BACKGROUND INFORMATION

FOR

THE ONCOLOGIC DRUGS ADVISORY COMMITTEE

13 July 2017

BIOLOGICS LICENSE APPLICATION FOR ABP 215

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1. EXECUTIVE SUMMARY

This document provides background information for the Oncologic Drugs Advisory Committee on the development of ABP 215 as a biosimilar product to the United States (US)-licensed reference product, Avastin® (bevacizumab [US]). Bevacizumab is approved for use in a number of oncologic indications. The mechanism of action, which is the same for all indications, is driven by the binding of bevacizumab to vascular endothelial growth factor type A (referred to in this document as VEGF), which prevents the interaction of VEGF with its receptors on the surface of endothelial cells. Through the neutralization of VEGF, angiogenesis required for the growth and persistence of solid tumors and their metastases is inhibited.

Amgen engaged with the Food and Drug Administration (FDA) throughout the development of ABP 215 to ensure that the development program followed the FDA-recommended stepwise approach to establish the biosimilarity of ABP 215 and bevacizumab. The totality of evidence, which includes analytical and nonclinical similarity assessments, a pharmacokinetic (PK) similarity study, and a clinical similarity study, has established that ABP 215 and bevacizumab are highly similar, with no clinically meaningful differences in terms of the efficacy, safety, and immunogenicity. The totality of evidence supports the approval of ABP 215 as a biosimilar to bevacizumab in each of the proposed indications.

Analytical Similarity

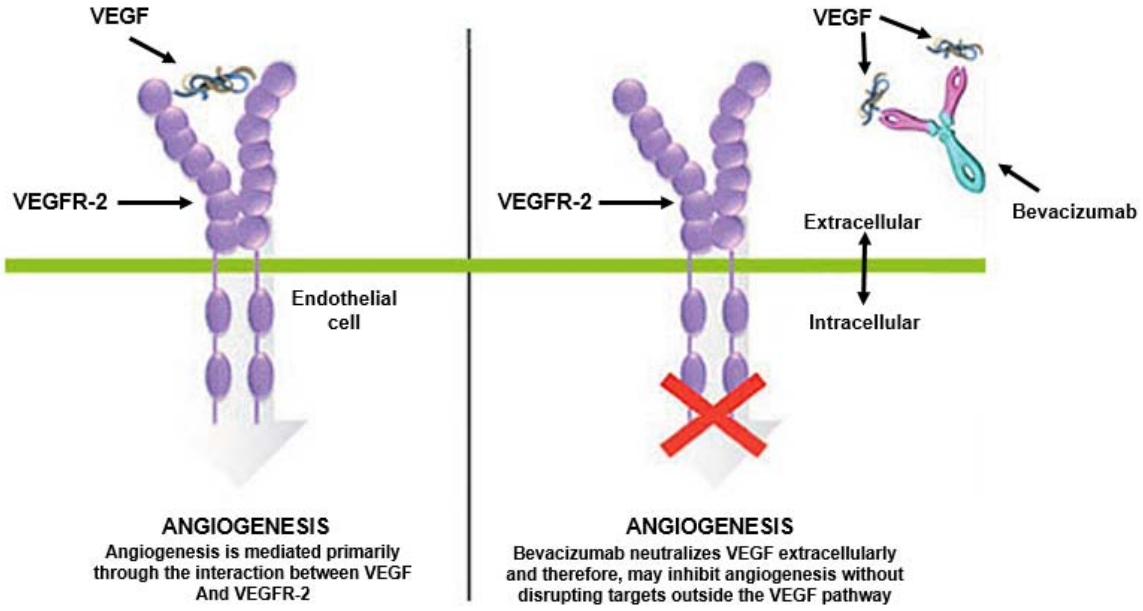
The design of the ABP 215 analytical similarity assessment was based on a comprehensive review of available literature regarding the structural and purity attributes, as well as the functional activities of bevacizumab. Amgen supplemented the literature findings with characterization studies of bevacizumab and knowledge gained from experience in manufacturing monoclonal antibodies. Using this information, a comprehensive analytical similarity plan that included approximately 100 attribute/assay combinations was defined to assess the similarity between the products. ABP 215 lots were compared with bevacizumab (US) and bevacizumab lots procured in the European Union [bevacizumab [EU]) for the analytical similarity assessment to support the global development program for ABP 215.

Analytical similarity was assessed with respect to structural and purity attributes using sensitive assays capable of distinguishing small differences in product attributes. Analytical similarity was demonstrated for an overwhelming majority of the attribute/assay combinations evaluated, but, as expected for a biosimilar, minor

analytical differences were observed in a small number of these attributes. Specifically, small differences in individual glycans, charge variants, and size variants were observed. Overall, the differences observed were unlikely to affect the comparative efficacy, safety, or immunogenicity of ABP 215 based on the existing knowledge of the clinical relevance for these attributes. This was confirmed by the comprehensive evaluation of functional activities, as well as nonclinical and clinical studies.

Analytical similarity was also assessed with respect to functional activities. Testing of ABP 215 and bevacizumab focused on the known fragment antigen binding (Fab)-related mechanism of action, ie, binding and neutralization of VEGF (Figure 1).

Figure 1. Bevacizumab Mechanism of Action



VEGF = vascular endothelial growth factor; VEGFR-2 = vascular endothelial growth factor receptor 2.
Adapted from https://minimednews.wordpress.com.

Furthermore, while not relevant to the mechanism of action, comparative binding to multiple fragment crystallizable (Fc) receptors and the first subcomponent of the C1 complex of the classical pathway of complement activation (C1q) were assessed to interrogate the similarity of the Fc domains of the products. Lastly, the expected lack of effector function by antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) was confirmed in multiple cell-based assays. The complete list of methods used to evaluate functional activities is provided in Table 1.

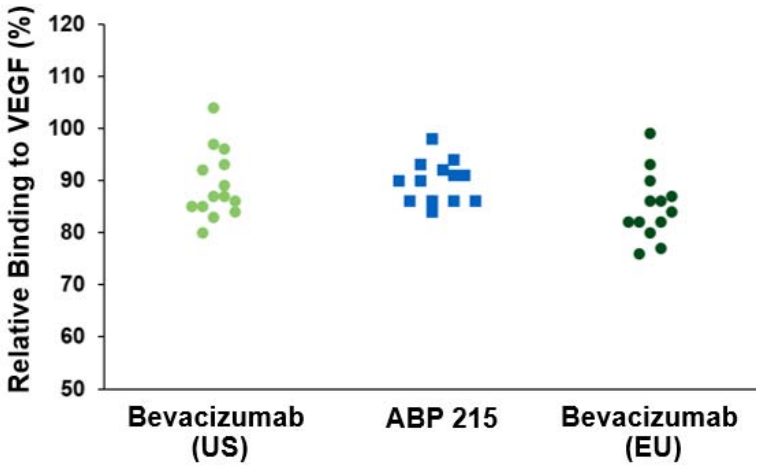
Table 1. Assays Performed to Assess Functional Activities

Fab-mediated Activities	Fc-mediated Characterization
Binding to VEGF	Binding to FcRn
Neutralization of VEGF-mediated proliferation in HUVEC (potency)	Binding to FcγRIa
Kinetic binding (VEGF)	Binding to FcγRIIa (131H)
Binding to VEGF isoforms	Binding to FcγRIIb
Inhibition of VEGFR-2 RTK autophosphorylation	Binding to FcγRIIIa (158V)
	Binding to FcγRIIIa (158F)
	Binding to FcγRIIIb
	Binding to C1q
Fab- and Fc-mediated Characterization	
Lack of ADCC activity	
Lack of CDC activity	

ADCC = antibody-dependent cell-mediated cytotoxicity; C1q = first subcomponent of the C1 complex of the classical pathway of complement activation; CDC = complement-dependent cytotoxicity; Fab = fragment antigen binding; Fc = fragment crystallizable; FcγR = Fc gamma receptor type; FcRn = neonatal Fc receptor; HUVEC = human umbilical vein endothelial cells; RTK = receptor tyrosine kinase; VEGF = vascular endothelial growth factor.

The functional testing supports the conclusion that ABP 215 and bevacizumab are highly similar. Notably, the binding and neutralization of VEGF were similar between ABP 215 and bevacizumab (Figure 2 and Figure 3, respectively).

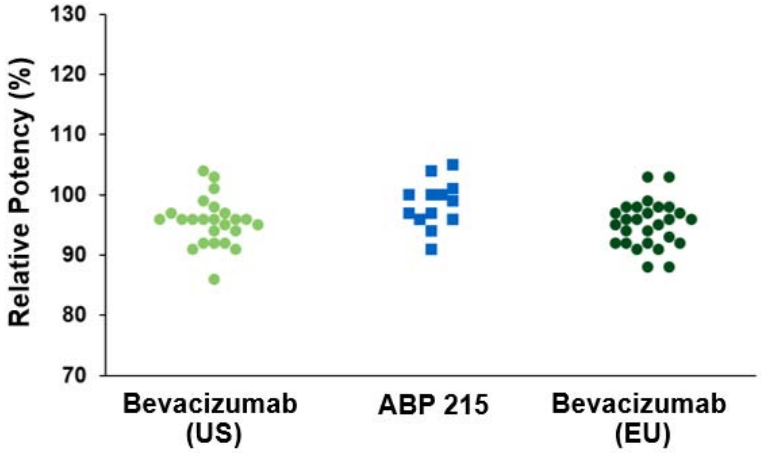
Figure 2. Relative Binding to VEGF



VEGF = vascular endothelial growth factor.

Binding was calculated relative to the ABP 215 reference standard.

Figure 3. Neutralization of VEGF-mediated Proliferation in HUVEC (Potency)



VEGF = vascular endothelial growth factor.

Potency was calculated relative to the ABP 215 reference standard.

The results of the analytical similarity assessment established that ABP 215 is highly analytically similar to bevacizumab. The analytical similarity results also form an essential element of the justification for extrapolation, ie, the justification that ABP 215 can be expected to perform similarly to bevacizumab in all its approved indications, including those that were not studied in the ABP 215 development program. Given that VEGF binding and neutralization is the mechanism of action across the different types of tumors, the analytical similarity between ABP 215 and bevacizumab provides compelling evidence in support of extrapolation.

Nonclinical Similarity

The nonclinical development program provided dose-response pharmacology assessments of ABP 215 and bevacizumab in addition to a toxicology evaluation, and consisted of:

- pharmacologic activity in 2 tumor xenograft models
  - conducted to explore the effects of ABP 215 and bevacizumab on tumor growth and tumor vasculature normalization
- pharmacologic activity in a mouse model of vascular permeability
  - conducted to compare the neutralization of recombinant human VEGF-induced vascular permeability
- toxicology in a 1-month study using the cynomolgus monkey
  - conducted to compare expected toxicities and toxicokinetic profiles, and to confirm a lack of unexpected toxicities with ABP 215 administration

Data from the tumor xenograft models support that ABP 215 inhibits tumor growth and tumor vascularization to a similar level as bevacizumab at multiple dose levels, and the results are consistent with the established bevacizumab mechanism of action. ABP 215 also inhibited VEGF-induced vascular permeability in a similar fashion to bevacizumab using an in vivo mouse model. ABP 215 and bevacizumab had similar toxicokinetics and both products induced the expected endochondral bone ossification (physeal dysplasia) in the cynomolgus monkey. Additionally, no unexpected toxicities were observed in the toxicology study. The nonclinical pharmacology, toxicokinetic, and toxicology data support the conclusion that ABP 215 is highly similar to bevacizumab and support the extrapolation to all bevacizumab indications.

Clinical Similarity

Amgen designed the clinical program, consisting of 2 studies (Table 2), to demonstrate clinical similarity with respect to PK, efficacy, safety, and immunogenicity. Amgen selected subject populations and clinical endpoints sensitive to detect any clinically meaningful differences between ABP 215 and bevacizumab, if such differences existed.

Table 2. ABP 215 Clinical Studies

Study Number	Population	Type of Study	Number of Subjects	Study Duration	Primary Endpoint
20110216 (Study 216)	Healthy male subjects	PK, safety, and immunogenicity	202	85 days	C_{max} and AUC_{inf}
20120265 (Study 265)	Non-small cell lung cancer subjects	Efficacy, safety, and immunogenicity	642	18 weeks[a]	ORR

AUC_{inf} = area under the serum concentration-time curve from time 0 extrapolated to infinite time; C_{max} = maximum observed drug concentration during a dosing interval; ORR = objective response rate; PK = pharmacokinetic.

[a] Expected duration from first dose of any investigational product to end-of-treatment visit. After completing the end-of-treatment visit, subjects were followed for disease progression/overall survival until the end of the clinical study, consent was withdrawn, they were lost to follow-up, died, or had proscribed therapy.

Study 216 in healthy subjects was designed to assess PK similarity in a study population that avoids potential effects of confounding medical conditions or concomitant medications, which can alter an individual PK or safety profile. Therefore, healthy subjects are considered a sensitive population to detect any potential exposure differences between ABP 215 and bevacizumab. Only male subjects were included due to the known risks of bevacizumab to the female reproductive system. A 3 mg/kg dose was used to minimize drug exposure in healthy subjects and is within bevacizumab's linear PK range of 1 mg/kg to 20 mg/kg making it an appropriate dose to assess PK

equivalence. The endpoints were the standard bioequivalence endpoints of area under the serum concentration-time curve from time 0 to infinity (AUC_{inf}) and maximum observed concentration (C_{max}). To establish PK similarity, the 90% confidence interval of the geometric mean ratio for each parameter was to be fully contained within the margins of 0.8 to 1.25.

Study 265 in subjects with advanced non-squamous non-small cell lung cancer (NSCLC) receiving first-line chemotherapy with carboplatin and paclitaxel was designed to demonstrate that there are no clinically meaningful differences between ABP 215 and bevacizumab in terms of efficacy, safety, and immunogenicity. The advanced non-squamous NSCLC patient population was considered appropriate and sensitive for the detection of potential differences between ABP 215 and bevacizumab given the demonstrated treatment effect with an objective response as an endpoint. The primary efficacy endpoint (objective response rate [ORR]) was chosen based on a statistically and clinically significant treatment effect demonstrated in placebo-controlled studies of bevacizumab in subjects receiving chemotherapy plus bevacizumab versus chemotherapy plus placebo for first-line treatment of unresectable advanced, metastatic, or recurrent NSCLC. Additionally, ORR was chosen as the primary endpoint to establish clinical similarity as it is a direct and objective measure of antitumor activity, and it has also been shown to be correlated with progression-free survival (PFS) and overall survival (OS). The primary efficacy analysis in Study 265 was based on the central, independent, blinded radiologists' review of radiographic images scored using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 on the intent-to-treat population. The clinical equivalence of ABP 215 and bevacizumab was assessed by the risk ratio of ORR. ORR was defined as the percent of subjects with a best response of either complete or partial response at any point in the study. To establish efficacy similarity, the confidence interval for risk ratio of ORR was to be fully contained within the pre-specified clinical equivalence margin of 0.67 and 1.50.

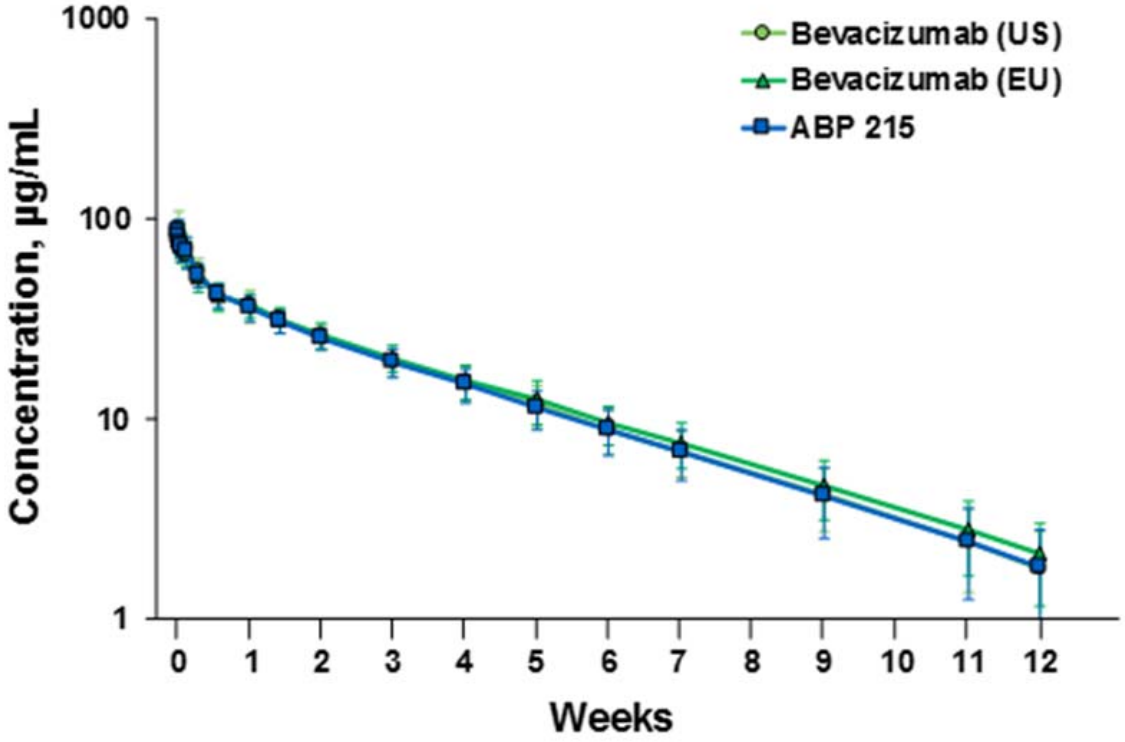
Clinical Pharmacology Results

The clinical pharmacology data to support the similarity of ABP 215 and bevacizumab consists of the PK similarity Study 216 and an assessment of trough concentrations of ABP 215 and bevacizumab in the NSCLC Study 265.

In Study 216, the 90% confidence intervals for the geometric mean ratios of C_{max} and AUC_{inf} were determined to be fully contained within the margin of 0.80 to 1.25, and thus,

PK similarity was demonstrated. The serum concentration-time profiles are presented in Figure 4.

Figure 4. Study 216 Mean (± SD) Serum Concentration-time Profiles



The adverse event profile for ABP 215 was similar to that of bevacizumab. No new safety signals were identified. Administration of ABP 215 or bevacizumab to healthy subjects did not result in the development of binding or neutralizing anti-drug antibodies.

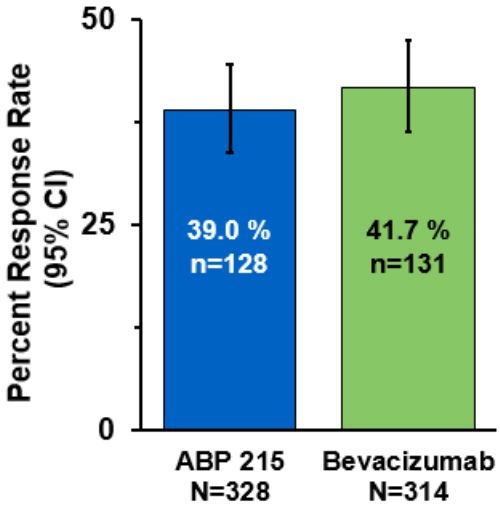
In addition to the PK similarity demonstrated in Study 216, trough concentrations of ABP 215 and bevacizumab were also assessed in the NSCLC Study 265. The trough PK results were consistent between the ABP 215 and bevacizumab groups, also indicating similar exposure between the 2 products in a patient population.

Clinical Similarity Study Results in Subjects with Advanced Non-squamous NSCLC

uct is licensed that wABP 215 and 41.7% for bevacizumab in the intent-to-treat population. The risk ratio was 0.93 with a 2-sided 90% confidence interval of (0.80, 1.09), which was within the pre-specified equivalence margin of (0.67, 1.50), concluding the equivalence in efficacy of ABP 215 and bevacizumab (Figure 5). Complete responses (CR) and partial responses (PR) were comparable across the ABP 215 and bevacizumab treatment groups. The percentage of subjects with a best response of CR

was 0.6% (2 subjects) for both groups, and for PR was 38.4% (126 subjects) for ABP 215 and 41.1% (129 subjects) for bevacizumab.

Figure 5. Study 265 Efficacy Results for the Primary Endpoint



Primary Analysis	ABP 215 vs Bevacizumab
RR (90% CI)	0.93 (0.80, 1.09)
Predefined equivalence margin	(0.67, 1.50)

CI = confidence interval; ECOG PS= Eastern Cooperative Oncology Group performance status; RR = risk ratio.

Primary analysis of ORR using RECIST 1.1 by central radiology review of ITT population.

Generalized linear model adjusted for randomization stratification factors; geographic region, ECOG PS, and gender.

Similar results were observed for all sensitivity analyses using different analysis populations or based on investigator's tumor assessments, as well as analyses adjusting for multiple baseline covariates, all supporting the primary analysis results. The results for secondary efficacy endpoints (risk difference for ORR, duration of response, and PFS) also support similar efficacy between ABP 215 and bevacizumab.

Similar safety profiles were observed between ABP 215 and bevacizumab. In the ABP 215 treatment group, the incidence of adverse events was 95.1% as compared to 93.5% in the bevacizumab treatment group. Across both the ABP 215 and bevacizumab treatment groups, the incidence, type, and severity of adverse events were comparable. The proportion of subjects who experienced grade ≥ 3 events was 42.9% in the ABP 215 group and 44.3% in the bevacizumab group. The most common grade ≥ 3 adverse events (≥ 5% in either of the ABP 215 and bevacizumab treatment groups, respectively) were neutropenia (12.3% and 11.7%), hypertension (6.5% and 5.2%), and thrombocytopenia (3.1% and 5.2%). The safety profiles observed for ABP 215 and bevacizumab in Study 265 were consistent with the known safety profile of

bevacizumab, including expected events such as gastrointestinal perforation, pulmonary hemorrhage, proteinuria, and wound healing complications.

During the study, the incidence of developing binding anti-drug antibodies post-baseline was 1.4% for ABP 215 and 2.5% for bevacizumab, and none were neutralizing antibodies. Similar immunogenicity was observed between ABP 215 and bevacizumab in advanced non-squamous NSCLC subjects.

The clinical data support the conclusion that ABP 215 is similar to bevacizumab and demonstrate that there are no clinically meaningful differences between ABP 215 and bevacizumab in terms of PK, efficacy, safety, and immunogenicity. The similarity in analytical structure and function, PK, efficacy, safety, and immunogenicity, combine to form the totality of evidence, and supports the conclusion that ABP 215 is highly similar to bevacizumab.

Extrapolation of Indications

The totality of evidence indicates that ABP 215 is expected to be as safe and efficacious as bevacizumab in all indications for which bevacizumab is approved. Specifically:

- A comprehensive analytical similarity assessment demonstrated similarity between ABP 215 and bevacizumab, including in functional assays reflecting the mechanism of action which is consistent across all approved indications.
- Equivalent PK profiles in healthy subjects as well as similar steady-state drug levels in the NSCLC study, and the consistent PK of bevacizumab across indications are predictive of equivalent PK in all indications.
- The immunogenicity observations were similar for ABP 215 and bevacizumab, and similar immunogenicity profiles are expected in all indications.
- ABP 215 and bevacizumab have shown similar safety and efficacy profiles, which were also similar to previously reported data for bevacizumab. These results are predictive of similar safety and efficacy of the 2 products when used in other indications.

Conclusion

The totality of evidence from the ABP 215 biosimilar development program leads to the conclusion that ABP 215 meets the scientific and statutory requirements for the demonstration of biosimilarity. Specifically, ABP 215 is highly analytically similar to bevacizumab notwithstanding minor differences in clinically inactive components, and there are no clinically meaningful differences between ABP 215 and bevacizumab in terms of PK, efficacy, safety, and immunogenicity.

The consistent mechanism of action across indications, demonstrated analytical similarity, PK equivalence, clinical similarity in a representative patient population, and the similarity within bevacizumab's conditions of use supports that ABP 215 will have similar clinical outcomes to bevacizumab regardless of tumor type or location. This conclusion supports the approval of ABP 215 as a biosimilar to bevacizumab in all of the bevacizumab indications for which licensure is sought. Amgen is therefore seeking approval of APB 215 in all indications of bevacizumab that are not subject to regulatory exclusivity:

- non-squamous NSCLC
- metastatic colorectal cancer
- glioblastoma
- metastatic renal cell carcinoma
- persistent, recurrent, or metastatic carcinoma of the cervix

2. BACKGROUND INFORMATION

2.1 Section 351(k) Regulatory Pathway for Biosimilars

The Biologics Price Competition and Innovation Act of 2009 created an abbreviated licensure pathway for biological products shown to be highly similar to an FDA-licensed biological product (also known as the reference product). Section 351(k) of the Public Health Service (PHS) Act allows a biosimilar sponsor to rely on existing scientific knowledge about the safety and efficacy of the reference product, and consequently enables a biosimilar biological product to be licensed based on less than a full complement of product-specific nonclinical and clinical data typically required under the section 351(a) regulatory pathway (ie, an innovative biologics license application).

Section 351(k) of the PHS Act defines the terms “biosimilar” or “biosimilarity” to mean that:

> the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components

and

> there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product

Statutory requirements mandate that a biosimilar product must have the same primary sequence, mechanism of action, route of administration, dosage form, and strength as the reference product. A 351(k) application for licensure of the biosimilar must contain, among other things, information demonstrating that the proposed product is highly similar to its reference product. This demonstration is typically based on data derived from comparative analytical studies, nonclinical studies, a human PK study, and at least 1 clinical similarity study in an appropriate condition of use. If the biological product meets the statutory requirements for licensure as a biosimilar product under section 351(k), the biosimilar can be licensed for additional conditions of use (eg, indications) for which the reference product is licensed that were not studied during the biosimilar development program. This concept is referred to as extrapolation and must be justified based on scientific evidence in the 351(k) application.

The development of a biosimilar product under section 351(k) differs from the development of an innovative biological product intended for submission under section 351(a) in regards to the intent, types, and scopes of studies performed. While both innovative and biosimilar development programs generate analytical, nonclinical, and clinical data, the number and types of studies conducted will differ based on the

differing goals and the different statutory requirements for licensure of each program. The purpose of an innovative development program is to establish the efficacy, safety, purity, and potency of the proposed product based on data derived from a full complement of quality, nonclinical, and clinical studies to ultimately establish the risk:benefit profile. The purpose of a biosimilar development program, in contrast, is not to independently establish or re-estimate a risk:benefit profile of the proposed biosimilar product in each indication, but instead to demonstrate that the proposed biosimilar product is highly similar to the reference product. Therefore, the clinical endpoints and study designs for biosimilars will typically differ from those of innovator development programs. Although the biosimilar nonclinical program is significantly reduced, the biosimilar quality program is the same as an innovator product with the addition of an extensive structural and functional characterization comparison between the biosimilar and reference product.

The underlying presumption justifying an abbreviated biosimilar development program is that a molecule shown to be structurally and functionally highly similar to a reference product will behave like the reference product in any clinical setting. However, biological products are structurally and functionally complex and certain clinical aspects (eg, immunogenicity) cannot be predicted with analytical and nonclinical comparative testing alone. Therefore, a clinical similarity study in a relevant and sensitive population is necessary for efficacy, safety, and immunogenicity comparisons to support biosimilar licensure. The sensitive population (ie, a population in whom potential differences between a biosimilar and reference product are likely to be detected, if such differences exist) is also informative when considering extrapolation to other indications of the reference product since it should be predictive of the biosimilars' clinical performance in other populations.

To demonstrate biosimilarity, the FDA has recommended that sponsors use a "stepwise approach" to develop the data and information needed for the biosimilar product's licensure.[1] The stepwise approach begins with an extensive structural and functional characterization of the proposed biosimilar product and the reference product, and serves as the foundation of the biosimilar development program, as well as informing extrapolation across indications. Based on the structural and functional characterization results, an analysis of any differences and their potential to be clinically meaningful is performed. Amgen conducted an extensive analytical similarity assessment comparing

[1] US FDA Biosimilar Guidances for Industry.

ABP 215 to bevacizumab to address each of the points above, and determined that ABP 215 is analytically highly similar to bevacizumab (Section 3).

In the next step, a nonclinical assessment is performed. The results must demonstrate a lack of unexpected effects with the biosimilar, as compared to the reference product, and provides supporting information in the determination of biosimilarity. Amgen conducted a nonclinical program and the results showed ABP 215 to be similar to bevacizumab (Section 4).

The last step in the stepwise approach is a targeted clinical program. Particularly, at least 1 clinical study in this step must be performed in a sensitive population and be designed to allow for the detection of any clinically meaningful differences. The purpose of the clinical program is to address any residual uncertainty remaining with respect to analytical differences and to confirm that such differences are not clinically meaningful. The clinical similarity of ABP 215 and bevacizumab was established, in terms of PK, efficacy, safety, and immunogenicity (Section 5).

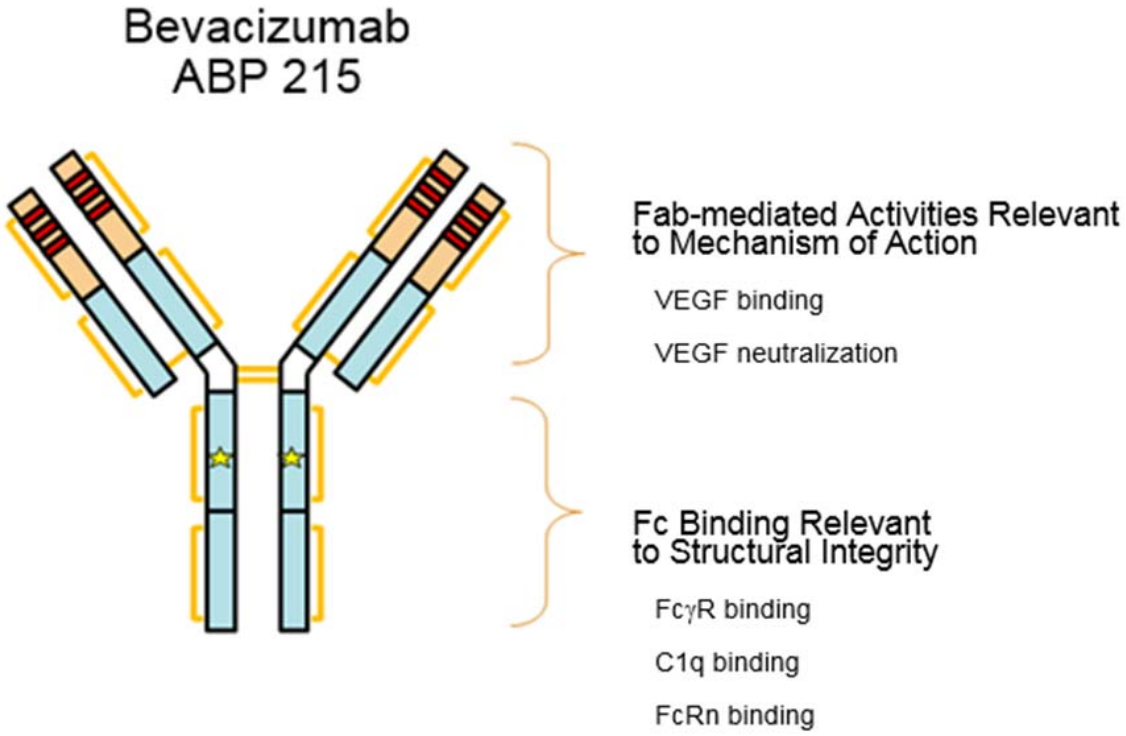
When the analytical, nonclinical, and clinical results are assessed holistically (ie, the "totality of the evidence"), the FDA can then evaluate whether an applicant has adequately demonstrated that a proposed biosimilar product meets the statutory requirement for biosimilarity to the reference product. During the evaluation, the FDA determines whether extrapolation to other indications approved for the reference product, that were not studied during the sponsor's biosimilar development program, is appropriate, if scientifically justified based on understanding of the mechanism of action and clinical considerations. Key points considered to support ABP 215's extrapolation to bevacizumab's approved indications are addressed in each section of this document, with an overall summary provided in Section 6.

2.2 Product Knowledge

2.2.1 Structural and Functional Characteristics

Bevacizumab and ABP 215 are recombinant humanized monoclonal IgG1 antibodies that bind to and neutralize the biologic activity of human VEGF. Both products are expressed in Chinese hamster ovary (CHO) cell lines, although each cell line is different and proprietary to the manufacturer. The antibodies consist of 2 heavy chains and 2 light chains (kappa subclass), including 32 cysteine residues that are involved in both intra-chain and inter-chain disulfide bonds. Each heavy chain contains an N-linked glycan at a consensus site in the Fc region, which is the site for glycosylation (Figure 6).

Figure 6. Bevacizumab and ABP 215 Functional Activities



C1q = complement component 1,q; Fab = fragment antigen binding; Fc = fragment crystallizable; FcγR = Fc gamma receptor; FcRn = neonatal Fc receptor; VEGF = human vascular endothelial growth factor A.

Yellow lines represent disulfide bonds. Red rectangles represent the complementarity-determining region. Yellow stars represent glycosylation sites.

An IgG1 antibody can generally be described as consisting of 2 main regions, the Fab region and the Fc region (Figure 6). Each region is responsible for mediating distinct functional activities. Binding to an antigen is mediated by the complementarity-determining region located in the Fab domain of the antibody. The Fc region, in contrast, is responsible for modulating antibody recycling via neonatal Fc receptor (FcRn) binding, and consequently affects the levels of antibody present in blood. The Fc region can also mediate effector functions (eg, ADCC and CDC) by binding to either Fcγ receptors on immune cells or to complement components and simultaneously engaging membrane resident antigens. However, ABP 215 and bevacizumab do not induce ADCC and CDC as the VEGF protein exists as a predominantly soluble protein (Wang et al, 2004). In the limited circumstances where VEGF is captured by neuropilin (NRP) co-receptor binding on the cell surface, the distance of bevacizumab from the cell membrane is too great to mediate effector function (Cleary et al, 2017). Both antibodies do exhibit binding to Fcγ receptors and to C1q, the first sub-component of complement. Therefore, regarding bevacizumab and

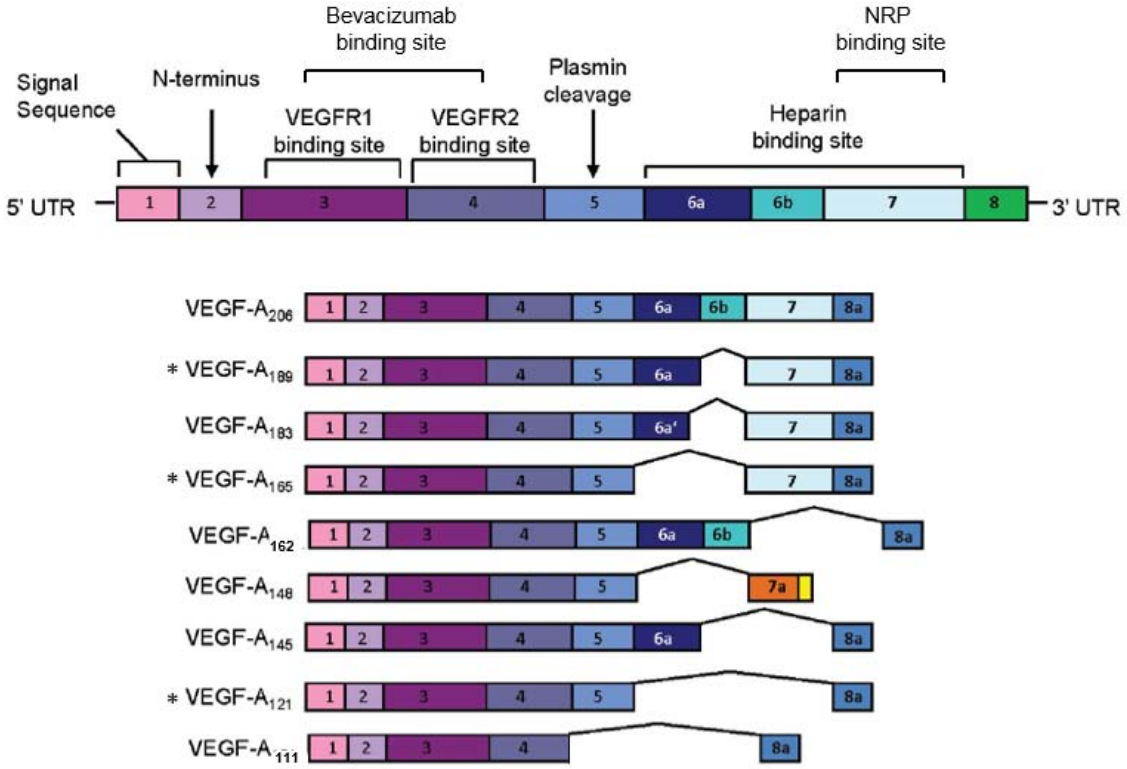
ABP 215, the Fab region is relevant to the mechanism of action, and the Fc region is not relevant to the mechanism of action. However, binding of the Fc region to FcRn may influence PK, and overall, characterizing the binding properties within this region of the molecule provides information regarding the structural integrity of the molecule.

In contrast to small molecules, which are generally a single chemical entity, monoclonal antibodies exhibit a degree of structural heterogeneity. This heterogeneity is caused by the complexity and inherent variability of protein production in a living system. Depending on the degree of the heterogeneity, and where structural variations occur in the antibody, this heterogeneity can either have no clinical impact, or it may affect the efficacy, safety, or immunogenicity of a product. Given that a degree of heterogeneity is expected in all biologic products, and because a biosimilar is manufactured using a different cell line and process than that of the reference product, minor differences in structural and purity attributes are expected between a biosimilar and its reference product.

2.2.2 Background on the Biology of VEGF

VEGF is important in angiogenesis and the neovascularization required to support solid tumor growth (Stimpfl et al, 2002). Several VEGF family members (eg, VEGF type B [-B], -C, -D, and placental growth factor [PlGF]) have been identified in the human genome, of which VEGF is the most potent promoter of angiogenesis (Shibuya, 2014). At least 9 pro-angiogenic VEGF isoforms are generated as a result of alternative splicing from a single gene (Arcondéguy et al, 2013 and Figure 7). The 121, 165, and 189 isoforms are the most commonly expressed VEGF isoforms (Ferrara et al, 2004).

Figure 7. Splice Isoforms of the VEGF Gene



NRP = neuropilin; VEGF-A = vascular endothelial growth factor type A; VEGFR1 = vascular endothelial growth factor receptor 1; VEGFR2 = vascular endothelial growth factor receptor 2; UTR = untranslated region.

* Denotes the most common isoforms.

Adapted from Arcondéguy et al, 2013 and Fearnley et al, 2013.

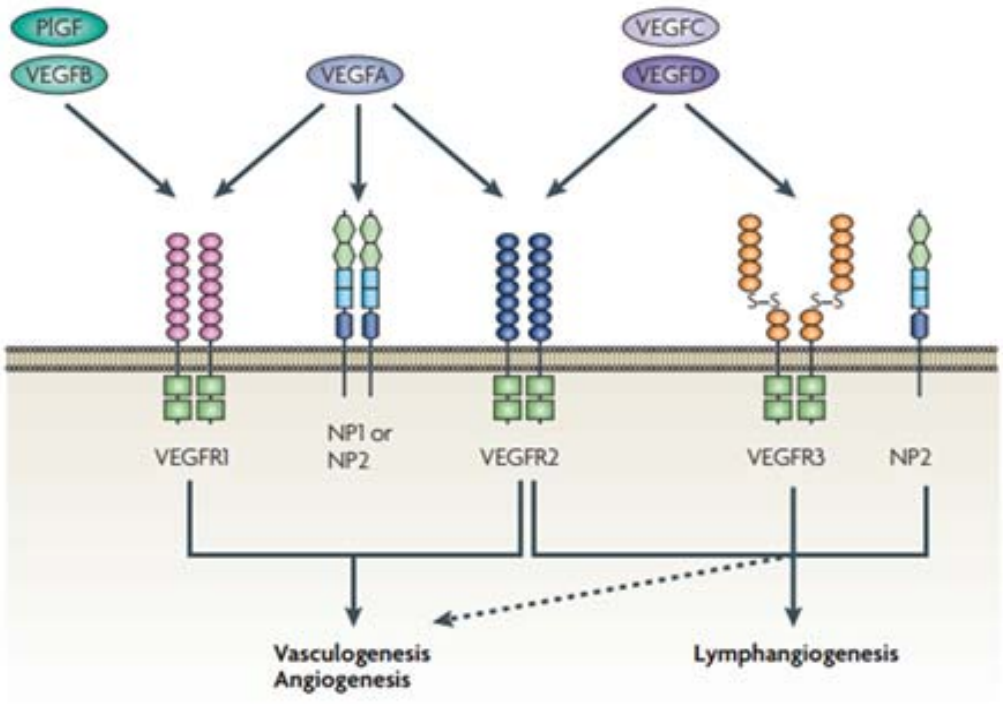
The bioavailability of VEGF family members is tightly regulated and expression is inducible and tissue-specific. The short forms of VEGF that lack the heparin binding site, $VEGF_{111}$ and $VEGF_{121}$, are freely diffusible while the longer forms, represented by $VEGF_{165}$, are tethered to the extracellular matrix or to neuropilin co-receptors (NRP-1/NRP-2) on the surface of cells unless they are released by proteases. VEGF gene expression is upregulated under hypoxic conditions, and the gene is expressed at high levels in most human tumors. VEGF also has basal tissue-specific expression with high levels of expression in healthy lung, kidney, heart, adrenal gland, and in vascular beds of the brain, and low but detectable levels in normal liver, spleen, and gastric mucosa (Holmes and Zachary, 2005; Hoeben et al, 2004). VEGF has been reported to be a paracrine factor (eliciting tumor effects) through NRP interactions by the long splice isoforms and an autocrine factor (eliciting endothelial cell effects) (Goel and Mercurio, 2013). It is important to note that bevacizumab only inhibits the autocrine functions of VEGF and does not disrupt paracrine signaling mediated through NRP binding.

Vascular endothelial growth factors bind to 3 receptor tyrosine kinases:

- vascular endothelial growth factor receptor (VEGFR)-1 (also known as Fms-like tyrosine kinase [Flt]-1)
- VEGFR-2 (also known as kinase insert domain receptor [KDR] and plays an important role in pathological neovascularization via its tyrosine kinase activity and growth promoting signals to blood vessels [Shibuya, 2014])
- VEGFR-3 (also known as Flt-4)

Vascular endothelial growth factor receptor co-receptors, NRP-1 and NRP-2, are also engaged by specific VEGF isoforms in tandem with the VEGFRs. The VEGFR family members are all expressed on vascular endothelium as well as in tissue-specific patterns depending on the developmental stage. Biological activity for each of the VEGF family members is derived from binding to specific cognate receptors. VEGF binds to VEGFR-2 and VEGFR-1, and the long forms (represented by $VEGF_{165}$) also bind NRP-1 and NRP-2; VEGF-B binds to VEGFR-1 and NRP-1; VEGF-C and VEGF-D bind VEGFR-2 and VEGFR-3; PlGF binds only to VEGFR-1 (Plein et al, 2014; Maynard and Karumanchi, 2011; Hicklin and Ellis, 2005; Holmes and Zachary, 2005) (Figure 8).

Figure 8. The VEGF Family of Ligands and Receptors



NP1 or 2 = neuropilin 1 or 2; PlGF= placental growth factor; VEGF = vascular endothelial growth factor; VEGF-A/BC/D = vascular endothelial growth factor A/BC/D; VEGFR1 = vascular endothelial growth factor receptor 1; VEGFR2 = vascular endothelial growth factor receptor 2; VEGFR3 = vascular endothelial growth factor receptor 3.

Reproduced from Ellis and Hicklin, 2008.

Vascular endothelial growth factor receptor-1 (VEGFR-1), when expressed alone, is unable to mediate cellular responses to VEGF and is only weakly autophosphorylated following VEGF binding. This may be due to a less efficient kinase domain as compared to VEGFR-2 or increased tyrosine phosphatase sensitivity, resulting in lower overall activity of VEGFR-1 (Waltenberger et al, 1994). Furthermore, VEGFR-1 is thought to act as a decoy receptor as it can be alternatively spliced as a soluble form with high affinity for VEGF, subsequently reducing the availability of VEGF to bind to VEGFR-2 (Kendall and Thomas, 1993). Evidence from knockout mice and recombinant expression studies demonstrate that VEGFR-2 expressed alone can mediate all known cellular effects of VEGF (Shalaby et al, 1995; Waltenberger et al, 1994). VEGF receptor heterodimers can generate a variety of signaling outcomes and the HUVEC used in the analytical similarity assays can form all of the potential receptor combinations implicated in cancer biology.

In summary, VEGF is the best-characterized and primary regulator of endothelial cell survival, angiogenesis, and vascular permeability, and current evidence suggests that VEGFR-2 is the primary receptor responsible for mediating VEGF functions involved in tumor promotion (Shibuya and Claesson-Welsh, 2006; Gerber and Ferrara, 2005; Tammela et al, 2005; Waltenberger et al, 1994).

2.2.3 Mechanism of Action for ABP 215 and Bevacizumab

The mechanism of action for bevacizumab and ABP 215 across indications is binding to soluble VEGF and preventing the interaction of VEGF to its receptors (VEGFR-1 and VEGFR-2) on the surface of endothelial cells, thus inhibiting endothelial cell proliferation, angiogenesis, and VEGF-induced vascular permeability (Table 3). VEGF is overexpressed in a majority of solid tumors and in some lymphomas and hematologic malignancies (Dvorak, 2002). VEGFR-2 is also often overexpressed in the vasculature of tumors that express VEGF. Normally, angiogenesis is a tightly regulated process, controlled by regulating the expression and bioavailability of VEGF, activity of VEGFR-2, and availability of VEGF-sequestering agents such as soluble VEGFR-1 (Eichmann and Simons, 2012). In the case of tumor growth and angiogenesis driven by pathologic overexpression of VEGF, an abnormal and dysregulated vascular network is formed (Goel et al, 2011; Ellis and Hicklin, 2008). VEGF expression strongly correlates with prognostic indicators across multiple solid tumor types in which bevacizumab has been studied, such as the metastatic colorectal carcinoma, non-small cell lung carcinoma, glioblastoma, metastatic renal cell carcinoma, and cervical cancer.

The ability of VEGF to mediate angiogenesis and abnormal vascular architecture underlies disease pathogenesis in all approved indications. The mechanism of action for bevacizumab and ABP 215 in all indications is driven by the binding to VEGF, which prevents the interaction of VEGF with its receptors on the surface of endothelial cells. Through the neutralization of VEGF, angiogenesis required for the growth and persistence of solid tumors and their metastases is inhibited (Table 3).

Table 3. VEGF Expression Across Tumor Types and Relation to Prognosis

Patient Population	VEGF Expression	Association of Expression and Prognosis	References
mCRC	Increased expression in tumor cells vs normal tissue	Intensity of expression higher in metastatic tumors than non-metastatic Higher plasma VEGF level correlates with higher metastasis and lower survival	Jurgensmeier et al, 2013; Wei et al, 2005; Hanrahan et al, 2003; Hyodo et al, 1998; Takahashi et al, 1995
NSCLC	Increased expression in tumor cells vs normal tissue	Most reports demonstrate high VEGF expression and vascularization correlated with shorter median survival time, early relapse, and metastasis	Seto et al, 2006; Lantuejoul et al, 2003; Ushijima et al, 2001; Yuan et al, 2000; Decaussin et al, 1999; Fontanini et al, 1997; Mattern et al, 1996
GBM	Increased expression in glioblastoma cells as compared to astrocytoma cells	Nuclear VEGF expression correlated with survival	Clara et al, 2014; Plate et al, 1992
mRCC	Increased expression in carcinoma vs normal kidney tissue	Increased VEGF expression associated with tumor stage High VEGF expression associated with lower survival	Minardi et al, 2015; Jacobsen et al, 2004; Paradis et al, 2000; Tomisawa et al,1999; Takahashi et al, 1994
Cervical cancer	Increased expression in adenocarcinoma as compared to squamous cell carcinoma	High VEGF associated with poor prognosis	Gadducci et al, 2013; Randall et al, 2009; Loncaster et al, 2000; Fujimoto et al, 1999; Tokumo et al, 1998; Dobbs et al, 1997

GBM = glioblastoma multiforme; mCRC = metastatic colorectal cancer; mRCC = metastatic renal cell carcinoma; NSCLC = non-small cell lung cancer; VEGF = vascular endothelial growth factor.

2.2.4 Incorporation of Bevacizumab Product Knowledge Into the ABP 215 Biosimilar Development Program

Information on the known mechanism of action in each indication, as well as bevacizumab product knowledge, was applied during the design of the analytical similarity testing plan for ABP 215. The first focus of the analytical similarity testing plan was to evaluate structural and purity attributes, which was followed by a comprehensive evaluation of functional activities.

To assess the similarity of ABP 215 to bevacizumab, testing focused on the mechanism of action, ie, binding and neutralization of VEGF. Furthermore, comparative binding to multiple Fc receptors and C1q was assessed. Lastly, the lack of effector function was confirmed using multiple cell lines. As discussed in Section 3, the analytical similarity assessment results form the basis for Amgen's conclusion that ABP 215 is highly similar to bevacizumab and that ABP 215 is expected to have similar clinical performance in all indications approved for bevacizumab.

2.3 ABP 215 Manufacturing Information

Amgen undertook a thorough process to develop a cell line for ABP 215 to ensure that ABP 215 would match the amino acid sequence and other important structural and functional characteristics of bevacizumab. In doing this, a large number of clones were screened before creating the ABP 215 cell bank.

The ABP 215 commercial manufacturing process was developed and implemented prior to the initiation of clinical studies. Manufacturing changes were minimized during development to reduce potential shifts in product quality that could confound a determination of biosimilarity. Notably, the commercial drug substance manufacturing process, site, and scale were used to manufacture all lots used in the clinical studies.

The ABP 215 drug substance manufacturing process consists of cell culture, harvest, and purification steps, including steps designed to inactivate or remove any potential viral contaminants and to reduce process-related impurities. The ABP 215 drug substance manufacturing process was validated and consistently meets process performance and product quality expectations.

Similar to bevacizumab, ABP 215 is supplied as a sterile, single-use, preservative-free solution for IV infusion in a vial containing 100 mg/4 mL or 400 mg/16 mL of ABP 215 (25 mg/mL). ABP 215 drug product is formulated to the same concentration, with the same excipients, and at the same pH as bevacizumab. Each vial contains 25 mg/mL of ABP 215, 60 mg/mL α,α-trehalose dihydrate, 51 mM sodium phosphate, 0.040% (w/v) polysorbate 20, pH 6.2. 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. The ABP 215 drug product manufacturing process was validated and consistently meets process performance and product quality expectations.

3. ANALYTICAL SIMILARITY PROGRAM

The analytical similarity assessment comparing ABP 215 and bevacizumab was conducted according to a testing plan that specified the analyses to be performed and the assessment criteria to be applied when evaluating the similarity between the products.

To perform the analytical similarity assessment, bevacizumab lots were procured over approximately 6 years to estimate the lot-to-lot variability of bevacizumab. Testing included 27 bevacizumab (US) and 29 bevacizumab (EU) lots (which included the lots used in the clinical studies). These were compared with drug product lots manufactured over approximately the same period, from 13 unique drug substance lots. At least 13 drug product lots were typically tested for attributes that could be influenced by the manufacturing process, such as purity tests and glycosylation. A reduced number of lots was tested for attributes considered insensitive to variations in process conditions, such as higher order structure. The ABP 215 lots used in the analytical similarity assessment included all lots used in the nonclinical and clinical studies, and process validation lots.

The discussion of analytical similarity provided in this section focuses on the comparison of ABP 215 to bevacizumab (US); however, Amgen used both bevacizumab (US) and bevacizumab (EU) in the clinical program (Section 5). Therefore, Amgen compared bevacizumab (US) and bevacizumab (EU) analytically and in a PK similarity study to establish a scientific bridge between the 2 products. For the analytical comparisons, ABP 215, bevacizumab (US), and bevacizumab (EU) were subjected to the same testing. The analytical similarity results between bevacizumab (US) and bevacizumab (EU) provided in the biologics license application, when combined with the 3-way PK similarity data (Section 5.1.1), established the requisite scientific bridge for bevacizumab sourced from the 2 regions.

The attributes studied in the analytical similarity assessment were selected based on knowledge regarding the structure, function, and heterogeneity of bevacizumab and ABP 215, including those characteristics critical to the biological activity and stability of the products (Section 2.2). The assessment included comparative evaluations and incorporated complementary structural, purity, and functional assays that measured the same attribute using different methodologies. All of the assays were appropriately qualified or validated, and determined to be suitable for their intended use.

3.1 Tiering of the Analytical Similarity Attributes

During the ABP 215 development program, the FDA recommended using a risk-ranking approach to tier attributes/assays. In accordance with FDA advice, each similarity attribute/assay was assigned to 1 of 3 tiers based on the relevance of the attribute to clinical outcomes and the nature of the data output from the analysis.

Tier 1 attributes/assays have the highest risk to clinical outcomes and include assays that evaluate the primary mechanism of action that is known to contribute to the safety and efficacy of the molecule. Specifically, potency (as determined by a proliferation inhibition bioassay) and binding to VEGF are directly relevant to the mechanism of action and were categorized as Tier 1 in agreement with FDA advice. Tier 1 attributes/assays were assessed using statistical equivalence. In this statistical approach, similarity is concluded when the 90% confidence interval for the difference in means between the products is contained within an equivalence acceptance criterion (EAC) of $\pm$ 1.5 times the standard deviation of the reference product dataset.

Tier 2 attributes/assays have a relatively lower risk to clinical outcomes and include structural attributes, protein concentration, product-related substances and impurities, and a subset of functional characterization assays. The Tier 2 attributes/assays were considered similar between the products when 90% of the ABP 215 lots fell within a pre-defined quality range established based on the reference product dataset. The quality range was defined as the mean of the reference product dataset $\pm$ 3 standard deviations. For Tier 2 attributes/assays where a change over time was observed at the recommended storage condition, all values were adjusted for material age prior to the quality range assessment. This was achieved by calculating the mean rate of change over time for the attribute, and then extrapolating all results to 24 months, to allow a comparison that would not be biased by sample age effects. For Tier 2 attributes/assays where the data were not amenable to statistical evaluation, individual values were compared to a pre-defined limit and similarity was demonstrated if every individual value satisfied the limit.

Tier 3 attributes/assays included those with the lowest risk to clinical outcomes and those where the data are qualitative or not suitable for numerical evaluations. Similarity of Tier 3 attributes was based on qualitative comparisons.

3.2 Analytical Similarity Results

The discussion of analytical similarity is split into 2 components. First, a summary of structural and purity results is presented (Section 3.2.1). Next, the functional activity results are provided (Section 3.2.2).

3.2.1 Structural and Purity Attributes

The structure and purity of ABP 215 and bevacizumab were compared using assays that assessed the following categories:

- primary structure
- higher order structure
- particles and aggregates
- product-related substances and impurities
- thermal stability and degradation
- general properties
- process-related impurities

A summary of the structural and purity results is presented in Table 4 which demonstrate that ABP 215 is analytically similar to bevacizumab. In the table, a check mark indicates that the pre-defined similarity assessment criteria were met. Where this was not the case, the observed difference is noted in the table. Additionally, representative analytical data that can be presented graphically are shown in Appendix 1.

Table 4. ABP 215 vs Bevacizumab (US) Analytical Similarity Assessment Results for Structural and Purity Characteristics

Category	Analytical Testing and Parameter	Tier - Similarity Assessment Approach	Assessment Criteria	ABP 215 Results	Demonstrated Similarity
Primary Structure	Intact molecular mass: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 32	√
	Intact molecular mass: Molecular weight	2 - Pre-defined limit	Observed mass should be within ± 50 ppm of the theoretical mass for the predominant species	Predominant species all within 50 ppm of the theoretical masses	√
	Reduced and deglycosylated molecular masses of HC and LC: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 33 and Figure 34	√
	Reduced and deglycosylated molecular masses of HC and LC: Molecular weight	2 - Pre-defined limit	Observed mass should be within ± 50 ppm of the theoretical mass	Observed mass was within 50 ppm of the theoretical mass	√
	Reduced peptide map: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 35	√
	Reduced peptide map: amino acid sequence	2 - Pre-defined limit	For the hybrid Ion Trap-Orbitrap Mass Spectrometer, the observed mass of the tryptic peptide fragments should be within ± 50 ppm of the theoretical mass.	Observed mass was within ± 50 ppm of the theoretical mass.	√
			For the linear ion trap mass spectrometer, the observed mass of the tryptic peptide fragments should be within ± 200ppm for peptide mass > 1000 Da, and within ± 500 ppm for peptide mass < 1000 Da when compared to the theoretical mass	Observed mass was within ± 200 ppm for peptide mass > 1000 Da, and within ± 500 ppm for peptide mass < 1000 Da when compared to the theoretical mass	

Abbreviations defined on last page of this table.

[a] Visually similar = no new peaks/species greater than the detection limit of the method.
[b] For Tier 2 attributes using a quality range, analytical similarity was demonstrated if at least 90% of the individual test lot values fell within the bevacizumab derived quality range.

Table 4. ABP 215 vs Bevacizumab (US) Analytical Similarity Assessment Results for Structural and Purity Characteristics

Category	Analytical Testing and Parameter	Tier - Similarity Assessment Approach	Assessment Criteria	ABP 215 Results	Demonstrated Similarity
Primary Structure	Non-reduced peptide map: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 36	√
	Non-reduced peptide map: Disulfide structure	2 - Pre-defined limit	Observed mass of the tryptic peptide fragments should be within ± 200 ppm for peptide mass > 2000 Da, and within ± 1000 ppm for peptide mass < 2000 Da	Observed mass was within ± 200 ppm for peptide mass > 2000 Da, and within ± 1000 ppm for peptide mass < 2000 Da	√
	Glycan map: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 37	Similar profile Minor quantitative differences in specific glycans (Section 3.2.1.1)
	Glycan map: % high mannose	2 - Quality range[b]	LOQ (0.1) to 1.2	1.2 to 2.7	
	Glycan map: % galactosylation	2 - Quality range[b]	1.2 to 26.7	17.1 to 29.4	
	Glycan map: % afucosylation	2 - Quality range[b]	0.9 to 3.5	1.2 to 1.7	√
	Glycan map: % sialylation	3 - Qualitative comparison	N/A	Both ABP 215 and bevacizumab have similarly low levels of sialylation at or near the LOQ (0.1%) of the assay	√
	cIEF: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 38	√
	cIEF: Isoelectric point	2 - Pre-defined limit	± 0.1 pH units	The average main peak pI value were within 0.1 pH units	√
	Extinction coefficient	2 - Pre-defined limit	± 10%	Within 10%	√
	Identity by ELISA	3 - Qualitative comparison	N/A	Similar	√

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Abbreviations defined on last page of this table.

[a] Visually similar = no new peaks/species greater than the detection limit of the method.

[b] For Tier 2 attributes using a quality range, analytical similarity was demonstrated if at least 90% of the individual test lot values fell within the bevacizumab derived quality range.

Table 4. ABP 215 vs Bevacizumab (US) Analytical Similarity Assessment Results for Structural and Purity Characteristics

Category	Analytical Testing and Parameter	Tier - Similarity Assessment Approach	Assessment Criteria	ABP 215 Results	Demonstrated Similarity
Higher Order Structure	FTIR: Spectral similarity	2 - Pre-defined limit	Spectral similarity is > 95%	Spectral similarity value of each individual lot is > 95% when compared to the reference spectrum	√
	FTIR: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 39 and Figure 40	√
	Near UV CD: Spectral similarity	2 - Pre-defined limit	Spectral similarity is > 95%	Spectral similarity value of each individual lot is > 95% when compared to the reference spectrum	√
	Near UV CD: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 41 and Figure 42	√
	DSC: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 43	√
	DSC: T_{m1}	2 - Quality range[b]	72.3 to 73.3	72.7 to 73.1	√
	DSC: T_{m2}	2 - Quality range[b]	82.7 to 84.3	83.2 to 83.7	√

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Abbreviations defined on last page of this table.

[a] Visually similar = no new peaks/species greater than the detection limit of the method.
[b] For Tier 2 attributes using a quality range, analytical similarity was demonstrated if at least 90% of the individual test lot values fell within the bevacizumab derived quality range.

Table 4. ABP 215 vs Bevacizumab (US) Analytical Similarity Assessment Results for Structural and Purity Characteristics

Category	Analytical Testing and Parameter	Tier - Similarity Assessment Approach	Assessment Criteria	ABP 215 Results	Demonstrated Similarity
Particles and Aggregates	HIAC: ≥ 2 μm particles ≥ 5 μm particles ≥ 10 μm particles ≥ 25 μm particles	3 - Qualitative comparison	N/A	Similar	√
	MFI: ≥ 5 μm particles	3 - Qualitative comparison	N/A	Similar	√
	MFI: ≥ 5 μm non-spherical particles	2 - Quality range[b]	0 to 3533	0 to 65	√
	FFF: Submicron particles	3 - Qualitative comparison	N/A	Similar	√
	DLS: Submicron particles	3 - Qualitative comparison	N/A	Similar, Figure 44	√
	AUC-SV: Monomer (%)	2 - Quality range[b]	96.4 to 99.4	98.0 to 99.3	√
	AUC-SV: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 45	√
	SE-HPLC-LS: Molar mass	2 - Pre-defined limit	Pre-peak within 10% and main peak within 5% of the expected molar masses	Pre-peak within 10% and main peak within 5% of the expected molar masses, Figure 46 and Figure 47	√

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Abbreviations defined on last page of this table.

[a] Visually similar = no new peaks/species greater than the detection limit of the method.
[b] For Tier 2 attributes using a quality range, analytical similarity was demonstrated if at least 90% of the individual test lot values fell within the bevacizumab derived quality range.

Table 4. ABP 215 vs Bevacizumab (US) Analytical Similarity Assessment Results for Structural and Purity Characteristics

Category	Analytical Testing and Parameter	Tier - Similarity Assessment Approach	Assessment Criteria	ABP 215 Results	Demonstrated Similarity
Product-related Substances and Impurities	SE-HPLC: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 48	Similar profile
	SE-HPLC: HMW	2 - Age adjusted quality range[b]	2.6 to 3.5	2.2 to 3.3	Minor differences in high molecular weight species (Section 3.2.1.4)
	rCE-SDS: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 49	Similar profile
	rCE-SDS: HC+LC	2 - Age adjusted quality range[b]	94.8 to 96.0	96.8 to 97.3	Minor differences in glycan occupancy and fragmented species (Section 3.2.1.4)
	rCE-SDS: NGHC	2 - Age adjusted quality range[b]	1.5 to 2.1	0.6 to 0.8	
	rCE-SDS: LMW + MMW	2 - Age adjusted quality range[b]	1.9 to 2.5	1.6 to 1.9	
	nrCE-SDS: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 50	Similar profile
	nrCE-SDS: Main peak	2 - Quality range[b]	96.5 to 97.5	96.1 to 97.7	Minor differences in partially reduced species (Section 3.2.1.4)
	nrCE-SDS: Pre-peaks	2 - Quality range[b]	2.1 to 2.8	2.0 to 3.8	

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Abbreviations defined on last page of this table.

[a] Visually similar = no new peaks/species greater than the detection limit of the method.
[b] For Tier 2 attributes using a quality range, analytical similarity was demonstrated if at least 90% of the individual test lot values fell within the bevacizumab derived quality range.

Table 4. ABP 215 vs Bevacizumab (US) Analytical Similarity Assessment Results for Structural and Purity Characteristics

Category	Analytical Testing and Parameter	Tier - Similarity Assessment Approach	Assessment Criteria	ABP 215 Results	Demonstrated Similarity
Product-related Substances and Impurities	CEX-HPLC: Profile	3 - Qualitative comparison	N/A	Visually similar[a], Figure 51	Similar profile
	CEX-HPLC: Acidic peaks	2 - Age adjusted quality range[b]	26.1 to 34.2	22.1 to 24.6	Minor differences in the levels of C-terminal lysine, C-terminal proline-amidation, deamidation, and N-terminal glutamic acid cyclization (Section 3.2.1.4)
	CEX-HPLC: Main peak	2 - Age adjusted quality range[b]	58.6 to 67.1	63.8 to 70.6	
	CEX-HPLC: Basic peaks	2 - Age adjusted quality range[b]	5.4 to 8.6	6.8 to 11.7	
Thermal Stability and Degradation	50°C Forced degradation	3 - Qualitative comparison	N/A	Similar	√
	40°C Stressed stability	3 - Qualitative comparison	N/A	Similar	√
	25°C Accelerated stability	3 - Qualitative comparison	N/A	Similar	√
General Properties	Protein concentration (mg/mL)	2 - Quality range[b]	23.3 to 26.4	24.4 to 25.8	√
	Volume	3 - Qualitative comparison	N/A	Similar	√
	Osmolality	3 - Qualitative comparison	N/A	Similar	√
	pH	3 - Qualitative comparison	N/A	Similar	√
	Appearance	3 - Qualitative comparison	N/A	Similar	√
	Color	3 - Qualitative comparison	N/A	Similar	√
	Clarity	3 - Qualitative comparison	N/A	Similar	√

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Abbreviations defined on last page of this table.

[a] Visually similar = no new peaks/species greater than the detection limit of the method.
[b] For Tier 2 attributes using a quality range, analytical similarity was demonstrated if at least 90% of the individual test lot values fell within the bevacizumab derived quality range.

Table 4. ABP 215 vs Bevacizumab (US) Analytical Similarity Assessment Results for Structural and Purity Characteristics

Category	Analytical Testing and Parameter	Tier - Similarity Assessment Approach	Assessment Criteria	ABP 215 Results	Demonstrated Similarity
Process-related Impurities	HCP- ELISA	3 - Qualitative comparison	N/A	Similar	√
	HCP analysis by LC-MS	3 - Qualitative comparison	N/A	Similar	√
	HCP analysis by 2D-DIGE	3 - Qualitative comparison	N/A	Similar	√
	Protein A - ELISA	3 - Qualitative comparison	N/A	Similar	√
	Residual DNA - qPCR	3 - Qualitative comparison	N/A	Similar	√

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2D-DIGE = 2 dimensional in-gel electrophoresis; AUC-SV = analytical ultracentrifugation sedimentation velocity; CEX-HPLC = cation exchange high performance liquid chromatography; cIEF = capillary isoelectric focusing; DLS = dynamic light scattering; DSC = differential scanning calorimetry; ELISA = enzyme linked immunosorbent assay; FFF = field flow fractionation; FTIR = fourier transform infrared spectroscopy; HC = heavy chain; HCP = host cell protein; HIAC = high accuracy light obscuration particle counting; HMW = high molecular weight; LC = light chain; LC-MS = liquid chromatography mass spectrometry; LMW = low molecular weight; LOQ = limit of quantitation; MFI = micro flow imaging; MMW = mid molecular weight; NGHC = non-glycosylated heavy chain; nrCE-SDS = non reduced capillary electrophoresis - sodium dodecyl sulfate; pI = isoelectric point; qPCR = quantitative polymerase chain reaction; rCE-SDS = reduced capillary electrophoresis - sodium dodecyl sulfate; SE-HPLC = size exclusion high performance liquid chromatography; SE-HPLC-LS = size exclusion high performance liquid chromatography with light scattering detection; UV CD = ultraviolet circular dichroism.

[a] Visually similar = no new peaks/species greater than the detection limit of the method.
[b] For Tier 2 attributes using a quality range, analytical similarity was demonstrated if at least 90% of the individual test lot values fell within the bevacizumab derived quality range.

3.2.1.1 Primary Structure

An analysis of the primary structure was performed which included assays to assess the amino acid sequence and glycosylation of ABP 215 and bevacizumab. Based on the results, Amgen concluded that ABP 215 has the same amino acid sequence as bevacizumab. The glycosylation profile was similar between ABP 215 and bevacizumab, and no new species were detected (Figure 37); however, some minor quantitative differences in specific glycans were observed.

Glycosylation

Non-human glycans, such as galactose-alpha-1,3-galactose (alpha-Gal) and N-glycolylneuraminic acid (NGNA), have been associated with immunogenicity in monoclonal antibody products produced in murine cell lines. Since ABP 215 and bevacizumab are manufactured in CHO cell lines, these glycans are not present in either product. However, other glycans may affect PK and/or biological functions, and therefore a robust assessment of the glycan profiles for ABP 215 and bevacizumab was conducted.

The following glycan groups were evaluated based on their potential to affect PK and/or biological functions including binding to C1q and Fc receptors:

- % high mannose, an afucosylated species, which has the potential to affect PK and FcγRIIIa binding
- % afucosylation, an afucosylated species other than high mannose, which has the potential to affect FcγRIIIa binding
- % galactosylation, which has the potential to affect C1q binding
- % sialylation, which is not expected to have an impact at the levels observed in bevacizumab

The glycosylation analysis indicated that ABP 215 has similar levels of afucosylation and sialyation. However, ABP 215 has slightly higher levels of high mannose and galactosylation compared to bevacizumab. These minor differences, at the levels observed, are not considered clinically meaningful since:

- High mannose levels below 5% have been previously documented to not impact IgG PK properties (Goetze et al, 2011).
- ABP 215 has a similar PK profile compared to bevacizumab (Section 5.1.1.2).
- ABP 215 and bevacizumab do not induce effector functions, such as ADCC and CDC that are mediated by binding to FcγRIIIa or C1q, respectively (Section 3.2.2.2).

3.2.1.2 Higher Order Structure

For the higher order structure evaluation (the 3-dimensional folding and assembly of the IgG1 chains), a number of spectroscopic techniques were used to assess the similarity of the secondary and tertiary structures. The results demonstrated similarity in all of the tests performed.

3.2.1.3 Particles and Aggregates

With respect to the particulates and aggregates, several methods were employed to assess different size ranges and morphologies. The results demonstrated similarity in all of the tests performed.

3.2.1.4 Product-related Substances and Impurities

Amgen determined that the main product-related substances and impurities for ABP 215 are charge and size variants. Levels of charge and size variants are known to differ between products manufactured using different cell lines and are known to be affected by the manufacturing process. Therefore, several highly sensitive assays were employed to detect potential differences.

Size Variants

The size variant profiles are similar between ABP 215 and bevacizumab, and no new species were present (Appendix 1). However, some quantitative differences were observed in low, medium, and high molecular weight variants. Overall, the quantity of size variants in both products are low, and the mean values for the different size variants in ABP 215 are all less than the mean values of the reference product. Since size variants are typically viewed as impurities, having slightly lower levels in ABP 215 is not considered likely to affect clinical safety or efficacy.

Size Exclusion - High Performance Liquid Chromatography

ABP 215 has a slightly lower level of high molecular weight species which does not affect the biological activity (Section 3.2.2) and does not negatively impact the safety profile of ABP 215.

Reduced Capillary Electrophoresis - Sodium Dodecyl Sulfate (rCE-SDS)

ABP 215 has a higher glycan occupancy than bevacizumab, as measured by rCE-SDS. ABP 215 has lower levels of non-glycosylated heavy chain (NGHC) and fragments (low molecular weight + mid molecular weight species) compared to bevacizumab, and a corresponding higher level of glycosylated structures indicated by % heavy chain + light chain in Table 4. NGHC levels could potentially affect effector functions if present.

However, since the magnitude of the difference is small, and ABP 215 and bevacizumab do not induce ADCC and CDC, the difference in glycan occupancy is not considered clinically meaningful (Reusch and Tejada, 2015; Jung et al, 2010; Tao and Morrison, 1989).

Non-reduced Capillary Electrophoresis - Sodium Dodecyl Sulfate (nrCE-SDS)

A non-reduced capillary electrophoresis technique was used to assess product-related impurities associated with variations in disulfide cross-linking. Partially reduced species, indicated as "% pre-peaks" in Table 4, are missing 1 or more disulfide bond cross-linkages. The observed differences are quantitatively small and were confirmed to have no effect on the relative potency of ABP 215 (Section 3.2.2).

Charge Variants

The charge profiles are similar between ABP 215 and bevacizumab, and no new species are present (Appendix 1). Some quantitative differences were observed for certain protein modifications; however, all of the modifications observed in both products are typical of monoclonal antibody products and do not pose a concern with respect to safety, efficacy, or immunogenicity.

Cation Exchange - High Performance Liquid Chromatography (CEX-HPLC)

CEX-HPLC separates proteins according to their surface charge, which can be influenced by the presence of variants such as deamidation, and C-terminal lysine. CEX-HPLC analysis showed that ABP 215 has a lower level of acidic variants and higher level of main peak compared to bevacizumab (US). The minor quantitative difference in the acidic peaks is attributed to deamidation and N-terminal glutamic acid cyclization. These variants do not occur in the complimentary-determining region, and are present in both ABP 215 and bevacizumab, which do not affect the biological activity (Section 3.2.2). In addition, both deamidation and N-terminal glutamic acid cyclization occur in monoclonal antibodies in vivo, and thus the minor quantitative difference is not clinically relevant (Liu et al, 2011).

ABP 215 has a slightly higher level of basic peaks compared to bevacizumab. The minor quantitative difference in the basic peaks can be attributed to unprocessed heavy chain C-terminal lysine and C-terminal proline-amidation, which do not affect the biological activity (Section 3.2.2). Variations in C-terminal lysine levels are common in monoclonal antibodies and are not considered to impact potency, safety, or immunogenicity. Furthermore, C-terminal lysine has been shown to be rapidly removed in vivo, soon after administration of an antibody (Cai et al, 2011). C-terminal

proline-amidation is a common post-translational modification resulting from enzymatic reactions, which involve basic carboxypeptidases, peptidylglycine α-hydroxylating monooxygenase, and peptidyl-α-hydroxyglycine α-amidating lyase. C-terminal proline-amidation of IgG antibodies is naturally occurring and has been found in serum and commercial monoclonal antibodies expressed in CHO cells (Tsubaki et al, 2013; Johnson et al, 2007).

3.2.1.5 Thermal Stability and Degradation

The product structure in thermal forced degradation experiments was assessed since the degradation behavior of a molecule may highlight structural differences that may not be apparent from other testing. The results showed that ABP 215 and bevacizumab have similar forced degradation behavior.

3.2.1.6 General Properties

General properties of the drug product were assessed, and similarity for all of the tested properties was demonstrated. ABP 215 has similar volume for both 100 mg/4 mL and 400 mg/16 mL vial presentations as compared to bevacizumab. ABP 215 is similar to bevacizumab for protein concentration.

3.2.1.7 Process-related Impurities

Process-related impurities were assessed, and the results demonstrated that these impurities are present at acceptably low levels in ABP 215.

3.2.2 Functional Activities

The biological assays performed were intended to evaluate analytical similarity and to support the extrapolation to all of the indications for which Amgen is seeking licensure for ABP 215. Therefore, multiple assays interrogating Fab-mediated activities were conducted (Table 5). Fc functionality was included in the testing plan to assess the structural integrity of the Fc domain. There is no contribution of Fc receptor binding to the clinical safety and efficacy profile of ABP 215 and bevacizumab. The results from these assays confirmed that ABP 215 and bevacizumab have similar functional activities.

Functional testing focused on the mechanism of action, binding and neutralization of VEGF. Furthermore, comparative binding to multiple Fc receptors and C1q were assessed to evaluate Fc-binding activities and the structural integrity of the Fc domain. The lack of ADCC and CDC effector functions was confirmed in multiple cell lines, including a cell line with cell surface captured VEGF.

The full list of methods used to evaluate functional activities between ABP 215 and bevacizumab is provided in Table 5. For Tier 1 and Tier 2 functional activities, 13 ABP 215 lots were tested along with a minimum of 10 bevacizumab (US) lots. Additional characterization assays were included to comprehensively assess the similarity of the products by testing a representative, but limited, set of ABP 215 and bevacizumab lots. Not all of the functional activity results are presented in this document, although they were included in the biologics license application. The results from the similarity testing in key assays are presented in the following sections, with information on the specific assays provided in Appendix 1.

Table 5. Functional Activity Assays

Method	Relevant Activity	Tier - Similarity Assessment Approach	Demonstrated Similarity
Fab-mediated Activities			
Binding to VEGF	VEGF	1 - Equivalence acceptance criterion	√
Neutralization of VEGF-mediated proliferation in HUVEC (potency)	VEGF	1 - Equivalence acceptance criterion	√
On and off bindrates (VEGF)	VEGF	3 - Qualitative comparison	√
Binding to VEGF isoforms	$VEGF_{121}$ and $VEGF_{165}$	3 - Qualitative comparison	√
Inhibition of VEGFR-2 RTK autophosphorylation	VEGF	3 - Qualitative comparison	√
Specificity by VEGFR-2 RTK autophosphorylation	VEGF-C and VEGF-D	3 - Qualitative comparison	√
Fc-mediated Characterization			
Binding to FcRn	FcR	2 - Quality range	√
Binding to FcγRIa	FcR	3 - Qualitative comparison	√
Binding to FcγRIIa (131H)	FcR	3 - Qualitative comparison	√
Binding to FcγRIIb	FcR	3 - Qualitative comparison	√
Binding to FcγRIIIa (158V)	FcR	2 - Quality range	Minor differences (Section 3.2.2.2)
Binding to FcγRIIIa (158F)	FcR	2 - Quality range	√
Binding to FcγRIIIb	FcR	3 - Qualitative comparison	√
Binding to C1q	C1q	2 - Quality range	√
Fab- and Fc-mediated Characterization			
Lack of ADCC activity	VEGF and FcR	3 - Qualitative comparison	√
Lack of CDC activity	VEGF and C1q	3 - Qualitative comparison	√

ADCC = antibody-dependent cell-mediated cytotoxicity; C1q = first subcomponent of the C1 complex of the classical pathway of complement activation; CDC = complement-dependent cytotoxicity; F = phenylalanine; Fc = fragment crystallizable; FcR = Fc receptor; FcRn = neonatal Fc receptor; FcγRIa = Fc gamma receptor Type Ia; FcγRIIa = Fc gamma receptor Type IIa; FcγRIIb = Fc gamma receptor Type IIb; FcγRIIIa = Fc gamma receptor Type IIIa; FcγRIIIb = Fc gamma receptor Type IIIb; H = histidine; HUVEC = human umbilical vein endothelial cells; RTK = receptor tyrosine kinase; V = valine; VEGF = vascular endothelial growth factor; $VEGF_{121}$ = vascular endothelial growth factor isoform 121; $VEGF_{165}$ = vascular endothelial growth factor isoform 165; VEGF-C = vascular endothelial growth factor type C; VEGF-D = vascular endothelial growth factor type D; VEGFR-2 = vascular endothelial growth factor receptor 2.

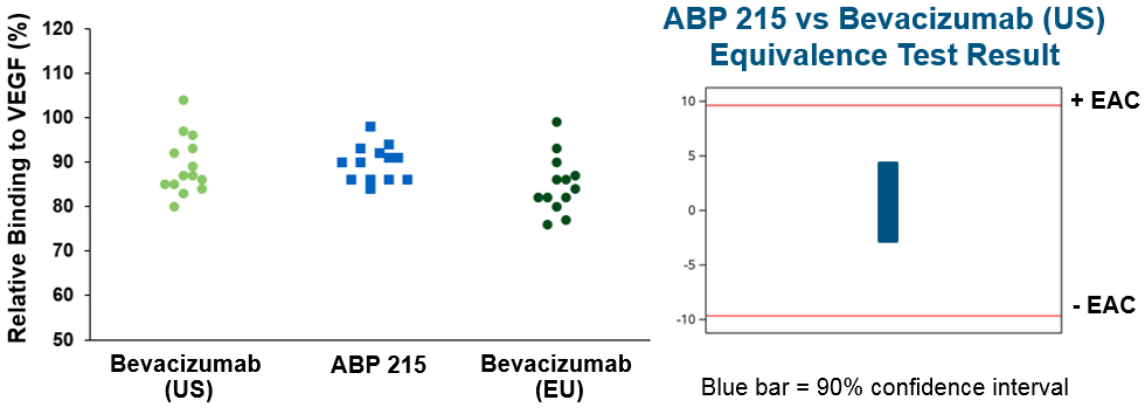
3.2.2.1 Assessment of Fab-mediated Activities

Binding to VEGF

Binding to VEGF is critical for the mechanism of action of bevacizumab. Binding to VEGF prevents its ability to signal through its receptors, resulting in downstream outcomes such as the inhibition of proliferation of endothelial cells and the formation of new blood vessels. There are multiple isoforms of VEGF due to the generation of alternative splice isoforms, and bevacizumab binds to all splice isoforms through a conserved epitope present in the N-terminus of all VEGF isoforms (Ferrara, 2004). Since binding and neutralization of VEGF is the mechanism of action in all indications for which ABP 215 licensure is being sought (Section 2.2.3), the binding results are critical when considering the totality of evidence for similarity and for extrapolation to indications not studied during the ABP 215 clinical program.

A solid phase enzyme-linked immunosorbent assay (ELISA) was used to determine the binding of ABP 215 and bevacizumab. The results are presented in Figure 9, along with a graph showing the confidence interval in relation to the bevacizumab (US)-derived Tier 1 EAC, as described in Section 3.1. Additionally, a representative dose-response curve for each product is shown in Figure 10. The 90% confidence interval for the difference in means falls within the EAC, and therefore, the VEGF binding of ABP 215 and bevacizumab (US) are statistically equivalent.

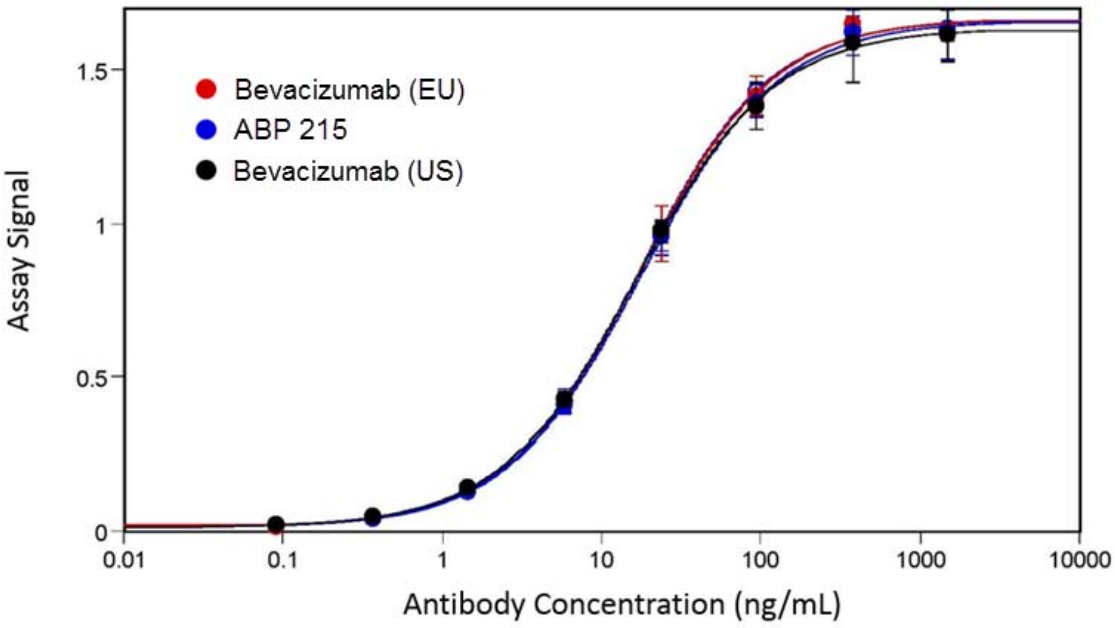
Figure 9. Relative Binding to VEGF



EAC = equivalence acceptance criterion; VEGF = vascular endothelial growth factor.

Binding is calculated relative to the ABP 215 reference standard.

Figure 10. Representative Dose-response Curve of Bevacizumab (EU), ABP 215, and Bevacizumab (US) in the VEGF Binding Assay

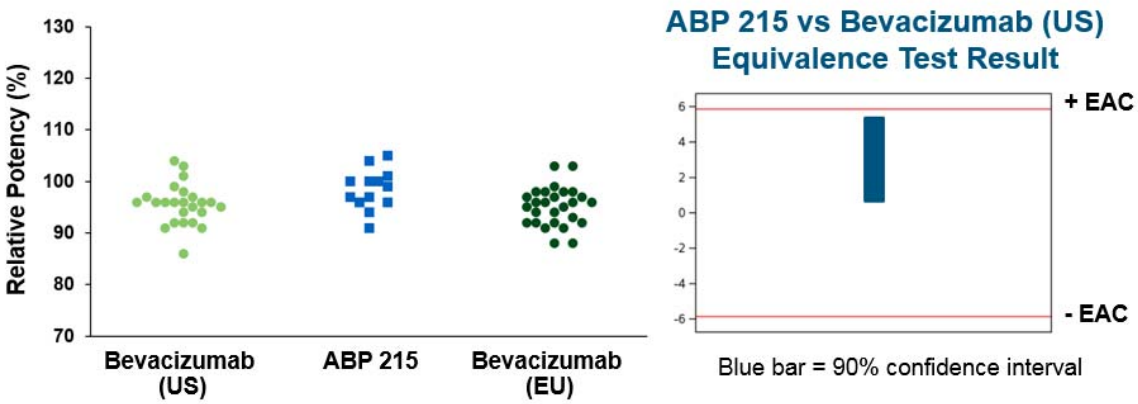


Neutralization of VEGF-mediated Proliferation in HUVEC

VEGF-induced activation of VEGFR-2 and the resulting signaling cascade causes a variety of cellular and tissue responses including vascular endothelial cell survival, proliferation, and migration ultimately leading to vasodilation and modulation of vascular permeability (Ferrara and Davis-Smyth, 1997). The most immediate and measurable effect, and therefore, the most relevant measure of similarity between ABP 215 and bevacizumab, is proliferation of HUVEC.

The primary potency assay assesses the neutralization of VEGF-mediated proliferation in HUVEC. HUVEC express all VEGF receptors (VEGFR-1, VEGFR-2, and VEGFR-3) in addition to the co-receptors NRP-1 and NRP-2, and proliferate in response to VEGF (Lee-Montiel et al, 2015; Aparicio et al, 2005). ABP 215 and bevacizumab were compared for their ability to inhibit the proliferation of HUVEC in a dose-dependent manner, and the results are presented in Figure 11, along with a graph showing the confidence interval in relation to the bevacizumab (US)-derived Tier 1 EAC, as described in Section 3.1. Additionally, a representative dose-response curve for each product is shown in Figure 12. The 90% confidence interval for the difference in means falls within the EAC, and therefore, the potency of ABP 215 and bevacizumab (US) are statistically equivalent.

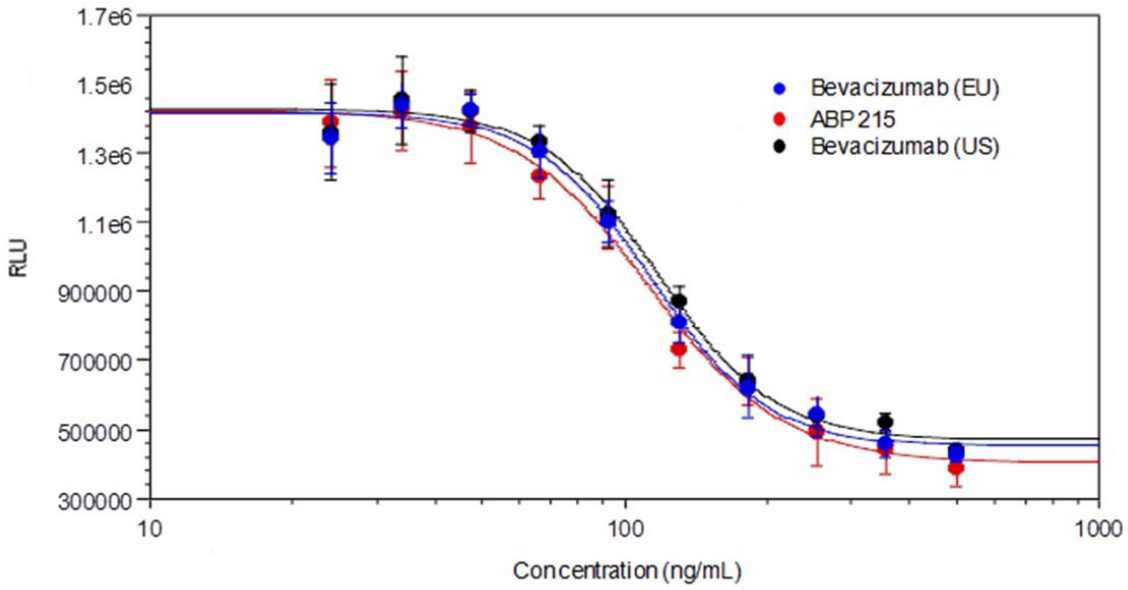
Figure 11. Neutralization of VEGF-mediated Proliferation in HUVEC (Potency)



EAC = equivalence acceptance criterion; VEGF = vascular endothelial growth factor.

Potency is calculated relative to the ABP 215 reference standard.

Figure 12. Representative Dose-response Curve of Bevacizumab (EU), ABP 215, and Bevacizumab (US) in the Proliferation Inhibition Bioassay (Potency)



RLU = relative lumincensence unit.

Kinetic Binding (VEGF)

To further characterize the binding of ABP 215 and bevacizumab to VEGF, surface plasmon resonance (SPR) was used. The kinetic data are valuable, because they not only provide the equilibrium binding constant, but also the rate constants, which characterize the dynamics of the interaction (Pollard, 2010). The association and dissociation rate constants (k_a, k_d) and the dissociation equilibrium binding constant (K_D) for binding of ABP 215 and bevacizumab to recombinant human VEGF are reported in Table 6. ABP 215 and bevacizumab demonstrate similar VEGF binding kinetics.

Table 6. Binding Kinetics and Affinity of VEGF

Lot	k_a $(M^{-1}s^{-1})\times10^5$	k_d $(s^{-1})\times10^{-6}$	K_D (pM)
EU B7115B10	3.5	8.0	22.9
EU B7003B03	3.5	6.5	18.4
EU B7108B02	3.4	8.2	23.4
ABP 0010095534	3.4	8.2	23.5
ABP 0010112870	3.5	7.7	22.6
ABP 0010133673	3.5	7.3	20.1
US 605024	3.4	8.1	23.4
US 616370	3.5	7.6	22.9
US 640016	3.4	7.8	23.9

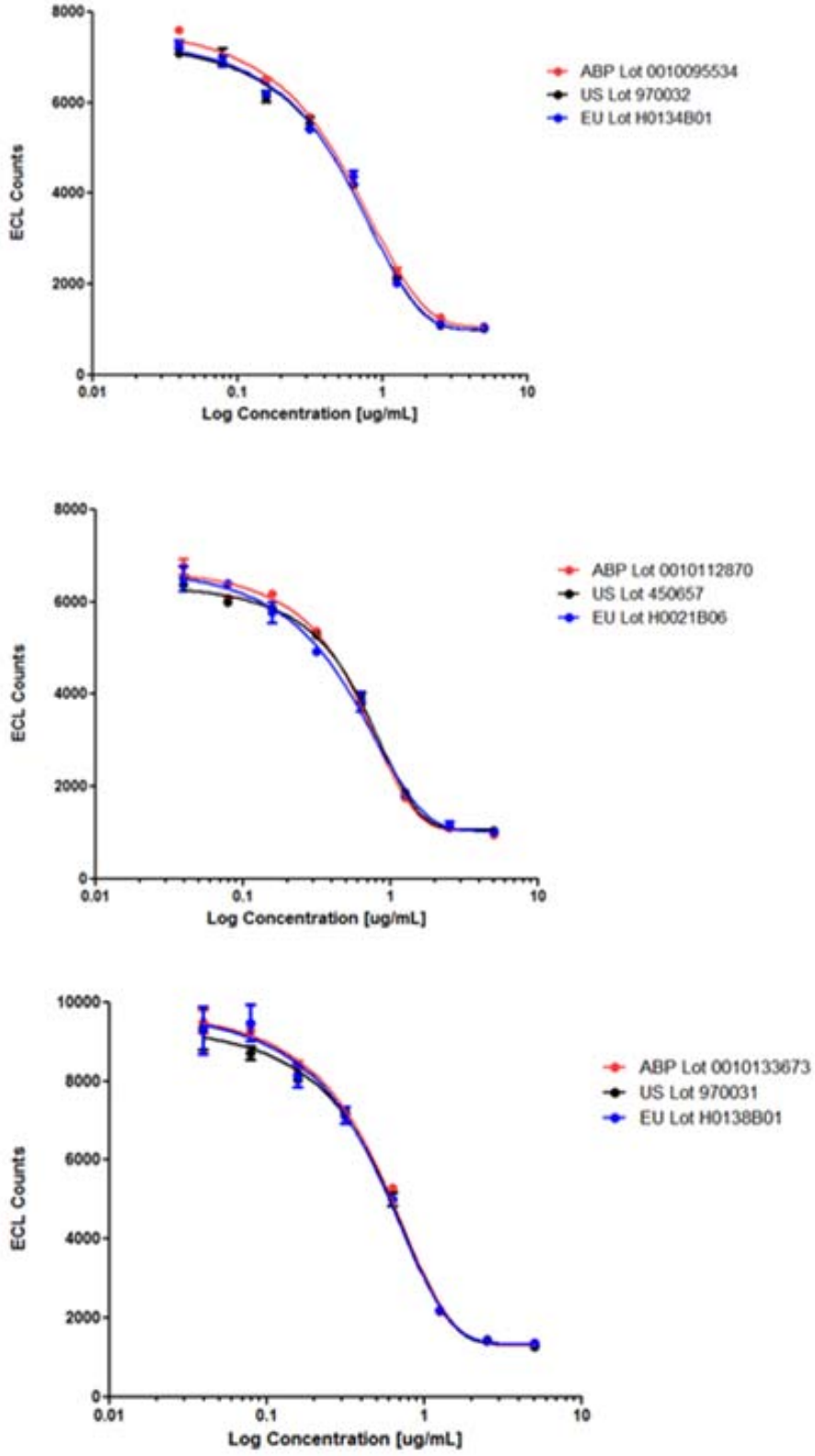
k_a = association rate constant; k_d = dissociation rate constant; K_D = the equilibrium dissociation constant, a ratio of k_d/k_a; pM = picomolar; VEGF = vascular endothelial growth factor.

Inhibition of VEGFR-2 RTK Autophosphorylation

VEGF is able to bind and activate both VEGFR-1 and VEGFR-2 as homodimers in addition to heterodimers containing VEGFR-1, VEGFR-2, and VEGFR-3, but the proliferation of vascular endothelial cells and angiogenesis is mediated predominantly through VEGF-mediated activation of VEGFR-2 (Gerber et al, 1998; Kroll and Waltenberger, 1997; Waltenberger et al, 1994). Binding of VEGF to VEGFR-2 expressed on endothelial cells results in the rapid autophosphorylation of the intracellular tyrosine residues of the VEGFR-2 receptor tyrosine kinase (RTK) (Feliers et al, 2005). The autophosphorylation of VEGFR-2 triggers downstream signaling cascades that result in cell proliferation and angiogenesis. The RTK phosphorylation method uses HUVEC, the same cell type used in the potency assay. ABP 215 and bevacizumab inhibit VEGF-mediated VEGFR-2 autophosphorylation in a dose-dependent manner.

Representative dose-response curves for ABP 215, bevacizumab (US), and bevacizumab (EU) inhibition of VEGF-mediated RTK autophosphorylation are shown in Figure 13. The results support the conclusion that ABP 215 has similar inhibition of VEGFR-2 by RTK autophosphorylation compared to bevacizumab.

Figure 13. Representative Inhibition in the RTK Phosphorylation Assay



ECL = electrochemiluminescent; RTK = receptor tyrosine kinase.

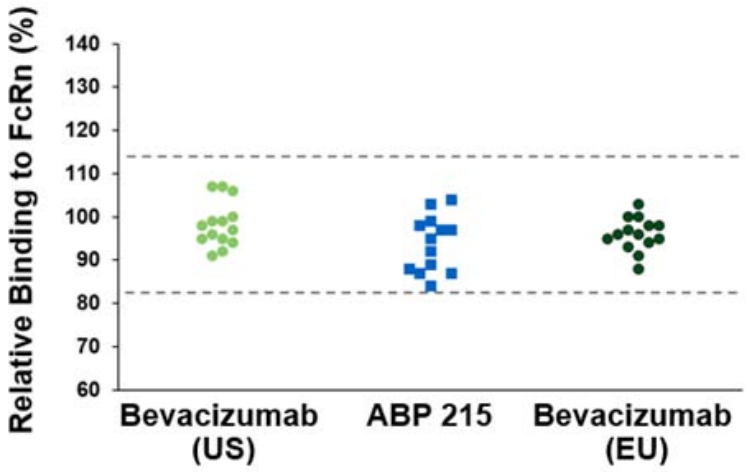
3.2.2.2 Assessment of Fc-mediated Binding

Binding to FcRn

FcRn binds to IgG1, IgG2, and IgG4 heavy chains in the Fc region of the IgG molecules. FcRn mediates IgG homeostasis in human adults by maintaining serum IgG levels. Consequently, FcRn binding is an important contribution to the assessment of analytical similarity as an orthogonal method to assess the primary and higher order structure of the Fc region.

ABP 215 has similar FcRn binding activity compared to bevacizumab (Figure 14). All ABP 215 lots are within the bevacizumab (US) Tier 2 quality range. Therefore, ABP 215 is similar to bevacizumab for FcRn binding activity.

Figure 14. Relative Binding to FcRn



FcRn = fragment crystallizable neonatal receptor.

Binding is calculated relative to the ABP 215 reference standard.

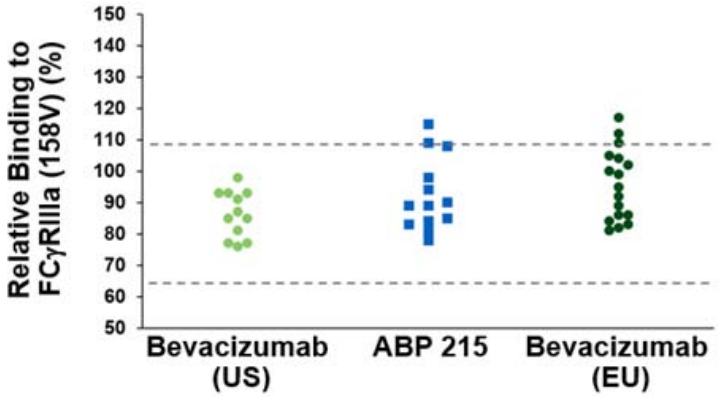
Binding to FcγRIIIa (158V)

FcγRIIIa is a pro-inflammatory receptor expressed on human natural killer cells and is involved in the induction of ADCC. Bevacizumab, a glycosylated IgG1, is capable of binding FcγRIIIa, although it does not induce ADCC. FcγRIIIa binding is highly sensitive to the glycan structure of the antibody. Therefore, FcγRIIIa binding is an important method to assess the primary and higher order structure of the Fc region in addition to the presence of key glycan structures. A genetic polymorphism in FcγRIIIa results in expression of either valine (V) or phenylalanine (F) at amino acid 158. The 2 isoforms differ in their affinity for IgG1, with the 158V isoform having the higher affinity.

A minor difference was observed between ABP 215 and bevacizumab with respect to FcγRIIIa (158V) binding, as presented in Figure 15. Eleven of 13 ABP 215 lots (84%)

are within the quality range. Since ABP 215 and bevacizumab do not induce ADCC, the differences are not considered clinically meaningful.

Figure 15. Relative Binding to FcγRIIIa (158V)

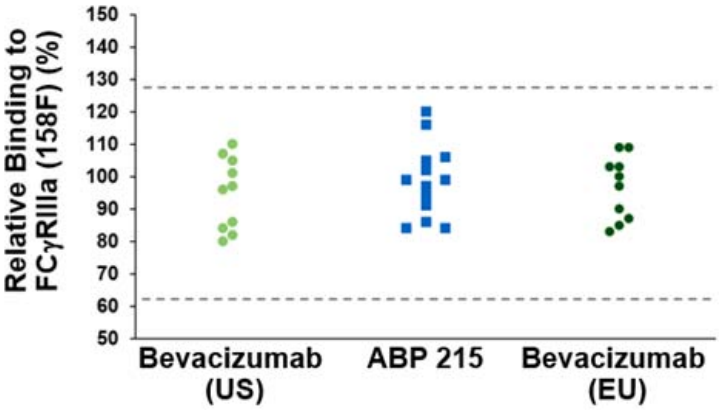


Binding is calculated relative to the ABP 215 reference standard.

Binding to FcγRIIIa (158F)

As mentioned above, FcγRIIIa (158F) is an allelic variant expressing phenylalanine at position 158 of FcγRIIIa that shows lower affinity binding to IgG as compared to the valine variant. ABP 215 and bevacizumab are similar with respect to FcγRIIIa (158F) binding, as presented in Figure 16. All ABP 215 lots are within the bevacizumab (US) Tier 2 quality range. Therefore, ABP 215 is similar to bevacizumab for FcγRIIIa (158F) binding activity.

Figure 16. Relative Binding to FcγRIIIa (158F)



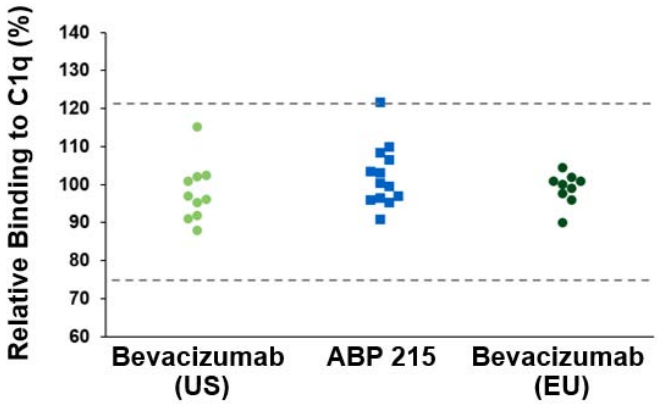
Binding is calculated relative to the ABP 215 reference standard.

Binding to C1q

C1q is the first sub-component of the classical complement pathway. The C1q binding assay is a characterization method that probes the presence or absence of binding as bevacizumab and ABP 215 do not exhibit CDC activity. ABP 215 have similar C1q

binding activity compared to bevacizumab (Figure 17). Twelve out of 13 ABP 215 lots (92%) are within the quality range. Therefore, ABP 215 is similar to bevacizumab for C1q binding activity.

Figure 17. Relative Binding to C1q



Binding is calculated relative to the ABP 215 reference standard.

3.3 Analytical Similarity Conclusions

The comprehensive analytical similarity assessment demonstrates that ABP 215 is highly analytically similar to bevacizumab. Additionally, the analytical component of the requisite scientific bridge was established. Some minor analytical differences in structural and purity attributes were observed between ABP 215 and bevacizumab, but based on bevacizumab product knowledge, these were not expected to affect the functional activities or PK of ABP 215. The minor differences were shown to have no effect on the functional activities relevant for the mechanism of action of bevacizumab and ABP 215. Importantly, all Tier 1 attributes/assays were demonstrated to be similar within the pre-specified assessment criteria. Furthermore, the minor structural differences were confirmed to have no effect on PK, efficacy, safety, or immunogenicity in the ABP 215 clinical studies (Section 5).

All of the approved indications for bevacizumab share a common mechanism of action according to prescribing information for bevacizumab and published studies (Section 2.2.3). The results of the comprehensive analytical similarity assessment support extrapolation to the other approved bevacizumab indications that were not studied in the ABP 215 clinical program.

4. NONCLINICAL DEVELOPMENT PROGRAM

The nonclinical development program provided dose-response pharmacology assessments of ABP 215 and bevacizumab in addition to a toxicology evaluation, and consisted of:

- pharmacologic activity in 2 tumor xenograft models
  - conducted to explore the effects of ABP 215 and bevacizumab on tumor growth and tumor vasculature normalization
- pharmacologic activity in a mouse model of vascular permeability
  - conducted to compare the neutralization of recombinant human VEGF-induced vascular permeability
- toxicology in a 1-month study using the cynomolgus monkey
  - conducted to compare expected toxicities and toxicokinetic profiles, and to confirm a lack of unexpected toxicities with ABP 215 administration

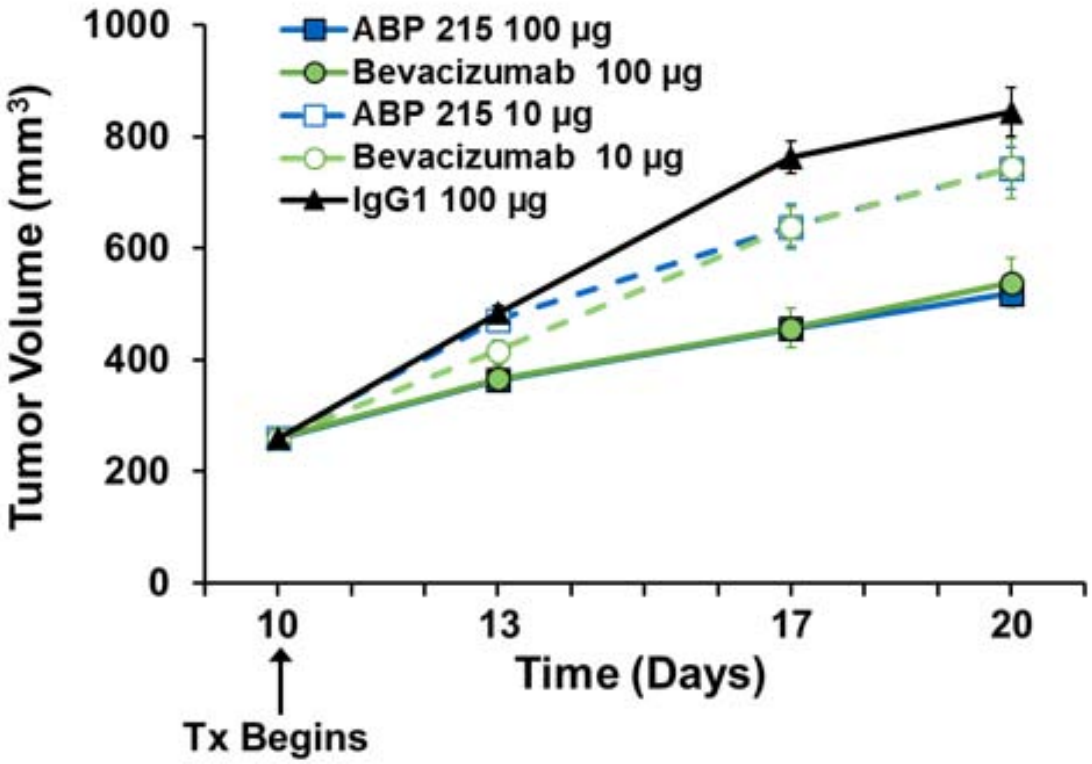
4.1 Pharmacology

For solid tumors to grow beyond 1- to 2 mm in size, new vessel growth must occur to provide the nutrients necessary to support expansion of tumors. VEGF is a key mediator of the angiogenesis process by signaling to promote survival of existing blood vessels feeding the tumor, increasing new vessel growth, and enhancing the permeability of the new blood vessels. ABP 215 and bevacizumab inhibit tumor angiogenesis by binding to VEGF and blocking its tumor promoting activities. The efficacy of bevacizumab has been evaluated in a number of preclinical models (Gerber and Ferrara, 2005). These models include a diverse range of tumor xenograft types used to assess effects on tumor growth as well as models evaluating tumor-induced vascularization and VEGF-induced vessel permeability (Vanqestel et al, 2011; Gerber and Ferrara, 2005; Ueda et al, 2005; Emanuel et al, 2004). Xenograft tumor models in A431 (human epithelial carcinoma cells expressing VEGF; historically shown to be sensitive to anti-VEGF therapy) and Colo205 (human colon cancer cells; representing a clinical indication of interest) were used to compare the ability of ABP 215 and bevacizumab to inhibit tumor growth and tumor vasculature. Both of these studies were dose-response evaluations, testing 2 dose levels of both ABP 215 and bevacizumab as compared to an IgG1 negative control.

The results from both xenograft studies were similar, thus only the results from the Colo205 colon cancer model are shown as an example. Briefly, athymic nude mice were injected subcutaneously with Colo205 tumor cells at a concentration of 2×10^6 cells per mouse. Ten days later, ABP 215 or bevacizumab (US) was

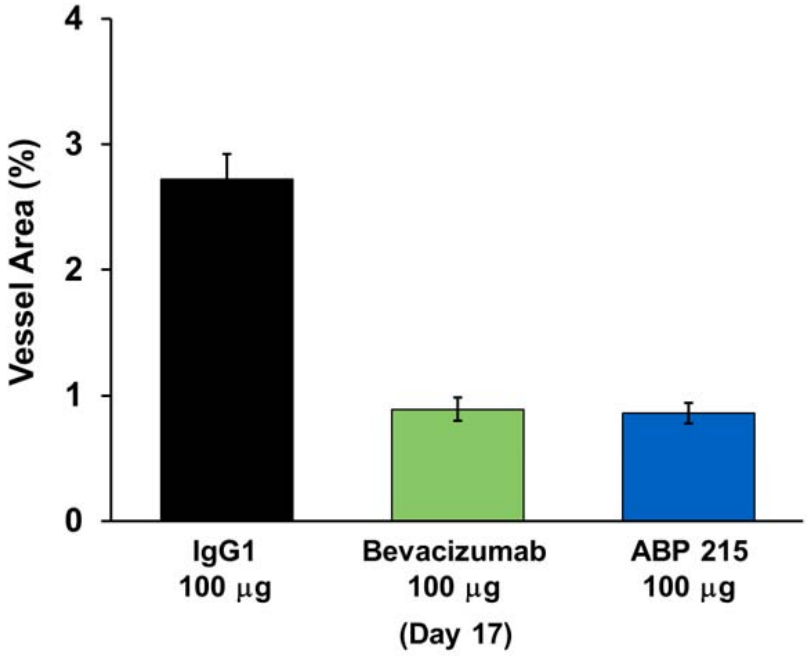
administered by intraperitoneal injection at doses of 10 or 100 μg twice weekly for 10 days. In the Colo205 model, ABP 215 and bevacizumab significantly and similarly inhibited tumor growth when compared to the control group (Figure 18). No statistically significant difference was observed when ABP 215 and bevacizumab treatment groups were compared to each other, at either the 10 μg or the 100 μg dose. Both ABP 215 and bevacizumab also resulted in a significant and similar decrease in vessel area measured by CD31+ staining as compared with the control group after 1 week of treatment (Figure 19).

Figure 18. Effect of ABP 215 and Bevacizumab on Colo205 Xenograft Tumor Growth



Colo205 = human colon cancer cells; IgG1 = immunoglobulin G1; SE = standard error; Tx = treatment.
Data represent mean ± SE for each group (N = 10/group).

Figure 19. Effect of ABP 215 and Bevacizumab on Vessel Area in Colo205 Tumors

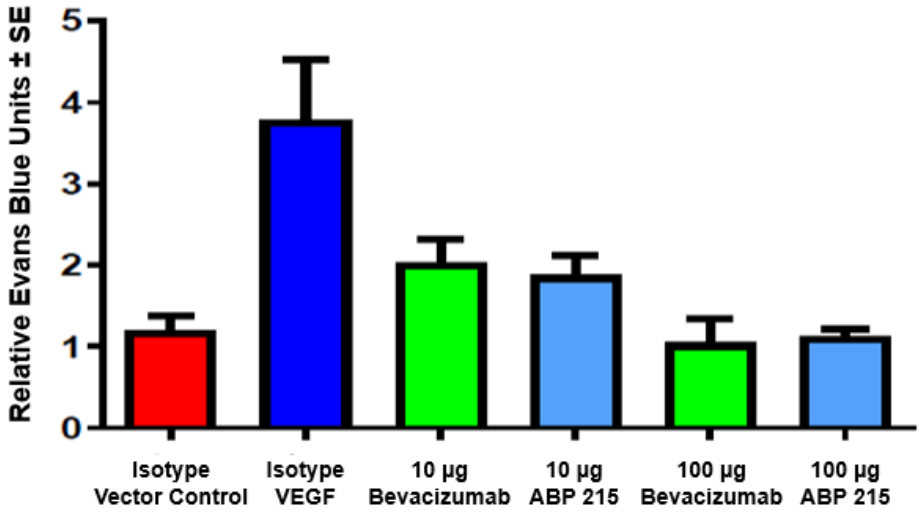


Colo205 = human colon cancer cells; IgG1 = immunoglobulin G1.

Blood vessel area in Colo205 tumors was measured from sections of tumor tissue stained for CD31. Data represent mean ± SE for each group (N = 10/group).

One of the early effects of VEGF on vascular endothelium is to induce vascular permeability (Senger et al, 1993). Bevacizumab binding to VEGF inhibits the established effect on vascular permeability. ABP 215 and bevacizumab were compared in a study grafting human embryonic kidney cells engineered to overexpress VEGF in mice. Vascular permeability in the skin adjacent to the grafted cells was assessed after administration of Evans Blue dye. ABP 215 inhibited recombinant human VEGF-induced vascular permeability in mouse skin vasculature in a similar fashion to bevacizumab (Figure 20).

Figure 20. Vascular Permeability Comparison Between ABP215 and Bevacizumab



4.2 Toxicology

The species, dose, regimen, and duration for the comparative toxicology study were selected to provide a meaningful toxicological comparison of ABP 215 and bevacizumab.

The cynomolgus monkey is considered a pharmacologically relevant species for ABP 215 and bevacizumab because the cynomolgus monkey VEGF protein sequence is identical to that of human VEGF (Shima et al, 1996). VEGF has an established role in endochondral ossification, a mechanism for formation of bone tissue that involves replacing calcified cartilage with a bone matrix (Ferrara, 2001). Bevacizumab has been reported to cause physeal dysplasia, a defect in endochondral ossification in the femur and humerus of young monkeys with open bone growth plates (Ferrara et al, 2004). Therefore, the toxicology profiles of ABP 215 and bevacizumab were compared in a cynomolgus monkey study (n = 3/sex/group) that included light microscopic evaluation of the femur for evidence of physeal dysplasia.

Selection of the dose and duration (50 mg/kg twice weekly for 4 weeks) for the study was based on bevacizumab historical data and enabled an evaluation of the expected effect on endochondral ossification and to identify any potential differences in any other toxicological effects (Avastin FDA Approval Package: Toxicology Data, 2004; Ryan et al, 1999). IV administration was used in the toxicology study because ABP 215 and bevacizumab are administered by that route clinically.

ABP 215 and bevacizumab were well tolerated and no unexpected toxicity was observed; no effects were observed on clinical signs, body weight, food consumption,

physiologic measurements (heart rate and body temperature), ophthalmic or electrocardiogram examinations, hematology, serum chemistry, coagulation, or urinalysis. The anticipated light microscopic finding of physeal dysplasia was observed in the femur of all animals dosed with either ABP 215 or bevacizumab and was primarily characterized by a thicker than expected physeal growth plate that contained long columns of large chondrocytes, consistent with a defect in the transition to a bone matrix. Femoral physeal dysplasia was mild in severity in both treatment groups and affected all animals, indicating ABP 215 and bevacizumab had similar effects. The changes in the femur were similar with those observed following bevacizumab treatment using monkeys in historical studies (Avastin FDA Approval Package: Toxicology Data, 2004; Ryan et al, 1999). The nonclinical study comparing ABP 215 and bevacizumab did not identify any new toxicologic findings.

The study demonstrated similar toxicokinetic parameters after repeat dosing of both ABP 215 and bevacizumab (Table 7), confirming that cynomolgus monkeys achieved similar exposures after treatment with either ABP 215 or bevacizumab.

Table 7. Comparative Assessment of Mean Systemic Antibody Exposure (Combined Sexes[a]) After IV Administration of ABP 215 and Bevacizumab

	C_{max} (μg/mL)		AUC_{0-72} (μg•hr/mL)	
Dose and Test Article	Day 1	Day 25	Day 1	Day 25
50 mg/kg ABP 215	1420	3750	60 400	196 000
50 mg/kg Bevacizumab	1340	3400	53 500	182 000

AUC_{0-72} = area under the concentration-time curve from time 0 to 72 hours; C_{max} = maximum observed concentration; IV = intravenous.

[a] N = 3/sex/group.

4.3 Nonclinical Program Conclusions

Data from the tumor xenograft models support that ABP 215 inhibits tumor growth and tumor vascularization to a similar level as bevacizumab at multiple dose levels, and the results are consistent with the established bevacizumab mechanism of action. ABP 215 also inhibited VEGF-induced vascular permeability in a similar fashion to bevacizumab using an in vivo mouse model. ABP 215 and bevacizumab had similar toxicokinetics and both products induced the expected endochondral bone ossification (physeal dysplasia) in the cynomolgus monkey. Additionally, no unexpected toxicities were observed in the toxicology study. The nonclinical pharmacology, toxicokinetic, and toxicology data support the conclusion that ABP 215 is highly similar to bevacizumab and support the extrapolation to all bevacizumab indications.

5. CLINICAL DEVELOPMENT PROGRAM

Amgen designed and conducted a biosimilar clinical program to confirm the similar PK, efficacy, safety, and immunogenicity of ABP 215 to bevacizumab. The program consisted of studies in sensitive populations (ie, a population in whom potential differences between ABP 215 and bevacizumab are likely to be detected, if such differences exist). The studies used clinically relevant and sensitive endpoints to evaluate similarity. This section describes the study designs and the results of the clinical program. The clinical evidence supporting the similarity of ABP 215 to bevacizumab includes results from the studies in Table 8.

Table 8. ABP 215 Clinical Studies

Study Number	Population	Type of Study	Number of Subjects	Study Duration	Primary Endpoint
20110216 (Study 216)	Healthy male subjects	PK, safety, and immunogenicity	202	85 days	C_{max} and AUC_{inf}
20120265 (Study 265)	Non-small cell lung cancer subjects	Efficacy, safety, and immunogenicity	642	18 weeks[a]	ORR

AUC_{inf} = area under the serum concentration-time curve from time 0 extrapolated to infinite time; C_{max} = maximum observed drug concentration during a dosing interval; ORR = objective response rate; PK = pharmacokinetic.

[a] Expected duration from first dose of any investigational product to end-of-treatment visit. After completing the end-of-treatment visit, subjects were followed for disease progression/overall survival until the end of the clinical study, consent was withdrawn, they were lost to follow-up, died, or had proscribed therapy.

5.1 Clinical Pharmacology

The PK profile of bevacizumab has been well characterized and provides relevant information as to the expected pharmacological profile of ABP 215. The PK profile of bevacizumab was assessed from available clinical trial data in subjects with solid tumors. Early dose-ranging studies showed that the PK of bevacizumab is linear at doses ranging from 1 mg/kg to 20 mg/kg (Lu et al, 2008; European Public Assessment Report, 2006). A population PK analysis of 491 subjects who received bevacizumab (1 to 20 mg/kg) weekly, every 2 weeks, or every 3 weeks also concluded a linear PK with an estimated half-life of approximately 20 days (range, 11 to 50 days). The predicted time to reach steady state was 100 days (Avastin United States Prescribing Information, 2016). Additional published information on the PK of bevacizumab in the indications of metastatic colorectal cancer, metastatic breast cancer, and NSCLC showed similar PK characteristics (European Public Assessment Report, 2006).

Based on results of a population PK analysis, no clinically relevant PK interaction of co-administered chemotherapy (interferon alfa-2a, irinotecan/5-fluorocuracil/and

leucovorin, 5-fluorouracil and leucovorin, carboplatin/paclitaxel, capecitabine, doxorubicin, or cisplatin/gemcitabine) on bevacizumab PK has been shown (Avastin Summary of Product Characteristics, 2017). In other clinical studies, results demonstrated no significant effect of bevacizumab on the PK of irinotecan or its active metabolite SN38, capecitabine or its metabolites, oxaliplatin, or interferon alfa-2a (Avastin United States Prescribing Information, 2016).

A subsequent population PK model was developed using a more comprehensive dataset from subjects with solid tumors including: NSCLC, hormone-refractory prostate cancer, breast cancer, colon cancer (adjuvant setting) and colorectal cancer, renal cell carcinoma, pancreatic cancer, and glioblastoma (Han et al, 2016), and confirmed the significant covariates identified by Lu et al (2008). This model evaluated additional covariates, including: indication (cancer type), ethnicity (Asian versus non-Asian), and baseline VEGF. The significant covariates identified in the updated model were body weight, gender, albumin, alkaline phosphatase, and interferon alpha treatment (Han et al, 2016). Notably, differences in ethnicity (Asian and non-Asian) and cancer indication were not significant covariates.

5.1.1 PK Similarity Study 216 in Healthy Subjects

5.1.1.1 Design of Study 216

PK similarity Study 216 was a single-dose, 3-arm parallel group study in healthy adult male subjects. The study was designed following FDA guidance to assess the PK similarity between ABP 215, bevacizumab (US), and bevacizumab (EU) by evaluating C_{max} and AUC_{inf} as the primary endpoints following single 3 mg/kg IV doses of each respective product. Subjects in the 2 study sites (US and United Kingdom) were randomized to receive ABP 215 or bevacizumab (bevacizumab [US] in the US and bevacizumab [EU] in the United Kingdom). Area under the serum concentration-time curve from time 0 to last quantifiable concentration (AUC_{last}) was assessed as a secondary endpoint. Standard FDA bioequivalence criteria were used, in which the 90% confidence intervals for the ratio of geometric means for C_{max} and AUC_{inf} are required to be contained entirely within the margin of (0.80, 1.25). Safety, tolerability, and immunogenicity of all 3 treatments were evaluated as secondary objectives. The 3-arm design enabled the study to be used as the PK component in establishing the requisite scientific bridge between bevacizumab (US) and bevacizumab (EU), enabling a subsequent 2-arm study to confirm efficacy, safety, and immunogenicity in NSCLC Study 265 (Section 5.2).

A healthy population in Study 216 avoids confounding medical conditions or concomitant medications that can alter individual PK or safety profiles, and therefore is a sensitive population for the assessment of PK similarity. The PK of bevacizumab was not studied in healthy subjects in the original submission or in subsequent submissions for the reference product (Avastin United States Prescribing Information, 2016; European Public Assessment Report, 2006; FDA, CDER, Approval Package for Avastin, Clinical Pharmacology/TOX Review, 2004). A 3 mg/kg dose was selected for Study 216 to minimize drug exposure in healthy subjects while ensuring sufficient exposure to enable PK parameter evaluations to assess PK equivalence. Additionally, this dose is within bevacizumab's linear PK range (1 to 20 mg/kg) and thus considered adequate to determine PK similarity between ABP 215 and bevacizumab and is predictive of clinical doses from 5 to 15 mg/kg.

Women were excluded from participating in Study 216 since bevacizumab studies have demonstrated an increased risk of ovarian failure, possible impaired female fertility, and the potential for fetal harm based on findings from animal studies and the drug's mechanism of action (Avastin United States Prescribing Information, 2016).
A parallel-group design with PK evaluation for 85 days was employed to account for the long half-life of bevacizumab (approximately 20 days).

5.1.1.2 PK Similarity Results for Study 216

In total, 202 subjects received investigational product and were followed up to 85 days:

- ABP 215, 68 subjects (63 completed study)
- bevacizumab (US), 67 subjects (64 completed study)
- bevacizumab (EU), 67 subjects (64 completed study)

The PK parameters were calculated from ABP 215, bevacizumab (US), and bevacizumab (EU) serum concentration data using noncompartmental methods. The calculated PK parameters and the geometric mean ratios and the associated 90% confidence intervals are shown in Table 9 and the serum concentration-time curves are shown in Figure 21. The 90% confidence intervals of the geometric least-squares mean ratios for the comparisons of ABP 215 to bevacizumab (US) and ABP 215 to bevacizumab (EU) for all 3 PK parameters (the primary parameters C_{max} and AUC_{inf}, and secondary parameter AUC_{last}) were contained within the standard bioequivalence margin of 0.80 to 1.25, thus demonstrating PK similarity. Additionally, the PK similarity of bevacizumab (US) to bevacizumab (EU) was demonstrated using the same criteria, which supports the similarity of bevacizumab sourced from both regions.

The bevacizumab results are consistent with those observed in the literature. Exposure to the 3 mg/kg single IV infusion was sufficient for the 85-day PK monitoring plan and resulted in greater than 97% of subjects having AUC_{last} values greater than 85% of the AUC_{inf}. Peak concentrations were observed approximately 1.5 to 4 hours after the start of the infusion, after which concentrations tended to decline in a biphasic manner.

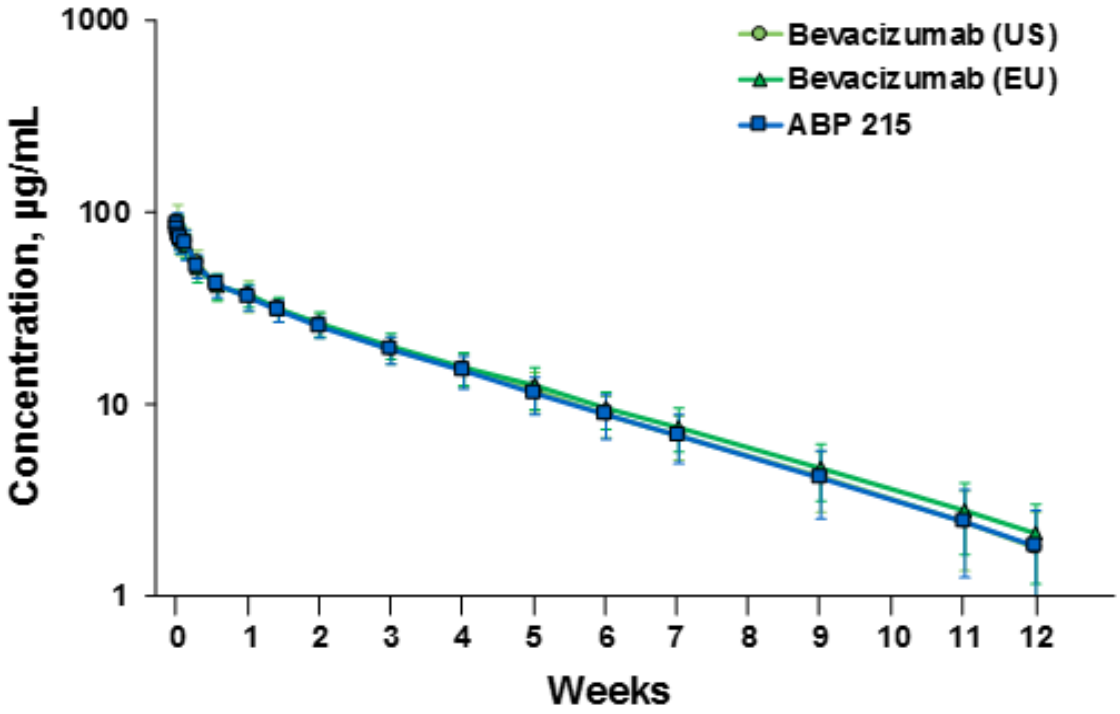
Table 9. Summary of Statistical Assessment of ABP 215, Bevacizumab (US), and Bevacizumab (EU) Pharmacokinetic Parameters (Study 216 PK Population)

Treatment and Comparison	C_{max} (μg/mL) Adjusted LS Geometric Mean [n]	AUC_{inf} (μg•h/mL) Adjusted LS Geometric Mean [n]	AUC_{last} (μg•h/mL) Adjusted LS Geometric Mean [n]
ABP 215	87.2 [67]	29400 [66]	28200 [62]
Bevacizumab (US)	89.1 [66]	29600 [66]	28500 [62]
Bevacizumab (EU)	84.7 [64]	30600 [66]	29400 [64]
	Ratio of Adjusted LS Geometric Means (90% CI)		
ABP 215 vs bevacizumab (US)	0.98 (0.933, 1.026)	0.99 (0.948, 1.042)	0.99 (0.946, 1.033)
ABP 215 vs bevacizumab (EU)	1.03 (0.982, 1.080)	0.96 (0.916, 1.006)	0.96 (0.920, 1.004)
Bevacizumab (US) vs bevacizumab (EU)	1.05 (1.004, 1.104)	0.97 (0.921, 1.012)	0.97 (0.930, 1.016)

AUC_{inf} = area under the serum concentration-time curve from time 0 to infinity; AUC_{last} = area under the serum concentration-time curve from time 0 to time of last quantifiable concentration; CI = confidence interval; C_{max} = maximum observed serum concentration; EU = European Union; LS = least squares; US = United States

Adjusted LS geometric means estimated from an analysis of covariance, adjusting for region.

Figure 21. Study 216 Mean (± SD) Serum Concentration-time Profiles



5.1.1.3 Safety Results for Study 216

The safety analysis was performed on the safety population, defined as all randomized subjects who received investigational product. The safety evaluations included adverse events and immunogenicity. Treatment-emergent adverse events were defined as adverse events that started or worsened after the start of treatment.

Treatment-emergent adverse events were reported for 47.1%, 32.8%, and 61.2% of subjects in the ABP 215, bevacizumab (US), and bevacizumab (EU) groups, respectively (Table 10). Treatment-emergent adverse events reported for greater than 5% of subjects in either treatment group were headache and nasopharyngitis (Table 11). There were no deaths, serious adverse events, or discontinuations due to adverse events reported in the study.

Table 10. Overall Summary of Treatment-emergent Adverse Events (Study 216 Safety Population)

Adverse Event Category	ABP 215 (N = 68) n (%)	Bevacizumab (US) (N = 67) n (%)	Bevacizumab (EU) (N = 67) n (%)
Any TEAE	32 (47.1)	22 (32.8)	41 (61.2)
Grade 1 TEAE	26 (38.2)	15 (22.4)	33 (49.3)
Grade 2 TEAE	4 (5.9)	7 (10.4)	7 (10.4)
Grade 3 TEAE	0	0	1 (1.5)
Grade 4 TEAE	2 (2.9)[a]	0	2 (1.0)
Grade 5 TEAE	0	0	0

CTCAE = common terminology criteria for adverse events; TEAE = treatment-emergent adverse event.

[a] Increased creatine kinase (n = 1), exercise-induced increase in muscle enzymes (n = 1); each unrelated to study drug.

Table 11. Treatment-emergent Adverse Events Reported in ≥ 5% of Subjects in Any Treatment Group by Preferred Term (Study 216 Safety Population)

Preferred Term	ABP 215 (N = 68) n (%)	Bevacizumab (US) (N = 67) n (%)	Bevacizumab (EU) (N = 67) n (%)
Subjects with any TEAE	32 (47.1)	22 (32.8)	41 (61.2)
Headache	6 (8.8)	10 (14.9)	16 (23.9)
Nasopharyngitis	4 (5.9)	0	11 (16.4)
Nausea	2 (2.9)	4 (6.0)	1 (1.5)
Pharyngitis	0	0	5 (7.5)
Vessel puncture site hematoma	4 (5.9)	0	1 (1.5)

TEAE = treatment-emergent adverse event.

The incidence of treatment-emergent adverse events varied across the treatment arms in the study overall. However, when the safety results were assessed by study site, no clinically meaningful differences in the incidence of any treatment-emergent adverse events between arms was observed (Table 12 and Table 13).

Table 12. Overall Summary of Treatment-emergent Adverse Events By Site (Study 216 Safety Population)

	Subjects, n (%)			
	US Site		EU Site	
AE Category	ABP 215 (N = 35)	Bevacizumab (N = 67)	ABP 215 (N = 33)	Bevacizumab (N = 67)
Any TEAE	13 (37.1)	22 (32.8)	19 (57.6)	41 (61.2)
Grade 1 TEAE	12 (34.3)	17 (25.4)	19 (57.6)	38 (56.7)
Grade 2 TEAE	2 (5.7)	7 (10.4)	3 (9.1)	7 (10.4)
Grade 3 TEAE	0	0	0	1 (1.5)
Grade 4 TEAE	2 (5.7)[a]	0	0	0
Grade 5 TEAE	0	0	0	0

CTCAE = common terminology criteria for adverse events; TEAE = treatment-emergent adverse event.

[a] Increased creatine kinase (n = 1), exercise-induced increase in muscle enzymes (n = 1); each unrelated to study drug.

Note: Subjects with multiple events in the same category our counted only once in that category. Subjects with events in more than 1 category are counted once in each of those categories.

Table 13. Treatment-emergent Adverse Events Reported in ≥ 5% of Subjects in Any Treatment Group by Preferred Term by Site (Study 216 Safety Population)

	Subjects, n (%)			
	US Site		EU Site	
Preferred Term	ABP 215 (N = 35)	Bevacizumab (N = 67)	ABP 215 (N = 33)	Bevacizumab (N = 67)
Subjects with any TEAE	13 (37.1)	22 (32.8)	19 (57.6)	41 (61.2)
Headache	4 (11.4)	10 (14.9)	2 (6.1)	16 (23.9)
Diarrhoea	2 (5.7)	1 (1.5)	1 (3.0)	1 (1.5)
Blood creatinine phosphokinase increased	2 (5.7)	0	0	0
Nausea	1 (2.9)	4 (6.0)	1 (3.0)	1 (1.5)
Nasopharyngitis	0	0	4 (12.1)	11 (16.4)
Pharyngitis	0	0	0	5 (7.5)
Vessel puncture site haematoma	0	0	4 (12.1)	1 (1.5)
Toothache	0	0	3 (9.1)	1 (1.5)
Acne	0	0	2 (6.1)	1 (1.5)
Dizziness	0	1 (1.5)	3 (9.1)	0

TEAE = treatment-emergent adverse event.

No new safety signals were identified with respect to either ABP 215 or bevacizumab. The adverse events seen in this study were consistent with typical adverse events seen in a healthy subject study.

5.1.1.4 Immunogenicity Results for Study 216

To detect anti-drug antibodies against ABP 215 and bevacizumab, Amgen developed and validated sensitive and drug tolerant assays. Blood samples for the determination of the presence of serum anti-drug antibodies were collected on day 1 prior to dosing and at the end of the study. All subjects tested were anti-drug antibody-negative at baseline and after drug treatment. In all subjects tested, drug levels at the end of study were well below levels demonstrated to potentially interfere with binding antibody detection and therefore anti-drug antibody detection was not affected by circulating drug.

5.1.2 Clinical Pharmacology Conclusions

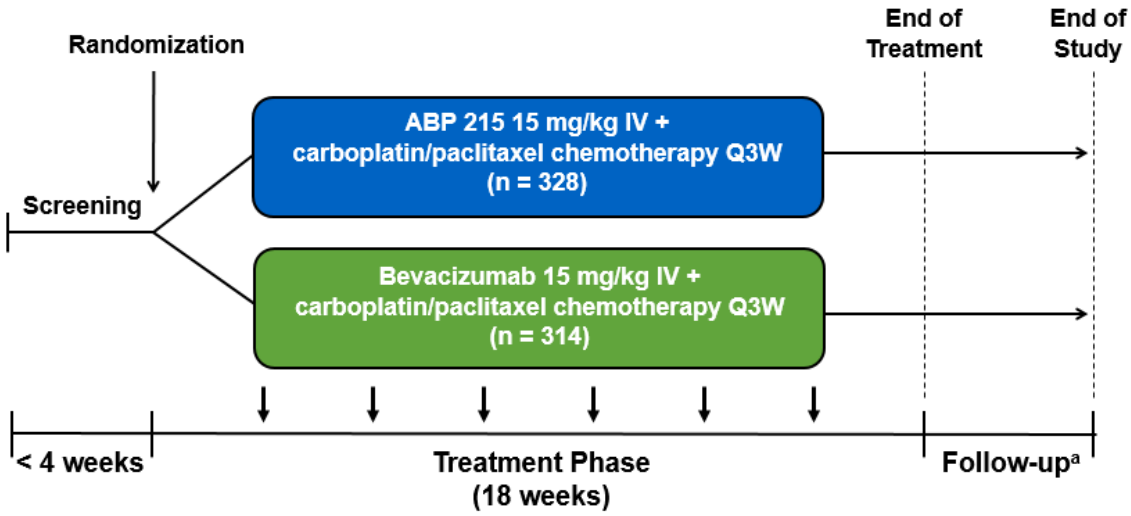
Based on the results from the PK similarity study, the PK of ABP 215 compared to bevacizumab (US), and of ABP 215 compared to bevacizumab (EU), were determined to be similar. Additionally, the PK of bevacizumab (US) and bevacizumab (EU) were determined to be similar. Study 216 also established the PK component of the requisite scientific bridge and, when combined with the analytical similarity assessment data for the products (see Section 3.2), these results justify the use of bevacizumab (EU) in Study 265.

5.2 Clinical Efficacy

5.2.1 Design of Study 265 in Advanced Non-squamous NSCLC Subjects

Study 265 was a comparative, randomized, double-blind, active-controlled study in adult subjects with advanced non-squamous NSCLC receiving first-line chemotherapy with carboplatin and paclitaxel. The study was designed to confirm that there are no clinically meaningful differences between ABP 215 and bevacizumab in terms of efficacy, safety, and immunogenicity. The study schema is shown in Figure 22.

Figure 22. Schema for Study 265 in Advanced Non-squamous NSCLC Subjects



IV = intravenous; NSCLC = non-small cell lung cancer; Q3W = every 3 weeks.

[a] Maintenance monotherapy not included.

5.2.1.1 Study Population and Key Eligibility Criteria

The subject population was chosen by considering the bevacizumab treatment effect in placebo-controlled studies at the time the ABP 215 clinical program was designed. As compared to other indications, the advanced non-squamous NSCLC patient population is considered sensitive for the detection of potential differences between the 2 products given the demonstrated treatment effect with objective endpoints (Section 5.2.1.3 and Section 5.2.1.4).

Study 265 limited the backbone chemotherapy to 1 regimen (ie, platinum-based chemotherapy, paclitaxel/carboplatin). The subjects were to have an Eastern Cooperative Oncology Group performance status 0 or 1 and were to initiate first line carboplatin/paclitaxel within 8 days of study entry. Males and females, ≥ 18 years to < 80 years of age, who had histologically- or cytologically-confirmed non-squamous NSCLC that was stage IV or recurrent metastatic disease, measurable according to RECIST v1.1, were enrolled into the study. Subjects with small cell lung cancer histology were excluded.

Differences between ethnic groups in bevacizumab clinical studies were not observed. Given the demonstrated analytical, nonclinical, and PK similarities between ABP 215 and bevacizumab, ABP 215 is also not expected to have any sensitivity to ethnic factors. Therefore, the advanced non-squamous NSCLC population in this study, originating from North America, Western Europe, Eastern Europe, and Asia Pacific is representative of NSCLC populations that would be expected to receive ABP 215 when it is marketed.

5.2.1.2 Study Dosing and Duration

Subjects were enrolled and randomized in a 1:1 ratio to receive either ABP 215 or bevacizumab, each administered as an IV infusion at 15 mg/kg every 3 weeks for 6 cycles. Dose adjustments were not permitted during the study. Subjects were also scheduled to receive carboplatin and paclitaxel chemotherapy every 3 weeks for 4 to 6 cycles. These treatments and their duration are the standard of care for first-line treatment of stage IV NSCLC (Reck et al, 2009; Sandler et al, 2006) and are consistent with the current bevacizumab prescribing information for the treatment for NSCLC with bevacizumab in combination with carboplatin and paclitaxel (Avastin United States Prescribing Information, 2016). The randomization was stratified by geographic region, Eastern Cooperative Oncology Group performance status (0 versus 1), and gender.

A subject was to remain in the treatment phase until 21 days after the last dose of investigational product or study-specified chemotherapy. Subjects were followed for disease progression and OS after completing the end-of-treatment visit until either the end of the study; consent was withdrawn; or they were lost to follow-up, died, or had proscribed therapy (eg, commercial bevacizumab, non-study anti-cancer treatment).

5.2.1.3 Study Endpoints

The primary efficacy endpoint selected in Study 265 was ORR (the percentage of subjects with a best overall response of PR or CR) as defined by RECIST v1.1 (Eisenhauer et al, 2009). As an endpoint, ORR is a direct and objective measure of anti-tumor activity. ORR is also an appropriate measure for a biosimilar assessment between ABP 215 and bevacizumab since it is sensitive to detect any potential clinical efficacy differences, if such differences were to exist. In bevacizumab clinical studies, when bevacizumab was added to chemotherapy, significant benefit with respect to ORR was demonstrated, in which the ORR effect size was larger than the PFS effect size (Botrel et al, 2011). In addition, results from meta-analyses of data in subjects with advanced NSCLC demonstrated that there was a correlation between ORR, PFS, and OS (Blumenthal et al, 2015, Clarke et al, 2015).

The best overall response is calculated from the best responses recorded for individual subjects throughout the study and is the recognized standard approach for defining tumor response for solid tumors. Notably, the best overall response was the approach used in the pivotal bevacizumab study in subjects with NSCLC (Sandler et al, 2006).

ORR measurements were based on tumor assessments using computed tomography or magnetic resonance imaging scans of the chest and abdomen, which were performed at

screening and regular intervals during the study. These are widely accepted methods to assess tumor response in subjects with NSCLC. The tumor assessments were performed at screening and weeks 7, 13, 19, and approximately every 9 weeks thereafter, regardless of treatment delays resulting from toxicity (to prevent any bias based on treatment delays). For all post-baseline assessments, the scan modality was the same as that used at baseline. The tumor assessments were reviewed by independent, centralized radiologists (for primary and sensitivity analyses) and study investigators (for sensitivity analysis) who were blinded to treatment. The primary analysis was based on risk ratio of ORR.

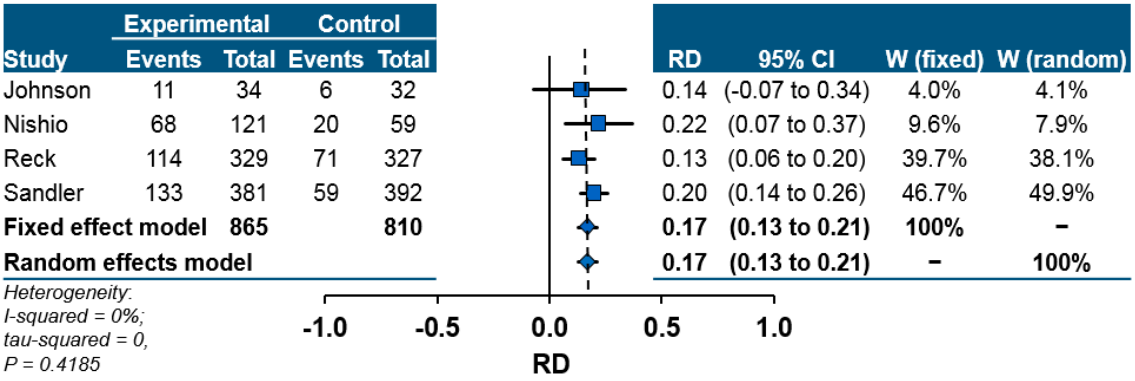
Secondary efficacy endpoints included the risk difference of ORR, PFS (defined as the time from randomization until the first occurrence of disease progression per RECIST v1.1 or death), and the duration of response (for subjects with an objective response; defined as the time from the first objective response [PR or CR] to disease progression per RECIST v1.1).

OS was evaluated as a safety endpoint. Safety and immunogenicity were also evaluated as secondary endpoints and are discussed in Section 5.4.

5.2.1.4 Margins and Sample Size

A meta-analysis of published data from bevacizumab NSCLC studies was conducted in which the lower 95% confidence interval for the ORR difference was 13% (Figure 23). Therefore, 12.5% was then used to establish an equivalence margin for Study 265. Risk ratio analysis was used due to its statistical properties compared to risk difference. Assuming that ORR would be approximately 38% for bevacizumab (calculated as the proportion of subjects with an objective response among all subjects in the studies reported in Botrel et al, 2011), a lower margin of 0.67 for risk ratio of ORR was derived since it corresponds to the margin of 12.5% for the ORR risk difference between ABP 215 and bevacizumab.

Figure 23. Meta-analysis of Published Data from NSCLC Studies with Bevacizumab



CI = confience interval; NSCLC = non-small cell lung cancer; RD = risk difference; W = weight.

An upper margin of 1.50 was then chosen to be symmetric with the lower margin of 0.67 on the log scale. An equivalence margin of (0.67, 1.50 [ie, 1/0.67]) is considered to be an appropriate margin to confirm no clinically meaningful differences in treatment effect for ABP 215 when compared to bevacizumab.

A study sample size was chosen to achieve > 90% power to demonstrate equivalence at a 2-sided significance level of 0.05 with the pre-specified equivalence margin. Note that to satisfy the equivalence definition using this margin, the observed difference in ORR between treatment arms can be no more than approximately 6 percentage points.

5.2.1.5 Statistical Methodology

Primary Efficacy Analysis

The primary analysis of ORR was based on the intent-to-treat (ITT) population (all randomized subjects), using data from the independent, central radiologists' reviews (central tumor assessment). Clinical equivalence of the primary endpoint was demonstrated by comparing the 2-sided 90% confidence interval of the risk ratio in ORR between ABP 215 and bevacizumab with an equivalence margin of (0.67, 1.50) (estimated using a generalized linear model adjusted for stratification factors).

To assess the robustness of the primary ORR analysis results, the primary analysis was repeated using the per-protocol population (central tumor assessment) and the tumor response set (central tumor assessment). These populations were defined as follows:

- Per-protocol population: subjects who completed the treatment period (6 cycles of ABP 215 or bevacizumab and at least 4 cycles of chemotherapy), or who discontinued investigational product or chemotherapy prior to completing the treatment period due to reasons that were allowed per protocol, and did not experience a protocol deviation that would affect an evaluation of the primary objective (determined based on blinded data review prior to database lock)

- Tumor response set: treated subjects who had measurable disease at screening as determined by the central radiology review

Additional sensitivity analyses were performed using data from the investigator's review for the ITT population (local tumor assessment) and adjusting for multiple baseline covariates in the ITT population (central tumor assessment). The consistency of the treatment effect on ORR among different subsets was examined by estimating risk ratio of ORR in sub-groups defined by various baseline covariates (geographic region, Eastern Cooperative Oncology Group performance status, gender, age group, weight loss in the last 6 months, stage IV versus recurrent disease at baseline, race, and smoking history).

A post hoc analysis of individual patient response data was also performed using the independent, central radiologists' evaluations to depict the magnitude of target lesion tumor response in the ABP 215 and bevacizumab treatment groups.

Secondary Efficacy Analyses

The secondary efficacy endpoints were risk difference of ORR, PFS, and duration of response, as described below:

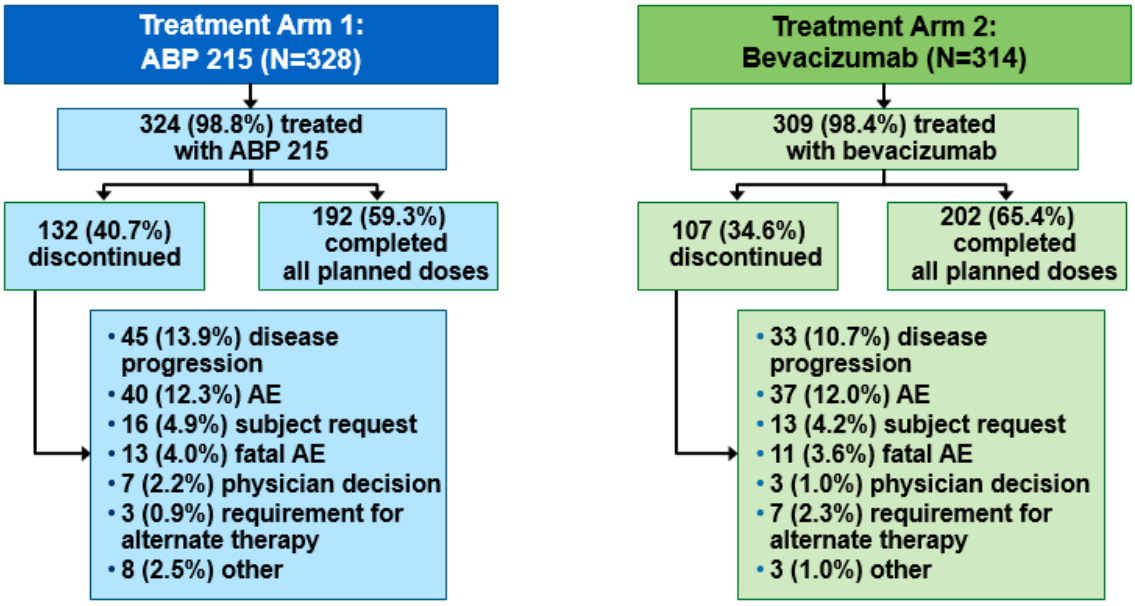
- Risk difference of ORR and corresponding 2-sided 90% confidence intervals were analyzed in a similar fashion to the risk ratio of ORR.
- PFS was summarized using the ITT population based on tumor assessments determined by independent, central radiologists, and investigators. Estimates of the hazard ratio and corresponding 2-sided 90% confidence intervals using the stratified Cox proportional hazards regression model are presented.
- Duration of response was analyzed in a similar fashion as the PFS analysis for the subset of subjects who had an objective response.

5.2.2 Efficacy Results for Study 265 in Subjects With Advanced Non-squamous NSCLC

5.2.2.1 Subject Disposition

A total of 642 subjects were randomized in the study, making up the ITT population: 328 subjects in the ABP 215 treatment group and 314 subjects in the bevacizumab treatment group (Figure 24). Of the randomized subjects in the ITT population, 324 (98.8%) and 309 (98.4%) received at least 1 dose of ABP 215 and bevacizumab, respectively.

Figure 24. Subject Disposition in Study 265 (ITT Population)



AE = adverse event; ITT = intent-to-treat.

With respect to the chemotherapy received in the ITT population, 321 (97.9%) subjects received at least 1 dose of carboplatin and 324 (98.8%) subjects received at least 1 dose of paclitaxel in the ABP 215 treatment group. In the bevacizumab treatment group, 309 (98.4%) subjects received at least 1 dose of carboplatin and 309 (98.4%) subjects received at least 1 dose of paclitaxel. The percentage of those who completed all planned doses was 55.5% for carboplatin and 54.6% for paclitaxel in the ABP 215 treatment group, and 61.8% for carboplatin and 61.5% for paclitaxel in the bevacizumab treatment group. The causes for discontinuing the carboplatin or paclitaxel treatment were most commonly adverse event and disease progression.

Subjects who were considered to have completed the study included those who completed the study per protocol (ie, were ongoing in the follow-up period when the study was terminated), died, or planned to continue therapy with a non-study anti-cancer therapy or commercial bevacizumab. The percentage of subjects who completed the study was similar between the ABP 215 and bevacizumab treatment groups (84.1% and 87.3%, respectively).

Overall, the number of subjects that received investigational product and chemotherapy was similar in the ABP 215 and bevacizumab treatment groups, as was the incidence of and reasons for discontinuation of investigational product and chemotherapy, and discontinuation of the study itself.

5.2.2.2 Demographic and Baseline Characteristics

Demographic and baseline disease characteristics were generally comparable across the 2 treatment groups. Key characteristics are summarized in Table 14.

Table 14. Summary of Demographic and Baseline Characteristics by Treatment (Study 265, ITT Population)

Variable	ABP 215 (N = 328)	Bevacizumab (N = 314)
Age, mean (SD) years	61.6 (9.09)	61.6 (8.88)
< 65 years	199 (60.7)	191 (60.8)
≥ 65 years	129 (39.3)	123 (39.2)
Weight, kg, mean (SD)	71.2 (14.7)	73.5 (15.3)
Race, white	315 (96.0)	300 (95.5)
Gender, male	196 (59.8)	188 (59.9)
Smoking status [n (%)]		
Never	65 (19.8)	76 (24.2)
Former	163 (49.7)	158 (50.3)
Current	100 (30.5)	80 (25.5)
Staging of original diagnosis [n (%)]		
≤ Stage IIIA	23 (7.0)	25 (8.0)
Stage IIIB	2 (0.6)	7 (2.2)
Stage IV	303 (92.4)	281 (89.5)
Stage IV/recurrent disease at baseline [n (%)]		
Stage IV	309 (94.2)	290 (92.4)
Recurrent disease	19 (5.8)	24 (7.6)
Weight loss in last 6 months [n (%)]		
0% to 5%	289 (88.1)	276 (87.9)
> 5% to 10%	39 (11.9)	37 (11.8)
ECOG performance status [n (%)]		
Grade 0	127 (38.7)	117 (37.3)
Grade 1	201 (61.3)	197 (62.7)

ECOG = Eastern Cooperative Oncology Group; ITT = intent-to-treat.

The demographic and baseline characteristics of subjects enrolled in this study were consistent with those found in the literature for NSCLC. In a meta-analysis of 14 clinical studies that included more than 12 500 subjects with advanced NSCLC (Blumenthal et al, 2015), demographic and baseline characteristics collected in those studies were similar overall to those characteristics in Study 265.

5.2.2.3 Primary Efficacy Analysis

The results of the primary efficacy analysis are summarized in Table 15. The results for the risk ratio of ORR demonstrated the equivalence of ABP 215 and bevacizumab. Across the ABP 215 and bevacizumab treatment groups, the percentage of subjects with a best overall response of CR (0.6% [2 subjects] for both groups) and PR (38.4% [126 subjects] and 41.1% [129 subjects], respectively) was comparable. The ORR was 39.0% (128 subjects) for ABP 215 and 41.7% (131 subjects) for bevacizumab. The risk ratio was 0.93 with a 2-sided 90% confidence interval of (0.80, 1.09), which was within the pre-specified equivalence margin of (0.67 and 1.50), indicating equivalence in efficacy between the 2 treatments. These results for ABP 215 and bevacizumab are consistent with the bevacizumab responses reported in the literature.

Table 15. Summary of Objective Response Rate (Study 265, ITT Population)

	ABP 215 (N = 328)	Bevacizumab (N = 314)
Best overall response [n (%)]		
Complete response	2 (0.6)	2 (0.6)
Partial response	126 (38.4)	129 (41.1)
Stable disease	144 (43.9)	137 (43.6)
Progressive disease	21 (6.4)	18 (5.7)
Not evaluable	35 (10.7)	28 (8.9)
ORR [n (%)][a]	128 (39.0)	131 (41.7)
95% CI (%)	(33.7, 44.5)	(36.2, 47.4)
Risk ratio (ABP 215/Bevacizumab)[b]	0.93	
90% CI	(0.80, 1.09)	

CI = confidence interval; ITT = intent-to-treat; ORR = objective response rate.

Note: For the primary efficacy analysis, objective response was determined by independent, central radiologists. Subjects without any post-baseline tumor assessment were included in the not-evaluable category per RECIST v1.1.

[a] Objective response rate is defined as the percentage of subjects with an objective response. Objective response is defined as the best overall response of partial response or complete response as defined by RECIST v1.1.

[b] Point estimate and CI are estimated using a generalized linear model adjusted for the randomization stratification factors geographic region, ECOG performance status, and gender.

Similar results were observed for all sensitivity analyses (Section 5.2.1.5, Table 16), supporting the primary efficacy analysis result.

Table 16. ORR Endpoint Sensitivity Analyses (Study 265)

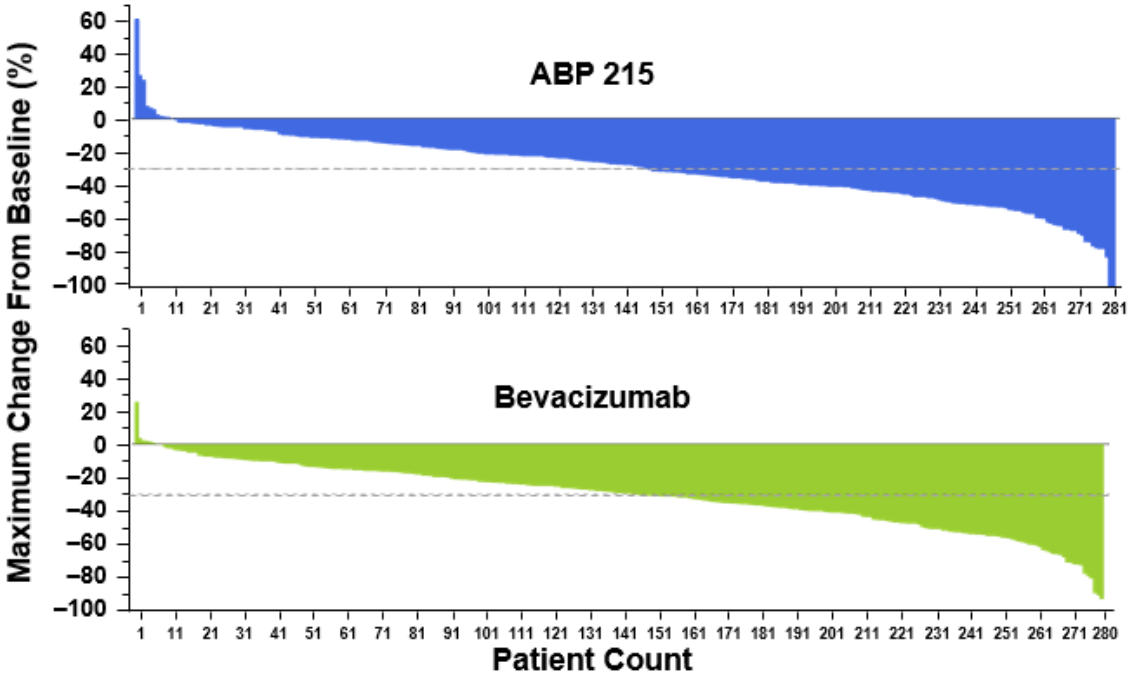
	ORR ABP 215	ORR Bevacizumab	RR (%) (90% CI)[a]	RD (%) (90% CI)[a]
ITT population (local tumor assessment)	47.9%	48.1%	1.01 (0.88, 1.16)	-0.68 (-7.11, 5.76)
Per protocol population (central tumor assessment)	43.1%	45.6%	0.94 (0.80, 1.10)	-2.82 (-9.73, 4.10)
Tumor response set (central tumor assessment)	40.4%	43.0%	0.93 (0.80, 1.09)	-2.78 (-9.27, 3.71)
Additional model covariates[a] in the ITT population (central tumor assessment)	39.0%	41.7%	0.90 (0.77, 1.05)	-3.24 (-9.54, 3.05)

ECOG = Eastern Cooperative Oncology Group; ITT = intent-to-treat; ORR = objective response rate; RD = risk difference; RR = risk ratio.

[a] Point estimate and CI are estimated using a generalized linear model adjusted for the randomization stratification factors geographic region, ECOG performance status, and gender.

A post hoc analysis of individual patient response data was performed using the independent, central radiologists' evaluations to depict the magnitude of tumor response in the ABP 215 and bevacizumab treatment groups. Imaging assessments of tumor response were performed using RECIST v1.1 to generate waterfall plots for the magnitude of target lesion response (Figure 25). In the figure, the horizontal reference line of 0 serves as a baseline measure with subject numbers along the axis; the vertical (y) lines are drawn for each subject, and the length of the line indicates the maximum percent change in target tumor burden from baseline. The horizontal dashed line at -30% represents the threshold for subjects to have a partial response for target lesions. Subjects with a best overall response of nonevaluable and subjects with missing percent reduction in sum of diameters of target lesions are excluded. The findings show comparable anti-tumor activity of ABP 215 and bevacizumab wherein the 2 groups had both a similar magnitude of response in addition to the similar rate of response.

Figure 25. Patient's Maximum Percent Reduction in Sum of Diameters of Target Lesions - Central Assessment (Study 265, ITT Population)



Based on central tumor review using RECIST v1.1.

5.2.2.4 Secondary Efficacy Analysis

The secondary efficacy endpoints were risk difference for ORR, PFS, and duration of response. Results from these secondary analyses support the conclusion of clinical similarity in efficacy between the ABP 215 and bevacizumab treatment groups. The key results from the secondary analyses are shown in Table 17. Details from these analyses are provided below.

Table 17. Secondary Efficacy Endpoints for Study 265 (ITT Population, Central Assessment)

Endpoint	ABP 215 N = 328	Bevacizumab N = 314
Risk Difference of ORR		
Responders, n (%)	128 (39.0)	131 (41.7)
Point estimate (90% CI)[a]	-2.90% (-9.26%, 3.45%)	
Progression-free Survival		
Disease progression or death, n (%)	131 (39.9)	125 (39.8)
HR (90% CI)[b]	1.03 (0.83, 1.29)	
Duration of Response		
Responders, n (%)	128 (39.0)	131 (41.7)
disease progression among responders, n (%)	43 (33.6)	45 (34.4)
HR (90% CI)[b]	0.76 (0.51, 1.14)	

HR = hazard ratio; ITT = intent-to-treat; ORR = objective response rate.

[a] Point estimate and CI are estimated using a generalized linear model adjusted for the randomization stratification factors geographic region, ECOG performance status, and gender.

[b] Hazard ratio for ABP 215 relative to bevacizumab, based on a Cox proportional hazards model stratified by randomization factors.

Risk Difference for Objective Response Rate

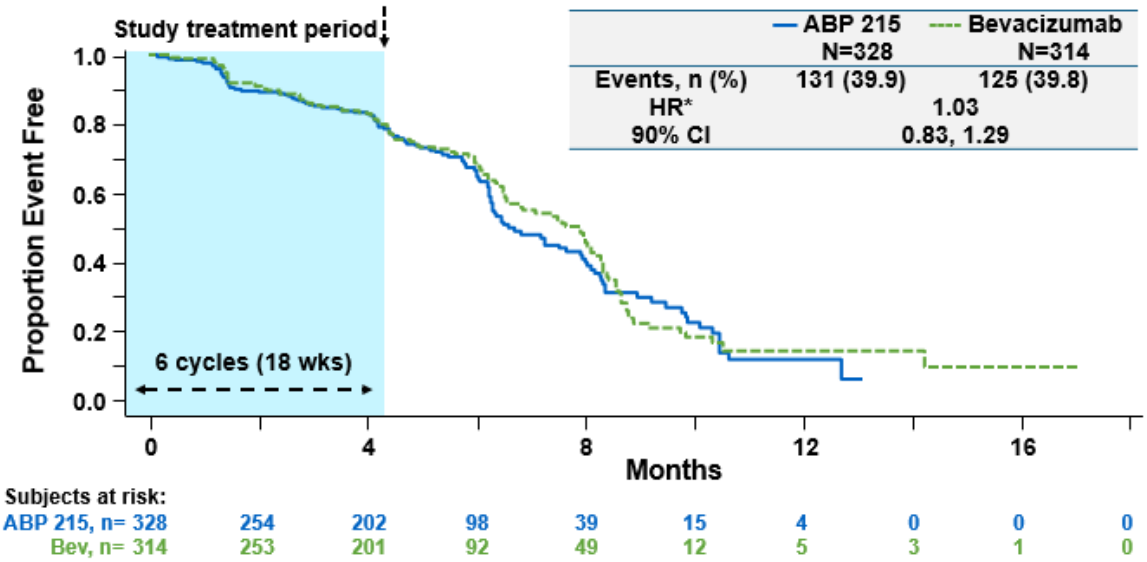
The risk difference in the ITT population (based on review of data by independent, central radiologists) between the ABP 215 and bevacizumab treatment groups was -2.90%. The 2-sided 90% confidence interval was (-9.26%, 3.45%). Analysis of the risk difference using the per-protocol population and the tumor response set showed similar risk differences. Analysis of the risk difference based on the investigator's assessment of disease showed that the 2 treatment arms were similar (Table 16). Overall, the results from analysis of risk difference for ORR support the clinical equivalence of ABP 215 to bevacizumab.

Progression-free Survival

In the ABP 215 treatment group, 131 (39.9%) subjects had progressed or died versus 125 (39.8%) subjects in the bevacizumab treatment group. The Cox proportional hazard ratio (ABP 215 versus bevacizumab) was 1.03 with 2-sided 90% confidence interval of (0.83, 1.29). The Kaplan-Meier plot of the PFS data is shown in Figure 26. The shaded blue box in Figure 26 was the controlled treatment period of the study. The study did not include maintenance treatment and therefore subjects ended the study if they went on to maintenance therapy, or they received any other anti-cancer treatment. To assess the robustness of the primary analysis results for PFS, a sensitivity analysis was conducted

post hoc using a piecewise Cox model with 6-week intervals for which the overall weighted hazard ratio was 1.01 with 2-sided 90% confidence interval of (0.81, 1.26), similar to the value obtained in the primary analysis of PFS. Findings were similar when PFS was analyzed based on the investigator's assessment of disease in the ITT population.

Figure 26. Kaplan-Meier Plot of Progression-free Survival (Study 265, ITT Population, Central Assessment)



Bev = bevacizumab; CI= confidence interval; HR = hazard ratio; ITT = intent-to-treat.

Duration of Response

Of the subjects with an objective response in the ABP 215 and bevacizumab treatment groups (128 [39.0%] and 131 [41.7%], respectively), 33.6% and 34.4% had subsequent disease progression, respectively. The Cox proportional hazard ratio (ABP 215 versus bevacizumab) was 0.76; the 2-sided 90% confidence interval was (0.51, 1.14). Findings were similar when duration of response was analyzed based on the investigator's assessment of disease in the ITT population, and when analyzed using the tumor response set.

5.2.3 Clinical Efficacy Conclusions

Clinical equivalence of ABP 215 and bevacizumab was demonstrated based on the risk ratio of ORR in subjects with advanced non-squamous NSCLC based on independent, central radiologist review of response data. Additional analyses of the robustness and sensitivity of the efficacy results confirmed the findings from the primary analysis.

Results from analyses of the secondary efficacy variables (risk difference of ORR, PFS, and duration of response) support the similarity between ABP 215 and bevacizumab.

5.3 Comparison and Analyses of PK Results in the NSCLC Study 265

Serial trough PK concentrations were measured in NSCLC Study 265. The PK analysis population (population that provide at least one serum concentration of ABP 215 or bevacizumab) consisted of 322 subjects in the ABP 215 arm and 308 subjects in the bevacizumab arm, and was used to summarize the PK concentration data (Table 18).

Table 18. Geometric Mean Summary for Serum Pharmacokinetics Concentration at Scheduled Visits (Study 265, PK Analysis Population)

Visit Statistic	ABP 215 (N = 322)	Bevacizumab (N = 308)
Week 4		
GeoMean [n[a]]	57143.81 [289]	61959.76 [285]
GMR (90% CI)	0.92 (0.86, 0.99)	
Week 7		
GeoMean [n[a]]	84685.34 [276]	91360.43 [273]
GMR (90% CI)	0.93 (0.85, 1.01)	
Week 13		
GeoMean [n[a]]	104686.78 [234]	118154.99 [233]
GMR (90% CI)	0.89 (0.80, 0.99)	
Week 19		
GeoMean [n[a]]	110334.50 [195]	114951.06 [208]
GMR (90% CI)	0.96 (0.85, 1.08)	

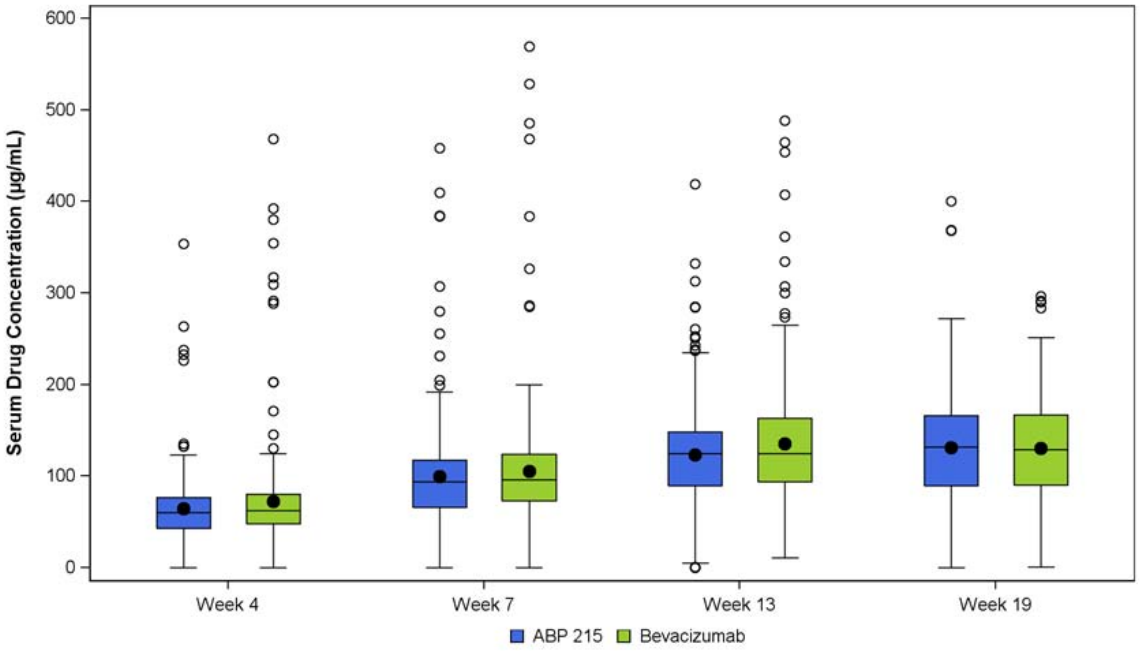
CI = confidence interval; GeoMean = geometric mean; GMR = geometric mean ratio; PK = pharmacokinetic.

Geometric mean, geometric mean ratio, and 90% CI are estimated based upon a generalized linear model with baseline weight as a covariate.

[a] PK concentrations below the lower limit of quantification are assigned a value of 0 and are excluded from the calculation of geometric mean (GeoMean) and GMR.

With repeated dosing of ABP 215 or bevacizumab at 15 mg/kg over the treatment period, serum concentrations in subjects in both treatment groups increased at each time point and remained consistent between the groups. Based on the median and geometric mean trough serum concentration values (Table 18 and Figure 27), steady state for ABP 215 and bevacizumab appeared to be reached by week 13, which is consistent with the predicted time for bevacizumab to reach steady state (approximately 100 days, Section 5.1). The trough serum concentrations were consistent between ABP 215 and bevacizumab throughout the treatment period (Figure 27), indicating that ABP 215 and bevacizumab have comparable PK following repeat dosing in advanced non-squamous NSCLC subjects.

Figure 27. Trough Concentration Comparison in Advanced Non-squamous NSCLC Subjects (Study 265)



IQR = interquartile range; NSCLC = non-small cell lung cancer.

Note: Boxes show mean (dots), median (lines), and 25th (bottom) and 75th (top) percentiles. Whiskers represent the lowest and highest values still within 1.5 times the respective IQR; data values that do not fall within 1.5 times the respective IQR are plotted as outliers.

In conclusion, with repeated therapeutic dosing of ABP 215 or bevacizumab, trough PK serum concentrations in subjects with advanced non-squamous NSCLC were comparable between the treatment arms, supporting the determination of PK similarity of ABP 215 to bevacizumab.

5.4 Safety and Immunogenicity

5.4.1 Safety

The safety analysis in the NSCLC Study 265 was performed on the safety population, defined as all randomized subjects who received at least 1 dose of investigational product. The safety evaluations included adverse events and immunogenicity. Treatment-emergent adverse events were defined as adverse events that began or increased in severity or frequency at or after the time of first treatment and on or prior to the end-of-treatment visit. Subjects in the NSCLC study were also followed for OS (defined as the time from date of randomization to date of death) throughout the treatment phase and regularly after the final visit until the end of the study, consent was withdrawn, they were lost to follow-up, died, or had proscribed therapy.

5.4.1.1 Subject Exposure

In the NSCLC Study 265, 324 subjects were exposed to ABP 215, and 309 subjets were exposed to bevacizumab. In addition to the number of subjects exposed to investigational product in Study 265, 629 subjects across both treatment groups received at least 1 dose of carboplatin, and 632 subjects overall received at least 1 dose of paclitaxel.

5.4.1.2 Overview of Safety

The safety profile for ABP 215 is consistent with the known safety profile of bevacizumab and no new safety signals were identified (Avastin United States Prescribing Information, 2016). An overview of safety in the PK similarity study in healthy subjects is provided in Section 5.1.1.3. In the NSCLC study, the incidences of treatment-emergent adverse events, grade ≥ 3 events, serious adverse events, adverse events leading to discontinuation of investigational product or chemotherapy, and adverse events of interest were similar between ABP 215 and bevacizumab (Table 19). Additionally, findings from the analysis of overall survival showed a similar percentage of deaths for ABP 215 and bevacizumab treatment groups during the study.

Table 19. Overview of Adverse Events in Study 265 (Safety Population)

Category	ABP 215 (N = 324) n (%)	Bevacizumab (N = 309) n (%)
Any TEAE	308 (95.1)	289 (93.5)
Any grade ≥ 3 TEAE	139 (42.9)	137 (44.3)
Any serious TEAE	85 (26.2)	71 (23.0)
Any fatal TEAE	13 (4.0)	11 (3.6)
TEAE leading to discontinuation of IP	61 (18.8)	53 (17.2)
TEAE leading to discontinuation of any component of chemotherapy	74 (22.8)	59 (19.1)
TEAE leading to dose delay of IP	73 (22.5)	69 (22.3)
TEAE leading to dose delay of any component of chemotherapy	86 (26.5)	83 (26.9)
TEAE leading to dose reduction of any component of chemotherapy	48 (14.8)	49 (15.9)
Any adverse event of interest	247 (76.2)	229 (74.1)

IP = investigational product; MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent adverse event.

Note: Adverse events were coded using MedDRA v18. Only TEAEs are summarized. For each category, subjects are included only once, even if they experienced multiple events in that category.

Treatment-emergent Adverse Events

The incidence of treatment-emergent adverse events in Study 265 was similar between ABP 215 and bevacizumab. The adverse events reported by preferred term (≥ 10% in either treatment group) are shown in Table 20. The most frequently reported adverse events were alopecia, nausea, and anemia, typical of patients receiving chemotherapy, and were reported at similar rates in the 2 groups. No trends in adverse events were seen, and the safety profile was consistent between ABP 215 and bevacizumab.

Table 20. Treatment-emergent Adverse Events by Preferred Term (≥ 10% of Subjects in Either Treatment Group) in Study 265 (Safety Population)

Preferred Term	ABP 215 (N = 324) n (%)	Bevacizumab (N = 309) n (%)
Subjects with any TEAE	308 (95.1)	289 (93.5)
Alopecia	140 (43.2)	127 (41.1)
Nausea	83 (25.6)	95 (30.7)
Anaemia	67 (20.7)	64 (20.7)
Neutropenia	60 (18.5)	61 (19.7)
Fatigue	59 (18.2)	59 (19.1)
Neuropathy peripheral	56 (17.3)	38 (12.3)
Decreased appetite	54 (16.7)	43 (13.9)
Hypertension	51 (15.7)	41 (13.3)
Asthenia	49 (15.1)	42 (13.6)
Thrombocytopenia	49 (15.1)	43 (13.9)
Epistaxis	45 (13.9)	39 (12.6)
Diarrhoea	42 (13.0)	56 (18.1)
Myalgia	39 (12.0)	44 (14.2)
Vomiting	38 (11.7)	42 (13.6)
Constipation	37 (11.4)	36 (11.7)
Paraesthesia	29 (9.0)	40 (12.9)

TEAE = treatment-emergent adverse event.

Grade ≥ 3 Treatment-emergent Adverse Events

The incidence of grade ≥ 3 adverse events was comparable between ABP 215 and bevacizumab in Study 265, as shown in (Table 19). The proportion of subjects who experienced grade ≥ 3 events was 42.9% in the ABP 215 group and 44.3% in the bevacizumab group. The most common grade ≥ 3 adverse events by preferred term (≥ 5% in either of the ABP 215 and bevacizumab treatment groups, respectively) were

neutropenia (12.3% and 11.7%), hypertension (6.5% and 5.2%), and thrombocytopenia (3.1% and 5.2%).

Serious Treatment-emergent Adverse Events

The incidence of serious adverse events in Study 265 was similar between the ABP 215 and bevacizumab treatment groups. Table 21 shows the incidence of serious adverse events (≥ 1% of subjects) reported in either treatment group.

Table 21. Serious Treatment-emergent Adverse Events in Study 265 (≥ 1% of Subjects in Either Treatment Group) (Safety Population)

Preferred Term	ABP 215 (N = 324) n (%)	Bevacizumab (N = 309) n (%)
Subjects with any SAE	85 (26.2)	71 (23.0)
Febrile neutropenia	11 (3.4)	8 (2.6)
Neutropenia	6 (1.9)	3 (1.0)
Pneumonia	6 (1.9)	5 (1.6)
Pulmonary embolism	5 (1.5)	6 (1.9)
Anemia	3 (0.9)	6 (1.9)
Dyspnoea	3 (0.9)	4 (1.3)
Hemoptysis	3 (0.9)	5 (1.6)

SAE = serious adverse event.

Fatal Treatment-emergent Adverse Events

In Study 265, fatal adverse events occurred at a similar rate in the ABP 215 and bevacizumab treatment groups: 4.0% and 3.6%, respectively (Table 19).

Treatment-emergent Adverse Events Leading to Discontinuation

Adverse events leading to discontinuation of investigational product in Study 265 were reported in 18.8% of subjects in the ABP 215 and 17.2% of subjects in the bevacizumab treatment group (Table 19). The most frequently reported adverse event leading to discontinuation of investigational product in both treatment groups was pulmonary embolism (1.5% for ABP 215 and 1.9% for bevacizumab). There was no observed trend in adverse events leading to discontinuation of investigational product.

Treatment-emergent Adverse Events of Interest

In the NSCLC Study 265, pre-specified adverse events of interest (EOIs) pertinent to the mechanism of action and known safety profile of bevacizumab were also analyzed. The pre-specified EOIs were those that are known safety risks for bevacizumab based on the Warnings and Precautions section of the Avastin United States Prescribing Information

(2016). These events overall and those grade ≥ 3 are listed and summarized in Table 22.

Table 22. Overall Summary of Adverse Events of Interest in Study 265 (Safety Population)

	ABP 215 (N = 324)		Bevacizumab (N = 309)	
Event of Interest	Grade ≥ 3 n (%)	All Grades n (%)	Grade ≥ 3 n (%)	All Grades n (%)
Any event of interest	102 (31.5)	247 (76.2)	99 (32.0)	229 (74.1)
Infusion reactions (occurring within 2 days of the 1st investigational product dose)	2 (0.6)	24 (7.4)	1 (0.3)	22 (7.1)
Peripheral sensory neuropathy	6 (1.9)	97 (29.9)	5 (1.6)	78 (25.2)
Neutropenia and infections	54 (16.7)	84 (25.9)	47 (15.2)	80 (25.9)
Thrombotic microangiopathy	13 (4.0)	76 (23.5)	20 (6.5)	59 (19.1)
Hemorrhages	11 (3.4)	72 (22.2)	6 (1.9)	65 (21.0)
Hypertension	22 (6.8)	61 (18.8)	17 (5.5)	49 (15.9)
Proteinuria	1 (0.3)	27 (8.3)	1 (0.3)	19 (6.1)
Hypersensitivity reactions	2 (0.6)	23 (7.1)	1 (0.3)	30 (9.7)
Reversible posterior leukoencephalopathy syndrome-related	2 (0.6)	16 (4.9)	2 (0.6)	9 (2.9)
Venous thromboembolic events	8 (2.5)	14 (4.3)	12 (3.9)	16 (5.2)
Pulmonary hemorrhage[a]	2 (0.6)	14 (4.3)	5 (1.6)	9 (2.9)
Arterial thromboembolic events	4 (1.2)	6 (1.9)	3 (1.0)	4 (1.3)
Gastrointestinal perforations	3 (0.9)	3 (0.9)	4 (1.3)	4 (1.3)
Surgery and wound healing complications	1 (0.3)	2 (0.6)	2 (0.6)	4 (1.3)
Non-gastrointestinal fistula formation	1 (0.3)	2 (0.6)	1 (0.3)	2 (0.6)
Congestive heart failure	1 (0.3)	2 (0.6)	1 (0.3)	1 (0.3)
Cardiac disorders	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Pulmonary hypertension	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)

[a] For 1 subject in the bevacizumab treatment group, the CTCAE toxicity grade was not provided by the investigator and is not included in this table.

The overall incidence of any EOI during the study was 76.2% in the ABP 215 treatment group and 74.1% in the bevacizumab treatment group. Overall, the incidence rates of adverse events of interest were generally balanced between ABP 215 and bevacizumab, and there were no clinically meaningful differences between the 2 groups.

Laboratory Values and Vital Signs

In Study 265, no clinically important changes were observed in the ABP 215 or bevacizumab groups in laboratory values (hematology, chemistry, urine protein) or vital signs (pulse, systolic and diastolic blood pressure) from baseline to the end of treatment.

Overall Survival

The analysis of OS supports the conclusion of similarity between the ABP 215 and bevacizumab treatment groups: 43 (13.3%) subjects and 36 (11.7%) subjects, respectively, died during treatment or during the follow-up period.

5.4.1.3 Safety Conclusions

The type, severity, and incidence of treatment-emergent adverse events, serious adverse events, adverse events of interest, as well as clinically significant changes in laboratory values, changes in vital signs, and overall survival with ABP 215 and bevacizumab were similar in the NSCLC Study 265. No new safety risks were identified and the safety of ABP 215 is consistent with the known safety profile of bevacizumab. Hence, it is concluded that there are no clinically meaningful differences with respect to the safety of ABP 215 and bevacizumab.

5.4.2 Immunogenicity

Immunogenicity data for the PK similarity Study 216 is summarized in Section 5.1.1.4. The validated assays used in the NSCLC Study 265 for anti-drug antibody detection were sensitive and robust to detect differences in immunogenicity between ABP 215 and bevacizumab. Samples that tested positive for binding antibodies were also tested for neutralizing activity.

Results from the immunogenicity assessments demonstrated that the incidence of anti-drug antibodies was low and similar for the ABP 215 and bevacizumab treatment groups. Three subjects (1.0%) in the bevacizumab group tested positive for pre-existing binding antibodies and none of these tested positive for neutralizing activity. Post-baseline, 4 subjects (1.4%) in the ABP 215 group and 7 subjects (2.5%) in the bevacizumab group tested positive for binding antibodies; for 3 of these subjects in each group, the results were transient, meaning negative results at the subject's last time point tested. None of the subjects developed neutralizing antibodies. Thus, it is concluded that there are no clinically meaningful differences in immunogenicity between ABP 215 and bevacizumab.

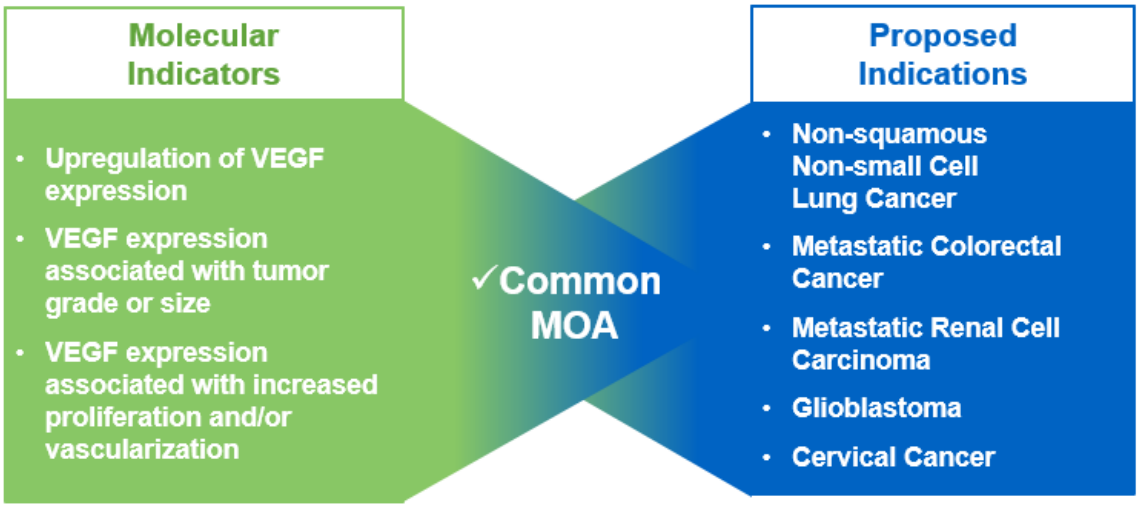
6. EXTRAPOLATION OF INDICATIONS

The purpose of this section is to summarize the scientific justification for extrapolating safety and efficacy claims for ABP 215 to conditions of use approved for bevacizumab that were not studied in the ABP 215 clinical program. In agreement with FDA guidance, this section considers the current knowledge of ABP 215 and bevacizumab, regarding the mechanism of action, PK, toxicities (safety profiles), immunogenicity, efficacy, and other factors that may affect the safety or effectiveness in each indication. This knowledge supports extrapolating the safety and efficacy of bevacizumab to ABP 215, as they are expected to have the same activity as each other in all indications.

A high degree of similarity in structural and purity attributes, and functional activities has been demonstrated (Section 6.1). PK similarity between ABP 215 and bevacizumab was established, and the PK profiles of bevacizumab in different patient populations are also similar (Section 6.2). Similar efficacy, safety, and immunogenicity has been demonstrated between ABP 215 and bevacizumab in a sensitive and representative patient population. Given the demonstrated similarity between ABP 215 and bevacizumab, and the commonalties in each of the indications of use, ABP 215 is expected to have similar risks and benefits in all the approved indications for bevacizumab.

As described in Section 2.2.3, the mechanism of action in all of the approved indications for bevacizumab is binding to soluble VEGF and preventing the interaction of VEGF with its receptors (VEGFR-1 and VEGFR-2) on the surface of endothelial cells, thus inhibiting endothelial cell proliferation, angiogenesis, and VEGF-induced vascular permeability. Bevacizumab is an effective treatment for a number of tumors and its mechanism of action is independent of tumor site (Avastin United States Prescribing Information, 2016). As illustrated in Figure 28, VEGF expression plays a similar role across the bevacizumab indications.

Figure 28. Mechanism of Action: VEGF Expression Plays a Similar Role Across Bevacizumab Indications



MOA = mechanism of action; VEGF = vascular endothelial growth factor.

6.1 Analytical Similarity

The ABP 215 analytical similarity assessment demonstrated a high degree of similarity with minimal analytical differences between ABP 215 and bevacizumab (Section 3.2). As noted in Section 2.2.3, the mechanism of action for bevacizumab is the same in all approved indications. Most importantly, the functional testing focused on the mechanism of action (binding and neutralization of VEGF), and the results between ABP 215 and bevacizumab were found to be statistically equivalent. Furthermore, comparative binding to multiple Fc receptors and C1q was evaluated, and the results demonstrated similarity between APB 215 and bevacizumab. Additionally, the lack of effector functions (ADCC and CDC) was confirmed in ABP 215 and bevacizumab. This high degree of analytical similarity, and the same mechanism of action in each indication, provides a key component of the scientific justification supporting extrapolation to all approved indications.

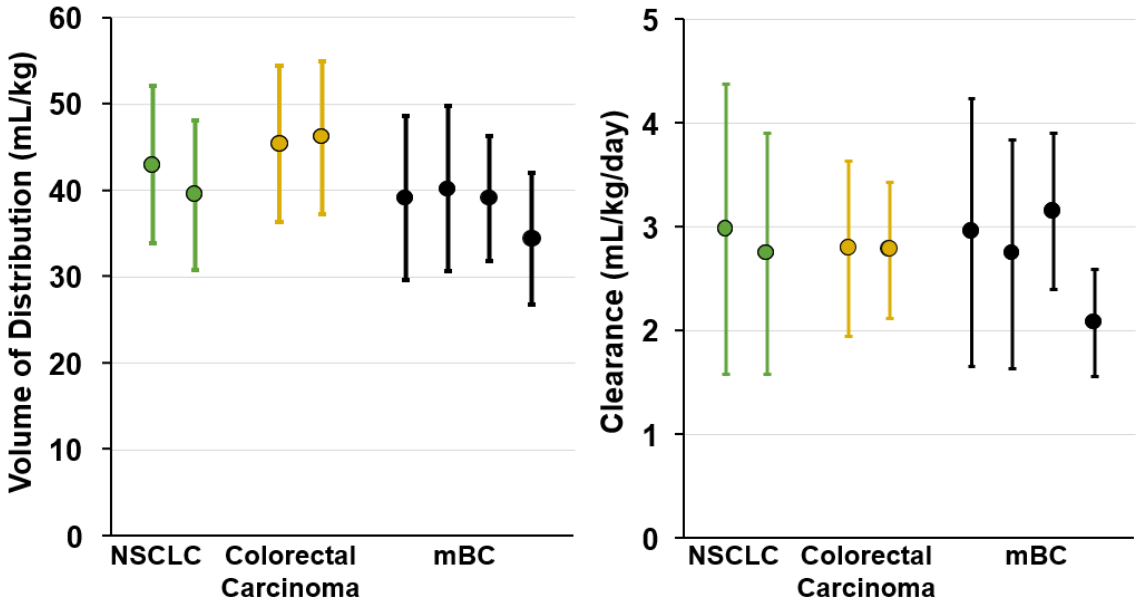
6.2 Clinical Pharmacology

The ABP 215 clinical pharmacology program included the PK similarity study, Study 216, that compared a single 3 mg/kg IV infusion of ABP 215, bevacizumab (US), and bevacizumab (EU) in healthy male subjects. All pairwise comparisons for Study 216 met the pre-specified acceptance criteria for PK similarity (Section 5.1.1.2). Study 265 in subjects with advanced non-squamous NSCLC also compared multiple 15 mg/kg IV infusions of ABP 215 and bevacizumab. Results from this study demonstrated similar trough PK concentrations between ABP 215 and bevacizumab throughout treatment

(Section 5.3). The PK comparisons of 3 mg/kg in PK Study 216 and 15 mg/kg in NSCLC Study 265 bracketed the range of 5 mg/kg to 15 mg/kg clinical doses. ABP 215 and bevacizumab serum concentrations were consistent across studies, indicating comparability between ABP 215 and bevacizumab, across all clinical doses.

A summary of the relevant PK properties of bevacizumab in different patient populations that have been studied with bevacizumab is shown in Figure 29. The figure shows that regardless of tumor type, bevacizumab exhibits similar volume of distribution and clearance.

Figure 29. Similarity of Distribution and Clearance of Bevacizumab Across Tumor Types

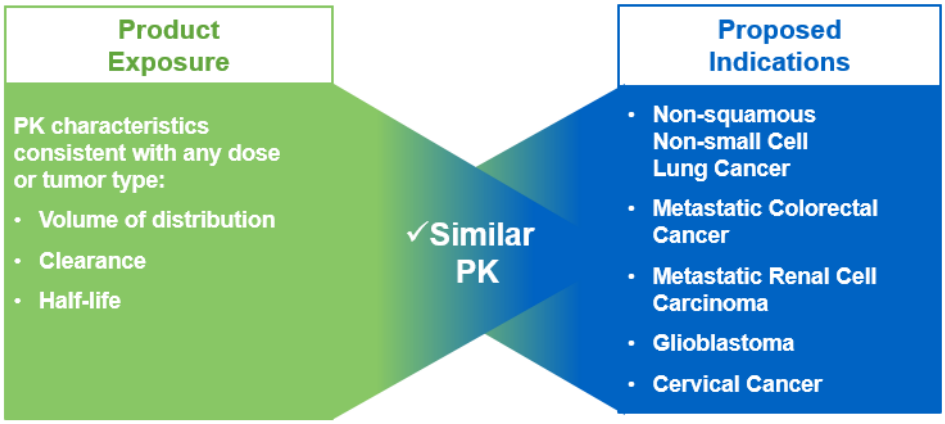


mBC = metastatic breast cancer; NSCLC = non-small cell lung cancer.

Note: mBC is not an approved indication in the US.

The comparative PK data, combined with the knowledge of the PK profiles of bevacizumab in different patient populations, indicate that ABP 215 will retain a PK profile similar to bevacizumab in all indications for which ABP 215 licensure is sought (Figure 30).

Figure 30. Similar PK Characteristics of Bevacizumab Across Tumor Types

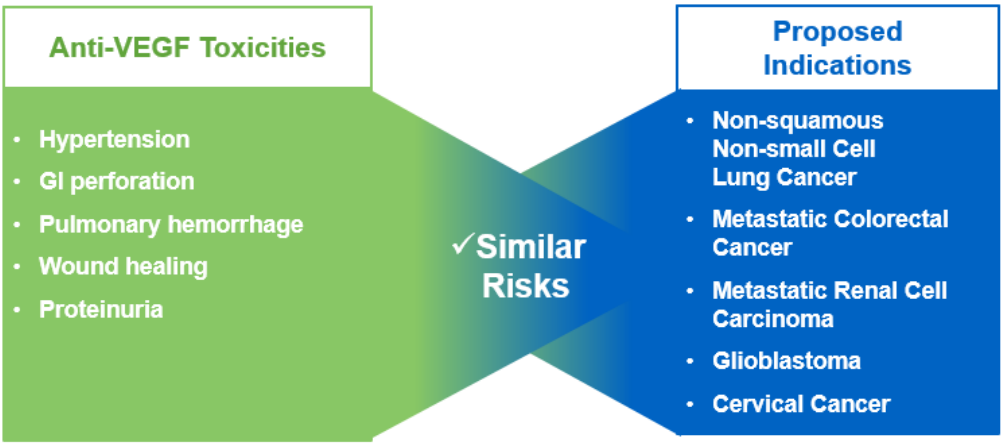


PK = pharmacokinetics.

6.3 Safety

Study 265 in subjects with advanced non-squamous NSCLC is considered sensitive and relevant for demonstrating similarity in safety profiles of ABP 215 and bevacizumab. As discussed in Section 5.4.1, no clinically meaningful differences in toxicities were observed between treatment groups in the NSCLC study, and the adverse events were consistent with the known safety profile of bevacizumab. The known risks associated with bevacizumab are common across approved indications and dosing regimens (Figure 31). Given the similarity in the safety profiles of ABP 215 with bevacizumab in the clinical similarity study (Study 265), the ABP 215 safety profile is expected to be the same as bevacizumab across all indications for which ABP 215 licensure is sought.

Figure 31. Similar Risks Associated With Bevacizumab Across Tumor Types



GI = gastrointestinal.

6.4 Immunogenicity

The results from the study in NSCLC patients confirm similar low immunogenicity of ABP 215 and bevacizumab in a patient population. The very low level of

immunogenicity of ABP 215 is expected to be similar to bevacizumab in all indications of use for which licensure is sought.

Bevacizumab is not an inherently immunogenic molecule and shows a low incidence of binding or neutralizing antibodies. Data from colorectal carcinoma trials demonstrated that 14 of 2233 evaluable subjects (0.63%) tested positive for treatment-emergent anti-bevacizumab antibodies as detected by an electrochemiluminescent (ECL)-based assay. Further analysis of those 14 subjects concluded that 3 subjects were positive for neutralizing antibodies against bevacizumab. The clinical significance of these anti-drug antibody responses is unknown (Avastin United States Prescribing Information, 2016).

6.5 Other Factors That May Affect Safety or Effectiveness

No clinically meaningful differences in efficacy or safety were observed in Study 265 when analyzed by sub-groups such as age, race, and gender. The demonstration of similar safety and efficacy profiles for ABP 215 and bevacizumab across various sub-groups of subjects with advanced non-squamous NSCLC is informative of the safety and efficacy in the other approved bevacizumab indications. Given the demonstrated similarity between ABP 215 and bevacizumab, the impact of ABP 215 on extrinsic factors such as PK interactions with other chemotherapeutic treatments, the potential complications with surgery before or after treatment, drug resistance, and long-term treatment is expected to be similar to that of bevacizumab.

6.6 Extrapolation Summary

The totality of evidence indicates that ABP 215 is expected to be as safe and efficacious as bevacizumab in all conditions of use for which bevacizumab is approved.

Specifically:

- A comprehensive analytical similarity assessment demonstrated similarity between ABP 215 and bevacizumab, including in functional assays reflecting the mechanism of action which is consistent across all approved indications.
- Equivalent PK profiles in healthy subjects as well as similar steady-state drug levels in the NSCLC study, and the consistent PK of bevacizumab across indications are predictive of equivalent PK in all indications.
- The immunogenicity observations were similar for ABP 215 and bevacizumab, and similar immunogenicity profiles are expected in all indications.
- ABP 215 and bevacizumab have shown similar safety and efficacy profiles, which were also similar to previously reported data for bevacizumab. These results are predictive of similar safety and efficacy of the 2 products when used in other indications.

7. CONCLUSION

This document summarizes the comprehensive results of analytical similarity, nonclinical, and clinical studies to establish the biosimilarity of ABP 215 to bevacizumab and supports approval in all indications for which Amgen is seeking licensure.

The ABP 215 analytical program demonstrated a high level of analytical similarity between ABP 215 and bevacizumab. The program included multiple assessments of VEGF functions that are common to all indications of use for bevacizumab. The nonclinical toxicokinetics, toxicology, and pharmacologic data also support the conclusion that ABP 215 is highly similar to bevacizumab.

Following the analytical and nonclinical similarity assessments, Amgen conducted a randomized, single-blind, single-dose PK study in healthy subjects. The study demonstrated PK similarity between ABP 215 and bevacizumab. Clinical similarity between ABP 215 and bevacizumab was then established in a randomized, double-blind, active comparator-controlled clinical similarity study (NSCLC Study 265) to complete the totality of evidence evaluation.

The totality of evidence from the ABP 215 biosimilar development program leads to the conclusion that ABP 215 meets the scientific and statutory requirements for the demonstration of biosimilarity. Specifically, ABP 215 is highly analytically similar to bevacizumab notwithstanding minor differences in clinically inactive components, and there are no clinically meaningful differences between ABP 215 and bevacizumab in terms of PK, efficacy, safety, and immunogenicity.

The consistent mechanism of action across indications, demonstrated analytical similarity, PK equivalence, clinical similarity in a representative patient population, and the similarity within bevacizumab's conditions of use supports that ABP 215 will have similar clinical outcomes to bevacizumab regardless of tumor type or location. This conclusion supports the approval of ABP 215 as a biosimilar to bevacizumab in all of the bevacizumab indications for which licensure is sought.

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Appendix 1. Supporting Method Descriptions and Data

This appendix provides supporting method descriptions and graphical data for a subset of the structural, purity, and functional methods. For the full results of all methods performed during the analytical similarity assessment, please refer to Section 3.2 in the briefing document.

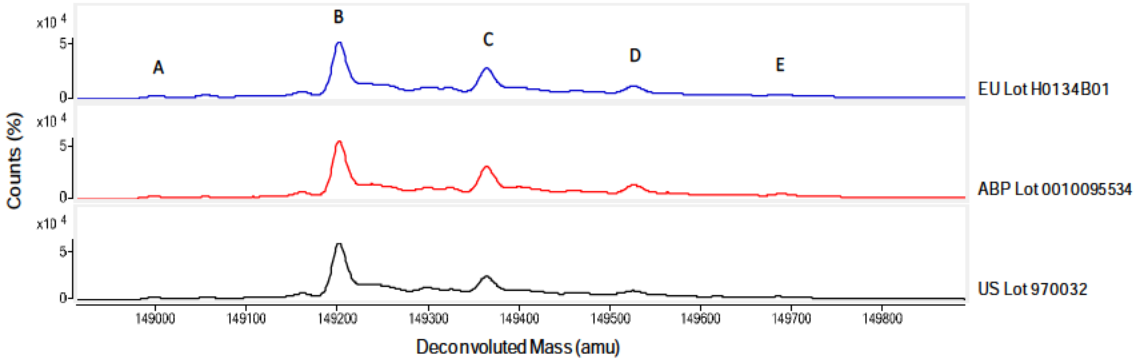
9. ANALYTICAL CHARACTERIZATION DATA

9.1 Primary Structure

Intact Molecular Mass

The intact molecular masses of ABP 215 and bevacizumab were determined by electrospray ionization-time of flight-mass spectrometer (ESI-TOF-MS) analysis. Samples were separated from buffer components and introduced to the mass spectrometer by size exclusion chromatography using a polyhydroxyethyl aspartamide column. The resulting summed ion spectra were deconvoluted to produce molecular mass profiles. The deconvoluted intact molecular mass profiles for ABP 215, bevacizumab (US), and bevacizumab (EU) are overlaid in Figure 32.

Figure 32. Intact Molecular Mass for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

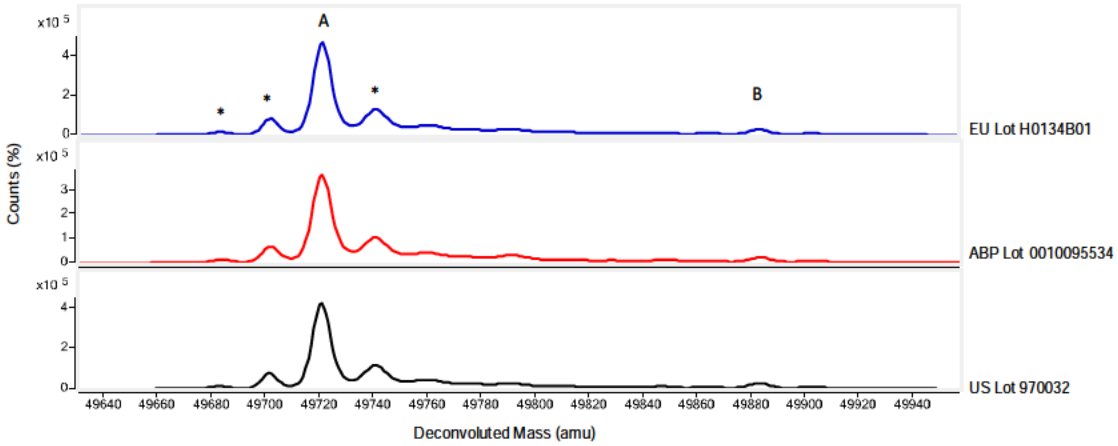


A = A2G0F:A1G0F; B = A2G0F:A2G0F; C = A2G0F:A2G1F; D = A2G1F:A2G1F or A2G0F:A2G2F; E = A2G1F:A2G2F.

Reduced and Deglycosylated Molecular Masses of Heavy Chain and Light Chain

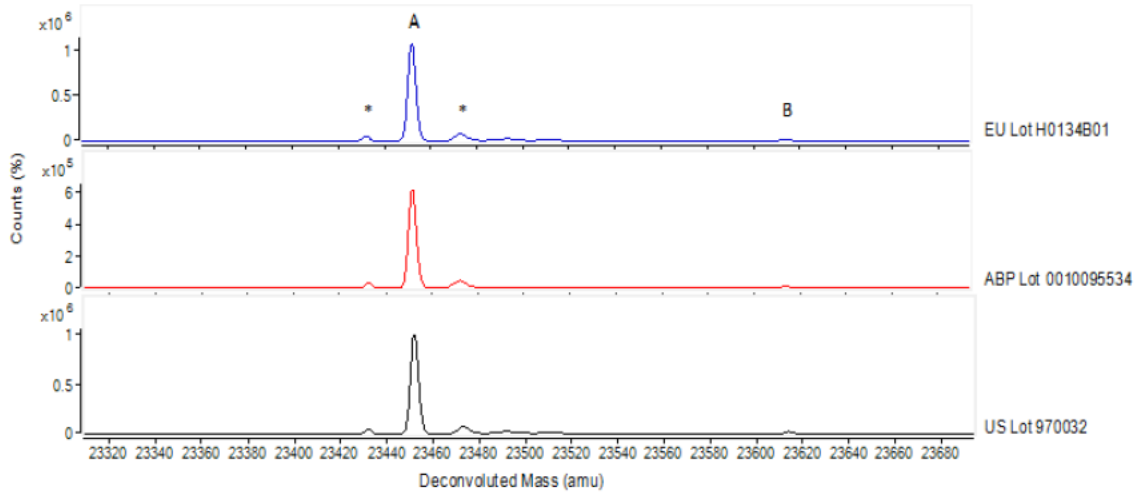
The molecular masses of reduced and deglycosylated ABP 215 and bevacizumab were evaluated using ESI-TOF-MS to provide further assurance that the polypeptide compositions were as expected. Samples were treated with PNGase F to remove N-linked glycans, subsequently denatured, and the disulfides reduced. The samples were then separated from buffer components and introduced to the mass spectrometer via reversed-phase chromatography. Summed ion spectra were deconvoluted to produce molecular mass profiles and compared with theoretical mass values. The deconvoluted reduced and deglycosylated mass profiles of ABP 215, bevacizumab (US), and bevacizumab (EU) are provided in Figure 33 (heavy chain) and Figure 34 (light chain).

Figure 33. Comparison of Reduced and Deglycosylated Heavy Chain Mass Profiles for Bevacizumab (EU), ABP 215, and Bevacizumab (US)



* Ionization artifact as a result of water loss (lower mass) and salt adduct (higher mass).

Figure 34. Comparison of Reduced and Deglycosylated Light Chain Mass Profiles for Bevacizumab (EU), ABP 215, and Bevacizumab (US)



* Ionization artifact as a result of water loss (lower mass) and salt adduct (higher mass).

Reduced Peptide Map

Peptide map analysis was conducted by enzymatic digestion with trypsin, followed by reduction with dithiothreitol and alkylation with sodium iodoacetic acid. The resulting cleavage fragments were separated by reversed-phase ultra performance liquid chromatography using an increasing gradient of acetonitrile in water. The eluted peaks were detected by ultraviolet (UV) absorbance at 214 nm. The peptides were identified by on-line mass spectrometry. The tryptic peptide map chromatograms of ABP 215, bevacizumab (US), and bevacizumab (EU) are overlaid in Figure 35.

Figure 35. Reduced Tryptic Peptide Map for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

(A) Time: 16 to 90 minutes

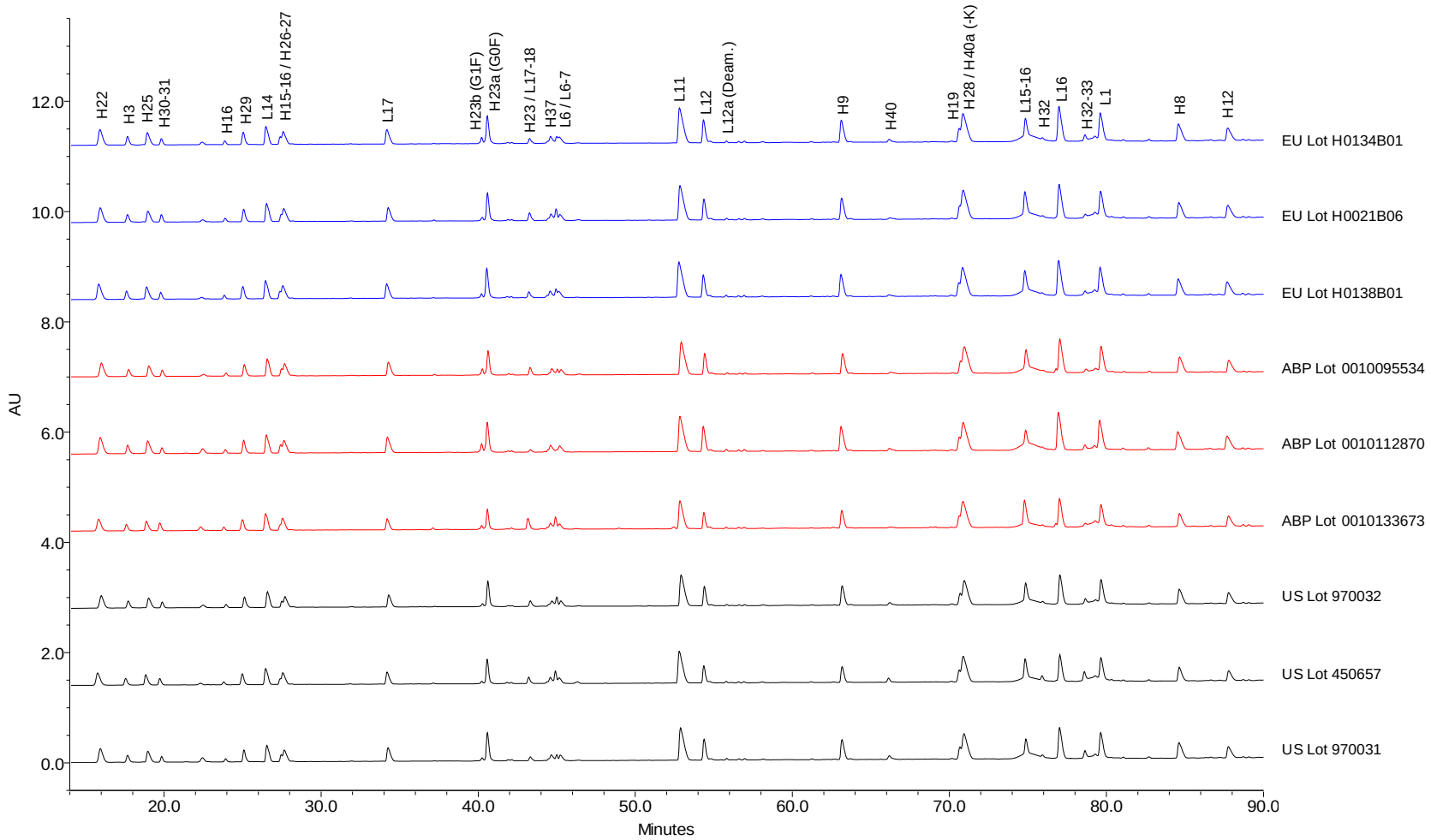
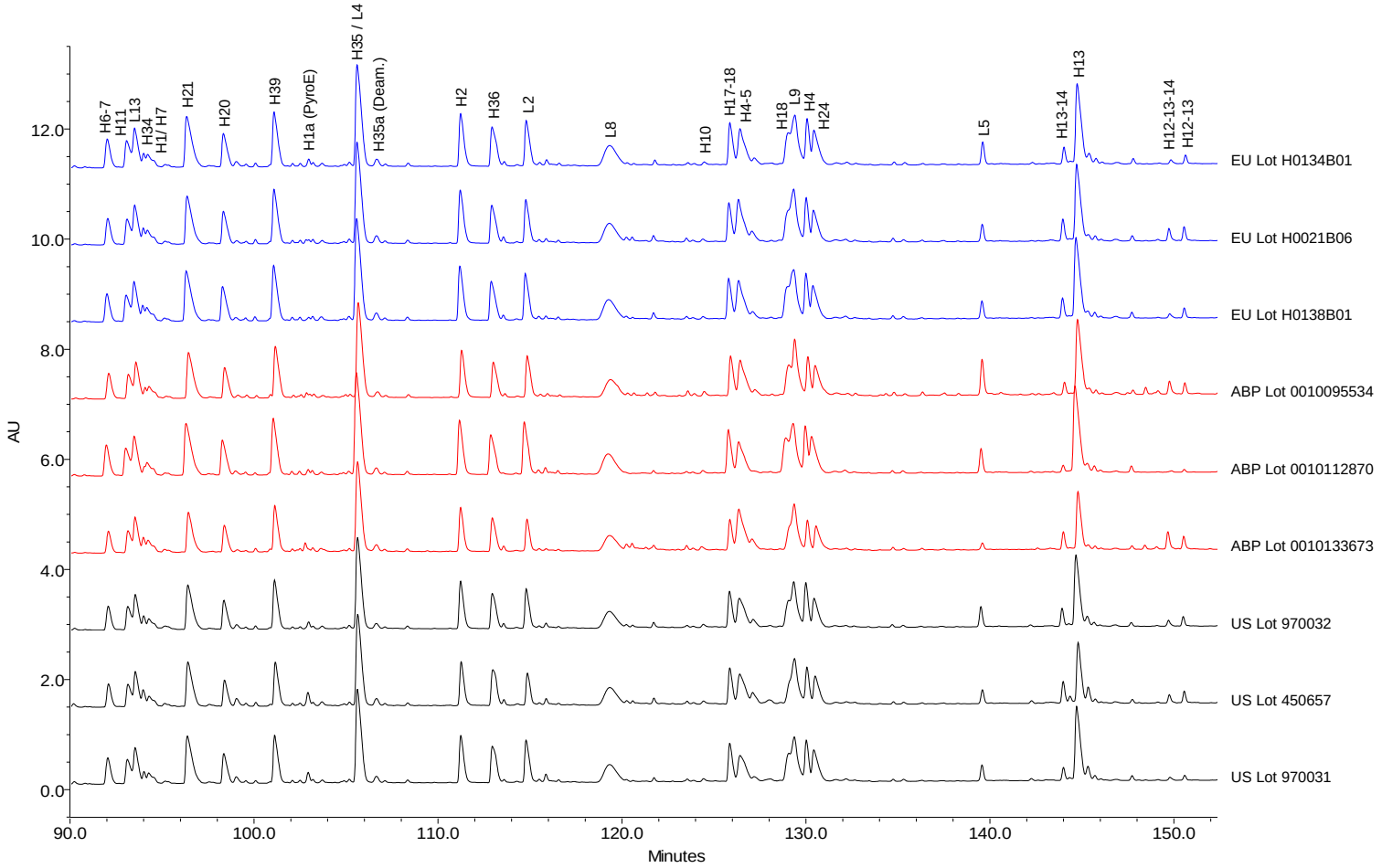


Figure 35. Reduced Tryptic Peptide Map for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

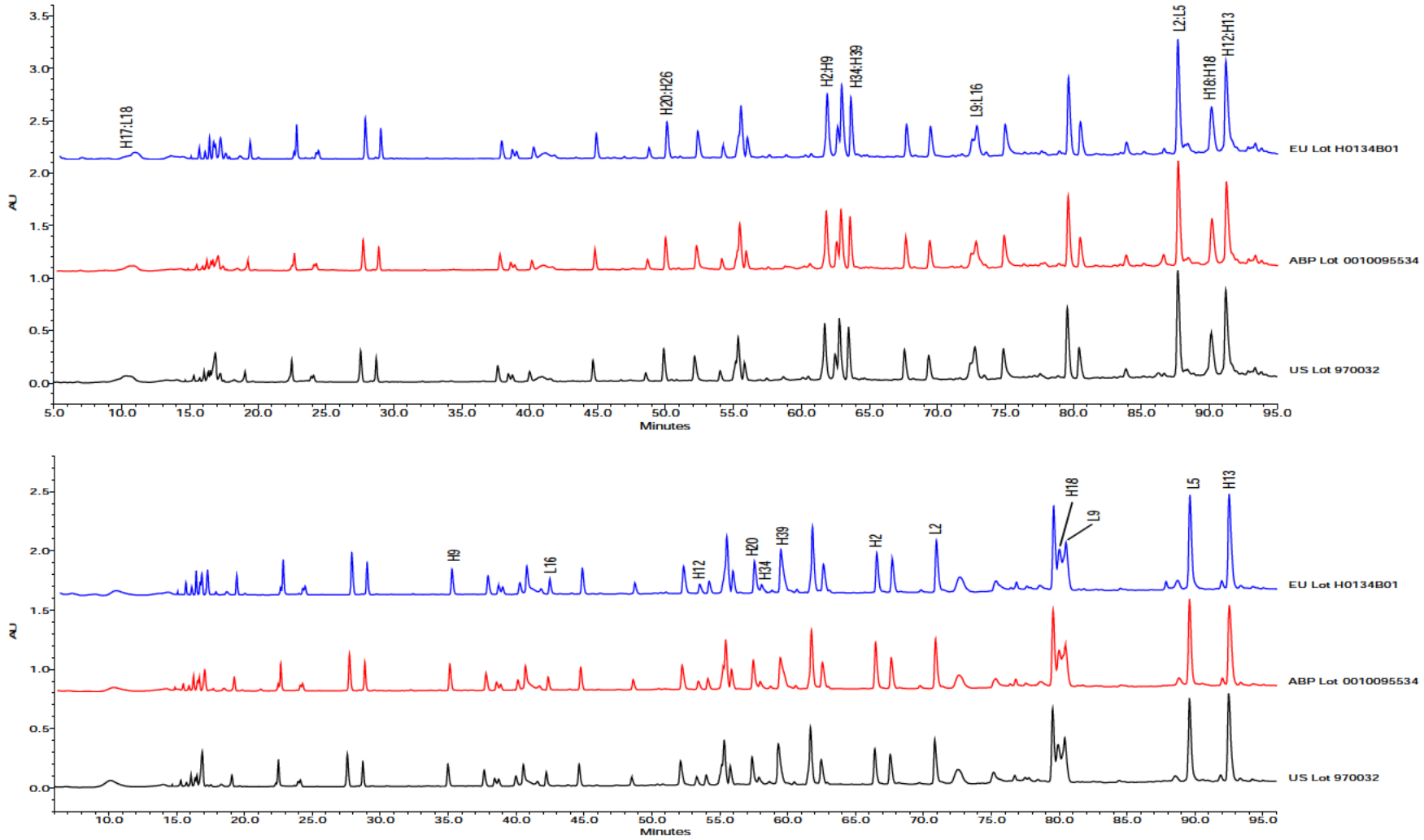
(B) Time: 90 to 162 minutes



Disulfide Structure - Non-reduced Peptide Map

Samples were digested with trypsin under denaturing, but non-reducing conditions. The resulting peptides were analyzed by reversed-phase chromatography using an increasing gradient of acetonitrile in water with UV light detection at 214 nm. The peptides containing disulfide bonds were identified by comparing peptides generated under reducing and non-reducing conditions. Confirmation of peptide identity was achieved using a high performance liquid chromatography (HPLC) system coupled through an electrospray interface to a high-resolution mass spectrometer, allowing the molecular mass determination for each peptide. The non-reduced and reduced tryptic peptide map chromatograms of ABP 215, bevacizumab (US), and bevacizumab (EU) are overlaid in Figure 36.

Figure 36. Non-reduced (Top) and Reduced (Bottom) Tryptic Maps for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

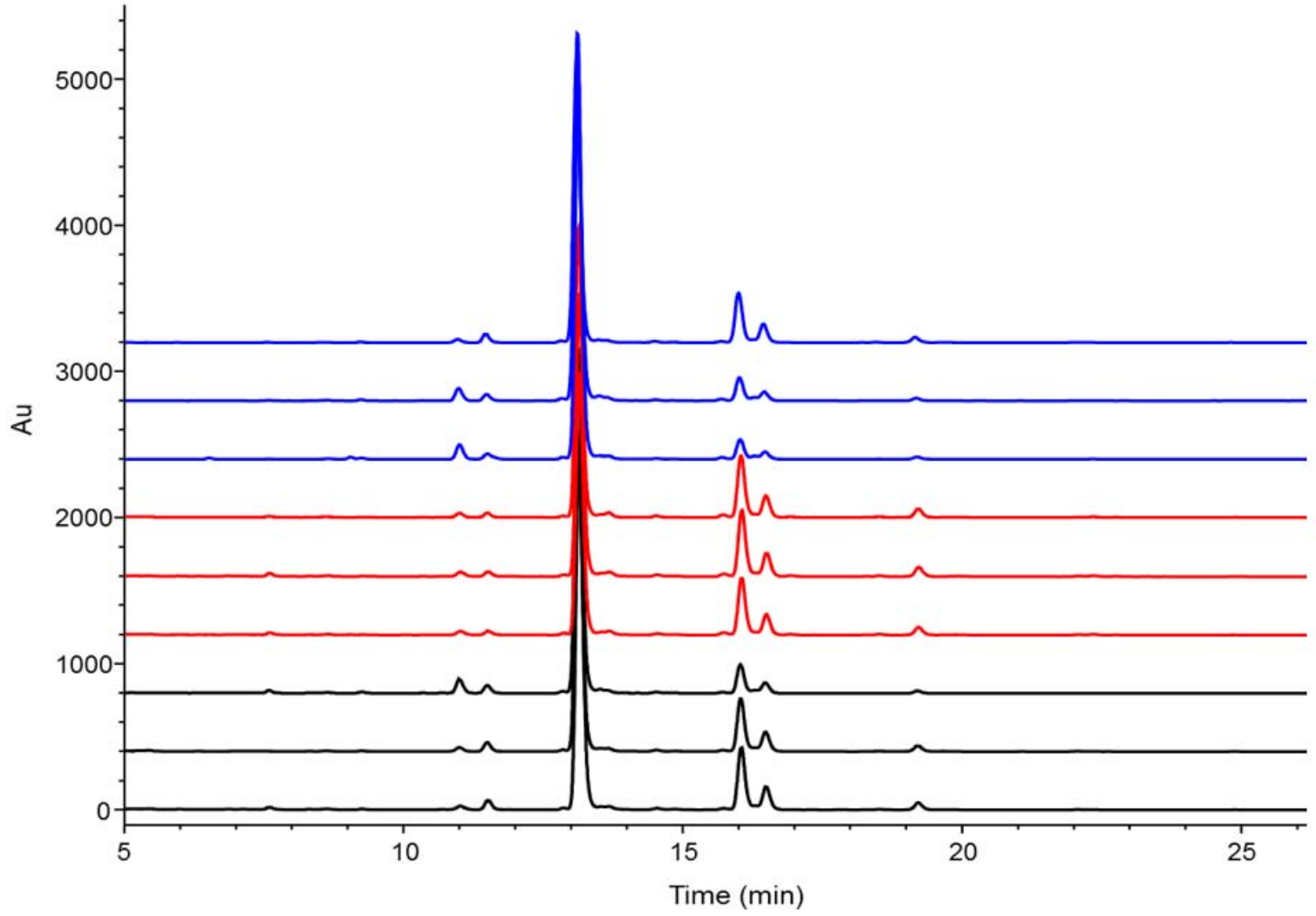


Only peptides involved in disulfide pairing are labeled.

Glycan Map

N-linked glycan profiles of ABP 215 and bevacizumab were evaluated by glycan map using hydrophilic interaction liquid chromatography (HILIC) with fluorescence detection. Glycan mapping involved release of N-glycans from ABP 215 and bevacizumab through treatment with PNGase F. The reducing termini of the released glycans were then labeled through reductive amination with a fluorescent tag (2-aminobenzoic acid), and the labeled glycans were separated by HILIC with fluorescence detection. The glycan map chromatograms of ABP 215, bevacizumab (US), and bevacizumab (EU) are overlaid in Figure 37.

Figure 37. HILIC Glycan Map for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

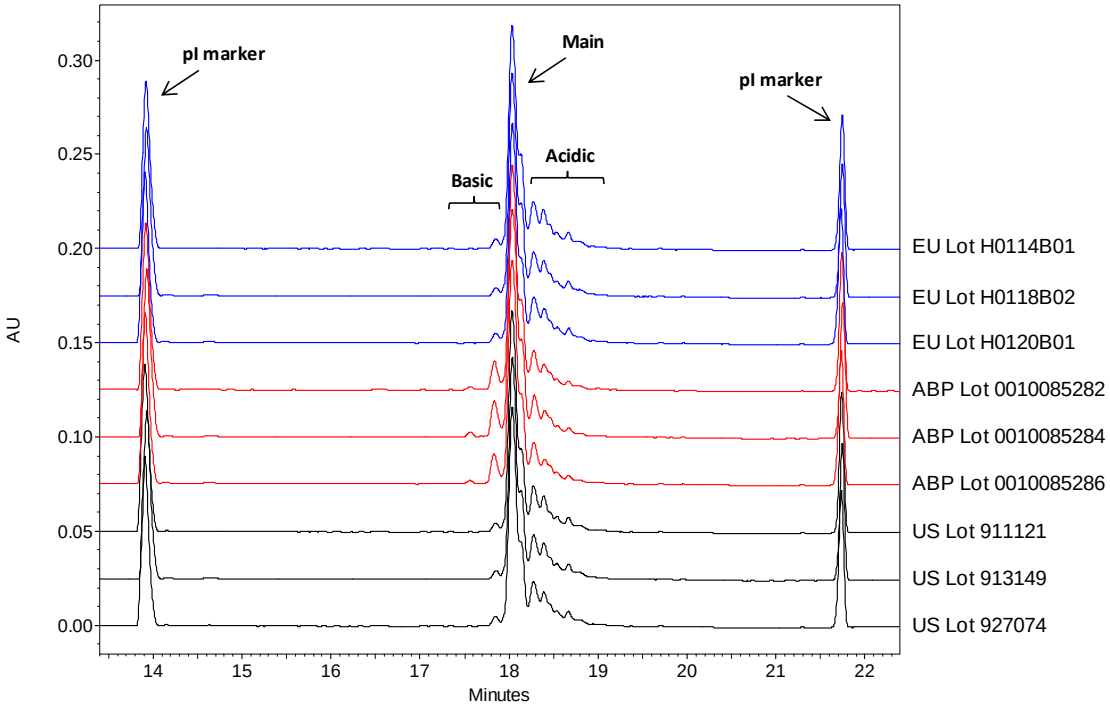


Blue = bevacizumab (EU), Red = ABP 215, Black = bevacizumab (US).

Isoelectric Point - Capillary Isoelectric Focusing (cIEF)

cIEF was performed by electrophoresis of proteins through a pH gradient in a capillary. cIEF was performed on a high resolution capillary electrophoresis separation instrument equipped with a neutral-coated capillary. The protein migrated through the pH gradient until it reached the pH equal to its isoelectric point (pI) and was then mobilized and detected by UV absorbance at 280 nm as it passed through a detection window in the capillary. To obtain the pI of the main peak, a linear regression between the 2 pI marker peaks was used. A comparison of the cIEF profiles for ABP 215, bevacizumab (US), and bevacizumab (EU) is provided in Figure 38.

Figure 38. Comparison of cIEF Profiles for Bevacizumab (EU), ABP 215, and Bevacizumab (US)



9.2 Higher Order Structure

Fourier-transform Infrared (FTIR) Spectroscopy

The FTIR measurements of ABP 215 and bevacizumab were performed using an FTIR spectrometer. The absorbance spectrum of the formulation buffer blank was subtracted from the protein absorbance spectra and the second derivative spectrum was calculated using a 9 point smoothing of the original spectra. The spectrum similarity of ABP 215 and bevacizumab (US) is shown in Figure 39. The spectrum similarity of ABP 215 and bevacizumab (EU) is shown in Figure 40.

Figure 39. Second Derivative FTIR Spectra Comparing ABP 215 and Bevacizumab (US)

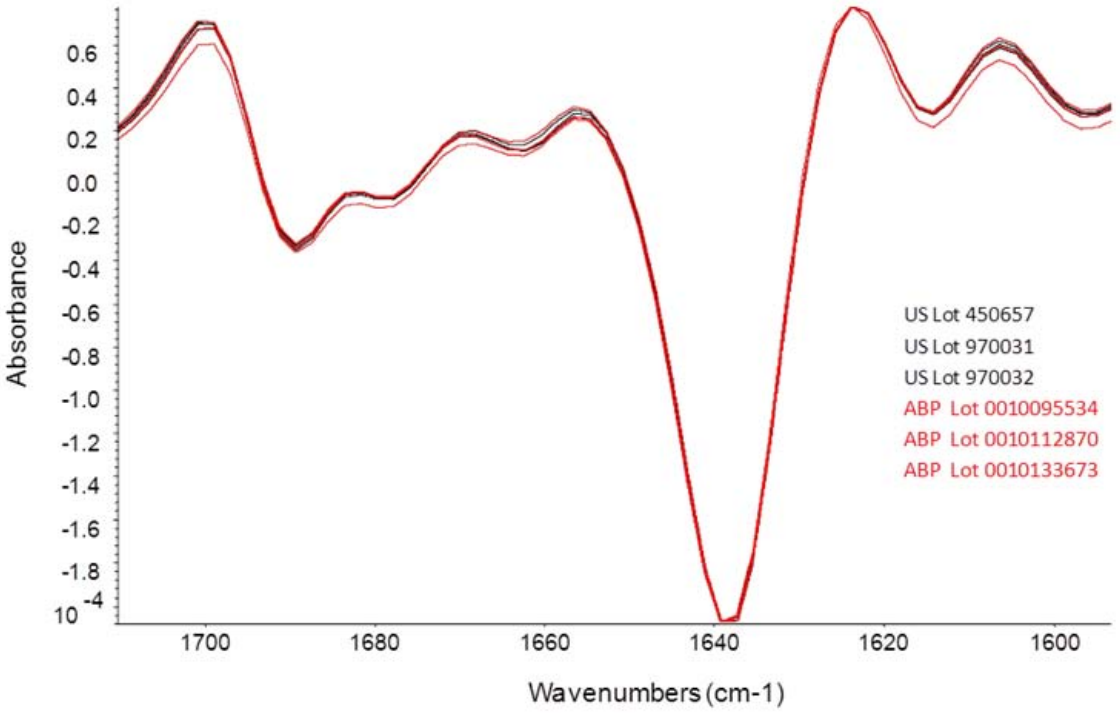
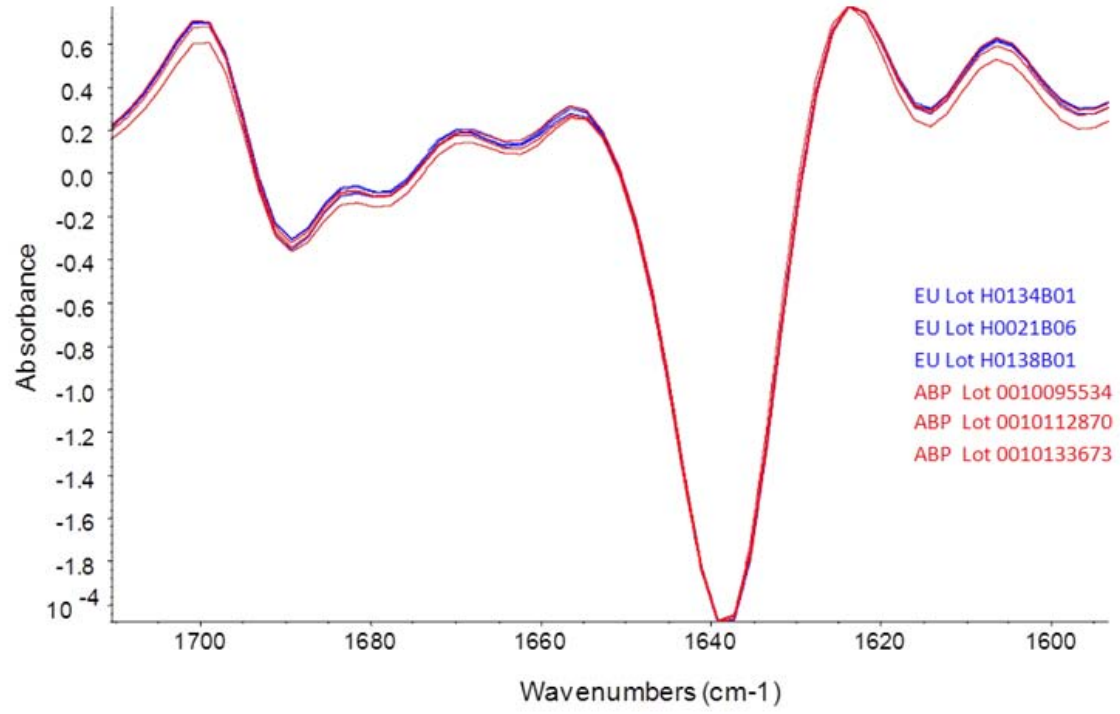


Figure 40. Second Derivative FTIR Spectra Comparing ABP 215 and Bevacizumab (EU)



Near Ultraviolet Circular Dichroism (UV CD) Spectroscopy

The near UV CD spectra of ABP 215 and bevacizumab samples were obtained on a spectropolarimeter at ambient temperature using cuvettes with a pathlength of 1 cm. The spectra were corrected for concentration and buffer contributions and the results were reported as CD ellipticity. The near UV CD spectra comparing ABP 215 and bevacizumab (US) are shown in Figure 41. The near UV CD spectra comparing ABP 215 and bevacizumab (EU) are shown in Figure 42.

Figure 41. Near UV CD Spectra Comparing ABP 215 and Bevacizumab (US)

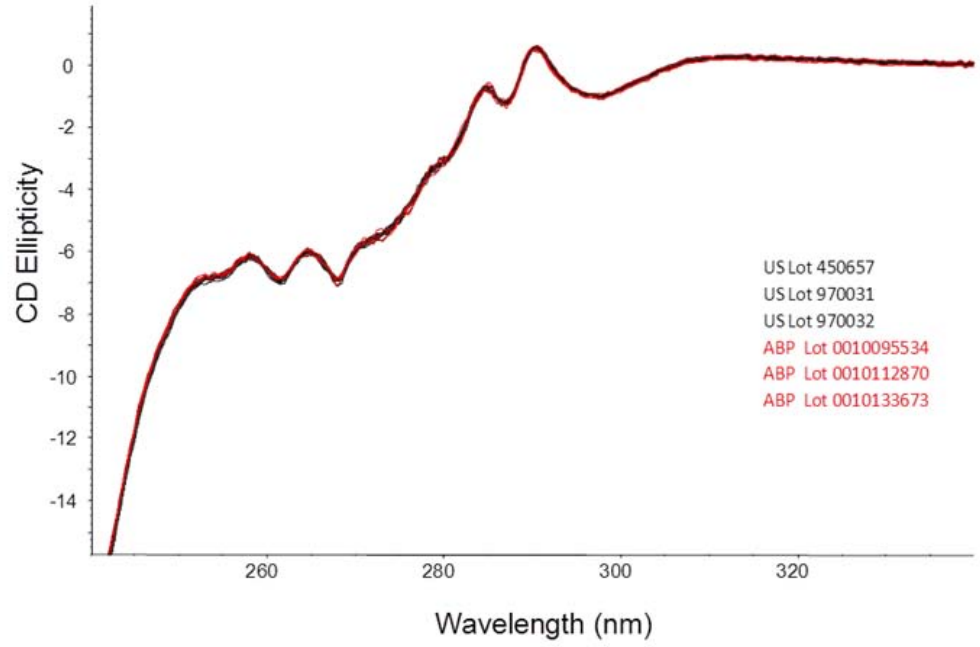
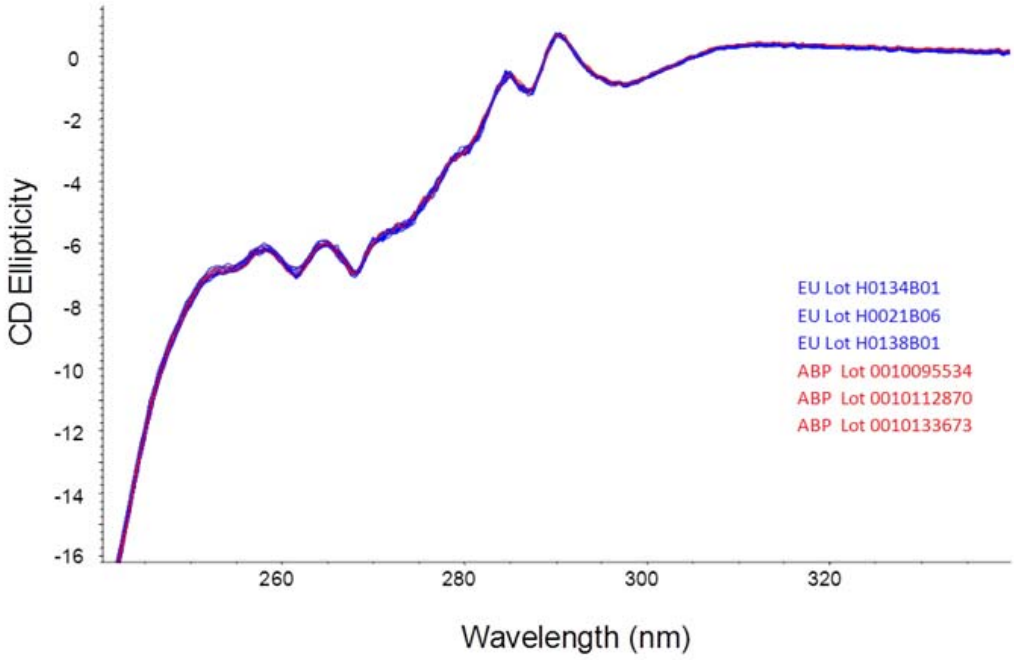


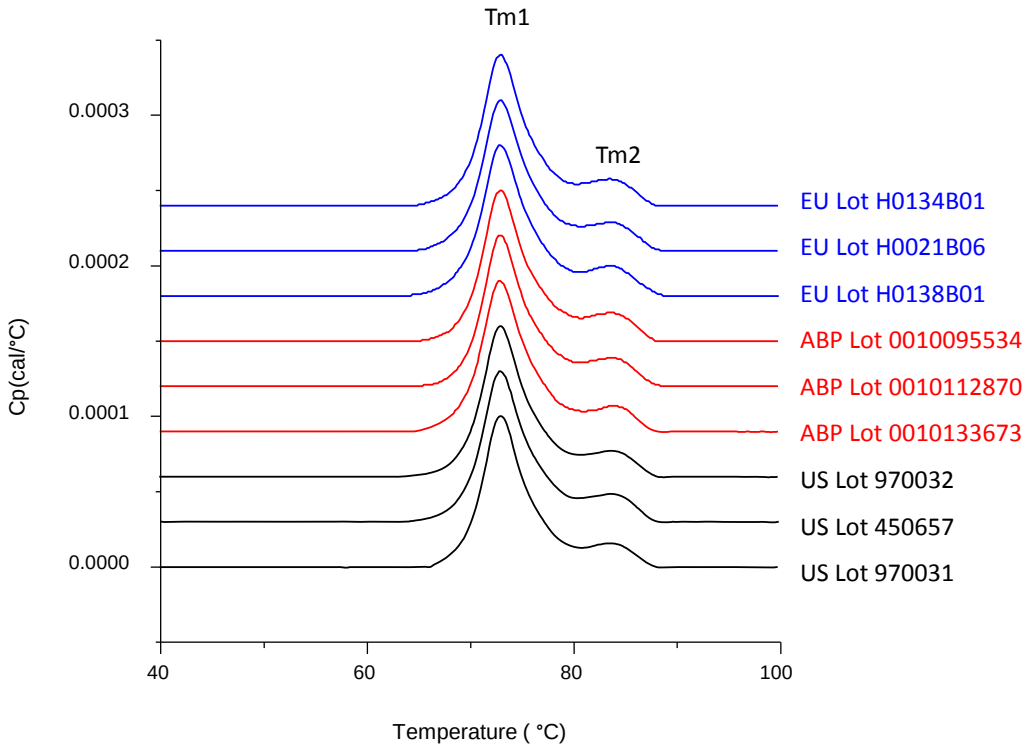
Figure 42. Near UV CD Spectra Comparing ABP 215 and Bevacizumab (EU)



Differential Scanning Calorimetry (DSC)

The thermal stability of the samples was assessed by DSC using a system in which temperature differences between the reference and sample cell are continuously measured and calibrated. The unfolding of the protein molecules appears as an endothermic transition on the DSC thermogram and can be characterized by the thermal melting temperatures (T_m). The protein concentrations used in the DSC experiments were approximately 0.5 mg/mL and obtained by diluting the original samples in formulation buffer. Overlays of the DSC scans for ABP 215, bevacizumab (US), and bevacizumab (EU) are shown in Figure 43.

Figure 43. DSC Scans of Bevacizumab (EU), ABP 215, and Bevacizumab (US)



9.3 Particles and Aggregates

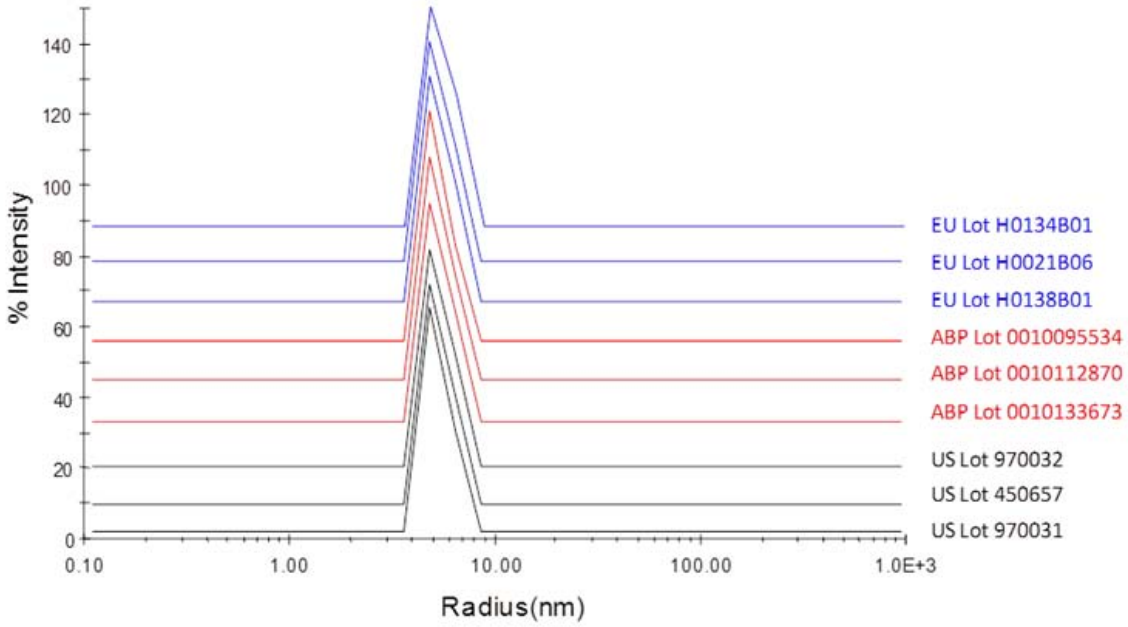
Microflow Imaging (MFI)

Particle characterization by MFI was performed with a particle imaging system containing a flow cell and a digital camera. The system characterizes particles through liquid sampling, image acquisition, and image analysis. Cumulative particle counts per mL for ≥ 5 μm particles were reported. To quantify product-related particles that are likely proteinaceous and thus have a higher risk for immunogenicity, the MFI data were further analyzed for the concentration of ≥ 5 μm non-spherical particles with an aspect ratio of < 0.85.

Dynamic Light Scattering (DLS)

Particle size distributions of ABP 215 and bevacizumab were determined on a DLS instrument at 25°C. The samples were measured after dilution in the drug product formulation buffer to approximately 1 mg/mL and the viscosity based on buffer composition was used for calculating the size distribution. The size distribution profiles of ABP 215, bevacizumab (US), and bevacizumab (EU) samples up to 1 μm are shown in Figure 44.

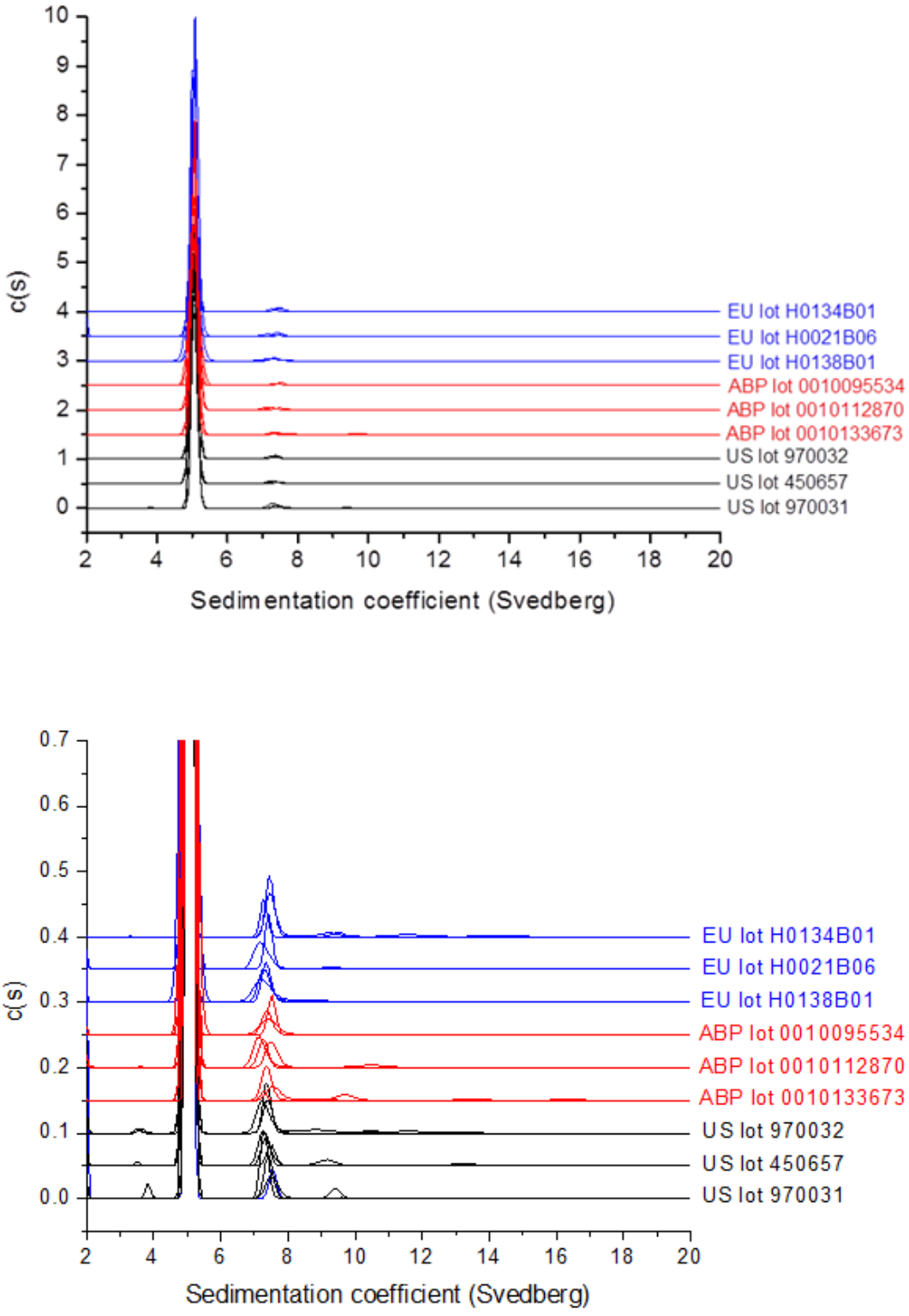
Figure 44. Dynamic Light Scattering Size Distribution Profiles for Bevacizumab (EU), ABP 215, and Bevacizumab (US)



Analytical Ultracentrifugation Sedimentation Velocity (AUC-SV)

ABP 215 and bevacizumab samples were diluted to approximately 0.5 mg/mL in buffer before measurements by AUC-SV. The sedimentation velocity experiments were performed at 45 000rpm, followed by absorbance measurement at 280 nm. The high-resolution sedimentation coefficient distribution of ABP 215 compared to bevacizumab (US) and bevacizumab (EU), as a function of the sedimentation coefficient, is shown in Figure 45.

Figure 45. AUC-SV Profiles for Bevacizumab (EU), ABP 215, and Bevacizumab (US)



Note: upper figure - full scale, lower figure - 10x magnified.

Size Exclusion High Performance Liquid Chromatography with Light Scattering Detection (SE-HPLC-LS)

The SE-HPLC-LS method is capable of detecting high molecular weight (HMW) species (multimer and aggregate). Results were expressed as the molar mass of individual peaks from monomer through HMW species. The SE-HPLC-LS method was coupled with a static light scattering (SLS) detector, a refractive index (RI) detector, and a UV detector with the wavelength set at 280 nm. For the molar mass calculation, a differential RI increment (dn/dc) value of 0.185 (mL/g) was used. The chromatograms comparing ABP 215 with bevacizumab (US) are presented in Figure 46. The chromatograms comparing ABP 215 with bevacizumab (EU) are presented in Figure 47.

Figure 46. SE-HPLC-LS - ABP 215 and Bevacizumab (US)

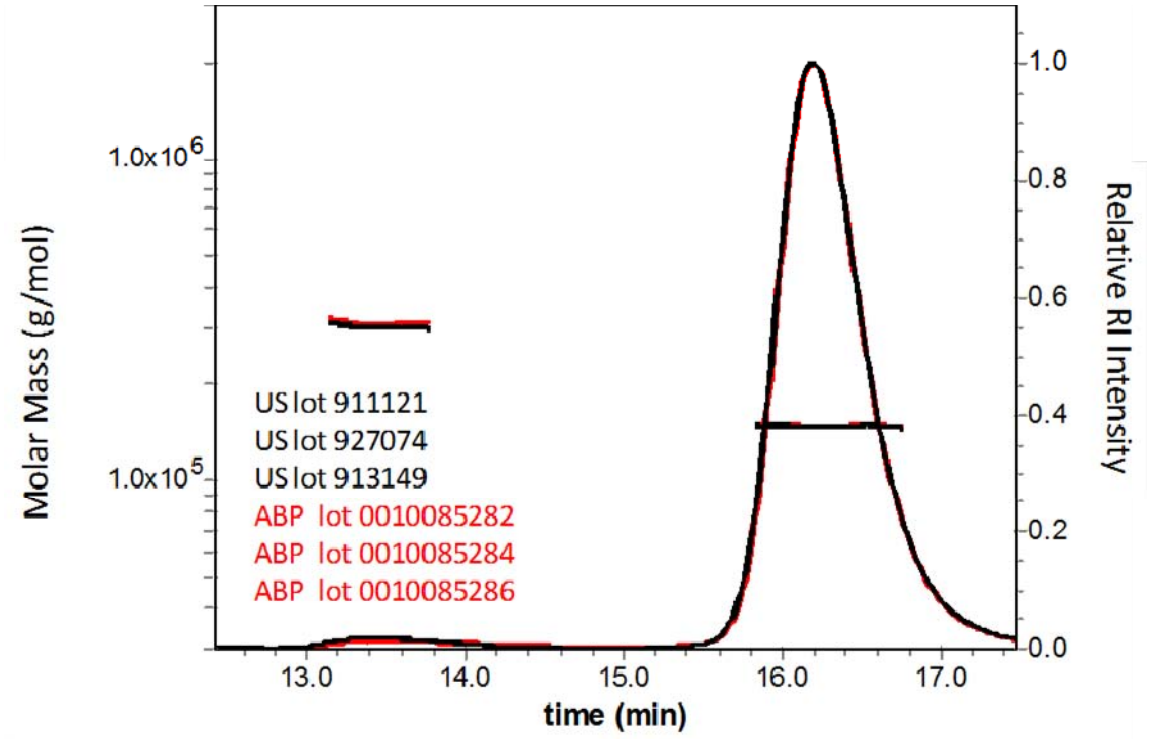
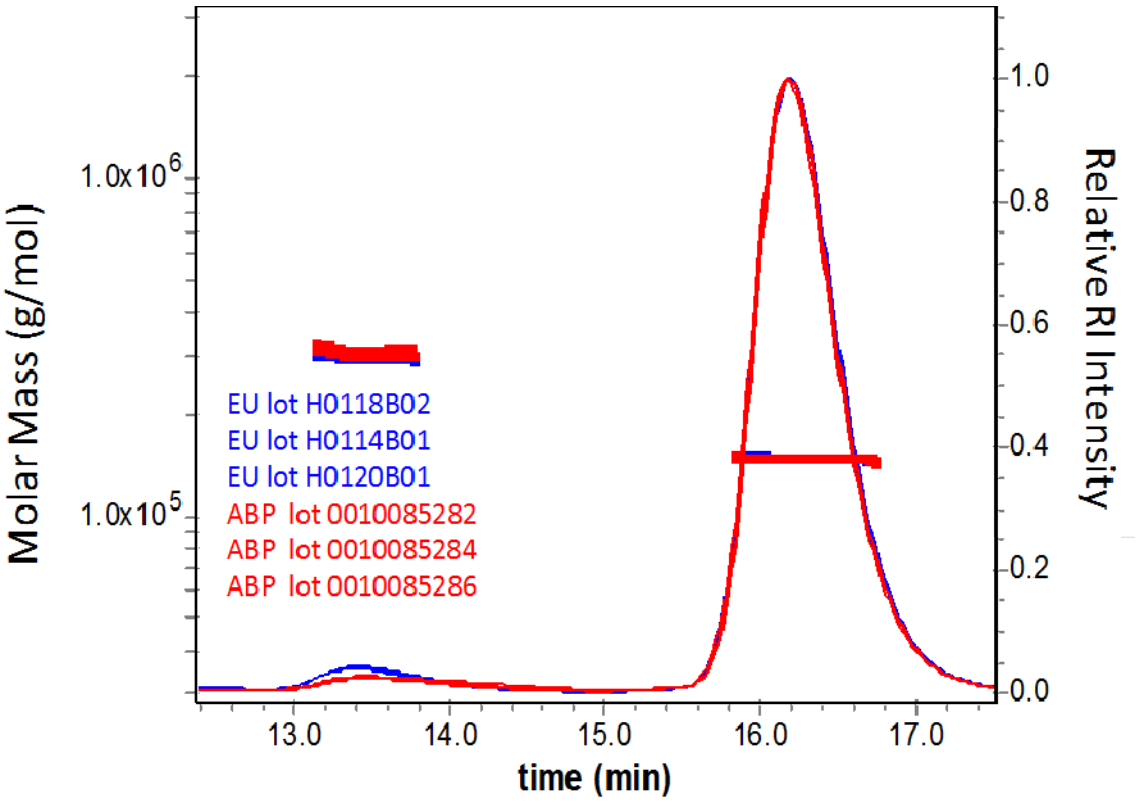


Figure 47. SE-HPLC-LS - ABP 215 and Bevacizumab (EU)



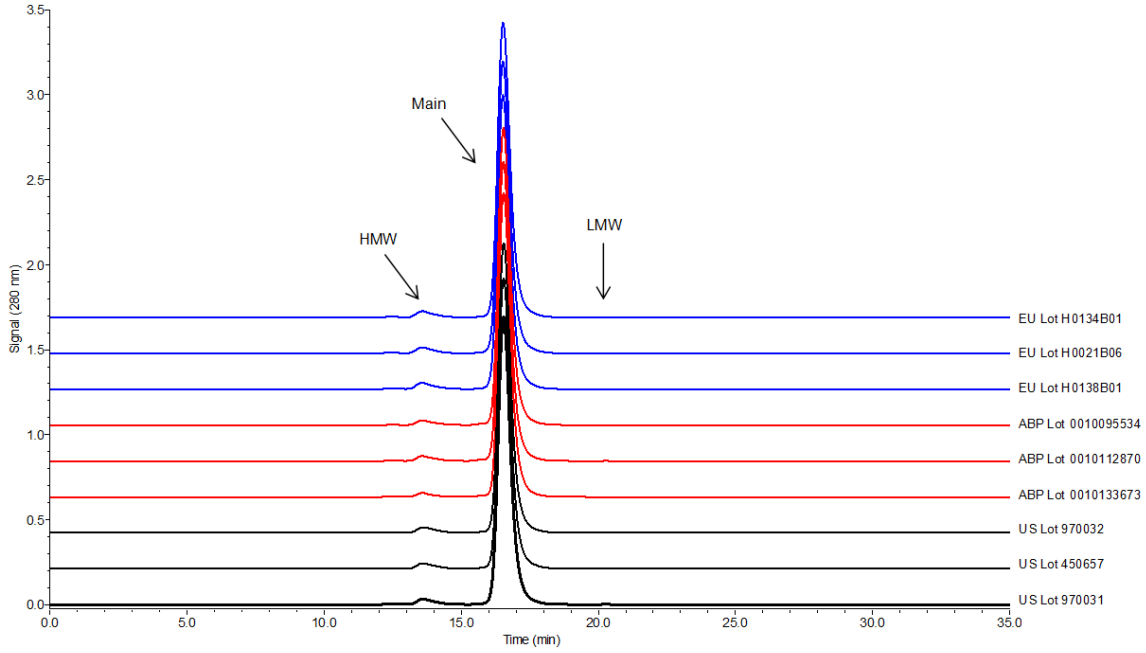
9.4 Product-related Substances and Impurities

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

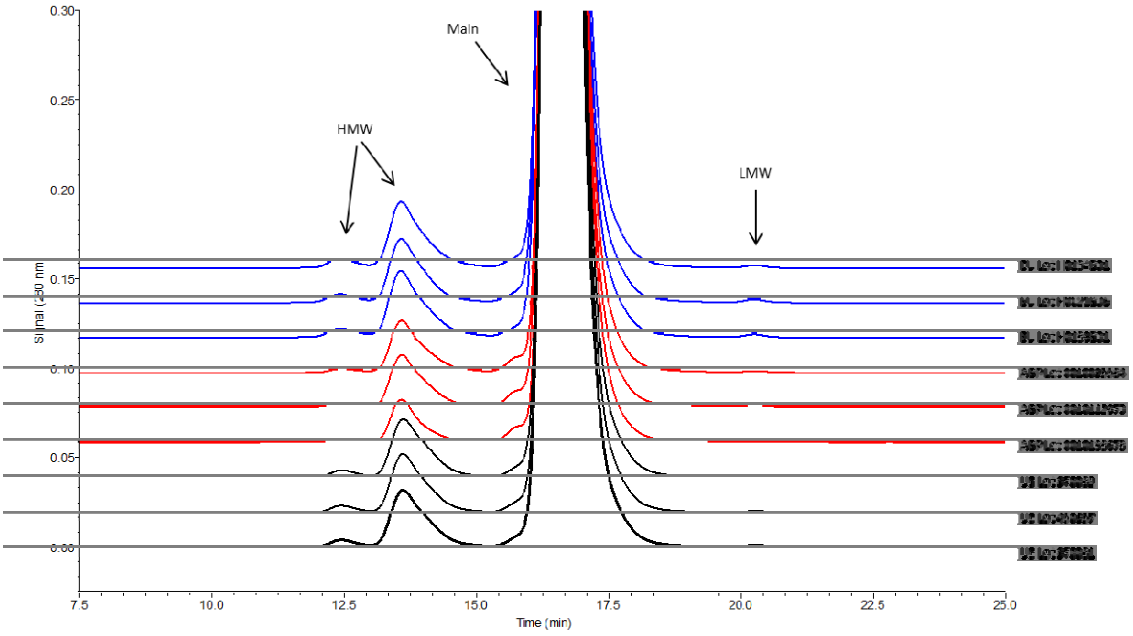
Samples were separated on an SE-HPLC column into HMW species, monomer (main peak), and low molecular weight (LMW) species based on their hydrodynamic size. A comparison of the SE-HPLC profiles for ABP 215, bevacizumab (US), and bevacizumab (EU) lots is provided in Figure 48.

Figure 48. SE-HPLC Profiles for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

Full View



Expanded View



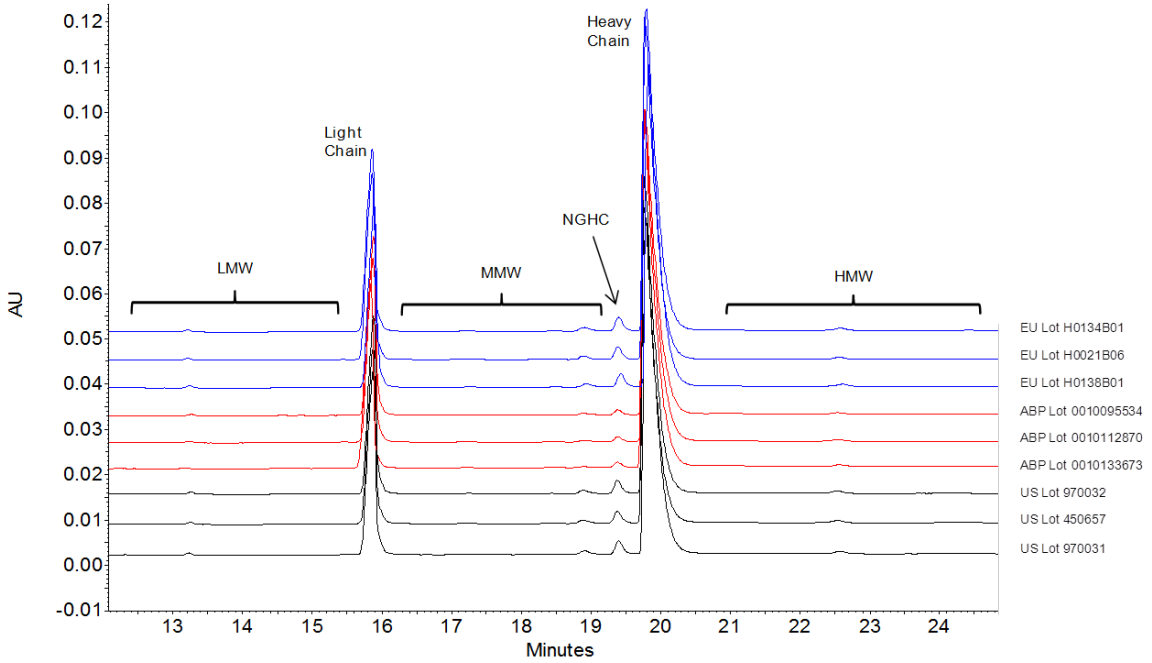
HMW = high molecular weight; LMW = low molecular weight.

Reduced Capillary Electrophoresis - Sodium Dodecyl Sulfate (rCE-SDS)

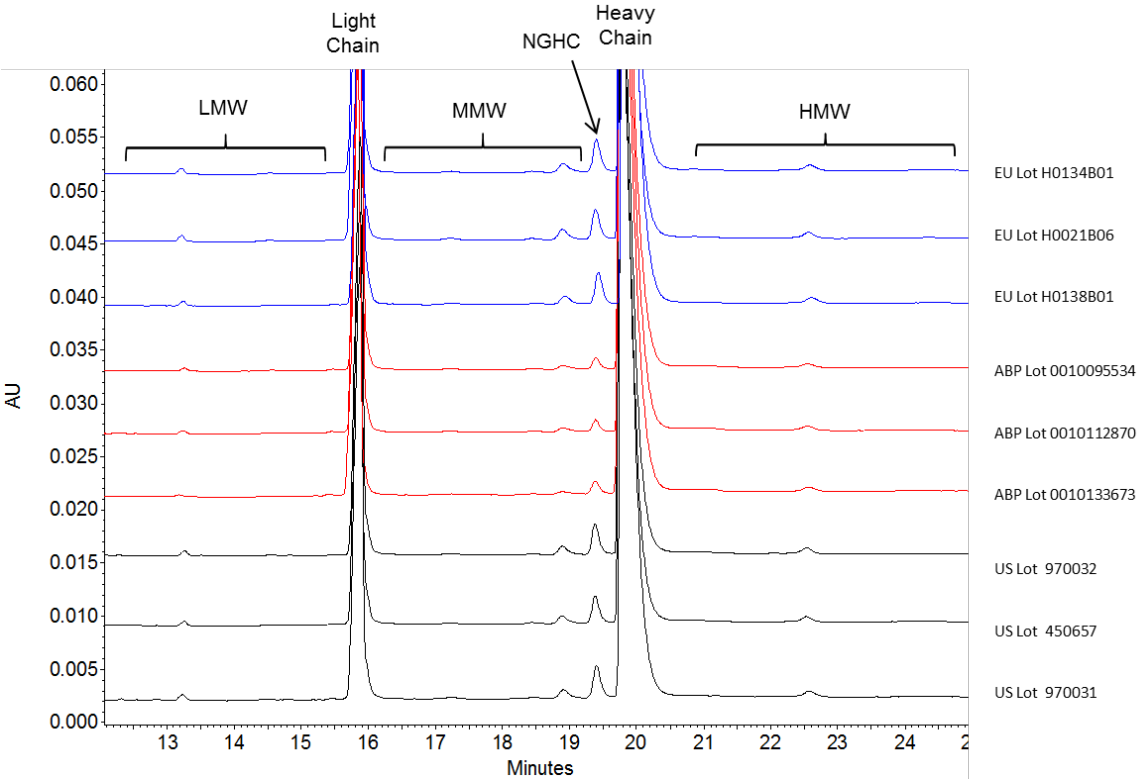
The reduced denatured proteins were separated based on hydrodynamic size where smaller size proteins migrate faster and larger size protein migrate slower. ABP 215 and bevacizumab were reduced using β–mercaptoethanol and denatured with SDS for the analysis. The procedure was suitable for quantifying the levels of fragments (low molecular weight and mid molecular weight), heavy chain and light chain, and non-glycosylated heavy chain. A comparison of the rCE-SDS profiles for ABP 215, bevacizumab (US), and bevacizumab (EU) lots is provided in Figure 49.

Figure 49. Comparison of Bevacizumab (EU), ABP 215, and Bevacizumab (US) rCE-SDS Profiles

Full View



Expanded View



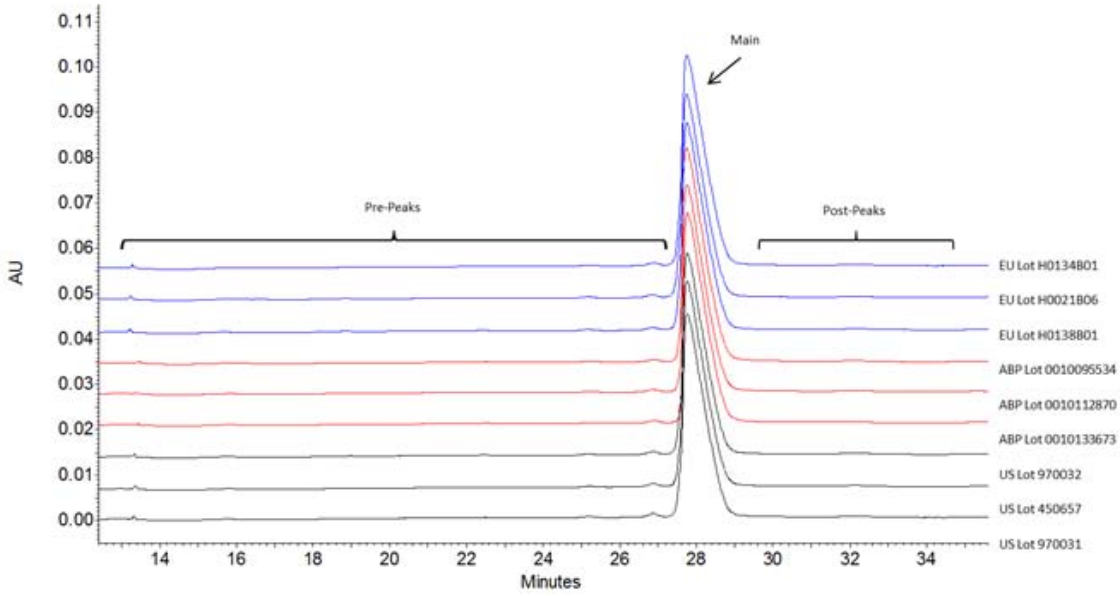
HMW = high molecular weight; LMW = low molecular weight; MMW = mid molecular weight; NGHC = non-glycosylated heavy chain.

Non-reduced Capillary Electrophoresis - Sodium Dodecyl Sulfate (nrCE-SDS)

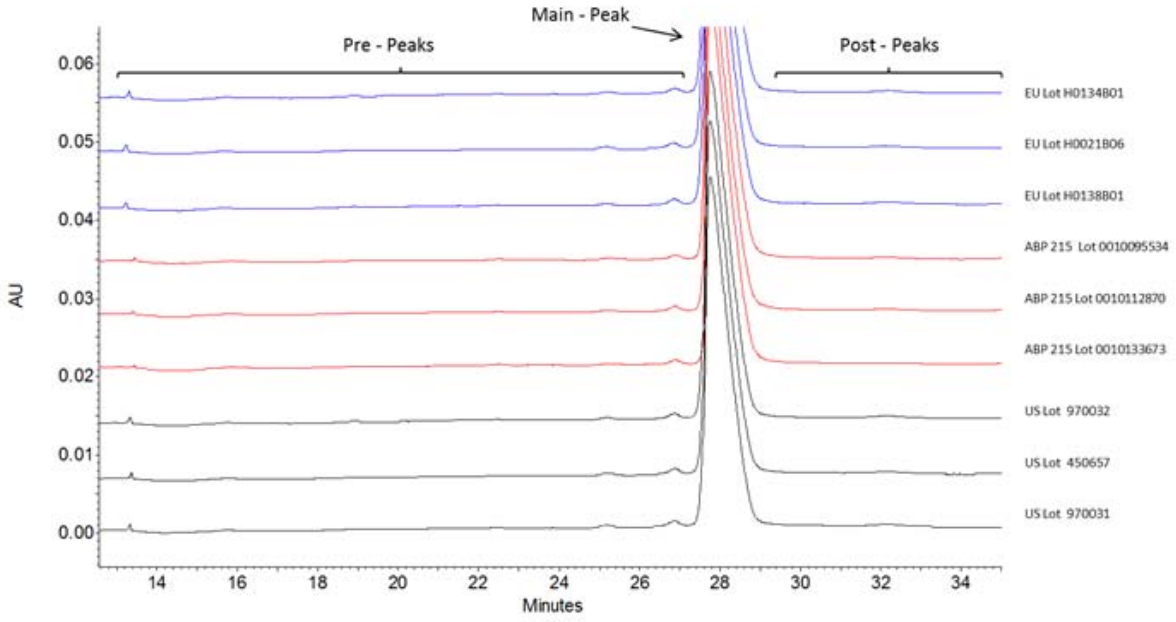
CE-SDS was performed under non-reducing conditions in order to evaluate the presence of non-monomeric species. The analysis is performed under denaturing conditions to unfold the protein and disrupt non-covalent associations, and is particularly useful for detection of partially reduced molecule species (eg, those lacking 1 or more of the 2 light chain and 2 heavy chain constituents expected of a monomeric antibody). A comparison of the nrCE-SDS profiles for ABP 215, bevacizumab (US), and bevacizumab (EU) lots is provided in Figure 50.

Figure 50. Comparison of Bevacizumab (EU), ABP 215, and Bevacizumab (US) nrCE-SDS Profiles

Full View



Expanded View

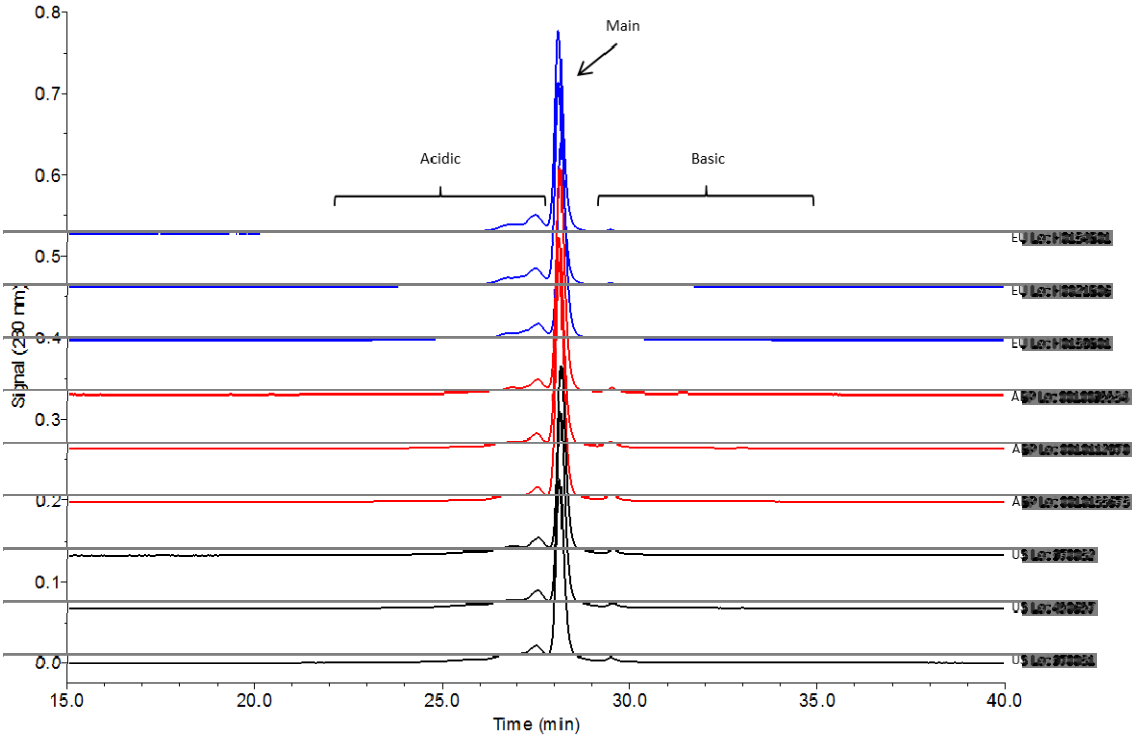


Cation Exchange - High Performance Liquid Chromatography (CEX-HPLC)

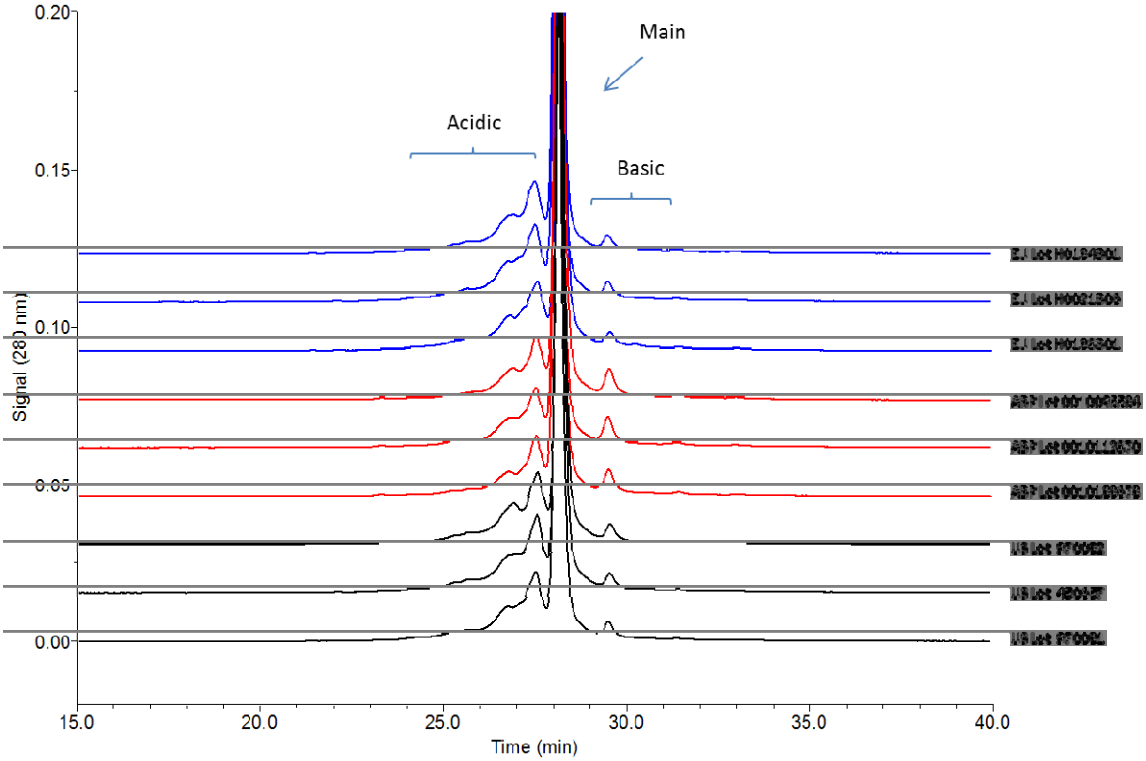
The CEX-HPLC method separated ABP 215 chromatographically from minor product-related charged variants (isoforms) that were eluted using a salt gradient. The CEX-HPLC method was developed to provide accurate quantitation and resolution of main product peak from acidic and basic species. A comparison of the CEX-HPLC profiles for ABP 215, bevacizumab (US), and bevacizumab (EU) lots is provided in Figure 51.

Figure 51. CEX-HPLC Profiles for Bevacizumab (EU), ABP 215, and Bevacizumab (US)

Full View



Expanded View



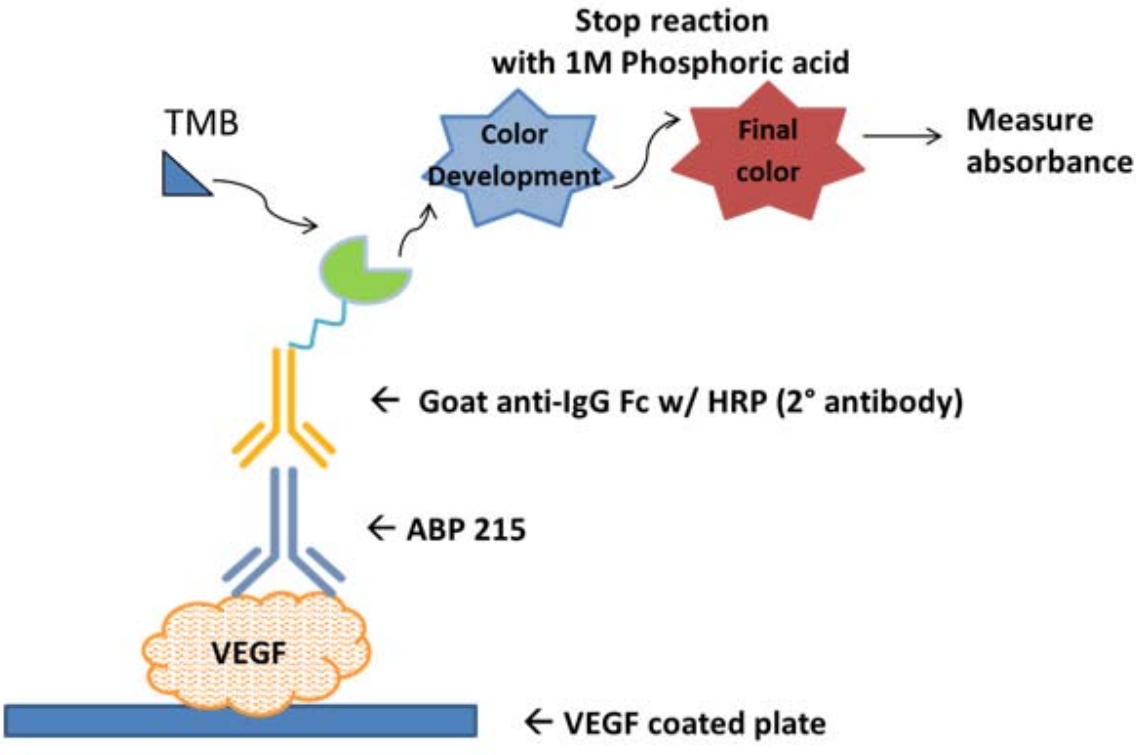
10. FUNCTIONAL ASSAY METHOD DESCRIPTIONS

Binding to VEGF

A solid phase ELISA was used to determine the binding of ABP 215 and bevacizumab to recombinant human VEGF.

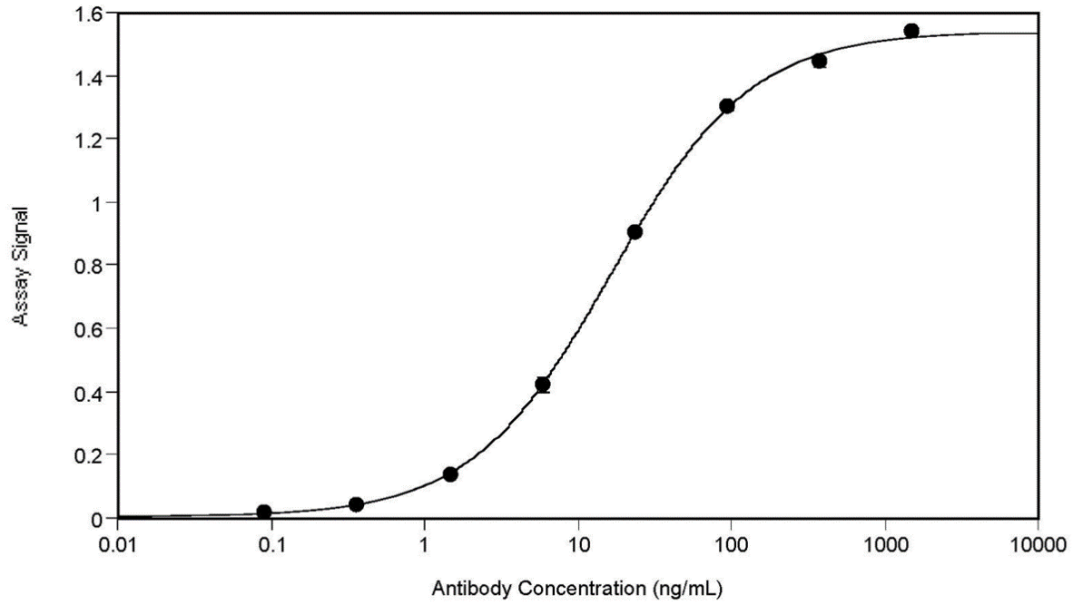
The principle of the ABP 215 VEGF binding assay is shown in Figure 52. Recombinant VEGF is coated onto the wells of microtiter ELISA plates. Serial dilutions of reference standard, control, and test sample(s) are added and incubated. Following a wash step, a goat anti-human IgG (Fc fragment) conjugated to horseradish peroxidase (HRP) is added to detect bound samples. After a final wash, a substrate/chromogen solution is added to the wells. The substrate changes color in the presence of HRP in proportion to the amount of ABP 215 or bevacizumab bound to VEGF. The reaction is stopped with 1.0 M phosphoric acid and absorbance is measured with a microplate reader. The relative binding of test samples is determined by comparing the test sample response to that of the ABP 215 reference standard response. Results are reportable by meeting assay acceptance criteria and the sample acceptance criteria for parallelism between test samples and the reference standard curve. A representative dose-response curve is shown in Figure 53.

Figure 52. Schematic for VEGF Binding Assay



Fc = fragment crystallizable; HRP = horseradish peroxidase; IgG = immunoglobulin G; TMB = 3,3',5,5'-tetramethylbenzidine; VEGF = vascular endothelial growth factor.

Figure 53. Representative Dose-response Curve for VEGF Binding



Assay Signal = optical density units.
Each point is a mean of 3 replicates ± standard deviation.

Neutralization of VEGF-mediated Proliferation in HUVEC

The principle of the ABP 215 proliferation inhibition bioassay is shown in Figure 54. In the assay, HUVEC are incubated with varying concentrations of ABP 215 reference standard, control, and test samples in the presence of a constant concentration of VEGF. After incubation, Cell-Titer Glo® Luminescent Cell Viability Assay Reagent is added to the assay plates. The addition of Cell-Titer Glo results in cell lysis and generation of a luminescence signal that is proportional to the amount of adenosine triphosphate (ATP) present. The quantity of ATP present is directly proportional to the number of viable cells in the culture and inversely proportional to the concentration of ABP 215 or bevacizumab present. The relative potency of test samples is determined by comparing the test sample response to that of the ABP 215 reference standard response. Results are reportable by meeting assay acceptance criteria and the sample acceptance criteria for parallelism between test samples and the reference standard curve. A representative dose-response curve of the ABP 215 proliferation inhibition bioassay is shown in Figure 55.

Figure 54. Principle of ABP 215 Proliferation Inhibition Bioassay

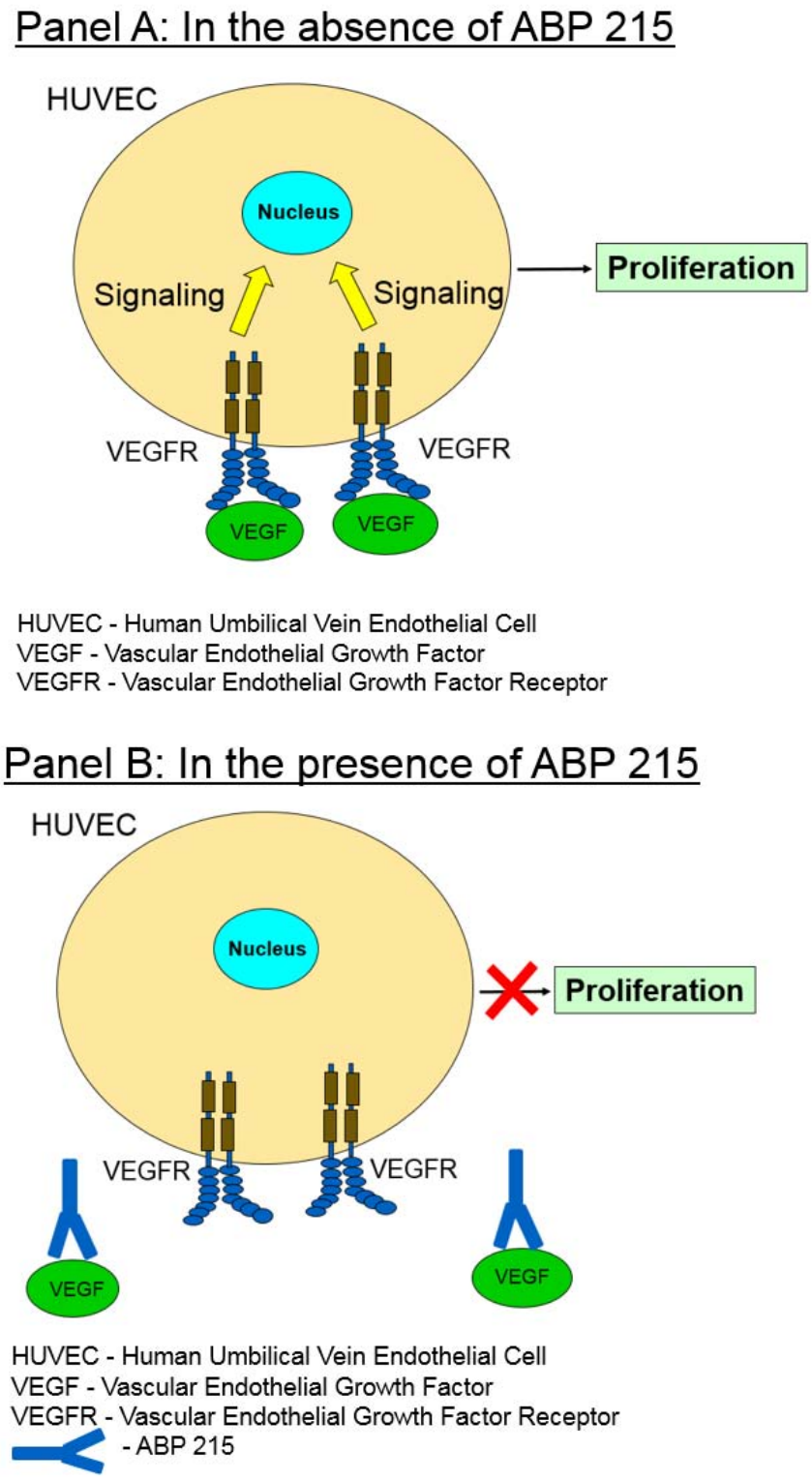
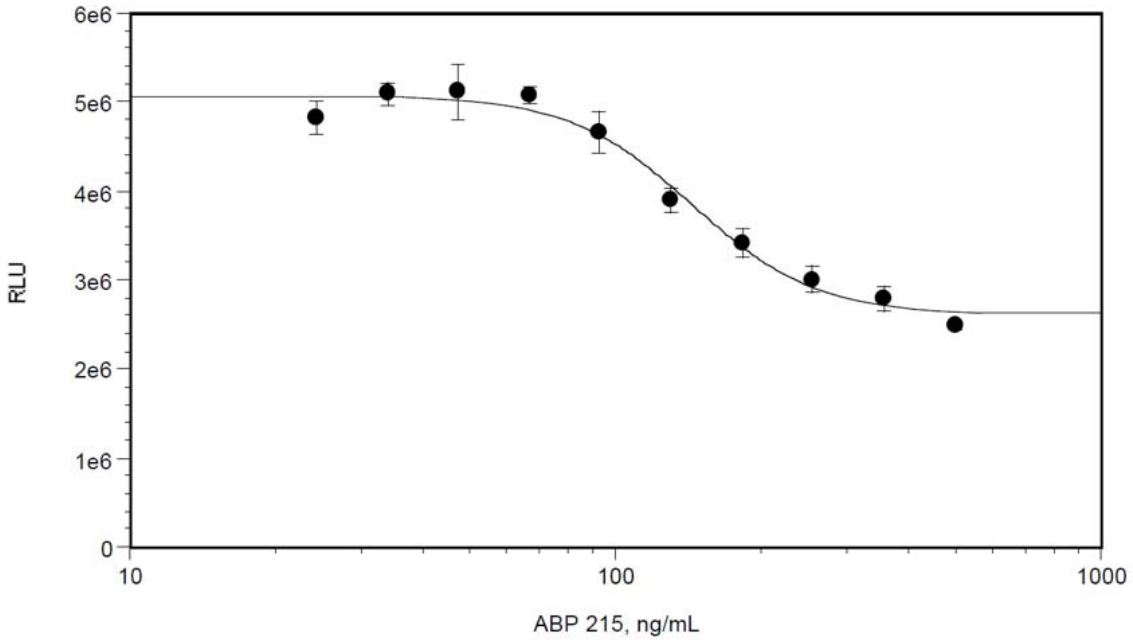


Figure 55. Representative Dose-response Curve for the Inhibition of Proliferation Assay



RLU = relative luminescence unit.
Each point is a mean of 3 replicates ± standard deviation.

Kinetic Binding (VEGF)

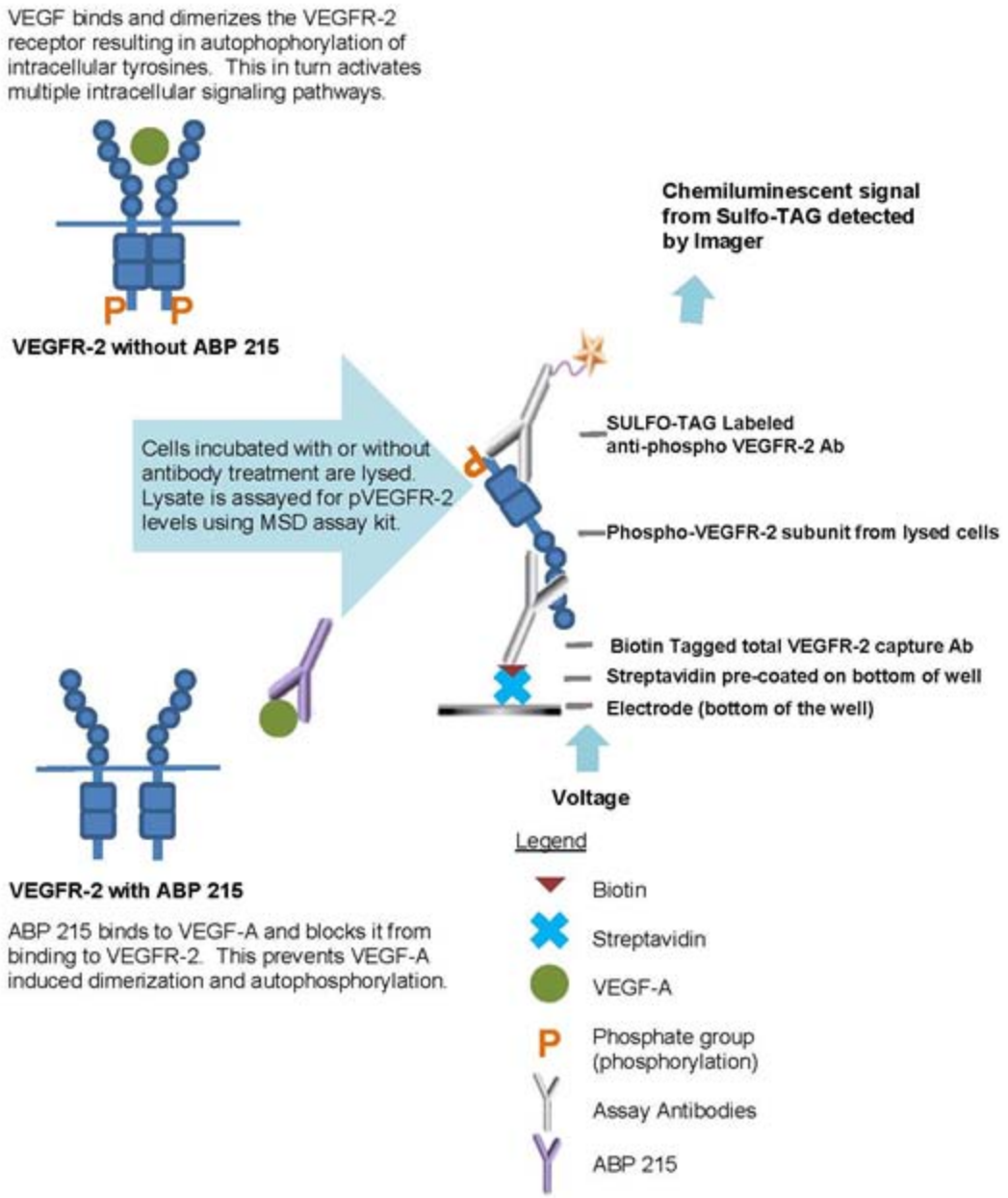
The SPR analysis was conducted at 25°C using a ProteOn XPR36 optical biosensor equipped with a general layer compact (GLC) sensor chip. ABP 215 and bevacizumab were captured on the GLC chip surface by a goat anti-human IgG capture antibody. Recombinant human VEGF was injected at concentrations ranging from 50.0 to 3.13 nM and analyzed in triplicate. Results are reported as the average of 3 intra-assay replicates per lot.

The association and dissociation phases for all VEGF concentrations were monitored for 240 seconds each. Additionally, a long dissociation phase experiment of 5400 seconds was performed using the 50.0 nM VEGF concentration in order to better assess the slow dissociation rate of the antibodies. The binding kinetics were fit using a 1:1 binding model.

Inhibition of VEGFR-2 RTK Autophosphorylation

HUVEC are incubated with varying concentrations of ABP 215 and bevacizumab in the presence of a constant concentration of VEGF. After a timed incubation, the cells are lysed. VEGFR-2 is captured from the lysate onto streptavidin-coated Mesoscale Discovery plates using a biotinylated antibody against the extracellular portion of VEGFR-2. This is followed by the addition of a murine anti-phosphotyrosine monoclonal antibody for detection of tyrosine phosphorylation on the captured VEGFR-2 and an anti-murine IgG conjugated with ruthenium for signal generation. The addition of a tripropylamine-containing buffer followed by electrical activation of the plate results in an ECL signal detected by a plate reader. The ECL signal counts are proportional to the level of VEGFR-2 tyrosine phosphorylation. A schematic of the method is shown in Figure 56.

Figure 56. Method Schematic of the VEGFR-2 RTK Autophosphorylation Assay



Binding to FcRn

A competitive binding assay has been developed to assess the binding of ABP 215 and bevacizumab to FcRn. The principle of the ABP 215 FcRn binding assay is shown in Figure 57. The assay is a bead-based amplified luminescent proximity homogeneous assay (AlphaScreen) that detects bimolecular interactions. The assay contains 2 bead types: an acceptor bead and a donor bead. The acceptor beads are coated with a hydrogel that contains a nickel chelate which binds to the histidine domain of histidine labeled FcRn (FcRn-His). The donor beads are coated with streptavidin, which binds to biotinylated CHO derived human Fc. When FcRn-His and the biotinylated human Fc bind together, they bring the acceptor and donor beads into close proximity. When laser light is applied to this complex, ambient oxygen is converted to singlet oxygen by the

donor bead and an energy transfer to the acceptor bead occurs, resulting in light emission, which is measured in a plate reader equipped for AlphaScreen signal detection. When ABP 215 is present at sufficient concentrations to inhibit the binding of FcRn-His to the biotinylated human Fc domain, a dose-dependent decrease in emission is observed. The relative binding of test samples is determined by comparing the test sample response to that of the ABP 215 reference standard response. Results are reportable by meeting assay acceptance criteria and the sample acceptance criteria for parallelism between test samples and the reference standard curve. A representative dose-response curve for ABP 215 is provided in Figure 58.

Figure 57. Schematic for the FcRn Binding Assay

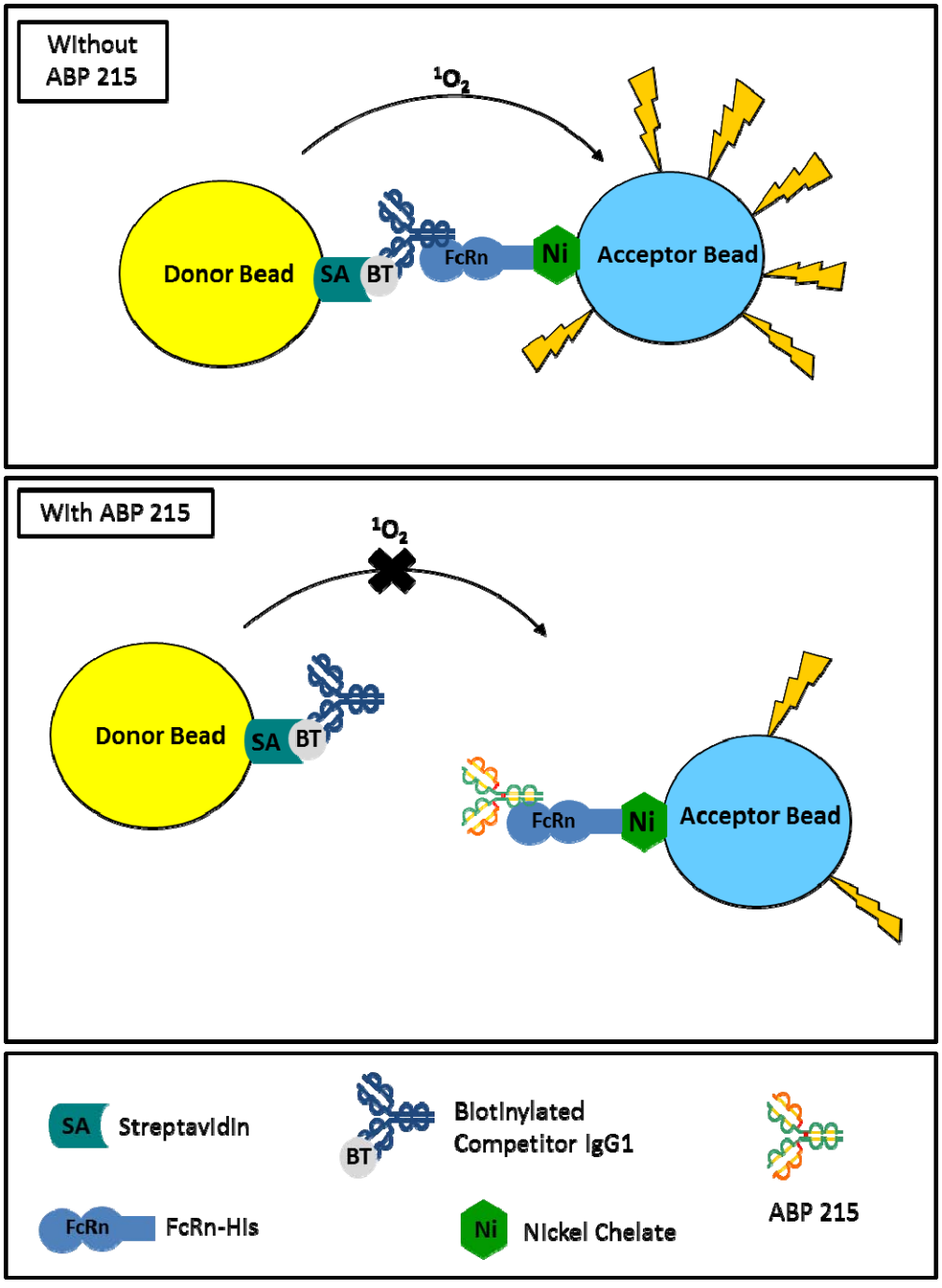
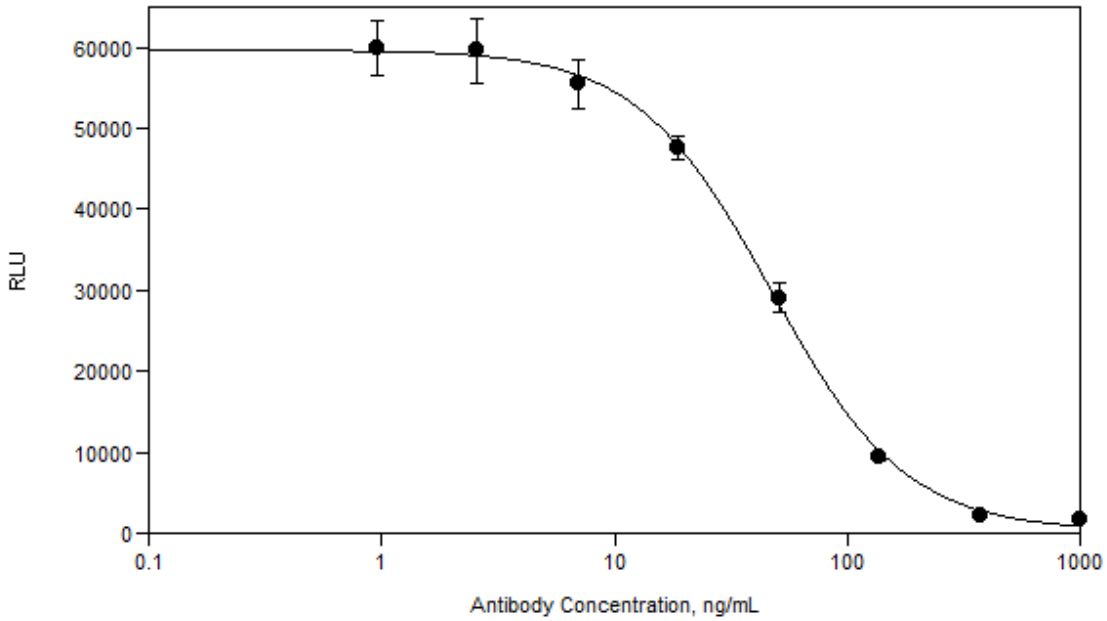


Figure 58. Representative Dose-response Curve for FcRn Binding



RLU = relative luminescence unit.
Each point is a mean of 3 replicates ± standard deviation.

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

FcγRIIIa is an activating Fc receptor expressed on natural killer cells which is involved in the induction of ADCC. There are at least 2 allelic variants in humans expressing either valine or phenylalanine at position 158 (158V and 158F, respectively). The 158V variant displays higher affinity binding to IgG1 antibodies as compared to the 158F variant.

A competitive binding assay was developed in order to assess the binding of ABP 215 to FcγRIIIa. The assay has been developed in 2 forms to assess both the 158V and 158F alleles for FcγRIIIa. The FcγRIIIa receptor binding assay is a bead-based amplified luminescent proximity homogeneous assay that detects bimolecular interactions.

A competitive AlphaLISA® binding assay was developed to assess the binding of ABP 215 and bevacizumab to FcγRIIIa (158V), using a biotinylated human IgG1 as a competitor to the test article. The method for FcγRIIIa (158F) is the same as described for FcγRIIIa (158V) except that the beads use FcγRIIIa (158F)-GST. The AlphaLISA assay contains 2 bead types, an acceptor bead and a donor bead. When FcγRIIIa-glutathione-S transferase (GST) and the biotinylated human IgG1 bind together, they bring the acceptor and donor beads into close proximity. When laser light is applied and the 2 beads are in proximity to one another, light production (luminescence) occurs, which is measured in a plate reader. When ABP 215 or bevacizumab are present at sufficient concentrations to compete for the binding of FcγRIIIa-GST to the biotinylated human IgG1, a dose-dependent decrease in luminescence is observed. A schematic of the FcγRIIIa (158V) binding assay is shown in Figure 59. A representative dose-response curves for ABP 215 and bevacizumab is shown in Figure 60.

Figure 59. Method Schematic for the FcγRIIIa Binding Assays

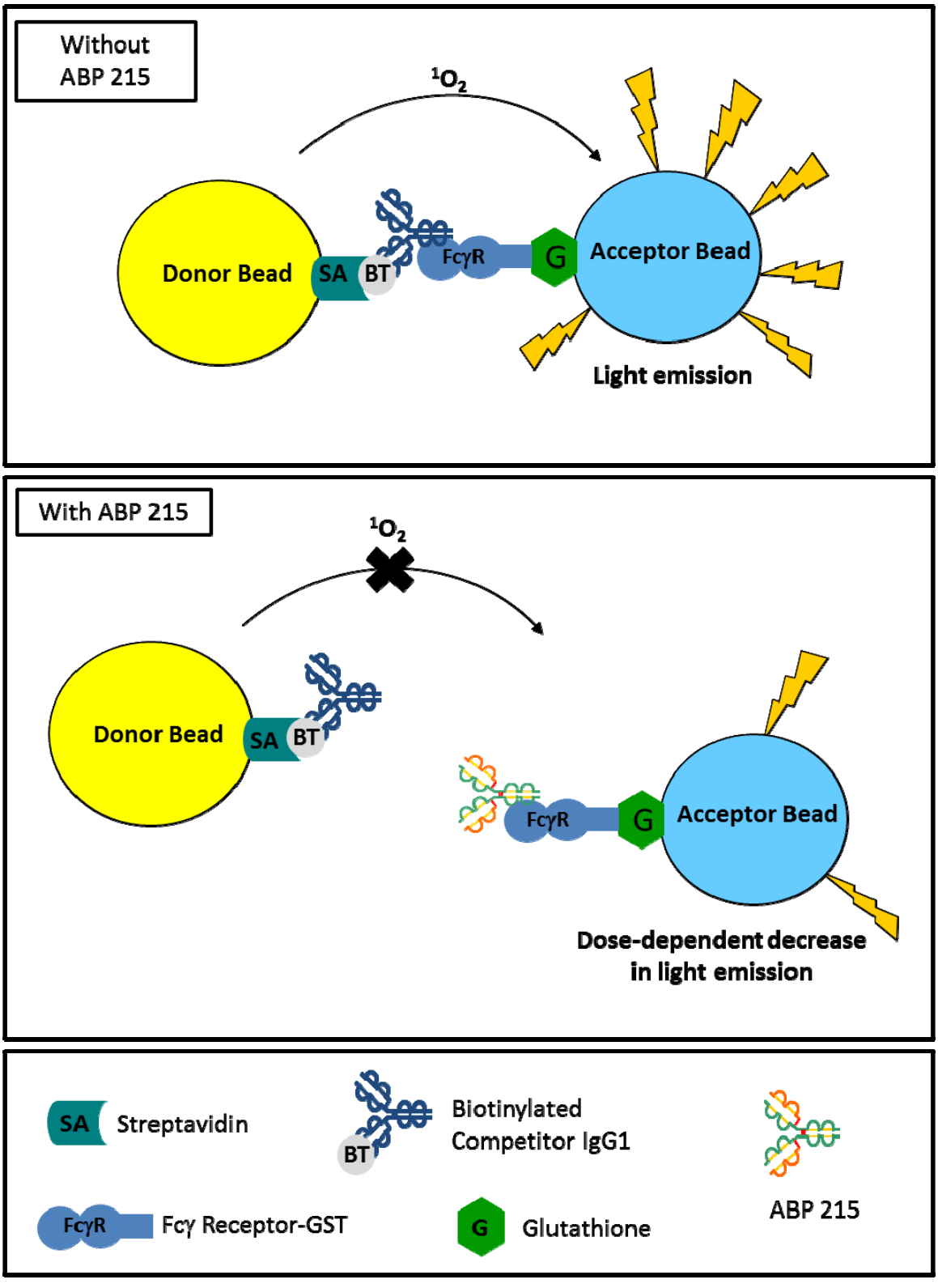
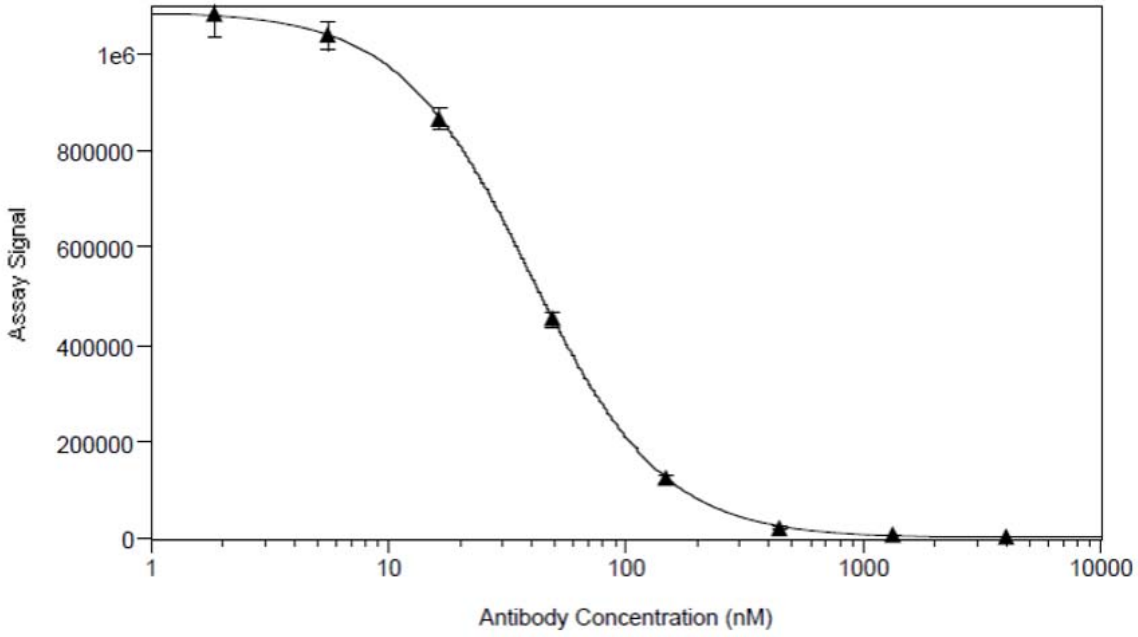


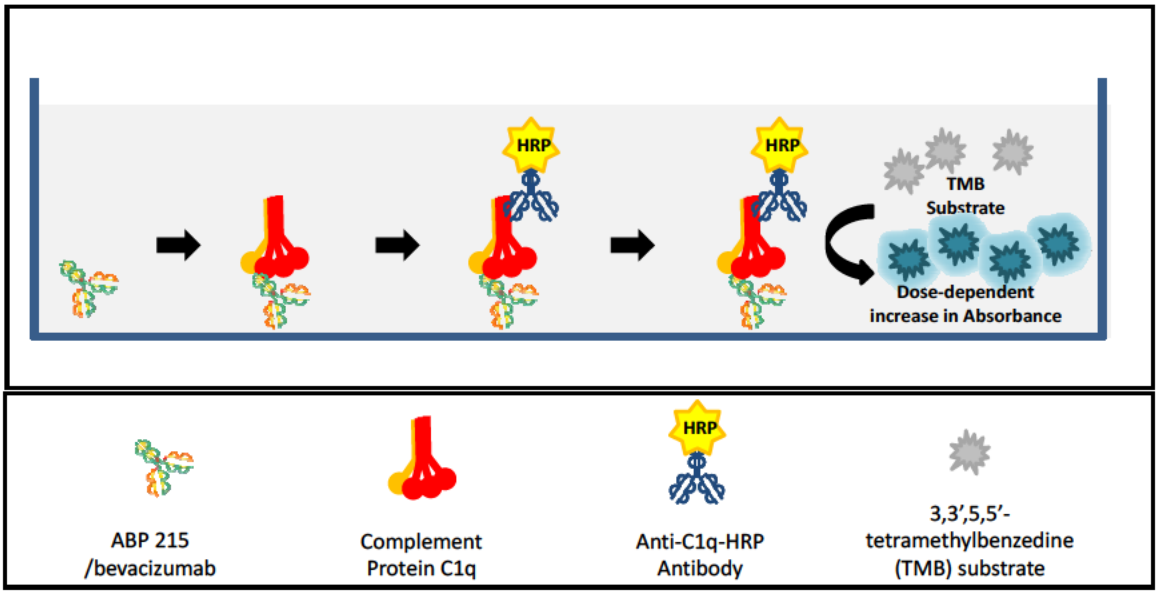
Figure 60. Representative Dose-response Curve for FcγRIIIa (158V) Binding



Binding to C1q

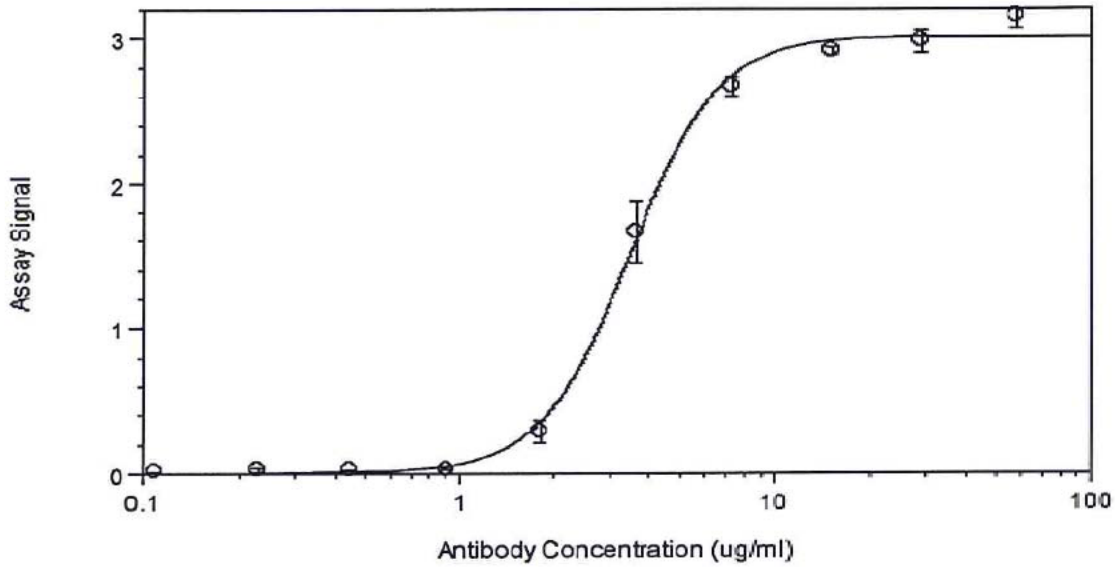
A direct binding ELISA method was developed to assess the binding of ABP 215 and bevacizumab to C1q. In this assay, bevacizumab or ABP 215 is adsorbed to a microtiter plate and incubated with C1q. Bound C1q is detected with an anti-C1q-HRP conjugated antibody. HRP activity is then detected using 3,3′,5,5′-Tetramethylbenzidine (TMB), a colorimetric substrate of the HRP enzyme, which generates a soluble blue product. The reaction is stopped by the addition of sulfuric acid, and the amount of colorimetric product is quantified by absorbance. Absorbance data from each well are recorded and analyzed. Data are plotted as dose-response curves and fit to a 4-parameter logistic model. The results, reported as percent relative binding (% relative binding), are determined by comparing test sample curves to the reference standard curve.
A schematic of the method is shown in Figure 61. A representative dose response curve for ABP 215 is provided in Figure 62.

Figure 61. Method Schematic for C1q Binding



Blue line represents the surface of a microtiter plate.

Figure 62. Representative ABP 215 Dose-Response Curve for C1q Binding



Assay Signal = optical density units.
Each point is a mean of 4 replicates ± standard deviation.