

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**761028Orig1s000**

**PRODUCT QUALITY REVIEW(S)**

**Office Of Pharmaceutical Quality Integrated Review**  
**BLA 761028 MVASI (bevacizumab-awwb)**  
**August 22, 2017**

**First Biosimilar for Bevacizumab**  
**Recommendation: BLA Approval**

The Office of Biotechnology Products, OPQ, CDER, recommends approval of BLA 761028 for MVASI (bevacizumab-awwb) manufactured by Amgen Inc. The data submitted in this application, including the analytical similarity assessment, are adequate to support the conclusion that:

- The biological product, bevacizumab-awwb, 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 MVASI is well-controlled and leads to a product that is pure and potent.

I recommend that this product be approved for human use under conditions specified in the package insert.

|                         |                                                                                                                     |
|-------------------------|---------------------------------------------------------------------------------------------------------------------|
| Drug Name/Dosage Form   | MVASI ( bevacizumab-awwb)/Injection                                                                                 |
| Strength                | 100 mg/4 mL and 400 mg/16 mL                                                                                        |
| Route of Administration | Intravenous                                                                                                         |
| Rx/OTC dispensed        | Rx                                                                                                                  |
| Indication              | All Indications Licensed for Avastin with the exception of those indications protected under the orphan exclusivity |
| Applicant/Sponsor       | Amgen, Inc.                                                                                                         |

**Product Overview**

Bevacizumab-awwb is a recombinant humanized IgG1 monoclonal antibody developed as a biosimilar to US-licensed Avastin. Bevacizumab-awwb binds to VEGFA isoforms that have been identified as regulators of developmental, physiological and pathological neovascularization, which support tumor growth and metastasis. Bevacizumab-awwb 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-awwb. Bevacizumab-awwb is produced in a CHO cell line. Drug product is manufactured to the same concentration, formulation, and presentation as US licensed Avastin, i.e. as a 25 mg/mL solution of bevacizumab-awwb in 100 mg/4 mL and 400 mg/16 mL preservative-free, single-dose vials.

**Quality Review Team**

| <b>Discipline</b> | <b>Reviewer</b> | <b>Branch/Division</b> |
|-------------------|-----------------|------------------------|
| Drug Substance    | Jee Chung       | OPQ/OBP/DBRRIV         |
| Drug Product      | Jee Chung       | OPQ/OBP/DBRRIV         |

|                            |                                                               |                                                |
|----------------------------|---------------------------------------------------------------|------------------------------------------------|
| Immunogenicity             | Jee Chung                                                     | OPQ/OBP/DBRRIV                                 |
| Labeling                   | Vicky Borders-Hemphill<br>Jee Chung                           | OPQ/OBP<br>OPQ/OBP/DBRRIV                      |
| Facility                   | Laura Fontan                                                  | OPQ/OPF/DIA                                    |
| Microbiology               | Scott Nichols (Drug Substance)<br>Dupeh Palmer (Drug Product) | OPQ/OPF/DMA-IV<br>OPQ/OPF/DMA-IV               |
| Team Lead                  | Chana Fuchs<br>Patricia Hughes<br>Peter Qiu                   | OPQ/OBP/DBRRI<br>OPQ/OPF/DMA-IV<br>OPQ/OPF/DIA |
| CMC RPBM                   | Keith Olin                                                    | OPQ/OPRO                                       |
| Application Technical Lead | Chana Fuchs                                                   | OPQ/OBP/DBRRIV                                 |

**Multidisciplinary Review Team:**

| <b>Discipline</b>            | <b>Reviewer</b> | <b>Office/Division</b> |
|------------------------------|-----------------|------------------------|
| RPM                          | Leah Her        | OHOP/DOP2              |
| Cross-disciplinary Team Lead | Steven Lemery   | OHOP/DOP2              |
| Medical Officer              | Sandra Casak    | OHOP/DOP2              |
| Pharm/Tox                    | Alex Putnam     | OHOP/DHOT              |
| Clinical Pharmacology        | Edwin Chow      | OCP/DCPV               |
| CMC Statistics               | Tianhua Wang    | OB/DBIV                |
| Clinical Statistics          | Vivian Yuan     |                        |

1. Names:

- a. Proprietary Name: MVASI
- b. Trade Name: MVASI
- c. Non-Proprietary/USAN: bevacizumab–awwb
- d. CAS registry number: 1438851-35-4
- e. Common name: ABP215
- f. INN Name: bevacizumab
- g. Compendial Name: Not applicable
- h. OBP systematic name: MAB HUMANIZED (IgG1) Anti-P15692 (VEGFA\_Human) [ABP 215]

**Submissions Reviewed:**

| Submission sequence number: | Document Date |
|-----------------------------|---------------|
| 0001                        | 11/14/16      |
| 0002                        | 12/07/16      |
| 0003                        | 12/22/16      |
| 0006                        | 2/10/17       |
| 0007                        | 2/22/16       |
| 0008                        | 2/23/17       |
| 0009                        | 3/28/17       |
| 0010                        | 4/7/17        |
| 0013                        | 5/26/17       |
| 0016                        | 6/9/17        |

|      |         |
|------|---------|
| 0017 | 6/16/17 |
| 0019 | 7/14/17 |
| 0020 | 7/20/17 |
| 0021 | 7/21/17 |
| 0022 | 7/28/17 |
| 0023 | 8/3/17  |
| 0024 | 8/8/17  |
| 0025 | 8/10/17 |
| 0026 | 8/15/17 |
| 0028 | 8/18/17 |

**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 <sup>1</sup> | Status <sup>2</sup> | Comments                                                                                                              |
|---------|----------|------------|-----------------|-------------------|---------------------|-----------------------------------------------------------------------------------------------------------------------|
| (b) (4) | III      | (b) (4)    | (b) (4)         | 3                 | N/A                 | Not reviewed. Sufficient Leachables and Extractables data and primary stability program in BLA.                       |
|         | III      |            |                 | 3                 | N/A                 | Sufficient Leachables and Extractables data and primary stability program in BLA. DMF reviewed on 28/4/16 by S. Olin. |
|         | V        |            |                 | 3                 | N/A                 | Sufficient data in the BLA for vial (b) (4)                                                                           |
|         | V        |            |                 | 3                 | N/A                 | Sufficient data in the BLA and from site inspections.                                                                 |

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

- **Consults:** None

## Executive Summary Integrated Review:

### I. Recommendations:

#### A. Recommendation and Conclusion on Approvability:

The Office of Biotechnology Products, OPQ, CDER, recommends approval of BLA 761028 for MVASI (bevacizumab-awwb) manufactured by Amgen Inc. The data submitted in this application, including the analytical similarity assessment, are adequate to support the conclusion that:

- The biological product, bevacizumab-awwb, 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-awwb 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 location:
  - Drug Substance: Amgen, Inc. (Amgen Thousand Oaks, ATO), 1 Amgen Center Drive, Thousand Oaks, CA 91320 (FEI.: 2026154)
  - Drug Product:
    - Manufacturing and fill: [REDACTED] (b) (4)
    - Labeling and packaging: Amgen Manufacturing Ltd., Juncos, Puerto Rico.
- Fill size and dosage form
  - 100 mg/4 mL single dose vial , Injection
  - 400 mg/16 mL single dose vial, Injection
- Dating period:
  - Drug Product: 36 months: 2-8°C
  - Drug Substance: (b) (4) months: (b) (4) °C
  - For packaged products: Not packaged
  - Stability:
    - 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 in accordance with 21 CFR 601.2a. MVASI is a specified product.

**C. Benefit/Risk Considerations:**

MVASI (bevacizumab-awwb) 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-awwb to US-licensed Avastin was evaluated using methods to assess physiochemical 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.

Additionally, 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 also provided pairwise analytical comparisons between bevacizumab-awwb and EU-approved bevacizumab and between US-licensed bevacizumab and EU-approved bevacizumab. Twenty-seven US-licensed Avastin and 29 EU-approved bevacizumab lots were used in this assessment, as compared to 19 bevacizumab-awwb drug product lots derived from 13 independent drug substance lots. Non-independent drug product lots were only used to assess attributes impacted by drug product manufacturing.

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. Issues with low endotoxin recovery (LER) have been dealt with through control of manufacturing, pyrogen testing at product release, and a post-marketing commitment to develop an assay that is not subject to LER (see item 4 in the section below). The DIA review recommends approval of the commercial manufacture of bevacizumab-awwb DS at Amgen Thousand Oaks and of bevacizumab-awwb DP at (b) (4) and at Amgen Manufacturing Ltd. 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:**

1. Re-evaluate the bevacizumab-awwb drug substance (DS) stability acceptance criteria for stability samples held at the (b) (4) °C condition after data from a minimum of 5 DS lots stored at (b) (4) °C are available. The final report should include the corresponding data, the analysis and statistical plan used to evaluate the results and acceptance criteria and any proposed changes to the approved criteria.

2. Perform method validation studies in support of the introduction of the glycan mapping method into the drug substance manufacturing control strategy.
3. Perform method validation studies in support of the introduction of the host cell protein detection method into the drug substance manufacturing control strategy.
4. Develop and implement a validated endotoxin detection method not subject to low endotoxin recovery for use in the release of the drug product.

## **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-awwb, 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 in the same formulation buffer. The totality of the analytical data provided is sufficient to support a demonstration that bevacizumab-awwb 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-awwb 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-awwb, US-licensed Avastin and EU-approved bevacizumab was demonstrated using 19 bevacizumab-awwb drug product lots, 27 US-licensed Avastin lots, and 29 EU-approved bevacizumab lots. The 19 bevacizumab-awwb drug product lots were derived from 13 independent drug substance lots and included development lots, lots used in the clinical studies, and process validation lots. US-licensed Avastin and EU-approved bevacizumab DP lots were collected over a period of six years and covered shelf-life span of 3 months to 27 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 study in healthy volunteers provided a 3-way comparison of bevacizumab-awwb, 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 mL<sup>1</sup>. The

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<sup>1</sup> U.S. Prescribing Information, U.S.-licensed Avastin. Accessed 8/25/2017 from [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2016/125085s317lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/125085s317lbl.pdf)

applicant is seeking approval for bevacizumab-awwb for the same strength as U.S.-licensed Avastin. Comparative in vitro potency, protein concentration (mg/mL), and extractable (deliverable) volume data reviewed as part of the analytical similarity assessment were used to inform the assessment of whether the proposed presentation of bevacizumab-awwb has the same strength 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-awwb, US-licensed Avastin and EU-approved bevacizumab used a comprehensive set of validated or qualified assays, as listed in Table 1 below:

**Table 1**

| <b>Category</b>                           | <b>Assays and Attributes by Analytical Techniques</b>                                                                                                                       |
|-------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Primary Structure                         | Intact molecular mass by ESI-TOF-MS                                                                                                                                         |
|                                           | Reduced and deglycosylated molecular masses of HC and LC by ESI-TOF-MS                                                                                                      |
|                                           | Amino acid sequence by reduced peptide map with on-line LC-MS/MS                                                                                                            |
|                                           | Disulfide structure by non-reduced peptide map with on-line LC-MS/MS                                                                                                        |
|                                           | Glycan map by HILIC HPLC<br>Glycan Map; % high mannose<br>Glycan Map; % afucosylation<br>Glycan Map; % galactosylation<br>Glycan Map; % sialylation<br>Glycan Map; profiles |
|                                           | Isoelectric point and profile by capillary isoelectric focusing                                                                                                             |
|                                           | Extinction coefficient by amino acid analysis/UV spectroscopy                                                                                                               |
|                                           | Identity by anti-idiotypic ELISA                                                                                                                                            |
| Higher Order Structure                    | Secondary structure by Fourier transform infrared spectroscopy (FTIR) – spectral similarity and profile                                                                     |
|                                           | Tertiary structure by near UV circular dichroism (CD) – spectral similarity and profile                                                                                     |
|                                           | Thermodynamic properties by differential scanning calorimetry (DSC)- T <sub>m1</sub> , T <sub>m2</sub> , and profile                                                        |
| Product-related Substances and Impurities | Size variants by SE-HPLC : HMW and profile                                                                                                                                  |
|                                           | Size variants by nrCE-SDS: main peak, pre-peaks, profile                                                                                                                    |
|                                           | Size variants by rCE-SDS: HC+LC, NGHC, LMW+MMW, profile                                                                                                                     |
|                                           | Charge variants by CEX-HPLC: acidic, main and basic peaks, profile.                                                                                                         |
| Particles and Aggregates                  | Subvisible particles by light obscuration (HIAC):<br>≥2µm particles<br>≥5µm particles<br>≥10µm particles<br>≥25µm particles                                                 |
|                                           | particles by microflow imaging (MFI):<br>≥5µm particles                                                                                                                     |

|                                          |                                                                                                                     |
|------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
|                                          | >5µm non-spherical particles                                                                                        |
|                                          | Subvisible Submicron particles by field flow fractionation (FFF)                                                    |
|                                          | particles by dynamic light scattering (DLS) – hydrodynamic radius                                                   |
|                                          | Submicron Size distribution by sedimentation velocity analytical ultracentrifugation(AUC-SV): % Monomer and profile |
|                                          | Molar mass determination by static light scattering (SLS) detector                                                  |
| Biological Activity – Fab mediated       | Potency by proliferation inhibition bioassay in HUVEC                                                               |
|                                          | VEGF-A <sub>165</sub> binding by ELISA                                                                              |
|                                          | VEGF-A <sub>111</sub> binding kinetics and affinity by SPR                                                          |
|                                          | Binding specificity to VEGF-A isoforms by SPR                                                                       |
|                                          | Inhibition of VEGFR-2 by RTK autophosphorylation                                                                    |
|                                          | Specificity for VEGF-A by RTK autophosphorylation                                                                   |
| Biological Activity – Fc mediated        | FcRn binding by AlphaLisa                                                                                           |
|                                          | FcγRIa binding by AlphaLisa                                                                                         |
|                                          | FcγRIIa (131H) binding by SPR                                                                                       |
|                                          | FcγRIIb binding by SPR                                                                                              |
|                                          | FcγRIIIa (158V) binding by AlphaLisa                                                                                |
|                                          | FcγRIIIa (158F) binding by AlphaLisa                                                                                |
|                                          | FcγRIIIb binding by SPR                                                                                             |
|                                          | C1q binding by ELISA                                                                                                |
|                                          | Lack of ADCC activity in Calu-6 and DLD-1                                                                           |
|                                          | Lack of ADCC activity in SKOV-3                                                                                     |
|                                          | Lack of CDC activity in Calu-6 and DLD-1                                                                            |
|                                          | Lack of CDC activity in SKOV-3                                                                                      |
| Thermal Stability and Forced Degradation | Forced degradation at 50°C by SE-HPLC, rCE-SDS, CEX-HPLC, and potency                                               |
|                                          | Stressed degradation at 40°C by SE-HPLC, rCE-SDS, CEX-HPLC, and potency                                             |
|                                          | Accelerated degradation at 25°C by SE-HPLC, rCE-SDS, CEX-HPLC, and potency                                          |
| General Properties                       | Protein concentration by UV absorbance                                                                              |
|                                          | Volume by content weight and density                                                                                |
|                                          | Osmolality by freezing point depression                                                                             |
|                                          | pH by potentiometric pH measurements                                                                                |
|                                          | Appearance by visual assessment                                                                                     |
|                                          | Color by visual assessment                                                                                          |
|                                          | Clarity by nephelometry                                                                                             |
| Process-related Impurities               | Residual HCP analysis by ELISA                                                                                      |
|                                          | Residual HCP analysis by LC-MS                                                                                      |
|                                          | Residual HCP analysis by 2D-DIGE                                                                                    |
|                                          | Residual DNA analysis by qPCR                                                                                       |
|                                          | Residual protein A analysis by ELISA                                                                                |

Each of the attributes described in Table 1 above met the pre-determined criteria for the pairwise comparison of bevacizumab-awwb 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.

- Noted differences in binding to FcγRIIIa receptor was specific for the 158V isotype of the receptor, while binding was similar to the 158F isotype. For bevacizumab the identified difference was assessed to not have a clinical impact based on our understanding of the mechanism of action of bevacizumab in that it binds a mainly soluble target which would not translate to significant ADCC activity. This was also supported by ADCC activity assays using an NK cell line and DLD-1 (adenocarcinoma) and Calu-6 (lung epithelial carcinoma cell line) as target cells. All three products demonstrated a lack of ADCC activity in the two VEGF secreting cell lines.
- Differences between bevacizumab-awwb and US-licensed Avastin and EU-approved bevacizumab were observed in the level of galactosylated N-linked glycans. Galactose levels can impact CDC activity. Difference in galactosylation was addressed by C1q binding studies and analysis of CDC activity in DLD-1, Calu-6, and SKOV3 cells using rabbit serum complement. 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.
- Differences between bevacizumab-awwb and US-licensed Avastin and EU-approved bevacizumab were observed in the level of high mannose N-linked glycans. Bevacizumab-awwb contains higher levels of high mannose content compared to US-licensed Avastin and EU-approved bevacizumab. Higher levels of high mannose glycans can result in faster clearance of product in vivo and therefore affect the PK of the product. The difference in high mannose was within ranges identified in the literature as not having impact on PK of various monoclonal antibodies studies. No impact on clearance was verified in the PK and clinical studies. Data also showed an increasing amount of high mannose on bevacizumab-awwb lots over time. The risk of this potential shift in mannose content that could ultimately impact bevacizumab-awwb PK was mitigated (b) (4)  
A PMC for validation of the glycan map method is part of the approval package (see Post-Marketing Commitments in Section ID of this memo)
- Differences between bevacizumab-awwb and US-licensed Avastin and EU-approved bevacizumab were observed in the level of aggregates and fragments. Apparent differences in aggregate and fragments were addressed through orthogonal methods which identified that all three products have low levels of particles and therefore have low risk to adversely impact safety. Bevacizumab-awwb has no new HMW species and lower levels of HMW species compared to some of the US-licensed Avastin and EU-approved bevacizumab lots. This is anticipated as HMW species are stability-related and the higher amounts are most likely due to the age of the US-licensed Avastin and EU-approved bevacizumab lots compared to bevacizumab-awwb.
- Differences between bevacizumab-awwb and US-licensed Avastin and EU-approved bevacizumab were observed in the level of charge variants. In order to determine the impact of the differences in charge variants fractions containing enhanced levels of acidic and basic peaks were isolated from all three products and characterized. Results identified that the same types of product variants are present for all three products, albeit in different amounts. Further analysis identified that the main difference in levels of basic variants was

due to higher levels of Heavy Chain C-terminal lysine residue in bevacizumab-awwb. 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. Additional contributors to difference in the basic species included the high mannose and galactosylated species discussed in the paragraph above, and MMW and LMW (N-terminal fragment of the HC CDR3 region) species. Characterization of the acidic peaks identify a significant amount of galactosylated species, LMW variants, as well as other minor product related species, and studies provided demonstrated that even dramatically enhanced levels had minimal impact on product in vitro potency. These results coupled with the clinical PK data support the conclusion that the differences identified in the amount of charge variants do not have clinical impact.

Review of the analytical similarity assessment identified that sponsor used VEGFA 111 as a substrate in the HUVEC potency assay. VEGFA 111 lacks both the neuropilin co-receptor binding site and the heparin binding site normally found on larger VEGFA isoforms. Additionally, based on data currently found in the literature, the VEGFA 111 isoform has not been identified as being expressed in normal human tissue or in tumors. To assess the acceptability of the VEGFA 111 isoform in the assessment of analytical similarity using the HUVEC assay, additional data were requested to demonstrate similar binding kinetics of each, bevacizumab-awwb, US-licensed Avastin and EU-approved bevacizumab, to VEGFA 165, VEGFA 121 and 111. Data were also requested to demonstrate that the HUVEC assay performance using VEGFA isoforms, 111,121, and 165 demonstrates similar sensitivity to detect changes in the product. Data provided identified that using Amgen's assays, the binding to all three VEGFA isoforms were similar among the three products, but the binding affinities to the VEGFA isoforms differed in that all three products bound to VEGFA 165 with higher affinities as compared to VEGFA 111 or 121. The HUVEC proliferation assay using 50°C stressed bevacizumab-awwb samples showed that for non-degraded product or for product mildly degraded over short time at 50°C, all three VEGFA isoform showed similar activity using the HUVEC assay. VEGFA 165 had slightly better sensitivity to detect product degradation after a more extended incubation at 50°C, but the difference compared to the ability of VEGFA 111 or 121 was no more than 13%. Given the totality of the data provided, the use of VEGFA 111 was found to be acceptable for use in Amgen's tier 1 bioassay analysis. It should be noted that the acceptability of use of VEGFA 111 is based on the specific assays used, and if sponsor decides to modify or add any additional assays for activity those assays would have to be assessed for the specific impact of using VEGFA 111 as compared to VEGFA 121 or 165.

The totality of the analytical data supports a demonstration that these products 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-awwb and US-licensed Avastin.

**B. Drug Substance bevacizumab-awwb Quality Summary**

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

| <b>CQA (type)</b>                                                                        | <b>Risk</b>                     | <b>Origin</b>                                                                                                                    | <b>Control Strategy</b> | <b>Other</b>                                                                                                                                          |
|------------------------------------------------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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, e.g. aggregates (HMW, MMW) and fragments (LMW) and Met oxidation in CEX-HPLC basic peaks. |
| Glycosylation - high mannose (product-related impurities)                                | PK/Efficacy                     | Intrinsic to molecule and cell culture. Affected by bioreactor conditions. Does not change during storage.                       |                         | At FDA request, sponsor added glycan mapping to the control strategy. See PMC for validation of glycan mapping method                                 |
| Glycosylation - sialic acid (product-related impurities)                                 | PK/Efficacy                     | Intrinsic to molecule and cell culture. Affected by bioreactor conditions. Does not change during storage.                       |                         |                                                                                                                                                       |
| High Molecular Weight (HMW)/Aggregates (product-related impurities)                      | Efficacy, PK and immunogenicity | Manufacturing process and storage conditions Minimal increase is expected on DS stability under controlled conditions.           |                         | Aggregates have reduced potency. Increased upon exposure to heat, light, and low 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 and light stress.                                                                                                     |

|                                                     |                                  |                                                                                       |         |                                                                                                                         |
|-----------------------------------------------------|----------------------------------|---------------------------------------------------------------------------------------|---------|-------------------------------------------------------------------------------------------------------------------------|
|                                                     |                                  |                                                                                       | (b) (4) |                                                                                                                         |
| 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) |         |                                                                                                                         |
| FcRn binding                                        | Efficacy Pharmacokinetics        | Bioreactor conditions and purification process                                        |         | Controlled by a (b) (4) during DS manufacturing                                                                         |
| Charge Variant Profile                              | Efficacy, PK                     | Bioreactor conditions and purification process, as well as storage                    |         | Potency impacted mostly by Acidic peaks 1-1 and 2 (HMW and fragments) and basic peaks 2, 3, 4 (with HMW and fragments). |

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

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

|                                               |                           |                                                           |         |         |
|-----------------------------------------------|---------------------------|-----------------------------------------------------------|---------|---------|
| impurity)                                     |                           | production cell line and the cell culture/harvest process | (b) (4) | (b) (4) |
| Residual Protein A (Process-related impurity) | Safety and Immunogenicity | Process-related impurity (b) (4)                          |         |         |
| (b) (4) (Process-related impurity)            | Safety                    | (b) (4)                                                   |         |         |
| (b) (4) (Process-related impurity)            | Safety                    | (b) (4)                                                   |         |         |
| (b) (4) (Process-related impurity)            | Safety                    | (b) (4)                                                   |         |         |
| (b) (4) (Process-related impurity)            | Safety                    | (b) (4)                                                   |         |         |
| Viruses (Contaminant)                         | Safety                    | Contamination during manufacture.                         |         |         |
| Mycoplasma (Contaminant)                      | Safety                    | Mycoplasma introduced during cell culture operations      |         |         |
| Leachables (Process-related impurity)         | Safety                    | (b) (4)                                                   |         |         |
| Visual appearance (Color and opalescence)     | Safety                    | Manufacturing process and formulation                     |         |         |

|                           |                     |                                                   |         |         |
|---------------------------|---------------------|---------------------------------------------------|---------|---------|
| (general)                 |                     |                                                   | (b) (4) |         |
| Protein content (general) | Impact on efficacy  | DS manufacture (b) (4)                            |         | (b) (4) |
| pH                        | Safety, efficacy    | Manufacturing process (formulation)               |         |         |
| Identity                  | Safety and Efficacy | NA                                                |         |         |
| Osmolality                | Safety, stability   | Manufacturing/ formulation                        |         |         |
| (b) (4)                   | Safety, stability   | Formulation raw material and formulation process. |         |         |

- **Description:**  
Bevacizumab-awwb 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-awwb DNA sequence was derived from commercially acquired lot of Avastin, and the deduced bevacizumab amino acid sequence was used as a template to synthesize the corresponding DNA sequences for the HC and LC variable regions. Bevacizumab-awwb has an average molecular weight of 149.2 kDa and a pI of ~7.9 (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 with 0 or 1 galactose residues. A non-consensus site glycosylation is present at approximately 1% on ABP 215 on HC residue Asn165 located on the CH1 region of the HC. This non-consensus site glycosylated variant can be detected as a pre-peak on the SE-HPLC chromatograms.
- **Mechanism of Action (MoA):**  
Bevacizumab-awwb 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-awwb is conserved in all splice isoforms.

- **Potency Assay:**

The proprietary potency assay is a cell based anti-proliferation assay using human umbilical vein endothelial cells (HUVEC). HUVECs are known to express VEGFA receptors VEGFR1 and VEGFR2, neuropilin co-receptors, as well as VEGFA, and proliferate in the presence of exogenously added VEGFA. Replicates of bevacizumab-awwb reference standard and sample dilutions are pre-incubated with VEGFA<sub>111</sub> followed by incubation with HUVEC for three days. At the end of the incubation period, HUVEC proliferation is measured by using an ATP specific luminescent reagent. The percent inhibition of proliferation by bevacizumab-awwb is determined and compared to the bevacizumab-awwb reference standard. Result of the sample is reported as % potency relative to the reference standard. There is an inverse relationship between levels of signal and levels of antibody in the sample such that the more bevacizumab present the more the signal decreases.

- **Reference Materials:**

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

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

The WRS was qualified against the PRS and was assessed by all the methods used for the PRS qualification. Following qualification, the working reference standard potency is assigned a value of (b) (4) %. Qualification parameters for future reference standards are included in the BLA with potency acceptance criterion based on a pre-determined confidence interval of the mean value in order to prevent drift in potency.

A description of the reference standard stability program is included in the BLA.

- **Critical starting materials or intermediates:**

(b) (4)

(b) (4)  
The MCB was adequately tested to ensure product safety from adventitious agents.

(b) (4)  
The WCB was adequately tested to ensure product safety from adventitious agents.

- Manufacturing Process Summary:

(b) (4)

- Container closure:

(b) (4)  
The container closure system is suitable for bevacizumab-awwb manufacturing based on stability data and maintenance of closure integrity

- Dating period and storage conditions:



(b) (4)

**C. Drug Product bevacizumab-awwb Quality Summary:**

Table 4, below, summarize the critical quality attributes and their control strategy that are relevant specifically to the Drug Product. 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**

| <b>CQA (type)</b>                                                                       | <b>Risk</b>                                                                                             | <b>Origin</b>                                                                                                        | <b>Control Strategy</b> | <b>Other</b>                                                                                                                                                           |
|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sterility (Contaminant)                                                                 | Safety and efficacy (due to degradation or modification of the product by contaminating microorganisms) | Contamination may be introduced throughout DP manufacturing process or by failure of the container closure integrity | (b) (4)                 | N/A                                                                                                                                                                    |
| Endotoxin (Contaminant)                                                                 | Safety (pyrogenic fever, increased immunogenicity risk) and purity                                      | Contamination may be introduced throughout DP manufacturing process in addition to coming from FDS                   |                         | The USP <85> release endotoxin test method is subject to low endotoxin recovery (LER). Submission of study data from test alternate methods will be addressed as a PMC |
| Container Closure Integrity (Contaminant)                                               | safety                                                                                                  | May be impacted by storage conditions.                                                                               |                         |                                                                                                                                                                        |
| Osmolality (general)                                                                    | Safety                                                                                                  | Formulation (Osmolality is a surrogate test for excipients levels)                                                   |                         | (b) (4)                                                                                                                                                                |
| 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                                              |         | (b) (4) |
| Subvisible Particulate Matter (product or process related impurities) | Safety and Immunogenicity               | Manufacturing process and CCS. Could be product or foreign particles. | (b) (4) |         |
| Color of solution (general)                                           | Safety and Efficacy                     | Formulation, contamination or degradation                             |         |         |
| Polysorbate 20 (general/critical excipient)                           | Safety and Efficacy (impacts aggregate) | Formulation                                                           |         |         |
| pH (general)                                                          | Safety                                  | Formulation                                                           |         | (b) (4) |
| Turbidity (general and process or product related impurities)         | Safety                                  | Manufacturing process and formulation                                 |         |         |
| Extractable Volume (general)                                          | Efficacy/Dosing                         | Manufacturing process                                                 |         | (b) (4) |
| 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:**

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

- **Strength:**

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

- 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-awwb has the same total content of therapeutic antibody as US-licensed Avastin. Bevacizumab-awwb 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-awwb is a sterile 25 mg/mL bevacizumab-awwb liquid filled into type I 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-awwb for intravenous infusion. The container closure system consists of a 6 cc (100 mg) or 20 cc (400 mg) Type I glass vial, elastomeric stopper, and aluminum seal with flip off cap.

- **List of Excipients:**

There are no further changes in excipients from DS. Each vial of bevacizumab-awwb contains 25 mg/mL of bevacizumab-awwb, 60 mg/mL  $\alpha,\alpha$ -trehalose dihydrate, 51 mM sodium phosphate, 0.040% (w/v) polysorbate 20, pH 6.2. 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-awwb drug substance. There is no formal drug product reference standard. Bevacizumab-awwb RS information is located in the drug substance section of the OBP Product Quality primary technical review in panorama.

- **Manufacturing process summary:**



(b) (4)



The data provided support a well-controlled process that will consistently produce a safe and effective product.

- **Container closure:**

The primary container closures are 6 mL and 20 mL (b) (4) glass vials supplied by (b) (4) sealed with 20 mm (b) (4) stoppers (b) (4) and 20 mm flip off caps (b) (4)

(b) (4) The secondary packaging material consists of a carton box to protect the drug product from damage and light.

- **Dating period and storage conditions:**

- Drug Product: 36 months at 2-8°C

- **List of co-package components, if applicable:** N/A

**D. Novel Approaches/Precedents:** None

**E. Any Special Product Quality Labeling Recommendations:**

Single dose vial, do not reuse. Store at 2-8°C. Protect from light.

**F. Establishment Information:**

| <b>Overall Recommendation:</b>                                                                                                                                                                           |                                                                                                                                  |                        |                                                                                                                                                                                                                        |                             |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| <b>DRUG SUBSTANCE</b>                                                                                                                                                                                    |                                                                                                                                  |                        |                                                                                                                                                                                                                        |                             |
| <b>Function</b>                                                                                                                                                                                          | <b>Site Information</b>                                                                                                          | <b>DUNS/FEI Number</b> | <b>Inspectional Observations</b>                                                                                                                                                                                       | <b>Final Recommendation</b> |
| Drug substance manufacture;<br><br>Drug substance in-process, lot release, stability testing;<br><br>Master cell bank and working cell bank manufacture and storage<br><br>Analytical similarity studies | Amgen, Inc. <sup>2</sup><br>Amgen Thousand Oaks Facility (ATO)<br>1 Amgen Center Drive<br>Thousand Oaks, CA 91320<br><br>(b) (4) | 039976196/<br>2026154  | pre-license inspection of the drug substance manufacturing and testing facility was conducted from May 8- 16, 2017. Analytical similarity data integrity was also inspected. Five 483 observations were made.<br>- VAI | Acceptable                  |

<sup>2</sup> AWA, and Amgen Thousand Oaks are the sites where the analytical similarity data were collected and analyzed. Similarity activities were evaluated during the PLI at ATO.

| Drug Substance lot release and stability testing                                                                                                               | Amgen Manufacturing Limited (AML)<br>Carr 31 KM 24 6 Juncos, Puerto Rico 00777 | 785800020/<br>1000110364 |                                                                                                                   | Acceptable (VAI on last inspection) |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------|-------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| Drug Substance lot release and stability testing                                                                                                               | Amgen Technology Ireland UC<br>Pottery Road Dun Laoghaire, Ireland             | 110004171/<br>3003359885 |                                                                                                                   | Acceptable (VAI on last inspection) |
| (b) (4)                                                                                                                                                        |                                                                                |                          |                                                                                                                   | Acceptable (VAI on last inspection) |
|                                                                                                                                                                |                                                                                |                          |                                                                                                                   | Acceptable (NAI on last inspection) |
|                                                                                                                                                                |                                                                                |                          |                                                                                                                   | Acceptable (NAI on last inspection) |
| <b>DRUG PRODUCT</b>                                                                                                                                            |                                                                                |                          |                                                                                                                   |                                     |
| <b>Function</b>                                                                                                                                                | <b>Site Information</b>                                                        | <b>DUNS/FEI Number</b>   | <b>Inspectional Observations</b>                                                                                  | <b>Final Recommendation</b>         |
| DP manufacture; in process release testing , RTRT, and release testing for sterility (appearance, color, clarity, identity, sterility, volume, pH, Osmolality) | (b) (4)                                                                        |                          | Last inspections<br>(b) (4)<br>NAI<br>VAI<br>VAI<br>VAI<br><br>BLA PLI waived                                     | acceptable                          |
| DP release and stability testing                                                                                                                               |                                                                                |                          | Amgen, Inc.<br>Amgen Thousand Oaks Facility (ATO)<br>1 Amgen Center Drive<br>Thousand Oaks, CA 91320<br>• (b) (4) | 039976196/<br>2026154               |

|                                                               |                                                                   |                        |                                                                                       |            |
|---------------------------------------------------------------|-------------------------------------------------------------------|------------------------|---------------------------------------------------------------------------------------|------------|
|                                                               | (b) (4)                                                           |                        | inspected.<br>Five 483 observations were made.<br>- VAI                               |            |
| DP labeling and packaging<br>DP release and stability testing | Amgen Manufacturing Ltd Carr 31 KM 24 6 Juncos, Puerto Rico 00777 | 785800020 / 1000110364 | Last inspections:<br>5/10/2017-VAI<br>1/23/2015-VAI<br>8/15/2013-VAI<br>3/29/2012-NAI | acceptable |
| DP release , stability testing                                | Amgen Technology Ireland UC, Pottery Road, Dun Laoghaire, Ireland | 896293920/ 3002808497  | Last insepctions<br>12/08/2015-VAI<br>4/02/2015-VAI<br>3/22/2013-VAI                  | acceptable |
| (b) (4)                                                       |                                                                   |                        | Last nspection<br>(b) (4)<br>NAI<br>NAI<br>NAI                                        | acceptable |

**G. Facilities:**

The pre-license inspection of the drug substance manufacturing facility at Amgen Inc., Thousand Oaks, CA was conducted May 8 - 16 , 2017 by Laura Fontan (CDER/OPQ/OPF/DIA), Jee Chung (CDER/OPQ/OBP/DBRRIV) and Sarah Arden (CDER/OPQ/OBP/DBRRII). The inspection covered the ABP215 drug substance manufacturing areas, QC laboratories, warehouse, and cell bank storage. The inspection also covered analytical similarity data, and the storage and tracking of the US-licensed Avastin and EU-approved bevacizumab vials used in the analytical similarity assessment. The inspection was system-based and covered Quality, Facilities and Equipment, Production, Materials, and Laboratories. This inspection was limited to the manufacturing of bevacizumab-awwb drug substance, QC assays used for drug substance and drug product release and stability testing, and the analytical similarity data and components.

A five-item Form FDA 483 was issued to the firm covering deficiencies in (b) (4)

(b) (4) Amgen addressed these issues in their response to the EIR. A final compliance status of voluntary action indicated (VAI) was given to the facility.

Manufacturing of bevacizumab-awwb drug product is performed at the (b) (4) (b) (4). The inspection of (b) (4) for DP manufacturing was waived for STN 761028/0. Based upon file review, the District recommended approval of the firm for its listed responsibilities in the application. Based on the firm's compliance history, its current acceptable status for profile SVS, (b) (4)

The other sites listed in the establishment table, above, were assessed for the relevance of the inspections previously conducted to the functions used for bevacizumab-awwb. Based on this analysis, all sites were found acceptable for the manufacturing activities of bevacizumab-awwb

## **H. Lifecycle Knowledge Management:**

### **a. Drug Substance:**

#### **i. Protocols approved:**

1. Annual stability protocol for drug substance
2. Stability protocol for the extension of drug substance shelf-life
3. Protocol for qualification of new primary and working reference material
4. (b) (4) validation protocols
5. (b) (4) validation protocols
6. (b) (4) validation protocols
7. (b) (4) validation protocols
8. Cell bank stability protocol

#### **ii. Outstanding review issues/residual risk:**

See Post Marketing Commitments numbers 1-3 in section ID.

#### **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:**

See Post Marketing Commitment number 4 in section ID.

#### **iii. Future inspection points to consider: None**

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

| #                                | Checklist                                                                                    | Yes                    | No | N/A |
|----------------------------------|----------------------------------------------------------------------------------------------|------------------------|----|-----|
| <b>Product Type</b>              |                                                                                              |                        |    |     |
| 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]                                                                 |                        | x  |     |
| <b>Regulatory Considerations</b> |                                                                                              |                        |    |     |
| 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 tem                                                       |                        | x  |     |
| 18.                              | SPOTS (special products on-line tracking system)                                             |                        | x  |     |
| 19.                              | USAN assigned name                                                                           | x                      |    |     |
| 20.                              | Other [fill in]                                                                              |                        |    |     |
| <b>Quality Considerations</b>    |                                                                                              |                        |    |     |
| 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                                                     |                        |    |     |
| 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}                                                                              |                        |    |     |



Chana  
Fuchs

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Joel  
Welch

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Patricia  
Hughes Troost

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Zhihao Peter  
Qiu

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

**LABELS AND LABELING REVIEW**

|                     |                                                                                                        |
|---------------------|--------------------------------------------------------------------------------------------------------|
| Date:               | September 7, 2017                                                                                      |
| Reviewer:           | Vicky Borders-Hemphill, PharmD<br>Labeling Review Specialist<br>Office of Biotechnology Products (OBP) |
| Through:            | Jee Chung, PhD, Product Quality Reviewer<br>OBP/Division of Biotechnology Review and Research IV       |
| Application:        | BLA 761028                                                                                             |
| Product:            | Mvasi (bevacizumab-awwb)                                                                               |
| Applicant:          | Amgen Inc.                                                                                             |
| Submission Date(s): | November 14, 2016, July 28, 2017, and September 5, 2017                                                |

**I) RECOMMENDATION**

The labels and labeling for Mvasi (bevacizumab-awwb) injection, 100 mg/4 mL and 400 mg/16 mL (25 mg/mL) single-dose vial for intravenous use submitted on July 28, 2017 (container label and carton labeling) and September 5, 2017 (prescribing Information) are acceptable from a quality perspective if the Applicant applies the FDA approved nonproprietary name suffix to the final labels and labeling.

**II) BACKGROUND AND SUMMARY DESCRIPTION**

The Applicant, Amgen Inc., submitted BLA 761028 Mvasi (bevacizumab-awwb) on November 14, 2016 as a proposed biosimilar to US-licensed Avastin.

Table 1 presents relevant product information for Mvasi (bevacizumab-awwb) that the Applicant submitted on November 14, 2016, and for U.S.-licensed Avastin.

| <b>Table 2. Relevant Product Information for Mvasi and U.S.-licensed Avastin</b> |                                                                                              |                              |
|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|------------------------------|
| <b>Product</b>                                                                   | <b>Biosimilar Product Name</b>                                                               | <b>U.S. licensed Avastin</b> |
| <b>Proprietary Name:</b>                                                         | Mvasi                                                                                        | Avastin                      |
| <b>Nonproprietary Name:</b>                                                      | bevacizumab-awwb                                                                             | bevacizumab                  |
| <b>Dosage Form:</b>                                                              | injection                                                                                    |                              |
| <b>Strength and Container-Closure:</b>                                           | 100 mg/4 mL and 400 mg/16 mL (25 mg/mL) single-dose vial                                     |                              |
| <b>Route of Administration:</b>                                                  | Intravenous                                                                                  |                              |
| <b>Storage and Handling:</b>                                                     | Refrigerate at 2–8°C (36–46°F). Vials should be protected from light. Do not freeze or shake |                              |

|                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>Indication:</b></p> <p><b>Dose and Frequency:</b></p> | <p><b><u>Metastatic colorectal cancer-</u></b></p> <p>5 mg/kg intravenously every 2 weeks with bolus-IFL</p> <p>10 mg/kg intravenously every 2 weeks with FOLFOX4</p> <p>5 mg/kg intravenously every 2 weeks or 7.5 mg/kg intravenously every 3 weeks with fluoropyrimidine-irinotecan or fluoropyrimidine-oxaliplatin based chemotherapy after progression on a first-line bevacizumab product containing regimen</p> <p><b><u>Non-squamous non-small cell lung cancer-</u></b></p> <p>15 mg/kg intravenously every 3 weeks with carboplatin/paclitaxel</p> <p><b><u>Glioblastoma-</u></b></p> <p>10 mg/kg intravenously every 2 weeks</p> <p><b><u>Metastatic renal cell carcinoma (mRCC)-</u></b></p> <p>10 mg/kg intravenously every 2 weeks with interferon alfa</p> <p><b><u>Persistent, recurrent, or metastatic carcinoma of the cervix-</u></b></p> <p>15 mg/kg intravenously every 3 weeks with paclitaxel/cisplatin or paclitaxel/topotecan</p> | <p><b><u>Metastatic colorectal cancer-</u></b></p> <p>5 mg/kg intravenously every 2 weeks with bolus-IFL</p> <p>10 mg/kg IV every 2 weeks with FOLFOX4</p> <p>5 mg/kg intravenously every 2 weeks or 7.5 mg/kg intravenously every 3 weeks with fluoropyrimidine-irinotecan or fluoropyrimidine-oxaliplatin based chemotherapy after progression on a first-line Avastin containing regimen</p> <p><b><u>Non-squamous non-small cell lung cancer-</u></b></p> <p>15 mg/kg intravenously every 3 weeks with carboplatin/paclitaxel</p> <p><b><u>Glioblastoma-</u></b></p> <p>10 mg/kg intravenously every 2 weeks</p> <p><b><u>Metastatic renal cell carcinoma (mRCC)-</u></b></p> <p>10 mg/kg intravenously every 2 weeks with interferon alfa</p> <p><b><u>Persistent, recurrent, or metastatic carcinoma of the cervix-</u></b></p> <p>15 mg/kg intravenously every 3 weeks with paclitaxel/cisplatin or paclitaxel/topotecan</p> <p><b><u>*Platinum-resistant recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer-</u></b></p> <p>10 mg/kg intravenously</p> |
|-------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

|  |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|--|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  |  | <p>every 2 weeks with paclitaxel, pegylated liposomal doxorubicin or weekly topotecan<br/> 15 mg/kg intravenously every 3 weeks with topotecan given every 3 weeks<br/> <b><u>*Platinum-sensitive recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer-</u></b><br/> 15 mg/kg intravenously every 3 weeks in combination with carboplatin/paclitaxel for 6-8 cycles, followed by 15 mg/kg intravenously every 3 weeks as a single agent<br/> 15 mg/kg intravenously every 3 weeks in combination with carboplatin/gemcitabine for 6-10 cycles, followed by 15 mg/kg intravenously every 3 weeks as a single agent</p> |
|--|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

\* US-licensed Avastin’s indication for recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer that is platinum-resistant in combination with paclitaxel, pegylated liposomal doxorubicin, or topotecan is protected by orphan drug exclusivity expiring on November 14, 2021. US-licensed Avastin’s indication for recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer that is platinum-sensitive in combination with carboplatin and paclitaxel or in combination with carboplatin and gemcitabine is protected by orphan drug exclusivity expiring on December 6, 2023. See the Orphan Drug Designations and Approvals database at <http://www.accessdata.fda.gov/scripts/opdlisting/oopd/index.cfm>. The Applicant is not seeking licensure for these indications in their application.

### III) **MATERIALS REVIEWED**

We considered the materials listed in Table 2 for this review.

**Table 1: Materials Considered for this Label and Labeling Review**

| <b>Materials Reviewed</b>                                             | <b>Appendix Section</b> |
|-----------------------------------------------------------------------|-------------------------|
| Proposed Labels and Labeling                                          | A                       |
| Other                                                                 | B                       |
| Relevant Code of Federal Regulations and CDER Labeling Best Practices | C                       |
| Acceptable Labels and Labeling                                        | D                       |

n/a = not applicable for this review

#### **IV) DISCUSSION**

We evaluated the proposed labels for compliance with the applicable requirements in the Code of Federal Regulations, United States Pharmacopeia (USP) standards, and CDER Labeling Best Practices (see Appendix C).

#### **V) CONCLUSION**

The prescribing information, container labels, and carton labeling for Mvasi (bevacizumab-awwb) injection, 100 mg/4 mL and 400 mg/16 mL (25 mg/mL) single-dose vial for intravenous use were reviewed and found to comply with the following regulations: 21 CFR 610.60 through 21 CFR 610.67; 21 CFR 201.2 through 21 CFR 201.25; 21 CFR 201.50 through 21 CFR 201.57; 21 CFR 201.100, USP standards, and CDER Labeling Best Practices.

The container labels and labeling submitted on July 28, 2017 (container label and carton labeling) and September 5, 2017 (prescribing Information) is acceptable (see Appendix D) from a quality perspective if the Applicant applies the FDA approved nonproprietary name suffix to the final labels and labeling.

**Appendix C: Applicant Code of Federal Regulations and CDER Best Labeling Practices**

**Table 2:** Label<sup>1,2</sup> and Labeling<sup>3</sup> Standards

**Container<sup>4</sup> Label Evaluation**

**100 mg/4 mL vial container label**

| Regulations                                                                                       | Conforms |    |     | Comments and Recommendations           |
|---------------------------------------------------------------------------------------------------|----------|----|-----|----------------------------------------|
|                                                                                                   | Yes      | No | n/a |                                        |
| <u>Proper Name</u><br>21 CFR 610.60<br>21 CFR 201.50<br>21 CFR 201.10                             |          |    | x   | We consider this to be a partial label |
| <u>Manufacturer name, address, and license number</u><br>21 CFR 610.60                            |          |    | x   | We consider this to be a partial label |
| <u>Lot number or other lot identification</u><br>21 CFR 610.60<br>21 CFR 201.18<br>21 CFR 201.100 |          |    | x   | We consider this to be a partial label |
| <u>Expiration date</u><br>21 CFR 610.60<br>21 CFR 201.17                                          |          |    | x   | We consider this to be a partial label |
| <u>Multiple dose containers (recommended individual dose)</u><br>21 CFR 610.60                    |          |    | x   | Single-dose                            |
| <u>Statement: "Rx only"</u><br>21 CFR 610.60<br>21 CFR 201.100                                    | x        |    |     |                                        |
| <u>Medication Guide</u><br>21 CFR 610.60                                                          |          |    | x   |                                        |

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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.

<sup>4</sup> 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.

| Regulations                                            | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|--------------------------------------------------------|----------|----|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                        | Yes      | No | n/a |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 21 CFR 208.24                                          |          |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <u>No Package for container</u><br>21 CFR 610.60       |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <u>Partial label</u><br>21 CFR 610.60<br>21 CFR 201.10 |          | x  |     | <p>See DMEPA review of nonproprietary name suffix.<sup>5</sup></p> <p>We consider this a partial label. Consider omitting the words "Manufactured by" in the manufacturer's statement to allow space for other important information such as the appropriate presentation of strength in the same visual field as the proprietary and proper names and dosage form.</p> <p>Consider adding the dosage form "Injection" to appear underneath the proper name. To accommodate this, you may consider omitting the license number, which is not required information for a partial label (see 21 CFR 610.60(c)).</p> <p>For example<br/>           TRADENAME<br/>           (bevacizumab-xxxx)<br/>           Injection<br/>           100 mg/4 mL (25 mg/mL)<br/>           Amgen Inc.</p> <p><i>Applicant omitted the dosage form but included the license number.</i></p> <p><i>The Applicant's revision is acceptable</i></p> |
| <u>No container label</u><br>21 CFR 610.60             |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <u>Ferrule and cap overseal</u>                        |          | x  |     | <p>Confirm there is no text on the ferrule and cap overseal of the vials to comply with a revised United States Pharmacopeia (USP), General Chapters: &lt;1&gt; Injections, Packaging, Labeling on Ferrules and Cap Overseals.</p> <p><i>Applicant responded: Amgen confirms that there is no text on the ferrule and cap overseal of the vials.</i></p> <p><i>Applicant's response is acceptable.</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

<sup>5</sup> Stewart J. Nonproprietary name suffix memorandum. RCM # 2017-1352 BLA 761028. Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 2017 August 18. P3.

| Regulations                                                      | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|------------------------------------------------------------------|----------|----|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                  | Yes      | No | n/a |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Visual inspection</u><br>21 CFR 610.60                        |          | x  |     | <p>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).</p> <p><i>Applicant responded: Amgen confirms that there is sufficient area on the container to allow for visual inspection. When the label is affixed to the vial, the label does not overlap and there is space between the edges to allow for visual inspection.</i></p> <p><i>Applicant's response is acceptable.</i></p>                       |
| <u>NDC numbers</u><br>21 CFR 201.2<br>21 CFR 207.35              | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Route of administration</u><br>21 CFR 201.5<br>21 CFR 201.100 | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Preparation instructions</u><br>21 CFR 201.5                  |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Package type term</u><br>21 CFR 201.5                         |          | x  |     | <p>Add the statement "Single-dose vial. Discard unused portion." to ensure that the package type term and adequate directions of use are provided on the container label per 21 CFR 201.5 (g) and USP chapter &lt;659&gt; Packaging and Storage Requirements (see Draft Guidance: 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 Guidance for Industry)</p> <p><i>Applicant revised as requested.</i></p> |
| <u>Drugs Misleading statements</u><br>21 CFR 201.6               |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Strength</u><br>21 CFR 201.10<br>21CFR 201.100                |          | x  |     | <p>Relocate the strength presentation from the side panel to appear beneath the proper name and dosage form in the same visual field so that the expression as strength per total volume is the primary and prominent expression on PDP followed in close proximity by strength/mL enclosed by parenthesis see USP General Chapters &lt;7&gt; Labeling (Strength per total volume for single dose and multiple dose injectable drug</p>                                                                                                                         |

| Regulations                                                                     | Conforms |    |     | Comments and Recommendations                                                                                                                                                                            |
|---------------------------------------------------------------------------------|----------|----|-----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                 | Yes      | No | n/a |                                                                                                                                                                                                         |
|                                                                                 |          |    |     | products)<br><br><i>The Applicant's revision is acceptable</i>                                                                                                                                          |
| <u>Drugs</u><br><u>Prominence of required label statements</u><br>21 CFR 201.15 | x        |    |     |                                                                                                                                                                                                         |
| <u>Bar code label requirements</u><br>21 CFR 201.25<br>21CFR 610.67             | x        |    |     |                                                                                                                                                                                                         |
| <u>Net quantity</u><br>21 CFR 201.51                                            | x        |    |     |                                                                                                                                                                                                         |
| <u>Usual dosage statement</u><br>21 CFR 201.55<br>21 CFR 201.100                |          |    | x   | We consider this to be a partial label                                                                                                                                                                  |
| <u>Inactive ingredients</u><br>21 CFR 201.100                                   |          |    |     | We consider this a partial label thus this information must appear on the carton, PI, and IFU (if applicable).                                                                                          |
| <u>Storage requirements</u>                                                     |          | x  |     | Relocate the storage information to appear on the side panel to accommodate the appropriate strength presentation on the principal display panel.<br><br><i>The Applicant's revisions is acceptable</i> |
| <u>Dispensing container</u><br>21 CFR 201.100                                   |          |    | x   |                                                                                                                                                                                                         |

#### **400 mg/16 mL vial container label**

| Regulations                                                            | Conforms |    |     | Comments and Recommendations                                               |
|------------------------------------------------------------------------|----------|----|-----|----------------------------------------------------------------------------|
|                                                                        | Yes      | No | n/a |                                                                            |
| <u>Proper Name</u><br>21 CFR 610.60<br>21 CFR 201.50<br>21 CFR 201.10  | x        |    |     | Ensure the nonproprietary name contains the suffix as determined by DMEPA. |
| <u>Manufacturer name, address, and license number</u><br>21 CFR 610.60 | x        |    |     |                                                                            |
| <u>Lot number or</u>                                                   | x        |    |     |                                                                            |

| Regulations                                                                         | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------------------------------------------------------------------------|----------|----|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                     | Yes      | No | n/a |                                                                                                                                                                                                                                                                                                                                                                                                            |
| <u>other lot identification</u><br>21 CFR 610.60<br>21 CFR 201.18<br>21 CFR 201.100 |          |    |     |                                                                                                                                                                                                                                                                                                                                                                                                            |
| <u>Expiration date</u><br>21 CFR 610.60<br>21 CFR 201.17                            | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                            |
| <u>Multiple dose containers (recommended individual dose)</u><br>21 CFR 610.60      |          |    | x   | Single-dose vial                                                                                                                                                                                                                                                                                                                                                                                           |
| <u>Statement: "Rx only"</u><br>21 CFR 610.60<br>21 CFR 201.100                      | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                            |
| <u>Medication Guide</u><br>21 CFR 610.60<br>21 CFR 208.24                           |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                            |
| <u>No Package for container</u><br>21 CFR 610.60                                    |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                            |
| <u>Partial label</u><br>21 CFR 610.60<br>21 CFR 201.10                              |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                            |
| <u>No container label</u><br>21 CFR 610.60                                          |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b><u>Ferrule and cap overseal</u></b>                                              |          | x  |     | <p>Confirm there is no text on the ferrule and cap overseal of the vials to comply with a revised United States Pharmacopeia (USP), General Chapters: &lt;1&gt; Injections, Packaging, Labeling on Ferrules and Cap Overseals.</p> <p><i>Applicant responded: Amgen confirms that there is no text on the ferrule and cap overseal of the vials.</i></p> <p><i>Applicant's response is acceptable.</i></p> |
| <u>Visual inspection</u><br>21 CFR 610.60                                           |          | x  |     | <p>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).</p> <p><i>Applicant responded: "Amgen confirms that there is sufficient area on the container to allow for visual</i></p>                                                                      |

| Regulations                                                      | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|------------------------------------------------------------------|----------|----|-----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                  | Yes      | No | n/a |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                  |          |    |     | <p><i>inspection. When the label is affixed to the vial, the label does not overlap and there is space between the edges to allow for visual inspection."</i></p> <p><i>Applicant's response is acceptable.</i></p>                                                                                                                                                                                                                                                                                                                                                                                                             |
| <u>NDC numbers</u><br>21 CFR 201.2<br>21 CFR 207.35              | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Route of administration</u><br>21 CFR 201.5<br>21 CFR 201.100 | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Preparation instructions</u><br>21 CFR 201.5                  |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Package type term</u><br>21 CFR 201.5                         |          | x  |     | <p>Add the statement "Single-dose vial. Discard unused portion." to ensure that the package type term and adequate directions of use are provided on the container label per 21 CFR 201.5 (g) and USP chapter &lt;659&gt; Packaging and Storage Requirements (see Draft Guidance: 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 Guidance for Industry)</p> <p><i>Applicant revised as requested.</i></p>                                                                 |
| <u>Drugs Misleading statements</u><br>21 CFR 201.6               |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Strength</u><br>21 CFR 201.10<br>21CFR 201.100                |          | x  |     | <p>Relocate the strength presentation from the side panel to appear beneath the proper name and dosage form in the same visual field so that the expression as strength per total volume is the primary and prominent expression on PDP followed in close proximity by strength/mL enclosed by parenthesis see USP General Chapters &lt;7&gt; Labeling (Strength per total volume for single dose and multiple dose injectable drug products). Add the dosage form "Injection" to appear underneath the proper name.</p> <p>For example<br/>           TRADENAME<br/>           (bevacizumab-xxxx)<br/>           Injection</p> |

| Regulations                                                           | Conforms |    |     | Comments and Recommendations                                                                                                                                                                           |
|-----------------------------------------------------------------------|----------|----|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                       | Yes      | No | n/a |                                                                                                                                                                                                        |
|                                                                       |          |    |     | 400 mg/16 mL (25 mg/mL)<br>Manufactured by Amgen Inc.<br>US License No. 1080<br><br><i>Applicant omitted the dosage form but included the license number. The applicant's revision is acceptable</i>   |
| <u>Drugs Prominence of required label statements</u><br>21 CFR 201.15 | x        |    |     |                                                                                                                                                                                                        |
| <u>Bar code label requirements</u><br>21 CFR 201.25<br>21CFR 610.67   | x        |    |     |                                                                                                                                                                                                        |
| <u>Net quantity</u><br>21 CFR 201.51                                  | x        |    |     |                                                                                                                                                                                                        |
| <u>Usual dosage statement</u><br>21 CFR 201.55<br>21 CFR 201.100      |          |    | x   | Space considerations and this information must appear on the carton, PI, and IFU (if applicable).                                                                                                      |
| <u>Inactive ingredients</u><br>21 CFR 201.100                         |          |    | x   | Space considerations and this information must appear on the carton, PI, and IFU (if applicable).                                                                                                      |
| <u>Storage requirements</u>                                           |          | x  |     | Relocate the storage information to appear on the side panel to accommodate the appropriate strength presentation on the principal display panel.<br><br><i>The Applicant's revision is acceptable</i> |
| <u>Dispensing container</u><br>21 CFR 201.100                         |          |    | x   |                                                                                                                                                                                                        |

## Package Label<sup>6</sup> Evaluation

| Regulations                                                                | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------------------------------------------|----------|----|-----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                            | Yes      | No | n/a |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <u>Proper name</u><br>21 CFR 610.61<br>21 CFR 201.50<br>21 CFR 201.10      | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <u>Manufacturer name, address, and license number</u><br>21CFR 610.61      |          | x  |     | Relocate the US license number to appear underneath the manufacturer's address and before the country of origin statement.<br><br>Consider revising the country of Origin statement from "Product of U.S. origin" to read "Product of U.S."<br><br><i>The Applicant revised as requested</i>                                                                                                                                                                                                                                                                                  |
| <u>Lot number or other lot identification</u><br>21 CFR 610.61             | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <u>Expiration date</u><br>21 CFR 610.61<br>21 CFR 201.17                   | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <u>Preservative</u><br>21 CFR 610.61                                       | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <u>Number of containers</u><br>21 CFR 610.61                               | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <u>Strength/volume</u><br>21 CFR 610.61<br>21 CFR 201.10<br>21 CFR 201.100 |          | x  |     | Revise all strength presentations from "xx mg/vial" to an expression as strength per total volume followed in close proximity by strength/mL enclosed by parenthesis which is an expression appropriate for the dosage form see USP General Chapters <7> Labeling (Strength per total volume for single dose and multiple dose injectable drug products).<br><br>Remove the word "i (b) (4)" and add the appropriate dosage form "Injection" per USP nomenclature to appear underneath the proper name on all carton panels.<br><br><i>The Applicant revised as requested</i> |

<sup>6</sup> 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.

| Regulations                                                                      | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                     |
|----------------------------------------------------------------------------------|----------|----|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                  | Yes      | No | n/a |                                                                                                                                                                                                                  |
| <u>Storage temperature</u><br>21 CFR 610.61                                      | x        |    |     |                                                                                                                                                                                                                  |
| <u>Handling: "Shake Well", "Do not Freeze" or equivalent</u><br>21 CFR 610.61    |          | x  |     | Add the statement "Do not Freeze or Shake" after the storage statement to ensure instructions for handling appear on the carton labeling per 21 CFR 610.61 (i).<br><br><i>The Applicant revised as requested</i> |
| <u>Multiple dose containers (recommended individual dose)</u><br>21 CFR 610.61   |          |    | x   |                                                                                                                                                                                                                  |
| <u>Route of administration</u><br>21CFR 610.61<br>21 CFR 201.5<br>21 CFR 201.100 | x        |    |     |                                                                                                                                                                                                                  |
| <u>Known sensitizing substances</u><br>21CFR 610.61                              | x        |    |     | This statement "This Product Contains Dry Natural Rubber." appears on the PDP.                                                                                                                                   |
| <u>Antibiotics added during manufacturing</u><br>21 CFR 610.61                   |          |    | x   |                                                                                                                                                                                                                  |
| <u>Inactive ingredients</u><br>21 CFR 610.61<br>21 CFR 201.100                   | x        |    |     |                                                                                                                                                                                                                  |
| <u>Adjuvant, if present</u><br>21 CFR 610.61                                     |          |    | x   |                                                                                                                                                                                                                  |
| <u>Source of the product</u><br>21 CFR 610.61                                    |          |    | x   |                                                                                                                                                                                                                  |
| <u>Identity of each microorganism used in manufacturing</u><br>21 CFR 610.61     | x        |    |     | The statement "Bevacizumab-xxxx is produced in a mammalian cell (Chinese Hamster Ovary) expression system." in PI section 11 is adequate.                                                                        |
| <u>Minimum potency of product</u><br>21 CFR 610.61                               | x        |    |     |                                                                                                                                                                                                                  |
| <u>Rx only</u>                                                                   | x        |    |     |                                                                                                                                                                                                                  |

| Regulations                                                           | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-----------------------------------------------------------------------|----------|----|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                       | Yes      | No | n/a |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 21CFR 610.61<br>21 CFR 201.100                                        |          |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <u>Divided manufacturing</u><br>21 CFR 610.63                         |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <u>Distributor</u><br>21 CFR 610.64                                   |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <u>Bar code</u><br>21 CFR 610.67<br>21 CFR 201.25                     | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <u>NDC numbers</u><br>21 CFR 201.2<br>21 CFR 207.35                   | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <u>Preparation instructions</u><br>21 CFR 201.5                       |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <u>Package type term</u><br>21 CFR 201.5                              |          | x  |     | Revise the term "Single dose vials" to read as singular "single-dose vial" since there is only one vial per carton.<br><br>Add the statement "Discard unused portion." to appear after the statement "Single-dose vial"<br><br><i>The Applicant revised as requested</i>                                                                                                                                                                                                                                                                                                                                           |
| <u>Drugs Misleading statements</u><br>21 CFR 201.6                    |          |    | x   | See recommendation regarding strength presentation for statement "100 mg/vial" appearing on the carton panels.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <u>Drugs Prominence of required label statements</u><br>21 CFR 201.15 |          | x  |     | Reduce the prominence of the blue and green ribbon graphic per 21 CFR 201.15 (b)(1)<br><br><i>The Applicant responded: "Amgen proposes to keep the blue and green ribbon graphic as the logo proposed for use with [Mvasi] is consistent with the logo approved by the FDA for Amjevita (BLA # 761024). Amgen prefers to keep consistency across our biosimilar portfolio and believes that with the modifications made to the prominence of the trade name and other Agency requests, the ribbon graphic will not present a distraction from the text."</i><br><br><i>The Applicant's response is acceptable.</i> |
| <u>Net quantity</u><br>21 CFR 201.51                                  | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <u>Usual dosage</u>                                                   | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

| Regulations                                        | Conforms |    |     | Comments and Recommendations |
|----------------------------------------------------|----------|----|-----|------------------------------|
|                                                    | Yes      | No | n/a |                              |
| statement<br>21 CFR 201.55<br>21 CFR 201.100       |          |    |     |                              |
| Dispensing<br>container<br>21 CFR 201.100          |          |    | x   |                              |
| Medication Guide<br>21 CFR 610.60<br>21 CFR 208.24 |          |    | x   |                              |

## Prescribing Information and Patient Labeling Evaluation

| Labeling Standards                                  | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                               |
|-----------------------------------------------------|----------|----|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                     | Yes      | No | n/a |                                                                                                                                                                                                                                                            |
| <b>PRESCRIBING INFORMATION</b>                      |          |    |     |                                                                                                                                                                                                                                                            |
| <b>Highlights of prescribing information</b>        |          |    |     |                                                                                                                                                                                                                                                            |
| PRODUCT TITLE<br>21 CFR 201.57(a)(2)                |          | x  |     | Methods of administration (intravenous infusion) should not appear in product title as best labeling practice<br><br><i>This recommendation was not incorporated into the recommended revisions by the review team.</i>                                    |
| DOSAGE AND ADMINISTRATION<br>21 CFR 201.57(a)(7)    | x        |    |     |                                                                                                                                                                                                                                                            |
| DOSAGE FORMS AND STRENGTHS<br>21 CFR 201.57(a)(8)   |          | x  |     | Dosage form must be included per 21 CFR 201.57(a)(8)<br><br><i>The Applicant revised as requested</i>                                                                                                                                                      |
| <b>Full Prescribing Information</b>                 |          |    |     |                                                                                                                                                                                                                                                            |
| 2 DOSAGE AND ADMINISTRATION<br>21 CFR 201.57(c)(3)  |          | x  |     | Add statement under preparation subsection "TRADENAME is a colorless to pale yellow solution. Do not use vial if solution is cloudy, discolored, or contains particulate matter."<br><br><i>The Applicant revised as requested</i>                         |
| 3 DOSAGE FORMS AND STRENGTHS<br>21 CFR 201.57(c)(4) |          | x  |     | Dosage form and identifying characteristics must be included per 21 CFR 201.57(c)(4). Recommends revising the strength presentation as 100 mg/4 mL (25 mg/mL) and 400 mg/16 mL (25 mg/mL) or similar:<br>"<Proprietary name> is a colorless to pale yellow |

| Labeling Standards                                                     | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|------------------------------------------------------------------------|----------|----|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                        | Yes      | No | n/a |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                        |          |    |     | <p>solution available as:<br/>Injection:</p> <ul style="list-style-type: none"> <li>• 100 mg/4mL (25 mg/mL)</li> <li>• 400 mg/16 mL (25 mg/mL)"</li> </ul> <p>Note: According to USP Chapter &lt;7&gt;, the primary expression of strength is the strength per total volume (total drug content supplied in container) followed in close proximity by a parenthetical that includes the strength/mL.</p> <p><i>The Applicant's revision is acceptable</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 6.2<br>IMMUNOGENICITY                                                  |          | x  |     | <p>Relocated and revised the language for this standard statement based on our current labeling best practice see draft Guidance for Industry: Labeling for Biosimilar Products)<br/>"As with all therapeutic proteins, there is a potential for immunogenicity. The detection of antibody formation is highly dependent on the sensitivity and the specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies in the studies described below with the incidence of antibodies in other studies or to other bevacizumab products may be misleading."</p> <p><i>The Applicant revised as requested</i></p> |
| 11 DESCRIPTION<br>21 CFR<br>201.57(c)(12)                              |          | x  |     | <p>Revise the identifying characteristic from (b) (4)" to "pale yellow".</p> <p><i>The Applicant revised as requested</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 16 HOW SUPPLIED/<br>STORAGE AND<br>HANDLING<br>21 CFR<br>201.57(c)(17) |          | x  |     | <p>Added dosage form, appropriate presentation of strength, and identifying characteristics per 21 CFR 201.57(c)(17).</p> <p><i>The Applicant revised as requested</i></p> <p>This biosimilar has a statement on their carton "This product contains dry natural rubber". U.S.-licensed Avastin carton does not have that statement on the</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

| Labeling Standards                                                     | Conforms |    |     | Comments and Recommendations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|------------------------------------------------------------------------|----------|----|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                        | Yes      | No | n/a |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                        |          |    |     | <p>carton or in the labeling, (unable to confirm at this time if U.S.-licensed Avastin vial has a dry natural rubber stopper).</p> <p>Per 21 CFR 610.61(I) known sensitizing agents should appear on the package label or reference an enclosed circular. Per 21 CFR 801.437 Medical devices containing rubber produced by the DNR process that contacts humans should include the following statement in bold print in their labeling: "This Product Contains Dry Natural Rubber." See User Labeling for Devices that Contain Natural Rubber (21 CFR 801.437); Small Entity Compliance Guide; Guidance for Industry, April 2003</p> <p>We added the statement to specify the component by adding statement "the vial stopper contains dry natural rubber" although this will differ from U.S.-licensed Avastin.</p> <p><i>The Applicant revised as requested</i></p> <p>Detailed storage conditions for reconstituted and diluted products should be described in the DOSAGE AND ADMINISTRATION section rather than the HOW SUPPLIED/STORAGE AND HANDLING section. Relocated to section 2.</p> <p><i>The Applicant revised as requested</i></p> |
| Manufacturer information                                               | x        |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>MEDICATION GUIDE, INSTRUCTIONS FOR USE, AND PATIENT INFORMATION</b> |          |    |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Title (names and dosage form)                                          |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Storage and Handling                                                   |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Ingredients                                                            |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Manufacturer Information                                               |          |    | x   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |



Vicky  
Borders-Hemphill

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Jee  
Chung

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Center for Drug Evaluation and Research  
WO Bldg 22  
10903 New Hampshire Ave.  
Silver Spring, MD 20993

**Date:** August 7, 2017  
**To:** Administrative File, STN 761028/0  
**From:** Dupeh Palmer, Ph.D., Reviewer, OPQ/OPF/DMA/BIV  
**Through:** Patricia Hughes, Ph.D., Acting Branch Chief, OPQ/OPF/DMA/BIV  
**Subject:** New Biologic License Application (BLA)  
**Applicant:** Amgen Inc.  
**US License Number:** 1080  
**Facility:** [REDACTED] (b) (4)  
(FEI: [REDACTED] (b) (4))  
**Product:** Bevacizumab-atto (ABP 215)  
**Indication:** Treatment for metastatic colorectal cancer; Non-squamous non-small cell lung cancer; Glioblastoma; Metastatic renal cell carcinoma; Persistent, recurrent, or metastatic carcinoma of the cervix  
**Dosage Form:** Sterile, preservative-free 25 mg/mL solution for intravenous infusion supplied in single-use vials to deliver either 4 mL or 16 mL of the drug product  
**FDA Receipt Date:** 14 November 2016  
**Action Date:** 14 September 2017

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**Recommendation for Approvability:** BLA 761028 is recommended for approval from a sterility assurance and microbiology product quality perspective pending the evaluation of additional information requested under review addendum below.

The following post-marketing commitment has been communicated to the applicant:

1. Develop and implement a validated endotoxin detection method for the release of the drug product not subject to low endotoxin recovery.

**REVIEW SUMMARY:**

Amgen Inc. submitted BLA 761028 to license ABP 215 for the treatment of various cancer. The application was submitted in eCTD format. This review contains the assessment of the ABP 215 drug product 3.2.P section of the BLA from a sterility assurance and microbiological quality perspective. The review of drug substance section of the BLA was conducted by Dr. Scott Nichols.

### **Drug Product Quality Microbiology Information Reviewed**

| Sequence number | Date             | Description                          |
|-----------------|------------------|--------------------------------------|
| 0001            | 12 November 2016 | Original BLA                         |
| 0007            | 22 February 2017 | Response to Information Request (IR) |
| 0016            | 9 June 2017      | Response to IR                       |
| 0017            | 16 June 2017     | Response to IR                       |
| 0021            | 21 July 2017     | Response to IR                       |
| 0023            | 28 July 2017     | Response to IR                       |
| 0024            | 31 July 2017     | Response to IR                       |
| 0028            | 24 August 2017   | Response to IR                       |

## **Module 3.2**

### **P.1 Description and Composition of the Drug Product**

The ABP 215 drug product (DP) is provided as a 100 mg or a 400 mg sterile, preservative-free solution in single use vials to deliver 4 mL or 16 mL for intravenous infusion. Both presentations contain 25 mg/mL of ABP215 and have a pH of 6.2. The table below lists the components of the DP and was copied from section P.1.

Table 1. Quantitative and Qualitative Composition of ABP 215

| Component                               | Grade                 | Function          | Concentration       | Quantity per 100 mg presentation | Quantity per 400 mg presentation |
|-----------------------------------------|-----------------------|-------------------|---------------------|----------------------------------|----------------------------------|
| ABP 215                                 | In house <sup>a</sup> | Active ingredient | 25 mg/mL            | 100                              | 400                              |
| $\alpha,\alpha$ -trehalose dihydrate    | USP, PhEur            | (b) (4)           | 60 mg/mL            | 240                              | 960                              |
| Sodium phosphate monobasic, monohydrate | USP, PhEur,           | (b) (4)           | 5.8 mg/mL           | 23.2                             | 92.8                             |
| Sodium phosphate dibasic, anhydrous     | USP, PhEur            | (b) (4)           | 1.2 mg/mL           | 4.8                              | 19.2                             |
| Polysorbate 20                          | NF, PhEur             | (b) (4)           | 0.040% (w/v)        | 1.6                              | 6.4                              |
| Water for injection                     | USP, PhEur            | (b) (4)           | qs to target volume | qs to target volume              | qs to target volume              |

<sup>a</sup> Tested to internal specifications as described in 3.2.S.4.1 (Specification).

### **P.2 Pharmaceutical Development Container Closure System**

The vial and stopper used for the DP are listed in Table 1 from this section of the BLA and the table is copied and pasted from the submission below.

Table 1. Compendial Compliance for the Primary Container Closure System

| Component | Material     | Quality Standard |
|-----------|--------------|------------------|
| Vial      | Type I glass | (b) (4)          |
| Stopper   | Elastomer    | (b) (4)          |

**Reviewer's comment:** *The description of the CCS is satisfactory.*

**Microbiological Attributes**

The integrity of the 6 cc and the 20 cc vial container closure system was assessed using a vacuum decay method. The method is described in section 3.2.P.5.2 (Container Closure Integrity). Container closure integrity test (CCIT) is a vacuum decay method which was qualified using commercially representative stoppers and vials filled with a surrogate solution. A study summary is in section 3.2.P.2 of the submission. It is more fully described in the pdf document entitled “Method – MET-001354-Container Closure Integrity” in section 3.2.R. The study used the same 6 mL and 20 mL vials indicated for commercial batches. Vials were filled at the manufacturing facility with 4 mL or 16 mL of an aqueous surrogate solution instead of the DP which is a viscous solution. (b) (4)

Capped vials were visually examined for damage then subjected to vacuum of 750 mbar inside a test chamber at room temperature (i.e., 20°C to 25°C). The test chamber was then isolated from the vacuum source and the rate of vacuum decay was monitored by transducers over the predetermined test cycle. The negative control was a "solid" vial with no leaks that was tested 5 times to establish a background vacuum decay rate (Pa/s) level which was determined as  $\leq (b) (4)$  Pa/second. The sensitivity limit for the test was established using a positive control vial with a (b) (4)  $\mu$ m breach. The (b) (4)  $\mu$ m-hole leak limit is based on the limit of sensitivity reported in ASTM F2338-09. A leak flow rate of (b) (4) mL/minute reported for the breach positive control corresponded with a vacuum decay rate of (b) (4) Pa/second. This vacuum decay rate was used as a pass/fail criterion to differentiate non-defective from defective samples. The acceptance criteria for the test were: (1) each evaluated non-defective vial must pass the leak rate of  $\leq (b) (4)$  Pa/s; (2) the Quality Index (Qu) value type must be  $\geq (b) (4)$ . The Qu was computed as follows:  
 $Qu = (U - X) / s$ ; where  $U =$  upper specification limit;  $s =$  standard deviation and  $X =$  mean leak rate calculated for required number of vials tested.

Results from the validation study are shown below in Table 1 in section 3.2.P.2 of the submission. Results for all test units were reported as “Pass” indicating that the calculated Qu value was  $\geq (b) (4)$  and the leak rate was  $\leq (b) (4)$  Pa/second. In contrast, the breached positive control failed to meet these acceptance criteria.

**Table 1. CCI Testing for 6 cc and 20 cc Vial Systems by Vacuum Decay**

| System            | Number of Samples | Outcome           |
|-------------------|-------------------|-------------------|
| 6 cc vial         | 270               | Pass <sup>a</sup> |
| 20 cc vial        | 270               | Pass <sup>a</sup> |
| Positive controls | 5 <sup>b</sup>    | Fail              |
| Negative controls | 5 <sup>b</sup>    | Pass <sup>a</sup> |

<sup>a</sup> Pass = all tested vials had a leak rate  $\leq$  (b) (4) Pa/s.  
<sup>b</sup> The positive control and negative control are each tested 5 times to check the system suitability of the method prior to the testing.

Defective and non-defective commercially representative units were tested during method qualification and the summary results are shown below in Table 1 from section 3.2.P.5.3 of the submission.

**Table 1. Container Closure Integrity Validation Results**

| Performance Characteristic | Acceptance Criteria | Results | Pass/Fail |
|----------------------------|---------------------|---------|-----------|
| Intermediate precision     |                     | (b) (4) | Pass      |
|                            |                     |         | Pass      |
|                            |                     |         | Pass      |
| Repeatability              |                     | (b) (4) | Pass      |
|                            |                     |         | Pass      |

*Reviewer's comment: The results demonstrate that the DP container closure system is capable of preventing microbial contamination.*

**SATISFACTORY**

**P.3 Manufacture**



(b) (4)



Patricia  
Hughes Troost

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Palmer-Ochieng

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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:** 08/18/2017  
**To:** Administrative File, STN 761028/0  
**From:** Laura Fontan, Consumer Safety Officer, CDER/OPQ/OPF/DIA  
**Endorsement:** Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA  
**Subject:** Original BLA  
**US License:** 1080  
**Applicant:** Amgen, Inc.  
**Drug Substance:** Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320 (FEI: 2026154)  
**Drug Product:** [REDACTED] (b) (4)  
(FEI: [REDACTED] (b) (4))  
**Product:** Anti-VEGF monoclonal antibody, ABP215, (Biosimilar to Avastin®)  
**Dosage:** 100 mg/4 mL, 400 mg/16 mL (25mg/ml) vial  
**Indication:** Treatment of Metastatic colorectal cancer; Non-squamous non-small cell lung cancer; Glioblastoma; Metastatic renal cell carcinoma; Persistent, recurrent or metastatic carcinoma of the cervix.  
**Due Date:** 09/13/2017

**RECOMMENDATION:** The compliance status of drug substance site, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320 (FEI: 2026154) is acceptable based on the inspection for this product in May 2017. The compliance status of drug product site, [REDACTED] (b) (4) (FEI: [REDACTED] (b) (4)) is also acceptable based on inspection and compliance history.

Final recommendation from a facility perspective for the proposed manufacturing and testing sites is to approve BLA 761028 for the drug substance manufacturing at Amgen Inc. (FEI: 2026154) and drug product manufacturing at [REDACTED] (b) (4) (FEI: [REDACTED] (b) (4)).

## SUMMARY

The subject BLA proposes manufacture of ABP 215, Anti-VEGF monoclonal antibody DS and DP at the following facilities. The drug substance is manufactured at Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320 (FEI: 2026154). The drug product is filled into vials at 25mg/ml at [REDACTED] (b) (4).

[REDACTED] (FEI: [REDACTED] (b) (4)) A complete list of facilities associated with ABP 215 manufacturing is provided in Summary tables 1 and 2.

Anti-VEGF monoclonal antibody (ABP215) DP is provided in one strength, 25 mg/mL.

**ASSESSMENT-DRUG SUBSTANCE FACILITIES**

**3.2.S.2.1 DS Manufacturers**

The sites proposed for ABP 215 DS manufacture, and testing are presented below.

**Summary 1a: Proposed Sites for ABP 215, Anti-VEGF monoclonal antibody DS Manufacture and Testing Operations (DS and DP) .**

| Facility/address                                                                   | FEI        | Responsibilities                                                                                                         | Inspection History/profile                                                                                                                      |
|------------------------------------------------------------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| AMGEN INC<br>1 AMGEN CENTER DR<br>THOUSAND OAKS, CA US<br>91320 1730               | 2026154    | STORER:<br>STABILITY AND<br>RELEASE TESTER:<br>MANUFACTURER: ABP215-DS<br><br>STABILITY AND<br>RELEASE TESTER: ABP215-DP | CBI<br>4/19/2017-NAI<br>3/17/2017-NAI<br>9/1/2016-NAI<br>6/6/2017 (PAI)-VAI<br>3/14/2016-NAI<br>2/12/2016-NAI<br><b>PAI May 8- 16, 2017-VAI</b> |
| AMGEN MANUFACTURING<br>LIMITED<br>PO BOX 4060<br>RD 31 KM 24.6<br>JUNCOS, PR 00777 | 1000110364 | STABILITY TESTER:<br>RELEASE TESTER:<br>ABP215-DS and DP<br><br>PACKAGER:                                                | SVS<br>5/10/2017-VAI<br>1/23/2015-VAI<br>8/15/2013-VAI<br>3/29/2012-NAI                                                                         |
|                                                                                    |            |                                                                                                                          | (b) (4)                                                                                                                                         |
| AMGEN TECHNOLOGY<br>IRELAND<br>POTTERY ROAD, DUN<br>LAOGHAIRE, IRELAND             | 3002808497 | RELEASE AND STABILITY<br>TESTER: ABP215-DS                                                                               | CTL<br>12/08/2015-VAI<br>4/02/2015-VAI<br>3/22/2013-VAI<br>(b) (4)                                                                              |
|                                                                                    |            |                                                                                                                          | (b) (4)                                                                                                                                         |

**Reviewer Comment:** *The facilities for manufacture of ABP 215 DS are adequately described.*

—Satisfactory—

**Prior Inspection History for DS Manufacturing and Testing Sites:**

**Amgen Inc (FEI: 2026154) ABP 215 DS Manufacturing and IPC, release and stability testing (DS and DP).**

- This was a Pre-license Inspection performed 05/08/2017 – 05/16/2017 by CDER-DIA and CDER-OBP. The inspection was in support of STN 761028/0, ABP 215 drug substance manufacturing. The Quality, Production, Laboratory, Raw Materials, Facilities and Equipment Systems were covered for cell culture and purification areas. The inspection

BLA 761028 ABP 215, Anti VEGF Monoclonal antibody DS and DP Manufacture

concluded with 5 FDA-483 observations which included a gap in cleaning validation program (potent product inactivation), exceeding validated product hold times, (b) (4), inadequate swab and rinse recovery study, and lack of specific instructions to perform operations. OPF/DIA reviewed the firm’s response to the observations and concurs with the VAI recommendation for BLA 761028. See CMS WA# 168826.

- This inspection was completed 06/06/2016 by CDER DIA, CDER-DMA, CDER-OBP, and LOS-DO. The inspection was in support of STN 761024/0, ABP 501 drug substance manufacturing. Quality, materials, production, laboratory control, facilities, and equipment systems were assessed for bioreactor and purification areas. The inspection resulted in the issuance of a 2-item FDA Form 483 regarding inadequate risk assessment for potential contamination from a potent mAb, AMG 103, and inadequate controls for preventing microbial contamination of the (b) (4). The firm’s responses were considered adequate and the inspection was classified VAI.
- This was a CDER initiated inspection completed 4/13/2016 conducted in accordance with compliance program 7348.810, Sponsors, Contract Research Organizations and Monitors. This inspection was for human drug ABP 501 (a proposed biosimilar to US licensed Humira, BLA 761024) to review Amgen records and activities related to two separate but related protocols (Protocols 20120262 and 20120263) for human drug ABP 501. No objectionable conditions were observed and no FDA-483 was issued to the firm. The inspection was classified as NAI.

**Summary 1b-Testing and packaging sites:**

| Site Name                                                                       | FEI        | Inspection dates/compliance outcome                                                                           | Status            |
|---------------------------------------------------------------------------------|------------|---------------------------------------------------------------------------------------------------------------|-------------------|
| AMGEN MANUFACTURING LIMITED<br>PO BOX 4060<br>RD 31 KM 24.6<br>JUNCOS, PR 00777 | 1000110364 | Testing DS and DP, Packager and Labeller:<br>5/10/2017-VAI<br>1/23/2015-VAI<br>8/15/2013-VAI<br>3/29/2012-NAI | <b>acceptable</b> |
| (b) (4)                                                                         |            |                                                                                                               | <b>acceptable</b> |
| (b) (4)                                                                         |            |                                                                                                               | <b>acceptable</b> |
| AMGEN TECHNOLOGY IRELAND<br>POTTERY ROAD, DUN LAOGHAIRE, IRELAND                | 3002808497 | CTL<br>12/08/2015-VAI<br>4/02/2015-VAI<br>3/22/2013-VAI                                                       | <b>acceptable</b> |
| (b) (4)                                                                         |            |                                                                                                               | <b>acceptable</b> |

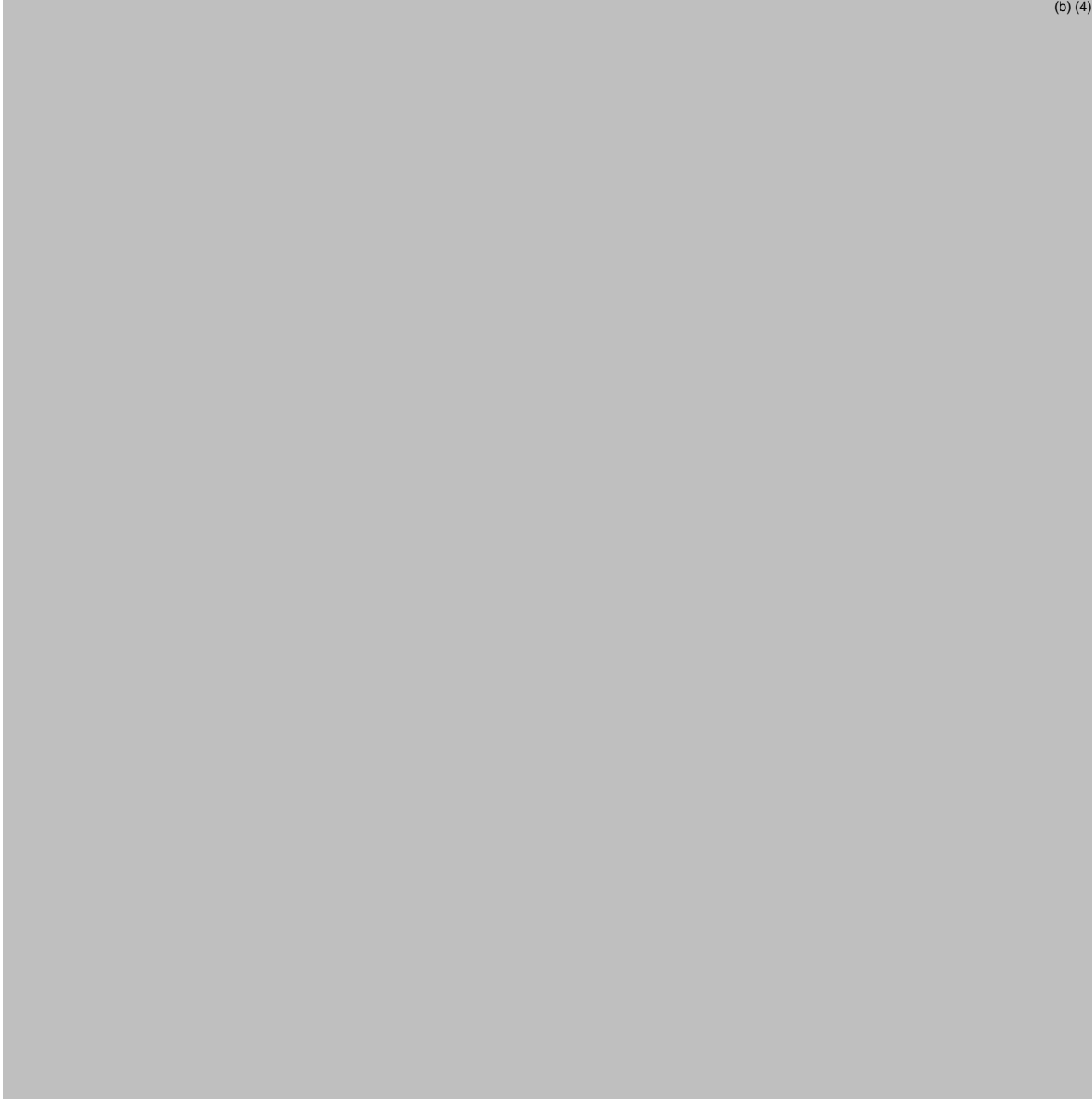
**Reviewer Comment:** The sites proposed for ABP 215 DS manufacture and DS and DP testing are adequate from the standpoint of inspectional assessment. As noted a PAI was conducted in

BLA 761028 ABP 215, Anti VEGF Monoclonal antibody DS and DP Manufacture

*support of the subject BLA for the ATO site proposed for DS manufacture. The inspection resulted in a VAI conclusion.*

*—Satisfactory—*

**ABP 215 Drug Substance Manufacturing Operations at Amgen Inc.**



(b) (4)

(b) (4)

—Satisfactory—

## CONCLUSION

Adequate descriptions were provided for the following sites proposed for ABP 215 DS and DP manufacture:

Amgen Inc., Thousand Oaks, CA FEI: 2026154

(b) (4) FEI: (b) (4)

Final recommendation for the proposed manufacturing and testing sites is to approve BLA 761028.

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Fontan -S

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Laura Fontan  
Consumer Safety Officer  
OPF Division of Inspectional Assessment  
Branch 1

Zhihao Qiu -S

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



Laura  
Fontan

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Zhihao Peter  
Qiu

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**BLA STN 761028**

**Product name: ABP 215**

**Amgen, 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

(Includes only information updated since the initial review was finalized on August 8, 2017)

1. **BLA#:** STN 761028
2. **REVIEW #:** 2 (Addendum 1)
3. **REVIEW DATE:** August 15, 2017
4. **REVIEWER(s):** Jee Chung, Ph.D., Primary Reviewer  
Chana Fuchs, Ph.D., Team Leader  
Joel Welch, Ph.D., Acting Review Chief
5. **COMMUNICATIONS WITH SPONSOR AND SUPPORTING DOCUMENTS SINCE THE FINALIZATION OF THE INITIAL REVIEW:**

| Communication/Document | Date    |
|------------------------|---------|
| Information Request-1  | 8/10/17 |
| Teleconference         | 8/11/17 |

6. **SUBMISSION(S) REVIEWED UNDER THIS ADDENDUM:**

| Submission   | Date Received | Review Completed (Yes/No) |
|--------------|---------------|---------------------------|
| Amendment 27 | 8/14/17       | Yes                       |

7. **STRENGTH/POTENCY:** 100 mg/4mL and 400 mg/16 mL

8. **ADMINISTRATIVE**

A. Signature Block (See electronic signatures at the end of document)

Jee Chung, Ph.D.  
Product Quality reviewer, OBP/DBRRIV

Chana Fuchs, Ph.D.  
Product Quality Team Lead, OBP/DBRRIV

Joel Welch, Ph.D.  
Review Chief, OBP/DBRRIV

## SUMMARY OF QUALITY ASSESSMENTS

Section V, Review Of Common Technical Document-Quality Module 3.2, below, lists the CMC issues that were not resolved by the GRMP due date for the finalization of the primary review (August 10, 2017). Responses to the initial CMC information requests were not sufficient and led to subsequent information requests sent on August 10, 2017, August 16, 2017, and a teleconference with Sponsor on August 11, 2017. Responses were emailed on 8/14/17 and 8/17/17 and final updates to the eCTD was submitted on 8/15/2017 and 8/18/17. Amgen's responses to these CMC issues and eCTD updates were reviewed and found to be acceptable. A review of the sponsor's responses is provided in this addendum.

### I. Primary Reviewer Summary Recommendation

We recommend approval of STN 761028 for ABP215 (MVASI, bevacizumab-xxxx<sup>1</sup>) manufactured by Amgen, Inc. The data submitted in this application are adequate to support the conclusion that the manufacture of MVASI (bevacizumab-xxxx) 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 ABP215 drug substance when stored at (b) (4)°C

We recommend an expiration dating period of 36 months for ABP215 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/Requirement (preliminary language)

1. To perform method validation studies in support of the introduction of the glycan mapping method into the drug substance manufacturing control strategy.
2. To perform method validation studies in support of the introduction of the host cell protein detection method into the drug substance manufacturing control strategy
3. To reassess the drug substance stability specifications acceptance criteria for drug substance stored at (b) (4)°C after an appropriate number of data points have been accumulated.

### V. Review Of Common Technical Document-Quality Module 3.2

The initial primary review of module 3.2 is provided in the original review document. At the time of the finalization of the primary review, several deficiencies communicated to the

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<sup>1</sup> MVASI is a provisional name pending final approval. A four letter suffix for the nonproprietary name for ABP 215 has not been determined. FDA is using "-xxxx" as a placeholder for the suffix. Since the proper name for ABP 215 has not yet been determined, ABP 215 is used throughout this review in place of the nonproprietary name for this product.

sponsor on 7/31/17 and 8/4/17 were not adequately addressed. The remaining deficiencies were communicated to the sponsor on 8/10/17 and sponsor responses were received 8/14/17. Based on the review of the responses, one additional information request was sent on 8/16/17 and sponsor response was received on 8/17/17. Review of the additional information received since the time of finalization of the initial primary review is provided below. See Appendix for the revised drug substance post-approval stability protocol and the stability protocols for the master cell bank, working cell bank, the primary reference standard, and the working reference standard, and the final drug substance and drug product release specifications.

(b) (4)





Jee  
Chung

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Chana  
Fuchs

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Joel  
Welch

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Center for Drug Evaluation and Research  
WO Building 22  
710903 New Hampshire Ave.  
Silver Spring, MD 20993

## PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

**Date:** August 8, 2017  
**To:** Administrative File, 761028/0  
**From:** Scott Nichols, Ph.D., Reviewer, OPQ/OPF/DMA/BIV  
**Through:** Patricia Hughes, Ph.D., Quality Assessment Lead, OPQ/OPF/DMA/BIV  
**Subject:** New 351(k) Biosimilar Biologics License Application (BLA)  
**US License:** 1080  
**Sponsor:** Amgen Inc.  
**Facilities:** Amgen, Inc. (Amgen Thousand Oaks, ATO), 1 Amgen Center Drive, Thousand Oaks, CA 91320  
(FEI: 2026154)  
**Product:** Bevacizumab, ABP 215  
**Dosage:** Sterile liquid (25 mg/mL) for intravenous infusion in a single use vial (100 mg/4 mL, 400 mg/16 mL)  
**Indication:** for the treatment of patients with metastatic colorectal cancer; non-squamous non-small cell lung cancer; glioblastoma; metastatic renal cell carcinoma, persistent, recurrent, or metastatic carcinoma of the cervix  
**Action Date:** September 14, 2017

### Conclusion and Approvability Recommendation:

The drug substance portion of BLA 761028, as amended, is recommended for approval from a microbial control and a microbiological product quality perspective. Please refer to the review memo from Dr. Dupeh Palmer for an assessment of sterility assurance and microbiological product quality for the drug product (DP) portions of the BLA.

24 Page(s) have been Withheld in Full as B4 (CCI/TS) immediately following this page



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Patricia  
Hughes Troost

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**BLA STN 761028**

**Product name: ABP 215**

**Amgen, 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#:** STN 761028
2. **REVIEW DATE:**
3. **PRIMARY REVIEW TEAM:**
  - CDTL:** Steven Lemery
  - Medical Officer:** Sandra Casak
  - Pharm/Tox:** Alex Putnam
  - Product Quality Team:** Jee Chung  
Chana Fuchs (Application Technical Lead)
  - Immunogenicity:** Jee Chung
  - Microbiology Drug Substance:** Scott Nichols
  - Microbiology Drug Product:** Dupeh Palmer
  - Facilities:** Laura Fontan
  - CMC Statistics:** Tianhua Wang
  - Clinical Pharmacology:** Edwin Chow
  - Clinical Statistics:** Vivian Yuan
  - OBP Labeling:** Vicky Borders-Hemphill
  - RPM:** Leah Her  
Keith Olin
4. **MAJOR GRMP DEADLINES**
  - Filing Meeting:** 1/13/17
  - Mid-Cycle Meeting:** 5/1/17
  - Wrap-Up Meeting:** 8/14/17
  - Primary Review Due:** 8/10/17
  - Secondary Review Due:** 8/17/17
  - CDTL Memo Due:** 8/24/17
  - PDUFA Action Date:** 9/14/17

5. **COMMUNICATIONS WITH SPONSOR AND OND:**

| Communication/Document | Date     |
|------------------------|----------|
| Information Request-1  | 12/04/16 |
| Information Request-2  | 12/16/16 |
| Information Request-3  | 1/31/17  |
| Information Request-4  | 3/21/17  |
| Information Request-5  | 4/4/17   |
| Information Request-6  | 6/29/17  |
| Information Request-7  | 7/7/17   |
| Information Request-8  | 7/31/17  |
| Information Request-9  | 8/4/17   |
| Information Request-10 | 8/9/17   |

6. **SUBMISSION(S) REVIEWED:**

| Submission                       | Date Received | Review Completed (Yes/No) |
|----------------------------------|---------------|---------------------------|
| Original Application             | 11/14/16      | Yes                       |
| Amendment 02                     | 12/07/16      | Yes                       |
| Amendment 03                     | 12/22/16      | Yes                       |
| Amendment 07                     | 2/22/16       | Yes                       |
| Amendment 08                     | 2/23/17       | Yes                       |
| Amendment 09                     | 3/28/17       | Yes                       |
| Amendment 10                     | 4/7/17        | Yes                       |
| Amendment 13                     | 5/26/17       | Yes                       |
| Amendment 20                     | 7/14/17       | Yes                       |
| Amendment 21                     | 7/20/17       | Yes                       |
| Amendment ?? (received by email) | 8/4/17        | Yes (email)               |
| Amendment ?? (received by email) | 8/7/17        | Yes (email)               |
| Amendment ??                     | Pending       | No                        |

7. **DRUG PRODUCT NAME/CODE/TYPE:**

- a. Proprietary Name: MVASI
- b. Non-Proprietary/USAN: bevacizumab-xxxx <sup>1</sup>(pending)
- c. CAS name: 1438851-35-4
- d. INN Name: bevacizumab-xxxx (pending)
- e. Compendial Name: Not applicable
- f. OBP systematic name: MAB HUMANIZED (IgG1) Anti-P15692 (VEGFA\_Human) [ABP 215]

8. **PHARMACOLOGICAL CATEGORY:** Therapeutic recombinant human monoclonal antibody to VEGFA

9. **DOSAGE FORM:** Injection

10. **STRENGTH/POTENCY:** 100 mg/4mL and 400 mg/16 mL

11. **ROUTE OF ADMINISTRATION:** Intravenous

12. **REFERENCED MASTER FILES:**

| DMF # | HOLDER | ITEM REFERENCED | Letter of Cross-Reference | COMMENTS (STATUS)                                                            |
|-------|--------|-----------------|---------------------------|------------------------------------------------------------------------------|
|       |        | (b) (4)         | Yes                       | Sufficient leachables and extractables and primary stability data in the BLA |
|       |        |                 | Yes                       | Sufficient leachables and extractables and primary stability data in the BLA |

<sup>1</sup> A four letter suffix for the nonproprietary name for ABP 215 has not been determined. FDA is using “-xxxx” as a placeholder for the suffix. Since the proper name for ABP 215 has not yet been determined, ABP 215 is used throughout this review in place of the nonproprietary name for this product.

|         |     |                           |
|---------|-----|---------------------------|
| (b) (4) | Yes | To be reviewed by OPF/DMA |
|---------|-----|---------------------------|

**13. INSPECTIONAL ACTIVITIES**

The pre-license inspection of the drug substance manufacturing facility at Amgen Inc., Thousand Oaks, CA was conducted from May 8<sup>th</sup>, 2017 to May 16<sup>th</sup>, 2017 by Laura Fontan (CDER/OPQ/OPF/DIA), Jee Chung (CDER/OPQ/OBP/DBRRIV) and Sarah Arden (CDER/OPQ/OBP/DBRRII). The inspection covered the ABP215 drug substance manufacturing areas, QC laboratories warehouse, cell bank storage, and the storage areas of US-licensed Avastin and EU-approved bevacizumab.

The inspection was system-based and covered Quality, Facilities and Equipment, Production, Materials, and Laboratories. The inspection was limited to the manufacturing of ABP 215 DS and the analytical similarity data and components.

A five-item Form FDA 483 was issued to the firm covering deficiencies in operational procedures, insufficient cross-contamination control, exceeding validated ranges, and insufficient information to support site activities. Amgen addressed these issues in their response to the EIR.

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 all that apply):

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

**15. PRECEDENTS**

None.

**17. ADMINISTRATIVE****A. Signature Block**

| Name and Title                                                                                                                                                                       | Signature and Date |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| Jee Chung, Ph.D.<br><br>Product Quality Reviewer<br>Division of Biotechnology Review and Research IV<br>Office of Biotechnology Products<br>Office of Pharmaceutical Quality, FDA    |                    |
| Chana Fuchs, Ph.D.<br><br>Product Quality Team Lead<br>Division of Biotechnology Review and Research IV<br>Office of Biotechnology Products<br>Office of Pharmaceutical Quality, FDA |                    |
| Joel Welch, Ph.D.<br><br>Acting Review Chief<br>Division of Biotechnology Review and Research IV<br>Office of Biotechnology Products<br>Office of Pharmaceutical Quality, FDA        |                    |

**B. CC Block**

| Recipient                                                               | Date |
|-------------------------------------------------------------------------|------|
| Clinical Division BLA RPM                                               |      |
| Division of Biotechnology Review and Research IV<br>File/BLA STN 761028 |      |

## SUMMARY OF QUALITY ASSESSMENTS

### I. Primary Reviewer Summary Recommendation

We recommend approval of STN 761028 for MVASI (bevacizumab-xxxx) manufactured by Amgen, Inc. pending sponsor agreement with proposed modifications to the specifications and IPCs, the DS stability protocol, reference standard stability protocol, and the cell bank stability protocol. The data submitted in this application are adequate to support the conclusion that the manufacture of MVASI (bevacizumab-xxxx) 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. Upon sponsor agreement with the proposed modifications specified above, 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 ABP215 drug substance when stored at (b) (4) °C

We recommend an expiration dating period of 36 months for ABP215 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 once pending items are finalized.

### III. List Of Post-Marketing Commitments/Requirement (Pending sponsor agreement)

1. To perform method validation studies in support of the introduction of the glycan mapping method into the drug substance manufacturing control strategy.
2. To perform method validation studies in support of the introduction of the host cell protein detection method into the drug substance manufacturing control strategy.

### IV. Review Of Common Technical Document-Quality Module 1

#### A. Environmental Assessment Or Claim Of Categorical Exclusion

##### Environmental Assessment Or Claim Of Categorical Exclusion

A categorical exclusion is claimed from 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.4 Analytical Similarity Assessment

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

The sponsor conducted an analytical similarity assessment with their proposed biosimilar product, ABP 215, against US-Avastin and EU-Avastin. The analytical assessment also included a comparison between US-Avastin and EU-Avastin in order to provide a scientific bridge to support the use of EU-Avastin as a comparator in the clinical studies.

#### **Terminology**

The sponsor's proposed biosimilar product is referred to as ABP 215 in the submission as well as in the BLA review. The reference product (RP) used in the similarity studies, US-licensed bevacizumab, is referred to in the submission as bevacizumab (US) by the sponsor. 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.

### 3.2.R.4.1 Testing Plan and Assessment Criteria

#### Drug Product Information

ABP 215, 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 the active ingredient. The formulation buffer for ABP 215 contains the same excipients as the US-Avastin and EU-Avastin products and consists of  $\alpha$ ,  $\alpha$ -trehalose dehydrate, sodium phosphate, and polysorbate 20, pH 6.2. The container closure system is also the same and consists of 6 cc or 20 cc Type I glass vials with elastomeric stopper and aluminum seal with flip-off cap. Both 100 mg and 400 mg presentations were used in the similarity study and the data from 100 and 400 mg vials were pooled to represent the characteristic of the active ingredient independent of the vial and fill size. The sponsor justified the pooling of the 100 mg and 400 mg data is acceptable because the active ingredient and the formulation buffer are the same. Fill volume was the only attribute that was not pooled for this assessment.

**Reviewer Comment:** *The pooling of the data from 100 mg and the 400 mg presentation assumes the product characteristics are the same irrespective of the volume and the container closure system. However, this is not always the case and product stability could vary depending on the vial size as surface to volume ratio could change. For the similarity study, the stability of the RP and EU-Avastin lots are already factored into the expiration date. Therefore, the equivalence of either 100 mg and 400 mg presentations should be demonstrated through real-time stability studies. Stability data from both presentations were comparable (see section 3.2.P.8) and support pooling of the data points from either presentation.*

#### Lots Tested in the Analytical Similarity Assessment

A total of 19 ABP 215 DP lots from 13 independent DS lots were used in the analytical similarity exercise. The 13 ABP 215 DS lots include development (manufactured at Amgen Washington (AWA)), clinical (Amgen Thousand Oaks (ATO)), and process validation lots (ATO) and the 19 DP lots include development (ATO), clinical (ATO), and process validation ( (b) (4) ) lots. Comparability studies were conducted between AWA and ATO (see section 3.2.S.2.6). US-Avastin and EU-Avastin DP lots were collected over a period of six years and covered shelf-life span of 3 months to 27 months till expiry. A total of 27 US-Avastin and 29 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 manufacturing dates and labeled expiry dates were provided in Tables 1 (ABP 215), 2 (US-Avastin), and 3 (EU-Avastin) of the Testing Plan and Assessment Criteria document (tables not shown in the review). All lots used in the clinical studies were included in the analytical similarity assessment.

**Reviewer comment:** *A slight discrepancy in the number of lots of bevacizumab (US) and bevacizumab (EU) exists in the submission. The text states that 26 and 28 US-Avastin and EU-Avastin lots, respectively, were used in the similarity study, but the tables provided list 27 and 29 lots, respectively. CMC statistics sent an IR regarding this discrepancy and the sponsor confirmed that the number of lots in the table is correct and it was an error on their part of the text. (IR response 2/23/17)*

#### Methods used in the Analytical Similarity Assessment

The sponsor states that the methods selected to perform the analytical similarity exercise were chosen based on product knowledge regarding the structure, function, heterogeneity, and stability of their product and the reference product. Therefore, the analytical similarity exercise was grouped into eight categories that evaluated biological activity, primary structure, higher order structure, particles and aggregates, product-related substances and impurities, thermal stability and forced degradation, general properties, and process-related impurities. The sponsor states that the method used were appropriately validated or qualified and deemed suitable to use for its intended purpose.

**Reviewer Comment:** Review of validated methods used in the similarity assessment can be found in section 3.2.S.4.2. The characterization methods used in the similarity assessment, such as ADCC, CDC, Fcγ receptor binding assays, were qualified to demonstrate suitability for its intended use in the analytical similarity assessment. The qualification parameters for specific assays varied, but all assays were qualified for accuracy and precision. For cell-based assay as well as higher order structure methods, the methods were also evaluated for specificity. All assay qualifications were deemed acceptable.

### Overall Approach to Assessment

#### 1. Testing Design

The sponsor utilized three testing procedures for the similarity assessment: 1) side-by-side, 2) independent, or 3) age-dependent. Side-by-side testing was employed for methods that required a comparison to an internal standard (e.g. Fourier-transform infrared [FTIR] spectroscopy) or to reduce the effect of method variability in the interpretation of the similarity results. Independent testing was employed for methods with adequate intermediate precision. Age-dependent testing was applied to attributes that were expected to change over time and consisted of independent testing of the sample over time, i.e. gathered data from multiple time points to generate a regression model for age-adjusted similarity assessment. The sponsor “presumed” 24 month shelf life for the RP and derived the RP age at the time of testing using the following equation:

$$\text{Material age (months)} = \text{testing date} - [\text{expiry date of individual lot} - 24 \text{ months}]$$

**Reviewer Comment:** The sponsor should not “presume” a 24 month shelf-life for the RP because the data interpretation could be compromised if the actual shelf-life is longer or shorter than the 24 months, i.e. if the data shows similar degradation profile with RP that is truly older or younger, would mean the proposed biosimilar is not similar because of a faster or better degradation profile, respectively. The age-adjusted similarity data was also provided in the submission and evaluated without the age-adjustment.

#### 2. Analytical Similarity Acceptance Criteria

The sponsor established analytical similarity acceptance criteria based on the criticality of the product quality attributes (tier groups), method capability, and lot-to-lot variability of the RP. For product quality attributes placed in Tier 1, an equivalence test was used where the proposed biosimilar is considered equivalent to the RP if the calculated 90% two-sided confidence interval of the mean of the proposed biosimilar product is within  $\pm \delta$ . The  $\delta$  is based on the standard deviation of all the RP lots tested and multiplied by 1.5.

Tier 2 group similarity acceptance criteria are categorized into analysis by quality range (QR), age-adjusted quality range, and expectation criteria. The similarity acceptance criterion for a Tier 2 QR based analysis is that greater than 90% of ABP 215 lots must fall within the QR. Tier 2 QR is calculated from the following equation:

$$\text{QR} = \text{RP}_{\text{mean}} \pm \text{K} \text{S}_{\text{RP}}$$

$\text{S}_{\text{RP}}$  = standard deviation of RP lots  
 $\text{K}$  = standard deviation multiplier

The sponsor used 3 as the multiplier in the QR equation for all attributes evaluated with QR. The justification provided states that because the true RP lot variability is likely to be underrepresented

because of the limited time for RP lot acquisition (six years) and the linkage between the DP lots and DS lots are unknown for the RP lots.

*Reviewer Comment: The justification provided appears to be reasonable for setting 3 as the multiplier. However, my determination of similarity will not be solely based on meeting the QRs and will also consider the closeness of the means between ABP 215 and RP, the age of the lots used and the quality attribute, and the impact to product safety and efficacy.*

Age-adjusted QR was determined for values derived from stability-indicating methods, such as CEX-HPLC method, for acidic, main, and basic peaks. The sponsor generated values for quality attributes at the “presumed” 24 month expiry by the following steps:

- Each RP lot and the EU-Avastin lot was tested at multiple time points from the time of acquisition. Similarly ABP 215 lots were tested at multiple time points.
- The values derived from testing at multiple time points were plotted against the calculated age of the product based on the given expiry date, the date of testing, and the “presumed” 24 months expiry time, e.g. if a lot of RP has an expiry of 12/2017 and testing occurred on 6/2016, 9/2016, and 12/2016, the calculated age at the time of testing would be 6, 9, and 12 months. For lots tested at the time of expiry the data were not age-adjusted. For ABP 215, the age was calculated based on the date of manufacture.
- Calculate the slope from all lots plotted
- Use the following equation: Age-adjusted Value = Intercept (lot-specific) + slope (product specific) x 24M(common time point)

The age-adjusted values of ABP 215, RP, and EU-Avastin were used to calculate the mean and the standard deviation. The QRs were derived from the age-adjusted value standard deviation.

Tier 2 expectation approach was applied to attributes that were not expected to vary, such as molecular weight. Therefore, expectation approach is not a statistical approach by compares the measured value against the theoretical value and the difference must fall within method capability.

Tier 3 acceptance criteria are qualitative comparisons of chromatograms, gels, etc, that were obtained side-by-side. Thermal stability chromatograms as well as effector function assays such as antibody-dependent cell-mediated cytotoxicity (ADCC) assay and complement-dependent cytotoxicity (CDC) assay were evaluated qualitatively.

### 3. Tier Assignments of Analytical Similarity Assays/Attributes:

The product quality attributes evaluated in the analytical similarity study were grouped into three tiers according to their potential to impact activity, pharmacokinetics/pharmacodynamics, safety, and immunogenicity as well as other factors such as the level of product attribute in the RP and ABP 215, the observed variability of the RP, and method capability. Sponsor stated that the product quality attributes with the highest likelihood/risk to impact activity, pharmacokinetics/pharmacodynamics, safety, and immunogenicity, and with the method capability that allows for tier 1 statistical assessment were labeled Tier 1 attributes and for product quality attributes with moderate and low risks were labeled Tier 2 and Tier 3 attributes, respectively. Tier 3 also includes data that are qualitative, such as chromatographic profile comparisons. The table below shows sponsor’s tiered quality attributes:

**Table 8. ABP 215 Analytical Similarity Assessment Program – Proposed Testing Plan and Tiers for Assays/Attributes**

| Attribute                                 | Analytical Test / Parameter                                        | Tier | Testing Design | Similarity Assessment Approach |
|-------------------------------------------|--------------------------------------------------------------------|------|----------------|--------------------------------|
| <b>Biological Activity – Fab-mediated</b> |                                                                    |      |                |                                |
| Potency                                   | Inhibition of proliferation in HUVEC (potency)                     | 1    | Independent    | Equivalence test               |
| Binding to VEGF                           | Binding to VEGF-A <sub>121</sub> by ELISA                          | 1    | Independent    | Equivalence test               |
| Binding to VEGF                           | On and off rates (VEGF-A <sub>121</sub> )                          | 3    | Side-by-side   | Qualitative comparison         |
| Binding to VEGF                           | Binding to VEGF-A isoforms                                         | 3    | Side-by-side   | Qualitative comparison         |
| Binding to VEGF                           | Inhibition of VEGFR-2 RTK autophosphorylation                      | 3    | Side-by-side   | Qualitative comparison         |
| Binding to VEGF                           | Specificity for VEGF-A by VEGFR-2 RTK autophosphorylation          | 3    | Side-by-side   | Qualitative comparison         |
| <b>Biological Activity – Fc-mediated</b>  |                                                                    |      |                |                                |
| Binding to FcRn                           | FcRn binding                                                       | 2    | Independent    | Quality range                  |
| Binding to FcγRIa                         | FcγRIa binding                                                     | 3    | Side-by-side   | Qualitative comparison         |
| Binding to FcγRIIa                        | FcγRIIa (131H) binding                                             | 3    | Side-by-side   | Qualitative comparison         |
| Binding to FcγRIIb                        | FcγRIIb binding                                                    | 3    | Side-by-side   | Qualitative comparison         |
| Binding to FcγRIIIa                       | FcγRIIIa (158V) binding                                            | 2    | Independent    | Quality range                  |
| Binding to FcγRIIIa                       | FcγRIIIa (158F) binding                                            | 2    | Independent    | Quality range                  |
| Binding to FcγRIIIb                       | FcγRIIIb binding                                                   | 3    | Side-by-side   | Qualitative comparison         |
| Binding to C1q                            | C1q binding                                                        | 2    | Independent    | Quality range                  |
| ADCC activity                             | Lack of ADCC                                                       | 3    | Side-by-side   | Qualitative comparison         |
| CDC activity                              | Lack of CDC                                                        | 3    | Side-by-side   | Qualitative comparison         |
| Attribute                                 | Analytical Test / Parameter                                        | Tier | Testing Design | Similarity Assessment Approach |
| <b>Primary Structure</b>                  |                                                                    |      |                |                                |
| Molecular weight                          | Intact molecular mass                                              | 2    | Side-by-side   | Expectation approach           |
|                                           | Intact molecular mass; profiles                                    | 3    | Side-by-side   | Qualitative comparison         |
|                                           | Reduced and deglycosylated molecular masses of HC and LC           | 2    | Side-by-side   | Expectation approach           |
| Amino acid sequence                       | Reduced and deglycosylated molecular masses of HC and LC; profiles | 3    | Side-by-side   | Qualitative comparison         |
|                                           | Reduced peptide map                                                | 2    | Side-by-side   | Expectation approach           |
|                                           | Reduced peptide map; profiles                                      | 3    | Side-by-side   | Qualitative comparison         |
| Post translational modifications          | Reduced peptide map; Post-translational modifications              | 3    | Side-by-side   | Qualitative comparison         |
| Disulfide structure                       | Non-reduced peptide map                                            | 2    | Side-by-side   | Expectation approach           |
|                                           | Non-reduced peptide map; profiles                                  | 3    | Side-by-side   | Qualitative comparison         |
| Glycosylation                             | Glycan Map; % high mannose                                         | 2    | Independent    | Quality range                  |
|                                           | Glycan Map; % afucosylation                                        | 2    | Independent    | Quality range                  |
|                                           | Glycan Map; % galactosylation                                      | 2    | Independent    | Quality range                  |
|                                           | Glycan Map; % sialylation                                          | 3    | Independent    | Qualitative comparison         |
|                                           | Glycan Map; profiles                                               | 3    | Side-by-Side   | Qualitative comparison         |

| Attribute                                        | Analytical Test / Parameter                       | Tier | Testing Design | Similarity Assessment Approach |
|--------------------------------------------------|---------------------------------------------------|------|----------------|--------------------------------|
| <b>Primary Structure</b>                         |                                                   |      |                |                                |
| Isoelectric point                                | Capillary isoelectric focusing: Isoelectric point | 2    | Side-by-side   | Expectation approach           |
|                                                  | Capillary isoelectric focusing: Profile           | 3    | Side-by-side   | Qualitative comparison         |
| Extinction coefficient                           | Amino acid analysis/UV spectroscopy               | 2    | Side-by-side   | Expectation approach           |
| Identity                                         | Identity by ELISA                                 | 3    | Independent    | Qualitative comparison         |
| <b>Higher Order Structure</b>                    |                                                   |      |                |                                |
| Secondary structure                              | FTIR: Spectral similarity                         | 2    | Side-by-side   | Expectation approach           |
|                                                  | FTIR: Profile                                     | 3    | Side-by-side   | Qualitative comparison         |
| Tertiary structure                               | Near UV CD: Spectral similarity                   | 2    | Side-by-side   | Expectation approach           |
|                                                  | Near UV CD: Profile                               | 3    | Side-by-side   | Qualitative comparison         |
|                                                  | DSC: T <sub>m1</sub>                              | 2    | Side-by-side   | Quality range                  |
|                                                  | DSC: T <sub>m2</sub>                              | 2    | Side-by-side   | Quality range                  |
|                                                  | DSC: Profile                                      | 3    | Side-by-side   | Qualitative comparison         |
| <b>Particles and Aggregates</b>                  |                                                   |      |                |                                |
| Subvisible particles                             | HIAC: ≥ 2 μm particles                            | 3    | Independent    | Qualitative comparison         |
|                                                  | HIAC: ≥ 5 μm particles                            | 3    | Independent    | Qualitative comparison         |
|                                                  | HIAC: ≥ 10 μm particles                           | 3    | Independent    | Qualitative comparison         |
|                                                  | HIAC: ≥ 25 μm particles                           | 3    | Independent    | Qualitative comparison         |
|                                                  | MFI: ≥ 5 μm particles                             | 3    | Independent    | Qualitative comparison         |
|                                                  | MFI: ≥ 5 μm non-spherical particles               | 2    | Independent    | Quality range                  |
| <b>Particles and Aggregates</b>                  |                                                   |      |                |                                |
| <b>Product-related Substances and Impurities</b> |                                                   |      |                |                                |
| Submicron particles                              | FFF: Submicron particles                          | 3    | Side-by-side   | Qualitative comparison         |
|                                                  | DLS (Hydrodynamic Radius)                         | 3    | Side-by-side   | Qualitative comparison         |
| Solution-state size distribution                 | AUC-SV: % Monomer                                 | 2    | Side-by-side   | Quality range                  |
|                                                  | AUC-SV: Profile                                   | 3    | Side-by-side   | Qualitative comparison         |
| Molar mass of size variants                      | SLS: Molar mass                                   | 2    | Side-by-side   | Expectation approach           |
| <b>Product-related Substances and Impurities</b> |                                                   |      |                |                                |
| Size variants                                    | SE-HPLC: HMW                                      | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | SE-HPLC: Profile                                  | 3    | Side-by-side   | Qualitative comparison         |
|                                                  | rCE-SDS: HC+LC                                    | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | rCE-SDS: NGHC                                     | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | rCE-SDS: LMW + MMW                                | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | rCE-SDS: Profile                                  | 3    | Side-by-side   | Qualitative comparison         |
|                                                  | nrCE-SDS: Main peak                               | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | nrCE-SDS: Pre-peaks                               | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | nrCE-SDS: Profile                                 | 3    | Side-by-side   | Qualitative comparison         |
| Charge variants                                  | CEX-HPLC: Acidic peaks                            | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | CEX-HPLC: Main peak                               | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | CEX-HPLC: Basic peaks                             | 2    | Age-dependent  | Age-adjusted quality range     |
|                                                  | CEX-HPLC: Profile                                 | 3    | Side-by-side   | Qualitative comparison         |

| Attribute                                                      | Analytical Test / Parameter                   | Tier | Testing Design | Similarity Assessment Approach |
|----------------------------------------------------------------|-----------------------------------------------|------|----------------|--------------------------------|
| <b>Forced Degradation, Accelerated, and Stressed Stability</b> |                                               |      |                |                                |
| 50°C Forced Degradation                                        | SE-HPLC: HMW degradation                      | 3    | Side-by-side   | Qualitative comparison         |
|                                                                | rCE-SDS: HC + LC degradation                  | 3    | Side-by-side   | Qualitative comparison         |
|                                                                | rCE-SDS: LMW + MMW degradation                | 3    | Side-by-side   | Qualitative comparison         |
|                                                                | CEX-HPLC: Main peak degradation               | 3    | Side-by-side   | Qualitative comparison         |
|                                                                | CEX-HPLC: Acidic peaks degradation            | 3    | Side-by-side   | Qualitative comparison         |
|                                                                | CEX-HPLC: Basic peaks degradation             | 3    | Side-by-side   | Qualitative comparison         |
|                                                                | Proliferation inhibition bioassay degradation | 3    | Side-by-side   | Qualitative comparison         |
| 40°C Stressed Stability                                        | SE-HPLC: HMW degradation                      | 3    | Independent    | Qualitative comparison         |
|                                                                | rCE-SDS: HC + LC degradation                  | 3    | Independent    | Qualitative comparison         |
|                                                                | rCE-SDS: LMW + MMW degradation                | 3    | Independent    | Qualitative comparison         |
|                                                                | CEX-HPLC: Main peak degradation               | 3    | Independent    | Qualitative comparison         |
|                                                                | CEX-HPLC: Acidic peaks degradation            | 3    | Independent    | Qualitative comparison         |
|                                                                | CEX-HPLC: Basic peaks degradation             | 3    | Independent    | Qualitative comparison         |
|                                                                | Proliferation inhibition bioassay degradation | 3    | Independent    | Qualitative comparison         |
| <b>General Properties</b>                                      |                                               |      |                |                                |
| Protein Concentration                                          | UV absorbance                                 | 2    | Independent    | Quality range                  |
| Volume                                                         | Gravimetric determination                     | 3    | Independent    | Qualitative comparison         |
| Osmolality                                                     | Freezing point depression                     | 3    | Independent    | Qualitative comparison         |
| pH                                                             | Potentiometric pH measurements                | 3    | Independent    | Qualitative comparison         |
| Appearance                                                     | Visual assessment                             | 3    | Independent    | Qualitative comparison         |
| Color                                                          | Visual assessment                             | 3    | Independent    | Qualitative comparison         |
| Clarity                                                        | Nephelometry                                  | 3    | Independent    | Qualitative comparison         |
| <b>Process-related Impurities</b>                              |                                               |      |                |                                |
| Residual host cell protein                                     | ELISA                                         | 3    | Independent    | Qualitative comparison         |
|                                                                | LC-MS                                         | 3    | Side-by-side   | Qualitative comparison         |
|                                                                | 2D-DIGE                                       | 3    | Side-by-side   | Qualitative comparison         |
| Residual Protein A                                             | ELISA                                         | 3    | Independent    | Qualitative comparison         |
| Residual DNA                                                   | qPCR                                          | 3    | Independent    | Qualitative comparison         |

**Reviewer Comments:**

*In general, the sponsor’s risk ranking of the quality attributes and placement into tiers 1, 2, and 3 are appropriate for the similarity study when both the potential for clinical impact and method capabilities are considered. However, a few quality attributes and their placement warrant further justification and/or data from the sponsor. Information requests were sent regarding the following issues:*

- *Binding specificity to VEGF C and D were evaluated in the receptor tyrosine kinase autophosphorylation assay. However, binding to VEGF family members, VEGFB and placental growth factor (PlGF) were not evaluated. As VEGFB and PlGF are known to have distinct as well as common functions as VEGFA, e.g. VEGFB is involved in the normal development of the heart as well*

as it plays a role in neuron survival by binding to VEGF receptor 1 (VEGFR1), that differs from angiogenesis or vasculogenesis by VEGFA (mediated mainly by VEGFR2 homodimer or VEGFR1 and VEGFR2 heterodimer), the sponsor was asked to provide binding specificity data for the other VEGFA family members as part of the similarity assessment. IR was sent on 1/31/2017 and the sponsor provided a response that stated that VEGFB and PLGF were not evaluated because those growth factors specifically signal through VEGFR1 and VEGFA signals through VEGFR2 as the predominant receptor to induce angiogenesis. In addition, sponsor stated that bevacizumab was shown to be unable to bind to VEGFB and PLGF. A literature reference was not provided but found independently from pubmed search that a study conducted by Papadopoulos, N. et. al. (2012, *Angiogenesis* 15: 171-185) showed that bevacizumab was unable to bind to VEGFB and PLGF. In conclusion, the justifications provided by the sponsor for the lack of VEGFB and PLGF specificity data is acceptable based on the VEGFC and VEGFD specificity data, the amino acid sequence data, and the higher order structure data that were all similar to US-Avastin. Therefore, it is unlikely that ABP 215 will bind to VEGFB and PLGF.

- Binding to VEGFA isoform 165 is tier 3, but our scientific assessment for biosimilar products to US-Avastin is for both VEGFA isoform 121 and 165 binding assessment to be placed in tier 1 because of the different functionalities of 121 (soluble) and 165 (heparin bound) isoforms in vivo. However, with justification, the binding assessment could be tier 2. The sponsor did not provide data or a justification for why VEGFA isoform 165 was ranked and evaluated as tier 3. An IR was sent to ask for data to support the placement of VEGFA 165 in tier 3 rather than in tier 1 (IR sent 1/31/17). See review of the IR 2/22/17 response in the Biological Activity section below.
- Regarding the potency bioassay, Amgen originally stated that they performed the HUVEC proliferation assay using VEGFA 121. However, in the response to IR sent 1/31/17, the sponsor indicated that they performed the HUVEC assay with VEGFA 111 isoform. In addition, the VEGFA isoforms used in the other biological activity assays were also revised to indicate the assays were conducted with VEGF111. For full details see Biological Activity section below. Several OBP internal meetings were held to determine the adequacy of the VEGFA 111 for use in the bioassay. The HUVEC proliferation assay using VEGFA 165 isoform had been identified by FDA as a highly critical assay based on literature reports that established the 165 isoform as the predominant and physiologically relevant isoform and because this isoform, unlike the smaller isoforms, may be cell associated through its heparin binding domain. The justification implied in the clinical summary section for extrapolation to other clinical indications by the sponsor states that the binding site for bevacizumab is the same among VEGFA isoforms (111, 121 and 165). The conclusions from the internal meetings were that sponsors can use VEGFA isoforms other than 165 to demonstrate analytical similarity, provided that the alternative isoform is demonstrated to have similar binding and kinetics using ABP215, US-Avastin and EU-Avastin, and is shown to have a similar sensitivity as the VEGFA 165 isoform in the bioassay for the purposes of detection of differences/change in the molecule. As this could be assay specific, depending on assay design, the relevance of isoforms other than VEGFA 165 would have to be justified by each applicant for their specific assay.

#### **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.

| BLA 761028 ABP 215 Similarity Exercise Summary Table |                |                                                    |      |                                                 |                                           |                                               |                                            |                                                                                                                                                                                                                                                                                                                           |
|------------------------------------------------------|----------------|----------------------------------------------------|------|-------------------------------------------------|-------------------------------------------|-----------------------------------------------|--------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Category                                             | Test           |                                                    | Tier | Number of Lots (US-Avastin: ABP215: EU-Avastin) | US-Avastin Range                          | ABP215 Range                                  | EU-Avastin Range                           | ABP215:US-Avastin/ ABP215:EU-Avastin/ EU-Avastin:US-Avastin                                                                                                                                                                                                                                                               |
| Primary Structure                                    | Molecular Mass | Intact Mass: Profile <sup>1</sup>                  | 3    | 4:4:4                                           | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass<br><sup>1</sup> Note that they also did quantitative difference from the theoretical mass as a tier 2 attribute                                                                                                                                                                                            |
|                                                      |                | Reduced/ Deglycosylated Mass: Profile <sup>1</sup> | 3    | 4:4:4                                           | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass                                                                                                                                                                                                                                                                                                            |
|                                                      |                | Reduced Peptide Map Profile <sup>1</sup>           | 3    | 9:9:9                                           | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass<br>No new peptides observed compared to US-licensed Avastin; however, sequence coverage is 98.9% for HC and 97.2% for LC due to small undetected peptides in all three products; 100% coverage was confirmed using ABP215 characterization data and bevacizumab sequence from drug bank database (DB00112) |
|                                                      |                | Non-Reduced Peptide Map Profile <sup>1</sup>       | 3    | 2:2:2                                           | Visually Similar to                       | Visually Similar to US-Avastin and            | Visually Similar to                        | Pass/Pass/Pass<br>All 16 disulfide bonds                                                                                                                                                                                                                                                                                  |

|            |                   |     |          |  | ABP215 and EU-Avastin | EU-Avastin    | US-Avastin and ABP 215 | with correct linkages detected.                                                                                                                                                                                                                                                                     |
|------------|-------------------|-----|----------|--|-----------------------|---------------|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Glycan Map | % High Mannose    | 2   | 23:13:25 |  | 0.3-1.3               | 1.2-2.7       | 0.4-1.2                | Fail/Fail/Pass<br>Outside QR for US and EU Avastin. However, based on difference in clinical PK data. The difference observed is acceptable.<br>US PK lot: 0.5<br>EU PK lot: 0.7<br>ABP PK lot: 1.5<br>ABP Clinical lots: 1.7, 2.3, 2.7<br>EU Clinical lots: 1.1, 0.9, 1.0, 0.9, 0.9, 1.0, 0.8, 1.1 |
|            | % Afucosylation   | 2   | 23:13:25 |  | 1.6-3.8               | 1.2-1.7       | 1.9-2.6                | Pass/Fail/Pass<br>Although ABP meets US QR, it is on the lower end of the QR range. Implies weaker ADCC if ABP had ADCC activity; less of a safety risk for unwanted ADCC activity                                                                                                                  |
|            | % Galactosylation | 2   | 23:13:25 |  | 10.0-21.8             | 17.1-29.4     | 7.8-21.2               | Fail/Fail/Pass<br>ABP is outside US and EU Avastin QR. Based on no effector functions, C1q binding and CDC activity, the difference is acceptable                                                                                                                                                   |
|            | % Sialylation     | 2   | 23:13:25 |  | 0.1-0.2               | 0.2-0.3       | 0.1-0.2                | Pass/Pass/Pass                                                                                                                                                                                                                                                                                      |
| pI         | cIEF              | 2/3 | 3:3:3    |  | 7.9/Visually          | 7.9/ Visually | 7.9/                   | Pass/Pass/Pass                                                                                                                                                                                                                                                                                      |

|                                 |                                                        |                                                                            |     |          |                                                                                                                                |                                                                                                                                     |                                                                                                                              |                                                                                                                                                                          |
|---------------------------------|--------------------------------------------------------|----------------------------------------------------------------------------|-----|----------|--------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                 |                                                        |                                                                            |     |          | Similar to ABP215 and EU-Avastin                                                                                               | Similar to US-Avastin and EU-Avastin                                                                                                | Visually Similar to US-Avastin and ABP 215                                                                                   | <i>Profile showed ABP had higher levels of basic species due to more unclipped lysine; see below for process related variants</i>                                        |
|                                 | Extinction Coefficient                                 | Amino Acid Analysis                                                        | 2   | 1:1:1    | 1.8                                                                                                                            | 1.7                                                                                                                                 | 1.8                                                                                                                          | Pass/Pass/Pass                                                                                                                                                           |
|                                 | Identity                                               | ELISA                                                                      | 3   | 3:13:3   | All Positive                                                                                                                   | All Positive                                                                                                                        | All Positive                                                                                                                 | Pass/Pass/Pass                                                                                                                                                           |
| <b>Higher Order Structure</b>   | Secondary Structure: $\alpha$ helix and $\beta$ sheets | FTIR<br>Tier 2: Spectral similarity calc against US-licensed Avastin       | 2/3 | 6:6:6    | 98.5/<br>Visually Similar to ABP215 and EU-Avastin                                                                             | 98.7/ Visually Similar to US-Avastin and EU-Avastin                                                                                 | 98.3/<br>Visually Similar to US-Avastin and ABP 215                                                                          | Pass/Pass/Pass                                                                                                                                                           |
|                                 | Tertiary Structure                                     | Near UV CD<br>Tier 2: Spectral similarity calc against US-licensed Avastin | 2/3 | 6:6:6    | 99.0/<br>Visually Similar to ABP215 and EU-Avastin                                                                             | 99.0/ Visually Similar to US-Avastin and EU-Avastin                                                                                 | 98.4/<br>Visually Similar to US-Avastin and ABP 215                                                                          | Pass/Pass/Pass                                                                                                                                                           |
|                                 | Melting Temperature                                    | Differential Scanning Calorimetry                                          | 2/3 | 12:11:12 | T <sub>m1</sub> : 72.8-73.1<br>T <sub>m2</sub> : 83.2-83.9<br>Visually Similar to ABP215 and EU-Avastin                        | T <sub>m1</sub> : 72.7-73.1<br>T <sub>m2</sub> : 83.2-83.7<br>Visually Similar to US-Avastin and EU-Avastin                         | T <sub>m1</sub> : 72.5-73.1<br>T <sub>m2</sub> : 83.0-84.0<br>Visually Similar to US-Avastin and ABP 215                     | Pass/Pass/Pass                                                                                                                                                           |
| <b>Particles and Aggregates</b> | Subvisible Particles                                   | HIAC                                                                       | 3   | 14:19:15 | $\geq 2 \mu\text{m}$ : 75-4749<br>$\geq 5 \mu\text{m}$ : 14-755<br>$\geq 10 \mu\text{m}$ : 3-112<br>$\geq 25 \mu\text{m}$ : 0- | $\geq 2 \mu\text{m}$ : 320-5736<br>$\geq 5 \mu\text{m}$ : 48-1488<br>$\geq 10 \mu\text{m}$ : 16-219<br>$\geq 25 \mu\text{m}$ : 0-96 | $\geq 2 \mu\text{m}$ : 77-15493<br>$\geq 5 \mu\text{m}$ : 24-688<br>$\geq 10 \mu\text{m}$ : 8-224<br>$\geq 25 \mu\text{m}$ : | Pass/Pass/Pass<br><i>In general, ABP 215 was similar to slightly higher in numbers compared to US and EU. Only 5 <math>\mu\text{m}</math> size was noticeably higher</i> |

|                                           |                     |                                                                                                                             |                                            |          |                                           |                                               |                                              |                                                                                                                                                                                         |
|-------------------------------------------|---------------------|-----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|----------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                           |                     |                                                                                                                             |                                            |          | 32                                        |                                               | 0-32                                         | <i>for ABP 215 and this size was further characterized by MFI.</i>                                                                                                                      |
|                                           |                     | MFI                                                                                                                         | 3 spherical                                | 11:19:12 | ≥ 5 µm particles: 42-3439                 | ≥ 5 µm particles: 16-218                      | ≥ 5 µm particles: 51-588                     | Spherical:<br>Pass/Pass/Pass                                                                                                                                                            |
|                                           |                     |                                                                                                                             | 2 non-spherical                            |          | ≥ 5 µm non-spherical: 6-2157              | ≥ 5 µm non-spherical: 0-65                    | ≥ 5 µm non-spherical: 8-430                  | Non-Spherical:<br>Pass/Pass/Pass                                                                                                                                                        |
|                                           | Submicron Particles | Field Flow Fractionation                                                                                                    | 3                                          | 6:6:6    | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215   | Pass/Pass/Pass                                                                                                                                                                          |
|                                           |                     | Dynamic Light Scattering                                                                                                    | 3                                          | 6:6:6    | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215   | Pass/Pass/Pass                                                                                                                                                                          |
|                                           | Size Distribution   | Sedimentation Velocity Analytical Ultracentrifugation                                                                       | 2/percent Monomer                          | 6:6:6    | Monomer: 97.3-98.6                        | Monomer: 98.0-99.1                            | Monomer: 97.3-98.6                           | Pass/Pass/Pass                                                                                                                                                                          |
|                                           |                     |                                                                                                                             | 3/profile                                  |          | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215   | Pass/Pass/Pass<br><i>Visually, no new HMW species in ABP 215 compared to US and EU. US lots had more HMW species likely associated with age of the product.</i>                         |
|                                           |                     | Static Light Scattering                                                                                                     | 2/molar mass of main and pre-main pks peak | 3:3:3    | Main Pk: 148<br>Pre-Main Pk: 300-301      | Main Pk: 148<br>Pre-Main Pk: 297-304          | Main Pk: 148<br>Pre-Main Pk: 294-298         | Pass/Pass/Pass                                                                                                                                                                          |
|                                           |                     |                                                                                                                             | 3/profile                                  |          | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215   | Pass/Pass/Pass                                                                                                                                                                          |
| Product Related Substances and Impurities | SE-HPLC             | %HMW<br>Raw data ranges from initial purchase time test point or t=0 time point for ABP lots are shown (no age-adjustments) | 2                                          | 24:13:26 | 2.4-3.3                                   | 1.3-2.3                                       | 2.3-3.4                                      | Fail/Fail/Fail<br><i>ABP215 has less aggregates and this difference is acceptable.</i>                                                                                                  |
|                                           |                     | Profile                                                                                                                     | 3                                          |          | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215   | Pass/Pass/Pass<br><i>ABP 215 Dev lots had a slight shoulder peak off the HMW peak that was missing in US and EU, but gone with clinical lots. Also US and EU HMW peaks were higher.</i> |
|                                           | rCE-SDS             | %HC+LC                                                                                                                      | 2                                          | 24:13:26 | 95.4-96.6                                 | 97.9-98.4                                     | 95.2-96.3                                    | Fail/Fail/Pass                                                                                                                                                                          |
|                                           |                     | %NGHC                                                                                                                       |                                            |          | 1.7-2.1                                   | 0.6-0.9                                       | 1.6-2.1                                      | Fail/Fail/Pass                                                                                                                                                                          |
| %LMW + % MMW                              |                     | 1.2-2.3                                                                                                                     |                                            |          | 0.5-0.9                                   | 1.4-2.0                                       | Fail/Fail/Pass<br><i>Mainly due to lower</i> |                                                                                                                                                                                         |

|  |          |                                                                                                                     |   |          |                                           |                                               |                                            |                                                                                      |
|--|----------|---------------------------------------------------------------------------------------------------------------------|---|----------|-------------------------------------------|-----------------------------------------------|--------------------------------------------|--------------------------------------------------------------------------------------|
|  |          | Raw data ranges from initial purchase time test point or t=0 time point for ABP lots are shown (no age-adjustments) |   |          |                                           |                                               |                                            | levels of NGHC and purer product. Failed even after age-adjustment                   |
|  |          | Profile                                                                                                             | 3 |          | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass                                                                       |
|  | nrCE-SDS | %Main Pk                                                                                                            | 2 | 24:13:28 | 96.9-97.9                                 | 96.7-98.4                                     | 96.3-98.2                                  | Fail/Fail/Fail<br>The differences are small and unlikely to have a clinical impact.  |
|  |          | % Pre-Pk                                                                                                            |   |          | 1.8-2.6                                   | 1.5-2.9                                       | 1.8-3.0                                    | Fail/Pass/Fail<br>Raw data shows ABP is outside of US and EU, but EU is close to US. |
|  |          | Raw data ranges from initial purchase time test point or t=0 time point for ABP lots are shown (no age-adjustments) |   |          |                                           |                                               |                                            |                                                                                      |
|  |          | Profile                                                                                                             | 3 |          | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP215  | Pass/Pass/Pass                                                                       |
|  | CEX-HPLC | % Acidic Pk                                                                                                         | 2 | 24:13:26 | 24.2-29.5                                 | 17.5-20.1                                     | 25.7-30.7                                  | Fail/Fail/Pass<br>ABP215 has lower levels of acidic peaks.                           |
|  |          | % Main Pk                                                                                                           |   |          | 64.6-70.1                                 | 69.1-75.2                                     | 62.8-68.9                                  | Fail/Fail/Pass<br>ABP215 has higher main peak due to differences in acidic peaks.    |

|                     |                |                                                                                                                                       |   |                           |                                           |                                                        |                                            |                                                                                                                                                                                                                                                                                                    |
|---------------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------|---|---------------------------|-------------------------------------------|--------------------------------------------------------|--------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                     |                | % Basic Pk<br><br>Raw data ranges from initial purchase time test point or t=0 time point for ABP lots are shown (no age-adjustments) |   |                           | 5.3-7.2                                   | 6.6-11.5                                               | 5.0-7.4                                    | Fail/Fail/Fail<br><i>ABP has higher lysine, pro-amidation, galactosylation, and high mannose glycans that contribute to basic peak levels.</i><br><br><i>The differences in acidic, main, and basic were shown not to impact potency, effector functions, and PK and is considered acceptable.</i> |
|                     |                | Profile                                                                                                                               | 3 |                           | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin          | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass                                                                                                                                                                                                                                                                                     |
| Thermal Degradation | 50°C (2 weeks) | SE-HPLC<br>rCE-SDS<br>CEX-HPLC<br>Potency                                                                                             | 3 | 6:6:6 (3:3:3) for potency | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-approved Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass<br><i>ABP degradation plot shows parallel lines indicating similar degradation rate to US.</i>                                                                                                                                                                                      |
|                     | 40°C (2 weeks) | SE-HPLC<br>rCE-SDS<br>CEX-HPLC<br>Potency                                                                                             | 3 | 1:2:1                     | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin          | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass<br><i>Similar rate observation for SE-HPLC plot as stated above.</i><br><i>No new species/peaks. Potency doesn't change much for US and EU but decrease is apparent in ABP. Could be assay variability since the change is ~10% for</i>                                             |

|                    |                               |                                           |   |                                 |                                           |                                               |                                            |                                                                                                                                                                                                                                                                                                                                            |
|--------------------|-------------------------------|-------------------------------------------|---|---------------------------------|-------------------------------------------|-----------------------------------------------|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                    |                               |                                           |   |                                 |                                           |                                               |                                            | <i>ABP and less for US and EU. Also sponsor states that bc not done side-by-side small differences could be due to method variability.</i>                                                                                                                                                                                                 |
|                    | 25°C<br>(3 months)            | SE-HPLC<br>rCE-SDS<br>CEX-HPLC<br>Potency | 3 | 1:2:1                           | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass<br><i>Similar rate observation for SE-HPLC plot as stated above.<br/>No new species/peaks. Potency doesn't change much for ABP and EU but increase for US. Could be assay variability since the change is ~15%. Also sponsor states that bc not done side-by-side small differences could be due to method variability.</i> |
| General Properties | Protein Concentration (mg/mL) |                                           | 2 | 24.13:26                        | 24.0-25.9                                 | 24.4-25.8                                     | 24.5-25.8                                  | Pass/Pass/Pass                                                                                                                                                                                                                                                                                                                             |
|                    | Deliverable Volume (mL)       |                                           | 3 | 6:7:5 (100mg)<br>6:12:8 (400mg) | 4.2-4.3<br>16.2-16.6                      | 4.1-4.3<br>16.3-16.5                          | 4.4-4.7<br>16.3-16.8                       | Pass/Pass/Pass                                                                                                                                                                                                                                                                                                                             |
|                    | Osmolality (mOsm/kg)          |                                           | 3 | 3:13:3                          | 273-275                                   | 275-282                                       | 272-277                                    | Pass/Pass/Pass<br><i>ABP trends higher, but sponsor states bc it is diluted prior to administration, no effect on safety or efficacy</i>                                                                                                                                                                                                   |
|                    | pH                            |                                           | 3 | 3:13:3                          | 6.2                                       | 6.0-6.2                                       | 6.2                                        | Pass/Pass/Pass<br><i>A difference of 0.2 is noted for ABP but could be due to limited lots.</i>                                                                                                                                                                                                                                            |

|                                            |                                                                              |   |          |                                                                                        |                                                                                    |                                                                                        |                                                                                                                                                                                                                  |
|--------------------------------------------|------------------------------------------------------------------------------|---|----------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                            | Appearance                                                                   | 3 | 3:13:3   | No particles<br>Practically<br>free from<br>particles                                  | No particles<br>Practically free<br>from particles                                 | No<br>particles<br>Practically<br>free from<br>particles                               | Pass/Pass/Pass                                                                                                                                                                                                   |
|                                            | Color                                                                        | 3 | 3:13:3   | Colorless to<br>slightly<br>yellow                                                     | Colorless to<br>slightly<br>yellow                                                 | Colorless                                                                              | Pass/Pass/Pass                                                                                                                                                                                                   |
|                                            | Clarity                                                                      | 3 | 3:13:3   | < Ref III                                                                              | < Ref III                                                                          | < Ref III                                                                              | Pass/Pass/Pass                                                                                                                                                                                                   |
| <b>Process-<br/>Related<br/>Impurities</b> | Host Cell Protein-ELISA (ng/mg)                                              | 3 | 3:13:3   | 6                                                                                      | 4-12                                                                               | 5-6                                                                                    | Pass/Pass/Pass                                                                                                                                                                                                   |
|                                            | Host Cell Protein-2D LC/MS (LOQ<br>13 ppm or ng/mg)                          | 3 | 3:13:3   | NA                                                                                     | NA                                                                                 | NA                                                                                     | Pass/Pass/Pass                                                                                                                                                                                                   |
|                                            | Host Cell Protein-2D in gel<br>electrophoresis                               | 3 | 1:1:0    | Visually<br>Similar to<br>ABP215                                                       | Visually Similar to<br>US-Avastin                                                  |                                                                                        | Pass/Pass/<br>Pass                                                                                                                                                                                               |
|                                            | Protein A-ELISA (ng/mg)                                                      | 3 | 3:13:3   | <1                                                                                     | <1                                                                                 | <1                                                                                     | Pass/Pass/Pass                                                                                                                                                                                                   |
|                                            | Residual DNA (pg/mg)                                                         | 3 | 3:13:3   | <1                                                                                     | <1                                                                                 | <1- <2                                                                                 | Pass/Pass/Pass<br><i>For EU the &lt;2 might<br/>be due to assay LOQ<br/>at the time of analysis.<br/>The current LOQ is<br/>stated as &lt;1.<br/>Regardless the levels<br/>are all very low and<br/>similar.</i> |
|                                            |                                                                              |   |          |                                                                                        |                                                                                    |                                                                                        |                                                                                                                                                                                                                  |
| <b>Biological<br/>Activity</b>             | Proliferation Inhibition Bioassay<br>(with VEGF 111)<br>(Relative Potency %) | 1 | 24:13:27 | 86-104                                                                                 | 91-105                                                                             | 88-103                                                                                 | Pass/Pass/Pass<br>(equivalency testing)                                                                                                                                                                          |
|                                            | VEGFA 165 ELISA Binding                                                      | 1 | 14:13:13 | 80-104                                                                                 | 84-98                                                                              | 76-99                                                                                  | Pass/Pass/Pass<br>(equivalency testing)                                                                                                                                                                          |
|                                            | VEGFA 121 Kinetics (SPR)                                                     | 3 | 3:3:3    | k <sub>i</sub> : 3.4-3.5<br>k <sub>d</sub> : 7.6-8.1<br>K <sub>D</sub> : 22.9-<br>23.9 | k <sub>i</sub> : 3.4-3.5<br>k <sub>d</sub> : 7.3-8.2<br>K <sub>D</sub> : 20.1-23.5 | k <sub>i</sub> : 3.4-3.5<br>k <sub>d</sub> : 6.5-8.2<br>K <sub>D</sub> : 18.4-<br>23.4 | Pass/Pass/Pass                                                                                                                                                                                                   |
|                                            | VEGFA 121 and 165 Binding by<br>SPR (sensogram comparison)                   | 3 | 1:1:1    | Visually<br>Similar to<br>ABP215 and<br>EU-Avastin                                     | Visually Similar to<br>US-Avastin and<br>EU-Avastin                                | Visually<br>Similar to<br>US-<br>Avastin<br>and ABP<br>215                             | Pass/Pass/Pass                                                                                                                                                                                                   |
|                                            | Inhibition of VEGFR2-RTK                                                     | 3 | 3:3:3    | Visually                                                                               | Visually Similar to                                                                | Visually                                                                               | Pass/Pass/Pass                                                                                                                                                                                                   |

|                                                                                |   |          |                                           |                                               |                                            |                                                                                                                                                                       |
|--------------------------------------------------------------------------------|---|----------|-------------------------------------------|-----------------------------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Autophosphorylation                                                            |   |          | Similar to ABP215 and EU-Avastin          | US-Avastin and EU-Avastin                     | Similar to US-Avastin and ABP 215          |                                                                                                                                                                       |
| Specificity to VEGFA and not too VEGF C and D by VEGFR2-RTK-P inhibition       | 3 | 1:1:1    | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass                                                                                                                                                        |
| FcRn Binding (%)                                                               | 2 | 14:13:14 | 91-107                                    | 84-104                                        | 88-103                                     | Pass/Pass/Pass                                                                                                                                                        |
| FcgRIa Binding (%)                                                             | 3 | 3:3:3    | 83-90                                     | 90-98                                         | 99-102                                     | Pass/Pass/Pass                                                                                                                                                        |
| FcgRIIa (131H) Binding (%)                                                     | 3 | 3:3:3    | 89-91                                     | 83-101                                        | 80-90                                      | Pass/Pass/Pass                                                                                                                                                        |
| FcgRIIb Binding (%)                                                            | 3 | 3:3:3    | 90-94                                     | 92-98                                         | 87-91                                      | Pass/Pass/Pass                                                                                                                                                        |
| FcgRIIIa (158V) Binding (%)                                                    | 2 | 12:13:17 | 76-98                                     | 78-115                                        | 81-117                                     | Fail/Pass/Fail<br><i>BC there is no ADCC activity the small difference is not likely to have a clinical impact</i>                                                    |
| FcgRIIIa (158F) Binding (%)                                                    | 2 | 10:13:10 | 80-107                                    | 84-120                                        | 83-109                                     | Pass/Pass/Pass<br><i>These binding to results could be due to higher high mannose levels in ABP</i>                                                                   |
| FcgRIIIb Binding (%)                                                           | 3 | 3:3:3    | 80-85                                     | 89-92                                         | 67-88                                      | Pass/Pass/Pass<br><i>ABP looks like higher binding but the slight difference is acceptable because target is mostly soluble and no effector function is observed.</i> |
| C1q Binding (%)                                                                | 2 | 10:13:9  | 88-115                                    | 91-122                                        | 90-104                                     | Pass/Pass/Pass                                                                                                                                                        |
| Absence of ADCC Activity in DLD-1 and Calu-6 cells NK-92M1/CD16 Effector Cells | 3 | 1:1:1    | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass                                                                                                                                                        |
| Absence of ADCC Activity in SKOV3 cells PBMC effector cells                    | 3 | 1:1:1    | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass                                                                                                                                                        |
| Absence of CDC Activity in DLD-1 and Calu-6 cells                              | 3 | 1:1:1    | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass                                                                                                                                                        |
| Absence of CDC in SKOV3 cells                                                  | 3 | 1:1:1    | Visually Similar to ABP215 and EU-Avastin | Visually Similar to US-Avastin and EU-Avastin | Visually Similar to US-Avastin and ABP 215 | Pass/Pass/Pass                                                                                                                                                        |

### 3.2.R.4.1 Primary Structure

The sponsor used several direct and indirect/orthogonal methods to determine the primary structure of ABP 215, US-Avastin, and EU-Avastin. The combination of results from intact mass, reduced and deglycosylated mass, reduced peptide map followed by mass spectrometry analysis (MS/MS), and determination of the extinction coefficient along with confirmation of disulfide linkages, glycan map, and identity by ELISA methods to determine the primary structure of the three products. The results of each testing approach and the similarity of the three products are reviewed below.

#### Intact Molecular Mass

Four lots of ABP 215, US-Avastin, and EU-Avastin each were used to determine the intact molecular mass. ABP 215 lots represented DP lots derived from three development lots and one clinical lot. The sponsor justified the use of only four lots each of the three products for the similarity assessment being sufficient because there were no process changes to the ABP 215 DS manufacturing process that could warrant a change in intact molecular mass. The samples were run side-by-side on an electrospray ionization-time of flight-mass spectrometer (ESI-TOF-MS) with a mass accuracy of 50 ppm.

**Reviewer Comments:**

- *The DS manufacturing process changed going from the development site at Amgen Washington state site to Amgen Thousand Oaks site in California. Small facility fit changes occurred and comparability study was conducted prior to the initiation of clinical studies (for details see 3.2.S.2.6). Drug substance was found to be comparable between AWA and ATO manufacturing sites (see IND 111960 original review for details). The site change did result in changes to the glycosylation profile that could affect intact molecular weight. Although the justification is faulty that no process changes occurred and that intact molecular mass is not expected to change, the sponsor also analyzed glycan profiles in the development lots and clinical lots to help address any differences in mass. Therefore, the number of lots used is acceptable considering the method capability and the inclusion of both the ABP 215 development and clinical lots.*
- *Mass accuracy of 50 ppm for intact monoclonal antibody with approximate weight of 150 kDa translates to  $\pm 7.5$  Da. Therefore, the results are accurate representation of the intact mass of the three products measured.*

The observed intact molecular mass of the three products was compared against the theoretical mass. The theoretical mass was determined from the expected amino acid sequence of the RP without C-terminal lysine residue, with the assumption that all disulfides are intact, and with the expected post-translational modifications, e.g. glycosylation at the consensus Asn303 residue. The deconvoluted mass profiles are evaluated as tier 3 attribute and the mass difference between the observed and theoretical of less than  $\pm 50$  ppm is evaluated as a tier 2 attribute.

A total of five peaks (A through E) were shown in all three products with similar intensities (see figures below). The different peaks represent different glycan structures. Peak E is higher in ABP 215 development lots compared to US-Avastin and EU-Avastin. Peak E represents glycan species A2G1F:A2G2F (A2 stands for asialylated bi-antennary core with 2 N-acetylglycosamine residues and G stands for galactose and F stands for fucose residue). The level of peak E is reduced in the clinical ABP 215 lot and similar to US and EU-Avastin products. The mass of all three products were less than  $\pm 50$  ppm and the deconvoluted intact molecular mass profiles for all three products are similar with no new peaks.

**Figure 1. Intact Molecular Mass for Bevacizumab (EU), ABP 215 (Development), and Bevacizumab (US)**

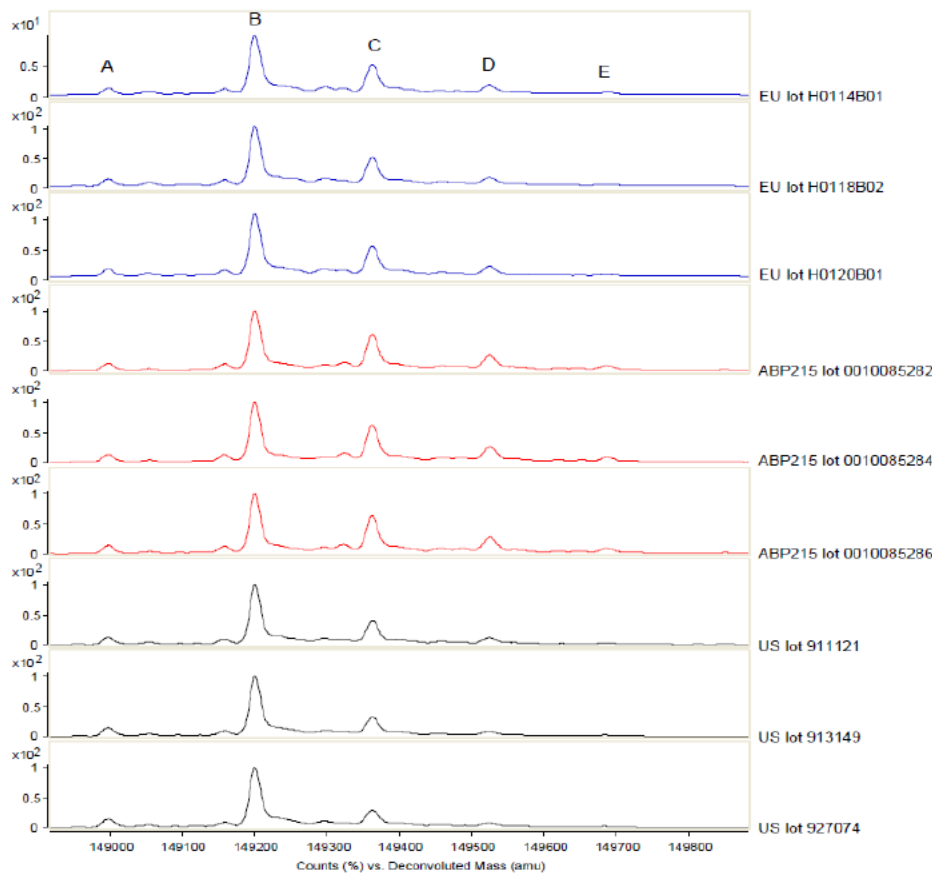
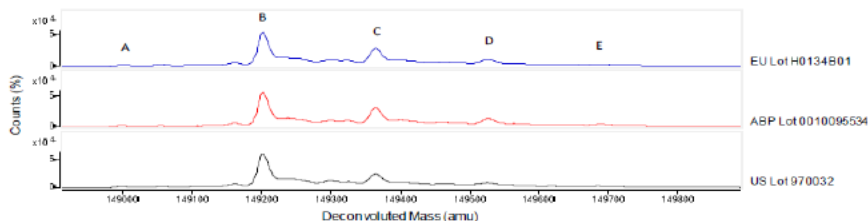


**Figure 2. Intact Molecular Mass for Bevacizumab (EU), ABP 215 (Clinical), and Bevacizumab (US)**



**Reviewer Comment:** The difference in peak E levels correlates to higher galactosylation levels in ABP 215 (see glycan map analysis below). Sponsor justifies the slightly higher level is acceptable given that the three products do not have effector functions, e.g. CDC activity and therefore, will not impact product safety or efficacy. The sponsor's justification is acceptable, and is supported by the lack of CDC activity. Therefore, this difference between the products is not clinically significant. The intact mass of all three products are similar to each other and the products have similar microheterogeneity.

#### Reduced and Deglycosylated Molecular Masses of Heavy Chain and Light Chain

The same four lots of each product and methodology (ESI-TOF-MS) that were used to analyze intact molecular mass were used to determine the mass of reduced and deglycosylated mass of the three products with the exception of the additional steps to remove glycans and disulfide bonds. The additional steps for this analysis included the PNGase F treatment to remove the N-linked glycans and reduction to remove disulfide bonds. The deconvoluted mass profiles are evaluated as tier 3 attribute and the mass difference between the observed and theoretical of less than  $\pm 50$  ppm is evaluated as a tier 2 attribute.

Deconvoluted profiles of the heavy chain (HC) and light chain (LC) of all three products contained one additional peak (peak B) that corresponded to glycosylated HC and LC. The intensity of the peak B was very low and similar in all three products. In addition, no new peaks were observed in any of the three products.

**Reviewer Comment:** *The reduced and deglycosylated mass of all three products were similar and passed both tier 2 and tier 3 criteria.*

#### Reduced Peptide Map

Nine lots of ABP 215, US-Avastin, and EU-Avastin each were used to generate the reduced peptide maps. ABP 215 lots represented DP derived from three development, three clinical, and three process validation lots. The peptide maps were generated from samples treated with trypsin followed by reduction with dithiothreitol and alkylation with sodium iodoacetic acid. The analysis using process validation samples were done without glycans, i.e. the samples were further treated with PNGase F. All samples were run side-by-side and analyzed using RP-HPLC followed by MS/MS for peptide identification. However, the analysis performed with ABP 215 development lots used a linear ion trap MS/MS with 200 ppm mass accuracy for peptides that are greater than 1000 Da and 500 ppm for peptides that are less than 1000 Da, whereas the analysis performed on the clinical and process validation lots were performed on hybrid Ion Trap-Orbitrap MS/MS with a mass accuracy of 50 ppm. The RP-HPLC chromatograms are evaluated as tier 3 attribute and the mass difference between the observed and theoretical is evaluated as a tier 2 attribute.

**Reviewer Comment:** *For a 200 ppm for a peptide of 1000 Da (approximately eight amino acid residues) the mass accuracy is  $\pm 0.2$  Da. Similarly, a 500 ppm mass accuracy for a peptide of less than 1000 Da translates to a mass accuracy is  $\pm 0.5$  Da. Therefore, both MS/MS machines provide sufficient accuracy for peptide map analysis and determination of the primary structure.*

The chromatograms of all 27 samples were similar with no new peaks above 3.3 times the noise level. In addition, all observed peptides were similar to the theoretical masses and within the mass accuracy of the methods used. Sponsor states, (not observable by eye in the chromatograms), that there are six low abundance peptides in all three products as well as one new peak below 3.3 times the noise level observed for ABP 215 only. The new peak represents a sequence S121A variant due to a point mutation in at least one copy of the ABP 215 LC gene. The S121A variant exists at < 1% and is stated by the sponsor to have no impact on safety and efficacy.

**Reviewer Comment:** *The existence of the S121A variant was known from the beginning of ABP 215 development. The Agency advised the sponsor to provide characterization data to determine impact to safety and efficacy or provide adequate control over the S121A variant so that the level does not exceed the level used in the clinical studies (~1%). The sponsor provided in the characterization section (3.2.S.3.1) the basis for their conclusion that the sequence variant S121A will not impact safety and efficacy. While the impact to efficacy could be demonstrated by potency comparison of all three products, its impact on safety is not as clear as this variant could have an impact on immunogenicity. However, based on the immunogenicity data provided in the submission, the sponsor's statement is acceptable and the existence of low levels of S121A variant does not impact safety.*

The recovered peptides from all three products provided 98.9% HC (448 residues determined/453 HC residues) and 97.2% LC (208 residues determined/214 LC residues) protein sequence coverage. The missing residues are from the trypsin cleavage sites, i.e. cleavage after lysine (K) or arginine (R) residues; hence the missing residues were two to three residues long and contained either K or R.

**Reviewer Comment:** Although the sequence coverage was not 100% for the HC and LC due to the trypsin cleavage sites, the missing sequences were the same for all three products. Orthogonal studies such as the disulfide structure determination and extinction coefficient determination (see below), provide data to demonstrate that all three products have identical protein sequence for the areas covered. Coverage of 100% of ABP215 sequence was presented as part of the characterization studies in section 3.2.S.3.1 using different enzymes to digest ABP 215. Comparison of the sequence to the known bevacizumab sequence (from <https://www.drugbank.ca/drugs/DB00112>) identified that it is identical.

#### Disulfide Structure-Non-reduced Peptide Map

Two lots of ABP 215 (one development and one clinical), US-Avastin, and EU-Avastin were used to generate peptide maps under non-reducing conditions with trypsin. The resulting peptides were separated with RP-HPLC and the mass of peptides determined using electrospray MS. The peptide map analysis was performed side-by-side. The peptide map chromatograms are evaluated as tier 3 attribute and the peptide mass difference between the observed and theoretical of less than  $\pm 200$  ppm (for peptides  $> 1000$  Da) and  $\pm 500$  ppm (for peptides  $< 1000$  Da) is evaluated as a tier 2 attribute.

All 16 disulfide (12 intrachain and 4 interchain) linkages were confirmed by comparing the non-reduced condition peptide map data with the reduced peptide map data. The observed masses of the non-reduced disulfide containing peptides and reduced cysteine containing peptides were all within 200 ppm and 500 ppm for the EU-Avastin lots. However, one US-Avastin and one development ABP 215 lot did not meet the acceptance criterion of 200 ppm for peptide, L16 (V191-207), under reduced condition.

**Reviewer Comment:** The ABP 215 (development) and US-Avastin lots for peptide L16 was 220 ppm different than the theoretical mass. L16 peptide theoretical mass is 1819.1 Da. The 220 ppm amounts to 0.4 Da difference from the theoretical mass for the peptide. The sponsor states that the 220 ppm difference is still within the method capability. Given the fact that difference from the theoretical is very small and the clinical ABP 215 as well as the second US-Avastin lot passed the acceptance criterion, the failure to meet the acceptance criterion for L16 for the development lot is probably due to method error and not a true difference in ABP 215 to the RP. In addition, the chromatograms of the non-reduced and reduced peptide maps were similar with no new peaks above the integrity limit of 3.3 times the background noise. Therefore, all three products passed both tier 3 and tier 2 acceptance criteria for disulfide linkages.

#### Glycan Map

Thirteen ABP 215, 23 US-Avastin, and 25 EU-Avastin lots were used to determine the glycan content present on all three products. Hydrophilic interaction liquid chromatography (HILIC) method was used with fluorescence and on-line MS detect the glycan species. In addition exoglycosidase was used to determine sialic acid content.

The type and amount of different glycan species were quantified using glycan reference standards. The glycan species were grouped based on content of mannose, sialic acid, fucose, and galactose residues because of their ability to affect pharmacokinetics (PK) and binding to FcγRIIIa, and C1q, respectively. The following formulas were used to calculate glycan levels with high mannose, afucosylation, galactosylation, and sialylation:

$$\% \text{ High Mannose} = \%M5 + \%M6 + \%M7 + \%M8a + \%M8b$$

$$\% \text{ Afucosylation} = \%A1G0 + \%A2G0 + \%A2G1$$

$$\% \text{ Galactosylation} = \%A1G1F + \%A2G1 + \%A2G1Fa + \%A2G1Fb + \%A3G1F + \%A2G2F + \\ \%A2S1G1F + \%A2S1G2F$$

$$\% \text{ Sialylation} = \% A2S1G1F + \%A2S1G2F$$

The comparison of the levels of high mannose, afucosylated, and galactosylated glycans were evaluated as tier 2 quality attribute with the acceptance criteria that 90% of the ABP 215 and EU lots must be within the QR of the US lots and 90% of the ABP 215 lots must be within the QR of the EU lots. The HILIC chromatograms were evaluated as tier 3 attribute. In addition, sialylation was evaluated as a tier 3 attribute due to the low levels observed on US-Avastin product and therefore its low risk for impact to the biological function of the US-Avastin, i.e. its impact on PK.

The results showed that ABP 215 contained higher levels of high mannose (ABP 215 mean = 2.0, US-Avastin = 0.6 and EU-Avastin = 0.8) and galactose (ABP 215 mean = 23.6, US-Avastin = 14.0, and EU-Avastin = 15.0) content compared to US-Avastin and EU-Avastin. US-Avastin and EU-Avastin contained higher levels of afucosylated glycans compared to ABP 215 (ABP 215 mean = 1.5, US-Avastin = 2.2, and EU-Avastin = 2.3). Sialic acid content was similarly low in all three products (ABP 215 mean = 0.2, US-Avastin = 0.1, and EU-Avastin = 0.1). The scatter plots of the glycan levels are shown below:

Figure 16. High Mannose Results for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

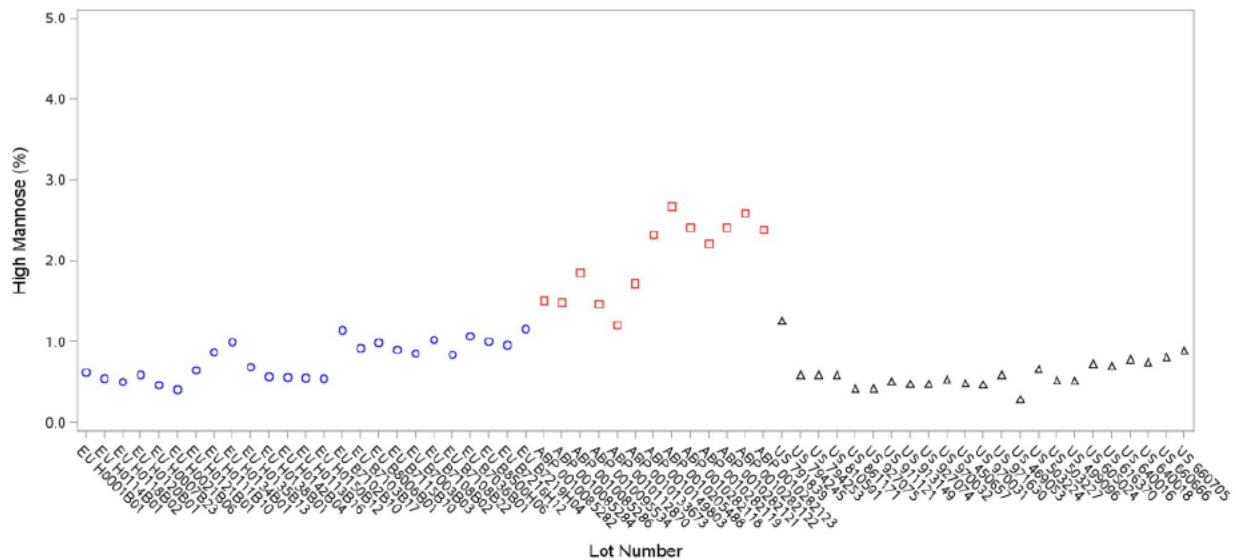


Figure 17. Afucosylation Results for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

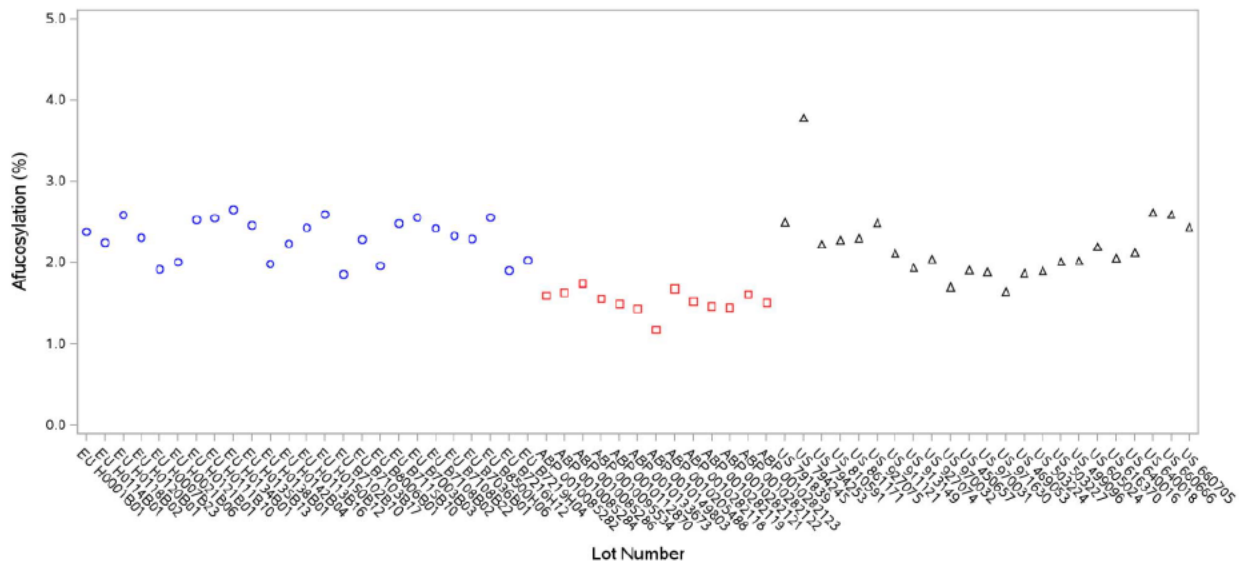


Figure 18. Galactosylation Results for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

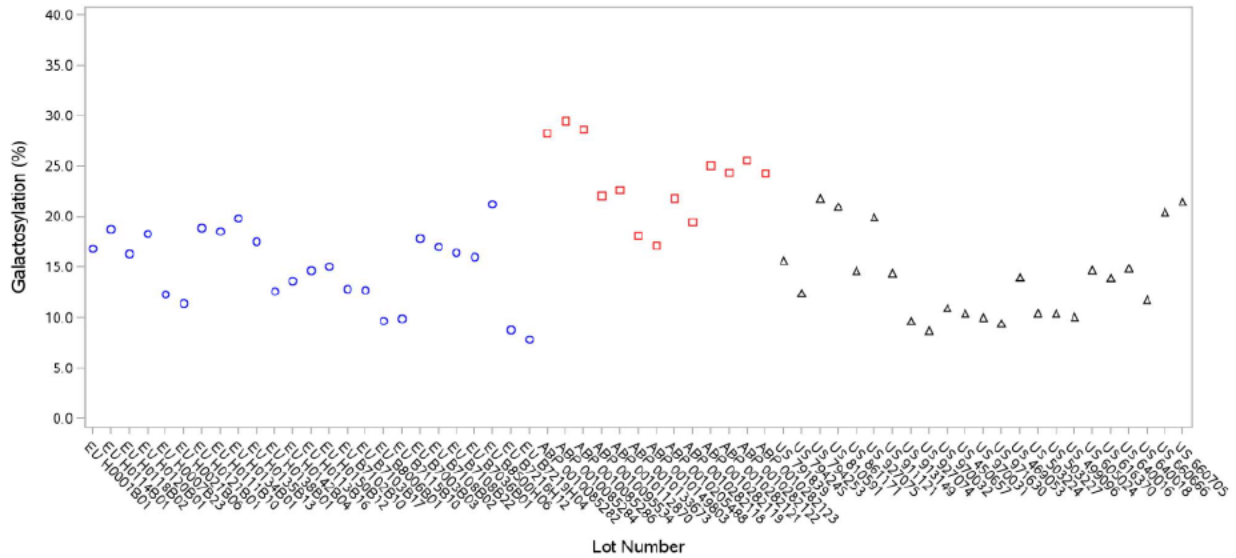
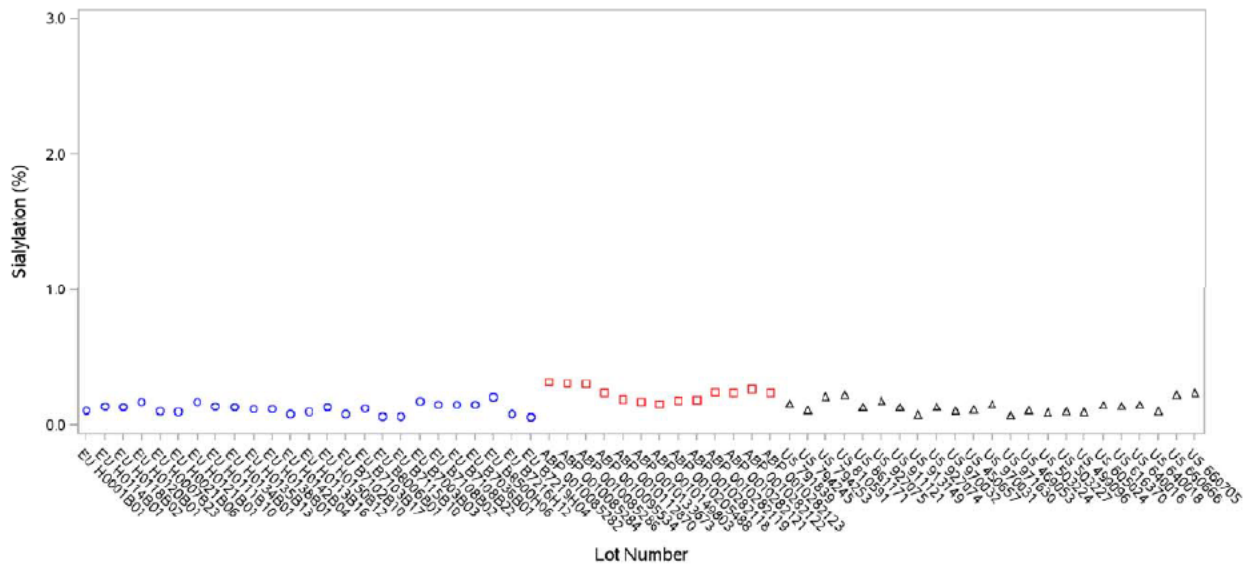
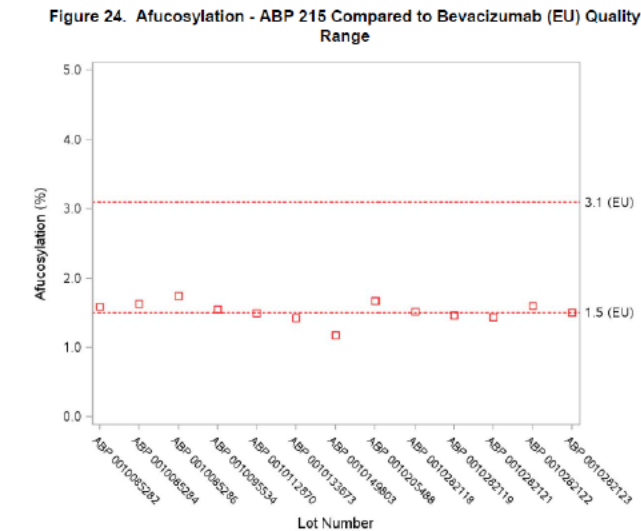
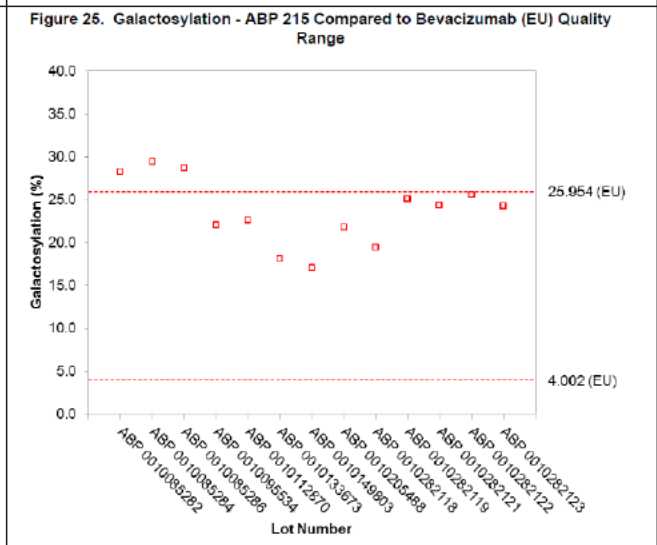
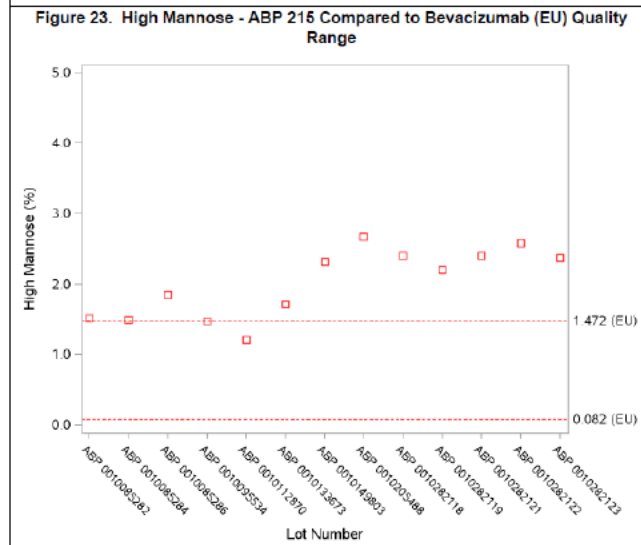
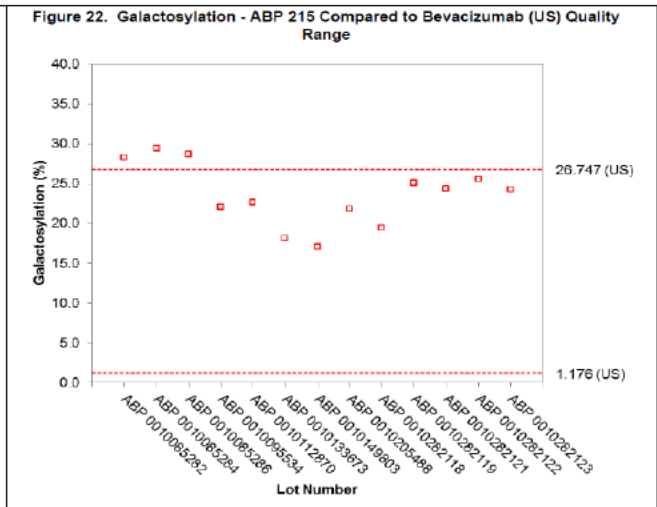
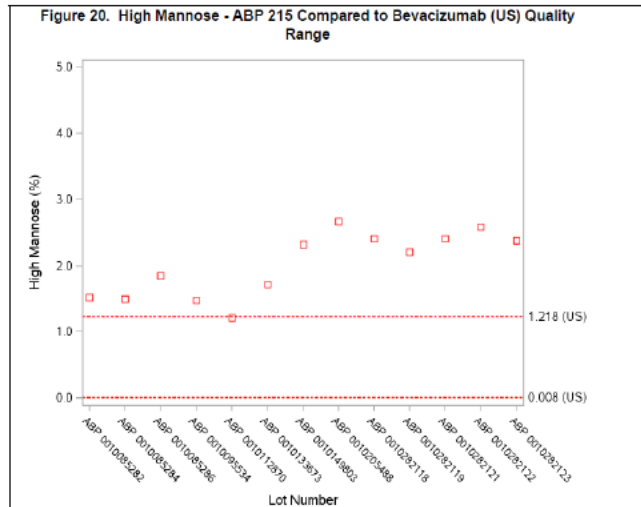


Figure 19. Sialylation Results for Bevacizumab (EU), ABP 215, and Bevacizumab (US)



The EU-Avastin lots met the similarity acceptance criteria against US-Avastin QRs for high mannose, galactosylation, and afucosylation. However, the ABP 215 lots failed to meet the US and EU QRs for high mannose and galactosylation. In addition ABP 215 failed to meet EU QR for afucosylation (see figures below).



**Reviewer Comments:**

- *The similarity assessment demonstrated US-Avastin and EU-Avastin lots have similar glycan profile and content.*
- *The HILIC chromatograms showed similar profiles. However, low levels of peaks, representing mannose glycan (M5, M7, and M8) are present on ABP 215, but not on US-Avastin or EU-Avastin.*
- *The failure of ABP 215 to meet the tier 2 similarity acceptance criteria for galactosylation, and afucosylation, was considered acceptable by the sponsor given the lack of effector functions demonstrated for both ABP 215 and US- and EU-Avastin products (see biological functions section for data). For example the higher galactosylation and afucosylation could affect C1q and FcγRIIIa binding and lead to differences in CDC and ADCC activities, respectively. However, all three products did not demonstrate any effector function activities. Therefore, the higher levels of galactosylation and lower levels of afucosylation are not considered likely to lead to clinically meaningful differences.*
- *High mannose glycans can lead to faster clearance of product in vivo and therefore affect the PK of the product. The sponsor justifies the difference in high mannose content between ABP 215 and US-Avastin and EU-Avastin lots as not likely to impact PK based on the results of their PK study in healthy volunteers using all three products (Study 20110216). In addition, the sponsor referenced Flynn, G., et al., 2010 Mol Immunol 47:2074 paper to state that high mannose content below ~3% is not likely to impact PK. However, the reference article was irrelevant to the sponsor's justification and an IR was sent to provide the correct literature reference.*
  - *Amgen submitted the correct literature reference and supports their justification.*
- *To determine if the sponsor's justification is acceptable, the high mannose content of the ABP 215 lot used in the PK study was compared against all the lots manufactured to date to determine if the PK lot is representative of the ABP-215 lots. Analysis of the ABP 215 lots for high mannose levels showed that the ABP 215 lot used in the PK study had lower levels of high mannose compared to the more recently manufactured ABP 215 lots (the PK lot had 1.5% and the process validation lots had 2.4%, 2.6%, and 2.4% high mannose content), i.e. an increasing trend in high mannose levels over time (scatter plot figure 16 above) is observed. The sponsor did use in their NSCLC study (Study 20120265) three lots with higher levels of mannose glycans (clinical lots used had 1.7%, 2.3%, and 2.7%). Input from clinical pharmacology reviewer was sought and based on the trough level PK data from the clinical study in NSCLC, the residual uncertainty regarding the analytical difference observed in the high mannose content was demonstrated to be not clinically meaningful.*
- *The difference in the high mannose levels is acceptable. However, the sponsor will need to explain the increasing trend in high mannose content and develop a control strategy that will tightly control and/or monitor the high mannose content so that the levels do not exceed the levels used in the clinical studies. An IR was sent to ask the sponsor to provide data and an explanation for the increasing trend for high mannose content for ABP 215.*
  - *Sponsor provided in their response to IR (received 2/22/17) the high mannose content data of the DS lots used to make the DP lots. A difference in the levels of high mannose content (~0.5%, e.g. 1.9% versus 2.4%) in the DS and DP lots were observed. The difference in the levels was stated to be due to assay variability. Because glycan species are stable and in general do not change going from DS to DP, the difference in the high mannose levels was likely due to assay performance and this was confirmed during inspection of ATO facility, where the glycan mapping method testing site changed from AWA to Amgen Cambridge, MA site. Therefore, the difference observed was probably not due to a change in the manufacturing process. (b) (4)*
- *In conclusion, the glycan content of ABP 215 showed differences when compared to US-Avastin and EU-Avastin that failed to meet the analytical similarity criteria. However, the residual uncertainties based on the analytical differences in glycan species were adequately justified by biological effector*

*function data, clinical PK data, and literature references. In addition, the control strategy implemented by the sponsor should ensure that the current glycan content will not drift further from the RP.*

#### Isoelectric Point-Capillary Isoelectric Focusing (cIEF)

Three lots of ABP 215, US-Avastin, and EU-Avastin each were used to determine the pI of the main peak. Only ABP 215 development lots were used because the sponsor states the orthogonal CEX method included assessment with clinical and process validation lots. The method was performed side-by-side and the similarity acceptance criterion for the pI was based on the expectation approach, i.e. method validation data that demonstrated intermediate precision standard deviation of 0.1 pH units. The comparison of the main peak pI is evaluated as a tier 2 attribute and the cIEF profiles as a tier 3 attribute.

The results showed the main peak pI for all three products was 7.9. The cIEF electrophoretograms showed similar profiles, but ABP215 contained higher levels of basic species.

**Reviewer Comment:** *The justification for ABP 215 lot selection is acceptable given that the all three types, development, clinical and process validation lots were tested by the CEX method, which also separates based on charge. Any differences in the main peak retention times would be noted and evaluated for effect on main peak pI value. The higher level of basic peak in ABP215 was attributed to C-terminal lysine residue variant, which is known to not affect in the vivo function or PK of monoclonal antibodies administered by the intravenous route, as this residue is quickly removed from the antibody once introduced into the circulation (Liu, H. et. al., 2015 Mabs 6(5):1145). In conclusion, the sponsor adequately demonstrated similarity in main peak pI and charge isoforms.*

#### Extinction Coefficient

One lot each of ABP 215, US-Avastin, and EU-Avastin was used to determine the extinction coefficient by amino acid analysis. The sponsor justified using only one lot of each product because the amino acid sequence was shown to be the same for all three products. The similarity acceptance criterion for the extinction coefficient is based on the expectation approach and is less than 10% different between each lot based on intermediate precision for the amino acid analysis method. The extinction coefficient is evaluated as a tier 2 attribute.

The experimentally determined extinction coefficients for ABP 215, US-Avastin, and EU-Avastin were 1.8, 1.7, and 1.8 mg<sup>-1</sup>cm<sup>-1</sup>mL, respectively. The results show ABP 215 has similar extinction coefficient as US-Avastin and EU-Avastin.

**Reviewer Comment:** *The justification for the number of lots used is acceptable and the results show all three products have similar if not identical extinction coefficients. This supports protein concentration determination with the same extinction coefficient is appropriate.*

#### Identity by ELISA

Thirteen ABP 215, three US-Avastin, and three EU-Avastin lots were used to determine the identity by the ELISA method. Because identity by ELISA is part of the release specification, data from all thirteen ABP 215 lots were used in the similarity assessment. Only three lots each of the US-Avastin and EU-Avastin used because identity, based on amino acid sequence, is not expected to vary from lot to lot. The ELISA method uses a mouse anti-ABP 215 monoclonal antibody to capture ABP 215, US-Avastin, and EU-Avastin products onto 96-well plates followed by detection using anti-ABP 215 antibody conjugated to horseradish peroxidase.

The results showed all three products share the same identity and are recognized by the in-house sponsor generated anti-ABP 215 monoclonal antibody.

**Reviewer Comment:** *The justification for the number of lots used is acceptable and the results confirm all three products share same identity as defined by the recognition of the same epitope by the monoclonal antibody generated against ABP 215 product. The assay also confirms that the epitope to which the anti-ABP 215 antibody is targeted is the same in all three products.*

### 3.2.R.4.2 Higher Order Structure

The sponsor used three different methods to determine the secondary and tertiary structures of ABP 215, US-Avastin, and EU-Avastin. The combination of results from Fourier transform infrared (FTIR) spectroscopy, near ultraviolet circular dichroism (CD), 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.

#### FTIR Spectroscopy

Six lots of ABP 215 (three development and three clinical), US-Avastin, and EU-Avastin each were used to determine the secondary structure. The sponsor justified the use of six lots each of the three products for the similarity assessment being sufficient because there were no process changes to the ABP 215 DS manufacturing process that could warrant a change in the secondary structure. The samples were run side-by-side with formulation buffer blank. The FTIR spectra were analyzed using Thermo OMNIC software to determine quantitatively the percent similarity of the spectra from the three products. The FTIR spectra are evaluated as tier 3 attribute and quantitative percent similarity is evaluated as a tier 2 attribute.

The quantitative assessment of the FTIR spectra showed greater than 98% similarity for ABP 215 and EU-Avastin against the US-Avastin spectra. Similarly, the ABP 215 and US-Avastin spectra were greater than 98% similar compared to EU-Avastin spectra. All three products passed the acceptance criterion of > 95% similarity in the spectra to conclude spectra similarity. The acceptance criterion was based on method capability. The FTIR spectra for all three products provided showed a signal for beta-sheet structure at wavelengths 1639  $\text{cm}^{-1}$  and 1689  $\text{cm}^{-1}$ .

**Reviewer Comment:** *The justification for the number of lots used is acceptable and the results confirm all three products have similar secondary beta sheet structure.*

#### Near Ultraviolet Circular Dichroism

Six lots of ABP 215 (three development and three clinical), US-Avastin, and EU-Avastin each were used to determine the tertiary structure. The sponsor justified the use of six lots each of the three products for the similarity assessment being sufficient because there were no process changes to the ABP 215 DS manufacturing process that could warrant a change in the secondary structure. The samples were diluted and run side-by-side. The near UV spectra were analyzed using Thermo OMNIC software to determine quantitatively the percent similarity of the spectra from the three products. The near UV spectra are evaluated as tier 3 attribute and quantitative percent similarity is evaluated as a tier 2 attribute.

The quantitative assessment of the near UV spectra showed greater than 98% similarity for ABP 215 and EU-Avastin against the US-Avastin spectra and greater than 98% similar compared to EU-Avastin spectra. All three products passed the acceptance criterion of > 95% similarity in the spectra to conclude spectra similarity. The acceptance criterion was based on method capability. The near UV spectra for all three products provided showed signals representative of aromatic amino acids and disulfide bonds in environments that represented similar folding.

**Reviewer Comment:** *The justification for the number of lots used is acceptable and the results confirm all three products have similar tertiary structure.*

### Differential Scanning Calorimetry

Eleven lots of ABP 215 (3 development, 3 clinical, and 5 validation), 12 lots of US-Avastin, and 12 lots of EU-Avastin were used to determine the thermal transition temperatures, i.e. unfolding or melting temperatures of the three products to deduce tertiary structures. Three lots of ABP 215 (development and clinical) were run side-by-side with US-Avastin and EU-Avastin lots. The five ABP 215 process validation lots were tested as part of the DP comparability exercise (section 3.2.P.2.3) and run side-by-side with three lots of US-Avastin and EU-Avastin. The ABP 215 PV lot results were combined with the development and clinical ABP 215 lots. The DSC profiles are evaluated as tier 3 attribute and the comparison of the melting temperatures are evaluated as a tier 2 attribute.

All three products showed two melting transition temperatures that correspond to the unfolding of the Fab/CH2 and CH3 domains. The first and second DSC melting temperatures for ABP 215, US-Avastin, and EU-Avastin were all 72.8°C and 83.5°C, respectively. The DSC method tier 2 quality attribute has the acceptance criteria that 90% of the ABP 215 and EU lots must be within the QR of the US lots and 90% of the ABP 215 lots must be within the QR of the EU lots. All three products passed the tier 2 criteria for DSC.

**Reviewer Comment:** *The results confirm that all three products have similar tertiary structure based on the near identical DSC melting temperatures.*

### **3.2.R.4.3 Particles and Aggregates**

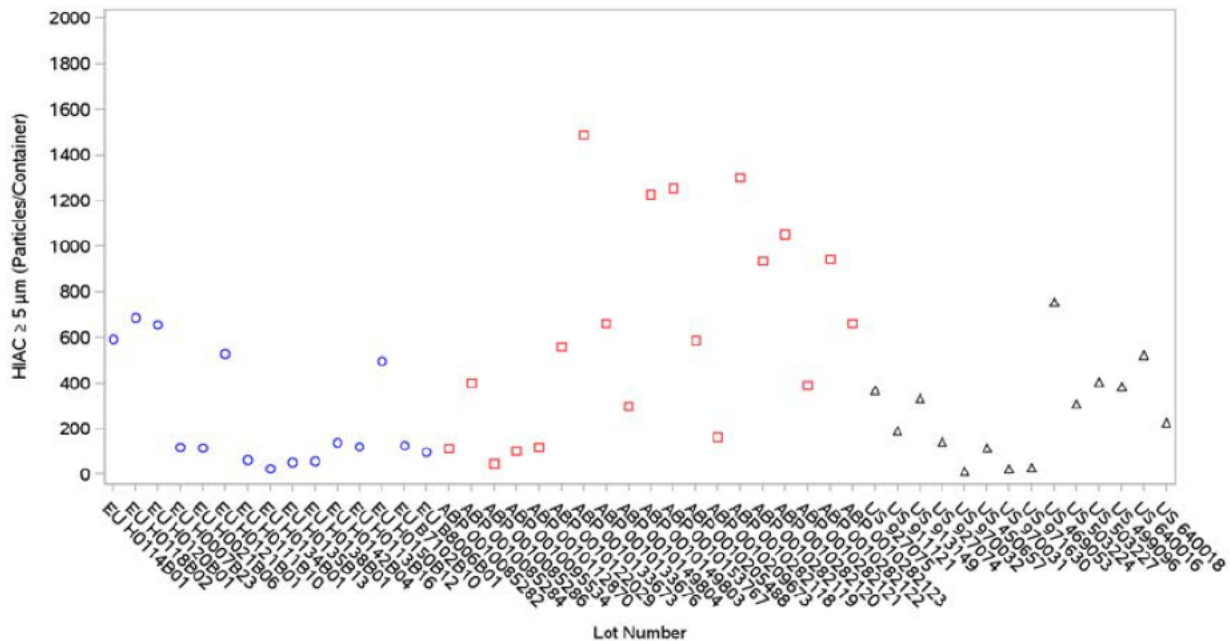
The sponsor evaluated the similarity of particle and aggregate contents in ABP 215, US-Avastin, and EU-Avastin using six different methods, light obscuration (HIAC), microflow imaging (MFI), field flow fractionation (FFF), dynamic light scattering (DLS), analytical ultracentrifugation sedimentation velocity (AUC-SV), and SE-HPLC-static light scattering (SLS). The different methods helped to target different particle sizes (subvisible versus submicron) as well as to characterize spherical and non-spherical particles, e.g. proteinaceous particles from silicon droplets. The results of each testing approach and the similarity of the three products are reviewed below.

#### Subvisible Particle Analysis

Two methods, the HIAC and MFI, were used to quantify subvisible particles. HIAC measured particles that were grouped into four sizes,  $\geq 2 \mu\text{m}$ ,  $\geq 5 \mu\text{m}$ ,  $\geq 10 \mu\text{m}$ , and  $\geq 25 \mu\text{m}$ . MFI further evaluated particles that were  $\geq 5 \mu\text{m}$  for the number of spherical versus non-spherical particles. The HIAC method used 19 ABP 215 lots, 14 lots of US-Avastin, and 15 lots of EU-Avastin. The MFI method used 19 ABP 215 lots, 11 US-Avastin lots, and 12 EU-Avastin lots. Particle assessment was evaluated as a tier 3 attribute. The HIAC results are evaluated as a tier 3 attribute and the MFI analysis for non-spherical particles was evaluated as a tier 2 attribute, because of its potential to affect immunogenicity and safety.

The results from the HIAC analysis showed all three products had low levels of  $\geq 10 \mu\text{m}$ , and  $\geq 25 \mu\text{m}$  that met the USP <788> specification. However, ABP 215 lots showed higher numbers of  $\geq 5 \mu\text{m}$  size particles compared to US-Avastin and EU-Avastin, (mean ABP 215 = 647, US-Avastin = 273, and EU-Avastin = 258) (see data below).

Figure 2. HIAC  $\geq 5 \mu\text{m}$  Subvisible Particles Results for Bevacizumab (EU), ABP 215, and Bevacizumab (US)



The sponsor further analyzed the  $\geq 5 \mu\text{m}$  size particle using MFI. The results showed that ABP 215 had lower numbers of proteinaceous particles compared to US-Avastin and EU-Avastin (mean ABP 215 = 22, US-Avastin = 279, and EU-Avastin = 94). The MFI tier 2 quality attribute has the acceptance criteria that 90% of the ABP 215 and EU lots must be within the QR of the US lots and 90% of the ABP 215 lots must be within the QR of the EU lots. All three products passed the tier 2 criteria for MFI.

**Reviewer Comments:**

- *Subvisible particles are not expected to be similar as the particle numbers are dependent on DP filling operations and do not necessarily reflect the protein product but may be associated with other particles e.g. fibers, glass, etc. However, the general order of magnitude of the number of proteinaceous versus non-proteinaceous particles is expected to be within range of each other so as not to affect safety. The HIAC analysis showed all three products had similar low numbers of particles, i.e. the differences were less than an order of magnitude. However, the  $\geq 5 \mu\text{m}$  size particle numbers were sufficiently different to warrant further analysis by the sponsor using the MFI method to determine if these could be attributed to higher proteinaceous particles, which could affect immunogenicity of the product.*
- *The MFI analysis showed differences in order of magnitude, e.g. 22 versus 279 particles for ABP 215 and US-Avastin, respectively. However, the standard deviation for the US-Avastin lots was 654, mainly due to two lots that were up to two orders of magnitudes higher than the majority of the US lots. Similarly, the same two US-Avastin lots showed higher amounts of total  $\geq 5 \mu\text{m}$  size particles that led to a standard deviation of 1020 versus 60 and 160 for ABP 215 and EU-Avastin, respectively. The two US-Avastin lots are likely outliers (see data tables below highlighted in yellow below). Therefore, the MFI numbers are similar when the two lots are considered outliers. Table 5 below is the total number of MFI particles and Table 6 below shows a subset of the total number of particles that were counted as non-spherical particles. The non-spherical particles were considered by the sponsor to represent proteinaceous particles as worse case because non-spherical could also include fibers.*

**Table 5. MFI ≥ 5 μm Results for Bevacizumab (EU), ABP 215, and Bevacizumab (US)**

| Lot Number  | Particles/<br>mL | Lot Number     | Particles<br>/mL | Lot Number | Particles/<br>mL |
|-------------|------------------|----------------|------------------|------------|------------------|
| EU H0114B01 | 70               | ABP 0010085282 | 39               | US 911121  | 70               |
| EU H0118B02 | 132              | ABP 0010085284 | 30               | US 913149  | 103              |
| EU H0120B01 | 147              | ABP 0010085286 | 16               | US 927074  | 139              |
| EU H0007B23 | 362              | ABP 0010095534 | 81               | US 970032  | 106              |
| EU H0021B06 | 85               | ABP 0010112870 | 46               | US 450657  | 63               |
| EU H0111B10 | 304              | ABP 0010122029 | 124              | US 970031  | 64               |
| EU H0134B01 | 57               | ABP 0010133673 | 74               | US 971630  | 1066             |
| EU H0135B13 | 218              | ABP 0010133676 | 106              | US 469053  | 3439             |
| EU H0138B01 | 51               | ABP 0010149804 | 218              | US 503227  | 110              |
| EU H0113B16 | 588              | ABP 0010149803 | 60               | US 640016  | 236              |
| EU B7102B10 | 168              | ABP 0010153767 | 178              | US 640018  | 42               |
| EU B8006B01 | 84               | ABP 0010205488 | 102              |            |                  |
|             |                  | ABP 0010209673 | 84               |            |                  |
|             |                  | ABP 0010282118 | 204              |            |                  |
|             |                  | ABP 0010282119 | 154              |            |                  |
|             |                  | ABP 0010282120 | 152              |            |                  |
|             |                  | ABP 0010282121 | 46               |            |                  |
|             |                  | ABP 0010282122 | 46               |            |                  |
|             |                  | ABP 0010282123 | 66               |            |                  |
| EU mean     | 189              | ABP mean       | 96               | US mean    | 494              |
| EU SD       | 160              | ABP SD         | 60               | US SD      | 1020             |

**Table 6. MFI ≥ 5 μm Non-spherical Results for Bevacizumab (EU), ABP 215, and Bevacizumab (US)**

| Lot Number  | Particles/<br>mL | Lot Number     | Particles/m<br>L | Lot Number | Particles/mL |
|-------------|------------------|----------------|------------------|------------|--------------|
| EU H0114B01 | 8                | ABP 0010085282 | 25               | US 911121  | 22           |
| EU H0118B02 | 14               | ABP 0010085284 | 26               | US 913149  | 21           |
| EU H0120B01 | 29               | ABP 0010085286 | 11               | US 927074  | 33           |
| EU H0007B23 | 276              | ABP 0010095534 | 65               | US 970032  | 53           |
| EU H0021B06 | 16               | ABP 0010112870 | 41               | US 450657  | 13           |
| EU H0111B10 | 198              | ABP 0010122029 | 12               | US 970031  | 27           |
| EU H0134B01 | 19               | ABP 0010133673 | 19               | US 971630  | 684          |
| EU H0135B13 | 60               | ABP 0010133676 | 20               | US 469053  | 2157         |
| EU H0138B01 | 26               | ABP 0010149804 | 24               | US 503227  | 26           |
| EU H0113B16 | 430              | ABP 0010149803 | 30               | US 640016  | 26           |
| EU B7102B10 | 28               | ABP 0010153767 | 40               | US 640018  | 6            |
| EU B8006B01 | 24               | ABP 0010205488 | 28               |            |              |
|             |                  | ABP 0010209673 | 12               |            |              |
|             |                  | ABP 0010282118 | 24               |            |              |
|             |                  | ABP 0010282119 | 10               |            |              |
|             |                  | ABP 0010282120 | 16               |            |              |
|             |                  | ABP 0010282121 | 2                |            |              |
|             |                  | ABP 0010282122 | 0                |            |              |
|             |                  | ABP 0010282123 | 6                |            |              |
| EU mean     | 94               | ABP mean       | 22               | US mean    | 279          |
| EU SD       | 135              | ABP SD         | 16               | US SD      | 654          |

- The MFI results indicate the higher levels of ≥ 5 μm size particles by HIAC is not a safety risk because the MFI analysis showed lower if not similar levels of proteinaceous particles for ABP215 as compared to US-Avastin and EU-Avastin.
- In conclusion, the subvisible particle analysis by HIAC and MFI support the conclusion that all three products have low levels of particles and therefore have low risk to adversely impact safety.

Submicron Particle Analysis

Six lots of ABP 215 (three development and three clinical), US-Avastin, and EU-Avastin each were used to analyze the submicron ( $\leq 1 \mu\text{m}$ ) size particle distribution with FFF and DLS methods. The profiles were evaluated as a tier 3 attribute.

The chromatograms provided for FFF showed all three products had single a single peak representing particles less than 100 nm in size. No additional peaks were present. Similarly, DLS chromatograms showed a single peak representing the monomer species.

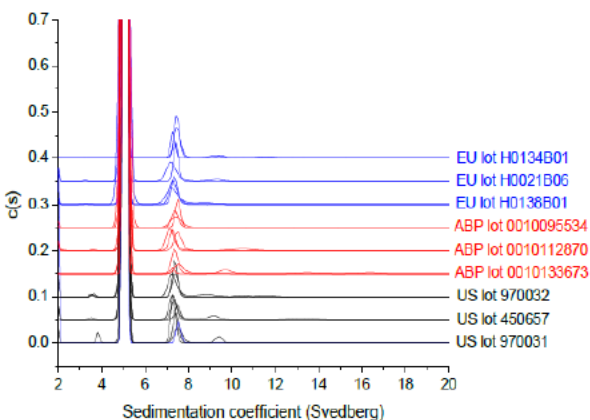
**Reviewer Comment:** *The results indicate all three products have similar submicron size particle distribution.*

#### Molecular Size and Aggregates Analysis by Sedimentation Velocity Analytical Ultracentrifugation

Six lots of ABP 215 (three development and three clinical), US-Avastin, and EU-Avastin each were used to analyze for the presence of higher molecular weight species. The AUC-SV method was used as an orthogonal method to SE-HPLC method. The sponsor justified the use of only six lots each of the three products for the similarity assessment being sufficient because there were no process or formulation changes to the ABP 215 manufacturing process from clinical to PV lots that could warrant a change in the product's submicron particle distribution. The samples were run side-by-side and the profiles were evaluated as a tier 3 attribute, whereas the percent monomer was evaluated as a tier 2 attribute.

The results showed all three products had similar profiles with the chromatograms showing a large monomer peak and a smaller higher molecular weight peaks. ABP 215 had less HMW species compared to US-Avastin and EU-Avastin (see representative chromatogram for some of the lots, below). The sponsor states that the HMW species were all less than the method LOQ (3.7%).

Figure 15. AUC-SV Profiles for Bevacizumab (EU), ABP 215 (Clinical), and Bevacizumab (US)



The results also showed that all ABP 215 and EU-Avastin lots met the US-Avastin QR for monomer peak and ABP 215 met EU-Avastin QR. The percent monomer was approximately 1% higher for the ABP 215 product (98.7% versus 97.9% and 97.8% for US-Avastin and EU-Avastin, respectively).

#### **Reviewer Comments:**

- *The number of lots used is acceptable as this is an orthogonal method to SE-HPLC method that also measures the amount of HMW species. However, the justification that PV lots were not needed because there were no process changes that would warrant a difference between clinical and PV lots is not correct as there were site-specific changes between ATO and (b) (4) that could have resulted in differences in HMW species. The lack of PV lot data is acceptable for this analysis as the DP*

comparability exercise used SE-HPLC method to determine that the levels of HMW species in ATO and (b) (4) DP lots were comparable.

- The AUC-SV chromatograms as well as the tabular data indicate that ABP 215 has no new HMW species and that it has lower levels of HMW species compared to some of the US-Avastin and EU-Avastin lots. This is anticipated as HMW species are stability-related and the higher amounts are mostly likely due to the age of the US-Avastin and EU-Avastin lots compared to ABP 215.

#### Molar Mass Determination by Static Light Scattering Detection

Three lots of ABP 215, US-Avastin, and EU-Avastin each were used to determine the molar mass of the monomer and HMW species. The sponsor stated that only three development lots of ABP 215 were sufficient to characterize the different size variants because there were no significant process changes that would affect molar masses of monomer and HMW species. The SE-HPLC-SLS method was used and samples were run side-by-side. The SE-HPLC-SLS profiles were evaluated as a tier 3 attribute and the molar mass of each size variant was evaluated as a tier 2 attribute.

The sponsor states that the expected method accuracy of SE-HPLC-SLS is dependent on multiple factors including the relative abundance and number of size variants in a sample. The sponsor states and refers to published literature that the method has an accuracy of 90% of the expected molar mass. The sponsor used the 10% margin of error for the pre-peak and a 5% error margin for the main peak due to the higher abundance of the monomer peak. Therefore, the acceptance criterion for similarity for a dimer was  $298,398 \pm 29,840$  Da and for a monomer was  $149,199 \pm 7,460$  Da. The results showed that all three products had two predominant size variants, a pre-peak and a main peak. The molar mass of the pre-peak was calculated to be approximately 300 kDa and the monomer peak was approximately 148 kDa for all three products and met the method accuracy expectation criteria for similarity. The chromatograms provided for all three products were similar in profile with similar retention times of the two size variants, and with no new peaks.

**Reviewer Comment:** *The number of lots used is acceptable as this is an orthogonal method to SE-HPLC method that also detects the relative distribution of different size variants. However, the justification that the use of only the development lots was appropriate because there were no process changes that would warrant a difference in the size variants in other lots is not correct as there were site-specific process changes between ATO and (b) (4) that could have resulted in differences the number of size variants. The lack of additional ABP 215 lot data is acceptable for this analysis as the DP comparability exercise used SE-HPLC method as well as other methods to conclude that lots manufactured at the two sites were comparable. Therefore, the SE-HPLC-SLS data supports the conclusion that all three products have similar molecular size variants.*

#### **3.2.R.4.4 Product Related Substances and Impurities**

The sponsor used four purity methods, SE-HPLC, reduced capillary electrophoresis (rCE-SDS), non-reduced (nr)CE-SDS, and cation exchange (CEX)-HPLC to determine the levels of product-related substances and impurities of ABP 215, US-Avastin, and EU-Avastin. In addition, free thiol levels were evaluated using the nr-CE-SDS method and CEX acidic, main, and basic fractions were further characterized using carboxypeptidase treatment and peptide mapping to evaluate the types of post-translational modifications and their effects on potency.

The sponsor stated that the product-related substances and impurities detected as size and charge variants typically change with time. Therefore, in order to perform the similarity exercise with market-purchased US-Avastin and EU-Avastin lots of different age based on expiration dates, the sponsor performed age-adjusted calculations of the quality attributes measured with the SE-HPLC, r-CE-SDS, and nr-CE-SDS methods.

**Reviewer Comment:** As discussed above in the 3.2.R.4.1 Testing Plan and Acceptance Criteria, the age-adjustment was based on a “presumed” 24 month expiry of the US-Avastin and EU-Avastin lots. As this approach is not considered appropriate and presumption of a 24 month shelf life cannot be verified, only the raw data were evaluated in assessment of analytical similarity.

The combination of results from the methods used to evaluate the size and charge variants helped to evaluate the similarity of the levels of product-related substances and impurities. The results of each testing approach and the similarity of the three products are reviewed below.

#### Size Exclusion-High Performance Liquid Chromatography (HPLC)

Thirteen lots of ABP 215, 24 US-Avastin lots, and 26 EU-Avastin lots were used to determine the levels of monomer and HMW species. The percent HMW species was evaluated as a tier 2 attribute and the profiles of the SE-HPLC chromatograms were evaluated as a tier 3 attribute.

The raw data for HMW species showed ABP 215 contained less HMW compared to US-Avastin and EU-Avastin lots. The calculated mean, standard deviation (SD), median and QRs based 3SD as well as the minimum and maximum ranges of %HMW species, with the associated lot ages of the extreme range values for ABP 215, US-Avastin, and EU-Avastin based on sponsor’s age calculation formula (Material age (months) = testing date – [expiry date of individual lot – 24 months]) are shown in the table below:

| % HMW Species     | Mean (%) | SD   | Median (%) | QR (3SD)   | Min/Max Range (Est Age months)     |
|-------------------|----------|------|------------|------------|------------------------------------|
| <b>ABP 215</b>    | 1.72     | 0.27 | 1.7        |            | 1.3 to 2.3 (0) <sup>a</sup>        |
| <b>US-Avastin</b> | 2.72     | 0.27 | 2.7        | 1.9 to 3.5 | 2.4 to 3.3 (20 to 1) <sup>b</sup>  |
| <b>EU-Avastin</b> | 2.95     | 0.20 | 3.0        | 2.3 to 3.6 | 2.3 to 3.4 (16 to 13) <sup>b</sup> |

<sup>a</sup>Months from date of manufacture

<sup>b</sup>Months to expiry

#### **Reviewer Comments:**

- *ABP 215 and EU-Avastin failed to meet the age-adjusted calculation of the US-Avastin QR. Similarly ABP 215 failed to meet the age-adjusted calculation of the EU-Avastin QR. The similarity exercise using the raw data calculated mean, SD, and QRs also showed that ABP 215 fails to meet the similarity acceptance criteria against the US-Avastin QR and the EU-Avastin QR. EU-Avastin lots failed to meet US-Avastin QR. However, EU falls within US QR calculated from the raw data.*
- *The raw data show that ABP 215 contains less HMW species 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 HMW species. HMW species are known to increase during storage of the product, e.g. stability data from accelerated condition (25°C) show increase in HMW peak from 1.5% to 2.3% (ABP 215 lot 0010133673) at one week, and indicates similar starting levels of HMW that would likely increase to similar amounts as the RP and EU-Avastin (see also Thermal Degradation similarity assessment section below).*
- *The chromatograms of the ABP 215 development lots showed a shoulder peak off the HMW peak that was not as apparent in the US-Avastin and EU-Avastin lots. However, the shoulder peak disappears in the ABP 215 clinical and validation lots (see figures below). In addition, a minor pre-peak is present on ABP 215 that is potentially present in the US-Avastin and EU-Avastin lots. The pre-peak has been characterized in ABP 215 by the sponsor as a non-consensus glycosylation HC variant that has an additional glycosylation site in the Asn165 in the CHI region, and quantitated the amount as ~1% (3.2.S.3.1 page 55). The orthogonal SE-HPLC-SLS and AUC-SV data of the development lots did not show any significant additional size variants compared to the US-Avastin and EU-Avastin lots and as discussed in the 3.2.S.3.1 Characterization section, the additional minor non-consensus site glycosylated variant is not likely to impact product safety and efficacy. Nevertheless, an IR was sent*

to determine whether the US-Avastin and/or EU-Avastin lots also contained Asn165 glycosylation variant.

- In the IR response received 7/14/17, the sponsor provided data from rCE-SDS method that showed a better separation of the non-consensus site glycosylation peak for all three products. The data showed similar levels of the non-consensus site glycosylation peak for all three products (0.3% to 0.5%). The IR also asked whether any specific control strategies were in place to control and limit the non-consensus site glycosylation variant. The sponsor stated that the non-consensus site glycosylation at Asn165 is considered a non-critical quality attribute based on the location of the glycosylation in the CH1 region that does not interfere with antigen binding. In addition, the Asn165 is within a conserved NSGAL motif in CH1 that is known to be frequently glycosylated in IgG1 and IgG2 antibodies (Valliere-Douglas, J.F. et al. 2009 JBC 284: 32493-32506). Therefore, the risk for increased immunogenicity due to the presence of this variant is unlikely because this type of glycosylation is frequent among IgG1 and IgG2 antibodies. Lastly, the sponsor provided data of the variant in LIVCA cells and showed the levels remained comparable to the reference product.
- Based on the location of the non-consensus site variant, similar levels in all three products, and limit of in vitro cell age (LIVCA) data, the non-consensus site glycosylation variant does not impact the conclusion that the three products are similar. Additionally, the general manufacturing and testing control strategy appears to sufficiently control that this variant would not significantly increase in future lots. Specifically, two methods, the SE-HPLC and rCE-SDS, can detect the non-consensus glycosylation peak and per SOP the chromatogram and electrophoretic profiles must be compared to the reference standard profile and any differences or unexpected change in the peak would be identified and evaluated.
- Therefore, taken together, the SE-HPLC profiles of all three products are similar.

**Figure 1. SE-HPLC Profiles for Bevacizumab (EU), ABP 215 (Development), and Bevacizumab (US)**

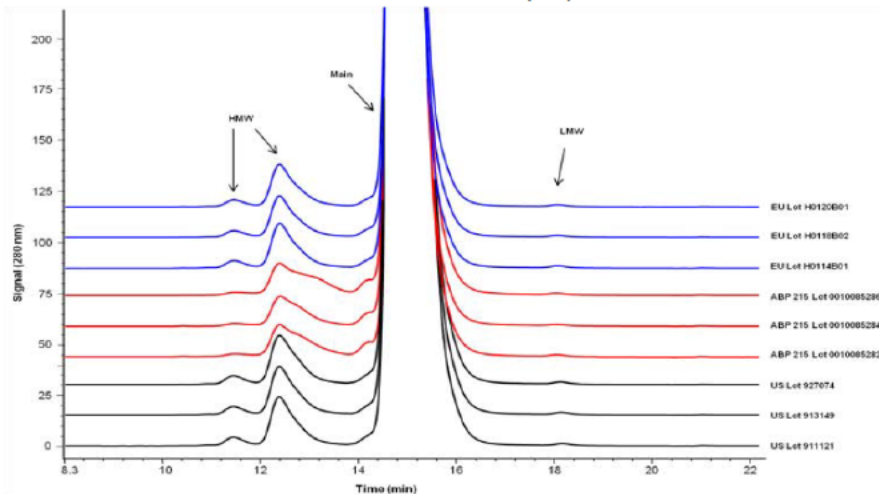
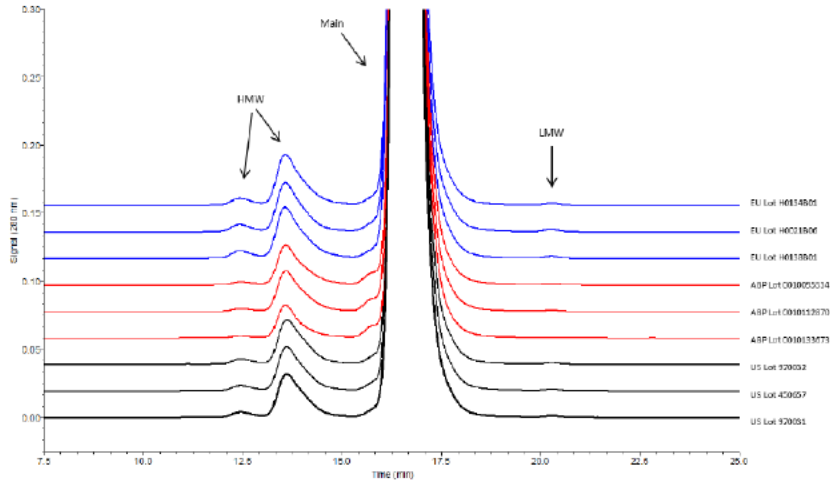


**Figure 2. SE-HPLC Profiles for Bevacizumab (EU), ABP 215 (Clinical), and Bevacizumab (US)**



- In conclusion, the lack of similarity in the levels of HMW is acceptable given that this attribute is lower in the ABP 215 product compared to the RP because of the nature of the measured attribute, i.e. HMW species 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 product potency and immunogenicity. In addition, US-Avastin and EU-Avastin lots have similar amounts of HMW species that correlate with the estimated age of the products which supports the scientific-bridge for the use of EU-Avastin in the clinical study.*

**Reduced Capillary Electrophoresis (CE)-Sodium dodecyl Sulfate (SDS)**

Thirteen lots of ABP 215, 24 US-Avastin lots, and 26 EU-Avastin lots were used to determine the levels of heavy chain plus light chain (HC + LC), non-glycosylated HC (NGHC), low molecular weight species plus middle molecular weight species (LMW + MMW), and the percentage of glycosylated HC. The percent HC + LC, NGHC, and LMW + MMW were evaluated as tier 2 attributes and the profiles of the rCE-SDS electropherograms were evaluated as a tier 3 attribute.

The %NGHC and %LMW + MMW in ABP 215 are less compared to US-Avastin and EU-Avastin lots, which lead to higher levels of %HC + LC. The calculated mean, SD, median and QRs based 3SD as well as the minimum and maximum ranges of the three species and the lot ages of the extreme range values for ABP 215, US-Avastin, and EU-Avastin based on sponsor’s age calculation formula are shown in the table below:

| % HC + LC         | Mean (%) | SD   | Median (%) | QR (3SD)     | Min/Max Range (Est Age months)       |
|-------------------|----------|------|------------|--------------|--------------------------------------|
| <b>ABP 215</b>    | 98.19    | 0.17 | 98.2       |              | 97.9 to 98.4 (1 to 0) <sup>a</sup>   |
| <b>US-Avastin</b> | 96.13    | 0.29 | 96.1       | 95.3 to 97.0 | 95.4 to 96.6 (3 to 17) <sup>b</sup>  |
| <b>EU-Avastin</b> | 95.94    | 0.25 | 96.0       | 95.2 to 96.7 | 95.2 to 96.3 (13 to 17) <sup>b</sup> |
| % NGHC            | Mean (%) | SD   | Median (%) | QR (3SD)     | Min/Max Range (Est Age months)       |
| <b>ABP 215</b>    | 0.78     | 0.1  | 0.8        |              | 0.6 to 0.9 (0 to 0) <sup>a</sup>     |
| <b>US-Avastin</b> | 1.83     | 0.1  | 1.8        | 1.5 to 2.1   | 1.7 to 2.1 (17) <sup>b</sup>         |
| <b>EU-Avastin</b> | 1.87     | 0.12 | 1.9        | 1.5 to 2.2   | 1.6 to 2.1 (13) <sup>b</sup>         |
| % LMW + MMW       | Mean (%) | SD   | Median (%) | QR (3SD)     | Min/Max Range (Est Age months)       |
| <b>ABP 215</b>    | 0.68     | 0.11 | 0.7        |              | 0.5 to 0.9 (1 to 0) <sup>a</sup>     |
| <b>US-Avastin</b> | 1.54     | 0.22 | 1.5        | 0.9 to 2.2   | 1.2 to 2.3 (19 to 1) <sup>b</sup>    |

|                   |      |      |     |            |                                   |
|-------------------|------|------|-----|------------|-----------------------------------|
| <b>EU-Avastin</b> | 1.69 | 0.16 | 1.7 | 1.2 to 2.2 | 1.4 to 2.0 (17 to 6) <sup>b</sup> |
|-------------------|------|------|-----|------------|-----------------------------------|

<sup>a</sup>Months from date of manufacture

<sup>b</sup>Months to expiry

**Reviewer Comments:**

- The NGHC variant is lower in ABP 215 compared to US-Avastin and EU-Avastin, whereas US-Avastin and EU-Avastin have similar levels. Based on the raw data as well as the age-adjusted data provided by the sponsor, NGHC does not increase due to the age of the product and the range of values reflects the manufacturing process of the three products. In general NGHC could affect product efficacy by its potential to affect effector functions, stability, or immunogenicity. However, ABP 215 does not exhibit effector functionality, similarly to US-Avastin and EU-Avastin. Additionally, the potency of the three products is similar (see Biological Activity similarity assessment section below). Lastly, the potential to affect immunogenicity is low given the very low levels of the NGHC variant, i.e. <1% of the NGHC variant in the ABP 215 product. Therefore, the difference in the level of NGHC is acceptable.
- Due to lower levels of NGHC and LMW + MMW species, the %HC + LC is higher in ABP215 as compared to US-Avastin and EU-Avastin. The justification and acceptability of the lower levels in the percent LMW + MMW variants in ABP 215 is similar to that for the HMW species justification, i.e. that accelerated stability data show that the LMW+MMW increases from 0.6% to 0.9% at one week up to 1.6% by one month upon acceleration of aging (see also Thermal Degradation similarity section below). Therefore, the differences in the rCE-SDS peaks do not affect the conclusion that ABP 215 is analytically similar to US-Avastin and EU-Avastin.
- The raw data show that the EU-Avastin range is similar to the US-Avastin range for all three attributes measured by rCE-SDS. Therefore, the raw data supports the adequacy of the scientific-bridge to use EU-Avastin in the clinical study. This conclusion is also supported by age-adjusted data provided by sponsor.
- The electropherogram profiles of all three products are similar with no new peaks observed in the ABP 215 lots. However, ABP 215 had less LMW, MMW, and NGHC, which is acceptable as discussed above.

**Non-reduced CE-SDS**

Thirteen lots of ABP 215, 24 US-Avastin lots, and 28 EU-Avastin lots were used to determine the levels of main peak and pre-main peak. The percent main peak and pre-main peak were evaluated as a tier 2 attribute and the profiles of the nr-CE-SDS electropherograms were evaluated as a tier 3 attribute.

The raw data for %main peak and pre-main peak in ABP 215 are outside of the US-Avastin QR. The calculated mean, SD, median and QRs based 3SD as well as the minimum and maximum ranges of the two species and the lot ages of the extreme range values for ABP 215, US-Avastin, and EU-Avastin based on sponsor’s age calculation (Material age (months) = testing date – [expiry date of individual lot – 24 months]) are shown in the table below:

| <b>%Main Peak</b> | <b>Mean (%)</b> | <b>SD</b> | <b>Median (%)</b> | <b>QR (3SD)</b> | <b>Min/Max Range (Est Age months)</b> |
|-------------------|-----------------|-----------|-------------------|-----------------|---------------------------------------|
| <b>ABP 215</b>    | 98.05           | 0.52      | 98.3              |                 | 96.7 to 98.4 (5 to 0) <sup>a</sup>    |
| <b>US-Avastin</b> | 97.58           | 0.22      | 97.6              | 96.9 to 98.2    | 96.9 to 97.9 (2 to 17) <sup>b</sup>   |
| <b>EU-Avastin</b> | 97.42           | 0.39      | 97.5              | 96.2 to 98.6    | 96.3 to 98.2 (2 to 17) <sup>b</sup>   |
| <b>% Pre-Peak</b> | <b>Mean (%)</b> | <b>SD</b> | <b>Median (%)</b> | <b>QR (3SD)</b> | <b>Min/Max Range (Est Age months)</b> |
| <b>ABP 215</b>    | 1.75            | 0.41      | 1.6               |                 | 1.5 to 2.9 (0 to 5) <sup>a</sup>      |
| <b>US-Avastin</b> | 2.10            | 0.17      | 2.1               | 1.59 to 2.60    | 1.8 to 2.6 (14 to 2) <sup>b</sup>     |
| <b>EU-Avastin</b> | 2.20            | 0.32      | 2.15              | 1.23 to 3.2     | 1.8 to 3.0 (17 to 2) <sup>b</sup>     |

<sup>a</sup>Months from date of manufacture

<sup>b</sup>Months to expiry

**Reviewer Comments:**

- *ABP 215 and EU-Avastin failed to meet age-adjusted US-Avastin QR for main peak and pre-peak. ABP 215 met the age-adjusted EU-Avastin QR for pre-peaks but not for the main peak. QRs determined from the raw data resulted in the same results as with the age-adjusted QRs with the exception that ABP 215 met the EU-Avastin QR for main peak in addition to meeting the pre-peak similarity acceptance criterion.*
- *The pre-peak for ABP 215 measured in nr-CE-SDS method contain covalent aggregates as well as partially reduced species. The raw data for ABP 215 indicate the range of 1.5 to 2.9% pre-peak represents manufacturing variability as the ages of the lots are close and method precision is high. For the US-Avastin and EU-Avastin lots, the pre-peak levels are higher in older products compared to younger products, which indicates that pre-peak levels increase over time under the approved storage conditions. Similarly, ABP 215 lots analyzed over a period of time showed increases in the pre-peak levels, e.g. lot 0010122029 has 2.9% pre-peak at 5 months and 3.8% pre-peak at 24 months; Lot 0010149803 has 1.5% pre-peak at 0 months and 2.0% pre-peak at 24 months (raw data from Table 15 of the Product-related Substances and Impurities Appendix. The sponsor did not generate ABP 215 DP stability data using nr-CE-SDS; sponsor's justification for not collecting routine stability data with nrCE-SDS because it was orthogonal to SE-HPLC for aggregates and r-CE-SDS for fragments). The justification is acceptable.*
- *Analysis of the raw data provided and of the DS and DP release data show that the development lots are outside of the younger US-Avastin and EU-Avastin lots shown in Table 15. For example, 13 of 15 ABP215 DS lots showed a pre-peak release data range of 1.2% (clinical) to 3.1% (development lot 0010094760). Note that the DP lot 0010122029 mentioned above is derived from DS lot 0010094760. If 3.1% of the development lot is, or if the development lots are, not included in the pre-peak analysis, the DS range becomes 1.2% to 1.7%, and clinical DP range for % pre-peaks is 1.4% to 2.1% while process validation DP lots range from 1.5% to 1.7%. The release data for the clinical and process validation DP lots (1.4% to 2.1%) are similar to the US-Avastin and EU-Avastin lots that represent the younger lots. Therefore, if development lots versus clinical and process validation lots are taken into consideration, as well as the fact that these show no difference in the potency, then it is reasonable to conclude that ABP 215 is analytically similar to US-Avastin and EU-Avastin lots for both main and pre-peaks.*
- *The raw data for EU-Avastin and US-Avastin show that US-Avastin lot variability was smaller than that of EU-Avastin, but the mean and the median values are very close, which supports the sponsor's justification for similarity failure between EU-Avastin and US-Avastin lots being due to the narrow QR derived from the small SD and not a real difference in the two products. In addition, the potencies of the two products are similar. Therefore, the raw data supports the adequacy of the scientific-bridge to use EU-Avastin in the clinical study.*
- *The electropherogram profiles of all three products are similar with no new peaks observed in ABP 215 lots.*

**Cation Exchange-HPLC**

Thirteen lots of ABP 215, 24 US-Avastin lots, and 26 EU-Avastin lots were used to determine the levels of acidic, main, and basic peaks by CEX-HPLC. The percent acidic, main, and basic peaks were evaluated as tier 2 attributes and the profiles of the CEX-HPLC chromatograms were evaluated as a tier 3 attribute.

The raw data for % acidic peaks in ABP 215 shows lower levels of % acidic peaks as compared to US-Avastin and EU-Avastin lots, whereas the %main and %basic peaks were greater than US-Avastin and

EU-Avastin. The calculated mean, SD, median, and QRs based +3SD, as well as the minimum and maximum ranges of the three species and the lot ages of the extreme range values for ABP 215, US-Avastin, and EU-Avastin according to sponsor’s age calculation formula are shown in the table below:

| % Acidic          | Mean (%) | SD   | Median (%) | QR (3SD)     | Min/Max Range (Est Age months)       |
|-------------------|----------|------|------------|--------------|--------------------------------------|
| <b>ABP 215</b>    | 19.06    | 0.72 | 19.4       |              | 17.5 to 20.1 (0 to 1) <sup>a</sup>   |
| <b>US-Avastin</b> | 27.21    | 1.62 | 27.3       | 22.3 to 32.1 | 24.2 to 29.5 (18 to 16) <sup>b</sup> |
| <b>EU-Avastin</b> | 27.32    | 1.15 | 27.3       | 23.9 to 30.8 | 25.7 to 30.7 (15 to 9) <sup>b</sup>  |
| % Main            | Mean (%) | SD   | Median (%) | QR (3SD)     | Min/Max Range (Est Age months)       |
| <b>ABP 215</b>    | 72.38    | 1.61 | 72.8       |              | 69.1 to 75.2 (0 to 0) <sup>a</sup>   |
| <b>US-Avastin</b> | 66.73    | 1.69 | 68.85      | 61.7 to 71.8 | 64.6 to 70.1 (16 to 18) <sup>b</sup> |
| <b>EU-Avastin</b> | 66.47    | 1.51 | 66.6       | 61.9 to 71.0 | 62.8 to 68.3 (9 to 15) <sup>b</sup>  |
| % Basic           | Mean (%) | SD   | Median (%) | QR (3SD)     | Min/Max Range (Est Age months)       |
| <b>ABP 215</b>    | 8.56     | 1.61 | 8.7        |              | 6.6 to 11.5 (0 to 0) <sup>a</sup>    |
| <b>US-Avastin</b> | 6.07     | 0.49 | 6.05       | 4.6 to 7.5   | 5.4 to 7.2 (19 to 14) <sup>b</sup>   |
| <b>EU-Avastin</b> | 6.18     | 0.68 | 6.2        | 4.2 to 8.2   | 5.0 to 7.4 (17 to 12) <sup>b</sup>   |

<sup>a</sup>Months from date of manufacture

<sup>b</sup>Months to expiry

In order to elucidate the differences observed in the acidic, main, and basic peaks, the sponsor treated one lot each of the three products (ABP 0010095534, US lot 970032, and EU lot H0134B01) with carboxypeptidase B prior to CEX-HPLC analysis, and purified the CEX-HPLC acidic, main, and basic peaks to determine activity and variant content by peptide map (LC/MS) analysis. The carboxypeptidase B treatment removes C-terminal lysine. Results showed that the ~2% higher levels of basic peaks in ABP 215 are due to the presence of C-terminal lysine variant (see data below).

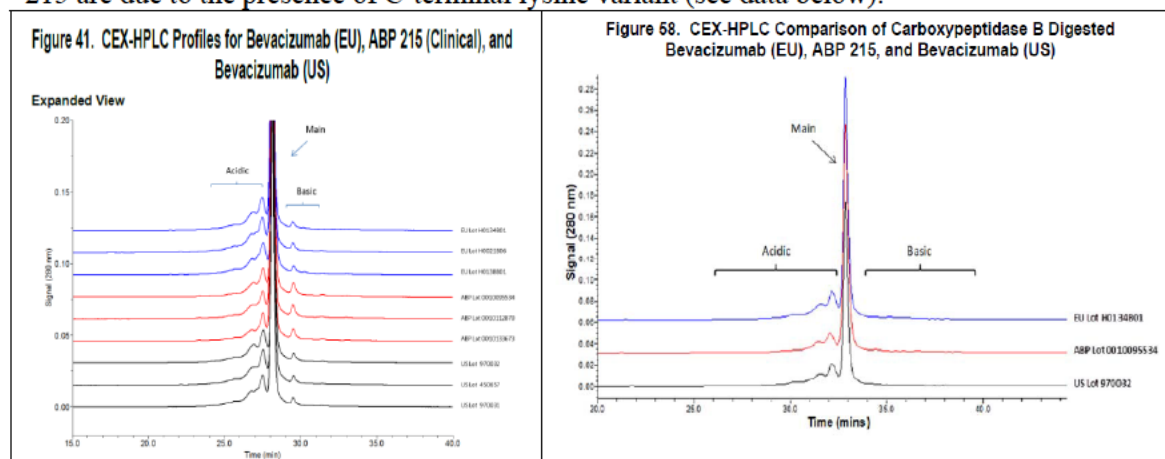


**Table 10. CEX-HPLC Results of Carboxypeptidase B Digested Bevacizumab (EU), ABP 215 (Development), and Bevacizumab (US)**

| Sample             | Before Digestion |             |               | After Digestion |             |               |
|--------------------|------------------|-------------|---------------|-----------------|-------------|---------------|
|                    | % Acidic Peaks   | % Main Peak | % Basic Peaks | % Acidic Peaks  | % Main Peak | % Basic Peaks |
| EU Lot H0134B01    | 28.8             | 65.8        | 5.4           | 29.3            | 65.5        | 5.2           |
| ABP Lot 0010095534 | 20.3             | 72.3        | 7.3           | 21.3            | 73.6        | 5.0           |
| US Lot 970032      | 29.2             | 65.4        | 5.5           | 30.4            | 65.3        | 4.3           |

**Reviewer Comments:** Carboxypeptidase treatment decreased basic peaks of all three products. ABP 215 had the highest amount of C-terminal lysine variant as compared to US-Avastin and EU-Avastin. The digest with carboxypeptidase lowered the basic peaks in ABP 215 to levels that were similar to the levels of US-Avastin and EU-Avastin.

The potency data showed an average of 16% and 20% reduction in activity of the purified acidic and basic peaks, respectively in comparison to the purified main peak. The contribution of the acidic and basic peaks to the overall potency was approximately 10%, e.g. US-Avastin potency was 94% compared to purified main peak, which was 105% (see data table below).

**Table 11. Summary of Bevacizumab (EU), ABP 215, and Bevacizumab (US) Relative Potencies for CEX-HPLC Fractions**

| Lot                | % Relative Potency             |              |           |             |
|--------------------|--------------------------------|--------------|-----------|-------------|
|                    | Starting Material <sup>a</sup> | Acidic Peaks | Main Peak | Basic Peaks |
| EU Lot H0120B01    | 96                             | 89           | 108       | 82          |
| ABP Lot 0010095534 | 90                             | 83           | 98        | 83          |
| US Lot 861171      | 94                             | 90           | 105       | 87          |

<sup>a</sup> Potencies of the starting materials were determined in side-by-side assays with the chromatography fractions.

**Reviewer Comment:** The differences in the potency values are within the validated assay variability of 5% CV. The reduction in potency values for the acidic and basic peaks can be considered similar for the three products. In addition, the peaks could be considered product-related substances as the potency differs at most by 20% compared to the purified main peak and the levels are within the release specifications of 80% to 125%.

The peptide map (LC/MS) analysis of the purified CEX-HPLC peaks showed that in all three products the acidic peak had enriched amounts of deamidated variants (non-CDR region Asn390 and Asn395) and cyclization of the N-terminal Glu residue (pyroglutamate). The basic peaks showed enriched levels of C-terminal lysine variant as well as C-terminal proline amidation in all three products. The peptide map data table is shown below:

**Table 12. Modifications Observed in CEX-HPLC Fractions for Bevacizumab (EU), ABP 215, and Bevacizumab (US)**

| Modifications                                               | EU Lot H0120B01 |           |             | ABP 215 Lot 0010095534 |           |             | US Lot 861171 |           |             |
|-------------------------------------------------------------|-----------------|-----------|-------------|------------------------|-----------|-------------|---------------|-----------|-------------|
|                                                             | Acidic Peaks    | Main Peak | Basic Peaks | Acidic Peaks           | Main Peak | Basic Peaks | Acidic Peaks  | Main Peak | Basic Peaks |
| N-term pE <sup>1</sup> (%)                                  | 5               | 1         | 2           | 4                      | 1         | 1           | 5             | 1         | 2           |
| W <sup>52</sup> Kyneurin (%)                                | 1               | 1         | 1           | <1                     | <1        | <1          | <1            | <1        | 1           |
| N <sup>52</sup> Deamidation (%)                             | <1              | <1        | 1           | <1                     | <1        | <1          | <1            | <1        | <1          |
| N <sup>64</sup> and Q <sup>52</sup> Total Deamidation (%)   | 4               | 1         | 1           | 2                      | 1         | 1           | 2             | 1         | 1           |
| W <sup>106</sup> Oxidation (%)                              | 1               | <1        | 2           | <1                     | <1        | 1           | <1            | 1         | 3           |
| M <sup>258</sup> Oxidation (%)                              | 6               | 5         | 6           | 5                      | 3         | 5           | 5             | 4         | 8           |
| N <sup>360</sup> and N <sup>395</sup> Total Deamidation (%) | 11              | 5         | 4           | 7                      | 4         | 3           | 9             | 3         | 3           |
| M <sup>434</sup> Oxidation (%)                              | 4               | 4         | 4           | 4                      | 2         | 3           | 2             | 2         | 4           |
| C-term K <sup>453</sup> (%)                                 | <1              | <1        | 14          | <1                     | <1        | 18          | <1            | <1        | 13          |
| C-term proline amidation P <sup>451</sup> (%)               | <1              | <1        | 1           | <1                     | <1        | 7           | <1            | <1        | 1           |

**Reviewer Comments:**

- The CEX-HPLC data show lower levels of the acidic variant peak in the ABP 215 compared to US-Avastin and EU-Avastin, which corresponds to higher levels of the main peak. Analysis of only the initial testing time points from the raw data for all three products shows a difference of approximately 8% between the ABP 215 (19%) and the US-Avastin (~27%) and EU-Avastin (~27%)

products. Similarly, the sponsor's age-adjusted data showed a difference of ~7% (ABP 215 mean was 23.6% and US-Avastin mean was 30.2%). The difference in acidic peaks was further analyzed by peptide mapping and the conclusion was no difference in the content of variants in the acidic peaks (data shown in Table 12). However, the data shown in Table 12 is difficult to interpret, in that it is not clear how the variants detected make up the difference in the acidic peak levels. For example, the difference in the deamidated variants are lower in ABP 215 by 2-4% and the difference in the pyroglutamate was only 1%. However, the difference of 3-5% in the acidic variant levels could average out higher if more lots were analyzed.

- Similarly, the difference in basic peaks by peptide map is approximately 4% higher (41% versus 37% ABP215 versus US-Avastin) for ABP 215, but the overall basic peak difference is ~2% (8% versus 6% ABP215 versus US-Avastin) of the total product. However, the carboxypeptidase treatment experiment supports the similarity of the basic peaks in ABP 215 compared to US-Avastin and EU-Avastin.
- The raw data as well as sponsor's age-adjusted data show that ABP 215 fails to meet the similarity acceptance criteria for US-Avastin and EU-Avastin for acidic, main and basic peaks. The sponsor stated that the age-adjusted data indicate that the acidic peak in ABP 215 will be lower by the 24 months timepoint and the basic peak will be higher by the 24 months timepoint as compared to the US-Avastin and EU-Avastin lots. In agreement with the sponsor's conclusion and data, the ABP 215 primary stability data at accelerated temperature shows that the acidic levels in ABP 215 did not reach 27% even at the 1 month time point. Therefore, the data indicate that levels of acidic peaks are higher in US-Avastin and EU-Avastin at release and that this is not only due to a difference in the age of the product. However, the difference in CEX acidic, main, and basic peak levels were shown not to impact ABP 215 potency. Additional characterization showed that the contents of the purified peaks are similar, with lower levels of acidic variants and increased C-terminal lysine variant (known not to impact efficacy) and C-terminal proline amidation. Therefore, it is reasonable to conclude that the differences observed in the levels of the acidic, main, and basic peaks will not have a clinical impact. Regarding safety, the higher levels of basic peaks in ABP 215 could result in difference in immunogenicity, but the clinical data did not show a difference in the immunogenicity rates between ABP 215 and EU-Avastin.
- Another point to consider is that within the basic peaks, ABP215 characterization studies showed that high mannose and galactosylated glycans are also present. Higher amounts of these glycan species contributes to the higher levels of basic peaks in ABP215 in comparison to US-Avastin and EU-Avastin.
- CEX-HPLC analysis showed that the chromatogram profiles are similar for all three products.
- The raw data and the sponsor's age-adjusted data support the similarity of the EU-Avastin lots to US-Avastin lots. Therefore, the data supports the scientific-bridge to use EU-Avastin in the clinical study.

#### 3.2.R.4.5 Thermal Degradation

The sponsor selected temperature stress to delineate and compare the similarity of the degradation pathways for ABP 215, US-Avastin, and EU-Avastin using four methods, the SE-HPLC, rCE-SDS, CEX-HPLC, and potency. The three temperatures chosen for the stress studies were 50°C, 40°C, and 25°C. The combination of results from the methods used to evaluate the degradation pathways helped to evaluate the similarity of the three products. The results of each thermal stress condition studies are reviewed below.

##### Forced Degradation Study at 50°C

The temperature stress at 50°C was performed with the goal of producing a reduction in the main peak by approximately 10% with concomitant increase in degradants by 10%. The sponsor stated that product degradation beyond ~10% would result in loss of method precision as peaks widen and merge with other

peaks. Therefore, for SE-HPLC, rCE-SDS, and potency methods, the duration of the thermal degradation study was 14-15 days, whereas for the CEX-HPLC method, the duration was only up to four days. The comparison of the degradation profiles is evaluated as a tier 3 attribute.

**Reviewer Comment:** *The justification provided by the sponsor for the duration lengths at 50°C is acceptable not because method precision would decrease as this is not a tier 2 attribute and the peaks are not quantified, but because as the peaks widen and merge, it would be difficult to assess the similarity of the peak profiles.*

Six lots of ABP 215 (development and clinical), US-Avastin, and EU-Avastin were tested side-by-side. The 50°C stress study using ABP development lots was performed for up to 15 days, whereas the ABP 215 clinical lots were tested with sampled held under the stressed condition for up to 14 days and with less frequency as compared to the development lots (daily for development lots compared to days 0, 3, 7, 10, and 14 for clinical lots) for all methods, with the exception of the CEX-HPLC method that was concluded after four days. The approximate age of the US-Avastin and EU-Avastin lots are similar, whereas the ABP 215 development lots are approximately four months younger than clinical lots (see Table 1 below).

**Table 1. Drug Product Lots Used in the 50°C Forced Degradation Study**

| Lot                         | Estimated Material Age at Study Initiation (Months) |                                         |
|-----------------------------|-----------------------------------------------------|-----------------------------------------|
|                             | Side-by-side with ABP 215 Development Lots          | Side-by-side with ABP 215 Clinical Lots |
| EU H0120B01                 | 10                                                  | N/A                                     |
| EU H0118B02                 | 10                                                  | N/A                                     |
| EU H0114B01                 | 13                                                  | N/A                                     |
| EU H0021B06                 | N/A                                                 | 15                                      |
| EU H0134B01                 | N/A                                                 | 11                                      |
| EU H0138B01                 | N/A                                                 | 11                                      |
| ABP 0010085286 <sup>a</sup> | 3                                                   | N/A                                     |
| ABP 0010085284 <sup>a</sup> | 3                                                   | N/A                                     |
| ABP 0010085282 <sup>a</sup> | 4                                                   | N/A                                     |
| ABP 0010095534 <sup>b</sup> | N/A                                                 | 8                                       |
| ABP 0010112870 <sup>b</sup> | N/A                                                 | 7                                       |
| ABP 0010133673 <sup>b</sup> | N/A                                                 | 0                                       |
| US 927074                   | 10                                                  | N/A                                     |
| US 913149                   | 11                                                  | N/A                                     |
| US 911121                   | 11                                                  | N/A                                     |
| US 970032                   | N/A                                                 | 12                                      |
| US 450657                   | N/A                                                 | 11                                      |
| US 970031                   | N/A                                                 | 11                                      |

N/A: not applicable.

<sup>a</sup> ABP 215 development lots.

<sup>b</sup> ABP 215 clinical lots.

**Reviewer Comment:** *The clinical lot degradation assessment up to 14 days as well as reduced frequency of testing compared to the 15 days is acceptable because the clinical ABP 215 lot stress duration and sampling frequencies are sufficient to capture product degradation over time for comparison purposes.*

The results showed that the profiles of the SE-HPLC chromatograms for the ABP 215, US-Avastin, and EU-Avastin lots were similar with no difference in the number and positions of the peaks. The 14 day study showed slightly reduced peak heights compared to the 15 day study, but the number of peaks was the same and the retention times of the peaks were the same among the three products although slightly shifted compared to the 15 day study. The degradation rates were plotted for the HMW species and main

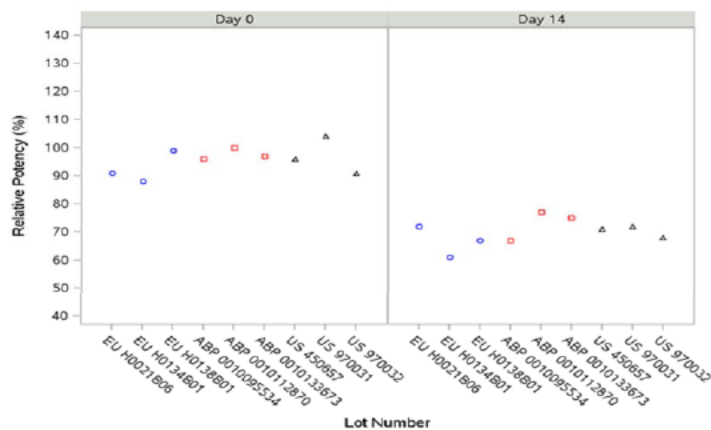
peak. The chromatogram overlays and the degradation rate plots are presented in the BLA and support similarity of the products.

Similar to the results for SE-HPLC method, the rCE-SDS showed the same number of peaks and peak positions within the same run. No difference other than peak heights was observed for the 15 day versus 14 day study, where there were lower peak heights at 14 days due to less product degradation for all three products. The degradation rates were plotted for the %HC + LC and % LMW + MMW. The electrophoretograms overlays and rate plots are found in the submission.

The results from the CEX-HPLC runs of samples collected up to four days showed same number of peaks and retention times within runs. The degradation rates were plotted for the acidic, main, and basic peaks. The chromatogram overlays and degradation rate plots are found in the submission and support a similar profile.

Only clinical ABP 215 lots were used to perform the potency assay side-by-side with US-Avastin and EU-Avastin lots. The results show that all three products lost approximately 20% in potency after 14 days at 50°C. The results are shown below:

Figure 19. Relative Potency Degradation Profile for Bevacizumab (EU), ABP 215 (Clinical), and Bevacizumab (US) on Day 0 and Day 14 at 50°C



**Reviewer Comment:** The results from the SE-HPLC, rCE-SDS, CEX-HPLC, and potency analysis of the 50°C degraded samples show that the degradation profiles are similar for all three products, which indicates that the three products are similar in structure and supports a demonstration of highly similar.

#### Forced Degradation Study at 40°C (Stressed Condition)

Two ABP 215 lots (one development (at 3 months (M)) and one clinical lot (0 M)), one US-Avastin lot (12 M), and one EU-Avastin lot (7 M) were used to determine the degradation profiles at 40°C. All methods evaluated samples that were incubated up to 14 days at 40°C. The sponsor noted that the variabilities of the degradation rates could be attributed to the fact that the 40°C was not conducted side-by-side. Only the degradation rate plots were provided for the four methods and the results are consistent with the 50°C condition study. Therefore, the plots are not reproduced in the review.

#### Reviewer Comments:

- The degradation at 40°C is less compared to at 50°C and approaches the 10% degradation amount only for CEX-HPLC method at 14 days.
- Contrary to the higher levels of degradants found in the US-Avastin and EU-Avastin lots, the potency for ABP 215 appears to decrease over time compared to US-Avastin lot. It is likely that the decrease in potency observed for ABP 215 is likely not significant as the test was not performed side-by-side and the result at 14 days is within the method variability of 5%.

- *Lack of chromatograms and electropherograms in the BLA for the 40°C samples are not an issue in this case as the 50°C condition is considered worse case based on the data for product degradation and no new peaks were observed in the 50°C condition chromatograms and electropherograms, and this worse case still gave very clear results, i.e. was not overly degraded to make assessment impossible.*
- *The 40°C thermal stress study result supports the 50°C study results in that there are similar patterns in the reduction in the main peak and increases in degradants, which support the conclusion that the three products are structurally similar and supports a demonstration of highly similar.*

#### Thermal Stability Study at 25°C (Accelerated Condition)

The same two ABP 215 lots (one development (3M) and one clinical lot (0 M)), one US-Avastin lot (12 M), and one EU-Avastin lot (7 M)) were used to determine the degradation profiles at 25°C. All methods evaluated samples that were incubated at the 25°C condition for up to 3 M. The sponsor also noted that the variabilities of the degradation rates could be attributed to the fact that the 25°C thermal stability study was not conducted side-by-side. Only degradation rate plots were provided for the four methods and the results are consistent with the 50°C condition study. Therefore, the plots are not reproduced in the review.

#### **Reviewer Comments:**

- *The degradation at 25°C is less compared to at 40°C and 50°C. The incubation time period extends to 3M in order to observe a degradation that is near 10% for CEX-HPLC method.*
- *Similar to the potency results at 40°C, the potency for ABP 215 as well as EU-Avastin lot appear to decrease over time compared to US-Avastin lot, which appear to increase over time. It is likely that the decrease in potency trend observed for ABP 215 and EU-Avastin lots and the increasing trend for US-Avastin lot are likely not significant as the result was not performed side-by-side, but no additional data or justification were provided by sponsor.*
- *Lack of chromatograms and electrophoretograms for the 25°C samples are acceptable as 50°C temperature is considered worse case based on the data for product degradation and no new peaks were observed in the 50°C condition raw data.*
- *In conclusion, the thermal stress studies showed that the three products are structurally similar and have similar degradation profiles.*

#### **3.2.R.4.6 General Properties**

The general properties of ABP 215, US-Avastin, and EU-Avastin lots were evaluated for similarity. Protein concentration was the only attribute in the general properties that was evaluated as a tier 2 attribute due to its impact on patient dosing and efficacy. All other attributes, such as the formulation properties, pH, osmolality, appearance, color, and clarity as well as volume were evaluated as a tier 3.

#### Protein Concentration

Thirteen ABP 215 lots, 24 US-Avastin, and 26 EU-Avastin lots were measured for protein concentration using ultraviolet spectrophotometer at 280 nm. The extinction coefficient used was 1.7 mL mg<sup>-1</sup>cm<sup>-1</sup> that was theoretically and experimentally determined. The sponsor experimentally determined the extinction coefficient from 1 lot each of ABP215, US-Avastin, and EU-Avastin. Results of 1.8 mL mg<sup>-1</sup>cm<sup>-1</sup> for US/EU and 1.7 mL mg<sup>-1</sup>cm<sup>-1</sup> for ABP 215 were within the sponsor's acceptance criterion of 10%.

The mean values for protein concentration for ABP 215, US-Avastin, and EU-Avastin were 25.0, 24.8, and 25.3 mg/mL, respectively. ABP 215 met US-Avastin and EU-Avastin QRs and EU-Avastin met the US-Avastin QR.

**Reviewer Comment:** *The data provided supports the conclusion that the three products are similar in protein concentration and supports a demonstration of highly similar.*

#### Volume

The volume for the 100 mg and 400 mg presentations of ABP 215, US-Avastin, and EU-Avastin were determined using the USP <1> compendial method for determining extractable volume. For the 100 mg presentation, seven ABP 215 lots, six US-Avastin lots, and five EU-Avastin lots were measured and for the 400 mg presentation, 12 lots of ABP 215, six US-Avastin lots, and eight EU-Avastin lots were measured.

The mean volumes for the 100 mg presentation were 4.2, 4.3, and 4.4 mL for ABP 215, US-Avastin, and EU-Avastin lots, respectively. The mean volumes for the 400 mg presentation were 16.4, 16.3, and 16.5 mL for ABP 215, US-Avastin, and EU-Avastin, respectively.

**Reviewer Comment:** *The data provided supports the conclusion that the three products are similar in extractable volume for both DP presentations.*

#### Osmolality

USP <785> compendial method was used to measure osmolality of thirteen ABP 215 lots, three US-Avastin, and three EU-Avastin lots. The range of osmolality values for ABP 215 was 275 to 282, and the individual results for the US-Avastin and EU-Avastin lots were, 275, 273, 274, and 275, 272, 277 mOSm/kg, respectively.

**Reviewer Comment:** *The osmolality results for the three products were within 10 mOSm/kg of each other and support the conclusion that the osmolality of the three products are similar.*

#### pH

USP <791> compendial method was used to measure the pH of thirteen ABP 215 lots, three US-Avastin, and three EU-Avastin lots. The range of pH values for ABP 215 was 6.0 to 6.2, and the median value was 6.1. The pH for US-Avastin and EU-Avastin lots were all at 6.2. The Avastin label states the pH is 6.2 (b) (4). A difference up to 0.2 pH units is stated by the sponsor to not impact product safety or efficacy, given that the product is diluted prior to intravenous administration.

**Reviewer Comment:** *A difference in pH could affect product stability. A formulation study that examined the effect of pH range (b) (4) on ABP 215 did show (b) (4), but the increase over time was not different within the range of pH (b) (4) when stored at 4°C (real time storage condition). In addition, the majority of the 13 ABP 215 lots (11 out of 13) were at pH 6.1. Therefore, a difference of 0.1 to 0.2 pH units is not significant and the impact to product safety and efficacy is considered low.*

#### Appearance, color, and clarity

USP <1>, <631>, and <855> compendial methods for appearance, color, and clarity were used on thirteen ABP 215 lots, three US-Avastin, and three EU-Avastin lots. The results met the requirement of “no particles” or “practically free from particles” for appearance, “colorless” or “colorless to slightly yellow” for color, and “< Ref III” for clarity for all tested lots.

**Reviewer Comment:** *The sponsor demonstrated that all three products had similar protein concentration, volume, osmolality, pH, appearance, color, and clarity.*

### 3.2.R.4.7 Process-Related Impurities

The similarity in the levels of process-related impurities such as host cell protein (HCP), host cell DNA, and protein-A, were evaluated for any significant differences in ABP 215 that could impact product safety. (b) (4)

the process-related impurities were evaluated as tier 3 attributes.

**Reviewer Comment:** *The sponsor's justification for tier 3 category form the measured attributes and the purpose of the similarity exercise for process-related impurities are acceptable.*

#### Host Cell Protein

The sponsor applied three different techniques to determine the HCP levels and content. Specifically, ABP 215, US-Avastin, and EU-Avastin lots were evaluated with ABP 215 product-specific HCP ELISA, 2-dimensional RP-HPLC coupled with online MS with data-independent (MS<sup>E</sup>) acquisition (2D-LC-MS<sup>E</sup>) method, and a 2-D differential in-gel electrophoresis (2D-DIGE) method. Thirteen ABP 215 lots were used to evaluate HCP using the ELISA, whereas for the 2D-LC-MS<sup>E</sup> and 2D-DIGE methods, only two and one lot(s) of ABP 215 were used, respectively as these methods are considered for characterization purposes only. For the US-Avastin and EU-Avastin lots, the sponsor used three, two, and one lot(s) were used for the ELISA, 2D-LC-MS<sup>E</sup>, and 2D-DIGE methods, respectively.

The results from the HCP ELISA showed a mean value of 6 ng/mg for all three products. The 2D-LC-MS<sup>E</sup> method was not able to detect HCPs above the method LOQ, which is stated to be 13 ng/mg for any individual protein in all three products. Lastly, the 2D-DIGE gels showed similar staining patterns that when scanned and quantified for differences between the ABP 215 and US-Avastin lots, showed a similarity of 98.8%.

**Reviewer Comment:** *All three methods showed similar levels and profiles of HCPs in ABP 215, US-Avastin, and EU-Avastin lots and supports a demonstration of highly similar..*

#### Protein A

Thirteen ABP 215, three US-Avastin, and EU-Avastin lots were used to measure residual protein A levels with a qualified ELISA method. All samples from the three products are reported as < 1 ng/mg, which indicates the lot values were zero after rounding to the nearest whole number (LOQ is reported as 0.125 ng/mg).

**Reviewer Comment:** *All three products showed similar levels of residual protein A.*

#### Residual DNA-Quantitative Polymerase Chain Reaction

Thirteen ABP 215, three US-Avastin, and EU-Avastin lots were used to measure residual host cell DNA levels with the qPCR method. All samples from the three products are reported as < 1 pg/mg, with the exception of two EU-Avastin lots that were < 2 pg/mg (LOQ is reported as 1.0 pg).

#### **Reviewer Comments:**

- *All three products showed similar levels of residual host cell DNA levels.*
- *In conclusion, all three products showed similar levels of process-related impurities that further supports the overall similarity of the three products.*

### 3.2.R.4.8 Biological Activity

The sponsor evaluated the similarity of the biological functions for ABP 215, US-Avastin, and EU-Avastin that included the examination of binding to the target antigen, VEGFA, and the inhibition of endothelial cell proliferation. As both VEGFA binding and anti-proliferation of endothelial cells

represent the proposed mechanism of action for Avastin, the VEGFA binding and endothelial proliferation were categorized as tier 1 attributes for their critical impact to product efficacy. As part of the VEGFA binding studies, the similarity in the binding kinetics to VEGFA121 and the specificity of binding to VEGFA family members, VEGFC and VEGFD, and VEGFA isoforms, VEGFA121 and VEGFA165, were also performed. The additional VEGFA binding, kinetics, and specificity studies were evaluated as tier 3 attributes.

Other biological activity assays examined in the analytical similarity study included comparisons of the binding activities of the Fc portion of ABP 215, US-Avastin, and EU-Avastin to the neonatal Fc receptor, FcRn, to the complement cascade component protein, C1q, and to FcγRIIIa. These were evaluated as tier 2 attributes because of their potential impact to PK and safety, the latter due to unwanted effector functions leading to antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). The sponsor 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 were evaluated as tier 3 attributes.

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

#### Proliferation Inhibition Bioassay (Potency)

Thirteen ABP 215, 24 US-Avastin, and 27 EU-Avastin lots were used to determine the inhibition of endothelial cell proliferation. Human umbilical vein endothelial cells (HUVEC) that express VEGF receptors, VEGFR1, VEGFR2, and VEGFR3 as well as VEGF co-receptors, Neuropilin 1 and 2, were used in the proliferation assay. The VEGFA isoform used to induce the proliferation of the HUVECs was originally stated to be VEGFA121, but then corrected to VEGFA111 in a response to IRs (received 2/22/17), and supplemental data justifying its use was provided and is considered appropriate. The sponsor also indicated in IR response (3/28/17) that the proliferation of HUVECs stimulated by VEGFA111 is the current potency assay for release and stability testing of ABP 215 and was used throughout development.

#### **Reviewer Comments:**

- *In response to CMC stats IR submitted on 2/13/17, the sponsor indicated that the number of lots for EU-Avastin was 27 lot and not 25. The data for the proliferation inhibition assay has been updated below as of 2/23/17. The additional 2 lots of EU-Avastin did not change the outcome of the tier 1 equivalency results.*
- *In response submitted on 1/31/17 regarding the use of VEGFA 121 and 165 isoforms in SPR binding as tier 3 attribute (see below review section) the sponsor updated the isoform used in several biological activity assays (IR response sent 2/22/17). As stated above, the VEGFA isoform used in the proliferation inhibition assay is VEGFA 111. VEGFA isoforms 165 and 121 are often identified as the major isoforms detected in various solid tumors, with a shift in relative ratios of these 2 isoforms when compared to normal tissue (Vempati, P. et.al. 2014 Cytokine & Growth Factor Rev 25: 1-19). One critical difference between VEGFA isoforms 165 and 121 is the presence of the heparin-binding site in isoform 165, which increases its association with the cell surface. An additional difference is the ability to bind the neuropilin co-receptors thereby modifying the intensity of the signal through the VEGF receptors. VEGFA 111 lacks both the neuropilin co-receptor binding site and the heparin binding site. Additionally, based on data currently found in the literature, the VEGFA 111 isoform has not been identified as being expressed in normal human tissue or in tumors. To assess the acceptability of the VEGFA 111 isoform in the assessment of analytical similarity using the HUVEC assay, additional data were requested to demonstrate similar binding kinetics of each, ABP215, US-Avastin and EU-Avastin, to VEGFA 165, VEGFA 121 and 111. In addition, data were requested to*

demonstrate that the HUVEC assay performance using all three VEGFA isoforms, 111,121, and 165 demonstrates similar sensitivity to detect changes in the product (IRs sent 3/21/17 and 4/4/17).

- Based on the data provided on 3/28/17 and 5/26/17, the binding to all three VEGFA isoforms were similar among the three products, but the binding affinities to the VEGFA isoforms differed in that all three products bound to VEGFA 165 with higher affinities as compared to VEGFA 111 or 121. The HUVEC proliferation assay using 50°C stressed ABP 215 samples showed that at for undegraded product or for product mildly degraded over short time at 50°C, all three VEGFA isoform showed similar activity using the HUVEC assay. VEGFA 165 had slightly better sensitivity to detect product degradation after a more extended incubation at 50°C, but the difference compared to the ability of VEGFA 111 or 121 was no more than 13%. Given the totality of the data provided, the use of VEGFA 111 was found to be acceptable for use in the tier 1 bioassay analysis. It should be noted that the acceptability of use of VEGFA 111 is based on the specific assays used, and if sponsor decides to modify or add any additional assays for activity those assays would have to be assessed for specific impact of using VEGFA 111 as compared to VEGFA 121 or 165.

As a tier 1 attribute, the sponsor applied equivalency testing to determine if the ABP 215 results were statistically equivalent to US-Avastin and to EU-Avastin, and if EU-Avastin was statistically equivalent to US-Avastin. The results shown below demonstrate statistical equivalence of ABP 215 and EU-Avastin to US-Avastin and ABP 215 to EU-Avastin.

Table 2. Equivalence Test Results for Relative Potency - ABP 215 to Bevacizumab (US)

| Assay/Attribute  | Bevacizumab (US) Mean | ABP 215 Mean | Estimated Difference Across Means | Lower Bound of 90% Confidence Interval on Difference in Means | Upper Bound of 90% Confidence Interval on Difference in Means | EAC    | Conclusion               |
|------------------|-----------------------|--------------|-----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------|--------------------------|
| Relative potency | 95.49                 | 98.43        | 2.94                              | 0.67                                                          | 5.22                                                          | ± 5.87 | Statistically equivalent |

Figure 2. Equivalence Graphs for Relative Potency - ABP 215 to Bevacizumab (US)

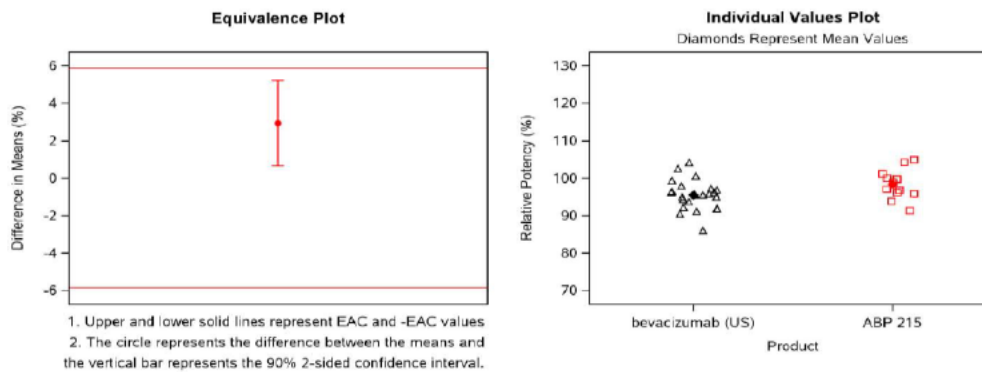


Table 3. Equivalence Test Results for Relative Potency - ABP 215 to Bevacizumab (EU)

| Assay/Attribute  | Bevacizumab (EU) Mean | ABP 215 Mean | Estimated Difference Across Means | Lower Bound of 90% Confidence Interval on Difference in Means | Upper Bound of 90% Confidence Interval on Difference in Means | EAC    | Conclusion               |
|------------------|-----------------------|--------------|-----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------|--------------------------|
| Relative Potency | 95.18                 | 98.43        | 3.25                              | 1.05                                                          | 5.45                                                          | ± 5.54 | Statistically Equivalent |

Figure 3. Equivalence Graphs for Relative Potency - ABP 215 to Bevacizumab (EU)

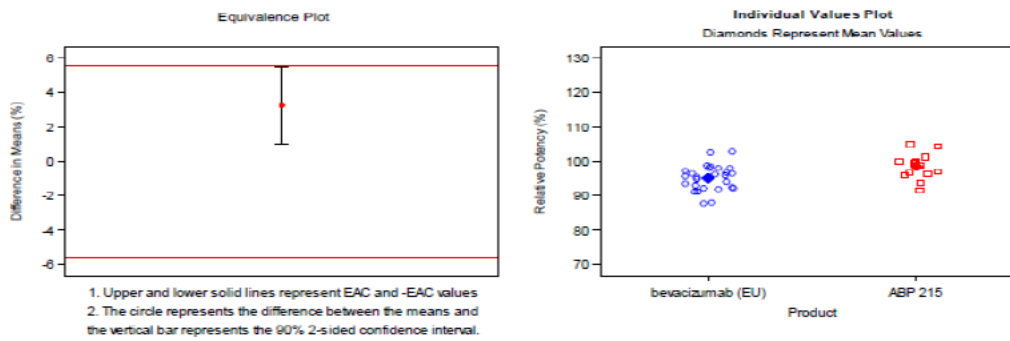
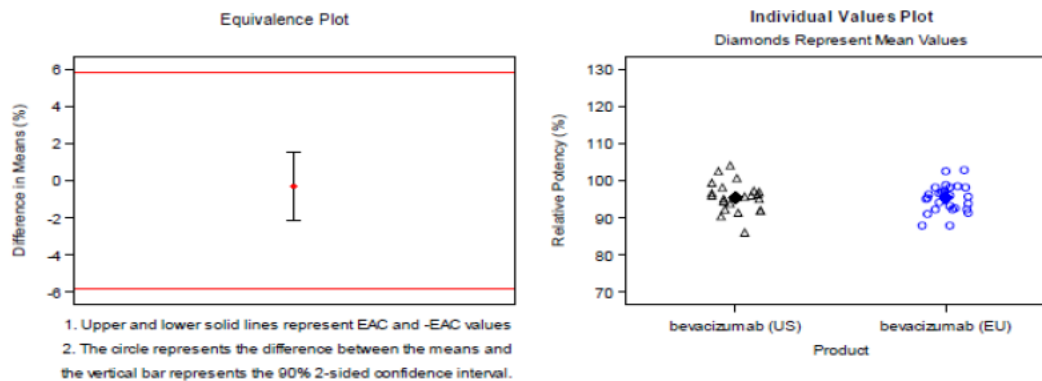


Table 4. Equivalence Test Results for Relative Potency – Bevacizumab (EU) to Bevacizumab (US)

| Assay/Attribute  | Bevacizumab (US) Mean | Bevacizumab (EU) Mean | Estimated Difference Across Means | Lower Bound of 90% Confidence Interval on Difference in Means | Upper Bound of 90% Confidence Interval on Difference in Means | EAC    | Conclusion               |
|------------------|-----------------------|-----------------------|-----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------|--------------------------|
| Relative Potency | 95.49                 | 95.18                 | -0.31                             | -2.10                                                         | 1.49                                                          | ± 5.87 | Statistically Equivalent |

Figure 4. Equivalence Graphs for Relative Potency – Bevacizumab (EU) to Bevacizumab (US)



**Reviewer Comments:**

- The statistics used to determine equivalence was found acceptable by CDER’s CMC statisticians.
- The results indicate similar anti-proliferative effects by all three products using VEGFA111.

VEGFA-165 Binding by ELISA

Thirteen ABP 215, 14 US-Avastin, and 13 EU-Avastin lots were used to determine binding to recombinant VEGFA165. An ELISA format that uses VEGFA165 to coat wells and capture binding antibodies was used in the binding study. The bound antibodies are detected by a secondary anti-IgG Fc antibody conjugated to horseradish peroxidase. The relative binding of test samples is calculated against a dose-response curve of the ABP 215 reference standard.

**Reviewer Comment:** In response to an IR, the VEGFA isoform used in this study has been updated by the sponsor from VEGFA 121 to VEGFA 165. Use of VEGFA 165 in this tier 1 assay helps establish that the binding activities of the three bevacizumab products to the more prevalent VEGFA isoform is similar for all three products. However, binding activity alone would be inadequate to demonstrate high similarity to US-Avastin and bioassay, specifically the HUVEC bioassay, data are necessary in the similarity exercise.

As a tier 1 attribute, the sponsor applied equivalency testing to determine if the ABP 215 results were statistically equivalent to US-Avastin and EU-Avastin as well as if EU-Avastin was statistically

equivalent to US-Avastin. The results shown below demonstrate statistical equivalence of ABP 215 and EU-Avastin to US-Avastin and ABP 215 to EU-Avastin.

**Table 6. Equivalence Test Results for VEGF-A Binding - ABP 215 to Bevacizumab (US)**

| Assay/Attribute  | Bevacizumab (US) Mean | ABP 215 Mean | Estimated Difference Across Means | Lower Bound of 90% Confidence Interval on Difference in Means | Upper Bound of 90% Confidence Interval on Difference in Means | EAC    | Conclusion               |
|------------------|-----------------------|--------------|-----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------|--------------------------|
| Relative binding | 89.11                 | 89.85        | 0.74                              | - 2.77                                                        | 4.25                                                          | ± 9.63 | Statistically equivalent |

**Figure 6. Equivalence Graphs for VEGF-A Binding - ABP 215 to Bevacizumab (US)**

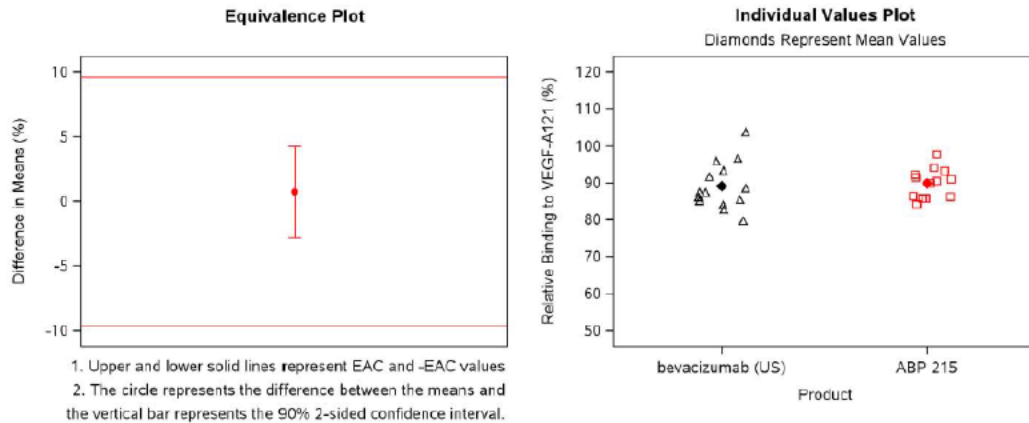


**Table 7. Equivalence Test Results for VEGF-A Binding - ABP 215 to Bevacizumab (EU)**

| Assay/Attribute  | Bevacizumab (EU) Mean | ABP 215 Mean | Estimated Difference Across Means | Lower Bound of 90% Confidence Interval on Difference in Means | Upper Bound of 90% Confidence Interval on Difference in Means | EAC    | Conclusion               |
|------------------|-----------------------|--------------|-----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------|--------------------------|
| Relative binding | 84.92                 | 89.85        | 4.93                              | 1.35                                                          | 8.52                                                          | ± 9.51 | Statistically equivalent |

**Figure 7. Equivalence Graphs for VEGF-A Binding - ABP 215 to Bevacizumab (EU)**

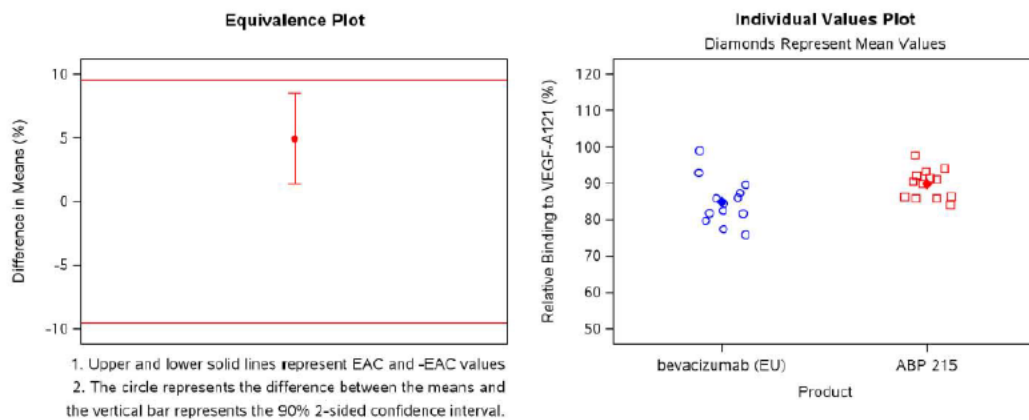
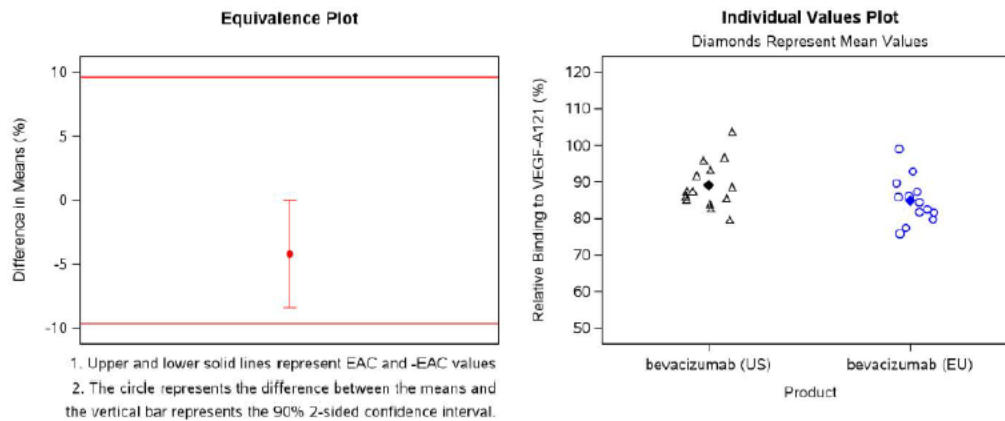


**Table 8. Equivalence Test Results for VEGF-A Binding - Bevacizumab (EU) and Bevacizumab (US)**

| Assay/Attribute  | Bevacizumab (US) Mean | Bevacizumab (EU) Mean | Estimated Difference Across Means | Lower Bound of 90% Confidence Interval on Difference in Means | Upper Bound of 90% Confidence Interval on Difference in Means | EAC    | Conclusion               |
|------------------|-----------------------|-----------------------|-----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------|--------------------------|
| Relative binding | 89.11                 | 84.92                 | -4.20                             | -8.39                                                         | 0.00                                                          | ± 9.63 | Statistically equivalent |

Figure 8. Equivalence Graphs for VEGF-A Binding – Bevacizumab (EU) to Bevacizumab (US)



**Reviewer Comments:**

- As originally stated in the BLA, only VEGFA121 was identified as used for tier 1 attribute assays. The sponsor stated that VEGFA121 was chosen as the “common” isoform. However, as discussed above and based on biological relevance and potential differences due to differences in a heparin binding site and neuropilin binding, the current expectation is to use both VEGFA 165 and 121 isoforms in evaluation of the tier 1 potency and binding assays. However, with adequate justification, if not using one of these in a tier 1 assay, VEGFA 165 or 121, as relevant, could also be used in a tier 2 binding assay. In this case, the sponsor placed binding to VEGFA 165 and 121 isoforms by SPR in tier 3. An IR was sent to justify the placement of VEGFA 165 in tier 3 rather than in tier 1.
- In response to the IR, the sponsor reassessed the isoforms used in their assays and updated the BLA with the fact that the isoform used in the ELISA binding assay was VEGFA 165 (identified as 121 in Error). Additional updates the VEGFA isoforms used in the Biological Activity studies are shown the table below:

**Table 1. Comparison of VEGF-A Isoforms used in Fab-mediated Assays**

| Assay Name                                    | Tier | Isoform Listed in Original Filing               | Isoform Used in Assay                           |
|-----------------------------------------------|------|-------------------------------------------------|-------------------------------------------------|
| Proliferation inhibition bioassay (potency)   | 1    | VEGF-A <sub>121</sub>                           | VEGF-A <sub>111</sub>                           |
| VEGF-A binding by ELISA                       | 1    | VEGF-A <sub>121</sub>                           | VEGF-A <sub>165</sub>                           |
| VEGF-A binding and kinetics by SPR            | 3    | VEGF-A <sub>121</sub>                           | VEGF-A <sub>111</sub>                           |
| Binding specificity to VEGF-A isoforms        | 3    | VEGF-A <sub>121</sub> and VEGF-A <sub>165</sub> | VEGF-A <sub>121</sub> and VEGF-A <sub>165</sub> |
| Inhibition of VEGFR2 RTK auto-phosphorylation | 3    | VEGF-A <sub>121</sub>                           | VEGF-A <sub>111</sub>                           |

- The isoforms used in the assays were verified during inspection at the facility.
- The statistics used to determine equivalence was found acceptable by CMC statisticians.
- The results indicate similar relative binding by all three products to VEGFA165.
- The sponsor stated that the justification to use VEGFA 121 and 165 by SPR as a tier 3 attribute was because this assay is considered supportive data to the ELISA binding assay. Sponsor claims that a single isoform is also sufficient for the ELISA tier 1 assay because both ABP 215 and bevacizumab

*bind to a common epitope on all VEGFA isoforms and because bevacizumab was shown in literature to not interfere with VEGFA long isoforms ability to bind to heparins or the neuropilin co-receptors. The justification is acceptable.*

#### VEGFA-111 Binding Kinetics and Affinity by SPR

Surface plasmon resonance (SPR) method was used to determine the binding kinetics of ABP 215, US-Avastin, and EU-Avastin. The SPR method was performed on three lots each of ABP 215, US-Avastin, and EU-Avastin. The method employed a ProteOn XPR36 optical biosensor with a GLC sensor chip. The GLC sensor chip is coated with goat anti-human IgG antibody to bind, capture, and cross-link the products onto the chip. Recombinant VEGFA111 was injected into the reaction chamber and the concentration range used was 50.0 to 3.13 nM. The assay was performed in triplicates and the results are reported as three intra-assay replicates per lot.

Table 9 below shows the association and dissociation rate constants ( $k_a$  and  $k_d$ ) and the dissociation equilibrium binding constant ( $K_D$ ) of ABP 215, US-Avastin, and EU-Avastin are similar.

**Table 9. Binding Kinetics and Affinity of VEGF-A121 to Bevacizumab (EU), ABP 215, and Bevacizumab (US)**

| Lot         | $k_a$<br>( $M^{-1}s^{-1}$ ) $\times 10^5$ | $k_d$<br>( $s^{-1}$ ) $\times 10^{-6}$ | $K_D$<br>(pM) | Lot            | $k_a$<br>( $M^{-1}s^{-1}$ ) $\times 10^5$ | $k_d$<br>( $s^{-1}$ ) $\times 10^{-6}$ | $K_D$<br>(pM) | Lot       | $k_a$<br>( $M^{-1}s^{-1}$ ) $\times 10^5$ | $k_d$<br>( $s^{-1}$ ) $\times 10^{-6}$ | $K_D$<br>(pM) |
|-------------|-------------------------------------------|----------------------------------------|---------------|----------------|-------------------------------------------|----------------------------------------|---------------|-----------|-------------------------------------------|----------------------------------------|---------------|
| EU B7115B10 | 3.5                                       | 8.0                                    | 22.9          | ABP 0010095534 | 3.4                                       | 8.2                                    | 23.5          | US 605024 | 3.4                                       | 8.1                                    | 23.4          |
| EU B7003B03 | 3.5                                       | 6.5                                    | 18.4          | ABP 0010112870 | 3.5                                       | 7.7                                    | 22.6          | US 616370 | 3.5                                       | 7.6                                    | 22.9          |
| EU B7108B02 | 3.4                                       | 8.2                                    | 23.4          | ABP 0010133673 | 3.5                                       | 7.3                                    | 20.1          | US 640016 | 3.4                                       | 7.8                                    | 23.9          |

$k_a$  = association rate constant;  $k_d$  = dissociation rate constant;  $K_D$  = the equilibrium dissociation constant, a ratio of  $k_d/k_a$ ; pM = picomolar; VEGF-A121 = recombinant human vascular endothelial growth factor 121 isoform.

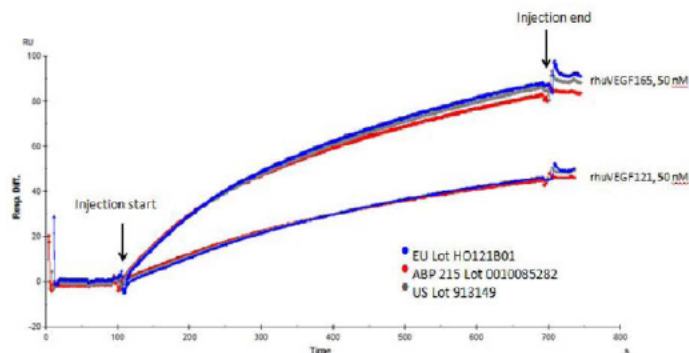
**Reviewer Comment:** *The sponsor noted that due to differences in coating efficiencies the results of the binding experiment should be analyzed across the row and not down the column. However, the binding kinetics are sufficiently similar even if analyzed down the column considering that one EU lot is somewhat of an outlier from the other two. In conclusion, the binding kinetics data show similar on/off rates and  $K_D$  values to VEGFA111 by all three products.*

#### Binding Specificity to VEGFA Isoforms 121 and 165 by SPR

The sponsor selected VEGFA isoforms 121 and 165 to further characterize the binding properties by the three products using SPR. Only one lot each of ABP 215, US-Avastin, and EU-Avastin was used in the SPR assay. Unlike the SPR experiment used in the binding kinetics analysis, the sponsor used Biacore. ABP 215, US-Avastin, and EU-Avastin, each were immobilized onto Biacore CM5 chip and 50 nM of VEGFA 121 or 165 were channeled over the chip to determine binding capabilities of the three products.

The overlay of the sensograms showed similar binding curves for both VEGFA isoforms by all three products (see below).

Figure 3. A Representative Graphic Overlay of the Net Binding Sensorgrams of rhuVEGF-A 121 and rhuVEGF-A 165 to ABP215, US and EU Bevacizumab-Immobilized Biosensor Chip Surfaces



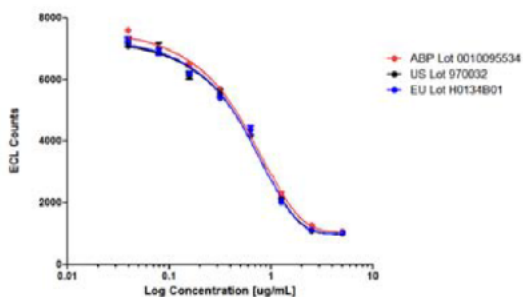
**Reviewer Comment:** A slight difference is noted towards the end of the injection period. The difference could be due to slightly less amount of product immobilization on the chip. The qualification report R20120017 provided in response to an IR (2/22/17) showed that ABP 215 had 977.2 response units (RU) compared to 1002.3 RU and 981.5 RU for US-Avastin and EU-Avastin, respectively. Therefore, this is probably not a true difference in binding by ABP 215. The SPR results support the tier 1 ELISA binding results showing that the three bevacizumab-products have similar binding characteristics to VEGFA 165 and to VEGFA 121, but also show that the affinities of all three bevacizumab products to VEGFA 165 and to VEGFA 121 are different. Both the similar binding to each of the isoforms and the same differences in affinities to the two isoforms support the similar binding kinetics of the three bevacizumab products.

#### Inhibition of VEGFR-2 Receptor-Tyrosine-Kinase (RTK) Autophosphorylation

The ability of ABP 215, US-Avastin, and EU-Avastin to inhibit VEGFR2 RTK autophosphorylation was evaluated with three lots of each product. The VEGFR2 RTK autophosphorylation assay was performed using HUVEC cells and VEGFA 111. The phosphorylation of VEGFR2 was detected using murine anti-phosphotyrosine residue on VEGFR2 captured on streptavidin-coated Mesoscale Discovery (MSD) plates via biotinylated antibody against the extracellular portion of VEGFR2.

The results from all three lots of each product showed similar dose dependent decrease in the amount of VEGFR2 phosphorylation by all three products. The result from one lot of each product is shown below as a representative example from the three lots tested:

Figure 11. Representative Inhibition by ABP 215, Bevacizumab (US), and Bevacizumab (EU) in the RTK Phosphorylation Assay



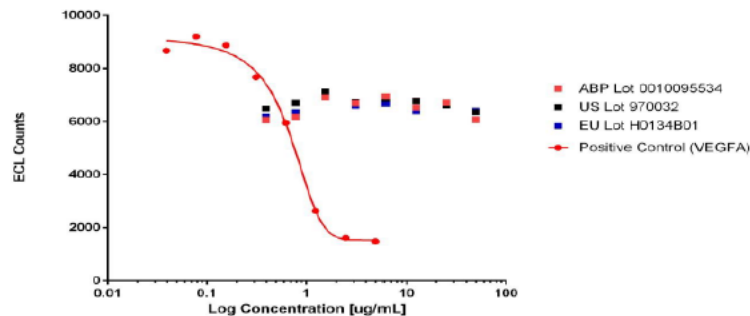
**Reviewer Comment:** The VEGFR2 RTK autophosphorylation assay results showed similar inhibitory activity by all three products. The VEGFR2 RTK assay supports the results from the tier 1 HUVEC anti-proliferation assay.

### Specificity to VEGFA (using VEGFA 111) by VEGFR2 RTK Autophosphorylation.

This study was done to evaluate the specificity of ABP215, US-Avastin and EU-Avastin to the VEGFA family as compared to VEGFC and D family members. The sponsor used the same MSD assay format described above. Only one lot each of the three products was used in the specificity study. The assay was performed with 10-fold higher concentration of VEGFC and VEGFD ligands compared to VEGFA111 in the previous study above.

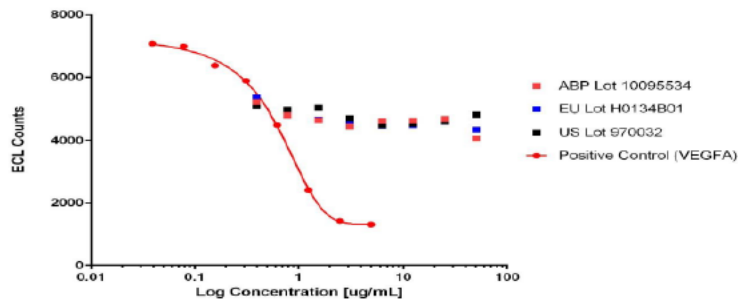
The results showed that all three products failed to decrease the RTK phosphorylation levels at all concentrations tested (see concentration response curves below). In addition, the results showed that even with 10-fold higher concentration of VEGFC and VEGFD, the signal is weaker as compared to VEGFA (ECL counts for VEGFC, D, and A were 6000, 5500, and approximately 8000, respectively).

Figure 12. Plot of ABP 215, Bevacizumab (US), and Bevacizumab (EU) in the RTK Autophosphorylation Assay with VEGF-C



ECL = electrochemiluminescent; RTK = receptor tyrosine kinase; VEGF-A = Vascular Endothelial Growth Factor family member A; VEGF-C = Vascular Endothelial Growth Factor family member C

Figure 13. Representative Plot of ABP 215, Bevacizumab (US), and Bevacizumab (EU) in the RTK Phosphorylation Assay with VEGF-D



ECL = electrochemiluminescent; RTK = receptor tyrosine kinase; VEGF-A = Vascular Endothelial Growth Factor family member A; VEGF-D = Vascular Endothelial Growth Factor family member D.

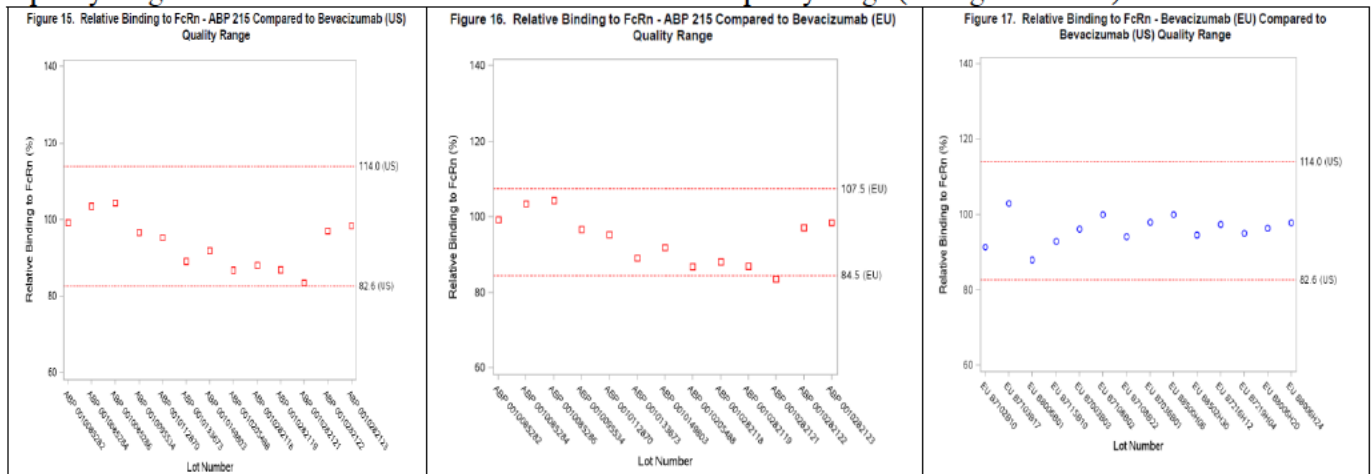
### **Reviewer Comments:**

The sponsor did not test the specificity ABP 215 against two other remaining VEGF family members, VEGFB and PlGF. An IR was sent for additional data showing lack of binding to VEGFB and PlGF. The sponsor stated in the IR response (2/22/17) that they did not test for specificity to VEGF family members B and PlGF because the sponsor only focused on the ability to interfere with VEGFR-2 signaling and not through VEGFR1. VEGF family members B and PlGF signal through VEGFR-1. In addition, the sponsor stated that a paper by Papadopoulos, N. et al., (2012 Angiogenesis) showed that bevacizumab did not bind to VEGFB and PlGF by SPR and because ABP 215 share the same sequence as bevacizumab the binding specificity should be the same. The response provided is acceptable.

### Tier 2 Binding Studies: FcRn, FcγRIIIa (158V and 158 F), and C1q Binding

#### FcRn Binding:

Thirteen ABP 215 lots, 14 US-Avastin lots and 14 EU-Avastin lots were used to determine relative FcRn binding activity against ABP 215 reference standard. The method used in the FcRn binding study was a competitive Amplified Luminescent Proximity Homogenous Assay (Alpha) Screen binding assay. The principle of the AlphaScreen assay utilizes two beads, an acceptor bead and a donor bead that contain interacting partners, such as FcRn and Fc, that will emit singlet oxygen species from the donor bead to the acceptor bead. The energy transfer between the donor bead and the acceptor bead is detected as light and only when the beads are in tight proximity such as that resulting from protein binding. ABP 215, US-Avastin, and EU-Avastin products were each introduced into the reaction mixture, where they competed for FcRn binding with the donor bead. The reduction in light is plotted per concentration and the samples curves are compared with an ABP-215 reference standard curve to determine relative binding. The results of the FcRn binding showed that ABP 215 and EU-Avastin lots were within the US-Avastin quality range and ABP 215 lots were within the EU-Avastin quality range (see figures below):

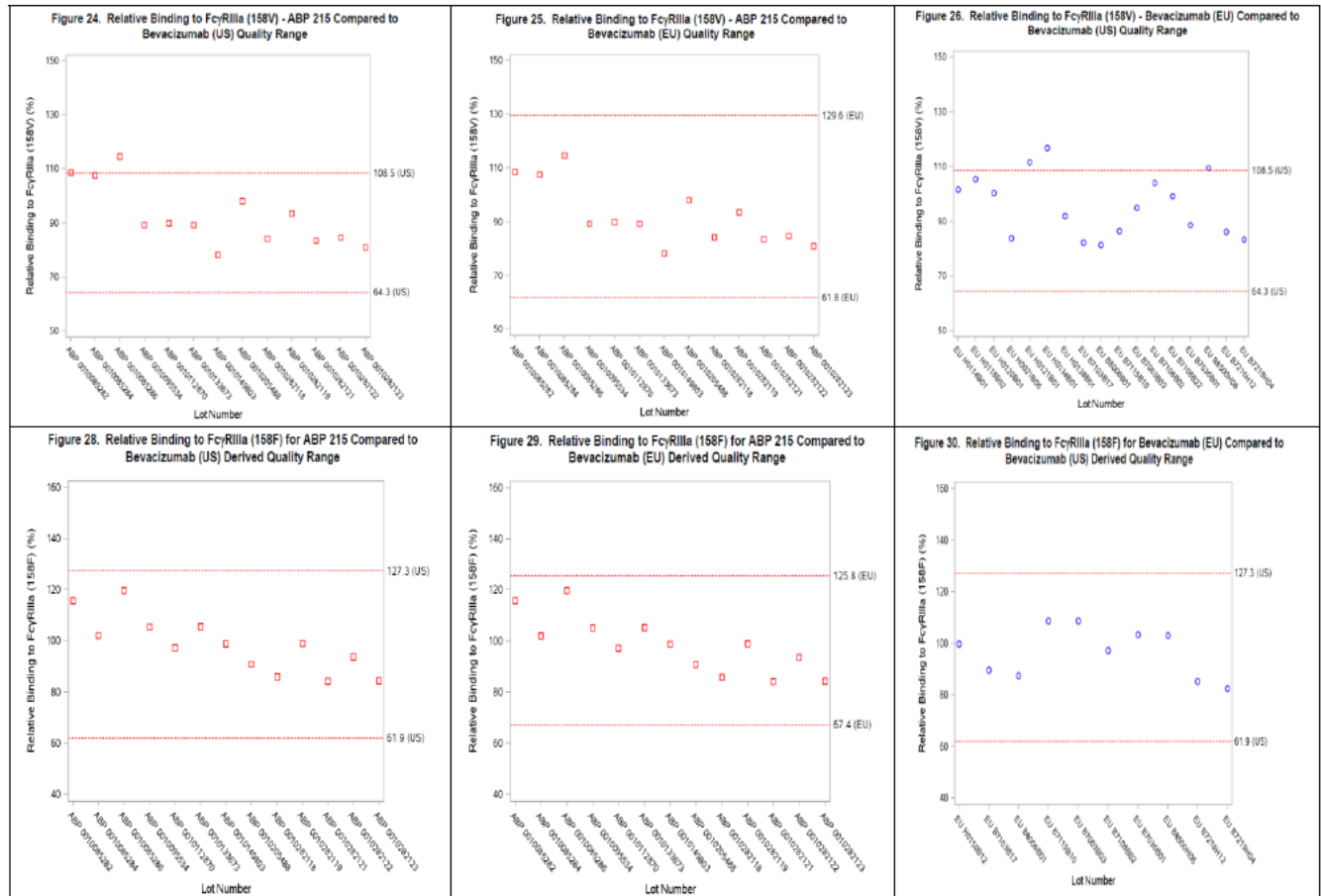


**Reviewer Comment:** The FcRn binding results indicate that all three products have similar FcRn binding properties.

**FcγRIIIa (158V and 158F) Binding:**

Thirteen ABP 215 lots were used to study the relative binding levels to two FcγRIIIa variants, the high affinity receptor valine at amino acid 158 variant and the low affinity receptor phenylalanine at amino acid 158 variant. Similar to the FcRn binding study, a competitive Alphascreen method was also used in the FcγRIIIa binding study. Twelve and 10 US-Avastin lots and 17 and 10 EU-Avastin lots were used in the FcγRIIIa (158V) and (158F) binding assays respectively.

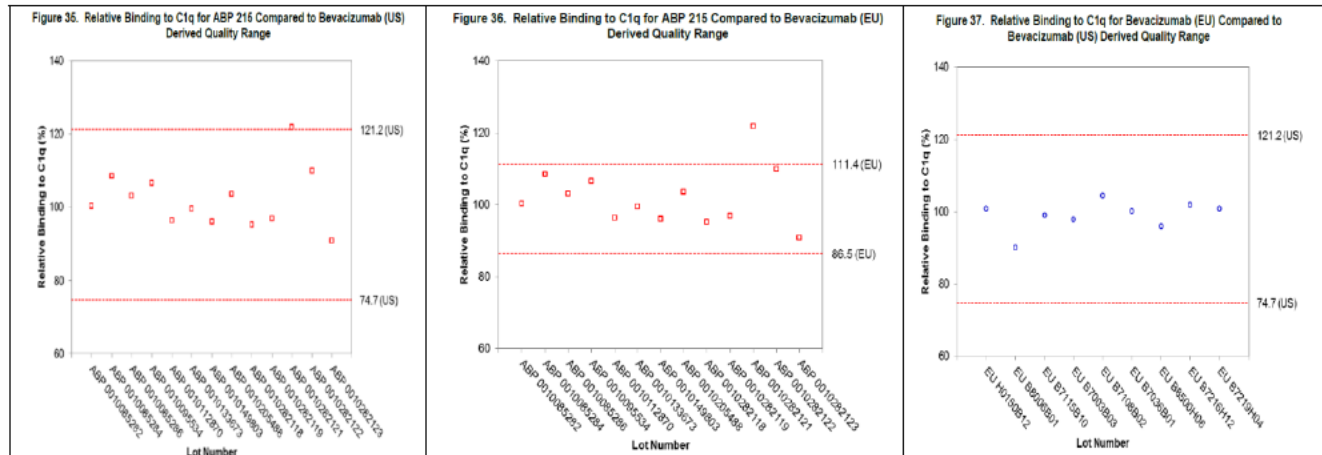
The results for FcγRIIIa (158V) binding showed that 11 out of 13 ABP 215 lots (84%) and 14 out of 17 EU-Avastin lots (82%) were within US-Avastin quality range, and that both products failed to meet the similarity acceptance criterion (i.e. 90% of the lots must be within US QR) for FcγRIIIa (158V) binding. Only ABP 215 lots met the EU QR for FcγRIIIa (158V) binding (see results below). The results for FcγRIIIa (158F) binding showed that all ABP 215 and EU-Avastin lots met US QR as well as all ABP 215 lots met EU-Avastin QR (see figures below).



**Reviewer Comments:** *The sponsor states that the failure to meeting the similarity acceptance criterion for the high affinity FcγRIIIa receptor (Figure 24, above) is acceptable based on the known mechanism of action of bevacizumab, which is to neutralize soluble VEGFA, and therefore, it is expected that the activity is unrelated to ADCC activity. Based on our understanding of the mechanism of bevacizumab in binding a mainly soluble target, as well as on the results of ADCC activity assay which support the lack of ADCC activity, the failure of a few lots to meet the QR for binding to FcγRIIIaR(158V) would not have a clinical impact.*

**Complement C1q Binding:**

Thirteen ABP 215 lots, 10 US-Avastin lots, and 9 EU-Avastin lots were used to determine relative binding to complement cascade component protein, C1q. An ELISA method was used in which ABP 215 or US-Avastin, or EU-Avastin proteins are coated onto 96-well plates and incubated with C1q. Bound C1q protein was detected using anti-C1q-HRP antibody. The sample binding curves were compared to ABP 215 reference standard curve and the results were reported as percent relative binding. The results of the C1q binding showed that ABP 215 and EU-Avastin lots were within the US-Avastin quality range and ABP 215 lots was within the EU-Avastin quality range (see figures below). Note that Sponsor’s acceptance criterion was that products are similar if 90% of the lots are within the QR.



**Reviewer Comments:**

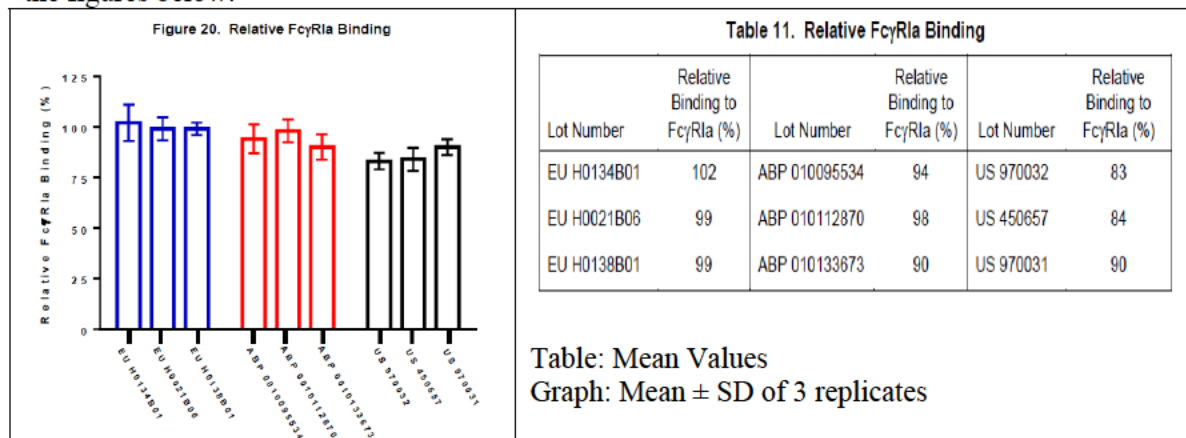
- The C1q binding results indicate that all three products have similar C1q binding properties.
- The development lots used in this study showed higher galactosylation levels compared to clinical lot (see intact mass data above in Primary Structure analysis section). However, the binding to C1q of the development lots and the clinical lots are comparable, which supports that difference in galactose levels do not translate to differences in C1q binding activity for these products.

**Tier 3 Binding Studies: FcγRIa, FcγRIIa (131H), FcγRIIb, and FcγRIIIb Binding**

**FcγRIa Binding:**

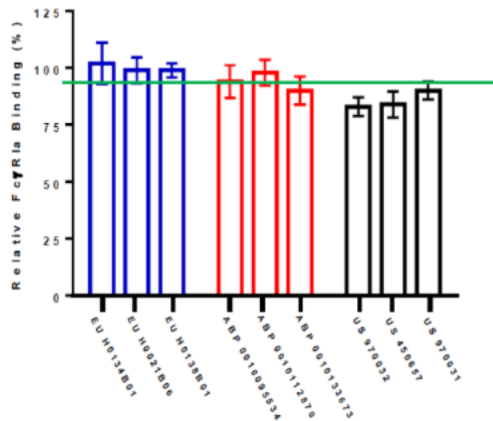
Three lots each of ABP 215, US-Avastin, and EU-Avastin were used in a competitive AlphaScreen assay to determine binding to FcγRIa. The sample binding curves are compared to ABP 215 reference standard curve and the results are reported as percent relative binding. The sponsor states that if the binding ranges overlap, the results are considered similar.

The FcγRIa binding mean ranges are 90-98%, 83-90%, and 99-102% for ABP 215, US-Avastin, and EU-Avastin, respectively. The results of three lots analyzed in triplicates for the three products are shown in the figures below:



**Reviewer Comment:** The FcγRIa binding by ABP 215 lots overlaps with US-Avastin lots when the standard deviations (SD) are also factored into the analysis. Similarly, EU-Avastin lots appear to overlap with one US-Avastin lot when the largest SD of the EU-Avastin lot, H0134B01, is factored into the evaluation. Because the sponsor did not provide SD values, a green line is drawn for visual comparison (see green line in figure below).

Figure 20. Relative FcγRIa Binding



Even with the SD, the results indicate that EU-lots have higher binding activity compared to US-Avastin lots, but this could be due to the insufficient number of lots that were analyzed for this receptor. Given the similarity demonstrated thus far between EU-Avastin and US-Avastin lots, the difference observed in the FcγRIa binding assay is not likely to lead to clinically meaningful difference.

**FcγRIIa (131H) Binding**

Three lots each of ABP 215, US-Avastin, and EU-Avastin were used to determine binding to FcγRIIa (131H) (also known as “high responder” with higher affinity for IgG than the 131R variant). The sponsor used BiaCore SPR method, where FcγRIIa (131H) was immobilized onto CM5 chip and the  $K_D$  was determined using the steady state affinity model. The data are plotted as percent relative binding by dividing the sample  $K_D$  by the ABP 215 reference standard  $K_D$  value and the binding is considered similar if the binding ranges of overlap.

The FcγRIIa (131H) mean binding ranges were 83-101%, 89-91%, and 80-90% for ABP 215, US-Avastin, and EU-Avastin lots, respectively. The results of three lots analyzed in duplicates for the three products are shown below:

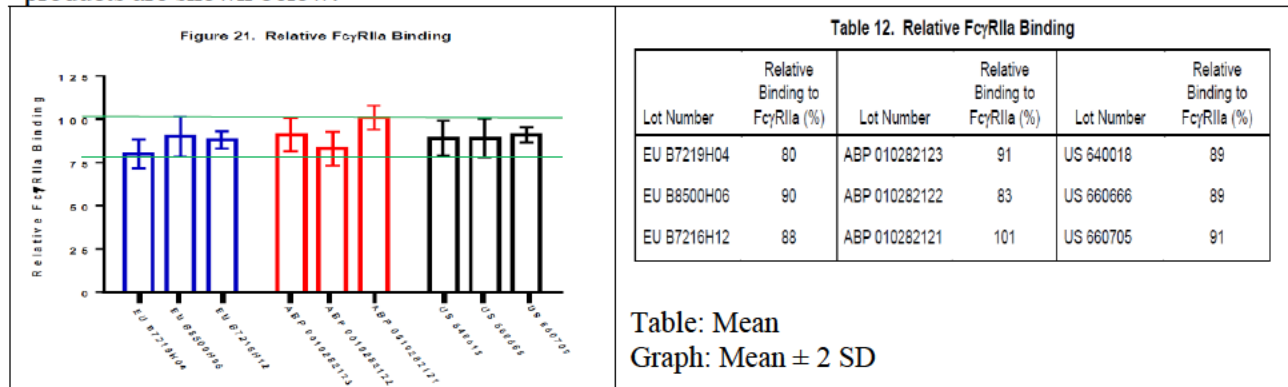


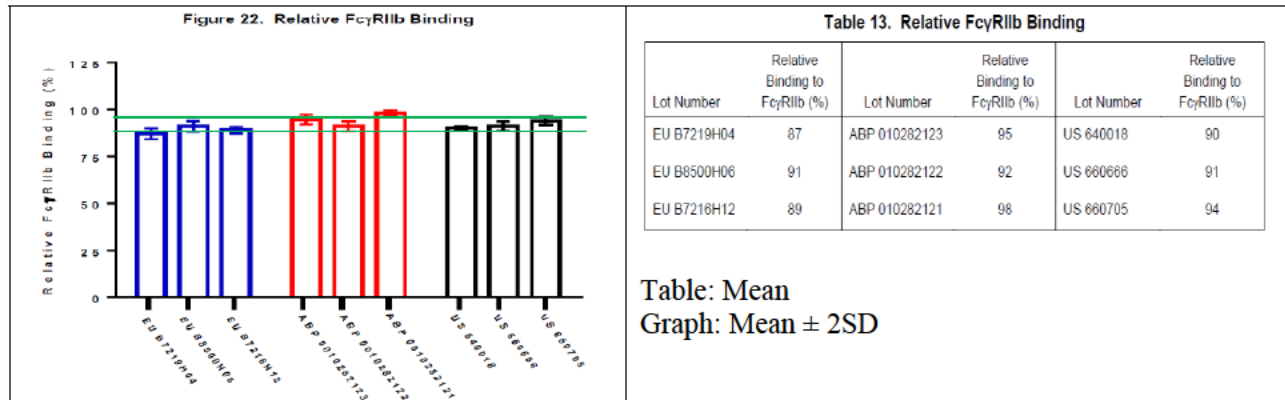
Table: Mean  
Graph: Mean ± 2 SD

**Reviewer Comment:** The green line above was added to show the highest and lowest SD of the US-Avastin lots for visual comparison because the sponsor did not provide the actual SD values. Based on the data above all three products have similar binding properties.

**FcγRIIb Binding**

Three lots each of ABP 215, US-Avastin, and EU-Avastin were used to determine binding to FcγRIIb. The same method and calculations for percent binding as FcγRIIa was used to determine K<sub>D</sub> values of the samples and the ABP 215 reference standard.

The relative binding ranges for FcγRIIb were 92-98%, 90-94%, and 87-91% for ABP 215, US-Avastin, and EU-Avastin, respectively. The results of three lots analyzed in duplicates for the three products are shown below:

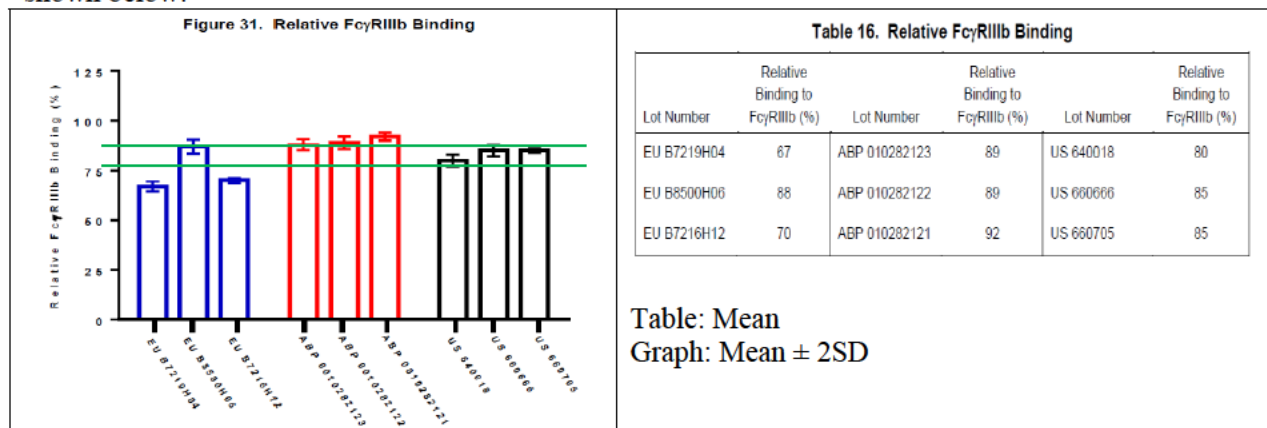


**Reviewer Comment:** Based on the data provided, all three products have similar binding properties to FcγRIIb.

**FcγRIIb Binding**

Three lots each of ABP 215, US-Avastin, and EU-Avastin were used to determine binding to FcγRIIb. The same method and calculations for percent binding as FcγRIIa was used to determine K<sub>D</sub> values of the samples and the ABP 215 reference standard.

The relative binding ranges for FcγRIIb were 89-92%, 80-85%, and 67-88% for ABP 215, US-Avastin, and EU-Avastin, respectively. The results of three lots analyzed in duplicates for the three products are shown below:



**Reviewer Comment:** The results indicate that overall, ABP 215 has slightly higher binding activity to FcγRIIb compared to US-Avastin and EU-Avastin lots. Two EU-Avastin lots out of three show binding outside of both ABP 215 and US-Avastin lots, whereas two out of three ABP 215 lots are within the US-Avastin lot SD (shown by the green lines). The sponsor acknowledges the difference in binding for ABP 215 compared to US- and EU-Avastin lots, but states that the difference is not clinically meaningful because the VEGF is a soluble factor, thus no effector functions are likely to occur. FcγRIIb is known to be involved in antibody-dependent cellular phagocytosis (ADCP). The sponsor also states on page nine of

the Biological Activity document that phagocytosis was not evaluated based on the demonstration of a lack of ADCC activity by ABP 215.

In order to help evaluate the difference, method qualification report was requested in an IR. However, method qualification data to Fc $\gamma$ RIIIb binding was not provided in the response to the IR (2/22/17), by what appears to be an error because the sponsor linked the method qualification report for Fc $\gamma$ RIIa for this method as well as for the Fc $\gamma$ RIIb. Therefore, the results could not be assessed as to whether they are due to method capability, e.g. due to differences in chip coating efficiency or to insufficient number of lots analyzed.

A discussion with Dr. Swisher regarding the argument that no ADCC activity would imply that there will be no ADCP activity identified that this is an incorrect assumption. In fact, Dr. Swisher pointed out that ADCP is an easier mechanism or bar to initiate than ADCC. However, since antibody mediated immune effector functions depend on binding a cell-associated target, it is highly unlikely that ADCP activity is clinically relevant.

#### ADCC Activity in DLD-1 and Calu-6 Cells

DLD-1 (adenocarcinoma) and Calu-6 (lung epithelial carcinoma cell line) cells were used to determine whether ADCC activity levels induced by ABP 215, US-Avastin, and EU-Avastin are similar, for activity through binding to VEGFA that is secreted by these cell lines. NK cell line, NK-92M1/CD16, was used as the effector cells. A B lymphocyte cell line, WIL2-S, was used as a negative control for ABP 215, US- and EU-Avastin because it is not known to secrete VEGFA, and as a positive control for rituximab induced ADCC via CD20 expressed on WIL2-S cells. One lot each of ABP 215, US- and EU-Avastin lots were used in the ADCC study.

**Reviewer Comment:** All three products demonstrated a lack of ADCC activity in the two VEGF secreting cell lines. 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.

#### ADCC Activity in SKOV3 Cells

The SKOV3 cell line was chosen by the sponsor to demonstrate a lack of ADCC activity by ABP 215 because this cell line has been shown in literature (Salvador et al, 2008; ref provided by sponsor) to produce both soluble as well as potentially cell-associated isoforms of VEGFA, e.g. VEGF165. The effector cells used with SKOV3 cells were peripheral blood mononuclear cells. As positive control for the ADCC assay, the sponsor used trastuzumab, which bind to HER2 expressed on SKOV3 cells. One lot each of ABP 215, US-Avastin, and EU-Avastin were used in the ADCC study with SKOV3 cells. The results showed all three products failed to induce ADCC activity in SKOV3 cells, whereas trastuzumab was able to induce ADCC activity in SKOV3 cells

#### **Reviewer Comments:**

- The sponsor was asked to provide method qualification report to determine whether SKOV3 lines they used actually expressed cell surface bound VEGFA, as suggested. The sponsor stated in the 2/22/17 IR response that all three products had been shown to bind to SKOV3 cells in the method qualification report. However, the method qualification report did not provide the data to show that all three products were able to bind to SKOV3 cells.
- In order to evaluate the results of the ADCC experiment with SKOV3 cells, which have been reported to express VEGFA 121 isoform (70% versus 25% VEGFA 165) (Vempati, P. et al., 2014), the sponsor should provide data to demonstrate that the SKOV3 cells are suitable for determination of ADCC activity mediated by interaction of the three products to the cell surface bound VEGFA isoform.
- During the inspection at ATO, development data was provided that showed ABP 215, US-Avastin, and EU-Avastin were able to bind to cell-surface associated VEGFA on SKOV3 cells.

#### CDC Activity in DLD-1 and Calu-6 Cells

The ability of ABP 215, US-Avastin, and EU-Avastin to induce CDC activity in DLD-1 and Calu-6 cells was studied using rabbit complement. Rituximab and WIL2-S cells were used as positive control for CDC activity. One lot each of ABP 215, US-Avastin, and EU-Avastin were used in the CDC activity study.

The results showed that all three products failed to induce CDC activity in DLD-1 and Calu-6 cells, whereas rituximab was able to induce CDC activity.

**Reviewer Comment:** *All three products demonstrated a lack of CDC activity in the two VEGF secreting cell lines. 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.*

#### CDC Activity in SKOV3 Cells

Induction of CDC activity by ABP 215, US-Avastin, and EU-Avastin was investigated using SKOV3 cells and rabbit serum complement. Although not provided in the Biological Activity document, the sponsor used as positive control, membrane bound TNF $\alpha$  expressing CHO cells with adalimumab (data provided in method qualification report submitted in response to IR (2/22/7).

The results showed that all three products failed to induce CDC activity in SKOV3 cells

#### **Reviewer Comments:**

- *Similar comments to apply to this CDC activity study as ADCC study regarding the demonstration that SKOV3 cells express cell-surface bound VEGFA isoform.*
- *Therefore, based on the development data provided during the ATO inspection, the results support the conclusion that all three products are not able to induce CDC activity in SKOV3 cells.*

*Overall conclusion regarding the similarity assessment is that based on the similarity assessment results from primary and higher order structures, biological activities, product-related variant and impurities levels, taken together support a determination of highly similar for the three products notwithstanding minor differences that have been shown to be not clinically meaningful. Additionally, The similarity data related to protein concentration and extractable volume support a demonstration of same strength. The sponsor provided adequate scientific bridge to support the use of EU-Avastin in the clinical study 20120265.*

#### **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 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 ABP215 product is labeled with biotin and a second set of ABP215 product is labeled with ruthenium and both sets of ABP215 are incubated with patient sample that potentially contains ADAs to ABP215. The ADA acts to bridge the biotin-ABP215 with ruthenium-ABP215. The biotin-ABP215-ADA-ABP215-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 the ABP215 reagents, the patient sample is treated with 300 mM acetic acid to dissociate any antibody complexes. In addition, the sample incubation mix contains soluble VEGF R1 to bind any VEGFA that may be present in the sample and lead to false positives by bridging ABP215-

ruthenium to ABP215-biotin. For the confirmatory assay, the incubation also contains unlabeled ABP215 to bind to ADAs and prevent binding to labeled ABP215, thereby decreasing the signal.

**Reviewer Comment:** *The neutralizing activity assay method description and validation study were provided in the submission. However, based on the clinical results of the screening assay, the neutralizing activity results were unnecessary. Therefore, only the screening assay validation study is described and reviewed below.*

#### **ECL Method Validation Report for the Detection of Antibodies Against ABP215 in Human Serum (MVR-000465)**

ECL method validation study for the detection of ADAs to ABP 215 was evaluated for cut-point in non-small cell lung carcinoma (NSCLC), screening and specificity sensitivity, drug tolerance limit, positive control antibody stability, soluble drug target, serum factor, and non-specific antibody interference/cross-reactivity, and assay precision. The low positive control (LPC) and high positive control (HPC) was also determined during the validation study.

As part of assay validation study, the biotin and ruthenium labeled ABP 215 were characterized by SE-HPLC and Biacore methods to determine whether the labeling procedure affected its ability to bind to VEGFA. The results showed minimal effect of the labeling process on binding to VEGFA by Biacore. A slight broadening and shift in retention time of the main peak is observed by ruthenium labeled ABP215.

**Reviewer Comment:** *The change in the main peak of ABP215 did not alter the binding ability of ABP215 as well as the recognition by the positive control antibody. Therefore, the labeled reagents are suitable for the detection of ADAs in patient serum samples.*

#### **Cut-point Determination:**

The assay cut-point (ACP) to analyze serum samples from the PK study (20110216) was determined using 46 human serum samples (23 females and 23 males) from normal donor population. The ACP to analyze serum samples from the NSCLC clinical study (20120265) was determined using 53 human serum samples from drug-naïve NSCLC donor population. The normal donor serum sample population samples were analyzed by two analysts using two MSD instruments, whereas for the NSCLC drug-naïve donor samples were analyzed by two analysts, using two different lots of plates, two lots of labeled ABP215, and two negative control (NC) lots.

For each donor sample, an S/N ratio was determined as the sample response. The S/N ratio is equal to the average ECL signal from an individual sample divided by the average ECL signal from the NC. The NC is neat pooled normal human serum (PNHS) and represents the background response of the assay. The ACPs are stated to be established by calculating the upper bound of a one-sided 95% prediction interval for the distribution of the S/N values. The ACP for PK study was calculated to be S/N=1.25 and for the NSCLC study the ACP for the NSCLC study, the S/N=1.16. No donor samples in the normal donor serum population were excluded in the ACP calculation. In the NSCLC drug naïve donor population samples, five donors were excluded from the ACP calculation as biological outliers and eight additional replicates from other donors were excluded as replicate outliers.

#### ***Reviewer Comments:***

- *The outliers were selected based on statistical analysis. The approach taken to remove the outliers is acceptable.*
- *The statistical approach taken by the sponsor is different than what is recommended in the current 2016 FDA guidance document for immunogenicity assay development and validation. Therefore, Dr. Shen from CMC statistical team was asked to help evaluate whether the approach taken by the*

*sponsor will result in a 5% false positive result that will be further tested in a confirmatory assay with unlabeled drug.*

- *Dr. Shen provided her analysis of the data provided by the sponsor using the approach recommended in our guidance document. The resulting ACP was 1.12 rather than 1.16.*
- *Based on the assurance that ACP of 1.12 includes a 5% false positive rate, a difference of 0.04 in the sponsor's calculated ACP is acceptable and is unlikely to change the result of the clinical study ADA response. It should also be noted that the validation study pre-dates the guidance document and despite the different statistical approach taken, the ACP results are similar.*
- *In order to confirm the adequacy of the ACP determined during the validation study, the sponsor should provide information whether a 5% false positive rate was achieved. An IR was sent to determine whether a 5% false positive rate was achieved with the actual clinical study patient samples.*
  - *In the IR response received 7/14/17, the sponsor provided data to confirm that a 5% false positive rate was achieved with the ACP. Specifically, the screening assay resulted in 30 out of 626 (4.8%) untreated baseline patient samples tested positive.*
  - *Based on the IR response the ACP was adequately established.*

#### Confirmatory Assay Cut-point (Specificity)

The confirmatory cut-point referred to by the sponsor as the depletion cut-point (DCP) was established by the addition of 10 µg/mL of unlabeled ABP215 into the donor serum samples. The DCP was established as the lower bound of a one-sided 99.9% prediction interval for the distribution of the percent ratio of the drug treated (T) S/N to its untreated (U) S/N, i.e. T/U (S/N) values. The DCP for the PK study was 30% and for the NSCLC the DCP was 26%.

#### Assay Sensitivity

The screening assay sensitivity is defined as the lowest concentration of antibody detected with a S/N at the ACP. The screening assay sensitivity was determined using a rabbit anti-ABP215 polyclonal antibody serially diluted in PNHS. The concentration range used for the sensitivity determination ranged from 640 ng/mL to 5 ng/mL. The average screening assay sensitivity for the PK ACP was 25 ng/mL and for the NSCLC ACP was 27 ng/mL.

The specificity assay sensitivity is defined as the lowest concentration of antibody detected that produces a signal at the DCP value, i.e. antibody level that generates a 26% decrease in signal and is above the ACP value in the screening assay. The average specificity assay sensitivity, i.e. in the presence of 10 µg/mL unlabeled ABP 215, for the PK and the NSCLC assays were 43 ng/mL and 60 ng/mL anti-ABP215 positive control antibody, respectively.

#### Hook Effect Analysis

A dose-response curve was generated using anti-ABP215 antibody at concentrations that ranged from 0.78 to 100 µg/mL to determine whether a hook effect is observed for the ECL method at the higher concentrations. The results showed that a hook effect was not observed up to 100 µg/mL.

#### Assay System Suitability

A low positive control (LPC) was determined by the sponsor to use as a system suitability control. The LPC established by the sponsor equals the concentration of the positive control antibody that will produce a S/N value two times the ACP used for the PK study. The LPC for the NSCLC study was statistically established by the sponsor to represent a failure rate of 1% for both the ACP and the DCP.

The LPC for the PK study ACP was calculated to be 160 ng/mL and for the NSCLC ACP the LPC was calculated to be 53.9 ng/mL anti-ABP 215. The LPC for the NSCLC DCP was calculated to be 110

ng/mL (rounded from 109.5 ng/mL). The sponsor stated that the LPC was selected as 110 ng/mL in order to meet the system suitability for both the screening and confirmatory assays. The high positive control (HPC) concentration was selected as 500 ng/mL.

#### Assay Precision

The intra-assay precision was determined by running the NC, LPC, and HPC in quadruplicates per plate. A total of nine plates were evaluated for the PK repeatability assessment. The results showed %CV ranged from 3 to 8%, 0 to 5%, and 1 to 5% for NC, LPC, and HPC, respectively.

The assay inter-assay precision was determined from results obtained from the NC, LPC, and HPC samples performed by two different analysts, using two different instruments, and over four days. The results showed %CV for the average ECL signals for the NC, LPC, and HPC was 10%, 13%, and 14%, respectively.

The intra-assay precision for the NSCLC study ECL method was determined by running the NC, LPC, and HPC in quadruplicates per plate. A total of 11 plates were used. The results showed %CV ranged from 1 to 7%, 1 to 6%, and 0 to 6% for NC, LPC, and HPC, respectively. For the inter-assay precision, the NC, LPC, and HPC samples were tested by two analysts, using two plate lots, two labeled ABP215 lots, two pooled serum lots, two instruments, and over 11 days. The results showed %CV for the average ECL signals for the NC, LPC, and HPC was 14%, 18%, and 20%, respectively.

#### Lower Limit of Reliable Detection

The lower limit of reliable detection (LLRD) is defined as the lowest concentration of the positive control antibody that can be reliably detected in any given serum sample. The sponsor tested three concentrations of anti-ABP215 (40, 80, and 110 ng/mL) spiked into 8 donor serum samples selected to represent low, middle, and high S/N values to determine which concentration of positive control antibody in the donor serum sample will meet both ACP and DCP. The results from the study showed that 110 ng/mL successfully met both the ACP and DCP (see data table below). The 110 ng/mL LLRD was then spiked into 36 NSCLC serum samples for confirmation of the results obtained from the 8 donors. The results from 36 NSCLC serum samples all produced a positive signal at 110 ng/mL concentration of anti-ABP215. Therefore, the LLRD was established as 110 ng/mL.

**Table 22. Determination of a Potential Lower Limit of Reliable Detection: Results for Screening and Specificity Assay Using Anti-ABP 215**

| Donor Serum | 40 ng/mL ADA |                 | 80 ng/mL ADA |                 | 110 ng/mL ADA |                 |
|-------------|--------------|-----------------|--------------|-----------------|---------------|-----------------|
|             | S/N          | % S/N Depletion | S/N          | % S/N Depletion | S/N           | % S/N Depletion |
| (b) (6)     | <b>1.19</b>  | <b>7</b>        | 1.43         | <b>22</b>       | 1.73          | 39              |
|             | <b>1.14</b>  | <b>3</b>        | 1.53         | 36              | 1.79          | 47              |
|             | 1.87         | <b>21</b>       | 2.12         | 30              | 2.35          | 38              |
|             | 1.42         | 27              | 1.63         | 30              | 1.87          | 42              |
|             | 1.40         | 30              | 1.69         | 44              | 1.89          | 50              |
|             | 1.48         | 32              | 1.73         | 38              | 2.00          | 47              |
|             | 1.44         | <b>24</b>       | 1.76         | 39              | 2.25          | 52              |
|             | 1.38         | 30              | 1.65         | 44              | 1.90          | 49              |
|             | 1.32         | <b>22</b>       | 1.57         | 31              | 1.75          | 36              |
|             | 1.42         | 30              | 1.81         | 46              | 1.98          | 49              |

ADA: anti-ABP 215 antibody

(b) (6)

**Bold, highlight indicates values  $\leq$  CP (S/N = 1.16) or  $\leq$  DCP (26%)**

Drug Tolerance

The screening assay drug tolerance limit is defined as the approximate drug concentration at which the S/N value is above the ACP at a given antibody concentration. The LPC and HPC antibody concentrations were evaluated for the drug tolerance level. The results for the PK study ECL, the drug tolerance level was 50  $\mu$ g/mL and 75  $\mu$ g/mL ABP 215 for the LPC (160 ng/mL) and HPC (500 ng/mL), respectively. For the NSCLC study ECL method, the drug tolerance level was 25  $\mu$ g/mL and 150  $\mu$ g/mL ABP 215 for the LPC (110 ng/mL) and HPC (500 ng/mL).

The confirmatory assay drug tolerance level was 10  $\mu$ g/mL and 50  $\mu$ g/mL ABP215 for the LPC and the HPC, respectively. These concentrations of ABP215 would be in addition to the 10  $\mu$ g/mL ABP215 used in confirmatory assay.

*Reviewer Comment: The drug tolerance levels for the NSCLC screening and confirmatory assay is higher than or at the average trough levels of ABP 215 found in the NSCLC study (average 64  $\mu$ g/mL for ABP215 arm and 73  $\mu$ g/mL for EU-Avastin). The drug tolerance level is acceptable. Note that for the single injection PK study, samples were taken at the end of the study, day 85, at which point the concentration of ABP215 was below the drug tolerance level of the assay (~20  $\mu$ g/mL).*

Assay Specificity: Soluble VEGF and Related Factors

The assay specificity regarding the potential of VEGFA or VEGF family members B, C, D, VEGFR2-Fc chimera, and neuropilin to interfere with the ability to detect anti-ABP215 antibodies was determined. Sponsor states that the average serum concentration of VEGFA in healthy humans was reported to be less than 300 pg/mL, whereas in NSCLC patient samples, the levels are unknown. Therefore, the sponsor spiked in 0 to 800 ng/mL VEGFA 165 isoform into NC serum with or without 160 ng/mL anti-ABP215 antibody (LPC for the PK study ECL) to determine the effect of the VEGFA in the serum.

The results showed that starting at 400 ng/mL VEGFA, a false positive result was generated in the NC without 160 ng/mL LPC. In the NC containing the LPC, all samples were above the ACP and DCP (see results below).

**Table 27. MET-003362 v1.0 Specificity: Soluble Drug Target**

| VEGF-A <sub>165</sub><br>(ng/mL) | 0 ng/mL anti-ABP 215 |                    |                       | 160 ng/mL anti-ABP 215 |                    |                       |
|----------------------------------|----------------------|--------------------|-----------------------|------------------------|--------------------|-----------------------|
|                                  | Untreated<br>S/N     | % S/N<br>Depletion | Specificity<br>Result | Untreated<br>S/N       | % S/N<br>Depletion | Specificity<br>Result |
| 800                              | <b>10.44</b>         | <b>81%</b>         | <b>Positive</b>       | <b>10.94</b>           | <b>83</b>          | <b>Positive</b>       |
| 400                              | <b>1.69</b>          | 29%                | Negative              | <b>2.86</b>            | <b>59</b>          | <b>Positive</b>       |
| 200                              | 1.22                 | N/A                | Negative              | <b>2.45</b>            | <b>57</b>          | <b>Positive</b>       |
| 100                              | 1.10                 | N/A                | Negative              | <b>2.31</b>            | <b>58</b>          | <b>Positive</b>       |
| 50                               | 0.94                 | N/A                | Negative              | <b>2.25</b>            | <b>58</b>          | <b>Positive</b>       |
| 10                               | 0.95                 | N/A                | Negative              | <b>2.10</b>            | <b>52</b>          | <b>Positive</b>       |
| 1                                | 0.95                 | N/A                | Negative              | <b>2.17</b>            | <b>54</b>          | <b>Positive</b>       |
| 0                                | 0.95                 | N/A                | Negative              | <b>2.04</b>            | <b>52</b>          | <b>Positive</b>       |

Bold S/N values are greater than the CP (S/N = 1.25) and/or greater than the DCP (30%).

**Reviewer Comment:** *The lower concentrations of VEGFA were likely bound up by the 500 ng/mL VEGFR1 that is part of the incubation mix to prevent false positive results. Based on the literature report the normal serum concentration of VEGFA is less than 0.3 pg/mL, which is much less than the level of VEGFA that would result in false positives.*

Similarly for the NSCLC study ECL method, the NC containing with or without 110 ng/mL (LPC) anti-ABP215 was spiked with 1 ng/mL or 10 ng/mL of VEGF family members, VEGFR2-Fc chimera, and neuropilin. The results showed NC samples without anti-ABP215 but spiked with the different VEGF family members, chimera, and neuropilin did not produce a false positive signal. In addition, the 10 ng/mL highest concentration of VEGFA used did not produce a false positive result, which is consistent with the data in Table 27 (above).

#### Assay Specificity: Serum Factors

A similar approach was taken as described above for evaluating the effect of serum factors in the ECL methods. Intralipid (1:20 dilution), conjugated and unconjugated bilirubin (0.2 mg/mL each), and hemoglobin (5 mg/mL). The results showed that the serum factors did not interfere with assay readout of the LPC spiked samples as well as it did not cross-react with the assay components leading to false positives (see data below). The lipid, bilirubin, and hemoglobin levels used in the study are higher than or at the level observed in “disease” populations, e.g. 0.05 mg/mL, 0.15 mg/mL, 5 mg/mL, and 5 mg/mL levels of conjugated bilirubin, unconjugated bilirubin, triglycerides, and hemoglobin.

**Table 30. MET-003362 v2.0 Specificity: Serum Factors**

| Serum Factor                | Anti-ABP 215 conc. (ng/mL) | Untreated S/N | Treated S/N | % S/N Reduction | Result   |
|-----------------------------|----------------------------|---------------|-------------|-----------------|----------|
| Conj Bilirubin, 0.2 mg/mL   | 0                          | 0.94          | 1.03        | -10             | Negative |
|                             | 110                        | 2.15          | 1.00        | 54              | Positive |
| Unconj Bilirubin, 0.2 mg/mL | 0                          | 1.00          | 1.04        | -4              | Negative |
|                             | 110                        | 2.13          | 1.03        | 52              | Positive |
| Intralipid, 1:20 dilution   | 0                          | 0.98          | 1.05        | -7              | Negative |
|                             | 110                        | 2.14          | 1.00        | 53              | Positive |
| Hemoglobin, 5 mg/mL         | 0                          | 0.91          | 0.89        | 1               | Negative |
|                             | 110                        | 1.92          | 0.97        | 49              | Positive |

Unconj.: unconjugated; Conj.: conjugated, Conc.: concentration

Assay Specificity: Non-Specific Antibodies and Therapeutics

NC with and without LPC (110 ng/mL) was spiked with high concentrations of un-related antibodies, specifically anti-ABP 980, anti-AMG 181, anti-AMG 139, and therapeutic products, AMG 139 and AMG 181. The results showed that presence of unrelated protein products did not result in assay readout of the LPC spiked samples as well as it did not cross-react with the assay components leading to false positives (see data below).

**Table 31. Specificity: Non-specific Antibodies and Therapeutics**

| Non-specific Antibody/Therapeutic | Cross-reactivity (anti-ABP 215 at 0 ng/mL) |              | Interference (anti-ABP 215 at 110 ng/mL) |         |             |              |
|-----------------------------------|--------------------------------------------|--------------|------------------------------------------|---------|-------------|--------------|
|                                   | U (S/N)                                    | Final Result | U (S/N)                                  | T (S/N) | % S/N Depl. | Final Result |
|                                   | anti-ABP 980, 5 µg/mL                      | 0.95         | Negative                                 | 1.85    | 1.03        | 45           |
| anti-AMG 181, 5 µg/mL             | 1.00                                       | Negative     | 1.79                                     | 1.08    | 39          | Positive     |
| anti-AMG 139, 5 µg/mL             | 1.00                                       | Negative     | 1.92                                     | 1.04    | 46          | Positive     |
| AMG 139, 20 µg/mL                 | 0.96                                       | Negative     | 1.88                                     | 1.05    | 44          | Positive     |
| AMG 181, 20 µg/mL                 | 0.94                                       | Negative     | 1.85                                     | 0.97    | 48          | Positive     |

U: untreated; T: treated; Depl.: Depletion

Specificity Assay Validation

In order to determine the suitability of the chosen 10 µg/mL of ABP215 used as the unlabeled ABP215 in the confirmatory assay, the sponsor spiked six NSCLC donor samples with LLRD (110 ng/mL) and 1000X LLRD (110 µg/mL) of anti-ABP215 antibody. The results showed that high concentration of positive antibody is still able to produce signal above the DCP (see data below).

**Table 32. Specificity: Donors Spiked to LLRD and 1000x LLRD**

| Donor   | 110 ng/mL anti-ABP 215 (LLRD) |         |             |              | 110 µg/mL anti-ABP 215 (1000x LLRD) |         |             |              |
|---------|-------------------------------|---------|-------------|--------------|-------------------------------------|---------|-------------|--------------|
|         | U (S/N)                       | T (S/N) | % S/N Depl. | Final Result | U (S/N)                             | T (S/N) | % S/N Depl. | Final Result |
| (b) (6) | 1.77                          | 0.89    | 50          | Positive     | 2783.63                             | 8.99    | 100         | Positive     |
|         | 1.83                          | 0.86    | 53          | Positive     | 2770.86                             | 9.01    | 100         | Positive     |
|         | 1.68                          | 0.82    | 51          | Positive     | 2970.84                             | 9.43    | 100         | Positive     |
|         | 1.77*                         | 0.80    | 55          | Positive     | 3378.59                             | 10.25   | 100         | Positive     |
|         | 1.71                          | 0.72    | 58          | Positive     | 2834.43                             | 8.99    | 100         | Positive     |
|         | 1.67                          | 0.73    | 56          | Positive     | 2712.64                             | 8.79    | 100         | Positive     |

U: untreated; T: treated; Depl.: Depletion

\*Note: untreated % CV was greater than 20%, but was not repeated.

**Cross-Reactivity of Anti-ABP215 Antibody to Avastin**

Sixteen health donor serum samples were spiked with anti-ABP215 at LPC and HPC concentrations. The spiked samples were incubated with 10 µg/mL of US-Avastin, EU-Avastin or ABP215. The results showed cross-reactivity of the positive control antibody to all three products and the reactivity level was similar (see data below).

**Table 39. MET-003362 v4.0 Cross Reactivity of anti-ABP 215 Antibody to Avastin US and Avastin EU**

| Donor        | LPC-spiked        |                     | HPC-spiked        |                     | LPC-spiked        |                     | HPC-spiked        |                     |
|--------------|-------------------|---------------------|-------------------|---------------------|-------------------|---------------------|-------------------|---------------------|
|              | % Inhibit (Av-US) | % Inhibit (ABP 215) | % Inhibit (Av-US) | % Inhibit (ABP 215) | % Inhibit (Av-EU) | % Inhibit (ABP 215) | % Inhibit (Av-EU) | % Inhibit (ABP 215) |
| (b) (6)      | 34.9              | 42.5                | 73.5              | 68.7                | 41.0              | 39.2                | 72.6              | 71.1                |
|              | 33.3              | 34.8                | 72.8              | 70.3                | 38.8              | 39.0                | 71.0              | 70.2                |
|              | 36.3              | 40.1                | 70.4              | 69.3                | 38.1              | 38.9                | 71.5              | 71.3                |
|              | 35.9              | 41.3                | 71.3              | 71.0                | 38.2              | 41.7                | 72.2              | 70.7                |
|              | 36.5              | 40.8                | 67.8              | 68.4                | 41.6              | 38.7                | 73.1              | 72.0                |
|              | 34.4              | 35.6                | 69.0              | 69.9                | 39.8              | 35.4                | 72.1              | 72.5                |
|              | 33.4              | 40.3                | 65.7              | 75.2                | 34.2              | 38.6                | 71.2              | 73.0                |
|              | 32.9              | 42.7                | 72.6              | 69.8                | 41.5              | 42.6                | 72.9              | 73.6                |
|              | 39.9              | 36.1                | 72.0              | 71.3                | 39.7              | 39.2                | 72.1              | 73.3                |
|              | 39.7              | 34.1                | 72.1              | 71.1                | 37.9              | 37.3                | 71.9              | 71.9                |
|              | 37.5              | 33.7                | 71.6              | 72.4                | 39.4              | 38.9                | 69.4              | 71.3                |
|              | 37.4              | 35.2                | 72.1              | 72.4                | 37.6              | 38.2                | 69.7              | 72.9                |
|              | 35.1              | 37.8                | 70.6              | 72.1                | 38.8              | 39.0                | 67.9              | 70.4                |
|              | 38.0              | 40.1                | 70.5              | 72.3                | 38.3              | 37.9                | 67.6              | 70.0                |
|              | 41.1              | 44.4                | 69.0              | 74.0                | 36.9              | 43.4                | 67.7              | 72.4                |
|              | 41.3              | 40.2                | 69.5              | 72.4                | 35.8              | 42.1                | 67.2              | 69.8                |
| Mean         | 36.7              | 38.7                | 70.7              | 71.3                | 38.6              | 39.4                | 70.6              | 71.7                |
| % Difference | -5.2              |                     | -0.9              |                     | -2.0              |                     | -1.4              |                     |

Av-US: Avastin US treated; Av-EU: Avastin EU treated

**Reviewer Comments:**

*Based on the validation study data, the screening and confirmatory assays have adequate cut-points, sensitivity, precision, and specificity. In addition, serum factors were evaluated for potential interference and cross-reactivity. In conclusion, the screening and confirmatory immunogenicity assays are suitable to use in the evaluation of clinical samples.*



Jee  
Chung

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