

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761088Orig1s000

PRODUCT QUALITY REVIEW(S)

Recommendation: Approval

BLA Number: 761088

Review Number: Second round

Review Date: October 30, 2018

Drug Name/Dosage Form	Truxima (rituximab-abbs) injection, for intravenous use
Strength/Potency	100 mg/10 mL, 500 mg/50 mL (10 mg/mL concentration)
Route of Administration	Intravenous
Rx/OTC dispensed	Rx
Indication	In the BLA resubmission, the applicant (b) (4) is seeking to include only 3 of the 4 Non-Hodgkin's Lymphoma indications and none of the other the multiple indications approved for US-Rituximab.
Applicant/Sponsor	Celltrion, Inc.

This BLA was initially submitted on April 28, 2017. A Complete Response (CR) letter for clinical, product quality, and facility deficiencies was issued on February 28, 2018 (see my Executive Summary for the original BLA review cycle, dated 2/28/2018). On May 29, 2018, Celltrion submitted responses to address the deficiencies identified in the CR letter.

An Oncology Drug Advisory Committee (ODAC) meeting was held on October 10, 2018. The ODAC agreed that the totality of analytical, pharmacologic, immunogenicity and clinical evidence supported the licensure of CT-P10 for the proposed indications based on the high degree of similarity, except for minor components, between CT-P10 and U.S.-licensed Rituxan.

Product Overview

Note: There is no change in this section from my Executive Summary filed 2/28/2018.

Rituximab-abbs (CT-P10) is a chimeric monoclonal IgG1 antibody, and the rituximab-abbs drug product, Truxima, has been developed as a proposed biosimilar to U.S.-licensed Rituxan. Rituximab-abbs binds to CD20 antigen expressed on the surface of pre-B and mature B-lymphocytes, and malignant B cells. Upon binding to CD20, rituximab-abbs mediates B-cell lysis. Possible mechanisms of cell lysis include complement dependent cytotoxicity (CDC), antibody dependent cell mediated cytotoxicity (ADCC), and antibody dependent cellular phagocytosis (ADCP), which are applicable to mechanisms of action of rituximab-abbs in B cell malignancies and in autoimmune diseases.

The rituximab-abbs molecule is constructed as a chimera of mouse variable regions binding human CD20 on human IgG1 backbone, and has one N-linked glycosylation site on each heavy chain. The Fc regions of the heavy chains retain Fc effector functions important in mechanism of action of rituximab.

Rituximab-abbs is produced in genetically engineered CHO (b) (4) cells. Rituximab-abbs drug product, Truxima, is manufactured to the same concentration, formulation, and presentation as U.S.-licensed Rituxan. Truxima is a sterile, preservative-free, colorless to pale yellow, clear to opalescent solution for intravenous infusion and supplied in single-dose vials containing CT-P10 at 100 mg/10 mL or 500 mg/50 mL.

Quality Review Team

Discipline	Reviewer	Branch / Division
Drug Substance	Haoheng Yan	OPQ/OBP/DBRR IV
Drug Product	Haoheng Yan	OPQ/OBP/DBRR IV
Drug Substance Microbiology	Scott Nichols	OPQ/OPF/DMA IV
Drug Product Microbiology	Candace Gomez-Broughton	OPQ/OPF/DMA IV
Facility	Thuy Thanh Nguyen	OPQ/OPF/DIA
Immunogenicity assay	Haoheng Yan	OPQ/OBP/DBRR IV
Analytical Similarity	Nina Brahme	OPQ/OBP/DBRR IV
Labeling	Vicky Borders Hemphill	OPQ/OBP
Product quality Team Lead	Bazarragchaa Damdinsuren	OPQ/OBP/DBRR IV
Microbiology QAL	Reyes Candau-Chacon	OPQ/OPF/DMA IV
Facility Branch Chief	Peter Qiu	OPQ/OPF/DIA
CMC RPBM	Oumou Barry	OPQ/OPRO
Application Team Lead	Bazarragchaa Damdinsuren	OPQ/OBP/DBRR IV

Multidisciplinary Review Team:

Discipline	Reviewer	Office/Division
RPM	Esther Park	OHOP/DHP
Cross-disciplinary Team Lead	Vishal Bhatnagar	OHOP/DHP
Medical Officer	Rachel ErshlerStefanie Freeman, Nikolay Nikolov (Team Lead)	OHOP/DHP ODEII/DPARP
Pharm/Tox	Michael Manning	OHOP/DHP
Clinical Pharmacology	Sang Chung	ODEII/DPARP
CMC Statistics	Yu-Ting Weng	OHOP/DHP
Clinical Statistics	Jingjing Ye James Travis	OHOP/DHP ODEII/DPARP

Names:

- Proprietary Name: Truxima
- Non-Proprietary/USAN/INN: Rituximab-abbs/rituximab
- CAS Registry number: 174722-31-7
- Company/Laboratory code: CT-P10
- OBP systematic name: MAB CHIMERIC (IGG1) ANTI P11836 (CD20_HUMAN) [CT-P10]
- Other Names: None

Submissions Reviewed in BLA Resubmission Review Cycle:

Submission(s) Reviewed /sequence number	Document Date
STN 761088 /34 (BLA resubmission)	5/29/2018
STN 761088 /36 (response to IR #1)	8/2/2018
STN 761088 /43 (response to IR #2)	10/22/2018
STN 761088 /44 (response to IR #3)	10/25/2018

Quality Review Data Sheet

1. Legal Basis for Submission: 351(k)

2. Related/Supporting Documents: *No change for information in this section (refer to my Executive Summary filed on 2/28/2018).*

3. Consults: None

Executive Summary

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

The Office of Biotechnology Products, OPQ, CDER, recommends approval of STN 761088 for TRUXIMA (rituximab-abbs) manufactured by Celltrion, Inc. The data submitted in this application are adequate to support the conclusion that the manufacture of TRUXIMA is well-controlled and leads to a product that is pure and potent. We recommend that this product be approved for human use under conditions specified in the package insert.

The analytical similarity assessment as presented in the BLA is acceptable to support the conclusions that the biological product, rituximab-abbs, is highly similar to U.S.-licensed Rituxan.

Finally, each of the product quality, microbiology, and facility deficiencies and additional comments described in the February 28, 2018, Complete Response letter were adequately addressed by Celltrion, Inc. in the May 29, 2018 resubmission.

B. Approval Action Letter Language:

- Manufacturing location:
 - Drug Substance: Celltrion, Inc., Incheon, Republic of Korea
 - Drug Product: Celltrion, Inc., Incheon, Republic of Korea
- Fill size and dosage forms:
 - 100 mg/10 mL (10 mg/mL concentration), single-dose vial, injection
 - 500 mg/50 mL (10 mg/mL concentration), single-dose vial, injection
- Dating period:
 - 100 mg/10 mL Drug Product: 24 months when stored at 2-8 °C
 - 500 mg/50 mL Drug Product: 48 months when stored at 2-8 °C
 - Drug Substance: (b) (4) when stored at (b) (4) including (b) (4)
 - For packaged products: Not packaged
 - Stability Option: We have approved the stability protocol in your license application for the purpose of extending the expiration dating of your drug product under 21 CFR 601.12.
- Exempt from lot release:
 - Yes. Truxima is a specified product exempted according to 21 CFR 601.2a.

C. Benefit/Risk Considerations:

Truxima (rituximab-abbs, CT-P10) is a proposed biosimilar to U.S.-licensed Rituxan and proposed for treatment of Non-Hodgkin's lymphoma which is a selected indication approved for U.S.-Rituxan. The analytical similarity data provided in the initial BLA support the conclusion that CT-P10 is highly similar to U.S.-licensed Rituxan. No new analytical similarity data were submitted in the May 29, 2018 BLA resubmission.

The deficiencies related to manufacturing and controls described in the Complete Response letter dated February 28, 2018 have been addressed in the resubmission. Overall, the OPQ review of manufacturing and controls in resubmission and in initial BLA submission has

identified that the methodologies and processes used for drug substance and drug product manufacturing, release and stability testing are sufficient to assure a consistent and safe product.

The commercial manufacture of rituximab-abbs drug substance and of drug product at Celltrion, Inc. (Incheon, Republic of Korea) is recommended for approval by DIA, OPF, as a pre-license inspection performed during the resubmission review cycle was classified as VAI. A pre-license inspection was not conducted during the first review cycle due to the facility's OAI status.

The technical assessments of the resubmission for OBP product quality, DMA microbiological drug product, DIA facility, and OBP labeling are located as separate documents in Panorama (see list in the end of this review).

D. Recommendation on Phase 4 (Post-Marketing) Commitments:

None.

II. Summary of Quality Assessments:

A. Analytical Similarity Assessment

No change for information in this section (refer to my Executive Summary dated 2/28/2018). Note that in the resubmission (in response to CR comment 7), Celltrion re-analyzed the similarity data for afucosylation, galactosylation and high mannose due to updated controls for glycans (drug substance specifications). The reanalysis results do not change the analytical similarity conclusion from the initial BLA assessment.

B. Critical Quality Attribute (CQA) Identification, Risk, and Lifecycle Knowledge Management

In the resubmission, Celltrion provided the following in response to CR deficiencies:

- {CR comment 9} Data to support that FcγRIIIa binding affinity is not likely to change under drug product (DP) long-term storage condition; therefore, the current testing strategy for ADCC activity as tested by FcγRIIIa binding affinity at drug substance (DS) release and DS long term stability is acceptable.
- {CR comment 5} Information and data to support implementation of (b) (4)

The control strategy for product fragments is acceptable.
- {CR comment 6} Justification and data supporting that the proposed control strategy for Asn55 deamidation in the complementarity-determining regions is adequate.
- {CR comment 7} Data supporting updated DS specifications with acceptance criteria to control all main afucosylated glycans.

No other changes to the application are introduced in this section (refer to my Executive Summary dated 2/28/2018).

C. Drug Substance, rituximab-abbs, Quality Summary

Drug Substance CQA Identification, Risk, and Lifecycle Knowledge Management

In the resubmission, Celltrion provided the following in response to CR deficiencies:

- {CR comment 10} Data to justify that polysorbate 80 (b) (4) rejection limit (b) (4) DP manufacturing is adequate.

The following information was updated in the resubmission (refer to my Executive Summary dated 2/28/2018 for a complete description of CQA identification, risk, and lifecycle knowledge management):

- **Critical starting materials or intermediates:** (b) (4)
 (b) (4)
 (b) (4)
 (b) (4)
 (b) (4)
- **Manufacturing process summary:** The drug substance manufacturing consists of production in cell culture and purification of rituximab-abbs using standard antibody manufacturing processes.

(b) (4)

The rituximab-abbs drug substance manufacturing process development is based on minimal process characterization and process validation studies. In response to CR comments, Celltrion provided:

- {CR comment 1} Data supporting control over an updated (b) (4) during manufacture,
- {CR comment 2} Additional process characterization and commercial scale production data to justify the proposed ranges of the purification process parameters,

- {CR comments 3 and 4} Updated accurate information in the manufacturing process description.

Overall, the control strategy is acceptable.

- **Dating period and storage conditions:** A shelf-life of (b) (4) when stored at (b) (4) including (b) (4)

D. Drug Product, Truxima, Quality Summary:

Drug Product-specific CQA Identification, Risk, and Lifecycle Management

In the resubmission, Celltrion provided the following in response to CR deficiencies:

- {CR comment 12} Results of media fill studies supporting the (b) (4) manufacturing process for 10 mL vial presentation of the CT-P10 drug product,
- {CR comment 13} Updated container closure integrity test to include positive controls with adequate breach size,
- {CR comment 14} Provided bacterial growth promotion study data to demonstrate consistent growth of the test organism to support the bacterial retention study,
- {CR comment 15} Updated description of equipment sterilization validation data to support (b) (4) sterilization of the (b) (4).

- **Manufacturing process summary:** The Truxima drug product manufacturing includes the following steps: (b) (4)

The filled vials are stoppered, and capped, 100% visually inspected, labeled and secondary packaged, and stored at 5 ± 3 °C.

(b) (4)

Truxima drug product manufacturing process development is based on minimal process characterization and process validation studies. The overall control strategy is acceptable.

- **Dating period and storage conditions:** A shelf-life of 24 months when stored at 5 ± 3 °C is acceptable for 100 mg/10 mL Truxima based on available stability data from the process II (commercial) lots. A shelf-life of 48 months when stored at 5 ± 3 °C is acceptable for 100 mg/10 mL Truxima.

E. Novel Approaches/Precedents: None.

F. Any Special Product Quality Labeling Recommendations: None.

G. Establishment Information:

DRUG SUBSTANCE				
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Final Recommendation
DS manufacturing, release and stability testing, MCB and WCB Storage	Celltrion, Inc. 23, Academy-ro Plants 1 and 2 Yeonsu-gu Incheon, S. Korea	3005241015	Withhold *	Approve
Viral testing of unprocessed bulk; Testing of the MCB, WCB and (b) (4)	(b) (4)		n/a	Approve based on facility profile
Sterility testing of (b) (4)			n/a	Approve based on facility profile
Mycoplasma testing of unprocessed bulk; Testing of the MCB, WCB, and (b) (4)			n/a	Approve based on facility profile
MCB and WCB storage			n/a	Approve based on facility profile
Production of the MCB and WCB			n/a	No Further Evaluation
DRUG PRODUCT				
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Final Recommendation
DP manufacturing, release and stability testing	Celltrion, Inc. 23, Academy-ro Plants 1 and 2 Yeonsu-gu Incheon, S. Korea	3005241015	Withhold *	Approve

DS - drug substance, DP - drug product, QC – quality control, IPC – in-process control, MCB - Master Cell Bank, WCB - Working Cell Bank, UPB – Unprocessed bulk

* See section H. Facilities.

H. Facilities:

The BLA proposes manufacture of Truxima drug substance and drug product at Celltrion, Inc. (FEI 3005241015). A pre-license inspection (PLI) was not conducted during the first review cycle due to the facility's OAI status with a Warning Letter (WL 320-18-28, issued on January 26, 2018). The OAI status is cleared by OMQ after a for-cause inspection conducted in July, 2018.

During the current review cycle, a PLI of Celltrion, Inc. was conducted from August 20 to 28, 2018 by OPQ/OFP/DIA and OPQ/OBP. This inspection covered the drug substance and drug product manufacturing, the testing laboratories and similarity assessment data. No issue was observed for similarity assessment data. A FDA 483 with seven observations was issued at the completion of the inspection, and the initial recommendation of this inspection was withhold. The sponsor responded to the FDA 483 observations that resulted in the final facility recommendation for approval.

I. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols approved (section):

1. Requalification of the MCB and WCB (3.2.S.2.3.2)
2. (b) (4)
3. (b) (4)
4. (b) (4) Stability Study (committed in January 3, 2018 response to IR comment 8)
5. Requalification of the Primary and Working Reference Standards (3.2.S.5)
6. Ongoing Stability Study (3.2.S.7.2)
7. Post-Approval Annual Stability Protocols (3.2.S.7.2, at recommended storage condition of (b) (4))

ii. Outstanding review issues/residual risk: None.

iii. Future inspection points to consider: None.

b. Drug Product

i. Protocols approved (section):

1. Ongoing Stability Studies:
 - a. 100 mg/10 mL and 500 mg/50 mL drug product lots (3.2.P.8.2)
 - b. Drug product lot 17C2C09 with polysorbate 80 content of (b) (4) % (committed in October 22, 2018 response to IR comment 5)
2. Ongoing Leachables Studies for 100 mg/10 mL drug product in inverted position (committed in January 3, 2018 response to IR comment 21)
3. Post-Approval Annual Stability Protocols (3.2.P.8.2)

ii. Outstanding review issues/residual risk: None.

iii. Future inspection points to consider: None.

Review documents related to this Executive Summary (links to documents in Panorama):

- Product quality review by Haoheng Yan, MD, PhD (OPQ/OBP/DBRR IV) [link](#)
- Drug product microbiology review by Candace Gomez-Broughton, PhD (OPQ/OPF/DMA IV) [link](#)
- Facility review by Thuy Thanh Nguyen, MPH (OPQ/OPF/DIA) [link](#)
- Labeling review by Vicky Borders-Hemphill, PhD (OPQ/OBP) pending in [link](#)

J. Quality Assessment Summary Tables

No change for information in this section (refer to my Executive Summary dated 2/28/2018).



Bazarragchaa
Damdinsuren

Digitally signed by Bazarragchaa Damdinsuren
Date: 10/31/2018 09:16:17AM
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Thuy Thanh
Nguyen

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Date: 10/31/2018 09:22:38AM
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Reyes
Candau-Chacon

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Date: 10/31/2018 10:26:16AM
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Christopher
Downey

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BLA STN 761088 Re-submission

Truxima (rituximab-abbs)

Manufacturer: Celltrion, Inc.

Drug Substance Quality Reviewer: Haoheng Yan, M.D., Ph.D.
Drug Product Quality Reviewer: Haoheng Yan, M.D., Ph.D.
Analytical Similarity Reviewer: Nina Brahme, Ph.D., M.P.H.
CMC Application Team Lead: Bazarragchaa Damdinsuren, M.D., Ph.D.
Review Chief: Christopher Downey, Ph.D.

**Division of Biotechnology Review and Research IV
Office of Biotechnology Products**

OBP CMC Review Data Sheet

1. **BLA#:** STN 761088

2. **REVIEW DATE:** October 25, 2018

3. **PRIMARY REVIEW TEAM:**

Product Quality Team:

Drug substance quality reviewer: Haoheng Yan (OPQ/OBP)

Drug product quality reviewer: Haoheng Yan (OPQ/OBP)

Analytical similarity reviewer: Nina Brahme (OPQ/OBP)

Drug substance microbiology reviewer: Scott Nichols (OPQ/OPF/DMA)

Drug product microbiology reviewer: Candace Gomez-Broughton (OPQ/OPF/DMA)

Microbiology QAL: Reyes Candau-Chacon (OPQ/OPF/DMA)

Facility reviewer: Thuy Thanh Nguyen (OPQ/OPF/DIA)

Facility lead: Zhihao Peter Qiu (OPQ/OPF/DIA)

OBP Labeling reviewer: Vicky Borders Hemphill (OPQ/OBP)

OPQ RBPM: Oumou Barry (OPQ/OPRO)

CMC Application Team Lead (ATL): Bazarragchaa Damdinsuren (OPQ/OBP)

CMC Statistics: Yu-Ting Weng, Meiyu Shen (OTS/OB)

Medical Officers: Rachel Ershler, Vishal Bhatnagar (DHP)

Pharm/Tox: Michael Manning (DHP)

Clinical Pharmacology: Sang Chung and Sarah Schrieber (DPARP)

Statistics: Cindy Gao (DHP)

RPM (clinical): Esther Park (DHP)

4. **MAJOR GRMP DEADLINES**

Planning/Filing Meeting	June 13, 2018
Mid-Cycle Meeting	August 30, 2018
Primary Reviews Complete (target)	October 25, 2018
Wrap-up Meeting	November 6, 2018
Secondary Reviews Complete (target)	October 30, 2018
Complete CDTL Memo (target)	November 4, 2018
Division Director Memo	November 6, 2018
BsUFA Goal Date	Target: November 6, 2018 Due: November 29, 2018

5. **COMMUNICATIONS WITH APPLICANT:**

Communication/Document	Date
Information Request #1	7/31/2018
Information Request #2	10/12/2018
Information Request #3	10/23/2018

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

Submission	Date Received	Review Completed
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		(responsible group)
STN 761088 /34	5/29/2018	Yes (OBP/DMA/DIA)
STN 761088 /36 (response to IR #1)	8/2/2018	Yes (OBP/DMA/DIA)
STN 761088 /43 (response to IR #2)	10/22/2018	Yes (OBP)
STN 761088 /44 (response to IR #3)	10/25/2018	Yes (OBP)

7. DRUG PRODUCT NAME/CODE/TYPE:

- a. Proprietary Name: Truxima
- b. Non-Proprietary/USAN/INN: rituximab-abbs
- c. CAS Registry number: 174722-31-7
- d. Company/Laboratory code: CT-P10
- e. OBP systematic name¹: MAB CHIMERIC (IGG1) ANTI P11836 (CD20_HUMAN) [CT-P10]
- f. Other Names: None

8. PHARMACOLOGICAL CATEGORY: Recombinant chimeric monoclonal IgG1 antibody directed against human CD20

9. DOSAGE FORM: Injection: 100 mg/10 mL (10 mg/mL) or 500 mg/50 mL (10 mg/mL) solution in single-dose vials

10. STRENGTH/POTENCY:

- (i) Truxima Drug Product: 500 mg/50 mL, 100 mg/10 mL
- (ii) Types of potency assays: 1. Complement-Dependent Cytotoxicity (CDC) Assay is a cell-based cytotoxicity assay used as an *in vitro* measure of CT-P10 CDC activity
2. FcγRIIIa Binding Affinity using Surface Plasmon Resonance (SPR) is an *in vitro* binding assay to measure an attribute which modulates antibody-dependent cell-mediated cytotoxicity (ADCC).

11. ROUTE OF ADMINISTRATION: Intravenous (i.v.)

12. REFERENCED MASTER FILES:

DMF #	HOLDER	ITEM REFERENCED	Letter of Cross-Reference	COMMENTS (STATUS)
(b) (4)			Yes	Not reviewed. Information provided in the BLA is sufficient for a conclusion of its product compatibility.

¹ The OBP systematic name allows searching for related products in OBP's database and in the Document Archiving, Reporting & Regulatory Tracking System (DARRTS) for safety reasons and it is different from the nonproprietary name. The tag at the end is used to separate products from different sponsors and it is generally the name used by sponsors to refer to the proposed product in their submissions.

(b) (4)	Bacterial Endotoxin Reduction Validation Process Validation	Yes	Validation for (b) (4) Defer to DMA reviewer.
	10mL (b) (4) Vials (used as 100mg drug product container)	Yes	Not reviewed. Information provided in the BLA is sufficient for a conclusion of its product compatibility.
	Glass Vials (used as 500mg drug product container)	Yes	Not reviewed. Information provided in the BLA is sufficient for a conclusion of its product compatibility.

13. INSPECTIONAL ACTIVITIES

The pre-approval inspection of Celltrion, Inc. in Incheon, Republic of Korea, was conducted from August 20 to 28, 2018. This inspection includes the drug substance and drug product manufacturing site inspection as well as the inspection for similarity assessment data. The inspection was initially classified as OAI, and upon review of the response to FDA 483 form updated to VAI as a final recommendation. No issue was observed for similarity assessment data. The team consisted of Thuy Nguyen, AQAL (OPQ/OPF/DIA), Viviana Matta, Consumer Safety Officer (OPQ/OPF/DIA), Riley Myers, Biologist (OPQ/OBP) and Haoheng Yan (OPQ/OBP).

14. CONSULTS REQUESTED BY OBP: None

15. QUALITY BY DESIGN ELEMENTS: None

16. PRECEDENTS: First biosimilar to US-licensed Rituximab

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

Name and Title	Signature and Date
Christopher Downey, Ph.D. Review Chief Division of Biotechnology Review and Research IV (DBRR IV) Office of Biotechnology Products (OBP) Office of Pharmaceutical Quality (OPQ)	See attached
Bazarragchaa Damdinsuren, M.D., Ph.D. Team Leader DBRR IV, OBP, OPQ	See attached
Haoheng Yan, M.D., Ph.D. Product Quality Reviewer DBRR IV, OBP, OPQ	See attached
Nina Brahme, Ph.D., M.P.H. Product Quality Reviewer DBRR IV, OBP, OPQ	See attached

B. CC Block

Recipient	Date
Esther Park Clinical Division RPM	
OBP/DBRR IV File/BLA STN 761088	

SUMMARY OF QUALITY ASSESSMENTS

I. Primary Reviewer Summary Recommendation

I recommend approval of BLA 761088 for Truxima (rituximab-abbs) manufactured by Celltrion Inc. The data submitted in this Biologics License Application adequately support the conclusion that the manufacture of Truxima (rituximab-abbs) is well controlled and leads to a product that is pure and potent.

I recommend approval of the proposed lot release and stability specifications and stability protocols for rituximab-abbs drug substance and drug product. I recommend an expiry period of (b) (4) for rituximab-abbs drug substance when stored at (b) (4) °C which includes (b) (4). I recommend expiry periods of 48 months for Truxima 500 mg/50 mL drug product and 24 months for Truxima 100 mg/10 mL drug product when stored at 5 ± 3 °C.

Celltrion has employed an array of orthogonal, sensitive methods to characterize structural and functional similarities between the proposed biosimilar Truxima and its reference product, US-licensed Rituxan. The data in the analytical similarity assessment adequately support the conclusion that Truxima (rituximab-abbs) is highly similar to US-licensed Rituxan.

It is recommended that Truxima (rituximab-abbs) be approved for human use and under conditions specified in the package insert.

II. List Of Deficiencies To Be Communicated: *There are no CMC-related deficiencies precluding approval of this BLA.*

III. List Of Post-Marketing Commitments/Requirements: *None*

IV. Review Of Common Technical Document-Quality Module 1

A. Environmental Assessment Or Claim Of Categorical Exclusion

Celltrion claims a categorical exclusion per 21 CFR Part 25.31(a), as stated "action on an application for marketing approval of a biologic product 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. The request for categorical exclusion is acceptable.

V. Primary Container Labeling Review

The CMC labeling review was performed by CAPT Vicky Borders-Hemphill, CDER/OPQ/OBP. The review memo is uploaded to Panorama separately.

VI. Review Of Common Technical Document – Quality Module 3.2

The review of 1.11.1 Quality Information Amendment (Response to CR and additional comments), 3.2.S. Drug Substance, 3.2.P. Drug Product and 3.2.R Regional information (method validation reports) and 3.2.R Regional Information (analytical similarity) are included in this document.

VII. Review of Immunogenicity Assays – from Module 5.3.1.4

No additional immunogenicity assay information was submitted in the re-submission.

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Unless otherwise noted, figures and tables in the review are adapted or copied directly from the submission. Some of the figures and tables in the submission were updated during the review cycle, the review contains only the updated final versions. The reviewer's comments are distinguished by use of italic font.

Product Quality Complete Response (CR) Comment 1

CR Comment:

The cell culture process (b) (4)

However, the characterization and assessment of (b) (4)

Therefore, your control over (b) (4) during manufacture is inadequate. To ensure product quality and cell culture process consistency (b) (4)

Sponsor Response:

(b) (4)

Reviewer's Comment: The proposed range (marked by red frame) are close to or narrower than the Min-Max range of the commercial manufacturing experience. In addition, the proposed ranges are sufficiently narrow to ensure [REDACTED] (b) (4). These proposed ranges are incorporated into 3.2.S.2.2 Description of Manufacturing Process. The response to CR comment 1a is acceptable.

b and c. The sponsor revised the proposed range for [REDACTED] (b) (4). [REDACTED] data was submitted (in response to 1c) because they addressed comments 1a and 1b. The response to CR comment 1b is acceptable.

FDA Assessment:

The sponsor adequately resolved product quality CR comment 1.

Product Quality CR Comment 2

CR Comment:

Your proposed ranges for certain process parameters are unacceptable, given that their values exceed those evaluated during process validation and that adequate process characterization data supporting their limits were not provided. For the following parameters, revise sections 3.2.S.2.2 to limit the proposed acceptable ranges for the commercial process to the validation ranges or to commercial manufacturing experience. Alternatively, provide additional process characterization data to justify the proposed ranges for these parameters.

(b) (4)

Reviewer's Comment: *In the re-submission, the sponsor chose to "provide additional process characterization data to justify the proposed ranges of these parameters." The sponsor and Agency had discussed this CR comment during the Type 1 meeting held on April 16, 2018. In the meeting package, the sponsor provided preliminary data obtained from small scale models (for chromatography steps) to support the proposed ranges for these process parameters. The Agency repeatedly communicated with the sponsor "that if Celltrion intends to use data obtained from scale-down process models to support the commercial scale process parameters, additional data are necessary to demonstrate that the scale-down models are representative of the commercial scale process."*

During the meeting the Agency also clarified that (from the meeting minutes):

"In order for the sponsor to use data derived from small scale study to support the proposed process parameter at commercial scale, additional data to support you need to provide, at minimum, the items discussed in a - c to justify the suitability of the scale-down models, and provide details on the operational parameters and set points for the model runs in comparison to the commercial runs."

(b) (4)

My assessment of the response to this CR comment focused on the following questions:

- Are there sufficient data to support the small scale models are representative of the commercial scale process. If so,*
- Are the data derived from the small scale studies sufficient to support the proposed process ranges in the commercial process?*

Sponsor Response:

Celltrion provided additional process characterization data from small scale studies to justify the proposed ranges of the parameters, along with additional qualification data to demonstrate that the scale-down models are representative of the commercial scale process. The information consists of:

- Small-scale Process Characterization Study (PCS) – the initial process characterization study using small scale models. *Data were submitted and reviewed in the original BLA.*
- The 1st downstream Small-Scale Model Qualification Study (SSMQ1). *Data were submitted and reviewed in the original BLA.*
- The 2nd downstream Small-Scale Model Qualification Study (SSMQ2) that was conducted to support the SSMQ1. *New data provided in the re-submission.*
- Small-scale Range Confirmation Study (RCS) - additional process characterization study conducted using the small scale models. *New data in the re-submission.*

The new and updated data are provided in Section 3.2.S.2.5.4 and Appendix 3.2.S.2.5-11 (in more detail) of Section 3.2.S.2.5. The sponsor states the SSMQ studies were conducted using representative feed materials manufactured at commercial-scale.

Reviewer's Comment: *To clarify, the sponsor did not change the small-scale models, they submitted additional model qualification data (SSMQ2) to support that data from PCS and RCS, both based on the small scale models, can be used to justify the commercial process.*

To evaluate if the small scale model (SSMQ1 and SSMQ2) for each chromatographic column can represent the commercial process, I compared the process parameters and performance indicators between the models and the commercial process.

Small Scale Model Qualification (information from Appendix 3.2.S.2.5-11):

(b) (4)

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(b) (4)

FDA assessment:

The sponsor acceptably resolved product quality CR comment 2.

Product Quality CR Comment 3

CR Comment:

In the responses received on November 21 and 28, 2017, to the Agency's information request (IR) comments 1e, 1f, 1i and 16 dated November 13, 2017, Celltrion included descriptions of certain process parameters. However, section 3.2.S.2.2 which describes the conditions of routine manufacture was not updated to reflect this information. Update section 3.2.S.2.2 to be consistent with the information received on November 21 and 28, 2017, for the following parameters:

(b) (4)

Sponsor Response:

- a. The sponsor included an equation that defines (b) (4) frequency in section 3.2.S.2.2.

(b) (4)

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

(b) (4)

FDA Assessment:

The sponsor adequately resolved product quality CR comment 3.

Product Quality CR Comment 4**CR Comment:**

In the response received on November 28, 2017, to our IR regarding the acceptable ranges for the (b) (4) for the chromatography columns, Celltrion stated that these ranges are based on the (b) (4). This does not provide sufficient control for consistency during routine manufacture. Parameters associated with (b) (4) should be assigned for the target values set in the (b) (4) and justified using data obtained in the PPQ and commercial manufacturing runs. If wider ranges are desired than those established during PPQ and commercial manufacturing runs, data should be provided to justify the proposed ranges.

Sponsor Response:

The sponsor clarifies the (b) (4) for the chromatography columns are as stated and used in the validation runs, and the target values set in the (b) (4). The acceptable ranges for these (b) (4) are based on (b) (4)

(b) (4)

Reviewer's Comment: The following are my assessment points:

- The small-scale models of the chromatographic columns can be considered reflective of the commercial scale process. See my review of CR comment 2.
- The range confirmation studies included the following worst-case (from the product quality/purity perspective) combinations of the proposed (b) (4):
(b) (4)
- No impact to product quality and yield was observed from the range confirmation study results when different combinations of (b) (4) are employed (data provided in Tables 2-4 of the response).

FDA Assessment:

The sponsor fully resolved product quality CR comment 4.

Product Quality CR Comment 5

CR Comment:

Product fragments are identified as a critical quality attribute (CQA) for CT-P10 and monitored (b) (4). However, the impact of the (b) (4) process on product fragment levels was not assessed during process development. Therefore, it is not clear if proposed critical process parameters are sufficient to control levels of fragments during routine manufacture. To adequately control for product fragments:

- Identify (b) (4) process unit operations that impact the levels of product fragments,
- (b) (4)
- Provide a complete summary and description of your control strategy for product fragments.

Reviewer's Comment: The sponsor and Agency had discussed this CR comment during the Type 1 meeting held on April 16, 2018. It is agreed that Celltrion would include (b) (4) to control product fragments. FDA also stated that in the submission, Celltrion should include (1) justification for proposed (b) (4) assay validation data to support assay suitability for the (b) (4) testing.

FDA assessment:

The sponsor adequately resolved product quality CR comment 5.

Product Quality CR Comment 6

The assessment of the response to Comment 6 was done by Dr. Nina Brahme.

CR Comment:

Deamidation at the heavy chain Asn55 (HC Asn55) site is identified as a CQA and controlled (b) (4). To support the proposed control strategy, provide data showing that changes in deamidation at HC Asn55 can be reliably detected by the (b) (4) method.

Reviewer comment: *Section 1.11.1 Quality information amendment includes the sponsor's response to our CR comment #6. This comment was also one of the subjects of a BPD1 meeting, where the Agency clarified our expectations for how Celltrion can address the issue described in the comment (see final meeting minutes uploaded to DARRTS on 5/16/2018 and OBP's review memo uploaded on 5/10/2018).*

In the resubmission, the sponsor appropriately relies on data from the forced degradation study and literature to assign deamidation a moderate criticality score. From the Agency's perspective, deamidation at HC Asn55 has high risk because this residue is within the CDR region. HC Asn 388 is located outside of the binding sites but is the more susceptible residue to deamidation. The forced degradation study data reviewed within the original BLA submission and BPD1 meeting package

showed that deamidation at HC Asn 388 varied more than deamidation at HC Asn 55, but that the two correlated with changes in the sum of acidic peaks. From the Agency's perspective based on totality of knowledge of HC Asn 55 from its occurrence, changes during manufacturing, storage, forced degradation conditions, and feasibility of analytical controls (see details below), control strategy of deamidation at HC Asn 55 can be based on (b) (4)

Nonetheless, the sponsor took a conservative approach and classified deamidation at all six sites as a CQA, which they claim is controlled (b) (4)

CT-P10 historical data were evaluated including lot release testing, similarity, stability, and shipping validation studies. To demonstrate acceptable levels of deamidation at HC Asn55 throughout CT-P10 shelf-life, additional testing was performed using DP lots of 15-40 months age. In addition, testing of samples derived from an agitation stress was conducted to evaluate the impact of mechanical/shear stress on product quality during shipping (see response to CR comment 10) in which deamidation levels at HC Asn55 following worst-case shipping conditions were examined.

The forced degradation study demonstrated that deamidation increases under high temperature and basic pH conditions. During the BPD1 meeting, the Agency clarified that a risk assessment related to comparison of forced degradation conditions and process conditions / controls can support the response to CR comment 6. In the resubmission, the sponsor noted that the conditions applied in the forced degradation studies are much more severe than are encountered during the manufacturing process. For example, while high temperature stress of 50°C was used in forced degradation, the manufacturing process environment is (b) (4). While basic stress conditions of pH 10.5 for 48 hours and 96 hours were applied during forced degradation, the most basic condition encountered during the manufacturing process is (b) (4)

Reviewer comment: I agree with the sponsor's risk assessment and the manufacturing process conditions are unlikely to trigger increased deamidation of CT-P10 at HC Asn 55. In addition, the storage and handling conditions are nowhere close to the studied degradation conditions, thus unlikely to trigger increased deamidation at HC Asn 55 (see stability data below).

DS and DP batch release data support low heterogeneity in acidic peak percentage. As expected, the small variation that exists does not appear to have any correlation to CDC activity ($R^2=0.089$). Characterization study on three DS lots and three DP lots compared deamidation at HC Asn 55 and Asn 388 (by peptide mapping). The data support that the DP manufacturing process does not have an impact on deamidation at HC Asn 55, variation in acidic peaks, CDC activity, or CD20 binding.

The sponsor's data comparing (estimated) lot age of Rituxan, CT-P10 DP, and MabThera to each lot's deamidation at HC Asn 55 does not show observable variation of deamidation at HC Asn 55 with shelf life of rituximab (Figure 2). Additional testing of CT-P10 DP performed at the end of product shelf life is summarized in Table 4.

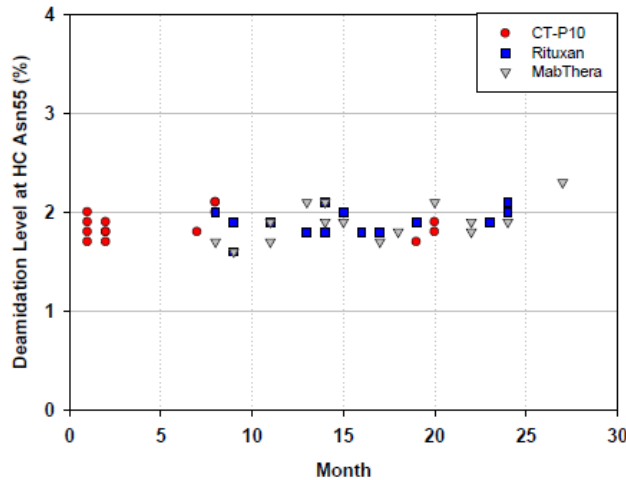


Figure 2: Trend Analysis of HC Asn55 Deamidation Levels as a Function of Lot Age

Table 4: Results of Peptide Mapping of Deamidation Level at HC Asn55 and HC Asn388 for CT-P10 Drug Product (15 – 40 Months)

Modification	Site	Reference Standard	15 Months	40 Months		
		(b) (4)	17C2C01	14C2C002	14C2C003	14C2C004
Deamidation	HC Asn55	2.5%	2.9%	2.9%	2.8%	3.0%
	HC Asn388	1.8%	2.2%	2.2%	2.1%	2.2%

¹ CT-P10 working reference standard (Section 3.2.S.5)

Reviewer comment: In Figure 2 and Table 5, the HC Asn 55 deamidation levels did not show CT-P10 age related change, although the reported levels were slightly different (~1.9% vs. ~2.9%, data in Figure 2 vs. Table 4).

In the following reviewer-generated table, I compared the 8-month (from Table 3 in the submission) and 40-month (Table 4) HC Asn 55 data for lots 14C2C002 - 14C2C004. Although a slight increase of Asn55 deamidation is seen, such minor increase is unlikely to impact product binding.

		deamidation at HC Asn 55	
		8M (%)	40M (%)
CT-P10 DP 500 mg	14C2C002	2.1	2.9
	14C2C003	2	2.8
	14C2C004	2.1	3

In the response, the sponsor discussed (b) (4)

The sponsor does not have any remaining samples from the shipping validation study performed to support transportation from the CT-P10 DP manufacturing site to the labelling and packaging site for US distribution (Section 3.2.P.3.5) and so cannot analyze the HC Asn 55/388 deamidation under those

conditions. Instead, the impact of shipping on deamidation was assessed by mechanical/shear stress. Samples derived from the (b) (4) formulation study (F3 formulation see response CR comment 10) were vortexed at 3,000 rpm for 4 hours at 25°C (data are presented in Table 5).

Table 5: Results of Peptide Mapping (LC-MS) of Deamidation Level at HC Asn55 and HC Asn388 under the Vortex Condition for (b) (4) Formulation Study

Modification	Site	No Agitation	Vortexing (3,000 RPM for 4 hr at 25°C)		
		Control	Sample 1	Sample 2	Sample 3
Deamidation	HC Asn55	2.6%	2.5%	2.4%	2.7%
	HC Asn388	2.1%	2.0%	2.1%	2.1%

Note: F3 samples (as the same formulation of CT-P10) were additionally tested in triplicate (Complete Response Product Quality 10).

Reviewer comment: The data support that a vortex/agitation stress does not appear to trigger increased deamidation at HC Asn 55, which is indirectly suggest that shipping likely will not affect deamidation levels.

Reviewer comment: In the response, Celltrion provided additional justifications and data supporting that changes in deamidation at HC Asn 55 are likely relatively minor over the course of manufacture, shipping, and storage. Therefore, the current control strategy for deamidation at HC Asn 55 is acceptable. We do not fully agree with the sponsor's conclusion "Based on the totality of the data, CELLTRION concludes that the current control strategy employing (b) (4) for CT-P10 drug substance and drug product to monitor HC Asn55 deamidation, as well as deamidation at other residues is adequate."

FDA assessment:

The sponsor adequately addressed product quality CR comment 6.

Product Quality CR Comment 7

CR Comment:

The data provided in section 3.2.R.4.1.5 Afucosylation show that increased afucosylated glycans (G0+G1) can affect FcγRIIIa binding and, consequently ADCC activity of CTP10. Therefore, the proposed DS specification for glycans of G0 = (b) (4)% and Unidentified Peaks (b) (4)% does not provide adequate control of ADCC, as it does not provide sufficient control of G1 content. Provide the BLA with the following:

- Propose DS specifications with acceptance criteria to control all main afucosylated glycans,
- Provide assay validation data to confirm the suitability of the oligosaccharide profile test for the proposed specifications for afucosylated glycans,
- Provide all available DS lot release and stability data to justify the revised specifications and the acceptance criteria.

Sponsor's Response:

- a. and c. The sponsor proposed to include G0+G1 in the DS release specification (in the section 3.2.S.4.1). The acceptance criteria for all glycosylation related attributes are revised as shown in Table 1.

Table 1: Proposed Specification of Oligosaccharide Profile for CT-P10 Drug Substance

Quality Attribute	Test Item	Method	Proposed Acceptance Criterion	
			Before	Newly Proposed
Glycosylation	Oligosaccharide Profile	In-House Bio-LC	(b) (4)	
			(b) (4)	

Justification of DS specification limits, connection to the clinical batches, and my assessment are included in the following reviewer-generated table:

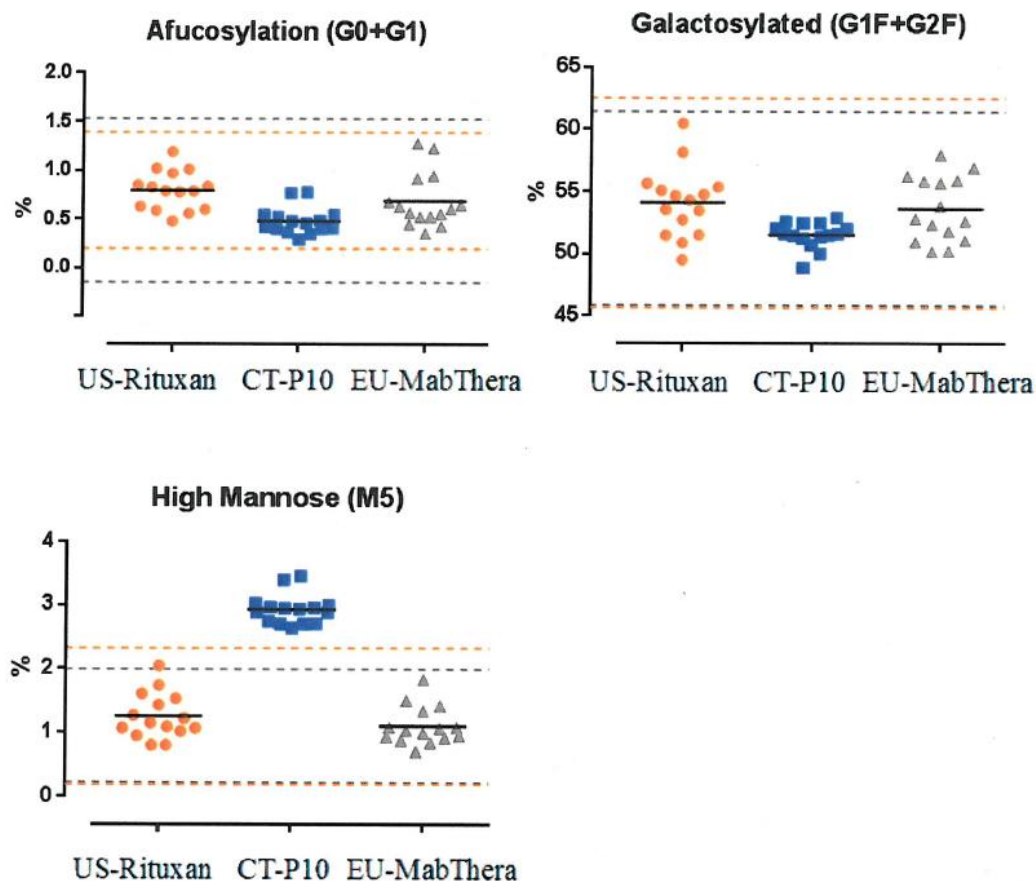
Test attribute	Acceptance limit	Justification for Specification (b) (4) of 23 process C&D lots]	Range of additional 41 lots (Appendix 1 of CR comment 1)	Range of DS Stability Data at (b) (4) C *	Quality Range of US-Rituxan ** (mean±3SD)
G0+G1	(b) (4)	(b) (4)	0.2 - 1.0%	0.13 – 1.44%	0.2 - 1.4%
The galactose content			0.56 – 0.61	0.57 – 0.66	0.53 - 0.77
Man5			2.4 - 4.1%	2.14 – 4.56%	0.19 - 2.32%
Sum of Unidentified Peaks (U1 to U4)			2.9 – 3.9%	2.19 – 4.25%	1.3 - 3.3%

* Glycosylation is not part of DS annual stability test, the sponsor provided data to support the proposed acceptance criteria. Because product glycosylation is not expected to change during the two storage conditions, I included the data in the table to show assay variability.

** Data from the reanalyzed glycoform data submitted in 1.11.1 CR product quality 7-appendix Table 1.

Reviewer's Comment: The proposed N-glycan DS specification is acceptable.

The sponsor re-analysed the N-glycosylation similarity data based on the revised control strategy for afucosylated species. Note that the similarity dataset is the same as reviewed in the original BLA. Dr. Nina Brahme put the results into scatter plots shown here.



The black horizontal bars represent the mean percentages. The dotted orange lines and grey lines represent the QR limits based on mean $\pm$ 3SD of the US-Rituxan and EU-MabThera lots, respectively.

Reviewer's Comment: Both afucosylation and galactosylation met the quality range limits defined by US-Rituxan and EU-MabThera lots. As reviewed in the original BLA, CT-P10 showed higher high mannose compared to US-Rituxan. High mannose content can impact ADCC activity and drug serum clearance; however, such minor difference (2.93% vs. 1.25%) is unlikely to result in significant difference in serum clearance. These re-analyzed results still do not preclude a demonstration of similarity between CT-P10 and US-Rituxan.

b. Assay validation

This re-validation report includes previous validation data and additional data from spike-in (with highly afucosylated species) experiment. In the re-evaluation exercise, the oligosaccharide peaks were re-classified into 2 groups, major peaks (G0F, G1F and G2F) and minor peaks (G0, G1, Man5 and unidentified peaks 1 – 4) shown in the following Figure 1.

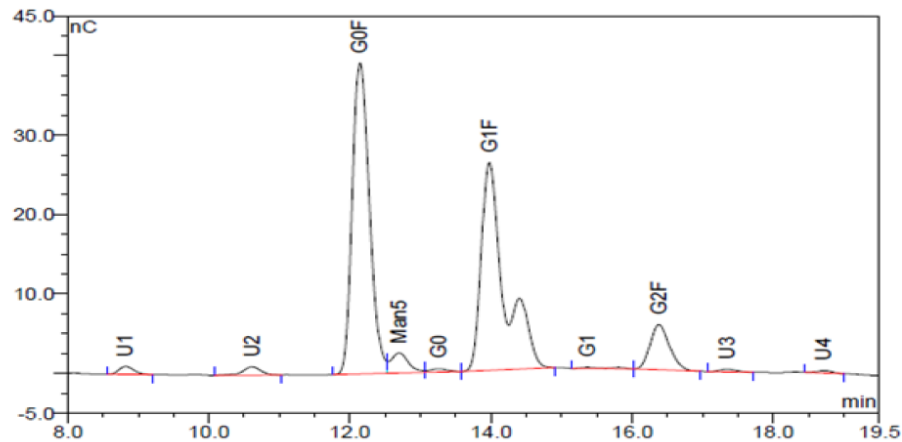


Figure 1: Representative Chromatogram of CT-P10 Oligosaccharide Profile

Reviewer's Comment: During initial BLA review, the assay performance was deemed to be acceptable for quantifying G0F, G1F, G2F, G0 and Man5 peaks, therefore the current assessment of the method validation data focuses on G1 and U1-U4 peaks (see summary table below).

In the spike-in experiments, samples were tested in 5 spike-in levels of G1 and G2:

	Level 1, (unspiked DS)	Level 2	Level 3	Level 4	Level 5
G0 (%)	0.2	0.9	1.6	2.3	3.0
G1 (%)	0.12	0.54	0.96	1.39	1.80

	Re-evaluation in Validation Report	G0, G1 spike-in study	Reviewer's Comment
Repeatability	DS samples are tested at 50%, 100%, and 150% relative concentration, %RSD for the %peak area for U1-U4 are $\leq 14\%$.	%RSD $\leq 4\%$ (at levels 1, 3, and 5)	The results are acceptable.
Intermediate precision (analyst to analyst, day to day)	%RSD for the %peak area: U1-U4 $\leq 9\%$, <u>G0, G1 = 31 - 48%</u>	Not tested	Note that G0 and G1 are $< 0.5\%$. For such low abundance forms, this level of variance is common and not expected to interfere with data integration and the control of afucosylated species.
Accuracy	DS samples are tested at 50%, 100%, and 150% relative concentration, % recovery of Man5 and U1-U4 peak areas are 81 - 110%	% Recovery G0=92-107%, G1=90-98% (at level 1, 3, and 5)	The results are acceptable.
Linearity	DS samples are tested at 50%, 75%, 100%, 125% and 150% of relative concentration, R^2 of the U1-U2 peaks areas are 0.96-0.99, regression charts are provided.	$R^2=0.98-0.99$ (at all 5 levels)	The results are acceptable.

FDA Assessment:

The sponsor adequately resolved product quality CR comment 7.

Product Quality CR Comment 8**CR Comment:**

In the response to IR comment 4 received on January 3, 2018, Celltrion described the stability testing plan and specifications for the CT-P10 master and working cell banks; however, the information was not submitted in relevant section of Module 3. Update section 3.2.S.2.3.2 with the information provided in the response.

Sponsor Response:

CELLTRION has updated Section 3.2.S.2.3.2 with the information regarding stability testing specifications for the CT-P10 master and working cell banks:



performed.

An IR was sent on 10/12/2018 requesting the sponsor to include the frequency of cell bank stability testing. In response, the sponsor updated section 3.2.S.2.3.2 stating that

[REDACTED]

FDA assessment:

The sponsor adequately addressed product quality CR comment 8.

Product Quality CR Comment 9**CR Comment:**

FcγRIIIa binding affinity is included in the DS release and stability specifications to control CT-P10 ADCC activity. Celltrion states that testing of DS is sufficient to ensure FcγRIIIa binding affinity of CT-P10 DP throughout its shelf life. However, no data is provided for FcγRIIIa binding affinity for DP stored under the long term storage condition of 5±3°C. Celltrion should provide data for FcγRIIIa

binding affinity during DP storage to support the current testing strategy, or alternatively, include FcγRIIIa binding affinity testing in the DP stability program.

Sponsor's Response:

The sponsor provided available data of FcγRIIIa binding affinity for CT-P10 500 mg and 100 mg DP lots at long-term storage condition of 5±3°C (Table 2).

Table 2: Long-term Stability Data of FcγRIIIa Binding Affinity for CT-P10 500 mg and 100 mg (5±3°C)

Lot No.		Proposed Acceptance Criterion for DS Release Testing (Section 3.2.S.4.1) (b) (4)	Time Point (Month)										
			0	1	2	3	6	12	18	24	30	36	48
CT-P10 500 mg (Process 3)	13C1C003		93	102	96	101	101	100	99	97	96	109	92
	13C1C004		101	98	100	105	105	98	100	97	98	110	94
	14C1C001		96	98	105	101	103	97	95	94	98	104	93
CT-P10 500 mg (Process 4)	14C2C002		NT	100	106	98	102	101	111	101	106	103	NT
	14C2C003		103	97	106	96	99	101	104	100	103	103	NT
	14C2C004		107	100	108	102	101	105	108	107	97	103	NT
CT-P10 100 mg (b) (4)	15C3B02		102	NT	NT	93	98	103	105	98	96	NT	NT
	15C3B03		102	NT	NT	104	98	99	112	97	95	NT	NT
	15C3B04		102	NT	NT	105	103	106	107	100	98	NT	NT

NT: Not Tested

Reviewer's Comment: Note that data for 100 mg DP is from (b) (4) lots (DP process 1) which are from earlier DS process A. Stability data from these lots are not sufficient to support stability of DP from commercial process (see original BLA review).

No change beyond the assay variability or trending are observed from the stability data from 500 mg DP at the recommended storage condition.

Forced degradation study results under various conditions were provided in Table 3, note that a CT-P10 DS lot was tested in this study.

Table 3: FcγRIIIa Binding Affinity Data from the Forced Degradation Study (Section 3.2.P.8.3.1.8)

Forced Degradation Condition	Rituxan® (590472)			15C2C04			MabThera® (N7029)		
	Control	Condition 1	Condition 2	Control	Condition 1	Condition 2	Control	Condition 1	Condition 2
Oxidative Stress (0.005 % and 0.01 % H ₂ O ₂ , for 6 hr)	106%	101%	102%	101%	102%	101%	100%	111%	111%
UV Light Stress (20 w/m ² , for 10 and 20 hr)	106%	102%	97%	101%	99%	96%	100%	109%	105%
High Temperature Stress (50 °C, 24 and 60 hr)	106%	111%	109%	101%	111%	108%	100%	112%	115%
Acidic Stress (pH 3.3, 10 and 20 hr)	106%	99%	98%	101%	100%	101%	100%	101%	104%
Basic Stress (pH 10.5, 48 and 96 hr)	106%	108%	106%	101%	100%	103%	100%	120%	110%

Reviewer's Comment: Overall, the sponsor provided sufficient data to support that FcγRIIIa binding affinity is not likely to change under DP long-term storage condition. Therefore, the current testing strategy for FcγRIIIa binding affinity (as testing at DS release and DS long term stability) is acceptable.

FDA assessment:

The sponsor adequately addressed product quality CR comment 9.

Product Quality CR Comment 10

CR Comment:

In the IR response received on January 3, 2018, you proposed an acceptance criterion of polysorbate 80 (PS80) concentration (b) (4)

. Provide data to support the adequacy of the proposed PS80 lower limit.

Sponsor's Response:

(b) (4)

FDA Assessment:

The sponsor acceptably resolved product quality CR comment 10.

Product Quality CR Comment 11**CR Comment:**

The response received on January 3, 2018, to the Agency's IR comment 26 dated December 21, 2017, indicates that a small portion of the 100 mg CT-P10 DP vials from the PPQ and commercial runs fail based on the (b) (4) fill weight check and these vials do not meet the requirement of extractable volume per USP<1>. Section 3.2.P.3.5 should be revised to reflect that these vials are quarantined and discarded.

Sponsor's Response:

The sponsor revised Section 3.2.P.3.3.2.2.5 to include "Any vials whose filling weight is out of acceptance criterion, (b) (4) mg, are quarantined and discarded." A same statement for 500mg DP lots failing the acceptance criterion of (b) (4) mg is included in Section 3.2.P.3.3.1.2.5. The sponsor states that the three 100mg DP lots (16C3C02, 16C3C03, and 16C3C04) manufactured prior to the filling weight acceptance criteria change and had some vials failed to meet the acceptance criterion will not be supplied to the US market.

FDA Assessment:

The response is acceptable. The sponsor adequately resolved the CR issue 11.

Additional Comments:**Additional Comment 1:**

The review of the response to Additional Comment 1 was conducted by Dr. Nina Brahme.

In 3.2.R.3.2.1.5 Peptide Mapping by LC-MS Celltrion stated that the peptide sequence coverage was 100% for both the heavy chain and light chain for all samples. Additionally, you state that "MS/MS data (data not shown) confirmed that the amino acid sequences of Rituxan, CT-P10, and MabThera matched and sequence coverage by MS/MS was also 100% (data not shown)." We note the summarized information in Table 3.2.R.3-6 described the trypsin- and Asp-N- digested peptides. However, the heavy chain residues 419 and 420 were not provided. Clarify this discrepancy.

Reviewer comment: *In the original submission, the sponsor provided summarized information from trypsin and Asp-N digests of all three products (Table 3.2.R.3-6). We noted that heavy chain residues 419 (S) and 420 (R) were not identified by the trypsin digest and the summarized Asp-N digest information did not cover these residues.*

In the resubmission, the sponsor states that these residues were unintentionally left out of Section 3.2.R.3.2.1.5 and they have now included a peptide resulting from a Lys-C digest (HYS35). Table 1 of the document titled "Complete Response Product Quality Additional Comments 1" under section 1.11.1

summarizes this additional peptide. This peptide is the same across all three drug products and provides coverage for heavy chain residues 419-420, which were not detected in the trypsin digest. The sponsor also updated section 3.2.R.3 with new summary tables for the peptide mapping data. While they are re-organized compared to the originally submitted data, they most importantly include the new HYS35 peptide. Trypsin, Asp-N, and Lys-C digests are summarized in tables 3.2.R.3-6 through 3.2.R.3-8 for Rituxan, CT-P10 DP, and MabThera, respectively.

This response adequately addresses our additional comment regarding primary sequence coverage by peptide mapping.

Additional Comment 2:

In the complete response submission, provide updated data from the following studies that are ongoing:

- The commercial (b) (4) (in section 3.2.S.2.5),
- The commercial (b) (4) study (in section 3.2.S.2.5),
- The ongoing CT-P10 DS and DP stability studies (in sections 3.2.S.7 and 3.2.P.8),
- The leachable study on 100 mg DP lots committed in your January 3, 2018 response to IR comment 21,
- The stability study on three (b) (4) lots for up to 12 months, committed in your January 3, 2018 response to IR comment 8. Provide the control strategy of (b) (4) used in the manufacturing of CT-P10.

Sponsor's Response:

- Celltrion updated the results of the commercial (b) (4) study (shown in table below).
Table 3.2.S.2.5-46: (b) (4)

(b) (4)

Reviewer comment: All (b) (4) performance results met the pre-specified acceptance criteria, and no trend was observed.

(b) (4)

- c. Celltrion updated the stability results of CT-P10 DS and DP. *Note that no additional stability lot data were reported, although 2017 and 2018 manufactured lots supposedly should be included in the stability program. The current dataset is acceptable considering that the additional lot data will be very minor portion of the cumulated stability data.* For the DS and DP commercial lots, up to 36 month of DS stability data at (b) (4) °C storage, up to 36 months of 500mg DP stability data and up to 12 months of 100mg DP data at 5°C storage condition. In addition, up to 48 months of stability data from process 3 500mg DP is provided. *All stability results are within the corresponding acceptance criteria. A slight increase of %Acidic peaks is observed: 16% to 19% (from 0 to 36 months). The 500 mg DP process 3 (used to manufacture clinical lots) is representative of process 4 (commercial) and the DP materials were comparable, therefore the process 3 data are used to support 500 mg DP shelf life (from the initial BLA review). Based on available data the shelf life of 500mg DP is set as 48 months when stored at 5±3°C.*

Regarding the shelf life of 100 mg DP, the sponsor proposed (b) (4) This approach is not acceptable, and the following IR was sent to the sponsor on 10/12/2018:

(b) (4)

The BLA contains 12 months long term stability data of 100 mg DP manufactured by the commercial process (process II) and 30 months long term stability data for 100 mg DP manufactured at (b) (4) (process I). In order to use the stability data from 100 mg DP manufactured by process I to support the shelf-life of the commercial 100 mg DP, provide the following:

- Justification that the process I is fully representative of and simulating the process II,
- Graphical presentation of all available stability results under long term, accelerated and stressed conditions for each quality attribute to support assessment of comparability between the 100 mg process I and process II DPs.

Alternatively, submit updates of the stability data at 5 ± 3 °C of the process II DP lots to support the shelf-life beyond 12 months for 100 mg presentation.”

In the response, the sponsor repeated the information submitted in the 3.2.P.2.3 for the comparison the Process I and II process parameters and IPC results. *No new information was submitted in this regards.* The graphical presentation of the stability data comparison of process I and process II lots included IEC-HPLC, reduced and non-reduced CE-SDS, SEC-HPLC, CDC, sub-visible particles assay results under 5±3°C, 25±2°C/60±5%RH (accelerated), and 40±2°C/75±5%RH (stressed) conditions. *The scales of the graphs were not visual optimal to assess the data/trends. However, it appears to show a slight difference in degradation trend of the H+L results (by CE-SDS) under accelerated and stressed conditions (see yellow highlights in the Figures below).*

Considering the differences in manufacturing sites and scales between the process I and II, and the described uncertainties for the comparability data, the stability data of process I 100mg DP lots cannot adequately support the shelf life of process II lots.

In the IR response, the sponsor submitted up to 24 months long-term stability data for process II 100mg DP lots. All results are within the DP stability acceptance criteria. A slight increase of %

acidic peaks is seen over the period of 24 months (from 16.5% to 18.6%). Based on available stability data from process II (commercial) DP lots, the shelf life of 100mg DP is set as 24 months when stored at 5±3°C storage condition.

- d. Celltrion updated the data (0, 1, and 3 month) from the 100mg DP leachable study (at inverted position). The samples were tested using RP-HPLC to detect elements, inductive coupled plasma (ICP) to detect UV active leachables, and gas chromatography (GC-MS) to detect volatile and semi-volatile leachables. Analytical detection limits for these tests are 0.1 – 1.0 ppm for RP-HPLC-UV, 1.0 – 10 ppm for GC-MS and 0.01 – 5 ppm for ICP-MS, respectively. No leacheables were detected at 1 and 3 month time points. The study will continue for 48 months. *The response is acceptable.*

(b) (4)

Additional Comparative Stability Data

The additional stability data comparing CT-P10 and US-Rituxan are submitted in 3.2.S.7.3 of the BLA, which was assessed by Dr. Nina Brahme.

Reviewer comment: *The sponsor submitted comparative accelerated (25°C for 6 months) and stressed (40°C for 3 months) stability data for CT-P10 and US-Rituxan. Although for some quality attributes at*

some stability time points, the analytical values slightly differ between the two products (e.g., sum of basic peaks), the overall trends are similar.

Additionally, in-use stability data comparing analytical profiles of CT-P10 and US-Rituxan diluted to 1 mg/mL or 4 mg/mL in either 250 mL polyethylene or polyvinyl bags with 5% dextrose were provided. Data was compared at time 0, after 24 hours of hold at 5°C, and after 24 subsequent hours of hold at 30°C. The in-use stability data for CT-P10 and for the analyzed Rituxan lots do not show any sign of incompatibility (degradation, quality changes), and further the products appear to have comparable characteristics under the studied conditions. Together, these data support a demonstration that these products are analytically similar.

Additional Issues discovered during Pre-licensure inspection:

(b) (4)



Haoheng
Yan

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Nina
Brahme

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Christopher
Downey

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Date: August 31, 2018
STN: 761088/0 Resubmission
Reviewer: Candace Gomez-Broughton, Ph.D. Microbiologist CDER/OPQ/OPF/DMA/Branch IV
Endorsed: Reyes Candau-Chacon, Ph.D. Quality Assessment Lead CDER/OPQ/OPF/DMA/Branch IV
Subject: Original Biological License Application
Applicant: Celltrion, Inc.
License #: 1996
Facilities: Celltrion, Inc. Plant 2 Incheon, Republic of Korea
FEI: 3005241015
Product: Truxima® (CT-P10)
Dosage: 500 mg/50 mL, 100 mg/10 mL
Indication: Treatment for non-Hodgkin's lymphoma, (b) (4)
Action Date: November 29, 2018

Recommendation: This BLA supplement is recommended for approval from a sterility assurance and microbiology product quality perspective.

The applicant, Celltrion Inc., has resubmitted BLA 761088 for CT-P10. The applicant has addressed the deficiencies listed in the Complete Response (CR) letter issued on 28 February 2018. Reference is made to the review memo dated 19 January 2018 which covered the drug product manufacturing process from a microbiological perspective. This review will cover the applicant's responses to CR items and an additional comment related to sterility assurance of the drug product manufacturing process.

Complete Response Item #12

The media fill simulation provided in the BLA was not conducted using the primary container closure system specific for the CT-P10 100-mg process (10-mL (b) (4) glass vial and 20mm (b) (4) rubber stopper). Conduct three media fill simulations using the CT-PT10 100-mg primary container closure system and include the study report upon submission.

Response

The applicant performed media fill studies consisting of three runs using the 10-mL vial which is the primary container for the CT-P10 100 mg DP presentation. Study report P2-MFR-60247 was submitted to the application.

Three consecutive media fill runs were completed. The media fill strategy consisted of (b) (4)

The three consecutive media fills were successfully completed. The runs that were completed using the primary container for CT-P10 (10-mL (b) (4) glass vial and 20mm (b) (4) rubber stopper) were completed from 21 April 2018 to 10 May 2018. Media fills were done using a filling speed of (b) (4) which is the same filling speed used during routine operation.

(b) (4) Growth promotion test were done using *S. aureus*, *B. subtilis*, *P. aeruginosa*, *A. brasiliensis*, *Burkholderia* Sp. and *C. albicans*. Vials are turned over to wet all surfaces then incubated for 7 days at 20-25°C

then 7 additional days at 30-35°C. Vials are inspected for growth on the 7th and 14th days. The media fill studies are summarized in the following table.

Media Fill Study for 10-mL Primary Container			
	Run 1	Run 2	Run 3
Total Vials Filled/Incubated	(b) (4)		
# Acceptable Vials			
Rejected Vials			
Contaminated Vials			
Total filling time			
Growth Promotion Result	Growth	Growth	Growth

Results show that there were no contaminated units from any of the runs. (b) (4)
 . Results were reported as "Pass" for each category.

Interventions used during the media fills included (b) (4)

Review comments: The media fill studies successfully supported the (b) (4) manufacturing process for 10 mL vial presentation of the CT-P10 drug product.

Complete Response Item #13

Vials used as positive controls in the dye penetration container closure integrity test were breached using a 34-gauge needle (80-190 µm). The positive control breach size is approximately 12-fold larger than the test method limit of detection. Modify the container closure integrity test to include positive controls with breach size close to the limit of detection.

Response

The dye penetration test method was updated. The test will now include positive controls with 10 µm laser drilled holes. Section 3.2.P.8.3.1.9.2 has been updated accordingly.

Reviewer comment: The CCIT has been adequately modified.

Complete Response Item #14

Information and data supporting the use of the drug product sterilizing filter is insufficient. The bacterial retention study included in the BLA showed a loss in cell viability after 8 hours of contact with the drug product. Therefore, the study was conducted by inoculating the bacteria during the last 8 hours of total contact time (b) (4). However, no evidence is provided to indicate that cell viability is maintained throughout the 8-hour challenge period. Provide bacterial growth study data to demonstrate consistent growth of *B. diminuta* for up to 8 hours of contact time with the drug product.

Response

The applicant has submitted data from the viability test showing sustained viability of the test organism for up to 8 hours of contact with the CT-P10 drug product at 20-25°C. Data showed no reduction in viability compared to the control sample at the 8-hour timepoint. However, after 24 hours of incubation there was a > 1.7 log reduction in viability in the test samples. Growth remained consistent in the control sample. The results from the viability study are provided in the following table.

	Sample	Acceptance Criteria	0 min	5 min	30 min	60 min	4 hours	8 hours	24 hours
Control Sample	Initial Concentration of Test Organism	< 1 log reduction	100 CFU/0.1 mL	NA					
	Viability of Test Organism		NA	109 CFU/plate	112 CFU/plate	113 CFU/plate	111 CFU/plate	109 CFU/plate	108 CFU/plate
	Log Reduction		NA	0 log reduction					
CT-P10 DP	Initial Concentration of Test Organism	< 1 log reduction	100 CFU/0.1 mL	NA					
	Viability of Test Organism		NA	106 CFU/plate	111 CFU/plate	109 CFU/plate	95 CFU/plate	83 CFU/plate	2 CFU/plate
	Log Reduction		NA	0 log reduction	0 log reduction	0 log reduction	0 log reduction	0.1 log reduction	1.7 log reduction

Reviewer comment: The data demonstrates sustained cell viability in CT-P10 DP for up to 8 hours. However, there is clear loss of cell viability in the next time-point that was taken after 24 hours of growth. . The applicant's strategy to inoculate the DP during the last 8 hours of the bacterial retention study appears to be acceptable.

Complete Response Item #15

The equipment sterilization validation data to support (b) (4) sterilization of the (b) (4) were not sufficiently described. (b) (4)

Response

The applicant has provided the requested information regarding (b) (4) validation studies. (b) (4)

Reviewer comments: The applicant's response to this item is satisfactory. (b) (4) validation studies demonstrate that

equipment sterilization procedures are sufficient. Sterilization study parameters and results were reviewed with the original BLA and were adequate.

Additional Comment #3

Results from the endotoxin spiking study indicate that the CT-P10 drug product is affected by low endotoxin recovery (LER) when tested using the kinetic chromogenic method. Therefore, the applicant submitted data to qualify the Gel Clot method as an alternative method for endotoxin detection. The data submitted in Amendment 0028 demonstrates that no LER effects are observed when the Gel Clot method is used. Update the BLA to include the use of the gel clot method as a release test. A description of the method, specifications, and method qualification studies should be included in the appropriate sections of the application.

Response

Reference is made to the applicant's response to drug product Information Request 15 that was submitted to the BLA on 3 Jan 2018 where the applicant committed to employ the gel clot method for endotoxin at release.

Method verification studies were completed in accordance with USP <85> *Bacterial Endotoxins Test*. Three lots of DP were used in these studies (15C2C08, 15C2C09, and 15C3C10). Inhibition and enhancement studies were performed using two concentrations (1/10 and 1/100 dilutions) for each batch, yielding 6 results. Acceptance criteria for the studies required the positive product control and positive water control to be no less than 0.5λ and no greater than 2λ. In addition, the difference between the positive product control and positive water control must be equal to or less than 2 times. Results from the studies met the acceptance criteria, demonstrating that there was no inhibition or enhancement. Maximum valid dilution was determined to be 10.

The endotoxin limit based on a maximum dose of 15 mg/kg and the 10mg/mL drug product concentration is (b) (4) EU/mL. However, the applicant has proposed a specification of (b) (4) EU/mL for the CT-P10 drug product based on the assay limit of detection and the historical lot data. Ten lots of DP were tested using the proposed gel clot method and all results met the proposed (b) (4) EU/mL acceptance criteria.

The applicant has updated the following sections:

- 3.2.P.2.3 – development history updated for specification and analytical method
- 3.2.P.5.1, 3.2.P.5.4, and 3.2.P.5.6 – updated specification and justification of specification
- 3.2.P.5.2 and 3.2.P.5.3 – updated to include and description of the analytical procedure and verification data for release and stability study
- 3.2.P.8.1 and 3.2.P.8.2 – updated for introduce the acceptance criteria for stability studies

Reviewer comment: The gel clot method procedure and verification study data was submitted to the BLA. The revised and tightened endotoxin specification has been adequately justified.

CONCLUSION

- I. This BLA is recommended for approval from sterility assurance and microbiology product quality perspective
- II. Information and data in this BLA not related to microbial control of the drug product should be reviewed by the OBP reviewer.
- III. Refer to Panorama for GMP status of the relevant facilities.



Candace
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Broughton

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Reyes
Candau-Chacon

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Date: 10/23/2018 02:46:53PM
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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

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

Date: 10/9/2018
To: Administrative File, BLA 761088-ORIG-1-RESUB-34
From: Thuy T. Nguyen, Facility Reviewer, CDER/OPQ/OPF/DIA
Endorsement: Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA
Subject: Original BLA
US License: 1996
Applicant: Celltrion, Inc.
Mfg. Facility: Drug Substance and Drug Product: Celltrion, Inc.
FEI 3005241015

Product: Truxima
Dosage: 500 mg/ 50 mL, 100 mg/ 10 mL
Indication: Non-Hodgkin's lymphoma, chronic lymphocytic leukemia, (b) (4)

Due Date: 11/29/2018

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

SUMMARY

The subject BLA proposes manufacture of Truxima drug substance (DS) and drug product (DP) at Celltrion, Inc. (FEI 3005241015). A post-approval for (b) (4) and GMP surveillance inspection was conducted from May 22 – June 02, 2017 resulted in an Official Action Indicated (OAI) status with Warning Letter issued to the firm. A pre-license inspection was not conducted during the first review cycle due to the facility's OAI status. During the previous review cycle, we recommended withhold approval for this application (see review memo dated 12/4/2017 uploaded in panorama under the original submission)

A pre-licence inspection was conducted during this review cycle BLA 761088-ORIG-1-RESUB-34. The inspection was classified as VAI. The firm adequately address the FDA-483, Inspectional

Observations. CDER/DIA recommends approval for Celltrion, Inc. (FEI 3005241015). All listed facilities are currently in state of compliance and the application is recommended for approval from a facilities assessment standpoint.

CONCLUSION

As amended, all manufacturing, packaging and testing sites listed in the submission are recommended for approval from a facilities assessment standpoint.

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Thuy T. Nguyen, M.P.H.
Facility Reviewer
OPF Division of Inspectional Assessment
Branch 1

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



Thuy Thanh
Nguyen

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Zhihao Peter
Qiu

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Recommendation: Complete Response

BLA Number: 761088
Review Number: First round
Review Date: February 21, 2018

Drug Name/Dosage Form	Truxima (rituximab-abbs) injection, for intravenous use
Strength/Potency	100 mg/10 mL, 500 mg/50 mL (10 mg/mL concentration)
Route of Administration	Intravenous
Rx/OTC dispensed	Rx
Indication	(b) (4) Non-Hodgkin's lymphoma, (b) (4)
Applicant/Sponsor	Celltrion, Inc.

Product Overview

Rituximab-abbs (CT-P10) is a chimeric monoclonal IgG1 antibody, and the rituximab-abbs drug product, Truxima, has been developed as a proposed biosimilar to U.S.-licensed Rituxan. Rituximab-abbs binds to CD20 antigen expressed on the surface of pre-B and mature B-lymphocytes, and malignant B cells. Upon binding to CD20, rituximab-abbs mediates B-cell lysis. Possible mechanisms of cell lysis include complement dependent cytotoxicity (CDC), antibody dependent cell mediated cytotoxicity (ADCC), and antibody dependent cellular phagocytosis (ADCP), which are applicable to mechanisms of action of rituximab-abbs in B cell malignancies and in autoimmune diseases.

The rituximab-abbs molecule is constructed as a chimera of mouse variable regions binding human CD20 on human IgG1 backbone, and has one N-linked glycosylation site on each heavy chain. The Fc regions of the heavy chains retain Fc effector functions important in mechanism of action of rituximab.

Rituximab-abbs is produced in genetically engineered CHO (b) (4) cells. Rituximab-abbs drug product, Truxima, is manufactured to the same concentration, formulation, and presentation as U.S.-licensed Rituxan. Truxima is a sterile, preservative-free, colorless to pale yellow, clear to opalescent solution for intravenous infusion and supplied in single-dose vials containing CT-P10 at 100 mg/10 mL or 500 mg/50 mL.

Quality Review Team

Discipline	Reviewer	Branch/Division
Drug Substance	Haoheng Yan	OPQ/OBP/DBRR IV
Drug Product	Haoheng Yan	OPQ/OBP/DBRR IV
Drug Substance Microbiology	Scott Nichols	OPQ/OPF/DMA IV
Drug Product Microbiology	Candace Gomez-Broughton	OPQ/OPF/DMA IV
Facility	Thuy Thanh Nguyen	OPQ/OPF/DIA
Immunogenicity assay	Haoheng Yan	OPQ/OBP/DBRR IV
Analytical Similarity	Nina Brahme	OPQ/OBP/DBRR IV
Labeling	Vicky Borders Hemphill	OPQ/OBP
Product quality Team Lead	Bazarragchaa Damdinsuren	OPQ/OBP/DBRR IV
Microbiology QAL	Reyes Candau-Chacon	OPQ/OPF/DMA IV
Facility Branch Chief	Peter Qiu	OPQ/OPF/DIA
CMC RPBM	Oumou Barry, Keith Olin	OPQ/OPRO
Application Team Lead	Bazarragchaa Damdinsuren	OPQ/OBP/DBRR IV

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Multidisciplinary Review Team:

Discipline	Reviewer	Office/Division
RPM	Esther Park	OHOP/DHP
Cross-disciplinary Team Lead	Nicole Gormley	OHOP/DHP
Medical Officer	Andrea Baines, Anand Shah Stefanie Freeman, Nikolay Nikolov (Team Lead)	OHOP/DHP ODEII/DPARP
Pharm/Tox	Michael Manning	OHOP/DHP
Clinical Pharmacology	Sang Chung	ODEII/DPARP
CMC Statistics	Yu-Ting Weng	OHOP/DHP
Clinical Statistics	Jingjing Ye James Travis	OHOP/DHP ODEII/DPARP

Names:

- Proprietary Name: Truxima
- Non-Proprietary/USAN/INN: Rituximab-abbs/rituximab
- CAS Registry number: 174722-31-7
- Company/Laboratory code: CT-P10
- OBP systematic name: MAB CHIMERIC (IGG1) ANTI P11836 (CD20_HUMAN) [CT-P10]
- Other Names: None

Submissions Reviewed:

Submission(s) Reviewed /sequence number	Document Date
STN 761088 /2	4/28/2017
STN 761088 /4 (response to IR #1)	5/24/2017
STN 761088 /12 (response to IR #2)	7/25/2017
STN 761088 /17 (response to IR #3)	8/18/2017
STN 761088 /22 (response to IR #3)	10/31/2017
STN 761088 /23 (response to IR#3)	11/15/2017
STN 761088 /21 (response to IR #4)	10/23/2017
STN 761088 /24 (response to IR #5)	11/21/2017
STN 761088 /25 (response to IR #5)	11/28/2017
STN 761088 /27 (response to IR #5)	12/18/2017
STN 761088 /28 (response to IR #5)	12/22/2017
STN 761088 /29 (response to IR #6)	1/3/2018
STN 761088 /30 (response to IR #6)	1/31/2018
STN 761088 /31 (response to IR #6)	2/9/2018

Quality Review Data Sheet

1. Legal Basis for Submission: 351(k)

2. Related/Supporting Documents:

A. DMFs:

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

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		(b) (4)	(b) (4)				Not reviewed. Sufficient information related to compatibility with the product is in the BLA.
(b) (4)	III			3	N/A	N/A	
	III		10 mL (b) (4) Vials	3	N/A	N/A	
	V		Bacterial Endotoxin Reduction Validation Process Validation	3	N/A	N/A	Defer to DMA reviewer. Contains validation for (b) (4)

1. Action codes for DMF Table:

- 1- DMF Reviewed; Other codes indicate why the DMF was not reviewed, as follows: 2 - Reviewed previously and no revision since last review; 3- Sufficient information in application; 4- Authority to reference not granted; 5- DMF not available; 6- Other (explain under "comments")
2. Adequate, Adequate with Information Request, Deficient, or N/A (There is enough data in the application; therefore, the DMF did not need to be reviewed.)

B. Other documents: IND 120885

3. Consults: None

Executive Summary

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

The Office of Biotechnology Products, OPQ, CDER, has completed review of STN 761088 for TRUXIMA (rituximab-abbs) manufactured by Celltrion, Inc. The data submitted in this application are not sufficient to support a conclusion that the manufacture of TRUXIMA is well-controlled and will lead to a product that is pure and potent for the duration of the shelf-life. From a CMC standpoint, OBP is recommending a Complete Response letter be issued to Celltrion to outline the deficiencies noted below and the information and data that will be required to support approval.

Additionally, this application cannot be approved during this review cycle due to facility deficiencies. A pre-license inspection of TRUXIMA drug substance and drug product manufacturing facility could not be conducted due to Official Action Indicated (OAI) status associated with a Warning Letter (WL 320-18-28) issued to Celltrion. The Division of Inspectional Assessment, OPF, OPQ is recommending a withhold status, and a Complete Response letter be issued to Celltrion to outline the deficiency noted below.

The Division of Microbiology Assessment, OPF, OPQ is recommending a Complete Response letter be issued to Celltrion to outline the deficiencies noted below and the information and data that will be required to support approval.

The analytical similarity assessment as presented in the BLA is acceptable to support the conclusions that:

- The biological product, rituximab-abbs, is highly similar to U.S.-licensed Rituxan,
- A sufficient scientific bridge was established to support the use of E.U.-approved MabThera as a comparator in clinical trials supporting this application.

B. Summary of Complete Response (CR) Issues:

Product Quality CR comments (provided by OBP):

1. The cell culture process (b) (4)

(b) (4)
However, the characterization and assessment of (b) (4)

(b) (4)
Therefore, your control (b) (4) during manufacture is inadequate. To ensure product quality and cell culture process consistency,

(b) (4)

(b) (4)

2. Your proposed ranges for certain process parameters are unacceptable, given that their values exceed those evaluated during process validation and that adequate process characterization data supporting their limits were not provided. For the following parameters, revise sections 3.2.S.2.2 to limit the proposed acceptable ranges for the commercial process to the validation ranges or to commercial manufacturing experience. Alternatively, provide additional process characterization data to justify the proposed ranges for these parameters.

(b) (4)

3. In the responses (received on 11/21/2017 and 11/28/2017) to the Agency's information request (IR) comments 1e, 1f, 1i and 16 (11/13/2017), Celltrion included descriptions of certain process parameters. However, section 3.2.S.2.2 which describes the conditions of routine manufacture was not updated to reflect this information. Update section 3.2.S.2.2 to be consistent with the information received on 11/21/2017 and 11/28/2017 for the following parameters:

(b) (4)

4. In the response received on 11/28/2017 to our IR regarding the acceptable ranges for the (b) (4) for the chromatography columns, Celltrion stated that these ranges are based on (b) (4). This does not provide sufficient control for consistency during routine manufacture. Parameters associated with (b) (4) should be assigned for the target values set in the (b) (4) and justified using data obtained in the PPQ and commercial manufacturing runs. If wider ranges are desired than those established during PPQ and commercial manufacturing runs, data should be provided to justify the proposed ranges.

5. Product fragments are identified as a critical quality attribute (CQA) for CT-P10 and monitored (b) (4)

However, the impact of the (b) (4) process on product fragment levels was not assessed during process development. Therefore, it is not clear if proposed critical process parameters are sufficient to control levels of fragments during routine manufacture. To adequately control for product fragments:

- a. Identify (b) (4) process unit operations that impact the levels of product fragments,

- b. (b) (4)

- c. Provide a complete summary and description of your control strategy for product fragments.

6. Deamidation at the heavy chain Asn55 (HC Asn55) site is identified as a CQA and controlled by (b) (4) To support

- the proposed control strategy, provide data showing that changes in deamidation at HC Asn55 can be reliably detected by the (b) (4) method.
7. The data provided in section 3.2.R.4.1.5 Afucosylation show that increased afucosylated glycans (G0+G1) can affect FcγRIIIa binding and, consequently ADCC activity of CT - P10. Therefore, the proposed DS specification for glycans of G0 = (b) (4) % and Unidentified Peaks (b) (4) % does not provide adequate control of ADCC, as it does not provide sufficient control of G1 content. Provide the BLA with the following:
 - a. Propose DS specifications with acceptance criteria to control all main afucosylated glycans,
 - b. Provide assay validation data to confirm the suitability of the oligosaccharide profile test for the proposed specifications for afucosylated glycans,
 - c. Provide all available DS lot release and stability data to justify the revised specifications and the acceptance criteria.
 8. In the response to IR comment 4 (received on 1/3/2018), Celltrion described the stability testing plan and specifications for the CT-P10 master and working cell banks; however, the information was not submitted in relevant section of Module 3. Update section 3.2.S.2.3.2 with the information provided in the response.
 9. FcγRIIIa binding affinity is included in the DS release and stability specifications to control CT-P10 ADCC activity. Celltrion states that testing of DS is sufficient to ensure FcγRIIIa binding affinity of CT-P10 DP throughout its shelf life. However, no data is provided for FcγRIIIa binding affinity for DP stored under the long term storage condition of 5±3°C. Celltrion should provide data for FcγRIIIa binding affinity during DP storage to support the current testing strategy, or alternatively, include FcγRIIIa binding affinity testing in the DP stability program.
 10. In the IR response received on 1/3/2018, you proposed an acceptance criterion of polysorbate 80 (PS80) concentration (b) (4)

 _____ Provide data to support the adequacy of the proposed PS80 lower limit.
 11. The response (received on 1/3/2018) to the Agency's IR (12/21/2017) comment 26 indicates that a small portion of the 100 mg CT-P10 DP vials from the PPQ and commercial runs fail based on (b) (4) fill weight check and these vials do not meet the requirement of extractable volume per USP<1>. Section 3.2.P.3.5 should be revised to reflect that these vials are quarantined and discarded.

Microbiology CR comments (provided by OPF/DMA IV):

12. The media fill simulation provided in the BLA was not conducted using the primary container closure system specific for the CT-P10 100-mg process (10-mL (b) (4) glass vial and 20mm (b) (4) rubber stopper). Conduct three media fill simulations using the CT-PT10 100-mg primary container closure system and include the study report upon resubmission.
13. Vials used as positive controls in the dye penetration container closure integrity test were breached using a 34-gauge needle (80-190 μm). The positive control breach size is approximately 12-fold larger than the test method limit of detection. Modify the container closure integrity test to include positive controls with breach size close to the limit of detection.

14. Information and data supporting the use of the drug product sterilizing filter is insufficient. The bacterial retention study data included in the BLA showed a loss in cell viability after 8 hours of contact with the drug product. Therefore, the study was conducted by inoculating the bacteria during the last 8 hours of total contact time (b) (4). However, no evidence is provided to indicate that cell viability is maintained throughout the 8-hour challenge period. Provide bacterial growth study data to demonstrate consistent growth of *B. diminuta* for up to 8 hours of contact time with the drug product.
15. The equipment sterilization validation data to support (b) (4) sterilization of the (b) (4) were not sufficiently described. (b) (4)

Facility CR comment (provided by OPF/DIA):

16. During a recent inspection of the Celltrion, Inc. (FEI 3005241015) manufacturing facility, the field investigator observed objectionable conditions at the facility and conveyed that information to the representative of the facility at the close of the inspection. Satisfactory resolution of the observations is required before this BLA may be approved.

Immunogenicity assay draft CR comment (provided by OBP to clinical division):

(b) (4)

C. Summary of Non-CR Issues (provided by OBP):

1. In 3.2.R.3.2.1.5 Peptide Mapping by LC-MS you stated that the peptide sequence coverage was 100% for both the heavy chain and light chain for all samples. Additionally, you state that "MS/MS data (data not shown) confirmed that the amino acid sequences of Rituxan, CT-P10, and MabThera matched and sequence coverage by MS/MS was also 100% (data not shown)." We note in the summarized information in Table 3.2.R.3-6 describing the trypsin-digested peptides and limited information about the Asp-N-digested peptides that heavy chain residues 419 and 420 are unaccounted for. Clarify this discrepancy.
2. In the Complete response submission, provide updated data from the following studies that are ongoing:
 - a. The commercial scale (b) (4) (in section 3.2.S.2.5),
 - b. The commercial (b) (4) study (in section 3.2.S.2.5),
 - c. The ongoing CT-P10 DS and DP stability studies (in sections 3.2.S.7 and 3.2.P.8),
 - d. The leachable study on 100 mg DP lots committed in your 1/3/2018 response to IR comment 21,
 - e. The stability study on three (b) (4) lots for up to 12 months, committed in your 1/3/2018 response to IR comment 8. Provide the control strategy of (b) (4) (b) (4) used in the manufacturing of CT-P10.

Summary of Non-CR Issue (provided by OPF/DMA):

3. Results from the endotoxin spiking study indicate that the CT-P10 drug product is affected by low endotoxin recovery (LER) when tested using the kinetic chromogenic method. Therefore, the applicant submitted data to qualify the Gel Clot method as an alternative method for endotoxin detection. The data submitted in Amendment 0028 demonstrates that no LER effects are observed when the Gel Clot method is used. Update the BLA to include the use of the gel clot method as a release test. A description of the method, specifications, and method qualification studies should be included in the appropriate sections of the application.

D. Benefit/Risk Considerations:

Truxima (rituximab-abbs, CT-P10) is a proposed biosimilar to U.S.-licensed Rituxan and proposed for use in (b) (4) Non-Hodgkin's lymphoma,

(b) (4)

To support a demonstration that CT-P10 is highly similar to U.S.-licensed Rituxan, and to establish a 3-way scientific bridge to justify the use of data generated from use of E.U.-approved MabThera, Celltrion performed an analytical similarity assessment using a sufficient number of CT-P10, U.S.-licensed Rituxan, and E.U.-approved MabThera drug product lots. Rituximab molecular attributes are collectively assigned to the correct tiers, and the attribute similarity data package was generated and evaluated using appropriate analytical and statistical methods. The data provided in the BLA support the conclusion that CT-P10 is highly similar to U.S.-licensed Rituxan. In addition, the data support the adequacy of the analytical component of scientific 3-way bridge to justify the use of clinical data generated using E.U.-approved MabThera.

The OPQ review of manufacturing has identified that the methodologies and processes used for drug substance and drug product manufacturing, release and stability testing as submitted in the initial BLA submission are not sufficient to assure a consistent and safe product (see CR comments 1-11 in section I B). The drug substance manufacturing process is robust for inactivation and removal of adventitious agents.

The BLA is not recommended for approval from a sterility assurance and microbiology product quality perspective by DMA-IV, OPF due to deficiencies related to drug product manufacturing (see CR comments 12-15 in section I B).

The commercial manufacture of rituximab-abbs drug substance and of drug product at Celltrion, Ltd. (Incheon, Republic of Korea) is recommended for withhold status by DIA, OPF, as a pre-license inspection could not be conducted until the facility OAI status is resolved (see CR comment 16 in section I B).

The immunogenicity assays are not sufficiently sensitive to detect anti-drug antibodies (ADA) and neutralizing antibodies (NAb) in presence of rituximab-abbs at plasma concentrations of Advanced Stage Follicular Lymphoma (AFL) patients (see draft CR comment 17 in section I B); however, the assays are acceptable for testing of ADAs and NAb in rheumatoid arthritis patients' samples. It is deferred to the clinical division to decide whether the immunogenicity data in AFL population is required for approval of the BLA.

The technical assessments for OBP drug substance and drug product quality and immunogenicity assay, DMA microbiological drug substance and drug product, DIA facility, and OBP analytical similarity are located as separate documents in Panorama (see list in the end of this review).

II. Summary of Quality Assessments:

A. Analytical Similarity Assessment

To support a demonstration that Truxima (rituximab-abbs, CT-P10) is highly similar to the U.S.-licensed Rituxan reference product, and to establish a 3-way scientific bridge, Celltrion performed an analytical similarity assessment using 15 lots of each CT-P10 drug product, U.S.-licensed Rituxan, and E.U.-approved MabThera. Rituximab molecular attributes are collectively assigned to the correct tiers and attribute similarity was evaluated using appropriate statistical methods (Table 1). CDC and ADCC activities were assigned into tier 1 similarity assessment based on primary mechanisms of action of rituximab. The similarity data package (analytical data provided in the original submission and in response to information requests) was generated using methods that are considered appropriately validated or qualified for their intended purpose. CT-P10 meets the statutory "same strength" requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.

The data as presented in the BLA submission support the conclusion that CT-P10 is highly similar to U.S.-licensed Rituxan. In addition, the data support the adequacy of the analytical component of scientific 3-way bridge to justify the use of clinical data generated using E.U.-approved MabThera.

Table 1: Summary of Similarity Assessment

¹ - Equivalence for tier 1 attributes: Equivalence margin (EM) was determined as 1.5σR of US-Rituxan data and results were determined as 90 % CI of mean difference between two products.

- % within quality range (QR) for tier 2 attributes: The QR limits were set based on the range of the values obtained for US-Rituxan variation, expressed as X times SD. High similarity was considered to have been demonstrated if 90% of data points were within the QR.

- Visual similarity for tier 3 attributes.

² OBP assessment on the highlighted comparison failing similarity.

³ X multiplier = 2.

⁴ X multiplier = 3.

⁵ 92% of commercial CT-P10 product lots from Process 4 were within the quality range of Rituxan.

Activity	Clinical Relevance	Tier	Assay	Measurement	Analytical Similarity ¹ US-Rituxan vs CT-P10 DP
Physicochemical Analysis					
Primary Structure	Efficacy, Safety and Immunogenicity	3	Peptide Mapping (HPLC)	Comparison of tryptic peptide map by visual inspection	Similar
		3	Amino Acid Analysis	Determination of amino acid composition	Similar
		2	Molar Absorptivity	Determination of molar absorptivity and extinction coefficient	Molar absorptivity: 93% ⁴ Extinction coefficient: 93% ⁴
		3	N-terminal Sequencing	Comparison of N-terminal sequences	Similar
		3	C-terminal Sequencing	Comparison of C-terminal sequences	Similar
		3	Intact Mass (LC-MS)	Molecular Weight	Similar
Post Translational Modification	Efficacy, PK, Safety and Immunogenicity	2	Peptide Mapping (LC-MS)	Deamidation	% at HC Asn55: 100% ⁴ % at HC Asn290: 100% ⁴ % at HC Asn319: 100% ⁴ % at HC Asn365: 80%⁴ % at HC Asn388: 87%⁴ % at LC Asn136: Similar
			² Whether and How Residual Uncertainty is Addressed: Yes. The actual difference is very small. Peak characterization study, forced degradation study, potency assays, and risk assessment based on location of post-translational modification (PTM, not involved with epitope or Fc receptor binding) addressed uncertainty.		
		3	Peptide Mapping (LC-MS)	Oxidation	Similar
		3		N-terminal glutamine	Similar
		3		C-terminal lysine	Similar
Higher Order Structure	Efficacy, Safety and Immunogenicity	3	FTIR	Comparison of secondary structure	Similar
		2	DSC	Evaluation of thermal stability and determination of thermal transition temperatures	Transition 1 (°C): 100% ⁴ Transition 2 (°C): 100% ⁴ Transition 3 (°C): 100% ⁴
		3	CD	Comparison of secondary and tertiary structures	Similar
		2	Free Thiol Analysis	Comparison of the amount of free sulfhydryl groups	100% ⁴
		3	Disulfide Bonds	Comparison of disulfide bond location	Similar
Content	Efficacy	2	Protein Concentration	Protein Concentration (UV280)	80%^{4,5}
			² Whether and How Residual Uncertainty is Addressed: Yes. CT-P10 DP Process 4 included a process change to better target the correct		

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			<i>protein concentration. This assessment included 12 Process 4 lots and 3 Process 3 lots. Additionally, the protein concentration is the same between the 100 mg and 500 mg presentations, thus similarity data supporting protein content from the 500 mg CT-P10, Rituxan, and MabThera lots alone is sufficient.</i>		
		3	Extractable volume	Extractable volume (mL)	Similar
Purity/Impurity	Efficacy, Safety and Immunogenicity	2	SEC-HPLC	Determination of aggregate content and monomeric purity	Monomer (%): 60%⁴ HMW (%): 40%⁴ LMW (%): 60%⁴
			²Whether and How Residual Uncertainty is Addressed: Yes. Lower aggregate content in CT-P10 is a low risk difference with no impact on biological activity assays. SEC-MALS and AUC data also provided.		
		2	SEC-MALS	Determination of aggregate/monomeric content and molecular weight	Monomer (% , UV): 100% ⁴ HMW (% , UV): 100% ⁴ Monomer (% , MALS): 100% ⁴ HMW (% , MALS): 100% ⁴ Monomer (MW, kDa): 100% ⁴
		3		Determination of molecular weight (HMW)	Similar
		2	AUC	Determination of aggregate/monomeric contents	Monomer (s-value): 100% ⁴ Monomer (% Area): 100% ⁴ Dimer (s-value): 100% ⁴ Dimer (% Area): 100% ⁴
	Efficacy	2	Non-reduced CE-SDS	Determination of electrophoretic mobility and purity under non-reducing conditions	% Peak 1+2+3+4+5: 87%⁴ % Intact IgG: 87%⁴
			²Whether and How Residual Uncertainty is Addressed: Yes. Lower content of fragmented species in CT-P10 is a low risk difference with no impact on biological activity assays.		
		2	Reduced CE-SDS	Determination of electrophoretic mobility and purity under reducing conditions	% Non-glycosylated HC: 7%⁴ % H+L: 7%⁴
			²Whether and How Residual Uncertainty is Addressed: Yes. Biological activity assays showed no differences. The actual difference is small.		
	Safety and Immunogenicity	3	Residual Host Cell Protein	Determination of the level of residual HCP	Similar
		3	Residual Host Cell DNA	Determination of the level of residual host cell DNA	Similar
		3	Residual rProtein A	Determination of the level of residual rProtein A	Similar
		3	MFI	Comparison of the numbers of sub-visible particles	Similar
		3	HIAC		Similar
Charge Variants	Efficacy	3	IEF	Comparison of isoelectric point(s)	Similar
		2	IEC-HPLC	Comparison of charge variant distribution (Peak 1+2+3)	7%⁴
			²Whether and How Residual Uncertainty is Addressed: Yes. Peak characterization study did not identify any one or few species driving differences in sums of acidic (Peak 1+2+3) or basic peaks. However, because the peaks have comparable functional activity to each other, it does not appear that differences in acidic or basic peak sums impacts biological activity.		
		3	IEC-HPLC	Comparison of charge variant distribution (Peak	Peak 4: Similar Basic Peaks: Not similar (see

				4 and sum of basic peaks 5, 6, 7)	<i>above)</i>
Glycosylation	Efficacy	2	Oligo-saccharide Profiling	Comparison of glycosylation patterns	% G0F: 100% ⁴ % Man5: 0%⁴ % G0: 100% ⁴ % G0+Man5: 53%⁴ % G1F: 100% ⁴ % G2F: 100% ⁴
		2	N-linked Glycan Analysis	Comparison of oligosaccharide structures, attachment sites and distribution	% G0F: 100% ⁴ % G1F: 100% ⁴ % G2F: 100% ⁴ % Man5: 0%⁴ %G0: 100% ⁴ %G0+Man5: 0%
			² Whether and How Residual Uncertainty is Addressed: Yes. The difference is due to high mannose (M5) levels. Comparative PK results showing no difference addressed residual uncertainty. Other MOA concerns are addressed by glycosylation and biological activity studies. Specifically, additional characterization studies showed that although afucosylation (G0, G1, etc.) levels should be controlled, small differences in total afucosylated species (G0+Man5) level (like 2.6% versus 4.0%) do not impact ADCC activity (as measured using a reporter- or PBMC-based method) or other functional assays (FcγRIIIa, C1q, FcRn, CD20 binding).		
		3	N-linked Glycan Analysis	Comparison of oligosaccharide structures, attachment sites and distribution	% G1F+NANA: Similar %G2F+NANA: Similar %G2F+2NANA: Similar
		3	Sialic Acid Analysis	Determination of sialic acid content	Similar
		3	Mono-saccharide Analysis	Comparison of neutral and amino sugar composition	Similar
		Biological Analysis			
Fab Binding	MoA and Efficacy	2	Cell-based CD20 Binding (CELISA)	% Relative binding by (%, EC50)	100% ³
		2	Apoptosis using Raji cell (FACS)	% Relative apoptotic cells (%, apoptotic cells)	At 0.13 µg/mL: 100% ⁴ At 0.04 µg/mL: 100% ⁴ At 0.01 µg/mL: 100% ⁴
Fab-Fc Mediated Activities	MoA and Efficacy	1	CDC using WIL2-S cell	% Relative CDC potency (%, EC50)	Within EM
		1	ADCC using PBMC	% Relative ADCC potency (%, cytotoxicity)	At 0.122 µg/mL: Within EM At 0.035 µg/mL: Within EM At 0.010 µg/mL: Within EM
		2	ADCC Reporter Assay	% Relative reporter activity (%, EC50)	100% ³
	Putative MoA and Efficacy	2	ADCP using Raji cell	% Relative ADCP activity (%, phagocytosis)	At 25.0 ng/mL: 100% ⁴ At 6.25 ng/mL: 100% ⁴ At 1.56 ng/mL: 100% ⁴
Fc Binding	MoA and Efficacy	2	C1q Binding (ELISA)	% Relative binding affinity (%, EC50)	100% ⁴
		2	FcγRIIIa-V Binding Affinity (SPR)	% Relative binding affinity (%, KD)	100% ⁴
		2	FcγRIIIa-F Binding Affinity (SPR)	% Relative binding affinity (%, KD)	100% ⁴
	Immune System Mediator	2	FcγRIIIb Binding Affinity (SPR)	% Relative binding affinity (%, KD)	100% ⁴

	Putative MoA and Efficacy	2	FcγRIIa Binding Affinity (SPR)	% Relative binding affinity (% , KD)	100% ⁴
	Immune System Mediator	2	FcγRIIb Binding Affinity (SPR)	% Relative binding affinity (% , KD)	100% ⁴
	Immune System Mediator	3	FcγRI Binding Affinity (SPR)	% Relative binding affinity (% , KD)	Similar
	PK	2	FcRn Binding Affinity (SPR)	% Relative binding affinity (% , KD)	100% ⁴

B. CQA Identification, Risk and Lifecycle Knowledge Management

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

CQA type	CQA	Risk	Origin and process linkage	Control Strategy
Potency	Potency - CDC	Bioactivity, Safety	Intrinsic to the molecule	(b) (4)
	Potency - ADCC	Bioactivity, Safety	Intrinsic to the molecule	
Identity	* Identity	Bioactivity, Safety	Intrinsic to the molecule	
Size-Related Variants	Aggregation	Bioactivity, PK (FcRn), Immunogenicity, Safety	Manufacturing process	
	Fragmentation	Bioactivity, PK	Manufacturing process	
Charge variants and Deamidation	Asn55 deamidation in CDRs	Bioactivity	Manufacturing process. (b) (4)	
	* Deamidation in non-CDRs	PK	Manufacturing process. (b) (4)	
Glycosylation	Aglycosylation	ADCC, CDC, PK	Cell culture process	
	Afucosylation	ADCC, PK	Cell culture process	
	High Mannose	PK	Cell culture process	

DS - drug substance, DP - drug product

* Sponsor did not name these attributes as CQA, however these were tested as part of DS and DP release.

Note that binding to CD20, ADCC and apoptosis are not considered as CQAs. These can be controlled through manufacturing process consistency.

C. Drug Substance, rituximab-abbs, Quality Summary

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CQA Identification, Risk, and Lifecycle Knowledge Management

Table 3: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management.
(Additional to API CQAs shown in Table 2)

CQA type	CQA	Risk	Origin and process linkage (manufacturing experience)	Control Strategy
Process-related impurities	Residual DNA	Safety (gene transfer)	Cell culture (b) (4)	(b) (4)
	Residual Host Cell Proteins (HCP)	Safety, Immunogenicity	Cell culture (b) (4)	
	* Leached Protein A	Immunogenicity, Safety	(b) (4)	
	(b) (4)	Safety	Cell culture (b) (4)	
	* Raw Materials (subset), Leachables	Immunogenicity, Safety	Raw materials, manufacturing process	
Adventitious Agents	Viral Safety	Safety	Raw materials and manufacturing process	
	* Mycoplasma	Safety	Raw materials and manufacturing process	
Bioburden	Bioburden	Safety, Purity, and Efficacy (degradation or modification of the product by contaminating microorganisms)	Raw materials and manufacturing process	
Endotoxin	Endotoxin	Safety, Purity	Raw materials and manufacturing process	
Composition and Strength	* Protein Concentration	Bioactivity	Manufacturing process (b) (4)	
	* pH	Bioactivity	Formulation (b) (4)	
	* Appearance (color, clarity)	Bioactivity, Safety	Product formulation	
	(b) (4)	Bioactivity, stability	Raw material control, Manufacturing process	

DS - drug substance, DP - drug product, IPC – in-process control

* Sponsor did not name these attributes as CQA, however these were tested as part of IPC, DS or DP release.

- (b) (4)

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- (b) (4)
- **Container closure:** CT-P10 drug substance is stored in (b) (4)
 - **Dating period and storage conditions:** Celltrion proposed a shelf-life of (b) (4) months when stored at (b) (4) °C (long term). (b) (4)

D. Drug Product, Truxima, Quality Summary:

Table 4 provides a summary of the identification, risk, and lifecycle knowledge management for drug product specific CQAs that derive from the drug product manufacturing process and general drug product attributes. Active pharmaceutical ingredient and drug substance CQAs apply to drug product (see Tables 2 and 3).

Table 4: Drug Product-specific CQA Identification, Risk, and Lifecycle Management
(Additional to API CQAs shown in Table 2)

CQA type	CQA	Risk	Origin	Control Strategy
Particles	* Subvisible Particles	Safety, Immunogenicity	DP manufacturing process, CCS and product	(b) (4)
	* Visible Particles (foreign)	Safety	DP manufacturing process, CCS	
Vial Content	* Extractable Volume	Efficacy	DP manufacturing process (fill)	
Sterility	Sterility	Safety, Purity, and Efficacy (degradation or modification of the product by contaminating microorganisms)	Contamination may be introduced throughout the DP manufacturing process	
Container closure integrity	* Container closure integrity	Safety	Container closure breaches during storage	
Endotoxin	Endotoxin	Safety, Purity and Immunogenicity	Raw materials, contamination	

			throughout the DP manufacturing process	(b) (4)
Composition and Strength	* Protein Concentration	Bioactivity	Manufacturing process (b) (4)	
	* Appearance (color, clarity)	Bioactivity, Safety	Product, formulation	
	* Osmolality	Bioactivity	Formulation	
	* Uniformity of dosage units	Bioactivity	DP manufacturing process (b) (4)	

DS - drug substance, DP - drug product, IPC – in-process control

* Sponsor did not name these attributes as CQA, however these were tested as part of IPC, DS or DP release.

- **Potency and Strength:** 500 mg/50 mL, 100 mg/10 mL (10 mg/mL concentration)
- **Summary of Product Design:** A sterile, colorless to pale yellow, clear to opalescent solution for intravenous administration and contains no preservatives. Presented in single-dose vials.
- **List of Excipients:** Sodium chloride, tri-sodium citrate dihydrate, polysorbate 80.
- **Reference Standards:** Same as the CT-P10 drug substance RSs.
- **Manufacturing process summary:** The Truxima drug product manufacturing includes the following steps: (b) (4)

The filled vials are stoppered, and capped, 100% visually inspected, labeled and secondary packaged, and stored at $5 \pm 3^\circ\text{C}$.

Truxima drug product manufacturing process development is based on minimal process characterization and process validation studies. The overall control strategy is inadequate (see details in Table 2).

- **Container closure:** 50 mL or 10 mL (b) (4) glass vial (b) (4) with a rubber stopper (b) (4) and a 20 mm flip-off seal (b) (4)
- **Dating period and storage conditions:** Celltrion proposed (b) (4) months of shelf life when stored at $5 \pm 3^\circ\text{C}$, protected from light.

E. Novel Approaches/Precedents: None

F. Any Special Product Quality Labeling Recommendations:

Labeling review was not performed given that the BLA will not be approved.

G. Establishment Information:

Overall Recommendation:				
DRUG SUBSTANCE				
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Final Recommendation
DS manufacturing, release and stability testing, MCB and WCB Storage	Celltrion, Inc. 23, Academy-ro Plants 1 and 2 Yeonsu-gu Incheon, S. Korea	3005241015	Unacceptable due to OAI status	Withhold
Viral testing of unprocessed bulk; Testing of the MCB, WCB and (b) (4)	(b) (4)	(b) (4)	n/a	Approve based on facility profile
Sterility testing of (b) (4)			n/a	Approve based on facility profile
Mycoplasma testing of unprocessed bulk; Testing of the MCB, WCB, and (b) (4)			n/a	Approve based on facility profile
MCB and WCB storage			n/a	Approve based on facility profile
Production of the MCB and WCB			n/a	No Further Evaluation
DRUG PRODUCT				
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Final Recommendation
DP manufacturing, release and stability testing	Celltrion, Inc. 23, Academy-ro Plants 1 and 2 Yeonsu-gu Incheon, S. Korea	3005241015	Unacceptable due to OAI status	Withhold

DS - drug substance, DP - drug product, QC – quality control, IPC – in-process control, MCB - Master Cell Bank, WCB - Working Cell Bank, UPB – Unprocessed bulk
* See section G. Facilities.

H. Facilities:

The subject BLA proposes manufacture of Truxima drug substance and drug product at Celltrion, Inc. (FEI 3005241015). A post-approval inspection for (b) (4) and GMP surveillance inspection was conducted on May 22 – June 02, 2017 and resulted in an Official Action Indicated (OAI) status with Warning Letter Recommendation. The Warning Letter (WL 320-18-28) was issued to Celltrion on January 26, 2018. A pre-license inspection cannot be conducted until the OAI status is cleared by OMQ. Please refer to the table above for other facilities described in the table (section F), which are not inspected within scope of this BLA. The Celltrion facility is recommended for Withhold from a facilities assessment standpoint (see CR comment 16 in section I B).

I. Lifecycle Knowledge Management:

a. Drug Substance:

- i. Protocols approved:** None
- ii. Outstanding review issues/residual risk:** See CR comments in section I B.
- iii. Future inspection points to consider:** PLI inspection will be considered based on CR re-submission.

b. Drug Product

- i. Protocols approved:** None
- ii. Outstanding review issues/residual risk:** See CR comments in section I B.
- iii. Future inspection points to consider:** PLI inspection will be considered based on CR re-submission.

Review documents related to this Executive Summary (links to document in Panorama):

- Drug substance quality review by Haoheng Yan, MD, PhD (OPQ/OBP/DBRR IV) [link](#)
- Drug product quality review by Haoheng Yan, MD, PhD (OPQ/OBP/DBRR IV) [link](#)
- Drug substance microbiology review by Scott Nichols, PhD (OPQ/OPF/DMA IV) [link](#)
- Drug product microbiology review by Candace Gomez-Broughton, PhD (OPQ/OPF/DMA IV) [link](#)
- Analytical similarity review by Nina Brahme, PhD, MPH (OPQ/OBP/DBRR IV) [link](#)
- Facility review by Thuy Thanh Nguyen, MPH (OPQ/OPF/DIA) [link](#)
- Immunogenicity assay review by Haoheng Yan, MD, PhD (OPQ/OBP/DBRR IV) [link](#)

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

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



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Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment

Date: January 19, 2018
STN: 761088/0
Reviewer: Candace Gomez-Broughton, Ph.D. Microbiologist CDER/OPQ/OPF/DMA/Branch IV
Endorsed: Reyes Candau-Chacon, Ph.D. Quality Assessment Lead CDER/OPQ/OPF/DMA/Branch IV
Subject: Original Biological License Application
Applicant: Celltrion, Inc.
License #: 1996
Facilities: Celltrion, Inc. Plant 2 Incheon, Republic of Korea
FEI: 3005241015
Product: Truxima® (CT-P10)
Dosage: 500 mg/50 mL, 100 mg/10 mL
Indication: Treatment for non-Hodgkin's lymphoma, chronic lymphocytic leukemia, (b) (4)
Action Date: February 28, 2018

Recommendation: The drug product section of the BLA, as amended, is not recommended for approval from a microbiology product quality perspective. The following deficiency has been identified:

Media fill studies are inadequate. Media fill studies for the 100-mg presentation were not performed using the primary container for the drug product.

Additional Comments:

- Breach sizes used for positive controls (34-gauge) in the dye penetration test are too large. Positive controls with smaller breach sizes (closer to the limit of detection) should be used for this study
- Bacterial retention studies are insufficient. The current study states that the test organism displayed sufficient growth for up to 8-hours. A growth curve should be submitted to support this statement and support the conclusions made from the study results.
- Data from the (b) (4) validation studies was insufficient. (b) (4)
- The application should be updated to reflect the use of the gel clot method for endotoxin detection.
- Provide the flush volume used in the (b) (4) filter integrity test

Introduction

Celltrion Inc. has submitted a 351 (k) biological license application (BLA) for the approval of Truxima® (CT-P10). CP-10 was developed as a biosimilar to the US-licensed Rituxan®. CT-P10 is a chimeric human-murine immunoglobulin G1 (IgG1) monoclonal that is produced in a CHO cell line. The antibody binds to CD20 expressed B-cells which leads to cell lysis.

The review covers the drug product manufacturing process. The drug substance process is covered in a separate review completed by Scott Nichols, Ph.D.

Amendments Reviewed

Sequence 0028: Response to information request sent 21 Dec 2018

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

CT-P10 drug product (DP) is a sterile liquid for intravenous injection that will be available in two presentations; 500 mg protein/50 mL and 100 mg protein/10mL. Both presentations also consist of the following components:

- 154 mM sodium chloride,
- 25 mM tri-sodium citrate dehydrate
- 0.07% polysorbate 80
- sterile water for injection

P.2 Pharmaceutical Development

(b) (4)



Candace
Gomez-
Broughton

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Reyes
Candau-Chacon

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BLA Review Addendum

STN:	BLA761088
Subject:	Review memo for information (IR response) submitted after the primary review due date of 1/19/2018
Submission Date:	1/31/2018 and 2/9/2018
Review Date:	January 21, 2018
Primary Reviewer:	Haoheng Yan, MD, PhD Product Quality Reviewer, OPQ/OBP/DBRR IV
Secondary Reviewer:	Bazarragchaa Damdinsuren, MD, PhD Team Leader, OPQ/OBP/DBRR IV
Tertiary Reviewer:	Joel Welch, PhD Review Chief, OPQ/OBP/DBRR IV
Applicant:	Celltrion, Inc.
Product:	Truxima (CT-P10), proposed biosimilar to US-licensed Rituxan
Indications:	(b) (4) Non-Hodgkin's lymphoma, (b) (4) (b) (4) (b) (4)
Action Due Date:	February 28, 2018

Summary

This addendum to the Drug Product review covers the information (IR responses) submitted to the BLA after the primary review due date (January 19, 2018). The responses pertain to CT-P10 100mg drug product (DP) filling and secondary packaging process. The responses are deemed acceptable.

Review

The reviewer's comments are distinguished by use of italic font.

Amendment (eCTD Sequence No. 0029) submitted on 1/31/2018

The sponsor submitted response to an IR request sent on 12/21/2017, Question 25:

25. [REDACTED] (b) (4)
[REDACTED]
[REDACTED]. Clarify how [REDACTED] (b) (4)
[REDACTED] or provide data showing that
composition (for example, protein concentration) is not changed in the first filled vials in routine
manufacturing.

In the response, the sponsor evaluated the protein concentration of the initial filled vials in a
commercial production run (Lot No. 18C3C02 manufactured on January 3, 2018). Data is
provided in Table 1 of the submission. The protein concentration of the initial vial is [REDACTED] (b) (4)
[REDACTED] at all testing vials [REDACTED] (b) (4)

Reviewer's Comment: The response is acceptable.

The submission contained also updates to the drug substance stability data relevant to
the revised specification of unidentified peaks (with acceptance criterion [REDACTED] (b) (4) %) in Section
3.2.S.7.3. The submitted data does not change our assessment and conclusion, because the
proposed specification for glycans of unidentified peaks [REDACTED] (b) (4) % (and G0 = [REDACTED] (b) (4) %) does not
provide adequate control of ADCC, as it does not provide sufficient control of G1 content (see
details in CR comment 7 included in the CMC Executive Summary in Panorama).

Amendment (eCTD Sequence No. 0030) submitted on 2/9/2018

The sponsor submitted response to an IR request sent on 12/21/2017, Question 29:
29. In section 3.2.P.7.2.2 Secondary Packaging Components for CT-P10 100 mg Drug Product,
you stated "The dimensions and drawing of carton box for CT-P10 100mg Drug Product are in
draft and will be firmed up during BLA review period." Submit the final secondary packaging
for the 100 mg DP and provide your evaluation on packaging process validation and shipping
validation related to the final configuration.

In the response, the sponsor states that 3 packaging runs were performed for the CT-P10
100mg DP. In each secondary packaging run, the DP vials were exposed to the typical labeling
and packaging condition [REDACTED] (b) (4)
[REDACTED], to simulate the maximum
labeling and packaging processing time of up to [REDACTED] (b) (4) hrs. The product quality was evaluated per
current DP specifications. Results were provided in Table 1.

Reviewer's Comment: The results of all 3 runs showed no significant difference in product
quality of samples before and after the secondary packaging. The secondary packaging process
was appropriately validated for the CT-P10 100mg DP. The response is acceptable.



Haoheng
Yan

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Bazarragchaa
Damdinsuren

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Joel
Welch

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BLA STN 761088

Rituximab-a bbs

Manufacturer: Celltrion, Inc.

Drug Substance Quality Reviewer: Haoheng Yan, M.D., Ph.D.
Drug Product Quality Reviewer: Haoheng Yan, M.D., Ph.D.
Analytical Similarity Reviewer: Nina Brahme, Ph.D., M.P.H.
ATL Reviewer: Bazarragchaa Damdinsuren, M.D., Ph.D.
Review Chief: Joel Welch, Ph.D.

Division of Biotechnology Review and Research IV

OBP CMC Review Data Sheet

1. **BLA#:** STN 761088.
2. **REVIEW DATE:** January 19, 2018
3. **PRIMARY REVIEW TEAM:**
Medical Officers: Andrea Baines, Anand Shah, Nicole Gormley (DHP) and Stefanie Freeman, Nikolay Nikolov (DPARP)
Pharm/Tox: Michael Manning (DHP)
Product Quality Team:
Drug substance quality reviewer: Haoheng Yan (OPQ/OBP)
Drug product quality reviewer: Haoheng Yan (OPQ/OBP)
Immunogenicity assay reviewer: Haoheng Yan (OPQ/OBP)
Analytical similarity reviewer: Nina Brahme (OPQ/OBP)
Drug substance microbiology reviewer: Scott Nichols (OPQ/OPF/DMA)
Drug product microbiology reviewer: Candace Gomez-Broughton (OPQ/OPF/DMA)
Microbiology QAL: Reyes Candau-Chacon (OPQ/OPF/DMA)
Facility reviewer: Thuy Thanh Nguyen (OPQ/OPF/DIA)
Facility lead: Zhihao Peter Qiu (OPQ/OPF/DIA)
OBP Labeling reviewer: Vicky Borders Hemphill (OPQ/OBP)
OPQ RPM: Oumou Barry (OPQ/OPRO)
CMC Application Team Lead (ATL): Bazarragchaa Damdinsuren (OPQ/OBP)
CMC Statistics: Yu-ting Weng
Clinical Pharmacology: Sang Chung and Sarah Schrieber (DPARP)
Statistics: James Travis (DPARP) and Jingjing Ye (DHP)
Biometrics: Yu-Ting Weng (OTS/OB/DBVI)
RPM (clinical): Esther Park (DHP)

4. MAJOR GRMP DEADLINES

Filing Meeting	June 2, 2017
Filing Date	June 27, 2017
Day 74	July 11, 2017
Mid-Cycle Meeting	September 18, 2017
Primary Reviews Complete (target)	January 19, 2018
Wrap-up Meeting	January 22, 2018
Secondary Reviews Complete (target)	January 28, 2018
Complete CDTL Memo (target)	February 5, 2018
Division Director Memo	February 5, 2018
BsUFA Goal Date	February 28, 2018

5. COMMUNICATIONS WITH APPLICANT:

Communication/Document	Date
Information Request #1	5/19/2017
Information Request #2	7/7/2017

Information Request #3	8/7/2017
Information Request #4	10/13/2017
Information Request #5	11/13/2017
Information Request #6	12/21/2017

6. SUBMISSION(S) REVIEWED:

Submission	Date Received	Review Completed (responsible group)
STN 761088 /2	4/28/2017	Yes (OBP/DMA/DIA)
STN 761088 /4 (response to IR #1)	5/24/2017	Yes (DMA/DIA)
STN 761088 /12 (response to IR #2)	7/25/2017	Yes (DMA)
STN 761088 /17 (response to IR #3)	8/18/2017	Yes (OBP)
STN 761088 /22 (response to IR #3)	10/31/2017	Yes (OBP)
STN 761088 /23 (response to IR#3)	11/15/2017	Yes (OBP)
STN 761088 /21 (response to IR #4)	10/23/2017	Yes (OBP/DMA)
STN 761088 /24 (response to IR #5)	11/21/2017	Yes (OBP)
STN 761088 /25 (response to IR #5)	11/28/2017	Yes (OBP)
STN 761088 /27 (response to IR #5)	12/18/2017	Yes (OBP)
STN 761088 /28 (response to IR #5)	12/22/2017	Yes (OBP)
STN 761088 /22 (response to IR #6)	1/3/2018	Yes (OBP/DMA)

7. DRUG PRODUCT NAME/CODE/TYPE:

- a. Proprietary Name: Truxima
- b. Non-Proprietary/USAN/INN: Rituximab-abbs
- c. CAS Registry number: 174722-31-7
- d. Company/Laboratory code: CT-P10
- e. OBP systematic name¹: MAB CHIMERIC (IGG1) ANTI P11836 (CD20_HUMAN) [CT-P10]
- f. Other Names: None

8. PHARMACOLOGICAL CATEGORY: Recombinant chimeric monoclonal IgG1 antibody directed against human CD20**9. DOSAGE FORM:** Concentrate for solution for infusion (liquid in single-use vial)**10. STRENGTH/POTENCY:**

- (i) Truxima Drug Product: 500 mg/50 mL, 100 mg/10 mL
- (ii) Types of potency assays: 1. Complement Dependent Cytotoxicity (CDC) Assay is a cell-based cytotoxicity assay used as an *in vitro* measure of CT-P10 CDC activity

¹ The OBP systematic name allows searching for related products in OBP's database and in the Document Archiving, Reporting & Regulatory Tracking System (DARRTS) for safety reasons and it is different from the nonproprietary name. The tag at the end is used to separate products from different sponsors and it is generally the name used by sponsors to refer to the proposed product in their submissions.

2. FcγRIIIa Binding Affinity using Surface Plasmon Resonance (SPR) is an *in vitro* binding assay to measure an attribute which modulates antibody dependent cell-mediated cytotoxicity (ADCC).

11. ROUTE OF ADMINISTRATION: Intravenous (i.v.)

MASTER FILES:

		ITEM REFERENCED	Letter of Cross-Reference	COMMENTS (STATUS)
	(b) (4)	(b) (4)	Yes	<i>Not reviewed. Information provided in the BLA is sufficient for a preliminary conclusion of its product compatibility. Extended study is requested through additional comment in the CR letter.</i>
		Bacterial Endotoxin Reduction Validation Process Validation	Yes	<i>Validation for (b) (4) Defer to DMA reviewer.</i>
		10mL (b) (4) Vials (used as 100mg drug product container)	Yes	<i>Not reviewed. Information provided in the BLA is sufficient for a preliminary conclusion of its product compatibility. Extended study is requested through additional comment in the CR letter.</i>
		Glass Vials (used as 500mg drug product container)	Yes	<i>Not reviewed. Information provided in the BLA is sufficient for a preliminary conclusion of its product compatibility. Extended study is requested through additional comment in the CR letter.</i>

L ACTIVITIES

Drug Substance Manufacturing Facility Inspection: *No prelicensure inspection conducted due to facility OAI status.*

Drug Product Manufacturing Facility Inspection: *No prelicensure inspection conducted due to facility OAI status.*

14. CONSULTS REQUESTED BY OBP: None

15. QUALITY BY DESIGN ELEMENTS: None

16. PRECEDENTS: None

17. ADMINISTRATIVE**A. Signature Block**

Name and Title	Signature and Date
Joel Welch, Ph.D. Review Chief Division of Biotechnology Review and Research IV (DBRR IV) Office of Biotechnology Products (OBP) Office of Pharmaceutical Quality (OPQ)	See attached
Bazarragchaa Damdinsuren, M.D., Ph.D. Team Leader DBRR IV, OBP, OPQ	See attached
Haoheng Yan, M.D., Ph.D. Product Quality Reviewer DBRR IV, OBP, OPQ	See attached
Nina Brahme, Ph.D., M.P.H. Product Quality Reviewer DBRR IV, OBP, OPQ	See attached

B. CC Block

Recipient	Date
Esther Park Clinical Division BLA RPM	
OBP/DBRR IV File/BLA STN 761088	

SUMMARY OF QUALITY ASSESSMENTS

I. Primary Reviewer Summary Recommendation

I do not recommend approval of this 351(k) BLA application for CT-P10 as a biosimilar to U.S.-licensed Rituxan from the Product Quality perspective due to the deficiencies listed below.

II. List Of Deficiencies To Be Communicated (Draft)

1. The cell culture process (b) (4)

(b) (4)

(b) (4) However, the characterization and assessment (b) (4)

(b) (4)

(b) (4) Therefore, your control over (b) (4) during manufacture is inadequate. To ensure product quality and cell culture process consistency,

(b) (4)

2. Your proposed ranges for certain process parameters are unacceptable, given that their values exceed those evaluated during process validation and that adequate process characterization data supporting their limits were not provided. For the following parameters, revise sections 3.2.S.2.2 to limit the proposed acceptable ranges for the commercial process to the validation ranges or to commercial manufacturing experience. Alternatively, provide additional process characterization data to justify the proposed ranges for these parameters.

(b) (4)

3. In the responses (received on 11/21/2017 and 11/28/2017) to the Agency's information request (IR) comments 1e, 1f, 1i and 16 (11/13/2017), Celltrion included descriptions of certain process parameters. However, section 3.2.S.2.2 which describes the conditions of routine manufacture was not updated to reflect this information. Update section 3.2.S.2.2 to be consistent with the information received on 11/21/2017 and 11/28/2017 for the following parameters:

4. In the response received on 11/28/2017 to our IR regarding the acceptable ranges for the (b) (4) (b) (4) for the chromatography columns, you stated that these ranges are based on the (b) (4). We clarify that the acceptable range of (b) (4) should be assigned based on the target values set in the (b) (4) the target values used in the PPQ and commercial manufacturing runs. If wider ranges are desired, provide data to justify the proposed ranges.
5. Product fragments are identified as a critical quality attribute (CQA) for CT-P10 and monitored (b) (4). However, the impact of the (b) (4) process on product fragment levels was not assessed during process development. Therefore, it is not clear if proposed critical process parameters are sufficient to control levels of fragments during routine manufacture. To adequately control for product fragments:
- Identify (b) (4) process unit operations that impact the levels of product fragments,
 - (b) (4)
 - Provide an overall summary and description of your control strategy for product fragments.
6. Deamidation at the heavy chain Asn55 (HC Asn55) site is identified as a CQA and controlled by (b) (4). To support the proposed control strategy, provide data showing that changes in deamidation at HC Asn55 can be reliably detected by the (b) (4) method.
7. The data provided in section 3.2.R.4.1.5 *Afucosylation* show that increased afucosylated glycans (G0+G1) can affect FcγRIIIa binding and, consequently ADCC activity of CT-P10. Therefore, the proposed DS specification for glycans of G0 = (b) (4)% and Unidentified Peaks (b) (4)% does not provide adequate control of ADCC, as it does not provide sufficient control of G1 content. Provide the BLA with the following:
- Assign DS specifications with acceptance criteria to control all main afucosylated glycans,
 - Provide assay validation data to confirm the suitability of the oligosaccharide profile test for the proposed specifications for afucosylated glycans,
 - Provide all available DS lot release and stability data to justify the revised specifications and the acceptance criteria.
8. In the response to IR comment 4 (received on 1/3/2018), Celltrion described the stability testing plan and specifications for the CT-P10 master and working cell banks; however, the information

was not submitted in relevant section of Module 3. Update section 3.2.S.2.3.2 with the information provided in the response.

9. FcγRIIIa binding affinity is included in the DS release and stability specifications to control CT-P10 ADCC activity. Celltrion states that testing of DS is sufficient to ensure FcγRIIIa binding affinity of CT-P10 DP throughout its shelf life. However, no data is provided on FcγRIIIa binding affinity for DP stored under long term storage condition of 5±3°C. Provide data for FcγRIIIa binding affinity during DP storage to support the current testing strategy, or alternatively, include FcγRIIIa binding affinity testing in the DP stability program.
10. In the IR response received on 1/3/2018, you proposed an acceptance criterion of polysorbate 80 (PS80) concentration (b) (4)
(b) (4)
(b) (4)
(b) (4) Provide data to support the adequacy of the proposed PS80 lower limit.
11. Your response (received on 1/3/2018) to the Agency's IR (12/21/2017) comment 26 indicates that a small portion of the 100 mg CT-P10 DP vials from the PPQ and commercial runs fail based on (b) (4) fill weight check and these vials do not meet the requirement of extractable volume per USP<1>. Section 3.2.P.3.5 should be revised to reflect that these vials are quarantined and discarded.

Additional comments that are not approvability issues:

1. In 3.2.R.3.2.1.5 *Peptide Mapping by LC-MS* you stated that the peptide sequence coverage was 100% for both the heavy chain and light chain for all samples. Additionally, you state that "MS/MS data (data not shown) confirmed that the amino acid sequences of Rituxan, CT-P10, and MabThera matched and sequence coverage by MS/MS was also 100% (data not shown)." We acknowledge the summarized information in Table 3.2.R.3-6 describing the trypsin-digested peptides and limited information about the Asp-N-digested peptides and where residues were confirmed only through Asp-N digestion and analysis, however heavy chain residues 419 and 420 are unaccounted for. Clarify this discrepancy.
2. In the Complete response submission, provide updated data from the following studies that are ongoing:
 - a. The commercial scale (b) (4) (in section 3.2.S.2.5),
 - b. The commercial (b) (4) study (in section 3.2.S.2.5),
 - c. The ongoing CT-P10 DS and DP stability studies (in sections 3.2.S.7 and 3.2.P.8),
 - d. The leachable study on 100 mg DP lots committed in your 1/3/2018 response to IR comment 21,
 - e. The stability study on three (b) (4) lots for up to 12 months, committed in your 1/3/2018 response to IR comment 8. Provide the control strategy of (b) (4) used in the manufacturing of CT-P10.

III. List Of Post-Marketing Commitments (PMCs) – *None, this BLA will not be approved.*

IV. Review Of Common Technical Document-Quality Module 1

A. Environmental Assessment Or Claim Of Categorical Exclusion

Celltrion claims a categorical exclusion per 21 CFR Part 25.31(a), as stated "action on an application for marketing approval of a biologic product 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. The request for categorical exclusion is acceptable.

V. Primary Container Labeling Review

Labeling review was not performed given that the BLA will not be approved.

VI. Review Of Common Technical Document – Quality Module 3.2

3.2.S. Drug Substance, 3.2.A Adventitious Agents Safety Evaluation and 3.2.R Regional information (method validation reports): this document.

3.2.P. Drug Product: reviewed by Dr. Haoheng Yan, a separate review is uploaded in Panorama

3.2.R Regional Information (analytical similarity): reviewed by Dr. Nina Brahme, a separate review is uploaded in Panorama.

VII. Review of Immunogenicity Assays – from Module 5.3.1.4

Refer to review by Dr. Haoheng Yan, a separate review is uploaded in Panorama.

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Unless otherwise noted, figures and tables in the review are adapted or copied directly from the submission. Some of the figures and tables in the submission were updated during the review cycle, the review contains only the updated final versions. The reviewer's comments are distinguished by use of italic font.

S. Drug Substance

3.2.S.1 General information

3.2.S.1.2 Structure

CT-P10 is a chimeric monoclonal IgG1 antibody, which has one N-link glycosylation on each heavy chain (HC). Each HC consists of 450 amino acids with 11 cysteine residues and each light chain (LC) consists of 213 amino acids with 5 cysteine residues. The amino acid sequence of the molecule is provided in Figure 3.2.S.1.2-1. The theoretical molecular weight is 144,252g/mol (deglycosylated) and the theoretical extinction coefficient is 1.60mL/mg/cm.

Heavy Chain:	
1	QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWVKQT PGRGLEWIGA
51	IYPGNGDTSY NQKFKGKATL TADKSSSTAY MQLSSLTSED SAVYYCARST
101	YYGGDWYFNV WGAGTTVTVS AASTKGPSVF PLAPSSKSTS GGTAALGCLV
151	KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSV TVPSSSLGTQ
201	TYICNVNHKP SNTKVDKAE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK
251	PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
301	N ³ STYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
351	QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTTP
401	VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG
451	(K*)
K* = C terminal lysine which is predominantly removed post-translationally	
Light Chain:	
1	QIVLSQSPAI LSASPGKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT
51	SNLASGVFVR FSGSGSGTSY SLTISRVEAE DAATYYCQW TSNPPTFGGG
101	TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYF REAKVQWKVD
151	NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
201	SSPVTKSFNR GEC

³ Glycosylation site is Asn301 of the heavy chain.

Yellow and blue color highlights the sequence of variable and constant regions, respectively.

3.2.S.1.3 General Properties

CT-P10 is colorless to pale yellow, clear to opalescent solution.

3.2.S.2 Manufacture

3.2.S.2.1 Manufacturer(s)

T low:

Table 3.2.S.2.1-1: CT-P10 Drug Substance Manufacturers

Company	Responsibility
(b) (4)	Production of the MCB and WCB
	Storage of the MCB and WCB
	- Testing of the MCB, WCB (b) (4) - Viral testing of CT-P10 unprocessed bulk
	- Testing of the MCB, WCB (b) (4) - Mycoplasma testing of CT-P10 unprocessed bulk
	Sterility testing of the (b) (4)
CELLTRION, Inc., Plant (b) (4) 23, Academy-ro, Yeonsu-gu, Incheon, 22014, Republic of Korea	- Release testing of CT-P10 drug substance - Stability testing of CT-P10 drug substance - Storage of the MCB and WCB
CELLTRION, Inc., Plant (b) (4) 20, Academy-ro 51 beon-gil, Yeonsu-gu, Incheon, 22014, Republic of Korea	- Production of CT-P10 drug substance - Release testing of CT-P10 drug substance - Release of CT-P10 drug substance - Stability testing of CT-P10 drug substance - Testing of MCB, WCB (b) (4) - Storage of the MCB and WCB

Note that Celltrion (Incheon, Republic of Korea) is the sole manufacturing and QC testing facility of CT-P10 DS.

(b) (4)

3.2.R Regional Information (USA)

This section includes Analytical similarity information (reviewed by Dr. Nina Brahme and uploaded in Panorama in a separate review), manufacturing batch records (will be reviewed during the PLI), and method validation reports (reviewed with information in section 3.2.S.4.3).



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BLA STN 761088

Rituximab-abbs

Drug Product Quality Review

Manufacturer: Celltrion, Inc.

Drug Substance Quality Reviewer: Haoheng Yan, M.D., Ph.D.

Drug Product Quality Reviewer: Haoheng Yan, M.D., Ph.D.

Analytical Similarity Reviewer: Nina Brahme, Ph.D., M.P.H.

ATL Reviewer: Bazarragchaa Damdinsuren, M.D., Ph.D.

Review Chief: Joel Welch, Ph.D.

Division of Biotechnology Review and Research IV

STN:	BLA761088
Subject:	Drug Product Review
Submission Date:	June 23, 2017
Review Date:	January 19, 2018
Primary Reviewer:	Haoheng Yan, MD, PhD Product Quality Reviewer, OPQ/OBP/DBRR IV
Secondary Reviewer:	Bazarragchaa Damdinsuren, MD, PhD Team Leader, OPQ/OBP/DBRR IV
Tertiary Reviewer:	Joel Welch, PhD Review Chief, OPQ/OBP/DBRR IV
Applicant:	Celltrion, Inc.
Product:	Truxima (CT-P10), proposed biosimilar to U.S.-licensed Rituxan
Indications:	(b) (4) Non-Hodgkin's lymphoma, (b) (4) (b) (4) (b) (4)
Action Due Date:	February 28, 2018

Product Quality Review Team:

Drug substance quality reviewer: Haoheng Yan (OPQ/OBP)
 Drug product quality reviewer: Haoheng Yan (OPQ/OBP)
 Immunogenicity assay reviewer: Haoheng Yan (OPQ/OBP)
 Analytical similarity reviewer: Nina Brahme (OPQ/OBP)
 Drug substance microbiology reviewer: Scott Nichols (OPQ/OPF/DMA)
 Drug product microbiology reviewer: Candace Gomez-Broughton (OPQ/OPF/DMA)
 Microbiology QAL: Reyes Candau-Chacon (OPQ/OPF/DMA)
 Facility reviewer: Thuy Thanh Nguyen (OPQ/OPF/DIA)
 Facility lead: Zhihao Peter Qiu (OPQ/OPF/DIA)
 OBP Labeling reviewer: Vicky Borders Hemphill (OPQ/OBP)
 OPQ RPM: Oumou Barry (OPQ/OPRO)
 CMC Application Team Lead (ATL): Bazarragchaa Damdinsuren (OPQ/OBP)

The BLA summary information, OBP CMC Review Data Sheet and Summary of Quality Assessments, sections 3.2.A Appendices and 3.2.R Regional Information (Assay Validation) are in the Drug Substance (DS) Review which can be found in Panorama.

3.2.R Regional Information (Similarity Assessment) is reviewed by Dr. Nina Brahme. The Similarity Review can be found in Panorama.

The DP related Complete Response (CR) and additional comments (draft) to be included in the action letter can be found in the DS review.

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P DRUG PRODUCT

3.2.P Truxima - Concentrate for solution for infusion – CELLTRION, INC

3.2.P.1 DESCRIPTION AND COMPOSITION OF THE DRUG PRODUCT

There are two dosage forms for CT-P10 DP: 500mg and 100mg, respectively in 50ml or 10ml (b) (4) glass vials with 20 mm rubber stoppers and 20 mm flip-off seals. Both are formulated as a sterile liquid solution at a CT-P10 concentration of (b) (4) mg/mL, 154 mM sodium chloride, 25 mM tri-sodium citrate dihydrate, and 0.07 % polysorbate 80, pH 6.5 for intravenous (IV) administration. The compositions of the CT-P10 500mg and 100mg DPs are summarized in the following Table (partially copied from the submission):

Tables 3.2.P.1-1 and 3.2.P.1-2: Compositions of the
CT-P10 500 mg DP

Table 3.2.P.1-2: Composition of the CT-P10 100 mg Drug Product

Ingredient	Ingredient	Quantity/Vial	Function	Grade
CT-P10	CT-P10	100 mg	Active ingredient	In-house
Sodium Chloride	Sodium Chloride	90 mg	(b) (4)	USP/Ph. Eur.
Tri-Sodium Citrate Dihydrate	Tri-Sodium Citrate Dihydrate	73.5 mg		USP/Ph. Eur.
Polysorbate 80	Polysorbate 80	7 mg		Ph. Eur/NF.
Water for Injection	Water for Injection	Q.S. to 10 mL		USP/Ph. Eur.

USP: United States Pharmacopoeia
Ph. Eur.: European Pharmacopoeia
NF: National Formulary
Q.S: Quantum Satis

3.2.P.2 Pharm

3.2.P.2.1 Components of the Drug Product

3.2.P.2.1.1.1 and 3.2.P.2.1.2.1 Drug Substance

The CT-P10 (b) (4)

3.2.P.2.1.1.2 and 3.2.P.2.1.2.2 Excipients

The excipients are listed in the Tables 3.2.P.1-1 and 3.2.P.1-2 above.

3.2.P.2.2 Drug Product

3.2.P.2.2.1.1 Formulation Development

(b) (4)



Overall, the proposed DP specification is acceptable.

3.2.P.6 Reference Standards or Materials

The same Reference Materials as discussed in section 3.2.S.5 Reference Standards or Materials are used for DP.

3.2.P.7 Container Closure System

DP primary CCS: Clear, 50 mL or 10 mL (b) (4) glass vial (b) (4) with a rubber stopper (b) (4) and a 20 mm flip-off seal ((b) (4)) The glass vial and rubber stopper meet USP and Ph. Eur. requirements.

CT-P10 DP vials for use in commercial supply are kept in a carton box (1 vial/carton) in order to prevent exposure to light.

The sponsor provided the primary CCS specification, critical dimension, technical drawings and representative CoA in the submission.

In the initial submission, the sponsor stated that dimensions and drawing of carton box for CT-P10 100mg DP are in draft and will be firmed up during BLA review period. *In the IR response received on 1/3/2018 (comment 29), the sponsor confirmed the draft was the final version of the carton box for CT-P10 100mg DP.*

Reviewer's Comment:

The container closure systems for CT-P10 DP are adequately described and deemed fit for purpose. Note that CCS leachable study is described in P.2.4.

3.2.P.8 Stability

3.2.P.8.1 STABILITY SUMMARY AND CONCLUSION

The sponsor proposed a (b) (4)-months of shelf life for commercial CT-P10 DP, both 500mg and 100mg, when stored at 5 ± 3 °C. The proposal is based on stability data listed in the following table (summarized by the reviewer).

Table: Current DP stability data at 5 ± 3 °C

Product	Manufacturing Site, Process	Available stability data at 5 ± 3 °C	Noteworthy Findings & <i>Reviewer's Comment</i>
500mg DP	(b) (4) process 2	36m / 3 lots	Due to the process differences (process 2 vs proposed commercial process [4]), the stability data from process 2 material cannot be used to support the proposed shelf life of the DP. Note: H2L2% by CE-SDS (non-reduced) is 92% at 18m and 24m for Lot11C0404. Current proposed stability AC is (b) (4)%.
	CLT2, process 3, clinical process	36m / 3 lots	There are slight differences between the manufacturing process 3 and 4 but are comparable (see comparability in P.2.3). The stability data from process 3 lots can be used to support the proposed shelf life of commercial DP.
	CLT2, process 4, clinical and commercial process	24m / 3 lots	
100mg DP	(b) (4) Process I	18m / 3 lots	1. It is not acceptable to use 500mg DP stability data to justify the shelf life claim for 100mg DP due to CCS and process differences. 2. The DP process I and II differ in site and scale. Additional characterization data is need to support the comparability between the DPs from Process I and II. Thus, the process I data cannot be used to support the proposed shelf life of the DP. Therefore, the provided data supports only 6-month shelf life for the 100mg DP based on the stability data from process II lots (commercial).
	CLT2, Process II, commercial process	6m / 3 lots	

Reviewer's Comments:

Overall, the proposed shelf life of (b) (4) months for the 500mg DP can be supported, however, for the 100mg DP the submitted data supports only 6 months of shelf life. We will include an additional comment in the CR letter to request stability update in the BLA resubmission.

3.2.P.8.2 POST-APPROVAL STABILITY PROTOCOL AND STABILITY COMMITMENT

Sponsor committed to complete the stability studies per description in P.8.1.

A minimum of one production batch for each CT-P10 500 mg and 100 mg DP will be added to the long-term stability program each year according to the protocol listed in Table 3.2.P.8.2-1. The end-of-shelf-life specification for CT-P10 500 mg is shown in Table 3.2.P.8.2-2. The specification for CT-P10 100 mg DP (in Table 3.2.P.8.2-3 of the submission) is the same as for 500 mg, except for extractable volume (≥ 10.0 mL).

Table 3.2.P.8.2-1: Post-Approval Stability Protocol for CT-P10 Drug Product (500 mg and 100 mg) ($5 \pm 3^{\circ}\text{C}$)

Test Item	Time Point (Month)								
	0	3	6	9	12	18	24	36	48
Clarity	T	T	T	T	T	T	T	T	T
Color	T	T	T	T	T	T	T	T	T
Visible Particles	T	T	T	T	T	T	T	T	T
pH	T	T	T	T	T	T	T	T	T
Osmolality	T	T	T	T	T	T	T	T	T
Extractable Volume	T	–	–	–	T	–	T	T	T
Uniformity of Dosage Unit	T	–	–	–	T	–	T	T	T
Sub-visible Particles	T	T	T	T	T	T	T	T	T
IEF	T	T	T	T	T	T	T	T	T
IEC-HPLC	T	T	T	T	T	T	T	T	T
CE-SDS (Reduced)	T	T	T	T	T	T	T	T	T
CE-SDS (Non-reduced)	T	T	T	T	T	T	T	T	T
SEC-HPLC	T	T	T	T	T	T	T	T	T
Protein Concentration (UV_{280})	T	T	T	T	T	T	T	T	T
CDC	T	T	T	T	T	T	T	T	T
Endotoxin	T	–	–	–	–	–	T	–	T
Methylene Blue Dye Penetration	T	–	–	–	T	–	T	T	T

Table 3.2.P.8.2-2: End-of-shelf-life Specification of CT-P10 500 mg Drug Product

Test Item	Acceptance Criterion
General	
Clarity	Clear to opalescent solution
Color	Colorless to pale yellow
Visible Particles	Free of visible particles
pH	(b) (4)
Extractable Volume	
Osmolality	
Uniformity of Dosage Units	
Sub-visible Particles	
Safety	
Endotoxin	(b) (4)
Identity	
IEF	(b) (4)
Purity/Impurity	
IEC-HPLC ¹	(b) (4)
CE-SDS (Non-reduced)	
CE-SDS (Reduced)	
SEC-HPLC	
Quantity	
Protein Concentration (UV ₂₈₀)	(b) (4)
Potency	
Complement Dependent Cytotoxicity (CDC)	(b) (4)
Container Closure Integrity	
Methylene Blue Dye Penetration	(b) (4)

Reviewer's Comments:

The post-approval stability protocol and end of shelf life specifications are acceptable. However, a CR comment regarding data supporting *FcγRIIIa* binding affinity at DP stored at long term storage will be included in the action letter.

3.2.P.8.3 STABILITY DATA**3.2.P.8.3.1 Stability Data - CT-P10 500 mg DP**

Stability data for process 3 and 4 DP lots are summarized by the reviewer.

- Long-term stability at 5 ± 3 °C (upright position): 36-months data from process 3 DP lots and 24-months data from process 4 DP lots:
 1. All results are within the proposed DP stability AC.
 2. A slight increase of %acidic peaks (peaks 1-3 by IEC-HPLC) is observed. Process 3 lots: from 16% to 19% at 36th month. Process 4 lots: from 16% to 18% at 24th month.
 3. The results of CE-SDS (non-reduced) seem fluctuate between 94-97%, such variation is uncharacteristic for the method.
- Long-term stability at 5 ± 3 °C (inverted position): from Lot No. 15C2C06 (process 4, 12 months) and Lot No. 11C0404 (process 2, 36 months; note that the data will not be considered):
 1. All results are within the proposed DP stability AC.
 2. The sponsor should collect additional long-term stability data from Lot No. 15C2C06 in inverted position to examine any impact of product quality from leachables from stopper.

Of note: For lot 11C0404, testing of charge variants used an earlier version of the IEC-HPLC method which measures the % sum of lysine and glutamine variants, the results did not show significant trend over the 36-months period.
- Accelerated stability at 25 ± 2 °C / 60 ± 5 % RH for 6 months from 3 process 4 DP and 3 process 3 DP lots:
 1. The acidic peaks by IEC-HPLC showed upward trends (from ~16% to ~31%) resulting in (b) (4). Corresponding downward trends were observed for the main and basic peaks.
 2. Downward trends are seen in H+L % by reduced CE-SDS (from 99% to 97%) resulting in (b) (4).
 3. Downward trends are seen in H2L2 % by non-reduced CE-SDS (from 96% to 93%).
 4. Downward trends are seen in potency by CDC assay (from $\sim 1.0 \times 10^4$ U/mg to 0.7×10^4 U/mg) resulting in (b) (4). Results of CD20 binding (by CELISA, additional test) correlated with the CDC assay results.
 5. The trends are similar between the process 3 and process 4 lots.
- Stress stability at 40 ± 2 °C / 75 ± 5 % RH for 6 months from 3 process 4 DP and 3 process 3 DP lots:

The stressed condition data showed amplified and faster changes for all changes seen in the accelerated condition. In addition, increase in visible and subvisible particles after 1st or 2nd months and decrease of monomer (by SEC-HPLC) are observed in the stressed condition.

- Confirmatory Photostability: with exposure to 1,200,000 Lux hr Vis. and 200 Whr/m² UV under 25 ± 2 °C / 60 ± 5 % RH for 70hrs:

When the DP vials are kept in secondary packaging, all stability results are within the DP AC, however multiple attributes changed beyond the stability AC when the DP vials are exposed to the light. The study confirms that CT-P10 DP in the primary packaging is photosensitive and 2nd packaging is necessary to ensure product stability. The limit of light exposure during DP manufacturing process should be reviewed during the PLI.

Overall, the CT-P10 500mg DP is stable for up to 36 months at long-term storage condition of 2-8°C protected from light. The analytical methods used to assess stability is sufficient. The IEC-HPLC (for charge variants) seems to be most sensitive method to detect product degradation under elevated temperature conditions. The reduced and non-reduce CE-SDS (fragments) and CDC assay are also the

stability indicating. The potential degradation pathway under elevated temperatures is through change in product charge variants likely due to deamidation.

- 3.2.P.8.3.1.8 Forced Degradation Study for CT-P10 and Reference Product
This section is reviewed as part of similarity assessment in section 3.2.R.

3.2.P.8.3.2 Stability Data - CT-P10 100 mg DP

The sponsor submitted 18 months stability data from process I DP lots, these data cannot be used to support the DP shelf life. Stability data for process II DP lots are summarized by the reviewer.

- Long-term stability at $5 \pm 3^\circ\text{C}$: 6 months stability data from 3 process II DP lots:
 1. *All results are within the proposed DP stability AC.*
 2. *A slight increase of % acidic peaks (peaks 1-3 by IEC-HPLC) is observed, as seen in 500mg DP lots.*
- Long-term stability at $5 \pm 3^\circ\text{C}$ (in inverted position) for 3 months from Lot No. 16C3C04 (process II):
 1. *All results are within the proposed DP stability AC and no trend is observed.*
 2. *The sponsor should collect additional long-term stability data from 100 mg DP in inverted position to examine any impact of product quality from leachables from stopper.*
- Accelerated stability at $25 \pm 2^\circ\text{C} / 60 \pm 5\% \text{ RH}$: 3 months data from 3 process II DP lots:
Similar trends compared to 500mg DP lots are seen in IEC-HPLC, CE-SDS (reduced and non-reduced) and CDC results for all lots.
- Stress Stability at $40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$: 3 months data from 3 process II lots
Similar trends are observed as in the 500mg DP lots in the stress conditions.

Overall, the provided stability data from process II lots show that the CT-P10 100mg DP is stable for up to 6 months in long-term storage at $5 \pm 3^\circ\text{C}$.



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Memorandum of Review

STN:	BLA761088
Subject:	Immunogenicity Assay Review
Submission Date:	June 23, 2017
Review Date:	January 17, 2018
Primary Reviewer:	Haoheng Yan, MD, PhD Product Quality Reviewer, OPQ/OBP/DBRR IV
Secondary Reviewer:	Bazarragchaa Damdinsuren, MD, PhD Team Leader, OPQ/OBP/DBRR IV
Tertiary Reviewer:	Joel Welch, PhD Review Chief, OPQ/OBP/DBRR IV
Applicant:	Celltrion, Inc.
Product:	Truxima (CT-P10), proposed biosimilar to US-licensed Rituxan
Indications:	(b) (4) Non-Hodgkin's lymphoma, (b) (4) (b) (4)
Action Due Date:	February 28, 2018

Summary

The sponsor used an anti-drug antibody (ADA) binding assay and a neutralizing antibody (NAb) assay for testing samples from the comparative clinical studies (CT-P10 3.2 in rheumatoid arthritis (RA) patients and CT-P10 3.3 in Advanced Stage Follicular Lymphoma (AFL) patients). The ADA binding assay consists of an ECL based bridging screening assay, a confirmatory assay and a titer assay. In the confirmatory and titer assays, excessive amount ofatumumab (monoclonal antibody against CD20, binding to a different epitope of CD20) was included in the reaction mix to reduce interference from circulating CD20 cell membrane fragments. The NAb assay is based on assessment of neutralization of the CDC activity on WIL2-S cells in response to rituximab.

Overall, the ADA binding and NAb assays are adequate for use in the study CT-P10 3.2 (in RA patients) to detect ADAs and NABs in 24 and 48-week patient samples in which the serum drug concentrations are close to the baseline. However, the ADA and NAb rates are perhaps under-reported at 8 and 16-week sample points, due to insufficient drug tolerances at higher serum drug concentrations.

The ADA binding and the NAb assays are inadequate for use in the study CT-P10 3.3 (in AFL patients), because the assay sensitivity is insufficient under the high serum drug concentrations observed at all sampling points in the study. Specifically, the assays are only capable of detecting 250 ng/mL ADA and 9600 ng/mL NAb in the presence of serum drug concentrations up to 20 µg/mL and 7 µg/mL, respectively. However, the observed average serum drug concentrations are approximately 60 µg/ml and 106 µg/ml at the two post-treatment ADA sampling points in the core study period (after cycle 4 and at EOT1), respectively. Therefore, the ADA and NAb rates are likely under-reported (with high false negative results) in the CT-P10 3.3 study. The clinical immunogenicity results show that the post-treatment ADA positive rate is lower than the pre-treatment rate and all ADAs are with high titers (4 – 11, log2 value). Four of the five ADA positive samples were NAb positive. These findings corroborate the assay performance conclusion that the assay drug tolerances are insufficient in the AFL study. The BLA review team plans to send a CR comment to develop and validate ADA and NAb assays that are sufficiently sensitive to detect ADAs and NAb in the presence of CT-P10 and U.S.-Rituxan levels that are expected to be present in the serum at the time of patient sampling. The sponsor should test the immunogenicity samples in the study CT-P10 3.3 using the re-developed assays.

DRAFT CR Comment by OBP (See the final CR comment in the issued action letter)

(b) (4)

Review

- Unless otherwise noted, figures and tables in this review are copied directly from the submission.
- The review sequence of the individual aspect of the assay validation may not follow the exact sequence in the submission.
- The "guidance" cited in the review refers to "FDA Draft Guidance to Industry: Assay Development for Immunogenicity Testing of Therapeutic Proteins, April 2016" that can be found at <http://www.fda.gov/downloads/Drugs/.../Guidances/UCM192750.pdf>

Assay Development History, Validation Review Summary

The sponsor used an ADA binding assay and a NAb assay for testing samples from the comparative clinical studies (CT-P10 3.2 for RA and CT-P10 3.3 for AFL). The ADA binding assay consists of an ECL-based bridging screening assay, a confirmatory assay and a titer assay. The NAb assay is based on assessment of neutralization of CDC activity on WIL2-S cells in response to rituximab.

At first, the patient samples were tested in an ADA binding screening assay. The samples that screened positive were tested by the confirmatory assay. The confirmed positive samples were tested for titer and for NAb. Figure 3 (copied from Integrated Immunogenicity Summary) outlines the testing strategy for CT-P10 3.2 and 3.3 studies.

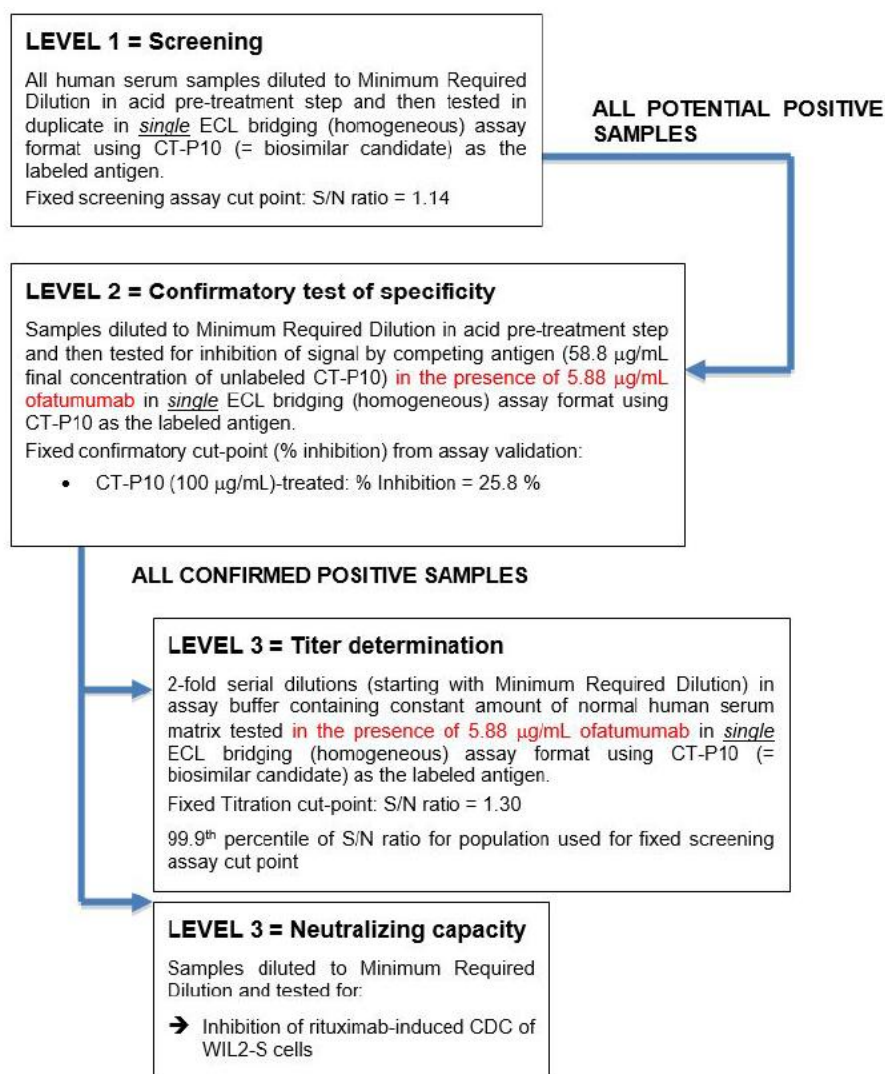


Figure 3: Hierarchical ADA Testing Strategy for Phase 3 Studies

Reviewer's Comments:

The sponsor uses a tiered approach to evaluate ADA, which aligns with the current guidance).

Note that the confirmatory and titer assays include usage of excessive ofatumumab to reduce the target interference of CD20 cell membrane fragments in these assays. The screening assay does not include ofatumumab, which is rather unusual since all three assays are based on the same format and all are susceptible to the target interference (this is discussed in section [confirmatory assay](#) of this review).

In the BLA, the sponsor submitted multiple validation reports for the ADA binding and NAb assays. I used information provided in the Integrated Immunogenicity Summary to identify the assays used in testing of clinical samples, and my review covers the validation data relevant to the assays used in comparative clinical studies CT-P10 3.2 and CT-P10 3.3; namely, HOX2, HOX4 and HOX9 for the ADA binding assay, and IOX2 and IOX3 for the NAb assay.

Anti-drug Antibody Binding Assay [from validation reports HOX2, HOX4 and HOX9]

Screening Assay

The ADA screening assay is an ECL based bridging assay. In brief, human plasma samples are diluted to 20% human serum with 300mM acetic acid to enable antibody complex dissociation prior to analysis. The diluted/acid dissociated samples are incubated in a mixture of neutralizing buffer, biotinylated CT-P10 and sulfa labeled CT-P10 in a nonbinding microtiter plate. The formed immune complex is then added to a streptavidin coated plate and allowed to bind via biotin-streptavidin interaction. After washing, the bound immune complexes are detected by adding substrate to the reaction. The ECL signal is analyzed MSD 6000 plate reader. Samples with a signal-to-noise (S/N) value greater than or equal to the assay cut point are deemed screened positive.

Screened positive samples are tested in the confirmatory assay which is based on the cold competition with excess CT-P10 (58.8µg/mL) in the presence of 5.88µg/mL ofatumumab. The aim to include ofatumumab is to remove the effect of CD20 positive cell membrane fragments (CD20+ CMFs) shown to induce positive signals (see [Matrix Interference section](#) below).

The ADA assay format and related validation reports (HOX) are summarized by the reviewer below:

	Screening Assay	Confirmatory Assay	Titer Assay
Study CT-P10 1.1 and 1.3	w/o ofatumumab, HOX2, 4	w/o ofatumumab HOX2, 4	w/o ofatumumab HOX2, 4
Study CT-P10 3.2 and 3.3	w/o ofatumumab, HOX2, 4	w/ ofatumumab HOX9	w/ ofatumumab HOX9

Reviewer's Comment:

The review focuses on the assay validation data related to the study CT-P10 3.2 and 3.3, because only these studies are used to support this BLA.

The sponsor also developed assays using E.U.-MabThera and U.S.-Rituxan tagged reagents and tested samples from Study CT-P10 3.2 (main study). Based on the tag concordances demonstrated among the 3 tags, the ADA analysis in the rest of Part 1 (up to Week 48), Part 2 of Study CT-P10 3.2 and the entire Study CT-P10 3.3 was performed using only CT-P10 tag. The overall immunogenicity results are based on assay with CT-P10 tagged reagents.

Reviewer's Comment:

It is OBP's general recommendation that the biosimilar-tagged reagents should be used in assessing immunogenicity in both, biosimilar- and reference product- treated patients. Therefore, it is adequate to report results generated by assays using CT-P10 tagged reagents only. Accordingly, only these assays are reviewed in the memo.

Essential Reagents and Concentrations

Capture Agents:	Biotinylated CT-P10 and Sulfo-tag-CT-P10
Positive Control (PC):	Anti-rituximab rat mAb by AbD serotec. It is a protein G-purified rat anti-human rituximab IgG2a mAb that was raised using the F(ab)2 fragment of rituximab as immunogen.
Low positive control (LPC):	20.0ng/mL PC
Medium positive control (MPC):	250ng/mL PC
High positive control (HPC):	1,000ng/mL PC
Normal human plasma:	K ₂ EDTA by Seralab, United Kingdom
Negative Control (NC):	Pooled human plasma collected from at least 10 healthy subjects with no detectable ADA.

Screening Cut Point Determination (HOX2)

Fifty (50) individual drug naïve human serum sample lots, twenty one (21) drug free RA serum sample lots, and twenty five (25) drug free non-Hodgkin's lymphoma (NHL) serum sample lots were used in cut point determination. Data were compiled from 27 independent assays performed over 12 days by 2 analysts. Assay readouts were normalized as S/N ($ECL_{\text{sample}}/ECL_{\text{NC}}$) ratios, based on the plate NC, to determine the screening cut point. Twenty-four outliers were identified using Tukey's Outlier Filter approach and were removed from further consideration. Neither of the S/N or log S/N data follows normal distribution. Therefore, a non-parametric approach was applied to the screening cut point determination at 95th percentile level, which resulted in

Assay Cut Point (S/N ratio) = 1.14

Reviewer's Comments:

Samples from different populations can have different background activity in ADA assays. Therefore, prior to pooling screening assay data the sponsor should show that the assay background results from these three populations are comparable.

In the statistical report of HOX2, the sponsor provided a table showing the screening data distributions (before outlier exclusion) of the three groups (Table 2 below). I found that the median levels of S/N are comparable between the three groups, i.e., no disease specific assay interference. Although the SD values and, at lesser extent, the means are different between the three populations, one should also consider that SDs and means are largely affected by the outliers that we cannot confirm from the provided data. This is also considered in cut point determination of the confirmatory assay.

Table 2. Normality Test Results for Complete Original Data

Population	Panel	N	Mean	SD	SE	CV%	Median	p-value for Normality Test
NHL	All Panels	50	1.12	0.528	0.075	47.3	0.98	<0.001
	Panel 1	25	1.07	0.321	0.064	29.9	0.98	<0.001
	Panel 2	25	1.16	0.680	0.136	58.6	1.00	<0.001
Normal	All Panels	100	1.31	1.345	0.135	103.0	1.01	<0.001
	Panel 1	50	1.26	1.224	0.173	96.8	1.00	<0.001
	Panel 2	50	1.35	1.468	0.208	108.8	1.01	<0.001
RA	All Panels	42	1.08	0.242	0.037	22.5	0.99	<0.001
	Panel 1	21	1.09	0.264	0.058	24.3	1.00	<0.001
	Panel 2	21	1.06	0.224	0.049	21.1	0.99	<0.001
Note: if Shapiro-Wilk normality test p-value>0.05, it indicates data are normally distributed; otherwise, data are not normally distributed.								

Nevertheless, the assay can consistently detect ADA at 20ng/mL level (i.e., LPC) with the cut point $S/N = 1.14$ (Table 22 Integrated Summary of Immunogenicity), which demonstrates that the assay is capable of detecting ADAs at a level recommended in the guidance (100ng/mL). Therefore, the identified cut point for screening assay is acceptable.

Assay Precision

NC, LPC, MPC and HPC were used to assess intra-assay precision (n=6) and inter-assay precision (8 plates). The intra-assay precision (%CV) for the NC, LPC, MPC, and HPC are 5.66%, 2.30%, 2.36%, and 1.08%, respectively, and the inter-assay precision (%CV) are 6.44%, 19.2%, 17.3%, and 19.1%, respectively based on RLU results (results are in Table 4 of the submission).

Reviewer's Comment:

The inter-assay %CV for the PCs are slightly higher than the recommended %CV <15%. Since the assay cut point is based on S/N value, the sponsor could have used S/N to evaluate assay precision which would further reduce the %CVs. The assay precision is acceptable.

Assay Sensitivity

Reviewer's Comment:

FDA recommends that screening and confirmatory ADA assays achieve sensitivity of at least 100ng/mL (per guidance). Because the assay can consistently detect ADA at 20ng/mL level (the LPC), the assay sensitivity in drug free serum is acceptable.

Drug Tolerance

To determine drug tolerance limits for the assay, the sponsor evaluated ability of the assay to detect ADA in the presence of circulating CT-P10 and established drug tolerance limits for the assay. The result is provided in the following (Table 13).

Table 13. Drug Tolerance

Inhibitor Conc. (µg/mL)			0.100			1.00			5.00			10.0		
ADA level	Inhibitor	Run ID	RLU	%CV	S/N	RLU	%CV	S/N	RLU	%CV	S/N	RLU	%CV	S/N
AIL	Rituximab	34HOX2	322	1.54	3.87	220	2.57	2.65	114	2.48	1.37	97.0	0.00	1.17*
	CT-P10	35HOX2	322	4.62	3.71	245	0.868	2.82	121	4.68	1.39*	94.5	2.24	1.09
AIM	Rituximab	34HOX2	2902	4.53	34.9	1814	1.25	21.8	508	1.53	6.11	254	2.51	3.05
	CT-P10	35HOX2	3318	0.938	38.2	1916	0.369	22.1	541	0.784	6.23	264	0.805	3.04
AIH	Rituximab	34HOX2	12835	1.86	154	7251	3.57	87.2	1830	0.927	22.0	735	2.79	8.84
	CT-P10	35HOX2	12407	0.849	143	7389	0.478	85.1	2026	0.279	23.3	814	0.521	9.37

Inhibitor Conc. (µg/mL)			20.0			50.0			100			500		
ADA level	Inhibitor	Run ID	RLU	%CV	S/N	RLU	%CV	S/N	RLU	%CV	S/N	RLU	%CV	S/N
AIL	Rituximab	34HOX2	83.5	5.93	1.00	80.5	0.878	0.968	82.5	0.857	0.992	79.5	2.67	0.956
	CT-P10	35HOX2	82.5	0.857	0.950	77.5	4.56	0.893	86.5	0.817	1.00	77.0	1.84	0.887
AIM	Rituximab	34HOX2	137	0.518	1.65	125	5.66	1.50*	83.5	0.847	1.00	84.0	1.68	1.01
	CT-P10	35HOX2	136	1.04	1.57*	89.0	1.59	1.02	78.0	3.63	0.898	75.5	2.81	0.869
AIH	Rituximab	34HOX2	301	0.706	3.62	132	1.07	1.59	95.5	2.22	1.15*	86.5	2.45	1.04
	CT-P10	35HOX2	314	0.901	3.62	122	1.75	1.40*	88.5	0.799	1.02	76.5	6.47	0.881

Legend:

* Highest concentration of inhibitor that still produces a positive response above the cut point of the assay

AIL = LPC (20ng/mL), AIM = MPC (250ng/mL), AIH = HPC (1000ng/mL)

Reviewer's Comments:

Based on results in Table 13 and cut point of 1.14, the assay can detect 20ng/mL (LPC), 250ng/mL (MPC) and 1000ng/mL (HPC) ADAs in the presence of up to 5 µg/mL, 20µg/mL, and 50µg/mL serum CT-P10, respectively. In the Integrated Immunogenicity Summary, the sponsor showed drug serum concentrations in the RA studies compared to the assay drug tolerance (Table 13 below). The presented results indicate that serum drug concentration (ranges) at week 8 will interfere with detection of 250ng/mL ADA (and likely higher) in this assay. At weeks 16, the serum drug concentration (ranges) may not interfere with detection of 250ng/mL or above ADAs, but likely will interference with detection of up to 20ng/mL ADA. At week 24, the drug serum concentrations are reduced to levels that will not interfere to detect all tested ADA PC levels. In real testing, the ADA positive rate would be largely under reported at week 8, and might be under reported at week 16.

Table 13: Drug Tolerance Level for ECL Assay

Study	Drug Tolerance Level ¹			Treatment	Drug concentration in serum at Week 8		Drug concentration in serum at Week 16		Drug concentration in serum at Week 24	
	LPC (20.0 ng/mL)	MPC (250 ng/mL)	HPC (1,000 ng/mL)		Mean (µg/mL)	Range (µg/mL)	Mean (µg/mL)	Range (µg/mL)	Mean (µg/mL)	Range (µg/mL)
CT-P10 3.2 Part 1	5 µg/mL	20 µg/mL	50 µg/mL	CT-P10	31.8 µg/mL	0.49 – 71.8 µg/mL	6.42 µg/mL	<0.04 ² - 12.8 µg/mL	0.36 µg/mL	<0.04 ² - 3.25 µg/mL
				Rituxan®	32.7 µg/mL	<0.04 ² – 8.1.5 µg/mL	3.37 µg/mL	<0.04 ² - 17.2 µg/mL	0.44 µg/mL	<0.04 ² - 2.9 µg/mL
				MabThera®	34.0 µg/mL	0.14 - 82 µg/mL	4.17 µg/mL	<0.04 ² - 24.2 µg/mL	0.49 µg/mL	<0.04 ² - 4.18 µg/mL
CT-P10 1.1	5 µg/mL	20 µg/mL	50 µg/mL	CT-P10	34.5 µg/mL	<0.02 ³ – 7.5.8 µg/mL	3.35 µg/mL	<0.02 ³ - 13.4 µg/mL	0.42 µg/mL	<0.02 ³ - 2.84 µg/mL
				MabThera®	35.7 µg/mL	5.4 – 84.0 µg/mL	2.77 µg/mL	<0.02 ³ - 8.6.8 µg/mL	0.32 µg/mL	<0.02 ³ - 1.9 µg/mL

Sources: HOX2, HOX15 and CSR Study CT-P10 3.2 Listing 16.2.6.1, CSR Study CT-P10 1.1 Listing 16.2.6.1

¹ The drug tolerance levels of LPC, MPC and HPC determined using CT-P10 reagent are presented.

² <0.04 µg/mL is regarded as BLOQ and calculated as 0.04.

³ <0.02 µg/mL is regarded as BLOQ and calculated as 0.02.

ECL: Electrochemiluminescence, HPC: High positive control, LPC: Low positive control, MPC: Medium positive control

The clinical study results in Table 24 below (from Integrated Immunogenicity Summary) showing low ADA positive rates at week 8 and 16 compared to the rates at week 0, which corroborate with the drug tolerance results (see the example in yellow highlight added by the reviewer, note that pre-treatment positive subjects were not excluded from the analysis). The week 8 and 16 data were not pre-specified in the protocol, and analysis of immunogenicity is based on data at 24th week and after (per communication with clinical reviewer, Dr. Stefanie Freeman), therefore the drug tolerance profile of the assay is fitting the purpose of the assay for use in the RA study.

Table 24: Summary of Testing for ADA and NAb Up to Main Week 48 in Study CT-P10 3.2 (Part 1): Safety Population and Safety Population – 2nd Treatment Course in Main Study Period Subset

Immunogenicity Test	Safety Population			
	CT-P10 (N=64)	Rituxan® (N=65)	MabThera® (N=60)	Rituxan® + MabThera® (N=125)
	n (%)			
Week 0				
ADA Positive	7/64 (10.9)	6/65 (9.2)	7/60 (11.7)	13/125 (10.4)
NAb Positive (as % of ADA positive)	0	0	0	0
Week 8				
ADA Positive	1/57 (1.8)	2/59 (3.4)	1/55 (1.8)	3/114 (2.6)
NAb Positive (as % of ADA positive)	0	0	0	0
Week 16				
ADA Positive	1/57 (1.8)	5/55 (9.1)	4/51 (7.8)	9/106 (8.5)
NAb Positive (as % of ADA positive)	1 (100)	0	0	0
Week 24				
ADA Positive	8/59 (13.6)	14/60 (23.3)	16/58 (27.6)	30/118 (25.4)
NAb Positive (as % of ADA positive)	0	0	0	0

For the AFL studies, the immunogenicity samples are drawn at baseline, after 4th cycle, and at end of treatment (EOT1) of the core study period. According to the clinical pharmacology reviewer, Dr. Sang Chung, the C_{trough} after 4th cycle is 60.395µg/mL for CT-P10 and 64.256µg/mL for US-Rituxan, the C_{trough} at EOT1 (cycle 8) is 106.435µg/mL for CT-P10 and 95.523µg/mL for US-Rituxan. These data show that the level of on board drug would interfere with the detection of all tested ADA levels (i.e., up to 1000ng/mL).

Overall, the drug tolerance of the ADA screening assay is adequate to evaluate immunogenicity in CT-P10 3.2 study in RA patient samples at week 24. The assay is inadequate to evaluate the immunogenicity in CT-P10 3.3 study in AFL patients due to insufficient drug tolerance.

Stability

Stability of LPC and HPC were evaluated under the following conditions: six freeze/thaw cycles, 24hr at room temperature, 256 days at -70 °C. The stability samples are tested in the screening assay and results are compared to results of freshly prepared LPC and HPC. The results showed no significant difference in all conditions (differences are less than 10%).

Reviewer's Comment:

Sample stability is appropriately validated.

Matrix Interference and Selectivity

The sponsor conducted studies evaluating the matrix interference and assay selectivity (after they observed high ADA positive rates in pre-treatment patients in CT-P10 1.1 and 1.3 studies).

RA matrix interference

To study the matrix interference of patient plasma, the sponsor analyzed 10 RA samples fortified with the LPC, MPC and HPC, the results are shown in Table 2A (*the boxes are added by the reviewer*).

Table 2A. Matrix Selectivity

HPC level											
	SPF 1 (RLU)	SPF 4 (RLU)	SPF 7 (RLU)	SPF 10 (RLU)	SPF 13 (RLU)	SPF 16 (RLU)	SPF 19 (RLU)	SPF 22 (RLU)	SPF 25 (RLU)	SPF 28 (RLU)	SPF 31** (RLU)
Run ID	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	11HOX4	11HOX4	11HOX4
S/N	24800	41800	43700	22800	23800	43100	23500	81400	27400	25500	25900
%C.V.	234	394	412	215	225	407	222	768	226	211	214
% Recovery	0.703	1.90	2.52	0.961	5.17	2.11	0.174	0.847	0.0620	1.92	5.25
	95.8	161*	169*	88.0	91.9	166*	90.7	314*	106	98.5	100
MPC level											
	SPF 2 (RLU)	SPF 5 (RLU)	SPF 8 (RLU)	SPF 11 (RLU)	SPF 14 (RLU)	SPF 17 (RLU)	SPF 20 (RLU)	SPF 23 (RLU)	SPF 26 (RLU)	SPF 29 (RLU)	SPF 32** (RLU)
Run ID	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	11HOX4	11HOX4	11HOX4
S/N	7020	18000	17600	6150	6100	18200	5740	15100	8750	6900	7540
%C.V.	66.2	170	166	58.0	57.5	172	54.2	142	72.3	57.0	62.3
% Recovery	1.67	5.17	5.48	2.16	1.03	2.71	5.11	0.276	2.05	2.42	0.685
	93.1	239*	233*	81.6	80.9	241*	76.1*	200*	116	91.5	100
LPC level											
	SPF 3 (RLU)	SPF 6 (RLU)	SPF 9 (RLU)	SPF 12 (RLU)	SPF 15 (RLU)	SPF 18 (RLU)	SPF 21 (RLU)	SPF 24 (RLU)	SPF 27 (RLU)	SPF 30 (RLU)	SPF 33** (RLU)
Run ID	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	11HOX4	11HOX4	11HOX4
S/N	774	3190	2920	499	820	1060	603	1680	1010	699	737
%C.V.	7.30	30.1	27.4	4.71	7.74	10.0	5.69	15.8	8.35	5.78	6.09
% Recovery	0.731	4.73	0.242	1.28	6.90	6.81	0.938	1.01	0.422	0.405	0.480
	105	433*	396*	67.7*	111	144*	81.8	228*	137*	94.8	100
Unspiked											
	SP 1 (RLU)	SP 2 (RLU)	SP 3 (RLU)	SP 4 (RLU)	SP 5 (RLU)	SP 6 (RLU)	SP 7 (RLU)	SP 8 (RLU)	SP 9 (RLU)	SP 10 (RLU)	SP 11** (RLU)
Run ID	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4	10HOX4
S/N	103	112	111	90.5	587	103	93.5	786	154	98.0	123
%C.V.	0.972	1.06	1.05	0.854	5.54	0.972	0.882	7.42*	1.45	0.925	1.16
	2.07	2.53	3.82	5.47	3.01	6.21	0.756	1.26	4.15	7.22	0.577

Legend:

* Outside acceptance criteria

** Control samples

SP=unspiked samples, SPF=PC spiked in samples

Each column represents one sample lot.

Reviewer's Comments:

- The sponsor performed similar matrix interference study using 10 healthy volunteer sample lots, no interference is observed (data in Table 12B in HOX2 report).
- Table 2A shows matrix interference in at least 4 of 10 RA serum lots (highlighted in red frames). The matrix interference is defined by the sponsor as % recovery <80% or >120%. Despite the interference, all PCs still resulted "positive" in RA sera.

- Four of 10 treatment naïve RA samples are scored positive in the assay based on the cut point of 1.14 (blue boxes), strongly suggesting that the screening assay might have a high false positive rate in RA samples.

Rheumatoid Factor (RF) Interference in normal and RA serum

Reviewer's Comment:

RF is generally an IgM antibody that recognizes Fc portion of IgG. It is known that a significant portion of RA patients have elevated RF (normal level is less than 15 IU/mL). Thus RF binding to Fc regions of a therapeutic protein product making it appear that specific antibody to the therapeutic product (i.e. ADA). Per guidance, FDA recommends incorporate approaches to minimize interference from RF, such as treatment with aspartame and careful optimization of reagent concentrations to reduce background binding.

To evaluate whether RF interferes with the assay, RF purchased from a commercial source was spiked at 10 IU/mL (low), 100 IU/mL (mid), and 1000 IU/mL (high) concentrations into 5 normal human serum and 5 RA serum samples. The results are presented in Tables 3 and 4 of HOX4 report.

Table 4. RF Interference in RA Human Serum (continued)

RF Concentration PC level	1000 IU/mL 0.00 ng/mL					1000 IU/mL 20.0 ng/mL				
	UNK 6 (RLU)	UNK 7 (RLU)	UNK 8 (RLU)	UNK 9 (RLU)	UNK 10 (RLU)	UNK 26 (RLU)	UNK 27 (RLU)	UNK 28 (RLU)	UNK 29 (RLU)	UNK 30 (RLU)
Run ID	18HOX4	18HOX4	18HOX4	18HOX4	18HOX4	19HOX4	19HOX4	19HOX4	19HOX4	19HOX4
Result	92.5	249	573	109	95.0	246	488	818	360	238
S/N	0.665	1.79	4.12	0.784	0.683	1.80	3.56	5.97	2.63	1.74
%C.V.	8.41	2.56	4.08	1.96	1.49	3.74	4.64	2.42	0.197	2.97
% Recovery	N/A	N/A	N/A	N/A	N/A	84.0	167	279	123	81.2
%Inhibition	2.70	65.2	84.7	22.1	11.1	64.0	82.6	89.6	76.5	64.1
RF Concentration PC level	0.0 IU/mL 0.00 ng/mL					0.0 IU/mL 20.0 ng/mL				
	UNK 1 (RLU)	UNK 2 (RLU)	UNK 3 (RLU)	UNK 4 (RLU)	UNK 5 (RLU)	UNK 21 (RLU)	UNK 22 (RLU)	UNK 23 (RLU)	UNK 24 (RLU)	UNK 25 (RLU)
Run ID	18HOX4	18HOX4	18HOX4	18HOX4	18HOX4	19HOX4	19HOX4	19HOX4	19HOX4	19HOX4
Result	98.5	262	567	98.0	88.5	294	559	931	414	282
S/N	0.709	1.88	4.08	0.705	0.637	2.15	4.08	6.80	3.02	2.06
%C.V.	6.46	8.64	0.249	1.44	2.40	3.85	4.43	2.43	0.513	2.01
% Recovery	N/A	N/A	N/A	N/A	N/A	100	191	318	141	96.2
%Inhibition	9.64	66.0	84.5	14.3	3.95	72.8	86.1	91.0	80.3	68.8

Legend:

N/A Not applicable

Run 19HOX4 Control = mean LPC (293 RLU)

Majority of the RF spiked samples (3-4 of 5 samples at each level of RF spike) showed recoveries outside of the acceptable range of 80% to 120%. The sponsor states that "the inability of individual matrix samples to meet the acceptance criteria was independent of the level of RF factor added to the sample, indicating effects of specific constituents of the matrix sample."

Reviewer's Comment:

The sponsor did not provide any explanation and action on the observation that the %recovery failed to meet the sponsor assigned acceptance criteria (80-120%). However, the S/N values do not correlate to the level of RF spike-in (comparing the S/N values between the red

frames added by the reviewer). The RF spike experiments demonstrate that RF does not cause false positive results.

Human Anti-murine Antibody (HAMA)

Reviewer's Comment:

Based on the ADA assay format, HAMA can also be a potential confounder causing false positive results (namely HAMA in human serum can bind to the murine part of the drug and form immunocomplex). It is reported that at least 10 percent of the general population has been observed to carry some form of antibodies against other animal species, most often against mice, due to the preponderance of medical agents made from the serum of animals (Klee GG, Arch Pathol Lab Med. 2000 Jun;124(6):921-3.).

To evaluate the impact of HAMA on the assay response, 10 serum samples (RA and normal) were analyzed in the presence of 250µg/mL mouse IgG. Note that the sample size of 10 seems insufficient to screen an event with occurrence rate of 10%. The sponsor states if HAMA is interfering with the assay, recoveries observed for LPC spiked samples would be reduced when in the presence of IgG. Results are presented in the following Table 5 (red notes added by the reviewer). The sponsor states “the addition of mouse IgG raised the background response of the assay, including the response of the negative control. Therefore, HAMA is unlikely to be the main cause of failed recovery experiments in RF serum.”

Table 5. Detection of ADA in the Presence of Mouse IgG

LPC spiked in samples	Run ID	16HOX4					17HOX4				
		SPF 1	SPF 2	SPF 3	SPF 4	SPF 5	SPF 6	SPF 7	SPF 8	SPF 9	SPF 10
Without IgG (16HOX4-A, 17HOX4-A)	Signal (RLU)	667	705	249	455	323	206	350	566	383	321
	% Recovery	246	260	91.9	168	119	81.7	139	225	152	127
	%C.V.	3.18	0.903	2.84	3.42	0.00	3.43	4.04	1.13	5.54	0.662
With IgG (16HOX4-B, 17HOX4-B)	Signal (RLU)	809	883	442	660	467	369	502	701	593	527
	% Recovery	167	183	91.5	137	96.7	86.3	117	164	139	123
	%C.V.	5.07	10.7	2.24	0.536	7.12	3.45	5.35	2.73	3.10	3.76

Non-spiked in samples	Run ID	16HOX4					17HOX4				
		SP 1	SP 2	SP 3	SP 4	SP 5	SP 6	SP 7	SP 8	SP 9	SP 10
Without IgG (16HOX4-A, 17HOX4-A)	Signal (RLU)	101	94.5	85.5	235	229	85.0	100	86.0	90.5	147
	S/N	0.800	0.749	0.677	1.86	1.81	0.690	0.811	0.698	0.734	1.19
With IgG (16HOX4-B, 17HOX4-B)	Signal (RLU)	337	327	286	455	275	299	297	273	265	291
	S/N	0.916	0.889	0.777	1.24	0.747	0.905	0.899	0.827	0.802	0.881

Legend:

Control for run 16HOX4 = mean LPC (271 RLU)

Control for run 17HOX4 = mean LPC (252 RLU)

Reviewer's Comments:

Judging from the experimental design and provided rationale, I think the sponsor intended to use excessive mouse IgG to neutralize the effect of HAMA. They state that “if HAMA is interfering with the assay, recoveries observed for samples spiked with the LPC that are elevated would be reduced when in the presence of IgG.” In my opinion, using excessive mouse IgG which is a non-specific blocking agent may not be appropriate strategy to study this effect. A

study with HAMA spike-in might be more informative. The submitted data cannot support any conclusion whether HAMA would interfere with the assay.

On the other hand, I note that 3 of 10 unspiked serum samples (SP4, 5, and 10) were initially screened positive, and the excessive IgG did reduce the S/N values in all 3 samples. This result might be indicative of that HAMA is contributing to the high positive rates seen in the pre-treatment clinical sample results. However, if the high positive rate is caused by the HAMA in the tested population, one would expect patients who are ADA-positive pre-treatment would be ADA-positive throughout the whole treatment duration. By examining the longitudinal data on baseline positive patients, most of them became negative in the subsequent testing points, which indicates HAMA is not the major contributor for the high positive rate at pre-treatment.

CD 20 cell membrane fragments:

The sponsor conducted a CD20+ cell membrane fragment (CD20+ CMFs) spike-in study to demonstrate assay interference from CD20 fragments. Figure 7 in the Integrated Immunogenicity Summary shows that CD20+ CMFs induced positive signal in the current ADA assay and pre-incubation with ofatumumab inhibited this signal significantly. Moreover, it is investigated whether ofatumumab could selectively inhibit signals from CD20+ CMFs without interfering with true ADA signals by comparing the PC (LPC, MPC, and HPC) responses in the presence and absence of ofatumumab. The results indicate that ofatumumab effectively inhibited the signals produced by CD20+ CMFs at all concentration tested (0.1 to 10 µg/mL of ofatumumab) and had no impact on all levels of PC signals (Figure 7). The sponsor also referenced literature publication by Chen et al (False-positive immunogenicity responses are caused by CD20+ B cell membrane fragments in an anti-ofatumumab antibody bridging assay. J Immunol Methods. 2013; 394:22-31) to support the interference of CD20+ CMF, and the use of anti-CD20 antibody to reduce the interference.

(A) Plate design

				Dilution of CMF									Arzerra in Rxn Buffer
Production NC/PCs		Inhibited Production NC/PCs		Lot 150519				Lot 150527					
1	2	3	4	Dil 3	Dil 6	Dil 20	Dil 80	Dil 3	Dil 6	Dil 20	Dil 80		
A	NC	NC	NC-I-C	AI 1	AI 2	AI 3	AI 4	AI 5	AI 6	AI 7	AI 8	0 µg/mL	
B	NC	NC	NC-I-C	AI 1	AI 2	AI 3	AI 4	AI 5	AI 6	AI 7	AI 8		
C	LPC 20.0 ng/mL	LPC 20.0 ng/mL	LPC-I-C 20.0 ng/mL	AI-I-A 1	AI-I-A 2	AI-I-A 3	AI-I-A 4	AI-I-A 5	AI-I-A 6	AI-I-A 7	AI-I-A 8	0.1 µg/mL	
D	LPC 20.0 ng/mL	LPC 20.0 ng/mL	LPC-I-C 20.0 ng/mL	AI-I-A 1	AI-I-A 2	AI-I-A 3	AI-I-A 4	AI-I-A 5	AI-I-A 6	AI-I-A 7	AI-I-A 8		
E	MPC 250 ng/mL	MPC 250 ng/mL	MPC-I-C 250 ng/mL	AI-I-B 1	AI-I-B 2	AI-I-B 3	AI-I-B 4	AI-I-B 5	AI-I-B 6	AI-I-B 7	AI-I-B 8	1 µg/mL	
F	MPC 250 ng/mL	MPC 250 ng/mL	MPC-I-C 250 ng/mL	AI-I-B 1	AI-I-B 2	AI-I-B 3	AI-I-B 4	AI-I-B 5	AI-I-B 6	AI-I-B 7	AI-I-B 8		
G	HPC 1000 ng/mL	HPC 1000 ng/mL	HPC-I-C 1000 ng/mL	AI-I-C 1	AI-I-C 2	AI-I-C 3	AI-I-C 4	AI-I-C 5	AI-I-C 6	AI-I-C 7	AI-I-C 8	10 µg/mL	
H	HPC 1000 ng/mL	HPC 1000 ng/mL	HPC-I-C 1000 ng/mL	AI-I-C 1	AI-I-C 2	AI-I-C 3	AI-I-C 4	AI-I-C 5	AI-I-C 6	AI-I-C 7	AI-I-C 8		

Source: NB11252

AI: Analyzed uninhibited, AI-I: Analyzed inhibited with ofatumumab, Arzerra: ofatumumab, CMF: CD20+ cell membrane fragments, Dil: Dilution, HPC: High positive control, LPC: Low positive control, MPC: Medium positive control, NC: Negative control, PC: Positive control, Rxn: Reaction

(B) Result

				Dilution of CMF								Arzerra in Rxn Buffer	
Production NC/PCs		Inhibited Production		Lot 150519				Lot 150527					
				Dil 3	Dil 6	Dil 20	Dil 80	Dil 3	Dil 6	Dil 20	Dil 80		
	1	2	3	4	5	6	7	8	9	10	11	12	
A	67	70	64	56	923	2496	5825	3348	2555	5933	8689	4850	0 µg/mL
B	68	70	63	56	788	2305	5306	3056	2308	5265	8555	5565	
C	866	798	819	684	864	1827	1013	221	2203	4573	2669	472	0.1 µg/mL
D	940	865	830	804	784	1679	909	213	2239	4756	2832	455	
E	10221	8986	8790	9167	231	103	90	65	279	123	85	70	1 µg/mL
F	10027	8470	7811	9869	231	108	93	65	315	138	85	70	
G	30611	29455	30109	30952	88	81	70	62	86	82	71	67	10 µg/mL
H	35189	32700	32474	32009	83	83	71	63	86	81	71	64	

Source: NB11252

Arzerra: ofatumumab, CMF: CD20+ cell membrane fragments, Dil: Dilution, NC: Negative control, PC: Positive control, Rxn: Reaction

Figure 7: Investigation of False Positive Signal using CD20+ CMF and Ofatumumab

Reviewer's Comment:

I agree with the sponsor's conclusion that CD20+ CMFs contributes in false positive results in the ADA assay, and excessive ofatumumab at the level of 10µg/mL can effectively inhibit the false positive signal without interfering with the ADA signal (from spiked-in PCs).

The effect of ofatumumab addition to baseline ADA results is shown in the following Table 7 (from Integrated Immunogenicity Summary).

Table 7: Number of Screened and Confirmed Positive Samples at Baseline in Studies CT-P10 1.1, CT-P10 3.2 and CT-P10 3.3

Protocol ¹	No. of baseline samples	Screened positive		Confirmed positive
		w/o ofa (%)	w/ ofa ² (%)	
Study CT-P10 1.1	153	41 (26.8 %) ³	N/A ³	30 (19.6 %) ³
Study CT-P10 3.2	372	69 (18.5 %) ⁴	49 (13.2 %)	39 (10.5 %)
Study CT-P10 3.3	119	59 (49.6 %) ⁴	12 (10.1 %)	12 (10.1 %)

Source: EPY, RBNP, RBNR

¹ Studies CT-P10 1.1 and CT-P10 3.2 for RA indication, Study CT-P10 3.3 for AFL indication

² Applied screening cut point with ofatumumab is obtained from HOX9.

³ ADA test result for Study CT-P10 1.1 was evaluated using screening and confirmatory cut point without ofatumumab.

⁴ All samples that screened positive in the absence of ofatumumab were analyzed in the confirmatory assay in the presence of ofatumumab

AFL: Advanced follicular lymphoma, No.: number, RA: Rheumatoid arthritis, w/o ofa: without ofatumumab, w/ ofa: with ofatumumab

Reviewer's Comments:

Adding ofatumumab effectively reduced the false positive rate in the screening assay, especially with higher rate in the AFL samples. Although ofatumumab is not used in the screening assay for testing, the confirmatory assay (with ofatumumab) did achieve the same effect of reducing false positive rate.

In the reported clinical data, the pre-treatment ADA positive rate (~10%) is higher than the published pre-treatment ADA rates (~3%) in U.S.-Rituxan studies (IMAGE by Tak et al., 2011 and SERENE study by Emery et al., 2010). In addition, the ADA-positivity in the most cases was transient, and the transient ADAs usually were with low titers. These observations (i.e., higher pre-treatment positive rates and transient ADAs with low titers) are indicative that re-adjusting to slightly higher cut point may help to interpret the clinical immunogenicity data (e.g., clinical effect of ADA), which can be achieved by stratifying the ADA-positive cases by titer.

Reviewer's Comment: Overall, matrix interference and selectivity studies are acceptable.

Confirmatory Assay

The confirmatory assay is based on the cold completion with excessive CT-P10 (58.8µg/mL) in the presence of 5.88µg/mL ofatumumab (mAb against CD20). A partial validation is provided in report HOX9. In brief, the confirmatory assay uses the same format as the screening assay, except that [ofatumumab (5.88µg/mL)] or [ofatumumab (5.88µg/mL) + CT-P10 (58.8µg/mL)] is added to the screening assay reaction mix. The confirmatory cut point is based on % inhibition:

$$\% \text{ Inhibition} = 100 * [1 - \text{value with CT-P10} / \text{value without CT-P10}]$$

Reviewer's Comment:

In HOX9 report, the sponsor determined the screening cut point and confirmatory cut point for the assay with ofatumumab. However, the screening assay with ofatumumab was not used in clinical sample testing. It is unclear why the sponsor did not replace the original screening assay with the new screening assay with ofatumumab knowing that CD20+ CMF can cause false

positive results. Nevertheless, as I noted in section Matrix Interference and Selectivity, although ofatumumab is not used in the screening assay for testing, the confirmatory assay (with ofatumumab) did achieve the same effect of reducing false positive rate.

Since the confirmatory and titer assays are different from the screening assay, and the comparison of the screening assay with and without ofatumumab is an important piece of data, I reviewed the screening assay cut point determination and assay precision as part of the confirmatory and titer assay validation.

Screening Cut Point Determination (with ofatumumab, from HOX9)

Cut points were determined for a population of 50 healthy human serum lots, 50 RA serum lots, and 30 NHL serum lots in the presence of ofatumumab. All lots are individual human and drug-naïve samples. I summarized the cut point determination in the following table:

	# of independent Assays	# of analysts	Total # of sample values	# of outliers identified* and removed	Statistical Method for cut point determination (see analysis flow chart)	Cut point value (S/N)
Healthy	9	2	150	20	non- parametric approach on non-transformed data, 95 th percentile	1.22
RA	12	3	150	9		1.24
NHL	6	2	90	3		1.13

* The outliers are identified using Tukey's outlier filter

Reviewer's Comment:

The experimental design for determining the cut points are acceptable. The assay can consistently detect ADA at level of 8.86ng/mL (a sponsor's defined cut point positive control), the cut points are acceptable.

Confirmatory Cut Point Determination

The confirmatory assay cut points (CCP) determined from the analysis of 50 individual drug-naïve healthy human serum lots, 50 individual drug-naïve rheumatoid arthritis serum lots, and 30 individual drug-naïve non-Hodgkin lymphoma serum lots analyzed in the presence of 10µg/mL ofatumumab and 100µg/mL CT-P10 (in the reaction buffer). % inhibition value is calculated for each sample for cut point determination.

$$\% \text{ Inhibition} = 100 * [1 - \text{value with CT-P10} / \text{value without CT-P10}]$$

The confirmatory cut points (i.e. % inhibition) for healthy volunteer, RA and NHL population are determined to be 27.1%, 25.5% and 20.3%, respectively. The sponsor combined the data from the 3 populations and determined a cut point of 25.8% which was used in CT-P10 3.2 and 3.3 studies.

Reviewer's Comment:

Although it is not recommended to combine data from different population to calculate cut point, the % inhibition of 25.8% is not significantly different from the values obtained for each population. In addition, % inhibition for LPC (20ng/mL) is 91.9%, i.e. the cut point confirms the ADA at 20ng/mL concentration. Therefore, the confirmatory cut point of 25.8% is acceptable.

The ofatumumab and CT-P10 concentrations used in the confirmatory assay validation are 10µg/mL and 100µg/mL, respectively. In the confirmatory testing of clinical samples, 5.88µg/mL ofatumumab and 58.8µg/mL CT-P10 were used due to calculation error. The sponsor

provided data to support the changes of reagent concentration do not impact the assay performance (in HOX9, data not copied in here).

Reviewer's Comment:

I reviewed the supporting data, and the change of atumumab and CT-P10 concentrations is not likely to affect the assay results (Table 10).

Table 10: Summary of Assay Cut Points for Study CT-P10 3.2 and Study CT-P10 3.3

Assay Cut Point	CT-P10	Rituxan®	MabThera®
Screening cut point	1.14	1.27	1.17
Source	Appendix D of HOX2	Appendix B of HOX7	Appendix B of HOX3
Statistical Approach	Non-parametric approach		
Confirmatory cut point ¹	25.8%	26.1%	30.7%
Source	Appendix C of HOX9	Appendix C of HOX8	
Statistical Approach	Non-parametric approach		

¹ Inhibitory drug was the same as each tagged reagent. (i.e. CT-P10 tag and CT-P10 as an inhibitory drug, Rituxan® tag and Rituxan® as an inhibitory drug, MabThera® tag and MabThera® as an inhibitory drug).

Assay precision (screening assays with ofatumumab)

Inter-assay precision was calculated from the NC and PCs in validation runs 1HOX9 through 24HOX9. %CV for NC, LPC, MPC and HPC are all less than 15%. Confirmatory assay precision is evaluated using HPC, where %CV is 5.97%.

Reviewer's Comment:

The LPC could be included in the evaluation of confirmatory assay precision, but we do not expect significant different results. The assay precisions are acceptable.

Titer Assay

In the titer assay, confirmed ADA positive samples are 2-fold serially diluted and tested by the screening assay with ofatumumab addition in the mix. The titer values of the CT-P10 tagged assay are transformed using a $[\log_2(x)] + 1$ transformation. The titer cut point is calculated based on 99.9th percentile of S/N ratio which is 1.30.

Reviewer's Comment:

The titer cut point and titer calculation are acceptable.

Overall ADA assay Assessment

In regards to high pre-treatment ADA positive rate (~10%) in the ADA assays, the sponsor provided historical ADA data from public information in biologic treatment-naïve RA patients: ADAs to rituximab were detected in 1.4% and 3.6% of placebo treated patients with active RA in the IMAGE (Tak et al., 2011) and SERENE study (Emery et al., 2010), respectively. The sponsor states "Nevertheless, approximately 10% of baseline positivity was still observed after modification of the method in Studies CT-P10 3.2 and CT-P10 3.3, which may also be attributed

to the effect of other binding factors. Thus, multiple binding factors are thought to be involved in this phenomenon and the relative quantitative importance may vary across and within different clinical populations”.

Reviewer's Comment:

In general, I agree with the sponsor that certain factors in patient serum may contribute to the high base positive rate. The fact that the ADA base rate is similar between the RA and AFL populations indicates the contributing factor is not disease specific. Also, I note here that the addition of ofatumumab in the ADA assay is effective in reducing false positive rate (Table 7 in Matrix Interference and Selectivity).

After discussing my concerns for the high pre-treatment positive rate with the clinical reviewer, Dr. Stefanie Freeman, and the clinical pharmacology reviewer, Dr. Sang Chung, I reviewed the [patient clinical dataset](#) in the submission (which includes ADA test results for each patient at each testing point) in seek of the meaning of the phenomena. Most of the pre-treatment ADA positive subjects become negative during the treatment, in the other words, the ADA positivity is transient. In fact, most of the ADAs are transient throughout the dataset, and the transient ADA positive samples are in low titer. These observations suggest that the sponsor may have set the cut points too low which can also cause high baseline positive rate. Large number of transient and low titer ADA may dilute the true clinical effect (e.g impact on PK) of ADAs in the data analysis, therefore we recommend sponsor use titer to stratify ADA levels and assess their impact on safety and efficacy. ADA titer is evaluated and reported in the submission. Therefore, the current ADA assay combined with titer analysis are adequate to evaluate the ADA status in the RA studies.

- Overall, the ADA binding assay is adequate for use in the study CT-P10 3.2 (in RA), although ADA rate is likely under-reported at 8-week and 16-week sample points, due to insufficient drug tolerances at higher serum drug concentration.*
- The ADA binding assay is inadequate for use in the study CT-P10 3.3 (in AFL), because the assay sensitivity (drug tolerance) is insufficient with high serum drug concentrations observed at all sample points in this study (due to higher dosing in AFL indication).*

The clinical immunogenicity data for the AFL study is presented in Table 36 of the Integrated Immunogenicity Summary:

Table 36: Summary of Testing for ADA and NAb Up to Core Cycle 8 [24 Weeks] in Study CT-P10 3.3 (Part 2): Safety Population

	CT-P10 (N=70)	Rituxan® (N=70)
Immunogenicity Test	n (%)	
Screening		
ADA Positive	5 (7.1)	8 (11.4)
NAb Positive (as % of ADA positive)	0	0
Core Cycle 4		
	CT-P10 (N=70)	Rituxan® (N=70)
Immunogenicity Test	n (%)	
ADA Positive	2 (2.9)	1 (1.4)
NAb Positive (as % of ADA positive)	2 (100.0)	1 (100.0)
EOT1		
ADA Positive	1 (1.4)	1 (1.4)
NAb Positive (as % of ADA positive)	0	1 (100.0)

Source: CSR CT-P10 3.3 Post-text Table 14.3.3.6

Note: The proportion of ADA positive patients was calculated using safety population as a denominator. The proportion of NAb positive patients was re-calculated using ADA positive patients as a denominator

ADA: Anti-drug antibody, EOT: End-of-treatment, NAb: Neutralizing antibody, n: the number of subjects with the event, N: the number of patients in each treatment group

Reviewer's Comments:

The post-treatment ADA positive rate is lower than the pre-treatment rate and all ADAs are with high titers (titer=4-11, log2 value). Four of the five ADA positive samples are NAb positive too. These findings corroborate with the assay performance conclusion that the ADA and NAb rates are likely under-reported in AFL study. See NAb assay review below.

Neutralizing Antibody Assay:

The validation reports for the assays used for testing NAb samples are IOX2 (for screening assay validation) and IOX3 (for confirmatory assay validation).

Method

NABs for CT-P10/rituximab are measured in human serum by complement-dependent cytotoxicity (CDC) assay. This assay is based on complement system activation by binding of CT-P10/rituximab to CD20 antigen on WIL2-S cells. The activation of the complement system leads to cell lysis and death of WIL2-S cells. In the presence of NAb, the CDC effect of CT-P10/rituximab is reduced.

The method procedure: controls and samples are heat inactivated at 56 °C prior to incubation with CT-P10. Incubated samples are treated with a 1:2 dilution of normal human serum(NHS) complement in the presence of WIL2-S cells. Cell liability is measured by quantifying the ATP presence using CellTiter-Glo® reagent via luminescence signal (by Wallac Victor plate reader). A sample is considered “reactive” for neutralizing activity if the screening assay value (SAV) is equal to or greater than the assay cut point. The reactive screening assay samples are serially diluted to determine the NAb titer.

In the Integrated Immunogenicity Summary, the sponsor provides justification on the CT-P10 concentration of 219ng/mL (in well) in the assay, which was selected based on ED50 and ED70 values from clinical samples of healthy, RA and NHL populations. In addition, the WIL2-S cell passage was restricted from 4 to 20 cells because used at passage lower than 30 cell passages the assay generated consistently and reproducibly lower mean RLU values with PC/DC (drug control) ratios above the cut point compared to mean RLU values and PC/DC ratios in assays using the cells at passage numbers higher than 30.

Although NAb assays using CT-P10 or rituximab were included in the validation, only the NAb assay using CT-P10 is used in the clinical sample testing.

Reviewer's Comment:

The CDC based NAb assay reflects the rituximab MoA, and it is an appropriate format to evaluate the NAb for CT-P10 and U.S.-Rituxan. The selection of CT-P10 concentration in the assay is justified.

It is acceptable to use only the biosimilar product in the ADA assays to test clinical samples regardless of treatment drug groups. Since only the CT-P10 NAb assay is used in the clinical sample testing, my review is focused only on validation parameters of the CT-P10 NAb assay.

The validation data are from reports IOX2 and IOX3.

Essential Reagents and Concentration:

WIL2-S Cell Line: Derived from ATCC (CRL-8885)

Affinity Purified Rat Anti-Rituximab, by ABD Serotec

Complement: Pooled NHS Complement, Innovative Research Product (No. IPLA-CSER)

Pooled normal human serum (PNHS): by Bioreclamation

Settings of Controls	
Positive Control (PC)	WIL2-S Cells + diluted NHS complement + CT-P10 +LPC/MPC/HPC in the presence of PNHS.
Negative Control (NC)	WIL2-S Cells + PNHS; represents the background signal from WIL2-S cells in the presence of PNHS
Complement Control (CC)	WIL2-S Cells + diluted NHS complement in the presence of PNHS.
Drug Control (DC)	WIL2-S Cells + diluted NHS complement + CT-P10 in the presence of PNHS.

MRD (Minimum required dilution): 1:4 for NHS and 1:8 for RA and NHL

PCs for NHS: LPC: 1200ng/mL, MPC: 1600ng/mL, HPC: 2000ng/mL

PCs for RA and NHL: LPC: 2400ng/mL, MPC: 3200ng/mL, HPC: 4000ng/mL

Cut Point Determination

Screening Assay Cut Point (IOX2)

The sponsor determined the cut points for each population using individual serum samples. The calculation is based on screening assay value (SAV).

$$\text{Assay Value (AV)} = \frac{\text{Mean RLU of Sample}}{\text{Mean RLU of DC}}$$

I summarized the statistical analysis and results in the following table:

	# of serum samples	# of assays	# of analysts	Total # of sample values	# of outliers	Statistical Method for cut point determination	Cut point value
Healthy	30	3	2	89	0	Parametric or non-parametric method at 95 th percentile	1.22
RA	30	3	2	90	6		1.20
NHL	31	4	2	114	6		1.11

Reviewer's Comment:

The NAb assay screening cut point is acceptable.

Confirmatory Assay Cut Point (IOX3)

In order to confirm the increase in signal observed in samples is due to antibodies and not due to non-specific matrix components, samples are immunodepleted using protein A/G. The confirmatory assay cut point is the % depletion below which a sample is defined to be “negative” (or at or above which it is defined to be “positive” for the presence of NAb). *I summarized the statistical analysis and results in the following table:*

	# of serum samples	# of assays	# of analysts	Total # of sample values	# of outliers	Statistical Method for cut point determination	Cut point value (% depletion)
Healthy	30	3	2	88	2	Parametric or non-parametric method at 99 th percentile	32.0%
RA	30	3	2	88	2		30.2%
NHL	31	3	2	83	4		23.3%

Reviewer's Comment:

Based on the results from assay selectivity with LPC spike-in data, the confirmatory cut point is appropriate.

Selectivity/Recovery (IOX2 and IOX3):

IOX2: Selectivity of the NAb screening assay

Selectivity was evaluated by analyzing the PCs diluted into 10 individual RA, and NHL serum samples. RA serum samples were spiked at LPC, MPC and HPC levels (2400, 3200, and 4000 ng/mL, respectively). Only LPC of 2400ng/mL were used for NHL serum samples due to limited serum availability. All 10 of 10 and 9 of 10 unspiked RA and NHL serum samples, respectively, showed negative results (SAV < cut point), and all (10 of 10 for each) spiked RA serum showed “reactive” results for the PCs (SAV ≥ cut point).

IOX3: Selectivity of the NAb screening and confirmatory assay

The procedure is the same as described in IOX2, but this time all levels of PCs were used for both RA and NHL. The results from the RA samples are shown in Table 5B (partial copy).

Table 5B. Recovery in RA – CT-P10

Unspiked														
Screening Assay							Confirmatory Assay							
Sample ID	Run ID	Raw Response (RLU)			%CV	SAV	Sample Result	Raw Response (RLU)			%CV	% Depletion	Sample Result	Final Result
		Dup 1	Dup 2	Mean				Dup 1	Dup 2	Mean				
SPa 1	09IOX3	58747	67122	62935	9.41	1.01	Negative	60806	61138	60972	0.385	3.12	Negative	Negative
SPa 2	09IOX3	68198	63662	65930	4.86	1.05	Negative	56430	48919	52675	10.1	20.1	Negative	Negative
SPa 3	09IOX3	88756	92386	90571	2.83	1.45	Reactive	55645	56350	55998	0.890	38.2	Positive	Positive
SPa 4	09IOX3	66675	72347	69511	5.77	1.11	Negative	59309	51164	55237	10.4	20.5	Negative	Negative
SPa 5	09IOX3	57899	64064	60982	7.15	0.975	Negative	62573	61475	62024	1.25	-1.71	Negative	Negative
SPa 6	09IOX3	67052	67486	67269	0.456	1.08	Negative	50748	51524	51136	1.07	24.0	Negative	Negative
SPa 7	18IOX3	85856	92913	89385	5.58	1.29	Reactive	89022	78928	83975	8.50	6.05	Negative	Negative
SPa 8	18IOX3	82669	104910	93790	16.8	1.35	Reactive	101287	100455	100871	0.583	-7.55	Negative	Negative
SPa 9	18IOX3	108165	115946	112056	4.91	1.62	Reactive	113248	115706	114477	1.52	-2.16	Negative	Negative
SPa 10	18IOX3	121497	131847	126672	5.78	1.83	Reactive	98621	106649	102635	5.53	19.0	Negative	Negative

Low Spike (2,400 ng/mL)														
Screening Assay							Confirmatory Assay							
Sample ID	Run ID	Raw Response (RLU)			%CV	SAV	Sample Result	Raw Response (RLU)			%CV	% Depletion	Sample Result	Final Result
		Dup 1	Dup 2	Mean				Dup 1	Dup 2	Mean				
SPFa 1	09IOX3	100784	111212	105998	6.96	1.69	Reactive	57953	57980	57967	0.0329	45.3	Positive	Positive
SPFa 2	09IOX3	116227	118048	117138	1.10	1.87	Reactive	54572	53790	54181	1.02	53.7	Positive	Positive
SPFa 3	09IOX3	115251	126143	120697	6.38	1.93	Reactive	62906	56238	59572	7.91	50.6	Positive	Positive
SPFa 4	09IOX3	103755	114356	109056	6.87	1.74	Reactive	59231	57647	58439	1.92	46.4	Positive	Positive
SPFa 5	09IOX3	99860	112031	105946	8.12	1.69	Reactive	62052	53333	57693	10.7	45.5	Positive	Positive
SPFa 6	09IOX3	135406	144657	140032	4.67	2.24	Reactive	54397	55683	55040	1.65	60.7	Positive	Positive
SPFa 7	18IOX3	134188	150771	142480	8.23	2.05	Reactive	112720	113408	113064	0.430	20.6	Negative	Negative
SPFa 8	18IOX3	162167	166763	164465	1.98	2.37	Reactive	107459	97856	102658	6.61	37.6	Positive	Positive
SPFa 9	18IOX3	190434	178339	184387	4.64	2.66	Reactive	114975	105703	110339	5.94	40.2	Positive	Positive
SPFa 10	18IOX3	169190	173093	171142	1.61	2.47	Reactive	88310	92736	90523	3.46	47.1	Positive	Positive

Reviewer' Comments:

The results show that 5 of 10 unspiked RA samples showed “reactive” results (noted by red arrow), while the results in IOX2 showed that all were negative. Only 1 out of the 5 unspiked samples is confirmed as “positive”.

All 10 of 10 LPC spiked-in RA samples showed “reactive” results in the NAb screening assay, from which 9 were confirmed as “positive” (the unconfirmed one is marked with an arrow).

The % depletion of LPC in RA serum is ~45%.

For NHL serum samples, 8 of 10 unspiked samples were confirmed “negative,” and 10 of 10 spiked samples at each level were confirmed “positive”

Overall, the NAb assay (screening and confirmatory) selectivity is acceptable.

Assay Sensitivity

Relative assay sensitivity was determined by the lowest concentration at which the PC consistently produces a response greater than or equal to the assay cut point. The PC was prepared by 2-fold dilution in range of 6400 - 25 ng/mL in the NC pool at 6400, 3200, 1600, 800, 400, 200, 100, 50.0, and 25.0 ng/mL and analyzed in duplicate runs. In the initial runs, the assay sensitivity was determined to be 3200ng/mL, which is 4 times higher than the sensitivity observed during assay development. The sponsor later determined that high cell passage affected

the assay sensitivity. When the cells are used at lower passage, the assay sensitivity was determined to be 800ng/mL.

Reviewer's Comment:

Based on the above finding the sponsor has restricted the cell passage to be 4-20 in clinical sample testing. The assay sensitivity as 800ng/mL is acceptable for the NAb assay.

Precision:

The intra-assay precisions (%CV of SAVs from 5 samples/PC/plate, 6 plates in total) of the 1200ng/mL LPC, 1600ng/mL MPC, and 2000ng/mL HPC are 17.4 %, 13.2 %, and 17.4 %, respectively.

The inter-assay precisions (%CV of SAVs from 21 total runs) of the LPC, MPC, and HPC are 14.6 %, 14.4 %, and 19.2 %, respectively.

Reviewer's Comment:

The assay precisions are acceptable for this NAb assay.

Drug Tolerance

The sponsor evaluated the ability of the assay to detect NAb in the presence of circulating drug and established drug tolerance limits for the assay. The results are provided in Table 12:

CT-P10
Run 90IOX2

PC ng/mL	Drug ng/mL	Mean	%CV	SAV	Sample Result
9600	14000	104177	2.15	3.68	Reactive
4800	14000	4420	6.70	0.156	Negative
1200	14000	3597	5.43	0.127	Negative
800	14000	3660	12.3	0.129	Negative
9600	7000	102056	4.45	3.61	Reactive
4800	7000	66658	5.73	2.36	Reactive
1200	7000	7373	12.2	0.260	Negative
800	7000	7295	1.87	0.258	Negative
9600	3500	103182	1.76	3.65	Reactive
4800	3500	127720	4.75	4.51	Reactive
1200	3500	44862	0.984	1.59	Reactive
800	3500	39607	9.66	1.40	Reactive
9600	1750	102278	2.10	3.61	Reactive
4800	1750	124863	5.29	4.41	Reactive
1200	1750	104196	0.0753	3.68	Reactive
800	1750	87769	3.24	3.10	Reactive

Rituximab
Run 91IOX2

PC ng/mL	Drug ng/mL	Mean	%CV	SAV	Sample Result
9600	14000	11194	17.6	0.647	Negative
4800	14000	3859	3.55	0.223	Negative
1200	14000	3015	3.96	0.174	Negative
800	14000	2736	1.47	0.158	Negative
9600	7000	107566	3.95	6.22	Reactive
4800	7000	42627	10.4	2.46	Reactive
1200	7000	6609	13.0	0.382	Negative
800	7000	5570	3.99	0.322	Negative
9600	3500	106547	2.36	6.16	Reactive
4800	3500	124700	6.62	7.21	Reactive
1200	3500	33907	1.54	1.96	Reactive
800	3500	24125	7.12	1.39	Reactive
9600	1750	99217	0.809	5.73	Reactive
4800	1750	122093	2.87	7.06	Reactive
1200	1750	99210	1.74	5.73	Reactive
800	1750	74971	5.96	4.33	Reactive

Cut point in NHS = 1.22 for both

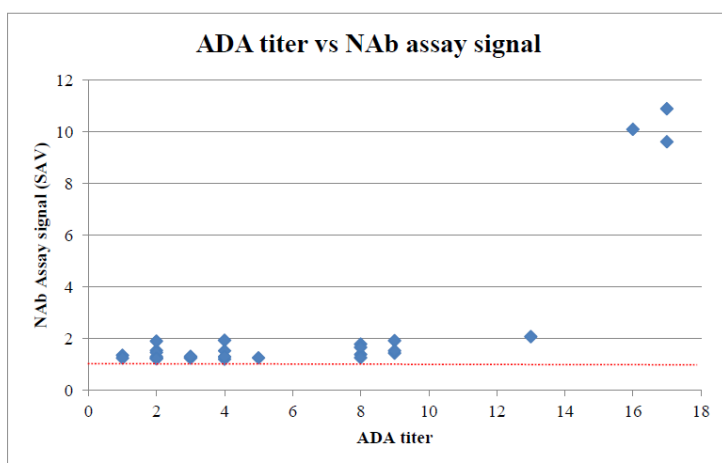
Reviewer's Comments:

In the RA studies, the mean serum drug (CT-P10, US-Rituxan and EU-MabThera) concentrations are 31.8-34.0µg/mL, 3.37-6.42µg/mL and 0.36-0.49µg/mL at treatment week 8, week 16 and week 24, respectively (see details in Reviewer's Comment in Drug Tolerance Section of the ADA binding assay). The results presented in the table above indicate that the expected drug serum concentrations at week 8 will interfere with the detection of up to

9600ng/ml of NABs, and likely for even higher concentrations, in this assay. At week 16, the assay's ability to detect up to 1200ng/ml NABs might be interfered by the drug concentrations in samples (drug tolerance level is close to the serum drug level). At week 24, the drug serum concentrations are low enough that this will not interfere with the detection even of 800ng/ml of NABs.

In the AFL studies, the immunogenicity samples are drawn at baseline, after 4th cycle, and EOT1. According to the clinical pharmacology reviewer, Dr. Sang Chung, the C_{trough} is 60.395µg/mL for CT-P10 and 64.256µg/mL for US-Rituxan after 4th cycle. This CT-P10 concentration is beyond the 14µg/mL, the highest drug concentration tested in this drug tolerance study; nevertheless, the above results showed that the 14µg/mL on board drug will interfere with detection of ADA concentrations for up to 9600ng/mL. Therefore, the sensitivity of the NAb assay is insufficient under the expected serum drug concentrations observed in AFL studies.

The sponsor attempted to justify the sensitivity of the NAb assay by examining the ADA titer of the NAb positive samples (the results are show in Figure 6 of the Integrated Immunogenicity Summary). The sponsor states that “from the assessment of the relationship between results in ADA titer and NAb SAV for all subjects who were classified as “NAb positive” in Study CT-P10 1.1 presented in Figure 6 below, it was shown that the very low titer of ADA positive samples can be detected as the NAb positive in this cell-based NAb assay method.”



Sources: EPY, FPY

Note: To estimate the magnitude of the ADA positive response, 2-fold dilutions beyond the MRD of 1:5 was applied but not counted in final titer value. In addition, the red line indicates 1.20 of NAb assay cut point.

SAV: Screening assay value

Figure 6: Relationship between Result in ADA Titer and NAb SAV for All Subjects who were classified as “NAb Positive”

Reviewer's Comment:

Note that the study CT-P10 1.1 used a titer assay without ofatumumab, while study CT-P10 3.2 and 3.3 used the titer assay with ofatumumab. The sponsor already determined that excessive CD20 fragments cause false positive results in the ADA screening assay. Since the titer assay is essentially the same as the screening assay, the titer determination from the assay (shown in Figure 6) may not represent the true titer of the ADA. Therefore, the data cannot fully support the sponsor's conclusion the very low titer of ADA positive samples can be detected as NAb positive in this cell-based NAb assay method.

The drug tolerance of the NAb assay is insufficient.

Stability (IOX2 and IOX3)

Stability of LPC and HPC were evaluated under the following conditions: 5 freeze/thaw cycles, 24hr at room temperature, and 207 days at -25°C and -70°C. The stability samples are tested in the NAb assay and the results are compared to the freshly prepared LPC and HPC. The results show that the differences between the stability and fresh samples are less than 20%.

Reviewer's Comment:

Sample stability is appropriately validated.

Substance Interference Testing

Hemolysis

Five individual NHS samples were spiked with LPC and HPC and analyzed. Hemolyzed whole blood was added to the samples at increments of 0 %, 0.5 %, 5 %, and 10 %. All 5 unspiked samples showed “negative” results at all levels of hemolysis. The LPC-spiked samples with 0 %, 0.5 %, and 5 % hemolysis resulted in “reactive” results, while only 1 had “reactive” results with 10 % hemolysis. HPC-spiked samples at all hemolysis levels had five of five (5/5) samples “reactive” results.

The sponsor states “samples with levels of hemolysis greater than 5% for CT-P10 cannot be analyzed with this method.” (in IOX2 report).

Reviewer's Comment:

I agree that hemolysis may cause false negative results in this NAb assay (clinical pharmacology reviewers need to consider hemolysis level in the clinical samples in their assessment).

Lipemia

Five individual lipemia serum samples were spiked with LPC (1200 ng/mL) and HPC (2000 ng/mL) and analyzed. All unspiked samples showed “negative” and all spiked samples showed “reactive” results.

Reviewer's Comment:

No interference from lipemia serum is observed.

Assay Acceptance Criteria

1. Cell Passage restrictions: 4 to 20
2. CC/NC $\geq$ 0.917, which is based on [mean - 3xSD] from all acceptable runs in the validation studies.
3. CC/DC $\geq$ 1.98, which is the lowest ratio obtained from all acceptable runs in the validation studies.
4. The HPC/DC, MPC/DC and LPC/DC ratios must be $\geq$ 1.22 (cut point).

Reviewer's Comment:

The described assay acceptance criteria are acceptable for the NAb assay.

- Overall, the NAb assay is adequate for use in the study CT-P10 3.2 (in RA), although NAb rate are perhaps under-reported at 8-week and 16-week sample points, due to insufficient drug tolerances at high serum drug concentration. This assay is adequate to detect NABs in the RA samples at 24- and 48-week time points (24 weeks/cycle).
- The NAb assay is inadequate for use in the study CT-P10 3.3 (in AFL), because the assay sensitivity is insufficient with high serum drug concentrations observed at all sample points in this study (due to higher dosing in AFL indication).

During the internal meeting on 1/16/2018, the review team discussed the suitability of the immunogenicity assays for the RA and the AFL study, as well as the feasibility of using the immunogenicity data from the CT-P10 3.2 study (RA) to extrapolate conclusion to the oncology indications. The DHP clinical team stated that the sponsor should revise the immunogenicity assays and use adequate assays to test the clinical samples in the AFL study and in ongoing study on low tumor burden follicular lymphoma. (b) (4)

[REDACTED]

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Welch

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Date: 1/19/2018 04:33:36PM

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Memorandum of Review

STN:	BLA761088
Subject:	Similarity Review
Submission Date:	April 28, 2017
Review Date:	January 10, 2018
Primary Reviewer:	Nina Brahme, PhD, MPH Product Quality Reviewer, OPQ/OBP/DBRR IV
Secondary Reviewer:	Bazarragchaa Damdinsuren, MD, PhD Team Leader, OPQ/OBP/DBRR IV
Tertiary Reviewer:	Joel Welch, PhD Review Chief, OPQ/OBP/DBRR IV
Applicant:	Celltrion, Inc.
Product:	Truxima (CT-P10), proposed biosimilar to US-licensed Rituxan
Indications:	(b) (4) Non-Hodgkin's lymphoma, (b) (4) (b) (4)
Action Due Date:	February 28, 2018

Review sections are organized to facilitate the assessment of analytical similarity with the section titles remaining the same as those in the BLA submission for ease of reference.

Similarity Review Summary:

To support a demonstration that CT-P10 is highly similar to the U.S.-licensed Rituxan reference product, and to establish a 3-way scientific bridge, Celltrion performed an analytical similarity assessment using a sufficient number of CT-P10, U.S.-licensed Rituxan, and E.U.-approved MabThera drug product lots. Rituximab molecular attributes are collectively assigned to the correct tiers and attribute similarity was evaluated using appropriate statistical methods. The similarity data package (analytical data provided in the original submission and in response to information requests) was generated using methods that are considered appropriately validated or qualified for their intended purpose. CT-P10 meets the statutory “same strength” requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act. Due to facility compliance issues, a pre-license inspection could not be performed. Pending an inspection that includes an assessment of similarity data integrity, the data provided support the conclusion that CT-P10 is highly similar to U.S.-licensed Rituxan. In addition, the data support the adequacy of the analytical component of scientific 3-way bridge to justify the use of clinical data generated using E.U.-approved MabThera.

Review

3.2.R.2.3 Criticality Ranking

The sponsor states that the criticality of each attribute and activity is a function of both the severity (S) of clinical impact (on efficacy, PK/PD, safety, immunogenicity or biological activities linked to known MOA) brought on by a change in attribute, and the likelihood of that severity (L). The sponsor employed a numerical criticality ranking for all physicochemical attributes and biological activities, regardless of whether statistical analyses are feasible. Ranking was based on literature, prior knowledge, and additional data.

A detailed description of the quantitative assignment for each level of severity (S) and likelihood (L) can be found in *Regional Information (RI) 2: Description of Tiered Approach*, and are summarized in Table 3.2.R.2-2.

Table 3.2.R.2-2: Table Showing Factors Included in Criticality Ranking for Physicochemical/Biological Test Data

Factor	Physicochemical/Biological Test Scoring System
Factor 1 (S) Severity of Clinical Impact	100: Clinical impact was considered very high 50: Clinical impact was considered high 25: Clinical impact was considered moderate 10 : Clinical impact was considered low 1: Clinical impact was considered very low
Factor 2 (L) Likelihood of Severity	5: It is known that a minor change in this attribute compared to the RP/RMP could have a potential impact on efficacy, safety, identity, strength, purity and quality. 3: It is known that a moderate change in this attribute compared to RP/RMP could have a potential impact on efficacy, safety, identity, strength, purity and quality. 1: It is known that a large change in this attribute compared to RP/RMP would be required to have a potential impact on efficacy, safety, identity, strength, purity and quality.

The final criticality score was calculated by multiplying the severity score and likelihood score to generate a “criticality determination matrix” (Table 3.2.R.2-3). *The specific physicochemical attribute and biological assay criticality rankings are summarized in detail in Tables 3.2.R.2-4 and 3.2.R.2-5 of the submission. Every tier 2 attribute has at least a “moderate” criticality score.*

Table 3.2.R.2-3: Criticality Determination Matrix

Factor 2. Likelihood \ Factor 1. Severity	5	3	1
100	500 (Very High)	300 (High)	100 (Moderate)
50	250 (High)	150 (Moderate)	50 (Moderate)
25	125 (Moderate)	75 (Moderate)	25 (Low)
10	50 (Moderate)	30 (Low)	10 (Very Low)
1	5 (Very Low)	3 (Very Low)	1 (Very Low)

Reviewer comment: Overall, we find that criticality ranking for all attributes is justified and acceptable. Molecular attributes were placed into the appropriate tiers and are in line with OBP expectations.

3.2.R.2.1 Description of Tiered Approach

Tier 1: Equivalence Test with Null Hypothesis

Briefly, the sponsor calculated the variability of Rituxan lots in CDC and ADCC potency assays and assay variance was used to establish a pre-determined equivalence margin (defined as $\pm 1.5 \times \text{SD}$ of Rituxan for each key assay). The sponsor calculated that to have 90% power to detect a difference in means between Rituxan and CT-P10 that exceeds the equivalence margin, a sample size of 15 CT-P10 lots are required.

Reviewer comment: *Fifteen lots of each product were included in all tier 1 similarity studies. The CMC Stats group reviewed the adequacy of the sponsor's power calculation as well as their statistical approach for assessing tier 1 similarity. The CMC stats group concluded that the sponsor's approach to evaluating equivalence is acceptable.*

Tier 2: Quality Range Approach

The quality range (QR) is defined as the mean value for Rituxan $\pm X \times \text{SD}$ of Rituxan for each tier 2 attribute. The sponsor states that the multiplier, X, is determined for each tier 2 attribute based on the variability of each assay and the relative importance of the attribute to safety and efficacy. High similarity is demonstrated when 90% of the test product data points are within the QR of Rituxan and MabThera.

Reviewer comment: *The sponsor gives general and assay/attribute-specific justifications of multipliers. Almost all the tier 2 assays use a multiplier of 3. Only the CD20 binding CELISA and the ADCC reporter assay have a multiplier of 2; the justification for this multiplier is due to the criticality of these attributes. I reviewed the justification for each multiplier, and a detailed summary of the justifications can be found in Table 3.2.R.2-7: Summary of Final Quality Range for Each Tier 2 Analytical Method in RI 2 of the submission. As needed, I elaborated on the appropriateness of the criticality score or multiplier in select methods below. Overall, I found the justification for each multiplier acceptable with one exception: QR for afucosylated species. This is discussed in more detail below in section titled "Oligosaccharide Profiling using HPAEC-PAD".*

The sponsor provides analytical similarity data for U.S.-licensed Rituxan, E.U.-approved MabThera, and CT-P10 and the statistical summary includes a 3-way similarity assessment. Below I summarized the methods and results of the 3-way similarity assessment (see Table 3.2.R.3-1).

Tier 3: Presentation of Raw/Graphical Data

This allows comparison of quality attributes with a low criticality ranking or methods with qualitative results. Statistical analysis is not applied. *This is acceptable.*

3.2.R.1.2 Lots Used in Similarity Program (Summary of Lot Age Information)

Of the 15 lots of CT-P10 DP lots used in the similarity assessment, 3 lots were manufactured using Process C DS and 12 lots were manufactured using Process D DS. *CT-P10 lot selection included clinical lots and is considered appropriate.* All Rituxan lots were sourced from the U.S. and all MabThera lots were sourced from the E.U. *The full summary information can be found in 2.3.R.2.* Regarding the analytical similarity testing, all test items were performed in a side-by-side manner except SEC-HPLC, CE-SDS, IEF, IEC-HPLC, and oligosaccharide profiling; lot release data from these assays were used for the similarity assessment.

The lot age at the time of the similarity assessment for lots of Rituxan, CT-P10, and MabThera DP lots can be found in Table 3.2.R.1-2 of the submission. Rituxan, CT-P10, and

MabThera lot age ranged from ~7-25 months, <1-21 months, and ~8-29 months, respectively. CT-P10 DP was tested within 1 month of manufacture for each of the above-mentioned release assays. *The sponsor investigated the potential effect of lot age at testing by comparing the CT-P10 DP release and long term stability data, and available Rituxan and MabThera data from the similarity assessment for these assays. Six process 3 and process 4 CT-P10 DP lots were used and the sponsor generated scatter plots that can be found in Figures 3.2.R.1-1 through 3.2.R.1-6 of the submission.*

Reviewer comment: *The sponsor used longitudinal data (repeated testing) for 6 CT-P10 lots on stability and non-longitudinal data (a single time testing) for Rituxan and MabThera lots to draw inferences about CT-P10, Rituxan, and MabThera product quality profiles over time as measured by IEC-HPLC, SEC, and CE-SDS. Except for the levels of intact IgG, none of the attributes had a clear trend over time. As shown in Figure 3.2.R.1-5, intact IgG levels for Rituxan and MabThera but not CT-P10 seem to show a very minor decrease over a 20-month period. However, the sponsor does not use lot age to justify any aspect of the analytical similarity assessments reviewed below and so effects of Rituxan and MabThera lot age on the similarity data are not considered necessary to interpret analytical similarity data.*

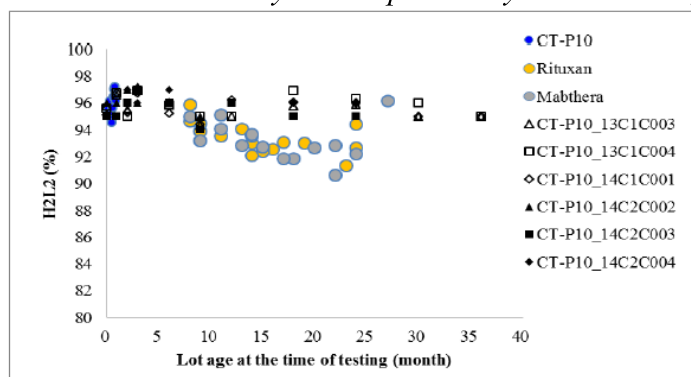


Figure 3.2.R.1-5: Scatter Plot for the Intact IgG (H2L2) in Non-reduced CE-SDS

The submission was unclear regarding the testing sites involved in similarity testing (IR comment #1 was sent on 8/7/2017). In the response (received 8/18/2017) to our IR, the sponsor clarified that the similarity and batch release/stability testing was performed in CLT^(b)₍₄₎ Celltrion plant^(b)₍₄₎ in either the R&D or QC departments, depending on the method. Only the AUC analysis was performed by an external contract research organization. This is acceptable.

3.2.R.3 The 3-way Similarity Assessment Summary

Table 3.2.R.3-1 is the executive summary table of the 3-way similarity study results using Rituxan, CT-P10, and MabThera. *The reviewer has highlighted attributes and comparisons that did not meet similarity criteria, all were in tier 2.*



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Rituximab-abbs

Table 3.2.R.3-1: Summary of 3-Way Similarity Assessment

Activity	Clinical Relevance	Level of Criticality	Tier	Assay	Measurement	Analytical Similarity Rituxan [®] vs CT-P10 DP	Analytic Bridge Rituxan [®] vs MabThera [®]	Analytic Bridge MabThera [®] vs CT- P10
						(Equivalence ¹ / % of samples within QR ² / visual similarity)		
Physicochemical Analysis								
Primary Structure	Efficacy, Safety and Immunogenicity	High	3	Peptide Mapping (HPLC)	Comparison of tryptic peptide map by visual inspection	High	High	High
			3	Amino Acid Analysis	Determination of amino acid composition	High	High	High
			2	Molar Absorptivity	Determination of molar absorptivity and extinction coefficient	Molar absorptivity: 93 ⁴ Extinction coefficient: 93 ⁴	Molar absorptivity: 93 ⁴ Extinction coefficient: 93 ⁴	Molar absorptivity: 100 ⁴ Extinction coefficient: 100 ⁴
			3	N-terminal Sequencing	Comparison of N-terminal sequences	High	High	High
			3	C-terminal Sequencing	Comparison of C-terminal sequences	High	High	High
			3	Intact Mass (LC-MS)	Molecular Weight	High	High	High
Post Translational Modification	Efficacy, PK, Safety and Immunogenicity	Moderate	2	Peptide Mapping (LC-MS)	Deamidation	% Deamidation at HC Asn55: 100 ⁴ % Deamidation at HC Asn290: 100 ⁴ % Deamidation at HC Asn319: 100 ⁴ % Deamidation at HC Asn365: 80 ⁴	% Deamidation at HC Asn55: 100 ⁴ % Deamidation at HC Asn290: 100 ⁴ % Deamidation at HC Asn319: 100 ⁴ % Deamidation at HC Asn365: 80 ⁴	% Deamidation at HC Asn55: 100 ⁴ % Deamidation at HC Asn290: 100 ⁴ % Deamidation at HC Asn319: 100 ⁴ % Deamidation at HC Asn365: 100 ⁴



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Activity	Clinical Relevance	Level of Criticality	Tier	Assay	Measurement	Analytical Similarity Rituxan [®] vs CT-P10 DP	Analytic Bridge Rituxan [®] vs MabThera [®]	Analytic Bridge MabThera [®] vs CT-P10
						(Equivalence ¹ / % of samples within QR ² / visual similarity)		
						% Deamidation at HC Asn388: 87 ⁴ % Deamidation at LC Asn136: High	% Deamidation at HC Asn388: 93 ⁴ % Deamidation at LC Asn136: High	% Deamidation at HC Asn388: 87 ⁴ % Deamidation at LC Asn136: High
		Low	3		Oxidation	High	High	High
		Very Low	3		N-terminal glutamine	High	High	High
		Very Low	3		C-terminal lysine	High	High	High
Higher Order Structure	Efficacy, Safety and Immunogenicity	Moderate	3	FTIR	Comparison of secondary structure	High	High	High
		Moderate	2	DSC	Evaluation of thermal stability and determination of thermal transition temperatures	Transition 1 (°C): 100 ⁴ Transition 2 (°C): 100 ⁴ Transition 3 (°C): 100 ⁴	Transition 1 (°C): 100 ⁴ Transition 2 (°C): 100 ⁴ Transition 3 (°C): 100 ⁴	Transition 1 (°C): 100 ⁴ Transition 2 (°C): 100 ⁴ Transition 3 (°C): 100 ⁴
		Moderate	3	CD	Comparison of secondary and tertiary structures	High	High	High
		Moderate	2	Free Thiol Analysis	Comparison of the amount of free sulfhydryl groups	100 ⁴	100 ⁴	100 ⁴
		Moderate	3	Disulfide Bonds	Comparison of disulfide bond location	High	High	High
Content	Efficacy	High	2	Protein Concentration	Protein Concentration (UV ₂₈₀)	80 ^{4,5}	100 ⁴	80 ⁴



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Activity	Clinical Relevance	Level of Criticality	Tier	Assay	Measurement	Analytical Similarity Rituxan [®] vs CT-P10 DP	Analytic Bridge Rituxan [®] vs MabThera [®]	Analytic Bridge MabThera [®] vs CT-P10
						(Equivalence ¹ / % of samples within QR ² / visual similarity)		
				(UV ₂₈₀)				
		High	3	Extractable volume	Extractable volume (mL)	High	High	High
Purity/ Impurity	Efficacy, Safety and Immunogenicity	Moderate	2	SEC-HPLC	Determination of aggregate content and monomeric purity	Monomer (%): 60⁴ HMW (%): 40⁴ LMW (%): 60⁴	Monomer (%): 100 ⁴ HMW (%): 100 ⁴ LMW (%): 100 ⁴	Monomer (%): 87⁴ HMW (%): 47⁴ LMW (%): 33⁴
		Moderate	2	SEC-MALS	Determination of aggregate/monomeric content and molecular weight	Monomer (% , UV): 100 ⁴ HMW (% , UV): 100 ⁴ Monomer (% , MALS): 100 ⁴ HMW (% , MALS): 100 ⁴ Monomer (MW, kDa): 100 ⁴	Monomer (% , UV): 100 ⁴ HMW (% , UV): 100 ⁴ Monomer (% , MALS): 100 ⁴ HMW (% , MALS): 100 ⁴ Monomer (MW, kDa): 100 ⁴	Monomer (% , UV): 100 ⁴ HMW (% , UV): 100 ⁴ Monomer (% , MALS): 100 ⁴ HMW (% , MALS): 100 ⁴ Monomer (MW, kDa): 100 ⁴
			3		Determination of molecular weight (HMW)	High	High	High
		Moderate	2	AUC	Determination of aggregate/monomeric contents	Monomer (s-value): 100 ⁴ Monomer (% Area): 100 ⁴ Dimer (s-value): 100 ⁴	Monomer (s-value): 100 ⁴ Monomer (% Area): 100 ⁴ Dimer (s-value): 100 ⁴	Monomer (s-value): 100 ⁴ Monomer (% Area): 93 ⁴ Dimer (s-value): 100 ⁴



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Activity	Clinical Relevance	Level of Criticality	Tier	Assay	Measurement	Analytical Similarity Rituxan [®] vs CT-P10 DP	Analytic Bridge Rituxan [®] vs MabThera [®]	Analytic Bridge MabThera [®] vs CT-P10	
						(Equivalence ¹ / % of samples within QR ² / visual similarity)			
						Dimer (% Area): 100 ⁴	Dimer (% Area): 100 ⁴	Dimer (% Area): 93 ⁴	
	Efficacy	Moderate	2	Non-reduced CE-SDS	Determination of electrophoretic mobility and purity under non-reducing conditions	% Peak 1+2+3+4+5: 87 ⁴ % Intact IgG: 87 ⁴	% Peak 1+2+3+4+5: 100 ⁴ % Intact IgG: 100 ⁴	% Peak 1+2+3+4+5: 100 ⁴ % Intact IgG: 100 ⁴	
		Moderate	2	Reduced CE-SDS	Determination of electrophoretic mobility and purity under reducing conditions	% Non-glycosylated HC: 7 ⁴ % H + L: 7 ⁴	% Non-glycosylated HC: 93 ⁴ % H + L: 93 ⁴	% Non-glycosylated HC: 87 ⁴ % H + L: 87 ⁴	
	Safety and Immunogenicity	Moderate	3	Residual Host Cell Protein	Determination of the level of residual host cell protein	High	High	High	
		Moderate	3	Residual Host Cell DNA	Determination of the level of residual host cell DNA	High	High	High	
		Moderate	3	Residual rProtein A	Determination of the level of residual rProtein A	High	High	High	
		Moderate	3	MFI	Comparison of the numbers of sub-visible particles	High	High	High	
		Moderate	3	HIAC		High	High	High	
	Charge Variants	Efficacy	Very Low	3	IEF	Comparison of isoelectric point(s)	High	High	High
			Moderate	2	IEC-HPLC	Comparison of charge variant distribution (Peak	7 ⁴	100 ⁴	20 ⁴



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Activity	Clinical Relevance	Level of Criticality	Tier	Assay	Measurement	Analytical Similarity Rituxan [®] vs CT-P10 DP	Analytic Bridge Rituxan [®] vs MabThera [®]	Analytic Bridge MabThera [®] vs CT-P10
						(Equivalence ¹ / % of samples within QR ² / visual similarity)		
						1+2+3)		
		Very Low	3		Comparison of charge variant distribution (Peak 4, 5, 6, 7)	High	High	High
Glycosylation	Efficacy	Moderate	2	Oligo-saccharide Profiling	Comparison of glycosylation patterns (ex. G0, Man5, G0F, G1F and G2F)	% G0F: 100 ⁴ % Man5: 0 ⁴ % G0: 100 ⁴ % G0+Man5: 53 ⁴ % G1F: 100 ⁴ % G2F: 100 ⁴	% G0F: 100 ⁴ % Man5: 100 ⁴ % G0: 100 ⁴ % G0+Man5: 100 ⁴ % G1F: 100 ⁴ % G2F: 100 ⁴	% G0F: 100 ⁴ % Man5: 0 ⁴ % G0: 100 ⁴ % G0+Man5: 33 ⁴ % G1F: 100 ⁴ % G2F: 100 ⁴
		Moderate	2	N-linked Glycan Analysis	Comparison of oligosaccharide structures, attachment sites and distribution (%G0F, %G1F, %G2F, %G0, %Man5)	% G0F: 100 ⁴ % G1F: 100 ⁴ % G2F: 100 ⁴ % Man5: 0 ⁴ %G0: 100 ⁴ %G0+Man5: 0 ⁴	% G0F: 100 ⁴ % G1F: 100 ⁴ % G2F: 100 ⁴ % Man5: 100 ⁴ %G0: 100 ⁴ %G0+Man5: 100 ⁴	% G0F: 100 ⁴ % G1F: 100 ⁴ % G2F: 100 ⁴ % Man5: 0 ⁴ %G0: 100 ⁴ %G0+Man5: 7 ⁴
		Low	3		Comparison of oligosaccharide structures, attachment sites and distribution (%G0F+NANA, %G1F+NANA, %G2F+NANA)	% G1F+NANA: High %G2F+NANA: High %G2F+2NANA: High	% G1F+NANA: High %G2F+NANA: High %G2F+2NANA: High	% G1F+NANA: High %G2F+NANA: High %G2F+2NANA: High



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Rituximab-abbs

Activity	Clinical Relevance	Level of Criticality	Tier	Assay	Measurement	Analytical Similarity Rituxan [®] vs CT-P10 DP	Analytic Bridge Rituxan [®] vs MabThera [®]	Analytic Bridge MabThera [®] vs CT-P10
		(Equivalence ¹ / % of samples within QR ² / visual similarity)						
		Low	3	Sialic Acid Analysis	Determination of sialic acid content	High	High	High
		Low	3	Mono-saccharide Analysis	Comparison of neutral and amino sugar composition	High	High	High
Biological Analysis								
Fab Binding	MoA and Efficacy	Very High	2	Cell-based CD20 Binding (CELISA)	% Relative binding by (% , EC ₅₀)	100 ³	100 ³	100 ³
		Moderate	2	Apoptosis using Raji cell (FACS)	% Relative apoptotic cells (% , apoptotic cells) At 0.13 µg/mL At 0.04 µg/mL At 0.01 µg/mL	100 ⁴ 100 ⁴ 100 ⁴	100 ⁴ 100 ⁴ 100 ⁴	100 ⁴ 100 ⁴ 100 ⁴
Fab-Fc Mediated Activities	MoA and Efficacy	Very High	1	CDC using WIL2-S cell	% Relative CDC potency (% , EC ₅₀)	Within EM	Within EM	Within EM
		Very High	1	ADCC using PBMC	% Relative ADCC potency (% , cytotoxicity) At 0.122 µg/mL At 0.035 µg/mL At 0.010 µg/mL	Within EM Within EM Within EM	Within EM Within EM Within EM	Within EM Within EM Within EM
			2	ADCC Reporter Assay	% Relative reporter activity (% , EC ₅₀)	100 ³	100 ³	100 ³



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Rituximab-abbs

Activity	Clinical Relevance	Level of Criticality	Tier	Assay	Measurement	Analytical Similarity Rituxan [®] vs CT-P10 DP	Analytic Bridge Rituxan [®] vs MabThera [®]	Analytic Bridge MabThera [®] vs CT-P10
						(Equivalence ¹ / % of samples within QR ² / visual similarity)		
	Putative MoA and Efficacy	High	2	ADCP using Raji cell	% Relative ADCP activity (% phagocytosis) At 25.0 ng/mL At 6.25 ng/mL At 1.56 ng/mL	100 ⁴ 100 ⁴ 100 ⁴	100 ⁴ 100 ⁴ 100 ⁴	100 ⁴ 100 ⁴ 100 ⁴
Fc Binding	MoA and Efficacy	High	2	C1q Binding (ELISA)	% Relative binding affinity (% EC ₅₀)	100 ⁴	100 ⁴	100 ⁴
	MoA and Efficacy	High	2	FcγRIIIa-V Binding Affinity (SPR)	% Relative binding affinity (% K _D)	100 ⁴	100 ⁴	100 ⁴
			2	FcγRIIIa-F Binding Affinity (SPR)	% Relative binding affinity (% K _D)	100 ⁴	100 ⁴	100 ⁴
	Immune System Mediator	Moderate	2	FcγRIIb Binding Affinity (SPR)	% Relative binding affinity (% K _D)	100 ⁴	100 ⁴	100 ⁴
	Putative MoA and Efficacy	Moderate	2	FcγRIIa Binding Affinity (SPR)	% Relative binding affinity (% K _D)	100 ⁴	100 ⁴	100 ⁴
	Immune System Mediator	Moderate	2	FcγRIIb Binding Affinity (SPR)	% Relative binding affinity (% K _D)	100 ⁴	100 ⁴	100 ⁴
	Immune System	Low	3	FcγRI Binding Affinity (SPR)	% Relative binding affinity (% K _D)	High	High	High



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Rituximab-abbs

Activity	Clinical Relevance	Level of Criticality	Tier	Assay	Measurement	Analytical Similarity Rituxan [®] vs CT-P10 DP	Analytic Bridge Rituxan [®] vs MabThera [®]	Analytic Bridge MabThera [®] vs CT-P10
						(Equivalence ¹ / % of samples within QR ² / visual similarity)		
	Mediator							
	PK	Moderate	2	FcRn Binding Affinity (SPR)	% Relative binding affinity (% , K _D)	100 ⁴	100 ⁴	100 ⁴

¹ EM was determined as 1.5 σ_R of Rituxan data and results were determined as 90 % CI of mean difference between two products.

² The QR limits were set based on the range of the values obtained for reference product variation, expressed as X times SD. High similarity was considered to have been demonstrated if 90 % of data points were within the QR.

³ X=2.

⁴ X=3.

⁵ 92% of commercial CT-P10 product lots from Process 4 were within the quality range of Rituxan.

The following is a reviewer-generated table summarizing the details surrounding the highlighted “activities” or attributes that initially failed Tier2 similarity assessment and the strategy used to address residual uncertainty.



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Rituximab-abbs

Assay	Measurement	Mean±SD			% of samples within QR			Whether and How Residual Uncertainty is Addressed
		Rituxan	CT-P10	MabThera	Rituxan vs CT-P10	Rituxan vs MabThera	MabThera vs CT-P10	
Peptide Mapping (LC-MS)	Deamidation: HC Asn365 HC Asn388	0.1 ± 0.03 2.0 ± 0.15	0.1 ± 0.04 1.8 ± 0.19	0.1 ± 0.04 2.1 ± 0.18	80 87	80 93	100 87	Yes. Peak characterization study, forced degradation study, potency assays, and risk assessment based on location of post-translational modification (PTM, not involved with epitope or Fc receptor binding) addressed uncertainty. The actual difference is very small.
Protein Conc-entration	UV280	10.2 ± 0.17	10.1 ± 0.28 10.1 ± 0.27 (P4 lots)	10.4 ± 0.19	80 92 (P4 lots)	100 NA	80 92 (P4 lots)	Yes. CT-P10 DP Process 4 included a process change to better target the correct protein concentration. Total number of Process 4 lots = 12. Additionally, the protein concentration is the same between the 100 mg and 500 mg presentations, and so similarity data supporting protein content from the 500 mg CT-P10, Rituxan, and MabThera lots alone is sufficient.
SEC-HPLC	size purity: monomer HMW LMW	98.81 ± 0.166 1.07 ± 0.142 0.12 ± 0.029	99.30 ± 0.102 0.53 ± 0.190 0.15 ± 0.154	99.01 ± 0.146 0.89 ± 0.129 0.10 ± 0.022	60 40 60	100 100 100	87 47 33	Yes. Lower aggregate levels in CT-P10 is a low risk difference with no impact on biological activity assays. SEC-MALS and AUC data also provided.
non-reduced CE-SDS	purity: sum Peaks 1-5 intact IgG	6.60 ± 1.180 93.40 ± 1.178	4.23 ± 0.771 95.78 ± 0.769	6.79 ± 1.439 93.21 ± 1.441	87 87	100 100	100 100	Yes. Lower levels of fragmented species in CT-P10 is a low risk difference with no impact on biological activity assays.
reduced CE-SDS	purity: NGHC H+L	0.43 ± 0.079 99.57 ± 0.079	0.80 ± 0.114 99.20 ± 0.114	0.47 ± 0.162 99.53 ± 0.164	7 7	93 93	87 87	Yes. Biological activity assays showed no differences.



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Rituximab-abbs

IEC-HPLC	charge purity: peak 1 peak 2 peak 3 sum Peaks 1-3	2.9 ± 0.30 7.7 ± 0.45 10.3 ± 0.55 20.93 ± 1.02	2.3 ± 0.51 6.2 ± 0.26 8.1 ± 0.12 16.65 ± 0.71	3.0 ± 0.30 7.5 ± 0.48 10.2 ± 0.55 20.76 ± 1.23	sum P1-3: 7	sum P1-3: 100	sum P1-3: 20	Yes. Peak characterization study did not identify any one or few species driving differences in sums of acidic or basic peaks. However, because the peaks have comparable functional activity to each other, it does not appear that differences in acidic or basic peak sums impacts biological activity.
Oligo-saccharide profiling	afucosylated: Man5 G0 G0+Man5	2.14 ± 0.278 1.45 ± 0.324 3.59 ± 0.347	3.65 ± 0.174 1.01 ± 0.194 4.66 ± 0.257	2.08 ± 0.381 1.40 ± 0.299 3.48 ± 0.332	0 100 53	100 100 100	0 100 33	Yes. The difference is due to M5 levels. Comparative PK results addressed residual uncertainty. Other MOA concerns are addressed by glycosylation and biological activity studies.
N-linked glycan analysis	afucosylated: Man5 G0 G0+Man5	1.8 ± 0.26 0.8 ± 0.23 2.6 ± 0.22	3.6 ± 0.27 0.4 ± 0.08 4.0 ± 0.32	1.8 ± 0.27 0.8 ± 0.22 2.6 ± 0.29	0 100 0	100 100 100	0 100 7	Specifically, additional characterization studies showed that although afucosylation (G0, G1, etc.) levels should be controlled, small differences in total afucosylated species (G0+Man5) level (like 2.6% versus 4.0%) do not impact ADCC activity (as measured using a reporter- or PBMC-based method) or other functional assays (FcγRIIIa, C1q, FcRn, CD20 binding).

Reviewer comment: The results of each of these assessments is discussed in detail in the relevant sections of the review below.

3.2.R.6 Analytical Method Qualification

Several methods used in the similarity assessment are also used for DS/DP release/stability, including UV280, extractable volume (USP<1>), SEC-HPLC, CE-SDS (reduced and non-reduced), IEC-HPLC, oligosaccharide profiling, CD20 CELISA, peptide mapping (HPLC), host cell protein ELISA, host cell DNA PCR, residual protein A ELISA, IEF, and HIAC (USP<788> and Ph. Eur. 2.9.40), and CDC. As an additional control for ADCC activity for release and stability, FcγRIIIa binding was added to the control strategy during the BLA review. The validations/qualifications of these most methods are reviewed by Dr. Haoheng Yan, while CDC and FcγRIIIa binding were reviewed by Dr. Nina Brahme (see reviews in section 3.2.S.4.3).

Reviewer comment: The physicochemical assays used in analytical similarity testing (and in CT-P10 characterization as well) are qualified for their intended use in analytical similarity testing. The qualification data is summarized in Table 3.2.R.6.1 of the submission and the extent of method qualification is summarized in the reviewer-generated table below.

Method	Test Attribute	Extent of Qualification
amino acid analysis	primary aa sequence	robustness of aa
N-terminal sequencing	primary aa sequence	repeatability, intermediate precision (inter-day), accuracy
C-terminal sequencing	primary aa sequence	
intact mass (LC-MS)	primary aa sequence	specificity, accuracy, repeatability, intermediate precision (inter-day)
FTIR	secondary structure	repeatability, intermediate precision (inter-day) concentration-dependent measurement
CD	secondary and tertiary structure	range, intermediate precision
native and reduced peptide mapping	disulfide bonds	repeatability, intermediate precision (inter-day) accuracy
MFI	particulates	Prior to use, the MFI equipment was calibrated using 2 µm, 5 µm, 10 µm COUNT-CALTM Particle Size standards (Thermo scientific). The particle standards of each size were tested in triplicate and the average was evaluated for system suitability (± 10% of the stated particle number of 3,000/mL for each particle size).
monosaccharide analysis	monosaccharide	specificity, range, linearity, accuracy, repeatability, intermediate precision (inter-day)
sialic acid analysis	sialic acids	specificity, linearity, accuracy, range, precision for standard, repeatability, intermediate precision (inter-day, inter-analyst)

Reviewer comment: These assays are sufficiently qualified for their intended use in analytical similarity testing.

The following reviewer-generated table summarizes the extent of validation or qualification data available for the remaining biological assays used in analytical similarity testing (and characterization). The qualification data is summarized in Table 3.2.R.6.2 in the submission. The



CDC assay was previously discussed and the ADCC assay is discussed in more detail in the Tier 1 similarity data section below.

Method	Test Attribute	Extent of Validation/Qualification
FcγR binding by SPR	FcγRIIIa (V-type) binding	[§] Validated for accuracy, specificity, precision (repeatability, intermediate precision), goodness of fitness, range, robustness (target ligand level variation, sample stability) using DP samples.
FcγR and FcRn binding by SPR	FcγRIIIa (F-type) binding	Qualified for specificity, accuracy, precision
	FcγRIIIb binding	
	FcγRIIIa Binding	
	FcγRIIIb Binding	
	FcRn Binding	
	FcγRI Binding	
CD20 Binding (CELISA)	CD20 binding	[#] Validated for accuracy, precision (repeatability and intermediate precision), linearity, range, specificity, robustness (cell passage number, cell plate incubation, fixation time, blocking time, sample/secondary incubation time), and interlaboratory comparability. Validation studies include samples from DS, DP, DP diluted for infusion, and stressed sample.
C1q Binding (ELISA)	C1q binding	Qualified for precision, accuracy, linearity, specificity
apoptosis using Raji cell by FACS	apoptosis	Qualified for precision, accuracy, linearity, range, specificity
ADCC reporter assay	ADCC	
ADCP using Raji cell	ADCP	
PBMC ADCC using Raji cell	ADCC	
Apoptosis using MEC-2B cells as target cells	Apoptosis (extrapolation)	
CDC using B cells* as target cells	CDC (extrapolation)	
ADCC using B cells* as target cells	ADCC (extrapolation)	
ADCP using B cells* as target cells	ADCP (extrapolation)	

[§] Validation report is submitted in 3.2.R.1-27.

[#] Validation report submitted in 3.2.R.1-24 through 3.2.R.1-26.

*Used B cells purified from PBMCs of healthy, NHL, CLL patients as target cells

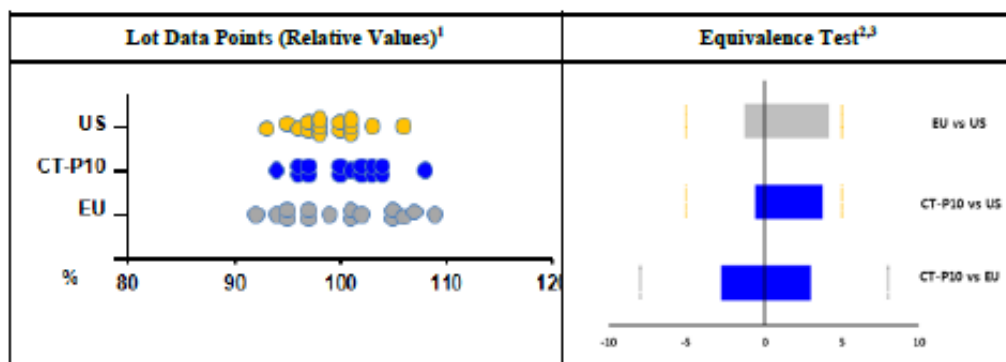
Reviewer comment: *These biological assays are sufficiently qualified for their intended use in analytical similarity testing. The ADCC (PBMC) assay is discussed in the next section.*

Tier 1 CDC and ADCC Assays and Similarity Results

This section combines information found in Regional Information (RI) 2: Description of tiered approach, RI 3: Executive summary of 3-way similarity study, RI 5: Method description, and RI 6: Method qualification. The sponsor uses CDC and ADCC potency assays in tier 1, 3-way similarity assessment between Rituxan, CT-P10, and MabThera.

CDC

The CDC assay is a validated potency assay for DS and DP release and stability testing. Assay descriptions and validation details can be found in sections 3.2.S.4.2 and 3.2.S.4.3 of the review. The summary for the data and testing is below, and the CMC Stats group assessed the statistical approach used for this assay as acceptable.



¹ Relative potency was determined against CT-P10 in-house reference standard.

² EM for Equivalence Acceptance Criterion was determined as $1.5\sigma_R$ of Rituxan® or MabThera® data, accounting for lot-to-lot variability. The dotted yellow line and grey lines represent the EM of Rituxan® and MabThera®, respectively.

³ Results are presented as 90 % CI of mean difference between 2 products.

Figure 3.2.R.3-40: Scatter Plot and Equivalence Test Results for CDC

Table 3.2.R.3-50: CDC Results (partial copy)

Statistical Analysis Results using Tier 1 approach		
		Relative Potency (%, EC50)
EM ($\pm 1.5 \sigma$) from Rituxan®		(-4.9, 4.9)
EM ($\pm 1.5 \sigma$) from MabThera®		(-7.9, 7.9)
90 % CI of mean difference	Rituxan® vs CT-P10 DP	(-0.68, 3.75)
	Rituxan® vs MabThera®	(-1.33, 4.13)
	MabThera® vs CT-P10 DP	(-2.73, 3.00)

Reviewer comment: The CDC potency as measured by this cell-based assay is similar for all three products. The similarity data show that the CT-P10 lots fall well within the equivalence margins derived from Rituxan and MabThera testing, respectively. CT-P10 meets tier 1 similarity criteria for CDC potency.

ADCC (PBMC)

The ADCC assay was carried out using the Raji cell line (CD20 positive B lymphoblast cells) as a target cell and human PBMCs as effector cells. Prior to incubation with antibodies, the target Raji cells were labelled with Calcein-AM using assay medium for 30 minutes at 37°C. The target cells were then incubated with therapeutic antibodies for 30 minutes at 3 concentrations (10, 35, and 122.4 ng/mL). Then PBMCs derived from a single healthy volunteer (Cellular Technology Ltd, Lot#HHU20120627) were incubated at E:T ratio of 16:1 with antibody-bound target cells in 37°C CO₂ incubator for 4 hours. The cell cytotoxicity was measured by calcein release, determined by fluorescence at 488 nm / 521 nm / 515 nm according to the following formula:

$$\text{Cell Cytotoxicity (\%)} = \left(\frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Maximal release} - \text{Spontaneous release}} \right) * 100$$

The relative ADCC activity was determined compared to CT-P10 in-house RS. A schematic of the method is in Figure 3.2.R.5-2.

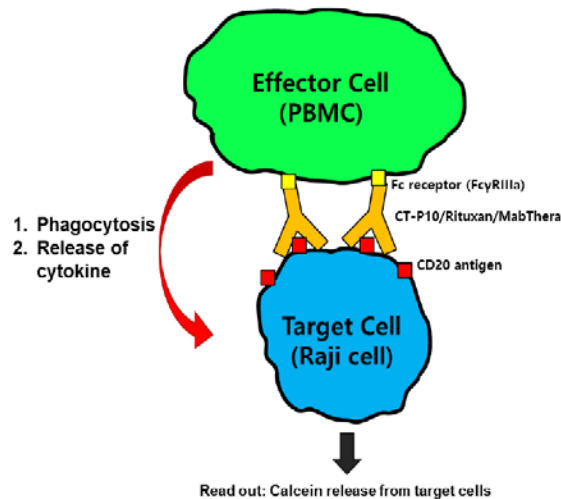


Figure 3.2.R.5-2: Illustration of CT-P10/Rituxan[®]/MabThera[®] Mediated ADCC using PBMC against Raji Cells

Reviewer comment: Because the ADCC potency assay assesses a potential MoA of rituximab (described in the Rituxan label) and is used as a tier 1 assay, we requested the assay qualification report (IR comment #6 of 8/7/2017). Attachment 1 of the response submission (8/18/2017) is the assay qualification report that supports the summarized assay performance qualification results described in 3.2.R.6.2 (see above). Attachment 2 of the response describes additional qualification for sensitivity of the assay to measure changes in afucosylated species (discussed in more detail in RI4: Glycosylation and Associated Biological Activities section below).

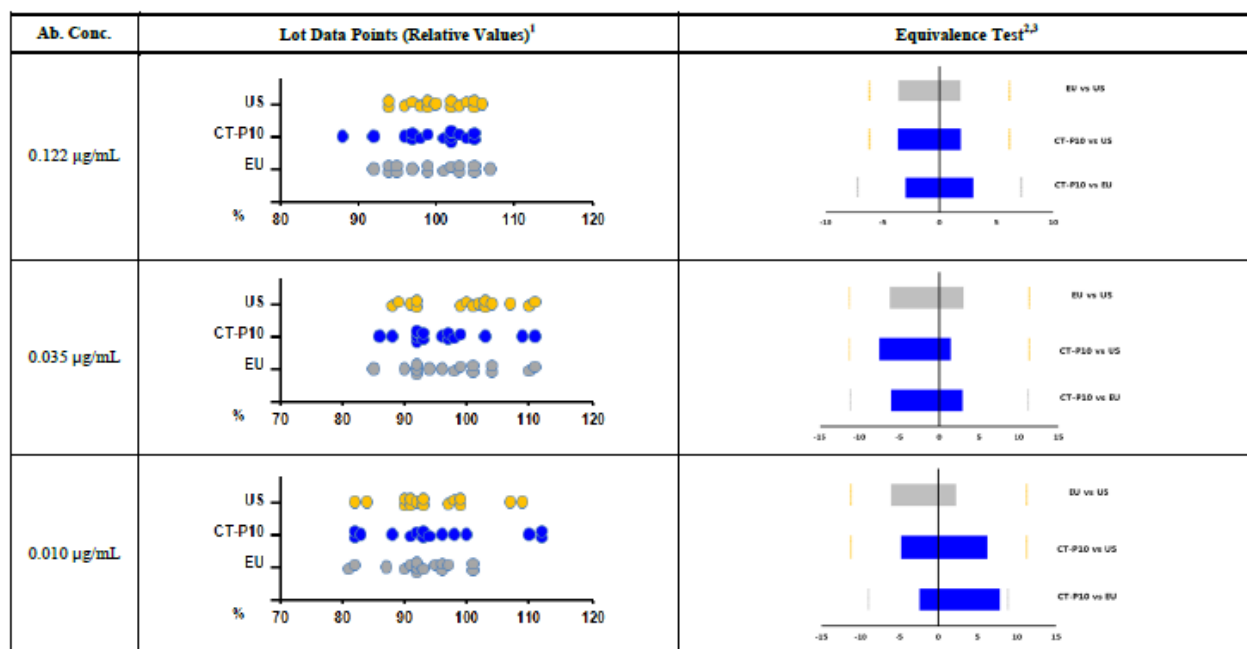
The details of these reports for the ADCC (PBMC) assay are summarized in the reviewer-generated table below.

	Qualification Detail	Reviewer's Comment
Notes	1. The sponsor screened donors for best PBMC effector cell performance in assay (largest signal window)	This strategy for identifying concentrations at which to

	2. Multiple dose ranges of antibody concentration were assessed to evaluate the linear range. 4-5 (of 8 total points on the dose response curve (DRC)) were within the linear range. 3. Three concentrations (within the linear range) were selected for ADCC activity evaluation. Assay qualification was performed at these three concentrations. 4. Additional method optimization was performed to identify: target (Raji cell) concentration, Calcein-AM concentration, antibody concentration, and effector:target cell ratio. 5. Qualification results are given for each concentration and/or [the mean of the three concentrations]	<i>test relative ADCC activity is acceptable.</i>
Samples	The in-house reference standards (b) (4) and (b) (4) were used as test samples for qualification studies, DS and DP samples were also tested. Negative controls: CT-P10 Fab fragment, Remicade, Herceptin, Human IgG1 (isotype control)	<i>These control and test samples are acceptable.</i>
Precision (repeatability)	Intra-run variance: 205.7, 56.1, 57.8 Repeatability (%CV): 14.1, 7.2, 7.4 [8.2]	<i>Evaluated by 6 independent runs, by 2 scientists, at 3 independent times for each of the three concentrations.</i>
Intermediate (inter-day) precision	Inter-run variance: 290.0, 74.1, 17.3 %CV: 21.9, 10.9, 8.5 [12.3]	
Accuracy (bias)	%bias: 1.9, 4.3, 2.2 [2.8]	<i>The sponsor has not defined the full linear range, however they have provided a range in which the assay has a linear readout.</i>
Linearity	Optimized results show that three concentrations (10.0, 35.0, and 122.4 ng/mL) present in the linear part of the DRC can be used to measure relative ADCC activity. Mean cytotoxicity% at each concentration and each R² from standard and samples (three replicates) by two analysts on three different days. (mean 0.9635, SD 0.03, %CV 2.93)	
Range	Defined as the three points along the linear part of DRC (10-122.4 ng/mL)	
Specificity	Confirmed using the negative controls and test sample of (b) (4)	<i>This control testing confirms the specificity of the assay.</i>
Sensitivity	Evaluated using CT-P10 samples with various levels of afucosylated glycans and (b) (4) showing correlation between afucosylated glycans and ADCC activity.	<i>The DRCs for (b) (4) Afucose_SP1 through Afucose_SP4 (all with ≤6.38% afucosylated species) are indistinguishable. DRCs for Afucose_SP5 through Afucose_SP7 (12.67-34.88% afucosylated species) showed clear shifts in EC50.</i> <i>This is an acceptable demonstration of assay sensitivity.</i>

System Suitability	Condition for maximal release (MR, target cells + Triton X-100) and spontaneous release (SR, target cells + effector cells w/o antibody) on same plate. Ratio of MR/SR value and cytotoxicity ratio of high/low dose considered for assay run pass/fail. Historical results and established ranges are included in Attachment 1.	<i>These system suitability criteria are acceptable.</i>
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Overall the qualification of the ADCC (PBMC) assay is acceptable for use in similarity assessment. The summary for the data and testing is below, and the CMC Stats group assessed the statistical approach used for this assay as acceptable.



¹ Relative potency was determined against CT-P10 in-house reference standard.

² EM for Equivalence Acceptance Criterion was determined as $1.5\sigma_R$ of Rituxan® or MabThera® data accounting for lot-to-lot variability. The dotted yellow lines and grey lines represent the EM of Rituxan® and MabThera®, respectively.

³ Results are presented as 90 % CI of mean difference between 2 products.

Figure 3.2.R.3-41: Scatter Plot and Equivalence Test Results for ADCC

Table 3.2.R.3-51: ADCC Results (*partial copy*)

Statistical Analysis Results using Tier 1 approach				
		Relative Potency (% , cytotoxicity)		
		0.010 µg/mL	0.035 µg/mL	0.122 µg/mL
EM (± 1.5 σ) from Rituxan®		(-11.1, 11.1)	(-11.2, 11.2)	(-6.0, 6.0)
EM (± 1.5 σ) from MabThera®		(-8.8, 8.8)	(-11.1, 11.1)	(-7.1, 7.1)
90 % CI of mean difference	Rituxan® vs CT-P10 DP	(-4.74, 6.20)	(-7.56, 1.43)	(-3.62, 1.89)
	Rituxan® vs MabThera®	(-6.08, 2.21)	(-6.15, 3.08)	(-3.58, 1.85)
	MabThera® vs CT-P10 DP	(-2.42, 7.76)	(-6.00, 2.94)	(-2.98, 2.98)



Reviewer comment: The ADCC activities of Rituxan, CT-P10, and MabThera as measured by this cell-based assay are similar with substantial overlap even where means and standard deviations are not near identical. CT-P10 meets tier 1 similarity criteria for ADCC (using PBMCs) activity.

Tier 2 Similarity Results: Physicochemical Characteristics

This section combines information found in RI 2, RI 3, RI 5, and RI 6. The reviewer has grouped information by analytical method. A summary of the method qualification results can be found in RI 6 in Table 3.2.R.6-1 of the submission.

Molar Absorptivity/Extinction Coefficient

The Beer-Lambert equation was applied using the optical density (OD) and protein molarity derived from the amino acid analysis using the robust amino acids. Molar extinction coefficient was calculated using UV absorbance at 280 nm, concentration of protein and molecular weight (144,252 Da) of Rituxan, CT-P10 DP and MabThera.

Reviewer comment: The molar absorptivity and extinction coefficients were calculated for each of the 15 lots of Rituxan and CT-P10 and both drugs have means that are within 1% of each other. These tests/measures were used to characterize primary structure and primary amino acid sequence.

Peptide Mapping

This test was done to assess the deamidation profile at known HC asparagine sites (HC Asn's 55, 290, 319, 365, and 388). Note that the LC Asn deamidation site (Asn136) was considered Tier 3 since the deamidated Asn136 levels for all samples were found to be the same and are therefore not amenable to statistical analysis. The sponsor states that the MS/MS data confirmed that the amino acid sequences of Rituxan, CT-P10 DP, and MabThera matched and the sequence coverage by MS/MS was also 100% (the data was not provided, the data will be requested to verify the coverage as a non-CR comment in action letter).

Reviewer comment: The 3-way similarity results are summarized in the reviewer-generated table below (means are deamidation %). Although CT-P10 DP does not pass tier 2 criteria for HC Asn365 and Asn388, the overall levels of deamidation appear low and the difference between the deamidation profiles for CT-P10 and Rituxan appear minor.

HC Asn Residue	μ-Rituxan (%)	μ-CTP10 (%)	%CTP10 lots within Rituxan QR	%CTP10 lots within MabThera QR	%MabThera lots within Rituxan QR
55	1.9	1.9	100	100	100
290	0.2	0.2	100	100	100
319	0.5	0.5	100	100	100
365	0.1	0.1	80	100	80
388	2	1.8	87	87	93

To inform our risk assessment regarding importance of different PTMs, we sent an IR (on 8/7/2017, comment #7) requesting information about the locations of these PTMs and their proximity to epitope- or Fc-binding sites. The sponsor responded (on 8/18/2017) with residues mapped onto crystallographic structures of antibody domains alone or in complex with CD20 peptide or with Fc receptor domains. The sponsor's response and my comments are summarized in the following table.

Table 1: List of PTM in CT-P10 Detected by LC-MS Peptide Mapping

Type of Modification	Location & assessment
Deamidation	HC Asn55 Looks close to CD20 ligand, but unclear if directly involved in binding.
	HC Asn290 Looks close to FcRn, but perhaps unlikely to be directly involved in binding (side chain not outward-facing).
	HC Asn319 Looks close to FcRn, but not likely to be directly involved in binding (inside helix).
	HC Asn365 Not involved in any binding
	HC Asn388 Not involved in any binding
	LC Asn136 Not involved in any binding
Oxidation	HC Met34 Close to CD20, but probably not involved in binding
	HC Met81 Not involved in any binding
	HC Met256 Not involved in any binding
	HC Met432 Not involved in any binding
	LC Met21 Not involved in any binding
N-terminal glutamine variant	HC Gln01 Not involved in any binding
	LC Gln01 Not involved in any binding
C-terminal lysine variant	HC Lys451 Not involved in any binding

Reviewer comment: Judging from the structural images of Rituximab fragments with CD20 peptides, human IgG Fc with human FcRn, and human Fc with various human FcγRs, I conclude the following: None of the listed PTMs, except HC Asn55, have high chance of affecting biological MoA. From characterization perspective, it remains unclear whether HC Asn55 plays any role in CD20 binding.

Free Thiol Analysis

Ellman's assay was used to determine the free thiols in the three products. Results were reported as molar ratios (Free SH/IgG, $\mu\text{M}/\mu\text{M}$).

Reviewer comment: The average free SH/IgG for each of the three DPs was very similar and 100% of the 15 CT-P10 lots are within the Rituxan QR as well as the MabThera QR.

Differential Scanning Calorimetry (DSC)

This method was used to evaluate thermal stability of higher order structures. Three transitions corresponding to the unfolding of the CH2 domain, Fab domain, and CH3 domains can be seen in Table 3.2.R.3-14 of the submission.

Reviewer comment: The average transition temperatures for each of the three DPs were identical and 100% of the 15 CT-P10 lots are within the Rituxan QR as well as the MabThera QR.

Protein Concentration

Protein concentration read at UV280 was used to assess content. The theoretical extinction coefficient of CT-P10 of 1.60 (mg/mL) cm was used. This method is used in DS and DP batch release and stability testing (see section 3.2.S.4.2).

(b) (4)

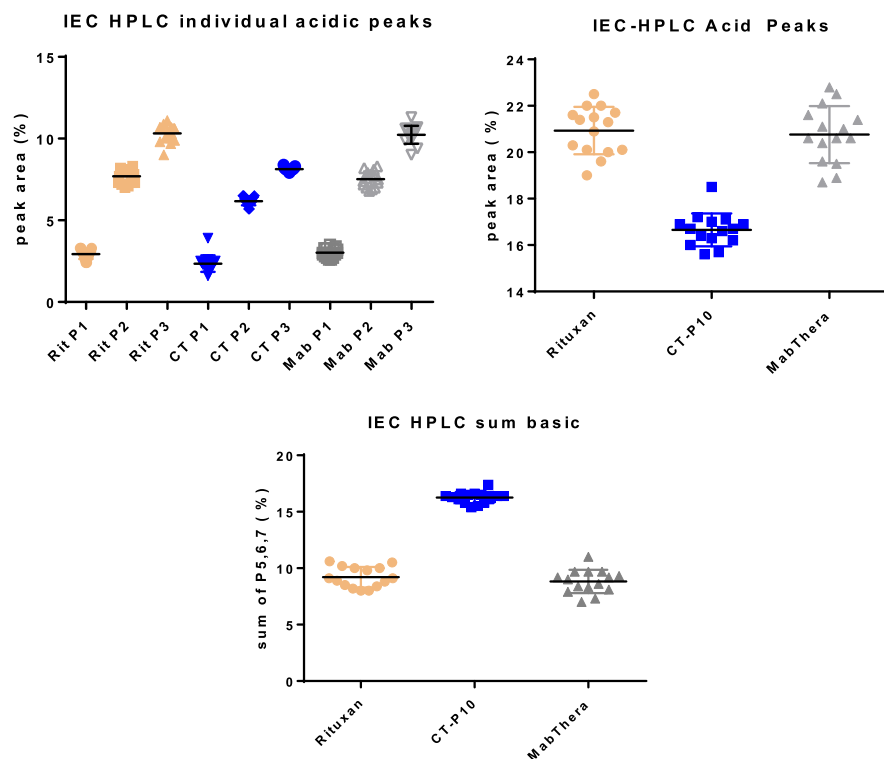
Reviewer comment: *The mean protein concentrations for each DP are very similar and are controlled during routine manufacture. 12 (80%) of 15 tested CT-P10 DP lots are within the Rituxan QR. However, if only the 12 Process 4 DP lots are considered, the % within Rituxan QR is 92% (11 lots). The same is true when calculating the %CT-P10 lots within the MabThera QR. Overall, I conclude that CT-P10 with additional manufacturing adjustment procedure for commercialization passes this tier 2 assessment for protein concentration.*

IEC-HPLC

Cation exchange chromatography was used to examine the distribution of charge variants. Seven peaks were separated and detected for integration; results are expressed as % peak area values. Sponsor's focus for tier 2 assessment is on acidic peaks (peaks 1-3) because the deamidated species (part of peaks 1-3) have "moderate" criticality. Other variants (main peaks 4 and basic peaks 5-7) have "very low" criticality score (species enriched in the basic peaks are N- and C-terminal variants) and so tier 3 similarity criteria are applied.

Reviewer comment: *The 3-way similarity assessment is summarized in the reviewer-generated table and plots below. The statistical assessment for acidic peaks failed tier 2 similarity criterion. In my examination of the data, I found that largely lower levels of peaks 2 and 3 are driving the difference in acidic peak profile between CT-P10 and Rituxan. Moreover, the sum of basic peaks was not similar between CT-P10 and Rituxan or MabThera (fails tier 3 similarity, see below).*

Species (% peak area)	μ-Rituxan (%)	μ-CTP10 (%)	%CTP10 lots within Rituxan QR	%CTP10 lots within MabThera QR	%MabThera lots within Rituxan QR
peak 1 (acidic)	2.9	2.3	7	20	100
peak 2 (acidic)	7.7	6.2			
peak 3 (acidic)	10.3	8.1			
peak 4	69.9	67.1	Fail Tier 3	Fail Tier 3	Fail Tier 3
peak 5 (basic)	5.6	9.6			
peak 6 (basic)	3.3	5.6			
peak 7 (basic)	0.3	1.1			
Sum P1+2+3	20.76	16.65			



3.2.R.3.2.5.3 IEC-HPLC Peak Characterization Study:

Tryptic peptide mapping by LC/MS was performed on the 8 peaks (*peaks 1-7, including 5a and 5b*) for the DPs to examine peak composition. Fractionated peaks were also tested in biological assays to examine relative functional activity.

Reviewer comment: *The peak fractionation study was done on one lot of each product and each of those lots appears to be representative with respect to charge distribution. The study showed:*

- *IEC-HPLC peak profiles (chromatograms) appear similar between the three products, appearing to vary only in peak height (see Figure 3.2.R.3-33 in the submission).*
- *SEC-HPLC analysis on each peak show that these are mostly monomeric species (see Table 3.2.R.3-32).*
- *IEF analysis showed that the pI values of each band in each peak are fairly similar (see Table 3.2.R.3-33 in the submission).*
- *Peptide mapping results showed that the molecular variants in each peak are conserved across the three DPs. The peptide mapping data is summarized in Figure 3.2.R.3-35.*

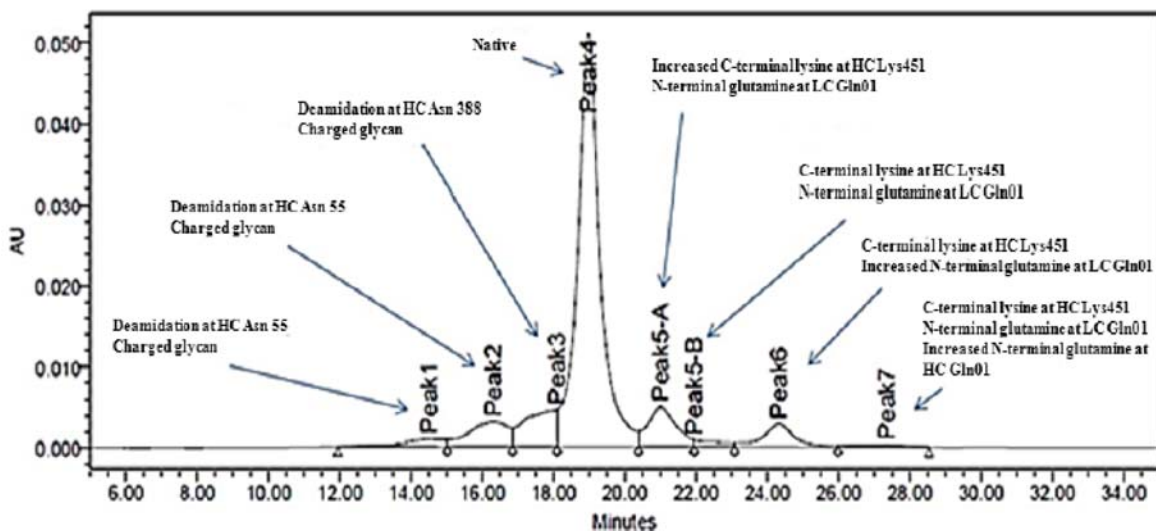


Figure 3.2.R.3-35: Peak Assignments for CT-P10 Drug Product (Lot No. 15C2C04)

Reviewer comment: I compared the %-change in relative abundance of each species in the unfractionated sample across the three drug products and identified those species with the largest %-change. Among those, I focused on species that were enriched in either acidic or basic peaks. Finally, I noted which were evaluated by tier 2 or tier 3 similarity criteria and the results of that assessment. In conclusion, this characterization data suggests:

- Charged glycans are found in acidic peaks and in lower levels in CT-P10 compared to levels in Rituxan/MabThera
- N- and C-terminal variants (HC/LC Gln01 and HC Lys450, respectively) are found in basic peaks and are the same or higher in CT-10 than in Rituxan/MabThera.

Assessment of relative biological activity of the peak fractions compared to intact sample in these 3 products with respect to FcRn binding affinity (by SPR), FcγRIIIa binding affinity (by SPR), C1q binding affinity (ELISA), cell-based CD20 binding affinity (CELISA), CDC activity, ADCC activity and apoptotic activity showed no significant difference considering assay variances (Tables 3.2.R.3-37 - 3.2.R.3-43 in the submission). In an IR (sent 8/7/2017, comment #4) we requested that the potency data be re-analyzed relative to a single standard across the three products to facilitate pair-wise comparisons between the three drug products. In the response (received 8/18/2017), the sponsor re-expressed the intact samples and relative IEC peak activity in reference to the in-house CT-P10 ^{(b) (4)}. This confirms that peaks 1-7 for CT-P10 have similar activity to the corresponding peaks of Rituxan and MabThera. This supports the conclusion that although there are differences in the species underlying various acidic and basic peaks for the three products, those differences do not appear to be significant enough to drive changes in biological activities as measured by in vitro assays (binding to FcRn, FcγRIIIa V-type, C1q, and CD20, and activity in CDC, ADCC, and apoptosis assays). Additionally, variants that may be driving differences appear unlikely to impact safety or potency.

Reviewer comment: In the same IR response (comment/amendment #2), the sponsor also clarified that only one primary in-house CT-P10 RS exists ^{(b) (4)} and stated that this lot is the only one used in the similarity assessment. As the similarity data includes some release assay results (for SEC, CE-SDS, IEF, and IEC) that could only be obtained using the RS in use at the time (for example, release data related to Process C-derived CT-P10 material), we know that the

similarity data includes comparison to more than one RS, however use of different RSs in these assays will not affect similarity results. For tier 1 testing, only (b) (4) was used for comparison. Of note, the data for the CT-P10 in-house RS submitted in 3.2.S.5 of the submission shows that the RS are comparable.

Size-Exclusion Chromatography-HPLC (SEC-HPLC)

This method was used to examine purity/impurity. Monomer and HMW content were used to assess aggregate profile, while the LMW content was used to assess fragment profile.

Reviewer comment: The 3-way similarity assessment is summarized in the reviewer-generated table below. Although CT-P10 does not meet tier 2 similarity criteria for size purity as measured by SEC-HPLC, the overall difference in species is very minor and CT-P10 has lower levels of HMW species. I do not believe this is a clinically-meaningful difference, especially when considered with the SEC-MALS and AUC data discussed below.

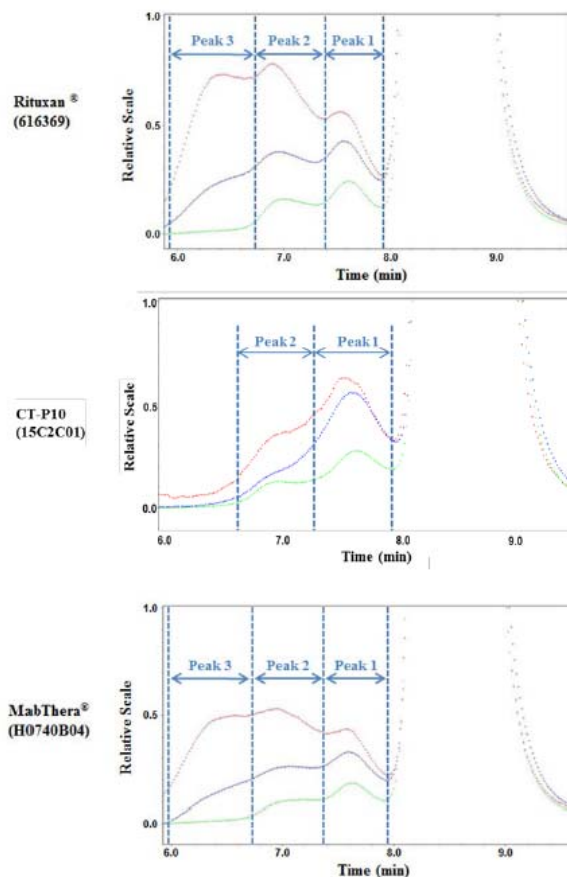
Species	μ-Rituxan (%)	μ-CTP10 (%)	%CTP10 lots within Rituxan QR	%CTP10 lots within MabThera QR	%MabThera lots within Rituxan QR
Monomer	98.81	99.3	60	87	100
HMW	1.07	0.53	40	47	100
LMW	0.12	0.15	60	33	100

SEC with Multi-Angle Laser Light Scattering (SEC-MALS)

This method was used as an orthogonal assessment of size purity and it provided molecular weight estimations for monomer and multimers. Molecular weight and the monomer and dimer content were determined with MALS and refractive index (RI) detectors. This method generates monomer% and HMW% as measured by UV and MALS and it generates an estimate (in kDa) for the molecular weight of the monomer and HMW species.

Reviewer comment: The size purity profiles appear similar and 100% of the 15 CT-P10 lots are within the Rituxan and MabThera QRs for monomer and HMW content measures. The summary of the 3-way similarity assessment is provided below in the reviewer-generated table. The average estimated monomer molecular weight was identical between Rituxan and CT-P10. The difference in estimated molecular weights for the HMW species between CT-P10 (smaller multimers) and Rituxan prompted further investigation (described below) that suggested statistical comparison was not applicable (N/A).

Species	μ-Rituxan (% or kDa)	μ-CTP10 (% or kDa)	%CTP10 lots within Rituxan QR	%CTP10 lots within MabThera QR	%MabThera lots within Rituxan QR
monomer (UV)	99.1	98.96	100	100	100
HMW (UV)	0.9	1.04	100	100	100
monomer (MALS)	97.03	97.32	100	100	100
HMW (MALS)	2.97	2.68	100	100	100
monomer (MW)	138	138	100	100	100
HMW (MW)	303	197	N/A	N/A	N/A



Peaks indicate different HMW forms; Detection: UV (green), RI (purple) and LS (red)

Figure 3.2.R.3-19: Magnified Images from SEC-MALS Analysis of Rituxan®, CT-P10 Drug Product and MabThera®

third peak, which might be comprised of dimers, (although it has different LS and RI spectral patterns between the DP's) does not appear to have much detectable protein content (near 0 UV reading). Overall, HMW species are < 3% of the total DP sample and less complex species are in CT-P10, thus I believe this difference is not one that affects safety, efficacy, or similarity.

Sedimentation Velocity Analytical ultracentrifugation (SV-AUC)

This method was used as an orthogonal assessment of size purity. Specifically, this method measured content and sedimentation value/coefficients of monomer and dimer. The sponsor states that no LMW protein peak signals were found in measurable amounts in any of the lots tested. The submission contains detailed information about centrifugation parameters, blanking, and model fitting. The resulting c(s) distribution profile was used to calculate the percentage of each species and the estimated molecular weights.

Reviewer comment: The size purity profiles appear very similar and 100% of the 15 CT-P10 lots are within the Rituxan QRs for monomer and dimer content and s-value measures. The summary of the 3-way similarity assessment is provided below in the reviewer-generated table.

To further investigate the HMW species, the sponsor examined magnified images of the HMW peaks from the SEC-MALS analysis using HPLC coupled with ultraviolet-refractive index-light scattering (UV-RI-LS) detector (*representative images shown in Figure 3.2.R.3-19*).

Because the molecular weight of HMW is an average of all HMW molecule weights, and CT-P10 does not have peak 3, the sponsor concluded that Rituxan and MabThera are comprised of more/different ratios of HMW forms than CT-P10 DP.

Reviewer comment: In my examination of this data, peaks 1 and 2 (smaller) appear similar between CT-P10 and Rituxan and the

Species	μ -Rituxan	μ -CTP10	%CTP10 lots within Rituxan QR	%CTP10 lots within MabThera QR	%MabThera lots within Rituxan QR
monomer (s-value)	6.17	6.18	100	100	100
monomer (%area)	98.05	98.18	100	93	100
dimer (s-value)	8.95	8.96	100	100	100
dimer (%area)	1.95	1.82	100	93	100

Reviewer comment: Overall, I conclude that the SEC-HPLC, SEC-MALS, and AUC data taken together support high similarity with respect to monomer/dimer/aggregate/HMW size purity between CT-P10 DP and Rituxan.

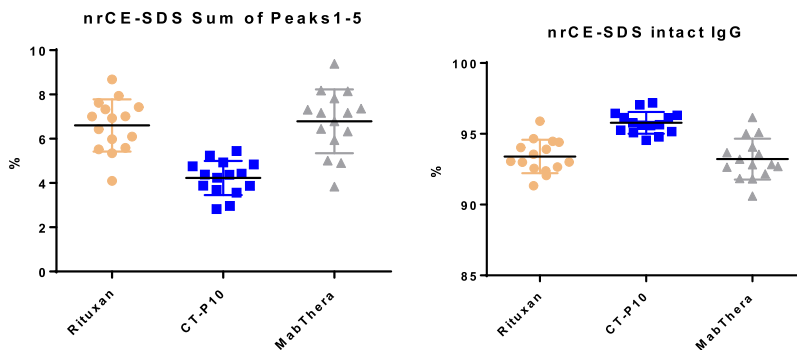
CE-SDS (reduced and non-reduced)

Capillary electrophoresis sodium dodecyl sulfate (CE-SDS) was used to assess purity/impurity with respect to fragments (nrCE-SDS) and aglycosylation (rCE-SDS). For determining the corrected peak area % HC+LC, and for non-glycosylated HC, samples were reduced and subjected to electrophoresis under reducing conditions. For determination of the corrected peak area % of intact IgG and quantification of non-assembled IgG molecules, samples were alkylated and subjected to electrophoresis under non-reducing conditions.

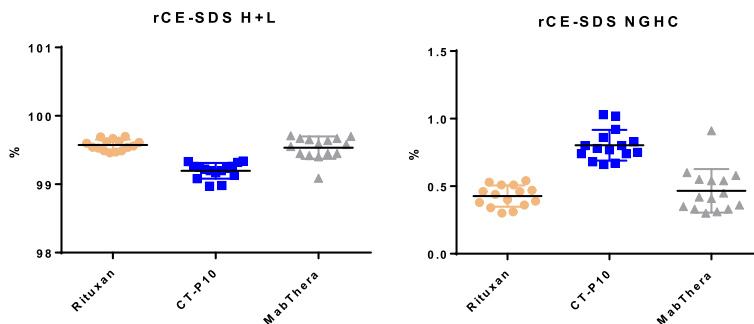
Reviewer comment: The 3-way similarity assessment is summarized in a reviewer-generated table and scatter plots below.

	Species	μ -Rituxan (%)	μ -CTP10 (%)	%CTP10 lots within Rituxan QR	%CTP10 lots within MabThera QR	%MabThera lots within Rituxan QR
nrCE-SDS (%)	peak 1 (L1)	1.59	0.87	87	100	100
	peak 2 (H1)	0.1	0.12			
	peak 3 (L2)	0.34	0.34			
	peak 4 (H1L1)	0.55	0.38			
	peak 5 (H2L1)	4.01	2.51			
	intact IgG	93.4	95.78	87	100	100
rCE-SDS (%)	NGHC	0.43	0.8	7	87	93
	H+L	99.57	99.2	7	87	93

Reviewer comment: Regarding the nrCE-SDS data, I found that there are some differences in averages (i.e., peaks 1, 4, and 5), which correlated with an approximately 2.4% difference in the average intact IgG between Rituxan and CT-P10. The sponsor performed a tier 2 similarity assessment based on the QR for intact IgG (Peak 6) and a QR based on the sum of Peaks 1, 2, 3, 4, and 5 and CT-P10 does not pass for either the sum of fragments or intact IgG because CT-P10 appears to have lower levels of fragments. This difference is not likely to pose a safety risk and so there is only a residual uncertainty about whether this difference impacts biological activity (to be discussed later in this section).

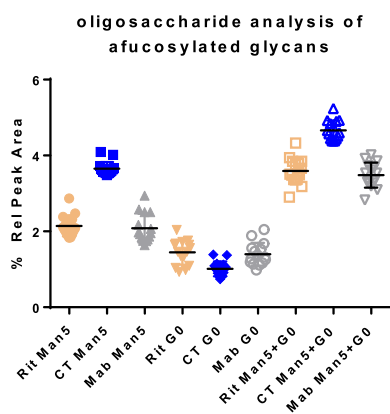


Reviewer comment: Regarding the rCE-SDS data, I found that there are some very minor differences in average non-glycosylated heavy chain (NGHC, 0.43% vs 0.8% respectively in Rituxan vs CT-P10), thus resulting in a small difference in total HC+LC (99.57% vs 99.2%). This small difference in NGHC likely will not impact pharmacokinetics (PK) or potency / MOA. The failure of tier 2 similarity for NGHC alone does not preclude a conclusion of similarity due to its very small content.



Oligosaccharide Profiling using HPAEC-PAD

High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to assess global oligosaccharide profile for each DP. PNGase F was used to cleave off oligosaccharides and HPAEC-PAD was used to separate carbohydrates based on glycan size, composition, and linkage.



Reviewer comment: The 3-way similarity assessment is summarized in a reviewer-generated table and scatter plot below. The fucosylated species meet tier 2 similarity criteria. Regarding the distribution of relative peak area % for afucosylated species (Man5 and G0), the more pronounced difference is in the Man5 species. See later discussion of whether the relevant biological assays can address this residual difference/uncertainty in potential impact on PK.

Species (% rel peak area)	μ-Rituxan (%)	μ-CTP10 (%)	%CTP10 lots within Rituxan QR	%CTP10 lots within MabThera QR	%MabThera lots within Rituxan QR

Man5	2.14	3.65	0	0	100
G0	1.45	1.01	100	100	100
total afucose (G0+Man5)	3.59	4.66	53	33	100
G0F	39.01	39.61	100	100	100
G1F	44.53	43.73	100	100	100
G2F	12.87	11.99	100	100	100
total galactose (G1F+G2F)	57.4	55.73	N/A		
galactose (moles/moles of HC)	0.7	0.68			

- Justification for Revising the Multiplier and QR for the sum of afucosylated glycans:

Based on publications and additional studies on glycosylation and ADCC activity (discussed and reviewed in detail in [RI 4: Glycosylation and Associated Biological Activities](#)), the sponsor concluded that the afucosylation levels outside the $\pm 3SD$ Rituxan QR have no impact on biological activities. They then performed a statistical analysis using a QR generated with a multiplier of 4. In this case, 93% of the CT-P10 lots were within the QR.

Reviewer comment: During IND review, OBP communicated to sponsor that G0 and Man5 can be grouped into one tier 2 attribute (from meeting minutes of 03/11/2016, discussion of Q2c). Afucosylated Man5 species content is the reason that CT-P10 does not meet tier 2 similarity criteria for afucosylated species.

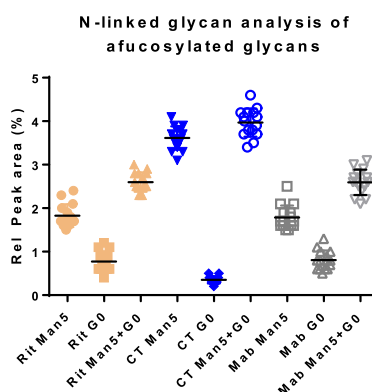
I do not agree with the sponsor's justification to use a multiplier of 4 for the sum of afucosylated glycans. Broadening of QR is not an appropriate approach. Based on our knowledge of the importance of afucosylated glycan species (Man5 in particular), failing similarity because of a difference in relative Man5 species has implications for altered FcγRIIIa binding and PK profile due to clearance by mannose receptors. The FcγRIIIa binding study (discussed later in this section) did not identify any difference between CT-P10 and Rituxan. Residual uncertainty on the clinical implication of this difference was addressed by the comparative PK study as the clinical pharmacology reviewers concluded that there were no differences in CT-P10 and Rituxan PK profiles.

N-linked Glycan Analysis

This was used as an orthogonal method to characterize the glycosylation of the products. LC-MS analysis of the peptides generated during peptide mapping was used to identify all sites of glycosylation. Samples were prepared as described in the peptide mapping by LC-MS section. Extracted ion chromatograms were used to quantify each oligosaccharide species. The percentage calculation was based on each glycosylation site. For each glycosylation site, all the detectable oligosaccharide structures were counted.

Reviewer comment: The 3-way similarity assessment is summarized in a reviewer-generated table and scatter plot below. This data confirms what was found using the HPAEC-PAD system: the fucosylated glycan and G0 profile between CT-P10, Rituxan, and MabThera is similar, however there is a higher level of Man5 in CT-P10 than in Rituxan or MabThera. CT-P10 fails tier 2 similarity assessment for afucosylated glycans.

Species (% rel peak area)	μ -Rituxan (%)	μ -CTP10 (%)	%CTP10 lots within Rituxan QR	%CTP10 lots within MabThera QR	%MabThera lots within Rituxan QR
G0F	44.9	46.2	100	100	100
G1F	42.9	42.1	100	100	100
G2F	8.1	6.8	100	100	100
Man5	1.8	3.6	0	0	100
G0F	0.8	0.4	100	100	100
G0+Man5	2.6	4	0	7	100



Tier 2 Similarity Results: Biological Characteristics

This section combines information found in RI 2, RI 3, RI 5, and RI 6. The reviewer has grouped information by analytical method.

Reviewer comment: These biological assays were measuring attributes with anywhere from “moderate” to “very high” criticality. The sponsor often claimed the assays were “reasonably sensitive” in their ability to detect a difference and the combination of the criticality and assay sensitivity usually yielded a multiplier of 3 for the QR. The CD20 binding CELISA and ADCC reporter assays had high criticality so in order to still confidently conclude similarity given assay capabilities, 2 was used for the multiplier to generate a QR. I found the justification for the multipliers for each attribute and assay acceptable.

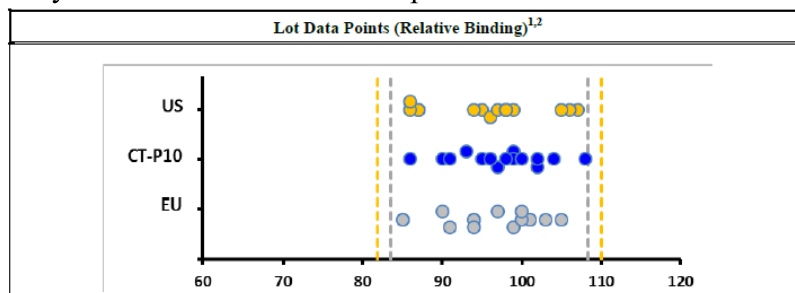
Regarding the 3-way similarity assessment as measured by these biological assays:

- 100% of CT-P10 lots are within the defined Rituxan QR
- 100% of MabThera lots are within the Rituxan QR
- 100% of the CT-P10 lots are within the MabThera QR

The following section describes the functional assays, summarizes the generated data, and highlights any reviewer concerns regarding the assay capabilities. A summary of the qualification results for Biological Assays can be found in RI 6 (see table 3.2.R.6-2).

Fab Binding by CD20 Binding Assay (CELISA)

This test is used to measure Fab binding. CHO-K1 cells expressing recombinant CD20 antigen were plated in a 96-well plate and after overnight incubation, cells were fixed and blocked. Serially diluted samples (from 0.24 ng/mL to 4,000 ng/mL) were added to the fixed cells. After 1 hour incubation, cells were washed and incubated with detection antibody, substrate was added, and plates were measured at 450 nm / 650 nm. The EC₅₀ was deduced from a four-parameter curve fitting of the sigmoid dose-response curves. The relative cell-based CD20 binding affinity was determined from comparison with the EC₅₀ value of the *in-house* RS.



¹ Relative binding affinity was determined against CT-P10 in-house reference standard

² The dotted yellow lines and grey lines represent the quality range limits based on mean $\pm$ 2SD of the Rituxan[®] and MabThera[®] lots, respectively.

Figure 3.2.R.3-39: Scatter Plot of Cell-based CD20 Binding Affinity (CELISA)

Fab Binding by Apoptosis using Raji Cells

This assay was used as an orthogonal method to measure Fab binding function. Detailed assay descriptions can be found in R15 of the submission. Briefly, this assay uses the Raji cell line which expresses CD20 on the cell surface and is prone to apoptotic cell death when rituximab binds to CD20 (Figure 3.2.R.5-6). Cells are incubated with therapeutic proteins for 48 hours, labeled with annexin-V and subjected to FACS analysis. The relative potency was determined at 0.13, 0.04, 0.01 μ g/mL concentrations that are within the linear range of the response and the results were compared with those of the CT-P10 in-house RS (*a partial Figure 3.2.R.3-43 is shown*).

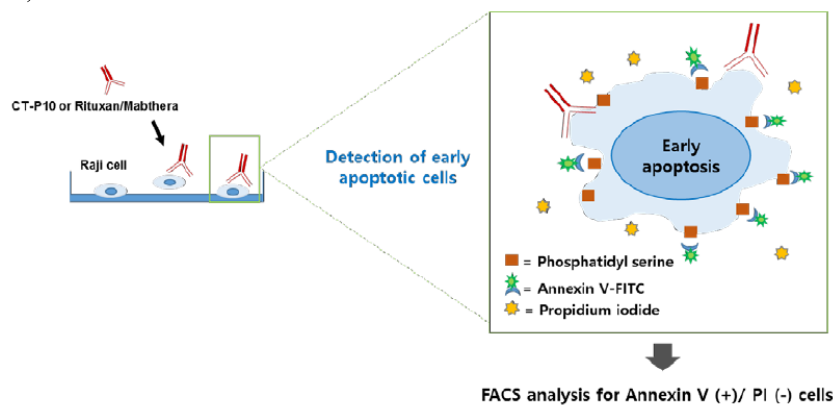


Figure 3.2.R.5-6: Illustration of CT-P10/Rituxan[®]/MabThera[®] Mediated Apoptosis

Ab. Conc.	Lot Data Points (Relative Values) ¹
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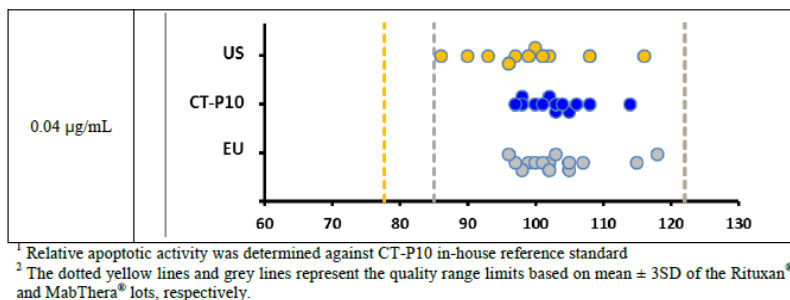


Figure 3.2.R.3-43: Scatter Plot of Apoptosis Results

ADCC Reporter Assay

This assay is used as an orthogonal read-out of *FcγRIIIa* binding affinity and activation of *NFAT*, which is reflective of part of the rituximab MOA. Detailed assay descriptions can be found in R15 of the submission. Briefly, this assay uses Raji cells overexpressing CD20 as target cells and Jurkat cells engineered to express NFAT-RE-Luciferase and *FcγRIIIa*-V158 (high affinity variant) as effector cells. The cross-linking of *FcγRIIIa* on the reporter cells by antibodies bound to target cells leads to activation of the NFAT response element and expression of luciferase (Figure 3.2.R.5-4). Raji cells were incubated with the therapeutic proteins first, then incubated with target cells (E:T ratio of 2:1), then ADCC activity was measured via luciferase signal. The relative potency of samples was determined by comparison of relative luminescence (defined by a four-parameter logistic curve) with the luciferase activity of CT-P10 in-house RS.

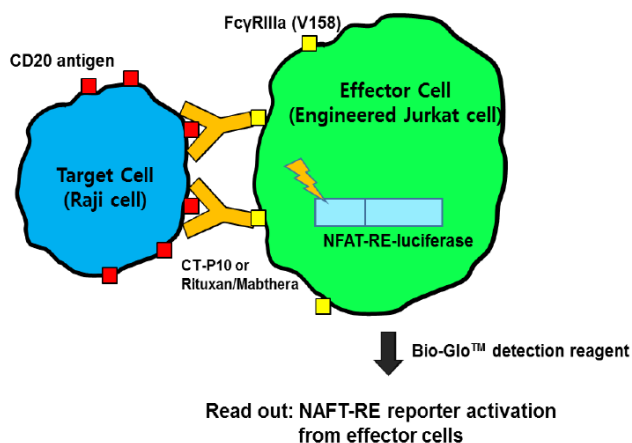


Figure 3.2.R.5-4: Illustration of CT-P10/Rituxan[®]/MabThera[®] Mediated Reporter Assay ADCC Activity

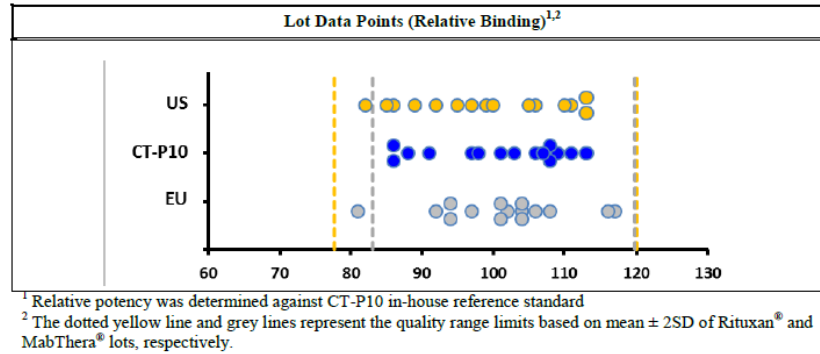


Figure 3.2.R.3-42: Scatter Plot of ADCC Reporter Assay Results

ADCP using Raji Cells

This assay measures phagocytosis of effector cells induced by the rituximab, however the phagocytosis is not a primary presumed MoA. Raji cells overexpressing CD20 on the surface were used as target cells while primary monocyte-derived macrophages were used as effector cells. Monocytes were isolated from human PBMCs and were differentiated into macrophages. Prior to incubation with antibodies, the target cells were labeled with a green fluorescence (FITC). The macrophages were added to incubation tubes at 1:1 E:T ratio. After a 3-hour incubation, the phagocytic activity was assessed by FACS analysis after staining macrophages with CD11b-APC (Figure 3.2.R.5-8). The % phagocytosis was calculated as the percentage of double-positive macrophages with respect to total target cells stained with green fluorescence as follows:

$$\text{Phagocytosis (\%)} = \left(\frac{\% \text{ double positive (Q2)}}{\% \text{ double positive (Q2)} + \% \text{ residual targets (Q4)}} \right) * 100$$

The relative phagocytic activity was determined at 1.56, 6.25, and 25 ng/mL concentrations and was compared to CT-P10 in-house RS (*a partial Figure 3.2.R.3-44 is shown*).

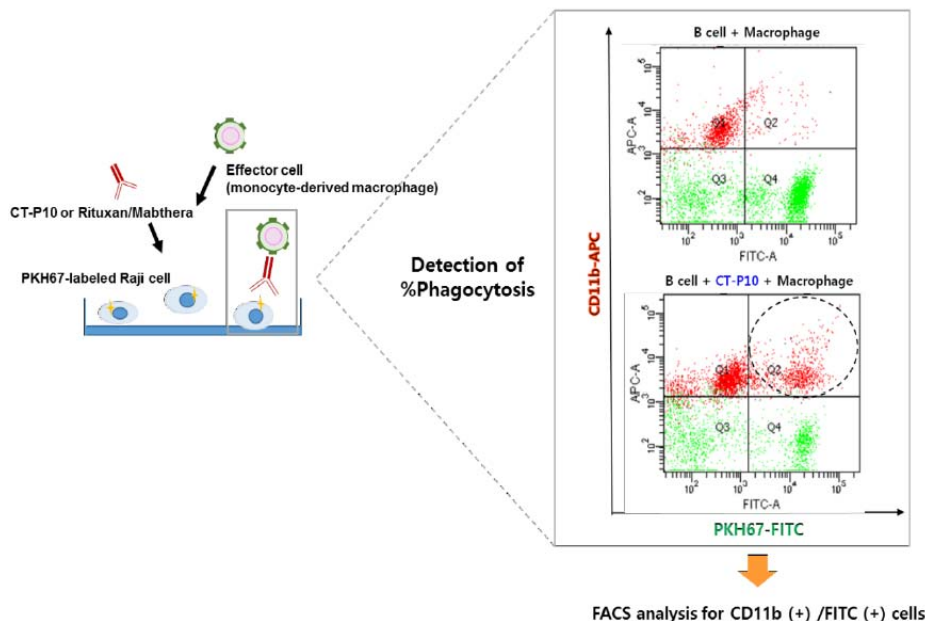


Figure 3.2.R.5-8: Illustration of CT-P10/Rituxan[®]/Mabthera[®] Mediated ADCP

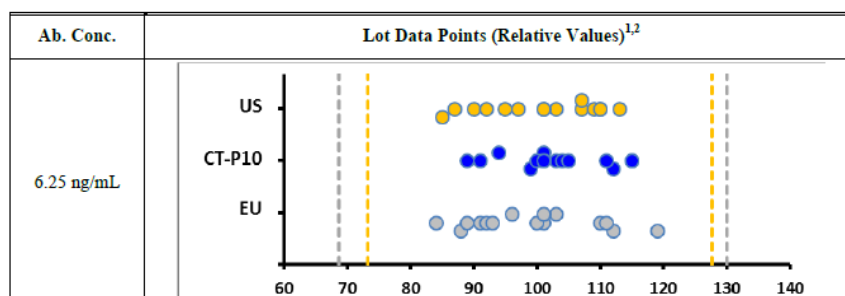


Figure 3.2.R.3-44: Scatter Plot of ADCP Results

Reviewer comment: Note that a separate set of Rituxan and MabThera lots (from those used for other attributes) are used in this 3-way similarity assessment for ADCP. Selection of the lots is acceptable.

In addition to directly measuring ADCP activity in this functional assay, the sponsor also compared Fc γ RIIIa and Fc γ RIIa binding between CT-P10 and Rituxan and MabThera using tier 2 similarity criteria (discussed below). The literature suggests that these receptor interactions are involved for ADCP.

C1q binding (ELISA)

This assay was used to measure one signaling step in the cascade that leads to CDC activity of rituximab. The C1q protein binds to the immunoglobulin CH2 domain and this interaction was detected by a sandwich ELISA. The ELISA signal is abrogated in the absence of C1q and enhanced at higher concentrations of IgG1 (in the presence of a constant saturating concentration of C1q). To coat the samples to each well the microplate was incubated serially diluted samples (30 μ g/mL - 0.013717 μ g/mL) at 2-8 $^{\circ}$ C. After blocking, C1q was added and

incubated. After wash, detection antibody, stopping substrate was added and was detected at 450 nm / 650 nm). The relative C1q binding affinity of samples was determined from comparison of the EC50 value (optical density fitted to a four-parameter curve) with the in-house RS.

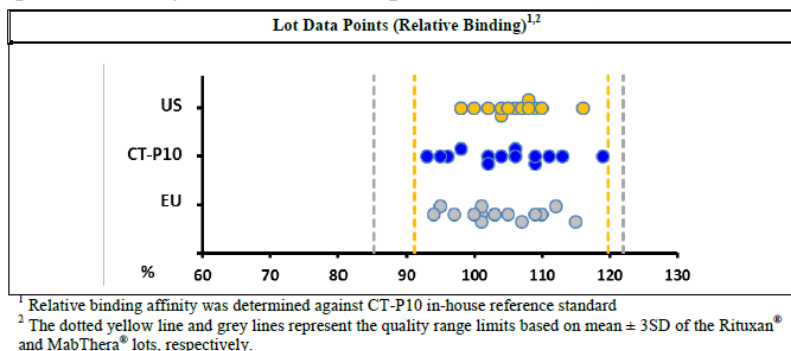


Figure 3.2.R.3-45: Scatter Plot of C1q Binding Affinity Results

Reviewer comment: The data show that C1q binding is similar between the three products.

Fc Receptor Binding Affinity by SPR

Because Fc modifications can influence Fc receptor binding (effector function or PK profiles), FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa (V type), FcγRIIIa (F type), FcγRIIIb, FcRn binding assays by SPR were used as orthogonal methods to detect variation in Fc modifications. Recombinant Fc receptors were coupled to a Biacore CM5 chip using regeneration solution. Details about the assays can be found in the RI 5: Method description, and they are summarized in Table 3.2.R.5-2 of the submission.

Reviewer comment: The relative binding affinity for each Fc receptor was determined against CT-P10 in-house reference standard. All data were assessed by tier 2 similarity (except FcγRI was assessed by tier 3). CT-P10 met the similarity criteria for each assay, supporting the conclusion that CT-P10 has similar Fc receptor binding properties to that of Rituxan and MabThera. The data plots can be found in the submission, only FcγRIIIa (V-type) and FcRn results are included below.

- FcγRIIIa V-type binding (SPR)

Although this FcγRIIIa V-type binding is in general a lower-affinity interaction, this is the variant with the higher affinity for IgG (as opposed to F-type binding).

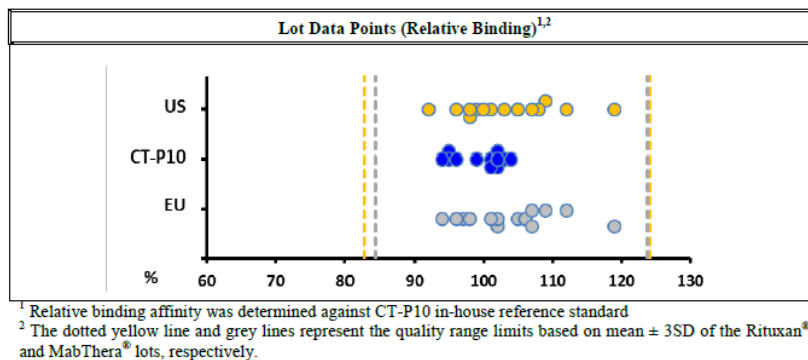
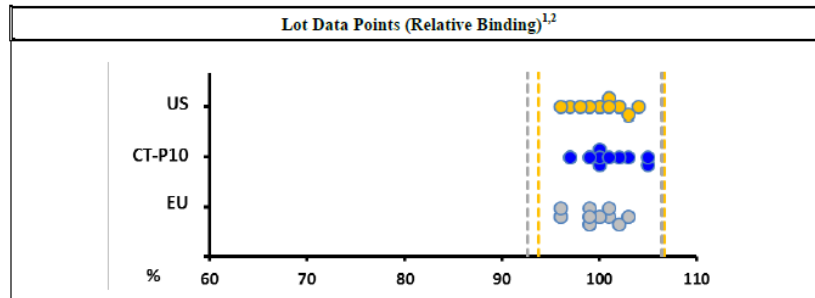


Figure 3.2.R.3-46: Scatter Plot of FcγRIIIa-V Binding Affinity Results

- FcRn binding (SPR)



¹ Relative binding affinity was determined against CT-P10 in-house reference standard.

² The dotted yellow line and grey lines represent the quality range limits based on mean $\pm$ 3SD of the Rituxan[®] and MabThera[®] lots, respectively.

Figure 3.2.R.3-52: Scatter Plot of FcRn Binding Affinity Results



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Tier 3 Similarity Results: Physicochemical and Biological Characteristics

The reviewer has generated the following executive summary of the Tier 3 attributes and assessments of similarity.

Attribute		Test/Assay	Measurement	3-way Similarity Notes
Primary Structure	primary aa sequence	Peptide mapping (HPLC)	peak profile	chromatograms look similar
Primary Structure	primary aa sequence	amino acid analysis	molar ratio	means and SDs of each AA are similar
Primary Structure	primary aa sequence	N-terminal sequencing	sequence identity	results from all products and lots conform to expected sequence(s)
Primary Structure	primary aa sequence	C-terminal sequencing	sequence identity	results from all products and lots conform to expected sequence(s)
Primary Structure	primary aa sequence	intact mass	molecular weight	molecular weights of each of the 4 species (G0F-G0F, G0F-G1F, G1F-G1F or G0F-G2F, and G1F-G2F) from each of the DPs were similar.
Post translational modifications	deamidation	Peptide mapping (LC-MS)	%deamidation (LC Asn136)	same %deamidation (0.1) across all samples and DPs
Post translational modifications	oxidation	Peptide mapping (LC-MS)	%oxidation (Met)	%oxidated Met is similar
Post translational modifications	N-terminal glutamine	Peptide mapping (LC-MS)	%N-terminal glutamine	%N-terminal glutamine on HC is similar. CT-P10 DP contains slightly higher levels of glutamine in the LC (Gln01, $4.3 \pm 0.54\%$ compared to $2.6 \pm 0.9\%$ for Rituxan and $2.4 \pm 0.73\%$ for MabThera), but this difference is low risk due to low amount. This also could be one contributing factor to observed basic peak (and therefore acidic peak) differences.



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Post translational modifications	C-terminal lysine	Peptide mapping (LC-MS)	%C-terminal lysine	%C-terminal lysine are similar.
Post translational modifications	Glycation	Peptide mapping (LC-MS)	% HC or LC glycation	Average % glycation for each chain was slightly lower for CT-P10, but overall similar to Rituxan and MabThera.
Higher order structure	secondary structure	FTIR	structure of protein by spectroscopy	the wave numbers for each structure are the same
Higher order structure	secondary and tertiary structure	CD	α -helical, β -sheet and unordered structures	the spectra of all tested samples have similar overlay
Higher order structure	Disulfide bonds	native and reduced peptide mapping	disulfide bond positions	each product has the same disulfide bond positions (listed in table 3.2.R.3-12)
Content	extractable volume	extractable volume	volume extracted (mL)	exactly the same (51 mL) between Rituxan and CT-P10. Seven lots of Rituxan were used due to unavailability of unopened vials. <i>Additional discussion of this attribute and expectations for similarity assessment are included below.</i>
Purity/Impurity	aggregates	SEC-MALS	HMW size	three products are similar in monomer%, HMW%, and monomer MW. Differences between Rituxan/MabThera and CT-P10 in HMW MW (data on p.87, Table 3.2.R.3-19). Chromatograms and biological assay results used to address residual uncertainty.
Purity/Impurity	HCP	residual HCP ELISA	HCP (ppm)	Similar. all < 3 ppm, ng/mg
Purity/Impurity	Host Cell DNA	residual host cell DNA PCR	DNA (ppb)	Similar. all tested samples were less than ~0.52 (except one lot of MabThera at 0.8)
Purity/Impurity	rProtein A	residual rProtein A ELISA	rProtein A (ppm)	Not similar, but acceptable because more pure. CT-P10 and MabThera had protein A < 0.06 while Rituxan ranged from 0.08-0.14 ppm



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Purity/Impurity	particulates	MFI	$1 \leq, < 100 \text{ (}\mu\text{m)}$ $2 \leq, < 100 \text{ (}\mu\text{m)}$ $5 \leq, < 100 \text{ (}\mu\text{m)}$ $10 \leq, < 100 \text{ (}\mu\text{m)}$ $15 \leq, < 100 \text{ (}\mu\text{m)}$ $25 \leq, < 100 \text{ (}\mu\text{m)}$ $40 \leq, < 100 \text{ (}\mu\text{m)}$ $50 \leq, < 100 \text{ (}\mu\text{m)}$ $70 \leq, < 100 \text{ (}\mu\text{m)}$ (cumulative counts/mL)	Similar. At every size interval, there is small variability between lots of the same DP, and at the lower sizes, CT-P10 seems to have fewer particles. nonetheless the mean counts at each interval are similar between Rituxan and CT-P10.
		HIAC	$\geq 2 \text{ (}\mu\text{m)}$ $\geq 5 \text{ (}\mu\text{m)}$ $\geq 10 \text{ (}\mu\text{m)}$ $\geq 25 \text{ (}\mu\text{m)}$ (cumulative counts/mL)	Similar. at every size interval, there is clear overlap between the DPs.
charge variants	charge variants	IEF	pI values of charge variants	Rituxan/MabThera Band0 and Band4 are "minor and very faint in intensity" and so are rarely detected. CT-P10 also has a rarely detectable Band4, but Band0 is detected a little more frequently. Overall very similar IEF profiles (pI's and gels).
charge variants	charge variants	IEC-HPLC	% peak 4 % peak 5 % peak 6 % peak 7	Main peak 4 is similar between products. Sum of basic peaks are not similar. Peak fractionation study used to address residual uncertainty.
Glycosylation	monosaccharide	monosaccharide analysis	Fuc GlcNAc GAL Man (monosaccharide/protein, mole/mole)	molar ratios for each species were similar between all products
Glycosylation	sialic acids	sialic acid analysis	NANA (sialic acid/protein, mol/mol)	similar



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Glycosylation	sialic acids	N-linked glycan analysis	%G0F+NANA %G1F+NANA %G2F+NANA	very minor differences, but still fairly similar (confirms peak fractionation study data)
Fc binding	FcγRI Binding	FcγRI Binding affinity (SPR)	Relative FcγRI binding affinity (%)	similar binding

Reviewer comment: *The strength of the U.S.-licensed Rituxan is labeled in mass per unit volume (100 mg/10 mL and 500 mg/50 mL). U.S.-licensed Rituxan is filled into a single-use vial. CT-P10 is seeking approval for the same strengths (presentations) as U.S.-licensed Rituxan. In an IR sent on 10/13/2017 (comment #1), we requested confirmation that only the 500 mg/50 mL strength of CT-P10 DP was used in the similarity assessment. The sponsor responded on 10/23/2017 with confirmation, and stated that they do not believe the use of one presentation in the similarity assessment will impact the validity and conclusions of the similarity study, which are equally applicable to the 100 mg/10 mL presentation. The sponsor provided a risk assessment summarizing potential quality impact due to differences in manufacturing process of the 500 mg and 100 mg presentations (Table 2 of amendment 1) and concluded that (besides extractable volume) there are no attributes expected to differ by strength/presentation.*

DP scale, filling, extractable volume and vial size are the main differences between the 100 mg and 500 mg presentations, and these aspects are addressed by process development, process validation/controls, and release testing. Between the two CT-P10 presentations, the protein concentration, vial material, and stopper are all the same. Additionally, the ratio of vial fill to vial volume is roughly the same between the two presentations. In our assessment of this proposed biosimilar, manufacturing capabilities address the identified presentation-specific attributes and therefore no additional presentation-specific similarity data are required for the similarity assessment. The sponsor’s approach to use only 500 mg/50 mL CT-P10 DPs in the similarity assessment, and extrapolate similarity for the 100 mg/10 mL presentation was discussed with OBP management, and we agreed that the approach is acceptable for biosimilars to Rituxan.

Comparative in vitro potency, protein concentration (mg/mL), and extractable volume data were reviewed as part of the similarity assessment of whether the proposed presentation of CT-P10 has the same strength as the presentation of U.S.-licensed Rituxan. Based on these comparative data, the CT-P10 500 mg/50 mL presentation has the same total content of DS in units of mass per unit volume as the 500 mg/50 mL presentation of U.S.-licensed Rituxan. The data combined with our assessment and justification for using only the 500 mg/50 mL presentation in the similarity assessment (described above) supports the conclusion that both CT-P10 presentations meet the statutory “same strength” requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.

Reviewer comment: *Tier 3 attributes are similar between CT-P10, Rituxan, and MabThera. I elaborate on a few key points related to this data below:*

- Regarding the SEC-MALS data measuring HMW size: this was discussed in detail earlier in this review (in SEC with Multi-Angle Laser Light Scattering section).*



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- *Regarding process-related impurities: CT-P10 had an impurities profile equal to or purer than Rituxan and/or MabThera and this is acceptable.*
- *Peptide mapping data that measured PTMs were used to partially resolve residual uncertainty about the differences in acidic and basic charge variants between CT-P10 and Rituxan/MabThera.*

Initially, glycation analyses were not included in the original BLA although it is considered a Tier 3 assay in the OBP standard language handbook for Rituximab biosimilars. In an IR (sent on 10/13/2017, comment #2), we requested this data. On 10/23/2017, the sponsor responded with similarity data that was used to make the conclusion summarized in the table above. Overall, CT-P10 meets tier 3 similarity criteria.

Regional Information 4: Glycosylation and Associated Biological Activities

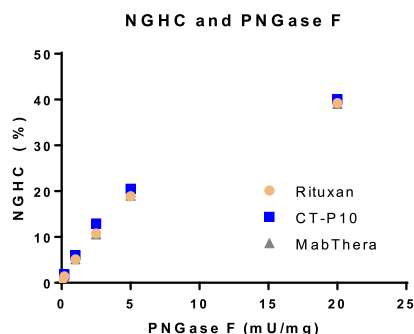
CT-P10 failed tier 2 similarity with respect to aglycosylated and afucosylated species. Because Fc glycans can influence binding interactions that relate to primary and potential MoAs for the U.S.-licensed Rituxan, the sponsor conducted additional biological studies to assess the relationship between glycosylation and biological activities as measured by various functional assays.

The impact of four “glycoparameters” (aglycosylation, agalactosylation, amannosylation, and asialylation) of Rituxan, CT-P10, and MabThera was assessed through evaluation of FcγRIIIa and FcRn bindings by SPR, and cell-based CD20 and C1q binding affinities by ELISAs. One lot of each DP (Rituxan lot 590472 (exp. Jun-2016), CT-P10 DP lot 15C2C04 (manuf. 31-May-2015), and MabThera lot N7029B05 (exp. Feb-2017) was used in this series of studies and a CT-P10 in-house RS (b) (4) was used to determine relative binding affinity. Note that all three lots were used in the 3-way similarity assessment, as well.

In a subsequent study, the correlation between afucosylated glycan and biological activity was investigated to determine whether the difference in the total amount of afucosylated glycan in CT-P10 and Rituxan/MabThera has a detectable effect in the FcγRIIIa binding affinity, and ADCC reporter assay.

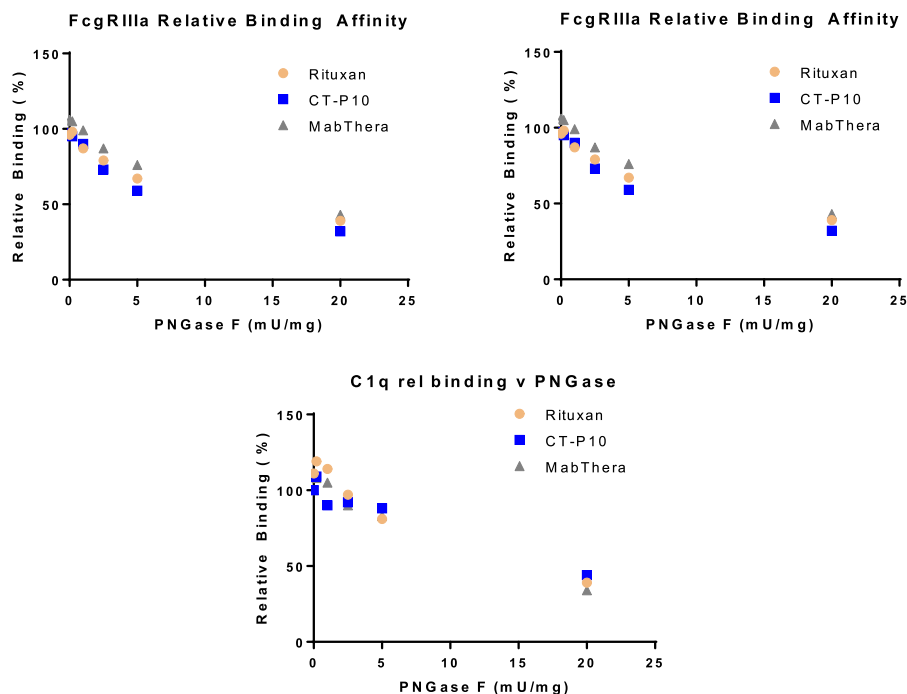
3.2.R.4.1.1 Aglycosylation

CT-P10 failed tier 2 similarity with respect to non-glycosylated HC species (CT-P10 had an average of 0.8% NGHHC while Rituxan/MabThera are around 0.4%). Sponsor studied the functional impact of increasing the aglycosylated levels (even a few percent) to address residual uncertainty of the difference in non-glycosylated species between CT-P10 and Rituxan/MabThera. Rituxan, CT-P10, and MabThera samples were prepared with different levels of aglycosylation (ranging from approximately 0.3 - 40% by reduced CE-SDS, see the reviewer-generated plot below) by treatment with different concentrations of PNGase F (concentrations ranged from 0.2 – 20 mU/mg).



The rCE-SDS data show that CT-P10 overall purity (glycosylated HC + LC) decreases (~60% as an effect of increase of non-glycosylated HC %) with increasing PNGase F concentration.

The FcγRIIIa, FcRn, C1q and CD20 bindings were analyzed in these samples. *The following reviewer-generated graphs summarize the data presented in the submission (in Tables 3.2.R.4-3 through 3.2.R.4-6).*



Reviewer comment: The following *in vitro* activity trends were observed for increasing levels of aglycosylation in CT-P10, Rituxan, and MabThera:

- *FcγRIIIa* binding is significantly reduced when the NGHC % goes higher than ~5% (seen at PNGase F ≥ 1 mU/mg), and drops below 50% at the highest NGHC%.
- *FcRn* binding is reduced significantly when the NGHC % goes higher than ~10% (seen at PNGase F ≥ 2.5 mU/mg), and drops to ~80% at the highest NGHC%.
- *C1q* binding is significantly reduced when the NGHC % goes higher than ~20% (seen at PNGase F ≥ 5 mU/mg), and drops below 50% at the highest NGHC%
- The data show no clear relationship between HC Fc glycosylation and CD20 binding (data in the submission), which is expected.

Overall, although strong relationships exist between (relatively high levels) of afucosylation and *FcγRIIIa*, *FcRn* and *C1q* binding, the ranges of the variability observed between CT-P10, Rituxan, and MabThera lots do not appear to overtly impact *FcγRIIIa*, *FcRn*, *C1q*, or CD20 binding.

3.2.R.4.1.2 Agalactosylation

CT-P10 passed tier 2 similarity criteria for Rituxan and MabThera G0F, G1F, and G2F levels. Rituxan, CT-P10, and MabThera with different levels of galactosylation were prepared by treatment with different concentrations (7.5 – 50 mU/mg) of β -1,4-galactosidase for 20 hours at 37 °C. The profiles of each sample were analyzed by Bio-LC for relative % of Man5, G0, G0F, G1F, and G2F, then the *FcγRIIIa*, *FcRn*, *C1q*, and CD20 binding were determined.

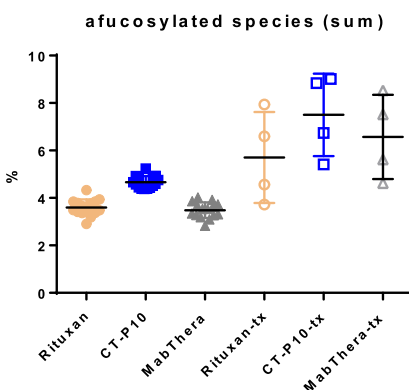
Table 3.2.R.4-7: Galactosylation Levels of Rituxan[®] (590472), CT-P10 Drug Product (15C2C04) and MabThera[®] (N7029B05) Following Galactosidase Treatment

Sample ID	Galactosidase (μU/mg)	G0F (%)	Man5 (%)	G0 (%)	G1F (%)	G2F (%)	Moles Galactose/Mole Heavy Chain
Rituxan [®] (590472)	50	92.06	3.80	4.14	0	0	0.000
	25	76.24	3.23	3.37	17.15	0	0.172
	15	55.30	2.38	2.18	35.37	4.76	0.449
	7.5	45.82	2.05	1.67	43.34	7.13	0.576
	0	36.95	1.82	1.26	47.32	12.64	0.726
CT-P10 Drug Product (15C2C04)	50	90.98	6.04	2.97	0	0	0.000
	25	85.07	5.82	3.02	6.10	0	0.061
	15	64.97	4.84	1.90	28.29	0	0.283
	7.5	49.40	4.10	1.31	38.95	6.24	0.514
	0	41.58	3.74	0.92	44.00	9.76	0.635
MabThera [®] (N7029B05)	50	91.47	3.74	4.79	0	0	0.000
	25	79.83	3.39	4.13	12.65	0	0.127
	15	61.29	2.63	2.99	30.04	3.05	0.361
	7.5	50.35	2.24	2.38	38.72	6.32	0.514
	0	41.45	1.97	1.81	44.68	10.09	0.649

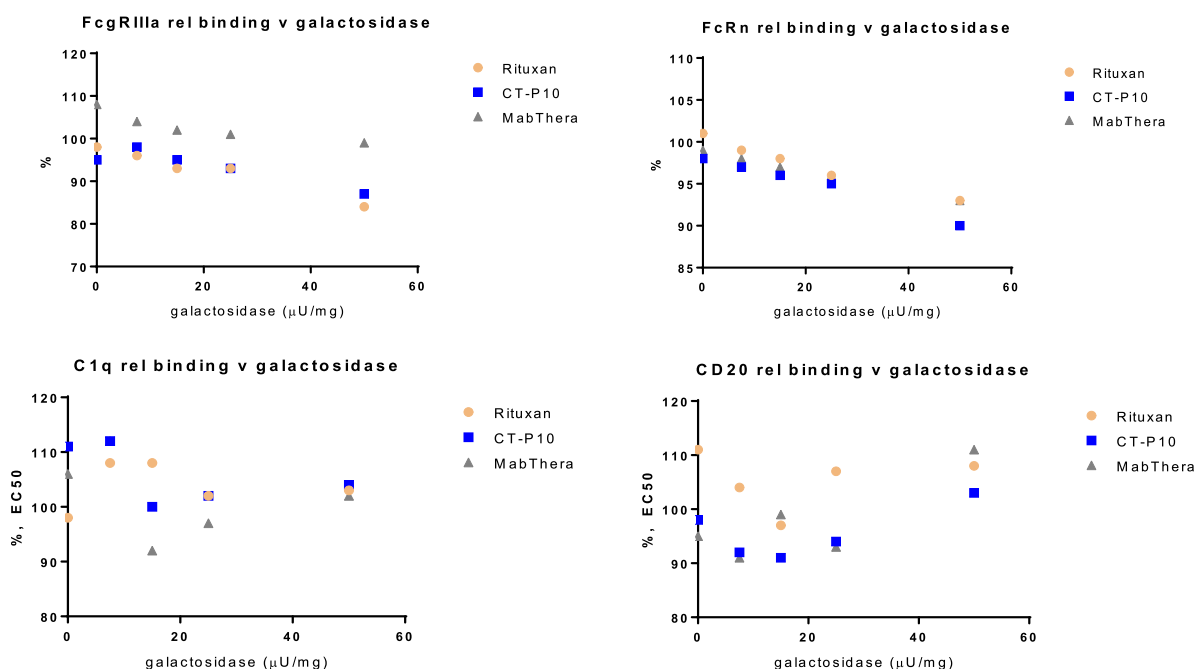
Reviewer comment: Galactose levels precipitously drop with increasing galactosidase treatment.

Note that in addition to increase of galactosylation with galactosidase treatment, the levels of afucosylated species (Man5 and G0) are increased. We think that this is not specific enzymatic reaction rather increase due to nonspecific afucosylation of the available single fucosylated species. These samples could also serve to study the functional impact of changes in afucosylated species, which failed tier 2 similarity.

The following graph titled “afucosylated species (sum)” includes the 3-way similarity data on the left and the sum of afucosylated species from this study to highlight how differences in afucosylation between CT-P10 and Rituxan are covered by the galactosidase-treated samples in this study.



The FcγRIIIa, FcRn, C1q and CD20 bindings were analyzed in these galactosidase-treated samples. *The following reviewer-generated graphs summarize the data presented in the submission (in Tables 3.2.R.4-8 through 3.2.R.4-11).*



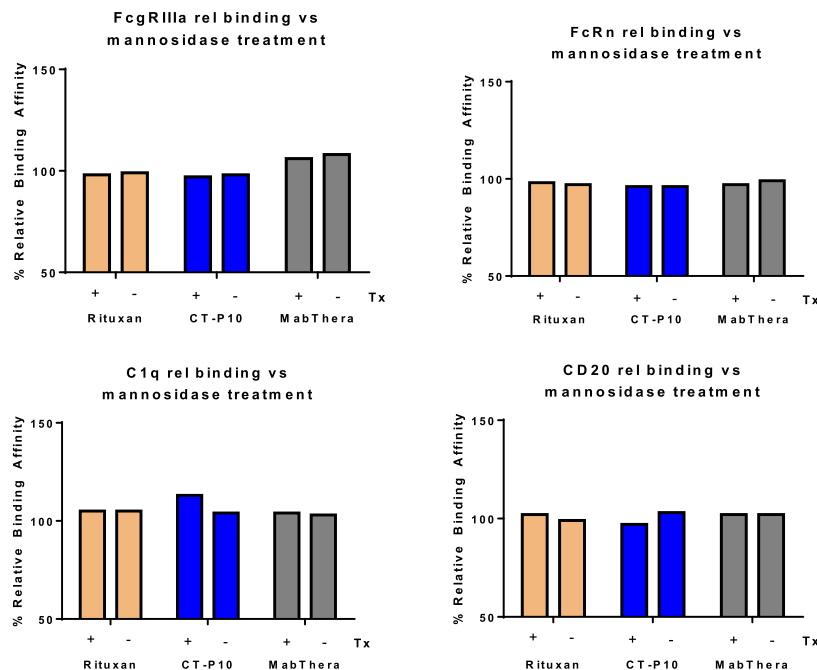
Reviewer comment: *The following in vitro activity trends were observed for increasing levels of agalactosylation in CT-P10, Rituxan, and MabThera:*

- *FcγRIIIa and FcRn very modestly decrease with increasing concentrations of galactosidase, where the activities are above 80% at the highest galactosidase treatment.*
- *C1q and CD20 bindings do not significantly change with galactosidase treatment. These data do not support relationship between terminal galactose and C1q or CD20 bindings for rituximab.*

Overall, in ranges relevant to the variability observed between CT-P10, Rituxan, and MabThera, small differences in agalactosylation levels and concomitant increases in afucosylated glycans (G0 and Man5) do not appear to overtly impact FcγRIIIa, FcRn, C1q, or CD20 binding.

3.2.R.4.1.3 Amannosylation

CT-P10 failed tier 2 similarity for levels of Man5 because they were higher than Rituxan and MabThera. These studies were to investigate the relationship between mannosylation and biological activity. Rituxan, CT-P10, and MabThera were treated with mannosidase (0.2 U/mg) at 37 °C for 20 hours. The profiles were analyzed by Bio-LC to determine the oligosaccharide profile and then the same binding assays were used to determine functional impact. Table 3.2.R.4-12 in the submission shows that the mannosidase treatment reduced the %Man5 to not detectable level (and not other oligosaccharides) in each of the Rituxan, CT-P10, and MabThera samples, respectively from 1.82%, 3.74%, and 1.97%. The FcγRIIIa, FcRn, C1q and CD20 bindings were analyzed in these samples. The following reviewer-generated graphs summarize the data (in Tables 3.2.R.4-13 through 3.2.R.4-16).



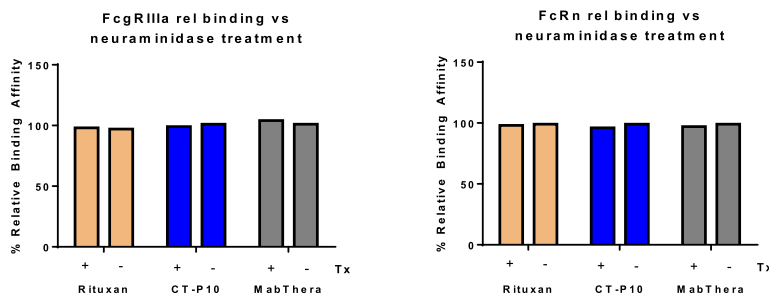
Reviewer comment: Mannosidase treatment (resulting in no detectable levels of Man5) had no impact on FcγRIIIa, FcRn, C1q and CD20 binding.

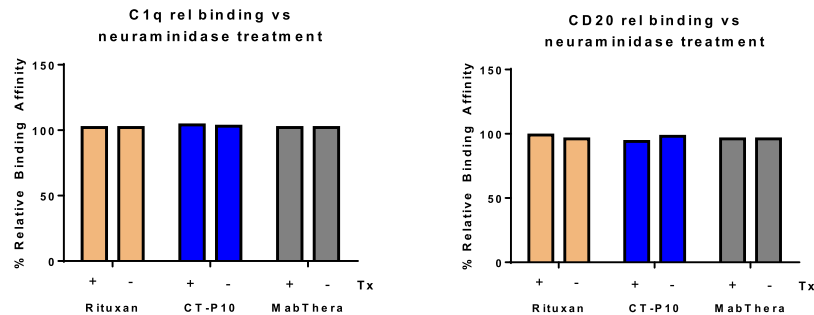
3.2.R.4.1.4 Asialylation

CT-P10 passed tier 3 similarity for sialic acid analysis although there were minor differences noticed in the 3-way similarity data (CT-P10 molar ratio for sialic acid was 0.08 while Rituxan and MabThera were 0.10, see Table 3.2.R.3-2 Summary Data for the 3-Way Biosimilarity Assessment, p.20 in section R13).

While this attribute has a low criticality and the difference is unlikely to be clinically significant, Celltrion conducted this study to address residual uncertainty. To investigate the relationship between terminal sialic acid and biological activity, the DP lots were treated with neuraminidase (166.7 U/mg) at 37 °C for 20 hours. This treatment reduced the sialic acid % to not detectable level in each of the Rituxan, CT-P10, and MabThera samples, respectively from 4.16%, 2.56%, and 2.28% (Table 3.2.R.4-17 in the submission).

The bindings were analyzed in these samples. The following reviewer-generated graphs summarize the data presented in the submission (in Tables 3.2.R.4-18 through 3.2.R.4-21).





Reviewer comment: The data show that asialylation or minor differences in sialic acid content between the three DPs has no apparent effect on FcγRIIIa, FcRn, C1q and CD20 binding.

3.2.R.4.1.5 Afucosylation

CT-P10 failed tier 2 similarity for afucosylated species largely because of the difference in Man5 levels. The sum of afucosylated species is known to be important for known and potential MOAs for Rituxan although it is unknown whether the differences observed between CT-P10 and Rituxan (as seen in the 3-way similarity data) are large enough to affect these MOAs. These studies were to investigate the relationship between afucosylated glycans and biological effect in ranges both relevant and well beyond the levels observed for the three products.

Highly afucosylated CT-P10 was generated by culturing cells in CT-P10 production conditions with a fucose analog designed to inhibit fucosylation. Highly afucosylated glycans were purified by MabSelect SuRe™ affinity chromatography. The afucosylation level was measured by HPAEC-PAD, and the total level was calculated as the sum of Man5, G0, G1, and G2. Table 3.2.R.4-22 compares the relative percent of various glycan species between the in-house CT-P10 RS and afucosylated CT-P10 sample.

Table 3.2.R.4-22: Oligoprofiling of Highly Afucosylated CT-P10 by HPAEC-PAD

Sample ID	Relative %							Total Afucosylated (Man5+G0+G1+G2)
	G0F	Man5	G0	G1F	G1	G2F	G2	
(b) (4)	42.06	3.47	0.65	44.29	N/D	9.54	N/D	4.12
Highly Afucosylated CT-P10	23.47	3.18	27.50	24.28	16.23	4.03	1.31	48.22

N/D: Not Detected

Eight samples of CT-P10 with a variety of levels of afucosylated glycans were generated by mixing the in-house RS (b) (4) with different amounts of the highly afucosylated CT-P10 sample as shown in Table 3.2.R.4-23.

Table 3.2.R.4-23: Mixing Ratio of In-House Reference and the Highly Afucosylated CT-P10 to Obtain Various Target Levels of Afucosylation

Sample ID	Target afucosylation (%)	Mixing Ratio	
		CT-P10 Reference (b) (4): 4.12% Afucosylated Glycans	Highly afucosylated CT-P10: 48.22% Afucosylated Glycans
Afucose_Control	4.1	1.00	0.00
Afucose_SP1	4.5	0.99	0.01
Afucose_SP2	5.0	0.98	0.02
Afucose_SP3	5.5	0.97	0.03
Afucose_SP4	7.0	0.93	0.07
Afucose_SP5	15.0	0.75	0.25
Afucose_SP6	25.0	0.53	0.47
Afucose_SP7	40.0	0.19	0.81

The level of afucosylated glycans was re-confirmed by HPAEC-PAD, and the sample integrity (intact IgG, HC+LC as measured by CE-SDS and monomer% as measured by SEC-HPLC) was confirmed (in Tables 3.2.R.4-24 and 3.2.R.4-25 of the submission). *Note that these samples have levels of afucosylation higher than any of the three DPs (see Table Table 3.2.R.3-2 in RI3).*

Reviewer comment: The Table 3.2.R.4-26 summarizes the afucosylation level (%) FcγRIIIa binding and ADCC Reporter assay activity for each engineered sample. While I could not find how relative ADCC %, EC50 is calculated, the characterization section 3.2.S.3.1 Elucidation of Structure and Other Characteristics (on page 59) describes that FcγRIIIa binding (%) is calculated as follows:

$$\text{Relative Binding Affinity (\%)} = \left(\frac{\text{Kd of in-house RS}}{\text{Kd of Sample}} \right) \times 100$$

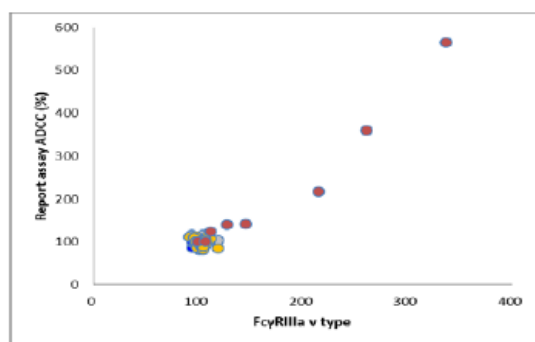
Reviewer comment: These afucosylated samples were also used in Attachment 2 pre-qualification report for ADCC (PBMC) biological assay to test the assay sensitivity. I have added the data from Table 5 of Attachment 2 to Table 3.2.R.4-26 below to inform the discussion about afucosylation and associated biological activities. The results are mostly the same where significant changes in ADCC activity are seen after sample Afucose_SP3 (>5.32% afucosylation level).

Table 3.2.R.4-26: FcγRIIIa Binding Affinity and ADCC Reporter Assay Results for CT-P10 Samples with Varying Afucosylation Levels (Modified by reviewer)

Sample ID	Afucosylation level (%)	FcγRIIIa Binding Affinity (% K _D)	Reviewer recalculated FcγRIIIa affinity [1/(%, K _D)]	ADCC Reporter Assay (% EC ₅₀)	ADCC PBMC Assay (% EC ₅₀)
Afucose_Control (b) (4)	4.0	100	100	100	100
Afucose_SP1	4.4	107	93	101	112

Afucose_SP2	5.0	112	89	124	118
Afucose_SP3	5.3	128	78	140	116
Afucose_SP4	6.4	146	68	142	143
Afucose_SP5	12.7	215	47	217	237
Afucose_SP6	20.6	261	38	359	407
Afucose_SP7	34.9	337	30	565	661

Reviewer comment: The data above show that afucosylated levels appear to correlate (although perhaps not linearly) with FcγRIIIa binding and ADCC reporter results. Samples with higher levels of afucosylation have better FcγRIIIa binding. While this data does not directly address whether 2.5% versus 4% afucosylated glycans (observed difference between Rituxan and CT-P10 DP batches) makes a measureable potency difference, the data show that ~0.25-0.55% increase in afucosylation above the CT-P10 average 4.75% level shows a substantial increase in ADCC reporter assay potency and FcγRIIIa binding (see Figure 3.2.R.4-6 below). This finding has implications for the CT-P10 control strategy with respect to afucosylated species specifications (see section 3.2.S.4.1 Specifications).



Rituxan®, CT-P10, MabThera® and samples of afucosylated CT-P10 are shown in yellow, blue, grey and red, respectively

Figure 3.2.R.4-6: Scatter Plot of FcγRIIIa V Type Binding Affinity versus Reporter Assay ADCC Activity with Samples of Variably Afucosylated CT-P10

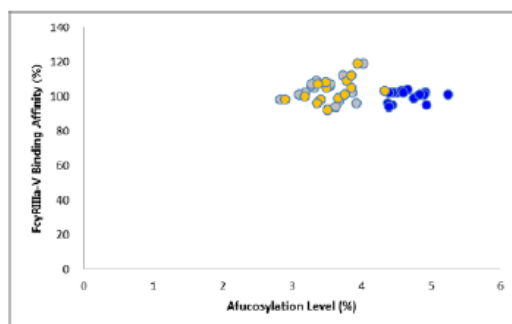
The more convincing justification for correlation between relevant levels of afucosylation and biological activities comes from an assessment of independent lots used in the similarity assessment.

The sponsor used the 3-way similarity data to generate and analyze Pearson correlations between (1) afucosylated glycan levels and reporter assay ADCC and (2) between FcγRIIIa V-type binding affinity and reporter assay ADCC both with and without the samples of variably afucosylated CT-P10. They also performed simple linear regression modeling.

Reviewer comment: Note that these analyses only determine the strength of a linear relationship between variables and it is possible that within some afucosylation ranges, the relationship is linear, while in other ranges there is no such relationship. The range most relevant for this BLA is approximately 2.5-5%.

The Figures 3.2.R.4-1 - 3.2.R.4-6 in the submission showed are scatter plots of the similarity lots with and without the engineered samples data for afucosylation levels in

correlation to FcγRIIIa-V binding and ADCC reporter assay (an example plot is copied below). Note that one CT-P10 lot (Lot 14CC002) has 5.24% afucosylated (Man5+G0) species by global oligosaccharide analyses.



Rituxan®, CT-P10 and MabThera® are shown in yellow, blue and grey, respectively.

Figure 3.2.R.4-1: Scatter Plot of Afucosylated Glycan Level versus FcγRIIIa V Type Binding Affinity without Samples of Variably Afucosylated CT-P10

Reviewer comment: I performed my own correlations restricted to the 12 Rituxan lots, 12 MabThera lots (included only lots included in the 3-way similarity assessment), and 15 CT-P10 lots that had oligosaccharide profiling, FcγRIIIa binding and ADCC reporter assay activity data. I found no evidence of linear correlation between afucosylation levels and FcγRIIIa or ADCC reporter assay activity. The engineered afucosylated sample experiment showed that these functional assays can detect the functional change by $\geq 1\%$ difference in the afucosylation. The correlative analyses only became statistically significant when the engineered samples (beyond $\sim 5.3\%$ afucosylated species) were included in the analysis. Therefore, the totality of studies related to afucosylated species show that the measured differences in Man5 levels between CT-P10 and Rituxan and MabThera may not have a functional impact on FcγRIIIa, FcRn, C1q, or CD20 binding.

The 3-way PK similarity study is used to address residual uncertainty of Man5 differences otherwise cannot be addressed by analytical similarity studies, given that Man5 level may affect PK (see Table 3.2.R.3-2 in RI3).

Overall, these glycosylation studies show that observed differences in glycan profiles between CT-P10, Rituxan, and MabThera do not impact biological functions related to FcγRIIIa, FcRn, C1q, or CD20 binding and do not preclude an assessment of being highly similar to Rituxan.

3.2.P.8.1.1.5 and 3.2.P.8.3.1.8 Forced Degradation Study for CT-P10 and Reference Product

In the initial BLA submission, the sponsor used only one DP lot of each product in the forced degradation study. In response to our IR, the sponsor performed the forced degradation study with additional DP lots. The studies are performed in a side-by-side setting. Below is a reviewer-modified table (from sponsor's Table 3.2.P.8.1-16) with a summary of lots used in the forced degradation studies.

**Lot Information for Forced Degradation Studies of CT-P10 500 mg Drug Product**

	EU-approved MabThera [®]	CT-P10 Drug Product	US-licensed Rituxan [®]
Lot No.	N7029 [±] , N7134B03, N7155B02	15C2C04 [§] , 15C2C04, 15C2C06	590472, 3055960, 3122526
Manufacturer	Roche	CELLTRION Plant (Korea) (b) (4)	Roche
Manufacturing Date	N/A	31May2015, 31May2015, 8Jun2015	N/A
Exp. Date	Feb. 2017, Jan 2019, Jun 2019	N/A	Jun. 2016, Apr 2018, Jan
Age at Study	12 M, 14 M, 9 M	2 M	20 M, 23 M, 14 M

Note: Forced degradation study was initiated on 19Aug2015. N/A: Not applicable

[±] Was used in clinical study

[§] This is manufactured by Process 4 DP and Process D DS.

Reviewer comment: In an IR sent on 8/7/2017 (comment #3), we requested: (A) justification for the chosen stress conditions and for excluding certain stresses (e.g., freeze/thaw and agitation) or biological assays from analysis (i.e., ADCC and CDC assays), and (B) that additional lots be tested to ensure a meaningful comparison between the products.

Regarding (A): The sponsor responded on 8/18/2017 describing that the types of stress tested in the forced degradation study are justified based on potential circumstances that could be encountered during production, shipping, and/or storage. The sponsor notes that some small-scale studies show no impact on product quality (freeze/thaw and mechanical stress) and so those conditions are not tested in the forced degradation study. Data in 3.2.S.2.5 confirmed no product quality impact from freeze/thaw. The sponsor also states that various levels of stress were screened for the comparative forced degradation study that are neither too harsh nor too mild to enable meaningful degradation rates and pathways to be detected. For some conditions, more than two intensities of stress were screened. The selected set of analytical methods for the forced degradation was used to detect, separate, and quantify any potential degradation products and so although ADCC and CDC assays are not employed here, attributes that impact that signaling (oligosaccharide profiling, and binding to CD20, FcγRIIIa, and C1q) were measured during this study. The response is acceptable for the similarity assessment. For product characterization purposes, additional clarification regarding mechanical stress will be requested.

Regarding (B): On 11/15/2017, the sponsor submitted Quality amendment 3 which includes forced degradation study results from “two additional lots” for each product. It appears that the sponsor may have re-tested one CT-P10 DP lot (15C2C04) for which forced degradation data was already submitted in the original BLA submission. Nonetheless, they do have at least 2 CT-P10 DP lots in their forced degradation studies. The study results are reviewed below. The response is acceptable.

The following reviewer-generated table gives a detailed summary of the forced degradation data from 2-3 lots of each product. The similarity is based on visual comparison of the provided data and graphs.

Test methods are IEC-HPLC, SEC-HPLC, Peptide Mapping (LC/MS), CE-SDS (non-reduced, reduced), IEF, Oligosaccharide profiling (Bio-LC), FcRn Binding Affinity, FcγRIIIa Binding



Affinity (both by SPR), C1q Binding Affinity (ELISA), and CD20 Binding Affinity (CELISA). In some cases, the sponsor claims the samples were stressed to the point that some biological interactions could not be evaluated due to atypical sigmoidal curves or lack of saturation (e.g., for CD20 binding or Fc receptor binding curves). Also, in an IR (sent on 8/7/2017, comment #3c), we requested the raw data for the biological assays that were considered inconclusive for the stressed samples. The sponsor provided binding curves (in corresponding amendments of 8/18/2017 and 11/15/2017 submissions). In these cases, where a numerical value was not obtained, I compared the binding curves to assess whether the data support similar degradation patterns between the stressed CT-P10, Rituxan, and MabThera samples. My assessments are included in the reviewer-generated table below.

Condition	Detailed findings	Support of similarity
Oxidative stress H ₂ O ₂ (0.005% or 0.01%) at 2 – 8 °C for 6 hr	↑↑HC Met256 oxidation ↑All other Met oxidation (HC Met34, Met81, Met432, and LC Met21) No major changes in other attributes Functional assays (FcRn, FcγRIIIa, C1q, CD20) do not show any overt trends, suggesting that this stress does not significantly affect Fab or Fc binding interactions. Reviewer comment: A range of conditions were tested. The oxidative stress study setting and results are acceptable.	Yes
UV stress 20 w/m ² , 25 °C, 60 % RH for 10 or 20 hr	↑↑acidic peaks and ↓↓main peak 4, slight ↓basic peaks ↓monomer and ↑HMW ↑↑HC Met256 and Met432 oxidation ↑All other Met oxidation (HC Met34, Met81, and LC Met21) slight ↓intact and LC+HC No overt difference in FcγRIIIa or C1q binding; FcRn could not be assessed because saturation was not reached. CD20 binding could not be assessed due to altered curves. No overt trends in other attributes. Reviewer comment: UV stress causes several molecular changes in all three products. In a preliminary test, IEC was performed for two chosen stress conditions. Modest to large shifts in IEC profiles are noted, perhaps affecting some functional interactions, rendering them not quantifiable. Comparison of CD20 and FcRn binding curves support similar degradation pathways and rates.	Yes
High Temperature Stress 50 °C for 24 or 60 hr	↑acidic peaks (~6% change in sum of acidic peaks), and ↓main peak 4 slight ↑deamidation of HC Asn55, Asn388 (50-100% change). Note that there are no deamidation changes when two additional lots of each DP are tested. No overt trends in FcRn, FcγRIIIa, or C1q binding. ↓CD20 binding (20% change in potency). Note that CD20 binding is not quantifiable for the two additional lots of each DP. No overt trends in other attributes. Reviewer comment: A range of time points were screened by SEC and IEC. Samples held for 24 hours have almost no discernable change in SEC or IEC when compared to control, yet CD20 binding was reduced or unevaluable.	Yes

	<i>Comparison of CD20 binding curves support similar degradation pathways and rates.</i>	
Acid stress pH 3.3, 25 °C for 10 or 20 hr	Slight ↓acidic peaks and ↑main peak ↓monomer and ↑↑HMW No overt difference in FcγRIIIa or C1q binding; FcRn curves did not reach saturation and CD20 binding could not be assessed. No overt trends in other attributes. Reviewer comment: <i>In a preliminary test, SEC was performed for two stress chosen conditions. Significant shifts in SEC profiles are noted, perhaps affecting some functional interactions, rendering them not evaluable/quantifiable. Comparison of CD20 and FcRn binding curves support similar degradation pathways and rates.</i>	Yes
Base Stress pH 10.5, 25 °C for 48 or 96 hr	↑↑acidic peaks and ↓↓main peak 4 slight ↓monomer, and slight ↑HMW ↑↑deamidation HC Asn388, slight ↑HC Asn55 slight ↑oxidation HC Met256. <i>No trend in the additional lots tested</i> slight ↓intact and LC+HC maybe slight ↓C1q binding FcRn could not be evaluated. No over trends in other attributes. Reviewer comment: <i>In a preliminary test, IEC was performed for incubation times ranging from 24-120 hr. Significant changes in peak profile are already observed by 24 hours. Comparison of FcRn binding curves support similar degradation pathways and rates.</i>	Yes

Reviewer comment: *The high temperature and base stress data suggest that deamidation of HC Asn55 located in the variable region (but not HC Asn388) may correlate with decreased CD20 binding. Deamidation at HC Asn55 and Ans388 do not appear to significantly affect FcγRIIIa or C1q binding. The impact that some of the described PMTs may have biological interactions is not clear because the sponsor claims that the samples were so stressed that quantification of binding affinities were not possible. Our evaluations of binding curves help address this gap in quantitative data. Overall, the forced degradation studies support that (in the conditions evaluated) CT-P10, Rituxan, and MabThera have similar degradation pathways and degradation rates.*

3.2.R.4.2 Biological activities supporting the extrapolation to NHL

(b) (4)

To support the extrapolation of the clinical results obtained from the RA indication

(b) (4)

(b) (4) Celltrion analyzed the ability of Rituxan, CT-P10, and MabThera to induce apoptosis, CDC, ADCC and ADCP using different cell lines and donor cells obtained from NHL and CLL patients. These studies were performed using 3 lots of each of Rituxan (lots 569328, 616346, 680572), CT-P10 (lots 15C2C04, 15C2C05, 15C2C06), and MabThera (lots H0688B04, H0739B03, N7029B05), which were also included in the 3-way similarity assessment. The relative potency was calculated relative to the CT-P10 in-house RS

(b) (4)

(b) (4). The RS was the same as was used for the studies of glycosylation and biological activity.



The sponsor states that, except for the cells used, the assays performed to support extrapolation were the same as those described in the similarity section. Sections 3.2.R.5.2.9 through 3.2.R.5.2.9 in RI 5 (Method Description) give additional information about the assays/reagents used in the extrapolation studies including assay parameters (concentrations, wavelengths, etc.), performance (linearity), how relative potency is expressed, and lot numbers of purchased patient cells.

Reviewer comment: *The data and assay details can be found in the submission. Briefly, the potency of the three lots of each DP tested overlapped at each concentration point tested for each of the four potency assays (Apoptosis, CDC, ADCC, and ADCP). From a CMC perspective, the main similarity assessment and assays described previously adequately reflect known primary and secondary MoA for Rituximab and so neither the CMC nor the clinical review divisions are relying on data submitted in this section to inform the similarity assessment.*



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Joel
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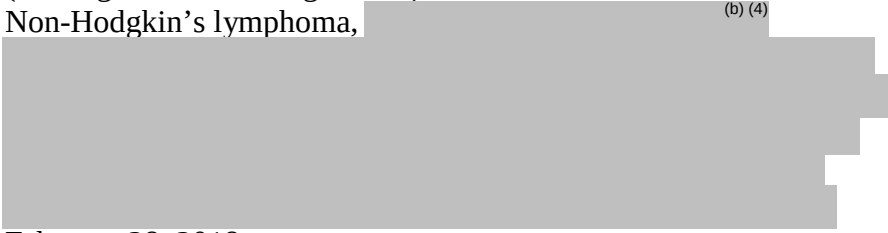


U.S. FOOD & DRUG
ADMINISTRATION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

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

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Date: January 9, 2018
To: Administrative File, 761088/0
From: Scott Nichols, Ph.D., Reviewer, OPQ/OPF/DMA/BIV
Through: Reyes Candau-Chacon, Ph.D., Quality Assessment Lead, OPQ/OPF/DMA/BIV
Subject: New 351(k) Biosimilar Biologics License Application (BLA)
US License Number: 1996
Sponsor: Celltrion, Inc.
Manufacturing Site: Celltrion, Inc. Plant II (CLT2), 20, Academy-ro 51 beon-gil, Yeonsu-gu, Incheon, 22014, Republic of Korea
Product: Truxima (proposed), CT-P10, CD20-directed cytolytic antibody
Dosage: sterile liquid for intravenous infusion (500 mg/50 mL, 100 mg/10 mL)
Indication: Non-Hodgkin's lymphoma, (b) (4)

Action Date: February 28, 2018

Conclusion and Approvability Recommendation:

The drug substance portion of BLA 761088, as amended, is recommended for approval from a microbial control and a microbiological product quality perspective. Please refer to the review memo from Dr. Candace Gomez-Broughton for an assessment of sterility assurance and microbiological product quality for the drug product (DP) portions of the BLA.

Product Quality Microbiology Assessment: Drug Substance

Drug Substance Quality Microbiology Information Reviewed

Sequence number	Date	Description
0001		Original BLA
0011	25 July 2017	Response to Information Request Sent on 7 July 2017
0020	23 October 2017	Response to Information Request Sent on 13 October 2017
0028	3 January 2018	Response to Information Request Sent on 21 December 2017

Review Narrative, Module 3.2

S DRUG SUBSTANCE

S.1 General Information

CT-P10 is a humanized monoclonal antibody of the IgG1 subclass that binds to CD20 that is expressed on the surface of pre-B and mature B-lymphocytes (but not haemopoietic stem cells, pro-B cells, normal plasma cells, or other normal cells). Ligation of CD20 by CTP-P10 promotes B-cell lysis. The mechanisms of action are apoptosis, complement-dependent cytotoxicity, and antibody-dependent cellular cytotoxicity. CT-P10 is expressed heterologously in Chinese Hamster Ovary (CHO) cells that secrete the antibody into the growth medium. (b) (4)

The DP is a sterile liquid for intravenous infusion.

S.2 Manufacture

S.2.1 Manufacturer(s)

The following facilities are used for the manufacture, release testing, and stability testing of CT-P10 drug substance:

- (b) (4)
Storage of the MCB and WCB
FEI: (b) (4)
- (b) (4) :
Testing of the MCB, WCB, (b) (4); Viral testing of the CT-P10 unprocessed bulk
FEI: (b) (4)
- (b) (4) : *Testing of the MCB, WCB, and (b) (4); Mycoplasma testing of the CT-P10 unprocessed bulk*
FEI: (b) (4)

- [REDACTED] (b) (4): *Sterility Testing of the* (b) (4)
FEI: [REDACTED] (b) (4)
- CELLTRION, Inc. Plant [REDACTED] (b) (4) 23, Academy-ro, Yeonsu-gu, Incheon, 22014, Republic of Korea: *Release of CT-P10 drug substance, Stability testing of CT-P10 drug substance, Storage of the MCB and WCB*
FEI: 3005241015
- CELLTRION, Inc. Plant [REDACTED] (b) (4) 20, Academy-ro 51 beon-gil, Yeonsu-gu, Incheon, 22014, Republic of Korea: *Production of CT-P10 drug substance, Release of CT-P10 drug substance, Release testing of CT-P10, Stability testing of CT-P10 drug substance, Testing of the MCB, WCB, and* [REDACTED] (b) (4)
FEI: 3005241015

Reviewer Comment:

The drug substance manufacturing facility, CELLTRION PLANT [REDACTED] (b) (4) FEI: 3005241015), has been adjudicated OAI (official action indicated) status after a 12-item FDA 483 Form was issued after a surveillance inspection conducted on 22-26 May 2017 and 29 May – 2 June 2017.

S.2.2 Description of Manufacturing Process and Controls

(b) (4)





Scott
Nichols

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Reyes
Candau-Chacon

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

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

Date: 12/04/2017
To: Administrative File, STN 761088
From: Thuy T. Nguyen, Facility Reviewer, CDER/OPQ/OPF/DIA
Endorsement: Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA
Subject: Original BLA
US License: 1996
Applicant: Celltrion, Inc.
Mfg. Facility: Drug Substance and Drug Product: Celltrion, Inc.
FEI 3005241015

Product: Truxima
Dosage: 500 mg/ 50 mL, 100 mg/ 10 mL
Indication: Non-Hodgkin's lymphoma, (b) (4)

Due Date: 1/8/2018

RECOMMENDATION: This submission is recommended for **Withhold** from a facility review perspective.

CR deficiency: During a recent inspection of the Celltrion, Inc. (FEI 3005241015) manufacturing facility for this BLA, our field investigator observed objectionable conditions at the facility and conveyed that information to the representative of the facility at the close of the inspection. Satisfactory resolution of the observations is required before this BLA may be approved.

SUMMARY

The subject BLA proposes manufacture of Truxima drug substance (DS) and drug product (DP) at Celltrion, Inc. (FEI 3005241015). A post-approval for (b) (4) and GMP surveillance inspection was conducted from May 22 – June 02, 2017 resulted in an Official Action Indicated (OAI) status with Warning Letter Recommendation. The Warning Letter is currently under review by senior management team in Office of Manufacturing Quality (OMQ). A pre-license inspection can not be

conducted until the OAI status is cleared by OMQ. Please refer to the table below for Master Cell Bank and testing facilities for DS and DP.

ASSESSMENT

DRUG SUBSTANCE AND DRUG PRODUCT FACILITIES

• 3.2.S.2.1 DS and DP Manufacturers.

The sites proposed for Truxima DS and DP manufacture, cell banking operations, and testing are presented below in **Table 1**.

Table 1: Proposed Sites for Truxima DS and DP Manufacture, Cell Banking and Testing Operations

Site Name/Location	FEI/ Profile	Responsibilities	Facility Status
Celltrion, Inc. 23, Academy-ro Plant 1 Yeonsu-gu Incheon, S. Korea	3005241015 CBI/SVS	Manufacturing Truxima of DS, DP, release and stability testing MCB and WCB Storage	Unacceptable due to OAI status
(b) (4)	(b) (4) CTL	Viral testing of CT-P10 unprocessed bulk; Testing of the MCB, WCB and (b) (4)	No Further Evaluation
(b) (4)	(b) (4) CTL	Sterility testing of (b) (4)	No Further Evaluation
(b) (4)	(b) (4) CTL	Mycoplasma testing of CT- P10 unprocessed bulk; Testing of the MCB, WCB, (b) (4)	No Further Evaluation
(b) (4)	(b) (4) CBI	MCB and WCB storage	No Further Evaluation
(b) (4)		Production of the MCB and WCB	No Further Evaluation

(b) (4)

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Reviewer Comment 1: *The facilities for manufacture of DS and DP are adequately described.*

- **Current Prior Approval Inspection Decisions**

- o Celltrion, Inc. Incheon, Republic of Korea DS and DP Truxima manufacturing and testing site.

- Profile CBI and SVS. A Pre-license inspection was not scheduled due to the facility's current OAI status as a result of a post-approval inspection conducted by ORA. The inspection resulted in a Warning Letter recommendation. As noted above under SUMMARY section, a pre-licensing inspection will not be scheduled until the facility's OAI status is cleared by OMQ.

Reviewer Comment 2: *OPF/DIA recommends Withhold of the manufacturing and testing facilities associated with the manufacture of Truxima due to Celltrion's current compliance status.*

- **3.2.S.2.2. Overview of Truxima DS Manufacturing Operations Conducted at Celltrion, Inc..**

(b) (4)

CONCLUSION

Adequate descriptions were provided for the Truxima DS and DP at the Celltrion facility but unable to verify by inspection. A post-approval for (b) (4) and GMP inspection was conducted from May 22 – June 02, 2017 resulting in Indicated (OAI) status with Warning Letter Recommendation. The Warning Letter is currently under review by senior management team in Office of Manufacturing Quality (OMQ). A pre-licensing inspection can not be conducted until the OAI status is cleared by OMQ. Master Cell Bank and testing facilities for DS and DP are acceptable. The Celltrion facility is recommended for WITHHOLD from a facilities assessment standpoint.

Thuy T. Nguyen, M.P.H.
Facility Reviewer
OPF Division of Inspectional Assessment
Branch 1

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



Thuy Thanh
Nguyen

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Zhihao Peter
Qiu

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/s/

ESTHER H PARK
11/28/2018