

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761086Orig1s000

PRODUCT QUALITY REVIEW(S)



US Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics
Division of Biometrics VI

STATISTICAL REVIEW AND EVALUATION

BLA NO.	761086
DATE RECEIVED BY THE CENTER	12/14/2018
DRUG NAME	ABP 710
DOSAGE FORM	A sterile, preservative-free, white to slightly yellow, lyophilized powder in a single-use vial for intravenous infusion
INDICATION	The same as Remicade (infliximab)
SPONSOR	Amgen
REVIEW FINISHED	5/6/2019
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SECONDARY REVIEWER	Meiyu Shen, Ph.D.
PROJECT MANAGER	Christine Ford

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1 Executive summary and recommendation

In this evaluation I reviewed the data and analyzed the similarity of two Tier 1 quality attributes, Relative Potency and sTNF α Binding by ELISA for ABP 710 compared with US-licensed Remicade, submitted by Amgen. There are no significant issues with the data quality.

My independent analysis showed that both attributes pass the statistical equivalence test and support a demonstration that ABP 710 is highly similar to US-licensed Remicade.

2 Introduction

On 12/14/2018, Amgen submitted to the U.S. Food and Drug Administration (FDA) a 351(k) BLA which included an analytical similarity assessment, comparing two Tier 1 quality attributes for ABP 710 with US-licensed Remicade. Below, I reviewed the data quality and evaluated the similarity of the two attributes.

3 The FDA Statistical reviewer's analysis

3.1 The equivalence test method

In this review, I used the equivalence test method used by the applicant, which was discussed and agreed during previous meetings between the applicant and the FDA. Below, I briefly discussed the method.

The equivalence test concerns testing the means of two normal distributions for Tier 1 QAs between a proposed biosimilar product (B) and a reference product (R). To test the equivalence in mean, the null and alternative hypotheses are formulated as follows:

$$H_0 : \mu_B - \mu_R \leq -\delta \text{ or } \mu_B - \mu_R \geq \delta,$$

and

$$H_a : -\delta < \mu_B - \mu_R < \delta,$$

where μ_B and μ_R are the mean responses of the proposed biosimilar and reference product lots, respectively, and $\delta > 0$ is the equivalence margin which will be specified later.

A test of the equivalence hypothesis can be conducted by requiring simultaneous rejection of the following two one-sided null hypotheses:

$$H_{10} : \mu_B - \mu_R \leq -\delta \text{ versus } H_{1a} : \mu_B - \mu_R > -\delta,$$

and

$$H_{20} : \mu_B - \mu_R \geq \delta \text{ versus } H_{2a} : \mu_B - \mu_R < \delta.$$

Let $(X_{B,j}, j = 1, \dots, n_B)$ and $(X_{R,j}, j = 1, \dots, n_R)$ be the two samples for the proposed biosimilar and reference product of sample size n_B and n_R respectively. It is assumed that for $I = B, R$, $X_{I,j} \sim_{IID} N(\mu_I, \sigma_I^2)$ are independent and identically distributed (IID) as a normal distribution with mean μ_I and standard deviation $\sigma_I > 0$.

In practice, $X_{I,j}$ represents the lot value, calculated as the sample mean of multiple replicated measurements for each drug product lot j of drug I . While there is no consensus on how many within-lot replicates should be used, it is known that under independent sampling, the more number of replicates are collected for each lot, the less the variability of lot-average will be.

It is also generally required that the same number of within-lot replicates should be obtained for all drug lots for both test and reference drug products, due to the following reasons. Note that in Tier 1 equivalence test for each drug product (DP) lot, the within-DP lot average is used, whose variance is a function of the number of within-DP lot replicates used in computing the average. Assume that $Y_{I,j,k}$ is the measured value for a QA from the k -th replicate from j -th DP lot for drug I , and $Y_{I,j,k} = \mu + r_j + \epsilon_{j,k}$, with $r_j \sim_{IID} N(0, \sigma_{dp}^2)$ and $\epsilon_{j,k} \sim_{IID} N(0, \sigma_\epsilon^2)$ and r_j and $\epsilon_{j,k}$ are mutually independent. Since $X_{I,j} = \frac{1}{K} \sum_{k=1}^K Y_{I,j,k}$, $\text{var}(X_{I,j}) = \sigma_{dp}^2 + \sigma_\epsilon^2/K$. Different number of within-lot replicates can lead to violation of IID assumption for lot value $X_{I,j}, I = B, R, j = 1, \dots, n_I$, used in the equivalence test.

Let $\hat{\mu}_I$ and $\hat{\sigma}_I^2$ be the sample mean and unbiased sample variance estimates respectively for $I = B, R$. The test statistics for the two one sided tests H_1 and H_2 are defined respectively as

$$\tau_1 = \frac{\hat{\mu}_B - \hat{\mu}_R + \delta}{\sqrt{\hat{\sigma}_B^2/n_B^* + \hat{\sigma}_R^2/n_R^*}},$$

and

$$\tau_2 = \frac{\hat{\mu}_B - \hat{\mu}_R - \delta}{\sqrt{\hat{\sigma}_B^2/n_B^* + \hat{\sigma}_R^2/n_R^*}},$$

where $n_B^* = \min\{1.5n_R, n_B\}$ and $n_R^* = \min\{1.5n_B, n_R\}$ are the adjusted sample sizes (Dong, Weng, and Tsong 2017). Then H_{10} is rejected if $\tau_1 > t_{1-\alpha, df^*}$ and H_{20} is rejected if $\tau_2 < t_{\alpha, df^*}$, where t_{α, df^*} is α -th upper quantile of the t-distribution with degree of freedom df^* , which is approximated by the Satterthwaite method with sample size adjusted and given as follows,

$$df^* = \frac{\left(\frac{\hat{\sigma}_B^2}{n_B^*} + \frac{\hat{\sigma}_R^2}{n_R^*}\right)^2}{\frac{1}{n_B-1} \left(\frac{\hat{\sigma}_B^2}{n_B^*}\right)^2 + \frac{1}{n_R-1} \left(\frac{\hat{\sigma}_R^2}{n_R^*}\right)^2}.$$

Equivalently, equivalence is accepted for the quality attribute if the following $100(1 - 2\alpha)\%$ two-sided confidence interval (CI) of the mean difference is within $(-\delta, \delta)$,

$$\left(\hat{\mu}_B - \hat{\mu}_R - t_{1-\alpha, df^*} \sqrt{\hat{\sigma}_B^2/n_B^* + \hat{\sigma}_R^2/n_R^*}, \hat{\mu}_B - \hat{\mu}_R + t_{1-\alpha, df^*} \sqrt{\hat{\sigma}_B^2/n_B^* + \hat{\sigma}_R^2/n_R^*} \right).$$

The equivalence margin is set as $\delta = c\sigma_R$, where c is the margin multiplier and is recommended to be 1.5 (Tsong, Dong, and Shen 2017). In this case, the test would yield a positive result if the 90% confidence interval about the difference in sample means lies within $(-1.5\sigma_R, 1.5\sigma_R)$. For known σ_R , if there were 10 biosimilar and 10 reference product lots, this test would have adequate power (at least 85%) to reject the null hypothesis in favor of equivalence when the true underlying mean difference between the proposed biosimilar and reference product lots is equal to $\sigma_R/8$, assuming a test with $\alpha = 0.05$. If the true difference between products is less than $\sigma_R/8$, the power will increase. In practice, σ_R in the proposed margin is unknown and estimated by the sample standard deviation of reference product lots estimated from the reference product lots available to the biosimilar applicant.

In the following analysis, the nominal size is set as $\alpha = 0.05$.

3.2 Data quality

3.2.1 ABP 710 Lots

The applicant included all ABP 710 drug product lots that were used in clinical studies in the similarity assessment. The applicant included the lots for the similarity assessment based on the following considerations:

- For parameters primarily influenced by the drug substance manufacturing process (eg, glycan profile and potency), each unique drug substance lot is represented once in the similarity assessment. A total of 14 ABP 710 drug substance lots were included in the similarity assessment represented by 12 drug product lots and 2 drug substance lots. The 2 drug substance lots were not filled into drug product and were therefore assessed directly. The ABP 710 drug substance lots include development lots, all lots used in clinical studies, and process validation lots.
- For parameters primarily influenced by the drug product manufacturing process (eg, protein content and reconstitution time), a total of 19 drug product lots were included in the analytical similarity assessment. This encompasses all lots used in clinical studies and 3 drug product process validation lots.
- For stability-indicating attributes (ie, product-related substances and impurities), drug product stability data were used to determine the product degradation rate. Stability-indicating assays showed no practically significant change at the recommended storage condition over the shelf life of the product; hence age-adjustment on the product quality attributes was not performed.

3.2.2 Infliximab Lots

The applicant sourced US-Remicade on a regular basis, over a period of approximately 6 years. A total of 28 US-Remicade were tested. The US-Remicade lots were evaluated as part of the analytical similarity assessment. For parameters expected to change as a function of time at their recommended storage condition, available infliximab lots were tested upon receipt and again at or near the end of shelf life. Note that all 28 US-Remicade lots were tested for Relative Potency. However, only 25 US-Remicade lots were tested for sTNF α Binding by ELISA. The applicant stated that three US-Remicade lots were not tested due to a combination of assay development timing and/or exhaustion of reference product sample.

3.3 Equivalence test results

3.3.1 Relative potency through inhibition of sTNF α -induced Apoptosis in U937

For Relative Potency, the data are illustrated in Figure 1 and the equivalence test result is summarized in Table 1, which shows that Relative Potency passed the equivalence test.

Table 1: Summary of equivalence test for Relative Potency (%) between ABP 710 and US-Remicade.

Product	# of Lots	Range	Mean	Std. Dev.	Mean Diff. (90% CI)	Margin	Pass ET?
ABP 710	14	(87-112)	100	6.8	-2.64 (-7.2, 1.91)	(-13.67,13.67)	Yes
US-Remicade	28	(78-117)	102	9.1			

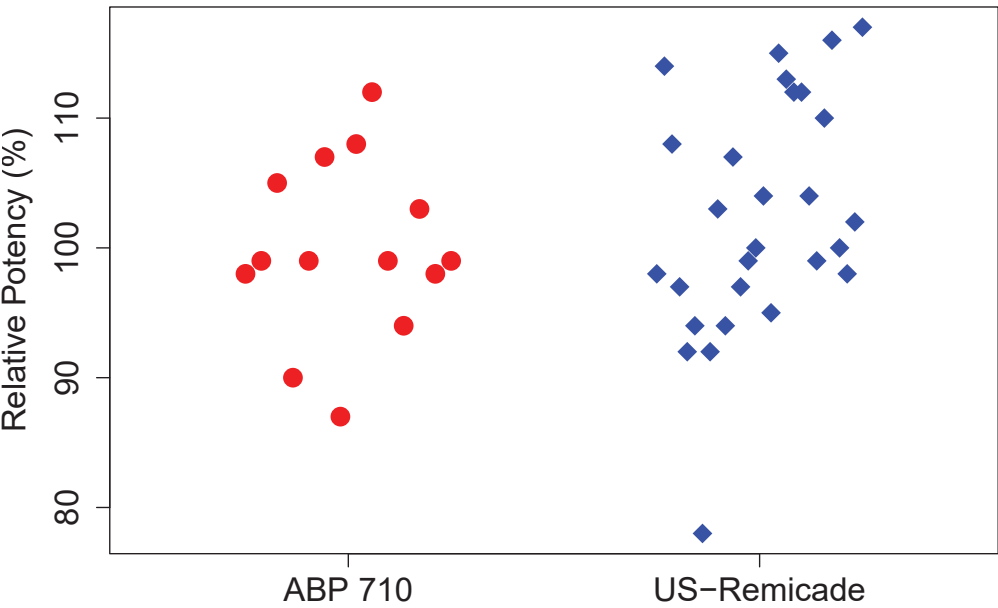


Figure 1: Scatter plot for Relative Potency.

3.3.2 sTNF α binding by ELISA

For sTNF α Binding by ELISA, the data are illustrated in Figure 2 and the equivalence test result is summarized in Table 2, which shows that sTNF α Binding by ELISA passed the equivalence test.

Table 2: Summary of equivalence test for sTNF α Binding by ELISA (%) between ABP 710 and US-Remicade.

Product	# of Lots	Range	Mean	Std. Dev.	Mean Diff. (90% CI)	Margin	Pass ET?
ABP 710	14	(88-109)	100	5.1	1.39 (-1.6, 4.39)	(-7.8,7.8)	Yes
US-Remicade	25	(88-112)	98	5.2			

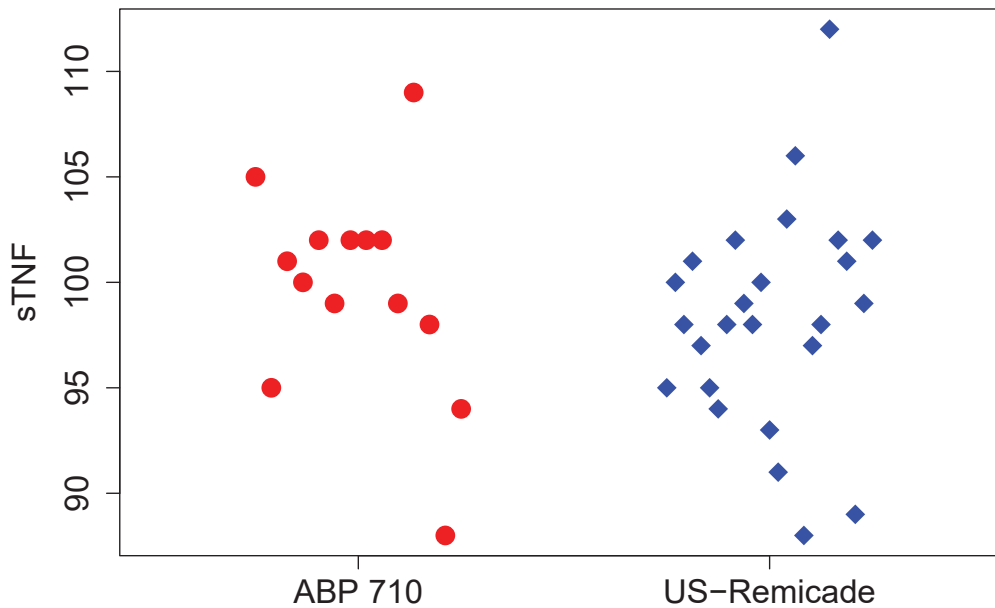


Figure 2: Scatter plot for sTNF α Binding by ELISA.

4 Conclusion and recommendations

In summary, I evaluated the data quality and similarity of two attributes, Relative Potency and sTNF α Binding by ELISA. There are no significant issues with the data quality and both attributes passed the equivalence test. This evaluation supports the demonstration that ABP 710 is highly similar to US-licensed Remicade.

Reference

- Dong, Xiaoyu, Yu-Ting Weng, and Yi Tsong. 2017. "Adjustment for Unbalanced Sample Size for Analytical Biosimilar Equivalence Assessment." *Journal of Biopharmaceutical Statistics* 27 (2). Taylor & Francis: 220–32.
- Tsong, Yi, Xiaoyu Dong, and Meiyu Shen. 2017. "Development of Statistical Methods for Analytical Similarity Assessment." *Journal of Biopharmaceutical Statistics* 27 (2). Taylor & Francis: 197–205.

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/s/

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10/28/2019 03:31:42 PM

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10/28/2019 09:22:23 PM

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11/01/2019 05:14:38 PM

First Approval for Indication/First Biosimilar/Expedited or Breakthrough Review: No

Recommendation: BLA Approval

BLA Number: 761086

Review Number: 1

Review Date: 08/14/2019

Drug Name/Dosage Form	Avsola—infliximab-axxq; ABP 710/lyophilized powder for injection
Strength/Potency	100 mg of lyophilized powder in a 20 mL vial to be reconstituted in 10 mL of sterile water for injection
Route of Administration	Intravenous infusion
Rx/OTC dispensed	Rx
Indication	All indications for US-licensed Remicade
Applicant/Sponsor	Amgen
US agent, if applicable	n/a

Product Overview

Avsola (ABP 710) is a chimeric IgG1κ monoclonal antibody proposed as a biosimilar to US-licensed Remicade. Avsola is supplied as a sterile, white lyophilized powder for intravenous infusion, following reconstitution with 10 mL of sterile Water for Injection.

Quality Review Team

Discipline	Reviewer	Branch/Division
Drug Substance	Yetao Jin	OPQ/OBP/DBRR II
Drug Product	Bruce Huang	OPQ/OBP/DBRR II
Immunogenicity	Bruce Huang	OPQ/OBP/DBRR II
Labeling	Scott Dallas	OPQ/OBP
Facility	Ziyang Su	OPQ/OPF/DIA
Microbiology	Max Van Tassell	OPQ/OPF/DMA
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OBP Tertiary Reviewer	Xianghong Jing	OPQ/OBP/DBRR II

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Discipline	Reviewer	Office/Division
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Clinical Pharmacology	Dipak Pisal / Anshu Marathe	OCP/DCPI
Statistics	James Travis / Lei Nie	OB/DB V
CMC Statistics	Chao Wang / Meiyu Shen	OB/DB IV

1. Names:

- Proprietary Name: ABP 710
- Trade Name: Avsola
- Non-Proprietary/USAN: infliximab-axxq
- INN Name: pending
- CAS Registry Number: 1446410-97-4
- OBP systematic name: MAB CHIMERIC (IGG1) ANTI P01375 (TNFA_HUMAN) [ABP 710]

Submissions Reviewed:

Submission(s) Reviewed	Document Date
761086/0001	12/14/2018
761086/0007 (response to DMA IR on 03/26/2019)	04/10/2019
761086/0008 (response to OBP IR on 04/11/2019)	04/25/2019
761086/0010 (response to DIA IR on 05/09/2019)	05/16/2019
761086/0012 (response to CMC Stats IR on 05/20/2019)	05/31/2019
761086/0013 (response to OBP IR on 05/21/2019)	06/04/2019
761086/0015 (response to DMA IR on 06/19/2019)	06/28/2019
761086/0016 (response to OBP & DMA IR on 07/19/2019)	08/01/2019
761086/0017 (response to OBP IR on 08/02/2019)	08/07/2019
761086/0019 (response to DMA IR on 07/19/2019)	09/18/2019
761086/0020 (response to OBP IR on 10/03/2019)	10/10/2019

Quality Review Data Sheet

1. Legal Basis for Submission: 351(k)

2. Related/Supporting Documents:

A. DMFs:

DMF #	DMF Type	DMF Holder	Item referenced	Code ¹	Status ²	Date Review Completed
(b) (4)	3	(b) (4)	(b) (4)	2	Adequate	N/A
	3			2	Adequate	N/A

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

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

B. Other documents: IND, Referenced Listed Drug (RLD), or sister application.

Document	Application Number	Description
IND	122136	Amgen-sponsored IND under which ABP 710 was developed and BPD meetings were held

3. Consults: None.

Executive Summary

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

The Office of Product Quality (OPQ), CDER, recommends approval of BLA 761086 for ABP 710 manufactured by Amgen, Inc. The data submitted in this application, including the analytical similarity assessment, are adequate to support the conclusion that:

- The manufacture of ABP 710 is well-controlled and leads to a product that is pure and potent;
- ABP 710 is similar to US-licensed Remicade notwithstanding minor differences in clinically inactive components.

It is recommended that this product be approved for human use under conditions specified in the package insert.

B. Approval Action Letter Language:

- Manufacturing location:
 - o Drug Substance:

Immunex Rhode Island Corporation (Referred to as Amgen Rhode Island or ARI)
40 Technology Way,
West Greenwich, Rhode Island 02817
USA
FEI: 3003359885
 - o Drug Product:

(b) (4)
- Fill size and dosage form:

100 mg lyophilized solid in a single-use 20 mL vial to be reconstituted with 10 mL sterile water for injection
- Dating period:
 - o Drug Product: 48 months: 2 to 8 °C
 - o Drug Substance: (b) (4) months: (b) (4) °C
 - o For packaged products: "Not packaged"
 - o Stability Option:

We have approved the stability protocol(s) in your license application for the purpose of extending the expiration dating of your drug substance and drug product under 21 CFR 601.12.
- Exempt from lot release
 - o Yes

- o Rationale, if exempted: Per FR notice 95-29960 well-characterized therapeutic recombinant DNA-derived and monoclonal antibody biotechnology products are exempt from 21 CFR 601.2a lot release requirement.
- Amgen claimed a categorical exclusion from the preparation of an environmental assessment for ABP 710 in accordance with 21 CFR 25.31 (c). The claim is because ABP 710 is considered “naturally occurring in the environment” and, when exposed to the environment, is not expected to significantly alter the concentration of the substance, its metabolites, or degradation products in the environment. No extraordinary circumstances exist.
The claim of a categorical exclusion is accepted.

C. Assessment Summary:

ABP 710 is a proposed biosimilar to US-licensed Remicade. ABP 710 has the same dosage form and route of administration as US-licensed Remicade. Amgen seeks licensure for the following indications:

- Crohn’s Disease (CD)
- Pediatric CD (6 years of age and older)
- Ulcerative Colitis (UC)
- Pediatric UC (6 years of age and older)
- Rheumatoid Arthritis (RA)
 - o In combination with methotrexate (MTX)
- Ankylosing Spondylitis (AS)
- Psoriatic Arthritis (PsA)
- Plaque Psoriasis (Ps)

The overall control strategy includes control of raw materials, facilities and equipment, manufacturing process, and adventitious agents. The control strategy combined with in-process, release, and stability testing ensure that the drug substance and drug product manufacturing processes are well controlled and lead to a product with the expected quality attributes and free of adventitious agents.

The data support the demonstration that ABP 710 is highly similar to US-Remicade, notwithstanding minor differences in clinically inactive components (refer to Section II of this memo for further details and discussion of the differences observed).

Analytical similarity between ABP 710 and US-Remicade was evaluated using a comprehensive array of analytical methods that were suitable to evaluate critical quality attributes of ABP 710 and US-Remicade. The numbers of lots tested and the statistical analyses were appropriate to allow for a meaningful evaluation of the results of the analytical studies.

EU-approved Remicade was not used as a comparator in the clinical studies. The analytical testing results from EU-approved Remicade submitted in the BLA were not assessed and the scientific bridging to US-licensed Remicade is deemed not necessary.

ABP 710 has the same dosage form, formulation, and route of administration as US-licensed Remicade, but has a different formulation. Comparative protein concentration (mg/mL) was assessed as part of the analytical similarity assessment. The deliverable volume (mL) and fill weight data were assessed (b) (4). The proposed presentations of ABP 710 have the same total content of drug substance in units of mass in a container as U.S.-licensed Remicade (100 mg/vial). The strength of ABP 710 (100 mg lyophilized powder in vial) is the same as that of US-Humira.

The microbial control and sterility assurance strategy is sufficient to support consistent manufacture of a sterile product. The BLA is recommended for approval from a sterility assurance and microbiology product quality perspective.

The DIA assessor is recommending approval of Immunex Rhode Island Corporation (Referred to as Amgen Rhode Island or ARI) Rhode Island, United States, FEI 3003359885, for commercial manufacture of ABP 710 DS (b) (4)

The OBP assessments including DS, DP, analytical similarity and validation of immunogenicity assays, DMA DS and DP microbiological assessments, and DIA facility technical assessment are located as separate documents in Panorama.

D. Recommendation on Phase 4 (Post-Marketing) Commitments, Requirements, Agreements, and/or Risk Management Steps, if approvable:

- 1. Develop and implement a control strategy for the Fc-domain-mediated effector function of antibody-dependent cell mediated cytotoxicity (ADCC) of ABP 710. The test format can either be a functional bioassay or the use of FcγRIIIa binding as a surrogate. The proposed control strategy and supporting validation data will be submitted to FDA following 21 CFR 601.12 (b).**

II. Comparative Analytical Assessment and Evaluation of the Analytical Component of the Scientific Bridge

A. Analytical Assessment Overview and Conclusions

The comparative analytical assessment between ABP 710 and US-licensed Remicade compared 19 lots of ABP 710 and 28 lots of US-licensed Remicade. The 19 ABP 710 lots included 17 drug product lots derived from 12 drug substance lots and 2 drug substance lots. Therefore, only 14 ABP 710 lots are considered independent lots. Data generated from studies using EU-approved Remicade were not used to support a demonstration of biosimilarity. Therefore, the analytical testing results from 20 lots of EU-approved Remicade submitted in the BLA were not assessed, as there was no need to establish an adequate scientific bridge.

Amgen used an acceptable risk-based approach for statistical evaluation of analytical results. Highest-ranked risk attributes tested using quantitative assays were evaluated using equivalence testing.

Moderate to high risk attributes tested using quantitative assays were evaluated using quality ranges calculated to account for reference product manufacturing variability and assay variability. Low risk attributes or attributes tested using qualitative assays were evaluated using visual display comparisons. The data analysis strategy used by Amgen is consistent with the recommendations FDA provided during product development. Results from method validation or qualification studies support the suitability of the methods used in the comparative analytical assessment.

The expiry dates of the US-licensed Remicade lots range from February 2015 to February 2020. The applicant tested US-licensed Remicade lots upon receipt and again at or near the end of shelf life for attributes expected to change over time at the recommended storage condition. Since no significant change over shelf life was observed, the initial time point testing results were used in the comparative analytical assessment.

The applicant also provided a comparison of stability under thermal forced degradation conditions of 40°C for the lyophilized and reconstituted products and accelerated conditions of 25°C for the lyophilized products.

Based on our assessment of the ABP 710 and US-licensed Remicade data, we determined that ABP 710 has been demonstrated to be highly similar to US-licensed Remicade, notwithstanding minor differences in clinically inactive components. ABP 710 has the same strength, dosage form, presentation, and route of administration as US-licensed Remicade. The applicant used a comprehensive array of analytical methods that were suitable to evaluate critical quality attributes of ABP 710 and US-licensed Remicade to support the demonstration that the products are highly similar. Numbers of lots tested and statistical analyses were appropriate to allow for a meaningful evaluation of the results of the comparative analytical studies. Observed differences do not preclude a demonstration that ABP 710 and US-licensed Remicade are highly similar.

B. Results of Comparative Analytical Assessment

The results of these analytical comparisons support a demonstration that ABP 710 is highly similar to US-licensed Remicade and the results are summarized in Table A below:

Table A. Quality Attributes Analyzed to Support a Demonstration of Highly Similar

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Supports a Demonstration of Highly Similar
<i>Primary Structure</i>	<i>Molecular weight (intact and reduced and deglycosylated of HC and LC)</i>	<i>Yes</i>
	<i>Amino acid sequence</i>	<i>Yes</i>
	<i>Methionine oxidation</i>	<i>Yes*</i>
	<i>Asparagine deamidation</i>	<i>Yes*</i>
	<i>Disulfide mapping</i>	<i>Yes</i>
	<i>Isoelectric point</i>	<i>Yes</i>
	<i>Identity by ELISA</i>	<i>Yes</i>
<i>Glycosylation</i>	<i>Afucosylation</i>	<i>Yes</i>
	<i>High mannose</i>	<i>Yes</i>

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Supports a Demonstration of Highly Similar
	<i>B</i> -galactosylation	<i>Yes</i>
	<i>α</i> -galactosylation	<i>Yes*</i>
	<i>Sialylation</i>	<i>Yes*</i>
<i>Higher Order Structure</i>	<i>Secondary Structure (FTIR)</i>	<i>Yes</i>
	<i>Tertiary Structure (near UV CD)</i>	<i>Yes</i>
	<i>DSC T_{m1} and T_{m2}</i>	<i>Yes</i>
<i>Product-related substances and impurities</i>	<i>HMW (SE-UHPLC)</i>	<i>Yes*</i>
	<i>Main peak (SE-UHPLC)</i>	<i>Yes*</i>
	<i>LMW (SE-UHPLC)</i>	<i>Yes</i>
	<i>Monomer and HMW (SV-AUC)</i>	<i>Yes</i>
	<i>Molar mass: main peak and HMW (SE-HPLC-SLS)</i>	<i>Yes</i>
	<i>HC + LC (rCE-SDS)</i>	<i>Yes*</i>
	<i>LMW + MMW (rCE-SDS)</i>	<i>Yes*</i>
	<i>NGHC (rCE-SDS)</i>	<i>Yes*</i>
	<i>HMW (rCE-SDS)</i>	<i>Yes</i>
	<i>Pre-peak (nrCE-SDS)</i>	<i>Yes*</i>
	<i>Main peak (nrCE-SDS)</i>	<i>Yes*</i>
	<i>Post-peaks (nrCE-SDS)</i>	<i>Yes</i>
	<i>Main peak (CEX-HPLC)</i>	<i>Yes</i>
	<i>Acidic peak (CEX-HPLC)</i>	<i>Yes*</i>
	<i>Basic peak (CEX-HPLC)</i>	<i>Yes*</i>
	<i>Profile treated with carboxypeptidase B</i>	<i>Yes</i>
<i>Bioactivity</i>	<i>Tumor neutralizing factor (TNF)-α neutralization (inhibition of sTNFα-induced apoptosis in U937 cells)</i>	<i>Yes</i>
	<i>Binding to sTNFα by ELISA</i>	<i>Yes</i>
	<i>Binding to sTNFα by SPR by SPR</i>	<i>Yes</i>
	<i>Binding kinetics to sTNFα</i>	<i>Yes</i>
	<i>Inhibition of sTNFα-induced IL-8 release in HUVEC</i>	<i>Yes</i>
	<i>Inhibition of sTNFα-induced cell death in L929</i>	<i>Yes</i>
	<i>Binding to mbTNFα by imaging cytometry</i>	<i>Yes</i>
	<i>Reverse signaling</i>	<i>Yes</i>
	<i>Binding to LTα by SPR</i>	<i>Yes</i>
	<i>Inhibition of LTα -induced IL-8 release in HUVEC</i>	<i>Yes</i>
	<i>Binding to FcγRIIIa (158V and 158F) by SPR</i>	<i>Yes</i>
	<i>Binding kinetics to FcγRIIIa (158V) by SPR /Kd (nM)</i>	<i>Yes</i>
	<i>Binding to primary NK cells by FACS</i>	<i>Yes</i>
	<i>Antibody-dependent cell-mediated cytotoxicity (ADCC) NK92 cell</i>	<i>Yes</i>
	<i>PBMC ADCC activity</i>	<i>Yes</i>
	<i>Binding to C1q by ELISA</i>	<i>Yes</i>
	<i>Complement dependent cytotoxicity (CDC) activity</i>	<i>Yes</i>
	<i>Binding to FcγRIIa (131R) by SPR</i>	<i>Yes</i>
	<i>ADCP activity</i>	<i>Yes</i>
	<i>Binding to FcγRIa by AlphaLISA</i>	<i>Yes</i>
	<i>Binding to FcγRIIb and FcγRIIIb by SPR</i>	<i>Yes</i>
	<i>Binding to FcRn by AlphaScreen</i>	<i>Yes</i>
	<i>Binding to FcRn by SPR</i>	<i>Yes</i>
<i>Drug Product Attributes</i>	<i>Protein content (UV absorbance)</i>	<i>Yes</i>

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Supports a Demonstration of Highly Similar
	<i>Reconstituted protein concentration</i>	<i>Yes</i>
	<i>Reconstitution time</i>	<i>Yes</i>

* Differences between ABP 710 and US-licensed Remicade were noted. However, these differences do not preclude a demonstration of highly similar. See section IV for additional information.

sTNF α binding and neutralization of sTNF α -induced apoptosis are generally regarded as the main mechanism of action for infliximab products. Two assays were conducted to probe these activities and the results were analyzed by equivalence testing. For the inhibition of sTNF α -induced apoptosis assay, the applicant provided data from 14 lots of ABP 710 and 28 lots of US-licensed Remicade. For the TNF- α binding assay by ELISA, the applicant provided data from 14 lots of ABP 710 and 25 lots of US-licensed Remicade. OBP reviewed the justification of lots selection and concluded that the lots selected for each product were representative. The CMC Statistics reviewer assessed statistical equivalence testing and determined that the data met the equivalence margins for both assays, supporting a demonstration that ABP 710 is highly similar to US-licensed Remicade.

Additional potential mechanisms of action have been proposed for infliximab products in the scientific literature. These include antibody dependent cell-mediated cytotoxicity against cells expressing membrane-bound TNF- α (mTNF- α), complement dependent cytotoxicity against mTNF- α positive cells, "reverse signaling" (signal transduction into cells by activation of mTNF- α), and induction of regulatory macrophages. It is likely that the relative role for each of these mechanisms differs between indications. Assays that are orthogonal to the sTNF α binding and the neutralization assays, assays that evaluate these additional potential mechanisms of action, and assays that evaluate purity, protein content, and other general properties of infliximab products were assigned for quality range or visual comparison assessment.

The protein biochemistry and biological activity attributes tested in Table A met the pre-defined comparative analytical acceptance criteria in the comparison of ABP 710 to US-licensed Remicade with exceptions discussed in Section D of this memo. The testing results from the comparison of the stability under stressed and accelerated conditions indicated that the stability of ABP 710 drug product and US-licensed Remicade are similar and any differences do not preclude a demonstration that ABP 710 is highly similar to US-licensed Remicade.

C. Analytical Studies to Support the Use of a Non-U.S.-Licensed Comparator Product

Not applicable.

D. Assessment of Comparative Analytical Study Results

Comparative analytical acceptance criteria were met for all attributes with the following exceptions:

- All ABP 710 lots have higher high molecular weight (HMW) levels (0.7-1.3%) than the quality ranges of US-licensed Remicade (0.1-0.4%). And the corresponding main peak levels (98.6-99.2%) are lower than the quality ranges of US-licensed Remicade (99.4-99.9%). The HMW fraction was characterized as predominantly non-covalent dimer (~60-86%). Non-covalent

dimers are reversible and may dissociate *in vivo*, making this already small difference effectively negligible. Difference in HMW species may impact product bioactivity. To evaluate whether the difference in HMW species impacted bioactivity, the HMW species fractions were isolated and their bioactivity was measured using cell-based potency assays for TNF- α neutralization and ADCC. The HMW fraction showed comparable bioactivity to the main peak in both the TNF- α neutralization assay and ADCC assay. TNF- α neutralization is the primary mechanism of action (MoA) of infliximab across all indications. ADCC activity is a potential MoA for certain indications. TNF- α neutralization assay and ADCC assay measure both bioactivities which are clinically relevant and are suitably representative of the drug's MoAs for this analysis. The total level of HMW species is low and the applicant proposed to control the levels of Main peak and HMW species for DS and DP release and stability. Based on the HMW species characterization study and the acceptable control strategy, the observed differences in HMW species do not preclude a demonstration of highly similar.

- The levels of size variants measured by rCE-SDS are out of the quality ranges of US-licensed Remicade as follows: 1) HC+LC% range (96.6-97.3%) is below the quality range of 98.3-99.1%; 2) LMW+MMW% range (0.7-1.0%) is slightly higher than quality range of 0.1-0.9%; 3) non-glycosylated heavy chain (NGHC%) range (1.6-2.1%) is higher than quality range of 0.4-0.6%. The LMW/MMW species are primarily fragments. The fragments and NGHC may have different biological activity compared with intact molecules. The bioactivity testing on the LMW/MMW fraction did not show any impact on the biological activity. The NGHC fraction was also tested for bioactivity and the results indicated the product biological activity was not impacted by the higher level of NGHC.

The %pre-peak (1.8-2.6) and % main peak (97.1-98.1%) measured by nrCE-SDS are out of the quality ranges of US-licensed Remicade of 0.7-2.1% and 97.8-99.3%, respectively. The difference for each attribute compared to the quality range was less than 1%. As characterized for the low molecular weight fractions in rCE-SDS, the minor differences did not impact biological activity and the levels of fragments have been controlled by controlling the levels of monomer and HMW at release and on stability. Therefore, the differences the levels of fragments observed in the rCE-SDS and nrCE-SDS assays do not preclude a demonstration of highly similar.

- The % basic peak in 7 out of 12 ABP 710 lots (30.0-36.1%) was below the quality ranges of US-licensed Remicade (32.3-60.2%). The carboxypeptidase B treatment study indicated that the basic species are the variant with C-terminal lysine. Since the C-terminal lysine will be immediately cleaved *in vivo*, it is not considered a critical quality attribute and bioactivity testing (TNF- α neutralization assay and ADCC assay) on the basic peak fraction did not show any impact on the biological functions representative of the product's MoA.

The %acidic peak in all batches of ABP 710 (19.5-23.6%) was above the quality ranges of US-licensed Remicade (8.4-17.8%). Characterization study of the ABP 710 acidic peak fractions showed that deamidation at HC CDR2 Asn⁵⁷ is higher in ABP 710 than US-licensed Remicade. Higher level of deamidation at the CDR may impact the protein bioactivity. The bioactivity of the purified acidic fractions from both products were tested using TNF- α neutralization assay and ADCC assay and the results demonstrated the acidic fractions have similar bioactivity to the main peak. The charge variants and post-translational modification differences observed between ABP 710 and US-licensed Remicade did not result in biological activity differences. Therefore, the differences noted in basic and acidic species do not preclude a demonstration of highly similar.

- US-licensed Remicade has low level of α -galactosylation (< 6.6%), which was not detected in ABP 710. US-licensed Remicade is produced in SP2/0 cells which express low level of terminal α -galactose. ABP 710 is produced in CHO cells which do not express terminal α -galactose. Increased galactosylation may increase C1q binding and CDC activity. However, this difference in α -galactosylation did not result in differences in CDC activity or C1q binding. Therefore, the observed differences in galactosylation do not preclude a demonstration of highly similar.
- Of the trace levels of sialylated glycans detected, N-acetylneuraminic acid (Neu5Ac) is the predominant sialic acid form in ABP 710 (< 1.6%) whereas N-glycolylneuraminic acid (Neu5Gc) is the predominant form in US-licensed Remicade (3.1-10.0%). This difference is also due to the different cell lines used for production. While sialylation has a low potential to impact Fc effector functions, it has the potential to impact other antibody functional domains and could result in differences in bioactivity related to the Fab arm functionality. However, comparative analyses of ABP 710 and US-licensed Remicade showed the difference of low-level sialylation had no observed effect on any receptor binding or functional assay activities, i.e., those indicative of Fab arm functionality. Therefore, the differences noted in glycosylation levels and species do not preclude a demonstration of highly similar.

The totality of the analytical similarity evidence supports the following conclusions:

- ABP 710 is highly similar to US-licensed Remicade, notwithstanding minor differences in clinical inactive components.
- For attributes where differences were observed between ABP 710 and US-licensed Remicade, the totality of the analytical data supports that the function, activity, and *in vitro* stability are not impacted. Specifically, the differences noted in size variants and charge variants were not reflected in functional assays such as TNF- α neutralization assay and ADCC assay. Therefore, these differences do not preclude a demonstration that ABP 710 is highly similar to US-licensed Remicade.

E. Same Strength:

ABP 710 has the same dosage form and route of administration as U.S.-licensed Remicade. Amgen is seeking approval of 100 mg ABP 710 in a vial. U.S.-licensed Remicade is available at this strength in a vial. Amgen is seeking approval for the same strength as U.S.-licensed Remicade. Comparative protein concentration upon reconstitution (mg/mL) was assessed as part of the comparative analytical assessment. The deliverable volume (mL) and fill weight data were assessed (b) (4)

The proposed presentation of ABP 710 has the same total content of drug substance in units of mass in a container as U.S.-licensed Remicade (100 mg). The strength of ABP 710 vial is the same as that of U.S.-licensed Remicade.

III. Summary of Quality Assessments:

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 1 below is a summary of critical quality attributes and the associated control strategies for attributes that are relevant to both Drug Substance and Drug Product. For additional information, see the primary

reviews, including the Drug Substance Quality Review and Drug Product Quality Review by OBP/DBRRII and the Drug Substance Microbiology Review and the Drug Product Microbiology Review by OPF/DMA.

Table 1: Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

CQA (type)	Risk	Origin	Control Strategy	Other
TNF-α binding and neutralization (potency)	Efficacy	Intrinsic to molecule	(b) (4)	
High-molecular weight species; (product-related impurity)	Efficacy, pharmacokinetics, and immunogenicity	Manufacturing process and storage conditions. Can form due to agitation, temperature, or light exposure.		HWM fraction has similar potency to monomer.
Fragmentation (product-related impurities)	Efficacy, pharmacokinetics, and immunogenicity	Manufacturing process and storage conditions. Fragmentation may be caused by agitation, temperature, or light		Characterization by non-reducing CE-SDS testing.
Acidic and basic variants (product-related variants and impurities)	Efficacy, pharmacokinetics, and immunogenicity	Fermentation, purification, deamidation during storage		
(b) (4) oxidation (CDR) (product related impurity)	Efficacy and safety	Manufacturing process and storage conditions		(b) (4)
Deamidation (CDR) (product related impurity)	Efficacy and safety	Manufacturing process and storage conditions		
Glycosylation	Efficacy and pharmacokinetics	(b) (4) process		

CQA (type)	Risk	Origin	Control Strategy	Other
Protein Concentration and Protein Content	Efficacy, pharmacokinetics, safety	Manufacturing process	(b) (4)	
ADCC	Efficacy	Intrinsic molecule to		PMC to add a functional or FcγRIIIa binding assay to DS release specification
CDC	Efficacy	Intrinsic molecule to		
Reverse signaling via mTNF-α	Efficacy	Intrinsic molecule to		
Identity (identity)	Safety, efficacy	Intrinsic molecule to		

B. Drug Substance [infliximab-axxq] Quality Summary

CQA Identification, Risk, and Lifecycle Knowledge Management

Table 2 below summarizes the critical quality attributes and their control strategy that are relevant specifically to the Drug Substance. For additional information, see the primary reviews, including the Drug Substance Quality Review and Drug Product Quality Review by OBP/DBRRII and the Drug Substance Microbiology Review and the Drug Product Microbiology Review by OPF/DMA.

Table 2: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management.

CQA (type)	Risk	Origin	Control Strategy	Other
Appearance (color and clarity)	Safety	Manufacturing process	(b) (4)	

CQA (type)	Risk	Origin	Control Strategy	Other
Host Cell Proteins (process-related impurity)	Safety and immunogenicity	Derived from host cell line	(b) (4)	
Host Cell DNA (process-related impurity)	Safety	Derived from host cell line		
(b) (4) (process-related impurity)	Safety and immunogenicity	Process-related impurity (b) (4)		
(b) (4) (process-related impurity)	Safety	(b) (4)		
(b) (4) (process-related impurity)	Safety			
(b) (4) (process-related impurity)	Safety			
(b) (4) (process-related impurity)	Safety			
(b) (4) (process-related impurity)	Safety			
(b) (4) (process-related impurity)	safety			
(b) (4) (process-related impurity)	Safety	From raw materials		
Virus Contamination (contaminant)	Safety	Contamination during manufacture		
Retrovirius like particles	Safety	Derived from CHO cell-substrate		

CQA (type)	Risk	Origin	Control Strategy	Other
(b) (4)	Safety, stability	Manufacturing process	(b) (4)	
(excipient)				
Endotoxin	Safety, Purity	Raw materials and manufacturing process		
Bioburden	Safety, Purity and Efficacy due to degradation or modification of the product by microbial contamination	Raw materials and manufacturing process		

- Description: ABP 710 is a recombinant human-murine chimeric monoclonal antibody of the immunoglobulin G1 (IgG1) subclass consisting of 2 heavy chains and 2 light chains of the Kappa subclass. ABP 710 contains 32 total cysteine residues involved in both intrachain and interchain disulfide bonds. Each heavy chain contains 450 amino acids with 4 intrachain disulfides and each light chain contains 214 amino acids with 2 intrachain disulfides. Each heavy chain contains an N-linked glycan at a consensus glycosylation site on asparagine 300. ABP 710 has a molecular weight of approximately 148.5 kDa. ABP 710 Drug Substance is formulated for Drug Product filling and is stored as a (b) (4)
- Mechanism of Action (MoA): ABP 710 binds specifically to tumor necrosis factor alpha (TNF-α) and blocks its interaction with receptors (TNFR-1 and TNFR-2). TNF-α, expressed by immune cells and other cells in response to infection or inflammation, is expressed both as a soluble cytokine (sTNFα) and a membrane-bound (mbTNFα) form. Elevated levels of TNF-α are found in the synovial fluid of patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. TNF-α plays an important role in both the pathologic inflammation and the joint destruction that are characteristic of these diseases. ABP 710 binds to mbTNFα on immune cells and induce apoptosis through “reverse signaling”. ABP 710 can induce multiple effector functions that dependent on binding to mbTNFα, including antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). These activities have been suggested to contribute to inflammatory bowel disease indications, although the significance of the contribution of any of these individual mechanisms not well established (based on literature review).
- Potency Assay: The potency assay measures inhibition of soluble TNFα-induced apoptosis in a human monocyte cell line that expresses TNF receptors (U-937 cells). The assay measures relative caspase-3 and caspase-7 levels, which are proportional to the apoptosis of cells, using a luminescence detection method. ABP 710 causes a dose-dependent loss

in luminescence signal. This activity is reported as a percentage of the activity of the reference standard.

- Reference Materials: A well characterized primary reference standard was derived from a clinical batch manufactured at Amgen Thousand Oaks (ATO) with process comparable to the commercial process. A working reference standard has been manufactured and qualification is ongoing. Amgen plans to qualify a working reference standard against this primary reference standard. A protocol is provided for the qualification of future primary and working reference standards. The protocol contains adequate testing and acceptance criteria. The implementation of a new reference standard will be reported in annual report. Both primary and working reference standards are re-tested (b) (4)

- Critical starting materials or intermediates: (b) (4)

(b) (4)

Raw materials used in commercial manufacture are of non-animal origin.

- Manufacturing process summary: (b) (4)

(b) (4)

- Container closure: (b) (4)
- Dating period and storage conditions: The applicant conducted real-time stability studies at (b) (4) °C for 4 clinical batches and 3 process validation batches. These data are also supported by accelerated and stressed stability studies. These data support a dating period of (b) (4) months at (b) (4) °C in (b) (4). The stability testing program is adequate and consistent with ICH Q5C recommendations.

C. Drug Product [Avsola] Quality Summary:

Table 3 provides a summary of the identification, risk, and lifecycle knowledge management for drug product CQAs that derive from the drug product manufacturing process and general drug product attributes.

Table 3: Drug Product CQA Identification, Risk, and Lifecycle Management

CQA (type)	Risk	Origin	Control Strategy	Other
Appearance (lyophilized cake)	safety	Manufacturing process	(b) (4)	
Appearance (color)	safety	Manufacturing process		
Appearance (visible particulates)	safety	Manufacturing process		
Appearance (clarity and color)	safety	Manufacturing process		
Content uniformity	Control of dosing	Manufacturing process		

CQA (type)	Risk	Origin	Control Strategy	Other
Sub-visible particles (general)	Safety	Manufacturing process, product degradation	(b) (4)	
Reconstitution time (general)	Safety, efficacy	Manufacturing process, storage conditions		
Moisture content (general)	Stability	Manufacturing process, loss of container closure integrity		
pH (general)	Safety, efficacy	Formulation		
Osmolality	Safety, stability	Formulation		
Filling volume (general)	Control of dosing	Manufacturing process		
Sterility (contaminant)	Safety (Infection), Purity and Efficacy (degradation or modification of products by contaminating microorganisms)	Contamination may be introduced throughout the manufacturing process or through failure of the container closure integrity		
Endotoxin (contaminant)	Safety, purity, and immunogenicity	Raw materials; contamination may be introduced throughout the DP manufacturing process		
Container closure integrity	Safety (maintenance of sterility during shelf-life)	Container closure breaches during storage. May be impacted by storage conditions.		

- Potency and Strength: Potency of ABP 710 is considered, for the purposes of this review, as the percent inhibition of TNF- α -induced apoptosis activity relative to the current reference standard. The potency assay is the same as those described in the Drug Substance section of this review.

ABP 710 is supplied as a 100 mg lyophilized solid in a single-use vial. The lyophilized powder in the vials is to be reconstituted in 10 mL sterile water for injection, yielding a 100 mg/10 mL solution. There is (b) (4)% overfill to assure that 10 mL reconstituted solution can be withdrawn per the product labeling. Comparative data for protein concentration demonstrate that ABP 710 (100 mg lyophilized solid in a single-use vial) has the same

total usable content of therapeutic antibody and the same concentration of antibody upon reconstitution as US-licensed Remicade. The proposed ABP 710 presentation (100 mg lyophilized solid in a single-use vial) meets the statutory same strength requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.

- **Summary of Product Design:** ABP 710 will be supplied as a sterile, preservative-free lyophilized powder in 20 mL vials. The lyophilized powder is to be reconstituted in sterile water for injection and further diluted into 0.9% sodium chloride solution in an infusion bag.
- **List of Excipients:** Excipients include dibasic sodium phosphate, anhydrous, monobasic sodium phosphate, monohydrate, sucrose and polysorbate 80. Except for the antibody itself, all ingredients meet compendial requirements (USP/NF, Ph. Eur. and/or JP) and are commonly used for formulation of biopharmaceuticals. No excipients are of human or animal origin.
- **Reference Materials:** The same reference standard is used for Drug Product as for Drug Substance. Refer to the Drug Substance reference standard section above.
- **Manufacturing process summary:** The manufacturing process for Drug Product includes the following steps: (b) (4)

- **Container closure:** The primary packaging material for ABP 710 Drug Product consists of a clear and colorless 20 mL (b) (4)
- **Dating period and storage conditions:** The sponsor conducted real-time, accelerated, and thermal-stressed stability studies on 3 commercial Drug Product lots and 5 clinical Drug Product lots. These data support a dating period of 48 months when stored at 2 to 8°C.

- List of co-package components: None.

D. Biopharmaceutics Considerations: None

E. Novel Approaches/Precedents: None

F. Any Special Product Quality Labeling Recommendations: None

G. Establishment Information:

Overall Recommendation:					
DRUG SUBSTANCE					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Drug substance manufacture Drug substance in-process, lot release, and stability testing Working cell bank storage Unprocessed bulk testing: mycoplasma Drug product lot release and stability testing	Immunex Rhode Island Corporation (Referred to as Amgen Rhode Island or ARI) Rhode Island, United States	968084785/ 3003359885	VAI	1. Inadequate procedure for investigating out of specification results. 2. Your quality unit failed to provide adequate oversight to ensure instrumentation is calibrated and maintained according to written procedures and an established schedule. 3. Your firm failed to conduct adequate deviation investigation. 4. Your quality unit fail to provide adequate oversight over critical manufacturing steps.	Approve
Drug substance in-process, lot	Amgen Inc	039976196/ 2026154	-----	Waived	Approve

release, and stability testing Master cell bank and working cell bank storage Working cell bank manufacture Drug product lot release and stability	(Referred to as Amgen Thousand Oaks or ATO) California, United States				
Drug substance in-process, lot release, and stability testing Drug product lot release and stability testing	Amgen Technology Ireland UC (Referred to as ADL) Dun Laoghaire, Ireland	896293920/3002808497	-----	Waived	Approve
Master cell bank storage Working cell bank storage	Amgen Inc. (Referred to as Louisville Distribution Center or LDC) Kentucky, United States	787632801/3003750095	-----	Waived	Approve
Mycoplasma testing (unprocessed bulk) Adventitious viral testing (unprocessed bulk)	(b) (4)		-----	Waived	Approve
DRUG PRODUCT					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Drug product manufacture, drug product in-process and lot release testing	(b) (4)		-----	Waived	Approve
Drug product packaging and labeling	Amgen Manufacturing Ltd (referred to as AML) Puerto Rico	785800020/1000110364	-----	Waived	Approve

	United States				
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H. **Facilities:** A pre-license inspection for ABP 710 drug substance manufacture was conducted on April 15-19, 2019 at ARI. A 4-item FDA Form 483 was issued and the initial recommendation is approval for the BLA. The final classification of the ARI pre-license inspection was acceptable.

The pre-license inspection of the drug product site (b) (4) was waived because of the site's compliance status and its recent surveillance inspection (b) (4)

I. Lifecycle Knowledge Management:

a. Drug Substance:

- i. Protocols approved: Stability protocol for the extension of shelf life, annual stability protocol, qualification of WCB, qualification of reference standards, annual stability testing for primary and working reference standards.
- ii. Outstanding review issues/residual risk: See PMC in section I. Recommendations
- iii. Future inspection points to consider: None

b. Drug Product

- i. Protocols approved: stability protocol for the extension of shelf life, annual stability protocol, comparability protocol for implementation of a new (b) (4) comparability protocol for the addition of Amgen Technology Ireland UC, Dun Laoghaire, Ireland as a manufacturing facility for ABP 710 drug product.
- ii. Outstanding review issues/residual risk: None
- iii. Future inspection points to consider: None

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

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

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/s/

YANMING AN
10/21/2019 02:23:18 PM

XIANGHONG JING
10/22/2019 08:22:23 AM

BLA STN 761086
Product: ABP 710
Proposed Trade Name: AVSOLA
Manufacturer: Amgen Inc.

Review of Comparative Analytical Assessment

Yetao Jin, Ph.D.
Drug Substance Assessor and Similarity Assessor

Yanming An, Ph.D., ATL

Office of Biotechnology Products
Division of Biotechnology Review and Research II

3.2.R Regional Information (USA)

COMPARATIVE ANALYTICAL ASSESSMENT

Assessor note:

Based on the review of analytical similarity data in Section 3.2.R, the conclusions below can be drawn:

- *ABP 710 is highly similar to US-licensed Remicade.*
- *Minor differences were observed for some impurity attributes, such as HMW, acidic variants and basic variants, between ABP 710 and US-licensed Remicade. The risks associated with these differences were mitigated by data from orthogonal analytical methods, such as potency and ADCC assays, supporting the notion that the potency and effector function activities, and in vitro stability are not impacted.*
- *The methods used in the comparative analytical assessment are scientifically sound and suitable to study the intended quality attributes according to the method validation or qualification results.*
- *The proposed quality ranges are deemed appropriate to be used as similarity acceptance criteria for the applicable quality attributes evaluated with Tier 2 statistical method. The quality ranges are generally based on a 3 standard deviation range unless otherwise noted.*
- *The strength of US-licensed Remicade is labeled as 100 mg of lyophilized infliximab. US-licensed Remicade is filled into a single-use vial to be reconstituted in 10 mL of Sterile Water for Injection, USP, for intravenous infusion. ABP 710 is seeking approval for the same strength as US-licensed Remicade. Comparative protein concentration upon reconstitution (mg/mL), reviewed as part of the comparative analytical assessment, and extractable volume and fill weight data reviewed (b) (4) were used to inform the assessment of whether the proposed presentation of ABP 710 has the same strength as the presentation of US-licensed Remicade. Based on these comparative data, the 100 mg of lyophilized ABP 710 in a single-use vial for intravenous infusion has the same total content of drug substance in units of mass in a container as the presentation of US-licensed Remicade. This presentation meets the statutory “same strength” requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.*

Overall, the analytical data provided support the conclusion that ABP 710 is highly similar to U.S.-licensed Remicade. In addition, E.U.-approved Remicade was only used to collect data as part of the global file and not specifically used for supporting U.S. license application of ABP710, therefore, scientific 3-way bridge to justify the use of clinical data generated using E.U.-approved Remicade is not within the scope of this review.

Note: Assessor comments are italicized. Unless otherwise stated, all tables and figures in this review have been copied from the BLA submission.

3.2.R.1 Comparative Physicochemical and Functional Assessment

3.2.R.1 .1 Selection of reference product

ABP 710 has been developed as a biosimilar product to the US-licensed Remicade® (infliximab) (BLA 103772, license is held by Janssen Biotech Inc.). Amgen used US-licensed Remicade as the comparator arm in the nonclinical and clinical program; thus, the comparative analytical assessment is performed between ABP 710 and US-licensed Remicade. Similarity data using EU-approved Remicade lots were collected as part of the global file and are presented for completeness.

The ABP 710 clinical program to support the application includes 2 studies listed in Table 3.2.R.1.

Table 3.2.R.1. ABP 710 Clinical Studies

Study Number	Subject Population	Type	Investigational Products
20140108	Healthy adult male and female subjects	PK similarity, safety, tolerability, immunogenicity	ABP 710 infiximab (US) infiximab (EU)
20140111	Adults subjects with moderate to severe RA	Efficacy, safety, immunogenicity	ABP 710 infiximab (US)

PK = pharmacokinetic; RA = rheumatoid arthritis

3.2.R.1.2 Comparative analytical assessment Approach for Assays/Attributes

A tier-based system was implemented for the assessment of analytical similarity, wherein each similarity assay/attribute is assigned to 1 of 3 tiers corresponding to the use of 3 different methods for comparing attributes.

- Tier 1 includes assays/attributes with the highest risk to clinical outcomes and includes assay(s) that evaluate clinically relevant, primary mechanism(s) of action.
- Tier 2 includes assays/attributes with moderate risk to clinical outcomes.
- Tier 3 contains assays/attributes with the lowest risk to clinical outcomes or where results are qualitative or not suitable for numerical evaluation, such as profile comparisons.

Refer to Table 3.2.R.7. below for the tier assignment of each test. The approaches to establishing similarity assessment criteria is summarized in Table 3.2.R.2.

3.2.R.1.3 Methods Selected

The methods used for the comparative analytical assessment were selected based on knowledge regarding the structure, function, and heterogeneity of the reference product and ABP 710, including those characteristics critical to the biological activity and stability of the product. The testing plan was organized into 7 categories (Table 3.2.R.7):

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

3.2.R.1.4 Overall Approach to Assessment

Testing Design

Data were generated in 1 of 2 testing designs: (1) side-by-side testing and (2) independent testing. The testing design is summarized in Table 3.2.R.7. The rationale for conducting the testing by each design was based on the nature of the attribute and method capability, as described below.

Side-by-side Testing

A side-by-side testing design was employed for analytical methods which required ABP 710 and reference product lots to be tested in a single experiment (e.g., chromatographic assays to generate profile overlays). Testing samples together in the same assay or study minimized any potential sources of variability. Additionally, side-by-side testing was employed when an assay required simultaneous testing of an internal standard in order to assess the similarity of the tested samples (e.g., Fourier-transform infrared [FTIR]).

Independent Testing

An independent testing design was employed for methods that demonstrate suitable intermediate precision to allow evaluation of quantitative results generated as lots were manufactured or procured over time. An example of an assay that was evaluated under the independent testing design is protein content.

Age-dependent Testing

For ABP 710 and reference product, the stability-indicating attributes did not show a practically significant change over the shelf life of 36 months of reference product. therefore, age adjustment was not performed for the similarity assessment. The initial time point tested for US-licensed Remicade was used to calculate the quality range. The lot release data for ABP 710 were assessed against the quality range of US-licensed Remicade.

Table 3.2.R.2. Summary of the Approach to Establishing Similarity Assessment Criteria (by the Assessor)

Approach	Description	Tier	Applicable Assays/Attributes
Equivalence Test	A statistical test of equivalence is used to assess that the difference between the means of the test product (TP) and the reference product (RP) is less than a pre-specified equivalence acceptance criterion (EAC).	Tier 1	Two Tier 1 tests: Inhibition of sTNF α -induced apoptosis in U937 (potency); Binding to sTNF α by ELISA.
Quality Range	The quality range of RP for a specific quality assay/attribute is defined as: $RP \text{ Mean} \pm K \times s_{RP}$, where K is the standard deviation multiplier and s_{RP} is the standard deviation of all RP lots. K is generally 3, unless the data suggest a different number should be chosen in order to derive an appropriate limit.	Tier 2	Binding to sTNF α by SPR; Binding to mbTNF α on CHO MT-3 cells by imaging cytometry; Reverse signaling in Jurkat mbTNF α line; Binding to Fc γ RIIIa (158V) by SPR; Binding to Fc γ RIIIa (158F) by SPR; NK92 ADCC activity; PBMC ADCC activity; Binding to C1q by ELISA; CDC activity; Binding to FcRn by AlphaScreen; Binding to FcRn by SPR; Glycan map: afucosylation; Glycan map: high mannose; Glycan map: beta-galactosylation; Tertiary structure by DSC: T _{m1} and T _{m2} ; <u>Size variants</u> : SE-UHPLC HMW and main peak; rCE-SDS HC + LC, LMW + MMW, and NGHC; nrCE-SDS pre-peak and main peak; <u>Charge variants</u> : CEX-HPLC main peak, acidic peak, and basic peak; Protein content; Reconstituted protein concentration.
Expectation Approach	For assays/attributes that have the potential to impact clinical outcome and where the data were best evaluated based on product knowledge, an expectation approach were employed to assess similarity.	Tier 2	Intact molecular mass; Reduced and deglycosylated molecular masses of HC and LC; Reduced peptide map; Non-reduced peptide map; Isoelectric point by cIEF, Secondary structure by FTIR: spectral similarity; Tertiary structure by Near UV CD: spectral similarity; Molar mass of size variants by SE-HPLC-SLS.
Qualitative Comparison	Results for assays/attributes are presented side-by-side to allow qualitative comparisons of the products.	Tier 3	Binding kinetics to sTNF α by SPR; Inhibition of sTNF α -induced IL-8 release in HUVEC; Inhibition of sTNF α -induced cell death in L929; Binding to LT α by SPR;

			Inhibition of LTα-induced IL-8 release in HUVEC; Binding kinetics to FcγRIIIa (158V) by SPR; Binding to primary NK cells by FACS; Binding to FcγRIIa (131R) by SPR; ADCP activity; Binding to FcγRIa by AlphaLISA; Binding to FcγRIIb by SPR; Binding to FcγRIIIb by SPR; Intact molecular mass: profile; Reduced and deglycosylated molecular masses of HC and LC: profiles; Reduced peptide map, profile; Reduced peptide map, post-translational modifications: levels of deamidation and oxidation; Non-reduced peptide map: profile; Glycan map: alpha-galactosylation, sialylation, and profile; cIEF: profile; Identity by ELISA; FTIR: profile; Near UV CD: profile; DSC: profile; SV-AUC: monomer, HMW, and profile; SE-HPLC-SLS: profile; SE-UHPLC: LMW and profile; rCE-SDS: HMW and profile; nrCE-SDS: post-peaks and profile; CEX-HPLC: profile; CEX-HPLC with carboxypeptidase B digestion: profile; 40°C forced degradation (reconstituted product): SE-UHPLC, rCE-SDS, nrCE-SDS, CEX-HPLC, Apoptosis inhibition bioassay; 40°C stressed stability (lyophilized product): SE-UHPLC, rCE-SDS, nrCE-SDS, CEX-HPLC, Apoptosis inhibition bioassay; 25°C accelerated stability (lyophilized product): SE-UHPLC, rCE-SDS, CEX-HPLC, Apoptosis inhibition bioassay; Reconstitution time.
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Assessor comment:

The sponsor has been developing ABP 710 as a biosimilar product to US-licensed Remicade. Because the sponsor used US-licensed Remicade as the comparator arm in the nonclinical and clinical programs and EU-approved Remicade was only used to collect data as part of the global file and presented for completeness, a scientific bridge to justify the use of clinical data generated using E.U.-approved Remicade is deemed unnecessary. Therefore, the comparative analytical assessment is performed between ABP 710 and US-licensed Remicade and the scope of this review does not include the evaluation of the analytical portion of the scientific bridge.

A series of state-of-the-art analytical methods was selected to assess similarity with respect to the function, structure, and heterogeneity of the US-licensed Remicade and ABP 710. The testing was performed either side-by-side or independently, based on the nature of the attribute and method capability. Because the stability-indicating attributes (e.g. size and charge variants) did not show a

significant change over the shelf life of US-licensed Remicade, age adjustment was not performed for the similarity assessment.

The sponsor took a tier-based strategy to assess the assay results for the comparative analytical assessment. The tier assignment is based on evaluations of the potential impact on efficacy, pharmacokinetics /pharmacodynamics (PK/PD), safety, and immunogenicity. This approach is acceptable. The TNF- α binding and apoptosis inhibition represent the primary mechanism of action and were categorized as high risk. Tier 1 statistical method -equivalence test was used for analyzing the results from these two assays. Quality Range was used as Tier 2 statistical method. Results from assays measuring quantitative quality attributes with high or moderate risk were analyzed with quality range method. Of note, the sponsor used "Expectation Approach" as a "Tier 2" statistical method to evaluate the data from assays, such as intact molecular mass, reduced and deglycosylated molecular masses of HC and LC, reduced peptide map, non-reduced peptide map, isoelectric point by cIEF, secondary structure by FTIR: spectral similarity, tertiary structure by near UV CD: spectral similarity, and molar mass of size variants by SE-HPLC-SLS. The quality attributes tested by these assays are primary and higher order structures, which are critical for the product mechanism of action. But the testing results are generally qualitative and graphical. Tier 3 graphical comparison method is more appropriate to assess the results from the above assays.

The overall design of similarity assessment appears reasonable and adequate.

3.2.R.1.5 Lots Tested in the comparative analytical assessment

Remicade drug product was sourced from both the US and EU over a period of approximately 6 years. A total of 28 US-licensed Remicade lots and 20 EU-approved Remicade lots, including all lots used in the clinical trials, were tested. The information on Drug Product Lot Number and Labeled Expiry Date was provided.

The US-licensed Remicade lots were evaluated as part of the comparative analytical assessment. For parameters expected to change as a function of time at their recommended storage condition, available Remicade lots were tested upon receipt and again at or near the end of shelf life.

All ABP 710 drug product lots that were used in clinical studies are represented in the similarity assessment. The lots included for the similarity assessment were based on the following considerations:

- For parameters primarily influenced by the drug substance manufacturing process (e.g., glycan profile and potency), each unique drug substance lot is represented once in the similarity assessment. A total of 14 ABP 710 drug substance lots were included in the similarity assessment represented by 12 drug product lots and 2 drug substance lots. The 2 drug substance lots were not filled into drug product and were therefore assessed directly. The ABP 710 drug substance lots include development lots, all lots used in clinical studies, and process validation lots.
- For parameters primarily influenced by the drug product manufacturing process (e.g., protein content and reconstitution time), a total of 19 drug product lots were included in the comparative analytical assessment. This encompasses all lots used in clinical studies and 3 drug product process validation lots.
- For stability-indicating attributes (i.e., product-related substances and impurities), drug product stability data were used to determine the product degradation rate.

ABP 710 lots used in the comparative analytical assessment and their site of manufacturing are provided in Table 3.2.R.3.

Table 3.2.R.3. ABP 710 Lots Included in the Comparative analytical assessment

Drug Product Lot Number	Drug Product Manufacturing Site	Drug Product Date of Manufacture	Drug Product Intended Use	Drug Substance Lot Number	Drug Substance Manufacturing Site	Drug Substance Manufacturing Scale	Drug Substance Date of Manufacture
0010189023 ^a	ATO	Mar 2014	Development, nonclinical, stability	0010194006 ^{b,c}	AWA	(b) (4)	Dec 2013
0010191102	ATO	Apr 2014	Development	0010201043	AWA		Mar 2014
0010190343 ^d	ATO	Jun 2014	Clinical, stability	0010191693	ATO		May 2014
0010194906	ATO	Jun 2014	Development	0010201044	AWA		May 2014
0010194907	ATO	Jun 2014	Development	0010201045	AWA		Jun 2014
0010190344	ATO	Aug 2014	Clinical, stability	0010201184	ATO		Jul 2014
0010222348 ^{e,f}	ATO	Apr 2016	Clinical	0010191693	ATO		May 2014
0010237659 ^e	ATO	Nov 2016	Clinical	0010267048	ATO		Jun 2016
0010314045 ^{e,f}	ATO	Feb 2017	Clinical	0010191693	ATO		May 2014
0010301972	ATO	Feb 2017	Clinical, stability	0010313936	ARI		Dec 2016
0010302149	ATO	Feb 2017	Clinical	0010313937	ARI		Dec 2016
0010337770 ^{e,f}	ATO	Jul 2017	Clinical	0010201184	ATO		Jul 2014
0010348408 ^{e,f}	ATO	Sep 2017	Clinical, stability	0010313936	ARI		Dec 2016
7i902	(b) (4)	Sep 2017	Development	0010307083	ARI		Oct 2016
0010328653 ^f	ATO	Sep 2017	Clinical	0010313936	ARI		Dec 2016
0010355582 ^{e,f}	ATO	Nov 2017	Clinical	0010313936	ARI		Dec 2016
0010367381	(b) (4)	Jan 2018	Process validation, stability	0010346358	ARI		Oct 2017
0010367383 ^f		Jan 2018	Process validation, stability	0010353290	ARI		Sep 2017
				0010346358	ARI		Oct 2017
				0010346359	ARI		Oct 2017
0010367384		Feb 2018	Process validation, stability	0010353290	ARI		Sep 2017
NA	NA	NA	NA	0010346359	ARI		Oct 2017
NA	NA	NA	NA	0010347635	ARI		Nov 2017

ARI = Amgen, Rhode Island, USA; ATO = Amgen Thousand Oaks, CA, USA; AWA = Amgen, W
(b) (4) NA = not applicable; PK = pharmacokinetic; WCB = on, USA;
g cell

bank

^a Lot used in nonclinical Study 118849.

^b Lot used to fill interim reference standard Lot 0010189002.

^c Lot was manufactured with pre-MCB. All subsequent lots were manufactured with WCB.

^d Lot used in PK Study 20140108.

^e Lot used in Rheumatoid Arthritis Study 20140111.

^f Lot used to assess attributes influenced by the drug product manufacturing process only.

Assessor comment:

28 US-licensed Remicade lots including all lots used in the clinical trials were used in the comparative analytical assessment. The labelled expiry dates ranged from February 2015 to February 2020 and these lots are deemed representative of the reference product.

A total of 19 ABP 710 drug product lots from 12 independent DS lots and 2 additional unfilled ABP 710 DS lots were used in the comparative analytical assessment, including all lots used in the clinical studies. The 12 independent DP lots and 2 additional independent DS lots were tested for parameters primarily influenced by the drug substance manufacturing process such as glycan profile and potency. All 19 DP lots were tested for parameters primarily influenced by the drug product manufacturing process such as protein content and reconstitution time. Drug product stability data were used to determine the product degradation rate for stability-indicating attributes including product-related substances and impurities. The selection and use of reference product lot and ABP 710 DP and DS lots in comparative analytical assessments are acceptable.

The OBP team participated in a pre-license inspection (PLI) of Immunex Rhode Island Corporation (also referred to as Amgen Rhode Island or ARI) at West Greenwich, RI from April 15-19, 2019. Overall, our assessment suggested that the similarity data and characterization assay descriptions presented in the BLA are accurate and representative of the raw data for ABP 710 and US-licensed Remicade collected by the sponsor.

3.2.R.1.6 Methods for comparative analytical assessment and validation/qualification

3.2.R.1.6.1 Methods for biological activity and validation/qualification

Table 3.2.R.4. Summary of methods used for biological activity and validation/qualification (prepared by the assessor)

Attribute	Attribute Test/ Parameter	Method Description	Qualification																		
Potency (apoptosis inhibition bioassay)	Inhibition of sTNF α -induced apoptosis in U937	The analytical procedure is the same as the method used for release testing.	RPT-080510: Method Validation Report for MET-003603. Refer to assessment in Section 3.2.S.4.																		
Binding to sTNF α	Binding to sTNF α by ELISA	The principle of the method is described in 3.2.S.3.1. In this ELISA, recombinant human TNF- α (rh TNF- α) is coated onto the wells of a microtiter ELISA plate. Dilutions of reference standard, control and test samples are added to bind to TNF- α adsorbed to the plate wells. Goat anti-human IgG conjugated to horseradish peroxidase (HRP) is added to bind to the ABP 710 or Remicade. A peroxidase substrate/ chromogen solution is added to the wells. The substrate changes color in the presence of HRP, in proportion to the amount of ABP 710 or Remicade bound to TNF- α . The reaction is stopped with 1.0 M phosphoric acid and absorbance is measured at 450 nm with a microplate reader. Software is used to analyze the data using a 4-parameter logical model. Relative binding activities of test samples are calculated based on the ratio of EC50 values of test samples relative to the ABP 710 reference standard. The results are reported as percent relative binding (% relative binding) values.	TRPT-029354: Method Qualification Report for TP-002064. The method has been qualified for the following performance characteristics with ABP 710 RS and rh TNF-α. The assay is qualified for method linearity over the binding range of 60% to 160% for ABP 710. The qualification target and results are shown below: <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>$r^2 \geq .98$</td><td>$r^2 = .9983$</td></tr><tr><td>Accuracy</td><td>The average % recovery between 80-120</td><td>The average % recovery 94.3</td></tr><tr><td>Intermediate precision</td><td>%CV ≤ 20</td><td>%CV = 6.6</td></tr><tr><td>Repeatability</td><td>% overall CV ≤ 20</td><td>% overall CV = 5.2</td></tr><tr><td>Specificity</td><td>No dose response with non-cognate control</td><td>No dose response with non-cognate control</td></tr></table> The method was also verified with US-licensed Remicade for linearity with binding levels in the range of 60-160%.	Performance Characteristics	Qualification Target	Qualification Result	Linearity	$r^2 \geq .98$	$r^2 = .9983$	Accuracy	The average % recovery between 80-120	The average % recovery 94.3	Intermediate precision	%CV ≤ 20	%CV = 6.6	Repeatability	% overall CV ≤ 20	% overall CV = 5.2	Specificity	No dose response with non-cognate control	No dose response with non-cognate control
Performance Characteristics	Qualification Target	Qualification Result																			
Linearity	$r^2 \geq .98$	$r^2 = .9983$																			
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Intermediate precision	%CV ≤ 20	%CV = 6.6																			
Repeatability	% overall CV ≤ 20	% overall CV = 5.2																			
Specificity	No dose response with non-cognate control	No dose response with non-cognate control																			

Binding to sTNFα	Binding to sTNFα by SPR	Binding was assessed by SPR using a Biacore T200 instrument with CM5 sensor chips. Signals were monitored at a flow rate of 50 μL/min. These conditions are applicable to all SPR methods in comparative analytical assessment unless otherwise mentioned. Anti-human IgG (Fc) antibody was immobilized on a chip surface. Samples were captured on the chip by anti-human IgG (Fc). Recombinant human (rh) sTNFα was used as the analyte. Each of the 5 sTNFα analyte concentrations from 30 to 0.370 nM (3-fold dilutions) were run in duplicate. The association and dissociation phases were monitored for 240 and 900 seconds, respectively. Results are reported as the average of 2 intra-assay replicates per lot. The data were analyzed using the Biacore T200 Evaluation software. The dissociation equilibrium binding constant (K_d) was determined using the 1:1 Langmuir binding model. The relative affinity of each sample relative to the reference standard (RS) was determined using the calculation: Relative Affinity (%RS) = (K_{d RS}/K_{d sample}) x 100 Results are reported as percent relative affinity values.	CLR-000252 for “qualification of the infliximab (ABP 710) -sTNF SPR binding assay using a Biacore T200” The method has been qualified for the following parameters with ABP 710 RS, Specificity Control (Rituximab), and rh sTNFα. Relative Affinity: <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Results</th></tr><tr><td>Specificity</td><td>No binding by the specificity control sample</td><td>No binding demonstrated by rituximab</td></tr><tr><td>Linearity</td><td>R² ≥ 0.9</td><td>0.9983</td></tr><tr><td>Accuracy</td><td>100±20%</td><td>100 ±17.0%</td></tr><tr><td>Range</td><td>50% - 200%</td><td>Qualified for a range of 50% - 200%</td></tr><tr><td>Intermediate Precision</td><td>%CV ≤20%</td><td>%CV ≤5.8%</td></tr><tr><td>Repeatability</td><td>% overall CV ≤20%</td><td>3.7 %</td></tr></table> Relative Binding <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Results</th></tr><tr><td>Linearity</td><td>R² ≥ 0.9</td><td>0.9673</td></tr><tr><td>Accuracy</td><td>100±20%</td><td>100 ±18.6%</td></tr><tr><td>Range</td><td>50% - 200%</td><td>Qualified for a range of 100% - 143%</td></tr><tr><td>Intermediate Precision</td><td>%CV ≤20%</td><td>%CV ≤6.7%</td></tr><tr><td>Repeatability</td><td>% overall CV ≤20%</td><td>3.2%</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Results	Specificity	No binding by the specificity control sample	No binding demonstrated by rituximab	Linearity	R ² ≥ 0.9	0.9983	Accuracy	100±20%	100 ±17.0%	Range	50% - 200%	Qualified for a range of 50% - 200%	Intermediate Precision	%CV ≤20%	%CV ≤5.8%	Repeatability	% overall CV ≤20%	3.7 %	Performance Characteristics	Qualification Target	Qualification Results	Linearity	R ² ≥ 0.9	0.9673	Accuracy	100±20%	100 ±18.6%	Range	50% - 200%	Qualified for a range of 100% - 143%	Intermediate Precision	%CV ≤20%	%CV ≤6.7%	Repeatability	% overall CV ≤20%	3.2%
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Repeatability	% overall CV ≤20%	3.2%																																								
	Binding kinetics to sTNFα by SPR	rh sTNFα was injected at 5 concentrations ranging from 33.3 to 0.412 nM (3-fold dilutions). Each of the 5 analyte sample concentrations were run in triplicate and in a mixed order. The association and dissociation phases were monitored 240 and 600 seconds, respectively. Results are reported as the average of 3 intra-assay replicates per lot. The data were analyzed using a SPR data processing and non-linear least squares regression fitting program. First, a dissociation rate coefficient (k_d) was determined by globally fitting the 600 second dissociation phase data to a simple exponential decay model. Second, this value was applied as a fixed parameter in the global fit of the 240 second association phase data to a 1:1 binding model to determine the association rate coefficient (k_a) and the K_d.	2018SE091555 for “Kinetic Rate Constants and Binding Constants for rhu TNFα Binding to ABP 710 and Infliximab (EU and US)” was provided to demonstrate the determination of the association and dissociation rate constants (k_a, k_d), and the dissociation equilibrium binding constant (K_d) for recombinant human (rh) TNFα binding to ABP 710 by surface plasmon resonance and to compare these affinities to those of Remicade sourced from the EU and US. All six lots of ABP 710 K_d values fall within the EU-approved Remicade K_d minimum and maximum values ± 20%, and all six lots of ABP 710 K_d values also lie within the US-licensed Remicade K_d minimum and maximum values ± 20%.																																							

Binding to sTNF α	Inhibition of sTNF α -induced IL-8 release in HUVEC	Human IL-8 homogeneous time resolved fluorescence (HTRF [®]) assay. Antibody dilutions were pre-incubated with sTNF α before transferring the mixture to the cells. Cells were incubated for 24 hours for IL-8 secretion. IL-8 detection reagent was added, and the plate was incubated in dark for 24 hours. The detection reagent consists of 2 non-competing anti-IL-8 antibodies labeled with a donor molecule (europium cryptate) on 1 and acceptor (allophycocyanin or XL665) on the other. When the 2 molecules are in close proximity, excitation of the donor antibody transfers energy to the acceptor antibody which fluoresces at a longer wavelength and is proportional to the amount of IL-8 present. Plates are read using an HTRF [®] compatible plate reader. Data are plotted as dose-response curves and fit to a 4-parameter logistic model. The percent relative binding of test samples is determined by comparing test sample curves to the reference standard curve. Results are reported as percent relative IL-8 inhibition.	R20150120 for “Qualification of the Method to Detect Neutralization of Human TNF α -induced Signaling in HUVEC by ABP 710 and Infliximab” Four independent qualification studies were run. Two of the assays used Remicade and two used ABP 710. The qualification target and results for each performance characteristic are shown below: <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Specificity</td><td>No significant activity with non-ABP 710 antibodies</td><td>No significant activity with non-ABP 710 antibodies</td></tr><tr><td>Linearity</td><td>R² $\geq$0.98</td><td>0.9917</td></tr><tr><td>Accuracy</td><td>The average % recovery between 80 to 120%.</td><td>97.8%</td></tr><tr><td>Intermediate Precision</td><td>%CV $\leq$20%</td><td>7.0%</td></tr><tr><td>Repeatability</td><td>overall %CV $\leq$20%</td><td>9.5%</td></tr></table> The potency range is 50-200%.	Performance Characteristics	Qualification Target	Qualification Result	Specificity	No significant activity with non-ABP 710 antibodies	No significant activity with non-ABP 710 antibodies	Linearity	R ² $\geq$ 0.98	0.9917	Accuracy	The average % recovery between 80 to 120%.	97.8%	Intermediate Precision	%CV $\leq$ 20%	7.0%	Repeatability	overall %CV $\leq$ 20%	9.5%
	Performance Characteristics	Qualification Target	Qualification Result																		
Specificity	No significant activity with non-ABP 710 antibodies	No significant activity with non-ABP 710 antibodies																			
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Intermediate Precision	%CV $\leq$ 20%	7.0%																			
Repeatability	overall %CV $\leq$ 20%	9.5%																			
Inhibition of sTNF α -induced cell death in L929	L-929 cells were plated and allowed time for attachment before exposure to actinomycin D to sensitize the cells to sTNF α . Antibody dilutions were pre-incubated with the cells after sensitization. sTNF α was then added to each well, and plates were placed in an incubator. After 24 hours, the medium was removed, and the number of remaining cells were lysed and quantified using an assay kit that measures ATP released from lysed cells. Luminescence correlated with cell number was measured using a plate reader and the data were plotted over the log of each antibody concentration. Data are plotted as dose-response curves and fit to a 4-parameter logistic model. The relative activity of test samples is determined by comparing test sample curves to the reference standard curve.	R20150121 for “Qualification of the Method to Detect ABP 710 Inhibition of Human TNF α -Induced Cytotoxicity in Sensitized L-929 Cells” The assay is qualified for use for method linearity over the potency range of 60% to 160% for ABP 710. The qualification target and results from 5 independent qualification studies are shown below: <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Specificity</td><td>No significant activity with non-ABP 710 antibodies</td><td>No significant activity with non-ABP 710 antibodies</td></tr><tr><td>Linearity</td><td>R² $\geq$0.98</td><td>0.997</td></tr><tr><td>Accuracy</td><td>The average % recovery between 80 to 120%.</td><td>100.8%</td></tr><tr><td>Intermediate Precision</td><td>% CV $\leq$20%</td><td>4.6%</td></tr><tr><td>Repeatability</td><td>% overall CV $\leq$20%</td><td>3.6%</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Specificity	No significant activity with non-ABP 710 antibodies	No significant activity with non-ABP 710 antibodies	Linearity	R ² $\geq$ 0.98	0.997	Accuracy	The average % recovery between 80 to 120%.	100.8%	Intermediate Precision	% CV $\leq$ 20%	4.6%	Repeatability	% overall CV $\leq$ 20%	3.6%	
Performance Characteristics	Qualification Target	Qualification Result																			
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Intermediate Precision	% CV $\leq$ 20%	4.6%																			
Repeatability	% overall CV $\leq$ 20%	3.6%																			

Binding to mbTNF α	Binding to mbTNF α on CHO MT-3 cells by imaging cytometry	The test sample competes with a fixed concentration of Alexa488-labeled ABP 710 for binding to the mbTNF α expressed on the engineered CHO cells. The dose-dependent decrease in cell-bound fluorescence was measured by an image cytometer. Data are plotted as dose-response curves and fit to a 4-parameter logistic model. The percent relative binding of test samples is determined by comparing test sample curves to the reference standard curve.	TRPT-024618 for “Method Qualification Report for the ABP 710 Cell-based Membrane Bound TNF alpha Binding Assay (TP-001686)” The results below indicate this assay was qualified for method linearity, precision, and accuracy over the binding range of 60% - 160%. The method is also specific and stability indicating. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>r² ≥ 0.98</td><td>r² = 0.9938</td></tr><tr><td>Accuracy</td><td>The average % recovery between 80-120</td><td>The average % recovery 105.4</td></tr><tr><td>Intermediate precision</td><td>%CV ≤20%</td><td>%CV = 7.4</td></tr><tr><td>Repeatability</td><td>%CV ≤20%</td><td>%CV = 4.9</td></tr><tr><td>Specificity</td><td>No dose response with non-cognate control</td><td>No dose response with non-cognate control</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Linearity	r ² ≥ 0.98	r ² = 0.9938	Accuracy	The average % recovery between 80-120	The average % recovery 105.4	Intermediate precision	%CV ≤20%	%CV = 7.4	Repeatability	%CV ≤20%	%CV = 4.9	Specificity	No dose response with non-cognate control	No dose response with non-cognate control
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	Reverse signaling in Jurkat mbTNF α line	An engineered Jurkat cell line constitutively expressing a non-cleavable version of mbTNF α was used to assess reverse signaling. Apoptosis is detected using fluorochrome-labeled Annexin V via flow cytometry. Propidium iodide (PI) was used for differentiation of membrane-compromised late stage apoptotic cells and dead cells (PI positive) from cells actively undergoing early stage apoptosis (PI negative, Annexin V positive). Data are plotted as dose-response curves and fit to a 4-parameter logistic model. The percent of relative reverse signaling for test samples is determined by comparing test sample curves to the reference standard curve. Results are reported as percent apoptotic cells.	TRPT-028522 for “Method Qualification Report for the Dose-Dependent Induction of Apoptosis by Anti-TNF α Antibodies in Jurkat. mbTNF Cells Assay (TP-002088) with ABP 710” The method was qualified for the potency range of 60% - 160% for ABP 710 and Remicade. In addition, the method is specific for anti-TNF α molecules such as ABP 710 and Remicade. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Specificity</td><td>No significant activity with antibodies which don't bind to TNFα</td><td>No significant activity with antibodies which don't bind to TNFα</td></tr><tr><td>Linearity</td><td>R² ≥0.98</td><td>0.9993</td></tr><tr><td>Accuracy</td><td>The average % recovery between 80 to 120%.</td><td>100.2%</td></tr><tr><td>Intermediate Precision</td><td>% CV ≤20%</td><td>5.3%</td></tr><tr><td>Repeatability</td><td>% overall CV ≤20%</td><td>4.8%</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Specificity	No significant activity with antibodies which don't bind to TNF α	No significant activity with antibodies which don't bind to TNF α	Linearity	R ² ≥0.98	0.9993	Accuracy	The average % recovery between 80 to 120%.	100.2%	Intermediate Precision	% CV ≤20%	5.3%	Repeatability	% overall CV ≤20%	4.8%
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Binding to LT α	Binding to LT α by SPR	ABP 710, Remicade (US or EU), or Amgen-manufactured etanercept (positive control) was captured on a chip surface using a goat-anti-human IgG1 antibody. A single saturating concentration of rh LT α (30 nM) was injected for 90 seconds followed by a dissociation time of 20 seconds. Immediately afterward, a saturating concentration of rh sTNF α (30 nM) was injected for 90 seconds followed by a dissociation time of 20 seconds. Each test sample was analyzed in duplicate. Capture and binding responses were assessed using Biacore software.	CLR-000217 for “qualification of ABP710 and commercial infliximab- LT α (TNF- β) negative binding assay using a BIACORE T200” The assay was qualified with the following results. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Results</th></tr><tr><td>Specificity</td><td>No significant binding of LTα to ABP 710 and commercial infliximab samples (<10% of binding response of the positive control TNF-α)</td><td>No apparent binding of commercial infliximab and ABP710 to LTα was observed (<10% of binding response of the positive control TNF-α)</td></tr><tr><td>Linearity (etanercept binding LTα)</td><td>R² ≥ 0.9</td><td>R² =0.95</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Results	Specificity	No significant binding of LT α to ABP 710 and commercial infliximab samples (<10% of binding response of the positive control TNF- α)	No apparent binding of commercial infliximab and ABP710 to LT α was observed (<10% of binding response of the positive control TNF- α)	Linearity (etanercept binding LT α)	R ² ≥ 0.9	R ² =0.95									
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Linearity (etanercept binding LT α)	R ² ≥ 0.9	R ² =0.95																			

Binding to LT α	Inhibition of LT α - induced IL-8 release in HUVEC	The method is analogous to the inhibition of sTNF α -induced IL-8 release in HUVEC bioassay except that LT α was substituted for sTNF α . A mouse IgG1 monoclonal anti-human LT α antibody was included in each assay run as a positive control.	R20150158 for “Qualification of the Method to Assess Specificity of ABP 710 on Lymphotoxin-a Mediated IL-8 Release in Human Umbilical Vein Endothelial Cells” This assay is not linear for ABP 710 as it does not inhibit LT α -mediated IL-8 signaling. This assay is specific, repeatable, and able to detect inhibition of IL-8 release when it occurs. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Specificity</td><td>Dose dependent activity with anti-LTα antibody</td><td>Dose dependent activity with anti-LTα antibody</td></tr><tr><td>Specificity</td><td>No significant activity with non-LTα antibodies</td><td>No significant activity with non-LTα antibodies</td></tr><tr><td>Repeatability</td><td>overall %CV $\leq$20%</td><td>2.2%</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Specificity	Dose dependent activity with anti-LT α antibody	Dose dependent activity with anti-LT α antibody	Specificity	No significant activity with non-LT α antibodies	No significant activity with non-LT α antibodies	Repeatability	overall %CV $\leq$ 20%	2.2%						
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Repeatability	overall %CV $\leq$ 20%	2.2%																			
Binding to Fc γ RIIIa (158V)	Binding to Fc γ RIIIa (158V) by SPR	rh Fc γ RIIIa (158V) was immobilized on a chip surface. Testing sample was injected at 6 concentrations ranging from 10 to 0.041 μ M (3-fold dilutions). Each of the 6 analyte sample concentrations were run in duplicate. The association and dissociation phases were monitored for 270 and 360 seconds, respectively. Results are reported as the average of 2 intra-assay replicates per lot. *Refer to the method for “Binding to sTNF α by SPR” for data analysis and report.	CLR-000226 for “qualification of ABP710 Fc γ RIIIa (158V and 158F alleles) binding assay using a BIACORE T200” The following qualification results support the suitability of this method for ABP710 and Fc γ RIIIa (158V) binding for sample concentrations between 0.123 μ M and 10 μ M. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Specificity</td><td>No binding with F(ab')₂ fragment lacking Fc region</td><td>No binding with F(ab')₂ fragment lacking Fc region</td></tr><tr><td>Linearity</td><td>R² $\geq$ 0.95</td><td>R²=0.99</td></tr><tr><td>Accuracy</td><td>100$\pm$20%</td><td>100.9-102.9</td></tr><tr><td>Repeatability</td><td>% overall CV $\leq$20%</td><td>%CV: $\leq$ 2.0</td></tr><tr><td>Intermediate precision</td><td>%CV $\leq$20%</td><td>%CV= 1.5</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Specificity	No binding with F(ab') ₂ fragment lacking Fc region	No binding with F(ab') ₂ fragment lacking Fc region	Linearity	R ² $\geq$ 0.95	R ² =0.99	Accuracy	100 $\pm$ 20%	100.9-102.9	Repeatability	% overall CV $\leq$ 20%	%CV: $\leq$ 2.0	Intermediate precision	%CV $\leq$ 20%	%CV= 1.5
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Binding to Fc γ RIIIa (158V)	Binding kinetics to Fc γ RIIIa (158V) by SPR	Each of the 7 rhu Fc γ RIIIa (158V) analyte concentrations ranging from 500 to 0.686 nM (3-fold dilutions) were run in triplicate. The association and dissociation phases were monitored for 120 and 240 seconds, respectively. Results are reported as the average of 3 intra-assay replicates per lot. The data were aligned and fit using an SPR data processing and non-linear least squares regression fitting program. The association and dissociation phase data were simultaneously, globally fit to a 1:1 binding model to determine k _a , k _d , and K _d .	CLR-000271 for “Kinetic Rate Constants and Binding Constants for rhu Fc γ RIIIa (158V) Binding to ABP 710 and Infliximab (EU and US)” was provided to demonstrate that the kinetic rate constants and binding constants of rhu Fc γ RIIIa (158V) for ABP 710 are similar to that of rhu Fc γ RIIIa (158V) for both Remicade (EU and US). All ten lots of ABP 710 K _d values fall within K _d minimum and maximum values $\pm$ 20% of the US-licensed or the EU-approved Remicade.																		

Binding to FcγRIIIa (158F)	Binding to FcγRIIIa (158F) by SPR	Similar to the method for Binding to FcγRIIIa (158V) by SPR above.	CLR-000226 The following qualification results support the suitability of this method for ABP710 and FcγRIIIa (158F) binding for sample concentrations between 0.123 μM and 10 μM <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Specificity</td><td>No binding with F(ab')2 fragment lacking Fc region</td><td>No binding with F(ab')2 fragment lacking Fc region</td></tr><tr><td>Linearity</td><td>R² ≥ 0.95</td><td>R²=0.99</td></tr><tr><td>Accuracy</td><td>100±20%</td><td>99.0-102.9</td></tr><tr><td>Repeatability</td><td>% overall CV ≤20%</td><td>%CV: ≤ 1.1</td></tr><tr><td>Intermediate precision</td><td>%CV ≤20%</td><td>%CV= 1.1</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Specificity	No binding with F(ab')2 fragment lacking Fc region	No binding with F(ab')2 fragment lacking Fc region	Linearity	R² ≥ 0.95	R²=0.99	Accuracy	100±20%	99.0-102.9	Repeatability	% overall CV ≤20%	%CV: ≤ 1.1	Intermediate precision	%CV ≤20%	%CV= 1.1
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Binding to FcγRIIIa	Binding to primary NK cells by FACS	Each test lot of ABP 710 or Remicade (US and EU) was serially diluted and combined with a fixed concentration of fluorescently labeled ABP 710. The mixture was incubated with freshly isolated PBMCs from 2 different FcγRIIIa (158V/F) heterozygous genotyped donors, allowing competition for FcγR binding to occur. After downstream processing, samples were analyzed by flow cytometry. NK cells (CD56-positive through antibody labeling) were assessed for competitive binding to cell surface FcγRIIIa between the fluorescently labeled ABP 710 and the serially diluted ABP 710 or Remicade (US and EU) test samples. Histograms representing the number of events and fluorescence intensity were plotted and presented as side-by-side overlays of 3-fold decreases in ABP 710 or Remicade (US and EU) lot protein concentrations which would show a dose-dependent decrease in fluorescently labeled ABP 710, CD56-positive NK cell populations. Histograms were generated using FlowJo data analysis software.	R20150195 for “Qualification of a Flow Cytometry Assay for Assessment of ABP 710 and Infliximab Binding to Fcγ Receptors on Primary Natural Killer Cells”. The following qualification results support that the method was fit-for-purpose for use as supportive data with a top assay concentration of 2357.5 μg/mL. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Specificity</td><td>Staining on expected cell types expressing FcγRs and lack of staining on unexpected cell types</td><td>Expected staining on NK cells populations was observed in PBMCs with no staining of CD56⁺ lymphocytes</td></tr><tr><td>Linearity</td><td>r² ≥ 0.9</td><td>r² = 0.91 for ABP 710 Reference Standard r² = 0.92 for infliximab</td></tr><tr><td>Reproducibility</td><td>Generation of similar dose response curves across test dates in a single donor</td><td>Dose response curves showed similar results across test dates</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Specificity	Staining on expected cell types expressing FcγRs and lack of staining on unexpected cell types	Expected staining on NK cells populations was observed in PBMCs with no staining of CD56 ⁺ lymphocytes	Linearity	r² ≥ 0.9	r² = 0.91 for ABP 710 Reference Standard r² = 0.92 for infliximab	Reproducibility	Generation of similar dose response curves across test dates in a single donor	Dose response curves showed similar results across test dates						
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NK92 ADCC	NK92 ADCC activity	The NK92 ADCC activity of ABP 710 was evaluated in a cell-based assay using CHO MT-3 cells as target cells (T) and NK92-M1 cells as effector cells (E). CHO MT-3 cells stably express a TNFα-converting enzyme (TACE)-resistant form of transmembrane TNFα. NK92-M1 cells were stably transfected with human CD16 (FcγRIIIa-158V). Target cells were labeled with 20 μM calcein-AM dye. The effector cells and labelled target cells were incubated at a 15:1 ratio with ABP 710 ranged from 0.01 ng/mL to 100,000 ng/mL. Upon ADCC-mediated target cell lysis, calcein is released into the culture medium and detected by a plate reader. The amount of culture supernatant fluorescence is proportional to the ADCC activity. Software is used to perform a 4-parameter data analysis and a constrained model curve fit to the dose-response data. ABP 710 test sample activity is determined by comparing the response to that of the ABP 710 reference standard and reported as percent relative ADCC activity.	TRPT-029332: 'ABP 710 Antibody Dependent Cellular Cytotoxicity (ADCC) Assay' Qualification Report (as updated in the IR response dated 7/29/2019) The following results (summarized by the Assessor) support that the method was fit for analyzing ABP 710 and Remicade ADCC activity. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>$r^2 \geq 0.98$</td><td>$R^2 = 0.993$</td></tr><tr><td>Accuracy</td><td>The average %</td><td>% overall recovery= 104.8</td></tr><tr><td>Intermediate Precision</td><td>% CV $\leq 20\%$</td><td>Overall %CV= 12.3</td></tr><tr><td>Repeatability</td><td>% overall CV $\leq 20\%$</td><td>Overall %CV= 7.0</td></tr><tr><td>Specificity (binding)</td><td>Specific for anti-TNF antibodies (ABP 710, Infliximab)</td><td>ABP 710 binds specifically to mbTNFα transfected CHO cells</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Linearity	$r^2 \geq 0.98$	$R^2 = 0.993$	Accuracy	The average %	% overall recovery= 104.8	Intermediate Precision	% CV $\leq 20\%$	Overall %CV= 12.3	Repeatability	% overall CV $\leq 20\%$	Overall %CV= 7.0	Specificity (binding)	Specific for anti-TNF antibodies (ABP 710, Infliximab)	ABP 710 binds specifically to mbTNF α transfected CHO cells
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PBMC ADCC	PBMC ADCC activity	PBMCs isolated from healthy donors, heterozygous for FcγRIIIa-158(V/F) are used as effector (E) cells. CHO cells constitutively expressing both a non-cleavable version of mbTNFα and a luciferase reporter gene are used as target (T) cells. Using previously determined donor-specific E:T ratios that would provide a suitable level of target death for the purposes of conducting a similarity assessment, target and effector cells are incubated with increasing concentrations of test samples for 20 to 22 hours. After incubation, Steady-Glo Luciferase Assay Reagent is added to the assay plates to measure intracellular luciferase activity released from membrane-compromised cells. The quantity of dead-cell luciferase in the medium is directly proportional to the number of dead cells in the culture. Data are plotted as dose-response curves and fit to a 4-parameter logistic model. The percent relative ADCC activity of test samples is determined by comparing test sample curves to the reference standard curve. Results are reported as percent relative ADCC activity.	CLR-000260 for “~~Draft Report:~~ Qualification of the ABP 710 and Infliximab ADCC (PBMC) Assay” (updated in the IR response dated 06/04/2019) The following qualification results support that the method was fit for the purpose of evaluating the ADCC activity of biosimilar and originator infliximab when samples are prepared at the same starting concentration, with a concentration range of 80% to 200%. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Accuracy</td><td>80% to 125%</td><td>80.9%-105.7%</td></tr><tr><td>Intermediate Precision</td><td>%CV $\leq 20\%$</td><td>Originator: 5%CV Biosimilar: 6%CV</td></tr><tr><td>Repeatability</td><td>%CV $\leq 20\%$</td><td>Originator: 13%CV Biosimilar: 5%CV</td></tr><tr><td>Dilutional Linearity</td><td>$R^2 \geq 0.98$</td><td>$R^2 = 0.9871$</td></tr><tr><td>Specificity</td><td>% death $\leq 40\%$</td><td>-16.84%</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Accuracy	80% to 125%	80.9%-105.7%	Intermediate Precision	%CV $\leq 20\%$	Originator: 5%CV Biosimilar: 6%CV	Repeatability	%CV $\leq 20\%$	Originator: 13%CV Biosimilar: 5%CV	Dilutional Linearity	$R^2 \geq 0.98$	$R^2 = 0.9871$	Specificity	% death $\leq 40\%$	-16.84%
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Binding to C1q	Binding to C1q by ELISA	A direct binding ELISA assay. ABP 710, US-licensed Remicade, or EU-approved Remicade is adsorbed to a microtiter plate and probed with C1q. Bound C1q is detected with an anti-C1q-horseradish peroxidase (HRP) conjugated antibody. HRP activity is then detected using a colorimetric substrate of the HRP enzyme. The reaction is stopped by 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 percent relative binding of test samples is determined by comparing test sample curves to the reference standard curve. Results are reported as percent relative C1q binding.	TRPT-030433 for “Method Qualification Report for ABP 710 C1q Binding Assay (TP-001975)”. The following results support that the method was fit for the purpose of evaluating the binding range of 60% -160%. The method was specific to binding of IgG1 samples with human C1q. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>$r^2 \geq 0.98$</td><td>$r^2 = 0.996$</td></tr><tr><td>Repeatability</td><td>% overall CV $\leq 20\%$</td><td>% overall CV = 7.2%</td></tr><tr><td>Accuracy</td><td>The average % recovery between 80-120</td><td>The average % recovery 101.2%</td></tr><tr><td>Intermediate precision</td><td>%CV $\leq 20\%$</td><td>%CV = 9.2%</td></tr><tr><td>Specificity</td><td>Specific for IgG1</td><td>Specificity demonstrated by lack of binding to IgG2/4 hybrid antibody</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Linearity	$r^2 \geq 0.98$	$r^2 = 0.996$	Repeatability	% overall CV $\leq 20\%$	% overall CV = 7.2%	Accuracy	The average % recovery between 80-120	The average % recovery 101.2%	Intermediate precision	%CV $\leq 20\%$	%CV = 9.2%	Specificity	Specific for IgG1	Specificity demonstrated by lack of binding to IgG2/4 hybrid antibody
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CDC	CDC activity	The principal of the method and method description is provided in 3.2.S.3.1. The CDC assay for ABP 710 uses the same target cells used in the ADCC assay. Target cells are loaded with calcein-AM, which readily enters the cells where it is cleaved by intracellular esterase to fluorescent calcein that remains trapped within the cells. Calcein-labeled target cells are opsonized with test antibody and incubated with baby rabbit complement. The supernatant is then removed to a fresh plate and the fluorescence measured. If CDC activity is present, lysed target cells release fluorescence calcein into the culture media. Therefore, the amount of supernatant fluorescence detected in a plate reader is proportional to the amount of cell death.	TRPT-029588: Method Qualification Report for ABP 710 and Infliximab Complement Dependent Cytotoxicity Assay (TP-001641) (as updated in the IR response dated 7/29/2019) The following results (summarized by the Assessor) support that the method was fit for analyzing ABP 710 and Remicade CDC activity. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>$R^2 \geq 0.98$</td><td>$r^2 = 0.9930$</td></tr><tr><td>Accuracy</td><td>The average % recovery 80 to 120</td><td>% overall recovery=98.3</td></tr><tr><td>Intermediate Precision</td><td>% CV $\leq 20\%$</td><td>Overall %CV= 8.0</td></tr><tr><td>Repeatability</td><td>% overall CV $\leq 20\%$</td><td>Overall %CV= 2.9</td></tr><tr><td>Specificity (binding)</td><td>Specific for anti-TNF antibodies (ABP 710, Infliximab)</td><td>ABP 710 binds specifically to mbTNFα transfected CHO cells</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Linearity	$R^2 \geq 0.98$	$r^2 = 0.9930$	Accuracy	The average % recovery 80 to 120	% overall recovery=98.3	Intermediate Precision	% CV $\leq 20\%$	Overall %CV= 8.0	Repeatability	% overall CV $\leq 20\%$	Overall %CV= 2.9	Specificity (binding)	Specific for anti-TNF antibodies (ABP 710, Infliximab)	ABP 710 binds specifically to mbTNF α transfected CHO cells
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Repeatability	% overall CV $\leq 20\%$	Overall %CV= 2.9																			
Specificity (binding)	Specific for anti-TNF antibodies (ABP 710, Infliximab)	ABP 710 binds specifically to mbTNF α transfected CHO cells																			

Binding to FcγRIIa (131R)	Binding to FcγRIIa (131R) by SPR	FcγRIIa (131R) was immobilized through standard amine coupling chemistry to a chip. Binding data were collected 4 seconds prior to the end of injection for each concentration of a duplicate sample dilution series. The Kd was determined using the steady state affinity model. The binding data generated from the assay run were used to determine the relative binding activity of the test samples in comparison to the reference standard.	CLR-000184 for “qualification of the infliximab FcγRIIa binding assay using a BIACORE T200” The following results (summarized by the Assessor) support that the method was fit for the purpose of evaluating the binding of a concentration ranges of 70% to 143% of the Reference Standard. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>r² ≥ 0.9</td><td>r² = 0 .9563</td></tr><tr><td>Repeatability</td><td>%CV ≤25.0%</td><td>%CV = 0.8%</td></tr><tr><td>Accuracy</td><td>±20%</td><td>11.2 to -3.5%</td></tr><tr><td>Specificity</td><td>lack of binding between ranibizumab and FcγRIIa</td><td>lack of binding to control ranibizumab</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Linearity	r ² ≥ 0.9	r ² = 0 .9563	Repeatability	%CV ≤25.0%	%CV = 0.8%	Accuracy	±20%	11.2 to -3.5%	Specificity	lack of binding between ranibizumab and FcγRIIa	lack of binding to control ranibizumab							
Performance Characteristics	Qualification Target	Qualification Result																							
Linearity	r ² ≥ 0.9	r ² = 0 .9563																							
Repeatability	%CV ≤25.0%	%CV = 0.8%																							
Accuracy	±20%	11.2 to -3.5%																							
Specificity	lack of binding between ranibizumab and FcγRIIa	lack of binding to control ranibizumab																							
ADCP	ADCP activity	CHO cells expressing non-cleavable human mbTNFα were labelled with eFluor670 for 10 minutes before dilution in growth medium. Labelled CHO cells were pelleted, suspended in FACS buffer, and combined with PBMCs in a 1:5 ratio. The PBMC were isolated from healthy donors with homozygous FcγRIIa (131H) allele. Titrated dilutions of ABP 710 or Remicade were added to each well. The cells were incubated with test sample, subsequently stained with anti-CD14 antibody for macrophages, and gated on the CD14 positive cell population. The ADCP-activity positive cell population was assessed based on both CD14 staining and the target cell label. Data were analyzed using FlowJo software, plotted as dose-response curves, and fitted to a 4-parameter logistic model. The percent relative ADCP activity of test samples is determined by comparing test sample curves to the reference standard curve and reported.	CLR-000221 for “Qualification of the infliximab ADCP (PBMC) Assay” The following results support that the method was fit for the purpose of evaluating the ADCP activity of a concentration ranges of 70% to 143% of the Reference Standard. <table><tr><th rowspan="2">Performance Characteristics</th><th rowspan="2">Qualification Target</th><th colspan="2">Qualification Result</th></tr><tr><th>ABP 710 RS</th><th>Infliximab</th></tr><tr><td>Linearity</td><td>r² ≥ 0.98 for a quantitative assay r² ≥ 0.9 for a qualitative assay</td><td>r² = 0.9975</td><td>r² = 0.9655</td></tr><tr><td>Accuracy</td><td>± 20%</td><td>± 9.7%</td><td>± 11.47%</td></tr><tr><td>Intermediate precision</td><td>%CV ≤ 20%</td><td>%CV = 5%</td><td>N/A</td></tr><tr><td>Repeatability</td><td>%CV ≤ 20%</td><td>%CV ≤ 9%</td><td>%CV = 0%</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result		ABP 710 RS	Infliximab	Linearity	r ² ≥ 0.98 for a quantitative assay r ² ≥ 0.9 for a qualitative assay	r ² = 0.9975	r ² = 0.9655	Accuracy	± 20%	± 9.7%	± 11.47%	Intermediate precision	%CV ≤ 20%	%CV = 5%	N/A	Repeatability	%CV ≤ 20%	%CV ≤ 9%	%CV = 0%
Performance Characteristics	Qualification Target	Qualification Result																							
		ABP 710 RS	Infliximab																						
Linearity	r ² ≥ 0.98 for a quantitative assay r ² ≥ 0.9 for a qualitative assay	r ² = 0.9975	r ² = 0.9655																						
Accuracy	± 20%	± 9.7%	± 11.47%																						
Intermediate precision	%CV ≤ 20%	%CV = 5%	N/A																						
Repeatability	%CV ≤ 20%	%CV ≤ 9%	%CV = 0%																						

Binding to FcγRIa	Binding to FcγRIa by AlphaLISA	The AlphaLISA binding assay is a bead-based amplified luminescent proximity homogeneous assay (Alpha) that detects bimolecular interactions. The assay contains 2 bead types, an acceptor (A) bead and a donor (D) bead. The A beads contain the fluorophore europium chelate and are coated with a hydrogel containing GSH to bind rh FcγRIa-GST. The D beads are coated with a hydrogel containing phthalocyanine (a photosensitizer) and streptavidin to capture biotinylated human IgG1. When FcγRIa-GST bind the biotinylated human IgG1, the A and D beads are brought into close proximity. An energy transfer to the A bead occurs when laser light is applied to this complex. The luminescence can be measured in a plate reader. With the presence of ABP 710 or Remicade (US or EU) at sufficient concentrations to inhibit the binding of FcγRIa-GST to the biotinylated human IgG1, a dose-dependent decrease in light emission occurs. Data are plotted as dose-response curves and fit to a 4-parameter logistic model. The percent relative FcγRIa binding of test samples is determined by comparing test sample curves to the reference standard curve and reported.	TRPT-030698 for “Qualification of TP-001698, FcGammaRIA AlphaLISA Binding Assay, for ABP 710 and Infliximab” The following results support that the method was fit for the purpose of evaluating the binding range of 50%-200%. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>R² ≥ 0.98</td><td>R² = 0.9986</td></tr><tr><td>Accuracy</td><td>Average % recovery between 80-120</td><td>Average % recovery = 100.5</td></tr><tr><td>Intermediate precision</td><td>%CV ≤ 20%</td><td>%CV = 3.7</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Linearity	R ² ≥ 0.98	R ² = 0.9986	Accuracy	Average % recovery between 80-120	Average % recovery = 100.5	Intermediate precision	%CV ≤ 20%	%CV = 3.7			
Performance Characteristics	Qualification Target	Qualification Result																
Linearity	R ² ≥ 0.98	R ² = 0.9986																
Accuracy	Average % recovery between 80-120	Average % recovery = 100.5																
Intermediate precision	%CV ≤ 20%	%CV = 3.7																
Binding to FcγRIIb	Binding to FcγRIIb by SPR	An SPR assay analogous to the FcγRIIa binding assay except that the chip was coated with rh FcγRIIb.	CLR-000185 for “qualification of the Remicade FcγRIIb binding assay using a BIACORE T200” The following results (summarized by the Assessor) support that the method was fit for the purpose of evaluating the binding of a concentration ranges of 80 - 125% of the Reference Standard. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>r² ≥ 0.9</td><td>r² = 0.9889</td></tr><tr><td>Repeatability</td><td>%CV ≤25.0%</td><td>%CV = 5.1 %.</td></tr><tr><td>Accuracy</td><td>±20%</td><td>-3.6 to -11.2%</td></tr><tr><td>Specificity</td><td>lack of binding between ranibizumab and FcγRIIb</td><td>lack of binding to contro ranibizumab</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Linearity	r ² ≥ 0.9	r ² = 0.9889	Repeatability	%CV ≤25.0%	%CV = 5.1 %.	Accuracy	±20%	-3.6 to -11.2%	Specificity	lack of binding between ranibizumab and FcγRIIb	lack of binding to contro ranibizumab
Performance Characteristics	Qualification Target	Qualification Result																
Linearity	r ² ≥ 0.9	r ² = 0.9889																
Repeatability	%CV ≤25.0%	%CV = 5.1 %.																
Accuracy	±20%	-3.6 to -11.2%																
Specificity	lack of binding between ranibizumab and FcγRIIb	lack of binding to contro ranibizumab																

Binding to FcγRIIIb	Binding to FcγRIIIb by SPR	An SPR assay analogous to the FcγRIIa (131R) binding assay except that the chip was coated with rh FcγRIIIb.	CLR-000223“qualification of the infliximab FcγRIIIb binding assay using a BIACORE T200” The following results support that the method was fit for the purpose of evaluating the binding of a concentration ranges of 70 - 143% of the Reference Standard. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Specificity</td><td>No binding with F(ab')2 fragment lacking Fc region</td><td>No binding with F(ab')2 fragment lacking Fc region</td></tr><tr><td>Linearity</td><td>R² ≥ 0.95</td><td>R²=0.98</td></tr><tr><td>Accuracy</td><td>100±20.0%</td><td>88.6-98.5%</td></tr><tr><td>Repeatability</td><td>% overall CV ≤20%</td><td>%CV: ≤ 5.3</td></tr><tr><td>Intermediate precision</td><td>%CV ≤20%</td><td>%CV= 4.1</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Specificity	No binding with F(ab')2 fragment lacking Fc region	No binding with F(ab')2 fragment lacking Fc region	Linearity	R ² ≥ 0.95	R ² =0.98	Accuracy	100±20.0%	88.6-98.5%	Repeatability	% overall CV ≤20%	%CV: ≤ 5.3	Intermediate precision	%CV ≤20%	%CV= 4.1			
Performance Characteristics	Qualification Target	Qualification Result																						
Specificity	No binding with F(ab')2 fragment lacking Fc region	No binding with F(ab')2 fragment lacking Fc region																						
Linearity	R ² ≥ 0.95	R ² =0.98																						
Accuracy	100±20.0%	88.6-98.5%																						
Repeatability	% overall CV ≤20%	%CV: ≤ 5.3																						
Intermediate precision	%CV ≤20%	%CV= 4.1																						
Binding to FcRn	Binding to FcRn by AlphaScreen	A competitive AlphaScreen binding assay. Refer to the description of the method for “Binding to FcγRIa by AlphaLISA” for details. The differences are that the A beads are coated with a hydrogel that contains a nickel chelate binding to FcRn-His and that the D beads are coated with streptavidin binding to biotinylated CHO-derived human Fc.	TRPT-030739: Method Qualification Report for the AlphaScreen FcRn Binding Assay for ABP 710 and Infliximab (TP-001359). (submitted in eCTD0013 dated 06/04/2019, replacement of the initially submitted TRPT-024695) The following results support that the method was fit for the purpose of evaluating analyzing ABP 710 and infliximab for FcRn binding. <table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Linearity</td><td>r² ≥ 0.98</td><td>r² = 0.9977</td></tr><tr><td>Range</td><td>60%-160%</td><td>60 - 160%</td></tr><tr><td>Accuracy</td><td>The average % recovery between 80-120</td><td>The average % recovery 97</td></tr><tr><td>Intermediate Precision</td><td>%CV ≤20%</td><td>Overall %CV= 8.3</td></tr><tr><td>Repeatability precision</td><td>%CV ≤20%</td><td>%CV ≤4.5%</td></tr><tr><td>Specificity</td><td>Binding is specific to Fc containing molecules</td><td>No binding observed to a control protein (BSA) without Fc domain</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Linearity	r ² ≥ 0.98	r ² = 0.9977	Range	60%-160%	60 - 160%	Accuracy	The average % recovery between 80-120	The average % recovery 97	Intermediate Precision	%CV ≤20%	Overall %CV= 8.3	Repeatability precision	%CV ≤20%	%CV ≤4.5%	Specificity	Binding is specific to Fc containing molecules	No binding observed to a control protein (BSA) without Fc domain
Performance Characteristics	Qualification Target	Qualification Result																						
Linearity	r ² ≥ 0.98	r ² = 0.9977																						
Range	60%-160%	60 - 160%																						
Accuracy	The average % recovery between 80-120	The average % recovery 97																						
Intermediate Precision	%CV ≤20%	Overall %CV= 8.3																						
Repeatability precision	%CV ≤20%	%CV ≤4.5%																						
Specificity	Binding is specific to Fc containing molecules	No binding observed to a control protein (BSA) without Fc domain																						

Binding to FcRn	Binding to FcRn by SPR	An SPR assay analogous to the FcγRIIa (131R) binding assay (Section 19.1) was developed, except that the CM5 chip was coated with rh FcRn.	CLR-000254 for “qualification of the ABP 710 FcRn binding assay using a BIACORE T200”																					
			The following results support that the method was fit for the purpose.																					
			<table><tr><th>Performance Characteristics</th><th>Qualification Target</th><th>Qualification Result</th></tr><tr><td>Accuracy</td><td>100 ±20%</td><td>88.6-96.8%</td></tr><tr><td>Range</td><td>50% to 200%</td><td>50% to 200%</td></tr><tr><td>Intermediate precision</td><td>%CV ≤20%</td><td>%CV ≤6.7%</td></tr><tr><td>Repeatability</td><td>% overall CV ≤20%</td><td>1.8%</td></tr><tr><td>Linearity</td><td>R² ≥ 0.95</td><td>R² = 0.95</td></tr><tr><td>Specificity</td><td>Lack of quantifiable binding to F(ab')2</td><td>No binding with F(ab')2 fragment lacking Fc region</td></tr></table>	Performance Characteristics	Qualification Target	Qualification Result	Accuracy	100 ±20%	88.6-96.8%	Range	50% to 200%	50% to 200%	Intermediate precision	%CV ≤20%	%CV ≤6.7%	Repeatability	% overall CV ≤20%	1.8%	Linearity	R ² ≥ 0.95	R ² = 0.95	Specificity	Lack of quantifiable binding to F(ab')2	No binding with F(ab')2 fragment lacking Fc region
			Performance Characteristics	Qualification Target	Qualification Result																			
			Accuracy	100 ±20%	88.6-96.8%																			
			Range	50% to 200%	50% to 200%																			
			Intermediate precision	%CV ≤20%	%CV ≤6.7%																			
Repeatability	% overall CV ≤20%	1.8%																						
Linearity	R ² ≥ 0.95	R ² = 0.95																						
Specificity	Lack of quantifiable binding to F(ab')2	No binding with F(ab')2 fragment lacking Fc region																						

Assessor comment:

The sponsor selected assays/attributes for similarity assessment of biological activities between ABP 710 and US-licensed Remicade based on the primary and potential mechanisms of action. The biological functions required for clinical efficacy are primarily mediated by the binding and neutralization of sTNFα bioactivity. Modulation of ADCC, CDC, and reverse signaling activity may be relevant to the efficacy in inflammatory bowel diseases (IBD) while the clinical relevance of ADCP activity is unknown. To demonstrate biological activities relevant to the primary and potential mechanisms are similar, ABP 710 and US-licensed Remicade were evaluated by a comprehensive set of functional assays and binding studies using a tier-based system. All methods appear appropriate for the intended uses. The following information requests were sent regarding method qualification:

Information Request (Question 1, sent on 05/21/2019)

CLR-000260 is a “Draft Report: Qualification of the ABP 710 and Infliximab ADCC (PBMC) Assay”. Provide the final report for Qualification of the ABP 710 and Infliximab ADCC (PBMC) Assay.

The sponsor’s response dated 06/04/2019:

The final report for “Qualification of the ABP 710 and Infliximab ADCC (PBMC) Assay” is provided (Method Qualification Report – CLR-000260 – PBMC ADCC).

No changes were made in content or information between the draft and final versions of the report. The only update is the status of the document.

Assessor comment:

The sponsor’s update with clarification is acceptable. The method qualification appears adequate.

Information Request (Question 2, sent on 05/21/2019)

In TRPT-024695 “Method Qualification Report for the AlphaScreen FcRn Binding Assay for Ig-Fc Containing Molecules Utilizing Solution Phase Binding (TP-001359)”, the “Qualification Targets for Performance Characteristics” were not included. Provide the “Qualification Targets for Performance Characteristics” and update this method qualification report.

The sponsor’s response dated 06/04/2019:

The ABP 710 method qualification report for the AlphaScreen FcRn Binding Assay is provided (Method Qualification Report – TRPT-030739 – FcRn binding). This report contains requested “Qualification Targets for Performance Characteristics”.

Please note that this report, TRPT-030739, replaces the initially submitted TRPT-024695 “Method Qualification Report for the AlphaScreen FcRn Binding Assay for Ig-Fc Containing Molecules Utilizing Solution Phase Binding (TP-001359)”. TRPT-024695 is an initial qualification report for the AlphaScreen FcRn binding method and is not specific for a particular molecule.

Assessor comment:

The sponsor’s response is acceptable.

Information Request (Question 1, sent on 07/22/2019)

In TRPT-029332: 'ABP 710 Antibody Dependent Cellular Cytotoxicity (ADCC) Assay' Qualification Report, the “Qualification Targets for Performance Characteristics” were not included. Provide the “Qualification Targets for Performance Characteristics” and update this method qualification report.

Assessor comment:

In the response dated 07/29/2019, the sponsor provided the qualification targets for performance characteristics for qualification of the ADCC method, which have been integrated in Table 3.2.R.4 above. The method in Module 3 has been updated accordingly. The method qualification is deemed adequate.

Information Request (Question 2, sent on 07/22/2019)

In TRPT-029588: Method Qualification Report for ABP 710 and Remicade Complement Dependent Cytotoxicity Assay (TP-001641), the “Qualification Targets for Performance Characteristics” were not included. Provide the “Qualification Targets for Performance Characteristics” and update this method qualification report.

Assessor comment:

In the response dated 07/29/2019, the sponsor provided the qualification targets for performance characteristics for qualification of the CDC method, which have been integrated in Table 3.2.R.4 above. The method in Module 3 has been updated accordingly. The method qualification is acceptable.

In conclusion, the methods for assessing biological activities of ABP 710 and US-licensed Remicade were appropriate and adequately qualified for the intended use.

3.2.R.1.6.2 Other methods used in comparative analytical assessment and validation/qualification

The following analytical methods used in the evaluation of quality attributes by Tier 2 or Tier 3 approaches are the same as those used for in-process and/or release testing for ABP 710 DS and DP. These methods have been validated and the evaluation of the method validation reports is in Section 3.2.S.4.2.

Table 3.2.R.5. Summary of other validated methods used in comparative analytical assessment (by the Assessor)

Quality attributes	Analytical methods	Parameters for Quality Range	Parameters for Graphic Comparison
Size variants	SE-UHPLC	HMW (%), main peak (%)	LMW (%), profile. All related parameters in Thermal Stability and Degradation studies.
	CE-SDS	HC +LC (%), LMW +MMW (%), NGHC (%)	HMW (%), profile. All related parameters in Thermal Stability and Degradation studies.

	nrCE-SDS	pre-peak (%), main peak (%)	post-peaks (%), profile. All related parameters in Thermal Stability and Degradation studies
Charge variants	CEX-HPLC	main peak (%), acidic peak (%), basic peak (%)	Profile. All related parameters in Thermal Stability and Degradation studies.
Glycosylation	glycan map	afucosylation (%), high mannose (%), β -galactosylation (%).	alpha-galactosylation (%), sialylation (%), profile
Protein content	UV absorbance	Protein content	
Reconstituted protein concentration	UV absorbance	Reconstituted protein concentration	
reconstitution time	Reconstitution time		Reconstitution time

Results from the following protein structure characterization methods are assessed by Tier 2 quality range or Tier 3 graphic comparison approach.

Table 3.2.R.6. Summary of other methods used in comparative analytical assessment with adequate information provided in the submission (by the Assessor)

Quality attributes	Analytical methods	Parameters for Tier 2 approach	Parameters for Tier 3 approach
Molecular weight	ESI-TOF-MS without or with PNGaseF treatment		Intact molecular mass and profile; Reduced and deglycosylated molecular masses of HC and LC and profile
Amino acid sequence	Reduced peptide map		Reduced peptide map, profile
Post-translational modifications	Reduced peptide map		levels of deamidation and oxidation
Disulfide structure	Non-reduced peptide map		Non-reduced peptide map and profile
Isoelectric point	cIEF		Main Peak pI and cIEF profile
Secondary structure	FTIR		spectral similarity (%) and profile
Tertiary structure	Near UV CD		spectral similarity (%) and profile
	DSC	T _{m1} and T _{m2}	DSC profile
Solution-state size distribution	SV-AUC		monomer (%), HMW (%), profile
Molar mass of size variants	SE-HPLC-SLS		molar mass and profile

Assessor comment:

Besides the methods for assessing biological activities of both products, other methods used in comparative analytical assessment are considered appropriately validated or qualified for their intended purpose. The provided information is deemed acceptable.

3.2.R.2. Results for Comparative analytical assessment

3.2.R.2.1. Summary of the comparative analytical assessment results

Table 3.2.R.7. Summary of Analytical Similarity Results (prepared by assessor)

Biological Activity							
Attribute	Test/Parameter	Tier	Number of lots (US: ABP)	Data range		Similarity Assessment Criteria (SAC) (EAC= equivalence acceptance criterion;	Met SAC? (ABP vs US)
				US-licensed Remicade (US-R)	ABP710		
Potency (apoptosis inhibition bioassay)	Inhibition of sTNF α -induced apoptosis in U937 (%)	1	28:14	78 to 117	87 to 112 90% CI vs US: (-6.69, 1.69) (-7.00, 2.00) ^a	Mean-based $\pm$ 13.58 (EAC)	Yes
Binding to sTNF α	Binding to sTNF α by ELISA (%)	1	25:14	88 to 112	88 to 109 90% CI vs US: (-1.64, 4.20) (-1.73, 4.29) ^a	Mean-based $\pm$ 7.85 (EAC)	Yes
	Binding to sTNF α by SPR (%)	2	10:10	90 to 105	92 to 109	84 to 108 (QR)	Yes
	Binding kinetics to sTNF α by SPR/ Kd (pM)	3	6:6	109.9 to 143.6	113.1 to 134.9	Qualitative	Yes
	Inhibition of sTNF α -induced IL-8 release in HUVEC (%)	3	3:3	98-116	97-102	Qualitative	Yes
	Inhibition of sTNF α -induced cell death in L929 (%)	3	3:3	100-119	94-104	Qualitative	Yes
Binding to mbTNF α	Binding to mbTNF α on CHO MT-3 cells by imaging cytometry (%)	2	25:14	91 to 114	92 to 108	89 to 119 (QR)	Yes
	Reverse signaling in Jurkat mbTNF α line (%)	2	24:14	90 to 114	95 to 109	86 to 119(QR)	Yes
Binding to LT α	Binding to LT α by SPR	3	3:3	no binding	no binding	Qualitative	Yes
	Inhibition of LT α -induced IL-8 release in HUVEC	3	3:3	No inhibition due to lack of binding to LT α	No inhibition due to lack of binding to LT α	Qualitative	Yes
Binding to Fc γ RIIIa (158V)	Binding to Fc γ RIIIa (158V) by SPR (%)	2	10:10	82 to 105	98 to 117	75 to 119(QR)	Yes
	Binding kinetics to Fc γ RIIIa (158V) by SPR /Kd (nM)	3		62.7-92.6	68.2-80.2	Qualitative	Yes
Binding to Fc γ RIIIa	Binding to Fc γ RIIIa (158F) by SPR (%)	2	10:10	86 to 108	88 to 119	74 to 117(QR)	Yes
Binding to Fc γ RIIIa	Binding to primary NK cells by FACS	3	3:3	Visually qualified profile	Visually similar ^b	Qualitative	Yes
NK92 ADCC	NK92 ADCC activity (%)	2	27:14	80 to 169	94 to 133	56 to 195(QR)	Yes
PBMC ADCC	PBMC ADCC activity (%)	2	10:10	82 to 119	90 to 118	66 to 135(QR)	Yes
Binding to C1q	Binding to C1q by ELISA (%)	2	25:14	60 to 93	83 to 104	52 to 105(QR)	Yes
CDC	CDC activity (%)	2	27:14	93 to 136	93 to 110	76 to 134(QR)	Yes

Binding to FcγRIIa (131R)	Binding to FcγRIIa (131R) by SPR (%)	3	3:3	98-99	101-104	Qualitative	Yes
ADCP	ADCP activity (%)	3	3:3	94-116	97-104	Qualitative	Yes
Binding to FcγRIa	Binding to FcγRIa by AlphaLISA (%)	3	3:3	92-95	92-99	Qualitative	Yes
Binding to FcγRIIb	Binding to FcγRIIb by SPR (%)	3	3:3	91-99	91-105	Qualitative	Yes
Binding to FcγRIIIb	Binding to FcγRIIIb by SPR (%)	3	10:10	91-111	96-117	Qualitative	Yes
Binding to FcRn	Binding to FcRn by AlphaScreen (%)	2	27:14	92-108	90-123	85 to 113(QR)	Yes
	Binding to FcRn by SPR (%)	2	10:10	93-134	91-109	76 to 157(QR)	Yes
Primary Structure							
Attribute	Test/Parameter	Tier	Number of lots (US: ABP)	Data range		Similarity Assessment Criteria (SAC) (QR=Quality Range)	Met SAC? (ABP vs US)
				US-licensed Remicade (US-R)	ABP710		
Molecular weight	Intact molecular mass	3	1:1	9 mass peaks (A-I) within 100 ppm of the theoretical masses	9 mass peaks (A-I) within 100 ppm of the theoretical masses	Observed mass should be within ± 100 ppm of the theoretical mass for the predominant species	Yes
	Intact molecular mass: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
	Reduced and deglycosylated molecular masses of HC and LC	3	1:1	Difference from theoretical mass: LC: 26ppm HC: 17ppm HC+K: 24ppm	Difference from theoretical mass: LC: 3ppm HC: 4ppm HC+K: 4ppm	Observed mass should be within ± 100 ppm of the theoretical mass	Yes
	Reduced and deglycosylated molecular masses of HC and LC: profiles	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
Amino acid sequence	Reduced peptide map	3	6:9	The observed molecular masses tryptic peptides were within ± 50 ppm of the theoretical masses expected for US-licensed Remicade.	The observed molecular masses tryptic peptides were within ± 50 ppm of the theoretical masses expected for US-licensed Remicade.	Observed mass of the tryptic peptide fragments should be within ± 50 ppm of the theoretical mass for US-licensed Remicade	Yes
	Reduced peptide map, profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes

Post-translational modifications	Reduced peptide map, post-translational modifications: levels of deamidation and oxidation	3	6:9	% modifications profile	Oxidation of HC Met ²⁵⁵ and Met ⁴³¹ , deamidation of HC Asn ⁵⁷ , and Hydroxylysine of HC Lys ⁵⁷ are higher than that of US-R. Others are similar to or lower than that of US-R.	Qualitative	Minor difference
Disulfide structure	Non-reduced peptide map	3	1:1	The expected disulfide linkages were present.	The expected disulfide linkages were present.	Observed mass of the peptide fragments should be within ± 50 ppm of the theoretical	Yes
	Non-reduced peptide map: profile	3	1:1	Visually qualified profile	Visually similar ^b	Qualitative	Yes
Glycosylation	Glycan map: afucosylation (%)	2	28:14	6.7 to 11.1	6.1 to 7.3	5.3 to 12.6(QR)	Yes
	Glycan map: high mannose (%)	2		3.0 to 5.5	3.2 to 4.2	2.1 to 6.6(QR)	Yes
	Glycan map: β -galactosylation (%)	2		25.9 to 52.0	25.1 to 34.2	16.0 to 51.9(QR)	Yes
	Glycan map: α -galactosylation (%)	3		1.6 to 6.6	N/A	Qualitative	Minor difference
	Glycan map: sialylation (%)	3		3.1 to 10.0	0.9 to 1.6	Qualitative	
	Glycan map: profile	3		Visually qualified profile	visually similar for the major glycans with minor differences observed in α -galactosylation and sialylation	Qualitative	
Isoelectric point	cIEF	3	1:1	Main Peak pI is 7.4 regardless of CPB treatment	Main Peak pI is 7.4 regardless of CPB treatment	± 0.1 pI unit	Yes
	cIEF: profile	3		Visually qualified profile	Visually similar ^b minor differences due to C-terminal lysine processing.	Qualitative	Yes

Identity	Identity by ELISA	3	3:14	Pass, all optical absorbance signal-to-background ratios for sample replicates of ≥ 10.0 .	Pass, all optical absorbance signal-to-background ratios for sample replicates of ≥ 10.0 .	Qualitative	Yes
Higher Order Structure							
Attribute	Test/Parameter	Tier	Number of lots (US: ABP)	Data range		Similarity Assessment Criteria (SAC) (QR=Quality Range)	Met SAC? (ABP vs US)
				US-licensed Remicade (US-R)	ABP710		
Secondary structure	FTIR: spectral similarity (%)	3	3:6	99.7-100	99.7-99.8	$\geq 95\%$ spectral similarity	Yes
	FTIR: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
Tertiary structure	Near UV CD: spectral similarity (%)	3	3:6	98.9-100	97.5-99.1	$\geq 95\%$ spectral similarity	Yes
	Near UV CD: profile	3		Visually qualified profile	Well overlapped with US-R	Qualitative	Yes
	DSC: T _{m1} (°C)	2	6:9	67.6 to 67.8	67.7 to 67.9	67.5 to 67.9 (QR)	Yes
	DSC: T _{m2} (°C)	2		83.3 to 84.2	83.3 to 84.3	82.5 to 85.1 (QR)	Yes
	DSC: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
Particles and Aggregates							
Attribute	Test/Parameter	Tier	Number of lots (US: ABP)	Data range		Similarity Assessment Criteria (SAC)	Met SAC? (ABP vs US)
				US-licensed Remicade (US-R)	ABP710		
Solution-state size distribution	SV-AUC: monomer (%)	3	3:3	98.9-99.7	98.4-99.4	Qualitative	Yes
	SV-AUC: HMW (%)	3		<LOQ (3.7%)	<LOQ (3.7%)	Qualitative	Yes
	SV-AUC: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
Molar mass of size variants	SE-HPLC-SLS: molar mass	3	3:3	Main Peak (kDa): 151-152 HMW (kDa): 292-319	Main Peak (kDa): 150-151 HMW (kDa): 284-287	HMW species within 10% and main peak within 5% of the expected molar masses	Yes
	SE-HPLC-SLS: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
Product-related Substances and Impurities							

Attribute	Test/Parameter	Tier	Number of lots (US: ABP)	Data range		Similarity Assessment Criteria (SAC) (QR=Quality Range)	Met SAC? (ABP vs US)
				US-licensed Remicade (US-R)	ABP710		
Size variants	SE-UHPLC: HMW (%)	2	28:12	0.2 to 0.3	0.7 to 1.3	0.1 to 0.4 (QR)	No
	SE-UHPLC: main peak (%)	2		99.3 to 99.8	98.6 to 99.2	99.4 to 99.9 (QR)	
	SE-UHPLC: LMW (%)	3		below the LOQ of the method (0.1%)	below the LOQ of the method (0.1%)	Qualitative	Yes
	SE-UHPLC: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
	rCE-SDS: HC +LC (%)	2	28:12	98.2 to 98.9	96.6 to 97.3	98.3 to 99.1 (QR)	No
	rCE-SDS: LMW +MMW (%)	2		0.3 to 1.1	0.7 to 1.0	0.1 to 0.9 (QR)	
	rCE-SDS: NGHC (%)	2		0.4 to 0.6	1.6 to 2.1	0.4 to 0.6 (QR)	
	rCE-SDS: HMW (%)	3		below the LOQ of the method (0.4%)	below the LOQ of the method (0.4%)	Qualitative	Yes
	rCE-SDS: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
	nrCE-SDS: pre-peak (%)	2	28:12	1.1 to 2.2	1.8 to 2.6	0.7 to 2.1 (QR)	No
	nrCE-SDS: main peak (%)	2		97.8 to 98.9	97.1-98.1	97.8 to 99.3 (QR)	
	nrCE-SDS: post-peaks (%)	3		below the LOQ of the method (0.4%)	below the LOQ of the method (0.4%)	Qualitative	Yes
	nrCE-SDS: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
Charge variants	CEX-HPLC: main peak (%)	2	28:12	31.7-51.7	43.5-47.7	29.7 to 51.6 (QR)	Yes
	CEX-HPLC: acidic peak (%)	2		9.9-15.8	19.5-23.6	8.4 to 17.8 (QR)	No
	CEX-HPLC: basic peak (%)	2		34.2-56.2	30.0-36.1	32.3 to 60.2 (QR)	
	CEX-HPLC: profile	3		Visually qualified profile	Visually similar ^b	Qualitative	Yes
	CEX-HPLC with carboxypeptidase B digestion: profile	3	3:3	Visually qualified profile	Visually similar ^b	Qualitative	Yes
General Properties							
Attribute	Test/Parameter	Tier	Number of lots (US: ABP)	Data range		Similarity Assessment Criteria (SAC) (QR=Quality Range)	Met SAC? (ABP vs US)
				US-licensed Remicade (US)	ABP710		

Protein content	UV absorbance (mg/vial)	2	26:19	97 to 102	96 to 99	95 to 104(QR)	Yes
Reconstituted protein concentration	UV absorbance (mg/mL)	2		9.2 to 9.8	9.1 to 9.6	9.1 to 10.0(QR)	Yes
Reconstitution time	Reconstitution time (s)	3	3:19	31-47	12-68	Qualitative	Yes

- An additional analysis was performed for ABP 710 and US-licensed Remicade using an equivalence test that implemented a correction for sample size imbalance. Sample sizes are considered imbalanced when the ratio of the number of reference lots tested to the number of comparator lots tested exceeds a 1.5-fold difference. The equivalence test taking into account sample size imbalance is outlined in 3.2.R Regional Information (Testing Plan and Assessment Criteria).
- Visually similar = no new peaks/species greater than the integrity limit of the method
- The cells were highlighted in yellow if the SAC was not met or minor differences were observed.

Assessor comment:

The attributes, tests/parameters, tier assignments, batch numbers, data ranges, assessment criteria, and similarity assessment results of the entire comparative analytical assessments are summarized in the table above. As assessed in this review previously, the selections of attributes, testing methods, ABP 710 and US-licensed Remicade lots, the tier assignment, and the approaches to establishing similarity assessment criteria (SAC) are generally acceptable. The results will be assessed in detail in the following section. During the assessment of analytical similarity, we sent an information request regarding the use of reference standard as follows:

Information Request (Question 3, sent on 05/21/2019)

You indicated that primary reference standard Lot 0010189002 has been used for all drug substance and drug product release testing during the ABP 710 development. Clarify/confirm whether the same reference standard Lot 0010189002 was used in the comparative analytical assessment for every assay that reports results as relative to a reference standard.

The sponsor's response dated 06/04/2019:

Amgen confirms that the same reference standard Lot 0010189002 was used in the comparative analytical assessment for every assay that reports results as relative to a reference standard.

Assessor comment:

The sponsor's clarification is acceptable.

3.2.R.2.2 Characterization Data

3.2.R.2.2.1 Comparative Assessment for Assays evaluated by Tier 1 (Equivalence Test)

The primary mechanism of action (MOA) of infliximab is the inhibition of TNF α via its Fab-mediated binding activity. Inhibition of sTNF α -induced apoptosis in U937 and Binding to sTNF α by ELISA, evaluated by equivalence test, were used to assess the similarity of ABP 710 and US-licensed Remicade in terms of the primary MOA.

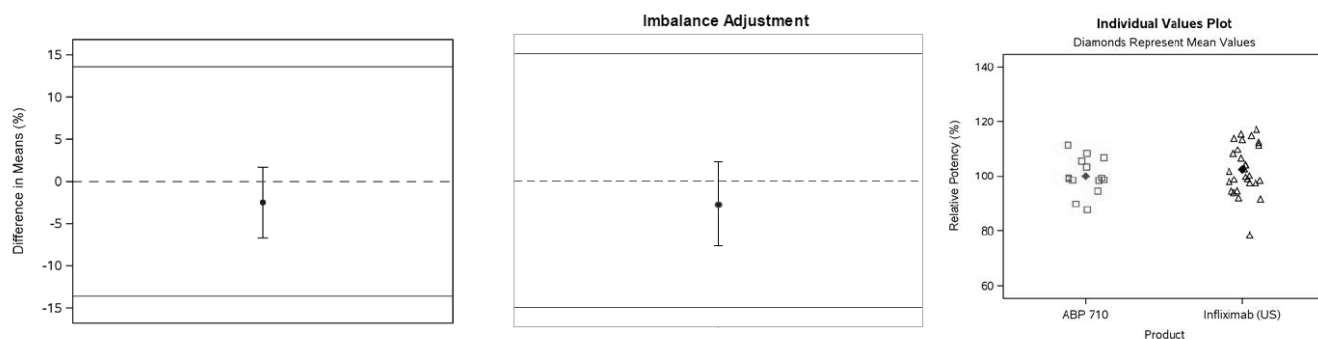
3.2.R.2.2.1.1 Inhibition of sTNF α -induced Apoptosis in U937 (Potency)

Tier 1 Equivalence test is used to assess the results of the apoptosis inhibition assay from both products. The statistical parameters used in the testing, the established EACs, and the upper and lower bounds of 90% confidence interval (CI) of Difference in Mean are summarized below.

Table 3.2.R.8 Equivalence Test Results for Relative Potency – ABP 710 to US-licensed Remicade

Assay/ Attribute	ABP 710 Mean	Infliximab (US) Mean	Estimated Difference Between Means	Degrees of Freedom Approximation	Degrees of Freedom	Standard Error	Lower Bound of 90% Confidence Interval on Difference in Means	Upper Bound of 90% Confidence Interval on Difference in Means	$\pm$ EAC	Conclusion
Relative potency (%)	99.98	102.48	-2.50	Satterthwaite approximation	33.90	2.48	-6.69	1.69	13.58	Equivalent
				Imbalance adjustment	37.26	2.67	-7.00	2.00	13.58	Equivalent

Figure 3.2.R.1. Equivalence Graphs for Relative Potency – ABP 710 to US-licensed Remicade



Upper and lower solid lines represent +EAC and -EAC values

The circle represents the different between the means and the vertical bar represents the 90% 2-sided confidence interval

Assessor comment:

The 90% Confidence Intervals (CIs) of the difference in mean relative potency between ABP 710 and US-licensed Remicade are well within the EACs. The TNF α inhibition activities are similar between ABP 710 and US-licensed Remicade. Refer to the CMC stats review for assessment of whether this demonstrates a satisfactory result for statistical equivalence testing.

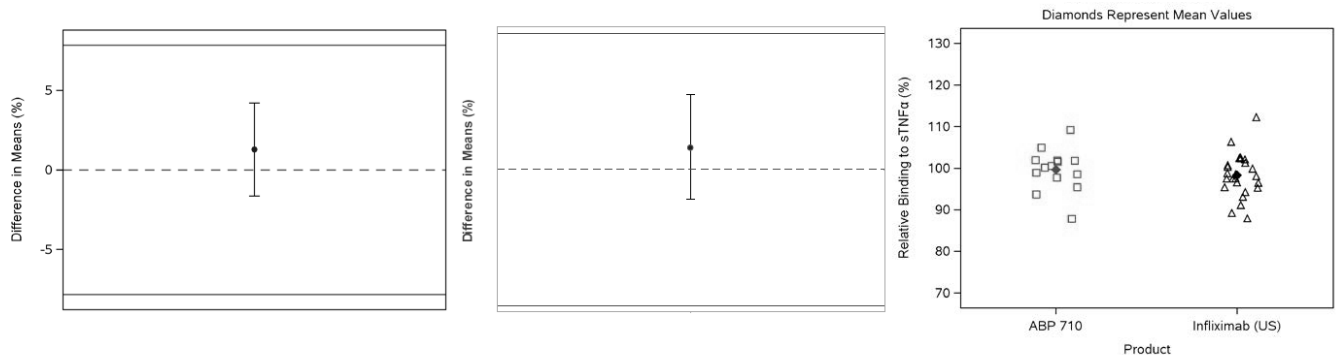
3.2.R.2.2.1.2 sTNF α Binding by ELISA

Results from binding to sTNF α by ELISA were assessed using Tier 1 Equivalence testing approach for comparative analytical assessment. The procedure to establish the equivalence acceptance criteria (EAC) was detailed in “Analytical Similarity Assessment – Testing Plan and Assessment Criteria” in 3.2.R – Regional Information. The statistical parameters used in the assessment, the established EACs, and the upper and lower bounds of 90% confidence interval (CI) of Difference in Means are summarized below.

Table 3.2.R.9 Equivalence Test Results for sTNF α Relative Binding by ELISA – ABP 710 to US-licensed Remicade

Assay/ Attribute	ABP 710 Mean	Infliximab (US) Mean	Estimated Difference Between Means	Degrees of Freedom Approximation	Degrees of Freedom	Standard Error	Lower Bound of 90% Confidence Interval on Difference in Means	Upper Bound of 90% Confidence Interval on Difference in Means	$\pm$ EAC	Conclusion
Relative binding to sTNF α (%)	99.59	98.31	1.28	Satterthwaite approximation	27.69	1.72	-1.64	4.20	7.85	Equivalent
				Imbalance adjustment	29.77	1.78	-1.73	4.29	7.85	Equivalent

Figure 3.2.R.2. Equivalence Graphs for sTNF α Relative Binding by ELISA – ABP 710 to US-licensed Remicade



Upper and lower solid lines represent +EAC and -EAC values

The circle represents the different between the means and the vertical bar represents the 90% 2-sided confidence interval

Assessor comment:

The 90% Confidence Intervals (CIs) of the difference in mean relative binding activity between ABP 710 and US-licensed Remicade is well within the established EACs. Therefore, TNF α binding activity is similar between ABP 710 and US-licensed Remicade. Refer to the CMC stats review for assessment of whether the results pass the statistical equivalence testing.

3.2.R.2.2.2 Similarity Assessment for Tier 2 (Quality Range Approach)

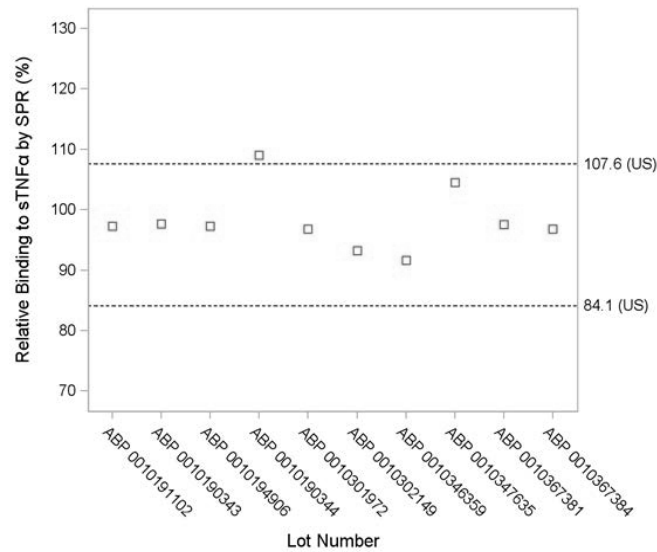
Assessor note:

In the comparative analytical assessment using Tier 2 approach, the quality range was established using mean $\pm$ 3SD from the results of US-licensed Remicade. Since the US-licensed Remicade lots used to establish the quality range may not represent the complete variability associated with Remicade over the entire history of the product, an acceptance criterion of 90% of the individual test product values is used as similarity assessment criteria (SAC).

3.2.R.2.2.2.1 sTNF α Binding by SPR

To further characterize sTNF α binding of ABP 710 and US-licensed Remicade, SPR was utilized. sTNF α relative binding by SPR allows for real-time collection of binding data enabling affinity and kinetic characterization, which informs the dynamics of the system. The sTNF α relative binding of 10 ABP 710 lots and US-licensed Remicade Quality Range are shown in Figure 3.2.R.3.

Figure 3.2.R.3. sTNF α Relative Binding by SPR – ABP 710 Compared to US-licensed Remicade Quality Range



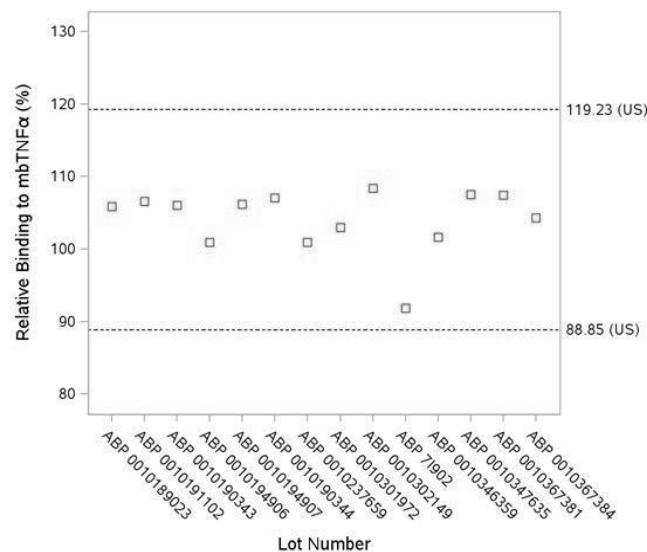
Assessor comment:

Nine of the 10 ABP 710 lots (90%) are within the quality range for sTNF α relative binding by SPR. This met the SAC for quality range evaluation. Therefore, ABP 710 is considered similar to US-licensed Remicade for sTNF α relative binding by SPR.

3.2.R.2.2.2.2 Binding to mbTNF α by Imaging Cytometry

TNF α exists as a membrane bound form (mbTNF α) prior to cleavage by proteases to generate soluble TNF α . Binding to mbTNF α by anti-TNF α antibodies has been shown to induce reverse signaling in some TNF α expressing cell types, leading to apoptosis. The induction of apoptosis, in the presence of ABP 710 or infliximab, may be involved in clinical efficacy in IBD indications. In addition, binding to mbTNF α -expressing cells by infliximab and ABP 710 can mediate effector functions (e.g., ADCC, ADCP, and CDC) in vitro.

Figure 3.2.R.4. Relative Binding to mbTNF α – ABP 710 Compared to US-licensed Remicade Quality Range



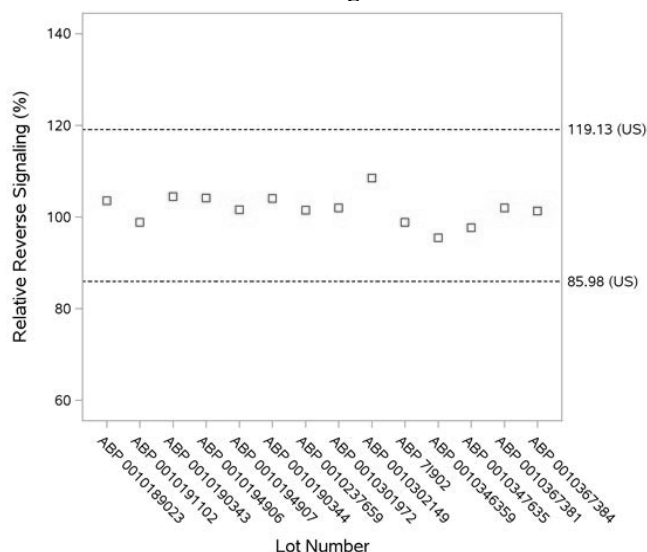
Assessor comment:

The Relative Binding to mbTNF α results for all ABP 710 lots are well within the quality range of the US-licensed Remicade. It can be concluded that ABP 710 has a similar mbTNF α binding activity to US-licensed Remicade.

3.2.R.2.2.3 Reverse signaling in Jurkat mbTNF α line

Apoptosis in monocytes and macrophages can occur through direct activation of an mbTNF α -specific reverse signaling pathway. The MOA may be involved in clinical efficacy in IBD indications. In vitro, infliximab also has the ability to induce apoptosis through reverse signaling in mbTNF α expressing cells through the fragment antigen binding (Fab). The sponsor assessed and compared the reverse signaling activity between ABP 710 and US-licensed Remicade utilizing Jurkat cells engineered to express a non-cleavable form of mbTNF α on the cell surface.

Figure 3.2.R.5. Relative Reverse Signaling – ABP 710 Compared to US-licensed Remicade Quality Range



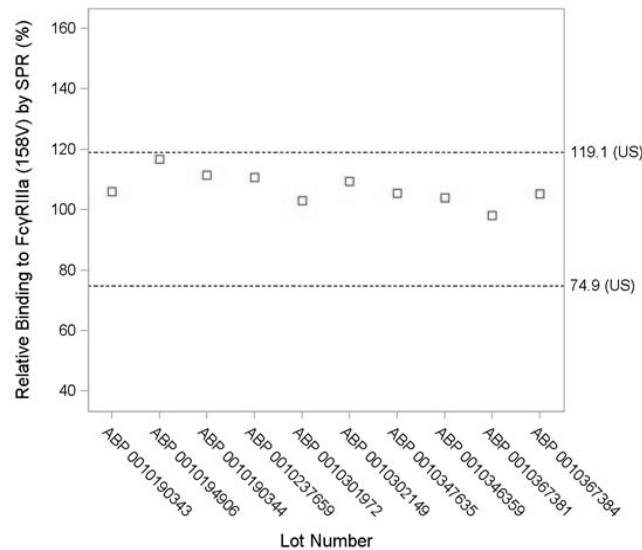
Assessor comment:

The relative reverse signaling activity of all ABP 710 lots are well within the quality range of the US-licensed Remicade. The results support the conclusion that ABP 710 and US-licensed Remicade have similar relative reverse signaling activity.

3.2.R.2.2.2.4 Binding to Fc γ RIIIa (158V) by SPR

ADCC occurs when antibodies bind to antigens expressed on target cells, and the antibody Fc domains simultaneously engage Fc receptors (e.g., Fc γ RIIIa) on the surface of effector cells. Fc γ RIIIa has 2 allelic variants that have been characterized as a high affinity variant (158V) and a low affinity variant (158F). Binding to both variants was evaluated when comparing ABP 710 to US-licensed Remicade. Figure 3.2.R.6. shows Fc γ RIIIa (158V) Relative Binding determined by SPR.

Figure 3.2.R.6. FcγRIIIa (158V) Relative Binding by SPR – ABP 710 Compared to US-licensed Remicade Quality Range



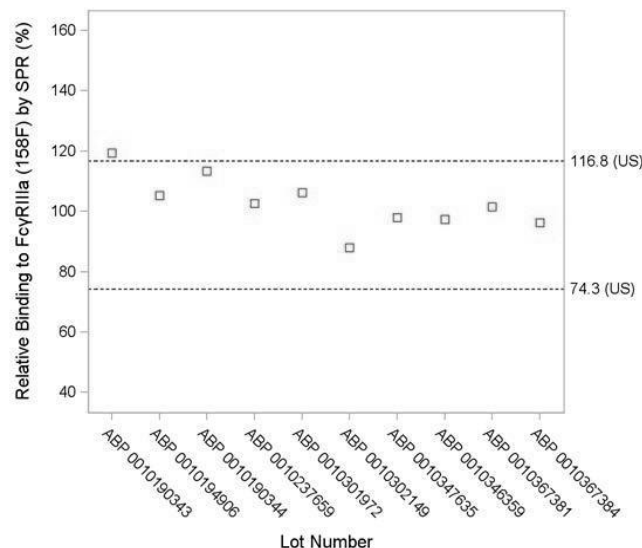
Assessor comment:

The results for all ABP 710 lots are within the quality range of the US-licensed Remicade. The FcγRIIIa (158V) relative binding by SPR results support the conclusion that ABP 710 is similar to US-licensed Remicade.

3.2.R.2.2.2.5 Binding to FcγRIIIa (158F) by SPR

FcγRIIIa has 2 common allelic variants that have been characterized as the high affinity variant (158V) and the low affinity variant (158F). This assay compares relative binding by SPR of ABP 710, infliximab (US), and infliximab (EU) to the low affinity variant, FcγRIIIa (158F).

Figure 3.2.R.7. FcγRIIIa (158F) Relative Binding by SPR – ABP 710 Compared to US-licensed Remicade Quality Range



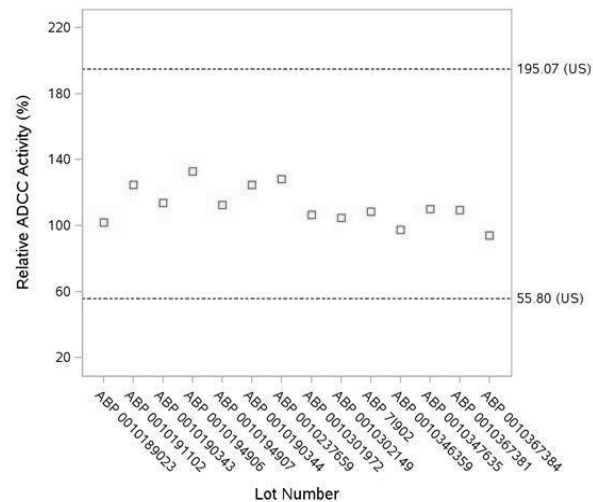
Assessor comment:

As shown in Figure 3.2.R.7, nine of the 10 ABP 710 lots (90%) are within the US-licensed Remicade quality range for FcγRIIIa (158F) relative binding. This met the SAC for quality range evaluation. ABP 710 is therefore considered similar to US-licensed Remicade for FcγRIIIa (158F) binding.

3.2.R.2.2.2.6 NK92 ADCC activity

ADCC occurs when an antibody binds to an antigen expressed on the surface of target cells and the antibody Fc domain simultaneously engages Fc gamma receptors (eg, FcγRIIIa) on the surface of effector cells (e.g., NK cells). This leads to the activation of the effector cells with concomitant granule exocytosis and target cell death mediated by perforins and granzyme. ADCC may be important in mediating efficacy of infliximab in inflammatory bowel disease (IBD) indications.

Figure 3.2.R.8. Relative NK92 ADCC Activity – ABP 710 Compared to US-licensed Remicade Quality Range



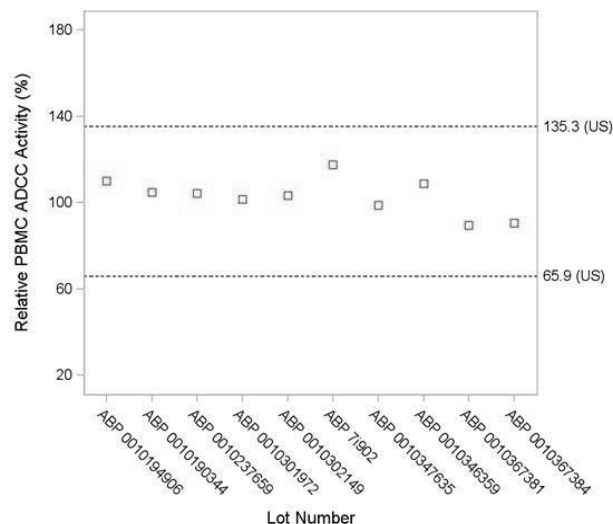
Assessor comment:

The results of relative NK92 ADCC activity for all ABP 710 lots are well within the quality range of the US-licensed Remicade, supporting the conclusion that ABP 710 has similar ADCC activity to US-licensed Remicade.

3.2.R.2.2.2.7 PBMC ADCC activity

A PBMC ADCC assay using cells from healthy donors heterozygous for position 158 in FcγRIIIa (FcγRIIIa 158 V/F) was developed to evaluate the ADCC activity of ABP 710 and US-licensed Remicade. PBMCs are more physiologically relevant cell type for ADCC assay. NK92 cells are engineered to overexpress the high affinity variant of FcγRIIIa (158V).

Figure 3.2.R.9. Relative PBMC ADCC Activity – ABP 710 Compared to US-licensed Remicade Quality Range



Assessor comment:

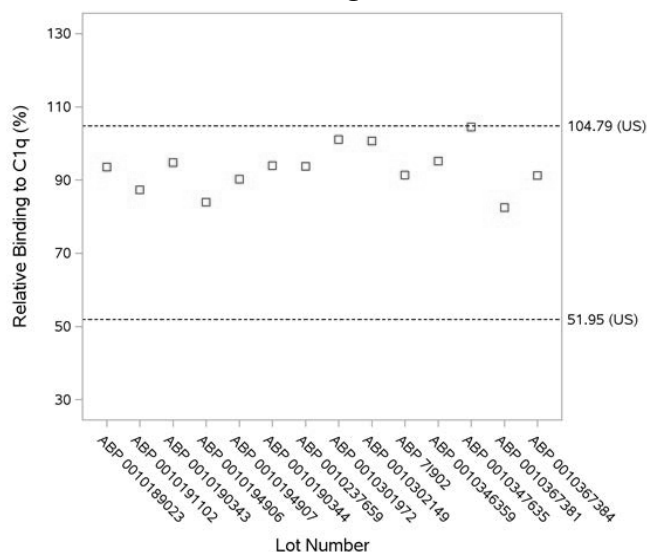
The ADCC activity results for all of the ABP 710 lots are well within the quality range of US-licensed Remicade for relative PBMC ADCC activity. Therefore, ABP 710 is considered similar to US-licensed Remicade for PBMC ADCC activity. Of note, the means of PBMC ADCC activity are similar between ABP 710 and US-licensed Remicade and the quality range is tighter than that of NK92 ADCC activity.

Overall, the results from both NK92 and PBMC ADCC assay support the conclusion that ABP 710 and US-licensed Remicade have similar ADCC activity. This observed similar ADCC activity aligns well with the similarity results from binding to FcγRIIIa (158V and 158F) by SPR (assessed above) and the similarity results from glycan map for Afucosylation, High Mannose, and β-galactosylation (assessed below).

3.2.R.2.2.2.8 Binding to C1q by ELISA

C1q is the first sub-component of the classical complement pathway. C1q binding is orthogonal to the CDC assay as it is the first step in complement-mediated cell lysis. An ELISA method was used to assess the binding of ABP 710 and US-licensed Remicade to C1q.

Figure 3.2.R.10. Relative Binding to C1q – ABP 710 Compared to US-licensed Remicade Quality Range



Assessor comment:

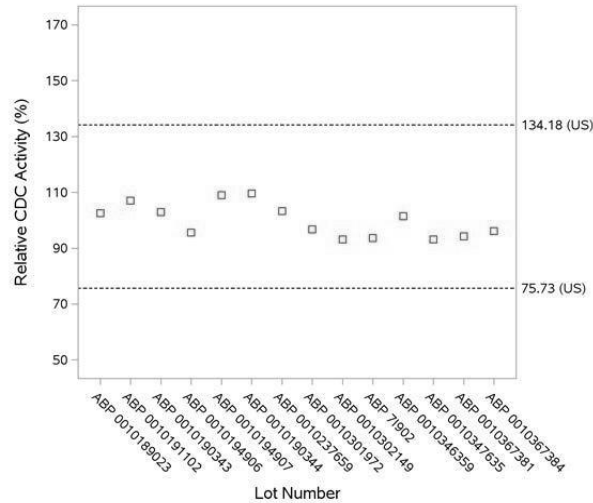
All of the ABP 710 lots are within US-licensed Remicade quality range for C1q relative binding. This met the SAC for quality range evaluation. A notion is that the mean C1q relative binding for ABP 710 is higher (93.2%) compared to that of US-licensed Remicade (78.4%). However, as assessed in the CDC activity section below, this difference did not lead to significant changes in CDC activity because ABP 710 and US-licensed Remicade showed similar means of CDC activities. Therefore, it can be concluded that the ABP 710 binding activity to C1q is similar to that of US-licensed Remicade.

3.2.R.2.2.2.9 CDC activity

CDC occurs when an antibody binds to an antigen on the surface of a target cell and the Fc domain of the antibody activates the complement cascade. The hexameric C1q protein binds to the heavy chain constant region (CH2) within the Fc domain of IgG molecules, mediating assembly of additional mediators of

complement activity. This leads to the formation of the membrane attack complex, which causes pore formation in the target cell membrane and results in cellular lysis. CDC is a mechanism of action that may be important for efficacy in IBD indications.

Figure 3.2.R.11. Relative CDC Activity – ABP 710 Compared to US-licensed Remicade Quality Range



Assessor comment:

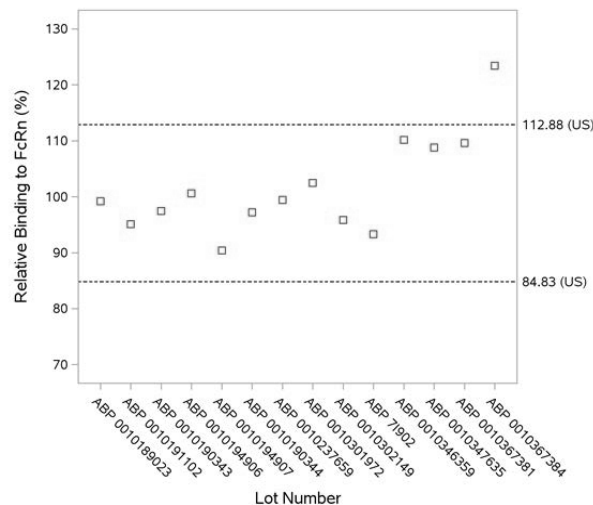
The relative CDC activity results of all ABP 710 lots are well within the quality range of the US-licensed Remicade. The relative CDC activity results support the conclusion that ABP 710 is similar to US-licensed Remicade.

Overall, the results from the orthogonal assay related to CDC activity, Binding to C1q by ELISA and CDC activity assay, support the conclusion that ABP 710 and US-licensed Remicade have similar CDC activity. This is also supported by the results from glycan map for β -galactosylation as assessed below which is believed correlated with CDC activity.

3.2.R.2.2.2.10 Binding to FcRn by AlphaScreen

FcRn binds to IgG1, IgG2, and IgG4 heavy chains in the Fc region of IgG molecules. FcRn mediates IgG homeostasis in human adults by maintaining serum IgG levels.

Figure 3.2.R.12. Relative Binding to FcRn by AlphaScreen – ABP 710 Compared to US-licensed Remicade Quality Range



Assessor comment:

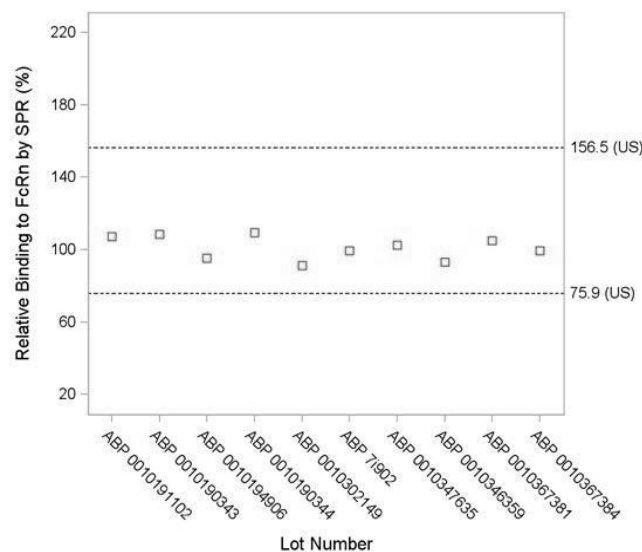
Thirteen of 14 ABP 710 lots (93%) are within the US-licensed Remicade quality range for FcRn binding by AlphaScreen. This met the SAC for quality range evaluation. The FcRn binding by AlphaScreen results support that ABP 710 is similar to US-licensed Remicade.

A notion is that the 4 ABP 710 validation lots (ABP 0010346359, ABP 0010347635, ABP 0010367381, and ABP 0010367384) trended higher on relative FcRn binding compared to that of US-licensed Remicade. An additional assessment of FcRn binding using an orthogonal analytical method, SPR, was carried out to further analyzed the trend.

3.2.R.2.2.2.11 Binding to FcRn by SPR

FcRn binding by SPR was assessed to characterize the affinity of FcRn to ABP 710 and US-licensed Remicade as slight differences in binding to FcRn between ABP 710 and US-licensed Remicade as measured by AlphaScreen were observed for a subset of ABP 710 lots. To evaluate whether this translated into a difference in affinity, the FcRn binding by SPR assay was performed.

Figure 3.2.R.13. Relative Binding to FcRn by SPR – ABP 710 Compared to US-licensed Remicade Quality Range



Assessor comment:

The results for all of the ABP 710 lots are within the quality range of US-licensed Remicade for FcRn binding by SPR. Therefore, ABP 710 and US-licensed Remicade are similar for FcRn binding by SPR. Taken together with the results of Binding to FcRn determined by AlphaScreen, it can be concluded that the FcRn binding results support the conclusion that ABP 710 is similar to US-licensed Remicade.

3.2.R.2.2.2.12 Glycan map: afucosylation; high mannose; beta-galactosylation;

The N-linked glycan assessment is important because of the possible impact on ADCC or CDC activities, which are the potential MoA for IBD indications. Afucosylated glycans can increase ADCC activity by enhancing binding to FcγRIIIa receptors. High mannose glycans can increase ADCC activity by enhancing binding to FcγRIIIa receptors, and can impact PK via differential clearance through binding to mannose-binding receptors and reducing antibody serum half-life. Galactosylated glycans are important for first sub component of the classical complement pathway (C1q) binding by IgG1 antibodies, which is a key step in the activation of complement mediated cellular cytotoxicity (CDC). Galactosylation also has a low potential to impact ADCC activity.

Figure 3.2.R.14. Afucosylation – ABP 710 Compared to US-licensed Remicade Quality Range

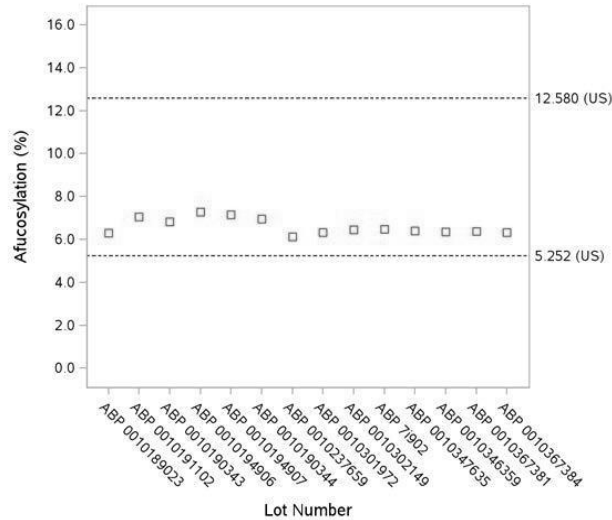


Figure 3.2.R.15. High Mannose – ABP 710 Compared to US-licensed Remicade Quality Range

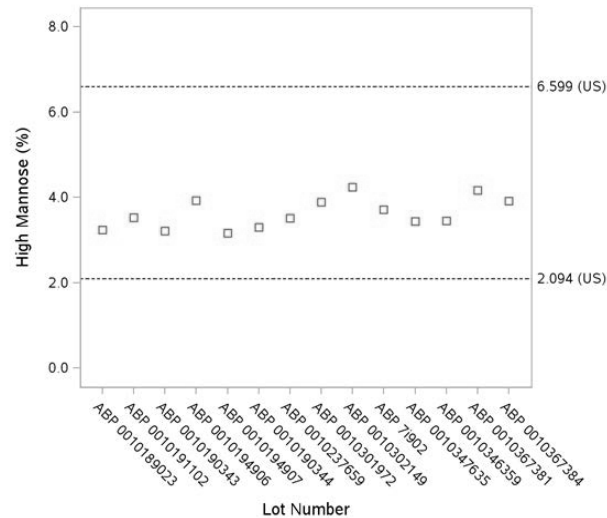
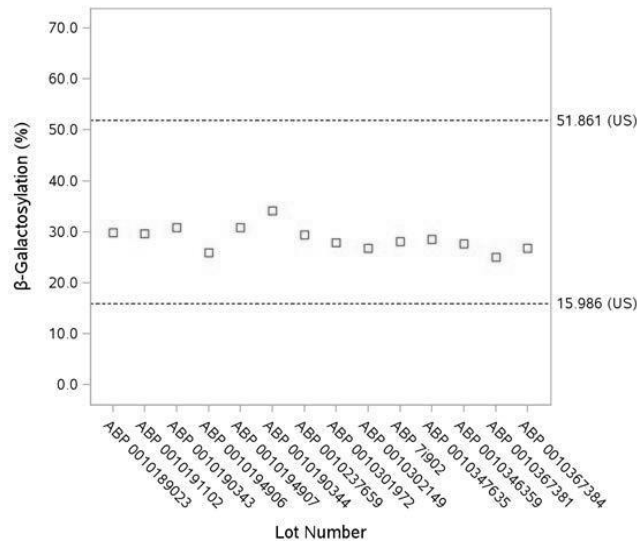


Figure 3.2.R.16. β -galactosylation – ABP 710 Compared to US-licensed Remicade Quality Range



Assessor comment:

The glycan map results for Afucosylation, High Mannose, and β -galactosylation of all ABP 710 lots are well within the quality ranges of the US-licensed Remicade. Though the afucosylation values of ABP 710 are close to the lower limit of the quality range, this does not significantly affect ADCC activity as assessed above. The results support that ABP 710 has similar Afucosylation, High Mannose, and β -galactosylation glycans compared to US-licensed Remicade.

3.2.R.2.2.13 Tertiary structure by DSC: T_{m1} and T_{m2}

The thermal stability of ABP 710 and US-licensed Remicade was assessed side-by-side 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 endothermic transitions on the DSC thermogram, and can be characterized by the thermal melting temperatures (T_m).

Figure 3.2.R.17. DSC T_{m1} – ABP 710 Compared to US-licensed Remicade Quality Range

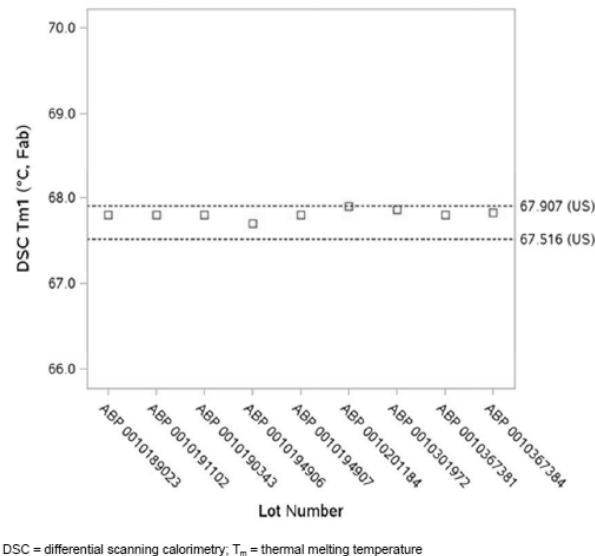
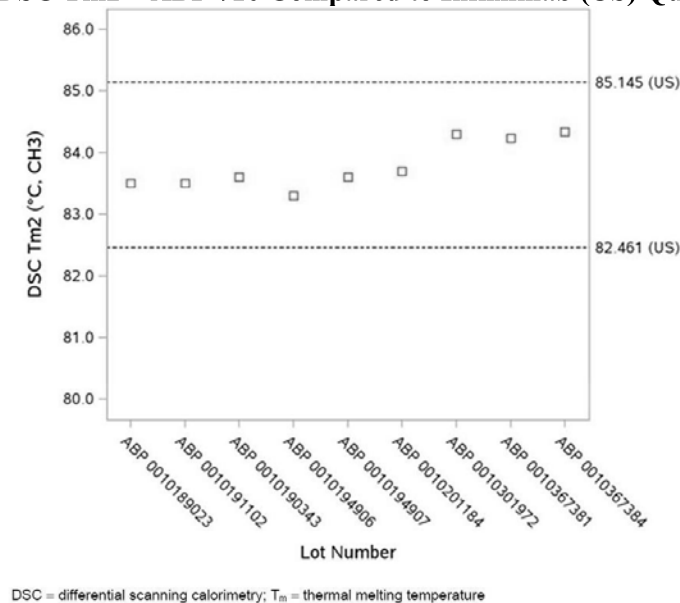


Figure 18. DSC T_{m2} – ABP 710 Compared to Infliximab (US) Quality Range



Assessor comment:

The DSC results for both Tm1 and Tm2 of all ABP 710 lots are well within the quality range of the US-licensed Remicade. The results demonstrate that ABP 710 has a similar conformation and thermal stability compared to US-licensed Remicade.

3.2.R.2.2.14 Size variants by SE-UHPLC: HMW and main peak

SE-UHPLC results demonstrate that ABP 710 has slightly higher levels of HMW compared to US-licensed Remicade and correspondingly slightly lower levels of main peak. Examples are shown below:

Figure 3.2.R.19. SE-UHPLC Profiles for ABP 710 (Clinical and Validation), US-licensed Remicade, and EU-approved Remicade

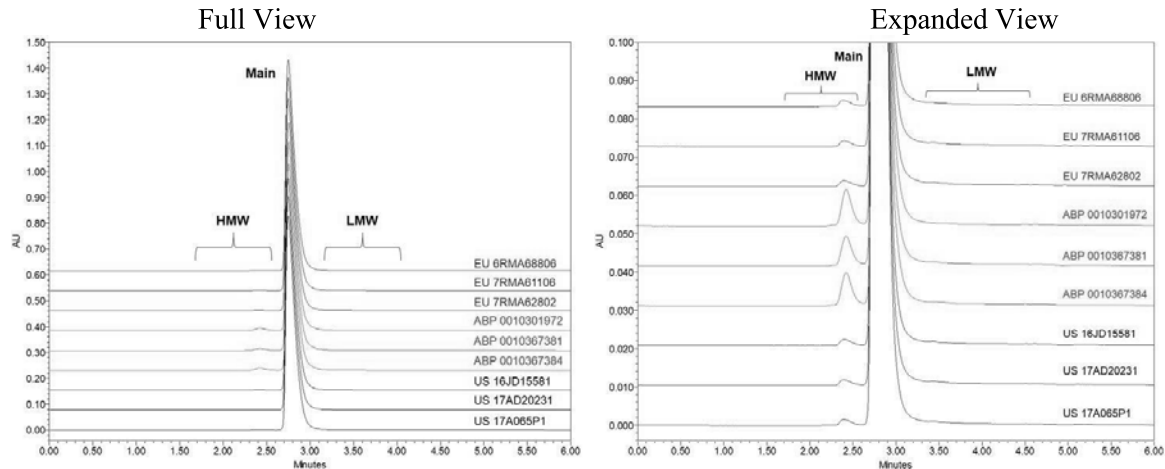


Figure 3.2.R.20. Comparison of SE-UHPLC HMW levels in ABP 710 to the Quality Range from US-licensed Remicade

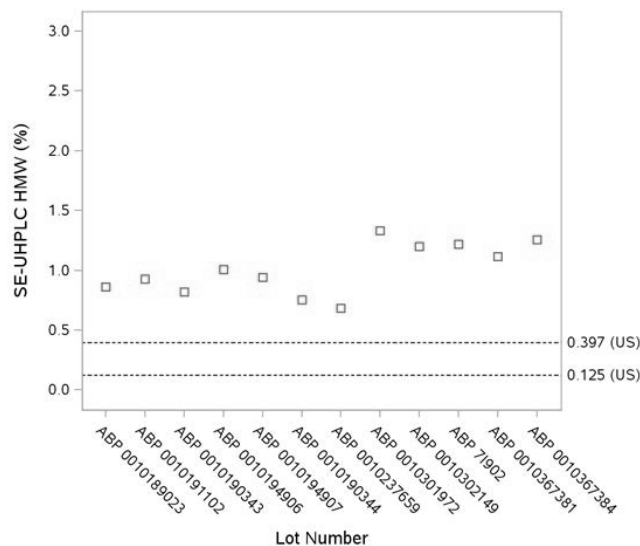
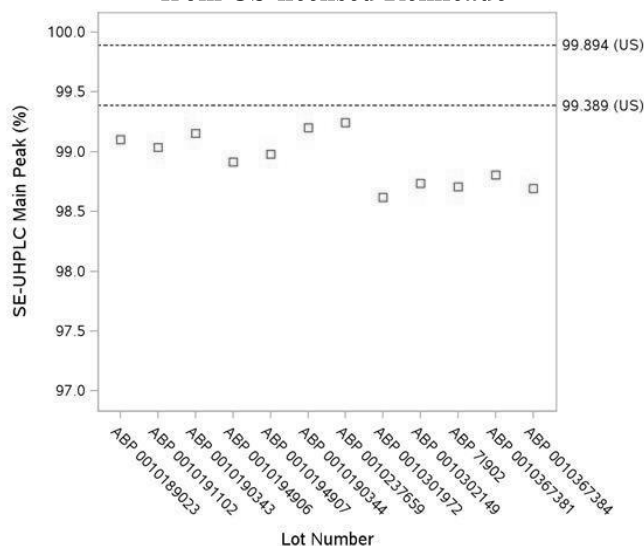


Figure 3.2.R.21. Comparison of SE-UHPLC Main Peak levels in ABP 710 to the Quality Range from US-licensed Remicade



Assessor comment:

SE-UHPLC: HMW (%) and main peak (%) were assessed against the quality ranges. In all tested ABP710 DP lots, the HMW levels were higher and the main peak levels were lower than the quality ranges, respectively. This did not meet the SAC for quality range evaluation.

The sponsor purified the HMW fractions from both products and characterized with analytical ultracentrifugation (SV-AUC) and denaturing SE-HPLC. The characterization elucidated that the HMW species is predominantly non-covalent dimer and that the levels are $\leq 1.3\%$ in ABP 710 lots. The major concern for the HMW species in drug product is the immunogenicity risk. Dimers have low risk for causing immunogenicity and the absolute level of HMW content in ABP 710 is still at very low level. The immunogenicity concern is low.

The purified HMW fraction demonstrated comparable potency and ADCC activity to the main peak in the characterization studies. The stability study and linear regression analysis results showed that the HMW species in ABP 710 did not change significantly over time at the recommended storage condition.

In addition, the sponsor has established adequate acceptance criteria to control the levels of HMW and main peak in DS and DP release and stability.

Overall, the sponsor's characterization studies and control strategy support that the quantitative differences in the HMW species and monomer main peak are not expected to preclude a determination that ABP710 is similar to US-licensed Remicade.

3.2.R.2.2.15 Size variants by rCE-SDS: HC +LC (%), LMW +MMW (%), and NGHC (%)

The rCE-SDS results demonstrate that ABP 710 has a slightly higher level of NGHC and correspondingly a slightly lower level of HC + LC and slightly higher levels of LMW + MMW compared to US-licensed Remicade. Examples are shown below:

Figure 3.2.R.22. rCE-SDS Profiles for ABP 710 (Clinical and Validation), US-licensed Remicade, and EU-approved Remicade

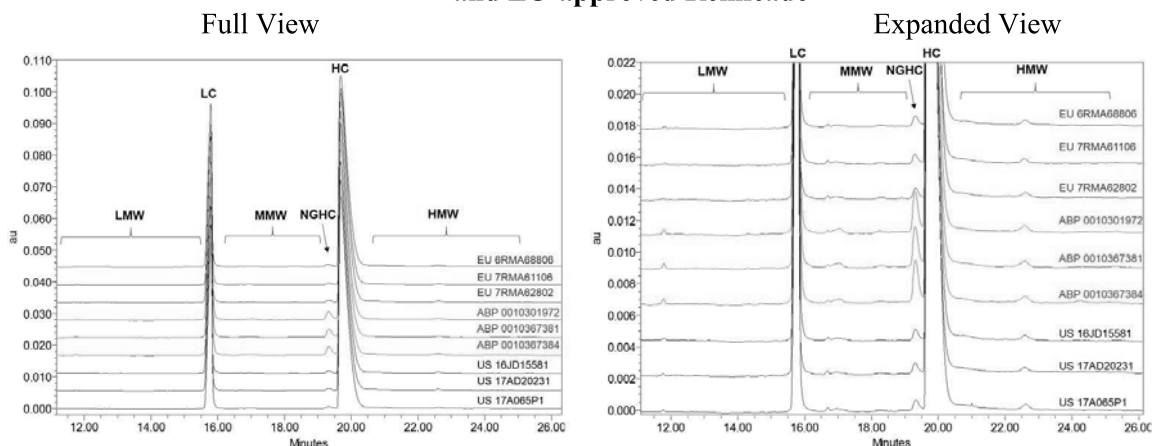


Figure 3.2.R.23. Comparison of rCE-SDS HC + LC in ABP 710 to the Quality Range from US-licensed Remicade

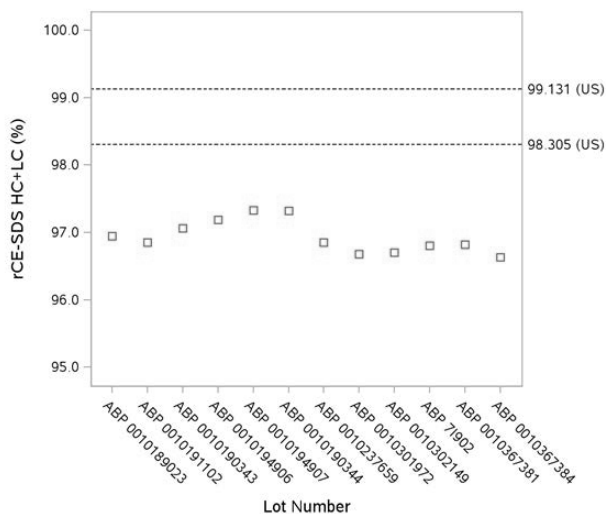


Figure 3.2.R.24. Comparison of rCE-SDS LMW + MMW in ABP 710 to the Quality Range from US-licensed Remicade

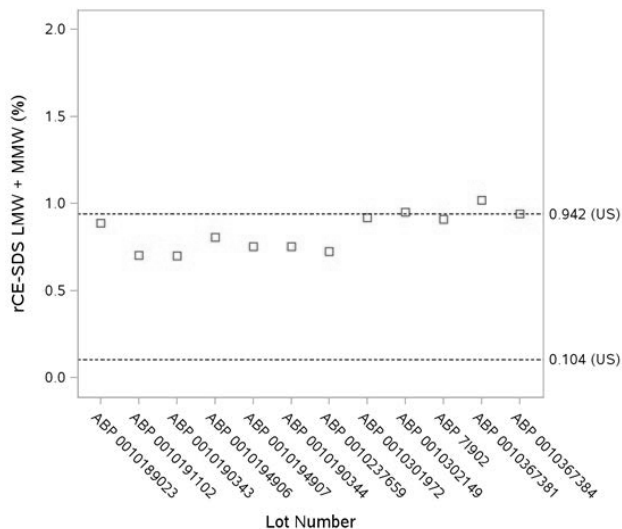
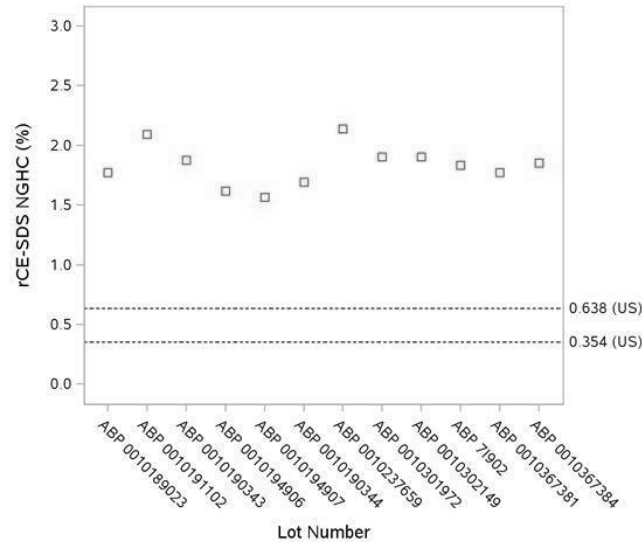


Figure 3.2.R.25. Comparison of rCE-SDS NGHC in ABP 710 to the Quality Range from US-licensed Remicade



Assessor comment:

The rCE-SDS: HC + LC (%), LMW + MMW (%), and NGHC (%) are assessed with quality ranges established from US-licensed Remicade. It was found that the levels of HC + LC (%) were below the quality range for all 12 ABP 710 lots and the level of NGHC was above the quality range in all 12 ABP 710 lots. This did not meet the SAC for quality range evaluation.

LMW/MMW species is derived from antibody fragmentation and increasing levels may decrease product potency. However, the observed slight difference between two products did not impact biological activity. In addition, no impact was observed on Fab- or Fc-region mediated activities in the forced degradation samples, which indicates that fragmentation at the present levels does not impact the structure of ABP 710.

NGHC level is driven by cell line and cell culture process and the level of HC + LC varies primarily due to the level of NGHC in ABP 710. The differences in both cases were less than 2% and the potency and ADCC assay results demonstrated that the low level of NGHC had no impact on biological activity.

The minor differences in rCE-SDS: HC + LC (%), LMW + MMW (%), and NGHC (%) between ABP 710 and US-licensed Remicade do not preclude a determination that ABP710 is highly similar to US-licensed Remicade.

3.2.R.2.2.2.16 Size variants by nrCE-SDS: pre-peak (%) and main peak (%)

The nrCE-SDS results demonstrate that ABP 710 has slightly lower level of main peak and correspondingly a slightly higher level of pre-peaks compared to US-licensed Remicade. Examples are shown below:

Figure 3.2.R.26. nrCE-SDS Profiles for ABP 710 (Clinical and Validation), US-licensed Remicade, and EU-approved Remicade

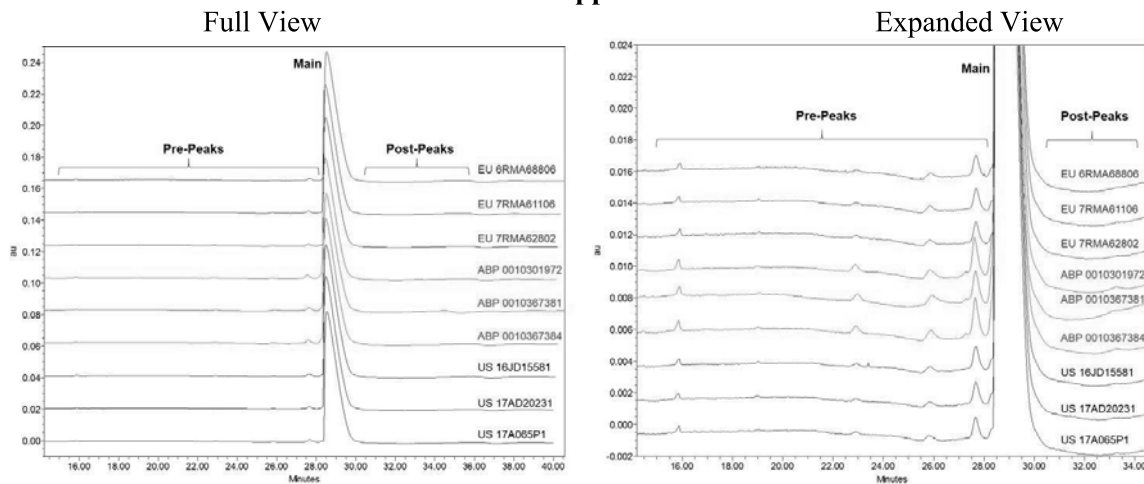


Figure 3.2.R.27. Comparison of nrCE-SDS Pre-peak in ABP 710 to the Quality Range from US-licensed Remicade

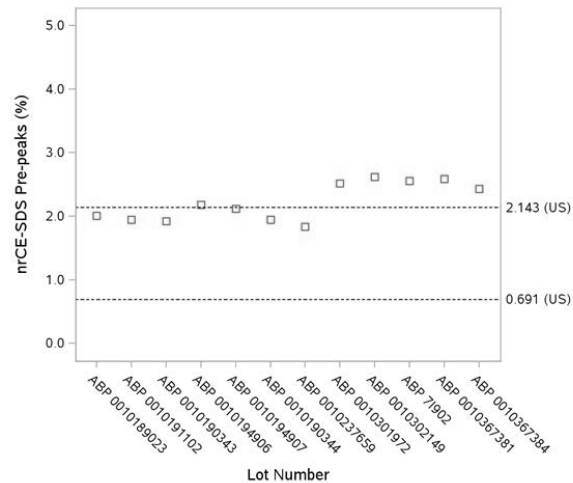
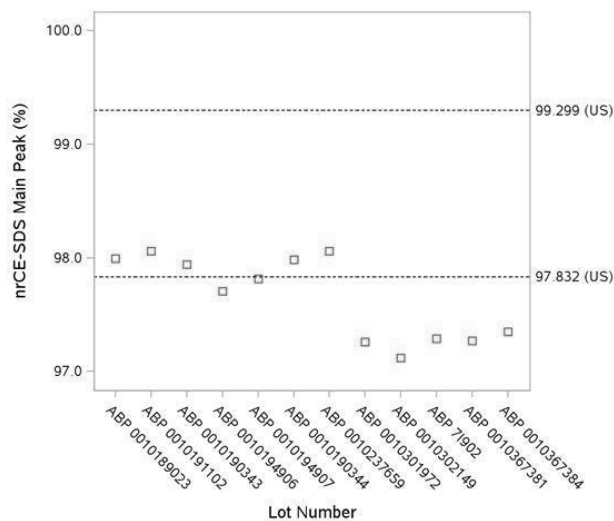


Figure 3.2.R.28. Comparison of nrCE-SDS Main Peak in ABP 710 to the Quality Range from US-licensed Remicade



Assessor comment:

The nrCE-SDS: pre-peaks (%) and main peak (%) did not meet the SAC for quality range evaluation because the levels of pre-peaks in 6 out of 12 ABP 710 lots were above the quality range and that levels of main peak in 7 out of 12 ABP 710 lots were below the quality range.

There is a slightly higher level of partially reduced species in ABP 710 compared to US-licensed Remicade under denaturing conditions. The differences for both pre-peaks (%) and main peak (%) were less than 1% and did not show a practically significant change over time at the recommended storage condition. In addition, ABP 710 and US-licensed Remicade have similar nrCE-SDS profiles and levels of post-peaks. The minor differences did not impact biological activity between the products and do not preclude a determination that ABP710 is similar to US-licensed Remicade.

3.2.R.2.2.17 Charge variants by CEX-HPLC: acidic peak (%) and basic peak (%) (Tier 2)

The results demonstrate that ABP 710 has similar levels of main peak, higher levels of acidic peaks, and lower levels of basic peaks, compared to US-licensed Remicade. Examples are shown below:

Figure 3.2.R.29. CEX-HPLC Profiles for ABP 710 (Clinical and Validation), US-licensed Remicade, and EU-approved Remicade

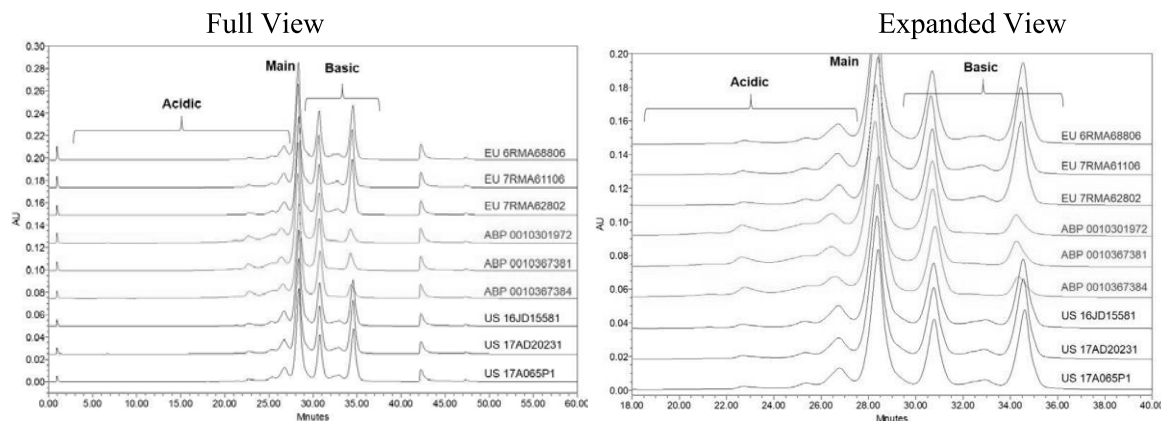


Figure 3.2.R.30. Comparison of CEX-HPLC Acidic Peak in ABP 710 to the Quality Range from US-licensed Remicade

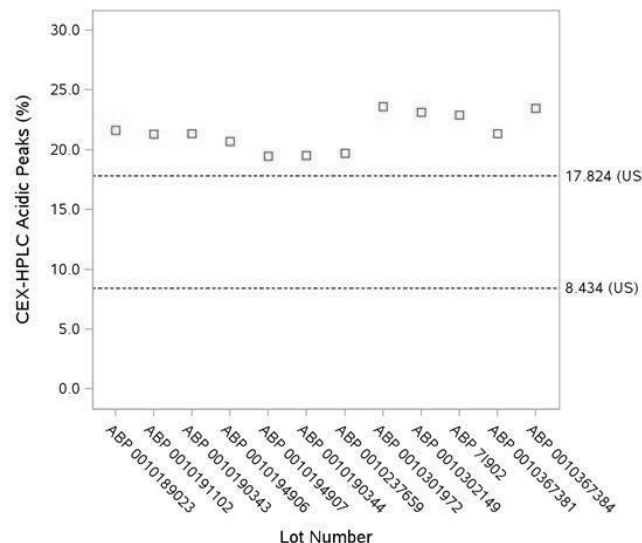
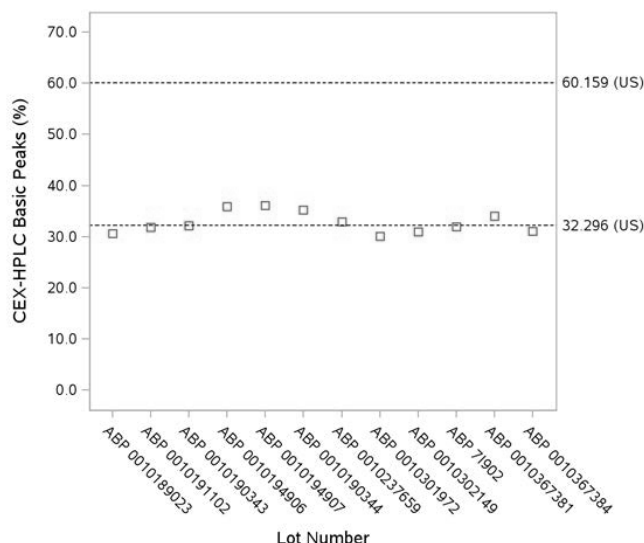


Figure 3.2.R.31. Comparison of CEX-HPLC basic Peak in ABP 710 to the Quality Range from US-licensed Remicade



Characterization of CEX-HPLC peaks:

1. Carboxypeptidase B treatment

ABP 710, US-licensed Remicade, and EU-approved Remicade were treated with Carboxypeptidase B to remove C-terminal lysine residues followed by CEX-HPLC analysis. The treated samples demonstrated similar levels of acidic, main, and basic peaks.

2. Characterization of CEX-HPLC Fractions

CEX-HPLC Fractions of ABP 710 drug substance, US-licensed Remicade, and EU-approved Remicade were purified and analyzed with Peptide Mapping, Glycan Analysis, Potency, and ADCC activity.

Peptide mapping results suggested that deamidation at Asn⁵⁷ and Asn³⁸⁷ was slightly enriched in the acidic peaks, the unprocessed heavy chain C-terminal lysine variants (HC Lys⁴⁵⁰ unprocessed) was significantly enriched in the basic peaks.

For ABP 710, the acidic variants have slightly lower levels of afucosylation and slightly elevated galactosylation. However, for US-licensed Remicade, and EU-approved Remicade, the enriched acidic variants have slightly higher levels of afucosylation and slightly decreased galactosylation. Higher levels of sialylation were observed in acidic variants and higher levels of high mannose were observed in basic variants.

Results for potency and ADCC activity are summarized below:

Table 3.2.R.8. Relative Potency of CEX-HPLC Fractions for ABP 710, US-licensed Remicade, and EU-approved Remicade

Lot Number	% Relative Potency			
	Starting Material ^a	Acidic Peaks	Main Peak	Basic Peaks
EU 4RM61803	118	95	105	102
ABP 0010313936	100	84	95	102
US EBD14013P1	106	81	104	100

^a Potencies of the starting materials were determined in side-by-side assays with the chromatography fractions.

Table 3.2.R.9. Relative NK92 ADCC of CEX-HPLC Fractions for ABP 710, US-licensed Remicade, and EU-approved Remicade

Lot Number	% Relative ADCC			
	Starting Material ^a	Acidic Peaks	Main Peak	Basic Peaks
EU 4RM61803	110	132	83	122
ABP 0010313936	107	95	114	133
US EBD14013P1	120	112	80	154

^a Potencies of the starting materials were determined in side-by-side assays with the chromatography fractions.

Assessor comment:

CEX-HPLC: main peak (%), acidic peaks (%), and basic peaks (%) are tested and the results were analyzed with Tier 2 quality range method. Though the CEX-HPLC profiles were visually similar between ABP 710 and US-licensed Remicade and no new or missing peaks were observed for ABP 710, the levels of acidic peak in all 12 ABP 710 lots were above the quality range and the levels of basic peaks in 6 out of 12 ABP 710 lots were below the quality range. This did not meet the SAC for quality range evaluation. The sponsor further evaluated the differences.

After Carboxypeptidase B treatment, CEX-HPLC main peak (%), acidic peaks (%), and basic peaks (%) are comparable between ABP 710 and the reference product, suggesting low levels of basic species in ABP 710 are from C-terminal lysine depletion. Since C-terminal lysine is cleaved in vivo shortly after intravenous injection, differences in its levels will not affect functions of the product and do not preclude a determination that ABP710 is similar to US-licensed Remicade.

The characterization of purified fractions of acidic peaks can be concluded as follows:

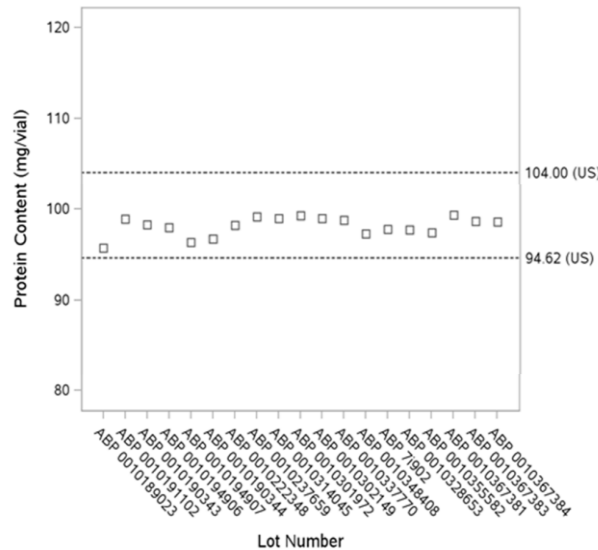
- The deamidation at HC Asn⁵⁷ and Asn³⁸⁷ is high in the acidic fractions of ABP 710, which causes acidic peak increase. These post-translational modifications are unlikely to affect binding to mbTNFα and FcγR based on the comparable potency and ADCC assay results from acidic fraction and main peak fraction.*
- Relative potency and ADCC activity of CEX-HPLC fractions of ABP 710 and US-licensed Remicade were comparable to the unfractionated materials, within the variability of the assay. Relative Potency and ADCC activity of each fraction were well within the acceptance criteria for release and stability.*
- The CEX-HPLC main peak (%), acidic peaks (%), and basic peaks (%) in ABP 710 and US-licensed Remicade did not change significantly over time at the recommended storage condition.*

Overall, the sponsor's justifications for the differences of acidic peaks (%) and basic peaks (%) in comparative analytical assessment are acceptable. In addition, the sponsor has included CEX-HPLC test in ABP 710 DS and DP release and stability specifications with justified numerical acceptance criteria for main peak, acidic peaks, and basic peaks to control the charge variant profile of the product. According to the above evaluation, the differences in the levels of acidic and basic peaks do not preclude a determination that ABP710 is similar to US-licensed Remicade.

3.2.R.2.2.2.18 Protein content

The lyophilized material was reconstituted with water for injection (WFI) and the mass of the reconstituted solution was determined. The protein concentration was determined by absorbance at UV 280 nm using the product extinction coefficient of 1.4 cm⁻¹(mg/mL)⁻¹. Calculate protein content using the following equation:

Figure 3.2.R.32. Protein Content – ABP 710 Compared to US-licensed Remicade Quality Range



Assessor comment:

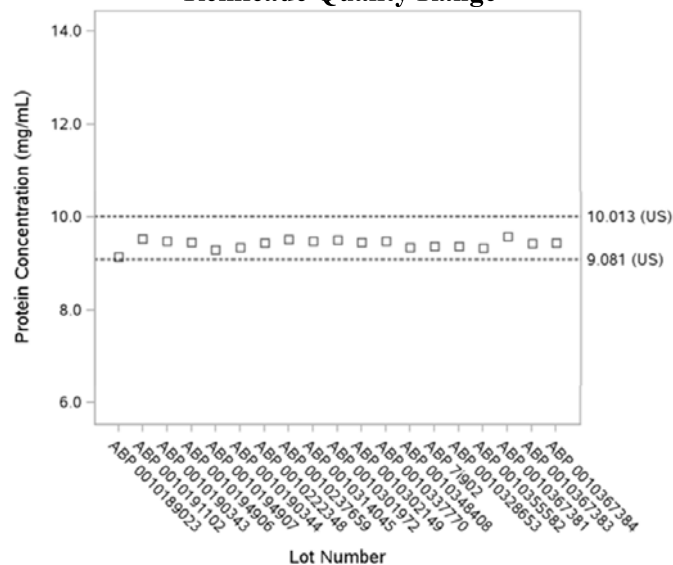
The protein content for all ABP 710 lots within the quality range of the US-licensed Remicade. The results support the conclusion that DP protein content is similar for ABP 710 and US-licensed Remicade.

3.2.R.2.2.2.19 Reconstituted protein concentration

Protein concentration is determined by the ultraviolet (UV) absorption of the sample at 280 nm. The average absorbance is calculated based on Beer's law. The extinction coefficient has been theoretically determined to be $1.4 \text{ mL cm}^{-1}(\text{mg/mL})^{-1}$.

$$\text{Protein Content (mg/vial)} = \frac{\text{Recon. Product Wt. (g/vial)}}{1.019 \text{ g/mL}} \times \text{Protein Concentration (mg/mL)}$$

Figure 3.2.R.33. Reconstituted Protein Concentration – ABP 710 Compared to US-licensed Remicade Quality Range



Assessor comment:

The reconstituted protein concentration for all ABP 710 lots is within the quality range of the US-licensed Remicade, supporting that protein concentration in reconstituted DP is similar for ABP 710 and US-licensed Remicade.

3.2.R.3.2.3 Similarity Assessment for Tier 3 Attributes (Raw Data/Graphical Comparison)

Assessor comment:

As listed in Table 3.2.R.2. assays/attributes for comparative analytical assessment with the lowest risk to clinical outcomes or where results are qualitative or not suitable for numerical evaluation, were assessed using Tier 3 Graphic Comparison approach. This category includes some assays/attributes for biological activity, primary structure, higher order structure, particles and aggregates, product-related substances and impurities, thermal stability and forced degradation, and general properties. For all attributes assessed using Tier 3 approach, results of the assays are presented side-by-side to allow qualitative comparisons between ABP710 and the reference product. As shown in Table 3.2.R.7 Summary of Analytical Similarity results, most attributes of this category met Similarity Assessment Criteria except that minor differences were observed for “Post-translational modifications using reduced peptide map” and “ α -galactosylation, sialylation, and profile of glycan map”, which will be further assessed in the following sections. These differences can be mitigated using data from other orthogonal methods. Therefore, they do not preclude a determination that ABP710 is similar to US-licensed Remicade. The overall results from assays/attributes assessed using Tier 3 graphic Comparison approach support the conclusion that ABP 710 is similar to US-licensed Remicade.

3.2.R.3.2.3.1 Differences in post-translational modifications using reduced peptide map

Table 3.2.R.10. Key Modifications in ABP 710 (Clinical and Validation) and US-licensed Remicade

Lot Number	Relative Abundance (%) ^a						
	Deamidation		Oxidation			Hydroxylysine	
	HC Asn ⁵⁷	HC Asn ³⁸⁷	HC Met ²⁵⁵	HC Met ⁴³¹	LC Met ⁵⁵	HC Lys ⁶⁷	HC Lys ¹²⁴
ABP 0010301972	2	1	3	1	1	2	1
ABP 0010367381	2	1	3	1	0	2	1
ABP 0010367384	2	1	3	1	1	2	1
US 16JD15581	1	1	1	0	0	0	0
US 17AD20231	1	1	1	0	0	0	0
US 17A065P1	1	1	1	0	0	0	0

^a Assessment of % modification with MS data by extracted ion chromatograms method is semi-quantitative due to variability in ionization efficiency of peptide ions. All estimated % modifications are rounded to whole numbers.

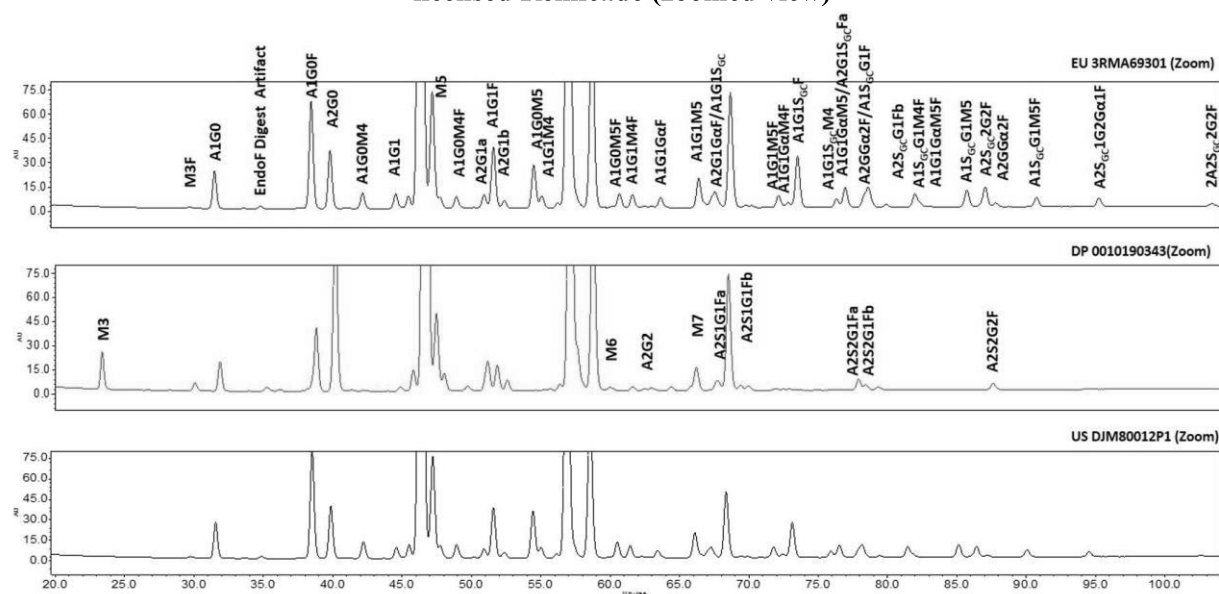
The post-translational modifications observed in ABP 710 are methionine oxidation at Met²⁵⁵ and Met⁴³¹ in the fragment crystallizable (Fc) region, Met⁵⁵ in the LC complementarity-determining region (CDR) 2, asparagine deamidation at Asn⁵⁷ in the HC CDR 2, Asn³⁸⁷ in the Fc region, hydroxylysine at Lys⁶⁷ in the HC CDR 2, and Lys¹²⁴ in the Fab domain.

Assessor comment:

The levels of post-translational modification in HC, including deamidation at Asn⁵⁷, oxidation at Met²⁵⁵ and Met⁴³¹, and hydroxylysine at Lys⁶⁷ and Lys¹²⁴ in ABP 710 are higher than that in US-licensed Remicade. However, the levels are low in both products and the differences were below 2% in clinical and validation batches. The fractionation analyses for CEX-HPLC peaks suggested that the differences in post-translational modifications did not adversely impact relative potency and ADCC activity. These differences do not preclude a determination that ABP710 is highly similar to US-licensed Remicade.

3.2.R.3.2.3.2 Differences in α -galactosylation, sialylation, and profile of glycan map

Figure 3.2.R.34. Glycan Map Profiles for EU-approved Remicade, ABP 710 (Clinical), and US-licensed Remicade (zoomed view)



The following differences were observed for Glycan Map in Figure 3.2.R.34:

α-galactosylation

US-licensed Remicade has low levels of α-galactosylation, but ABP 710 has no α-galactosylation. The level of α-galactosylation in US-licensed Remicade is below 6.6%. The presence of α-galactose species in US-licensed Remicade could potentially impact immunogenicity and PK if recognized by human antibodies to the α-galactose species.

Sialylation

The level of NANA sialylation in ABP 710 is below 1.6% and the level of NGNA sialylation in US-licensed Remicade is below 10.0%. The absence of non-human glycans in ABP 710 may potentially result in improved immunogenicity profile.

Glycan Profile

The glycan map profiles are visually similar across the 2 products for the major glycans with minor differences observed in α-galactosylation and sialylation due to the change in cell line used to manufacture ABP 710 compared to US-licensed Remicade. These differences in α-galactosylation and sialylation have no impact on biological activity

Assessor comment:

The glycan map results of ABP 710 was similar to that of US-licensed Remicade for afucosylation, high mannose, and β-galactosylation. However, differences were observed in α-galactosylation and sialylation. These two glycan forms and glycan profile were evaluated with qualitative assessment criteria. Unlike US-licensed Remicade, ABP 710 did not contain detectable levels of non-human glycans, such as N-glycolylneuraminic acid (NGNA) and α-galactosylation. ABP 710 contained minor levels of N-acetylneuraminic acid (NANA) sialylation, which was absent in US-licensed Remicade. These differences are expected because US-licensed Remicade is manufactured in a SP2/0 (mouse myeloma) cell line, whereas ABP 710 is manufactured using CHO cells. No impact to biological functions, including ADCC and CDC, was observed due to the differences in glycan profiles between ABP 710 and US-licensed Remicade. These differences do not preclude a determination that ABP710 is highly similar to US-licensed Remicade.

3.2.R.3.2.4 Comparative forced degradation studies

The degradation pathways of ABP 710 and US-licensed Remicade were evaluated by a set of thermal degradation studies as follows:

- Reconstituted Forced Degradation Study at 40°C
- Lyophilized Stressed Degradation Study at 40°C
- Lyophilized Accelerated Stability Study at 25°C

As part of the comparative analytical assessment, a thermal forced degradation study was performed side-by-side at 40°C on reconstituted drug product to aid in the comparison of ABP 710 and US-licensed Remicade. This forced degradation study compares changes in product size variant and charge variant profiles, and potency to confirm that the same degradation pathways are present in both ABP 710 and US-licensed Remicade. Additionally, accelerated (25°C) and stressed (40°C) stability studies were performed to compare ABP 710 lyophilized drug product to US-licensed Remicade lyophilized drug product. The reconstituted product was evaluated at 40°C because development studies indicated 50°C incubation of the lyophilized product resulted in insufficient degradation and temperatures slightly above 60°C resulted in a plasticized lyophilized cake. The data demonstrated that conducting forced degradation at the edge of this transition may not generate reproducible degradation. The results of the reconstituted 40°C forced degradation study, lyophilized 40°C stressed stability study, and lyophilized 25°C accelerated stability study are summarized below.

Table 3.2.R.11. Summary of Thermal Stability and Degradation Results (prepared by assessor)

Attribute	Test/Parameter	Tier	Number of lots (US: ABP)	Data range		Similarity Assessment Criteria (SAC)	Met SAC? (ABP vs US)
				US-licensed Remicade (US-R)	ABP710		
40°C forced degradation, for 3 days, 1 week, 2 weeks, and 3 weeks (reconstituted product)	SE-UHPLC: HMW degradation	3	2:2	Visually qualified profile	Visually similar degradation profiles and degradation rates	Qualitative	Yes
	rCE-SDS: HC +LC degradation	3		Visually qualified profile	Visually similar degradation profiles and degradation rates		
	rCE-SDS: LMW +MMW degradation	3		Visually qualified profile	Visually similar degradation profiles and degradation rates		
	nrCE-SDS: pre-peak degradation	3		Visually qualified profile	Visually similar degradation profiles and degradation rates		
	nrCE-SDS: main peak degradation	3		Visually qualified profile	Visually similar degradation profiles and degradation rates		
	CEX-HPLC: main peak	3		Visually qualified profile	Visually similar degradation profiles and degradation rates		
	CEX-HPLC: acidic peaks	3		Visually qualified profile	Visually similar degradation profiles and degradation rates		
	CEX-HPLC: basic peaks degradation	3		Visually qualified profile	Visually similar degradation profiles and degradation rates		
40°C stressed stability, for 0.5, 1, 3 and 6 months (lyophilized product)	Apoptosis inhibition bioassay (%)	3	1:1	Observed levels of potency after incubation at 40°C for 3 weeks	Potency level is similar	Qualitative	Yes
	SE-UHPLC: HMW degradation (%)	3		No significant degradation or trends in 6 months.	No significant degradation or trends in 6 months.		
	SE-UHPLC: main peak degradation (%)	3					

	rCE-SDS: HC +LC degradation	3		No significant degradation or trends in 6 months	No significant degradation or trends in 6 months.		
	rCE-SDS: LMW +MMW degradation (%)	3					
	CEX-HPLC: main peak	3		No significant degradation or trends in 6 months.	No significant degradation or trends in 6 months.		
	CEX-HPLC: acidic peaks degradation (%)	3					
	CEX-HPLC: basic peaks degradation	3					
	Apoptosis inhibition bioassay	3		No significant trends in 6 months	No significant trends in 6 months		
25°C accelerated stability, 1, 3, 6, and 9 months (lyophilized product)	SE-UHPLC: HMW degradation	3	1:1	No significant degradation or trends in 9 months	No significant degradation or trends in 9 months	Qualitative	Yes
	SE-UHPLC: main peak degradation	3					
	rCE-SDS: HC +LC degradation	3		No significant degradation or trends in 9 months	No significant degradation or trends in 9 months		
	rCE-SDS: LMW +MMW degradation	3					
	CEX-HPLC: main peak	3		No significant degradation or trends in 9 months	No significant degradation or trends in 9 months		
	CEX-HPLC: acidic peaks	3					
	CEX-HPLC: basic peaks degradation	3					
	Apoptosis inhibition bioassay	3		No significant trends in 9 months	No significant trends in 9 months		

Assessor comment:

The sponsor conducted three comparative forced degradation studies to assess the degradation pathways of ABP 710 and US-licensed Remicade.

The results from Reconstituted Forced Degradation Study at 40°C indicated that the thermal degradation profiles and rates of ABP 710 and US-licensed Remicade are visually similar for size variants monitored by SE-UHPLC, rCE-SDS, and nrCE-SDS, charge variants monitored by CEX-HPLC, and relative potency by apoptosis inhibition bioassay.

The results from Lyophilized Stressed Degradation Study at 40°C demonstrated that ABP 710 and US-licensed Remicade have similar degradation rates for size variants monitored by SE-UHPLC and rCE-SDS, charge variants by CEX-HPLC, and relative potency by apoptosis inhibition bioassay after incubation at 40°C for 6 months in lyophilized form.

The results from Lyophilized Accelerated Stability Study at 25°C suggested that ABP 710 and US-licensed Remicade) have similar degradation rates for size variants monitored by SE-UHPLC and rCE-SDS, charge variants by CEX-HPLC, and relative potency by apoptosis inhibition bioassay after incubation at 25°C for 9 months in lyophilized form.

Overall, the results from three comparative forced degradation studies support the conclusion that ABP 710 and US-licensed Remicade are similar on thermal stability, degradation pathways, and degradation rates.



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