

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125544Orig1s000

CHEMISTRY REVIEW(S)



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

U. S. Food and Drug Administration
Center for Drug Evaluation and Research
Division of Biotechnology Review and Research II
CDER/OPQ/OBP (HFD-123)
Silver Spring MD 20903

STN 125544 Product Quality Team Leader Summary

Date: March 9, 2016

From: Kurt Brorson, Ph.D., Acting Lab Chief LBPS, **Kurt A. Brorson -A**
CDER/OBP, Division 2

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Date: 2016.03.10 10:53:11 -05'00'

Through: David Frucht, MD, Acting Division Director,
CDER/OBP, Division 2

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Submission Type: BLA resubmission

Sponsor: Celltrion, Inc.

Contact: Jennifer Seiler, PhD., RAC (301) 634-8034

Product: CT-P13 (Infliximab-dyyb)

OBP Name: MAB CHIMERIC (IGG1) ANTI P01375 (TNFA_HUMAN) [CTP13]

Indications: rheumatoid arthritis, IBD, psoriasis and others

Purpose of Submission: Resubmission of BLA to address complete response (CR) deficiencies and questions for the ongoing development program for CT-P13. The Applicant seeks agreements that additional quality similarity assessment data for subvisible particulate analysis and ADCC are sufficient to demonstrate that CT-P13 and US Remicade® are highly similar in support of a 351(k) application.

Submission Date: October 5, 2015

Due Date: April 5, 2016

Recommendation: *Based on review of the additional data and other information provided in this submission, the analytical assessment supports the conclusion that CT-P13 is highly similar to US-licensed Remicade. This conclusion is consistent with the Feb 9, 2016 recommendation of the Arthritis Advisory Committee (AAC) that was reached by a twenty-one to three vote. The BLA can be approved from a Product Quality standpoint*

Product background/Overview:

Background: CT-P13 is a proposed biosimilar to US-licensed Remicade developed by Celltrion, Inc. Celltrion, Inc. initially submitted a 351(k) BLA in Aug 2014. Drug substance and product are manufactured by Celltrion, Inc. (Incheon, South Korea). The initial submission contained analytical similarity data comparing multiple lots of CT-P13 with US-licensed Remicade using methods to assess physicochemical and functional properties of the products. The conclusion of the Agency's review of the first submission was that the two products had been demonstrated to be analytically similar with respect to most important protein structure and functional aspects. However, the review concluded that there was residual uncertainty regarding differences vs. the innovator product in ADCC activity and in FcγRIIIa binding (see primary quality review by Dr. P. Adams, dated May 7, 2015). In addition, a separate immunogenicity review of a small, single dose trial revealed immunogenicity rates that were higher with CT-P13 administration than with the U.S.-licensed reference. This difference could have been, in theory, attributable to differences in subvisible particle content (see immunogenicity review by Dr. W. Hallett, dated 5/22/2015). A CR letter was issued on June 8, 2015 with directed questions requesting additional data and justifications to address these two potential differences between CT-P13 and the reference product.

Resolution of CR issues:

1. Celltrion provided results of additional subvisible particulate analysis from a large number of additional CT-P13, US-licensed Remicade[®], and EU-approved Remicade[®] lots using two orthogonal methods, MFI and HIAC. The additional testing revealed that levels of subvisible particles varied, but no consistent trend towards more or fewer particles were evident for any of the three products. Thus, no quality-related attribute that could increase the antigenicity of CT-P13 over US licensed Remicade appears to exist; this resolves the CR issue #1. Moreover, additional immunogenicity data from a new clinical study involving patients revealed no increase in immunogenicity in patients receiving CT-P13 vs. US-licensed Remicade[®], further mitigating this concern.

- 2a. Celltrion evaluated the ADCC activity of additional lots of CT-P13, US-licensed Remicade[®] and EU-approved Remicade[®]. The additional analysis revealed that >90% of

CT-P13 lots were within the quality range established by testing of the reference product, meeting expectations for a determination of “highly similar” for this product quality attribute. This resolves in part, the CR issue #2.

- 2b. Celltrion provided an evaluation of an exercise to identify and demonstrate control of product quality attributes that underlie ADCC activity in CT-P13. As a result of this exercise, it was determined that FcγRIIIa binding strength correlated with NK-cell mediated ADCC activity. Celltrion agreed to tighten their drug substance specifications for FcγRIIIa binding strength, ensuring that CT-P13 lots will be within the quality range for NK-cell mediated ADCC activity, as determined by testing of multiple lots of the reference product. This, together with the point above, resolves the CR issue #2.
- 2c. Celltrion provided an additional justification that the observed, small differences (~20%) in mean ADCC activity are probably not important for clinical activity. Most of these data were provided as a follow-up (see review by C. Agarabi, dated Jan 7, 2016) to a position paper (“Extrapolation of CT-P13 Data to Indications for which Licensure is sought”) submitted in the original BLA in section 5.3.5.4 (reviewed by C. Agarabi, dated Jan 5, 2016). These reviews, which included an independent CDER review of the pertinent scientific literature, concluded that reverse signaling together with TNF sequestration (antibody activities not dependent on the Fc portion of the molecule) likely predominate in the mechanism of infliximab function for all indications, including IBD. The response also addressed a question raise during a Type I meeting on Aug 5, 2015, regarding the lack of efficacy or effectiveness only for maintenance by antibodies or fusion proteins lacking or with attenuated Fc effector functions (e.g., Onercept, Enbrel, Cimzia, and CDP571). This observation suggests a role for intact Fc function and ADCC in IBD indications. Celltrion’s response countered this suggestion by providing other explanations aside from lack of ADCC activity for the clinical outcome of each of the products referenced above, such as structural features of the biomolecules or inadequate dosing or other aspects of clinical trial design. Overall, the response and the section 5.3.5.4 white paper support the resolution of CR issue #2.

Other relevant conclusions/updates from review:

- All DMFs associated with the BLA 125544 file have been reviewed by CDER at least twice in the past two years. These include DMF (b) (4) (type III) for (b) (4) Butyl Rubber Formulations; DMF (b) (4) (type V) for (b) (4) Butyl Rubber (b) (4) Stoppers, and DMF (b) (4) (type III) (b) (4) for Type I Borosilicate Vials, USP. The two (b) (4) DMFs were reviewed in 2015 by OPF in the context of other products (e.g., DMF (b) (4) was reviewed by C. Zhang on 10/20/2015, and DMF (b) (4) was reviewed by M. Cruz-Fisher on 8/4/2015). The (b) (4) DMF was reviewed by the OBP review team during the CR response cycle (see K. Brorson review 3/8/2016). No deficiencies were noted.

- Minor adjustments to the lyophilization cycle were implemented and validated. These were introduced to correct “(b)(4)” issues. These changes were found to be acceptable.
- Real-time stability data through 45 months for the CT-P13 drug product manufactured at CLT (b)(4) was provided. These data reflect the proposed marketed product held at the recommended storage conditions. Celltrion also provided 51 months supportive data for the lots produced by other drug product manufacturing sites. They propose a shelf-life of 51 months based on a six month extrapolation from the 45 months of CLT (b)(4) stability data, as it exhibited no trending predicting exceeding acceptance criteria over that timeframe. This expiry dating proposal is acceptable.



U. S. Food and Drug Administration
Center for Drug Evaluation and Research
Division of Biotechnology Review and Research II
CDER/OPQ/OBP (HFD-123)
Silver Spring MD 20903

Subject: STN 125544 (sponsor response to CR); Product Quality Review Memo

Date: March 10, 2016

To: Administrative File, STN 125544

From: Cyrus Agarabi, PharmD, Ph.D., Quality Reviewer LBPS, CDER/OBP, Division 2

Cyrus Agarabi -S

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Date: 2016.03.10 13:10:57 -05'00'

Through: Kurt Brorson, Ph.D., Acting Lab Chief LBPS, CDER/OBP, Division 2

Submission Type: BLA

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ou=People, 0.9.2342.19200300.100.1.1=1300078163,
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Sponsor: Celltrion, Inc.

Contact: Jennifer Seiler, PhD., RAC (301) 634-8034

Product: CT-P13 (Infliximab-dyyb)

OBP Name: MAB CHIMERIC (IGG1) ANTI P01375 (TNFA_HUMAN) [CTP13]

Indication: rheumatoid arthritis and others

Purpose of Submission: Resubmission of BLA to address complete response (CR) deficiencies and questions for the ongoing development program for CT-P13. The Applicant seeks agreements that additional quality similarity assessment data for subvisible particulate analysis and ADCC are sufficient to demonstrate similarity in quality between CT-P13 and US Remicade® in support of a 351(k) application

Submission Date: October 5, 2015

Due Date: April 5, 2016

Note: *Reviewer comments are indicated by italicized font. Reviewer summaries of the BLA contents are in regular font.*

Recommendation: *Based on review of the additional data and other information provided in this submission, the analytical assessment supports the conclusion that CT-P13 is highly similar to US-licensed Remicade. This conclusion is consistent with the recommendation of the Arthritis Advisory Committee (AAC).*

Product background/Overview:

Background: CT-P13 is a proposed biosimilar to US-licensed Remicade developed by Celltrion, Inc. Celltrion, Inc initially submitted a 351(k) BLA in Aug 2014. Drug substance and product are manufactured by Celltrion, Inc. Incheon South Korea. The initial submission contained analytical similarity data comparing CT-P13 with US-licensed Remicade using methods to assess physicochemical and functional properties of the products. A comparison of product-related substances and impurities was also conducted. A total of 7 to 13 lots of CT-P13 DP and 7 to 17 lots of US-licensed Remicade were evaluated using the various analytical methods in the first submission. The conclusion of the Agency review of the first submission was that the two products had been demonstrated to be analytically similar with respect to most important protein structure and functional aspects. However, there was residual uncertainty regarding differences vs. the innovator product in ADCC activity and in FcγRIIIa binding. In addition, in a small single dose trial immunogenicity rates were higher vs. the U.S.-licensed reference. A CR letter was issued on June 8, 2015 with directed questions requesting additional data and justification to address the two above noted differences between CT-P13 and the innovator product.

After the CR, a briefing package was submitted on June 19, 2015, in which Celltrion requested a type 1 meeting with Agency review staff and managers. This was held on August 5, 2015. The Applicant requested further clarification on specific points raised in the CR letter, as well as input regarding a path forward for CT-P13. After review and discussion at the meeting, the FDA indicated that a BLA resubmission with additional data would be an acceptable path for Celltrion.

Summary of submission content:

1. Celltrion provided results of additional subvisible particulate analysis from a large number of additional CT-P13, US-licensed Remicade[®], and EU-approved Remicade[®] lots.

- 2a. Celltrion evaluated the ADCC activity of additional lots of CT-P13, US-licensed Remicade[®] and EU-approved Remicade[®].
 - 2b. Celltrion provided an evaluation of an exercise to identify and demonstrate control of product quality attributes that underlie ADCC activity in CT-P13.
 - 2c. Celltrion provided an additional justification that the observed, small difference in mean ADCC activity is not relevant to clinical activity. Much of these data were provided as a follow-up to a position paper (“Extrapolation of CT-P13 Data to Indications for which Licensure is sought”) submitted in the original BLA in section 5.3.5.4. Also, during the review cycle, Celltrion agreed to tighten the specification for FcγRIIIa binding activity for DS, such that any released lot must be within the quality range of the reference product lots tested during the biosimilarity exercise
3. Additional appended information on quality and stability (expiry).

Specific responses to the CR letter:

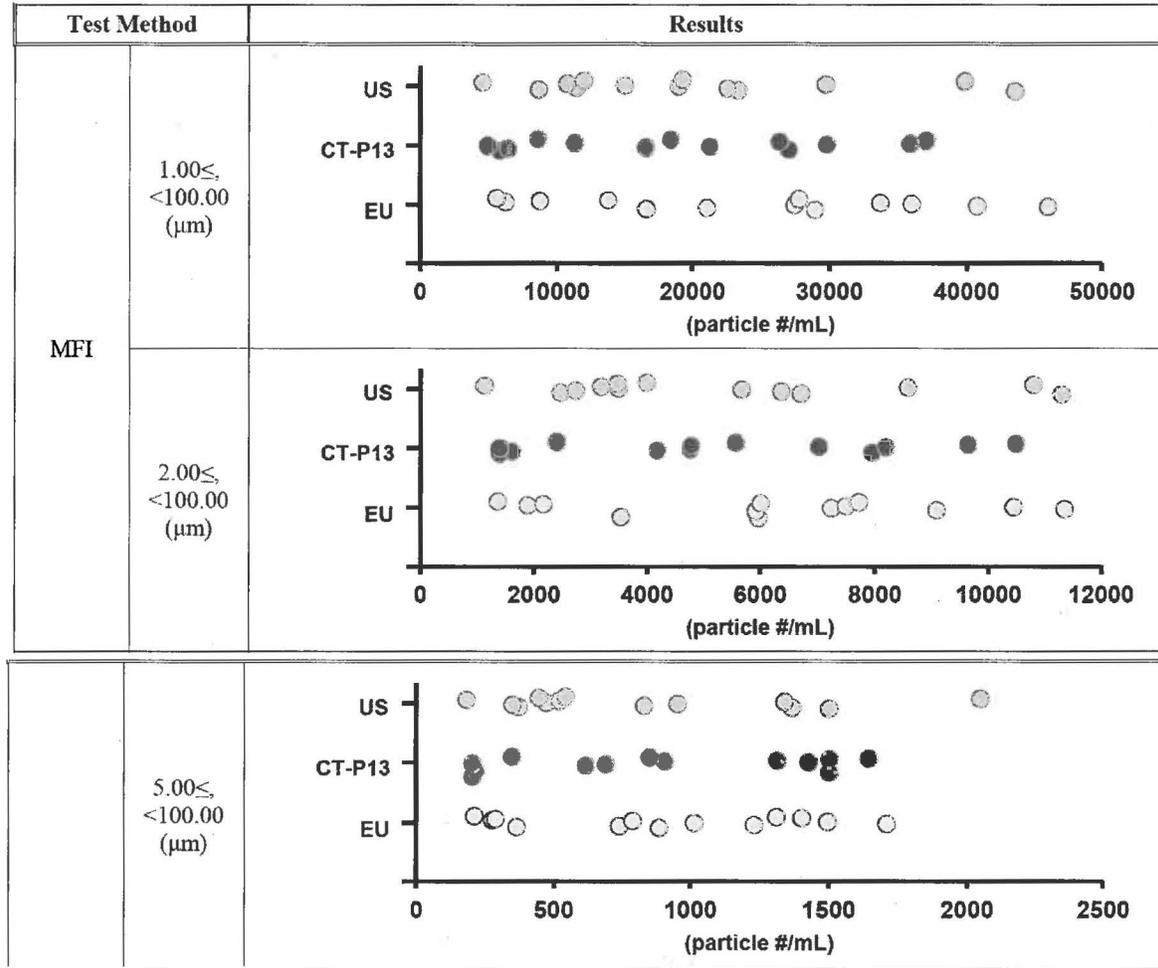
FDA CR question 1: “You provided data from a limited number of lots showing lower levels of subvisible particulates in the range of 1 to 5 microns in US-licensed Remicade compared to both CT-P13 and EU-approved Remicade. The observed differences may be due to the limited number of lots of CT-P13, US-licensed Remicade and EU-approved Remicade used to perform the analysis. However, these results suggest that analytical differences may exist between US-licensed Remicade and EU-approved Remicade, which, if confirmed, could impact the assessment of the adequacy of the analytical bridge between the three products. To address this concern, provide results of subvisible particulate analysis from an adequate number of additional CT-P13, US-licensed Remicade and EU-approved Remicade lots.”

FDA Review:

Purpose: As agreed upon as part of the Type I meeting on August 5, 2015, additional lots (13 lots total) of each product (US, EU, and CT-P13) were tested for subvisible particles by two orthogonal test methods, MFI and light obscuration (HIAC). This was performed to determine whether previously observed differences in the SVP content (1-5 micron range) between the US-licensed Remicade[®], EU-approved Remicade[®], and CT-P13 (which may have correlated with antibody responses in the above referenced single dose, small clinical trial) were due to random selection variation during testing of a limited number of lots (i.e., one or two lots from each).

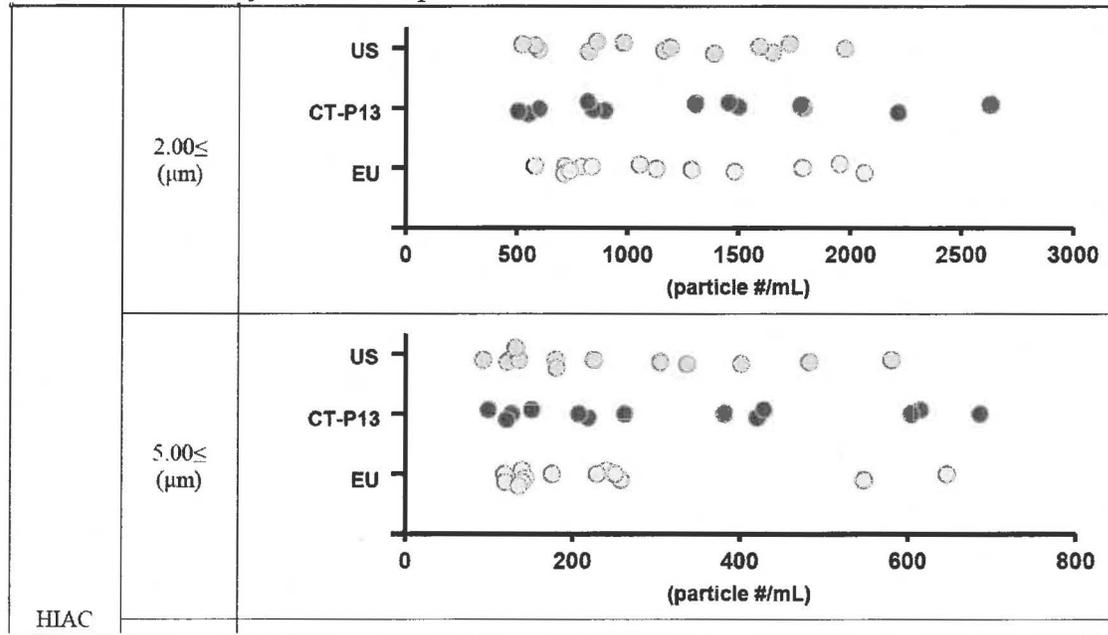
MFI

The results of the testing of additional lots can be seen in the scatter plots below. Although the levels of subvisible particles varied, no consistent trend towards more or fewer subvisible particles were evident for any of the three products.



HIAC

The results of the testing of additional lots can be seen in the scatter plots below. The levels of subvisible particles varied, but no consistent trend towards more or fewer subvisible particles were evident for any of the three products.



Reviewer's Comments: *The two orthogonal sub-visible particle methods both showed CT-P13 and US-licensed Remicade have similar particulate distributions. The increase in sample size has eliminated the chance that the original observed differences were meaningful, but instead reflected the small sample size and random chance. An additional SVP analysis of diluted CT-P13 held in IV bags and passed through in-line filters provided similar results to US- Remicade. This resolves the CR issue.*

FDA CR question 2: "You evaluated the analytical similarity of CT-P13 and US-licensed Remicade[®] using a variety of functional assays. Your data generated using a standard NK-cell based killing ADCC assay suggest that CT-P13 has ~20% lower ADCC activity compared to the reference product US-licensed Remicade, which correlates with differences in Fc γ RIIIa binding. The difference in ADCC leads to residual uncertainty about whether CT-P13 is highly similar to US-licensed Remicade, as the role of ADCC remains uncertain in the clinical activity of the reference product (e.g., in the setting of inflammatory bowel disease). Furthermore, you did not adequately justify the impact of the difference in ADCC on the analytical similarity assessment and did not identify the structural basis underlying this difference. For example, you should determine whether the H2L1 variant that is present at relatively high levels in CT-P13 compared to US-licensed Remicade plays a role in decreasing NK-dependent ADCC activity. On the other hand, the Agency has not excluded the possibility that analysis of additional lots of CT-P13, US-licensed Remicade, and EU-approved Remicade lots could overcome a statistical anomaly due to the analysis of a limited number of lots. To this point, we note that prior differences in glycan patterns were reduced when additional lots of CT-P13, US-licensed Remicade and EU-approved Remicade were analyzed. To address the current deficiency with respect to differences in ADCC

activity, we recommend that you repeat the evaluation of ADCC using additional lots to determine whether the ADCC difference you have reported was due to small sample size and decreases when additional lots are evaluated. If the difference in ADCC persists following analysis of additional lots, you should identify and demonstrate control of the product quality attributes that underlie ADCC activity in CTP13 (e.g., glycan pattern, contribution of H2L1 variant, etc.) and provide an adequate justification, including an evaluation of the role of ADCC particularly in the setting of inflammatory bowel disease, that the observed difference in ADCC is not relevant to clinical activity.”

Background. In the Complete Response dated 08 Jun 2015, FDA recommended that additional lots be included in NK ADCC assays. The goal was to determine whether the ~20% ADCC difference reported in the original BLA was an artifact of a small sample size. FDA also recommended that Celltrion identify and demonstrate control of the product quality attributes that could influence or underlie ADCC activity of CT-P13, including glycan pattern, H2:L1 variant, non-glycosylation, HMW species, and glycation. This recommendation was conveyed at the Type I meeting held on 05 Aug 2015 (Type I Meeting Minutes dated 26 Aug 2015).

Reviewers note: *The applicant chose to subdivide CR question #2 (stated in full above) into three subsections (2A,2B,2C) for the sake of response clarity; this subdivision approach is followed in this review.*

2A) ADCC

“Furthermore, you did not adequately justify the impact of the difference in ADCC on the analytical similarity assessment and did not identify the structural basis underlying this difference....we recommend that you repeat the evaluation of ADCC using additional lots to determine whether the ADCC difference you have reported was due to small sample size and decreases when additional lots are evaluated.”

Sponsor Response

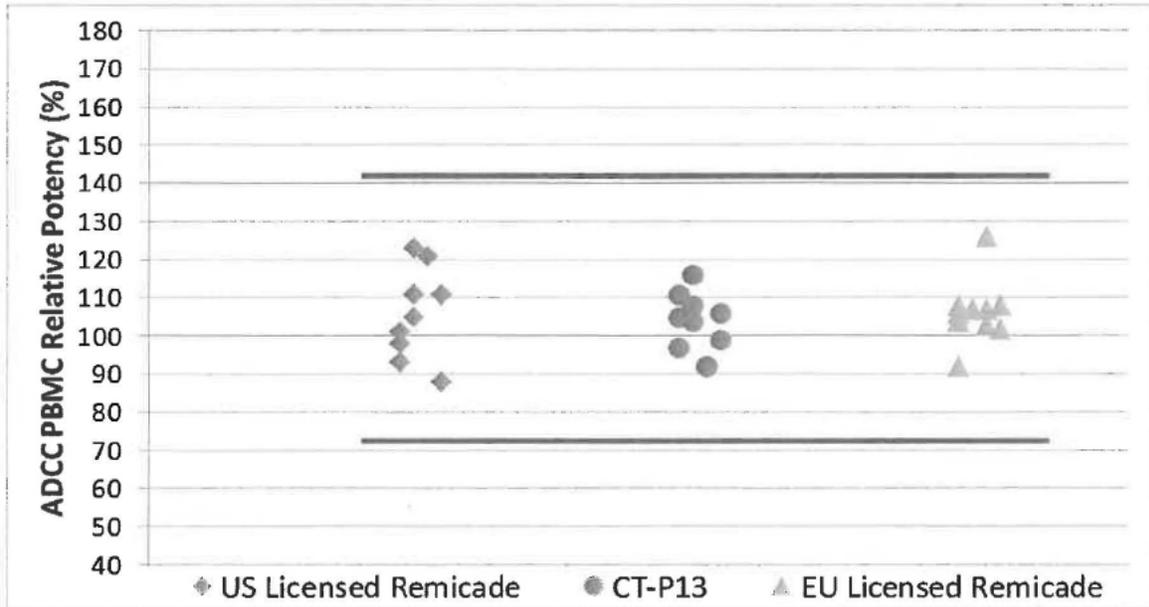
To respond to CR comment #2, Celltrion analyzed 22 additional lots of US-licensed Remicade®, 13 lots of CT-P13, and 20 lots of EU-approved Remicade®. The results of Celltrion’s quality range analysis are below:

Analysis	Data Presentation & Analysis	Conclusion of Statistical Analysis CT-13 vs US Remicade®
FcγRIIIa binding affinity (SPR)	Relative Binding Potency	85 % within QR V Type 61 % within QR F Type
	Absolute KD Values	77 % within QR V Type 70 % within QR F Type
NK ADCC	Relative Activity	> 96 % within QR at all 3 concentrations
	Absolute Cytotoxicity	92 % within QR at all 3 concentrations
Oligosaccharide Profile (HPAEC-PAD)	G0, Man 5, G0+Man 5	9 % within QR for G0, 100 % within QR for Man5, 100 % within QR for G0+Man5
	Other Glycans	100 % within QR
Intact forms, H+L and Non-glycosylated forms (CE-SDS)	Intact IgG	0 % within QR
	H+L	96 % within QR
	Non-glycosylated forms	96 % within QR
Monomer/HMW (SEC-HPLC)	Monomer	0 % within QR
Glycation (LC-ES-MS)	Total glycation (LC, HC)	0 % within QR
	Glycation site	Locations are the same

The Agency performed an independent quality range evaluation of ADCC activity data from all lots submitted since the original BLA. In some cases the same lot had been analyzed more than once; the mean value is shown and has been used in all statistical analyses performed by the Agency. For the purposes of this exercise, the quality range was defined at mean +/- 3 standard deviations of ADCC activity of the reference product (i.e., US-licensed Remicade).

PBMC ADCC

The infliximab induced ADCC activity using transfected transmembrane TNF- α Jurkat cells as target cells and PBMC from healthy donor as effector cells is shown in the scatter plot below for US Remicade, EU Remicade, and CT-P13.

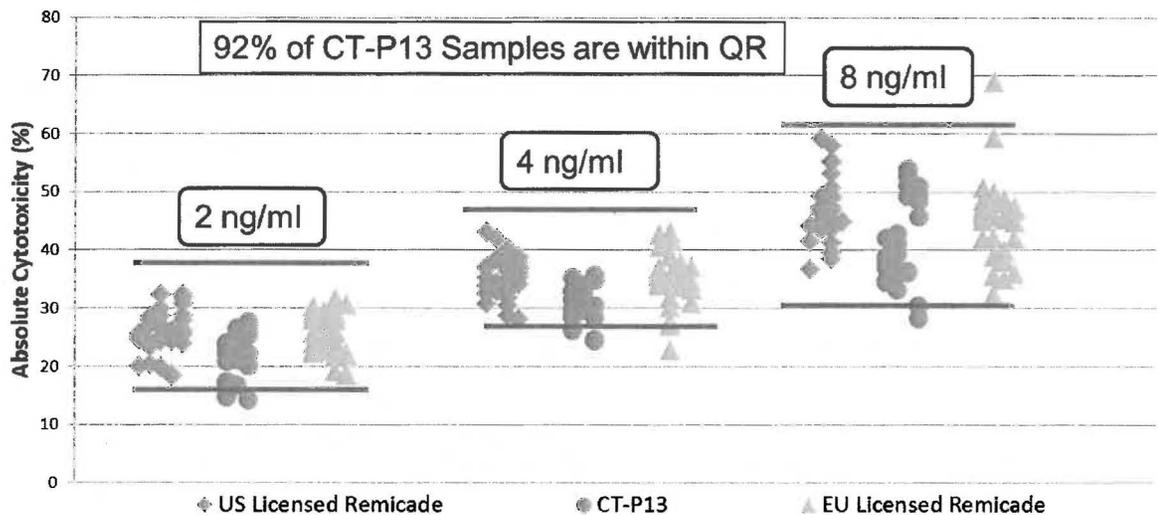


100% of the samples were within the quality range, defined as the mean of US Licensed Remicade +/- 3 standard deviations of RLD (red bars in graph).

Reviewer's comments: Peripheral blood mononuclear cells are a complex population of cells which would include NK cells. PBMC would also include other cell types that may also serve as effector cells as well as potential regulatory cells that may modulate NK cell activity. The Applicant argues that this population is more physiologically relevant than purified populations like enriched NK cells. As can be seen, 100% of the lots of the proposed biosimilar are within the quality range of the reference product, as defined by the mean plus or minus three standard deviations of reference values.

ADCC using NK Cells from Healthy Donors

The infliximab-induced ADCC activity using transfected tmTNF α Jurkat cells as target cells and NK cell purified from PBMC as effector cells was assessed at three different antibody concentrations (2, 4 and 8 ng/mL):



Reviewer's Comments: An assay format was developed using enriched NK cells as effectors as well. It is possible that this assay could more precisely measure the activity of the effector cell type most likely to mediate ADCC via infliximab, if this activity occurs or is important for down-modulating inflammation at diseased sites. Between 26 and 35 lots of the three antibodies were compared at three different concentrations. While considerable overlap exists between the lots of the products, a small downward shift is evident in ADCC activity in the assay format. Nevertheless, >90% of the lots of the proposed biosimilar are within the quality range of the reference product, which meets the acceptance criteria set by CMC statistics. This resolves CR comment 2a.

2B) Control Strategy CR Language

“If the difference in ADCC persists following analysis of additional lots, you should identify and demonstrate control of the product quality attributes that underlie ADCC activity in CT-P13 (e.g., glycan pattern, contribution of H2L1 variant, etc.)”

Response:

Celltrion performed an exercise where impurity-enriched CT-P13 preparations were evaluated for ADCC activity and Fc γ RIIIa binding. The goal was to see if any of the variants/impurities normally present in CT-P13 in low levels, such as afucosylation, deglycosylation, H2L1 forms, high molecular weight (HMW) forms and/or glycation are impacting ADCC activity. If so, the identified variant could be the structural basis in the antibody molecule underlying the difference in Fc γ RIIIa binding affinity and hence ADCC. These impurities were enriched by fractionation or stress/enzyme incubation. Of these, only glycan variants had a noticeable impact trend on ADCC activity (see table below).

The first aspect Celltrion evaluated were potential events in manufacture of DS and DP lots that would result in abnormal FcγRIIIa or NK ADCC activities. None were identified; this is probably an inherent characteristic of the molecule secreted by the CT-P13 expression cell line. A rough correlation was established between ADCC activity and FcγRIIIa binding but as can be seen the R² value is low (see scatter plot below). However, the majority of the CT-P13 lots fall in the bottom left quadrant of the scatter plot, arguing that by controlling the FcγRIIIa binding upwards, an up-shift in ADCC activity is the likely result.

Scatter plot of ADCC activity and FcγRIIIa binding of the various lot of CT-P13 and Remicade tested (CT-P13 is in blue).

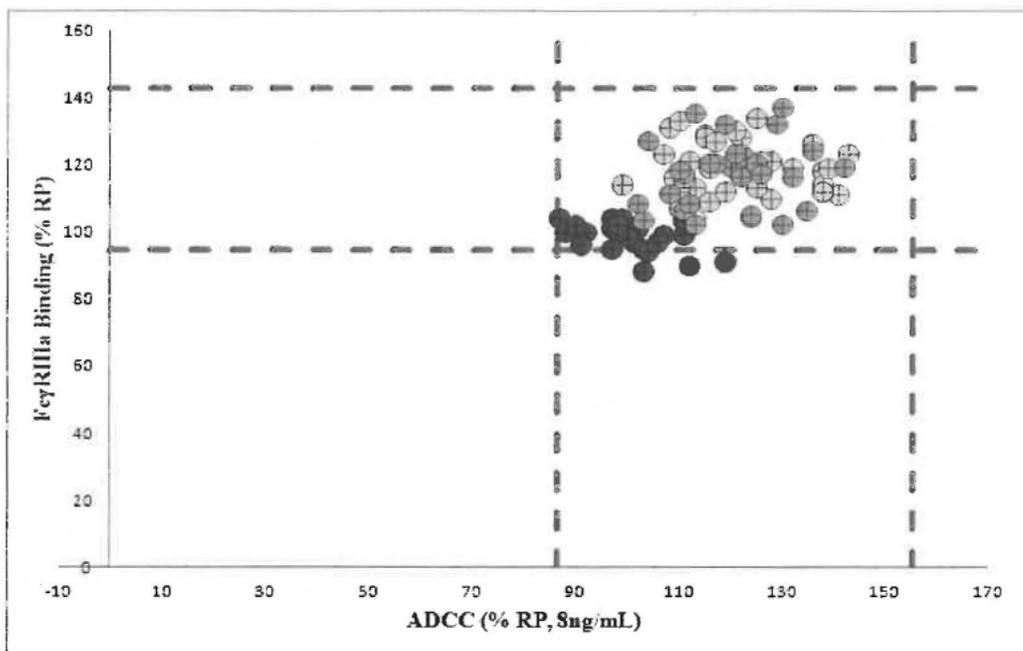
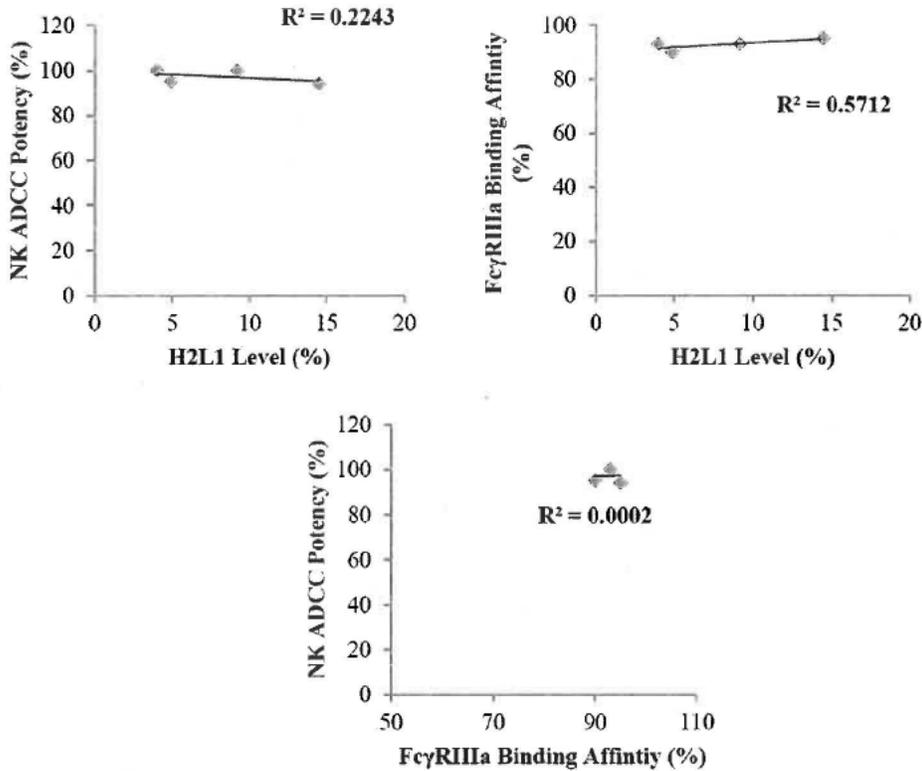


Table of attributes tested and potential correlations between each attribute and ADCC activity or FcγRIIIa binding.

Attribute	Samples	Correlation with FcγRIIIa Binding Affinity	Correlation with NK ADCC Activity
Afucosylated glycans	Highly afucosylated samples	Yes	Yes
	Levels seen in CT-P13 and Remicade®	No	No
Non-glycosylated forms	Highly non-glycosylated samples	Yes	Yes
	Levels seen in CT-P13 and Remicade®	No	No
H2L1 forms	High H2L1 samples	No	No
High molecular weight (HMW) forms	Highly HMW samples	No	No
Glycated forms	Highly glycated samples	No	No

As stated in the table, only glycan variants correlated with ADCC activity or FcγRIIIa binding strength. Instead of showing all negative data in this review, below is an example to illustrate the investigation procedure; impact of H2L1 forms on NK ADCC activity. This was performed using CT-P13 drug product and 4 samples of fractionated CT-P13 with different levels of H2L1. The isoforms were separated and enriched from CT-P13 drug product (Lot# 12B1C003) using two CHT (Ceramic Hydroxyapatite) chromatography steps and a size-exclusion chromatography step. As can be seen, there is no noticeable correlation between H2:L1 and NK-based ADCC activity.

Figure 21: NK ADCC Activity of CT-P13 Samples with Varying H2L1 Levels



As performed above for ADCC, the Agency performed an independent statistical analysis of the applicant's FcγRIIIa data from CT-P13 and US-licensed Remicade lots (see graph below). While CT-P13 drug product and drug substance were largely overlapping (with one DP outlier), a shift towards lower binding activity by CT-P13 was confirmed. Further, 15% of the CT-P13 DP lots were below the quality range set by the reference product.

Reviewer's Comments: *These observations suggest that a control strategy based on FcγRIIIa testing is warranted. While the correlation (R^2) between ADCC and FcγRIIIa is not strong (see scatter plot above), most CT-P13 lots were in the lower left hand quadrant (i.e., lower ADCC, lower FcγRIIIa binding) in the scatter plot. Furthermore, a role for FcγRIIIa binding as part of NK cell-dependent ADCC is considered established scientifically. For this reason, the applicant's proposed specifications for FcγRIIIa binding by DS of (b) (4) % of reference should be tightened to remove low ADCC outliers.*

Reviewers Comments: The applicant proposed criteria for FcγRIIIa of (b) (4) % for Drug Substance is not appropriate for CT-P13. When compared with the QR of the US Remicade, the lower bound of the range is (b) (4) %, therefore selection of (b) (4) % would permit the release of lots with unacceptable quality. Additionally, the use of (b) (4) is unacceptable to reject the rare occurrence of statistical outliers on the upper end. Therefore, the Agency determined that the specification should be changed to ensure that released CT-P13 lots remain in the quality range of US-licensed Remicade. An IR was sent 04 Jan 2016 that asked the firm to change their proposed specifications.

Comment conveyed to Celltrion on January 4, 2016:

“We have concerns regarding your control strategy in which you propose a drug substance (DS) acceptance criterion for FcγRIIIa V type of (b) (4) % (% relative potency). This value is based on mean values +/- 3SD generated from the analysis of CT-P13 lots that were assessed alongside US-licensed Remicade for NK ADCC activity. Further, your proposed acceptance criterion does not contain an upper bound. Develop a tighter control strategy, which includes an upper and lower bound, for FcγRIIIa binding for the drug substance such that the release specifications assure that the CT-P13 product is appropriately controlled within the variability of the reference product (e.g., within 3 SD of the reference product mean).”

IR Response: Celltrion responded to the IR on 08 Jan 2016 and accepted the proposed changes to the control strategy for the drug substance. See below table copied from the response.

Proposed Acceptance Criterion of FcγRIIIa Binding Affinity for CT-P13 Drug Substance at Batch Release

	Evaluation	Acceptance Criterion
Previously proposed on 05 Oct 2015	Mean-3SD of FcγRIIIa binding affinity (% relative potency) of CT-P13 lots excluding lots outside the QR for NK ADCC activity	(b) (4)
Newly Proposed	Mean±3SD of FcγRIIIa binding affinity (% relative potency) of all US-licensed Remicade® lots	

Reviewer's Comment: The newly proposed control strategy is acceptable and resolves CR comment 2B.

CR comment 2C

'If the difference in ADCC persists following analysis of additional lots, you shouldprovide an adequate justification, including an evaluation of the role of ADCC particularly in the setting of inflammatory bowel disease, that the observed difference in ADCC is not relevant to clinical activity'.

Celltrion response on ADCC and Clinical Impact

Celltrion updated their position paper in section 5.3.5.4 of the BLA to provide a comprehensive literature search on failed TNF-blockers (CDP571, etc.), expert opinions, and a new experimental report from the Department of Gastroenterology, Chaim Sheba Medical Center (Affiliated to the Tel-Aviv University, Sackler School of Medicine, Israel) on the ADCC activity of gut lamina propria mononuclear cells. The goal was to further 'provide an adequate justification, including an evaluation of the role of ADCC particularly in the setting of inflammatory bowel disease, that the observed difference in ADCC is not relevant to clinical activity'.

Reviewer's Comments:

See reviews of the Position paper on the "Extrapolation of CT-P13 Data to Indications for which Licensure is sought" submitted in section 5.3.5.4 in Aug 2014 and the position paper appendix submitted on 05 Oct 2015, uploaded on January 5, 2016 and January 7, 2016, respectively. Essentially, Celltrion argues that, based on their assessment of the scientific and medical literature as well as in-house experimentation, ADCC is probably not an important mechanism of action for infliximab in any indication. The arguments presented by Celltrion were reasonable. The Agency now considers ADCC to be a "plausible" mechanism of action of infliximab for which a similarity approach involving a quality range assessment is appropriate. ADCC activity between CT-P13 and US-licensed Remicade is highly similar based on this assessment. This addresses CR comment 2c.

Additional New Quality information:

Review of glass vial DMF

The type 1 borosilicate glass vials used for CT-P13 drug product are sourced from (b) (4). The DMF for the vials is DMF (b) (4), submitted May 29, 2008. Below is a summary of the DMF content:

- An org chart and company history was provided. The company was founded in (b) (4)
- Glass tubing is sourced from (b) (4). This raw material is inspected by (b) (4) for visible attributes, physical dimension and CoA testing by (b) (4) upon arrival.
- Engineering diagrams of (b) (4) mL vials were provided. The firm also makes (b) (4) diagrams of these were also provided,
- Manufacture of vials consist of the following steps:



- Environmental and safety information to comply with (b) (4) requirements.
- A sample CoA for vials and ampoules.
- Certificates from (b) (4) stating compliance with ISO 14001:2004 and ISO 9001:2000.

Adjustment of Lyophilization Cycle for (b) (4) for Manufacture of CT-P13

Previously, the applicant had experienced “(b) (4)”. This can occur when (b) (4). To correct this issue, the sponsor is proposing to adjust the (b) (4). Three simulated runs have successfully been performed that have validated a hold time of (b) (4).

Reviewers note: *This adjustment to the lyophilization cycle is acceptable. The validation data provided in the submission support the new cycle as minimizing (b) (4) during the (b) (4) step.*

Additional Stability data:

At time of submission of CR response:

The applicant has completed some long-term, accelerated, and stressed stability studies and some are on-going. In the submission, the following data have been provided within the acceptance criteria for the following conditions:

- Start and current (3 month) pull point data for the stability of batches stored under long-term conditions ($5\pm 3^{\circ}\text{C}$) for the final commercial process.
- Start and end (6 month) pull point data for the stability of batches stored under accelerated conditions ($25\pm 2^{\circ}\text{C}$ / $60\pm 5\%$ RH).
- Start and end (3 month) pull point data for the stability of batches stored under stress conditions ($40\pm 2^{\circ}\text{C}$ / $75\pm 5\%$ RH)

Submitted as an amendment on January 26, 2016:

An information amendment submitted at mid-cycle provided updated real-time stability data through 45 months for the CT-P13 drug product manufactured at CLT^{(b)(4)}. This represents the proposed marketed product held at the recommended storage conditions and can be used to set expiry. There are also 51 months supportive data for the lots produced by RB and ^{(b)(4)} sites. CELLTRION proposes to extend the shelf-life to 51 months for the drug product based on 45 months stability data are within the acceptance without significant trending from the drug product manufactured by CLT^{(b)(4)} with 6 months ^{(b)(4)} extrapolation. The extrapolation is supported by real-time data from additional lots of non-CLT^{(b)(4)} CT-P13 placed on stability. The current ongoing stability protocol is in alignment with this proposal, and data can be submitted in annual reports. Section 3.2.P.8.1 has an updated stability protocol to extend the shelf life post-approval.

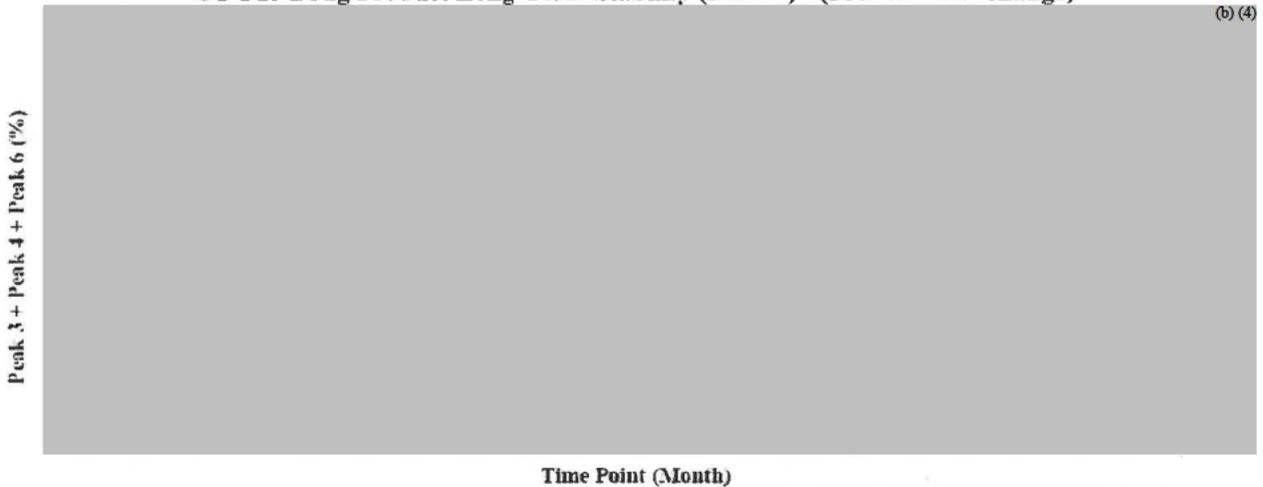
A summary of lots placed on stability is in the table below:

Batch	Date of Initiation of Study	Data Currently Available	Study Status	Container CI
CT-P13 Drug Product Manufactured at RB				20 mL Type I borosilicate glass vial, 20 mm stopper, 20 mm flip-off seal
09B9401	16 Dec 2009	60M	Complete	
09B9502	21 Dec 2009	60M	Complete	
10B9301	08 Jan 2010	60M	Complete	
10B0303	06 May 2010	60M	Complete	
10B0204	09 Jul 2010	60M	Complete	
10B0106	20 Dec 2010	54M	Ongoing	
B11R001	18 Apr 2011	51M	Ongoing	
B11R002	27 Apr 2011	51M	Ongoing	
B11R003	10 May 2011	51M	Ongoing	
CT-P13 Drug Product Manufactured at ^{(b)(4)}				

B11M004	18 Jul 2011	51M	Ongoing	
B11M005	22 Jul 2011	51M	Ongoing	
B11M006	25 Jul 2011	51M	Ongoing	
CT-P13 Drug Product Manufactured at CL1^(b)₍₄₎				
12B1C002	13 Feb 2012	45M	Ongoing	
12B1C003	18 Feb 2012	45M	Ongoing	
12B1C004	23 Feb 2012	45M	Ongoing	
CT-P13 Drug Product Manufactured at CL1^(b)₍₄₎ (Final Proposed Commercial Process for US Market)				
15B4C01	29 Mar 2015	3M	Ongoing	
15B4C02	02 Apr 2015	3M	Ongoing	
15B4C03	06 Apr 2015	3M	Ongoing	
EU-approved Remicade[®]				Native container closure of Remicade [®]
9RMA60401	08 Jan 2010	24M	Complete	
9RMA60902	08 Jan 2010	24M	Complete	

Stability trend of charge isoforms of product held under recommended storage conditions (i.e. 5°C)

**IEC-HPLC (%Peak 3 + Peak 4 + Peak 6) Trend Analysis (with EU approved Remicade)
CT-P13 Drug Product Long-Term Stability (5 ± 3°C) - (Pre- vs. Post- change)**



No charge isoform drift is evident when held under recommended storage conditions. Lots 09B9401, 09B9502, 10B9301 represent Process A (pre change) manufactured at RB facility, Lots 10B0303, 10B0204, 10B0106 represent Process B (post change) manufactured at the same RB facility, while Lots 9RMA60401 and 9RMA60902 are EU-approved Remicade lots. The IEC-HPLC profiles of the main peaks (Peak 3 + Peak 4 + Peak 6) are all within acceptance

criteria when held at the proposed long term storage condition (5°C) for (b) (4) months and show no a significant downward trend.

**IEC-HPLC (%Peak 5) Trend Analysis (with EU approved Remicade)
CT-P13 Drug Product Long-Term Stability (5 ± 3°C) - (Pre- vs. Post- change)**



The same lots as above, with similar observations for Peak 5, the minor peak. The pre-process change lots (i.e., process A) are near the specification limit of (b) (4)%. The post-change lots are within acceptance criteria with no significant trend. It should be noted that the EU Remicade lots have more of this variant (6-7%) than CT-P13 lots. Accelerated and stress condition studies of CT-P13 show an expected upward trend of Peak 5, but it remains within stability specifications.

Reviewers Comments:

The stability data, on-going study under protocol and non-CLI (b) (4) lot data support a 51 month expiration date. Small changes in (b) (4) made in 2015 to correct a minor protein content imbalance (3%) between CT-P13 and Remicade DPs are highly unlikely to impact stability and are not relevant to an expiry claim based on biochemically identical 2012 drug product manufactured at CLI (b) (4)

Reviewers Summary:

The data submitted in this package complete an extensive comparison of the functional, physicochemical, protein and higher order structure attributes of CT-P13 and US-licensed Remicade. The overall quality package lead us to the conclusion the proposed biosimilar is analytically “highly similar” to the reference product. The updated data package also support the conclusion that EU-approved and US-licensed Remicade products are sufficiently similar to allow an analytical bridge for extrapolation of clinical and non-clinical data using the EU product to the reference product. On February 9, 2016 the Rheumatoid Arthritis Advisory Committee voted 21-3 to approve CT-P13 as a biosimilar to Remicade for all proposed indications. BLA approval is recommended

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My Work Projects Reporting Requests Timesheet



BLA-125544-ORIG-1-RESUB-42 » Manufacturing Facility Inspection

Overall Manufacturing Inspection Recommendation

Edit Task | Task Actions

Task Summary Task Details Issues Updates **Inspection Management Form**

Inspection Management Form

As of 1:17 PM

Inspection Management Form

BLA-125544-ORIG-1-RESUB-42

- (b) (4) CTX CONTROL TESTING LABORATORIES "ALSO" (DRUGS) | Approve Facility -
- (b) (4) CTL CONTROL TESTING LABORATORY | Approve Facility -
- CELLTRION INC | 3005241015 | CBI BIOTECHNOLOGY DERIVED API (STERILE & NON-STERILE) | Approve Facility -
- CELLTRION INC | 3005241015 | SVL SMALL VOLUME PARENTERAL, LYOPHILIZED | Approve Facility -
- (b) (4) | CTX CONTROL TESTING LABORATORIES "ALSO" (DRUGS) | Approve Facility -
- (b) (4) | CTL CONTROL TESTING LABORATORY | Approve Facility -
- (b) (4) | CTL CONTROL TESTING LABORATORY | Approve Facility -
- (b) (4) | SVL SMALL VOLUME PARENTERAL, LYOPHILIZED | Approve Facility -

Overall Manufacturing Inspection Recommendation

- Approve
- Withhold

Cancel

Assigned To

OPF Reviewer

Wayne Seifert

IM - OPF Reviewer

Edit Assignment

This was done on
Mar 9, 2016
(12 days ago)

Status Complete

Requested by

DARRTS Integration

This task is waiting on Facilities

Last Update	Submitted On
Mar 9, 2016	Oct 6, 2015

Reference Number
5802113

Submission Manufacturing Facilities

Facility Status	Completion Date	Project Name	FBI	DUNS	Global ID	Facility Name	Profile Co
Approve Facility	3/9/2016	BLA-125544-ORIG-1-RESUB-42				(b) (4)	SVL SMAI
Approve Facility	1/6/2016	BLA-125544-ORIG-1-RESUB-42					CTL CONT
Approve Facility	1/6/2016	BLA-125544-ORIG-1-RESUB-42					CTX CON
Approve Facility	1/6/2016	BLA-125544-ORIG-1-RESUB-42					CTL CONT
Approve Facility	1/6/2016	BLA-125544-ORIG-1-RESUB-42					CTL CONT
Approve Facility	1/6/2016	BLA-125544-ORIG-1-RESUB-42					CTX CON
Approve Facility	1/6/2016	BLA-125544-ORIG-1-RESUB-42	3005241015	688836030	131388	CELLTRION INC	SVL SMAI
Approve Facility	1/6/2016	BLA-125544-ORIG-1-RESUB-42	3005241015	688836030	131388	CELLTRION INC	CBI BIOTE
Approve Facility	6/4/2015	BLA-125544-ORIG-1	3005241015	688836030	131388	CELLTRION INC	SVL SMAI
Approve Facility	6/4/2015	BLA-125544-ORIG-1	3005951160	688836030	131206	CELLTRION INC	CBI BIOTE
No Further Evaluation	6/4/2015	BLA-125544-ORIG-1	3009681457	687944645	131384	BINEX	CTL CONT



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

Date: 2/11/2016
To: Administrative File, STN 125544/0/38 (dated October 5, 2015)
From: Bo Chi, Ph.D., CDER/OPQ/OPF/DMA/Branch IV
Endorsement: Patricia Hughes, Ph.D., Acting Branch Chief, CDER/OPQ/OPF/DMA/Branch IV
Subject: 351(K) Biologic License Application (BLA) Resubmission
Applicant: Celltrion, Inc.
US License: 1996
Facility: CELLTRION, Inc., 20, Academy-ro 51 beon-gil, Yeonsu-gu, Incheon, Republic of Korea
FEI: 3005241015
Product: CT-P13
Dosage: Powder for solution, 100 mg/vial, intravenous
Indication: Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, ulcerative colitis, Crohn's disease, pediatric ulcerative colitis and pediatric Crohn's disease
BsUFA date: April 5, 2016

Recommendation: This review memo covers the shipping validation of unlabeled drug product vials. The shipping of unlabeled DP vials from Celltrion to (b) (4) has been adequately validated.

Review Summary

This is a resubmission to Biologics License Application (BLA) STN125544/0 for CT-P13. In the resubmission package, an additional labeling and packaging site at (b) (4) was added. The shipping validation data covering the shipping of unlabeled drug product vials from the DP facility at Celltrion, Incheon, Republic of Korea to (b) (4) were provided in amendment dated 2/5/2016 (Sequence 45). This review memo reviews these shipping validation data.

Assessment

(b) (4)



Reviewer comment: The shipping of unlabeled CT-P13 DP vials uses (b) (4)

The risk for temperature excursion is low. The shipping validation of the DP vials from the DP facility to the labeling and packaging facility (b) (4) is adequate.

Satisfactory

Conclusion

This review memo covers the shipping validation of unlabeled drug product vials. The shipping of unlabeled DP vials from Celltrion to (b) (4) has been adequately validated.

Cc: Chi
Hughes
Ton

Primary reviewer signature

Bo Chi -S



Digitally signed by Bo Chi -S
DN: c=US, o=U.S. Government,
ou=HHS, ou=FDA, ou=People,
cn=Bo Chi -S,
0.9.2342.19200300.100.1.1=13001
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Date: 2016.02.24 11:21:10 -05'00'

Secondary reviewer signature

Patricia F.
Hughestroost -S



Digitally signed by Patricia F.
Hughestroost -S
DN: c=US, o=U.S. Government, ou=HHS,
ou=FDA, ou=People,
0.9.2342.19200300.100.1.1=1300096547,
cn=Patricia F. Hughestroost -S
Date: 2016.02.24 12:41:56 -05'00'



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

U. S. Food and Drug Administration
Center for Drug Evaluation and Research
Division of Biotechnology Review and Research II
CDER/OPQ/OBP (HFD-123)
Silver Spring MD 20903

Subject: STN 125544: CTD module 5.3.5.4 Appendix; Product Quality Review Memo

Date: January, 5 2016

To: Administrative File, STN 125544

From: Cyrus Agarabi, PharmD, Ph.D., Quality Reviewer LBPS, CDER/OBP, Division 2

Through: Kurt Brorson, Ph.D., Acting Lab Chief LBPS, CDER/OBP, Division 2

Submission Type: BLA

Sponsor: Celltrion, Inc.

Contact: Jennifer Seiler, PhD., RAC

Product: CT-P13

Indication: rheumatoid arthritis and others

Executive Summary

Celltrion submitted a report, "Position paper on the Extrapolation of CT-P13 Data to Indications for which Licensure is sought" in their Aug 2014 BLA for CT-P13 (proposed infliximab biosimilar); it was placed in CTD module 5.3.5.4. The position paper presented an overview of

*C.A.
1/5/2016*

*KB
1-7-2016*

TNF biology, likely functions and mechanisms of action of the TNF antagonists (currently there are five licensed), their analytical biosimilarity exercise and a discussion of clinical data.

Celltrion's overall assertion was that their analytical biosimilarity exercise demonstrates that CT-P13 is highly similar to the US licensed reference product. They took a totality of evidence approach, based on the known (TNF binding and neutralization), likely (reverse signaling via mTNF on lamina propria T cells) and plausible (ADCC) mechanisms of action of the product. Their literature review concludes that TNF sequestration is the primary action of TNF blocker therapy, and for certain indications (i.e. IBD), inhibition of cytokine induction and/or apoptosis by reverse signaling via mTNF (a function not dependent on Fc function) is the most likely secondary mechanism. They acknowledge and addressed differences between the CT-P13 and the US-licensed Remicade for the glycans (% afucosylation), Fc γ RIII binding and associated biological activity (ADCC) but contend that the differences are small (~20% on average) and likely not clinically relevant given their review of the medical and scientific literature on the mechanism of TNF blockers.

In this appendix, submitted with the CR response on 5 Oct 2015, Celltrion provides some additional information:

- Expert assessments of Celltrion's position from three opinion leaders in the IBD field.
- A summary of known properties of other TNF blocking agents that failed to effectively treat IBD. These blocking agents, CDP571, Enbrel and Onercept, were referenced specifically in the Type I meeting held with FDA on 05 Aug 2015.
- An experimental report entitled "ADCC of intestinal cells from IBD patients by CT-P13 versus US-Remicade". It was written on 14 July 2015 by Shomron Ben-Horin M.D., Director, IBD Service & Gastro-Immunology Laboratory, Sheba Medical Center, Tel-Hashomer, Israel.

Expert Opinions. Three expert clinicians in the Rheumatology or IBD field reviewed Celltrion's position paper and batch data supporting extrapolation of the RA clinical data plus the finding of "high similarity" of the reference product to biosimilarity of CT-P13 for all indications

for infliximab. Each provided a summary of their assessment and supported extrapolation to all indications. They are:

-  (b) (4)
-
-

TNF blocking agents that failed to effectively treat IBD. In the Type I meeting held with FDA on 05 Aug 2015, four TNF blocking agents, Cimzia (certolizimab pegol), CDP571, Enbrel and Onercept, were referenced by CDER as potential examples why a functional Fc portion may be important for infliximab's mechanism of action in treating IBD. CDP571 is an IgG4 molecule that does not possess ADCC activity, while Enbrel and Onercept are TNFR:Fc fusion proteins with limited ADCC activity. Cimiza is a pegylated Fab fragment with no Fc portion; it is indicated for maintaining response in IBD patients, but not for induction. Celltrion provided an explanation for each why they believed that the subpar or missing IBD efficacy wasn't necessarily tied to limited or no ADCC activity.

- Cimiza is a pegylated Fab fragment developed by Celltech (now UCB), previously referred to as CDP870. It has no ADCC activity due to the absence of an Fc, but can reverse signal mTNF⁺ monocytes to down-modulate LPS-responsiveness (Nesbitt et al., 2007). Celltrion stated that some efficacy is evident in the maintenance effect and other favorable effects seen in CD patients, thus the therapeutic benefit of certolizumab pegol was considered clinically relevant  (b) (4). Nesbitt et al. proposed that the inhibition of LPS-induced cytokine release in gut associated monocytes is more important for TNF blocker efficacy in IBD vs. ADCC. Finally, Celltrion states "in the absence of direct head-to-head clinical studies between infliximab and certolizumab pegol, and because of methodological, study population (e.g. inclusion of infliximab-exposed patients in Cimzia® studies) and statistical differences between

ACCENT studies with Remicade® and PRECiSE studies with Cimzia®, it is challenging to draw conclusions on the impact of structural and functional differences between infliximab and certolizumab pegol translate on their efficacy profiles in IBD settings”.

- CDP571 (Humicade) was a product developed by Celltech (now UCB) in the 1990’s for IBD treatment. It is an IgG4, an isotype with limited ADCC activity. Celltrion pointed out that there were other factors that could have led to the lack of efficacy, including Fab-arm exchange, a known property of IgG4s, or low avidity. The low avidity of CDP571 was not reported in the published clinical literature, rather it was noted in the clinical pharmacology review for Cimzia in 2008. Apparently, CDP571 is 2-3 log₁₀ less potent at neutralizing TNF compared to CDP870 (Cimzia, certolizumab pegol). Celltrion also points out that the same limitations noted above about comparing different trials with different designs and study populations apply here as well.
- Enbrel (etanercept). Enbrel is a TNFR:Fc (p75 version of TNFR) fusion protein licensed for rheumatology indications, but not IBD. It has diminished (but not absent) ADCC activity relative to other TNF blockers like Remicade and Humira (Nesbitt et al., 2007). Celltrion pointed out that it also doesn’t reverse signal mTNF+ cells for either apoptosis or down-modulation of cytokine release. Celltrion also points out that the same limitations noted above about comparing different trials with different designs and study populations apply here as well.
- Onercept was a TNFR:Fc (p55 version of TNFR) fusion protein that failed during development. It was tested in two phase I/II trials for IBD, but was terminated after poor clinical results were seen. Because of the failed development, little data was released by the developing firm about its biological effects, except it did down-modulate IL-8 and adhesion molecule induction in endothelial cells stimulated by TNF. Celltrion held that the limitations noted above about comparing different trials with different designs and study populations, and the limited information released by the firm that developed this product renders any connection between ADCC and the observation that it didn’t work for IBD inconclusive.

Report by Shomron Ben-Horin M.D. This report, entitled “ADCC of intestinal cells from IBD patients by CT-P13 versus US-Remicade” extends in vitro studies performed by Celltrion using Remicade and the CT-P13 product by using intestinal lamina propria mononuclear cells (LPMC) from six IBD patients as target cells. LPMC would be the actual, tissue specific targets for ADCC by infliximab, if it were important for infliximab mechanism of action. This assay format is physiologically closer to the actual disease process than the format developed by Celltrion using LPS activated monocytes as targets, nor can it be argued to be artificial like using a transfectoma as a target population. Because of the highly specialized nature of the assay that required actual patient biopsies, it was contracted out to Dr. Ben-Horin’s lab in Sheba Medical Center, Israel. Dr. Ben-Horin’s lab found that infliximab-mediated ADCC of LPMC cells from the intestine of IBD patients was very low and similar to that exhibited in the presence of a control IgG1. No difference in ADCC of LPMC was observed between CT-P13 and US-Remicade at concentrations equivalent to high-end concentrations achieved in the blood of treated patients early after the infusions. His overall conclusion was that ADCC is probably not a mechanism of action of infliximab in IBD, either US-Remicade or CT-P13

Overall conclusion

Celltrion submitted cohesive arguments and evidence that ADCC is probably not a mechanism of action of infliximab in IBD, either US-Remicade or CT-P13.

References

Nesbitt, A., Fossati, G., Bergin, M., Stephens, P., Stephens, S., Foulkes, R., Brown, D., Robinson, M., & Bourne, T. (2007). Mechanism of action of certolizumab pegol (CDP870): in vitro comparison with other anti-tumor necrosis factor alpha agents. *Inflammatory bowel diseases*, 13(11), 1323-1332.

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/s/

CYRUS AGARABI
01/07/2016



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

U. S. Food and Drug Administration
Center for Drug Evaluation and Research
Division of Biotechnology Review and Research II
CDER/OPQ/OBP (HFD-123)
Silver Spring MD 20903

Subject: STN 125544: CTD module 5.3.5.4; Product Quality Review Memo

Date: January, 5 2016

To: Administrative File, STN 125544

From: Cyrus Agarabi, PharmD, Ph.D., Quality Reviewer LBPS, CDER/OBP, Division 2

Through: Kurt Brorson, Ph.D., Acting Lab Chief LBPS, CDER/OBP, Division 2

Submission Type: BLA

Sponsor: Celltrion, Inc.

Contact: Jennifer Seiler, PhD., RAC

Product: CT-P13

Indication: rheumatoid arthritis and others

C.A.
1/5/2016

K.B.
1/5/2016

Executive Summary

Celltrion submitted a report, “Position paper on the Extrapolation of CT-P13 Data to Indications for which Licensure is sought” in their Aug 2014 BLA for CT-P13 (Celltrion’s proposed infliximab biosimilar); it was placed in CTD module 5.3.5.4. The position paper presents an overview of Tumor necrosis factor- α (TNF) biology, likely functions and mechanisms of action of the TNF antagonists (currently there are five licensed), their analytical biosimilarity exercise and a discussion of clinical data.

Celltrion’s overall assertion is that their analytical biosimilarity exercise demonstrates that CT-P13 is highly similar to the US licensed reference product. They take a totality of evidence approach, based on the known (TNF binding and neutralization), likely (reverse signaling via mTNF on lamina propria T cells) and plausible antibody-dependent cell-mediated cytotoxicity (ADCC) mechanisms of action of the product. They acknowledge differences between the CT-P13 and the US-licensed Remicade for the glycans (% afucosylation), Fc γ RIII binding and associated biological activity (ADCC) but contend that the differences are small (~20% on average) and likely not clinically relevant given their review of the medical and scientific literature on the mechanism of TNF blockers. Thus, the ADCC activity differential would fall under the “notwithstanding minor differences in clinically inactive components” exemption for a designation of “highly similar” under the existing biosimilars guidance. Their literature review concludes that TNF sequestration is the primary action of TNF blocker therapy, and for certain indications (i.e., IBD), inhibition of cytokine induction and/or apoptosis by reverse signaling via mTNF (a function not dependent on Fc function) is the most likely secondary mechanism. While there is *in vivo* evidence from patients, biopsies and/or cultured cells from clinical samples that reverse signaling by TNF blockers may play a role in IBD efficacy, no direct evidence exist that ADCC plays a role. They conclude that ADCC of mTNF⁺ cells by TNF blockers is likely to be minimal *in vivo* and unlikely to be part of the mechanism of TNF blocker therapy. At the end of this review, an independent literature review of mechanisms of TNF blocker therapy is presented, including literature not evaluated by the Sponsor.

TNF Overview

Tumor necrosis factor- α (TNF) is a cytokine with a diverse array of biological functions; however its role at the nexus of chronic inflammatory response is most relevant in this context. TNF is expressed on the surface of a range of cell types and is released upon stimulation; it is functional both as a membrane bound (mTNF) form and soluble form (sTNF). Because both forms are active, signals may be passed locally from cell-to cell via mTNF, or more systemically through release of sTNF. The biological responses to TNF are mediated through two high affinity TNF receptors, TNF-R1 (p55) and TNF-R2 (p75). Most cells constitutively express TNF-R1 on their surface. In contrast, TNF-R2 is inducible and expressed preferentially on the hematopoietic and endothelial cells. The binding of TNF to TNF-R1 activates elements of the nuclear factor (NF)- κ B pathway. The NF- κ B pathway activates a range intracellular signaling pathways and release of proinflammatory cytokines. This flurry of events can lead to an inflammatory response, tissue damage and cytotoxicity. Experimental data along with clinical experience has consistently associated elevated TNF levels with the sites of inflammation in inflammatory diseases such as IBD, RA and others.

TNF and its role in chronic inflammatory diseases

Chronic inflammatory conditions result from a complex interaction between genetic and environmental factors. TNF has been shown to have a prominent role in the pathology of a number of chronic inflammatory conditions including the following:

- Rheumatoid Arthritis
- Ankylosing Spondylitis
- Psoriatic Arthritis
- Psoriasis
- Crohn's Disease and Ulcerative Colitis

With the exception of Crohn's disease and ulcerative colitis, these diseases are characterized by elevated serum levels of sTNF- α , arguing for a prominent role in eliciting the signs and symptoms of disease. In contrast, Crohn's disease differs from these diseases as they include localized granulomatous component hypothesized to involve both mTNF and sTNF- α . In the

position paper, Celltrion provides an overview of relevant medical literature on the role of TNF- α in the individual clinical indications currently approved for infliximab and the presumed mechanism impact of infliximab treatment (Table 1 excerpted from Celltrion position paper).

Table 1. Role of TNF α in disease, Genetic Association Between Diseases, Infliximab Target, Infliximab Mediated Effects and Impact of Treatment

Indication	Role of TNF α	Genetic Association Between Diseases	Primary Target for Infliximab	Infliximab Mediated Effects	Impact of infliximab treatment
RA	<p>Synovial inflammation: TNFα \rightarrow 1) \uparrow adhesion mol. + chemokines \rightarrow influx of leukocytes \rightarrow 2) \uparrow cytokines from activated T cells and MΦ.</p> <p>Cartilage damage: 1) cytokine-induced cytokine release. 2) activation of synovial fibroblasts \rightarrow TNFα and IL-1β \rightarrow further proinflammatory mediators (IL-1β, IL-6, IL-18 TNFα, vascular endothelial growth factor and matrix-degrading enzymes).</p> <p>Bone erosion: differentiation into mature osteoclasts driven by cytokines (TNFα and IL-1)</p>	Genome-wide association (GWAS) studies have shown a genetic overlap of disease pathways between AS, RA, Ps, PsA, CD and UC (Tsui <i>et al.</i> , 2014).	sTNF α	<ul style="list-style-type: none"> - Inhibits cytokine/selectin release - Inhibits osteoclast maturation - Inhibits recruitment of immune cells - Inhibits angiogenesis 	Reduce levels of Rheumatoid factors and markers of systemic inflammation, attenuate angiogenesis, decrease cytokine (e.g. IL-6, IL-1 β , TNF α and VEGF), chemokine and adhesion molecule expression in synovial tissue and fluid, diminish serum levels of cytokines and chemokines, and inhibit damage to cartilage and bone. Decrease the number of macrophages and T cells in synovial tissue of patients with RA (Smets <i>et al.</i> , 2003).
AS	Along with IL-1, TNF α induces downstream effects in response to proinflammatory cytokines	Genome-wide association (GWAS) studies have shown a genetic overlap of disease pathways between AS, RA, Ps, PsA, CD and UC (Tsui <i>et al.</i> , 2014).	sTNF α	<ul style="list-style-type: none"> - Neutralization of TNFα - Inhibits cytokine/selectin release - Inhibits osteoclast maturation - Inhibits recruitment of immune cells 	Robust attenuation of functional and pathological features of the disease is observed when TNF α is blocked. Downregulates T cell capacity for production of IFN and TNF α (Zou <i>et al.</i> , 2002).
PsA	Joint inflammation and bone erosion caused by similar processes as observed in RA patients.	have shown a genetic overlap between PsA, Ps, RA and IBD with some loci implicated in the TNF pathway (Bluett & Barton, 2012)	sTNF α	<ul style="list-style-type: none"> - Inhibits cytokine/selectin release - Cytokine/selectin release - Osteoclast maturation 	Decrease the number of leucocytes (predominantly T cells) in both synovial tissue and psoriatic lesions
Ps	Blocking TNF α signaling significantly reduces T cell numbers in the lesions in the skin of patients which leads to attenuation of disease development	Genome-wide association (GWAS) studies have shown a genetic overlap of disease pathways between AS, RA, Ps, PsA, CD and UC (Tsui <i>et al.</i> , 2014).	sTNF α	<ul style="list-style-type: none"> - Neutralization of TNFα. - Inhibits cytokine/selectin release - Inhibits T cell proliferation - Protects from apoptosis of keratinocytes 	Decrease the number of leucocytes (predominantly T cells) in psoriatic lesions
CD and UC	Implicated in the chronic inflammation evident in CD and UC patients Damage to epithelial cells via necrosis or apoptosis \rightarrow loss of epithelial barrier.	Shared genetic susceptibility factors (host immune response for example) (Ho <i>et al.</i> , 2011)	sTNF α	<ul style="list-style-type: none"> - Inhibits cytokine release - Inhibits myofibroblast stimulation - Inhibits MMP increase 	Binds to tmTNF α , hence affecting the downstream signaling cascade, making it effective in CD and UC patients; results in induction of regulatory macrophages which has been shown to be important in wound healing Exerts both proinflammatory action by blocking tmTNF α binding to its receptors and anti-inflammatory effects through stimulation of reverse signaling pathways.
	Inhibitory impact of TNF α on apoptosis of T lymphocytes residing in lamina propria \rightarrow immunodysregulation and perpetual mucosal inflammation.		tmTNF α	<ul style="list-style-type: none"> - Reverse signalling inhibits apoptosis and possibly cytokine release - Induction of regulatory macrophages 	
	Proposed as the initiating factor that drives mucosal degradation, ulceration and fistulas (Di Sabatino <i>et al.</i> , 2007).		tmTNF α (Fc mediated)	<ul style="list-style-type: none"> - Induction of regulatory macrophages 	

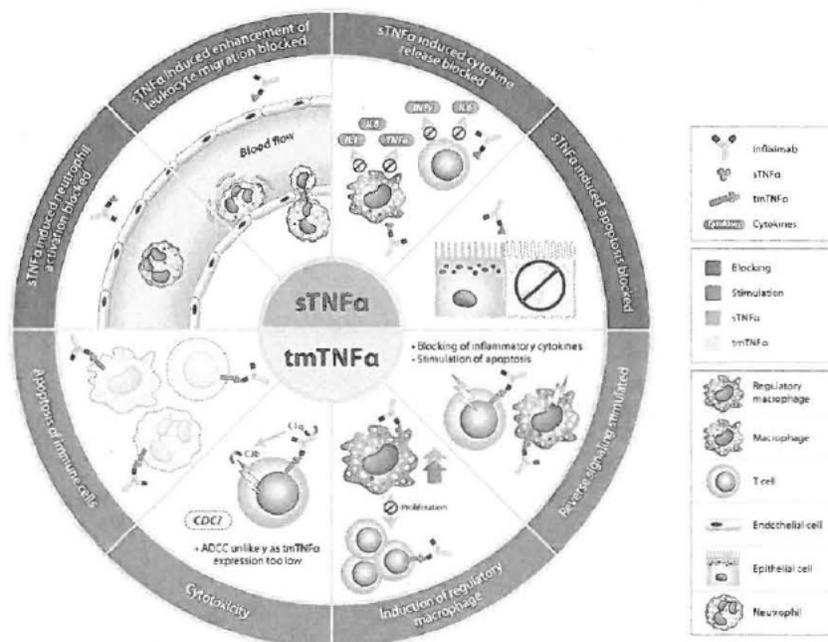
Presumed Mechanism of Action of Infliximab

In their position paper, Celltrion concluded based on their literature review that binding and neutralization of TNF- α (via sequestration) is the primary mechanism of action of infliximab for all the approved clinical indications. Binding and sequestration of sTNF- α removes it from sites of inflammation, suppressing further activation of proinflammatory components.

The binding of mTNF by infliximab could trigger other effects that may be relevant to the mechanism of action of some of the indications. Celltrion identified as also likely for IBD indications the prevention of binding of infliximab to mTNF and concomitant cellular downstream effects via reverse signaling including cytokine suppression and apoptosis. This contention is based on some published literature that suggest that infliximab may function this way in IBD patients, as supported by studies using *in vivo* immunoflorescent staining and/or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays of IBD patient biopsies as well as *in vitro* studies using cultured clinical isolates (Peake et al., 2013) ;(Atreya et al., 2014).

In contrast, Celltrion argues that ADCC is unlikely to play a role in the mechanism of action of infliximab. This is based on their review of the literature where no direct evidence exists from patients or patient samples that ADCC is induced after TNF blocker treatment. They also presented immunohistochemical staining data showing that expression of mTNF in inflamed IBD tissue is low, and unlikely to be at sufficient levels to trigger ADCC. The universe of potential cellular activities identified by Celltrion as possibly triggered by TNF and mTNF binding with infliximab are shown in Figure 1.below, excerpted from Celltrion position paper.

Figure 1. Potential cellular activities possibly triggered by TNF and mTNF binding



Biosimilarity: Structural Analysis and Functional Assays.

Celltrion has used a range of methods to demonstrate analytical similarity between CT-P13 and US licensed Remicade. They addressed primary structure, secondary and tertiary structure along with post-translational modifications and functional assay for both the V regions (antigen binding, reverse signaling) and the Fc domain (ADCC, etc.). Functional assays were chosen to measure that range of the possible mechanisms of action by infliximab, including antigen binding, reverse signaling, regulatory macrophage activity, induction of apoptosis, wound healing, complement fixation and ADCC.

Table 2 below is a summary of the functional assay data from 3-way enhanced similarity study subjected to a statistical analysis. While most functional assays demonstrated analytical similarity between CT-P13 and US-licensed Remicade, a subset directly related to Fc function do not (e.g., ADCC using fresh NK cells as effector cells, FcγRIIIa binding SPR).

Table 2. Summary Table of Equivalence Test Based on Statistical Analysis

Analytical Test Method	CT-P13 Vs US	EU Vs US
<i>In vitro</i> TNF α neutralization	Equivalent	Equivalent
TNF α Binding Affinity (ELISA)	Equivalent	Equivalent
Cell Based Binding Affinity	Equivalent	Equivalent
Inhibition of Cytokine Release by Reverse Signaling	Equivalent	Equivalent
Caco-2 (Cytokine Suppression)	Equivalent	Equivalent
FcRn Binding Affinity (SPR)	Equivalent	Equivalent
Induction of Apoptosis by Reverse Signaling (FACS)	Highly similar	Highly similar
Induction of Regulatory Macrophages	Highly similar	Moderately similar
T-cell Proliferation by Regulatory Macrophages	Highly similar	Moderately similar
Clq Binding Affinity (ELISA)	Highly similar	Highly similar
CDC	Highly similar	Highly similar
Fc γ RIIIa V Type Binding Affinity (SPR)	Low similarity	Highly similar
Fc γ RIIIa F Type Binding Affinity (SPR)	Low similarity	Highly similar
Fc γ RIIIb Binding Affinity (SPR)	Highly similar	Highly similar
Fc γ RIIa Binding Affinity (SPR)	Highly similar	Highly similar
Fc γ RIIb Binding Affinity (SPR)	Highly similar	Highly similar
Fc γ RI Binding Affinity (ELISA)	Highly similar	Highly similar
<i>Ex Vivo</i> Binding in 1% BSA with NK Cells	Low similarity	Highly similar
<i>Ex Vivo</i> Binding in 50% Serum with NK Cells	Highly similar	Highly similar
ADCC using PBMC (Healthy Donor)	Highly similar	Highly similar
ADCC using NK Cells (Healthy Donor)	Moderately similar	Highly similar

Binding TNF- α

Binding, sequestration and neutralization of soluble TNF- α represents the principal mechanism of action. Celltrion assessed the activity of CT-P13 and US-licensed Remicade using *in vitro* TNF- α neutralization assays and a TNF- α binding affinity ELISA. The results from this analysis are shown in Table 3 below. They support Celltrion's contention that CT-P13 is highly similar to the US licensed reference product in this aspect. The effects of afucosylation on the binding soluble and membrane-bound form of TNF- α were measured using an afucosylated form of CT-P13. This function is dependent on the complementarity determining region (CDR) surface part of the antibody, which is physically distant from the N-linked glycosylation site. Thus, removal of even all fucoses from the antibody would not be expected to have an effect on these functions.

Table 3. Neutralization of Soluble TNF α by CT-P13 and Remicade and Afucosylated CT-P13

Test Method	Purpose		US-licensed Remicade [§]	CT-P13 Drug Product	CT-P13 14.5% Afucosylation ¹	EU-approved Remicade [§]	Equivalence Margin ²	Equivalence Test ³
ELISA (TNF α binding affinity)	Comparison: TNF α binding affinity (Relative binding, %)	2-Way Similarity Study	-	94.8 \pm 5.6 (n=5)	102 (n=1)	95.7 \pm 3.2 (n=3)	(-20.00, 20.00)	CT-P13 vs. EU : (-7.85, 6.12)
		3-Way Similarity Study (including US-licensed Remicade [§])	100.1 \pm 3.5 (n=10)	98.9 \pm 2.2 (n=10)	-	97.4 \pm 3.5 (n=10)	(-10.43, 10.43)	US vs. CT-P13 : (-1.11, 3.43) EU vs. CT-P13 : (-3.80, 0.74) US vs. EU : (-0.01, 5.39)
ELISA (hTNF β binding affinity)	Comparison: TNF β binding affinity	2-Way Similarity Study	-	No binding affinity	-	No binding affinity	-	-
<i>In vitro</i> TNF α neutralization	Comparison: neutralizing effect on TNF α based upon the viability of mouse sarcoma WEHI 164 cell (Relative potency, %)	2-Way Similarity Study	-	101.6 \pm 4.8 (n=5)	108 (n=1)	104.7 \pm 6.5 (n=3)	(-20.00, 20.00)	CT-P13 vs. EU : (-10.79, 4.66)
		3-Way Similarity Study (including US-licensed Remicade [§])	103.5 \pm 3.6 (n=10)	102.3 \pm 4.3 (n=10)	-	102.7 \pm 4.0 (n=10)	(-10.85, 10.85)	US vs. CT-P13 : (-1.81, 4.33) EU vs. CT-P13 : (-2.83, 3.59) US vs. EU : (2.07, 3.83)

¹ A batch of CT-P13 produced with afucosylation levels of up to 14.5% (achieved by spiking with material from batches produced in the presence of a fucosylation inhibitor).

² For 2-way similarity study, Equivalence Margin (EM) in means for confidence interval was determined as $\pm 20\%$ for relative potency bioassays. For 3-way similarity study, EM was determined as 3SD of reference product.

³ Results are presented as 90% CI of mean difference between two products.

The interaction with membrane-bound TNF- α was also assessed using a cell-based assay with Jurkat cells that express membrane bound TNF- α . shown below in Table 4. Cell based assays tend to be less precise than assays that use purified proteins, like the ELISA described above. Nevertheless, the results of the analysis provided supportive evidence that CT-P13 is highly similar to the US licensed reference product in this aspect.

Table 4. Neutralization of mTNF α by CT-P13 and Remicade and Afucosylated CT-P13

Test Method	Purpose		US-licensed Remicade [§]	CT-P13 Drug Product	CT-P13 14.5% Afucosylation ¹	EU-approved Remicade [§]	Equivalence Margin ²	Equivalence Test ³
Cell based binding affinity	Comparison: cell based binding affinities (Relative binding, %)	2-Way Similarity Study	-	93 \pm 8.8 (n=5)	99 (n=1)	97 \pm 6.3 (n=3)	(-20.00, 20.00)	CT-P13 vs. EU : (-9.74, 1.83)
		3-Way Similarity Study	98 \pm 6.8 (n=10)	99 \pm 6.3 (n=10)	-	101 \pm 7.1 (n=10)	(-20.45, 20.45)	US vs. CT-P13 : (-6.19, 3.99) EU vs. CT-P13 : (-3.01, 7.41) US vs. EU : (-8.70, 2.01)

¹A batch of CT-P13 produced with afucosylation levels of up to 14.5% (achieved by spiking with material from batches produced in the presence of a fucosylation inhibitor).

²For the 2-way similarity study, Equivalence Margin (EM) in means for confidence interval was determined as $\pm 20\%$ for relative potency bioassays. For the 3-way similarity study, EM was determined as 3SD of Reference Product values.

³Results are presented as 90% CI of mean difference between two products.

IBD *in vitro* models.

Based on Celltrion's literature review, three potential tmTNF mediated mechanisms of action for infliximab have been established based on *in vivo* or clinical evidence: (1) reverse signaling suppressing secretion of IL-1, IL-10 and IL-12 from monocytes (Eissner G & E., 2000)(Nesbitt et al., 2007) (Shen et al., 2007)); (2) induction of regulatory macrophages ((Vos et al., 2011); (Vos et al., 2012)); (3) stimulation of apoptosis in monocytes ((Eissner et al., 2000)) Shen et al., 2007 and T cells ((Mitoma et al., 2005)) Specific assays that model these three mechanisms were used to measure activity of CT-P13 and US licensed Remicade. When feasible, these assays used intestinally derived cells like Caco-2 cell to model epithelial cells that line the surface of the intestinal mucosa.

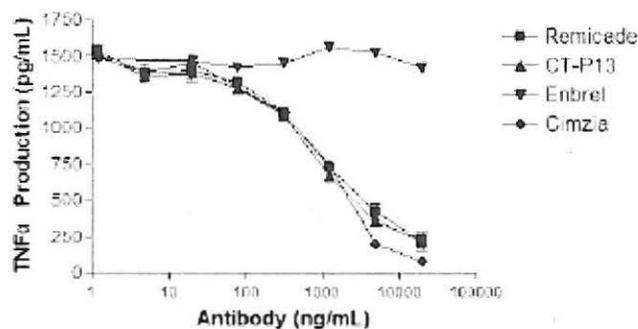
Reverse Signaling and Apoptosis

Reverse signaling is a cellular feedback which occurs when a molecule that is normally a signaling molecule like TNF- α that is present on the cell surface of immune cells (e.g., NK cells and monocytes) is bound by an antibody which transduces a signal to that cell. This reverse signal can transduce a cellular response in the mTNF⁺ cells leading to suppression of cytokine release and even apoptosis. Celltrion's reverse signaling assays use Caco-2 cells stimulated with a mixture of inflammatory cytokines and lipopolysaccharide (LPS) to induce a cellular response. The suppression of cytokine secretion and apoptosis by US-licensed Remicade and CT-P13 was

assessed. As discussed above, reverse signaling is described in the literature as important in the mechanism of action of TNF antagonists in inflammatory bowel diseases. Additional circumstantial evidence for this contention is provided by the observation that infliximab, adalimumab and certolizumab pegol (a Fab fragment that lacks an Fc portion) are able to reverse signal cytokine suppression in an *in vitro* assay, while etanercept does not (Nesbitt et al., 2007) The authors contended that this observation correlates with anti-IBD activity of the former three products, but not the latter.

Celltrion recapitulated this observation in Figure 2 of *in vitro* reverse signaling data in the position paper:

Figure 2 Reverse Signaling; Suppression of TNF α Release from LPS-Stimulated PBMCs by Cimzia, Enbrel, CT-P13 and Remicade



Celltrion provided *in vitro* assay-based evidence that US-licensed Remicade and CT-P13 possessed analytically similar reverse signaling activity using three separate assay formats:

- Apoptosis through reverse signaling measured in an assay using differentiated Caco-2 cells as well as PBMCs. For the Caco-2 cells, apoptosis activity was determined 24 hours following exposure to TNF α , IL-1 β , IFN γ and LPS and either US-licensed Remicade and CT-P13. The PBMC format used LPS. The data show that apoptosis induction is equivalent. Moreover, removal of fucoses from the antibody does not have an impact on apoptosis through reverse signaling.

- Cytokine suppression through reverse signaling was measured using Caco-2 cells activated by TNF α , IL-1 β , IFN γ and LPS. IL-8 release was suppressed equivalently in a dose dependent manner by US-licensed infliximab and CT-P13 in this assay.

Induction of Regulatory Macrophages – Mixed Lymphocyte Reaction (MLR) assay and Wound Healing

Wound healing is a putative outcome from infliximab therapy believed to be mediated by regulatory macrophages. IBD induces wounds in the intestinal lining such as fistulas that require closure for healing to take place. Presumed induction of the regulatory macrophages by infliximab is hypothesized to be mediated by activated T lymphocytes expressing mTNF with bound antibody which bridges via the Fc region with a macrophage (Vos et al., 2011)). Celltrion employed a mixed lymphocyte reaction assay to measure inhibition of T-cell proliferation and the induction of regulatory macrophages. Wound healing was also examined by an assay using physically manipulated *ex vivo* colorectal epithelium tissue. While data from these assays are largely regarded as semi-quantitative, US-licensed Remicade and CT-P13 possessed analytically similar bioactivity (data not shown).

Complement Binding and CDC Activity

Complement activity requires a series of serum proteins that are activated by cell surface antibody binding that leads to cell lysis via membrane pore formation, referred to as complement dependent cytotoxicity (CDC). This cascade begins with the binding of the first subcomponent of the C1 complex, C1q, to antibodies cross-linked to a cell surface via their Fc portions. This leads to the sequential activation of the other eight complement proteins; the last of which (C9) ultimately forms transmembrane pores in the target cell. In theory, complement dependent lysis of mTNF⁺ inflammatory cells could down-modulate a disease process at an inflamed site. Celltrion could not find any references in the medical or scientific literature providing *in vivo*/clinical evidence that CDC is a mechanism of action for infliximab in any of the approved

indications, although it was possible to develop *in vitro* assays using infliximab. CT-P13 and US-licensed Remicade were compared for C1q binding using a sandwich-ELISA assay, and complement dependent-cytotoxicity using a cell-based assay. CT-P13 and US-licensed Remicade were shown to be analytically similar in this activity (data not shown).

FcR Binding

Fc receptors are cell surface molecules that bind antibodies by their Fc portion. There are several types, including FcRn, FcγRI, FcγRII, and FcγRIII types, as well as subtypes (e.g., there are two subtypes, a and b, of FcγRII and FcγRIII). They are differentially expressed various cell types, for example, FcγRIIIa, also known as CD16, is predominantly expressed on NK cells (natural killer cells). Polymorphonucleated leukocytes (PMNs) express FcγRII. In ADCC, Fc receptor molecules allow bridging between effector cells and target cells with antibody bound to their surface. Because of this, the binding strength between infliximab and FcγRs may impact antibody function, especially if NK mediated ADCC of TNF⁺ inflammatory cells is a component of drug efficacy. Binding strength of CT-P13 and US-licensed Remicade to a panel of FcγRs was assessed and compared with either SPR or ELISA assays. The binding interaction between FcRn, FcγRI, FcγRIIa, and FcγRIIb receptor types and CT-P13 were shown to be analytically similar to US-licensed Remicade. In contrast, CT-P13 binding to both FcγRIIIa and FcγRIIIb was about 20-25% lower than that of US-licensed Remicade.

As mentioned above, FcγRIIIa is predominantly expressed on NK cells and key for their ADCC function. Because of this, they also evaluated binding of the two main FcγRIIIa genotypes, 158V and 158F in a FACS-based NK-cell binding assay. These studies were conducted using V/V (highest affinity), V/F and F/F (lowest affinity) NK cells from healthy and IBD patients. They were also conducted in the presence of either 1% BSA or 50% normal serum. They observed that Remicade had approximately 20-25% higher binding under the following circumstances:

- NK cells from either CD patients or healthy individuals with the higher affinity Fc γ RIIIa isotype, V/V, but not the low affinity F/F isotype.
- Assayed in the presence of 1% BSA, but not 50% serum.
- Antibody added at 1 and 10 ug/mL, but not at 100 ug/mL.

Celltrion provided arguments that the above assay results, while real, do not reflect physiological conditions at sites of inflammation. First, 50% serum is more complex, contains native antibodies which could also take up Fc γ R binding sites and is overall closer to the actual physiological composition of the interstitial fluid at an inflamed site vs. PBS with 1% BSA. Second, infliximab is administered at saturating levels level relative, thus the experiments conducted at 100 ug/mL antibody are a better model for an inflamed site in an infliximab-treated patient.

Reviewer's note: *The fluid compositions of actual inflamed sites in each of the infliximab indications are unknown, rendering their arguments speculative.*

ADCC activity

ADCC is an effector mechanism of antibodies where bridging between “effector cells”, often NK cells, eosinophils or other PMNs, and antibody bound “target cells” results in lysis of the target cells by releasing perforins and proteases in their proximity. This activity is particularly important in the immune response against viruses, where viral proteins on the surface of an infected cell will be bound by antibodies for ADCC targeting. It also can be important for clearance of helminth infections where IgE coat these parasites attracting eosinophil effector cells. FcRs, which bridge the effector cells to the antibody on the target cells, are key for this activity. FcRs are a large family of proteins, which are distributed differentially on effector cell types. For example, natural killer cells (NK cells) have Fc γ RIIIa on their surface, which is critical for ADCC activity. Similarly, eosinophils have Fc ϵ RI on their surface, while PMNs have Fc γ RII. Some FcRs do not mediate ADCC; for example Fc γ RIIB is inhibitory while FcRn transports IgG in neonates.

In theory, ADCC of inflammatory cells at disease sites could be a part of the mechanism of action of infliximab. Celltrion asserts that no clinical or *in vivo* studies in the literature exists to support this hypothesis (Peake et al., 2013), although infliximab ADCC activity can be measured in *in vitro* assays. Celltrion provided several arguments against the relevance of infliximab ADCC in IBD indications. Firstly, the studies that they found where infliximab mediated ADCC *in vitro* were evaluated using assays with artificial high expresser mTNF⁺ transfectomas as target cells (Nesbitt et al., 2007). In contrast, when naturally derived cells such as LPS-stimulated human monocytes are used as target cells, infliximab-mediated ADCC could not be detected (Shealy et al., 2010). Celltrion replicated this system using both US-licensed Remicade and CT-P13 and found a similar paucity of measurable ADCC activity when the monocyte targets are used. They also performed immunohistochemical staining of IBD tissue and found only low level expression of mTNF, 20-50 fold less than the level present on mTNF⁺ transfectomas where ADCC-targeting can be detected *in vitro*.

Reviewers' note: *It is unclear if either system modeled the actual inappropriately activated cell target for infliximab in IBD. Jurkat transfectomas are obviously artificial, but mTNF⁺ activated T-cells in the lamina propria are the most likely target for infliximab therapy, not monocytes.*

Celltrion's second argument against the involvement of ADCC in TNF-blocker efficacy for IBD indication cites the efficacy of certolizumab pegol, an anti-TNF Fab fragment lacking an Fc portion, in treating Crohn's disease and ulcerative colitis. Because this molecule lacks an Fc protein, certolizumab pegol is incapable of eliciting ADCC of mTNF⁺ cells, but it has been licensed for IBD indications nonetheless.

Reviewers' note: *Celltrion's assertion is partially correct in that certolizumab, which has no ADCC activity, was able to achieve a clinical response. However, this was only a response during induction rather than clinical remission achieved by other approved TNF antagonists. Although it is possible that other factors contributed to this outcome, such as inadequate dosing, it leaves open the question as to whether absence of ADCC activity could have played a role.*

ADCC activity mediated by CT-P13 and US-licensed Remicade were compared in three different formats. As noted above, the first format used LPS stimulated monocytes as target cells

and PBMC as effector cells. This format detected no activity, an observation Celltrion asserted as reflecting low mTNF expression in a physiologically relevant cell population. The other two formats used mTNF⁺ Jurkat cell transfectomas as target cells and either fresh human PBMC or freshly isolated human NK cells as effector cells. In both assay formats, infliximab-and CT-P13-mediated ADCC activity was measurable. In the first of these two assay formats, ADCC activity mediated by CT-P13 and US-licensed Remicade were equivalent. In the second format where isolated NK cells were used as effector cells, US-licensed Remicade was about 20-25% more active on average in eliciting ADCC. Celltrion argues that should not be viewed as evidence against CT-P13 as being “highly similar” to US-licensed Remicade on several grounds;

- The NK- cell based ADCC assay is an artificial system where isolated cells not representative of an actual physiological effector cell population is used to kill target cells expressing artificially high levels of TNF. They argue that PBMC, a mixed population of cells directly isolated from humans without isolation or manipulation, are more representative as the human effector cell population as a whole.

Reviewer’s note: *Celltrion’s point that other leukocyte type in the natural PBMC population could be involved with ADCC may be valid. For example, in a study of another monoclonal antibody, 2F8 the anti-tumor antibody, indicates that FcγRII⁺ PMN mediated ADCC or tumor targets was compared to FcγRIII⁺ NK mediated ADCC (Peipp et al., 2008). Both populations could be isolated from PBMC to elicit this activity. In contrast the NK-mediated ADCC, antibody fucosylation actually increased PMN mediated ADCC.*

- They argue that the LPS stimulated monocytes have a more representative level of mTNF expression than the Jurkat transfectoma cells, which in their studies has about 20-fold higher expression of mTNF than IBD tissue.
- Certolizumab pegol is effective for IBD treatment. This anti-TNF Fab molecular entity lacks an Fc portion, and thus cannot mediate ADCC.

Summary of Celltrion's position.

Based on a literature review, Celltrion has identified several potential mechanisms of action of TNF-blocker therapy. They contend that primary mode of action is TNF sequestration and neutralization via direct binding. They assert that likely secondary mechanisms of action in IBD indications are either apoptosis or cytokine inhibition via reverse signaling. The sole antibody effector activity where a quantitative difference between CT-P13 and US-licensed Remicade was found is ADCC. Celltrion provides arguments that this activity is probably not involved with TNF-blocker efficacy in any indication.

Reviewer Assessment:

Celltrion has provided an overview of existing medical and scientific literature on the mechanism of action of TNF-blockers as well as a summary of their analytical similarity data with CT-P13 and US-licensed Remicade. The goal of this position paper is to support extrapolation of positive clinical outcomes in RA and almost complete similarity to Remicade to IBD indications. As the sole antibody effector activity where a quantitative difference between CT-P13 and US-licensed Remicade was found was found to be ADCC, Celltrion focused their arguments on this activity not being involved with TNF-blocker efficacy in any indication.

A major focus on the position paper is the efficacy of the certolizumab pegol for the treatment patients with inflammatory bowel disease notwithstanding the absence of an Fc region. It should be noted that this was only a response during induction rather than clinical remission achieved by other approved TNF antagonists.

However, their justifications do not include analytical and clinical data in the literature that argue against conclusions regarding the importance of Fc effector functions (such as ADCC) in the mechanism of action of anti-TNF therapies in the setting of IBD. For example, while certolizumab pegol has labelling for treating IBD patients, it is only for a response during induction rather than clinical remission. They also did not comprehensively address experience with other TNF-alpha blockers that are lacking or having attenuated Fc function have failed clinical trials for the Crohn's disease indication as follows:

- a. Enbrel (Etanercept), an Fc-TNFRp75 fusion protein (lacking the CH1 domain) failed to show a clinical response in active Crohn's disease. It should be noted that Etanercept has been shown to be capable of inducing ADCC *in vitro* albeit at a reduced level compared to Remicade (Nesbitt et al., 2007) (Arora et al., 2009).
- b. CDP571 (Humicade), an IgG4 targeting TNFa failed to induce a statistically significant long term clinical response in moderate to severe Crohn's disease (Sandborn & Faubion, 2004).
- c. Onercept, a soluble TNFRp55 receptor protein failed to induce remission in patients with active Crohn's disease (Rutgeerts et al., 2006).

Regarding the argument that mTNF in IBD tissue is at such a low level that ADCC is unlikely to even occur during TNF-blocker treatment, published literature supporting an alternative conclusion was not discussed:

1. Potential differences that can occur in the cell type and expression level at the site of inflammation and may be relevant to the mechanism of action. There are published data showing that NK cells are reduced in the blood of patients with Crohn's disease and ulcerative colitis compared to healthy controls (Steel, Mela, Lindsay, Gazzard, & Goodier, 2011) which agrees with the Sponsor's conclusion. However, these same studies also showed that NK cells are present at significantly higher levels in the colonic lamina propria of patients with Crohn's disease and ulcerative colitis vs. healthy controls.
2. Published data show that the CD14⁺CD16⁺ monocytes are significantly increased in the lamina propria along with TNF- α levels (Koch, Kucharzik, Heidemann, Nusrat, & Luegering, 2010) in IBD patients vs. health individuals. Similarly, the TNF- α levels found in CD14⁺ macrophages isolated from the mucosa of Crohn's and ulcerative colitis patients are significantly higher than healthy controls (Kamada et al., 2008). Therefore, there is a disease-related increase in the TNF- α levels for cell types that could potentially be targets for ADCC.

3. Regarding the assertion that expression of tmTNF- α on activated monocytes is too low to trigger ADCC, the comparison of transfected mTNF in Jurkat cells and LPS-stimulated monocytes could have been skewed by the way that the experiment was conducted. For example, Celltrion tested monocytes at only one time point (approx. 4 hour following stimulation with LPS); the peak expression of mTNF in this system may occur later.
4. Finally, as discussed above, the likely dysregulated targets for infliximab-mediated ADCC would be mTNF⁺ activated T-cells, not monocytes.

In conclusion, while the white paper is comprehensive, Celltrion has not completely ruled out any possibility for a role for ADCC as a mechanism of action for infliximab. However, their contention that it is secondary at best is supported by their data and the bulk of literature on the topic.

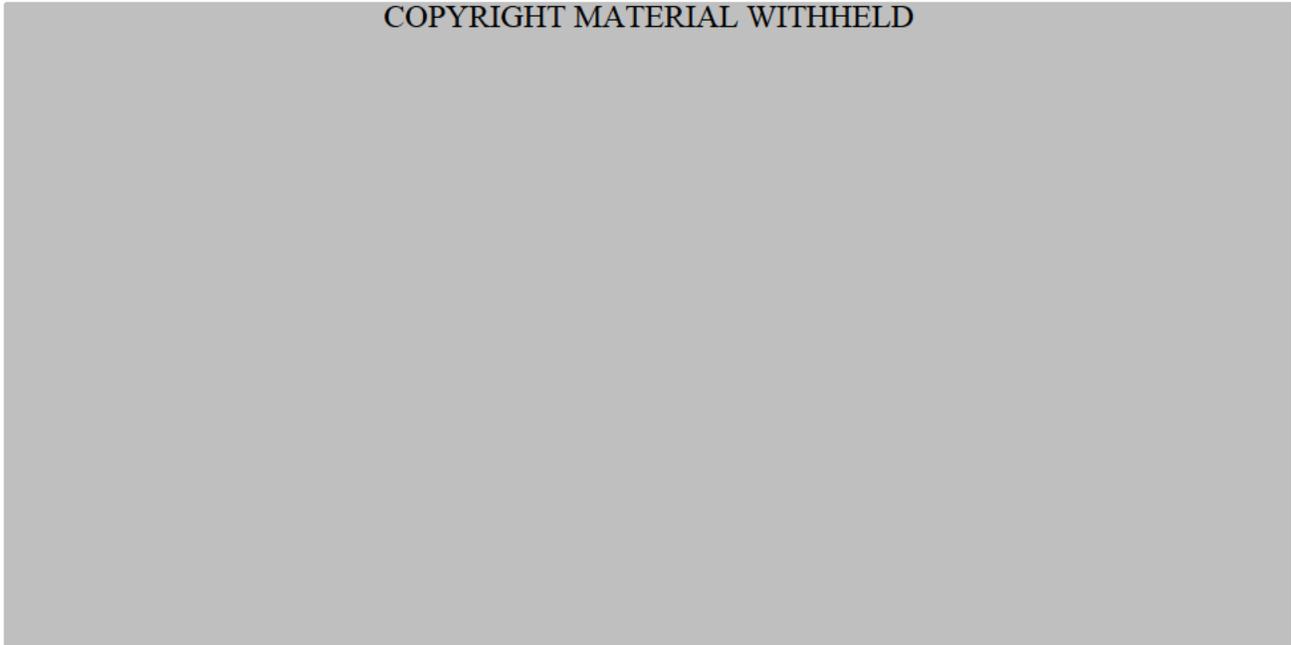
CDER assessment of the medical and scientific literature concerning the MoA of infliximab

TNF and infliximab overview

Tumor Necrosis Factor (TNF)- α is considered to be a master cytokine critical for the function of the immune system as well as inflammatory responses. It exists in both a soluble and membrane bound form that can be produced by a range of immune-related or other cell types. The consequences of effector functions of TNF- α are also varied and include tissue destruction, activation of pro-inflammatory cytokines and cell death. Thus, dysregulation of this master pro-inflammatory cytokine can have multiple clinical consequences in diseases like rheumatoid arthritis (RA) or inflammatory bowel disease (IBD).

Figure 3. TNF- α : A “Master” Cytokine, (Neurath, 2014)

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TNF- α exists in both in a 26 kDa membrane bound (mTNF- α) form and a 17 kDa soluble form (sTNF- α), both of which form non-covalently linked homo-trimers. Because both forms are

active, signals may be passed locally from cell-to-cell via mTNF:TNF-R interactions, or more distally through release of sTNF.

Infliximab is an IgG1 kappa monoclonal antibody, with a high avidity for TNF- α , both soluble and membrane-bound forms. It functions primarily via the variable region complementary determining region (CDR) surface by binding, neutralizing and sequestering excess sTNF- α produced in local inflammatory disease tissue sites. Another potential variable region-mediated mechanism of action is mediating reverse signaling via binding and cross-linking mTNF on inflammatory cells or induction of regulatory macrophages. Clinical and *in vivo* evidence has been presented in the scientific literature that reverse signaling could play a role in TNF blocker mechanism of action in IBD. Finally, there are some potential functions dependent on the Fragment crystallizable region (Fc) part of the antibody that may be important. These include antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) of lysis of mTNF⁺ inflammatory T-cells or other cells associated with particular disease states.

In summary, the relative importance of merely sequestering sTNF vs. eliciting other effector functions on mTNF⁺ cells has not been completely elucidated and may vary between disease states. A summary of the potential mechanisms of action of infliximab can be seen in Table 5 below. In this table we categorize them as “likely” or “plausible” based on whether there are or are not published (1) *in vivo* or biopsy immunofluorescent staining, (2) clinical biopsy TUNEL analysis, or (3) *in vitro* studies using cultured clinical isolates that suggest that infliximab may function this way in patients (discussed in detail below):

Table 5. Mechanism of Action of Remicade

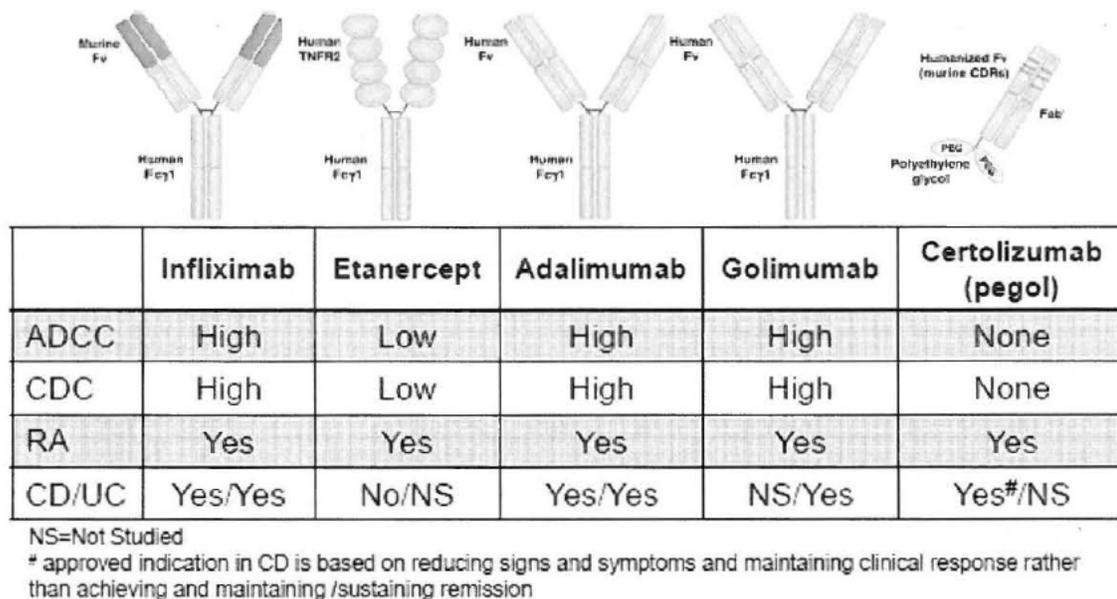
MOA of Remicade	Rheumatology Indications	IBD Indications ^a
Blocking TNFR1 and TNFR2 activity via binding and neutralization of s/tmTNF		
	Yes	Yes
Reverse (outside-to-inside) signaling via tmTNF:		
Apoptosis of lamina propria activated T cells	-	Likely
Suppression of cytokine secretion	-	Likely
Mechanisms involving the Fc region of the antibody:		
Induction of CDC on tmTNF-expressing target cells (via C1q binding)	-	Plausible
Induction of ADCC on tmTNF-expressing target cells (via FcγRIIIa binding expressed on effector cells)	-	Plausible
Induction of regulatory MΦ in mucosal healing	-	Plausible

Experience with other products

Evidence that removal of mTNF⁺ cells in inflamed areas of the gut is important for the mechanism of action exists for at least one TNF-blocker, Humira, as reported by (Atreya et al., 2014). In this study, molecular imaging was used to measure frequency of mTNF⁺ cells in the gut. The technique relies on *in vivo* topical antibody administration and visualization using confocal laser endomicroscopy. This was performed before administration of Humira to classify patients into those with high or low numbers of mTNF⁺ cells in inflamed areas. Those with high levels of mTNF⁺ cells responded to Humira therapy at a significantly higher rate vs. those with low mTNF⁺ levels (92% vs. 15%). Immunofluorescent staining of pre-treatment biopsies identified the mTNF⁺ cells as largely CD14⁺ macrophages and some CD3⁺ T-cells. Biopsies from responders were also taken after Humira therapy; these showed lower inflammatory scores while endoscopy from these patients showed mucosal healing. Overall this *in vivo* study of actual Crohn's disease patients provided fairly direct evidence that targeting mTNF⁺ cells was an important aspect of the mechanism of TNF-blocker therapy. It does not address whether the mechanism is reverse signaling, ADCC or CDC.

A hint that Fc related mechanisms for TNF blockers can be seen when the broad class of anti-TNF- α products are examined (Remicade, Enbrel, Humira, Simponi, Cimzia) is illustrated in the Figure 3 below.

Figure 3. The role of Fc in the anti-TNF- α Class mechanism(s) of action.(Arora et al., 2009; Kaymakcalan et al., 2009; Mitoma et al., 2005)



As shown in the third row, all listed TNF- α antagonists have demonstrated efficacy and are approved for the treatment of Rheumatoid Arthritis (RA). However, this is not true for all indications as shown in the bottom row reflecting efficacy in Crohn's Disease (CD) and Ulcerative Colitis (UC). Etanercept (Enbrel), which has low ADCC activity, is not approved for treatment. Published literature supports a lack of efficacy in CD based on a small study (N=49) using a dose approved in RA. In addition, certolizumab pegol (Cimzia), which has no ADCC activity, was only able to achieve clinical response during induction rather than clinical remission achieved by other approved TNF- α antagonists.

Finally, as noted above, an investigational anti-TNF antibody product that did not reach the market, the IgG4 CDP571 (Humicade), failed to induce a statistically significant long term clinical response in moderate to severe Crohn's disease (Sandborn et al., 2004). Human IgG4

antibodies do not possess the ability to elicit ADCC or CDC due to structural differences in the Fc portion versus the other IgG1 antibodies.

Overall, it is possible that other factors contributed to the observed clinical outcomes for certolizumab or CDP571, such as inadequate dosing or Fab arm or half body exchange in the case of CDP571 (Labrijn et al., 2009); (van der Neut Kolfshoten et al., 2007). However, it also raises a question as to whether absence of ADCC activity in certolizumab or CDP571 could have played a role.

Reverse signaling

Reverse signaling is a cellular feedback which occurs when a molecule that is normally a signaling molecule, like mTNF on immune cells (e.g., T cells and monocytes), is instead bound and/or cross-linked by an antibody transducing a signal back to that cell instead of forward to another cell. In theory, reverse signal by infliximab can signal to mTNF⁺ cells transducing a response like suppression of cytokine release or apoptosis.

There is some published literature suggesting that infliximab may function this way in IBD patients. For example, this contention is also supported by studies using immunofluorescent staining or TUNEL analysis of patient biopsies as well as *in vitro* studies using cultured clinical isolates (Peake et al., 2013). The most convincing studies rely on TUNEL staining of before- and after-treatment patient biopsies and/or annexin 5 staining of lamina propria T-cells treated *in vitro* (Di Sabatino et al., 2004); (ten Hove, van Montfrans, Peppelenbosch, & van Deventer, 2002). TUNEL analysis detects cells with DNA strand breaks of the type produced during apoptosis, while annexin 5 is a protein expressed and activated in apoptotic cells. Both studies observed an increase in TUNEL⁺ cells in Crohn's disease biopsies either 24 hours (ten Hove et al., 2002) or ten weeks post-treatment (Di Sabatino et al., 2004). In the second study as well as a third (Van den Brande et al., 2003), treatment of isolated lamina propria T-cells treated *in vitro* with infliximab induced apoptosis (annexin 5 staining) but not features of ADCC (granzyme B). Atreya et al., 2011 found similar results but only when the lamina propria T-cells were co-cultured with gut associated CD14⁺ cells.

Inhibition of cytokine production is a second potential outcome for reverse signaling of mTNF⁺ cells. The evidence for this hypothesis relies on *in vitro* experimentation, using cultured lamina propria T-cells from IBD patients or monocytes or PMNs from healthy PBMC. Agnholt and Kaltoft (2001) demonstrated that staphylococcus enterotoxine A (SEA) activated lamina propria T-cells from Crohn's disease patients could be reverse signaled by infliximab, resulting in down modulation of γ IFN production but not apoptosis. (Bourne, Fossati, & Nesbitt, 2008) proposed the hypothesis that IBD is caused or exacerbated by inappropriate cellular responses to normal gut bacterial flora such as generalized activation of monocytes in response to LPS. To test this, they developed assays for ADCC, CDC as well as reverse signaling to either induce apoptosis of neutrophils or down modulate cytokine production by LPS-activated monocytes. Each marketed TNF-blocker was tested for activity in these assay formats; only the cytokine production reverse signaling assay was predictive of IBD efficacy for each product (etanercept, infliximab, adalimumab, and certolizumab). Interestingly, certolizumab, unlike etanercept, was able to reverse signal, despite being monomeric and unable to cross-link mTNF molecules.

Certolizumab must bind the mTNF in a manner that still transmits a signal without cross-linking; presumably its antibody V region structure must cause conformational changes to the mTNF that are different that are caused by the more natural TNFR portion on etanercept.

In summary, while not conclusive, fairly reasonable and extensive clinical, *in vivo* and *in vitro* evidence exists that reverse signaling of mTNF⁺ cells is a "likely" mechanism of action for infliximab in Crohn's disease and ulcerative colitis.

***In vitro* evidence for ADCC**

ADCC is an immune function where effector cells like such as NK cells lyse target cells via antibody bound to the surface of the targets. The antibody Fc portion is able to recruit the effector cells via Fc γ R:Fc bridging. Fc γ RIIIa or CD16 is the main form of Fc γ R on NK cells, a highly potent type of immune cells that target antibody bound tumor or virally infected cells. Infliximab ADCC activity against mTNF⁺ target cells will vary with the strength of the Fc γ R:Fc bridging, which in turn seems to be dependent on the glycan composition on the antibody

ADCC by infliximab and other TNF blockers has been demonstrated *in vitro* ((Arora et al., 2009); (Bourne et al., 2008); (Scallon et al., 2002)) but no *in vivo* or clinical evidence exists that it plays a role in infliximab efficacy in IBD indications (beyond the above noted correlation between the presence of an Fc region and IBD efficacy of certolizumab and CDP571). It should be noted that some of these studies were able to detect low level ADCC activity by etanercept (Bourne et al., 2008) while others could not ((Arora et al., 2009)). Presumably subtle differences in assay format led to this disparity.

The above studies used ADCC assays using whole or CD14⁺ depleted PBMC, not enriched NK cells, as effector cells. In this format, there is more than one leukocyte type that could lyse the target cells. For example, in a study of another monoclonal antibody, 2F8 the anti-tumor antibody, indicates that FcγRII⁺ PMN mediated ADCC or tumor targets was compared to FcγRIII⁺ NK mediated ADCC (Peipp et al., 2008). Both populations could be isolated from PBMC to elicit this activity. In contrast the NK mediated ADCC, antibody fucosylation actually increased PMN mediated ADCC. This supports the contention that all effector cell types should be considered when designing an assay to determine mechanism of action and impact of product quality attributes.

FcγRIII genotyping and pharmacogenetics

Pharmacogenetics has been proposed to have the potential to provide clinical benefit to RA and IBD patients by predicting which will respond more effectively to TNF blocker therapy. Several potential marker polymorphisms, including FcγRIIIa polymorphisms, have been identified as potentially associated with clinical outcomes of infliximab therapy in IBD patients (Kooloos, de Jong, Huizinga, & Guchelaar, 2007).

In the case of FcγRIIIa, there are two alleles that are potentially important; the high affinity V allele and the lower affinity (by about 4-fold) F allele. In an early study comparing response rates of VV, FV and FF Crohn's disease patients to infliximab, a modest (83% vs 73%) trend towards higher clinical responsiveness (measured by Crohn's Disease Activity Index (CDAI)) was noted in the VV population vs. the others (E. Louis et al., 2004). These VV patients also had a statistically significant larger decrease in C-reactive protein (CRP), a serum marker for

inflammation, post-infliximab treatment. This was taken as evidence that FcγRIIIa binding strength, and thus NK-mediated ADCC activity improved infliximab effectiveness in patients. Subsequently, these observations of an association of allotype and efficacy were not confirmed when larger populations of patients were evaluated in controlled clinical trials (Edouard Louis et al., 2006). A subsequent study of Japanese Crohn's patients treated with infliximab also failed to find a significant difference in clinical response between the genotypes, although a transient difference in CRP was noted (Moroi et al., 2013).

In summary, FcγRIII pharmacogenetics did not consistently find an association between high and low binding alleles and infliximab clinical efficacy in IBD. Thus pharmacogenetics does not robustly support the notion that infliximab is more effective in IBD patients with high affinity alleles of FcγRIIIa.

Early clinical experience with CT-P13 in Europe

In 2014, CT-P13 was introduced into Europe for all indications currently treated by EU approved Remicade. The earliest results from this experience were reported in abstracts of the 2015 European Crohn's and Colitis Organization annual meeting. Some countries (Hungary, Poland) reported that CT-P13 displayed a similar clinical safety and efficacy profile as EU Remicade, albeit in the small number of IBD patients treated by their centers to this point (D. Jarzebicka, 2015) (K. Gecse, 2015); (T. Molnar, 2015). One site in Ireland reported a higher rate for the need for surgery and an actual increase in CRP post-initiation to CT-P13 in the small number of patients treated in 2014 (C. Murphy, 2015). All of these studies involved small numbers of patients and were not blinded/controlled. Thus these data do not provide sufficient evidence as to whether or not subtle Fc and ADCC activity differences between CT-P13 and Remicade influence *in vivo* activity by any mechanism of action.

Overall conclusion

The totality of evidence indicates that TNF binding and neutralization is the primary mechanism of action of infliximab in all indications. There is clinical and *in vivo* evidence that reverse signaling of mTNF⁺ cells in inflamed sites is likely to play a role in infliximab's mechanism of

down-modulating disease activity in IBD indications. It is plausible, based solely on *in vitro* evidence, that ADCC could play a role in infliximab's mechanism of action in IBD patients.

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/s/

CYRUS AGARABI
01/05/2016

BLA STN 125544

Product USAN name: TBD

CT-P13

Celltrion Inc.

Peter Adams, Ph.D., Senior Staff Fellow, DBRR I, OBP
Kurt Brorson, Ph.D., Acting Laboratory Chief, DBRR II, OBP
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OBP CMC Review Data Sheet

1. **351(k) BLA#:** STN 125544
2. **REVIEW DATE:** 5 May 2015
3. **PRIMARY REVIEW TEAM:**

Medical Officer:	Juwaria F. Waheed, CDER/DPARP
Clinical Reviewer	Nikolay Nikolov, CDER/DPARP
Pharm/Tox:	Matthew T. Whittaker, CDER/ODEII/DPARP
Product Quality Team:	Peter Adams, CDER /OBP/DBRR1 Kurt A. Brorson, CDER /OBP/DBRR2 Erik Read, former OBP (filing scan for completeness)
Micro Branch VI (Facilities):	Bo Chi, OPF/OPQ Maria Candauchacon, OPF/OPQ
Immunogenicity:	William Hallett/ Harold Dickensheets OBP
Clinical Pharmacology:	Lei He, CDER/OCP/DCPII
Statistics:	Gregory P. Levin, CDER Meiyu Shen, CDER
OBP Labeling:	Jibril Abdus-Samad, CDER/OPQ/OBP
RPM:	Nina Ton, CDER/ODEII/DPARP
4. **MAJOR 21st Century Review DEADLINES**

Filing Meeting: September 29, 2014
Filing Date: October 7, 2014
Day 74 letter date: October 21, 2014
Mid-Cycle Meeting: January 20, 2015
Wrap-Up Meeting: May 5, 2015
Primary Review Due: May 4, 2015
Secondary Review Due: May 11, 2015
CDTL Memo Due: May 18, 2015
Action Letter and action package: May 18, 2015
BsUFA Action Date: June 8, 2015

5. **COMMUNICATIONS WITH SPONSOR AND OND**

Communication/Document	Date
Information Request 1	February 13 th , 2015
Information Request 2	April 10 th , 2015

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

Submission	Date Received	Review Completed (Yes/No)
Original Application	August 8 th , 2014	Y
Response to FDA IR #01 (eCTD seq 0026)	March 9 th , 2015	Y
Response to FDA IR #01 (eCTD seq 0028)	March 31 st , 2015	Y
Response to FDA IR #01 (eCTD seq 0029)	April 2 nd , 2015	Y
Response to FDA IR #02 (eCTD seq 0032)	April 17 th , 2015	Y

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

- a. Code Name: CT-P13
- b. Trade Name: INFLECTRA
- c. Non-Proprietary/USAN: to be determined
- d. CAS name: 170277-31-3
- e. INN Name: infliximab
- f. Compdial Name: not applicable
- g. OBP systematic name: MAB HUMAN (IGG1) ANTI CAA26669 (TNF-alpha_HUMAN) [CT-P13]
- h. Other Names: ATC code L04AB02, DrugBank DB00065, UNII B72HH48FLU, KEGG D02598, ChEMBL1201581

8. **PHARMACOLOGY/Mechanism of Action:** Infliximab is a TNF blocker, which has a primary action of sequestration of TNF- α . During treatment of patients with rheumatoid arthritis, infliximab reduces infiltration of inflammatory cells into affected joints. In Crohn's disease infliximab reduces infiltration of inflammatory cells into inflamed areas of the intestine and reduces TNF- α production. In psoriatic arthritis patients, treatment with infliximab reduces the number T-cells, blood vessels, and macrophages in the synovium. In plaque psoriasis patients, infliximab treatment reduces the epidermal thickness and infiltration of inflammatory cells. A role of ADCC in the clinical activity of infliximab in the setting of certain clinical indications (e.g., inflammatory bowel disease) is possible.

Infliximab is administered with methotrexate (inhibitor of folate that results in lymphocyte apoptosis) for treatment of rheumatoid arthritis (b) (4)

- 9. **DOSAGE FORM:** sterile lyophilized powder in a 20 mL capacity vial
- 10. **STRENGTH/POTENCY:** 100 mg per vial
- 11. **ROUTE OF ADMINISTRATION:** intravenous after reconstitution in 10 mL sterile water.

12. REFERENCED MASTER FILES:

DMF #	HOLDER	ITEM REFERENCED	Letter of Cross-Reference
<div style="background-color: #cccccc; width: 100%; height: 100%; display: flex; align-items: center; justify-content: center;"> (b) (4) </div>		Butyl Rubber Formulations	Yes
		Butyl Rubber Stopper (b) (4)	Yes
		Type I Borosilicate Vials, USP	Yes

13. INSPECTIONAL ACTIVITIES

A PAI was conducted from February 19th, 2015 through March 7th. Information about the facility and the FDA personnel involved are shown below.

Firm:	Celltrion Inc
Location	23 Academy-ro, Yeonsu-gu, Incheon, 406-840, Republic of Korea
Phone	+82-32-850-5000
Fax	+82-32-850-6593
Dates of inspection	2/23/2015 – 02/27/2015 and 03/02/2015 to 03/06/2015
Days in facility	10
FDA Participants	Bo Chi, CDER/OPQ/OPF/DMA/MABIV Maria Candauchacon, CDER/OPQ/OPF/DMA/MABIV Kurt Brorson, CDER/OPQ/OBP/DBRR2 Peter Adams, CDER/OPQ/OBP/DBRR1 Chiang Syin, OGROP/OIP

A fifteen item 483 form was issued at the conclusion of the inspection. The final EIR document is pending.

14. **QUALITY BY DESIGN ELEMENTS**

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

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

Celltrion proposes a Quality by Design (QbD) control strategy for CT-P13 DS and this discussed in section 3.2.S.2.4

15. **PRECEDENTS:**

CT-P13 was developed as a biosimilar to the reference product, US-licensed Remicade (Janssen Inc.).

16. ADMINISTRATIVE

A. Signature Block

Name and Title	Signature and Date
Kurt A. Brorson, Ph.D. Acting Chief Lab 2 DBRR II, OBP	Kurt A. Brorson -A  Digitally signed by Kurt A. Brorson -A DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300078163, cn=Kurt A. Brorson -A Date: 2015.05.07 11:54:22 -04'00'
Peter Adams, Ph.D. Primary Reviewer DBRR I, OBP	Peter Adams -S  Digitally signed by Peter Adams -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Peter Adams -S, 0.9.2342.19200300.100.1.1=0011335608 Date: 2015.05.07 11:48:39 -04'00'

B. CC Block

Recipient	Date
David Frucht, M.D. Acting Director DBRR II, OBP	
Nina Ton, RPM DPARP, OND	
Division of Monoclonal Antibodies/Therapeutic Proteins File/BLA STN 125544	

Executive Summary of Quality Assessments

I. Primary Reviewer Summary Recommendation

CT-P13, a proposed biosimilar to US-licensed Remicade, is manufactured by standard bioprocessing and (b)(4) Drug Product fill/lyophilization, leading to a high quality Drug Substance and Drug Product. The testing and quality control programs are adequate to verify manufacture of a product that meets quality standards expected of a biotechnology product. Stability of the Drug Product has been demonstrated under refrigerated storage conditions to support expiry dating of at least 42 months.

However, I recommend that this 351 (k) BLA application not be approved at this time due to deficiencies in the biosimilarity assessment leading to residual uncertainty regarding the determination that this product is “highly similar” to the reference product, as outlined below.

II. List Of Deficiencies To Be Communicated (Proposed Language)

- In your submission, you evaluated the analytical similarity of CT-P13 and US-licensed Remicade using a variety of functional assays. Your data generated using a standard NK-cell based killing ADCC assay suggest that CT-P13 has ~20% lower ADCC activity compared to the reference product US-licensed Remicade, which correlates with

differences in FcγRIIIa binding. The difference in ADCC leads to residual uncertainty regarding the conclusion that CT-P13 is highly similar to US-licensed Remicade, as the role of ADCC remains uncertain in the clinical activity of the innovator product (e.g. in the setting of inflammatory bowel disease). Furthermore, you did not adequately justify the impact of the difference in ADCC on the analytical similarity assessment and did not identify the structural basis underlying this difference. For example, you should determine whether the H2L1 variant that is present at relatively high levels in CT-P13 compared to US-licensed Remicade plays a role in decreasing NK-dependent ADCC activity. On the other hand, the Agency has not excluded the possibility that analysis of additional lots of CT-P13 and innovator lots could overcome a statistical anomaly due to the analysis of a limited number of lots. To this point, we note that prior differences in glycan patterns were reduced when additional lots of CT-P13 and innovator product were analyzed. To address the current deficiency with respect to differences in ADCC activity, we recommend that you repeat the evaluation of ADCC using additional lots to determine whether the ADCC difference you have reported decreases when additional lots are evaluated (i.e., due to small sample size). If the difference in ADCC persists following analysis of additional lots, you should identify and demonstrate control of the product quality attributes that underlie ADCC activity in CT-P13 (e.g., glycan pattern, contribution of H2L1 variant, etc.) and provide an adequate justification, including an evaluation of the role of ADCC particularly in the setting of inflammatory bowel disease, that the observed difference in ADCC does not have clinical impact.

2. The current drug product stability data using Process B batches of CT-P13 supports an expiry date of 42, not (b) (4) months. To address this concern, adjust your proposed expiry dating to reflect existing data and provide a stability protocol to support post-approval expiry extension.
3. We acknowledge the plan outlined in your 10 Apr 2015 letter to develop and validate a revised version of the visible particle test for reconstituted drug product. The revised test will use (b) (4) and visual inspection of 20 reconstituted vials. Data supporting the assay revision have not been provided to the BLA. To address this concern, submit the assay SOP, validation report and revised specification to the Agency for review.

Note: An additional product quality-related deficiency was noted in the course of the immunogenicity review regarding differences in sub-visible particulates described in a response to an information request. Please refer to the immunogenicity review for specific details.

III. List of Post Marketing Commitments/Requirement- N/A at this time

IV. Review of Common Technical Document-Quality Module 1

A. Environmental Assessment Or Claim of Categorical Exclusion

A categorical exclusion is requested by Celltrion, Inc. under 21 CFR Part 25.31(a). As stated in 21 CFR Part 25.31(a), if the action does not increase the use of the active moiety. Approval of this submission will not increase the overall use of the active moiety. Therefore, we recommend approval of this exclusion.

V. Primary Container Labeling Review- N/A at this time

VI. Review of Common Technical Document- Quality Module 3.2- see below

VII. Review of Immunogenicity Assays
Refer to review by William Hallett and Harold Dickensheets

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QUALITY OF DRUG SUBSTANCE AND DRUG PRODUCT

S. DRUG SUBSTANCE

3.2.S.1.2 Structure

CT-P13 DS is a chimeric IgG1 κ monoclonal antibody designed to be identical in amino acid sequence to Remicade, or infliximab. It has an N-glycan attached to Asn300 within the CH2 domain of each heavy chain. Each antibody is comprised of two heavy chains (450 amino acids each) and two light chains (215 amino acids each) that are covalently linked by interchain cysteine disulfide bonds. Some heavy chains have an additional C-terminal lysine and deamidated variants of residues Asn57, Asn387, and Asn318 have been detected.

Heavy Chain				
1	EVKLEESGGG	LVQPGGSMKL	SCVASGFIFS	NHWMNWVRQS PEKGLEWVAE
51	IRSKSINSAT	HYAESVVRGRF	TISRDDSKSA	VYLQMTDLRT EDTGVVYCSR
101	NYYGSTYDYW	GQGTTLTVSS	ASTKGPSVFP	LAPSSKSTSG GTAALGCLVK
151	DYFPEPVTVS	WNSGALTSKV	HTFPAVLQSS	GLYSLSSVVT VPSSSLGTQT
201	YICNVNHKPS	NTRVDKKVEP	KSCDKTHTCP	PCPAPELLGG PSVFLFPPKP
251	KDILMISRTP	EVTGVVVDVS	HEDPEVKFNW	YVDGVEVHNA KTKPREEQYN ¹
301	STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS KAKGQPREPQ
351	VYTLPPSRDE	LTRNQVSLTC	LVKGFYPSDI	AVEWESNGQP ENNYKTTTPV
401	LDSGGSPFLY	SKLTVDKSRW	QQGNVFGCSV	MHEALHNHYT QKSLSLSPGK
Light Chain				
1	DILLTQSPAI	LSVSPGERVS	FSCRASQFVG	SSIHVYQORT NGSPRLLIKY
51	ASESMGIPIS	RFGSGSGGTD	FTLSINTVES	EDIADYVCOQ SHSWPFTFGS
101	GTNLEVKRTV	AAPSVFIFPP	SDEQLKSGTA	SVVCLLNNFY PREAKVQWKV
151	DNALQSGNSQ	ESVTEQDSKD	STYSLSSTLT	LSKADYEKHK VYACEVTHQG
201	LSSPVTKSEF	RGEC		

The theoretical extinction coefficient of CT-P13 (1.45 mL/mg/cm) was calculated based on the mass spectroscopy confirmed molecular mass of CT-P13 (145,878 g/mol) and known Cys, Thr and Trp content.

Reviewer note: *The extinction coefficient was experimentally measured by A280 and an amino acid analysis for CT-P13 and US-licensed Remicade; see response to Feb 13, 2015. This value is close to 1.45*

3.2.S.1.3 General Properties

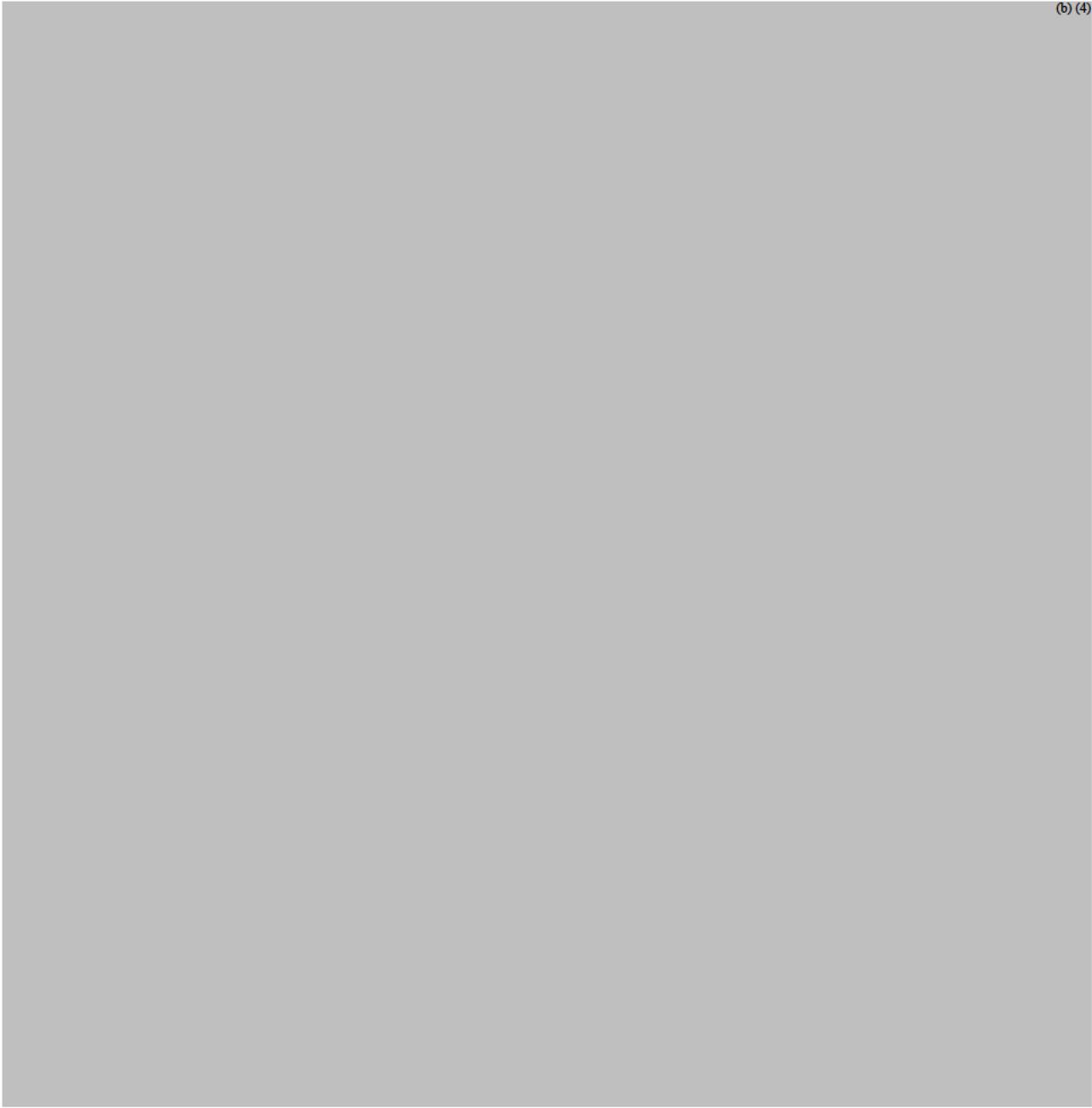
CT-P13 DS is visibly opalescent; colorless to light yellow. The liquid is an approximately pH 7.2, solution of (b) (4) mg/mL CT-P13 mAb (infliximab), in phosphate buffer, sucrose and polysorbate 80. Infliximab, the proposed USAN name for CT-P13 and the existing name for US licensed Remicade, is a chimeric human-murine IgG1 κ monoclonal antibody that binds with high affinity to soluble and membrane bound human tumor necrosis factor α (TNF- α).

Infliximab inhibits TNF- α 's ability to bind receptors and thus neutralizes the presumed biological activity of TNF- α . This may include cytokine induction (IL-1 and IL-6), increased

leukocyte migration, activation of neutrophil and eosinophil functions, induction of liver proteins, and induction of tissue degrading enzymes. Infliximab bound to the transmembrane form of TNF- α also induces cell lysis via ADCC (antibody-dependent cell cytotoxicity). The infliximab Fc domain can activate complement, and bind Fc-receptors on a variety of immune cells. CT-P13 is being developed as a biosimilar to the innovator infliximab DP (US-licensed Remicade), and would presumably possess similar TNF α binding as well as the above effector functions possibly important for in vivo activity.

3.2.S.2 Manufacture

(b) (4)



110 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page

3.2.R.3 Analytical Similarity

The analytical similarity data and information provided in this section was submitted by Celltrion. Unless otherwise noted, all figures and tables that have been referred in this section were excerpted from the Celltrion 351(k) BLA submission.

Global approach. To establish analytical similarity with the reference product, Celltrion has used a two part approach. Initially, the Sponsor had undertaken a 2-way analytical similarity assessment comparing CT-P13 against EU-approved infliximab to support approval in Europe (2013). Clinical trials comparing CT-P13 and EU-approved infliximab for the treatment of rheumatoid arthritis and ankylosing spondylitis were conducted to support a demonstration of biosimilarity for the EU market.

Reviewer's Comment: These studies had been conducted prior to the US IND submission in 2013. A full report of this data was included in the US IND submitted on 02 Oct 2013. A full analysis of the two-way biosimilarity exercise is included in the IND 118135 original submission review by G. Miesegeas, 13 Nov 2013. Thus, this BLA review will reference the IND review for the two-way study information re-submitted in the package.

To support a scientific bridge to justify the relevance of the data generated using EU-approved infliximab to an assessment of biosimilarity and establish an acceptable bridge to the US-approved Remicade, a 3-way analytical similarity assessment was performed using batches of US-licensed Remicade, EU-approved infliximab and CT-P13. Initially, the Sponsor had chosen the sample size of 7 as the minimum number to be used in the analysis based on 95% confidence interval for a statistical t-test distribution. In an addendum to the BPD Type 4 meeting July 2014 the Agency recommended using a statistical equivalence test, with non-equivalence as the null hypothesis, to support analytical similarity of the three products. The Sponsor initially determined that the inclusion of 10 lots would provide sufficient statistical powering based on assessment of the biological assay used in the two-way analysis comparing CT-P13 with EU-approved infliximab.

Reviewer's Comment: Additional lots were subsequently added for certain tests (see discussion of TNF ELISA analysis and CMC Stats review).

To establish analytical similarity for the three-way analysis, 7 lots were analyzed with physiochemical assays, and 10 lots were analyzed for the biological assays. Given the importance of TNF binding and potency measure by the bioassay for the mechanism of action, data from these two assays were defined as Tier 1 attributes and evaluated with a more rigorous statistical testing approach.

Lots used. A two-way similarity exercise was initially performed to gain EU clinical trial approval using lots of EU-approved infliximab and a selection of 2009-11 drug product lots of

CT-P13. As noted above, this study was conducted, to support approval in the EU, and data from this were also included in the US IND (for summary, see IND 118135 review from November 13th 2013).

Based on Agency advice in a post-meeting addendum following the BPD Type 4 meeting (June 13th, 2014), analytical similarity data for physico-chemical analysis in the initial BLA was generated using 7 lots of US-licensed Remicade, EU-approved infliximab and CT-P13. Analytical similarity data for biological assays were generated using 10 lots of US-licensed Remicade, EU-approved infliximab and CT-P13. A subset of the research-grade biological assays was undertaken using smaller numbers of lots (3 or 5 lots). These assays (Caco-2 assay, wound-healing, reverse signaling, etc.) are biologically complex and inherently variable. They were performed more to establish product mechanisms vs. providing a precise comparison of biological activity of the three products. Subsequently after an IR letter dated 13 Feb 2015, data from additional lots were added for certain assays (e.g., TNF binding, TNF neutralization, protein content), see discussion of protein content and CMC Stats review (May 1, 2015).

The expiration dates for lots of US-licensed Remicade used to determine analytical similarity range from February 2015 to March 2017, EU-approved infliximab 2014-2017 and the CT-P13 lots were manufactured in 2012.

Reviewer's Comment: *Celltrion has selected lots that cover a range of expiration dates. While there is an imbalance of lots of US-licensed Remicade that have a 2015 expiration date, this probably occurred due to the increased demand for statistical rigor as described above. It is unlikely to have a significant impact on the overall conclusion of the analytical similarity exercise and, furthermore, it may no longer be feasible to source materials that have an equal distribution of expiration dates.*

Table 3.2.R-2: Batch Selection for 3-way Similarity Assessment

Source	Sample Batch No.	Description	Manufacturing Date / Expiration Date	Purpose
US-licensed Remicade®	CBS13015P1	Remicade®	Feb. 2015 ¹	Initial IND (Abridged study)
	CBM14012P1	Remicade®	Feb. 2015 ¹	
	CHD56013P1	Remicade®	Aug. 2015 ¹	
	CHF59013P1	Remicade®	Aug. 2015 ¹	IND amendment (Enhanced study)
	CHM62015P1	Remicade®	Aug. 2015 ¹	
	CLM91012P1	Remicade®	Dec. 2015 ¹	
	CKS86016P1	Remicade®	Nov. 2015 ¹	
	CBM12011P1	Remicade®	Feb. 2015 ¹	
	CJM76016P1	Remicade®	Oct. 2015 ¹	
	DAD96011P1	Remicade®	Jan. 2016 ¹	BLA (Statistical powering)
	14A124P1	Remicade®	Mar 2017 ¹	
	ECD18012P1	Remicade®	Mar 2017 ¹	
ECD19016P1	Remicade®	Mar 2017 ¹		
CT-P13 Drug Product (CLT2)	12B1C002	CT-P13 DP	13 Feb 2012	Initial IND (Abridged study)
	12B1C003	CT-P13 DP	18 Feb 2012	
	12B1C004	CT-P13 DP	23 Feb 2012	
	12B1C015	CT-P13 DP	09 Nov 2012	IND amendment (Enhanced study)
	12B1C016	CT-P13 DP	13 Nov 2012	
	12B1C017	CT-P13 DP	18 Nov 2012	
	12B1C018	CT-P13 DP	21 Nov 2012	
	12B1C019	CT-P13 DP	26 Nov 2012	
	12B1C020	CT-P13 DP	30 Nov 2012	
	12B1C021	CT-P13 DP	04 Dec 2012	
	12B1C012	CT-P13 DP	17 Dec 2012	
	12B1C013	CT-P13 DP	21 Dec 2012	BLA (Statistical powering)
	12B1C014	CT-P13 DP	05 Nov 2012	
EU-approved	2RMA60103	Remicade®	Dec. 2014 ¹	IND amendment

Source	Sample Batch No.	Description	Manufacturing Date / Expiration Date	Purpose
Remicade®	1RMA64901	Remicade®	Jul. 2014 ¹	(Enhanced study)
	2RMA61905	Remicade®	Jan. 2015 ¹	
	3RMKA80702	Remicade®	Feb. 2016 ¹	
	1RMKA87103	Remicade®	Sep. 2014 ¹	Initial IND and IND amendment (Abridged and enhanced studies)
	1RMA65804	Remicade®	Aug. 2014 ¹	
	1RMA61310	Remicade®	Feb. 2014 ¹	
	0RMA62801	Remicade®	Mar. 2013 ¹	Initial IND (Abridged study)
	3RMA69701	Remicade®	Sep. 2016 ¹	BLA (Statistical powering)
	4RMKA80103	Remicade®	Jan. 2017 ¹	
	4RMA60601	Remicade®	Dec. 2016 ¹	

¹ Expiration date of Remicade®

The table from the original BLA submission lists the lots used for the similarity assessment.

Reviewer’s Comment: The column labeled “Purpose” denotes the sequence in which in the pre-BLA development the testing was performed (e.g., “initial” vs. “enhanced”). From the standpoint of the final similarity assessment, they can be grouped. Data from additional lots were submitted in an amendment dated Mar 10, 2015, see CMC Stats review.

3-Way Similarity Assessment: Summary of Analytical Procedures

The analytical procedures used to demonstrate the analytical similarity of CT-P13, US-licensed Remicade and EU-approved infliximab include the routine lot release tests, extended characterization tests and tests that can be best described as research-grade for establishment of product mechanisms. They can be grouped into seven categories based on the product quality characteristics that they assessed as follows: primary structure, higher order structure, content, purity, charged isoforms, glycosylation and biological activity. The analytical methods used are listed below.

Reviewer's Comment: Some methods yielded results that showed minor differences from the reference product; these are discussed in greater detail.

- Primary Structure and Posttranslational Modifications
 - Peptide Mapping (HPLC, LC-MS)
 - Intact Mass (Reduced) (LC-MS)
 - Amino Acid Analysis/Molar Absorptivity
 - N and C-terminal Sequencing
- Higher Order Structure
 - FTIR
 - DSC
 - CD
 - Free Thiol Analysis
 - Disulfide Bond
 - Antibody Array
- Protein Content
 - UV 280
- Purity
 - SEC-HPLC

- SEC-MALS
- AUC
- Reduced/Non-reduced CE-SDS
- Charge Forms
 - IEF
 - IEC-HPLC
- Glycosylation
 - Oligosaccharide Profiling
 - N-linked Glycan Analysis
 - Sialic Acid Analysis
 - Monosaccharide Analysis
- Biological Activity
 - *In vitro* TNF α Neutralization
 - Fc γ R receptor binding (both SPR and ELISA)
 - ADCC using PBMC and NK Cells
 - CDC
 - Apoptosis
 - Reverse signaling
 - Other functional activity (these include many R&D type assays)

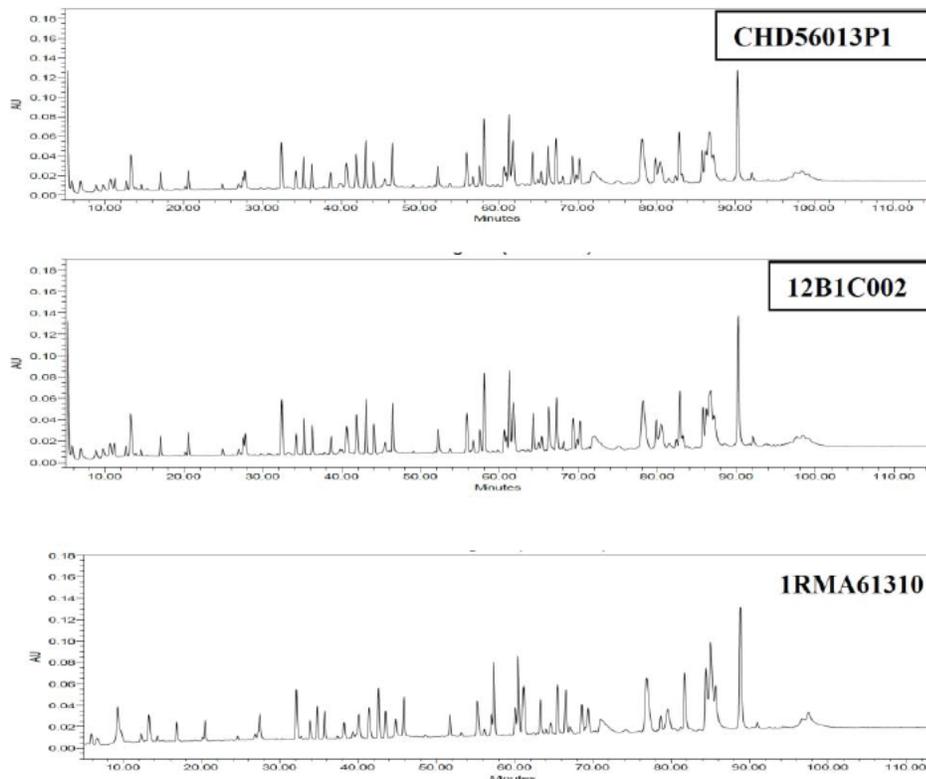
Primary Structure

Peptide Mapping

The Sponsor has used peptide mapping, intact antibody mass spectroscopy, and N & C-terminal sequencing to compare the primary structure in the three-way analysis. MS/MS analysis of fragments liberated from peptide mapping experiments was used to confirm the predicted primary sequence of the peptides and compare to a subset of post translational modifications.

Since C-terminal lysine content varies between antibodies, it was also compared in the three way study.

Tryptic peptide mapping. RP-HPLC based tryptic peptide mapping was performed to confirm sequence identity:



Peptide mapping analysis of lots of US-licensed Remicade (lot CHD56013P1), CT-P13 (lot 12B1C002) and EU-approved infliximab (lot 1RMA61310) exhibit similar patterns. Representative chromatograms from figure 3.2.R-4 are shown above (seven lots of each product had been analyzed by this method and reported in the BLA).

Reviewer's Comment: *The data have a similar peak profile, which provides evidence to demonstrate that the primary sequences of the three product types are identical.*

Peptide mapping in combination with MS/MS

MS analysis was performed on peaks drawn from the peptide mapping of HPLC of tryptic digested CT-P13, EU-approved infliximab and US-licensed Remicade. The Applicant claims peptide sequence coverage was 100% for both heavy and light chain. Data were not shown for this analysis, however, data from the two-way analysis was submitted in table 3.2.R-65 of the BLA submission.

The assay involves the reduction and alkylation of samples prior to treatment with trypsin or Asp-N. The peptides generated following cleavage are separated using a Water Acquity UPLC equipped with a C18 reverse phase column with a gradient of acetonitrile and formic acid. An AB SCIEX triple TOF 5600 mass spectrometer connected online to the UPLC system was used to collect mass spectra and fragment intact peptides for sequencing.

Reviewer's Comment: *The peptide mapping data and the resultant sequences derived from the MS/MS data in the two-way analysis of the CT-P13 and EU-approved infliximab, in addition to the data generated from peptide mapping comparing US-licensed Remicade confirms that the CT-P13 primary sequence is the same as the reference product.*

This method also measures post-translational amino acid modifications found at low levels (e.g., oxidation and deamidation). Data from all seven lots of each product type are listed in table 3.2.R-7. The technique measures deamidation of Asn57 HC, Asn318 HC, Asn364 and Asn387 and Asn41 LC as well as oxidation of Met255 HC. There were minor differences in the level deamidation of Asn57 HC CT-P13 and oxidation levels of Met 255. Neither was at high levels in either CT-P13 EU-approved infliximab or US-licensed Remicade. CT-P13 samples had slightly lower levels (34%) of the C-terminal lysine variant compared to the EU approved infliximab (38.7%) and the US licensed Remicade (37.9%).

Reviewer's Comment: *The Sponsor argues that the differences in the C-terminal lysine did not translate to a measurable impact on the biological activity in vitro and that it is clipped in vivo immediately after infusion. This is a reasonable conclusion.*

Reviewer's Comment: *Only minor differences in oxidation and deamidation have been identified. The Sponsor undertook additional analytical studies comparing CT-P13 and EU-approved infliximab under stress conditions and long term storage, see section 3.2.R-3.6. There did not appear to be significant difference in the results for US-licensed Remicade and EU-approved infliximab.*

Intact Mass Spectroscopy (reduced)

Mass spectroscopy was performed on the three product types reduced with DTT. For this procedure, the samples are treated with a reducing agent (dithiothreitol) followed by analysis using LC-ES-MS. In this case an Agilent 1100 HPLC is coupled to an Agilent 6530 Q-TOF mass spectrometer. Data from the analysis of the intact mass (reduced) was consistent across all batches and yielded a single mass for the light chain and a limited set (4) of masses for the heavy chain consistent with different glycan patterns corresponding to either G0, G0F, G1F and G2F in combination with C-terminal Lysine variants. Three glycan patterns were detected when C-terminal lysine was removed with carboxypeptidase; these corresponded to G0F, G1F, and G2F glycoforms of the antibody.

Amino Acid Analysis and Protein Content

An amino acid analysis was carried out on a subset of the batches of the CT-P13 (3), US-licensed Remicade (3) and EU-approved infliximab (3).

Amino acid analysis involves breaking-down of the protein to its constituent amino acids by acid hydrolysis. These are then derivatized with a fluorescent label to facilitate separation and quantitation using HPLC.

To perform the analysis, individual amino acids are liberated by hydrolysis in the presence of 6M HCl for 24 hours at 110°C. (**Note:** *This destroys some amino acids.*) Following hydrolysis, the amino acids are derivatized with OPA /FMOC and separated by reverse phase HPLC. Proline is derivatized via a reaction with FMOC-Cl and sarcosine is used as an internal standard. The resolvable individual amino acid peaks are quantified using a ratio based calibration curve using an internal standard (norvaline). A subset of 'robust' amino acids (i.e., those known not be harmed by the acid treatment) is used to calculate the concentration of amino acids (pmol/uL). In order to derive an experimental extinction coefficient, the optical density (OD₂₈₀) of the intact protein is measured. The Beer-Lambert equation is applied using protein concentration measured by the amino acid analysis.

Table 3.2.R-9: Results of Amino Acid Analysis of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Product		US-licensed Remicade [®]			CT-P13 Drug Product			EU-approved Remicade [®]		
Batch No.		CLM91 012P1	CKS860 16P1	CJM76 016P1	12B1C 015	12B1C0 16	12B1C01 7	1RMA 64901	1RMA6 5804	1RMK A87103
Amino Acid Analy sis (molar ratio) ¹	Aspartic acid	105.6	105.2	105.2	105.7	105	106.7	106.1	106.8	106.5
	Glutamic acid	129.6	128.9	128.8	130.3	130.1	130	129	129.4	129.3
	Serine	170.2	169.7	169	172.2	171.4	169.7	167.4	167.7	168.8
	Histidine	25.3	25.3	25.2	27.3	27.9	27.8	24.5	24.2	23.8
	Glycine	86.4	86.7	84.7	86.4	87.5	85.2	85.4	87.7	86.9
	Threonine	93.8	93.5	94.2	95.7	96.1	94.9	94.2	93.1	93.3
	Arginine	40.4	40.5	41.8	39.9	36.3	41	40.9	40.4	40.6
	Alanine	57.8	57	58	58.9	59.5	58.8	58.5	58.7	58.8
	Tyrosine	32.4	32.7	38.5	27.7	17.5	33.9	36.3	35.1	35.5
	Valine	111.9	111.4	111.4	111.5	112.4	114	110.3	110.6	110.7
	Methionine	14.4	14.2	15.3	13.6	11.3	13.1	14.9	14.7	14.7
	Phenylalanine	45.5	45.5	45.4	44.6	43.8	45.5	44.8	43.3	45.1
	Isoleucine	33.3	33.6	33.9	33.6	32.7	33.7	33.7	32.7	33.1
	Leucine	96	96	96	96	96	96	96	96	96
Lysine	84.4	88.9	89.4	78.9	81.4	82.5	86.8	84.6	88.6	
Proline	76.6	66.3	69.5	72.1	68.2	73.9	73.8	69.6	71.1	
Molar Absor ptivity	Molar Absorptivity (L·mol ⁻¹ ·cm ⁻¹)	204,000	195,000	201,000	219,000	212,000	215,000	194,000	200,000	196,000
	Extinction Coefficient (L·g ⁻¹ ·cm ⁻¹)	1.40	1.34	1.38	1.50	1.45	1.47	1.33	1.37	1.34

¹The variability of amino acid analysis is extremely low; therefore, three lots per product were tested.

Reviewer's Comment: The amino acid analysis results shown above in table 3.2.R-9 were largely similar between the three products, except in the case of Tyrosine and Methionine. In addition, it is evident that there was some variation in the measured molar absorptivity observed for CT-P13 that was not observed EU-approved infliximab and US-licensed Remicade. An IR letter was communicated to the Sponsor requesting an explanation for the inconsistencies in the data for Tyr levels and values obtained for the extinction coefficient.

Information Request Letter. An IR was sent to the Sponsor on 13th February 2014 that included questions regarding the amino acid analysis. Celltrion responded on March 9th, 2015 (Sequence No 026) and with a follow-up on April 2nd, 2015 (Sequence 029).

The question was multi-part:

“In your submission you included the results of the amino acid analysis and also determined the molar absorptivity (extinction coefficient) for each product lot as shown in Table 3.2.R-9. We note that the data obtained from the amino acid analysis of CT-P13, while largely matching those of US-licensed Remicade[®], differed in that tyrosine (Tyr) values were lower, and generally more variable, than the values obtained for both EU-approved infliximab and US-licensed Remicade[®]. Moreover, the Tyr value from the analysis of CT-P13 lot 12B1C016 was significantly less than the two other lots of CT-P13

(12B1C015 and 12B1C017). We further note that the values obtained for the molar absorptivity of the three CT-P13 lots reported in this table are higher than those obtained for both the EU-approved infliximab and US-licensed Remicade®. To address these concerns, we have the following requests:

- i. Provide a scientific explanation for the discrepancy in the Tyr data between CT-P13 and US-licensed Remicade®. Address whether this is due to assay method or if it reflects a true difference in amino acid content/sequence. Clarify whether the CT-P13 and US-licensed Remicade® test articles were tested side-by-side or on different days.”

Celltrion Response (summarized):

An investigation indicated that contractor lab error may have cause the variable Tyr and Met data. Upon receipt, not all samples were analyzed under the same conditions and not all samples were run on the same day (report provided, appendix 6 130768). The likely source of the variability in the Tyr data is variation in hydrolysis; Tyr is not considered to be a robust amino acid as the acid step degrades it slowly. The Sponsor noted that there was a correlation between the variability in both Tyr and Met among the lots which provided further evidence for oxidation playing a role in degradation. In the follow-up response, data from an improved analytical method (see table below) with less variability and improved recovery were submitted.

Table 1: Comparison of the Test Conditions for Amino Acid Analysis

Original Analysis (from 2009 AAA qualification report)	Improved Analysis (from 2015 AAA study report)
(b) (4)	

Additional batches of CT-P13 and reference product were compared in this procedure. The data were much tighter.

Reviewer’s Comment: *The explanation is scientifically sound, and the data have been presented to confirm the Sponsor’s conclusions. The improvement in the methodology resulted in a significant improvement in the data for Tyr recovery and reduced the variability.*

“ii. Explain the variability in Tyr values between different lots of CT-P13. If an explanation is not available, we recommend that additional lots be tested by amino acid analysis to establish the range and average value of Tyr.”

Response to Question 2a-ii:

See response to question 1; variability observed in Tyr levels observed between CT-P13 lots is due to hydrolysis and the assay method. Additional testing of 13 lots using the improved method was provided (April 2nd, 2015 - Sequence 029). This showed significant improvement in recovery and reduced variability. Using this approach the mean Tyr recovered for CT-P13 was 48 +/- 2.0% and 48.5 +/- 2.1% for US-licensed Remicade.

Reviewer's Comment: *The explanation is acceptable.*

“b. Provide a step-by-step description of the procedure and calculation used to conduct the amino acid analysis in Table 3.2.R-9, as well as the procedure and calculation used to determine the extinction coefficients

i. Address whether the acid-based amino acid liberation procedure that you used in your assay could damage individual amino acids like Tyr or Trp.”

Response to Question 2b-i:

See response to question 1; variability observed in Tyr levels observed between CT-P13 lots is due to hydrolysis. It is expected based on known chemistry that there will be losses due to acid hydrolysis for Tyr and Trp, which is destroyed during the analysis.

Reviewer's Comment: *The explanation is acceptable.*

“ii. Provide the equation of how these data were subsequently used to calculate the concentrations of US-licensed Remicade® and CT-P13 Drug Product.”

Response to Question 2b-ii:

The protein concentration is calculated based on Beer Lamberts law.

$$A=\epsilon Cl$$

Where A= Absorbance at 280nm, ϵ = extinction coefficient, C = concentration and *l* is the path length of the cuvette. A theoretical extinction coefficient (1.45) is used to calculate the protein concentration for routine lot testing. The approach used to generate the theoretical extinction coefficient is shown below in table 20 and table 21. The experimentally determined extinction coefficients of the individual lots were generated to confirm the theoretical extinction coefficient.

The Sponsor indicates that the experimental extinction coefficient was not used to calculate the protein concentration of CT-P13 or US-licensed Remicade in any of the analytical similarity assays or on a routine basis for lot or stability testing.

Table 20: Theoretical Molecular Weight of CT-P13

Atom	Molar Mass (g/mol)	Atomic Composition of Heavy Chain	Subtotal Mass of Heavy Chain	Atomic Composition of Light Chain	Subtotal Mass of Light Chain
C	12.0107	2,203	26,459.5721	1,028	12,346.9996
H	1.00794	3,411	34,38.08334	1,587	1,599.60078
N	14.00674	585	8,193.9429	279	3,907.88046
O	15.9994	682	10,911.5908	337	5,391.7978
S	32.066	16	513.056	6	192.396
Total Molecular Weight			49,516.24514		23,438.67464
		Subtotal Mass of Two Heavy Chain	99,032.49028	Subtotal Mass of Two Light Chain	46,877.34928
A: Sum of Mass of Two Heavy Chain and Two Light Chain					145,909.83956
Molecular Weight of CT-P13 (A-16·H ₂) ¹					145,878 (g/mol)

(b) (4)

Table 21: Theoretical Molar Extinction Coefficient of CT-P13

	Heavy Chain in CT-P13	Light Chain in CT-P13	One Molecule of CT-P13	Molar Extinction Coefficient (L mol ⁻¹ cm ⁻¹)
# of Trp	9	3	24	212,080
# of Tyr	20	8	56	
# of Cys	11	5	32	
Molecular weight of CT-P13				145,878
Extinction coefficient (cm ⁻¹)				1.45

Reviewer's Comment: *The approach outlined here ameliorates the concern that the experimentally derived extinction coefficients were used to determine the protein concentration. That practice would lead to differences in the amount of protein used and assay imprecision. The answer is acceptable.*

“iii. In section 3.2.R.5.1.5 you state that “The derivations of the molar absorptivity values were performed using the previously mentioned robust amino acids”. This list did not include Tyrosine or Tryptophan, which are generally considered to be the amino acids that contribute the most to protein absorptivity and extinction coefficients. Explain why this procedure was used if the absorptivity contribution of these two amino acids are not included in the final calculation of the extinction coefficient.”

Response to Question 2b-iii: See above, Celltrion uses the theoretical extinction coefficient to calculate the protein concentrations.

“iva. Provide a scientific explanation for the apparent difference in protein concentrations between U.S-licensed Remicade and CT-P13. Specifically address whether this difference could be explained by differences in your experimentally determined extinction coefficients.”

Response to Question 2b-iv: See above; the protein concentration is determined using the same theoretical extinction coefficient for CT-P13, US-licensed Remicade and EU-approved infliximab. The theoretical extinction coefficient falls within the range of the experimentally determined extinction coefficients.

The protein concentration was determined using ten lots of CT-P13 and 10 lots US-approved Remicade with mean values 9.6mg/mL and 9.3mg/mL respectively. Therefore the difference in protein content is approximately 3.2%. The drug product release criterion for protein content is (b) (4) mg/mL and this corresponds to a concentration of (b) (4) mg/mL (following reconstitution).

The data from the 3-way PK study (Study 1.4) did not show any differences in the values obtained for *in vivo* PK. The primary endpoints were within the equivalence margin (80-125%). Therefore the Sponsor contends that minor differences in the protein concentration did not have an impact.

Reviewer's Comment: *The 4% protein content difference is small, but probably not a statistical fluke. Celltrion plans to tighten (b) (4) to more closely match the US-Remicade protein content, see page 138 for discussion.*

“2b-iv) 1 Clarify whether you used one unified extinction coefficient to determine protein concentrations for all lots in your 3-way analysis, or whether you used experimentally-derived extinction coefficients, which vary between CT-P13, US-licensed Remicade and EU-approved infliximab.”

Response to Question 2b-iv)-1: As describe above, the theoretical extinction coefficient was used to determine protein concentration throughout for CT-P13, EU-approved infliximab and US-licensed Remicade.

Reviewer's Comment: *This is acceptable.*

“2b-v) Describe current measures taken to match CT-P13 protein content to US-licensed Remicade.”

Response to Question 2b-v: Celltrion has carried out additional studies of 13 lots of US-licensed Remicade and CT-P13 and data shows that there really is a difference of approximately 4% in protein content. Celltrion is proposing to adjust (b) (4) and other drug product processing parameters of CT-P13 to more closely match protein content of US-licensed Remicade.

Reviewer's Comment: *This is acceptable (see page 138 for discussion).*

“2b-vi. If further analysis reveals that the CT-P13 and US-licensed Remicade protein contents actually are consistently 3-4% different, describe plans to readjust the CTP13 ^{(b) (4)} process to allow its protein content to more closely match that of US-licensed Remicade.”

Response to Question 2b-vi: They proposed a plan to adjust and tighten ^{(b) (4)} of CT-P13 to match the US-licensed Remicade. This is summarized in table 9 below, excerpted from IR response.

Table 9: Outline of Plan to Adjust CT-P13 Product Protein Content

Item/In-process Control	Acceptance Criteria Before Change	Acceptance Criteria After Change
^{(b) (4)}		

The proposed revised specification for protein content will be (^{(b) (4)} Celltrion mg units/mL). This is within the quality range obtained from the lots of US-licensed Remicade. The Sponsor is proposing to manufacture 3 conformance lots at a commercial scale and assess tolerances during process validation. These data will be submitted as an amendment to the BLA in early May 2015. The three lots will be added to the on-going stability program that includes long-term, real and accelerated stability studies. An additional 10 lots will be manufactured following the approval to confirm the specifications.

Reviewer’s Comment: *The Sponsor is proposing to modify the DP manufacturing process. It is unclear if this can be accomplished in the remaining weeks of the BsUFA review cycle (ends June 6, 2015).*

“2b-vii. If protein content/concentration values of individual lots of US-licensed Remicade, EU- approved infliximab or CT-P13 require readjustment after re-analysis of the product extinction coefficients, we recommend statistical reanalysis of all assays where results are expressed as units per mg antibody (e.g., binding assays like FcRn, etc.).”

Response to Question 2b-vi: This question is moot in that Celltrion uses the same theoretical extinction coefficient to calculate protein concentration of all lots of all three product types.

Reviewer’s Comment: *The response is consistent with the data and rationale that has been presented previously.*

“2b-vii. Address whether additional lots of US-licensed Remicade, EU-approved infliximab and CT-P13 are available to compare protein content with a more representative number of batches. We recommend using reference lots covering various expiration periods to avoid clustering data from related lots. Address whether clinical batches are available for this purpose.”

Response to Question 2b-vii: See above. Inclusion of additional lots confirmed the small differential in protein content between CT-P13 and US licensed Remicade. This will be resolved by (b) (4).

Reviewer's Comment: This is acceptable.

N-terminal and C-terminal Sequencing. Seven lots each of CT-P13 DP, EU-approved and US licensed Remicade were subjected to peptide mapping in combination with MS/MS to determine the N- and C-terminal sequence. The N-terminal sequences are consistent amongst all the lots tested. The variability of the C-terminus of the light chain and heavy chains is consistent across all the lots (i.e. C-terminal Lys variants). The following N and C terminal sequences were obtained by peptide mapping in combination with MS/MS:

N-terminal sequence

Light chain: DILLTQSPAILSVPGER

Heavy chain: EVKLEESGGGLVQPGGSMK

C-terminal Sequence

Light chain: SFNRGEC

Heavy chain: SLSLSPGK or SLSLSPG

Reviewer's Comment: The experimental data from the N-terminal and C-terminal sequencing provide further evidence that the primary sequences of CT-P13 and the US-licensed Remicade are the same.

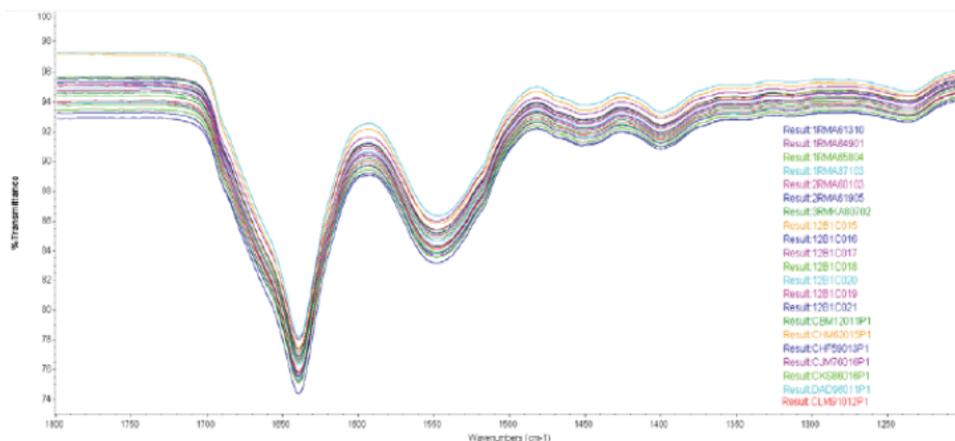
Higher Order Structure (i.e. secondary and tertiary structure)

A number of analytical approaches typically employed for antibodies were used to assess the secondary and tertiary structure of CT-P13, US-licensed Remicade and EU-approved infliximab. They included Fourier transform infrared spectroscopy (FTIR), circular dichroism (CD), differential scanning calorimetry (DSC), disulfide bond structure (free thiols) and an experimental approach called antibody array. No significant differences in the higher order structure were identified in the samples using these analytical approaches.

FTIR: Analysis of proteins with FTIR can yield nine characteristic bands that can be used to probe the secondary structure of the protein. The amide I and II bands are the major bands present in the spectrum characteristic of the protein secondary structure. The amide I band is associated with backbone conformation and amide II is sensitive to changes in conformation. The spectra were analyzed by comparing the location and shape of the prominent spectral peaks at 1640 (amide 1) and 1548 (amide 2) and three other bands occurring between 1000 and 1500 cm^{-1} .

The figure 3.2.R-6 shown below does not display significant differences between several lots of CT-P13, US-licensed Remicade and EU-approved infliximab.

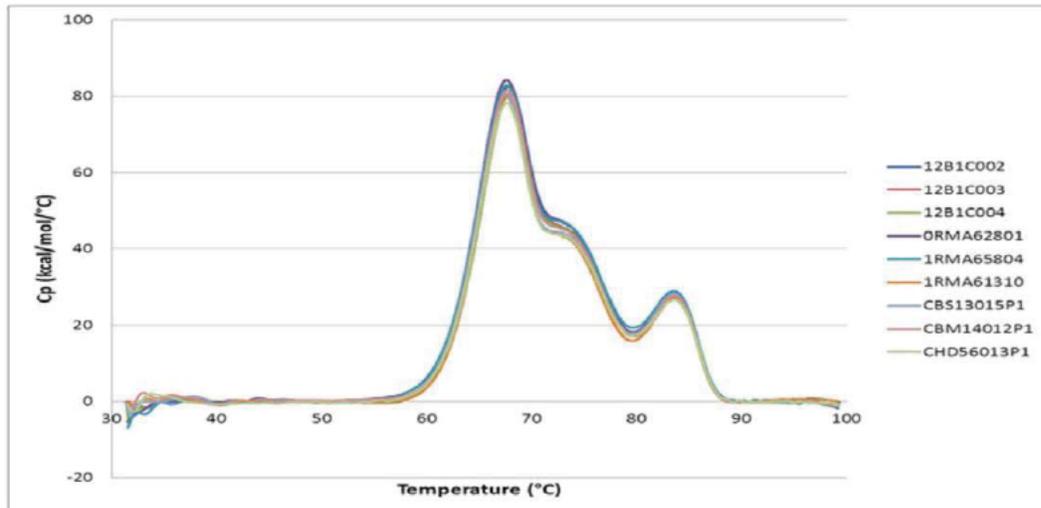
Figure 3.2.R-6: FTIR Difference Spectra of EU-approved Remicade[®], US-licensed Remicade[®] and CT-P13 Drug Product (Abridged Assessment Batches)



EU-approved Remicade[®] (2RMA60103, 1RMA64901, 2RMA61905, 3RMA680702, 1RMA65804, 1RMA67103, 1RMA61310), US-licensed Remicade[®] (CHF59013P1, CHM62015P1, CLM91012P1, CKS86016P1, CBM12011P1, CJM76016P1, DAD96011P1) and CT-P13 Drug Product (12B1C015, 12B1C016, 12B1C017, 12B1C018, 12B1C019, 12B1C020, 12B1C021) (Enhanced Assessment Batches)

Differential Scanning Calorimetry: This analytical technique is used to assess thermally induced transitions such as conformational (unfolding) changes in biological molecules as temperature is ramped up. Thermograms were generated using a Microcal VP-DSC instrument (GE Healthcare). For the three-way analysis samples were diluted with modified sample dilution buffer (5 mM histidine, 60 mM trehalose, 0.01% tween 20, and pH 6.0). The measurement is based on the difference in the heat flow between a sample and reference chamber as both chambers are slowly heated or cooled. Thermograms for CT-P13, US-licensed Remicade and EU-approved infliximab showed close agreement. The analysis shows 3 endothermic transitions at 68°C, 74°C and 83°C and tabulated data from the abridged and enhanced assessment batches shown in table 3.2.R-12 (see BLA). The data show that there does not appear to be significant difference between CT-P13, EU-approved infliximab and US-licensed Remicade.

An example of the DCS thermograms is shown below in figure 3.2.R-8



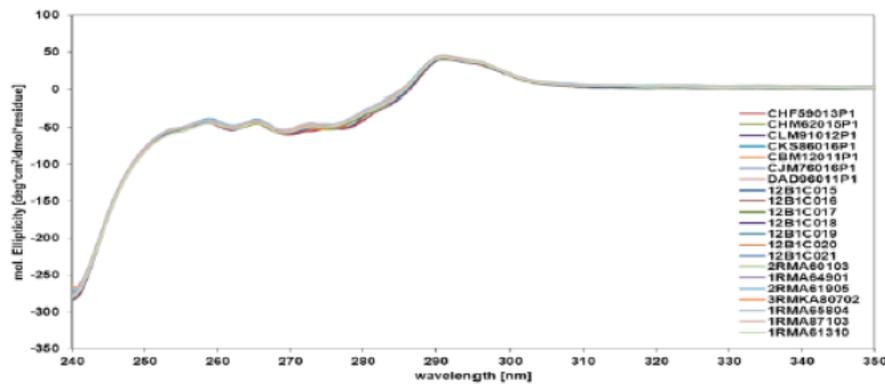
EU-approved Remicade[®](ORMA62801, 1RMA65804, 1RMA61310), US-licensed Remicade[®] (CBS13015P1, CBM14012P1, CHD56013P1) and CT-P13 Drug Product (12B1C002, 12B1C003, 12B1C004)

Figure 3.2.R-8: DSC Thermograms for EU-approved Remicade[®], US-licensed Remicade[®] and CT-P13 Drug Product (Abridged Assessment Batches)

Circular Dichroism: Tertiary and secondary structure were determined using near and far UV circular dichroism using a J-710 (JASCO) spectropolarimeter. No significant differences were noted in CT-P13, US-licensed Remicade and EU-approved infliximab in terms of the maximum and minimum wavelength of the spectral signal and the mean residue ellipticity (MRE).

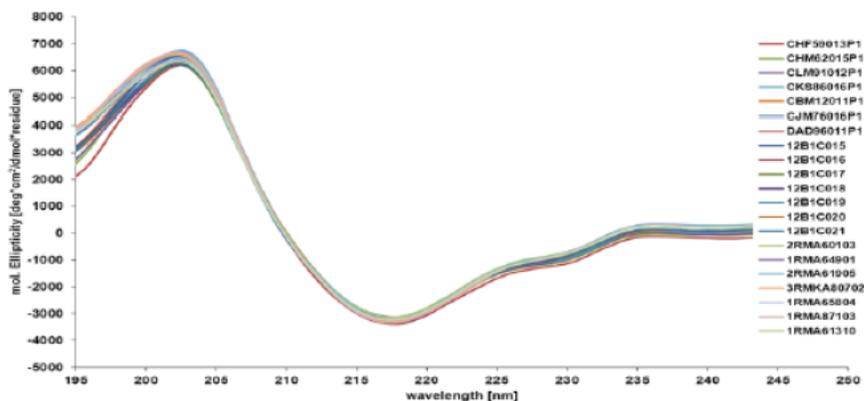
Examples of near and far-UV CD spectra of CT-P13, EU-approved infliximab and US-licensed Remicade are given below from figure 3.2.R-10 and 3.2.R-12.

Figure 3.2.R-10: Near-UV CD spectra of EU-approved Remicade[®], US-licensed Remicade[®] and CT-P13 Drug Product (Abridged Assessment Batches)



EU-approved Remicade[®] (2RMA60103, 1RMA64901, 2RMA61905, 3RMKA80702, 1RMA65804, 1RMKA87103, 1RMA61310), US-licensed Remicade[®] (CHF59013P1, CHM62015P1, CLM91012P1, CKS86016P1, CBM12011P1, CJM76016P1, DAD96011P1) and CT-P13 Drug Product (12B1C015, 12B1C016, 12B1C017, 12B1C018, 12B1C019, 12B1C020, 12B1C021) (Enhanced Assessment Batches)

Figure 3.2.R-12: Far-UV CD Spectra for EU-approved Remicade[®], US-licensed Remicade[®] and CT-P13 Drug Product (Abridged Assessment Batches)



EU-approved Remicade[®] (2RMA60103, 1RMA64901, 2RMA61905, 3RMKA80702, 1RMA65804, 1RMKA87103, 1RMA61310), US-licensed Remicade[®] (CHF59013P1, CHM62015P1, CLM91012P1, CKS86016P1, CBM12011P1, CJM76016P1, DAD96011P1) and CT-P13 Drug Product (12B1C015)

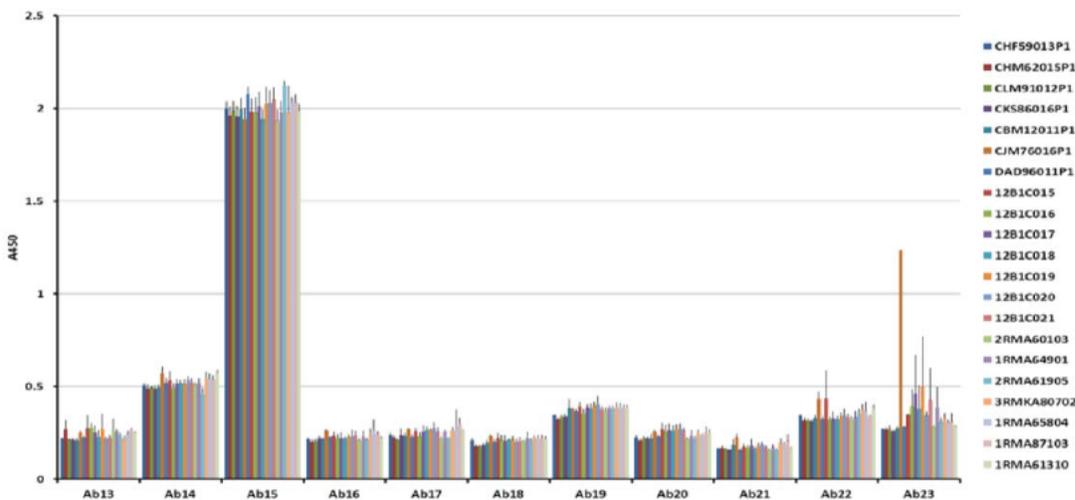
Free Thiol Analysis: High levels of free Thiols on Cys residues are a hallmark of improperly folded protein. The presence of free thiols in CT-P13, EU-approved infliximab and US-licensed Remicade was determined using an Ellman's reagent based assay. The DTNB reacts with the free thiol present on the free cysteine to yield TNB. The reaction is stoichiometric and the TNB

can be quantified in spectrophotometer by measuring the absorbance at 412nm. US-licensed Remicade (0.15 free SH/IgG mol/mol), CT-P13 Drug Product (0.14 free SH/IgG mol/mol) and EU-approved infliximab (0.14 free SH/IgG mol/mol) had similar, low levels of free thiols.

Conformational Array ELISA: The RemiBridge Protein Conformational Array ELISA is an R&D technique marketed by Array Bridge in St Louis.

Reviewer's Comment: This is a newly developed approach to compare higher order structures of proteins, but industry has not gained sufficient experience with the method to conclude it is sufficiently robust for regulatory purposes beyond preliminary characterization.

The array consists of 34 pools of polyclonal antibodies that have been raised against short segments of the peptide sequence of CT-P13. For a correctly folded protein the majority of the epitopes are buried and therefore not recognized. If there is misfolding or unfolded elements present, they may be detected by one or more of the antibodies on the array. The study was performed by (b) (4). The results from batches of CT-P13, US-licensed Remicade and EU-approved infliximab were largely consistent, with the exception of a single batch of the US-licensed Remicade (CJM76016P1, see last column in figure).



Reviewer's Comment: This is a new, untested R&D assay. There is no reason to believe that the one batch of US-licensed Remicade should be significantly different from other marketed batches of Remicade. This result is more likely due to assay imprecision.

Vial Protein Content

The protein content of reconstituted vials of CT-P13, US-licensed Remicade and EU-approved infliximab was determined using a spectrophotometer (i.e., A280). The batches used in the enhanced assessment (10 vials each) of EU-approved infliximab and US-licensed Remicade are the same average concentration (9.2 mg/mL). The concentrations of ten reconstituted vials of CT-P13 were found to be slightly higher on average (9.5 mg/mL). The extinction coefficient used to calculate these concentrations was $1.45 \text{ L}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}$

Reviewer’s Comment: Statistical equivalence testing revealed that the protein content difference of 0.3 mg/ml was statistically significant. This may have resulted because Celltrion tried to match the labeled content (100 mg) using their extinction coefficient when in reality the innovator may have used a different one. Using the Celltrion method (with an extinction coefficient of $1.45 \text{ L}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}$), US-licensed Remicade typically was determined to contain on average of 92 Celltrion mg units of protein.

This was the subject of an IR letter sent Feb 10, 2015 (see narrative description of letter and follow-up in “Amino Acid Analysis and Protein Content” section of the analytical similarity assessment. In summary, analysis of additional vials of CT-P13 and US-licensed Remicade confirmed the 3.2% difference in protein content. The firm will tighten their ^{(b) (4)} more closely match the actual protein content of US-licensed Remicade.

This was implemented in April 2015 and described in an amendment dated April 30, 2015. The process was adjusted as per the below table. They also revised the protein content specification to ^{(b) (4)} Celltrion mg units/vial to more closely match the actual content of the US-licensed Remicade vials that had been tested in their analysis:

Table 2: Adjustment of CT-P13 Drug Product Manufacturing Process

Item/In-process Control	Acceptance Criteria Before Change	Acceptance Criteria After Change
(b) (4)		

Three validation batches were produced according to this revised procedure; 15B4C01, 02 and 03. They passed acceptance criteria for In-Process testing for the ^{(b) (4)} process, as well as release testing. Protein content of the three lots was 9.3 mg/mL, 9.1 mg/mL and 9.4

mg/mL. These values are within the mean \pm 3SD (8.80 – 9.63 mg/mL) of the 30 batches of US-licensed Remicade that had been tested by Celltrion by that time:

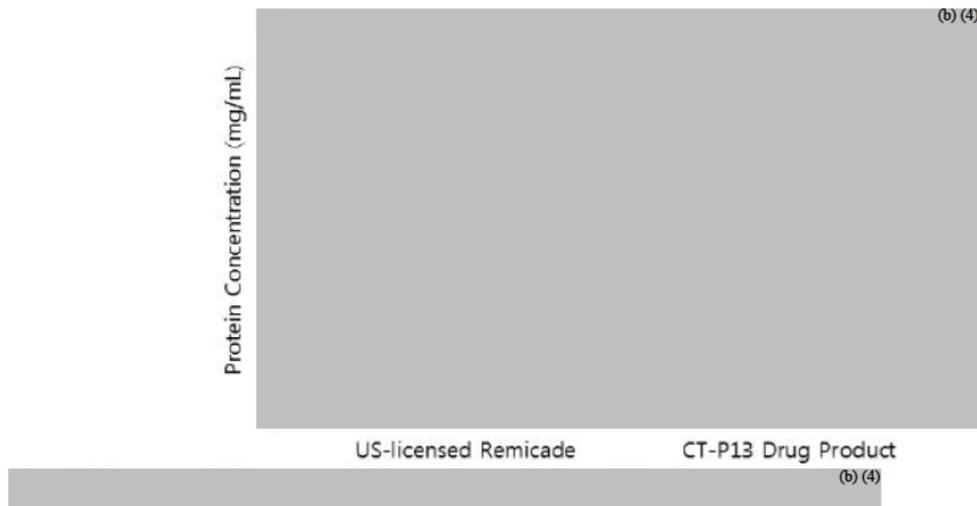


Figure 1: Comparison of Mean and Standard Deviation of Protein Concentration of CT-P13 and US-licensed Remicade[®] Batches

Reviewer's Comment: The (b) (4) process adjustments are adequate and have been validated. The adjustment of the protein content DP lot release specification to (b) (4) Celltrion mg units/vial is warranted given the actual average protein content of the reference product measured in a large number ($n=30$) batches of US-licensed Remicade using the Celltrion method. It can be inferred that the Celltrion method for determining concentration provides a value in units that differs from the assay used by the innovator. Using these adjusted parameters, Celltrion can match the concentration of the innovator product.

Purity/Impurity

The biochemical/biophysical purity (i.e. absence of aggregates) of CT-P13, EU-approved infliximab and US-licensed Remicade were assessed using SEC-HPLC, SEC-MALS and AUC. Overall, the data from the analysis show there are at most minor differences between the three products, and aggregate levels are routinely $<1\%$.

SEC-HPLC: Size exclusion chromatography was undertaken using HPLC equipped with a TOSOH TSK G3000_{SW} column with an aqueous buffer. Elution of the protein off the column was monitored with a UV detector set 214nm. An analysis using size exclusion chromatography has shown that batches of CT-P13, US-licensed Remicade and EU-approved infliximab included in the enhanced assessment predominantly elute as a single peak ranging from 99.5% to 99.9%.

EU-approved infliximab and US-licensed Remicade is on average 99.9% monomer, while CT-P13 is on average 99.4% monomer.

Table 3.2.R-18: Results of SEC-HPLC of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Purpose	Product	Batch No.	SEC-HPLC (Monomer, %)
Abridged Assessment Batches	US-licensed Remicade [®]	CBS13015P1	99.5
		CBM14012P1	99.5
		CHD56013P1	99.5
	CT-P13 Drug Product	12B1C002	99.1
		12B1C003	99.2
		12B1C004	99.1
	EU-approved Remicade [®]	0RMA62801	99.6
		1RMA65804	99.5
		1RMA61310	99.6
Enhanced Assessment Batches	US-licensed Remicade [®]	CHF59013P1	99.9
		CHM62015P1	99.8
		CLM91012P1	99.9
		CKS86016P1	99.9
		CBM12011P1	99.8
		CJM76016P1	99.8
		DAD96011P1	99.9
		Mean	99.9
		SD	0.1
		CT-P13 Drug Product	12B1C015
	12B1C016		99.4
	12B1C017		99.4

Purpose	Product	Batch No.	SEC-HPLC (Monomer, %)
		12B1C018	99.4
		12B1C019	99.4
		12B1C020	99.4
		12B1C021	99.4
		Mean	99.4
		SD	0.0
	EU-approved Remicade [®]	2RMA60103	99.9
		1RMA64901	99.9
		2RMA61905	99.9
		3RMKA80702	99.8
		1RMA65804	99.8
		1RMKA87103	99.8
		1RMA61310	99.9
		Mean	99.9
		SD	0.1

Reviewer’s Comment: SEC-HPLC results show that the CT-P13 has somewhat higher levels of HMW species (~0.5%). Aggregate levels were consistent with industry experience (< 1% HMW

species), and if they were immunogenic, a signal would be detected in the immunogenicity assessment (see immunogenicity review).

SEC-MALS: Size exclusion chromatography with multi-angle laser light scattering was undertaken to provide an estimation of the molecular weight of monomeric and multimeric protein species eluting from the size exclusion column. The columns used for this procedure included the TOSOH TSK G3000_{SW} column and a Superdex 200 10/300GL. The monitoring system included a DAWN[®] TREOS II[™] and an Optilab rEX An analysis using SEC-MALS. The data show that CT-P13, US-licensed Remicade and EU-approved infliximab predominantly elute as a single peak ranging from 99.5% to 99.8%. The molecular weight of the monomer calculated by the MALS software is elevated in CT-P13 (154.1kDa) vs. EU-approved infliximab (152.1kDa) and US-licensed Remicade (150.6kDa). As above, CT-P13 has a relatively greater amount of the multimer (0.5%) compared to 0.2% for both the EU-approved infliximab and US-licensed Remicade.

Table 3.2.R-19: Results of SEC-MALS of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Purpose	Product	Batch No.	SEC-MALS			
			% Monomer	% Dimer	MW (kDa) Monomer	MW (kDa) Multimer
Enhanced Assessment Batches	US-licensed Remicade [®]	CHF59013P1	99.8	0.2	154	341
		CHM62015P1	99.9	0.1	149	286
		CLM91012P1	99.8	0.2	151	322
		CKS86016P1	99.9	0.1	149	311
		CBM12011P1	99.8	0.2	150	547
		CJM76016P1	99.9	0.1	151	299
		DAD96011P1	99.8	0.2	150	426
		Mean	99.8	0.2	150.6	361.7
		SD	0.1	0.1	1.7	93.7
	CT-P13 Drug Product	12B1C015	99.5	0.5	157	268
		12B1C016	99.5	0.6	151	244
		12B1C017	99.4	0.6	156	320
		12B1C018	99.4	0.6	154	369
		12B1C019	99.6	0.4	155	564
		12B1C020	99.5	0.5	153	418
		12B1C021	99.5	0.5	153	355
		Mean	99.5	0.5	154.1	362.6
		SD	0.1	0.1	2.0	106.9
	EU-approved Remicade [®]	2RMA60103	99.8	0.2	150	288
		1RMA64901	99.8	0.2	151	307
		2RMA61905	99.9	0.1	151	514
		3RMKA80702	99.8	0.2	154	372
		1RMA65804	99.8	0.2	151	408
		1RMKA87103	99.8	0.2	153	537
		1RMA61310	99.8	0.2	155	275
		Mean	99.8	0.2	152.1	385.9
		SD	0.0	0.0	1.9	106.5

Reviewer’s Comment: The MALS software deconvolutes complex light scattering data to generate an estimated molecular weight of the main peak. It is generally understood in the light scattering field that the estimated molecular weight is not as accurate as that obtained by more precise assays (e.g., mass spectroscopy). It is also recognized that two peaks in close proximity, especially when one is much larger than the other, can be merged into one by the software to a

calculated values that includes contributions from both. Thus, it is not surprising that the estimated molecular weight of CT-P13, which contains somewhat higher levels of aggregates, is slightly higher than that of the innovator product.

AUC: Sedimentation Velocity Analytical Ultracentrifugation was performed using a Beckman Coulter XL-A AUC instrument. This method can detect weakly associated aggregates in the native formulation state that dissociate when run in HPLC systems. The protocol was optimized to ensure the experimental parameters were compatible for the test article and the instrumentation system. Initial scans showed that no large aggregates could be detected. Assessment of the data using SEDFIT program was undertaken to generate a profile of the sedimentation coefficient values. With this manipulation, more meaningful data could be ascertained. The data obtained from the analysis of the enhanced assessment lots of CT-P13, US-licensed Remicade and EU-approved infliximab using AUC are broadly consistent with the data obtained using SEC-MALS, given below in Table 3.2.R-20

Reviewer’s Comment: Given the nature of the assay, there is variability in the AUC data, particularly for the higher molecular weight species. AUC is generally considered to be a characterization test, which is not sufficiently precise or robust to be used for routine purposes like lot release.

Table 3.2.R-20: Results of AUC of US-licensed Remicade®, EU-approved Remicade® and CT-P13 Drug Product

Purpose	Product	Batch No.	AUC			
			Monomer (s value)	Monomer (%)	Higher species (s value)	Higher species (%)
Enhanced Assessment Batches	US-licensed Remicade®	CHF59013P1	4.6-4.8	97.1±3.4	9.0-16.2	2.9±3.4
		CHM62015P1	4.7-4.9	96.4±2.1	8.7-15.4	3.6±2.1
		CLM91012P1	4.5-5.3	99.7±0.5	12.5-15.6	0.3±0.4
		CKS86016P1	4.8-4.9	95.6±2.0	7.6-15.0	4.5±2.1
		CBM12011P1	4.8	98.6±2.1	9.4-15.4	1.5±2.1
		CJM76016P1	4.9	98.5±0.6	10.3-14.0	1.5±0.6
	DAD96011P1	4.5-4.9	98.3±0.8	10.1-14.6	1.7±0.8	
	CT-P13 Drug Product	12B1C015	4.6	99.8±0.4	11.0-15.2	0.2±0.3
		12B1C016	4.6-5.2	99.7±0.4	12.2-15.4	0.3±0.4
		12B1C017	4.6-5.0	98.2±0.8	9.2-13.2	1.9±0.8
		12B1C018	4.6	98.4±0.4	9.0-15.0	1.6±0.4
		12B1C019	4.9-5.0	95.4±6.4	7.3-15.8	4.6±6.4
		12B1C020	4.8	98.2±2.5	6.4-15.8	1.8±2.5
	12B1C021	4.8-5.0	98.8±0.6	9.2-15.2	1.3±0.7	
	EU-approved Remicade®	2RMA60103	4.8	97.0±4.2	8.6	3.0±4.2
		1RMA64901	4.6-5.6	97.7±3.3	8.8-15.6	2.3±3.3
		2RMA61905	4.8-5.0	94.2±8.2	8.2-14.6	5.8±8.2
		3RMKA80702	4.7-5.1	96.0±2.3	8.7-13.9	4.0±2.4
		1RMA65804	4.6-4.7	100.0±0.0	ND	0.0±0.0
		1RMKA87103	4.6-4.8	97.7±2.0	9.0-13.6	2.3±2.0
	1RMA61310	4.8-4.9	99.8±0.4	13.5-19.0	0.3±0.4	

Reduced/non-reduced CE-SDS: Capillary sodium dodecyl sulphate gel electrophoresis was carried out using a Beckman Coulter PA-800 capillary electrophoresis system. Analysis performed under non-reducing conditions was used to determine levels of intact IgG and

fragments. While the analysis performed under reducing conditions provided an estimate of the heavy and light chains. The Sponsor indicates that the results of the three-way analysis show that under reduced conditions the level of the intact IgG is slightly lower for the CT-P13 lots. The level of intact antibody in US-licensed Remicade (98.1%) and EU-approved-Remicade (97.9%) is greater than CT-P13 (94.9%).

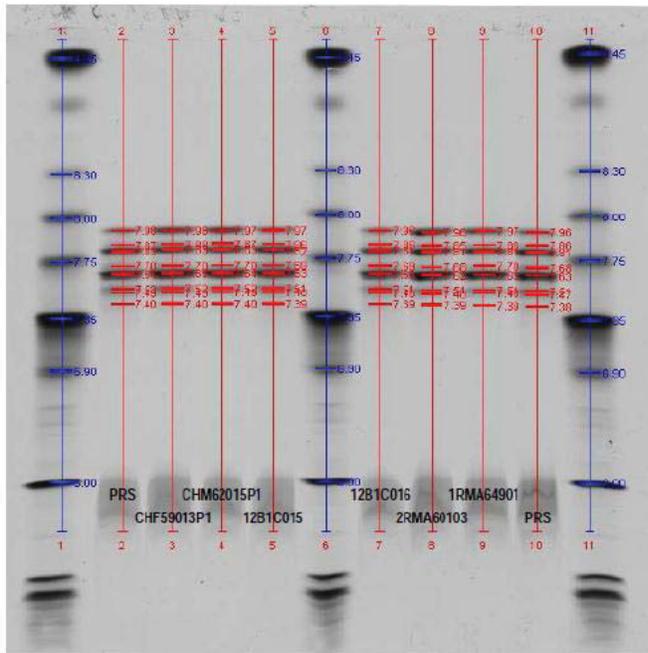
Reviewers Comment: *The lower levels of intact CT-P13 reflect higher levels of an H2L1 variant. This issue was partially addressed in the IND by the two-way analysis where the diminished intact antibody levels did not impact product potency in an in vitro assay, see discussion of these studies above in review of section 3.2.S.3.1. However, the contribution of the H2L1 variant to NK-cell mediated ADCC activity has not been addressed. In addition, it is not known whether an H2L1 variant would be more immunogenic than the intact monoclonal antibody.*

Charge Isoforms

IEF: This assay was performed using IsoGel agarose IEF plates in combination with a flatbed electrophoresis system (Multiphor II, Amersham). The charge pattern is consistent across the various lots of CT-P13, US-licensed Remicade and EU-approved infliximab. There are 8 bands resolved by the assay; band 8 variability is attributed to assay variability. Charge state identification from the lots was included in the enhanced assessment, as shown below.

Table 3.2.R-22: Results of IEF of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Purpose	Product	Batch No.	IEF							
			Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Band 7	Band 8
Enhanced Assessment Batches	US-licensed Remicade [®]	CHF59013P1	7.98	7.88	7.83	7.70	7.65	7.52	7.40	7.49
		CHM62015P1	7.97	7.87	7.83	7.70	7.64	7.52	7.40	7.49
		CLM91012P1	7.91	7.83	7.80	7.70	7.64	7.54	7.43	7.51
		CKS86016P1	7.90	7.83	7.80	7.70	7.64	7.54	7.43	7.51
		CBM12011P1	7.92	7.84	7.81	7.71	7.66	7.56	7.45	7.53
		CJM76016P1	7.92	7.84	7.81	7.72	7.66	7.56	7.46	7.53
		DAD96011P1	7.88	7.80	7.76	7.68	7.63	7.53	7.44	7.52
	CT-P13 Drug Product	12B1C015	7.97	7.86	7.82	7.69	7.63	7.51	7.39	7.48
		12B1C016	7.96	7.86	7.81	7.69	7.63	7.51	7.39	7.48
		12B1C017	7.91	7.83	7.79	7.70	7.64	7.54	7.43	7.51
		12B1C018	7.90	7.82	7.78	7.69	7.63	7.54	7.41	7.50
		12B1C019	7.92	7.84	7.80	7.71	7.66	7.56	7.45	7.53
		12B1C020	7.91	7.84	7.80	7.71	7.65	7.56	7.45	7.53
	EU-approved Remicade [®]	12B1C021	7.88	7.81	7.77	7.69	7.64	7.55	7.45	7.52
		2RMA60103	7.96	7.85	7.81	7.68	7.62	7.51	7.39	7.48
		1RMA64901	7.97	7.86	7.81	7.70	7.63	7.51	7.39	7.48
		2RMA61905	7.90	7.81	7.78	7.68	7.63	7.53	7.42	7.50
		3RMKA80702	7.90	7.82	7.78	7.69	7.64	7.53	7.42	7.50
		1RMA65804	7.91	7.84	7.80	7.71	7.65	7.55	7.45	7.53
		1RMKA87103	7.91	7.84	7.80	7.70	7.65	7.55	7.45	7.53
		1RMA61310	7.88	7.81	7.77	7.69	7.64	7.54	7.45	7.52



Representative IEF gel showing band density measurement procedure. The software capture pixel density is calculated in a defined area.

IEC-HPLC: This method was run on an HPLC system that was equipped with a Propac WCX-10 analytical column. The system was run at ambient temperature. The protein was eluted from the column by gradient elution with NaCl and detected by UV set 214nm. Six peaks elute from the IEC-HPLC for CT-P13, US-licensed Remicade and EU-approved infliximab, analogous to the six-seven charge variants seen by IEF. However, there is a shift in peak area from peak 5 and 6 towards 1, 2, & 4 in CT-P13 vs. EU-approved infliximab and US-licensed Remicade (see table below).

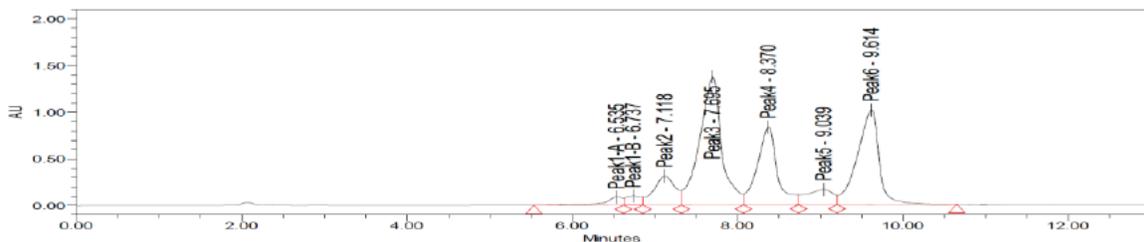
Table 3.2.R-23: Results of IEC-HPLC of US-licensed Remicade®, EU-approved Remicade® and CT-P13 Drug Product

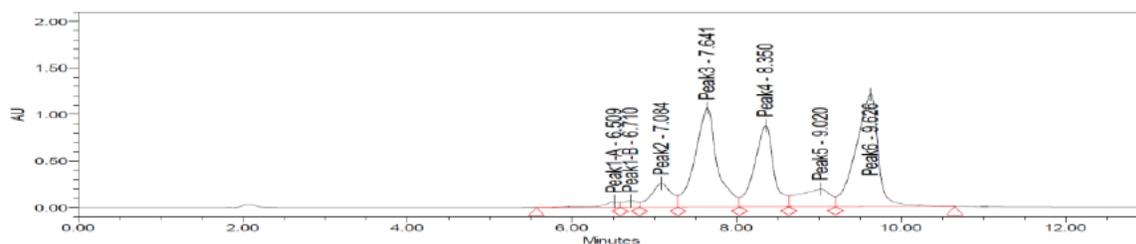
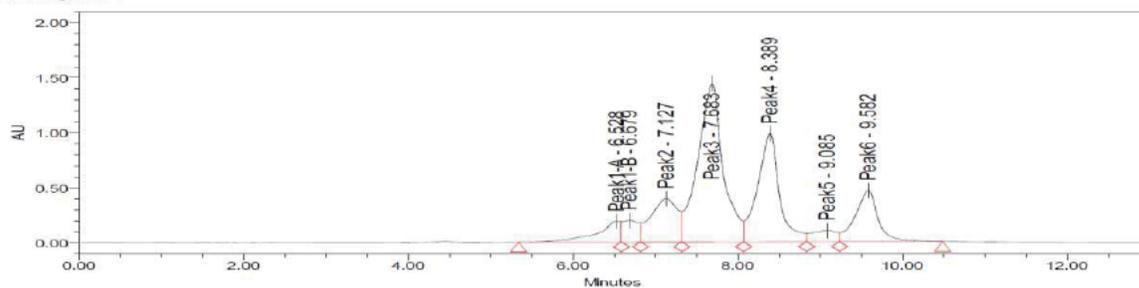
Purpose	Product	Batch No.	IEC-HPLC (%)						
			Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	
Abridged Assessment Batches	US-licensed Remicade®	CBS13015P1	3.7	8.0	36.4	20.0	5.3	26.6	
		CBM14012P1	3.9	8.6	38.5	21.2	4.7	23.2	
		CHD56013P1	6.0	12.3	41.9	18.2	4.4	17.2	
	CT-P13 Drug Product	12B1C002	8.5	11.8	40.9	24.1	2.8	11.8	
		12B1C003	8.4	11.4	40.7	24.4	2.9	12.2	
		12B1C004	8.1	11.3	40.1	24.8	3.0	12.6	
	EU-approved Remicade®	ORMA62801	2.9	6.2	26.2	20.3	8.7	35.6	
		1RMA65804	4.0	8.8	42.9	21.0	3.5	19.9	
		1RMA61310	2.6	6.5	29.4	20.8	7.5	33.3	
Enhanced Assessment Batches	US-licensed Remicade®	CHF59013P1	4.3	11.3	42.0	19.1	4.6	18.7	
		CHM62015P1	3.2	9.6	42.3	20.6	3.9	20.6	
		CLM91012P1	3.7	10.2	42.8	19.4	4.1	19.9	
		CKS86016P1	3.3	9.2	37.6	20.1	5.6	24.3	
		CBM12011P1	3.2	9.0	39.5	19.7	5.8	22.9	
		CJM76016P1	3.4	9.5	41.9	19.4	5.2	20.7	
		DAD96011P1	4.0	10.5	41.8	18.6	5.5	19.6	
		Mean	3.6	9.9	41.1	19.6	5.0	21.0	
		SD	0.4	0.8	1.9	0.7	0.8	2.0	
		CT-P13 Drug Product	12B1C015	6.3	12.2	39.8	25.0	3.7	13.2
			12B1C016	6.2	12.6	40.2	24.7	3.5	12.9
	12B1C017		5.6	11.9	39.5	25.5	3.8	13.8	
	12B1C018		5.8	11.9	39.4	25.4	3.8	13.8	
	12B1C019		6.2	11.9	39.3	25.1	3.8	13.6	
	12B1C020		5.7	11.3	39.0	25.8	3.8	14.5	
	12B1C021		6.6	12.2	40.0	24.6	3.6	12.9	
	Mean		6.1	12.0	39.6	25.2	3.7	13.5	
	SD	0.4	0.4	0.4	0.4	0.1	0.6		
	EU-approved Remicade®	2RMA60103	2.9	7.8	42.2	20.7	4.5	21.9	
		1RMA64901	3.1	9.2	45.4	21.1	3.2	18.0	
		2RMA61905	2.7	8.1	39.8	21.0	4.6	23.9	
		3RMKA80702	4.3	11.2	41.3	19.4	4.8	19.0	
		1RMA65804	3.2	9.2	42.9	20.0	4.6	20.2	
		1RMKA87103	2.7	7.6	36.9	19.7	6.3	26.8	
		1RMA61310	2.2	6.8	29.1	20.0	8.5	33.3	
		Mean	3.0	8.6	39.7	20.3	5.2	23.3	
		SD	0.7	1.4	5.4	0.7	1.7	5.3	

Table showing IEC-HPLC peak areas of CT-P13, and US & EU Remicade.

Examples of IEC-HPLC chromatograms are shown below (US-licensed Remicade, CBS13015P1, EU-approved infliximab 1RMA61310, CT-P13 12B1C002):

CBS13015P1



1RMA61310**12B1C002**

Reviewer's Comment: *The variability in the charge variants observed using this technique, largely from C-terminal Lysine heterogeneity, is unlikely to have a significant clinical impact given the fact that the C-terminal Lys is clipped in the serum (see Brorson K and Jia AY. 2014. Therapeutic monoclonal antibodies and consistent ends: terminal heterogeneity, detection, and impact on quality. Curr Opin Biotechnol 30:140-6).*

Glycosylation

Glycosylation was assessed using three assays: an LC-MS peptide mapping technique for site specific glycan analysis and an oligosaccharide analysis using HPAE-PAD. Monosaccharide analysis of neutral and amino sugars and sialic acid analysis were also undertaken.

Site Specific and N-linked Glycan analysis by LC-MS: This approach uses the same methodology and analytical approach that was outlined in the peptide mapping section. It was confirmed that CT-P13 has typical glycosylation pattern of antibodies: Asn300 is N-glycosylated, and there was no evidence for O-linked glycans. Use of selected ion monitoring enabled quantification of each of the glycan species, although this method should be considered semi-quantitative. The amounts of N-linked glycan present on CT-P13, EU-approved infliximab and US-licensed Remicade are shown below in table 3.2.R-24. Both G0 and G0F are the major glycan species; both EU-approved infliximab and US-licensed Remicade have slightly more of each. These forms are also more variable than CT-P13. The Sponsor indicates that this method is semi-quantitative vs. the HPAEC-PAD N-glycan profiling described below.

Table 3.2.R-24: Results of N-Linked Glycan Analysis of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Purpose	Product	Batch No.	N-linked Glycan Analysis: Ratio (%)							
			Man5	G0F-GlcNAc	G0	G0F	G1F	G2F	G1F 1NeuGe	G2F 1NeuGe
Abridged Assessment Batches	US-licensed Remicade [®]	CBS13015P1	5.1	10.7	2.4	50.5	26.5	3.5	0.9	0.5
		CBM14012P1	3.7	5.3	2.4	34.7	42.4	10.0	0.7	0.8
		CHD56013P1	5.4	9.0	2.2	41.7	32.6	7.1	1.0	1.0
	CT-P13 Drug product	12B1C002	4.0	6.0	1.0	41.2	38.5	5.6	2.1	1.7
		12B1C003	4.1	6.0	1.1	41.0	38.4	5.5	2.1	1.8
		12B1C004	4.1	6.3	1.0	41.2	37.7	5.8	2.0	1.7
	EU-approved Remicade [®]	0RMA62801	6.1	7.7	2.4	40.2	34.7	6.7	1.2	1.0
		1RMA65804	5.1	10.2	3.2	56.5	21.6	2.2	0.8	0.4
		1RMA61310	6.3	13.2	2.6	55.0	20.0	2.0	0.7	0.3
	Enhanced Assessment Batches	US-licensed Remicade [®]	CHF59013P1	5.5	12.0	2.2	37.5	33.4	7.7	0.8
CHM62015P1			4.3	12.6	2.0	47.8	27.7	4.6	0.6	0.5
CLM91012P1			6.0	14.3	2.2	47.3	24.9	4.2	0.7	0.5
CKS86016P1			5.3	11.8	1.8	37.5	34.0	8.2	0.6	0.7
CBM12011P1			3.9	10.3	2.1	37.9	36.2	8.4	0.6	0.7
CJM76016P1			4.7	13.9	2.3	48.3	26.1	3.7	0.7	0.4
DAD96011P1			6.6	15.1	2.2	47.5	24.0	3.5	0.6	0.5
Mean			5.2	12.9	2.1	43.4	29.5	5.8	0.7	0.6
SD			1.0	1.7	0.2	5.4	4.9	2.2	0.1	0.1
CT-P13 Drug Product			12B1C015	4.5	10.0	1.1	40.7	35.7	5.2	1.6
		12B1C016	4.6	9.6	1.1	41.2	35.4	5.0	1.7	1.4
		12B1C017	4.8	10.2	1.1	40.0	35.7	5.0	1.8	1.4
		12B1C018	4.5	10.2	1.0	40.3	35.7	5.2	1.7	1.4
		12B1C019	4.7	9.4	1.1	41.8	35.3	4.7	1.6	1.3
		12B1C020	4.5	9.9	1.2	42.1	34.6	4.7	1.8	1.3
		12B1C021	4.7	9.8	1.2	40.4	35.6	5.2	1.8	1.4
		Mean	4.6	9.9	1.1	40.9	35.4	5.0	1.7	1.4
		SD	0.1	0.3	0.1	0.8	0.4	0.2	0.1	0.1
		EU-approved	2RMA60103	3.3	12.3	2.5	49.0	28.1	3.9	0.6
Remicade [®]		1RMA64901	3.4	10.9	2.4	45.4	31.4	5.4	0.6	0.5
	2RMA61905	4.2	10.1	2.0	33.5	39.0	9.9	0.5	0.7	
	3RMKA80702	6.0	11.9	2.0	42.7	30.0	5.9	0.7	0.7	
	1RMA65804	5.4	14.9	2.9	51.5	22.2	2.3	0.6	0.3	
	1RMKA87103	4.7	13.8	2.3	47.6	27.0	3.8	0.5	0.3	
	1RMA61310	7.7	16.3	2.5	51.6	19.1	1.9	0.5	0.2	
	Mean	5.0	12.9	2.4	45.9	28.1	4.7	0.6	0.4	
	SD	1.6	2.2	0.3	6.3	6.5	2.7	0.1	0.2	

Data from MS-based glycan method.

Oligosaccharide Profiling of Optimized HPAEC-PAD. The optimized high pH anion exchange chromatography with pulsed amperometric detection (HPEAC-PAD) method starts with liberation of the glycan from the peptide chain by PNGase enzyme cleavage. The glycans are resolved on a Carpac analytical column and detected pulsed amperometric detection using an AgCl electrode. Quantitation of the glycans involves dividing individual peak areas by the sum of 7 routinely resolvable peaks [(G0F, Man5, G0, G1F, G2F, SA1 and SA2)]. This is multiplied by 100%.

Table 3.2.R-25: Results of Oligosaccharide Profiling of US-licensed Remicade®, EU-approved Remicade® and CT-P13 Drug Product

Purpose	Product	Batch No.	Oligosaccharide Profiling (%)										
			G0F	Man5	G0	G1F	G2F	SA1	SA2	G0 +Man5	G0F +G1F +G2F	SA1 +SA2	
Abridged Assessment Batches	US-licensed Remicade®	CBS13015P1	48.9	5.7	1.9	27.0	3.1	2.9	10.5	6.3	79.0	13.4	
		CBM14012P1	29.6	3.4	1.5	39.3	9.2	5.9	11.1	4.9	78.1	17.0	
		CHD56013P1	34.0	5.2	1.7	31.3	6.9	5.9	15.1	6.9	72.2	21.0	
	CT-P13 Drug Product	12B1C002	36.6	3.6	0.7	38.1	5.8	2.4	12.8	4.3	80.5	15.2	
		12B1C003	36.1	4.1	1.0	38.3	5.9	2.1	12.7	5.1	80.3	14.8	
		12B1C004	35.5	4.1	0.8	37.9	5.8	2.4	13.6	4.9	79.2	16.0	
	EU-approved Remicade®	0RMA62801	34.9	4.4	1.9	33.0	6.8	2.7	16.4	6.4	74.7	19.1	
		IRMA65804	51.7	6.6	2.7	25.6	3.0	1.8	10.7	9.3	80.3	12.5	
		IRMA61310	51.6	5.7	2.6	23.2	2.1	2.2	11.7	8.3	76.9	13.9	
		CHF59013P1	45.40	5.05	1.79	26.55	4.39	4.98	11.83	6.84	76.3	16.8	
Enhanced Assessment Batches	US-licensed Remicade®	CHM62015P1	35.00	4.44	1.27	34.38	7.93	4.58	12.40	5.71	77.3	17.0	
		CLM91012P1	34.42	4.26	1.38	33.87	7.19	5.06	13.82	5.64	75.5	18.9	
		CKS86016P1	45.71	3.68	1.28	30.47	4.80	3.44	10.62	4.96	81.0	14.1	
		CBM12011P1	34.22	3.60	1.16	37.25	7.69	4.74	11.34	4.76	79.2	16.1	
		CJM76016P1	47.02	4.36	1.43	28.18	3.64	4.00	11.37	5.79	78.8	15.4	
		DAD96011P1	47.01	5.46	1.75	25.49	3.29	3.25	13.74	7.21	75.8	17.0	
		Mean	41.25	4.41	1.44	30.88	5.56	4.29	12.16	5.84	77.7	16.5	
		SD	6.31	0.67	0.24	4.42	1.98	0.74	1.23	0.91	12.7	2.0	
		CT-P13 Drug Product	12B1C015	38.98	4.36	0.67	37.42	5.28	3.74	9.54	5.03	81.7	13.3
			12B1C016	37.76	4.75	0.75	36.39	5.17	4.44	10.74	5.5	79.3	15.2
	12B1C017		38.66	4.45	0.69	36.79	5.08	3.04	11.29	5.14	80.5	14.3	
	12B1C018		38.72	4.59	0.74	36.82	5.14	3.73	10.27	5.33	80.7	14.0	
	12B1C019		38.78	4.50	0.63	36.46	4.79	4.59	10.26	5.13	80.0	14.9	
	12B1C020		39.76	4.61	0.61	36.42	4.93	2.66	11.00	5.22	81.1	13.7	
	12B1C021		38.32	4.44	0.70	36.63	5.12	3.88	10.90	5.14	80.1	14.8	
	Mean		38.71	4.53	0.68	36.70	5.07	3.73	10.57	5.21	80.5	14.3	
	SD		0.61	0.13	0.05	0.36	0.16	0.69	0.59	0.18	1.1	1.3	
	EU-approved Remicade®		2RMA60103	49.82	3.55	1.61	30.70	4.07	2.94	7.32	5.16	84.6	10.3
		IRMA64901	44.35	3.25	1.40	32.33	5.16	3.25	10.27	4.65	81.8	13.5	
		2RMA61905	31.29	3.56	1.23	40.00	9.65	3.17	11.09	4.79	80.9	14.3	
3RMKA80702		39.96	4.47	1.44	30.25	5.61	4.55	13.71	5.91	75.8	18.3		
IRMA65804		52.75	5.06	2.07	24.00	2.41	3.59	10.11	7.13	79.2	13.7		
IRMKA87103		48.13	4.31	1.44	28.73	3.68	3.42	10.28	5.75	80.5	13.7		
IRMA61310		52.45	6.47	1.97	21.62	1.99	3.74	11.76	8.44	76.1	15.5		
Mean		45.54	4.38	1.59	29.66	4.65	3.52	10.65	5.98	79.9	14.2		
SD		7.75	1.12	0.31	5.96	2.57	0.53	1.93	1.43	16.3	2.5		

Results of 10 lots each of CT-P13, US-licensed Remicade and EU-approved infliximab using the HPAEC-PAD method. CT-P13 on average has ~39% G0F (the predominant form), while US-licensed Remicade & EU-approved infliximab had 41-46% G0F.

Reviewer’s Comment: *The pattern of the glycan data from the three way analysis differed from that in the two-way analysis in that a clear trend towards higher afucosylation (defined by G0 +Man5) in CT-P13 was not as obvious (compare table above to IND 118135 original submission review by G. Miesegaes, 13 Nov 2013). In the case of the three-way analysis, CT-P13 was 5.2% afucosylated, while US-licensed Remicade was 5.8%. The sponsor was asked to explain this in an IR letter dated 13th February 2014. Celltrion responded on March 18, 2015.*

“Fucosylation is an important attribute of CT-P13, because the degree of fucosylation affects binding of CT-P13 to Fc γ RIIIa and its effector function. We note that the data you provided in your submission are inconsistent in regard to fucose level in CT-P13, US-licensed Remicade and EU-approved infliximab. In table 3.2.R-124, you provided an estimate of the total proportion of afucosylated species by HPAEC-PAD. The data provided in the table show that EU-approved infliximab has higher fucosylation levels

than CT-P13. These findings contrast data from Table 3.2.R-25 showing fucose levels obtained by summing Man5 and G0 levels from an oligosaccharide profiling assay. The data provided in this table indicate that fucose levels are similar in CT-P13, US-licensed Remicade and EU-approved infliximab. Moreover, Table R-126 shows a monosaccharide analysis where fucosylation is similar in CT-P13, US-licensed Remicade and EU-approved infliximab.”

a. Provide an explanation for the inconsistencies in the fucosylation results and a detailed description of each of the three assays used to generate data depicted in the three tables. In addition, clearly indicate the purpose of each assay.

b. Provide a scientific rationale to justify that summing the amount of Man5 and G0 glycans will give an accurate estimate of afucosylation, as was performed in table R-25. Clarify which glycan nomenclature system you are using. Clarify why other glycans typically present on antibodies, such as G1 or Man6 were not included in the calculation. Justify why this method is used instead of the HPAEC-PAD as in the two-way analysis.”

Response to Question 1a. The difference between the pattern obtained in the early and smaller two-way analysis and larger three-way analysis performed later is probably due to variation in glycans in the reference product (i.e. US licensed Remicade) lots. The assay had not substantially changed since the two way analysis aside from (b) (4) modifications unlikely to impact peak resolution; thus an assay change could not explain the observed differences. Evidence of the reproducibility of the assay over time was provided from two lots of EU-approved infliximab that were included in both the two-way and the three-way studies (Abridged and Enhanced). Levels of Man5 are lower in the later analyses (shown in table 1); but both Man5 and G0 peaks are within the acceptance criteria that have been set for the assay. This was set as < 22% %RSD for minor peaks; see table 2, excerpted from the IR response.

Table 1: Oligosaccharide Profile Results for EU-approved Remicade® Batches Tested in Both 2-way (R&D) and 3-way Studies (QC)

Testing lab	Study	Oligosaccharide Profiling (%)									
		G0F	Man5	G0	G1F	G2F	SA1	SA2	G0+Man5	G0F+G1F+G2F	SA1+SA2
IRMA65804											
QC	Abridged*	51.70	4.49	2.70	25.56	3.02	1.84	10.69	12.53	51.70	4.49
QC	Enhanced	52.75	5.06	2.07	24.00	2.41	3.59	10.11	7.13	79.16	13.7
R&D	2-way	49.01	6.64	2.72	28.82	2.99	1.37	8.44	9.36	80.82	9.81
IRMA61310											
QC	Abridged*	51.57	6.59	2.62	23.20	2.12	2.22	11.68	13.90	51.57	6.59
QC	Enhanced	52.45	6.47	1.97	21.62	1.99	3.74	11.76	8.44	76.06	15.50
R&D	2-way	48.67	8.46	2.53	26.40	2.38	1.97	9.58	10.99	77.45	11.55

* Value corrected after submission of 3.2.R and now provided to 2 decimal places

Table 2: Assay Variation of Man5 and G0 for Oligosaccharide Profiles in the 2-way and 3-way Similarity Assessment Studies

	1RMA65804		1RMA61310	
	Man5	G0	Man5	G0
Mean	5.4	2.5	7.2	2.4
STD	1.1	0.4	1.1	0.3
%RSD	21	15	16	15
Acceptance criteria (intermediate precision)	< 22%	< 22%	< 22%	< 22%

Celltrion also provided an analysis of the standard deviation of the 2-way and 3-way data. The analysis shows that both EU-approved infliximab and US-licensed Remicade have higher variability compared to CT-P13 in both studies. See table 4 below, excerpted from the IR response.

Table 4: Standard Deviation for Glycans of US-Licensed Remicade®, CT-P13 and EU-Approved Remicade® in 3-way and 2-way Studies

Product	Study	G0F	Man5	G0	G1F	G2F	SA1	SA2	G0+ Man5
US	3-way	7.25	0.81	0.25	4.72	2.21	1.05	1.55	0.88
CT-P13	3-way	1.40	0.35	0.11	0.74	0.39	0.90	1.30	0.32
	2-way	1.97	0.29	0.12	0.93	0.68	0.39	0.67	0.34
EU	3-way	7.80	1.15	0.51	5.55	2.42	0.78	2.40	3.25
	2-way	1.33	0.72	0.29	1.64	0.5	0.53	0.78	0.72

Reviewer's Comment: *The Sponsor has provided evidence that assay variability probably does not account for the differences between the patterns of Man5 and G0 observed for the two and three-way analytical data. They also show that the Man5 and G0 glycan levels in the Originator lots (i.e., US-licensed Remicade and EU-approved infliximab) do appear to have higher variability compared CT-P13. Moreover, there is greater variability overall in the data from three-way analysis compared to the two-way analysis data, which analyzed fewer batches in general. The difference between afucosylation patterns observed in the two-way data and the three-way analysis for Man5+ G0 levels is not satisfactorily explained. As noted above (see review of DS lot testing), they will measure FcγRIIIa binding at drug substance lot release with a precise SPR assay. This is the functional attribute that can be impacted by levels of afucosylation.*

Response to Question 1b: HPAEC-PAD is considered in the bioanalytical world to be a robust and precise method for glycan analysis. Data were provided comparing it with an alternative method, NP-UPLC with 2AB-labeling. The NP-UPLC method starts with enzymatic treatment with PNGaseF to release N-linked glycans; these are then labelled with a fluorescent dye, 2-AB. The labelled glycans are separated by normal phase chromatography run on an Aquity UPLC system equipped with a fluorescence detector. The column is a BEH glycan column. The assignment of the peaks for the various glycans was confirmed by LC/MS. The afucose levels for the HPAEC-PAD method only included G0 and Man5 as other afucosylated glycans (e.g., G2, G1) are below the limit of quantification. Calculation of the afucose levels using the more

sensitive NP-UPLC method included G1 & G2 glycans as well. The two methodological approaches were compared by analyzing ten lots of US-licensed Remicade and ten lots of CT-P13. Three additional lots that were used in the 3-way PK study were also analyzed using both analytical methods. While the data from the NP-UPLC method were less variable, it is clear that the contributions from G1 and G2 did not contribute greatly to the overall % afucosylation assessment.

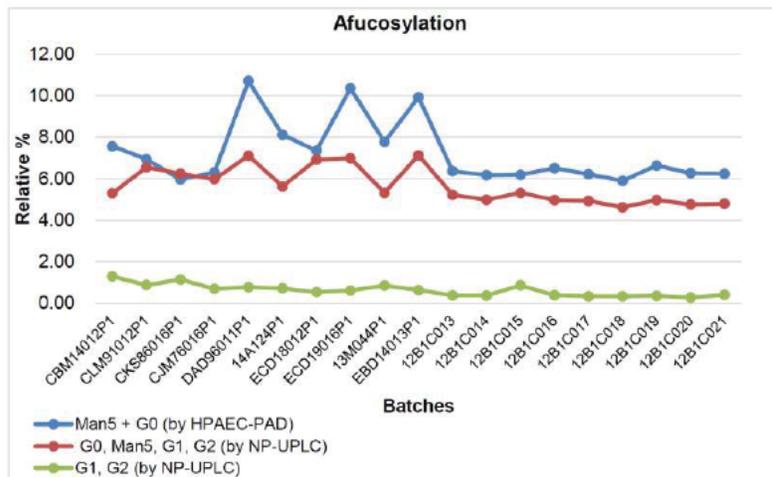


Figure 6: Comparison of Afucose Levels between HPAEC-PAD and NP-UPLC Chromatography for Additional 9 Batches of CT-P13 and 10 Batches of US-licensed Remicade®

Data were provided to correlate afucosylation, binding to FcγRIIIa receptor and ADCC using NK effector cells. Scatter plots of this correlation is shown below in figure 8 (excerpted from the IR response). Additional correlation was provided between FcγRIIIa binding, NK cell-mediated ADCC activity and Man5+G0% is shown Figure 9 (excerpted from the IR). The scatter plots show that a rough correlation could be made between G0/Man5 ratios and the functional activities of NK mediated ADCC and FcγRIIIa binding.

Reviewer's Comment: This observation does not resolve uncertainty regarding the underlying analytical difference in glycans controls ADCC and the observed differences between CT-P13 and the reference product in this critical functional aspect. In theory, other differences such as H2L1 could also play a role.

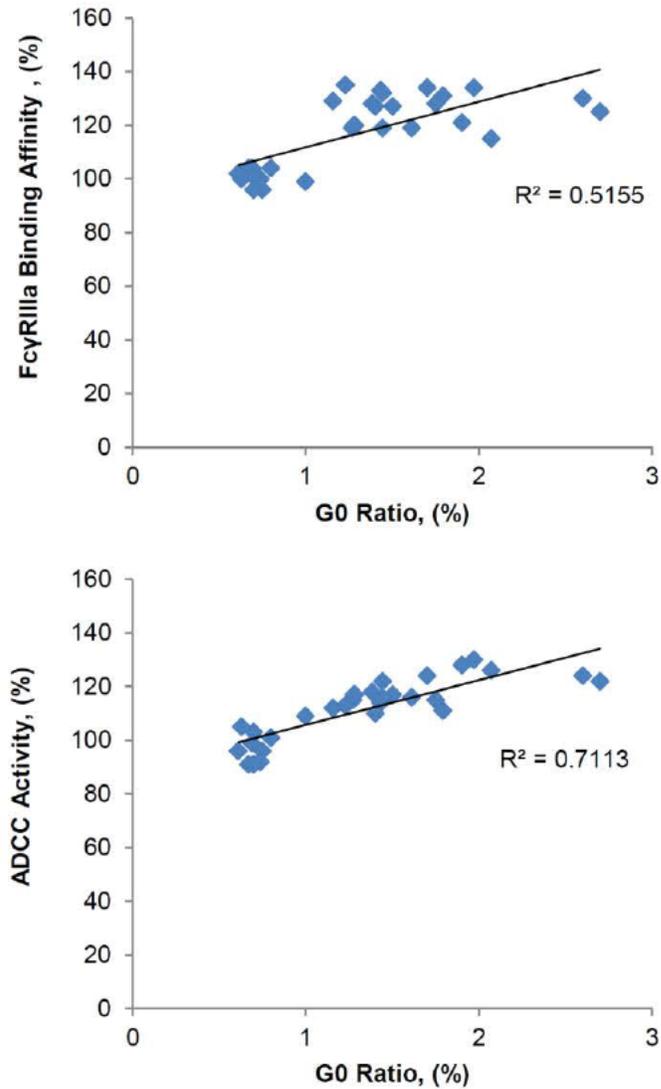


Figure 8. Correlation of G0 Ratio with FcγRIIIa Binding Affinity and ADCC Activity with NK Cells

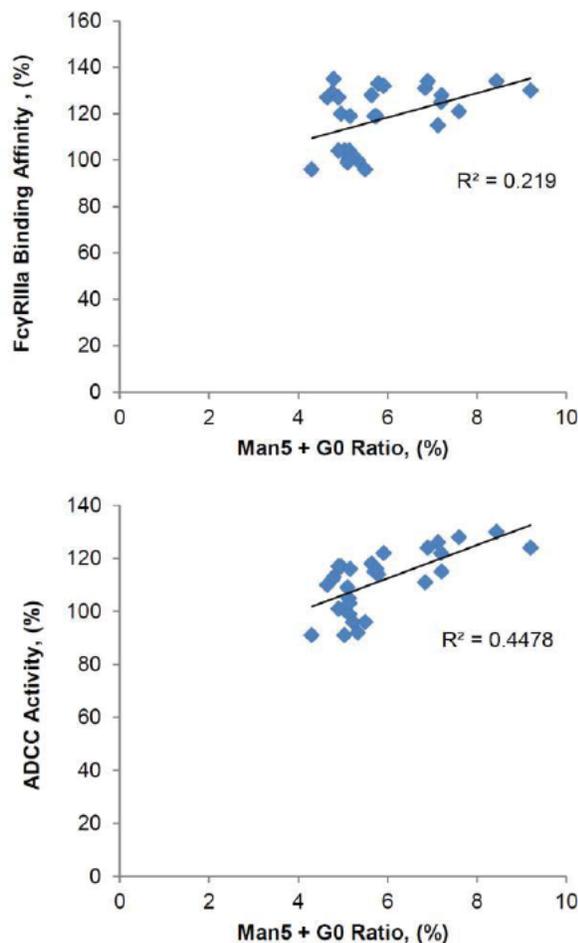


Figure 9: Correlation of Man5 + G0 Ratio with FcγRIIIa Binding Affinity and ADCC Activity with NK Cells

Reviewer's Comment: The Sponsor's analysis revealed that there is a rough correlation between FcγRIIIa binding and G0 ratio for CT-P13. The scatter plots show that the correlation washes out somewhat when Man5 is included, probably because FcγRIIIa binding is insensitive to the Man5 glycan variant. However, it should be noted that both functional activities tested are constrained to a tight range.

Sialic Acid analysis showed predominantly NGNA (N-glycaneuraminic acid) in the three products; all batches were within a similar range of 0.10-0.30mol/mol. In the monosaccharide analysis, the sugars residues identified in this analysis included fucose, N-acetylglucosamine, galactose and mannose. There were no significant differences between the overall levels measured for CT-P14, EU-approved Remicade and US-licensed Remicade.

Reviewers Comment: The monosaccharide analysis for fucose is consistent for all lots; which is general agreement with the pattern in the three-way analysis using HPAEC-PAD.

Reviewer's Comment: Overall, this analysis argues that inclusion of an Fc γ R1IIa binding SPR assay at lot release is a prudent measure to control Fc mediated aspects of product function.

Biological Activity.

Antibodies function by binding antigens; however they also have other functions such as ADCC or complement fixation mediated by the Fc part of the molecule. Celltrion's strategy to assess biological activity was to cover as many such functional aspects associated with antibody function as feasible, including Fc effector functions. They also included specific assays for the biological activity of a TNF antagonist. Ten lots of each product were included in the studies to facilitate statistical analysis. The results of the analysis were generally calculated for individual lots as a percentage relative to reference standard CT-P13.

Reviewer's Comment: Aside from the main WEHI bioassay, many of the cell based assays are R&D assays intended to explore and compare mechanisms of action. The level of precision and robustness expected for a lot release assay is not applicable for this type of assay.

TNF α -binding dependent assays.

In Vitro TNF α Neutralization Activity: TNF α is the antigen for CT-P13 and Remicade; binding TNF α is the main function of these antibodies. The neutralizing activity of the three forms of infliximab was assessed in a WEHI bioassay (also used in lot release, described in review of section 3.2.S.3.1). Results from the 3-way batch analysis shows that the 90% confidence intervals of the mean difference are within the equivalence margins; therefore statistically equivalent (see CMC stats review).

Results from TNF Bioassay

Product type	US- Remicade	CT-P13	EU-Remicade
% binding/activity	103.5 +/- 3.6%	102.3 +/- 4.2%	102.7 +/- 4.0%

In addition, a TNF- α Binding Assay (ELISA) and a cell based mTNF- α FACS binding assay were included as more direct assays to measure interaction with TNF. Ten lots of each antibody type were tested.

Results from TNF-binding ELISA

Product type	US- Remicade	CT-P13	EU-Remicade
% binding/activity	100.1 +/- 3.5%	98.9 +/- 2.2%	97.4 +/- 3.5%

Reviewer's Comment: *In the statistical analysis of TNF binding ELISA, the comparison between EU-approved infliximab and US-licensed Remicade did not meet statistical equivalence testing expectations, probably due to the limited number of lots studied. CT-P13 passed equivalence testing when compared to either US-licensed Remicade or EU-approved infliximab. This issue was the subject of an IR letter sent 13th February 2014. The issue was handled by the CMC Statistics reviewer.*

“The binding of TNF- α is a critical component of the mechanism of action of CT-P13, and in a risk ranking assessment, TNF- α binding is high in criticality ranking. Therefore, a wider range of lots should be analyzed by a statistical equivalency test for TNF- α binding. The results of the statistical equivalency test conducted by the FDA showed that the EU approved- infliximab did not meet the equivalency margins established based on analysis of US-licensed Remicade for this parameter. We believe that this result might be due to the limited number of lots you provided in your submission. Provide additional data on TNF- α binding assay for available lots of CT-P13, EU-approved infliximab and US-licensed Remicade.”

Response and Resolution: The response and resolution was reviewed by the CMC stats reviewer (see CMC stats review). In essence, Celltrion identified and included an additional five US-licensed Remicade and three EU-approved infliximab lots for analysis in the TNF binding ELISA. When reanalyzed using the 15 US-licensed Remicade and 13 EU-approved infliximab lots, the statistical analysis met equivalence testing expectations.

Cytokine and signal transduction assays:

Induction of Apoptosis by Reverse Signaling: The binding of the membrane-bound TNF- α by the antibody leads to the induction of apoptosis in Jurkat cells. The relative activities of CT-P13, US-licensed Remicade and EU-approved infliximab were compared at increasing concentrations (0.2 μ g/mL to 1 μ g/mL). There is a dose response observed; results are given as relative potency. The data were largely equivalent between CT-P13, EU-approved infliximab and US-licensed Remicade.

Suppression of cytokine release by reverse signaling: PBMC's were stimulated with LPS; the ability of increasing concentrations (1.1 μ g/mL to 5.3 μ g/mL) of CT-P13, US-licensed Remicade

and EU-approved infliximab to attenuate the release of cytokines (TNF- α) was assessed. No major differences were observed between the three products.

Inhibition of the secretion of cytokines (IL-8) from Caco-2 Cells: Caco-2 cells were stimulated and the release of inflammatory cytokines (IL-8) was evaluated to assess the response of the cells to CT-P13, US-licensed Remicade and EU-approved infliximab. Caco-2 is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells. This assay is a model for activity of this key cytokine for IBD. The analysis of the data by the Sponsor showed that there were not major differences between the three antibodies.

Reviewer's Comment: Results from these three assays support the contention that CT-P13, US-licensed Remicade and EU-approved infliximab v possess equivalent activity in these in vitro models of IBD and/or inflammatory disease pathology. As noted above these assays are all R&D assays suitable for product characterization, mechanism of action establishment or to support analytical similarity. Full validation as expected for a lot release assay is not warranted.

Regulatory macrophage based assays. The Sponsor has utilized three bioassays to assess the effect of CT-P13, US-licensed Remicade and EU-approved infliximab on the function of regulatory macrophages. This includes the induction of regulatory macrophages and the suppression of T cell proliferation.

FACS regulatory macrophage assay. The first assay is quantitation of infliximab-induced regulatory macrophages by FACS: The ability of CT-P13, US-licensed Remicade and EU-approved infliximab to induce regulatory macrophages in a mixed lymphocyte reaction (MLR) was assessed by the presence CD14/CD206 cell population. This assay was undertaken using a subset of the lots that included 5 US-licensed Remicade, 3 EU-approved infliximab and 5 CT-P13 lots. A linear relationship between increasing concentrations of the antibody (0.156 μ g/mL – 2.5 μ g/mL) and the induction of regulatory macrophages (1.9-3.6%) was shown. The three products were largely equivalent in this assay.

MLR assay. The second assay evaluates the effect of infliximab on the suppression of T Cell proliferation by regulatory macrophages in mixed lymphocyte reactions (MLR). PBMCs taken from healthy volunteers that expressed the Fc γ RIIIA V/V genotype receptor were used to demonstrate inhibition of proliferation. A linear relationship between inhibition and the concentration of the individual antibody (0.031-0.125 μ g/mL) was demonstrated, and was equivalent between CT-P13, US-licensed Remicade and EU-approved infliximab.

Wound healing. The final assay for regulatory macrophages evaluates wound healing *in vitro*. Wound healing is a potentially important mechanism of action for infliximab in IBD patients, where closure of fistulas and other inflamed areas is desirable. The *in vitro* bioassay assesses the ability of the antibody to induce closure of a simulated wound in a cell monolayer through the

activity of regulatory macrophages. CD14+ Regulatory macrophages were isolated from two healthy donors for use in this assay. The ability of the antibodies to induce closure of a physically created gash in a monolayer of HCT116 cells was used provide a measure of activity. Three lots of US-licensed Remicade, EU-approved Remicade and CT-P13 were pooled. The statistical analysis of the data indicates that there are no significant differences between CT-P13, US-licensed Remicade and EU-approved infliximab.

Reviewer's Comment: Results from these three assays support the contention that CT-P13, US-licensed Remicade and EU-approved infliximab possess equivalent activity in these in vitro models of IBD and/or inflammatory disease pathology. All three regulatory macrophage methods are R&D assays suitable for product characterization, mechanism of action establishment or to support analytical similarity. Full validation as expected for a lot release assay is not warranted.

Complement based assays: C1q Binding affinity and CDC activity. The affinity of CT-P13, US-licensed Remicade and EU-approved infliximab for C1q was assessed using an ELISA binding assay. C1q is the first component of complement that is activated by antibody binding and cross-linking on a cell surface. No significant difference between the three antibodies was observed (10 lots tested).

The complement dependent cytotoxicity of CT-P13, US-licensed Remicade and EU-approved infliximab was assessed. The complement system comprises an array of serum proteins that lyse foreign cells that are coated with antibodies. Complement activation is one of the main effector functions of IgG antibodies. No significant difference between CT-P13, US-licensed Remicade and EU-approved infliximab was observed using these assays (10 lots each tested).

Results from C1q and CDC assays

Product type	US- Remicade	CT-P13	EU-Remicade
% C1q binding/activity	96 +/- 6.4%	97 +/- 6.8%	101 +/- 7.4%
CDC activity %	94 +/- 4.6%	100 +/- 9.0%	92 +/- 8.8%

Fc Receptor Binding Affinity Studies

The three way similarity study includes an assessment of Fc receptor function. The Fc part of antibodies can interact with a class of proteins called Fc receptors (FcR), which when specific for IgGs are termed FcγRs. Via these receptors, antibodies mediate effector functions, like antibody

dependent cytotoxicity (ADCC), which helps the immune system to clear target cells (e.g., infected cells).

Surface plasmon resonance (SPR) based assays were used in most cases by Celltrion to evaluate FcR binding to CT-P13, US-licensed Remicade and EU-approved infliximab, with a few functions being measured by ELISA. The results are given as a relative binding affinity (K_D) relative to reference CT-P13. Celltrion evaluated the four main FcR types: FcRn, FcγRI, FcγRII, and FcγRIII. These can consist of different sub-types, e.g. FcγRIIIa and FcγRIIIb. Also, some of these receptors can have different alleles in the human population, i.e. FcγRIIIa (V allele) and FcγRIIIa (F allele). Celltrion evaluated binding of CT-P13, US-licensed Remicade and EU-approved infliximab to a large number of these sub-types..

FcγRIIIa (V type) Binding Affinity (SPR): The results from the SPR analysis of the interaction of the FcγRIIIa (V type) receptor with CT-P13, US-licensed Remicade and EU-approved infliximab showed statistically significant differences. For the enhanced assessment batches both the US-licensed Remicade and EU-approved infliximab had relative binding affinities of 126% and 127% respectively. In contrast, the CT-P13 drug product the mean relative binding affinity was reported to be 101%.

Table 3.2.R-40: Results of FcγRIIIa (V type) Binding Affinity of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Purpose	Product	Batch No.	FcγRIIIa V Type Binding Affinity (SPR)
			Relative Binding (%)
Abridged Assessment Batches	US-licensed Remicade [®]	CBS13015P1	121
		CBM14012P1	127
		CHD56013P1	134
	CT-P13 Drug product	12B1C002	96
		12B1C003	99
		12B1C004	104
	EU-approved Remicade [®]	1RMKA87103 ¹	119
		1RMA65804	125
		1RMA61310	130
Enhanced Assessment Batches	US-licensed Remicade [®]	CHF59013P1	131
		CHM62015P1	119
		CLM91012P1	128
		CKS86016P1	120
		CBM12011P1	129

		CJM76016P1	133
		DAD96011P1	128
		14A124P1	121
		ECD18012P1	130
		ECD19016P1	126
		Mean	127
		SD	4.9
	CT-P13 Drug Product	12B1C015	104
		12B1C016	96
		12B1C017	104
		12B1C018	100
		12B1C019	100
		12B1C020	102
		12B1C021	101
		12B1C012	101
		12B1C013	101
		12B1C014	103
			Mean
		SD	2.3
	EU-approved Remicade®	2RMA60103	119
		1RMA64901	127
		2RMA61905	135
		3RMKA80702	132
		1RMA65804	115
		1RMKA87103	119
		1RMA61310	134
		3RMA69701	124
		4RMKA80103	122
		4RMA60601	137
			Mean
		SD	7.7
Statistical Evaluation	90% CI of Mean Difference (EM : -20 ~ 20)	US vs. CT-P13	(22.33, 28.27)
		EU vs. CT-P13	(20.76, 29.64)
		US vs. EU	(-4.92, 5.12)

Reviewer’s Comment: Differences between the CT-P13 and the US-licensed Remicade and EU-approved infliximab lots exist (101% vs. 127 & 126%). Glycans, especially levels of afucosylated glycoforms, have a key role in this interaction between Fc and FcγRIIIa, although a consistent pattern of higher or lower % afucosylation is not clear when comparing CT-P13 and the reference product, US-licensed Remicade. This attribute will be monitored at the Drug substance lot release level for CT-P13.

Celltrion claims (1) this attribute will be controlled at lot release and (2) there should be no clinically significant impact in vivo as the antibody is administered in saturating amounts and (3) it isn’t clear if FcγRIIIa mediated ADCC is important for infliximab function (see ADCC discussion below).

FcγRIIIa (F type) Binding Affinity (SPR): The interaction between FcγRIIIa (F allele) receptor was also measured; binding assays conducted with CT-P13, US-licensed Remicade and

EU-approved infliximab yield results that are similar to the results obtained with the (V allele) receptor assay. The enhanced assessment lots of both US-licensed Remicade and EU-approved infliximab had relative binding affinities of 124% and 126% respectively. In contrast, for the CT-P13 drug product the mean relative binding affinity was reported to be 103%.

Table 3.2.R-41: Results of FcγRIIIa (F type) Binding Affinity of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Purpose	Product	Batch No.	FcγRIIIa F Type Binding Affinity (SPR)
			Relative Binding (%)
Enhanced Assessment Batches	US-licensed Remicade [®]	CHF59013P1	131
		CHM62015P1	112
		CLM91012P1	126
		CKS86016P1	124
		CBM12011P1	118
		CJM76016P1	123
		DAD96011P1	133
		14A124P1	122
		ECD18012P1	122
		ECD19016P1	124
		Mean	124
	SD	6.0	
	CT-P13 Drug Product	12B1C015	103
		12B1C016	104
		12B1C017	103
		12B1C018	100
		12B1C019	108
		12B1C020	102

		12B1C021	105	
		12B1C012	104	
		12B1C013	98	
		12B1C014	106	
		Mean	103	
	SD	2.8		
	EU-approved Remicade [®]	2RMA60103	123	
		1RMA64901	117	
		2RMA61905	128	
		3RMKA80702	124	
		1RMA65804	132	
		1RMKA87103	117	
		1RMA61310	135	
		3RMA69701	124	
		4RMKA80103	133	
		4RMA60601	129	
		Mean	126	
		SD	6.3	
		Statistical Evaluation	90% CI of Mean Difference (EM : -20 ~ 20)	US vs. CT-P13
	EU vs. CT-P13			(19.03, 26.57)
US vs. EU	(-7.46, 2.06)			

FcγRIIIb Binding Affinity (SPR): The interactions between FcγRIIIb receptor and CT-P13, US-licensed Remicade and EU-approved infliximab are similar to the results obtained for the FcγRIIIa receptor. The enhanced assessment batches of both US-licensed Remicade and EU-approved infliximab had relative binding affinities of 120% and 122% respectively. In contrast, the CT-P13 drug product the mean relative binding affinity was determined to be 101%.

Table 3.2.R-42: Results of FcγRIIIb Binding Affinity of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Purpose	Product	Batch No.	FcγRIIIb Binding Affinity (SPR)
			Relative Binding, (%)
Enhanced Assessment Batches	US-licensed Remicade [®]	CHF59013P1	129
		CHM62015P1	111
		CLM91012P1	127
		CKS86016P1	124
		CBMI2011P1	119
		CJM76016P1	122
		DAD96011P1	135
		14A124P1	109
		ECD18012P1	112

		ECD19016P1	115
		Mean	120
	SD	8.6	
	CT-P13 Drug Product	12B1C015	106
		12B1C016	100
		12B1C017	103
		12B1C018	96
		12B1C019	109
		12B1C020	94
		12B1C021	105
		12B1C012	99
		12B1C013	98
		12B1C014	99
		Mean	101
		SD	4.7
	EU-approved Remicade [®]	2RMA60103	120
		1RMA64901	121
		2RMA61905	130
		3RMKA80702	122
		1RMA65804	132
		1RMKA87103	113
		1RMA61310	137
		3RMA69701	111
4RMKA80103		119	
4RMA60601		119	
Mean	122		
SD	8.2		
Statistical Evaluation	90% CI of Mean Difference (EM : -20 ~ 20)	US vs. CT-P13	(14.02, 24.78)
		EU vs. CT-P13	(16.29, 26.71)
		US vs. EU	(-8.64, 4.44)

Other FcR binding assays. The pattern seen with FcγRIII (i.e., Remicade had 20% higher binding strength than CT-P13) was not seen with the other FcR types tested. See summary table below:

Results from other FcR binding assays

Product type	US- Remicade	CT-P13	EU-Remicade
FcγRIIIa	97 +/- 3.2%	98 +/- 3.7%	96 +/- 3.0%
FcγRIIb	96 +/- 6.6%	97 +/- 2.2%	95 +/- 4.8%
FcγRI	99 +/- 6.1%	100 +/- 3.5%	99 +/- 3.6%
FcRn	97 +/- 2.2%	101 +/- 3.5%	98 +/- 3.9%

FcγRIIIa Binding Affinity (SPR): Ten lots of each antibody type were tested. The results from the SPR analysis of the interaction of the FcγRIIIa receptor with CT-P13, US-licensed Remicade and EU-approved infliximab showed that there are no obvious differences between the three products. For the enhanced assessment batches both the US-licensed Remicade and EU-approved infliximab had relative binding affinities of 97% and 96% respectively. In contrast, the CT-P13 drug product the mean relative binding affinity was given as 98%.

FcγRIIb Binding Affinity (SPR): Ten lots of each antibody type were tested. The results from the SPR analysis of the interaction of the FcγRIIb receptor with the CT-P13, US-licensed Remicade and EU-approved infliximab showed that there are no obvious differences between the three products. For the enhanced assessment batches both the US-licensed Remicade and EU-approved infliximab had relative binding affinities of 96% and 95% respectively. In contrast, the CT-P13 drug product the mean relative binding affinity was given as 97%.

FcγRI Binding Affinity (ELISA): The interaction between FcγRI receptor and CT-P13, US-licensed Remicade and EU-approved infliximab was assessed using an ELISA based assay. Ten lots of each antibody type were tested. The results are given as a percentage compared to the reference standard; no obvious differences were observed between the three products.

FcRn Binding Affinity (SPR): The interaction between FcRn receptor and the CT-P13, US-licensed Remicade and EU-approved infliximab was assessed using SPR. Ten lots of each antibody type were tested. The results are given as a percentage compared to the reference standard; no obvious differences were observed between the three products.

Cell based *Ex Vivo* Binding Assay (NK Cells from Healthy PBMC) The above described SPR analysis observed differences in the affinity of FcγRIIIa and b for CT-P13 vs. EU-approved infliximab and US-licensed Remicade. To clarify if there are biological implications for the observed differences *in vivo*, additional cell based binding experiments have been carried out. To accomplish this, NK cells were isolated from healthy human volunteer PBMC's for a FACS analysis. The analysis was undertaken using three lots of each product. The FACS-based binding assay was performed in either the presence of 1% BSA or 50% normal human serum. When performed in the presence of BSA (essentially a neutral carrier protein), the assay found some difference between CT-P13 and US-licensed Remicade and EU-approved infliximab; 100% vs. 122% and 119% respectively. However, in the presence of 50% human serum (presumably containing high levels of human IgG antibodies), the assay yielded revealed no difference in the binding between CT-P13, US-licensed Remicade and EU-approved infliximab.

Reviewer's Comment: Celltrion argues that 50% serum is more representative of an actual tissue microenvironment in a human patient versus 1% BSA, and thus the analysis where there are no differences between CT-P13, US-licensed Remicade and EU-approved infliximab in binding strength is more meaningful. This may be true in the case of circulatory infliximab. A joint synovia or intestinal lumen microenvironment is not likely to be the same as either of the two assay conditions used by Celltrion, thus it is unclear if the data directly translate to either situation.

ADCC Function assays

ADCC is a cell killing activity mediated through FcγR receptors that is a potential mechanism of action for infliximab for some disease states. ADCC can be mediated by many types of effector cells, including NK cells, monocytes and other white blood cells. Different effector types may be more reliant on one receptor type or the other; NK cells mostly use FcγRIII to bridge to the target cells. These different cell types may be more predominant in one tissue (e.g., the gut) vs. others (e.g., joint synovia). Celltrion states that they thus used more than one effector and target cell type to design their *in vitro* ADCC assays as models for CT-P13 and Remicade function. ADCC assays utilize two cell types: (1) “effector cells” which possess FcRs on their surface that are activated to kill other cells and (2) “target cells”, which possess cell surface molecules (in the case mTNFα) which are bound by antibody that recruit the “effector” cells through their cell surface FcRs.

ADCC using PBMC from Healthy Donors: This ADCC assay utilized engineered Jurkat cells expressing mTNF-α as the target cells and PMBC obtained from healthy volunteers as effector cells. PBMC presumably contain many types of potential effector cells, including NK cells. The cells were mixed at a ratio of 1:16, and antibody was titrated in to determine an EC50. The assay

was performed with 10 batches each of CT-P13, US-licensed Remicade and EU-approved infliximab; no difference was observed. The Sponsor indicated that the presence of a mixed population of effector cells with multiple Fc receptors in the PBMC population may average out the overall effector cells response. They asserted that this more biologically relevant than assays using single effector cell types.

Table 3.2.R-49: Results of ADCC using PBMC of US-licensed Remicade®, EU-approved Remicade® and CT-P13 Drug Product

Purpose	Product	Batch No.	ADCC (PBMC)
			Relative Potency (%)
Abridged /Enhanced Assessment Batches	US-licensed Remicade®	CBS13015P1	98
		CBM14012P1	101
		CHD56013P1	93
		CHF59013P1	105
		CHM62015P1	111
		CKS86016P1	123
		CBM12011P1	111
		14A124P1	88
		ECD18012P1	111
		ECD19016P1	121
		Mean	106
		SD	11.4
	CT-P13 Drug product	12B1C002	97
		12B1C003	105
		12B1C004	111
		12B1C015	116
		12B1C017	104
		12B1C018	108
		12B1C021	92
		12B1C012	92
		12B1C013	99
		12B1C014	106
		Mean	103
		SD	7.9
	EU-approved Remicade®	0RMA62801	92
		1RMA65804	106
		1RMA61310	104
		2RMA60103	108
		1RMA64901	126
		3RMKA80702	102
		1RMKA87103	107
		3RMA69701	103
		4RMKA80103	107
4RMA60601		108	
Mean		106	
SD		8.4	
Statistical Evaluation	90% CI of Mean Difference (EM : -20 ~ 20)	US vs. CT-P13	(-4.42, 10.82)
		EU vs. CT-P13	(3.03, 9.63)
		US vs. EU	(-7.87, 7.67)

Table with results from the ADCC assay with PBMC effector cells

Reviewer’s Comment: *The Sponsor argued that the presence of a mixed population of cells with multiple Fc receptors is a more biologically relevant model of in vivo activity vs. an assay using an enriched or purified effector cell population. This assertion makes sense if the site of inflammation in all of the Remicade indications is in the blood circulation, but it is not clear if a potential effector cell population in the gut for example would have the same populations.*

ADCC using NK Cells from Healthy Donors: The ADCC assay has been performed with NK cells isolated from healthy donors as effector cells and the above described engineered Jurkat cells expressing TNF- α as target cell, at ratio of 2:1. 10 lots of each CT-P13, US-licensed Remicade and EU-approved infliximab were used in the analysis in a dilution series. The assay showed a linear response over the selected concentration range for the antibodies (2-8 ng/mL). The activities were about 25% different; CT-P13 (97%) vs. EU-approved infliximab (120%) and US licensed Remicade (115%).

Table 3.2.R-50: Results of ADCC using NK cell of US-licensed Remicade[®], EU-approved Remicade[®] and CT-P13 Drug Product

Purpose	Product	Batch No.	ADCC (NK cell) Relative Potency, (%)				
			2 ng/mL	4 ng/mL	8 ng/mL	Batch Mean	SD
Abridged Assessment Batches ¹	US-licensed Remicade [®]	CBS13015P1	119	138	128	128	9.5
		CBM14012P1	117	118	117	117	0.6
		CHD56013P1	125	123	125	124	1.2
	CT-P13 Drug product	12B1C002	92	91	91	91	0.6
		12B1C003	111	106	111	109	2.9
		12B1C004	98	105	99	101	3.8
	EU-approved Remicade [®]	IRMKA87103 ¹	117	120	116	118	2.1
		IRMA65804	120	128	117	122	5.7
		IRMA61310	134	117	122	124	8.7
	Enhanced Assessment Batches	US-licensed Remicade [®]	CHF59013P1	123	102	108	111
CHM62015P1			125	105	116	115	10.0
CLM91012P1			116	117	122	118	3.2
CKS86016P1			115	115	120	117	2.9
CBM12011P1			113	108	115	112	3.6
CJM76016P1			111	120	110	114	5.5
DAD96011P1			100	130	115	115	15.0
14A124P1			109	114	126	116	8.7
ECD18012P1			102	109	121	111	9.6
ECD19016P1			109	125	136	123	13.6
Product Mean			115				
SD			8.5				

	CT-P13 Drug Product	12B1C015	106	81	87	91	4.2
		12B1C016	100	97	82	96	3.5
		12B1C017	112	88	97	99	6.4
		12B1C018	98	89	88	92	0.7
		12B1C019	109	105	100	105	3.5
		12B1C020	92	93	102	96	6.4
		12B1C021	110	97	102	103	3.5
		12B1C012	92	102	97	97	5.0
		12B1C013	80	100	90	90	10.0
		12B1C014	95	96	111	101	9.0
		Product Mean	97				
	SD	8.3					
	EU-approved Remicade®	2RMA60103	124	104	120	116	10.6
		1RMA64901	111	114	104	110	5.1
		2RMA61905	119	107	113	113	6.0
		3RMKA80702	121	127	119	122	4.2
		1RMA65804	127	122	128	126	3.2
		1RMKA87103	107	112	128	116	11.0
		1RMA61310	120	135	136	130	9.0
		3RMA69701	102	119	136	119	17.0
		4RMKA80103	104	111	123	113	9.6
		4RMA60601	124	139	130	131	7.5
		Product Mean	120				
SD	10.5						
Statistical Evaluation	90% CI of Mean Difference (EM : -20 ~ 20)	US vs. CT-P13	(14.40, 22.20)				
		EU vs. CT-P13	(18.70, 26.50)				
		US vs. EU	(-8.20, -0.40)				

¹ 1RMKA87103 was close to expiry and was used for FcγRIIIa V type binding affinity, ADCC (NK cell) and *ex vivo* binding affinity instead of 0RMA62801 for abridged assessment batches.

Reviewer’s Comment: *The mean relative potency appears to be calculated by deriving the mean (vs. reference standard) from the total sum of readings across the full range of concentrations in the linear range of the dilution curve rather than from the values for each concentration. This calculation should not impact on the overall conclusion of this exercise.*

ADCC Using LPS-Stimulated Monocyte as Target Cells: The ability of LPS-stimulated monocytes isolated from healthy volunteers to serve as a target in the ADCC assay using PMBC and the same experimental set up was assessed. Seven lots of each of CT-P13, US-licensed Remicade and EU-approved infliximab were assayed; none had activity in this assay. In a separate study, more potent NK cells were used as effector cells; these also did not lyse the target monocyte targets (data not shown).

Purpose	Product	Batch No.	ADCC using LPS-stimulated Monocytes and NK Cell
Enhanced Assessment Batches	US-licensed Remicade®	CHF59013P1	No activity
		CHM62015P1	No activity
		CLM91012P1	No activity
		CKS86016P1	No activity
		CBM12011P1	No activity
		CJM76016P1	No activity
		DAD96011P1	No activity
	CT-P13 Drug Product	12B1C015	No activity
		12B1C016	No activity
		12B1C017	No activity
		12B1C018	No activity
		12B1C019	No activity
		12B1C020	No activity
		12B1C021	No activity
	EU-approved Remicade®	2RMA60103	No activity
		1RMA64901	No activity
		2RMA61905	No activity
		3RMKA80702	No activity
		1RMA65804	No activity
		1RMKA87103	No activity
		1RMA61310	No activity

Reviewer’s Comment: *The sponsor argues that this is the most representative of the three ADCC assay formats. This is based on the contention that the target cells in this assay are not an artificial transfectoma (i.e., mTNF₊ Jurkats), but rather primary leukocytes studied ex vivo. They further argue that the above results indicate that ADCC activity of infliximab in vivo is likely to be limited. To support this contention they evaluated tmTNF α Expression Level in IBD Patients (see below)*

Ex vivo tmTNF α Expression Level in IBD Patients: The Sponsor determined the expression levels for mTNF α on monocytes and macrophages present in biopsies isolated from five patients with mild to moderate ulcerative colitis or Crohn’s disease. This experiment was performed by FACS analysis of isolated Lamina Propria Mononuclear Cells (LPMC) from IBD patients with anti-CD13 and anti-HLA antibodies. LPS stimulated PBMC from healthy donors and mTNF⁺ Jurkats were used as positive staining controls, while unstimulated PBMC were defined as background. The Sponsor performed this analysis with the hypothesis that mTNF α must be expressed at high levels if infliximab actually promotes ADCC as a mechanism of action in IBD. The Sponsor asserts that monocytes and macrophages in the lamina propria of IBD patients express low levels of tmTNF α . The expression of mTNF α on the IBD monocytes/macrophages was approximately 5 fold lower than on a similar population of monocytes/macrophages present in the LPS stimulated PBMCs.

Data from FACS analysis of tmTNF α Expression Level in IBD Patients

Table 3.2.R-52: Summary of MFI for Expression Level of tmTNF α on LPS-Stimulated Monocytes (comparison of LPMC and LPS)

FITC Labeled Antibody	MFI Value						
	Monocyte within LPMC_#1	Monocyte within LPMC_#2	Monocyte within LPMC_#3	Monocyte within LPS-induced PBMC #1	Monocyte within LPS-induced PBMC #2	Monocyte within LPS-induced PBMC #3	Transfected tmTNF α Jurkat cell
CT-P13 Fab-FITC binding in Unstimulated Monocytes				101	184	238	
Human IgG-FITC	101	100	107	67	64	98	89
CT-P13 Fab-FITC	192	219	357	731	588	890	10,873
Fold induction	1.9	2.2	3.3	10.9	9.2	9.1	122.2
MFI Value for Specific Binding to tmTNFα and Relative Expression Level							

tmTNF α Specific Binding (CT-P13 Fab-Human IgG)	91	119	250	664	524	792	10784
Mean (n=3)	153			660			
Relative Expression Compared to tmTNF α Jurkat Cell (%)	0.8%	1.1%	2.3%	6.2%	4.9%	7.3%	100.0%
Mean (n=3)	1.4%			6.1%			
SD	0.79			1.24			

Reviewer’s Comment: *These results are supportive of the contention that IBD gut monocytes do not have high levels of mTNF, but a solid conclusion cannot be made given (1) the small number of patients that were included and (2) the unknown sensitivity of the assay, which may be lower than the amount of mTNF needed to activate ADCC.*

2-Way Similarity Study Summary

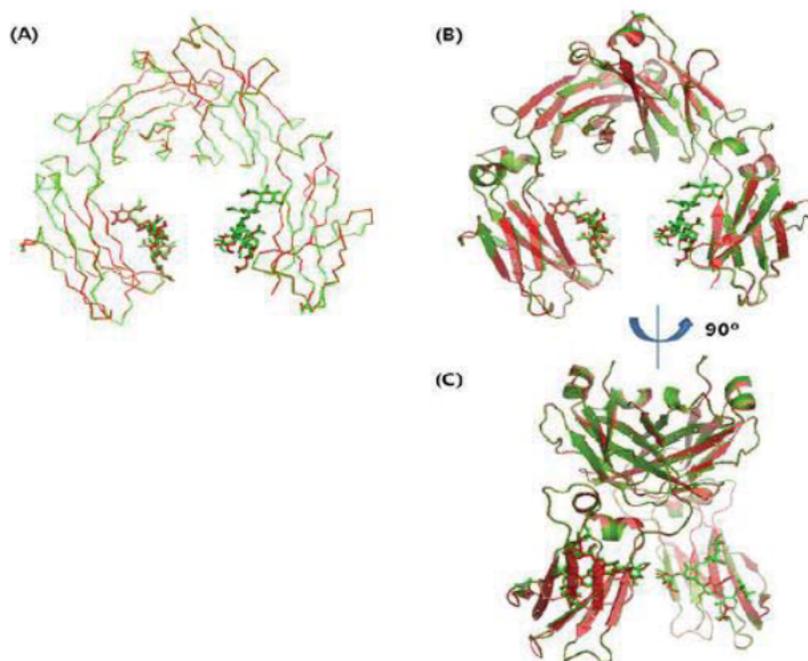
The two-way similarity study performed early this decade to support EU registration of CT-P13 has already been reviewed and summarized for IND 118135 (see review from November 13th 2013). A review of the information will not be duplicated in this review as many of the same analytical

and biochemical methods used in the three-way biosimilarity study were employed only with a smaller number of lots.

However, some of the methods used were unique to the two-way study and are summarized below. This includes the structural characterization of the Fc region using x-ray crystallography, characterization of the H2L1 fragment, effects of afucosylation on the ADCC.

Comparative Evaluation of Fc Structure Using X-ray Crystallography: Celltrion provided structural data for the Fc region generated using X-ray crystallography studies of one lot of CT-P13 drug product and a single lot of EU-approved infliximab. The Fc region of each antibody was generated following cleavage with papain and this was subsequently purified to enable crystallization. X-ray diffraction data collected in-house was used for molecular replacement and subsequent model building and refinement.

Reviewer's Comment: Comparing the structures of the Fc region of CT-P13 and EU-approved infliximab at 2.4Å resolution shows that this region of the molecule is structurally similar. The structural information, processing and refinement statistics provided by the Sponsor are reasonable.



RMSD for all Coatoms between 2 structures is less than 0.18 Å indicating that they adopt nearly identical 3D conformation. Two (2) different representations were used to show structure similarity between CT-P13 Fc and EU-approved Remicade[®] Fc; (A) is overlay of backbone trace of 2 structures as simple linear connection line, whereas (B) is overlay of backbone trace of 2 structures as a ribbon diagram to visualize secondary structure compositions. (C) is sideview of (B) by rotating 90°.

Non-reduced SDS-PAGE. Non-reduced SDS-PAGE of CT-P13 and EU-approved infliximab revealed six minor bands (see figure below). The individual bands were excised and run again on a non-reduced SDS Page Gel for identification. The mobility of the bands did not change, nor were there any differences between CT-P13 and EU-infliximab.

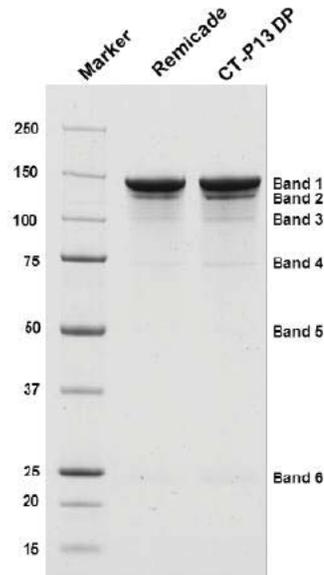


Figure 3.2.R-61: Non-Reduced SDS-PAGE Gel Image of CT-P13 Drug Product (B11R003, 4 µg) and EU-approved Remicade® (9RMA61401, 4 µg)

Each of the six individual bands was analyzed using LC-MS. The observed masses were consistent with combination of light chain and heavy chain variants. The Sponsor claims that the data show that both CT-P13 and EU-approved infliximab have a tendency to contain fragments (i.e., EU-approved infliximab can also form H2L1 variants). The amount of intact CT-P13 (95.1%) is slightly less than EU-approved infliximab (98.1%) as determined by reduced CE-SDS. Celltrion asserts that band 3 which constitutes the majority of all fragments and the non-assembled forms; LC-MS analysis identifies it as two heavy chains (2HC).

Table 3.2.R-94: Molecular Mass of Protein Eluted from Each Band of SDS-PAGE Gel for CT-P13 Drug Product and EU-approved Remicade®

Band	Observed Mass (Da)		Theoretical Mass or Mass Range, G0F~G2F (Da)	Expected Structure
	EU-approved Remicade®	CT-P13 Drug Product		
Band 1	149,074	149,052	148,795~149,443	2HC, 2LC
Band 2	125,750	125,744	125,357~126,005	2HC, LC
Band 3	102,251	102,158	101,919~102,567	2HC
Band 4	74,628	74,619	74,398~74,722	HC, LC
Band 5	51,295	51,276	50,960~51,284	HC
Band 6	23,519	23,520	23,438	LC

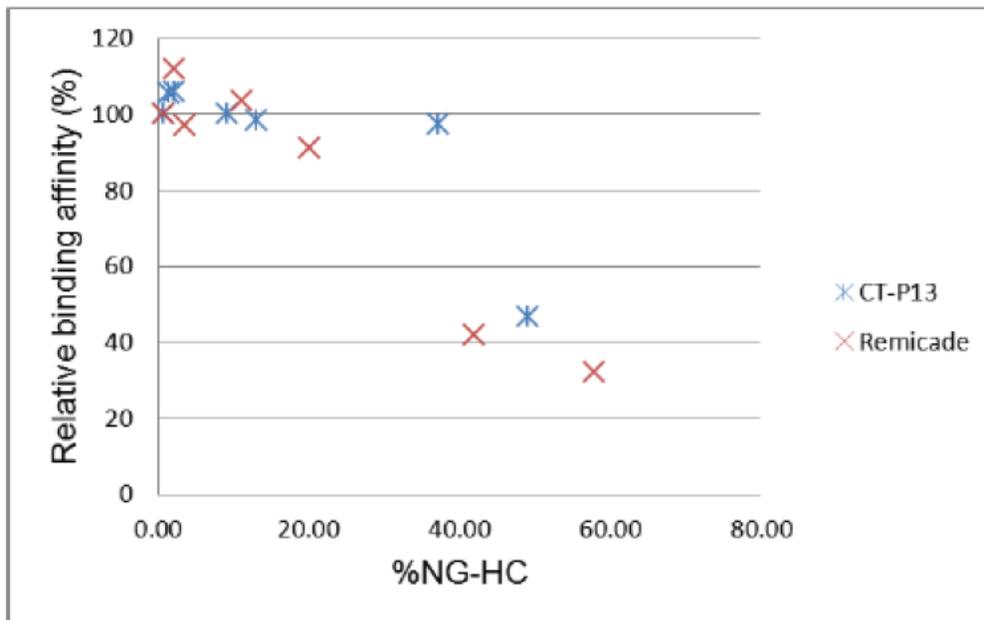
HC: Heavy chain, LC: Light chain

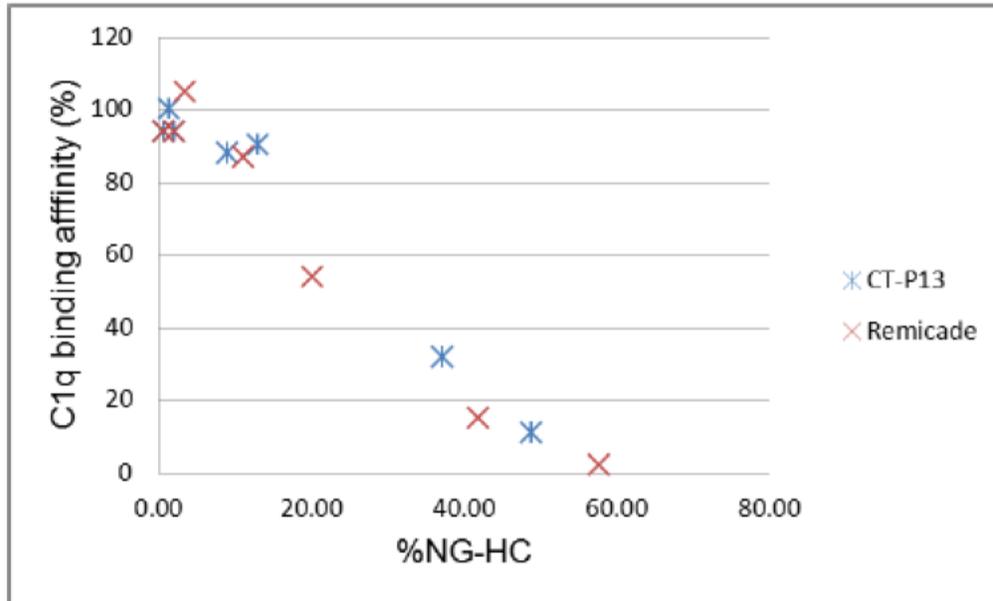
The Sponsor performed additional analytical studies to assess the impact of combinations of the various forms on the Fc effector functions of CT-P13. Increasing amounts of the 2HC variant (2.6, 37 and 5.7%) were added to intact CT-P13, and the impact on Fc mediated biological activity was assessed (see table 3.2R.100 below). The Sponsor claims that the data show that an increasing concentration of the 2HC variant did not have a significant impact on the bioactivity of CT-P13.

Table 3.2.R-100: Re-Purification Experiment and Comparison of Biological Activity

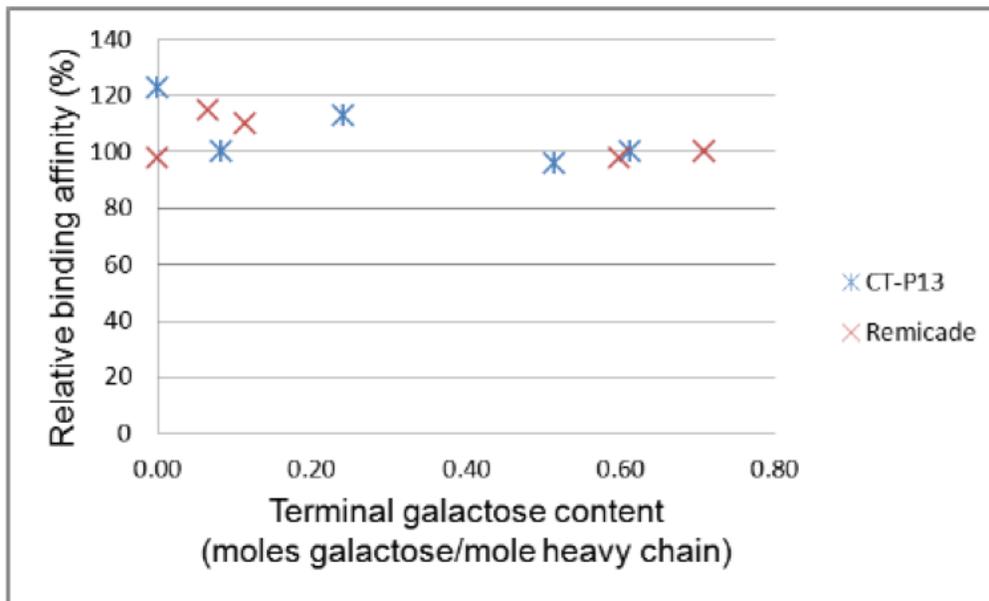
Sample	Intact IgG (%)	H2L1 (%)	TNF α Binding Affinity (ELISA, %)			Fc γ RIIIa Binding Affinity (SPR, %)			Fc γ RIIb Binding Affinity (SPR, %)			CDC (Relative Potency, %)		
			1 st	2 nd	Ave. (%)	1 st	2 nd	Ave. (%)	1 st	2 nd	Ave. (%)	1 st	2 nd	Ave. (%)
CT-P13 High	94.7	3.5	104.9	108.1	106.5	102	96	99	95	100	98	91	110	101
CT-P13 Middle	92.1	5.2	100.1	104.4	102.3	93	91	92	103	96	100	94	116	105
CT-P13 Low	87.4	7.6	96.4	101.9	99.2	98	93	96	109	102	106	101	106	104

Glycosylation and Associated Biological Activity: The Sponsor provided data that were obtained from a series of studies that were undertaken to assess the impact that glycosylation of the Fc region has on biological activity. The biological activity that was assessed included the binding with Fc γ IIIa measured using SPR and the C1q binding. Samples containing increasing concentrations of PNGase treated CT-P13 were assessed for interaction with Fc region of the antibody. The relationship between terminal galactose and biological activity was assessed using the same approach where PNGase was substituted for β -(1,4)-galactosidase. Removal of the terminal galactose did have an effect the binding to the Fc γ IIIa or C1q (see figures below)

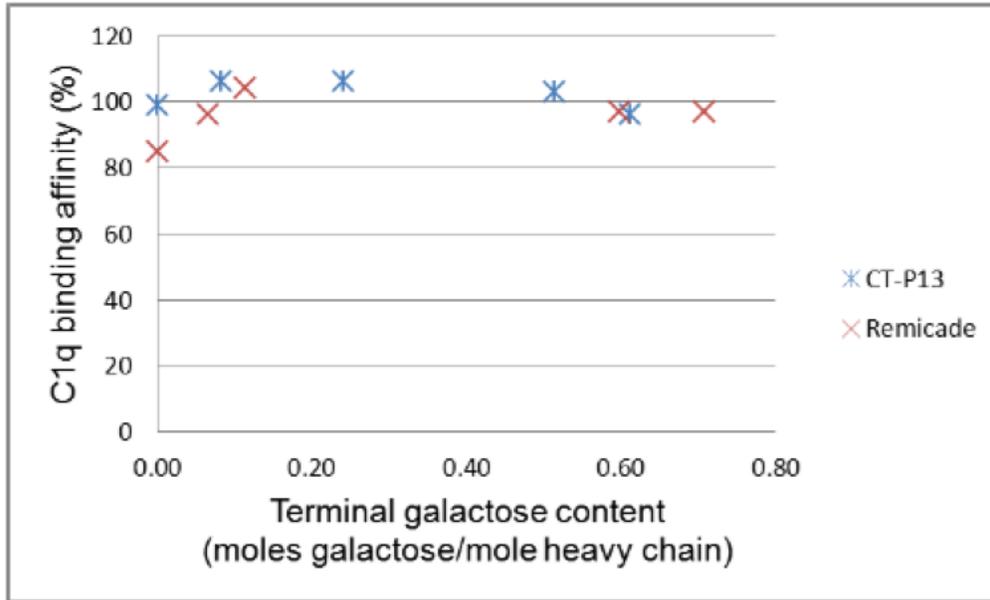




Scatter plot of data from PNGase F treated antibody



Scatter plot of functional data of β -(1,4)-galactosidase treated antibody



Scatter plot of functional data of β -(1,4)-galactosidase treated antibody

The Sponsor examined the correlation of microheterogeneity (afucosylation) of existing batches with binding to Fc γ RIIIa and activity in functional assays. The study used 6 lots CT-P13 DP and 6 lots EU-approved infliximab, see table below. The total proportion of afucosylated species present in the CT-P13 lots (5.46- 6.26%) in this case was reduced compared to EU-approved infliximab (8.87-10.99%). This reduction in afucosylated species correlated to reduced binding to Fc γ RIIIa, but there did not appear to be a significant difference in ADCC or CDC.

Table 3.2.R-138: Summary of Afucosylation, Fc γ RIIIa Binding Affinity, ADCC (w/normal PBMC and NK cells) and CDC in CT-P13 and EU-approved Remicade[®] Batches

Batch No.	Man5	G0	Total Proportion of Afucosylated Species (%) by HPAEC-PAD	Fc γ RIIIa Binding Affinity (R.P.%)				ADCC with PBMC (E/T=16:1) (R.P.%)			ADCC with NK (E/T=2:1) (R.P.%)			CDC (%)	
				Set 1	Set 2	Set 3	Mean	5 (ng/mL)	10 (ng/mL)	100 (ng/mL)	2 (ng/mL)	8 (ng/mL)	40 (ng/mL)		
PRS	5.36	0.76	6.12	100	100	100	100	100	100	100	100	100	100	100	100
10B0106	5.41	0.85	6.26	97	95	96	96	73	85	88	89	104	100	97	
10B9202	5.12	0.58	5.70	99	102	101	101	122	105	126	97	115	106	91	
10B9301	4.70	0.76	5.46	101	98	105	101	101	81	107	101	101	102	98	
B11M004	5.40	0.83	6.23	109	104	98	104	98	91	90	98	108	103	99	
B11M005	4.87	0.87	5.74	103	102	97	101	93	98	117	107	103	97	98	
B11M006	5.30	0.90	6.20	105	94	95	98	114	102	108	92	96	91	97	
0RMA60901	7.70	2.11	9.81	127	123	121	124	75	90	97	89	115	98	105	
0RMA61703	7.89	2.09	9.98	121	114	117	117	97	77	113	96	131	115	106	
1RMA61310	8.46	2.53	10.99	132	115	132	126	138	130	125	104	82	95	86	
1RMA65804	6.64	2.72	9.36	125	113	119	119	95	84	86	105	133	116	94	
0RMA64201	6.81	2.06	8.87	117	122	125	121	87	80	88	94	93	88	103	
1RMA62701	6.95	2.53	9.48	121	125	128	125	111	107	101	128	109	110	96	

Data from studies correlating glycosylation and biological activity in the two-way analysis

***Reviewer's Comment:** Results from the 2-way study provided evidence that glycosylation patterns differed substantially between EU-infliximab and CT-P13 (see table above). This pattern was not replicated in the three-way analysis and the subject of a Feb 10, 2015 IR letter (see summary above). Analysis of additional lots for this attribute submitted Mar 6, 2015 also did not corroborate the pattern seen in the two-way analysis. Ultimately, these observations provide support of the additional monitoring of Fc γ RIIIa binding at DS lot release agreed to by Celltrion, as this attribute can be measured more precisely and it is more functionally relevant.*

Overall Assessment of Analytical Similarity

The Sponsor has used a range of physiochemical and biological assays to characterize CT-P13, EU-approved infliximab and US-licensed Remicade. The biological assays cover a range of functions that may reflect a role in assessing biosimilarity with respect to potential mechanisms of action *in vivo*.

Some of the clinical studies used a non-US-licensed comparator product [European Union (EU)-approved infliximab]. To justify the use of these comparative clinical data to support a demonstration of biosimilarity of CT-P13 to US-licensed Remicade, the Applicant performed an analytical study to establish an adequate scientific bridge between CT-P13, EU-approved infliximab and US-licensed Remicade. The results of these initial studies showed that the comparison between US-licensed Remicade and EU-approved infliximab met expectations for analytical similarity, including pre-specified statistical criteria for the critical potency bioassays (TNF α binding and neutralization). Nevertheless, it should be noted that an assessment of subvisible particulates in the range of 1-10 microns was not included as part of the biosimilarity assessment reviewed in this document. These data, however, were requested by the reviewer responsible for evaluating immunogenicity. Data were provided for a limited number of lots showing an apparent difference in the levels of particulates between US-licensed Remicade and EU-approved infliximab, leading to residual uncertainty regarding the analytical bridge between EU-approved infliximab and US-licensed Remicade. Refer to the immunogenicity review for details regarding this concern.

In summary, the data show that within constraints of assessing a highly complex macromolecule that CT-P13 is analytically similar to the US-licensed Remicade. Data from two assays, WEHI cell based potency bioassay and TNF binding ELISA were subjected to a Tier One statistical equivalence test. The analysis met equivalence criteria expectations.

The following attributes match the reference product:

- Primary sequence
- TNF binding
- Secondary structure
- Overall tertiary structure
- Potency in a bioassay
- Effector functions in a variety of *in vitro* assays meant to establish mechanism of action
 - ADCC with PBMC
 - Regulatory macrophage function assays
 - TNF reverse signaling assays
 - CDC assays
 - Fc γ RI, Fc γ RII and FcRn binding

The analytical assessment of CT-P13 has revealed subtle shifts in glycan composition and other product-related variants (including H2L1). These in turn cause a 20% reduction in Fc γ RIIIa binding by CT-P13 relative to the reference product and similarly reduced *in vitro* activity in

ADCC assay that uses NK cells as effector cells. It should be noted that FcγRIIIa binding will be monitored at drug substance lot release.

CT-P13 also has more aggregated species (~0.6%) and a non-intact form (~5%) missing a light chain designated as H2L1. These levels are not more than allowed in other biotherapeutics. However, potential roles of these variants in modulating ADCC and immunogenicity cannot be excluded.

A minor difference in protein content (3%) between CT-P13 and the reference product will be corrected in early 2015 by tightening the (b) (4) process.

The current understanding of the roles of FcγRIIIa binding and subsequent ADCC of mTNF⁺ inflammatory cells in the clinical activity of infliximab is unclear. This leads to residual uncertainty regarding assigning the status of analytical highly similar, as it is not known whether product quality differences in FcγRIIIa binding and ADCC are clinically relevant. Assuming that the differences in FcγRIIIa binding/ADCC persist following analysis of additional lots, residual uncertainty will remain regarding a designation of “highly similar” in the absence of additional data (e.g., clinical data in the setting of IBD treatment).

3.2.R.3 Proposed Complete Response Comments

1. In your submission, you evaluated the analytical similarity of CT-P13 and US-licensed Remicade using a variety of functional assays. Your data generated using a standard NK-cell based killing ADCC assay suggest that CT-P13 has ~20% lower ADCC activity compared to the reference product US-licensed Remicade, which correlates with differences in FcγRIIIa binding. The difference in ADCC leads to residual uncertainty regarding the conclusion that CT-P13 is highly similar to US-licensed Remicade, as the role of ADCC remains uncertain in the clinical activity of the innovator product (e.g. in the setting of inflammatory bowel disease). Furthermore, you did not adequately justify the impact of the difference in ADCC on the analytical similarity assessment and did not identify the structural basis underlying this difference. For example, you should determine whether the H2L1 variant that is present at relatively high levels in CT-P13 compared to US-licensed Remicade plays a role in decreasing NK-dependent ADCC activity. On the other hand, the Agency has not excluded the possibility that analysis of additional lots of CT-P13 and innovator lots could overcome a statistical anomaly due to the analysis of a limited number of lots. To this point, we note that prior differences in glycan patterns were reduced when additional lots of CT-P13 and innovator product were analyzed. To address the current deficiency with respect to differences in ADCC activity, we recommend that you repeat the evaluation of ADCC using additional lots to determine whether the ADCC difference you have reported decreases when additional lots are evaluated (i.e., due to small sample size). If the difference in ADCC persists following analysis of additional lots, you should identify and demonstrate control of the product quality attributes that underlie ADCC activity in CT-P13 (e.g., glycan pattern, contribution of H2L1 variant, etc.) and provide an adequate justification, including an evaluation of the role of ADCC particularly in the setting of inflammatory bowel disease, that the observed difference in ADCC does not have clinical impact.
2. The current drug product stability data using Process B batches of CT-P13 supports an expiry date of 42, not (b) (4) months. To address this concern, adjust your proposed expiry dating to reflect existing data and provide a stability protocol to support post-approval expiry extension.
3. We acknowledge the plan outlined in your 10 Apr 2015 letter to develop and validate a revised version of the visible particle test for reconstituted drug product. The revised test will use (b) (4) and visual inspection of 20 reconstituted vials. Data supporting the assay revision have not been provided to the BLA. To address this concern, submit the assay SOP, validation report and revised specification to the Agency for review.

5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

Reviewer's Comment: Qualification of the immunogenicity assay will be reviewed by Will Hallett and Harold Dickensheets (Division 2, OBP). They will also perform an evaluation of the immunogenicity data.

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/s/

PETER L ADAMS
05/26/2015



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

Date: 1 May, 2015
To: Administrative File, STN 125544/0
From: Reyes Candau-Chacon, PhD. Reviewer, OPQ/OPF/DMA Maria D. Candau-Chacon -S
Through: Patricia Hughes, Ph.D., Acting Branch Chief, OPQ/OPF/DMA Patricia F. Hughestroost -S
Subject: New 351(k) Biologic License Application (BLA)
US License: 1996
Applicant: CELLTRION, Inc.
Facilities: CELLTRION, Inc. (FEI 3005241015), 23, Academy-ro, Yeonsu-gu, Incheon, 406-840, Republic of Korea
Product: CT-P13, proposed biosimilar to US-licensed Remicade (infliximab), INFLECTRA
Dosage: Sterile lyophilized powder in single-use 20-ml vials containing 100 mg/vial CT-P13 for reconstitution with 10 mL SWFI and further dilution with 0.9% sodium chloride for injection prior to IV infusion.
Indication: Proposed biosimilar for the treatment of Crohn's Disease, Pediatric Crohn's Disease, Ulcerative Colitis, Pediatric Ulcerative Colitis¹, Rheumatoid Arthritis, Ankylosing Spondylitis, Psoriatic Arthritis, and Plaque Psoriasis
Due date: June 8, 2014

Recommendation for Approvability: The drug substance part of BLA 125544 as amended is recommended for approval from a microbial control and microbiology product quality perspective.

Review Summary

CELLTRION, Inc. has submitted 351(k) BLA 125544 to obtain approval of CT-P13. CT-P13 is a chimeric monoclonal antibody of the IgG1 subclass; CT-P13 is a proposed biosimilar to US-licensed Humira (infliximab); infliximab binds to soluble and membrane-bound forms of TNF α .

¹ This reflects information for Inflectra that Celltrion submitted on August 8, 2014. We note that the indication for pediatric ulcerative colitis is protected by orphan drug exclusivity expiring on September 23, 2018. See the Orphan Drug Designations and Approvals database at <http://www.accessdata.fda.gov/scripts/opdlisting/oopd/index.cfm>.

BLA 125544 was submitted in eCTD on August 8, 2014. This review contains the assessment of the microbial quality attributes of the CT-P13 bulk drug substance from a microbiological quality perspective. For review of drug product aspects of the application, please see review by Dr. Bo Chi.

Amendments Reviewed for Drug Substance Quality

Information Request date	Question numbers	Amendment sequence	Amendment date
September 26, 2014	1 to 11	0006	October 27, 2014
December 10, 2014	1d, 2f, 2k, 4, 5b, 5c, 7b, 8, 9e, 10d	0015	December 29, 2014
February 6, 2015	1d, 2f, 2k, 5b	0024	February 24, 2015

Review Narrative

S DRUG SUBSTANCE

S.1 General Information

CT-P13 is a chimeric human-murine monoclonal antibody produced in Sp2/0-Ag14 (b)(4). CT-P13 is a glycoprotein with two heavy chains and two light chains; each heavy chain has 450 aminoacids, each light chain has 214 aminoacids.

Reviewer's comment:

Reference about the mechanism of action of CT-P13 is made to infliximab, the US licensed reference.

Satisfactory

S.2 Manufacture

S.2.1 Manufacturer(s)

The following facilities are used for the manufacture, release testing, and stability testing of CT-P13 drug substance:

- CELLTRION, Inc., 23, Academy-ro, Yeonsu-gu, Incheon, 406-840, Republic of Korea; Drug Substance manufacture, drug substance release and stability testing; storage of WCB.
FEI: 3005951160; DUNS: 688836030
- CELLTRION, Inc., 20, Academy-ro 51 beon-gil, Yeonsu-gu, Incheon, 406-840, Republic of Korea; Drug Substance release and stability testing.
FEI: not provided; DUNS: 688836030
- (b)(4)
Testing of CT-P13 unprocessed bulk.
FEI: (b)(4)
- (b)(4) Testing of CT-P13 unprocessed bulk.
FEI: (b)(4)

- [Redacted] (b) (4)

Production and storage of the MCB and WCB

FEI: [Redacted] (b) (4)

Reviewer comments:

[Redacted] (b) (4)

. Refer to the cGMP status section of this review for the compliance status of CELLTRION, Inc., Incheon, Korea.

S.2.2 Description of the Manufacturing Process and Process Controls

S.2.2.1 Batches and Scale Definition

[Redacted] (b) (4)

S.2.2.2

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(b) (4)

(b) (4)

S.7.3 Stability Data

All bioburden and endotoxin results are within specifications; bioburden was (b) (4) CFU/10 mL for all samples; endotoxin was (b) (4) EU/mL for all samples.

Reviewer Comments:

All results were within acceptance criteria; however, microbial quality results of stability batches are not necessarily representative of other batches. In

addition, stability samples were not stored in the commercial container closure.

Satisfactory

Environmental Assessment

A categorical exclusion for an action on a biologic product application, if the action does not increase the use of the active moiety is claimed under 21 CFR 25.31(a). The biosimilar product CT-P13 substitutes directly for an approved product for the approved indications; therefore, it is not considered to increase the use of the active moiety. In addition, CT-P13 is a protein and its metabolites will breakdown in the environment to carbon dioxide, water and nitrogen; therefore, no extraordinary circumstances exist as described in 21 CFR 25.21.

cGMP Status

Refer to Panorama for cGMP status of the relevant facilities.

Conclusion

- I. The drug substance section of this BLA as amended was reviewed from a microbial control and microbiology product quality perspective and is recommended for approval.
- II. The BLA should be reviewed by an OBP reviewer.
- III. A pre-license inspection was conducted at CELLTRION, Inc. from February 23rd to March 6th, 2015 by OPF/DMA (Bo Chi and Reyes Candau-Chacon), OBP (Kurt Brorson and Peter Adams) and OIP (Syin Chiang). A 15-observation 483 was issued.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Center for Drugs Evaluation and Research – Food and Drug Administration
Office of Biotechnology Products / Office of Pharmaceutical Quality
Division Two, WO Bldg. 72, HFD-123
10903 New Hampshire Ave., Silver Spring MD 20903

BLA 125544

CT-P13

Review Date: May 11, 2015

Recommendation: Complete Response

Drug Name/Dosage	CT-P13
Strength/Potency	100 mg lyophilized antibody
Route of	Intravenous infusion
Rx/OTC Dispensed	Rx
Indications	CT-P13 is proposed to be indicated for all US-licensed Remicade currently approved indications: <ul style="list-style-type: none"> • Rheumatoid arthritis in combination with methotrexate • Crohn’s disease • Pediatric Crohn’s disease • Ulcerative Colitis • Pediatric Ulcerative Colitis • Ankylosing Spondylitis • Psoriatic Arthritis • Plaque Psoriasis
Applicant/Sponsor	Celltrion Inc.

Submissions reviewed

Submission	Date Received	Review Completed*
Original Application	8/8/2014	Y
Amendment 1	9/5/2014	N/A
Amendment 2	9/22/2014	Y

Amendment 4	10/1/2014	Y
Amendment 5	10/20/2014	Y
Amendment 6	10/27/2014	Y
Amendment 15	12/29/2014	Y
Amendment 16	1/15/2015	Y
Amendment 17	1/22/2015	Y
Amendment 27	3/23/2015	Y
Amendment 28	3/31/2015	Y
Amendment 29	4/2/2015	Y
Amendment 30	4/17/2015	Y
Amendment 31	4/17/2015	Y
Amendment 32	4/17/2015	Y

* Review completed by OBP or related Quality reviewer (microbiology, immunogenicity, CMC Statistics).

Quality Review Team

DISCIPLINE	REVIEWER	BRANCH/DIVISION
Drug Substance	Peter Adams	DBRR I
Drug Product	Peter Adams	DBRR I
Analytical Similarity	Peter Adams	DBRR I
Immunogenicity	Will Hallett/ Harold Dickensheets	DBRR II
Labeling	Jibril Abdus-Samad	OBP
Facility and Microbiology	Bo Chi/ Maria Candauchacon	DMA/OPF
Secondary Reviewer	Kurt Brorson	DBRR II
Tertiary Reviewer	David Frucht	DBRR II

Multidisciplinary Review Team

DISCIPLINE	REVIEWER	OFFICE/DIVISION
RPM	Phuong Nina Ton	DPARP/OND
CDTL/Medical Officer	Nikolay Nikolov	DPARP/OND
Medical Officers	Juwaria Waheed, Rob Fiorentino, Gary Chiang, David Kettl	DPARP, DGIEP, DDP/OND
Pharm/Tox	Matt Whittaker, Tim Robison	DPARP

Clinical Pharmacology	Lei He, Satjit Brar	OTS/DCPII
Biostatistics	Greg Levin, Ruthie Davi	OTS/DBII
CMC Statistics	Meiyu Shen, Yi Tsong	OTS/DBVI
Legal	Janice Weiner	ORP/DRPI
Biosimilars Policy	Leah Christl, Sue Lim, Carla Lankford, Neel Patel, Tyree Newman	TBBT/OND

Signature Block

Name Title	Signature and Date
Kurt Brorson, Ph.D. Acting Lab Chief DBRR2, OBP	Kurt A. Brorson -A <small>Digitally signed by Kurt A. Brorson -A DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300078163, cn=Kurt A. Brorson -A Date: 2015.05.11 13:33:10 -04'00'</small>
David Frucht, M.D. Acting Division Director DBRR2, OBP	David M. Frucht -S <small>Digitally signed by David M. Frucht -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300168694, cn=David M. Frucht -S Date: 2015.05.11 13:28:27 -04'00'</small>

CC Block

Immunogenicity Reviewer:
CMC Statistics Reviewer:
Product Quality Reviewer:
OBP Office Director:
Medical Officer:
Clinical Deputy Division Director:
Division 2 File:

William Hallett, Ph.D.
 Meiyu Shen, Ph.D.
 Peter Adams, Ph.D
 Steven Kozlowski, M.D.
 Nikolai Nikolov, M.D.
 Sarah Yim, M.D.
 BLA STN 125544

Quality Review Data Sheet

1. LEGAL BASIS FOR SUBMISSION: 351(k)

2. RELATED/SUPPORTING DOCUMENTS:

A. DMFs:

DMF #	TYPE	HOLDER	ITEM REFERENCED	CODE ¹	STATUS ²	DATE REVIEW COMPLETED
(b) (4)	III	(b) (4)	Rubber stopper formulation	2	adequate	2/5/2015 (M. Haber)
	V		Westar rubber stopper process	2	adequate	5/28/2014 (J. Wells)
	III		Type I Borosilicate vials	6	Requested from DCC, Pending	Pending

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 sufficient data in the application, therefore the DMF did not need to be reviewed)

Consults:

DISCIPLINE/TOPIC	DATE REQUESTED	STATUS	RECOMMENDATION	REVIEWER
CDER/OTS/OB/DBVI -Statistical Equivalence of bioactivity and TNF binding	8/8/2014	Completed	Adequate	Meiyu Shen

Executive Summary

I. Recommendations

A. Recommendation and Conclusion on Approvability

The Office of Biotechnology Products, OPQ, CDER, recommends that this 351 (k) BLA application not be approved at this time due to deficiencies in the analytical similarity assessment leading to residual uncertainty regarding the determination that this product is “highly similar” to the reference product.

B. Action letter language

Manufacturing location:

Drug substance – Celltrion, Inc., Incheon South Korea

Drug product – Celltrion, Inc., Incheon South Korea

Fill sized and dosage form – 100 mg vial

Dating period:

Drug product – 42 months at $5 \pm 3^{\circ}\text{C}$

Drug substance – (b) (4) months at (b) (4) $^{\circ}\text{C}$ or (b) (4) months at (b) (4) $^{\circ}\text{C}$

Stability option: The stability protocol in the license application is approvable for the purpose of extending the expiration dating period of the drug product under 21 CFR 601.12.

Exempt from lot release– CT-P13 is a specified product as per 601.2a

C. Benefit/Risk Considerations

The data submitted in this application support the conclusion that the manufacture of CT-P13 is well controlled, and leads to a product that is sufficient to meet quality parameters recommended by FDA. The conditions used in manufacturing have been sufficiently validated, and a consistent product is produced from the multiple production runs presented. From a product quality perspective alone, this product would have been approvable for human use.

CT-P13 is a proposed biosimilar to US-licensed Remicade. Analytical similarity of CT-P13 to US-licensed Remicade was evaluated using methods to assess physicochemical and functional properties of the products. Comparison of product-related substances and impurities were also conducted. A total of 7 to 13 lots of CT-P13 DP and 7-17 lots of US-licensed Remicade were evaluated using the methods summarized below. The two products have been demonstrated to be analytically similar in terms of important protein structure and functional aspects as summarized below:

- Primary sequence
- TNF binding
- Secondary structure

- Overall tertiary structure
- Potency in a bioassay
- Effector functions in a variety of in vitro assays meant to establish mechanism of action
 - ADCC with PBMC
 - Regulatory macrophage function assays
 - TNF reverse signaling assays
 - CDC assays
 - FcγRI, FcγRII and FcRn binding

Yet-to-be defined shifts in relative percentages of N-linked glycans appear to appear to correlate with an approximately 20% lower FcγRIII binding strength in the CT-P13 product, as well as activity in NK-dependent ADCC assays.

CT-P13 also has more aggregated species (~0.6%) and a non-intact form (~5%) missing a light chain designated as H2L1. These levels are not more than allowed in other biotherapeutics. However, potential roles of these variants in modulating ADCC and immunogenicity cannot be excluded.

A minor difference in protein content (3%) between CT-P13 and the reference product was corrected in early 2015 by (b) (4) process. Strength of antibody products is generally defined by antibody protein content, which in the case of US-licensed Remicade is 100 mg/vial. Upon completion of (b) (4) process to ensure a protein content match to US-licensed Remicade, the statutory requirement for same strength, same route of administration (ROA), and same dosage form was met.

CT-P13 was also evaluated in various clinical studies using a non-US-licensed comparator product [European Union (EU)-approved infliximab]. To justify the use of these comparative clinical data to support a demonstration of biosimilarity of CT-P13 to US-licensed Remicade, the Applicant performed an analytical study to support a bridge between US and EU Remicade. These studies initially supported the analytical bridge, but additional data submitted in response to an immunogenicity-related information request brought this conclusion into question. These data involving a limited number of lots revealed a potential difference in subvisible particulate concentrations between US-licensed Remicade, EU-approved infliximab, and CT-P13. Data from additional lots will be requested to determine if this difference is statistically significant.

The totality of analytical data is sufficient to conclude that CT-P13 is “similar” to the reference product, but at this time it is premature to conclude that it is “highly similar”. In addition, the consensus of the Quality review team was that the analytical differences in FcγRIII binding and ADCC activity impart residual uncertainty when extrapolating to clinical indications that could involve antibody-dependent cellular cytotoxicity (ADCC).

D. Recommendation on Phase 4 , if Approvable: Not applicable

II. Summary of Quality Assessments

CQA Identification, Risk and Lifecycle Knowledge Management.

Some elements of a QbD-like strategy were implemented, starting from the development of a QTPP for the product. Risk assessments, as well as statistical approaches were used for lot testing and acceptance criteria setting. However, Celltrion did not prepare the BLA as a formal QbD submission.

Drug Substance Quality Summary

Names:

- Code Name: CT-P13
- Trade Name: INFLECTRA
- Non-Proprietary/USAN: to be determined
- CAS name: 170277-31-3
- INN Name: infliximab (proposed)
- Compendial Name: not applicable
- OBP systematic name: MAB HUMAN (IGG1) ANTI CAA26669 (TNF-alpha_HUMAN) [CT-P13]
- Other Names: ATC code L04AB02, DrugBank DB00065, UNII B72HH48FLU, KEGG D02598, ChEMBL1201581

Pharmacology category: TNF- α Blocker

Description: CT-P13 is a recombinant chimeric IgG1 monoclonal antibody that binds human TNF- α . It is a proposed biosimilar of US-licensed Remicade (a chimeric anti-TNF- α mAb currently marketed for RA, Crohn’s disease and other clinical indications). Biochemically, CT-P13 is an IgG1 κ monoclonal antibody with human amino acid sequence constant regions and murine variable regions, with a range of molecular

weights of 148.2 - 149.4 kilodaltons and isoelectric points between 7.4 and 7.9. The CT-P13 expression construct was reverse engineered from published Remicade sequences and is expressed in an SP2/0 based transfectoma. Amino acid sequencing and MS-based tryptic peptide mapping confirms the correct infliximab amino acid sequence for CT-P13.

Mechanism of action: TNF- α is a widely expressed cytokine that acts as a pro-inflammatory molecule with stimulatory activities for most cells of the immune system. It is also involved in an array of biologic responses including apoptosis, differentiation, and proliferation. Elevated levels of TNF- α over prolonged periods can result in chronic disease states. TNF- α is functional both in a 26 kDa membrane bound (mTNF- α F) form and a 17 kDa soluble form (sTNF- α), both of which form non-covalently linked homotrimers. Because both forms are active, signals may be passed locally from cell to cell via mTNF- α :TNF-R interactions, or more distally through release of sTNF- α . sTNF- α is generated following cleavage by members of a class of metalloproteinases called "sheddas", which include TNF-converting enzyme (TACE; ADAM17) and ADAM 10. While under normal physiological conditions, the concentration of TNF found in bodily fluids is usually very low, stimulation by external sources can increase concentrations 10,000 fold (10). Biological responses to TNF are mediated through two structurally distinct, cognate TNF- α receptors, TNF-R1 (p55) and TNF-R2 (p75). These high affinity receptors are present as preassembled trimers on the cell surface. Most cells constitutively express TNF-R1 on their surface; in contrast, TNF-R2 is inducible and expressed preferentially on the hematopoietic and endothelial cells.

CT-P13 has a high avidity for TNF, both soluble and membrane-bound forms. It functions primarily by binding, neutralizing and sequestering excess sTNF- α produced in local tissue sites during various inflammatory disease states. Other possible clinically-relevant activities include mediating antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) of mTNF- α + inflammatory T-cells or other cells associated with a disease state. The relative importance of sequestering sTNF- α vs. ADCC of mTNF+ cells has not been definitively established, and may vary between disease states as discussed in the clinical review [e.g., ADCC may play a greater role in the anti-inflammatory activity of infliximab in inflammatory bowel disease (IBD) vs. rheumatoid arthritis (RA)].

Potency Assay. The TNF neutralizing activity of the CT-P13 antibody is assessed with an *in vitro* assay in which the ability protect the WEHI-164 cell line from treatment with hTNF- α is evaluated. The relative potency of typical batches of CT-P13 DS and DP ranges from 89 – 109 %. This is within the proposed release test specification for this assay. CT-P13 binding affinity for TNF α can also be measured by SPR and ELISA. It was found to be close to the RS, within the precision of the assays.

Reference material(s): The current primary CT-P13 RS (1301-02) was produced from a subset of the (b) (4) L CT-P13 version one of process B DS lot 1000B03.

Critical starting materials or intermediates. Starting materials used to produce CT-P13 are typical of bioprocessing: protein free cell culture media, non-toxic chemicals, and water.

Manufacturing process summary. The antibody drug substance is manufactured using standard bioprocess technology at an approximately (b) (4) kg per batch scale and takes place at (b) (4) Celltrion Inc. in Incheon Korea.

CT-P13 is manufactured consistently by a robust process with precautions for contamination. Drug substance manufacture consists of (b) (4) production in bioreactors followed by (b) (4). Cell banks conform with virological safety testing requirements in ICH Q5A, 1998. (b) (4)

Residual levels of process-related impurities such as host cell proteins (HCP), host cell DNA (HCDNA) as well as other process-related impurities specific to the CT-P13 process were evaluated in CT-P13 drug substance. Data were provided to demonstrate that the manufacturing process of CT-P13 drug substance is able to reduce the levels of these impurities to very low levels (e.g., ppm for host cell protein (HCP) and pg/mg for host cell DNA). Each batch is approximately (b) (4) kg and stored at (b) (4) °C.

The manufacturing process for CT-P13 drug substance was scaled up and optimized during the clinical development program. To rule out evolution in product quality, Celltrion has provided data to demonstrate equivalent product quality of CT-P13 drug substances that were manufactured over the duration of process development. The drug product manufactured for commercial launch was also shown to be comparable to the drug product manufactured by the clinical process.

Container closure. The CT-P13 DS is filled into and stored (b) (4)

Dating period and storage conditions: The proposed CT-P13 DS storage at (b) (4) °C (NMT (b) (4) months) and at (b) (4) °C (NMT (b) (4) years):

C. Drug Product Quality Summary

Description: CT-P13 final drug product (FDP) will be supplied as a sterile, preservative-free lyophilized powder in vials. The sterile lyophilized powder in the vials is to be reconstituted in sterile water for injection and diluted into infusion bags. Because of the potential formation of visible particles, an in-line filter must be incorporated in the patient set during infusion. *The statutory requirements for same strength, same route of administration (ROA), and same dosage form was met.*

Strengths: 100 mg / vial. The statutory requirement for same strength as the reference product was met.

Summary of Product Design. CT-P13 was developed as a biosimilar to US-licensed Remicade and thus to target the quality profile of the reference product.

Excipients. The CT-P13 formulation has the same chemical constituents as US-licensed Remicade. The formulation components are as follows: 100 mg CT-P13 antibody, 500 mg sucrose, 2.2 NaH₂PO₄ monohydrate, Na₂HPO₄ dihydrate and 0.5 mg polysorbate 80; when reconstituted in 10 mL water the pH is 7.2. 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 material(s): The same reference material is used for DS and DP.

Manufacturing process summary: CT-P13 DP intended for distribution in the US will be manufactured at Celltrion Plant ^{(b) (4)} in Incheon, Korea. Overall, the manufacturing process is comprised of ^{(b) (4)}

The validated commercial lot size is about ^{(b) (4)} vials. Three drug product batches at this scale were analyzed to validate the vial fill manufacturing process. Product-and process-related contaminants are within proposed specification limits. Validated testing to assure microbial and viral safety (controlled during drug substance manufacture) is in place. Media fill simulations of the ^{(b) (4)} fill line was also performed. During the biosimilarity exercise, it was noted that US-licensed Remicade contains on average about 92 mg of antibody protein while CT-P13 contained 96 mg, more closely matching the labeled content of 100 mg. As a result, Celltrion tightened their ^{(b) (4)} criteria to more closely match the US licensed protein content and produced an additional three process validation lots in April 2015.

Container closure: The container closure system is comprised of USP Type I borosilicate 20 mL capacity glass vials, ^{(b) (4)} butyl rubber stoppers, and flip-off aluminum seals with a ^{(b) (4)} button.

Dating period and storage conditions: 42 months at 5 ± 3°C. Drug product lot testing and stability studies utilizes compendial or validated analytical assays appropriate for anti-TNF-α antibodies. It also includes a specific test for FcγRIII binding, an attribute that seems to vary somewhat from US licensed Remicade. Stability testing of the proposed commercial product has demonstrated adequate stability for 42 months when held under refrigerated conditions.

List of co-packaged components: not applicable

Novel Approaches/Precedents: none

Any Special Product Quality Labeling Recommendations

- [REDACTED] (b) (4)
- After reconstitution in water, dilute only with saline in infusion bags.

Establishment Information

Inspection of the Celltrion drug substance [REDACTED] (b) (4) and product [REDACTED] (b) (4) manufacturing buildings at the Incheon site was performed on 2/26 through 3/6/2015. The inspection resulted in a fifteen item 483 form noting deficiencies in the quality, data management and other systems. The firm responded to the 483 observations on 3/25/2015. The final EIR document and inspection resolution is pending.

Lifecycle Knowledge Management

a) Drug Substance

- Protocols approved: Stability protocol
- Outstanding review issues/residual risk: None besides biosimilarity assessment
- Future inspection points to consider – None

b) Drug Product

- Protocols approved: Stability protocol
- Outstanding review issues/residual risk: None
- Future inspection points to consider: None

Quality TL Analytic Similarity Assessment

The totality of analytical data is sufficient to conclude that CT-P13 is “similar” to the reference product, but at this time it is premature to conclude that it is “highly similar”.

Analytical similarity of CT-P13, US-licensed Remicade, and EU-approved infliximab was evaluated using multiple DP lots of each of the three products. Initially, seven lots of CT-P13, EU-approved infliximab and US- licensed Remicade were used for analysis with physicochemical methods. Ten lots each were used for analysis with biological assays. The expiration dates of the US-licensed Remicade lots and EU-approved infliximab lots that were analyzed spanned approximately 3 years and 4 years, respectively. The CT-P13 lots that were used for analysis were manufactured in 2012. The number of lots that were analyzed was initially chosen by the Applicant based on their assessment of the variability of the analytical method and availability of material. Subsequently, an additional three-five lots each of CT-P13, EU-approved infliximab and US- licensed Remicade were added to increase statistical power to the comparison.

Some of the clinical studies used a non-US-licensed comparator product [European Union (EU)-approved infliximab]. To justify the use of these comparative clinical data to support a demonstration of biosimilarity of CT-P13 to US-licensed Remicade, the Applicant performed an analytical study to establish an adequate scientific bridge between EU-approved infliximab and US-licensed Remicade. The results of these comparisons show that the three products (CT-P13, US-licensed Remicade, and EU-approved infliximab) met expectations for analytical similarity, including pre-specified statistical criteria for the critical potency bioassay (TNF binding and neutralization). However, additional data submitted in response to an immunogenicity-related information request brought this conclusion into question. These data involving a limited number of lots revealed a potential difference in subvisible particulate concentrations between US-licensed Remicade, EU-approved infliximab, and CT-P13. Data from additional lots will be requested to determine if this difference is statistically significant. If these additional data resolve the apparent subvisible particulate difference, an adequate analytical bridge between US-licensed Remicade and EU-approved infliximab could be established. Establishment of the analytical bridge would support the use of clinical data generated with EU-approved infliximab to support biosimilarity of US-licensed Remicade and CT-P13.

Analytical similarity of CT-P13, US-licensed Remicade and EU-approved infliximab was assessed using a comprehensive range of methods listed in the table below:

Quality attributes and methods used to evaluate analytical similarity of CT-P13, US-licensed Remicade and EU-approved infliximab

Quality Attribute	Methods
Primary structure	<ul style="list-style-type: none"> <li data-bbox="643 1738 1369 1812">• Peptide mapping with ultraviolet (UV) and mass spectrometry (MS) detection <li data-bbox="643 1822 922 1854">• Amino Acid Analysis

Quality Attribute	Methods
	<ul style="list-style-type: none"> • Post-translational modification (MS/MS) • Intact Mass Reduced (LC-MS) • Peptide mapping coupled with tandem mass spectrometry (MS/MS)
Protein content	<ul style="list-style-type: none"> • UV280
Higher order structure	<ul style="list-style-type: none"> • Far and Near UV circular dichroism • FTIR • Free thiols • Antibody Array • Liquid chromatography coupled with mass spectrometry (LC-MS)(disulfide bond characterization) • Differential scanning calorimetry
High molecular weight species/aggregates	<ul style="list-style-type: none"> • Size exclusion chromatography (HPLC) • Size exclusion chromatography (SEC-MALS) • CE-SDS (reduced and non-reduced) • Analytical Ultracentrifugation (AUC)
Charge	<ul style="list-style-type: none"> • IEF • IEC-HPLC
Glycosylation	<ul style="list-style-type: none"> • Oligosaccharide profiling • N-linked Glycan analysis • Sialic Acid analysis • Monosaccharide Analysis
Potency	<ul style="list-style-type: none"> • <i>In vitro</i> TNF-α neutralization assay
Binding assay – TNF	<ul style="list-style-type: none"> • ELISA • Cell based binding affinity
Binding assay – Fc	<ul style="list-style-type: none"> • FcγRIIIa V and F type binding affinity (SPR) • FcγRIIIb binding affinity (SPR) • FcγRIIa binding affinity (SPR) • FcγRIIb binding affinity (SPR) • FcγRI binding affinity (ELISA) • FcRn binding affinity (SPR) • C1q binding assay (ELISA)
Binding assay-complement	<ul style="list-style-type: none"> • C1q binding assay (ELISA)

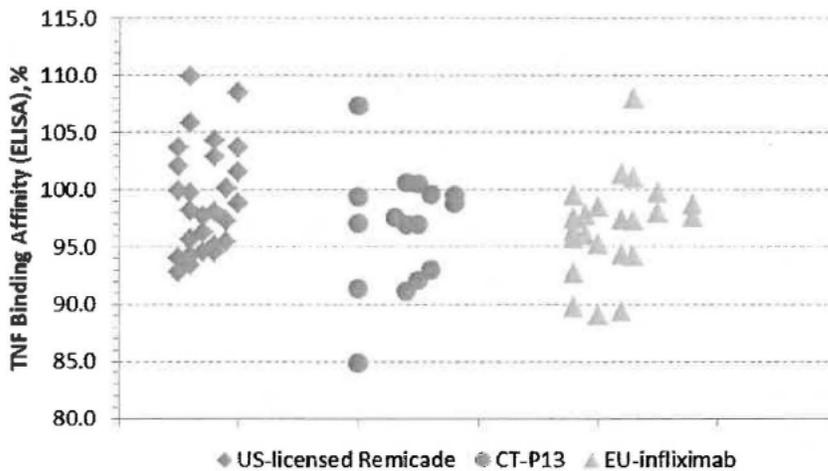
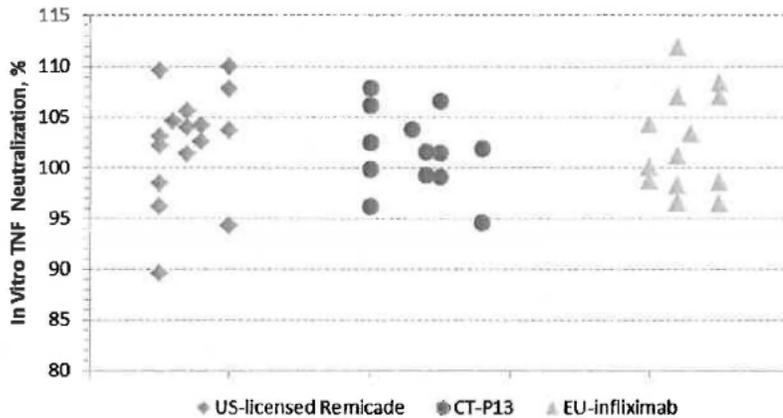
Quality Attribute	Methods
Bioassay/functional or Mechanism of action	<ul style="list-style-type: none"> • ADCC (PBMC) • ADCC (NK Cells) • ADCC (LPS-stimulated monocytes and NK cells) • CDC • Induction of apoptosis by reverse signaling • Inhibition of cytokine release by reverse signaling • Caco-2 (suppression of pro-inflammatory cytokines) • Wound healing (closure %) • Inhibition of T Cell proliferation • Induction of regulatory macrophages

The following attributes are similar or matched (i.e., amino acid sequence) the reference product:

- Primary sequence
- TNF binding (ELISA and bioassay)
- Secondary structure
- Overall tertiary structure
- Potency in a bioassay
- Effector functions in a variety of in vitro assays meant to establish mechanism of action
 - ADCC with PBMC
 - Regulatory macrophage function assays
 - TNF reverse signaling assays
 - CDC assays
 - FcγRI, FcγRII and FcRn binding

Tier One statistical equivalence testing was performed by the CDER CMC Stats reviewer on lot data from the TNF ELISA (direct TNF binding assay) and the WEHI bioassay (direct potency). 13 batches of CT-P13, 16 batches of US-licensed Remicade, and 13 batches of EU-approved infliximab are included in the in vitro TNF α neutralization activity dataset for the statistical equivalence testing. 16 batches of CT-P13, 27 batches of US-licensed Remicade, and 23 batches of EU-approved infliximab are included in the TNF α binding affinity (ELISA) dataset for the statistical equivalence testing. The statistical analysis using equivalence testing passed with the equivalence margin $\pm 1.5 \sigma_R$, with largely overlapping data from all three versions of the antibody, see figures below:

TNF binding activity of CT-P13, US- and EU- Remicade measured by two assays:



SE-HPLC analysis revealed that CT-P13 has somewhat more aggregated species (~0.6%) and cSDS analysis revealed that a non-intact form (~5%) missing a light chain called H2:L1. These levels are not more than allowed in other biotherapeutics. However, potential roles of these variants in modulating ADCC and immunogenicity cannot be excluded.

A minor difference in protein content (3%) between CT-P13 and the reference product was corrected in early 2015 by tightening the (b) (4) process. Upon completion, the statutory requirement for same strength, same route of administration (ROA), and same dosage form was met.

The analytical assessment of CT-P13, however, has revealed subtle shifts in glycan composition. These in turn cause a 20% reduction in FcγRIII binding by CT-P13 relative to the reference product and similarly reduced *in vitro* activity in an ADCC assay that uses NK cells as effector cells, see table below:

ADCC activity assay and FcγRIIIa binding (SPR)

Assay Format ^a	CT-P13	EU-infliximab	US-Remicade
In vitro functional			
ADCC % (PBMC effectors; transfected Jurkat targets)	106±7.9	106±8.4	106±11.4
ADCC % (NK effectors; transfected Jurkat targets)	97±8.3	120±10.5	115±8.5
ADCC (NK effectors; LPS monocytes targets)	No activity	No activity	No activity
Binding (SPR)			
FcγIIIa V type%	101±2.3	126±7.7	127±4.9
FcγIIIa F type%	103±2.8	126±6.3	124±6.0

- a. All data are expressed at % activity relative to a CT-P13 reference standard included in the same assay.

Additional data are required to demonstrate that CT-P13 and the referenced product, US-licensed Remicade, are similar from an immunogenicity perspective. There are two major concerns leading to the above conclusion. The first is that there is a difference in the subvisible particulate levels between US-licensed Remicade and EU-approved infliximab, providing an obstacle to the analytical bridging needed to support the use of the clinical immunogenicity data derived from studies involving EU-approved infliximab. Secondly, there is a statistically meaningful difference in the immunogenicity incidence rates between subjects administered CT-P13 compared to subjects administered US-licensed Remicade (Study CT-P13.4, single dose in healthy controls). Even if an analytical bridge can be established between US-licensed Remicade and EU-approved infliximab (likely following submission of additional subvisible particulate data), Study CT-P13.4 will still show a trend toward higher neutralizing immunogenic responses in subjects receiving CT-P13 compared to the pool of subjects receiving either EU-approved infliximab or US-licensed Remicade. These results are in contrast to those from larger immunogenicity studies involving arthritis patients receiving repeated doses of study drugs (CT-P13 1.1 and 3.1), in which the rates of immunogenicity between CT-P13 and EU-approved infliximab were similar.

To overcome these deficiencies, Celltrion will be requested to provide subvisible particulate data from additional lots to support analytical bridging. Moreover, they will be asked to address concerns regarding a trend toward increased immunogenic responses in subjects receiving CT-P13 in study 3.4, as well as provide a comprehensive argument

that their data support the similarity of the immunogenicity profiles of CT-P13 and US-licensed Remicade.

The current understanding of the clinical activity of infliximab is unclear with respect to FcγRIII binding and downstream ADCC of mTNF⁺ inflammatory. It is also possible that ADCC of mTNF⁺ cells may be more clinically relevant in the treatment of some disease indications (i.e., IBD) than others (e.g., RA). This leads to residual uncertainty regarding assigning the status of analytical highly similar.

At this time, it is not possible to categorically remove this uncertainty in the absence of additional data (e.g., clinical data in the setting of IBD treatment). Therefore, the totality of analytical data is sufficient to conclude that CT-P13 is “similar” to the reference product, but at this time it is premature to conclude that it is “highly similar”.

Summary Basis for Complete Response

- CT-P13 is manufactured by a robust process with precautions for contamination. It is manufactured consistently, leading to a safe and effective product for human use.
- The majority of analytical methods found that CT-P13 was similar to the reference product, US-licensed Remicade. However, two critical Fc functional attributes, FcγRIII binding and ADCC activity differed by about 20%. The clinical significance of the activity of CT-P13 to induce ADCC of mTNF⁺ cells during the treatment of some disease indications (i.e. IBD) is unknown. This leads to residual uncertainty regarding assigning the status of analytical highly similar. At this time, due to the 20% lower ADCC activity of CT-P13, it is not possible to categorically remove this risk in the absence of additional data (e.g, clinical data in the setting of IBD treatment). Therefore the totality of analytical data is sufficient to conclude that CT-P13 is “similar” to the reference product, but at this time it is premature to conclude that it is “highly similar”.
- **ENVIRONMENTAL ASSESSMENT OR CLAIM OF CATEGORICAL EXCLUSION.** CT-P13 qualifies for a categorical exclusion under 21 CFR 25.31(c), no extraordinary circumstances exist. There is minimal impact expected of CT-P13 manufacturing on the environment.

LIST OF DEFICIENCIES TO BE COMMUNICATED (Proposed Language)

- 1) You provided data from a limited number of lots showing lower levels of subvisible particulates in the range of 1 to 5 microns in US-licensed Remicade compared to both CT-P13 and EU-approved infliximab. These apparent differences may be due to the limited number of lots of CT-P13, US-licensed Remicade and EU-approved infliximab used to perform the analysis. However, these results suggest that analytical differences may exist between US-licensed Remicade and EU-approved infliximab, which, if confirmed, could impact the assessment the adequacy of the analytical bridge between

the three products. To address this concern, provide results of subvisible particles analysis from additional CT-P13, US-licensed Remicade and EU-approved infliximab lots.

- 2) You conducted comparative clinical study CT-P13 1.4 to assess the immunogenicity of CT-P13 and US-licensed Remicade. Even if you confirm an analytical bridge is between US-licensed Remicade and EU-approved infliximab (additional subvisible particulate data requested, see above), this study demonstrates a potential trend toward increased neutralizing immunogenic responses in CT-P13-treated subjects compared to the pooled group of subjects receiving either U.S.-licensed Remicade or EU-approved infliximab [27% vs. 19%, respectively (90% confidence Interval: -2.5%, +20%)]. This result is accompanied by differences in binding antibody titers (mean transformed titers 4.74 vs 3.63 in CT-P13 and US-Remicade samples, respectively) and neutralizing antibody titers (mean transformed titers 4.63 vs 2.63, respectively). Although it is possible that this trend could represent a statistical anomaly, is also possible that these data reflect a true clinical difference resulting from analytical differences between US-licensed Remicade and CT-P13. To resolve this deficiency, address these concerns and provide a rationale for why the results from study CT-P13 1.4 are in alignment with the conclusion that the immunogenicity profiles of CT-P13 and US-licensed Remicade are similar.
- 3) In your submission, you evaluated the analytical similarity of CT-P13 and US-licensed Remicade using a variety of functional assays. Your data generated using a standard NK-cell based killing ADCC assay suggest that CT-P13 has ~20% lower ADCC activity compared to the reference product US-licensed Remicade. The difference in ADCC leads to residual uncertainty regarding the conclusion that CT-P13 is highly similar to US-licensed Remicade, as the role of ADCC remains uncertain in the clinical activity of the innovator product (e.g., in the setting of inflammatory bowel disease). Furthermore, you did not adequately justify the impact of the difference in ADCC on the analytical similarity assessment and did not identify the structural basis underlying this difference. For example, you should determine whether the H2L1 variant that is present at relatively high levels in CT-P13 compared to US-licensed Remicade plays a role in decreasing NK-dependent ADCC activity. On the other hand, the Agency has not excluded the possibility that analysis of additional lots of CT-P13 and innovator lots could overcome a statistical anomaly due to the analysis of a limited number of lots. To this point, we note that prior differences in glycan patterns were reduced when additional lots of CT-P13 and innovator product were analyzed. To address the current deficiency with respect to differences in ADCC activity, we recommend that you repeat the evaluation of ADCC using additional lots to determine whether the ADCC difference you have reported decreases when additional lots are evaluated (i.e., due to small sample size). If the difference in ADCC persists following analysis of additional lots, you should identify and demonstrate control of the product quality attributes that underlie ADCC activity in CT-P13 (e.g., glycan pattern, contribution of H2L1 variant, etc.) and provide an adequate justification, including an evaluation of the role of ADCC particularly in the setting of inflammatory bowel disease, that the observed difference in ADCC does not have clinical impact.

- 4) The current drug product stability data using Process B batches of CT-P13 support an expiry date of 42, not (b) (4) months. To address this concern, adjust your proposed expiry dating to reflect existing data and provide a stability protocol to support post-approval expiry extension.
- 5) We acknowledge the plan outlined in your 10 Apr 2015 letter to develop and validate a revised version of the visible particle test for reconstituted drug product. The revised test will use (b) (4) and visual inspection of 20 reconstituted vials. Data supporting the assay revision has not been provided to the BLA. To address this concern, submit the assay SOP, validation report and revised specification to the Agency for review.



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

Date: 5/5/2015
To: Administrative File, STN 125544/0
From: Bo Chi, Ph.D., CDER/OPQ/OPF/DMA/Branch IV
Endorsement: Patricia Hughes, Ph.D., Acting Branch Chief, CDER/OPQ/OPF/DMA/Branch IV
Subject: New 351(K) Biologic License Application (BLA)
Applicant: Celltrion, Inc.
US License: 1996
Facility: CELLTRION, Inc., 20, Academy-ro 51 beon-gil, Yeonsu-gu, Incheon, Republic of Korea
FEI: 3005241015
Product: CT-P13
Dosage: Powder for solution, 100 mg/vial, intravenous
Indication: Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, ulcerative colitis, Crohn's disease, pediatric ulcerative colitis¹ and pediatric Crohn's disease
BsUFA date: June 8, 2015

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

Review Summary

Celltrion has submitted this Biologics License Application (BLA) under 351(k) of the Public Health Service Act for CT-P13, an anti-human tumor necrosis factor alpha (TNF α) human-murine IgG1 monoclonal antibody. This is a proposed biosimilar product to US licensed Remicade[®] (infliximab). The BLA seeks licensure for the same indications for which the reference product Remicade is currently approved. CT-P13 drug substance (DS) and drug product (DP) are manufactured at Celltrion, Incheon, Republic of Korea. This application contains CMC information in an eCTD format.

Assessment

Drug Product

Description of the Composition of the Drug Product (3.2.P.1):

¹ This reflects information for Inflectra that Celltrion submitted on August 8, 2014. We note that the indication for pediatric ulcerative colitis is protected by orphan drug exclusivity expiring on September 23, 2018. See the Orphan Drug Designations and Approvals database at <http://www.accessdata.fda.gov/scripts/opdlisting/oopd/index.cfm>.

CT-P13 drug product is supplied as lyophilized powder in a 20 mL type I borosilicate glass vial. Each drug product vial contains 100 mg CT-P13 protein, 2.2 mg sodium dihydrogen phosphate monohydrate, 6.1 mg di-sodium hydrogen phosphate dihydrate, 500 mg sucrose, and 0.5 mg polysorbate 80. There is no overfill in the vial. The lyophilized cake is reconstituted with 10 mL of sterile water for injection (SWFI) to yield a single dose containing 10 mg/mL CP-P13, 5 mM sodium phosphate, 5 % (w/v) sucrose, and 0.005 % polysorbate 80, at pH 7.2. The SWFI diluent is not provided.

Reviewer comment: The information on DP composition is adequately described.

Satisfactory

Pharmaceutical Development (3.2.P.2):

CT-P13 is a chimeric human-murine immunoglobulin G1 (IgG1) monoclonal antibody to human tumor necrosis factor alpha (TNF α). It inhibits binding of TNF α to its receptors and neutralizes the biological activity of TNF α . CT-P13 is a glycoprotein with 1 N-linked glycosylation site in the CH2 domain of each heavy chain. The primary sequence and structure of CT-P13 are identical to the reference product Remicade. CT-P13 is manufactured in Sp2/0-Ag14 cells.

Microbiological Attributes (3.2.P.2.5):

Container closure integrity (CCI) test

The BLA provided Table 3.2.P.2.5–1 below for the container closure integrity test results:

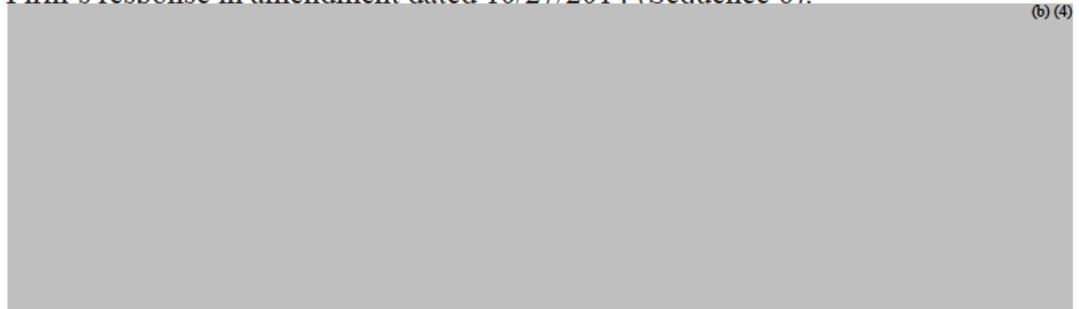
Table 3.2.P.2.5–1: Container Closure Integrity Test Result

Container	Test Method	No. of Vials	Acceptance Criteria	Result	Pass/Fail
20 mL Vial/Rubber Stopper/Flip-Off Seal	Microbial Immersion	200	Negative	Negative	Pass
	Dye Penetration	20*3	Negative	Negative	Pass
	Vacuum Pressure Decay	20*3	(b) (4)	All vials passed the test	Pass

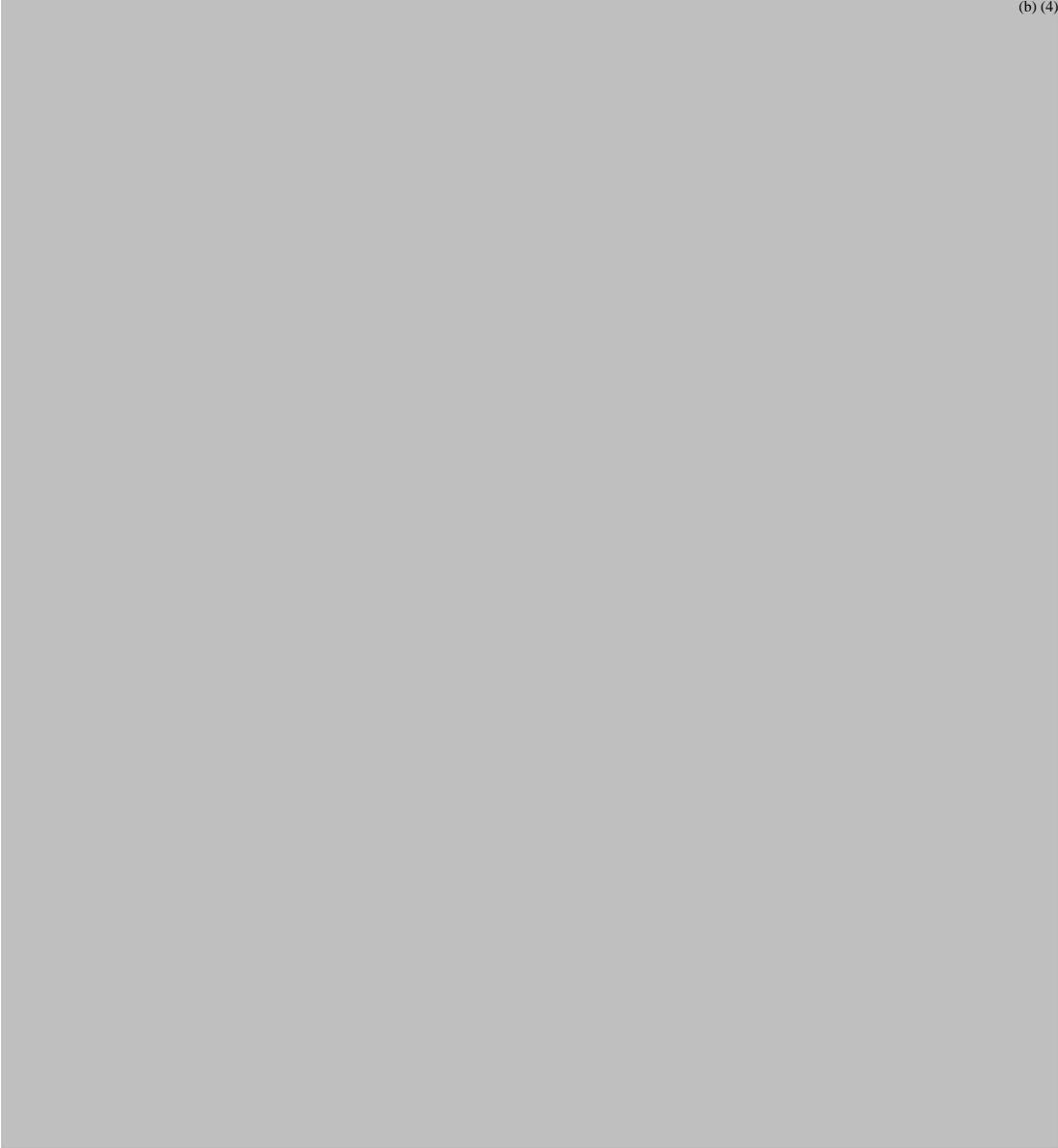
FDA question:

Provide summary validation data of the container closure integrity tests used to determine the integrity of the CT-P13 drug product primary container closure. Please include the sensitivity of the tests (smallest breach size the tests can detect), challenge conditions (pressure/vacuum applied and duration), and dye solution concentration and microbial challenge concentration. The sensitivity of the microbial ingress test should be correlated to that of the dye ingress test.

Firm’s response in amendment dated 10/27/2014 (Sequence 6):



(b) (4)

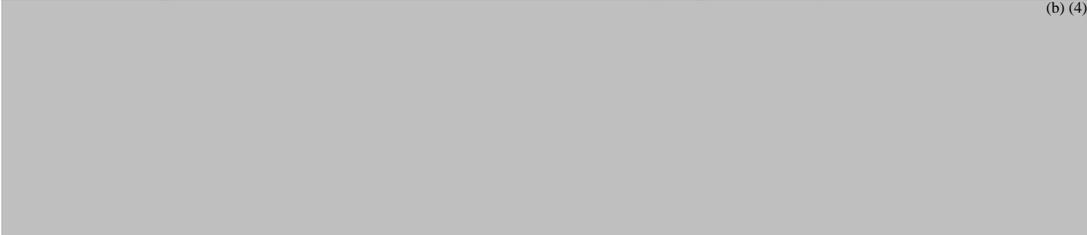


FDA follow-up question:

Please provide the sensitivity of the microbial ingress test and correlation to that of the dye ingress and pressure decay tests as soon as the information is available within the review cycle.

Firm's response in amendment dated 1/15/2015 (Sequence 16):

(b) (4)





Reviewer comment: The container closure integrity tests are adequately validated. Additional information on the vacuum decay test is provided below.

Satisfactory

Describe how the vacuum decay test is conducted and the challenges applied to the vials during the test.

Firm's response in amendment dated 1/15/2015 (Sequence 16):



Please explain how the positive controls are prepared for the studies provided in Table 3.2.P.2.5–1. Provide the results of the positive and negative controls for the studies.

Firm's response in amendment dated 1/15/2015 (Sequence 16):



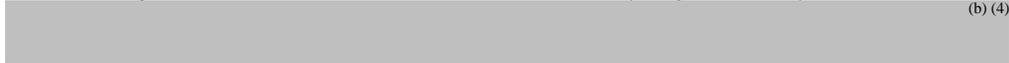
Reviewer comment: It appears that no positive controls were used for dye ingress or microbial ingress tests conducted for the studies provided in Table 3.2.P.2.5–1. However, a positive control with calibrated leak is used prior to each vacuum decay test conducted. In addition, the sensitivities of the microbial ingress test, dye ingress test, and vacuum decay test were demonstrated to be comparable in the validation studies. Therefore, the vacuum decay test results demonstrating integral container closure of CT-P13 DP vials are reliable.

During the pre-license inspection, the sponsor communicated that some of the data provided in Table 3.2.P.2.5-1 were generated using vials crimped at a site other than Celltrion. It was not clear if the crimping parameters used for those vials are applicable to the process at Celltrion.

FDA question:

During the pre-license inspection (PLI), it was communicated that some of the data provided in Table 3.2.P.2.5-1 were generated using vials crimped at a site other than Celltrion. It was not clear if the crimping parameters used for those vials are applicable to the process at Celltrion. Provide the relevant container closure integrity data using CT-P13 drug product vials manufactured at Celltrion.

Firm's response in amendment dated 4/17/2015 (Sequence 30):



(b) (4)

Reviewer comment: The integrity of the container closure for CT-P13 DP was adequately validated using the validated microbial immersion, dye penetration, and vacuum pressure decay tests.

Satisfactory

FDA question:

Provide summary container closure integrity test results from CP-P13 vials crimped using the worst-case crimping parameters (e.g., capping pressure, speed, head height). Explain how the positive controls for the routine container closure integrity tests are prepared.

Firm's response in amendment dated 10/27/2014 (Sequence 6):

(b) (4)

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immediately following this page

(b) (4)

Reviewer comment: The above results were obtained using an engineering batch (12B1C001) manufactured at Celltrion. The positive control for the vacuum decay method has a breach size of (b) (4) μm . Container closure integrity test is conducted for each DP batch manufactured.

FDA follow-up question:

You indicate that the target capping head height is (b) (4). Please provide the unit for head height.

Firm's response in amendment dated 1/15/2015 (Sequence 16) copied:

(b) (4)

Table 4).



Satisfactory

Manufacture (3.2.P.3):

Manufacturers (3.2.P.3.1):

CELLTRION, Inc.,
20, Academy-ro 51 beon-gil,
Yeonsu-gu, Incheon,
406-840, Republic of Korea
FEI: 3005241015

DP manufacturing, release and stability testing of DS and DP, secondary packaging and labeling, testing of MCB and WCB

CELLTRION, Inc. (b) (4)
23, Academy-ro,
Yeonsu-gu, Incheon,
406-840, Republic of Korea
FEI: 3005241015
Release and stability testing of DP

Description of the Manufacturing Process and Process Controls (3.2.P.3.3)



Stability (3.2.P.8)

The proposed shelf life for CT-P13 drug product is (b) (4) months when stored at 2-8°C. At least one commercial batch will be added to the stability program. Sterility test is conducted initially, at 12 month, and then every 6 months thereafter until expiry. Endotoxin is tested initially and every 6 months until expiry. A container closure integrity testing is not on the stability program.

FDA question:

We recommend conducting container closure integrity test in lieu of sterility test for stability samples annually and at expiry. Describe the container closure integrity test that will be used on the stability program, including the challenge conditions, sensitivity of the test (smallest breach size the test can detect), and how the positive controls will be

prepared.

Firm's response in amendment dated 1/15/2015 (Sequence 16):

Both sterility test and container closure integrity test (dye ingress test) will be conducted annually and at expiry. (b) (4)

The challenge conditions and sensitivity of the test are the same as described in 3.2.P.2.5 for the dye ingress test.

FDA follow-up question:

The positive controls for the container closure integrity tests use (b) (4) gauge needles. This provides a very large breach. Please use needles with smaller inner diameters, e.g., (b) (4) gauge needles for positive controls for the dye penetration test for samples on stability.

Firm's response in amendment dated 4/17/2015 (Sequence 30):

Satisfactory

Facilities and equipment (3.2.A.1)

CT-P13 drug product is manufactured at the Fill & Finish area at Celltrion Plant (b) (4). The facility is a multi-product facility. (b) (4). Diagrams for the area classifications and the flow of product, waste, personnel, and raw materials are provided and are adequate. A list of shared product-contact equipment is provided. The other products manufactured in the facility are (b) (4). No potent or toxic products are manufactured in the facility.

FDA question:

The labeling in the facility diagrams provided in Section 3.2.A.1, "Facilities and equipment" for the DP facility is not legible. Provide diagrams of better quality.

Diagrams of better quality were provided in amendment dated 1/15/2015 (Sequence 16).

Satisfactory

Conclusion

- I. The drug product section of the BLA, as amended, is recommended for approval from a sterility assurance and product quality microbiology perspective.
- II. Information and data in this submission not related to drug product sterility assurance

was not evaluated and should be reviewed by an OBP reviewer.

III. See panorama for GMP status of the relevant facilities.

Cc: Chi
Hughes
Ton

Primary reviewer signature

Bo Chi -A
Digitally signed by Bo Chi -A
DN: c=US, o=U.S. Government, ou=HHS,
ou=FDA, ou=People, cn=Bo Chi -A,
0.9.2342.19200300.100.1.1=1300194820
Date: 2015.05.07 10:16:10 -04'00'

Secondary reviewer signature

**Patricia F.
Hughestroost
-S**
Digitally signed by Patricia F.
Hughestroost -S
DN: c=US, o=U.S. Government,
ou=HHS, ou=FDA, ou=People,
0.9.2342.19200300.100.1.1=13000965
47, cn=Patricia F. Hughestroost -S
Date: 2015.05.07 10:24:27 -04'00'

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

BLA Number: STN 125544 **Applicant:** Celltrion **Stamp Date:** August 08, 2014

Established/Proper Name: Proposed biosimilar to infliximab/CT-P13 **BLA Type:** Biosimilar, standard

On **initial** overview of the BLA/NDA application for filing:

CTD Module 1 Contents	Present?	If not, justification, action & status
Cover Letter	Y	
Form 356h completed	Y	
<input type="checkbox"/> including list of all establishment sites and their registration numbers	Y	
Comprehensive Table of Contents	Y N	Not required
Environmental assessment or request for categorical exclusion (21 CFR Part 25)	Y	
Labeling:	Y	
<input type="checkbox"/> PI –non-annotated	Y N	
<input type="checkbox"/> PI –annotated	Y N	
<input type="checkbox"/> PI (electronic)	Y N	
<input type="checkbox"/> Medication Guide	Y N	
<input type="checkbox"/> Patient Insert	Y N	
<input type="checkbox"/> package and container	Y N	
<input type="checkbox"/> diluent	Y N	
<input type="checkbox"/> other components	Y N	
<input type="checkbox"/> established name (e.g. USAN)	Y N	
<input type="checkbox"/> proprietary name (for review)	Y N	

Examples of Filing Issues	Yes?	If not, justification, action & status
Content, presentation, and organization of paper and electronic components sufficient to permit substantive review?: Examples include:	Y	
<input type="checkbox"/> legible	Y	
<input type="checkbox"/> English (or translated into English)	Y	Batch records are in Korean; an English translation of the MBR is provided.
<input type="checkbox"/> compatible file formats	Y	
<input type="checkbox"/> navigable hyper-links	Y	
<input type="checkbox"/> interpretable data tabulations (line listings) & graphical displays	Y	
<input type="checkbox"/> summary reports reference the location of individual data and records	Y	
<input type="checkbox"/> all electronic submission components usable (e.g. conforms to published guidance)	Y	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
Companion application received if a shared or divided manufacturing arrangement	Y N	Not applicable

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	N	Not necessary
Introduction to the summary documents (1 page) [2.2]	Y	
Quality overall summary [2.3]	Y	
<input type="checkbox"/> Drug Substance	Y	
<input type="checkbox"/> Drug Product	Y	
<input type="checkbox"/> Facilities and Equipment	Y	
<input type="checkbox"/> Adventitious Agents Safety Evaluation	Y N	Defer to OBP
<input type="checkbox"/> Novel Excipients	Y N	Defer to OBP
<input type="checkbox"/> Executed Batch Records	Y N	Defer to OBP
<input type="checkbox"/> Method Validation Package	N	There is no method validation package in this section, but method qualification reports are included in Section 3.2.R; DS endotoxin and bioburden qualification reports are not included and will be asked during the review.
<input type="checkbox"/> Comparability Protocols	N	Not applicable

CTD Module 3 Contents	Present?	If not, justification, action & status
Module Table of Contents [3.1]	Y N	
Drug Substance [3.2.S]		
<input type="checkbox"/> general info	Y N	Defer to OBP
<input type="checkbox"/> nomenclature		
<input type="checkbox"/> structure (e.g. sequence, glycosylation sites)		
<input type="checkbox"/> properties		
<input type="checkbox"/> manufacturers (names, locations, and responsibilities of all sites involved)	Y	
<input type="checkbox"/> description of manufacturing process and process control	Y	Description of manufacturing process is adequate from a microbial control perspective
<input type="checkbox"/> batch numbering and pooling scheme		
<input type="checkbox"/> cell culture and harvest		
<input type="checkbox"/> purification		
<input type="checkbox"/> filling, storage and shipping		
<input type="checkbox"/> control of materials	Y N	Defer to OBP.

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
<ul style="list-style-type: none"> ○ raw materials and reagents ○ biological source and starting materials ○ cell substrate: source, history, and generation ○ cell banking system, characterization, and testing □ control of critical steps and intermediates <ul style="list-style-type: none"> ○ justification of specifications ○ stability □ process validation (prospective plan, results, analysis, and conclusions) 	<p>Y</p> <p>Y</p>	<p>Information on the microbial controls of critical steps are provided</p> <p>Validation data for the microbial control strategy, process hold times, and qualification for in-process bioburden and endotoxin tests are included. Validation of maximum hold times for microbial quality not included but it will be requested during the review process.</p>
<ul style="list-style-type: none"> □ manufacturing process development (describe changes during non-clinical and clinical development; justification for changes) 	Y N	Defer to OBP
<ul style="list-style-type: none"> □ characterization of drug substance □ control of drug substance <ul style="list-style-type: none"> ○ specifications <ul style="list-style-type: none"> ○ justification of specs. ○ analytical procedures ○ analytical method validation ○ batch analyses 	Y N Y	Defer to OBP
<ul style="list-style-type: none"> □ reference standards 	Y N	Defer to OBP
<ul style="list-style-type: none"> □ container closure system 	Y	
<ul style="list-style-type: none"> □ stability <ul style="list-style-type: none"> □ summary □ post-approval protocol and commitment □ pre-approval <ul style="list-style-type: none"> ○ protocol ○ results ○ method validation 	Y N Y	Defer to OBP
<ul style="list-style-type: none"> Drug Product [3.2.P] [Dosage Form] □ description and composition □ pharmaceutical development <ul style="list-style-type: none"> ○ preservative effectiveness ○ container-closure integrity □ manufacturers (names, locations, and responsibilities of all sites involved) 	<p>Y</p> <p>Y N</p> <p>Y</p> <p>Y</p>	<p>Not applicable.</p>

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status	
<input type="checkbox"/> batch formula	Y		
<input type="checkbox"/> description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y		
<input type="checkbox"/> controls of critical steps and intermediates	Y		
<input type="checkbox"/> process validation including aseptic processing & sterility assurance: <ul style="list-style-type: none"> <input type="checkbox"/> Filter validation <input type="checkbox"/> Component, container, closure depyrogenation and sterilization validation <input type="checkbox"/> Validation of aseptic processing (media simulations) <input type="checkbox"/> Environmental Monitoring Program <input type="checkbox"/> Lyophilizer validation <input type="checkbox"/> Other needed validation data (hold times) 	Y		
<input type="checkbox"/> control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin)	Y	N	Defer to OBP
<input type="checkbox"/> control of drug product (justification of specifications; analytical method validation; batch analyses, characterization of impurities)	Y		
<input type="checkbox"/> reference standards or materials	Y	N	Defer to OBP
<input type="checkbox"/> container closure system [3.2.P.7] <ul style="list-style-type: none"> <input type="checkbox"/> specifications (vial, elastomer, drawings) <input type="checkbox"/> availability of DMF & LOAs <input type="checkbox"/> administration device(s) 	Y	N	Not applicable
<input type="checkbox"/> stability <ul style="list-style-type: none"> <input type="checkbox"/> summary <input type="checkbox"/> post-approval protocol and commitment <input type="checkbox"/> pre-approval <ul style="list-style-type: none"> <input type="checkbox"/> protocol <input type="checkbox"/> results <input type="checkbox"/> method validation 	Y		

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
Diluent (vials or filled syringes) [3.2P']		Not applicable
<input type="checkbox"/> description and composition of diluent	Y N	
<input type="checkbox"/> pharmaceutical development	Y N	
<input type="checkbox"/> preservative effectiveness	Y N	
<input type="checkbox"/> container-closure integrity		
<input type="checkbox"/> manufacturers (names, locations, and responsibilities of all sites involved)	Y N	
<input type="checkbox"/> batch formula	Y N	
<input type="checkbox"/> description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y N	
<input type="checkbox"/> controls of critical steps and intermediates	Y N	
<input type="checkbox"/> process validation including aseptic processing & sterility assurance:	Y N	
<input type="checkbox"/> Filter validation		
<input type="checkbox"/> Component, container, closure depyrogenation and sterilization validation	Y N	
<input type="checkbox"/> Validation of aseptic processing (media simulations)	Y N	
<input type="checkbox"/> Environmental Monitoring Program		
<input type="checkbox"/> Lyophilizer sterilization validation	Y N	
<input type="checkbox"/> Other needed validation data (hold times)	Y N	
<input type="checkbox"/> control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin, other novel excipients)	Y N	
<input type="checkbox"/> control of diluent (justification of specifications; analytical method validation, batch analysis, characterization of impurities)	Y N	
<input type="checkbox"/> reference standards		
<input type="checkbox"/> container closure system		
<input type="checkbox"/> specifications (vial, elastomer, drawings)	Y N	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
<ul style="list-style-type: none"> ○ availability of DMF & LOAs <input type="checkbox"/> stability <ul style="list-style-type: none"> <input type="checkbox"/> summary <input type="checkbox"/> post-approval protocol and commitment <input type="checkbox"/> pre-approval <ul style="list-style-type: none"> ○ protocol ○ results 	Y N Y N	
Other components to be marketed (full description and supporting data, as listed above): <ul style="list-style-type: none"> <input type="checkbox"/> other devices <input type="checkbox"/> other marketed chemicals (e.g. part of kit) 	Y N Y N	Not applicable
Appendices for Biotech Products [3.2.A] <ul style="list-style-type: none"> <input type="checkbox"/> facilities and equipment <ul style="list-style-type: none"> ○ manufacturing flow; adjacent areas ○ other products in facility ○ equipment dedication, preparation, sterilization and storage ○ procedures and design features to prevent contamination and cross-contamination <input type="checkbox"/> adventitious agents safety evaluation (viral and non-viral) e.g.: <ul style="list-style-type: none"> ○ avoidance and control procedures ○ cell line qualification ○ other materials of biological origin ○ viral testing of unprocessed bulk ○ viral clearance studies ○ testing at appropriate stages of production <input type="checkbox"/> novel excipients 	Y Y N Y N	Defer to OBP Not applicable
USA Regional Information [3.2.R] <ul style="list-style-type: none"> <input type="checkbox"/> executed batch records <input type="checkbox"/> method validation package <input type="checkbox"/> comparability protocols 	Y N Y N N	Defer to OBP Defer to OBP Not applicable
Literature references and copies [3.3]	Y	

Examples of Filing Issues	Yes?	If not, justification, action & status
Includes production data on drug	Y	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
substance and drug product manufactured in the facility intended to be licensed (including pilot facilities) using the final production process(es)		
Includes data demonstrating consistency of manufacture	Y	
Includes complete description of product lots and manufacturing process utilized for clinical studies	Y N	Defer to OBP
Describes changes in the manufacturing process, from material used in clinical trial to commercial production lots	Y N	Defer to OBP
Data demonstrating comparability of product to be marketed to that used in clinical trials (when significant changes in manufacturing processes or facilities have occurred)	Y N	Defer to OBP
Certification that all facilities are ready for inspection	Y	
Data establishing stability of the product through the proposed dating period and a stability protocol describing the test methods used and time intervals for product assessment.	Y N	Defer to OBP
If not using a test or process specified by regulation, data is provided to show the alternate is equivalent (21 CFR 610.9) to that specified by regulation. List: <input type="checkbox"/> LAL instead of rabbit pyrogen <input type="checkbox"/> mycoplasma <input type="checkbox"/> sterility	Y N Y N Y	The sponsor has committed to provide the rabbit pyrogen test results in four months. Defer to OBP
Identification by lot number, and submission upon request, of sample(s) representative of the product to be marketed; summaries of test results for those samples	Y	
Floor diagrams that address the flow of the manufacturing process for the drug substance and drug product	Y	
Description of precautions taken to prevent product contamination and cross-contamination, including identification of other products utilizing the same	Y	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
manufacturing areas and equipment		

IS THE PRODUCT QUALITY SECTION OF THE APPLICATION FILEABLE? ___ Yes ___

If the application is not fileable from product quality perspective, state the reasons and provide comments to be sent to the Applicant.

N/A

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

BO CHI
09/30/2014

REYES CANDAU-CHACON
09/30/2014

PATRICIA F HUGHES TROOST
10/01/2014

PRODUCT QUALITY (Biotechnology)

FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)

BLA/NDA Number: 125544 Applicant: Celltrion Inc.

Stamp Date: 8/8/2014

Established/Proper Name: BLA/NDA Type: BLA
Infliximab

On **initial** overview of the BLA/NDA application for filing:

CTD Module 1 Contents	Present?	If not, justification, action & status
Cover Letter	Y N	
Form 356h completed	Y N	
<input type="checkbox"/> including list of all establishment sites and their registration numbers	Y N	
Comprehensive Table of Contents	Y N	
Environmental assessment or request for categorical exclusion (21 CFR Part 25)	Y N	
Labeling:	Y N	
<input type="checkbox"/> PI –non-annotated	Y N	
<input type="checkbox"/> PI –annotated	Y N	
<input type="checkbox"/> PI (electronic)	Y N	
<input type="checkbox"/> Medication Guide	Y N	
<input type="checkbox"/> Patient Insert	Y N	
<input type="checkbox"/> package and container	Y N	
<input type="checkbox"/> diluent	Y N	No diluent
<input type="checkbox"/> other components	Y N	No other components
<input type="checkbox"/> established name (e.g. USAN)	Y N	
<input type="checkbox"/> proprietary name (for review)	Y N	

Examples of Filing Issues	Yes?	If not, justification, action & status
Content, presentation, and organization of paper and electronic components sufficient to permit substantive review?: Examples include:	Y N	
<input type="checkbox"/> legible	Y N	
<input type="checkbox"/> English (or translated into English)	Y N	
<input type="checkbox"/> compatible file formats	Y N	
<input type="checkbox"/> navigable hyper-links	Y N	
<input type="checkbox"/> interpretable data tabulations (line listings) & graphical displays	Y N	
<input type="checkbox"/> summary reports reference the location of individual data and records	Y N	
<input type="checkbox"/> all electronic submission components usable (e.g. conforms to published guidance)	Y N	
Companion application received if a shared or divided manufacturing	Y N	No divided manufacture

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
arrangement		

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	Y N	
Introduction to the summary documents (1 page) [2.2]	Y N	
Quality overall summary [2.3]	Y N	
<input type="checkbox"/> Drug Substance	Y N	
<input type="checkbox"/> Drug Product	Y N	
<input type="checkbox"/> Facilities and Equipment	Y N	
<input type="checkbox"/> Adventitious Agents Safety Evaluation	Y N	
<input type="checkbox"/> Novel Excipients	Y N	
<input type="checkbox"/> Executed Batch Records	Y N	
<input type="checkbox"/> Method Validation Package	Y N	
<input type="checkbox"/> Comparability Protocols	Y N	

CTD Module 3 Contents	Present?	If not, justification, action & status
Module Table of Contents [3.1]	Y N	Sections 3.2.S and 3.2.P have sufficient TOCs for DS and DP, respectively
Drug Substance [3.2.S]		
<input type="checkbox"/> general info	Y N	
<input type="checkbox"/> nomenclature		
<input type="checkbox"/> structure (e.g. sequence, glycosylation sites)		
<input type="checkbox"/> properties		
<input type="checkbox"/> manufacturers (names, locations, and responsibilities of all sites involved)	Y N	
<input type="checkbox"/> description of manufacturing process and process control	Y N	
<input type="checkbox"/> batch numbering and pooling scheme		
<input type="checkbox"/> cell culture and harvest		
<input type="checkbox"/> purification		
<input type="checkbox"/> filling, storage and shipping		
<input type="checkbox"/> control of materials	Y N	
<input type="checkbox"/> raw materials and reagents		
<input type="checkbox"/> biological source and starting materials		
<input type="checkbox"/> cell substrate: source, history, and generation		
<input type="checkbox"/> cell banking system, characterization, and testing		
<input type="checkbox"/> control of critical steps and intermediates	Y N	

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
<ul style="list-style-type: none"> ○ Validation of aseptic processing (media simulations) ○ Environmental Monitoring Program ○ Lyophilizer validation ○ Other needed validation data (hold times) <input type="checkbox"/> control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin) <input type="checkbox"/> control of drug product (justification of specifications; analytical method validation; batch analyses, characterization of impurities) <input type="checkbox"/> reference standards or materials <input type="checkbox"/> container closure system [3.2.P.7] <ul style="list-style-type: none"> ○ specifications (vial, elastomer, drawings) ○ availability of DMF & LOAs ○ administration device(s) <input type="checkbox"/> stability <ul style="list-style-type: none"> <input type="checkbox"/> summary <input type="checkbox"/> post-approval protocol and commitment <input type="checkbox"/> pre-approval <ul style="list-style-type: none"> ○ protocol ○ results ○ method validation 	<p align="center">Y N</p>	
<p>Diluent (vials or filled syringes) [3.2P']</p> <ul style="list-style-type: none"> <input type="checkbox"/> description and composition of diluent <input type="checkbox"/> pharmaceutical development <ul style="list-style-type: none"> ○ preservative effectiveness ○ container-closure integrity <input type="checkbox"/> manufacturers (names, locations, and responsibilities of all sites involved) <input type="checkbox"/> batch formula <input type="checkbox"/> description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities) <input type="checkbox"/> controls of critical steps and intermediates 	<p align="center">Y N</p>	<p>No diluent accompanies the product</p>

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
<input type="checkbox"/> process validation including aseptic processing & sterility assurance: <ul style="list-style-type: none"> <input type="checkbox"/> Filter validation <input type="checkbox"/> Component, container, closure depyrogenation and sterilization validation <input type="checkbox"/> Validation of aseptic processing (media simulations) <input type="checkbox"/> Environmental Monitoring Program <input type="checkbox"/> Lyophilizer sterilization validation <input type="checkbox"/> Other needed validation data (hold times) 	<p>Y N</p> <p>Y N</p> <p>Y N</p> <p>Y N</p> <p>Y N</p> <p>Y N</p>	
<input type="checkbox"/> control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin, other novel excipients)	Y N	
<input type="checkbox"/> control of diluent (justification of specifications; analytical method validation, batch analysis, characterization of impurities)	Y N	
<input type="checkbox"/> reference standards	Y N	
<input type="checkbox"/> container closure system <ul style="list-style-type: none"> <input type="checkbox"/> specifications (vial, elastomer, drawings) <input type="checkbox"/> availability of DMF & LOAs 	Y N	
<input type="checkbox"/> stability <ul style="list-style-type: none"> <input type="checkbox"/> summary <input type="checkbox"/> post-approval protocol and commitment <input type="checkbox"/> pre-approval <ul style="list-style-type: none"> <input type="checkbox"/> protocol <input type="checkbox"/> results 	Y N	
Other components to be marketed (full description and supporting data, as listed above):		
<input type="checkbox"/> other devices	Y N	The DP is a lyophilized powder within a capped vial
<input type="checkbox"/> other marketed chemicals (e.g. part of kit)	Y N	
Appendices for Biotech Products [3.2.A]		
<input type="checkbox"/> facilities and equipment <ul style="list-style-type: none"> <input type="checkbox"/> manufacturing flow; adjacent areas 	Y N	

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
for inspection		
Data establishing stability of the product through the proposed dating period and a stability protocol describing the test methods used and time intervals for product assessment.	Y N	
If not using a test or process specified by regulation, data is provided to show the alternate is equivalent (21 CFR 610.9) to that specified by regulation. List: <input type="checkbox"/> LAL instead of rabbit pyrogen <input type="checkbox"/> mycoplasma <input type="checkbox"/> sterility	Y N Y N Y N	
Identification by lot number, and submission upon request, of sample(s) representative of the product to be marketed; summaries of test results for those samples	Y N	
Floor diagrams that address the flow of the manufacturing process for the drug substance and drug product	Y N	
Description of precautions taken to prevent product contamination and cross-contamination, including identification of other products utilizing the same manufacturing areas and equipment	Y N	

IS THE PRODUCT QUALITY SECTION OF THE APPLICATION FILEABLE? **Yes** No

If the application is not fileable from product quality perspective, state the reasons and provide comments to be sent to the Applicant.

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Product Quality Reviewer(s) Date

Branch Chief/Team Leader/Supervisor Date

Division Director Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ERIK K READ
09/30/2014

KURT A BRORSON
09/30/2014

KATHLEEN A CLOUSE STREBEL
09/30/2014