.g. Table 3.2.R.3.7-5, 3.2.R.3.7-7, 3.2.R.3.7-9, 3.2.R.3.7-10, and 3.2.R.3.7-11 in section 3.2.R.3.7 are missing the last three alpha-numeric codes, such as B08 for H0150. Because some EU-Avastin lots used in the analytical similarity studies have same four alpha-numeric codes at the beginning, but different three alpha-numeric codes at the end it is unclear which lot was used. Pfizer should provide and update tables with all seven alpha-numeric codes for all EU-Avastin lots used in the analytical similarity assessment.*

IR Response 10/2/18:

Sponsor updated the missing codes for EU-Avastin lots in the indicated tables above.

Reviewer Comment: *The response is acceptable.*

- *The sponsor did not provide a justification for why more EU-Avastin lots were used for the subunit analysis by LC/MS. However raw data were provided in the appendix for all the lots used in the subunit analysis and supports the conclusion that no new species are present in PF-06439535 lots and slightly higher amounts of high mannose residues are present compared to US-Avastin and EU-Avastin lots. Therefore, the lack of justification for differences in the tested lot numbers is acceptable regarding isoform types.*
- *Table 3.2.R.3.2.1-5 shows subunit isoform levels in PF-06439535, US-Avastin, and EU-Avastin. It is unclear based on the description provided in the submission if the quantified values are derived from one lot or as many as 13 lots for EU-Avastin. IR was sent for Pfizer to provide a clarification on the number of lots used to provide the quantified numbers (see response below). Note also that the difference that accounts for the large difference in G0F levels is the difference in G1F level in PF-06439535 compared to US-Avastin in addition to C-terminal lys.*

IR Response 10/2/18:

Sponsor stated that the data reported in Table 3.2.R.3.2.1-5 is from a single lot, specifically reference standard lot 128926-30). The additional lots of PF-06439535, US-Avastin, and EU-Avastin (4, 5, and 13 lots respectively) were for profile comparisons and were not used for quantitation.

Reviewer Comment: *As a characterization assay the use of a single lot is acceptable. Regarding glycoform differences observed, see comment under N-glycan analysis.*

- *Mass accuracy of 50 ppm for intact monoclonal antibody with approximate weight of 150 kDa translates to ± 7.5 Da. In addition, for subunit analysis, the mass accuracy of 2 ppm translates to ± 0.05 Da for a mass of 25 kDa. Therefore, the results are accurate representation of the intact mass and subunit masses of the three products.*
- *See Reviewer comments in sections below for Man5 and C-terminal lysine residue differences and why the higher levels found in PF-06439535 do not detract from the conclusion that PF-06439535 is analytically similar to US-Avastin.*
- *Methionine oxidation, deamidation, and succinimide intermediates are located on the same peptides in all three products. In addition, the levels are comparably low in all three products, with the exception of deamidation, which is likely due to the elevated pH for the digestion condition. Therefore, the Lys-C peptide mapping experiment confirms that no new post-translation modifications are observed in PF-06439535 compared to US-Avastin and EU-Avastin.*

3.2.R.3.2.2 Biological Activity and Mechanism of Action-Introduction

The sponsor evaluated the similarity of the biological functions for PF-06439535, US-Avastin, and EU-Avastin that included binding to the target antigen, VEGFA, and the inhibition of endothelial cell proliferation. As both VEGFA binding and endothelial cell anti-proliferative activity represent the proposed mechanism of action for Avastin, the VEGFA binding and endothelial proliferation were evaluated by equivalence test because of their high risk to impact product efficacy. As part of the VEGFA binding studies, the similarity in the binding to VEGFA isoforms 121, 189, and 206 were also performed. Binding to additional VEGFA isoforms were evaluated using a graphical comparison..

Other biological activity assays examined in the analytical similarity study included comparisons of the binding activities of the Fc portion of PF-06439535, US-Avastin, and EU-Avastin to the neonatal Fc

receptor, FcRn, to the complement cascade component protein, C1q, and to FcγRIIIa. Only binding to FcRn was evaluated by quality ranges because of the potential impact to PK. C1q and FcγRIIIa (158V and 158F) binding were originally evaluated by graphical comparison and later evaluated by quality ranges following an IR. Pfizer also performed cell-based effector function analysis to further characterize and confirm the similarity of activities for the three products. The cell-based effector function assays, antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) were evaluated by graphical comparison..

The results of each testing approach to determine biological functions and the similarity of the three products are reviewed below.

3.2.R.3.2.2 Biological Activity-Functional Characterization of the Fab Domain

Proliferation Inhibition Bioassay (Potency)

Ten PF-06439535, 46 US-Avastin, and 43 EU-Avastin lots were used to determine the inhibition of endothelial cell proliferation. Human umbilical vein endothelial cells (HUVEC) that express VEGF receptors, VEGFR1 and VEGFR2 on the cell surface were used. The VEGFA165 isoform was used in the potency assay as the ligand.

Equivalency testing was applied to the potency results. The results shown below demonstrate statistical equivalence of PF-06439535 to US-Avastin, PF-06439535 to EU-Avastin, and EU-Avastin to US-Avastin.

Table 3.2.R.3.2.2-2. Summary of Descriptive Statistics for Inhibition of Cell Growth Activity using PF-06439535 and Bevacizumab-EU Independent Lots

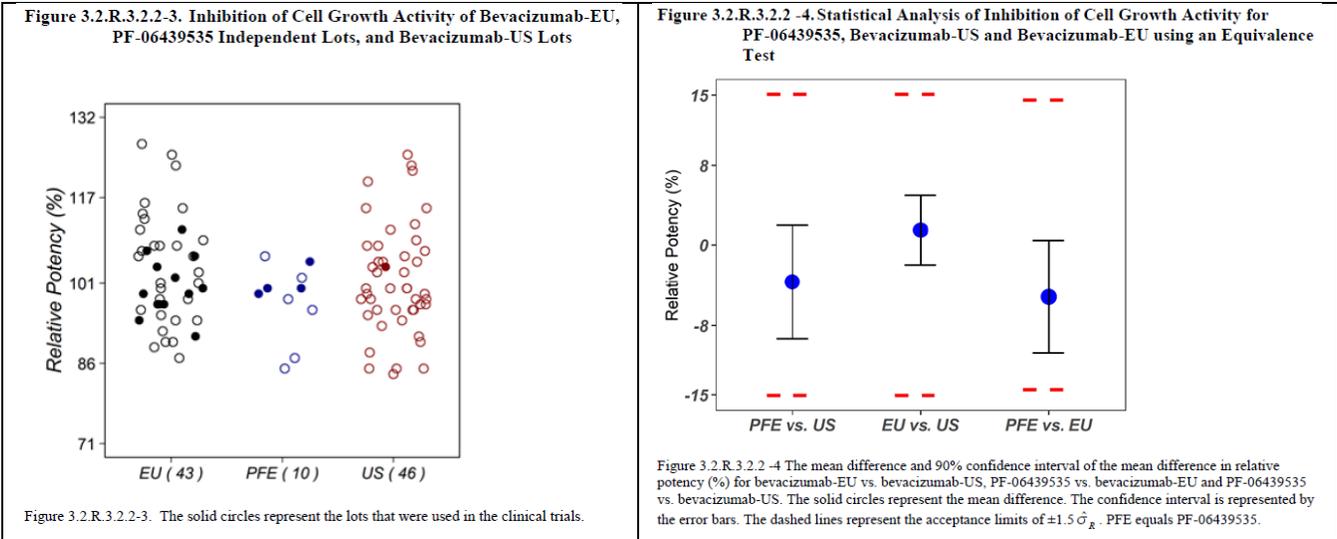
Relative Potency (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	43	103	9.7	9.4	87	127
Pfizer	10	98	6.9	7.1	85	106
US	46	101	10.0	9.9	84	125

N = sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Table 3.2.R.3.2.2 -3. Summary of Equivalence Testing for Inhibition of Cell Growth Activity (% Relative Potency)

Comparison	Reference SD (%)	Mean Difference	90% Confidence Interval of the Mean Difference	Equivalence margin of $1.5 \hat{\sigma}_R$	Pass or Fail Equivalence
PFE vs. US	10.04	-3.68	(-9.4 , 2.05)	15.06	Pass
EU vs. US	10.04	1.50	(-1.97 , 4.97)	15.06	Pass
PFE vs. EU	9.66	-5.18	(-10.79 , 0.43)	14.49	Pass

SD = standard deviation; PFE = PF-06439535; EU = Bevacizumab-EU; US = Bevacizumab-US



Reviewer Comments:

- The statistics used to determine equivalence will be reviewed by CDER’s CMC statisticians.
- The results indicate similar anti-proliferative effects by all three products.

VEGFA-165 Binding by ELISA

Ten PF-06439535, 25 US-Avastin, and 27 EU-Avastin lots were used to determine binding to recombinant VEGFA165. The method is an ELISA that uses VEGFA165 to coat wells and capture binding antibodies. The bound antibodies are detected by Peroxidase AffiniPure F(ab’)₂ Fragment Goat Anti-Human IgG Fcγ₁ Fragment Specific antibody. The relative binding of test samples is calculated against a dose-response curve of the PF-06439535 reference standard.

Equivalency testing was applied to the ELISA binding results. The results shown below demonstrate statistical equivalence of PF-06439535 to US-Avastin, PF-06439535 to EU-Avastin, and EU-Avastin to US-Avastin.

Table 3.2.R.3.2.2-4. Summary of Descriptive Statistics for VEGF₁₆₅ Binding Activity of Bevacizumab-EU, PF-06439535, and Bevacizumab-US*

Relative Potency (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	27	101	6.6	6.5	89	114
Pfizer*	10	103	9.6	9.3	90	115
US	25	106	8.0	7.6	89	119

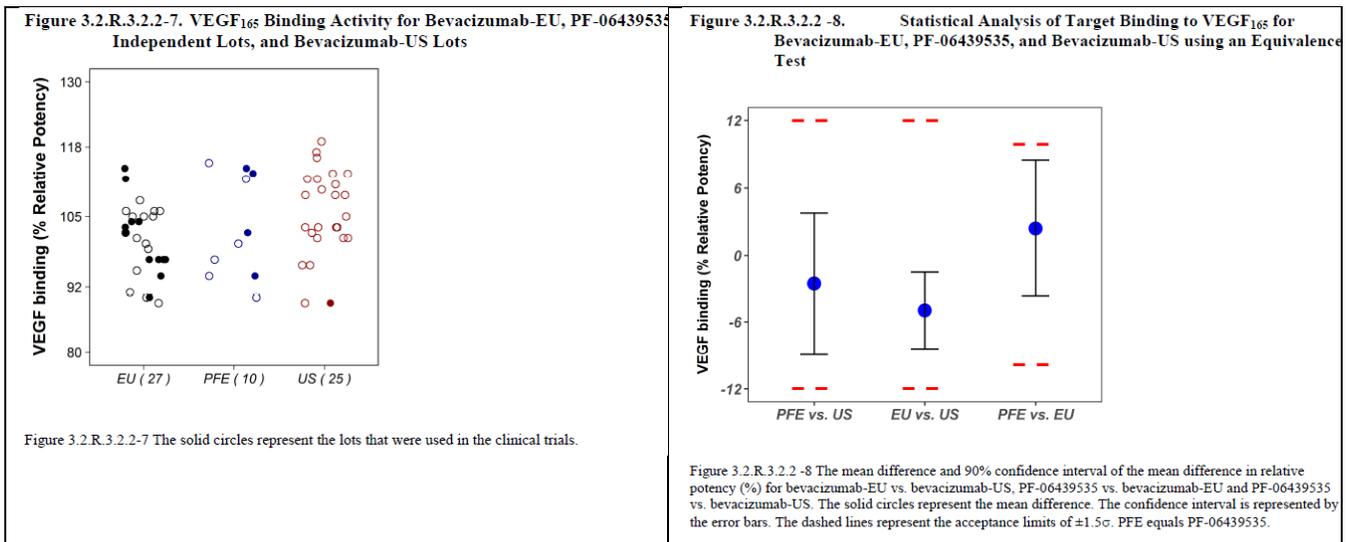
N = sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

*This table was modified by reviewer to insert Pfizer lot value of 10 and results from 10 lots and not the original 23 lots

Table 3.2.R.3.2.2 -6. Summary of Equivalence Testing for Target Binding to VEGF₁₆₅ (% Relative Potency) of PF-06439535, Bevacizumab-US, and Bevacizumab- EU

Comparison	Reference SD (%)	Mean Difference	Confidence Interval of the Mean Difference	Equivalence margin of $1.5 \hat{\sigma}_R$	Pass or Fail Equivalence
PFE vs US	7.99	-2.58	(-8.93 , 3.77)	11.98	Pass
EU vs. US	7.99	-4.98	(-8.4 , -1.56)	11.98	Pass
PFE vs. EU	6.57	2.40	(-3.68 , 8.47)	9.86	Pass

SD = standard deviation; PFE = PF-06439535; EU = Bevacizumab-EU; US = Bevacizumab-US



Reviewer Comments:

- The statistics used to determine equivalence will be reviewed by CDER’s CMC statisticians.
- The results indicate similar binding by all three products.

VEGFA Isoform Binding by ELISA

Initial data for binding to VEGFA isoforms 121, 189, and 206 were conducted using one lot each of PF-06439535, US, and EU-Avastin. The PF-06439535 used in the study is not stated in the review, but the US-Avastin and EU-Avastin lots used in the binding studies were, 453807 and lot H0150B08, respectively. The relative binding of test samples is calculated against a dose-response curve of the PF-06439535 reference standard, 128926-30, derived from the pilot scale lot 12P138K603.

Reviewer Comment: The use of a single lot is not acceptable as this is to demonstrate similarity for binding affinity to other VEGFA isoforms with different heparin binding affinities. Therefore, IR was sent to request additional data from additional lots and response was received 1/18/19. The data showed similar binding affinities by all three products for the VEGFA isoforms except for VEGFA 206 that showed a higher binding affinity by PF-06439535 lots by ~10% (see summary table above for results). The ~10% higher binding could result in a difference in effector function activities since this version of VEGFA is membrane associated. However, the results from the ADCC and CDC activities with SKOV3

cells showed an absence of effector functions. Thus, the higher binding by PF-06439535 to VEGFA 206 isoform would not preclude the conclusion that PF-06439535 is highly similar to US-Avastin.

The dose-response curves of the US-Avastin and EU-Avastin lots provided showed that the curves were superimposable to PF-06439535 reference standard lot 128926-30. Pfizer also included a duplicate assessment of PF-06439535, US-Avastin, and EU-Avastin lot to VEGFA165 that were not included in the equivalence statistical analysis. The binding data shown in Table 3.2.R.3.2.2-7 show high percent binding by PF-06439535 and EU-Avastin for VEGFA165 and VEGFA189. The EC50 results are similar among the three products for all four VEGFA isoforms.

Table 3.2.R.3.2.2-7. Percent Relative Potency of VEGF Binding Activity from PF- 06439535, Bevacizumab-US and Bevacizumab-EU for VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉, and VEGF₂₀₆.

Material Tested	Average Relative Potency (%) ^c			
	VEGF ₁₂₁	VEGF ₁₆₅ ^a	VEGF ₁₈₉	VEGF ₂₀₆
PF-06439535	98	117 ^b	114	96
Bevacizumab-US	98	99	99	97
Bevacizumab-EU	98	121 ^b	115	95

- a. VEGF₁₆₅ was included in this side-by-side experiment to investigate the binding of bevacizumab to the other major human VEGF isoforms in addition to the tier 1 similarity assessment. The VEGF₁₆₅ data from this experiment was not included in the tier 1 statistical analysis of VEGF₁₆₅ binding (section 3.2.R.3.2.2.1).
- b. Relative Potency (%) outside the max listed in Table 3.2.R.3.2.2-4.
- c. n= 3.

Table 3.2.R.3.2.2-8. EC₅₀ of VEGF Binding Activity from PF-06439535, Bevacizumab- US and Bevacizumab-EU for VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉, and VEGF₂₀₆.

Material Tested	Average EC ₅₀ (ng/mL) ^a			
	VEGF ₁₂₁	VEGF ₁₆₅	VEGF ₁₈₉	VEGF ₂₀₆
PF-06439535	31	24	25	24
Bevacizumab-US	31	27	28	24
Bevacizumab-EU	29	20	22	23

- a. n= 3.

Reviewer Comments:

- The PF-06439535 lot information was not provided, and an IR was sent to request the information.

IR Response 10/2/18:

Sponsor stated that the PF-0439535 lot used in the VEGFA isoform studies was the reference standard, 128926-30.

Reviewer Comment: The response supports the fact that only a single lot was used to determine binding affinities to VEGFA isoforms. IR will be sent to request additional VEGFA isoform study include more than a single lot. See comment above.

- *The high percent binding for VEGFA165 and VEGFA189 could still be within assay variability and also due to the use of a single lot. The standard deviation of VEGFA165 binding shown for the tier 1 analysis showed for 10 lots a value of 9.6%. In addition, the maximum value was recorded as 115%, which is similar to 117% reported in Table 3.2.R.3.2.2-7. Therefore, for the purpose of determining if PF-06439535 binds to other VEGFA isoforms similarly to US-Avastin, the single lot binding data would not be sufficient to determine if the PF-06439535 has similar binding activities. IR was sent to request data from additional lots to confirm similar binding affinities to other VEGFA isoforms (see above).*

3.2.R.3.2.2 Biological Activity-Functional Characterization of the Fc Domain

ADCC Activity in DLD-1

VEGFA secreting DLD-1 (adenocarcinoma) cells were used to determine whether ADCC activity could be induced by PF-06439535, US-Avastin, and EU-Avastin and if so determine if the levels of ADCC activities are similar. Peripheral blood mononuclear cells (PBMC) were used as the effector cells. HER2 expressing cells line, SKBR3 and an anti-HER2 monoclonal antibody (trastuzumab) were used as positive control. One lot each of PF-06439535, US- and EU-Avastin lots were used in the ADCC study.

Reviewer Comments:

- *All three products demonstrated a lack of ADCC activity. The results support the conclusion that the three products have similar lack of effector functions leading to ADCC activity in cells that are known to secrete VEGFA.*
- *IR was sent regarding ADCC activity in cells that are known to secrete membrane bound form of VEGFA.*

IR Response 12/14/18 Query#2: Human ovarian carcinoma cell line SKOV-3 was acquired byPfizer as it is known to express membrane-bound VEGFA to evaluate the ability of PF-06439535 (128926-30), US-Avastin (453807), and EU-Avastin (H0150B08) to induce ADCC activity. PBMC were used as the effector cells and trastuzumab was used as the positive control. The results showed ADCC was not induced by PF-06439535, US-Avastin, and EU-Avastin lots (see data below).

Figure 1. ADCC Activity (RLU) of PF-06439535, Bevacizumab-US, Bevacizumab-EU, and Trastuzumab (positive control)

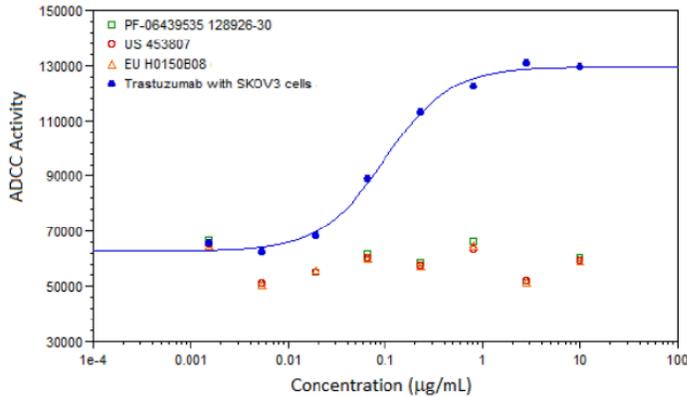


Figure 1. Bevacizumab materials tested were PF-06439535 reference material lot 128926-30, bevacizumab-US lot 453807 and bevacizumab-EU lot H0150B08. RLU = relative luminescence units.

Reviewer Comment: According to the lot information provided section 3.2.R.3.5-1, the US-Avastin lot and the EU-Avastin lots used in the ADCC study are expired. The US-Avastin lot 453807 expired November 2013 and EU-Avastin lot H0150B08 expired April 2014. A justification for using expired lots was not provided. In addition, method qualification report or technical report (b) (4) 100168142 did not include information on whether SKOV3 cells were confirmed to express membrane bound VEGFA (data were available for DLD for the presence of soluble VEGFA expression). IR was sent to justify the use of expired lots for the ADCC study (i.e. show maintenance of product quality) and for data to show that SKOV3 cells express membrane bound VEGFA.

IR Response 2/6/19 Query #3: Sponsor stated that large quantities of the US-Avastin lot 453807 and EU-approved bevacizumab lot H0150B08 were purchased and aliquoted into small volume prior to expiry and stored frozen between -60°C and -90°C to retain the product quality observed at the time of freezing. In addition, stability of the US and EU lots used at the time of ADCC and CDC assay experiment in SKOV3 cells were assessed and data from SE-HPLC, iCE, and CGE were provided. The results showed that the US and EU lots used in the recent ADCC and CDC experiments showed ≤1% increase in HMMS and fragments.

Reviewer Comment: The stability data provided supports the use of the expired lots in the ADCC and CDC assays. Although no binding data were provided in the IR response, the section containing data supporting sample handling did look at potency after freeze thaw and extended stability data were provided to show no impact. Additionally, freeze thaw studies showed that freeze/thaw did not impact glycoforms.

IR Response 2/13/19 Query #2 from IR sent 1/29/19: Sponsor provided data to show that the SKOV3 cells used in the ADCC and CDC assay expressed membrane bound VEGFA using flow cytometry.

Reviewer Comment: The response is acceptable.

Binding to FcγRIIIa (158V and 158F) by SPR

Binding to FcγRIIIa was evaluated by graphical comparison. One lot each of PF-06439535, US-Avastin, and EU-Avastin were used to determine binding kinetics to FcγRIIIa (158V and 158F). The results showed slightly higher binding affinity to FcγRIIIa 158F variant by PF-06439535 (K_D of 0.524 μM vs 0.707 μM for PF-06439535 and US-Avastin respectively), whereas the K_D values were similar for FcγRIIIa 158V variant.

Table 3.2.R.3.2.2-1. Binding of PF-06439535, Bevacizumab-US, and Bevacizumab-EU to FcγRIIIa 158V and 158F

Sample	FcγRIIIa 158F ^c				FcγRIIIa 158V ^c			
	k_a , 1/Ms	k_d , 1/s	K_D , M	% K_D	k_a , 1/Ms	k_d , 1/s	K_D , M	% K_D
PF-06439535 RM ^a	2.80E+04	1.44E-02	5.24E-07	100	1.21E+05	1.13E-02	1.03E-07	100
PF-06439535 IC ^b	2.79E+04	1.58E-02	5.70E-07	92	1.14E+05	1.04E-02	1.06E-07	97
Bevacizumab-US	2.63E+04	1.77E-02	7.07E-07	75	7.50E+04	6.44E-03	9.81E-08	105
Bevacizumab-EU	2.68E+04	1.63E-02	6.30E-07	83	6.68E+04	6.24E-03	1.04E-07	100

- a. Reference material PF-06439535 128926-30 was set as 100%.
- b. PF-06439535 128926-30 was tested against itself as internal control (IC).
- c. n= 3.

Table 3.2.R.3.2.2-2. Average Relative FcγRIIIa Binding Affinity (% K_D) of Bevacizumab-EU to Bevacizumab-US

Sample	Average Relative K_D (%)	
	FcγRIIIa 158F	FcγRIIIa 158V
Bevacizumab-US	100	100
Bevacizumab-EU	112	95

Note that this is reported relative to PF-06439535 reference standard

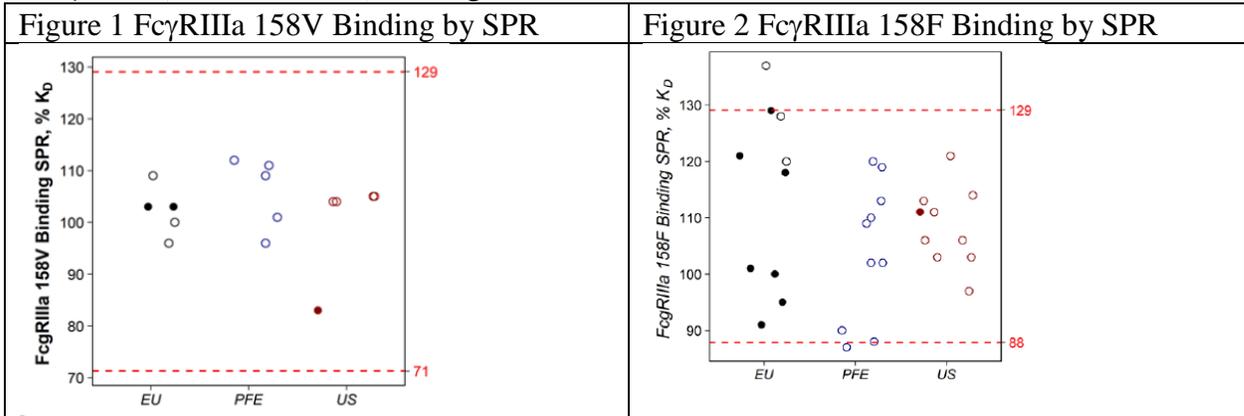
Reviewer Comments:

- Given the lack of method qualification data for the SPR method used to evaluate FcγRIIIa binding, and the use of a single lot of each product, it cannot be concluded that the three products have similar binding affinities.
- IR was sent to request binding affinity analysis to be evaluated by quality ranges because of the existence of membrane bound form of VEGFA and the potential safety concern due to unwanted effector functions.

IR Response 10/2/18 and 10/31/18:

Pfizer evaluated binding to FcγRIIIa (158F and 158V variants) by SPR and analyzed the data using quality ranges. A total of 5 lots of PF-06439535, US-Avastin, and EU-Avastin each were

evaluated for FcγRIIIa 158V variant and 10 lots each for FcγRIIIa 158F variant. The percent K_D results for FcγRIIIa (158V) were 106, 102, and 100 and for FcγRIIIa (158F) were 104, 114, and 109 for PF-06439535, EU-Avastin, and US-Avastin, respectively. Statistical analysis showed that PF-06439535 falls within US-Avastin quality ranges of 71-129% for FcγRIIIa 158V and 88-129%, determined using the mean \pm 3SD of US-Avastin lots. Unlike, FcγRIIIa 158V binding, the FcγRIIIa 158F binding showed a correlation with the level of afuscoylation. The scatter plots of FcγRIIIa (158V and 158F) binding are shown below.



Reviewer Comment: The response is acceptable. In addition, IR response 12/21/18 showed that PF-06439535 was within EU QR and EU-Avastin lots were within US-Avastin QR.

- IR was sent to also identify the site of FcγRIIIa testing because Table 3.2.R.3.7-12 did not identify the testing site of lots used in the study. See response in section 3.2.R.3.7
- IR was sent to request method qualification data for the SPR assay.

IR Response 10/2/18 Query #5: The method qualification report was provided and showed adequate precision and accuracy.

CDC Activity in DLD-1

VEGFA secreting DLD-1 (adenocarcinoma) cells were used to determine whether CDC activity could be induced by PF-06439535, US-Avastin, and EU-Avastin and if so determine if the levels of CDC activities are similar. Human complement was used in the CDC assay. Ramos cell line that express CD20 and rituximab (anti-CD20) monoclonal antibody were used as positive control. One lot each of PF-06439535, US- and EU-Avastin lots were used in the CDC study.

Reviewer Comments:

- All three products demonstrated a lack of CDC activity. The results support the conclusion that the three products have similar lack of effector functions leading to CDC activity in cells that are known to secrete VEGFA.
- IR was sent regarding CDC activity in cells that are known to secrete membrane bound form of VEGFA.

IR Response 12/14/18 Query #2: Human ovarian carcinoma cell line SKOV-3 was acquired byPfizer as it is known to express membrane-bound VEGFA to evaluate the ability of PF-06439535 (128926-30), US-Avastin (453807), and EU-Avastin (H0150B08) to induce CDC activity. The

results showed CDC was not induced by PF-06439535, US-Avastin, and EU-Avastin lots (see data below).

Figure 2. CDC Activity (RLU) of PF-06439535, Bevacizumab-US, Bevacizumab-EU, and Rituximab (positive control)

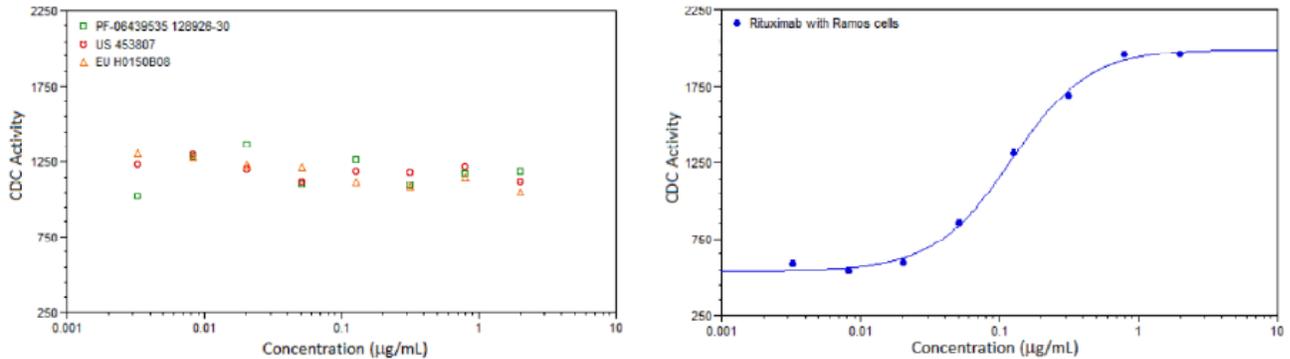


Figure 2. Bevacizumab materials tested were PF-06439535 reference material lot 128926-30, bevacizumab-US lot 453807 and bevacizumab-EU lot H0150B08. RLU = relative luminescence units.

Reviewer Comment: Similar comment as ADCC using membrane associated VEGFA cell line. Sponsor used expired US and EU-Avastin lots to conduct CDC activity assay. See IR response above under ADCC.

C1q Binding by Immunoassay (Meso Scale Discovery Technology (MSD))

MSD method was used in which PF-06439535 or US-Avastin, or EU-Avastin proteins are coated onto 96-well plates and incubated with C1q. Bound C1q protein was detected using goat anti-human C1q antibody followed by anti-goat sulpho tagged antibody. The sample binding curves were compared to PF-06439535 reference standard curve and the results were reported as percent relative binding. One lot each of PF-06439535, US- and EU-Avastin lots were used in the C1q binding study. The results showed superimposable binding curves and relative binding percentages of 98% and 101% for US-Avastin and EU-Avastin, respectively.

Reviewer Comments:

- The use of single lot each for C1q binding is insufficient to show similar binding affinities. IR was sent to request data from additional lots.

IR Response 2/6/19 Query #1: Sponsor provided data from 5 lots using a different qualified method, an ELISA. The results showed that PF-06439535, US-Avastin lots, and EU-approved bevacizumab lots were similar with 99-113%, 99-113%, and 103-112% of the reference material, respectively.

Reviewer Comment: The results demonstrate that the difference in total galactosylation do not lead to a difference in C1q binding.

- PF-06439535 reference standard binding curve and percent data to itself were provided. However, Table 3.2.R.3.7-12 shows that potentially another lot, 13J138K003, was used for C1q binding. IR was sent for clarification and to update the section with data from the 13J138K003 lot.

IR Response 10/2/18:

Sponsor confirmed that PF-06439535 lot 13J138K003 was used for CDC, C1q binding, and FcγR receptors (RI, RIIa, RIIb, and RIIIb) analysis and results were provided.

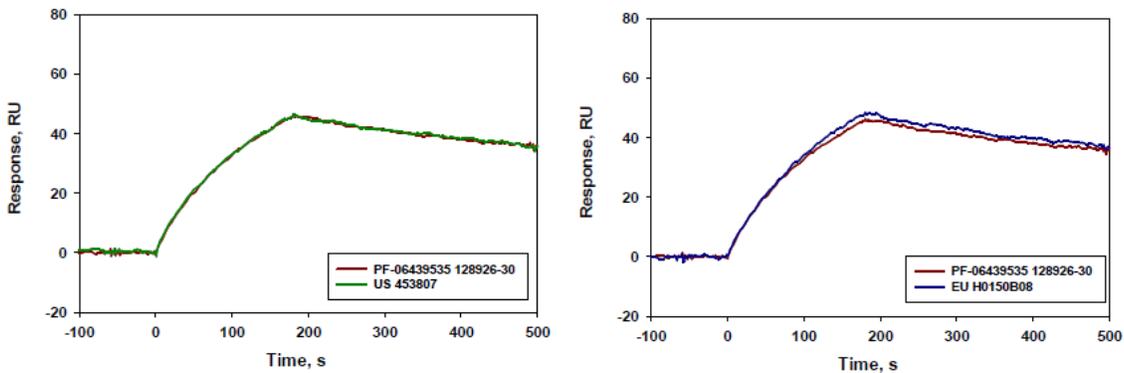
Reviewer Comment: *The results showed similar binding affinities for the Fcγ receptors and lack of CDC activity for the additional lot. In total, two PF-06439535 lots including the reference standard were used to assess CDC and C1q binding.*

Binding to Fcγ Receptors (RI, RIIa, RIIb, and RIIIb) by SPR

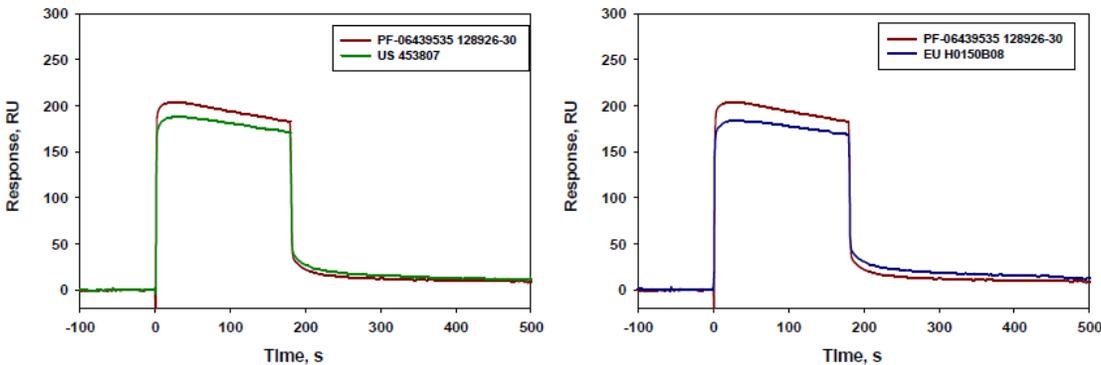
One lot each of PR-06439535, US-Avastin, and EU-Avastin were used to evaluate binding kinetics in triplicates for FcγRI and FcγRIIa (131H and 131R) and duplicates for FcγRIIb and FcγRIIIb. The lots used were PF-06439535 ref std, 128926-30, US-Avastin, 453807, and EU-Avastin, H0150B08 lots. Sensorgrams and K_D data showed higher association by US- and EU-Avastin for FcγRIIb and FcγRIIIb and the reverse results with FcγRIIa 131H and 131R, whereas binding to FcγRI was similar (see data below). In addition to K_D values, sponsor provided k_a , and k_d data for FcγRI binding.

Figure 3.2.R.3.2.2-5. SPR Sensorgrams of PF-06439535, Bevacizumab-US, and Bevacizumab-EU Binding to Fcγ Receptors

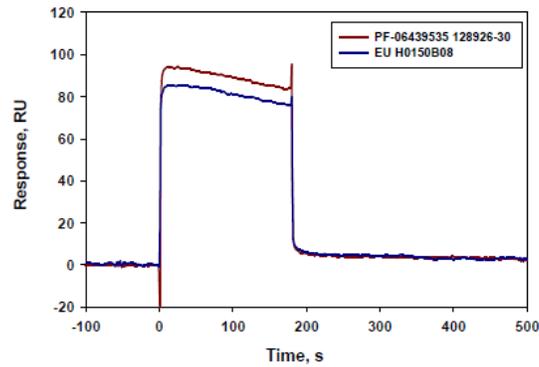
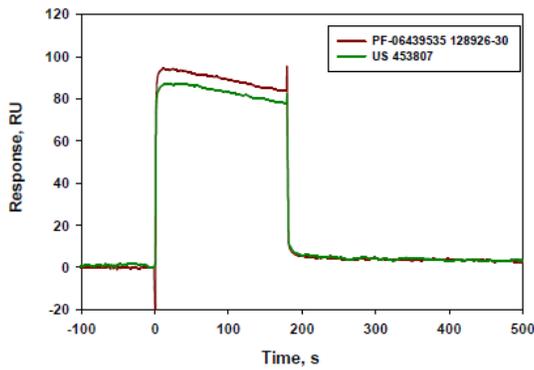
A. Binding to FcγRI



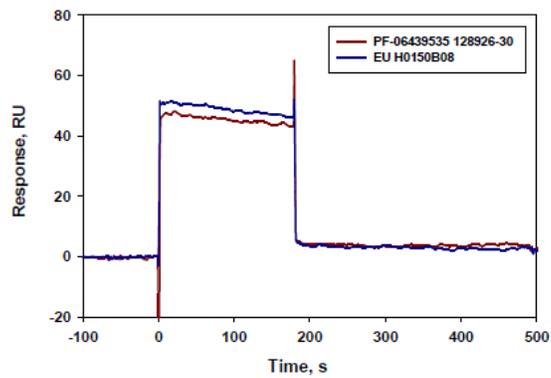
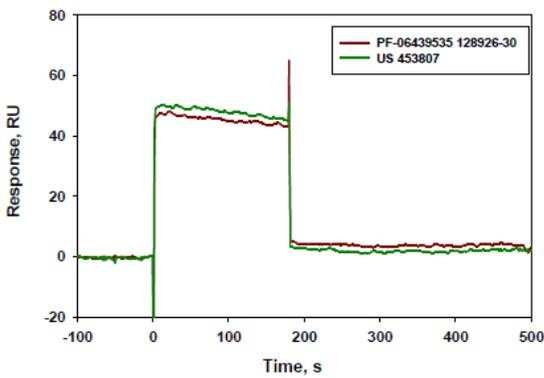
B. Binding to FcγRIIa 131H



C. Binding to FcγRIIa 131R



D. Binding to FcγRIIb



E. Binding to FcγRIIIb

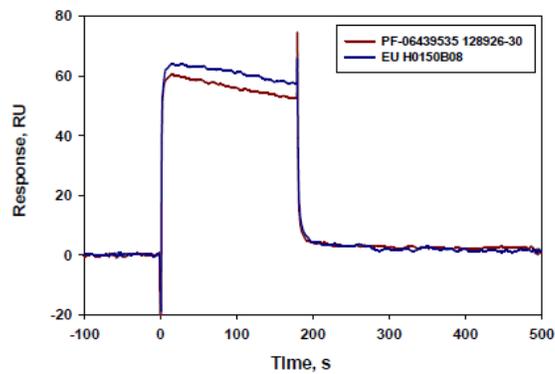
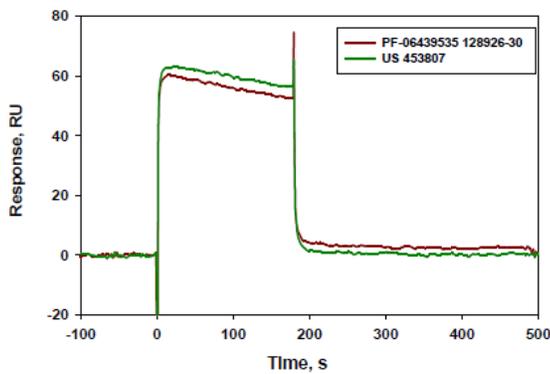


Figure 3.2.R.3.2.2-5. SPR sensorgrams of bevacizumab-US, and bevacizumab-EU with the reference material PF-06439535 128926-30 (100 nM for FcγRI, 2,500 nM for FcγRIIa 131H and 131R, 5,000 nM for FcγRIIb and FcγRIIIb).

Table 3.2.R.3.2.2-4. Binding of PF-06439535, Bevacizumab-US, and Bevacizumab-EU to FcγRI, FcγRIIa 131H and 131R, FcγRIIb, and FcγRIIIb

Sample	FcγRI ^a				FcγRIIa 131H ^{a,d}		FcγRIIa 131R ^{a,d}		FcγRIIIb ^{d,e,f}	FcγRIIIb ^{d,e,f}
	k _{on} , 1/Ms	k _{off} , 1/s	K _D , M	% K _D	K _D , M	% K _D	K _D , M	% K _D	K _D , M	K _D , M
PF-06439535 RM ^b	9.55E+04	9.05E-04	9.66E-09	100	3.00E-06	100	4.13E-06	100	> 1.0E-05	> 1.0E-05
PF-06439535 IC ^c	8.93E+04	9.12E-04	1.04E-08	94	2.80E-06	107	3.75E-06	110	> 1.0E-05	> 1.0E-05
Bevacizumab-US	9.47E+04	9.20E-04	9.93E-09	97	2.61E-06	115	3.80E-06	110	> 1.0E-05	> 1.0E-05
Bevacizumab-EU	9.61E+04	9.01E-04	9.52E-09	100	2.54E-06	118	3.76E-06	109	> 1.0E-05	> 1.0E-05

- a. n=3.
- b. Reference material PF-06439535 128926-30 was set at 100% K_D.
- c. PF-06439535 128926 was tested against itself as internal control (IC).
- d. Due to low affinity binding, steady state analysis was used as accurate kinetic measurement could not be obtained, which is expected for IgG1 subtype antibodies.
- e. Curves did not achieve saturation at highest concentration (10 μM), due to low affinity interaction with captured ligand, affinity constants reported as greater than 10 μM.
- f. n=2.

Reviewer Comments:

- *Similar to C1q binding, the PF-06439535 lot information is unclear. Based on the data provided in this section, only the reference standard appears to have been used, but according to Table 3.2.R.3.7-12 an additional PF-06439535 lot, 13J138K003 is also indicated to have been used for the binding study to the FcγRs. IR was sent to clarify if a second lot of PF-06439535 (13J138K003) was used and if so to provide the data.*

IR Response 12/21/18 Query “d”: Sponsor confirmed that only a single lot each of the three products were used to assess binding to FcγRI, RIIa, RIIb, and RIIIb.

- *No justifications were provided for why some receptor binding studies were done in triplicate and others as duplicates. IR was sent for clarification.*
- IR Response 12/21/18 Query “d”:** Sponsor stated that triplicates were performed for kinetic as well as steady state analysis to determine the binding affinity, i.e. K_D values for FcγRI and FcγRIIa, respectively to obtain accurate values. However, for FcγRIIb and FcγRIIIb, due to weak binding even steady state levels were not obtainable and results were reported as > 10 μM. Therefore, for FcγRIIb and RIIIb, only 2 runs were performed.

Reviewer Comment: *The response is acceptable.*

- *No justification for why kinetic (ka and kd) data for FcγRIIa 131H and 131R binding was provided. IR was sent for justifications.*

IR Response 12/21/18 Query “d”: See sponsor’s response above for triplicate and duplicate runs.

- *The use of only the reference standard lot is considered acceptable because the clinical/commercial scale comparability data show that the glycoforms are comparable and are unlikely to have an impact on Fc functionality. (see section 3.2.S.2.6 Manufacturing Process Development History). In addition, the use of only the reference standard lot is considered acceptable because Pfizer used the ref std, PF-06439535 lot 128926-30, to release subsequent clinical as well as the process validation lots, and therefore provides adequate assurance that it is representative of the PF-06439535 lots*

and justifies the use of only a single PF-06439535 lot in the Fc domain functional assays. However, use of single lots for the FcγR binding is a different issue (see comment bullet below).

- Single lots of PF-06439535, US-Avastin, and EU-Avastin to determine similar binding affinities. IR was sent to justify the use of single lot each for binding analysis.

IR Response 12/21/18 Query “d”: Sponsor stated that in their risk assessment for quality attributes to impact safety, efficacy, PK/PD, and immunogenicity, binding to Fcγ receptors were ranked low based on published knowledge of the reference product. Therefore, single lots were selected for FcγRI, IIa, IIb, and IIIb whereas, FcγRIIIa was evaluated with multiple lots as the receptor with the greatest potential to mediate ADCC activity compared to the other Fcγ receptors.

Reviewer Comment: The data provided support similar weak binding to FcγRI, IIa, RIIb, and RIIIb. However, a single lot is not sufficient for similarity assessment because as an IgG1 molecule, the ability to bind these receptors is present. Thus, more than a single lot is required to assess whether the products have similar affinities to the Fcγ RI, RIIa, RIIb, and RIIIb. IR was sent to request additional data.

IR Response 2/13/19 Query #1 from IR sent 1/29/19: Sponsor provided data from 2 additional lots of US-Avastin and EU-approved Avastin. and are shown below.

Table 1. Binding of PF-06439535, Bevacizumab-US, and Bevacizumab-EU to FcγRI, FcγRIIIa 131H and 131R, FcγRIIb, and FcγRIIIb (additional 2 lots requested)

Region	Sample	FcγRI				FcγRIIIa 131H ^a		FcγRIIIa 131R ^{a c}		FcγRIIb ^{a b}		FcγRIIIb ^{a b}	
		ka, 1/Ms	kd, 1/s	K _D , M	% K _D	K _D , M	% K _D	K _D , M	% K _D	K _D , M	% K _D	K _D , M	% K _D
PF-06439535	12P138K603 (RM) ^d	1.04E+05	7.79E-04	8.16E-09	100	3.57E-06	100	> 2.5E-06	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05	
	12P138K603 (IC) ^e	8.71E+04	8.03E-04	9.36E-09	89	3.51E-06	102	> 2.5E-06	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05	
	R59889-W	1.02E+05	8.41E-04	8.38E-09	99	3.67E-06	99	> 2.5E-06	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05	
	R64867-W	7.93E+04	7.67E-04	9.99E-09	84	3.62E-06	100	> 2.5E-06	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05	
Bevacizumab-US	3044096	1.04E+05	8.08E-04	7.89E-09	105	3.32E-06	108	> 2.5E-06	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05	
	3049679	7.76E+04	8.19E-04	1.08E-08	77	3.77E-06	95	> 2.5E-06	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05	
Bevacizumab-EU	B8004H04	1.11E+05	7.74E-04	7.02E-09	118	3.60E-06	100	> 2.5E-06	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05	
	B8018H09	8.49E+04	8.47E-04	1.01E-08	82	3.63E-06	99	> 2.5E-06	> 1.0E-05	> 1.0E-05	> 1.0E-05	> 1.0E-05	

- Due to low affinity binding, steady state analysis was used as accurate kinetic measurement could not be obtained, which is expected for IgG1 subtype antibodies.
- Curves did not achieve saturation at highest concentration (10 μM), due to low affinity interaction with captured ligand, affinity constants reported as greater than 10 μM.
- Curves did not achieve saturation at highest concentration (10 μM), due to low affinity interaction with captured ligand, affinity constants reported as greater than 2.5 μM.
- Batch 12P138K603, the source of the clinical reference material 128926-30 was used as RM and set at 100% K_D.
- Batch 12P138K603, the source of the clinical reference material 128926-30 was tested against itself as internal control (IC).

Reviewer Comment: The data provided supports the original single lot data and shows similar weak binding. The response is acceptable.

FcRn Binding by SPR

Twelve PF-06439535, 11 US-Avastin lots and 11 EU-Avastin lots were used to determine relative FcRn binding activity against PF-06439535 reference standard. The results of the FcRn binding showed that PF-06439535 and EU-Avastin lots were within the US-Avastin quality range (QR) (see descriptive statistics and QR figures below):

Table 3.2.R.3.2.2-5. Summary of Descriptive Statistics for FcRn SPR Binding Activity of Bevacizumab-EU, PF-06439535, and Bevacizumab-US*

Relative K _D (%)						
Region	N	Mean	SD	CV (%)	Min	Max

EU	11	105	6.4	6.2	97	117
Pfizer*	12	94	10.2	10.8	82	118
US	11	104	6.4	6.2	96	119

N = sample size, SD = standard deviation, CV= coefficient of variation, Min = minimum, Max = maximum

* This table was modified by the reviewer to insert Pfizer lot value of 12 and results from 12 lots and not the original 13 lots

Figure 3.2.R.3.2.2-8. Statistical Quality Range for FcRn SPR Binding Activity of Bevacizumab-EU, PF-06439535, and Bevacizumab-US

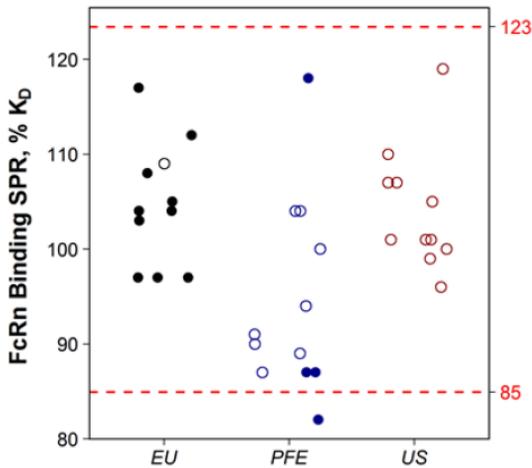


Figure 3.2.R.3.2.2-8 illustrates the distribution of results for FcRn binding (% Relative KD) as well as the upper and lower limits of the statistical quality range (the red dashed lines) from testing individual lots of bevacizumab-EU (11 lots), and bevacizumab-US (11 lots) with PF-06439535 (12 DS + DP). The solid circles represent the lots that have been used in the clinical trials. Tabulated data can be found in Appendix.

Reviewer Comments:

- EU-Avastin QR analysis was not provided. IR was sent to request the analysis and PF-06439535 is within EU-Avastin QR (IR response 12/21/18).
- The FcRn binding results indicate that all three products have similar FcRn binding properties.

3.2.R.3.2.3 N-Linked Glycan Structure

Glycan structures were evaluated by graphical comparison based on the known literature knowledge that the mechanism of action for US-Avastin was not through effector functions mediated through the Fc region. Therefore, Pfizer compared the profiles of glycan structures from PF-06439535, US-Avastin, and EU-Avastin lots. The methods used included HILIC/MS and sequential digestion with different exoglycosidases to determine the composition and linkages of monosaccharide residues. The in-depth characterization study using exoglycosidases was conducted with PF-06439535 ref std, 128926-30, US-Avastin lot 453807, and EU-Avastin lot H0150 and glycan structure determination using the HILIC release method was on multiple PF-06439535, US-Avastin, and EU-Avastin lots (10 for PF-06439535, 46 for UF-Avastin, and 51 for EU-Avastin).

Reviewer Comments:

- IR was sent regarding the data analysis method for glycan structures (see comment in section 3.2.R.3.1.1 regarding data analysis).

IR Response 10/2/18:

Sponsor provided total afucosylation, galactosylation, and high mannose glycan data from 46, 51, and 12 lots of US-Avastin, EU-Avastin, and PF-06439535, respectively. The following table shows the glycan species that contributed to the quantification of afucosylation, galactosylation, and high mannose levels.

Table 2. N-linked Glycans Identified for PF-06439535, Bevacizumab-US, and Bevacizumab-EU

Quality Attribute	Level	PF-06439535	Bevacizumab-US	Bevacizumab-EU
Total Afucosylation	Main Glycan	Man5	G0	G0
	Others:	G0 minus 2 GlcNAc, G0 minus GlcNAc, G0, Man6, Man7, Man8	G0 minus 2 GlcNAc, G0 minus GlcNAc, Man5, Man6, Man7, Man8	G0 minus 2 GlcNAc, G0 minus GlcNAc, Man5, Man6, Man7, Man8
Terminal Galactosylation	Main Glycan	G1F(a+b)	G1F(a+b)	G1F(a+b)
	Others:	G1F minus GlcNAc, G1-TriF, G2F	G1F minus GlcNAc, G1-TriF, G2F,	G1F minus GlcNAc, G1-TriF, G2F
Total High Mannose	Main Glycan	Man5	Man5	Man5
	Others:	Man6, Man7, Man8	Man6, Man7, Man8	Man6, Man7, Man8

The data provided showed that PF-06439535 lots had higher levels of afucosylation, terminal galactosylation, and high mannose content (see data tables and figures below) and failed to meet US-Avastin quality ranges.

Table 3. Summary of Statistical Analysis for Bevacizumab-EU, PF-06439535, and Bevacizumab-US Total Afucosylation

Total Afucosylation (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	51	3.0	0.47	15.5	2.2	4.3
PFE	12	3.6	0.80	22.6	2.3	4.6
US	46	2.7	0.21	7.9	2.4	3.3

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Table 4. Summary of Statistical Analysis for Bevacizumab-EU, PF-06439535, and Bevacizumab-US Terminal Galactosylation

Terminal Galactosylation (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	51	14.9	4.00	26.8	6.0	21.7
Pfizer	12	16.8	3.17	18.9	13.0	22.1
US	46	11.9	2.58	21.7	6.5	18.9

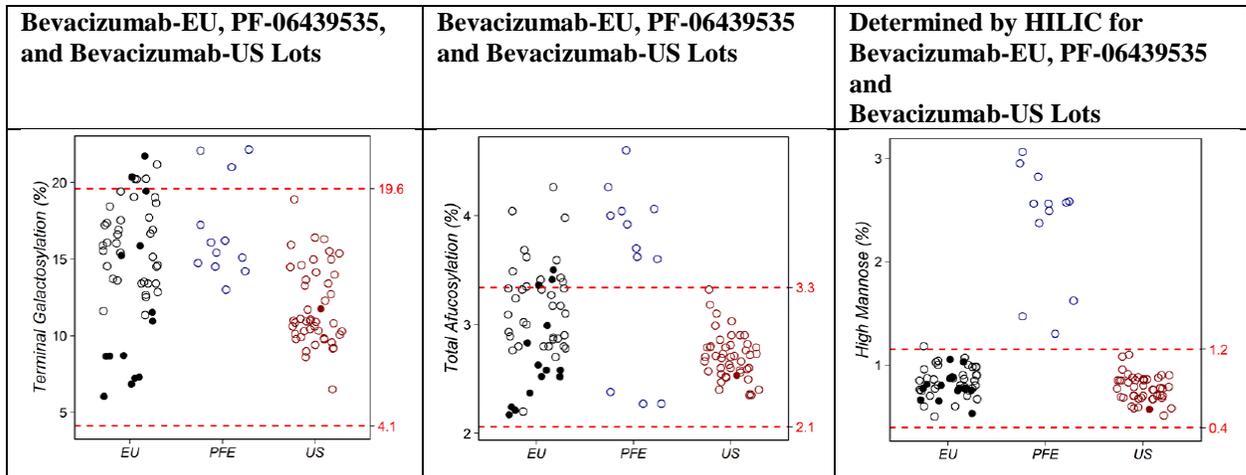
N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Table 5. Summary of Statistical Analysis for Bevacizumab-EU, PF-06439535, and Bevacizumab-US Total High Mannose

High Mannose (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	51	0.8	0.14	17.1	0.5	1.2
PFE	12	2.4	0.58	24.6	1.3	3.1
US	46	0.8	0.13	16.4	0.5	1.1

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Figure 2. Individual Values and Statistical Quality Range for Terminal Galactosylation for	Figure 3. Individual Values and Statistical Quality Range for Total Afucosylation for	Figure 4. Individual Values and Statistical Quality Range for High Mannose
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The results shown above indicate that both PF-06439535 and EU-Avastin are outside of US-Avastin quality ranges by 25% and 12% and fail to meet the acceptance criteria of greater 90% of the lots should be within US-Avastin quality ranges. Sponsor states that the differences are small and does not affect the biological activity, i.e. binding to VEGFA of PF-06439535. In addition, the three lots that had higher terminal galactose levels were due to (b) (4) and has been resolved. Similarly, the PF-06439535 and EU-Avastin lots fail to meet US-Avastin QR for afucosylation and only PF-06439535 lots are outside of US-Avastin QR for high mannose content. Sponsor stated that the difference in afucosylation does not affect similarity of PF-06439 because the biological activity is not affected and there are no differences in effector functions (ADCC and FcgRIIIa binding) observed. Regarding high mannose content, sponsor states that PK studies demonstrate that the small difference in high mannose content did not lead to a clinically meaningful difference in clearance.

Reviewer Comments:

- The response provided is acceptable and justified based on the data provided that show small numerical differences in total afucosylation, terminal galactosylation, and high mannose content, e.g. afucosylation, terminal galactosylation, and high mannose were higher in PF-06439535 by 1.3% (3.3% versus 4.6%), 3.2% (18.9% versus 22.1%), and 2.0% (1.1% versus 3.1%), respectively and lack of effector functions.
- Sponsor did not address how the difference in EU-Avastin lots and failure to meet US-Avastin QR affects the similarity data. Although the same justification applies to EU-Avastin lots as PF-06439535, IR was sent for Pfizer to discuss the results of the EU-Avastin for the three-way bridge.
- As with all quality range analyses, Pfizer neglected to provide EU-Avastin QR analysis and justification for any differences. IR sent and response is below.

IR Response 12/21/18 Query “a”: Pairwise analysis was provided and showed that EU-Avastin was outside of US-Avastin QR. Sponsor acknowledged that EU-Avastin lots were more heterogeneous and thus, EU-Avastin lots were outside of US-Avastin QR. However, sponsor justified that based on similar functional activities such as binding to

FcRn, FcgRIIIa, and lack of ADCC and CDC activities, the differences in total afucosylation, and galactosylation levels are not likely to have clinical impact.

Reviewer Comment: *The functional data including potency and FcRn binding supports sponsor’s conclusion. The response is acceptable.*

- IR was sent to determine if EU-Avastin lot H0150 for the in-depth characterization study is H0150B08 because the last three alpha-numeric code was not provided in section 3.2.R.3.7 Table 3.2.R.3.7-10.

IR Response 10/2/18:

Sponsor confirmed that the EU-Avastin lot that starts with H0150 is B08. Table 3.2.R.3.7-10 has been updated in section 3.2.R.3.7.

Reviewer Comment: *The response is acceptable.*

Representative HILIC glycan profiles of the three products that contain 7 major peaks are shown below followed by Table 3.2.R.3.2.3-1 that provide the quantified values for all glycan species observed.

Figure 3.2.R.3.2.3-1. Representative 2-AB HILIC N-Linked Glycan Mapping Profiles of PF-06439535, Bevacizumab-US, and Bevacizumab-EU

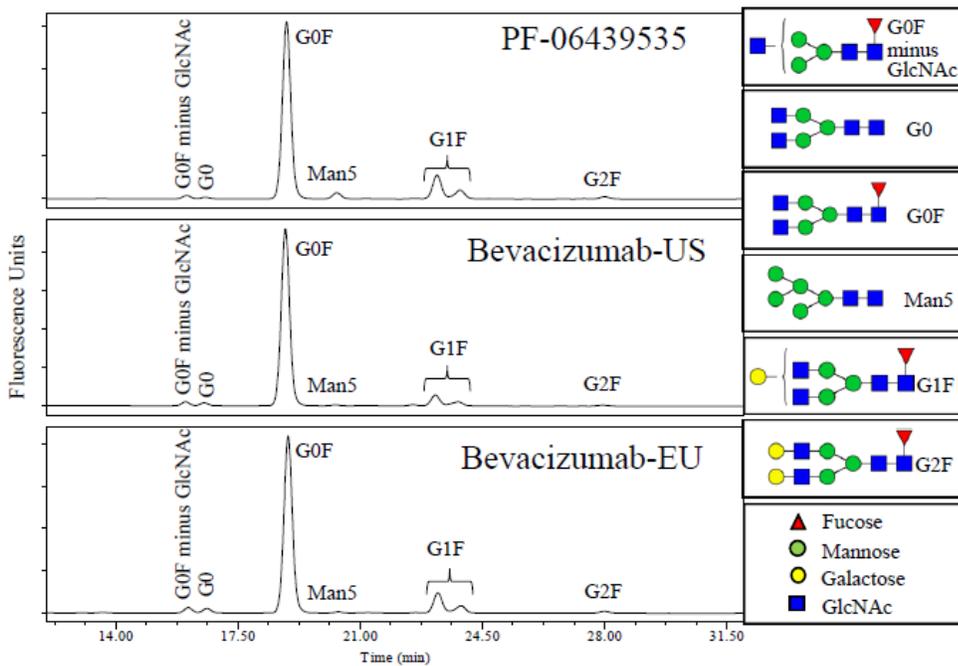


Table 3.2.R.3.2.3-1. Relative Peak Area Ranges of 2-AB Labeled N-Linked Glycans from PF-06439535, Bevacizumab-US and Bevacizumab-EU Lots

N-Linked Glycan	RPA(%) ^a		
	PF-06439535 (N=10)	Bevacizumab-US (N=46)	Bevacizumab-EU (N=51)

	Min.	Max.	Min.	Max.	Min.	Max.
G0 minus 2GlcNAc	≤QL	0.6	ND	ND	ND	ND
G0F minus 2GlcNAc	ND	ND	≤QL	≤QL	≤QL	≤QL
G0 minus GlcNAc	≤QL	0.5	≤QL	≤QL	≤QL	0.6
G0F minus GlcNAc	0.8	2.7	1.2	4.9	0.8	5.5
G0	0.8	1.3	1.3	2.3	1.2	2.8
G0F	73.2	79.2	75.5	85.6	72.4	85.9
Man5	0.9	2.3	0.4	0.9	0.4	1.1
G1F minus GlcNAc	≤QL	0.5	≤QL	0.6	≤QL	0.9
G0-TriF	≤QL	0.5	≤QL	0.7	≤QL	0.5
G1Fa	8.7	13.9	3.5	11.9	3.3	13.6
G1Fb	4.0	6.0	2.1	4.9	2.0	5.5
Man6	≤QL	≤QL	≤QL	≤QL	≤QL	≤QL
G1F minus GlcNAc+NeuAc	≤QL	≤QL	≤QL	≤QL	≤QL	≤QL
G1-TriF	≤QL	≤QL	≤QL	≤QL	≤QL	≤QL
G1F+NeuAc	≤QL	≤QL	≤QL	≤QL	≤QL	≤QL
G2F	0.9	1.9	0.4	1.4	0.4	1.8
Man7	≤QL	≤QL	≤QL	≤QL	≤QL	≤QL
G2F+NeuAc	≤QL	≤QL	≤QL	≤QL	≤QL	≤QL
Man8	≤QL	≤QL	≤QL	≤QL	≤QL	≤QL
G2F+2 NeuAc	≤QL	≤QL	≤QL	≤QL	≤QL	≤QL
Total Sialylation ^b	0.4	0.7	≤QL	≤QL	≤QL	≤QL

RPA = relative peak area of 2-AB labeled glycans
b. Combined peak areas of sialylated species, G1F minus GlcNAc+NeuAc, G1F+NeuAc, G2F+NeuAc and G2F+2NeuAc
≤QL = Less than or equal to the quantitation limit of the assay (0.3%)
ND = Not detected

Reviewer Comments:

- Given the absolute amount of high mannose, the higher level by approximately 2% is unlikely to alter PK based on literature (Goetze, A.M. et al 2011 Glycobiology 21: 949-59).
- The differences in afucosylation and galactosylation will require further analysis of FcgRIIIa binding and C1q binding data along with ADCC and CDC activities. IR was sent for additional analysis of FcgRIIIa binding and ADCC and CDC activity and responses provided show that all three products showed similar binding and lack of effector functions (see IR responses 12/14 and 12/21/18).
- Sialic acid content was also higher in PF-06439535 lots compared to US and EU-Avastin lots. However, the amount was less than 1% (0.4% to 0.7% min/max). Therefore, there is low risk to impact PK in comparison to FcRn binding. In addition, characterization of the sialic acid found that only N-acetylneuraminic acid (Neu5Ac) containing structures were detected, which is the predominant species in mammalian cells, hence would have lower risk for immunogenicity compared to N-glycolylneuraminic acid structure.
- The results from the exoglycosidases to show the composition and linkages of the glycans were provided and showed that the profiles of all three products were similar.

3.2.R.3.2.4 Charge Heterogeneity

Twelve lots of PF-06439535, 46 lots of US-Avastin, and 44 lots of EU-Avastin were used to determine the levels of acidic, main, and basic peaks by iCE. The percent acidic and basic peaks were evaluated by quality ranges. The main peak was evaluated using “descriptive statistics,” i.e. the mean, standard deviation, and the minimum and maximum numbers, and the profiles were compared and evaluated for similar charged species as part of additional characterization studies that included MS and carboxypeptidase digested peak analysis.

The chromatographic profiles provided for the three products are similar with the exception of an additional basic peak in the PF-06439535 compared to US-Avastin and EU-Avastin lots. The amount of the basic peaks is higher for PF-06439535 compared to US and EU-Avastin lots (see figure below) and is outside of US-Avastin QR as only 8% of PF-06439535 lots are within the US-Avastin QR. The % acidic peaks are similar among the three products and met the quality range acceptance criterion for similarity. The tabular data for the acidic, basic, and main peaks are shown below followed by graphical tier 2 analyses.

Figure 3.2.R.3.2.4-1. Representative iCE Profiles of PF-06439535, Bevacizumab-US, and Bevacizumab-EU

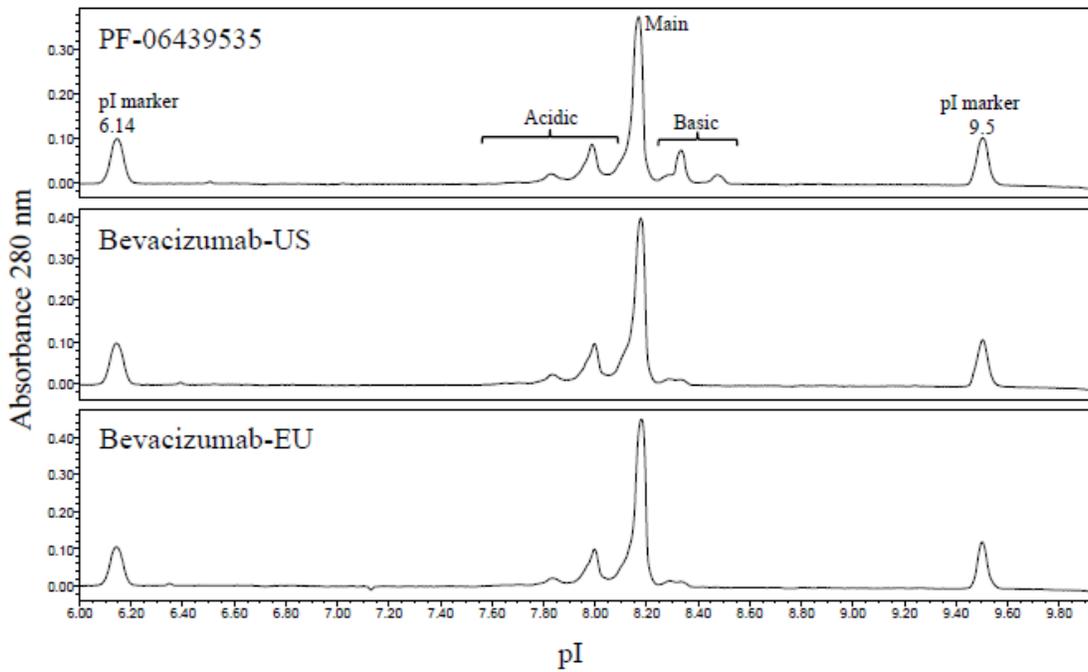


Table 3.2.R.3.2.4-3. Summary of Descriptive Statistics for Acidic Species using PF-06439535 and Bevacizumab-EU Independent Lots

Acidic Species (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	44	27.5	2.41	8.8	24.0	33.6
Pfizer	12	25.5	1.59	6.2	22.0	28.0
US	46	28.9	1.87	6.5	25.4	32.6

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Table 3.2.R.3.2.4-4. Summary of Descriptive Statistics for Basic Species using PF-06439535 and Bevacizumab-EU Independent Lots

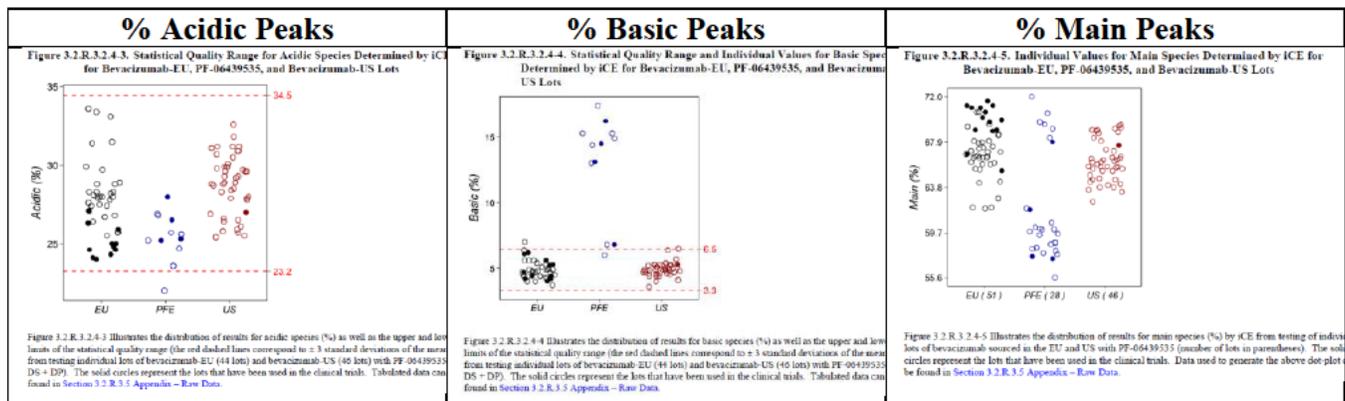
Basic Species (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	44	4.9	0.69	14.1	3.7	7.0
Pfizer	12	12.8	3.97	31.0	6.0	17.4
US	46	4.9	0.53	10.7	3.6	6.5

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Table 3.2.R.3.2.4-5. Summary of Descriptive Statistics for Bevacizumab-EU, PF-06439535, and Bevacizumab-US Main Species by iCE

Main Species (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	51	67.5	2.45	3.6	61.9	71.6
Pfizer	28	61.7	4.87	7.9	55.6	72.0
US	46	66.2	1.84	2.8	62.5	69.5

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum



In order to elucidate the differences observed in the basic peaks, Pfizer fractionated one lot each of the three products (PF-06439535 128926-30, US-Avastin lot 453807, and EU-Avastin lot H0150) using preparative CEX chromatography column for analysis using LC/MS. The following figure depicts the isolated fractions by CEX as well as peak identification using iCE method.

Figure 3.2.R.3.2.4-2. CEX-HPLC Individual Fraction Assignments for PF-06439535, Bevacizumab-US, and Bevacizumab-EU and Correlation to iCE Peaks

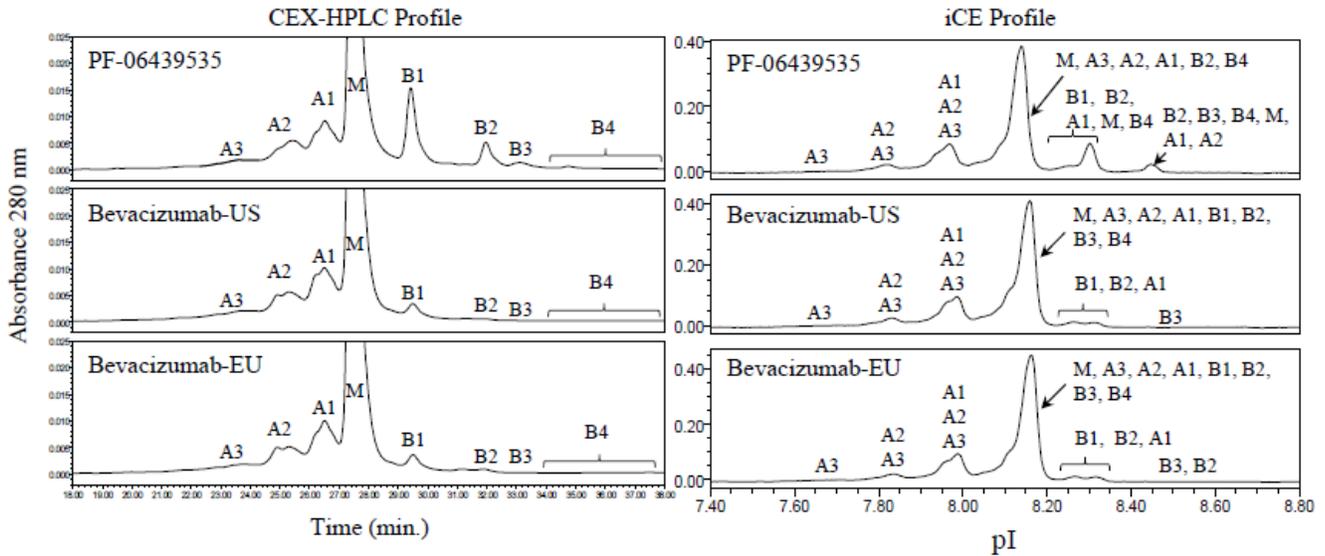


Figure 3.2.R.3.2.4-2 The various acidic (A), main (M), and basic (B) individual fractions isolated for in-depth characterization are noted on the chromatograms.

Table 3.2.R.3.2.4-1 shows the identities of the charge variants found in the CEX-HPLC fractions. All the peaks with the exception of the basic peaks B3 and B4 contained the same charged species in all three products. The basic peaks B3 and B4 were combined prior to analysis using LC/MS and the data showed that majority of the variants identified in PF-06439535 were also present in US and EU-Avastin, with the exception of a H chain VHS signal peptide extension.

Table 3.2.R.3.2.4-1. Assignment of Charge Isoforms for PF-06439535, Bevacizumab-US, and Bevacizumab-EU

CEX-HPLC Fractions	CEX-HPLC Peak Relative Abundance ^a	Isoform Identification ^b			Results
		PF-06439535	Bevacizumab-US	Bevacizumab-EU	
A1	PF-06439535: 5.2% Bevacizumab-US: 6.7% Bevacizumab-EU: 5.7%	Glycation. ^c Mono-sialylated N-linked glycans.	Glycation. ^c Mono-sialylated N-linked glycans.	Glycation. ^c Mono-sialylated N-linked glycans.	No difference, all three products contained glycation and mono-sialylated N-linked glycans.
A2	PF-06439535: 8.2% Bevacizumab-US: 9.2% Bevacizumab-EU: 8.1%	Glycation. ^c Mono-sialylated N-linked glycans. 1 truncated H chain [106-452].	Glycation. ^c Mono-sialylated N-linked glycans. 1 truncated H chain [106-452].	Glycation. ^c Mono-sialylated N-linked glycans. 1 truncated H chain [106-452].	No difference, all three products contained glycation, mono-sialylated N-linked glycans and truncation of H chain [106-452].
A3	PF-06439535: 10.7% Bevacizumab-US: 13.0% Bevacizumab-EU: 11.5%	Glycation. ^c Di-sialylated N-linked glycans.	Glycation. ^c Di-sialylated N-linked glycans.	Glycation. ^c Di-sialylated N-linked glycans.	No difference, all three products contained glycation and di-sialylated N-linked glycans.
Main ^b	PF-06439535: 58.1% Bevacizumab-US: 66.0% Bevacizumab-EU: 69.0%	4-chain antibody with lysine absent on C termini of both H chains	4-chain antibody with lysine absent on C termini of both H chains	4-chain antibody with lysine absent on C termini of both H chains	No difference, predominant isoform for all products is 4-chain antibody with lysine absent on C termini of both H chains.
B1	PF-06439535: 11.8% Bevacizumab-US: 3.6% Bevacizumab-EU: 3.5%	1 H chain with C-terminal lysine.	1 H chain with C-terminal lysine.	1 H chain with C-terminal lysine.	All three products contained 1 H chain with C-terminal lysine.
B2	PF-06439535: 3.6% Bevacizumab-US: 0.5% Bevacizumab-EU: 0.7%	2 H chains with C-terminal lysine. 4-chain mAb with one trisulfide bond.	2 H chains with C-terminal lysine. 4-chain mAb with one trisulfide bond.	2 H chains with C-terminal lysine. 4-chain mAb with one trisulfide bond.	All three products contained 2 H chains with C-terminal lysine and the 4-chain mAb with one trisulfide bond.
B3 ^d	PF-06439535: 1.5% Bevacizumab-US: 0.4% Bevacizumab-EU: 0.7%	1 L chain having a signal peptide extension, ³ VHS ⁻¹ . 1 H chain with a signal peptide sequence extension, ³ VHS ⁻¹ .	1 L chain having a signal peptide extension, ³ VHS ⁻¹ . 1 H chain with C-terminal lysine.	1 L chain having a signal peptide extension, ³ VHS ⁻¹ .	All three products contained 1 L chain with signal peptide extension, ³ VHS ⁻¹ . PF-06439535 only contained H chain with a signal peptide sequence extension, ³ VHS ⁻¹ .
B4 ^d	PF-06439535: 0.9% Bevacizumab-US: 0.7% Bevacizumab-EU: 0.9%	1 and 2 H chains with C-terminal lysine.			PF-06439535 and Bevacizumab-US contained H chain C-terminal lysine.

a. Relative peak area of each collected CEX-HPLC peak (i.e. based on the chromatographic UV profile).

b. The main charge isoform in all collected fractions of all three products has two L chains and 2 H chains with des-Lys at the C terminus, full disulfide bond connectivity, and two N-linked glycosylation consensus sites occupied with neutral N linked glycans (primarily G0F and G1F). All charge species have these N-linked glycan structures unless otherwise noted.

c. Due to low abundance, it was not possible to localize glycation.

d. CEX-HPLC fractions B3 and B4 were combined for heightened characterization by LC/MS due to their trace levels.

To confirm that the major difference in basic peak amount found in PF-06439535, 11 PF-06439535, nine US-Avastin, and 15 EU-Avastin lots were digested carboxypeptidase B prior to iCE analysis. The carboxypeptidase B treatment removes C-terminal lysine. Results showed that the ~9% higher levels of basic peaks in PF-06439535 are due to the presence of C-terminal lysine variant (see data below) as well as the peak profiles of the three products were similar (data provided but not shown in the review).

Table 3.2.R.3.2.4-2. Summary of Descriptive Statistics for Basic Species Peak by iCE after CPB Treatment

Region	N	Mean	SD	CV (%)	Minimum	Maximum
PF-06439535	11	3.8	0.9	24.3	3.1	5.6
Bevacizumab-US	9	3.7	1.0	25.9	2.6	4.9
Bevacizumab-EU	15	3.7	1.0	27.3	2.5	5.3

N = Sample size, SD = standard deviation, CV = coefficient of variation

In addition to the descriptive statistics provided above, Pfizer provided individual lot data of the basic peak levels before and after carboxypeptidase B treatment.

Figure 3.2.R.3.2.4-4. Comparison of iCE Basic Species in Untreated and Carboxypeptidase B Treated PF-06439535 Drug Substance Batches

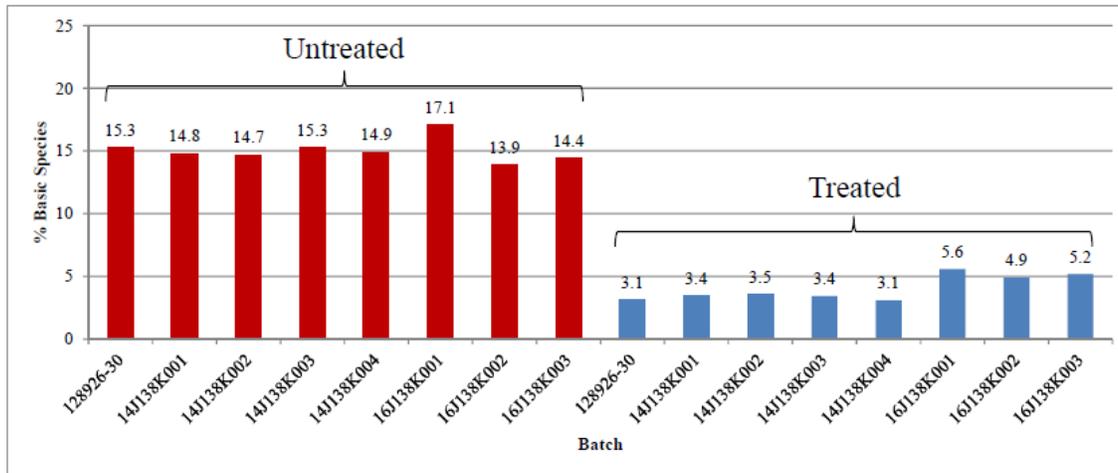
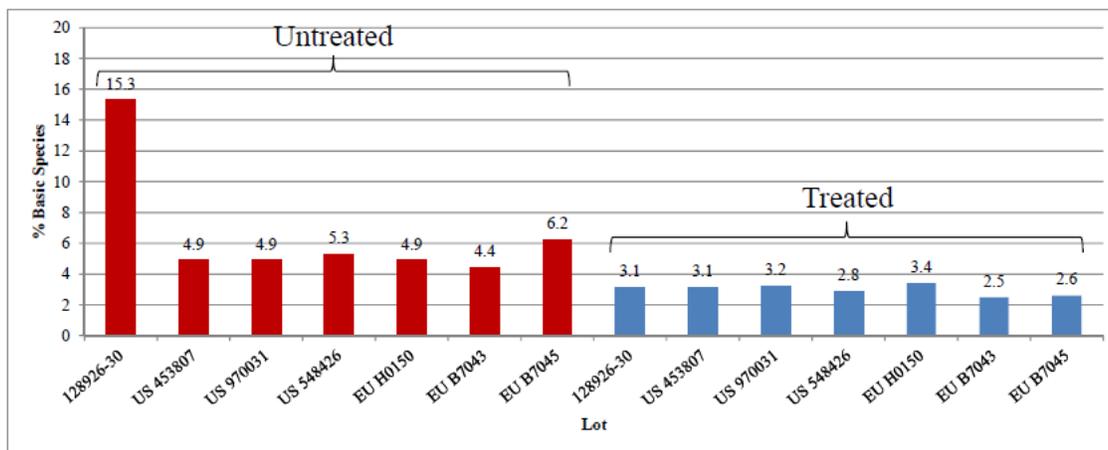


Figure 3.2.R.3.2.4-5. Comparison of iCE Basic Species in Untreated and Carboxypeptidase B Treated PF-06439535, Bevacizumab-US, and Bevacizumab-EU Lots



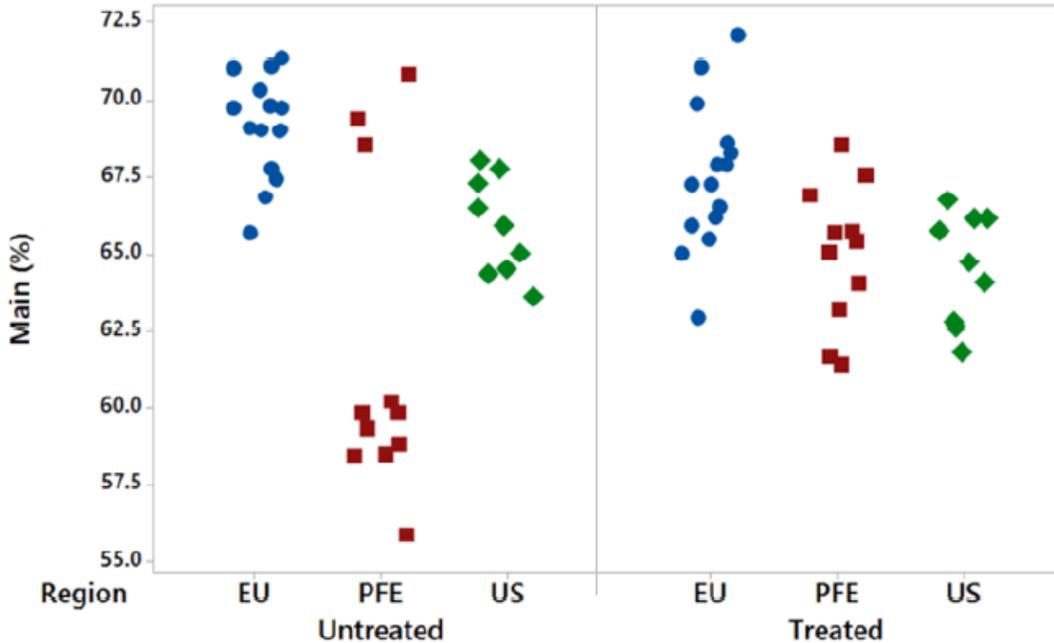
Reviewer Comments:

- By default, the main peak species would differ by the differences observed in the acidic and basic peaks. Therefore, the descriptive statistical analysis and main peak characterization provided for the main peak is sufficient to determine similarity of charged species.
- Figure 3.2.R.3.2.4-5 for the % main peak shows two populations of PF-0439535 product. An explanation was not provided for apparent difference in population of main peak. In addition, the main peak descriptive statistics includes data from 28 lots of PF-06439535 and contain redundant data. IR was sent to provide an explanation for the difference in PF-0439535 lots and data descriptive calculations that includes only independent PF-06439535 lots.

IR Response 12/21/18 Query “F”: The two populations are due to difference in basic species that resulted from equipment changes between manufacturing campaigns. The basic species difference is

the result of different levels of C-terminal lysine residue present on the IgG1. Data from CBP treatment main species are shown below.

Figure 1. Individual Values for Main Species Determined by iCE for Bevacizumab-EU, PF-06439535, and Bevacizumab-US Lots



In addition, data from independent lots of PF-06439535 and EU-Avastin were provided and the results are similar to the ones originally submitted with redundant lots included. Thus, the conclusion remains the same.

Reviewer Comment: *The response confirms that the major difference noted in the main peak was due to basic peak difference. The response is acceptable.*

- *Similar to FcRn tier 2 analysis, Pfizer did not provide QR analysis using EU-Avastin lots. IR was sent to provide data analysis using EU-Avastin QR. PF-06439535 is outside of EU-Avastin QR, but EU-Avastin is within US-Avastin QR. The results are acceptable based on the fact that the difference in charge variants are the result of age-related variants as well as differences in glycoforms which do not impact potency and FcRn binding.*
- *The profiles of the three products differ in that PF-06439535 has an extra peak in the basic peak region. However, the charge heterogeneity characterization study showed that US and EU-Avastin lots also have the extra peak, labeled as B3 in Figure 3.2.R.3.2.4-2 in the charge heterogeneity characterization document, but at trace levels. The MS data of the CEX fractionated peaks verified that the constituents of the basic peaks B3 and B4 with the exception of signal peptide extension of the H chain on PF-06439535, are the same for the three products. The level of the H chain signal peptide extension is low, but Pfizer should control the signal peptide variant as part of their control strategy, i.e. basic peak species acceptance criterion as part of release testing.*
- *The data from the CPB digested PF-06439535 PPQ lots appears to be consistently 2% higher compared to the other PF-06439535 lots analyzed. IR was sent to ask for an explanation cause of*

the 2% higher levels in the PPQ lots for basic peaks and the identity of the variant(s) that make up the 2% difference.

IR Response 12/21/18 Query “g”: Sponsor provided LC/MS subunit data to show that the difference in PPQ lots is due to higher amount of signal peptide VHS extension on the LC.

Reviewer Comment: *As this signal peptide variant is detectable in the basic peaks, Pfizer will be asked to tighten the basic peak acceptance criteria. The response is acceptable.*

3.2.R.3.2.5 Product Purity

Twelve lots of PF-06439535, 46 lots of US-Avastin, and 44 lots of EU-Avastin were used to determine the levels of HMMS and monomer by SE-HPLC. The percent HMMS and monomer peaks were evaluated as tier 2 attributes. Twenty-eight lots of PF-06439535, 46 US-Avastin lots, and 51 EU-Avastin lots were used to determine the levels of fragments, HC + LC, and intact IgG by reduced and non-reduced CGE. The fragments, HC + LC, and intact IgG were evaluated using “descriptive statistics,” i.e. the mean, standard deviation, and the minimum and maximum numbers, and the profiles were compared and evaluated for similar product-related species. In addition, SDS-PAGE and Western blots of one lot each of PF-06439535, US-Avastin, and EU-Avastin were evaluated for similar protein band patterns.

The chromatographic profiles, electropherograms, SDS-PAGE and Western blot scans provided for the three products are similar. The level of HMMS and monomer peaks are lower and higher, respectively, for PF-06439535 and outside of the US-Avastin QRs (see data below). The HMMS peak was further analyzed using SEC-MALS. The results from SEC-MALS showed that the HMMS peak consisted predominantly of dimers for all three products.

Figure 3.2.R.3.2.5-1. Representative SE-HPLC Profiles of PF-06439535, Bevacizumab-US and Bevacizumab-EU

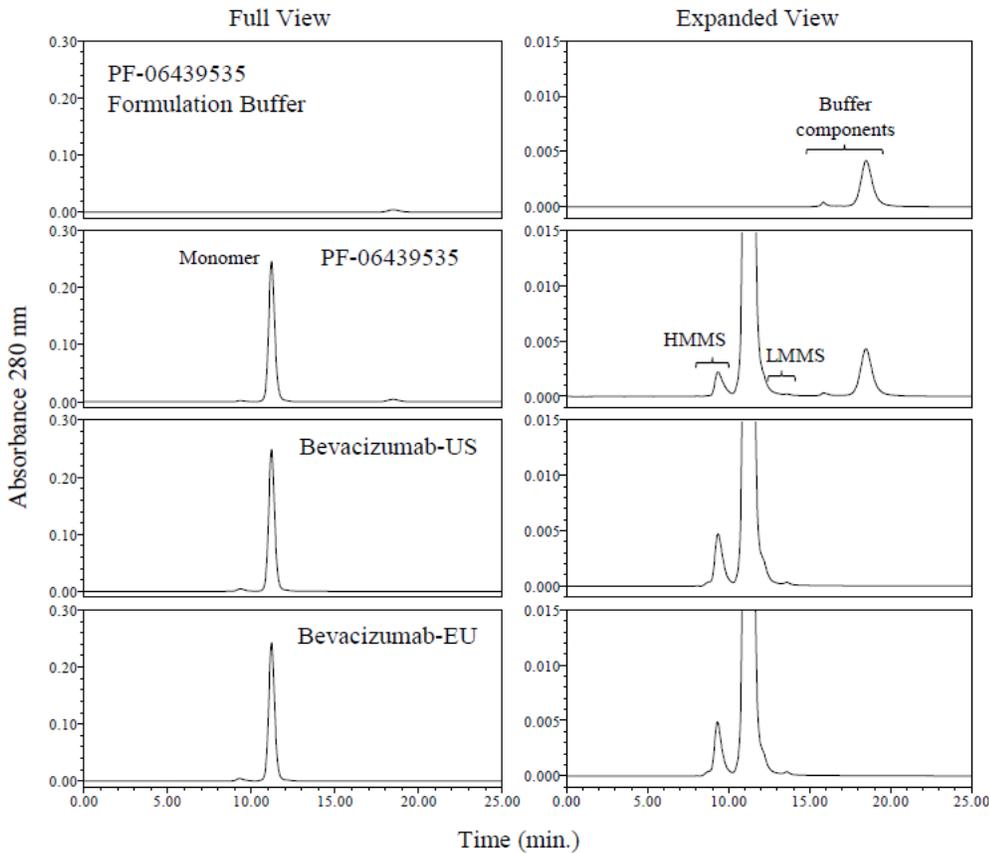


Figure 3.2.R.3.2.5-1 Representative SE-HPLC profiles of PF-06439535 formulation buffer, PF-06439535, bevacizumab-US, and bevacizumab-EU. The HMMS, monomer, and LMMS are noted on the chromatogram. The method is described in Section 3.2.S.4.2 Analytical Procedures.

Table 3.2.R.3.2.5-3. Summary of Descriptive Statistics for Monomer using PF-06439535 and Bevacizumab-EU Independent Lots

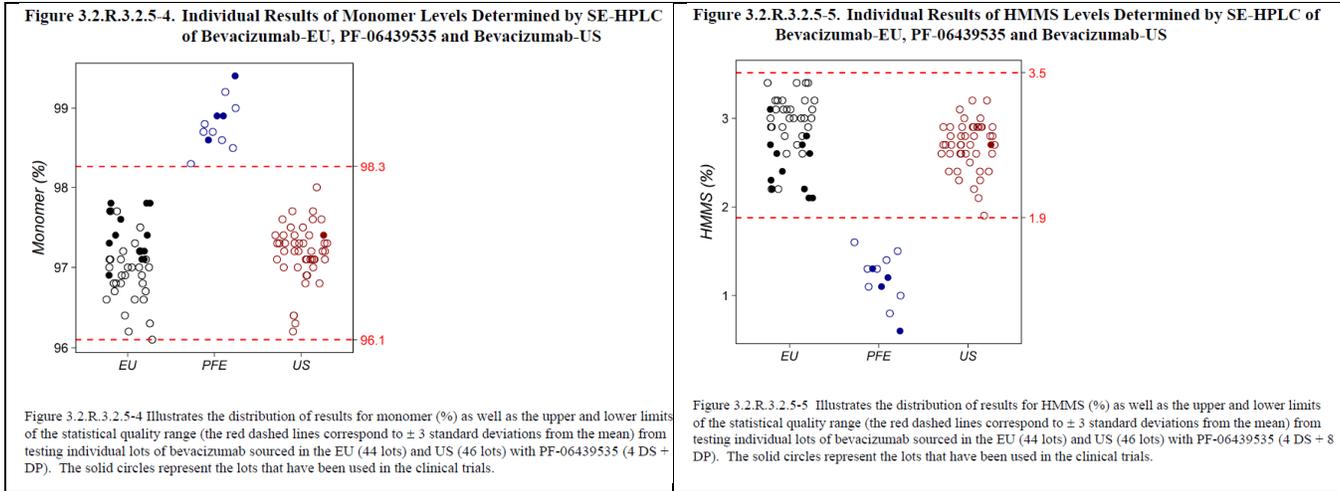
Region	N	Monomer (%)				Min	Max
		Mean	SD	CV (%)			
EU	44	97.1	0.42	0.4	96.1	97.8	
Pfizer	12	98.8	0.30	0.3	98.3	99.4	
US	46	97.2	0.36	0.4	96.2	98.0	

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Table 3.2.R.3.2.5-4. Summary of Descriptive Statistics for HMMS using PF-06439535 and Bevacizumab-EU Independent Lots

Region	N	HMMS (%)				Min	Max
		Mean	SD	CV (%)			
EU	44	2.8	0.37	13.1	2.1	3.4	
Pfizer	12	1.2	0.29	24.1	0.6	1.6	
US	46	2.7	0.27	10.1	1.9	3.2	

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum



Similar to HMMS, the results of the percent of fragments, HC+LC, and intact IgG showed that PF-06439535 lots contained less fragments and higher levels of HC+LC and intact IgG (see data tables below). The descriptive statistics were derived using 28 lots of PF-06439535 rather than from 12 independent lots.

Table 3.2.R.3.2.5-5. Summary of Descriptive Statistics for Bevacizumab-EU, PF- 06439535 and Bevacizumab-US Heavy + Light Chain by CGE (Reducing)

Heavy + Light Chain (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	51	96.9	0.58	0.6	95.3	97.8
Pfizer	28	98.5	0.28	0.3	97.7	98.9
US	46	97.0	0.51	0.5	95.7	97.9

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Table 3.2.R.3.2.5-6. Summary of Descriptive Statistics for Bevacizumab-EU, PF-06439535 and Bevacizumab-US Fragments by CGE (Reducing)

Fragment (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	51	2.7	0.49	18.4	1.9	4.2
Pfizer	28	0.9	0.09	10.3	0.7	1.0
US	46	2.6	0.40	15.5	1.7	3.6

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Table 3.2.R.3.2.5-7. Summary of Descriptive Statistics for Bevacizumab-EU, PF-06439535 and Bevacizumab-US Intact IgG by CGE (Non-Reducing)

Intact IgG (%)						
Region	N	Mean	SD	CV (%)	Min	Max
EU	51	96.5	0.59	0.6	94.8	97.4
PFE	28	97.4	0.35	0.4	96.6	98.0
US	46	96.7	0.40	0.4	95.7	97.7

N = Sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

Reviewer Comments:

- The raw data show that PF-06439535 contains less HMMS compared to US-Avastin and EU-Avastin. The results correlate with the estimated age of the materials tested, in that the newer product contains less HMMS and more monomer. Pfizer provided the following figure plotting the amounts of HMMS and monomer by months to expiry. The oldest, therefore highest amount of HMMS can be found on the left side of the graph, i.e. between months to expiry 0 and 5.

Figure 3.2.R.3.1.2-1. Assessment of Stability Indicating Attributes Against Bevacizumab-US and Bevacizumab-EU Lot Age at Time of Testing

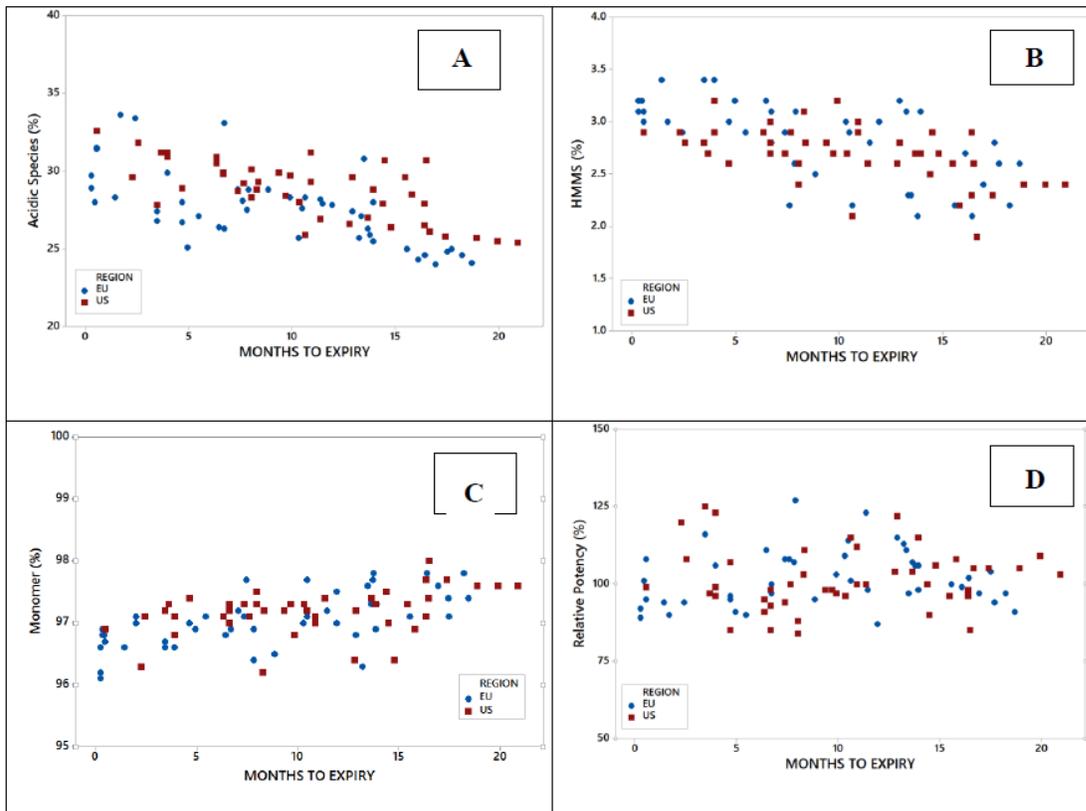


Figure 3.2.R.3.1.2-1 Months to expiry is calculated by subtracting the assay date from the licensed product expiry and divided by 30.5. Panel A displays acidic species from bevacizumab-EU and bevacizumab-US lots tested in the iCE method. Panel B displays HMMS from bevacizumab-EU and bevacizumab-US lots tested in the SE-HPLC method. Panel C displays monomer results from bevacizumab-EU and bevacizumab-US lots tested by reducing CGE. Panel D displays % relative potency from bevacizumab-EU and bevacizumab-US lots tested in the Inhibition of Cell Growth method.

- Similar trend in increase in HMMS and decrease in monomer are shown for PF-06439535, e.g. stability data from accelerated condition (25°C) show increase in HMMS peak from 1.3% to 1.6% (PF-06439535 lot R64867-W) at 3 month time point and at long term storage condition (5°C) HMMS increased from 0.6% to 1.1% (PF-06439535 lot H49500) at 36 month time point. Note that although the increasing trend in HMMS is also present for PF-06439535, the amounts are likely lower due to the difference in formulation buffer.
- In conclusion, the lack of similarity in the levels of HMMS is acceptable given that this attribute is lower in the PF-06439535 product compared to US-Avastin because of the nature of the measured

attribute, i.e. HMMS increases over time and is dependent on the age of the tested samples, and the fact that the difference in amounts noted did not have an impact on product potency and immunogenicity.

- *The sponsor did not provide PF-06439535 data analysis using EU-Avastin QRs. IR was sent to request EU-Avastin QR analysis in order to support the scientific-bridge for the use of EU-Avastin in the clinical study (see summary table above for results provided as part of IR response 12/21/18).*
 - *The descriptive statistics for fragments, HC+LC, and intact IgGs should include data from independent lots. IR was sent to request descriptive statistics derived from 12 independent lots.*
- IR Response 12/21/18 Query “h”:** Sponsor updated section 3.2.R.3.5 Product Purity with data from independent lots. The results of the independent lots showed that PF-06439535 contains less degradation, i.e. fragments and correspondingly higher levels of HC+LC and intact IgG peak.

Reviewer Comment: *The difference observed using CGE reduced and non-reduced CGE methods is acceptable for the same reason stated above for HMMS.*

3.2.R.3.2.6 Disulfide Bonds

One lot each of PF-06439535, US-Avastin, and EU-Avastin were used to determine disulfide bond linkages and free sulfhydryl. The disulfide bond linkages were determined using LC/MS-non-reducing peptide mapping (Lys-C) method. The products were treated initially denatured and alkylated to prevent disulfide bond scrambling. Free sulfhydryls were determined using Ellman’s reagent.

The results showed that all three products contained 16 disulfide bonds (12 intrachain and 4 interchain) located in the expected peptide. The chromatograms of the peptides were provided and were nearly identical. The amount of free sulfhydryls were low in all three products, 0.28, 0.39, and 0.40 mol SH/mol mAb for PF-06439535, US-Avastin, and EU-Avastin, respectively.

Reviewer Comment: *The results showed that all three products met the criteria for similarity for disulfide bonds and free sulfhydryl.*

3.2.R.3.2.7 Higher Order Structure

Pfizer used five different methods to determine the secondary and tertiary structures of PF-06439535, US-Avastin, and EU-Avastin. The combination of results from far-ultraviolet circular dichroism (UV-CD), Fourier transform infrared (FTIR) spectroscopy, near-UV CD, intrinsic fluorescence emission spectroscopy, and differential scanning calorimetry (DSC) helped to evaluate the similarity of the higher order structures of the three products. The results of each testing approach and the similarity of the three products are reviewed below. The raw data from the higher order structure analysis for all the lots analyzed were provided in section 3.2.R.3.5 Appendix-Raw data.

Far-UV CD and FTIR Spectroscopy

The secondary structures of the three products were compared using far-UV CD and FTIR. A total of four PF-06439535 lots, five US-Avastin lots, and 15 EU-Avastin lots were evaluated using far-UV CD and for FTIR analysis, a total of four PF-06439535 lots, four US-Avastin lots, and one EU-Avastin lot were used (Table 3.2.R.3.7-13 provided in section 3.2.R.3.7 contain specific lot number information and is not reproduced here). The results showed nearly superimposable spectrums for both far UV-CD and FTIR methods characteristic of β -sheet structure.

The sponsor also measured chemically denatured and heat-denatured PF-06439535 using far-UV CD and FTIR, respectively, to demonstrate method sensitivity to detect differences. Far UV-CD was shown to detect unfolded molecules when present at levels greater than 0.15% in a sample and FTIR was shown to detect unfolded molecules when present at levels greater than 2.0%.

Reviewer Comment: *The profiles of the spectrum were similar for all three products and the results confirm the presence of mainly beta sheet structure.*

Near-UV CD and Intrinsic Fluorescence Spectroscopy

The tertiary structures of the three products were compared using near-UV CD and intrinsic fluorescence spectroscopy. The same four PF-06439535 lots, five US-Avastin lots, and 15 EU-Avastin lots were evaluated using near-UV CD and for the intrinsic fluorescence spectroscopy, the same lots used for FTIR were also used (Table 3.2.R.3.7-13 provided in section 3.2.R.3.7 contain specific lot number information and is not reproduced here). The results of the near-UV CD absorption spectra of the three products showed similar absorption peak patterns produced by aromatic amino acid residues and disulfide bonds in environments that represents similar tertiary folding, whereas, the results of the intrinsic fluorescence showed similar tryptophan emission spectra.

The sponsor also measured absorption and emission spectra of denatured PF-06439535 using near-UV CD and intrinsic fluorescence to demonstrate method sensitivity to detect differences. The near-UV CD was shown to detect unfolded molecules when present at levels greater than 1.5% in a sample and intrinsic fluorescence was shown to detect unfolded molecules when present at levels greater than 0.59%.

Reviewer Comment: *The profiles of the spectrum were similar for all three products and confirm that all three products have similar tertiary structure.*

DSC

A total of four PF-06439535 lots, five US-Avastin lots, and 15 EU-Avastin lots were used to evaluate thermal stability as an orthogonal method to determine tertiary structure using DSC. The results showed similar thermograms for all three products with thermal transitions at ~74°C and ~85°C. Pfizer also used chemically denatured PF-06439535 to demonstrate method sensitivity to detect differences. The sensitivity of DSC was shown to be able to detect unfolded molecules when present at levels greater than 1.8% in a sample.

Reviewer Comment: *The profiles of the thermograms were similar for all three products and confirm that all three products have similar tertiary structure.*

3.2.R.3.2.8 Protein Concentration

Sixteen PF-06439535 DP lots, 46 US-Avastin lots, and 51 EU-Avastin lots were measured for protein concentration using ultraviolet spectrophotometer at 280 nm. The extinction coefficient used was 1.65 mL mg⁻¹cm⁻¹ that was experimentally determined. Pfizer experimentally determined the extinction coefficient using three methods as shown below.

- Method 1 UV absorption at 280 nm: Thirteen different US and EU-Avastin lots and back calculating the absorption coefficient based on the labeled claim of the RP, i.e. 25 mg/mL. The average of 13 lots of US and EU-Avastin was 1.65 with a SD of 0.04 mL mg⁻¹cm⁻¹. SE-HPLC

method was used to determine the effect of different formulation buffer of PF-06439535 on accuracy of the method. All three products used 1.65 absorption coefficient to determine the protein concentration of the three products in their respective buffers then based on the calculated protein concentrations, the required dilutions were made for injection into SE-HPLC for peak area ratio determination. The A280 total protein peak area ratios were calculated for US-Avastin and EU-Avastin relative to PF-06439535. The ratio results were 1.00, 0.97, and 0.94 for PF-06439535, US-Avastin, and EU-Avastin, respectively.

- Method 2 UV absorption at 280 nm and amino acid analysis: Two lots of PF-06439535 and one lot each of US-Avastin (45807) and EU-Avastin (H0150B08) were used to determine the absorption coefficient through amino acid analysis. The absorption coefficient values were 1.76, 1.56, and 1.64 mL mg⁻¹cm⁻¹ for PF-06439535, US-Avastin, and EU-Avastin, respectively.
- Method 3 Calculation based on Trp, Tyr, and cystine content: The absorption coefficient calculation is based on the absorption coefficient of the Trp, Tyr, and cysti residues in PF-06439535, which is the same as US-Avastin, and EU-Avastin (confirmed by amino acid sequencing results) divided by the molecular weight of the aglycosylated PF-06439535. The calculated absorption coefficient based on the Trp, Tyr, and cystine were 1.66 or 1.60 mLmg⁻¹cm⁻¹ by using slightly different absorption coefficients for Trp, Tyr, and cystine residues as shown in Pace et. al. (1995) or Gill Von Hippel, et al. (1989).

Based on the three methods used to determine the extinction coefficient, Pfizer selected 1.65 mL mg⁻¹cm⁻¹ for evaluation of the protein concentration. The mean values for protein concentration for PF-06439535, US-Avastin, and EU-Avastin were 24.6, 24.8, and 25.4 mg/mL, respectively. PF-06439535 and EU-Avastin met US-Avastin QRs.

Reviewer Comments:

- *The selection of 1.65 mL mg⁻¹cm⁻¹ as the absorption/extinction coefficient is in close agreement to the values obtained using methods 2 and 3 and is acceptable.*
- *The sponsor did not provide PF-06439535 data analysis using EU-Avastin QRs. IR was sent to request EU-Avastin QR analysis in order to support the scientific-bridge for the use of EU-Avastin in the clinical study.*

IR Response 12/21/18 Query “a”: The data provided shows that 37% of PF-06439535 lots (6 lots out of 16) were outside of EU-Avastin QR of 24.4-26.4 mg/mL (PF-06439535 min/max are 23.6/25.0 mg/mL). Sponsor justified that the small difference in protein content is not likely to have clinical impact because PK studies did not show a difference and also because the product is dosed to saturation the small difference in amount would not have a clinical impact.

Reviewer Comment: *Pfizer’s justification is plausible. In addition, potency was similar and the difference in protein concentration is small. The protein concentration of the three products are similar.*

3.2.R.3.3 Comparative Forced Degradation Study

Forced degradation studies were performed to determine whether the three products had similar degradation profiles and not necessarily same degradation rate due to the difference in formulation

buffer of the PF-06439535 compared to US-Avastin and EU-Avastin. The forced degradation conditions included heat stress (40°C) for 1 month, light exposure (5.1 klux) for 14 days, alkaline condition (phosphate buffer pH 7.5) at 40°C for 14 days, and different concentrations of peracetic acid treatments (0, 308, and 615 µM). The review of each forced degradation conditions are described below.

Reviewer Comment: Note that the incubation time and temperature for peracetic treatments were not stated in the submission. IR was sent to ask for the information and sponsor stated that the incubation time was for 2 hours at ambient temperature (IR response 12/21/18).

Elevated Temperature Study (40°C for 1 Month)

Twelve PF-06439535 DP lots, 6 US-Avastin lots, and 6 EU-Avastin lots were used in the 40°C heat stress study. Both DP presentations (100 mg/4 mL and 400 mg/16 mL) were used in the heat stress studies for all three products. The PF-06439535 DP lot information are shown below in Table 3.2.R.3.3-3 (note that the table has been modified to include DS lot information and clinical lot information; the different colors indicate different DS lots). The US-Avastin and EU-Avastin lot information was also provided but not shown in the review.

Table 3.2.R.3.3-3. Lots Enrolled in Elevated Temperature Study: Forced Degradation of Full-Scale Drug Product

PF-06439535 Drug Product Lots					
Source	Presentation	Lot Number ^a	DS Lot #	Date of Manufacture	Age at Time of Enrollment
Pfizer	400 mg/ 16 mL	I3SQ02	I3J138K003	Aug-13	10 months
Pfizer	400 mg/ 16 mL	H49500	I3J138K003 ^b	Sep-13	9 months
Pfizer	400 mg/ 16 mL	H90818-W	I3J138K004	Jan-14	6 months
Pfizer	400 mg/ 16 mL	J61289-W	I4J138K002 ^b	Jul-14	12 months
Pfizer	400 mg/ 16 mL	R59889-W	I6J138K002 ^b	Sep-16	3 months
Pfizer	400 mg/ 16 mL	J61289-W	I4J138K002 ^b	Jul-14	29 months at 2-8 C (Aged)
Pfizer	100 mg/ 4 mL	I12512-W	I3J138K003	Mar-14	3 months
Pfizer	100 mg/ 4 mL	I12513-W	I3J138K004	May-14	1 month
Pfizer	100 mg/ 4 mL	J76232-W	I4J138K002 ^b	Aug-14	11 months
Pfizer	100 mg/ 4 mL	R64866-W	I6J138K001	Oct-16	2 months
Pfizer	100 mg/ 4 mL	I12512-W	I3J138K003	Mar-14	33 months at 2-8 C (Aged)

a. Detailed information for Pfizer lots can be found in [Section 3.2.P.2.3 Manufacturing Process Development - Lot Genealogy and Usage.](#)

b. Clinical Lot

The results showed that all three products shared similar degradation patterns, e.g. all three products showed increased levels of acidic peaks, HMMS, and fragments with correspondingly decreasing amounts of main peak. A decrease in potency was consistently observed for the US-Avastin and EU-Avastin lots, whereas the potency varied for PF-06439535 lots. For example, 5 out of 6 lots of PF-06439535 at the 400 mg/16 mL strengths showed decreased potency after 1 month and no change for the

6th lot (H90818-W). For the 100 mg/4 mL strength PF-06439535 lots, 3 out of 5 lots showed slight increase (~1-2%, e.g. from 96% to 97% after 1 month for lot J12513-W, which is derived from the same DS as H90818-W) and the remaining 2 lots, which includes the aged sample (lot J12512-W) showed decreased potency. The results for the basic peaks showed a different pattern between PF-06439535 and the US-Avastin and EU-Avastin lots, e.g. for both US-Avastin and EU-Avastin, all lots increased in the amount of basic peaks, but the PF-06439535 showed little to no change in basic peak levels (see data tables below).

Table 3.2.R.3.3-12. Comparison Table for Bevacizumab-US, Bevacizumab-EU, and PF-06439535 400 mg/16 mL Lots after storage for 1 month at 40 °C, Change from T=0

Analytical Procedure	Evaluation Parameter	Change from T = 0											
		Bevacizumab-US, 400 mg/16 mL			Bevacizumab-EU, 400 mg/16 mL			PF-06439535, 400 mg/16 mL					
		Lot 616370	Lot 642289	Lot 548426	Lot B7102	Lot H0151	Lot B7034	Lot 13SQ02	Lot H49500	Lot H90818-W	Lot J61289-W	Lot R59889-W	Lot J61289-W (aged)
iCE	Acidic Species (%)	+30.3	+27.9	+27.9	+30.4	+30.5	+29.8	+28.5	+25.9	+29.4	+20.9	+22.9	+20.3
	Main Species (%)	-31.9	-29.9	-29.4	-32.7	-32.9	-31.7	-29.7	-25.3	-31.7	-18.8	-23.2	-19.7
	Basic Species (%)	+1.7	+1.0	+1.5	+2.4	+2.2	+1.8	+1.2	-0.5	+2.3	-2.1	+0.3	-0.8
Size Exclusion HPLC	Monomer (%)	-6.4	-5.3	-6.1	-6.3	-6.5	-6.9	-2.5	-2.6	-2.6	-2.8	-1.2	-0.9
	HMMS (%) ^a	+4.8	+3.8	+4.2	+4.0	+4.1	+5.3	+0.6	+0.7	+0.5	+0.6	+0.4	+0.2
CGE (reducing)	HC+ LC (%)	-6.5	-6.4	-3.2	-1.5	-1.5	-6.9	-1.2	-1.7	-1.2	-4.0	-3.2	-2.9
	Fragments (%)	+5.9	+5.8	+2.6	+1.3	+1.3	+6.0	+1.3	+1.6	+1.2	+3.7	+2.9	+2.7
Subvisible particles (Particles per mL)	≥ 2 µm	-179	-210	-72	-100	-83	+5	-177	-112	-346	-140	+9	+91
	≥ 5 µm	-17	-62	-27	-28	-22	0	-43	-31	-91	-38	+3	+15
	≥ 8 µm	-4	-18	-8	-6	-8	0	-14	-9	-28	-13	+2	+4
	≥ 10 µm	-2	-9	0	-3	-4	+1	-6	-2	-9	-5	+1	+4
	≥ 25 µm	0	0	0	0	-2	0	0	0	0	0	0	0

a. The lower amount of HMMS observed in PF-06439535 lots is not unexpected due to the optimized formulation.

Abbreviations: CGE = Capillary Gel Electrophoresis, iCE = imaged Capillary Electrophoresis, HMMS = High Molecular Mass Species. ND = No Data.

Table 3.2.R.3.3-13. Comparison Table for Bevacizumab-US, Bevacizumab-EU, and PF-06439535 100 mg/4 mL Lots after storage for 1 month at 40 °C, Change from T=0

Analytical Procedure	Evaluation Parameter	Change from T = 0										
		Bevacizumab-US, 100 mg/4 mL			Bevacizumab-EU, 100 mg/4 mL			PF-06439535, 100 mg/4 mL				
		Lot 504740	Lot 585982	Lot 640016	Lot B7105	Lot B7110	Lot B8000	Lot J12512-W	Lot J12513-W	Lot J76232-W	Lot R64866-W	Lot J12512-W (aged)
iCE	Acidic Species (%)	+29.2	+29.4	+30.1	+33.0	+29.4	+29.9	+28.2	+26.3	+20.5	+22.6	+23.5
	Main Species (%)	-31.9	-33.5	-31.8	-34.9	-31.4	-31.3	-30.0	-25.8	-18.8	-22.7	-25.5
	Basic Species (%)	+2.6	+4.0	+1.7	+1.8	+2.0	+1.5	+1.8	-0.5	-1.8	0.0	+2.0
Size Exclusion HPLC	Monomer (%)	-5.8	-5.0	-6.3	-8.3	-7.4	-6.1	-2.5	-2.4	-2.8	-1.2	-0.9
	HMMS (%) ^a	+3.6	+2.9	+4.6	+6.1	+5.7	+4.5	+0.5	+0.7	+0.6	+0.4	+0.3
CGE (reducing)	HC+ LC (%)	-1.6	-1.6	-6.5	-2.9	-7.1	-6.1	-1.3	-2.1	-4.0	-2.9	-2.9
	Fragments (%)	+1.4	+1.6	+5.8	+2.5	+6.2	+5.6	+1.2	+1.9	+3.7	+2.7	+2.7
Subvisible particles (Particles per mL)	≥ 2 µm	-174	-1280	-105	-669	-361	+53	-199	+330	-90	+123	-158
	≥ 5 µm	-133	-220	-64	-161	-99	-14	-45	+18	-33	+22	-29
	≥ 8 µm	-33	-55	-20	-53	-33	-14	-16	-8	-3	+10	-7
	≥ 10 µm	-16	-23	-9	-17	-15	-8	-8	-7	1	+4	-3
	≥ 25 µm	-1	-5	+2	+1	-2	-1	-1	-1	0	0	1

a. The lower amount of HMMS observed in PF-06439535 lots is not unexpected due to the optimized formulation.

Abbreviations: CGE = Capillary Gel Electrophoresis, iCE = imaged Capillary Electrophoresis, HMMS = High Molecular Mass Species. ND = No Data.

The elevated temperature storage for 1 month is stated in Table 3.2.R.3.3-14 to have increased truncation (H5 peptide amino acid residues 99-105), glutamic acid cyclization (H1 peptide amino acid residues 1-43), and deamidation (H2 amino acid residues 44-65 and H28 amino acid 377-398) levels in all three products.

Reviewer Comments:

- The elevated temperature stress study potency results are stated by Pfizer to have an overall decreasing trend for all three products with more dramatic decreases observed for US-Avastin and EU-Avastin lots. However, the data for the PF-06439535 lots are variable and appears to be within method variability of ~10% for reproducibility because the largest difference observed was a decrease of 11% for R-59889-W. It should be noted that the potency results could be in line with the significantly lower levels of HMMS compared to US-Avastin and EU-Avastin, e.g. an increase of approximately 0.02% to 0.7% of HMMS is observed versus 2.9% to 6.1%. However, the peptide mapping showed an increase in H5 peptide, which could impact potency (see comment below on modified peptide levels).
- The difference in basic peak pattern for elevated temperature between the US-Avastin/EU-Avastin lots compared to PF-06439535 is likely due to higher amounts of HMMS species in the US-Avastin and EU-Avastin lots because aggregates can appear in the basic regions of certain proteins. IR was sent to explain the difference noted for the basic peak patterns.

IR Response 12/21/18 Query “j”: Sponsor stated that additional lots of PF-06439535 were evaluated at elevated temperatures at 30°C as well as 35°C and observed that there was a consistent pattern of increases and decreases, i.e. fluctuations in basic species. Sponsor attributed the fluctuation, which were small in amount (0.5%/-1.4%, 1.1%/-2.6%, and -2.1%/2.3%, at 30°C, 35°C, and 40°C) to method variability on resolution of adjacent peaks.

Reviewer Comment: *The method variability based on validation study showed technical analytical error of 5.7% based on precision RSD of 6.34% and accuracy of 100.1%. However, iCE method was not likely validated at the time of analytical similarity and potential differences observed in the results above could be due to method.*

- *The peptide map chromatograms for elevated temperature storage study do show increased peak for H5, but the increases in peaks H1, H2, and H28 was not apparent by eye and Pfizer did not provide quantification data as this study results were ranked tier 3. As a tier 3, the profiles of the three products are similar and no new peaks can be detected in PF-06439535 compared to US-Avastin and EU-Avastin lots.*
- *Note that H5 truncation occurs in the third CDR region on the heavy chain. Therefore, the decrease in potency, for US-Avastin and EU-Avastin lots correlate with the increase in H5 peptide levels. Similarly, the PF-06439535 decrease in potency could also be attributed to the increase in H5 peptide, but for the elevated temperature study, some PF-06439535 lots did not show a decrease in potency. Note that for the peptide mapping study, only a single lot each were analyzed. IR was sent to request an explanation for the variability of the potency results given that H5 truncation increases during storage and could affect potency and any additional data to support a correlation between H5 truncation levels with potency, i.e. the lots which did not decrease in potency could have reduced levels of H5 peptide compared to the ones that have reduction in potency.*

IR Response 12/21/18 Query “b”: Sponsor stated that the decrease in potency by the US and EU-Avastin lots cannot all be attributed to increase in H5 peptide because the increase was 4-6% for all three products. However, other factors also increased more for US-Avastin and EU-Avastin lots such as HMMS and fragments which could impact potency, but not observed as much in PF-06439535 lots due to the difference in formulation. Data was provided for PF-06439535 lot reformulated into US-Avastin formulation and showed similar decrease in potency.

Reviewer Comment: *The response is acceptable.*

Light Exposure Study (5.1klux, 14 days, 25°C)

Two lots of PF-06439535 DP (one lot at each strength), one US-Avastin lot at 400 mg/16 mL (lot 548426), and one EU-Avastin lot at 100 mg/4 mL (lot B7105) were used to determine degradation pathways by light exposure. The results showed similar degradation patterns as elevated temperature study, i.e. increase in acidic peaks by iCE, HMMS by SEC, and fragments by reducing CE. Slight differences in results compared to the elevated temperature study is noted in potency in that all lots showed a decrease in activity, whereas, the basic peak levels decreased slightly for PF-06439535 and US-Avastin lot (percent change of -0.3% to -0.5%) and increased for EU-Avastin (percent change +0.4). Peptide map analysis showed increases in methionine oxidation in peptides H16ox (residues 255 and 280), H26 (residues 347 to 366), and H31 (residues 421 to 445) in all three products. There were no new degradant peaks detected in all three products.

Reviewer Comments:

- *The sponsor did not justify the use of the higher strength DP lot from US-Avastin and the lower strength DP lot from EU-Avastin. However, based on the elevated temperature study results, the*

two strengths degrade similarly for both US-Avastin and EU-Avastin lots. Therefore, there is no indication that both strengths from US-Avastin and EU-Avastin products is required.

- *The slight decrease and increase in basic species by iCE for PF-06439535, US-Avastin, and EU-Avastin lots are within method variability of 0.9%. Therefore, there is no change in basic species for the conditions of light stress studied.*
- *The decrease in potency could be attributable to higher levels of HMMS increase due to light stress compared to heat stress, e.g. the HMMS increased ~0.5% due to heat stress compared to ~3.5% due to light stress.*
- *The H26 peptide and potentially H26ox peptide for the US-Avastin lot appears to be higher compared to PF-06439535 and EU-Avastin lots. However, Pfizer did not provide quantification of the methionine oxidized levels because the purpose of the study was to determine similar types of degradants and not necessarily the rates or a direct comparison of levels due to the difference in the formulation buffer. In addition, FcRn binding studies were not performed for the light stress, but were conducted for peracetic treated samples (see below).*

Phosphate Buffer Exchange (alkaline buffer pH 7.5, 14 days, at 40°C)

Two lots of PF-06439535 DP (one lot at each strength), one US-Avastin lot at 400 mg/16 mL (lot 548426), and one EU-Avastin lot at 100 mg/4 mL (lot B7105) were used to determine degradation pathways by alkaline buffer exchange incubation condition. The results showed similar degradation patterns as elevated temperature study, i.e. increase in acidic peaks by iCE, HMMS by SEC, and fragments by reducing CE. However, the changes in acidic peaks, HMMS, and fragmentation were of similar magnitude, e.g. HMMS increased ~15% for all three products, whereas PF-06439535 lots had lower levels of HMMS increase compared to US-Avastin and EU-Avastin lots. In addition, the basic peaks for the three products all showed similar level of increases, e.g. ~1% increases. Pfizer concluded that potency changes varied based on one PF-06439535 lot (J12513-W) that increased whereas the remaining three lots all decreased (see data table below).

Table 3.2.R.3.3-21. Summary Table for Comparative Forced Degradation Study for PF-06439535 Drug Product, Bevacizumab-US, and Bevacizumab-EU, Phosphate Buffer Incubation

Analytical Procedure	Evaluation Parameter	PF-06439535 Lot H49500			PF-06439535 Lot J12513-W			Bevacizumab-US Lot 548426			Bevacizumab-EU Lot B7105		
		T=0 Days	Phosphate Buffer Incubation T=14 Days	Change from T=0	T=0 Days	Phosphate Buffer Incubation T=14 Days	Change from T=0	T=0 Days	Phosphate Buffer Incubation T=14 Days	Change from T=0	T=0 Days	Phosphate Buffer Incubation T=14 Days	Change from T=0
UV Spectroscopy	Protein Concentration (mg/mL)	16.4	17.0	+0.6	16.1	16.6	+0.5	16.0	16.5	+0.5	16.0	16.5	+0.5
iCE	Acidic Species (%)	29.5	65.4	+35.9	26.3	64.7	+38.4	31.6	70.5	+38.9	27.3	69.9	+42.6
	Main Species (%)	64.7	27.5	-37.2	67.1	28.1	-39.0	64.3	24.2	-40.1	69.6	25.6	-44.0
	Basic Species (%)	5.8	7.0	+1.2	6.6	7.2	+0.6	4.2	5.3	+1.1	3.1	4.4	+1.3
Size Exclusion HPLC	Monomer (%)	97.1	80.5	-16.6	97.5	80.8	-16.7	95.5	78.4	-17.1	95.3	77.8	-17.5
	HMMS (%)	2.7	17.7	+15.0	2.4	17.4	+15.0	4.3	19.5	+15.2	4.5	20.0	+15.5
CGE (reducing)	Heavy Chain + Light Chain (%)	98.5	92.2	-6.3	99.0	92.5	-6.5	97.5	90.2	-7.3	98.8	90.1	-8.7
	Fragments (%)	1.0	6.0	+5.0	0.7	5.8	+5.1	2.2	7.6	+5.4	0.8	7.8	+7.0
Cell-Based Bioassay	Relative Potency (%) ^a	106	86	N/A	84	92	N/A	91	88	N/A	92	76	N/A

a. Change from T=0 not calculated since this is an assay with a relative readout.
N/A = Not applicable.

Peptide map analysis showed increases in deamidation as shown in peptides H2 (residues 44-65), H4 (residues 77-98), H8H9 (residues 154-216), and H28 (residues 377-398) as well as increase in glutamic acid cyclization in H1(residues 1-43) peptide in all three products. There were no new degradant peaks detected in all three products.

Reviewer Comment: *The potency results for PF-06439535 lot J12513-W appears contrary to the results for the three other lots as well as to the level of HMMS that increased and increase in deamidated peptide H2 that occurs within the CDR2 of the Heavy Chain. Perhaps there is a typographical error where they switched the results? IR was sent to request an explanation for the difference in potency for J12513-W compared to the other three lots.*

IR Response 12/21/18 Query “k”: Sponsor stated that the difference of 8% (84% to 92%) is not reflective of a difference in potency compared to H49500. In addition, stability data of lot H90818-W (400 mg/m16 mL) strength was compared for signs of difference in product quality as H90818-W was made from the same DS as J12513-W and data showed similar potency ranges as H49500 at 2-8°C. The ranges were 90-120% (J12513-W) and 87-110% (H90818-W).

Reviewer Comment: *Based on the response, the likely cause of the difference in potency, increase rather than decrease in potency by J12513-W appears to be assay variability. The response is acceptable.*

Peracetic Acid Treatment (308 µM and 615 µM)

One lot of each product was used to generate oxidized degradants for effect on potency and FcRn binding. Specifically, PF-06439535 lot R64867-W, US-Avastin lot 3063923, and EU-Avastin lot

B7102B13 were used in the peracetic acid forced degradation study. Peptide map analysis showed increases in methionine oxidation in peptides H16, H26, and H31, which contain Met258, Met364, and Met434, respectively. No other changes were detected on HC Met 34 and 83 as well as on LC Met4.

Based on the results that showed highest level of methionine oxidation in H16 peptide that corresponds to Met258, Pfizer quantified the levels of H16ox. The results showed that H16ox increased from ~3% for untreated sample to ~98% in samples treated with 615 μ M peracetic acid. Binding studies conducted to determine the effect on FcRn binding showed a peracetic acid concentration dependent decrease in FcRn binding. Although there was a significant decrease in binding affinity to FcRn, the potency of the peracetic acid treated samples did not change.

Reviewer Comments:

- *Note that chromatograms showing an increase in H26ox is difficult to see, whereas for the light treated samples, a small increase in the H26ox peak could be seen. The difference here appears to be the Y-axis scale, which is enlarged for the light exposure compared to the peracetic acid treatment experiment.*
- *In conclusion, the forced degradation studies showed that the three products are structurally similar and have similar degradation profiles, but the degree of degradation differs for PF-06439535 due to the difference formulation buffer.*

3.2.R.3.7 Analytical Testing Sites

The sponsor provided the following table listing the analytical similarity testing sites.

Table 3.2.R.3.7-1. Sites and Responsibilities for Similarity Assessment of PF-06439535

Site	Site Abbreviations	Responsibility	
Drug substance manufacturing site: Pfizer Worldwide Global Supply located at Wyeth BioPharma Division of Wyeth Pharmaceuticals LLC ^a One Burt Road Andover, MA 01810 USA	AND_PGS	Release testing	PF-06439535 drug substance and drug product
Pfizer Worldwide Research and Development located at Wyeth BioPharma Division of Wyeth Pharmaceuticals LLC ^a One Burt Road Andover, MA 01810 USA	AND_QC, AND_BIT, AND_MICRO, AND	Release testing - cell-based assay	PF-06439535 drug substance
		Release testing	PF-06439535 drug product
		Similarity testing	Bevacizumab - US Bevacizumab - EU
		In-depth characterization	PF-06439535 Bevacizumab - US Bevacizumab - EU
		Statistical analysis and similarity assessment	PF-06439535 Bevacizumab - US Bevacizumab - EU
Pfizer Worldwide Research and Development located at Pfizer Inc. 700 Chesterfield Parkway West Chesterfield, MO 63017 USA	STL_QC, STL	Release testing	PF-06439535 drug substance
		Release testing	PF-06439535 drug product
		Similarity testing	Bevacizumab - US Bevacizumab - EU
(b) (4)	(b) (4)	Similarity testing	PF-06439535 Bevacizumab - US Bevacizumab - EU
(b) (4)	Not applicable	In-depth characterization – LC/MS/MS – Peptide Mapping with specialized bioinformatics	PF-06439535 Bevacizumab-US Bevacizumab -EU
(b) (4)	Not applicable	In-depth characterization – Peptide Mapping/ Edman Degradation	PF-06439535 Bevacizumab-US Bevacizumab -EU

a. Wyeth is a wholly owned subsidiary of Pfizer Inc.

All lot release testing is performed at Andover, MA facility, which is also the DS manufacturing site. In addition, majority of the characterization tests for biological functions such as binding to Fcg receptors, ADCC and CDC activities, as well as mass spectrometry for peptide map and molecular weight determinations were performed at Andover, MA. For physicochemical analyses as well as bioactivity, Table 3.2.R.3.7-4 listed all the testing site per analytical methods (7 in total; CGE reduced and non-reduced, UV280 for protein concentration, glycan, ICE, SEC, and activity) used in the similarity assessment. Based on Table 3.2.R.3.7-4, there are seven different combinations for the three testing locations, Andover, MA, (b) (4) and Chesterfield, MO. The following table was drafted to deconstruct how the testing sites were utilized per US-Avastin and EU-Avastin lots.

Testing Site and Method Combination	Number of US-Avastin lots	Number of EU-Avastin lots
<p>Andover, MA for all 7 assays</p> <p>Total number of lots (US + EU)=37</p>	<p>453807 952901 957823 548426 492879 503227 503229 3044096 3055965 3063923</p>	<p>H0150B08 H0108B07 H0109B06 H0130B08 H0131B10 H0110B01 H0111B01 H0151B06 B7109B09 B7200B06 B7108B22 B8503H04 B8504H11 B8004H04 B8013H03 B8013H04 B7227B08</p>
<p>(b) (4) for all 7 assays</p> <p>Total number of lots (US + EU)=19</p>	<p>642292 642293 640018 680576 3022680 extra glycan 3049679 extra glycan 684386 extra glycan</p>	<p>H0113B05 H0115B26 H0119B06 B7102B06 B7102B13 B7102B27 H0115B31 extra ICE B8000B19 B8001H15 B8003H05 B8500H04 extra glycan B8503H02 extra glycan</p>
<p>Andover, MA for all 6 except ICE at Middleton</p> <p>Total number of lots (US + EU)=13</p>	<p>450657 477504 488495 497080 970031 971630 469053 478201</p>	<p>B7102B03 H0133B06 H0121B03 H0114B34 B7100B06</p>
<p>Andover, MA for all 6 except bioactivity at (b) (4)</p>	<p>504740 521496 522210 529846</p>	<p>B7100B18 B7101B19 B7012B09 B7108B16 extra CGE red</p>

Total number of lots (US + EU)=17	535400 626307 640016 648510	B7104B13 B7106B04 B7110B03 B7045B01 B7043B01
(b) (4) for all 6 except bioactivity at Andover, MA Total number of lots (US + EU)=4	458726 470332	H0135B11 H0138B02
Chesterfield, MO for all except bioactivity at (b) (4) Total number of lots (US + EU)=13	541430 535443 557412 566296 569321 569327 579977 585982 605025 611118 616350	B7103B04 B7105B12
Chesterfield, MO for all except bioactivity at Andover, MA Total number of lots (US + EU)=3		B8507H02 B8018H09* B7222H08

*Andover performed also concentration by UV280, CGE-reduced

Reviewer Comments:

- Table 3.2.R.3.7.4 Testing Sites for Quality Attributes Used in Similarity and Statistical Analysis of Bevacizumab-US and Bevacizumab-EU methodology column list an assay called “Specific_Activity” and Table 3.2.R.3.7-17 Methodology Naming Convention states that “Specific_Activity” is also known as “Cell-based assay”. In addition, “Bioassay_cellular” is also stated as “Cell-based assay”. Pfizer should clarify if the “Bioassay_cellular” and “Specific_Activity” are the same assay as the proposed release and stability potency assay, which is the HUVEC proliferation assay. IR was sent for clarification.

IR Response 10/2/18:

Sponsor confirms that the above-mentioned terminology all refers to the validated HUVEC bioassay used for release and stability testing.

Reviewer Comment: The response is acceptable.

- *Table 3.2.R.3.7-12 Analytical Similarity Testing and In-Depth Characterization for Biological Activity Characterization did not list the testing site for binding to FcγRIIIa by SPR. IR was sent to request testing site information for FcγRIIIa.*

IR Response 10/2/18:

Sponsor states that the testing site for FcγRIIIa by SPR was Andover. In addition, the table 3.2.R.3.7-12 has been updated to include the testing site information.

Reviewer Comment: *The response is acceptable.*

Overall conclusion of the similarity assessment based on the results from primary and higher order structures, biological activities, product-related variant and impurities levels, support a determination of highly similar for the three products notwithstanding minor differences that were shown to be not clinically meaningful. Additionally, the similarity data related to protein concentration and manufacturing process capability support a demonstration of same strength. The sponsor provided adequate scientific bridge to support the use of EU-Avastin in the clinical studies.

5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

The sponsor developed and validated a screening, confirmatory, and a neutralizing activity (Nab) assays to screen patient samples for the development of anti-drug-antibodies (ADAs). The screening method is an electrochemiluminescence (ECL) immunoassay that uses the Meso Scale Discovery Sector Imager 6000 instrument (MSD) for signal detection. The ECL method utilizes an electric current to generate a luminescent signal in the presence of ruthenium and tripropylamine reagent. Specifically, one set of PF-06439535 or EU-Avastin product is labeled with biotin and a second set of PF-06439535 or EU-Avastin product is labeled with ruthenium and both sets are incubated with patient sample that potentially contains ADAs to PF-06439535 or EU-Avastin. The ADA acts to bridge the biotin-PF-06439535/EU-Avastin with ruthenium-PF-06439535/EU-Avastin. For example, the biotin-PF-06439535-ADA-PF-06439535-ruthenium complex is captured onto the ECL plate that is coated with streptavidin. After a washing step, an electric current is passed through the plate and a luminescent signal is read as a positive signal above the established cut-point of the ECL assay.

Prior to incubation with labeled PF-06439535, the patient samples undergo two acid dissociation steps. The first acid dissociation step is remove VEGF bound to PF-06439535. The acid dissociated samples are neutralized with Tris-base on anti-VEGF antibody coated plate to remove any potential endogenous VEGF interference. Following the removal of endogenous VEGF, the samples are treated to acetic acid for a second round of acid dissociation to dissociate bound anti-PF-06439535 from PF-06439535. Following second neutralization with Tris-base, the samples are incubated with ECL reagents, i.e. “master mix” that contains biotinylated-PF-06439535 and ruthenylated-PF-06439535, as well as anti-VEGF antibody to remove residual endogenous VEGF.

Reviewer Comments:

- *The ADA and Nab method procedures were provided in the submission but appear to be written prior to execution of the method validations. Therefore, ADA screening, confirmatory, and Nab method SOPs used to evaluate patient samples were requested and provided in IR response 2/14/19.*

- *Studies to show that labeling PF-06439535 with ruthenium and biotin did not alter the binding abilities were not provided in the validation report. IR was sent to request appropriateness of the labeled reagents.*

IR Response 2/14/19 Query 2A: Sponsor did not provide qualification data that assessed the impact of the labeling reagents on PF-06439535. However, sponsor claims that based on the PC results that showed high sensitivity (48 ng/mL using human anti-bevacizumab monoclonal antibody) of the assay using the labeled reagents indicates minimal impact on PF-06439535 due to the labeling process.

Reviewer Comment: *The response is acceptable.*

Two screening assays were validated and used for PK study B7391001. The ADA screening assay validation numbers are B7397001 and B7397006 and differ only in the biotinylated and ruthenated labeling reagents, i.e. B7397001 used PF-06439535 and B7397006 used EU-Avastin as the labeled reagent. Similarly, two validated Nab assays were used to test ADA confirmed positive samples. The Nab validation numbers are B7397002 and B7397004 and again differ only in the capture reagents used, i.e. B7397002 used PF-06439535 as the capture reagent and B7397004 used EU-Avastin as the capture reagent. Single immunogenicity assays were used to evaluate serum samples from the multi-dose clinical study, B7391003. Specifically, B7397001 and B7397002 assays were used for ADA and Nab, respectively.

Reviewer Comment: *The neutralizing activity assay method description and validation study were provided in the submission. However, based on the very low immunogenicity seen in the clinical results using the screening assay, the neutralizing activity results were not necessary. Therefore, only the screening assay validation study is described and reviewed below.*

Bioanalytical Method Validation Report (B7397001): The Validation of an ECL Assay for the Detection of Anti-Drug Antibodies (ADA) Directed Against PF-06439535 in Human Serum

The ADA screening and confirmatory assays were evaluated for method precision, specificity, dilution, linearity, sensitivity, drug tolerance, interference by VEGF, positive control cross-reactivity, and sample stability. Pooled normal human serum (from at least 50 donors) was used as the negative control (NC) and affinity purified human anti-bevacizumab monoclonal antibody (primary positive control) as well as affinity purified rabbit polyclonal anti-bevacizumab antibody were used as positive controls (PC). Stock solution of PC was derived using 1:50 dilution of the PC stock A (0.5 mg/mL) into human serum (10 µg/mL, stock B). The minimum required dilution (MRD) is stated as 1: 193.

Reviewer Comment: *The typical range for MRD is from 1:5 to 1:100 and should be determined from treatment-naïve subjects. The MRD of 1:193 is above the typical range and data to justify the MRD was not provided in the validation report. IR was sent to provide additional data and justification for the chosen MRD.*

IR Response 2/14/19 Query 2B: Sponsor states that an MRD of 1:193 was necessary due to high levels of VEGF dimers that led to false positive results. Additional data showing high false positive results at MRD 1:360 during assay development was provided. With the use of anti-VEGF antibodies, the MRD was reduced to 1:193.

Reviewer Comment: *The data provided justifies the chosen MRD as well as the use of NC serum samples rather an assay buffer for the matrix selectivity assessment. The response is acceptable.*

Precision for Human Anti-Bevacizumab Monoclonal Antibody PC

The screening assay precision was evaluated using PC response unit output (RU) obtained from intra-run, intra-cay, and inter-assay runs. The method is considered precise when the coefficients of variation (%CV) of PC end point log10 titers from each set of PC (intra-assay and inter-assay) runs are less than 25%. Both RU and end-point titer results were provided for the PCs. PC starting concentration used for precision was 10000 ng/mL, which was then serially diluted 1:3 until end point log10 titer was obtained.

Intra-run precision was determined from one set of NC and five sets of titrated PC on a single 96-well plate, i.e. single run, Run 32. The results showed mean RU for NC was 69.5 and maximum PC RU ranged from 2589 to 3034 with %CV as 6.4%. The end point log10 titers ranged from 5.10 to 5.45 with % CV of 2.4%. (see table below).

Table 2 Intra-Run Precision of the Human Anti-Bevacizumab Monoclonal Antibody for the Detection Assay in Human Serum

Cut Point Factor Applied: 1.06							
Human Anti-Bevacizumab mAb PC							
Run	Mean NC RU	Maximum PC RU			End Point Log ₁₀ Titer		
32	69.5	2589	N	5	5.32	N	5
		3013	Mean	2897	5.10	Mean	5.31
		2984	SD	184	5.32	SD	0.129
		2864	%CV	6.4	5.36	%CV	2.4
		3034			5.45		

The intra-day run precision was determined from one set of NC and five sets of titrated PC on four 96-well plates (Runs 28, 29, 30, and 31). The results showed mean RU for NC ranged from 68.0 to 72.0 and the mean of four plates was 69.1 and maximum PC RU ranged from 2189 to 2720 with %CV as 7.1%. The end point log10 titers ranged from 4.49 to 5.06 with % CV of 2.9%. (see table below).

Table 3 Intra-Day Precision of the Human Anti-Bevacizumab Monoclonal Antibody for the Detection Assay in Human Serum

Cut Point Factor Applied: 1.06							
Human Anti-Bevacizumab mAb PC							
Run	Mean NC RU	Maximum PC RU			End Point Log ₁₀ Titer		
28	68.9	2471	N	5	4.80	N	5
		2720	Mean	2588	4.67	Mean	4.66
		2574	SD	94.3	4.59	SD	0.0811
		2542	%CV	3.6	4.63	%CV	1.7
		2634			4.63		
29	72.0	2693	N	5	4.83	N	5
		2605	Mean	2528	4.64	Mean	4.76
		2493	SD	132	4.73	SD	0.194
		2343	%CV	5.2	4.56	%CV	4.1
		2504			5.06		
30	67.6	2196	N	5	4.63	N	5
		2439	Mean	2302	4.51	Mean	4.66
		2189	SD	137	4.84	SD	0.131
		2225	%CV	6.0	4.57	%CV	2.8
		2463			4.73		
31	68.0	2214	N	5	4.64	N	5
		2355	Mean	2279	4.83	Mean	4.65
		2251	SD	60.3	4.67	SD	0.122
		2330	%CV	2.6	4.62	%CV	2.6
		2245			4.49		
N	4	20			20		
Mean	69.1	2424			4.68		
SD	1.99	172			0.135		
%CV	2.9	7.1			2.9		

The inter-day precision was determined from 45 runs over 9 different days. The mean maximum PC RU values, mean PC end point titer, mean PC end point titer log₁₀, mean NC RU, plate cut point, and mean PC concentration (ng/mL) at assay cut point were reported (see table below). The %CV for mean maximum PC RU was 25.1%, the mean PC end point log₁₀ titer was 5.5%, and the mean NC RU was 6.8%. The relative assay sensitivity was determined using the data from inter-assay runs and the formula, mean PC concentration at assay cut point + 1.645*SD. The mean PC concentration at assay cut point was calculated using the formula, MRD x highest starting primary PC concentration in the assay)/end point titer, and is 24.86 ng/mL.

Table 6 Sensitivity and Inter-Assay Precision of the Human Anti-Bevacizumab Monoclonal Antibody Detection Assay in Human Serum

Cut Point Factor Applied: 1.06							
Run Date	Run	Mean PC Maximum RU	Mean PC End Point Titer	Mean PC End Point Log ₁₀ Titer	Mean Negative Control RU	Plate Cut Point	Mean PC Concentration (ng/mL) at Assay Cut Point ^b
9-Dec-13	1	2793	122000	5.09	61.1	64.8	15.82
9-Dec-13	2	2736	83400	4.92	66.4	70.4	23.14
9-Dec-13	3	2405	77200	4.89	64.5	68.4	25.00
10-Dec-13	4	3662	100000	5.00	72.1	76.5	19.30
10-Dec-13	5	3677	46600	4.67	67.6	71.7	41.42
10-Dec-13	6	3925	120000	5.08	69.9	74.1	16.08
10-Dec-13	7	3719	126000	5.10	79.1	83.9	15.32
11-Dec-13	8	3020	NR	NR	62.1	65.9	NC
11-Dec-13	9	3361	332000	5.52	66.1	70.1	5.81
11-Dec-13	10	3900	98900	4.99	62.8	66.5	19.51
11-Dec-13	11	3797	110000	5.04	66.8	70.8	17.55
11-Dec-13	12	3906	138000	5.14	71.0	75.3	13.99
11-Dec-13	13	3633	103000	5.01	65.1	69.0	18.74
12-Dec-13	14	1947	42900	4.63	61.6	65.3	44.99
12-Dec-13	15	1655	94500	4.98	68.4	72.5	20.42
12-Dec-13	16	1654	105000	5.02	67.8	71.8	18.38
12-Dec-13	17	1649	32600	4.51	70.3	74.5	59.20
12-Dec-13	18	2092	248000	5.39	65.0	68.9	7.78
12-Dec-13	19	1940	44600	4.65	71.0	75.3	43.27
13-Dec-13	20	1986	320000	5.51	57.5	61.0	6.03
13-Dec-13	21	1821	39600	4.60	62.0	65.7	48.74
13-Dec-13	22	1937	62400	4.80	69.5	73.7	30.93
13-Dec-13	23	1720	105000	5.02	61.8	65.5	18.38
16-Dec-13	24	3554	198000	5.30	71.9	76.2	9.75
16-Dec-13	25	3270	140000	5.15	78.4	83.1	13.79
16-Dec-13	26	3168	321000	5.51	71.4	75.7	6.01
16-Dec-13	27	3073	87100	4.94	75.9	80.4	22.16
18-Dec-13	28 ^a	2588	47000	4.66	68.9	73.0	41.06
18-Dec-13	29 ^a	2528	62900	4.76	72.0	76.3	30.68
18-Dec-13	30 ^a	2302	46800	4.66	67.6	71.7	41.24
18-Dec-13	31 ^a	2279	46100	4.65	68.0	72.1	41.87
18-Dec-13	32 ^a	2897	210000	5.31	69.5	73.7	9.19

Cut Point Factor Applied: 1.06							
Run Date	Run	Mean PC Maximum RU	Mean PC End Point Titer	Mean PC End Point Log ₁₀ Titer	Mean Negative Control RU	Plate Cut Point	Mean PC Concentration (ng/mL) at Assay Cut Point ^b
18-Dec-13	33	2468	45200	4.66	71.4	75.7	42.70
18-Dec-13	34	2530	46700	4.67	70.4	74.6	41.33
18-Dec-13	35 ^a	2271	52900	4.71	70.5	74.7	36.48
18-Dec-13	36 ^a	2305	41600	4.62	64.5	68.4	46.39
18-Dec-13	37 ^a	2983	83200	4.92	75.4	79.9	23.20
18-Dec-13	38	2940	115000	5.06	75.5	80.0	16.78
18-Dec-13	39	2604	88000	4.94	70.3	74.5	21.93
18-Dec-13	40	2927	229000	5.36	69.8	73.9	8.43
18-Dec-13	41	2803	90600	4.96	74.6	79.1	21.30
17-Dec-13	42	2991	134000	5.13	68.8	72.9	14.40
17-Dec-13	43	2476	45000	4.65	70.6	74.9	42.89
17-Dec-13	44	2603	95900	4.98	68.0	72.1	20.13
6-Jan-14	45	3707	156000	5.19	73.4	77.8	12.37
N		45	44	44	45	45	44
Mean		2760	112100	4.96	68.8	72.9	24.86
SD		693	77600	0.271	4.68	4.96	14.0
%CV		25.1	69.2	5.5	6.8	6.8	56.3
Relative Sensitivity ^c = 48 ng/mL							

a: Multiple replicates of PC were analyzed in this run.

The average Maximum RU and/or Endpoint Titer were reported.

b: MRD x highest starting primary PC concentration in the assay ÷ End Point Titer

c: Mean PC Concentration (ng/mL) at Assay Cut Point + 1.645*SD

NR: No Reportable titer (titer > 5.63)

NC: Not calculated

PC Acceptance Criteria: Mean PC End Point Log₁₀ Titer ± 3SD = 4.15 to 5.77

Reviewer Comments:

- The intra-run and intra-day assay precision are close in value and have low %CV. However, the inter-assay precision is quite variable. The %CV for inter-assay just passes the validation acceptance criterion of 25%. Note that of the 45 data points for the maximum PC RU, 8 data points are the mean from runs with multiple PC per plate, e.g. run 32 has 5 replicates of PC. Therefore, the number reported is the mean of replicates and is not a single value as the other 36 data points. If the 8 mean maximum PC values are deleted from the overall inter-assay precision calculation, the %CV increases to 26.5% and would not meet the validation acceptance criterion. The validation protocol states to determine the core inter-assay precision from 6 runs over three days and overall precision statistics from all accepted validation runs and used to specify the assay acceptance criteria for sample analysis, i.e. system suitability. If core inter-assay precision is calculated using data from Dec 9, 10, and 12, first 2 runs, the %CV decreases to 14.6%. Based on core inter-assay precision statistics along with intra-run and intra-day variability, the ADA assay can be considered to have adequate precision.

- *The overall inter-assay precision results from 45 runs do indicate that variability increases over multiple runs that span multiple days greater than 3. This could be due to various assay parameters. It is unclear whether assay robustness was investigated during development. IR was sent to determine whether robustness was evaluated and to provide the results.*

IR Response 2/14/19 Query 2C: Sponsor states that assay robustness is demonstrated by obtaining high precision from data obtained by using 4 analysts, over 45 runs, conducted over 30 days. In addition, assay robustness studies were conducted that assessed wash buffer lots, anti-VEGF lots, etc. The precision of the PC titers increased as the PC concentration moved close to the plate cut-point and demonstrates the ability of the assay to consistently detect potential positive ADAs.

Reviewer Comment: *The response is acceptable.*

- *The assay acceptance criteria for PC is stated as “Mean PC End Point Log10 Titer \pm 3SD” and is equal to 4.15 to 5.77 (see footnote above under Table 6). The acceptance criterion would allow mean end point titer to vary between 14,125.4 to 588,843.7 and the assay considered acceptable. The lowest end point titer observed over 9 days was 32,600 and the highest was 332,000. The acceptable range is 42-fold and is wide. High variability is expected because the assay acceptance criterion is based on end point titers, which are just above the cut-point value. Another way to think of this would be that assay is running as expected if the assay can detect as low as 3.28 ng/mL (588843 end point titer) to 136.6 ng/mL (14,125.4 end point titer). The high end of assay acceptance criterion should not be less than the established assay sensitivity of 48 ng/mL. IR was sent to justify the assay acceptance criterion of the lower end, 4.15 log10 or 14,125 end point titer, which amounts to 136.6 ng/mL.*

IR Response 2/14/19 Query 2D: Sponsor states that the assay acceptance criterion represents PC concentration of 4.57 ng/mL to 123 ng/mL for each run and falls within the assay sensitivity requirements of < 250 ng/mL per guidance at the time of assay validation. The assay acceptance criterion is also stated to meet the expectation of 1% assay failure rate near the cut-point stated in FDA guidance for immunogenicity assay development as 5 out of 337 runs failed (1.5%).

Reviewer Comment: *The response is acceptable.*

ADA Screening and Confirmatory Cut-Point Determinations:

The screening cut-point was determined using serum from diseased (solid tumor) and normal populations. A total of 28 solid tumor patient serum samples and 50 normal serum samples were used to determine the screening assay cut-point. The individual samples were analyzed over 4 days and in 4 different runs for screening cut-point and 2 independent runs for confirmation by at least 2 analysts.

The normal and solid tumor data were log10 transformed and evaluated for normal distribution. Outliers were identified using the box-plot method and removed. If the data are normally distributed, then the cut-point factor was calculated using the formula, [(mean response of individual sera + 1.645*SD)/mean response of NC] and if the data are not normally distributed, then the cut-point factor was calculated using the formula, [(95th percentile of response of individual sera)/mean response of NC]. A floating cut-point was established by determining a cut-point factor and using the formula, cut-point factor x mean response of NC on individual plate. The cut-point factor for both the normal serum samples and solid tumor samples was 1.06.

The confirmatory assay screening cut-point was determined by evaluating the response in the presence and absence of 12 µg/mL of PF-06439535 and PC at 10000 ng/mL (HPC) and 1000 ng/mL (LPC). The signal inhibition was determined using the formula, $\{100 \times [1 - (\text{spiked signal}) / (\text{unspiked signal})]\}$ and the confirmatory cut-point was calculated using the formula, $\% \text{ signal inhibition} + (\text{coefficient} \times \text{SD})$, where the coefficient was equal to 3.09 to achieve 0.1% false-positive rate. The cut-point based on normal serum samples was 11.6% and based on solid tumor samples was 13.9%.

Reviewer Comments:

- *Statistical analysis for normal distribution was provided and all but the first day of the normal serum samples were not normally distributed for screening assay cut-point.*
- *The statistical analysis of the data to justify the selection of a floating cut-point was not provided. IR was sent to provide the justification for a floating cut-point.*

IR Response 2/14/19 Query 2E: Sponsor provided statistical analysis data that showed that the means were different from 4 runs each of normal and solid tumor serum samples, but the variances were the same.

Reviewer Comment: *The response is acceptable.*

- *For disease serum samples, Pfizer did not use non-small cell lung cancer (NSCLC) patient samples, but used solid tumor patient samples. As NSCLC serum samples is also considered solid tumor, the cut-point determination using these samples is acceptable. However, IR was be sent to confirm a 5% false-positive rate in NSCLC patients.*

IR Response 2/14/19 Query 2F: Sponsor stated that 44 out of 705 baseline samples tested positive for ADA. The false-positive rate was 6.2% in the NSCLC study population.

Reviewer Comment: *The response is acceptable.*

- *FDA guidance does not recommend a false positive rate of 0.1% due to increased risk of false-negative results. IR was sent to assess the immunogenicity samples based on higher false-positive rate, e.g. 1%.*

IR Response 2/14/19 Query 2G: Sponsor stated that the 0.1% was the recommended false positive rate at the time of validation study (2013 to 2014). Therefore, Pfizer recalculated the confirmed positive numbers using the confirmatory cut-point based on 1% false positive rate. The use of the cut-point with 1% false positive rate led to an increase in the number of confirmed positive from 19 to 63 patients. The tables below show the 1% cut-point values and the additional samples that were confirmed positive.

Table 5. Bevacizumab ADA Assays B7397001 and B7397006 CCP Comparison

False-Positive Rate Used for CCP	B7397001 Normal Serum	B7397001 Solid Tumor	B7397006 Normal Serum
0.1% false –positive rate	11.60%	13.90%	EU-18% US-14.2%
1% false-positive rate	7.90%	9.50%	EU-12.9% US-10.7%

Source: [Module 5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies; Assays B7397001 and B7397006](#)

Abbreviations: ADA=anti-drug antibody; CCP= confirmatory cut-point; EU=European Union (Bevacizumab); US=United States (Bevacizumab).

Table 6. Comparison of Confirmed ADA Positive From Bevacizumab Studies B7391001 and B7391003 (Baseline and Post-Treatment) Using B7397001 ADA Assay (Anti-PF-6439535 Antibody)

Pfizer Beva Study	Confirmed ADA Positive Followed CCP Using 0.1% False-Positive Rate	Confirmed ADA Positive Followed CCP Using 1% False-Positive Rate	Difference in Confirmed ADA Positive
B7391001	14 (4 from PFE treated, 7 from EU treated, 3 from US treated)	27 (9 from PFE treated, 11 from EU treated, 7 from US treated)	13 (5 from PFE treated, 4 from EU treated, 4 from US treated)
B7391003	19 (6 from PFE treated, 13 form EU treated)	63 (29 from PFE treated, 34 form EU treated)	44 (23 from PFE treated, 21 from EU treated)

Source: Module 5.3.3.1 Reports of Bioanalytical and Analytical Methods for Human Studies; B7391001; Module 5.3.5.1 Reports of Bioanalytical and Analytical Methods for Human Studies; B7391003
Abbreviations: ADA=anti-drug antibody; CCP= confirmatory cut-point; Beva= Bevacizumab; EU=European Union (Bevacizumab); PFE= PF-06439535; US=United States (Bevacizumab).

Reviewer Comment: *The increase in confirmed positive patients were increased for both PF-06439535 and EU-approved Avastin groups. Therefore, although the numbers are higher, the occurrence of ADA are also similar. The adequacy of the new confirmed positive values will be assessed by clinical pharmacology reviewer.*

Sample Stability Assessment:

The stability of PC in human serum was determined to assess the sample stability during handling and processing. PC (10000 ng/mL) that were aliquoted and stored at -70°C were thawed and stored at room temperature up to 24 hours, then stored at -70°C until analysis. The end point log10 titer of the stability sample was within 0.48 (log10 of 3, the serial dilution factor) of each other, i.e. 4.75 (time zero sample) versus 4.99 (24 h sample).

The stability of PC in human serum was determined after 7 freeze/thaw cycles between room temperature and -20°C and -70°C. For the first cycle the PC samples were kept frozen up to 24 hours and subsequent cycles the samples were kept frozen for 12 hours and thawed at room temperature unassisted and left at room temperature for 1 hour before being refrozen. The end point log10 titer of the stability sample was within 0.48 (log10 of 3, the serial dilution factor) of each other, i.e. 4.75 (time zero sample) versus 4.70 (-20°C) and 4.65 (-70°C).

Reviewer Comment: *The stability of the PC as a surrogate for sample stability was adequately assessed and indicates that the samples can be thawed and stored up to 24 hours at room temperature as well as undergo up to 7 freeze/thaw cycles.*

Dilution Linearity:

The linearity of the response was determined using PC at 5000, 2500, and 1000 ng/mL that represents 5-fold, 10-fold, and 25-fold of the starting PC concentration of 25000 ng/mL. The high, medium, and low PC samples were titrated to obtain endpoint log10 titer values. The endpoint log10 titers were normalized to a concentration of 25000 ng/mL and the differences between the normalized endpoint log10 titers was less than 0.48 for the three concentration levels, i.e. 5.27 (5000 ng/mL), 5.08 (2500 ng/mL), and 5.52 (1000 ng/mL).

Matrix Selectivity (Recovery):

High (10000 ng/mL) and low (1000 ng/mL) PC was spiked into 10 normal and 10 solid tumor serum samples and the signal compared to non-spiked serum samples to determine the recovery of the PC signal in the presence of matrix components. The percent recovery was determined using the following formula, $[100 * (\text{Spiked} - \text{Unspiked}) / (\text{Mean Spiked NC} - \text{Mean Unspiked NC})]$. The results for HPC showed all but one lot of normal serum sample passed recovery acceptance criteria of 75-125% with a value of 68.3%. The results for LPC had recoveries within the acceptance criteria for normal serum samples, but for the solid tumor samples, the experiment was repeated because only 60%, i.e. 6 lots out of 10 passed the recovery acceptance criteria. The repeat testing of the LPC spiked samples was run in duplicates. The repeat testing of the LPC all met the recovery acceptance criteria.

Reviewer Comment: *Typically, the matrix selectivity is performed against buffer to determine if components in the serum samples affect the signal of the PC. The current matrix selectivity experiment is testing whether individual serum samples contain components that affect PC signal differently compared to the pooled normal serum samples that is used as the NC. The results that compare against assay buffer is similar to determining the MRD and stated in the 2008 Shankar et.al paper on immunogenicity assay buffer as a way to confirm that the selected MRD is appropriate. IR was sent to request signal compared to assay buffer, which may be part of their MRD assessment. See IR response 2/14/19 query 2B.*

Drug Interference (Tolerance):

Different concentrations of PF-06439535 (0, 0.8, 1.6, 3.2, 6.4, 12.5, 25, 50, 100 µg/mL) was incubated with HPC and LPC for 1 hour at room temperature prior to MRD. The results showed that both HPC and LPC could tolerate up to 100 µg/mL of PF-06439535.

Reviewer Comment: *The trough level for clinical study B7397003 was between 15 to 100 µg/mL. Therefore, the drug interference or tolerance up to 100 µg/mL is considered acceptable.*

VEGF Interference:

Different concentrations of VEGF (0, 100, 500, 1000, 3000, and 10000 pg/mL) was incubated with HPC and LPC for 1 hour at room temperature prior to analysis. Results showed no difference in the signal due to the presence of VEGF in the samples, i.e. no dose response reduction for the monoclonal HPC and LPC and rabbit LPC. The results for the rabbit HPC showed a decrease in signal of ~25% at all concentrations of VEGF tested.

Reviewer Comment: *There is a lack of dose response for the percent decreases in the signal for both PCs at low and high concentration. In addition, the ~25-30% decrease in signal by the HPC compared to the LPC does not appear to be due to a lack of linearity in the concentration of the VEGF used in the study and could be due to assay design for the HPC. Because the LPC control represents the worst-case and the VEGF concentration range covers serum concentrations of VEGF typically found in normal and cancer patients, e.g. around 173 pg/mL and 328 pg/mL (Kut, C. et. al. British J Cancer 2007, 97: 978), respectively. Therefore, adequate tolerance to VEGF was demonstrated.*

PF-06439535/VEGF Complex Interference:

HPC and LPC were spiked with two concentrations of VEGF (500 and 10000 pg/mL) and PF-06439535 (10 and 40 mg/mL) and pre-incubated for 1 hour at room temperature prior to analysis. The results

showed ~30 - 60% and ~30 - 75% decrease in RU for LPC and HPC, respectively. However, all RU were several folds (2 to 30 fold) above the plate cut-point.

Reviewer Comments:

- *The drop in signal is likely due to the presence of the drug, PF-06439535.*

Mass-based Assay Sensitivity:

The sensitivity of the screening assay defined as the concentration of the anti-bevacizumab antibodies (positive control) in human serum that results in a signal equal to the plate cut-point was determined as the 95th confidence interval of the mean from inter-assay precision and intra-assay precision run data for the monoclonal and rabbit polyclonal PC, respectively. Specifically, the assay sensitivity was calculated from the following formulas:

$$\text{Mean PC concentration at assay cut-point} = \frac{[\text{highest starting PC concentration in the assay}]}{\text{End Point Titer} \times \text{MRD}}$$

$$\text{Relative Assay Sensitivity} = \text{Mean PC concentration at assay cut-point} + (1.645 \times \text{SD})$$

The assay sensitivities were 48 ng/mL and 164 ng/mL for monoclonal and rabbit polyclonal PC, respectively.

Reviewer Comment: *The assay sensitivity is technically lower than what is reported because typically, the concentration that provides a signal at assay cut-point is not increased by factoring in a 95th confidence interval. However, even with the additional confidence interval factored in, the assay is still considered sensitive and is adequate.*

Conclusion: *The ADA and confirmatory assays, based on the validation data, are adequate to support the intended use.*



Jee
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STATISTICAL REVIEW AND EVALUATION

Biometrics Division: VI

BLA #	761099
Related IND #:	117038
Product Name:	PF-06439535 humanized immunoglobulin 1(IgG1) monoclonal antibody
Strength	100 mg/4 mL, 400 mg/16 mL (25mg/ml)
Indication(s):	All indications approved for bevacizumab excluding the indication of platinum-resistant recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer.
Applicant:	Pfizer
Dates:	01/26/2018
Statistical reviewer	Tianjiao Dai
Second reviewer	Tianhua Wang
Medical Officer	Sandra Casak, M.D.
Project Manager	Shubhangi (Gina) Mehta

Tianjiao Dai, Ph.D., Mathematical Statistician, CDER/OTS/OB/DB VI
 Tianhua Wang, Ph.D., Mathematical Statistician, CDER/OTS/OB/DB VI

Concur: _____

Meiyu Shen, Ph.D., Lead Mathematical Statistician, CDER/OTS/OB/DB VI
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I. EXECUTIVE SUMMARY AND RECOMMENDATION

The CMC statistical reviewer in the Office of Biostatistics analyzed the comparative results of two critical quality attributes: % Relative Potency as assessed by Inhibition of Cell Growth Assay and VEGF165 Binding by ELISA, which were recommended for equivalence testing analysis by the Office of Biotechnology. Tier 1 statistical equivalence testing was conducted using equivalence margins of $\pm 1.5\sigma_R$, where R represents the reference product US-licensed Avastin or comparator product EU-approved bevacizumab. 10 PF-06439535 lots (2 DS batches and 8 DP lots), 46 US-licensed Avastin lots, and 43 EU-approved bevacizumab lots were used for equivalence testing for % Relative Potency as assessed by Inhibition of Cell Growth Assay. The results are summarized in Table 1.

Table 1: Equivalence testing results for Inhibition of Cell Growth Activity (% Relative Potency)

Comparison	# of lots	Mean Difference, %	90% Confidence Interval for Mean Difference, %	Equivalence Margin, %	Pass the Equivalence Testing?
PF-06439535 vs. US	(10, 46)	-3.68	(-9.40, 2.05)	(-15.06, +15.06)	Yes
PF-06439535 vs. EU	(10, 43)	-5.18	(-10.79, 0.43)	(-14.49, +14.49)	Yes
EU vs. US	(43, 46)	1.50	(-1.97, 4.97)	(-15.06, +15.06)	Yes

*The 90% confidence interval is adjusted by the sample size imbalance.

Ten PF-06439535 lots (2 DS batches and 8 DP lots), 25 US-licensed Avastin lots and 27 EU-approved bevacizumab lots were used for equivalence testing for VEGF165 binding by ELISA. The results are summarized in Table 2.

Table 2: Equivalence testing results for the VEGF₁₆₅ Binding by ELISA

Comparison	# of lots	Mean Difference, %	90% Confidence Interval for Mean Difference, %	Equivalence Margin, %	Pass the Equivalence Testing?
PF-06439535 vs. US	(10,25)	-2.58	(-8.93, 3.77)	(-11.98, 11.98)	Yes
PF-06439535 vs. EU	(10,27)	2.40	(-3.68, 8.47)	(-9.86, 9.86)	Yes
EU vs. US	(27,25)	-4.98	(-8.40, -1.56)	(-11.98, 11.98)	Yes

*The 90% confidence interval is adjusted by the sample size imbalance.

CMC Statistical Review of BLA761099

As shown in Tables 1 and 2, the results from the statistical equivalence testing of % Relative Potency as assessed by Inhibition of Cell Growth Assay and VEGF₁₆₅ Binding by ELISA support a demonstration that the proposed biosimilar PF-06439535 is highly similar to US-licensed Avastin. The results also support the analytical portion of the scientific bridge to justify the relevance of EU-approved bevacizumab data from the comparative safety and efficacy study

II. INTRODUCTION

On January 26, 2016, Pfizer Inc. submitted to the U.S. Food and Drug Administration (FDA) a 351(k) BLA which included an analytical similarity assessment of comparing the Tier 1 quality attributes for PF-06439535, US-licensed Avastin, and EU-approved bevacizumab.

A total of ten DS batches and sixteen DP lots were produced at the commercial scale and two development scale DS batches of PF-06439535 were included in the similarity assessment. These batches were demonstrated to be representative and comparable to the commercial scale batches through their quality attributes and assessment of the manufacturing process details. However, independent lots for PF-06439535 were chosen resulting in 2 DS batches and 8 DP lots for a Tier 1 statistical analysis. The lot genealogy and independent lot selected for Tier 1 statistical analysis are shown in Table 3.

A total of 46 bevacizumab-US drug product lots (400 mg/16 mL and 100 mg/4 mL presentation) and 51 bevacizumab-EU drug product lots (400 mg/16 mL and 100 mg/4 mL presentation) were purchased on the open market by Pfizer Inc at set intervals with no predetermined criteria, and each lot was considered equally relevant in terms of reflecting the licensed product profile established to be safe and efficacious. These licensed product lots represent nearly the full 24-month expiry period. For statistical analysis of % Relative Potency, all of the 46 bevacizumab-US lots are used. However, independent lots for bevacizumab-EU were chosen resulting 43 bevacizumab-EU lots (only lots with unique identifiers using the first 5 characters are included in the statistical analysis). An information request was sent out by FDA CMC Statistical reviewer to Pfizer on August 21, 2018 (Query 7). FDA requested Pfizer to clarify the inconsistency between the selected bevacizumab-EU lots stated in section 3.2.R.3.1.2-2 and shown Table 3.2.R.3.5-1. Pfizer clarified the 43 bevacizumab-EU lots they used for % Relative Potency statistical analysis and updated their analysis results accordingly in their response received on August 30, 2018.

For statistical analysis of VEGF₁₆₅ binding activity by ELISA, 25 bevacizumab-US and 27 bevacizumab-EU drug product lots are used. FDA CMC Statistical reviewer sent out the information request letter to Pfizer on August 21, 2018 (Query 8). FDA requested Pfizer to provide the justification of Bevacizumab-EU and Bevacizumab-US lot selection for statistical analysis of VEGF₁₆₅ binding activity and explain the inclusion of the three Bevacizumab-US lots not listed in their raw data in Table 3.2.R.3.5-1. The response of the information request was received on August

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30, 2018. Pfizer justified the random selected subset of available bevacizumab-US and bevacizumab-EU lots were tested by the VEGF165 binding ELISA and provided the corresponding evolution of the similarity assessment strategy. Pfizer also explained the inclusion of the three lots which are newly purchased, and the test results were available only after the data lock for preparation of the BLA submission. The related tables in the application have been updated accordingly by sponsor along with the IR response.

Lots information and data of Inhibition of Cell Growth and VEGF₁₆₅ binding used in Statistical Analysis of PF-06439535, Bevacizumab-US, and Bevacizumab-EU Lots are shown in Table A and Table B respectively in Appendix.

Table 3: PF-06439535 Lots Used to Demonstrate Analytical Similarity

Drug Substance Batch	Drug Product Presentation (mg)	Drug Product Lot
12P138K601	N/A	N/A
12P138K603	N/A	N/A
• 13J138K002(G27608)	N/A	N/A
13J138K003(G27629)	100	J12512-W
	400	• H49500^a
13J138K004(G27631)	100	• J12513-W
	400	H90818-W
14J138K001(J32980)	100	• J76233-W
	400	J76228-W
14J138K002(J33002)	100	J76232-W
	400	• J61289-W^b
• 14J138K003(J33003)	N/A	N/A
14J138K004(J33004)	100	J76234-W
	400	• J76229-W^b
16J138K001(R12923)	100	• R64866-W
	400	R59890-W
16J138K002(R32728)	100	R64865-W
	400	• R59889-W^b
16J138K003(R32744)	100	• R64867-W
	400	R59891-W

- Bold, bulleted DS batches and DP lots were selected as independent lots for Tier 1 statistical analyses.

N/A = No drug product made from the drug substance

The Agency carefully evaluated the data for the % Relative Potency as assessed by Inhibition of Cell Growth Assay and VEGF165 Binding by ELISA provided in the BLA submission. Our comments regarding Pfizer, Inc.'s equivalence testing (Tier 1 approach) is provided in Section 3.

and our independent statistical equivalence testing analyses are presented in section 4.

III. APPLICANT'S STATISTICAL EQUIVALENCE TESTING

In this submission, Pfizer, Inc. conducted Tier 1 statistical equivalence testing with the margin defined as $(-1.5\sigma_R, 1.5\sigma_R)$ for % Relative Potency as assessed by Inhibition of Cell Growth Assay and VEGF₁₆₅ Binding by ELISA. Pairwise comparisons were used for the assessment of the Tier 1 quality attributes. Similarity is demonstrated if all the two-sided 90% confidence intervals of the difference between means for PF-06439535 vs. US-licensed Avastin, PF-06439535 vs. EU-approved bevacizumab, and EU-approved bevacizumab vs. US-licensed Avastin are within the EAC of $(-1.5\sigma_R, 1.5\sigma_R)$, where R represents the reference product US-licensed Avastin or EU-approved bevacizumab in each of the 3 pairwise comparisons. Pfizer, Inc. presumed unequal variances and used adjusted effective sample size to calculate the two-sided 90% confidence interval of the mean difference.

FDA CMC statistics reviewer's comments on Pfizer, Inc.'s equivalence testing are:

- 1) Pfizer, Inc.'s statistical approach is appropriate for Tier 1 analytical similarity assessment.
- 2) Pfizer, Inc.'s statistical analysis results matches with the results of the independent study conducted by FDA CMC statistical reviewer. The independent study conducted by FDA CMC statistical reviewer is present in section 4 below.

IV. FDA STATISTICAL ANALYSES

A Tiered approach is recommended by the Agency to evaluate analytical similarity. That is, product quality attributes are assigned to three tiered based on their criticality. The quality attributes with potential highest risk in product quality, efficiency, safety and PK/PD are assigned to Tier 1, in which analytical similarity is assessed by statistical equivalence test. Quality attributes with lower impact are assigned to Tier 2 and their analytical similarity is evaluated by Quality Range approach. That is, a high percentage of the biosimilar data should be covered by $(\text{Mean} - X \cdot \text{SD}, \text{Mean} + X \cdot \text{SD})$ defined by the reference product. Here, the multiplier X should be justified by the scientific

knowledge. The quality attributes with the lowest risk are assigned to Tier 3 and their analytical similarity is evaluated by side-by-side comparison using graphic display. This review focuses on the equivalence testing in Tier 1.

IV.I Statistical method

Let μ_T and μ_R be respectively the population means of the quality attribute for the test product and the population mean of the quality attribute for the US-licensed Avastin product. Let σ_R be the standard deviation of the quality attribute of interest for the US-licensed Avastin. In order to conclude the equivalence in the quality attribute of interest between the test product and the US-licensed Avastin product, we aim to reject the null hypothesis of the following null and alternative hypotheses:

$$H_0: \mu_T - \mu_R \leq \theta_1 \text{ or } \mu_T - \mu_R \geq \theta_2$$

$$H_1: \theta_1 < \mu_T - \mu_R < \theta_2$$

Here $\theta_1 = -1.5\sigma_R$, $\theta_2 = 1.5\sigma_R$, θ_1 and θ_2 are equivalence margins.

We reject H_0 if 90% confidence interval for the mean difference in the quality attribute of interest falls within $(-1.5\sigma_R, 1.5\sigma_R)$. In other words, we conclude that the equivalence in the quality attribute of interest between the test product and the reference product if 90% confidence interval for the mean difference in the quality attribute of interest falls

within $(-1.5\sigma_R, 1.5\sigma_R)$. This specific equivalence margin was set as 1.5 times the standard deviation of the quality attribute for the reference product to ensure an adequate power for the case in which a small but sufficient number of lots are available for testing. For example, the probability of rejecting H_0 in the above two one-sided tests procedure with the equivalence margin being $(-1.5\sigma_R, 1.5\sigma_R)$ is 87% if the true mean difference is $0.125\sigma_R$ for a sample size of 10 biosimilar lots and 10 reference lots. When the number of lots is smaller than 10, the test size may be relaxed somewhat, but agreement on this should be reached in advance with FDA scientists. First, we estimate σ_R by the sample variability of the reference product and then in the statistical analysis, θ_1 and θ_2 are treated as a constant, not a random variable.

Let X_{Tj} be the observed value of the quality attribute of interest for Batch j of the test product (the

proposed biosimilar product) and X_{Rj} be the observed value of the quality attribute of interest for Batch j of the US-licensed Avastin product. Since the two products are manufactured by two manufacturers, two groups are independent. $\bar{X}_i = \frac{\sum_{j=i}^{n_j} X_{ij}}{n_i}$ and $S_i^2 = \frac{\sum_{j=i}^{n_j} (X_{ij} - \bar{X}_i)^2}{(n_i - 1)}$, where n_j is the number of lots in the i th product, $i = T, R$.

Under the unequal variance of the test product and the US-licensed Avastin product, the $(1 - 2\alpha) \times 100\%$ confidence interval of the mean difference in the quality attribute of interest can be calculated as:

$$(\bar{X}_T - \bar{X}_R - t_\alpha(v) \sqrt{\frac{S_T^2}{n_T} + \frac{S_R^2}{n_R}}, \bar{X}_T - \bar{X}_R + t_\alpha(v) \sqrt{\frac{S_T^2}{n_T} + \frac{S_R^2}{n_R}}) \quad (1)$$

Here $t_\alpha(v)$ is the $(1 - \alpha)$ quantile and v is the degrees of freedom calculated by Satterthwaite's approximation.

If $n_R > 1.5n_T$, the $(1 - 2\alpha) \times 100\%$ confidence interval of the mean difference in the quality attribute of interest can be calculated as:

$$(\bar{X}_T - \bar{X}_R - t_\alpha(v^*) \sqrt{\frac{S_T^2}{n_T} + \frac{S_R^2}{n_R^*}}, \bar{X}_T - \bar{X}_R + t_\alpha(v^*) \sqrt{\frac{S_T^2}{n_T} + \frac{S_R^2}{n_R^*}}) \quad (2)$$

Here $n_R^* = \min(n_R, 1.5n_T)$ and $v^* = \frac{(\frac{S_T^2}{n_T} + \frac{S_R^2}{n_R^*})^2}{\frac{1}{n_T - 1} (\frac{S_T^2}{n_T})^2 + \frac{1}{n_R - 1} (\frac{S_R^2}{n_R^*})^2}$

If the number of biosimilar lots, n_T , is 50% more than the number of reference lots, n_R , we can apply a similar approach as above with $n_T^* = \min(n_T, 1.5n_R)$ for the confidence interval calculation. In the following analyses, we use $\alpha = 0.05$.

IV.II FDA statistical equivalence testing for Inhibition of Cell Growth Assay

The Inhibition of Cell Growth Assay data distributions of PF-06439535 lots, US -licensed Avastin and EU-approved bevacizumab are displayed in Figure 1. Ten PF-06439535 lots, 46 US- licensed Avastin lots, and 43 EU-approved bevacizumab are included in the Inhibition of cell growth assay dataset for statistical equivalence testing. Descriptive statistics for the Inhibition of cell growth assay data of PF-06439535 lots, US -licensed Avastin and EU-approved bevacizumab are listed in Table 4.

Figure 1: Scatter plots of Inhibition of cell growth assay for US-licensed Avastin, PF-06439535, and EU-approved bevacizumab.

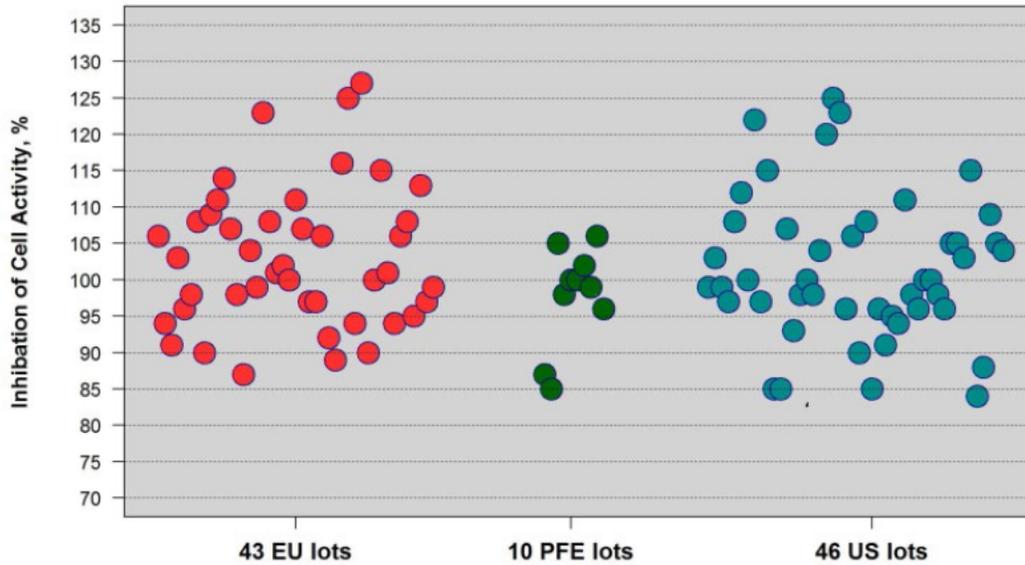


Table 4: Descriptive statistics for Inhibition of Cell Growth Assay data

Product	Number of lots	Sample mean, %	Sample standard deviation, %	Min, %	Max, %
PF-06439535	10	97.80	6.9	85	106
US-licensed Avastin	46	101.48	10.0	84	125
EU-approved bevacizumab	43	102.97	9.7	87	127

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Table 5 shows that the 90% confidence interval for the mean difference in the Inhibition of cell growth assay between PF-06439535 and US-licensed Avastin is (-9.40, 2.05)%. It falls entirely within the equivalence margin (-15.06, +15.06)%. Hence, the results of the Inhibition of Cell Growth Assay for PF-06439535 are equivalent to those for US-licensed Avastin.

It also shows that the 90% confidence interval for the mean difference in Inhibition of Cell Growth Assay between PF-06439535 and EU-approved bevacizumab is (-10.79, 0.43)%. It falls within the equivalence margin (-14.49, +14.49)%. Therefore, the results of the Cell Growth Inhibition Assay for PF-06439535 are equivalent to those for EU-approved bevacizumab.

The 90% confidence interval for the mean difference in Inhibition of Cell Growth Assay between EU-approved bevacizumab and US-licensed Avastin is (-1.97, 4.97)%, which falls entirely within the equivalence margin (-15.06, +15.06)%. Therefore, the results of the Inhibition of Cell Growth Assay for EU-approved bevacizumab are equivalent to those for US-licensed Avastin.

The statistical equivalence analyses support that relative potency as measured by the Inhibition of Cell Growth Assay of PF-06439535 is similar to that of US-licensed Avastin. The results of all three pairwise comparisons support the analytical portion of the scientific bridge to justify the relevance of the data obtained from clinical studies that compared EU-approved bevacizumab and the PF-06439535 product to support a demonstration of biosimilarity to US-licensed Avastin.

Table 5: Results of equivalence testing for Inhibition of Cell Growth Assay

Comparison	# of lots	Mean Difference, %	90% Confidence Interval for Mean Difference, %	Equivalence Margin, %	Pass the Equivalence Testing?
PF-06439535 vs. US	(10, 46)	-3.68	(-9.40, 2.05)	(-15.06, +15.06)	Yes
PF-06439535 vs. EU	(10, 43)	-5.18	(-10.79, 0.43)	(-14.49, +14.49)	Yes
EU vs. US	(43, 46)	1.50	(-1.97, 4.97)	(-15.06, +15.06)	Yes

IV.III FDA statistical equivalence testing for VEGF₁₆₅ Binding by ELISA

Scatter plots of the VEGF₁₆₅ Binding by ELISA for PF-06439535, US-licensed Avastin and EU-approved bevacizumab are shown in Figure 2. Ten batches of PF-06439535, 25 batches of US-licensed Avastin, and 27 batches of EU-approved bevacizumab are included in the VEGF₁₆₅ Binding by ELISA dataset for statistical equivalence testing. Descriptive statistics for the VEGF₁₆₅ Binding by ELISA data of PF-06439535, US-licensed Avastin, and EU-approved bevacizumab are listed in Table 6.

Figure 2: Scatter plots of VEGF₁₆₅ binding by ELISA for US-licensed Avastin, PF-06439535, and EU-approved bevacizumab

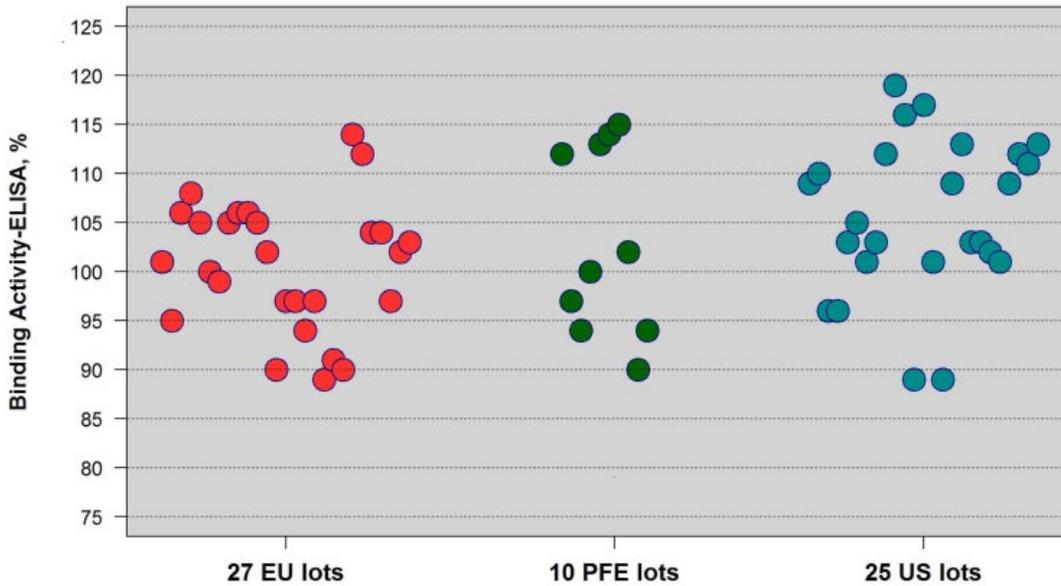


Table 6: Descriptive statistics for the VEGF₁₆₅ binding by ELISA data

Product	Number of lots	Sample mean, %	Sample standard deviation, %	Min, %	Max, %
PF-06439535	10	103.10	9.6	90	115
US-licensed Avastin	25	105.68	8.0	89	119
EU-approved bevacizumab	27	100.70	6.6	89	114

Table 7 shows that the 90% confidence interval for the mean difference in the VEGF₁₆₅ Binding by ELISA between PF-06439535 and US-licensed Avastin is (-8.93, 3.77)%. It falls entirely within the equivalence margin (-11.98, +11.98)%. Hence the VEGF₁₆₅ binding by ELISA of PF-06439535 is equivalent to the VEGF₁₆₅ binding by ELISA of US-licensed Avastin.

It also shows that the 90% confidence interval for the mean difference in the VEGF₁₆₅ binding by ELISA between PF-06439535 and EU-approved bevacizumab is (-3.68, 8.47)%. It falls within the equivalence margin (-9.86, +9.86)%. Therefore, the VEGF₁₆₅ binding by ELISA of PF-06439535 is equivalent to the VEGF₁₆₅ binding by ELISA of EU-approved bevacizumab.

The 90% confidence interval for the mean difference in VEGF₁₆₅ binding by ELISA between EU-approved bevacizumab and US-licensed Avastin is (-8.40, -1.56)%, which falls entirely within the equivalence margin (-11.98, +11.98)%. Therefore, the VEGF₁₆₅ binding by ELISA of EU-approved bevacizumab is equivalent to the VEGF₁₆₅ binding by ELISA of US-licensed Avastin.

The statistical equivalence analyses support that VEGF₁₆₅ binding measured by ELISA of PF-06439535 is similar to that of US-licensed Avastin. The results of all three pairwise comparisons support the analytical portion of the scientific bridge to justify the relevance of the data obtained from clinical studies that compared EU-approved bevacizumab and the PF-06439535 product to support a demonstration of biosimilarity to US-licensed Avastin.

Table 7: Equivalence testing results for the VEGF₁₆₅ Binding by ELISA

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Comparison	# of lots	Mean Difference, %	90% Confidence Interval for Mean Difference, %	Equivalence Margin, %	Pass the Equivalence Testing?
PF-06439535 vs. US	(10,25)	-2.58	(-8.93, 3.77)	(-11.98, 11.98)	Yes
PF-06439535 vs. EU	(10,27)	2.40	(-3.68, 8.47)	(-9.86, 9.86)	Yes
EU vs. US	(27,25)	-4.98	(-8.40, -1.56)	(-11.98, 11.98)	Yes

V. ADDITIONAL ANALYSIS UPON REQUEST

According to the request by OBP on n January 24, 2019, the data analysis of Tier 1 QAs are conducted after the four lots US 952901, EU H0114B34, EU B7100B06 and EU H0110B01 are removed. Site inspection showed that these 4 lots were tested multiple times and the sponsor claims that the conclusion is the same even if they took out these lots.

Results for Inhibition of Cell Growth Assay are shown in Table 7 and 8 and Results for VEGF165 Binding by ELISA are shown in Table 10 and 11. They still pass the equivalence tests for all comparisons. So, the conclusions of statistical analysis of Tier 1 QAs do not change.

Table 7. Descriptive statistics for Inhibition of Cell Growth Assay data (lots US 952901, EU H0114B34, EU B7100B06 and EU H0110B01 are removed)

Product	Number of lots	Sample mean, %	Sample standard deviation, %	Min, %	Max, %
PF-06439535	10	97.80	6.92	85	106
US-licensed Avastin	45	101.07	9.75	84	125
EU-approved bevacizumab	40	102.05	8.97	87	125

Table 8. Results of equivalence testing for Inhibition of Cell Growth Assay data (lots US 952901, EU H0114B34, EU B7100B06 and EU H0110B01 are removed)

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Comparison	# of lots	Mean Difference, %	90% Confidence Interval for Mean Difference, %	Equivalence Margin, %	Pass the Equivalence Testing?
PF-06439535 vs. US	(10, 45)	-3.27	(-8.90, 2.37)	(-14.63, +14.63)	Yes
PF-06439535 vs. EU	(10, 40)	-4.25	(-9.65, 1.15)	(-13.46, +13.46)	Yes
EU vs. US	(40, 45)	0.98	(-2.40, 4.36)	(-14.63, +14.63)	Yes

Table 9. Descriptive statistics for the VEGF₁₆₅ binding by ELISA data (lots US 952901, EU H0114B34, EU B7100B06 and EU H0110B01 are removed)

Product	Number of lots	Sample mean, %	Sample standard deviation, %	Min, %	Max, %
PF-06439535	10	103.10	9.6	90	115
US-licensed Avastin	24	105.54	8.13	89	119
EU-approved bevacizumab	24	100.17	6.74	89	114

Table 10. Equivalence testing results for the VEGF₁₆₅ Binding by ELISA (lots US 952901, EU H0114B34, EU B7100B06 and EU H0110B01 are removed)

Comparison	# of lots	Mean Difference, %	90% Confidence Interval for Mean Difference, %	Equivalence Margin, %	Pass the Equivalence Testing?
PF-06439535 vs. US	(10,24)	-2.44	(-8.82, 3.94)	(-12.19, 12.19)	Yes
PF-06439535 vs. EU	(10,24)	2.93	(-3.17, 9.04)	(-10.10, 10.10)	Yes
EU vs. US	(24,24)	-5.38	(-8.99, -1.76)	(-12.19, 12.19)	Yes

VI. CONCLUSION AND RECOMMENDATION

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The statistical equivalence analyses shown above regarding VEGF165 binding and the inhibition of cell growth of PF-06439535 support a demonstration that PF-06439535 is highly similar to US-licensed Avastin. They also support the analytical portion of the scientific bridge to justify the relevance of EU-approved bevacizumab data from the comparative safety and efficacy study.

VII. APPENDIX

Table A. Lot Information and Data of Inhibition of Cell Growth used in Statistical Analysis for PF-06439535 Bevacizumab-US, and Bevacizumab –EU

Methodology				Inhibition of Cell Growth
BATCH/ LOT NAME	SOURCE	TESTING START DATE ^a	EXPIRY DATE	(%) RELATIVE POTENCY
450657	US	02-Jul-2013	31-Oct-2013	99
453807	US	22-Mar-2013	30-Nov-2013	103
458726	US	14-Oct-2013	31-Oct-2013	99
469053	US	02-Jul-2013	30-Apr-2014	97
470332	US	14-Oct-2013	31-Dec-2013	108
477504	US	02-Jul-2013	31-May-2014	112
478201	US	02-Jul-2013	31-May-2014	100
488495	US	02-Jul-2013	31-Jul-2014	122
492879	US	10-Apr-2014	31-Jul-2014	97
497080	US	02-Jul-2013	31-Aug-2014	115
503227	US	10-Apr-2014	31-Aug-2014	85
503229	US	10-Apr-2014	31-Oct-2014	85
504740	US	10-Apr-2014	31-Aug-2014	107
521496	US	10-Apr-2014	31-Oct-2014	93
522210	US	10-Apr-2014	31-Oct-2014	98
529846	US	10-Apr-2014	30-Nov-2014	100
535400	US	10-Apr-2014	31-Jan-2015	98
548426 ^b	US	07-Feb-2014	31-Mar-2015	104
952901	US	22-Mar-2013	31-May-2013	120
957823	US	17-May-2013	31-Aug-2013	125
970031	US	02-Jul-2013	31-Oct-2013	123
971630	US	02-Jul-2013	31-Oct-2013	96
3022680	US	04-Feb-2016	30-Apr-2017	106
3044096	US	15-Jun-2016	31-Aug-2017	90

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3049679	US	04-Feb-2016	31-May-2017	108
3055965	US	15-Jun-2016	31-Oct-2017	85
3063923	US	15-Jun-2016	30-Sep-2017	96
535443	US	18-Aug-2014	28-Feb-2015	91
541430	US	18-Aug-2014	28-Feb-2015	95
557412	US	18-Aug-2014	31-Mar-2015	94
566296	US	18-Aug-2014	30-Apr-2015	111
569321	US	18-Aug-2014	31-May-2015	98
569327	US	18-Aug-2014	30-Jun-2015	96
579977	US	18-Aug-2014	31-Jul-2015	100
585982	US	18-Aug-2014	31-Oct-2015	100
605025	US	18-Aug-2014	31-Dec-2015	98
611118	US	18-Aug-2014	31-Dec-2015	96
616350	US	18-Aug-2014	31-Jan-2016	105
626307	US	01-Oct-2014	30-Apr-2016	105
640016	US	01-Oct-2014	30-Jun-2016	103
640018	US	11-Sep-2015	31-Jul-2016	115
642292	US	30-Apr-2015	31-Dec-2015	84
642293	US	30-Apr-2015	31-Dec-2015	88
648510	US	01-Oct-2014	31-May-2016	109
680576	US	11-Sep-2015	31-Jan-2017	105
684386	US	04-Feb-2016	28-Feb-2017	104
B7012B09	EU	01-Oct-2014	30-Nov-2015	106
B7043B01 ^b	EU	08-Dec-2014	31-May-2016	94
B7045B01 ^b	EU	08-Dec-2014	30-Jun-2016	91
B7100B06	EU	02-Jul-2013	30-Apr-2014	103
B7101B19	EU	10-Apr-2014	31-Aug-2014	96
B7102B03	EU	02-Jul-2013	31-Aug-2014	98
B7103B04	EU	18-Aug-2014	31-Mar-2015	108
B7104B13	EU	15-Dec-2014	31-May-2015	90

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B7105B12	EU	19-Aug-2014	30-Jun-2015	109
B7106B04	EU	15-Dec-2014	30-Jun-2015	111
B7108B16	EU	15-Dec-2014	31-Oct-2015	114
B7109B09 ^b	EU	07-Feb-2014	31-Mar-2015	107
B7110B03	EU	15-Dec-2014	30-Nov-2015	98
B7200B06	EU	02-Jun-2015	31-May-2016	87
B7222H08 ^b	EU	13-Oct-2016	31-Mar-2018	104
B7227B08 ^b	EU	25-Jan-2017	31-May-2018	99
B8000B19	EU	19-May-2015	30-Apr-2016	123
B8001H15	EU	11-Sep-2015	30-Apr-2016	108
B8003H05	EU	11-Sep-2015	31-Jul-2016	101
B8004H04 ^b	EU	15-Feb-2016	30-Jun-2017	102
B8013H03 ^b	EU	12-Apr-2016	31-Jul-2017	100
B8018H09 ^b	EU	19-Sep-2016	31-Oct-2017	111
B8500H04	EU	04-Feb-2016	30-Sep-2016	107
B8503H04 ^b	EU	02-Jun-2015	31-Oct-2016	97
B8504H11 ^b	EU	24-Jun-2015	31-Dec-2016	97
B8507H02 ^b	EU	06-Oct-2016	30-Nov-2017	106
H0108B07	EU	22-Mar-2013	31-Mar-2013	92
H0109B06	EU	22-Mar-2013	31-Mar-2013	89
H0110B01	EU	17-May-2013	31-Aug-2013	116
H0111B01	EU	17-May-2013	31-Aug-2013	125
H0113B05	EU	16-Dec-2013	28-Feb-2014	94
H0114B34	EU	02-Jul-2013	28-Feb-2014	127
H0115B26	EU	07-Jan-2014	28-Feb-2014	90
H0119B06	EU	07-Jan-2014	31-Jul-2014	100
H0121B03	EU	02-Jul-2013	31-Jul-2014	115
H0130B08	EU	17-May-2013	31-May-2013	101
H0131B10	EU	17-May-2013	30-Jun-2013	94
H0133B06	EU	02-Jul-2013	31-Oct-2013	106

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H0135B11	EU	14-Oct-2013	31-Oct-2013	108
H0138B02	EU	14-Oct-2013	31-Oct-2013	95
H0150B08	EU	22-Mar-2013	30-Apr-2014	113
H0151B06 ^b	EU	07-Feb-2014	31-Aug-2014	97
B7221B10 ^b	EU	25-Jan-2017	31-Mar-2018	99
I2P138K601 ^c	PFE	29-Nov-2012	N/A	100 ^e
I2P138K603 ^c	PFE	29-Nov-2012	N/A	103 ^e
I3J138K002 (G27608) ^c	PFE	3-May-2013	N/A	87
I3J138K003 (G27629)	PFE	16-May-2013	N/A	96
I3J138K004 (G27631)	PFE	23-May-2013	N/A	92
I4J138K001 (J32980)	PFE	6-May-2014	N/A	82
I4J138K002 (J33002)	PFE	15-May-2014	N/A	93
I4J138K003 (J33003) ^c	PFE	22-May-2014	N/A	85
I4J138K004 (J33004)	PFE	2-Jun-2014	N/A	93
I6J138K001 (R12923)	PFE	18-Jul-2016	N/A	103
I6J138K002 (R32728)	PFE	18-Jul-2016	N/A	109
I6J138K003 (R32744)	PFE	4-Aug-2016	N/A	80
H49500 ^{b, c}	PFE	3-Oct-2013	N/A	105
H90818-W	PFE	3-Feb-2014	N/A	107
J12512-W	PFE	11-Apr-2014	N/A	99
J12513-W ^c	PFE	9-Jun-2014	N/A	98
J61289-W ^{b, c}	PFE	22-Aug-2014	N/A	100
J76228-W	PFE	10-Nov-2014	N/A	101
J76229-W ^{b, c}	PFE	19-Dec-2014	N/A	100
J76232-W	PFE	8-Sep-2014	N/A	103
J76233-W ^c	PFE	30-Sep-2014	N/A	102
J76234-W	PFE	5-Dec-2014	N/A	97
R59889-W ^{b, c}	PFE	20-Dec-2016	N/A	99
R59890-W	PFE	21-Dec-2016	N/A	95

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R59891-W	PFE	21-Dec-2016	N/A	96
R64865-W	PFE	21-Dec-2016	N/A	94
R64866-W _c	PFE	21-Dec-2016	N/A	106
R64867-W _c	PFE	21-Dec-2016	N/A	96

- Testing start date is the date the testing was initiated.
- Lots used in the comparative clinical studies.
- Pfizer independent batch/lots for statistical analysis.
- Bevacizumab-EU lots excluded from statistical analysis based on lack of independence.
- Bioassay for information only since reference material was not established prior to analysis; result not used in statistical analysis. Abbreviations: NT = Not Tested

Table B. VEGF165 Binding ELISA Data used in Statistical Analysis of PF-06439535, Bevacizumab-US, and Bevacizumab-EU Lots

Source	Lot/Batch Number	Binding ELISA	
		Assay Date ^d	% Relative Potency
PFE	12P138K601 ^b		
PFE	12P138K603 ^b		
PFE	13J138K002 ^b	2-Jul-2013	112
PFE	13J138K003	2-Jul-2013	96
PFE	13J138K004	2-Jul-2013	106
PFE	14J138K001	24-Jul-2014	100
PFE	14J138K002	24-Jul-2014	99
PFE	14J138K003 ^b	24-Jul-2014	97
PFE	14J138K004	24-Jul-2014	91
PFE	H49500 ^{b,c}	31-Jul-2015	94
PFE	H90818-W	31-Jul-2015	107
PFE	J12512-W	31-Jul-2015	91
PFE	J12513-W ^b	31-Jul-2015	100
PFE	J61289-W ^{b, c}	31-Jul-2015	113
PFE	J76228-W	31-Jul-2015	112
PFE	J76229-W ^{b, c}	31-Jul-2015	114
PFE	J76232-W	31-Jul-2015	101
PFE	J76233-W ^b	18-Aug-2015	115
PFE	J76234-W	18-Aug-2015	111
PFE	R59890-W	17-Feb-2017	99
PFE	R59889-W ^{b, c}	17-Feb-2017	102
PFE	R59891-W	17-Feb-2017	94
PFE	R64866-W ^b	17-Feb-2017	90
PFE	R64867-W ^b	17-Feb-2017	94
PFE	R64865-W	17-Feb-2017	95
US	952901	23-Jul-2013	109 ^a
US	957823	23-Jul-2013	110
US	450657	18-Jul-2013	96
US	477504	18-Jul-2013	96
US	488495	23-Jul-2013	103
US	497080	23-Jul-2013	105

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US	970031	23-Jul-2013	101
US	453807	17-Jul-2013	103
US	971630	25-Jul-2013	112
US	469053	25-Jul-2013	119
US	478201	25-Jul-2013	116
US	548426 ^c	24-Jul-2014	89
US	503227	18-Aug-2015	117 ^a
US	503229	29-Jul-2015	101 ^a
US	492879	29-Jul-2015	89 ^a
US	640016	10-May-2016	109
US	3049679	10-May-2016	113
US	680576	10-May-2016	103
US	640018	10-May-2016	103
US	684386	10-May-2016	102
US	3022680	10-May-2016	101

- a. Sample tested in this assay was a -70 °C stored frozen liquid drug product aliquot that was frozen within expiry, but date of use was beyond the manufacturer's expiry date for the liquid product.
 - b. Pfizer independent batch/lot for statistical analysis.
 - c. Lot used in the comparative clinic study.
 - d. Assay date is the date the material was tested in the assay.
 - e. For steady-state binding, K_D values are provided.
- Gray cells are the lots not tested.

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SHEIN-CHUNG CHOW
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Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment
WO Building 22
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Silver Spring, MD 20993

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Reviewer: Jessica Hankins, Ph.D.
Quality Assessment Lead: Reyes Candau-Chacon, Ph.D.

BLA: 761099
Applicant: Pfizer, Inc.
US License Number: 2001
Submission Reviewed: Original 351(k) BLA
Product: PF-06439535 (proposed biosimilar to Avastin)
Indication: Metastatic colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, metastatic renal cell carcinoma, and cervical cancer
Dosage Form: Solution for intravenous infusion (25 mg/mL)
Manufacturing Sites: Pharmacia and Upjohn Company, LLC 7000 Portage Road
Kalamazoo, MI USA (FEI: 1810189)
FDA Receipt Date: 29 June 2018
Action Date:

Conclusion and Approvability Recommendation

The drug product portion of this BLA, as amended, is recommended for approval from a product quality microbiology and sterility assurance perspective.

Product Quality Microbiology Assessment: Drug Product

Drug Product Quality Microbiology Information Reviewed

Sequence number	Date	Description
------------------------	-------------	--------------------

0003	06/29/2018	Original BLA (resubmission after withdraw)
0017	12/03/18	IR response
0021	12/21/18	IR response
0024	01/15/19	IR response
N/A	01/31/19	T-con to discuss hold times
0029	02/08/19	IR response

The following Drug Master File was reviewed in support of the BLA:

DMF #	DMF Holder	Review Date	Review Number	Document Title	Conclusion
	(b) (4)	07/12/18	37	(b) (4)	Adequate

Module 1

1.14 Labeling

The DP is administered as an intravenous infusion over 90 minutes for the first infusion. The administration of subsequent infusions may be shortened if the drug is tolerated. The undiluted DP is stored refrigerated at 2-8°C. The DP is then diluted in a total volume of 100 mL of 0.9% sodium chloride injection, USP and may be stored at 2-8°C for up to 8 hours.

Reviewer comment: Data presented in P.2.6 supports storage of the diluted DP at the label's recommended storage conditions (2-8°C for 8 hours).

SATISFACTORY

Module 3.2

P.1 Description and Composition of the Drug Product

The DP is supplied as a liquid solution for infusion. The composition for the 100 mg/4 mL and 400 mg/16 mL DP is provided in the tables below, copied and pasted from the submission.

Table 3.2.P.1-1. Composition of PF-06439535 Drug Product, 25 mg/mL (100 mg/4 mL)

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	(b) (4)	85	340
Edetate Disodium Dihydrate (EDTA)	USP, Ph. Eur., JP	(b) (4)	0.05	0.2
Polysorbate 80	NF, Ph. Eur., JP	(b) (4)	0.2	0.8
				(b) (4)
Water for injection	USP, Ph. Eur., JP	(b) (4)	q.s. to 1.0 mL	q.s. to 4.0 mL

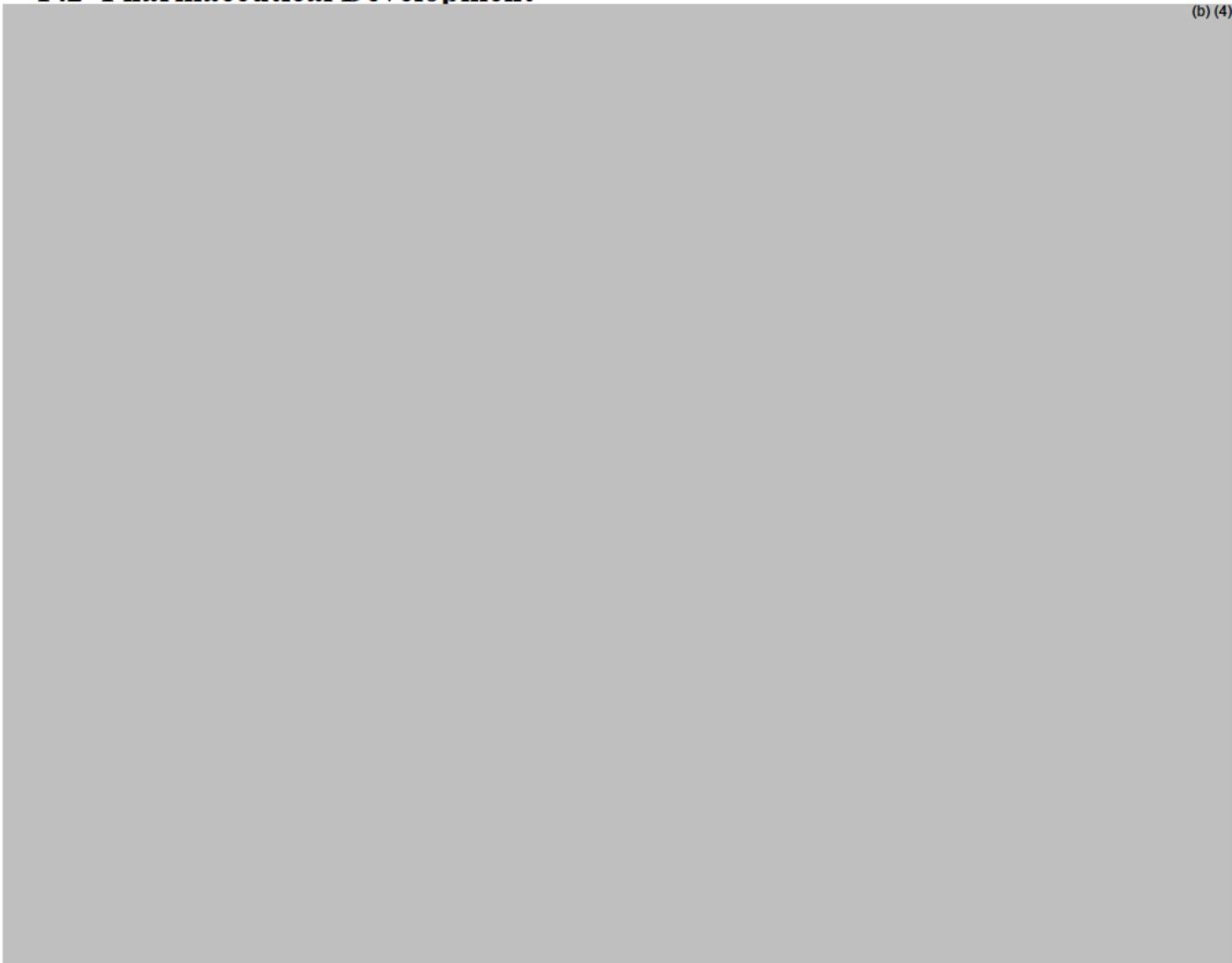
Table 3.2.P.1-2. Composition of PF-06439535 Drug Product, 25 mg/mL (400 mg/16 mL)

Name of Ingredients	Reference to Standard	Function	Unit Formula (mg/mL)	Unit Formula (mg/vial)
PF-06439535	In-house specification	Active ingredient	25	400
Succinic Acid	NF, JPE	(b) (4)	2.36	37.76
Sucrose	NF, Ph. Eur., JP		85	1360
Edetate Disodium Dihydrate (EDTA)	USP, Ph. Eur., JP		0.05	0.8
Polysorbate 80	NF, Ph. Eur., JP		0.2	3.2
Water for injection	USP, Ph. Eur., JP		q.s. to 1.0 mL	q.s. to 16.0 mL

The 100 mg/4 mL presentation is supplied in a 5 mL (b) (4) clear glass vial and includes an overfill of ~ (b) (4) mL. The 400 mg/16 mL presentation is supplied in a 20 mL (b) (4) clear glass vial and has an overfill of ~ (b) (4) mL. The DP is preservative-free and is single-use.

SATISFACTORY

P.2 Pharmaceutical Development



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P.8 Stability

The DP (100 mg/4 mL and 400 mg/16 mL) recommended long-term storage conditions are $5\pm 3^{\circ}\text{C}$. The stability program includes the recommended long-term condition, accelerated conditions ($25\pm 2^{\circ}\text{C}$, $60\pm 5\%$ relative humidity), thermal stress, and photostability. The stability program includes CCIT annually through expiry. Sterility and endotoxin testing are performed at release and at expiry for both DP presentations. CCIT results for lots on stability were 'pass', indicating no dye ingress occurred at the timepoints tested.

SATISFACTORY

CGMP Status

The assessment of manufacturing facilities is documented in panorama.

Conclusion

- I. The BLA, as amended, was reviewed from a sterility assurance and product quality microbiology perspective and is recommended for approval.
- II. Product quality aspects other than microbiology should be reviewed by OBP.
- III. Refer to Panorama for the CGMP status of the relevant manufacturing facilities.

DP Quality Microbiology Information Requests Sent and Date



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Hankins

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Reyes
Candau-Chacon

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Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment
WO Building 22
10903 New Hampshire Ave.
Silver Spring, MD 20993

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Reviewer: Aimee L. Cunningham, Ph.D., M.P.H.
Acting Quality Assessment Lead: Reyes Candau-Chacon, Ph.D.

BLA: 761099/0
Applicant: Pfizer
US License Number: 2060
Submission Reviewed: Original BLA
Product: Zirabev (PF-06439535), proposed biosimilar to US licensed Avastin (bevacizumab)
Indication: metastatic colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, metastatic renal cell carcinoma, and cervical cancer
Dosage Form: 25 mg/mL solution for i.v. infusion
Manufacturing Sites: Wyeth BioPharma in Andover, MA (FEI: 1222181)
FDA Receipt Date: 06/29/2018
Action Date: 06/29/2019

Conclusion and Approvability Recommendation

The drug substance portion of this BLA was reviewed from a microbial control and product quality microbiology standpoint and is recommended for approval.

Review Summary

Pfizer has submitted BLA 761099 to license Zirabev (PF-06439535, an Avastin / bevacizumab biosimilar), and its associated drug substance and drug product manufacturing processes. BLA 761099 was resubmitted via eCTD on 06/29/2018. This review contains the assessment of the manufacturing process of PF-06439535 bulk drug substance from a microbiological quality perspective. For review of the drug product aspects of this application, please see the review by Dr. Jessica Hankins.

Drug Substance Quality Microbiology Information Reviewed

Sequence number	Date	Description
0001	01/28/2018	Original BLA submission

Sequence number	Date	Description
0003	06/29/2018	BLA resubmission
0017	12/03/2018	IR responses
0029	02/08/2019	IR responses

Module 3.2

S.1 General Information

PF-06439535 is a proposed biosimilar to Avastin (bevacizumab). It is a recombinant humanized IgG1κ monoclonal antibody which binds and neutralizes VEGF (vascular endothelial growth factor). It is produced in CHO cells.

SATISFACTORY

S.2 Manufacture

S.2.1 Manufacturers

Firm	FEI	Responsibility
Wyeth BioPharma Division of Wyeth Pharmaceuticals Inc. One Burtt Road Andover, MA 01810 USA	1222181	Cell bank manufacture and storage; manufacture, in-process control testing, DS storage; release and stability testing
Pfizer Ireland Pharmaceuticals Grange Castle Business Park Clondalkin, Dublin 22 Ireland	3004145594	Cell bank storage
(b) (4)		In-process testing

SATISFACTORY

S.2.2 Description of the Manufacturing Process and Process Controls

Description of Process Steps

PF-06439535 DS is produced at Wyeth BioPharma using CHO cells. A flow diagram representing the DS manufacturing process is provided in the submission (Figure 3.2.S.2.2-1).

Batch and Scale Definition

(b) (4)

SATISFACTORY

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Reyes
Candau-Chacon

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Food and Drug
Administration Center for Drug
Evaluation and Research WO Bldg.
51,10903 New Hampshire Ave.
Silver Spring, MD 20993

Date: February 4, 2019
To: Administrative File, **STN 761099/0**
From: Michael Shanks, Biologist, CDER/OPQ/OPF/DIA
Endorsement: Zhihao Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA
Subject: Original BLA
US License: 2001
Applicant: Pfizer Inc.
Mfg. Facility: *Drug Substance:* Wyeth BioPharma, Division of Wyeth Pharmaceuticals, LLC, a subsidiary of Pfizer, Andover, MA, FEI: 1222181
Drug Product: Pharmacia and Upjohn Company LLC, Kalamazoo, MI, FEI: 1810189
Product: PF-06439535 (bevacizumab, biosimilar to Avastin[®]), Sterile Solution for intravenous infusion.
Strength: PF-06439535 drug product is provided as a 25 mg/mL concentrate for solution for intravenous (IV) infusion: 100 mg in a single-use 5 mL vial with a 4 mL nominal fill and, 400 mg in a single-use 20 mL vial with a 16 mL nominal fill.
Indication: PF-06439535, a proposed biosimilar to Avastin[®] (bevacizumab) for all indications approved for treatment by Avastin[®] in the US: metastatic colorectal cancer, first-line 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.
Goal Date: 6/29/2019

RECOMMENDATION

This submission is recommended for approval from a facility review perspective.

SUMMARY

PF-06439535 (bevacizumab, a proposed biosimilar to Avastin[®]) is a humanized recombinant IgG1 monoclonal antibody directed against the vascular endothelial growth factor (VEGF, and also VEGF-A). The known mechanism of action across all globally approved indications of Avastin is to bind soluble VEGF, thereby inhibiting the interaction of VEGF and its receptors (VEGFR-1 and VEGFR-2) on the surface of endothelial cells and downstream signaling pathways. Avastin has been shown to effectively inhibit VEGF-induced activities such as endothelial cell proliferation and new blood vessel formation in in vitro models of angiogenesis. PF-06439535 (bevacizumab) drug product (DP) is provided as a 25 mg/mL concentrate in 100 mg in a single-use 5 mL vial with a 4 mL nominal fill and

400 mg in a single-use 20 mL vial with a 16 mL nominal fill, Type clear glass vial sealed with a stopper and an aluminum seal with flip-off plastic cap, for intravenous infusion. The drug product contains no preservative.

The subject BLA proposes commercial manufacture of PF-06439535 (bevacizumab) drug substance (DS) and DP at Wyeth BioPharma, Division of Wyeth Pharmaceuticals, LLC (FEI 1222181) and Pharmacia and Upjohn Company LLC (FEI 1810189), respectively.

The scope of this review is primarily for the information included in Modules 3.2.S.2.1. *Manufacturers*, 3.2.P.3.1. *Manufacturers*, and 3.2.A.1. *Facilities and Equipment* of the BLA. As part of this review cycle, pre-approval inspections (PAI) were conducted at the Wyeth BioPharma, Division of Wyeth Pharmaceuticals, LLC DS manufacturing facility from 01/14/2019 to 01/18/2019, and the Upjohn Company LLC DP manufacturing facility from 9/10-2018 to 9/21/2018, both of which had a recommendations of approve for BLA 761099-ORIG-1-RESUB-3, PF-06439535 (Bevacizumab).

ASSESSMENT

DRUG SUBSTANCE FACILITIES

3.2.S Drug Substance [Substance – Manufacturer]

3.2.S.2. Manufacture

3.2.S.2.1. DS Manufacturer(s)



CONCLUSION

Adequate descriptions of the facilities, equipment, environmental controls, cleaning and contamination control strategy were provided for Pharmacia and Upjohn Company LLC, Kalamazoo, MI (FEI 1810189), proposed for PF-06439535 (bevacizumab, a proposed biosimilar to Avastin[®]) DS and DP manufacture. All proposed manufacturing and testing facilities are acceptable based on their currently acceptable CGMP compliance status and recent relevant inspectional coverage. This submission is recommended for approval from a facility.

Michael Shanks
Biologist
OPF Division of Inspectional Assessment
Branch 1

Zhihao Peter Qiu, Ph.D.
Branch Chief
OPF Division of Inspectional Assessment
Branch 1



Michael
Shanks

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Zihao Peter
Qiu

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