CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761091Orig1s000

PRODUCT QUALITY REVIEW(S)

Complete Response
BLA 761091
Trastuzumab-pkrb
Celltrion, Inc.

Summary of Quality Assessments

I. Primary Reviewer Summary Recommendation

This memo contains the review for the responses to the CR comments and additional comments in the CR letter for Drug Product. Trastuzumab-pkrb drug product is manufactured and supplied as a 420 mg/vial presentation, which is the same as U.S.-licensed Herceptin. The drug product is a sterile, lyophilized powder for intravenous infusion and is supplied as a multi-dose vial. The formulation is identical except for an increase in α,α-trehalose dihydrate (from 381 to 839 mg/vial), which is (b) (4) During review of the original application, it was determined that the drug product release specification must be adjusted to ensure that the recoverable protein content meets the minimum label claim of 420 mg, which would require adjustment of (b) (4) control strategy. The sponsor's newly proposed control strategy ensures that the label claim for protein content will be met. Responses to additional comments are acceptable. CMC recommends approval for BLA 761091.

II. List of Deficiencies to be Communicated for Drug Product

None. All information requests were answered satisfactorily during the review cycle.

Reviewer comment: *Refer to BLA 761091 Drug Substance review for the OBP CMC Review Data Sheet.*

Description of Drug Product

Review

1.11.1. Quality Information Amendment

AC#3
Responses to Complete Response Additional Comment 3: We acknowledge receipt of your process characterization studies performed in order to establish acceptable ranges for lyophilization process parameters on February 28, 2018. The acceptability of your updated control strategy (process parameters and associated acceptable ranges) for lyophilization will be a review issue that will be addressed in the next review cycle upon receipt of your resubmission.

Reviewer comment: *Four IR responses were received regarding defining the proven acceptable ranges and the critical process parameters for the lyophilization process: IR response SN0027 (dated 1/24/18), IR response SN0034 (dated 2/20/18), IR response SN0039 (dated 2/28/18), and CR response SN0041 (dated 6/15/18). IR responses SN0027 (dated 1/24/18) and SN0034 (dated 2/20/18) were reviewed during the review cycle of the original application in Section 3.2.P.3.5.9 Lyophilization of the Drug Product review. IR response SN0039 (dated 2/28/18) and CR response SN0041 (dated 6/15/18) are reviewed here.*

(b) (4)

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(b) (4)

AC#6
Responses to Complete Response Additional Comment 6: In your resubmission, provide updated data to the section indicated from the following ongoing studies: c. The ongoing CT-P6 DS and DP stability studies (in sections 3.2.S.7 and 3.2.P.8).

Reviewer comment: *CT-P6 DP stability studies in Section 3.2.P.8 submitted on 10/19/18 (SN0048) was reviewed here.*
Updated long-term stability studies (at 5±3°C) were submitted. For DP Process A lots (13A3C002, 13A3C003, 13A3C004), data was provided for 48 months during the review cycle of the original application. This updated submission provides data for the 60-month storage time.

Reviewer comment: *No adverse trend is observed for DP at the 60-month storage time compared to the 48-month storage time and earlier time points. IE-HPLC Peak 5, which is the active peak, is 64.4 - 64.8%, bioactivity is 1.0 x 10^4 U/mg, and HER2 binding affinity is 91.29 – 105.42% for the 60-month time point and met the acceptance criterion. Reconstitution time did not show any increase compared to the 48-month time point and met the acceptance criterion.*

For DP Process B lots (15A3C01, 16A3C01, 16A3C02), data was provided for 18 months during the review cycle of the original application. This updated submission provides data for the 24 months and 30 months-time points.

Reviewer comment: *No adverse trend is observed for DP at the 24- and 30-month storage time compared to earlier time points. IE-HPLC Peak 5 is 63.5 - 64.2%, bioactivity is 1.0 x 10^4 U/mg, and HER2 binding affinity is 99.30 – 107.16% for the 30-month time point and met the release acceptance criterion. Reconstitution time did not show any increase compared to the 18-month time point and met the acceptance criterion.*

Based on the long-term stability data, the sponsor is proposing a shelf-life at 5±3°C of 60 months, which is acceptable.

AC#6-d

Responses to Complete Response Additional Comment 6-d: In your resubmission, provide updated data to the section indicated from the following ongoing studies: d. The shipping validation of the finished CT-P6 drug product (in section 3.2.P.3.5).

Reviewer comment: *Section 3.2.P.3.5.12 Shipping Validation of Unlabeled/Finished Drug Product was updated for shipping validation and was reviewed here.*

(b) (4)

Reviewer comment: *Shipping validation for unlabeled DP and unlabeled diluent showed that the shipping container was maintained at 2 - 8°C during transportation. Product quality data showed that compared to the batch analyses data for 15A3C01 for CT-P6 420 mg and 13I1C001 for diluent, no*

adverse trend was observed in the unlabeled DP after shipping and container closure integrity was maintained.

(b) (4)

Reviewer comment: *Shipping validation for finished DP packaged with diluent showed that the shipping container was maintained at 2 - 8°C during transportation. Product quality data showed no adverse trend for the finished DP after shipment compared to the batch analyses data and container closure integrity was maintained.*

Regarding the potency assay in Table 3.2.P.3.5-36 and Table 3.2.P.3.5-39, which were listed as complement-dependent cytotoxicity, the sponsor confirmed that they were typographical errors and the potency assay was the in vitro bioactivity test (SN0054 received 11/27/18). The sponsor's response is acceptable.

AC#6-e

Responses to Complete Response Additional Comment 6-e: In your resubmission, provide updated data to the section indicated from the following ongoing studies: e. The stability study on three in-use PS20 lots for up to 12 months, committed in your 2/20/18 response (in Section 3.2.P.4).

The sponsor plans to conduct in-use stability study of the polysorbate 20 (PS20) to establish control strategy for PS20. The information on in-use stability samples and test time points currently available are provided in Table 1.

(b) (4)

Reviewer comment: *The studies to establish the control strategy for PS20 is acceptable. The study is ongoing.*

CR#2

Responses to Complete Response 2: Per the "Guidance for Industry: Allowable Excess Volume and Labeled Vial Fill Size in Injectable Drug and Biological Products" (https://www.fda.gov/ucm/groups /fdagovpublic /@fdagov-drugs-gen/documents/document/ucm389069.pdf), "In the case of drug products requiring reconstitution, the product should be designed to meet the label claim and acceptable overfill, and allow for correct dosing". Adjust the Drug Product (DP) release specification of (b) (4) mg/ml to ensure that the recoverable protein content at the lower limit of the acceptance criterion will consistently meet the label claim of 420 mg.

In order to meet the label claim of 420 mg, the sponsor has adjusted the (b) (4) to ensure a CT-P6 DP vial content of (b) (4) mg/vial (see review of CR#3 below). Historical release data from 12 DP lots (Table 1, not copied) had protein content range of (b) (4) mg/mL. Sections 3.2.P.2.3, 3.2.P.5.1, 3.2.P.5.4, 3.2.P.5.6, 3.2.P.8.1, 3.2.P.8.2, and 3.2.P.8.3 have been updated accordingly.

Reviewer comment: *The new acceptance criteria for protein content of (b) (4) mg/mL can be met by the sponsor's current DP manufacturing process and meets the label claim and is therefore, acceptable. See DS review for this complete submission for review of the updated DS manufacturing control strategy, as the (b) (4) was adjusted to meet this requirement.*

CR#3

Responses to Complete Response 3: To support the licensure of CT-P6 420 mg/vial, the CT-P6 DP manufacturing process and controls should be set to ensure the appropriate deliverable volume and protein concentration after reconstitution in each DP vial to meet the label claim. Insufficient information and data on the approach and method used to derive the overfill volume ((b) (4)%; Section 3.2.P.2.2) and fill weight limits ((b) (4) mg; Section 3.2.P.3.4) were provided in the BLA to support fill volume/weight control strategy. Provide additional information (e.g., capability of the filling machine) and data (e.g., from process development results and calculations used to derive the fill weight controls) to support current fill volume/weight limits.

Overfill

CT-P6 420 mg DP is filled into vials with a (b) (4)% overfill. The (b) (4)% overfill was determined based on the (b) (4) (as shown in Table 1). Eight DP vials were reconstituted with 20 mL of BWFI. The resulting DP total volume was (b) (4) mL, which was an approximate (b) (4)% volume increase due (b) (4).

(b) (4)

Reviewer comment: *The justification for the overfill amount is acceptable.*

(b) (4)

(b) (4)

(b) (4)

An IR was sent on 11/13/18 regarding the specification for extractable volume, which is currently set at (b) (4) mL. Because this specification could technically allow an extractable volume of (b) (4) mL, the sponsor was asked to provide data to at least one decimal place to support that the product is designed with an acceptable volume to meet the label claim and allow for adequate dosing. In addition, these data should be used to propose extractable volume acceptance criteria for drug product release that contain at least one decimal place. In the IR response received on 11/27/18, the sponsor was not able to provide data to one decimal place because extractable volume was evaluated with specification effective at the time of analysis. However, the extractable volume results from 12 DP lots show that extractable volume was 21 mL, indicating that all lots were above (b) (4) mL. The sponsor has agreed to update the specification for extractable volume and report data to one decimal place. Sections P.2.3, P.5.1, P.5.4, P.5.6, P.8.1, and P.8.2 have been updated with the new specification. The sponsor's response is acceptable.

Shadia Zaman

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Jennifer Swisher

Digitally signed by Jennifer Swisher
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Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Biotechnology Products

LABELS AND LABELING REVIEW

Date:	November 19, 2018
Reviewer:	Vicky Borders-Hemphill, PharmD Labeling Review Specialist Office of Biotechnology Products (OBP)
Through:	Shadia Zaman, PhD, Product Quality Reviewer OBP/Division of Biotechnology Review and Research I
Application:	BLA 761091
Applicant:	Celltrion, Inc
Submission Date:	June 15, 2018
Product:	Herzuma (trastuzumab-pkrb)
Dosage form(s):	for injection
Strength and Container-Closure:	420 mg/vial in a multiple dose vial
Background and Summary Description:	The Applicant submitted a biologics license application as a proposed biosimilar to US-licensed Herceptin (trastuzumab).
Recommendations:	The prescribing information (submitted on October 29, 2018), and container labels and carton labeling (submitted on November 2, 2018) were reviewed and found to be acceptable (see Appendix D) from an OBP labeling perspective.

Materials Considered for this Label and Labeling Review	
Materials Reviewed	**Appendix Section**
Proposed Labels and Labeling	A
Other	B (n/a)
Evaluation Tables	C
Acceptable Labels and Labeling	D

n/a = not applicable for this review

DISCUSSION and CONCLUSION

We evaluated the proposed labels and labeling for compliance with applicable requirements in the Code of Federal Regulations (21 CFR 610.60 through 21 CFR 610.67; 21 CFR 201.2 through 21 CFR 201.25; 21 CFR 201.50 through 21 CFR 201.57; 21 CFR 201.100) and evaluated against recommendations in FDA Guidance and United States Pharmacopeia (USP) standards (see Appendix C).

The prescribing information (submitted on October 29, 2018), and container labels and carton labeling (submitted on November 2, 2018) were reviewed and found to be acceptable (see Appendix D) from an OBP labeling perspective.

APPENDICES

Appendix A: Proposed Labeling

Prescribing Information (submitted June 15, 2018
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Container Labels (submitted June 15, 2018)

(b) (4)

Carton Labeling (submitted June 15, 2018)

Appendix B: Other (n/a)
Appendix C: Evaluation tables

Table 1: Label[1,2] and Labeling[3] Standards

Container[4] Label Evaluation

	Acceptable
Proper Name (21 CFR 610.60, 21 CFR 201.50, 21 CFR 201.10) *for container of a product capable of bearing a full label*	☐ No ☒ Yes ☐ N/A
Manufacturer name, address, and license number (21 CFR 610.60) *for container of a product capable of bearing a full label*	☐ No ☒ Yes ☐ N/A
Lot number or other lot identification (21 CFR 610.60, 21 CFR 201.18, 21 CFR 201.100)	☐ No ☒ Yes ☐ N/A
Expiration date (21 CFR 610.60, 21 CFR 201.17)	☐ No ☒ Yes ☐ N/A
Multiple dose containers (recommended individual dose) 21 CFR 610.60	☐ No ☒ Yes ☐ N/A
Statement: "Rx only" 21 CFR 610.60 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Medication Guide 21 CFR 610.60 21 CFR 208.24	☐ No ☐ Yes ☒ N/A
No Package for container 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
Partial label 21 CFR 610.60 21 CFR 201.10	☐ No ☐ Yes ☒ N/A
No container label 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
Ferrule and cap overseal	☐ No ☒ Yes ☐ N/A

[1] Per 21 CFR 1.3 (b) *Label* means any display of written, printed, or graphic matter on the immediate container of any article, or any such matter affixed to any consumer commodity or affixed to or appearing upon a package containing any consumer commodity.

[2] Per CFR 600.3(dd) *Label* means any written, printed, or graphic matter on the container or package or any such matter clearly visible through the immediate carton, receptacle, or wrapper.

[3] Per 21 CFR 1.3(a) *Labeling* includes all written, printed, or graphic matter accompanying an article at any time while such article is in interstate commerce or held for sale after shipment or delivery in interstate commerce.

[4] Per 21 CFR 600.3(bb) *Container* (referred to also as "final container") is the immediate unit, bottle, vial, ampule, tube, or other receptacle containing the product as distributed for sale, barter, or exchange.

Comment/Recommendation: Confirm there is no text on the ferrule and cap overseal of the vials to comply with a revised United States Pharmacopeia (USP), General Chapters: <7> Labeling (Ferrules and Cap Overseals).

Response: The Applicant would like to confirm that there is no text on the top of a ferrule and a cap overseal. Only the lot number appears on the side of the ferrule without distraction from the top surface.

This is acceptable per USP General Chapters:<7> Labeling, Other statements or features including, but not limited to, identifying numbers or letters, such as code numbers, lot numbers, company names, logos, or product names, etc., may appear on the side (skirt) surface of the ferrule on vials containing injectable products, but not on the top (circle) surface of the ferrule or cap overseal. The appearance of such statements or features on the skirt surface of the ferrule should not detract from, or interfere with, the cautionary statement on the top surface.

Visual inspection 21 CFR 610.60	☐ No ☒ Yes ☐ N/A

Comment/Recommendation: Confirm there is sufficient area on the container to allow for visual inspection when the label is affixed to the vial and indicate where the visual area of inspection is located per 21 CFR 610.60(e).

Response: The Applicant confirms that the container remains uncovered for its circumference and there is sufficient area on the container to allow for visual inspection when the label is affixed to the vial.
This is acceptable.

NDC numbers 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A
Route of administration 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Preparation instructions 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
Package type term 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
Drugs **Misleading statements** 21 CFR 201.6	☐ No ☒ Yes ☐ N/A
Strength 21 CFR 201.10 21CFR 201.100	☐ No ☒ Yes ☐ N/A
Drugs **Prominence of required label statements** 21 CFR 201.15	☐ No ☒ Yes ☐ N/A

Spanish-language (Drugs) 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
FD&C Yellow No. 5 and/or FD&C Yellow No. 6 21 CFR 201.20	☐ No ☐ Yes ☒ N/A
Phenylalanine as a component of aspartame 21 CFR 201.21	☐ No ☐ Yes ☒ N/A
Sulfites; required warning statements 21 CFR 201.22	☐ No ☐ Yes ☒ N/A
Bar code label requirements 21 CFR 201.25 21 CFR 610.67	☐ No ☒ Yes ☐ N/A
Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) 21 CFR 610.68, 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
Net quantity 21 CFR 201.51	☐ No ☒ Yes ☐ N/A
Usual dosage statement 21 CFR 201.55 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Inactive ingredients 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Comment/Recommendation: Although this is not considered a partial label, the container label lacks sufficient space for this information, thus this information must appear on the carton, PI, and IFU (if applicable). See carton recommendations below.	
Storage requirements	☐ No ☒ Yes ☐ N/A
Dispensing container 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
USP monograph for Bacteriostatic Water for Injection, USP vial	☐ No ☒ Yes ☐ N/A
Packaging and Storage: Preserve in single-dose or multiple-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass, of not larger than 30-mL size. Labeling: Label it to indicate the name(s) and proportion(s) of the added antimicrobial agent(s). Label it also to include the statement "Not For Use In Newborns", in boldface capital letters on the label immediately under the official name, printed in a contrasting color, preferably red. Alternatively, the statement may be placed prominently elsewhere on the label if the statement is enclosed within a box.	

Comment/Recommendation:
Relocate the statement "NOT FOR USE IN NEWBORNS" appears directly beneath the official name per the USP monograph labeling requirements.
The applicant revised as requested

Package Label[5] Evaluation

	Acceptable
Proper name (21 CFR 610.61, 21 CFR 201.50, 21 CFR 201.10)	☐ No ☒ Yes ☐ N/A
Manufacturer name, address, and license number 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Lot number or other lot identification 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Expiration date 21 CFR 610.61 21 CFR 201.17	☐ No ☒ Yes ☐ N/A
Preservative 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Number of containers 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Strength/volume 21 CFR 610.61 21 CFR 201.10, 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Storage temperature 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Handling: "Shake Well", "Do not Freeze" or equivalent (21 CFR 610.61)	☐ No ☒ Yes ☐ N/A
Multiple dose containers (recommended individual dose) 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Route of administration 21 CFR 610.61, 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Known sensitizing substances 21 CFR 610.61	☐ No ☐ Yes

[5] Per 21 CFR 600.3(cc) *Package* means the immediate carton, receptacle, or wrapper, including all labeling matter therein and thereon, and the contents of the one or more enclosed containers. If no package, as defined in the preceding sentence, is used, the container shall be deemed to be the package. Thus this includes the carton, prescribing information, and patient labeling.

	☒ N/A
Inactive ingredients 21 CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Source of the product 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Minimum potency of product 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Rx only 21 CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Divided manufacturing 21 CFR 610.63	☐ No ☐ Yes ☒ N/A
Distributor 21 CFR 610.64	☐ No ☒ Yes ☐ N/A
Bar code 21 CFR 610.67 21 CFR 201.25	☐ No ☒ Yes ☐ N/A
Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) 21 CFR 610.68, 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
NDC numbers 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A
Preparation instructions 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
Package type term 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
Drugs **Misleading statements** 21 CFR 201.6	☐ No ☒ Yes ☐ N/A
Drugs **Prominence of required label statements** 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
Spanish-language (Drugs) 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
FD&C Yellow No. 5 and/or FD&C Yellow No. 6 21 CFR 201.20	☐ No ☐ Yes ☒ N/A
Phenylalanine as a component of aspartame 21 CFR 201.21	☐ No ☐ Yes

	☒ N/A
Sulfites; required warning statements 21 CFR 201.22	☐ No ☐ Yes ☒ N/A
Net quantity 21 CFR 201.51	☐ No ☒ Yes ☐ N/A
Usual dosage statement 21 CFR 201.55 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Dispensing container 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
Medication Guide 21 CFR 610.60 21 CFR 208.24	☐ No ☐ Yes ☒ N/A

Prescribing Information Evaluation

	Acceptable
PRESCRIBING INFORMATION	
Highlights of prescribing information	
PRODUCT TITLE 21 CFR 201.57(a)(2)	☐ No ☒ Yes ☐ N/A
DOSAGE AND ADMINISTRATION 21 CFR 201.57(a)(7)	☐ No ☒ Yes ☐ N/A
DOSAGE FORMS AND STRENGTHS 21 CFR 201.57(a)(8)	☐ No ☒ Yes ☐ N/A
Full Prescribing Information	
2 DOSAGE AND ADMINISTRATION 21 CFR 201.57(c)(3)	☐ No ☒ Yes ☐ N/A
3 DOSAGE FORMS AND STRENGTHS 21 CFR 201.57(c)(4)	☐ No ☒ Yes ☐ N/A
11 DESCRIPTION (21 CFR 201.57(c)(12), 21 CFR 610.61 (m), 21 CFR 610.61(o), 21 CFR 610.61 (p), 21 CFR 610.61 (q))	☐ No ☒ Yes ☐ N/A
16 HOW SUPPLIED/ STORAGE AND HANDLING 21 CFR 201.57(c)(17)	☐ No ☒ Yes ☐ N/A
MANUFACTURER INFORMATION 21 CFR 610.61, 21 CFR 610.64	☐ No ☒ Yes ☐ N/A

APPENDIX D. Acceptable Labels and Labeling

Prescribing Information (submitted on October 29, 2018 \\cdsesub1\evsprod\bla761091\0049\m1\us\draft-labeling-text.pdf)

(b) (4)

Carton Labeling (submitted on November 2, 2018)

(b) (4)

Vicky Borders-Hemphill

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Shadia Zaman

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Date: 11/19/2018 09:12:12AM
GUID: 583dce940076eea0edb730e401622d6d

Recommendation: Approval

BLA Number: 761091
Review Number: Second round
Review Date: November 19, 2018

Drug Name/Dosage Form	Herzuma (trastuzumab-pkrb)/ for injection
Strength/Potency	420 mg/vial
Route of Administration	Intravenous infusion
Rx/OTC dispensed	Rx
Indication	Treatment of HER2-overexpressing breast cancer
Applicant/Sponsor	Celltrion, Inc.

The BLA was initially submitted on May 30, 2017. A Complete Response (CR) letter for facility and product quality deficiencies was issued on March 30, 2018 (see CMC Executive Summary for initial BLA 761091 review cycle, dated 2/23/2018). On June 15, 2018, Celltrion submitted responses to address the deficiencies identified in the CR letter.

Product Overview

Note: There is no change in this section from my Executive Summary filed on 2/23/2018 in Panorama.

Herzuma (trastuzumab-pkrb) is a proposed biosimilar to US-Herceptin (trastuzumab). It is a humanized IgG1κ monoclonal antibody produced in CHO cells. Trastuzumab targets human epidermal growth factor receptor 2 (HER2) and when bound to HER2 on HER2-expressing cells, trastuzumab 1) inhibits HER2 receptor dimerization and downstream signaling, 2) increases destruction of the endocytic portion of the HER2 receptor 3) inhibits HER2 extracellular domain shedding, and 4) activates cell-mediated immune defenses such as ADCC activity through concomitant binding to Fcγ receptors on immune effector cells.

Trastuzumab-pkrb is produced in genetically engineered CHO (b) (4) cells. Trastuzumab-pkrb drug product, Herzuma, is manufactured to the same concentration and presentation as U.S.-licensed Herceptin at 420 mg/vial; the formulation is identical except for an increase in α,α-trehalose dihydrate (from 381 to 839 mg/vial), which is (b) (4) Herzuma drug product is supplied at 420 mg/vial as a sterile, lyophilized powder for intravenous infusion; the 420 mg presentation is a multi-dose vial. Herzuma is proposed as a treatment for HER2-overexpressing breast or metastatic gastric cancer.

Quality Review Team

Discipline	Reviewer	Branch/Division
Drug Substance	Riley Myers	OPQ/OBP/DBRR I
Drug Product	Shadia Zaman	OPQ/OBP/DBRR I
Drug Substance Microbiology Drug Product Microbiology	Scott Nichols Candace Gomez-Broughton	OPQ/OPF/DMA IV OPQ/OPF/DMA IV
Facility	Thuy Thanh Nguyen	OPQ/OPF/DIA
Immunogenicity assay	Shadia Zaman	OPQ/OBP/DBRR I
Analytical Similarity	Riley Myers	OPQ/OBP/DBRR I
Labeling	Vicky Borders Hemphill	OPQ/OBP
Product Quality Team Lead	Jennifer Swisher	OPQ/OBP/DBRR I

Microbiology QAL Facility Branch Chief	Reyes Candau-Chacon Peter Qiu	OPQ/OPF/DMA IV OPQ/OPF/DIA
CMC RPBM	Andrew Shiber	OPQ/OPRO
Application Team Lead	Jennifer Swisher	OPQ/OBP/DBRR I

Multidisciplinary Review Team:

Discipline	Reviewer	Office/Division
RPM	Rajesh Venugopal	OND/OHOP/DOP-1
Cross-disciplinary Team Lead	Sanjeeve Balasubramaniam	OND/OHOP/DOP-1
Medical Officer	Jennifer Gao	OND/OHOP/DOP-1
Pharm/Tox	Wei Chen/Tiffany Ricks (TL)	OND/OHOP/DHOT
Clinical Pharmacology	Christy John, Salaheldin Hamed/Nam Atiqur Rahman (TL)	OTS/OCP/DCPV
CMC Statistics Clinical Statistics	Erik Bloomquist /Shenghui Tang (TL) Chao Wang/Meiyu Shen (TL)	OTS/OB/DBV OTS/OB/DBIV

Names:

- Proprietary Name: Herzuma
- Non-Proprietary/USAN/INN: Trastuzumab-pkrb
- CAS Registry number: 180288-69-1
- Company/Laboratory code: CT-P6
- OBP systematic name: MAB HUMANIZED (IGG1) ANTI P04626 (ERBB2_HUMAN) [CT-P6]
- Other Names: None

Submissions Reviewed in BLA Resubmission Review Cycle:

Submission(s) Reviewed /sequence number	**Document Date**
STN 761091/42	6/15/2018
STN 761091/43 (response to IR #1)	8/02/2018
STN 761091/49 (Stability Update)	10/19/2018
STN 761091/52 (response to IR#2)	11/07/2018
STN 761091/54 (response to IR #3)	11/14/2018
STN 761091/ (response to IR #5)	11/27/2018
STN 761091/ (response to IR #4)	11/28/2018

Quality Review Data Sheet

1. Legal Basis for Submission: 351(k)

2. Related/Supporting Documents: *No change in information for this section; refer to Executive Summary filed on 2/23/2018.*

3. Consults: None

Executive Summary

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

The Office of Biotechnology Products, OPQ, CDER, recommends approval of STN 761091 for HERZUMA (trastuzumab-pkrb) manufactured by Celltrion, Inc. The data submitted in this application are adequate to support the conclusion that the manufacture of HERZUMA 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 adequate to support the conclusion that the biological product, trastuzumab-pkrb, is highly similar to U.S.-licensed Herceptin.

B. Approval Action Letter Language:

- Manufacturing Location:
  - Drug Substance:
    - Manufacturing: Celltrion, Inc., Incheon, Republic of Korea
  - Drug Product:
    - Manufacturing, fill, labeling, and packaging: Celltrion, Inc., Incheon, Republic of Korea
  - Bacteriostatic Water for Injection (BWFI):
    - Manufacturing, fill, labeling, and packaging: Celltrion, Inc., Incheon, Republic of Korea
- Fill size and dosage forms:
  - Trastuzumab-pkrb: 420 mg/vial for injection, multi-dose vial
  - BWFI: 20 mL/vial
- Dating period:
  - Drug Product: 60 months when stored at 2-8°C
  - Drug Substance: (b) (4) months when stored at ≤(b) (4) °C
  - Packaged Component:
    - BWFI: 60 months when stored at 2-8°C
- Exempt from lot release:
  - Yes. Herzuma is a specified product exempted according to 21 CFR 601.2a.

C. Benefit/Risk Considerations:

Herzuma (trastuzumab-pkrb) is proposed as a biosimilar to US-Herceptin (trastuzumab) for treatment of HER2 overexpressing breast cancer. The analytical similarity assessment provided in the original submission support the conclusion that CT-P6 is highly similar to U.S.-licensed Herceptin. No new analytical similarity data were submitted in the June 15, 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 the resubmission and in the initial BLA submission have confirmed that the processes and methods 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 trastuzumab-pkrb drug substance and of drug product at Celltrion, Inc. (Incheon, Republic of Korea) is recommended for approval by DIA, OPF, as a prelicense inspection performed during the resubmission review cycle (August 20-28, 2018) was classified as VAI. A prelicense inspection was not conducted during the first review cycle due to the OAI status of the facility.

The primary assessments of the resubmission for product quality, microbial safety, facility issues, and labeling are located as separate documents in Panorama.

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

None.

II. Summary of Quality Assessments:

A. Analytical Similarity Assessment

There was no change to this information in the resubmission and there were no changes to the conclusions regarding similarity from those communicated in the CMC Executive Summary regarding the BLA original submission (dated 2/23/2018 in Panorama).

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

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

CR Items #2 and 3: In order to ensure that the labeled amount of drug product can be withdrawn from the vial within the current DP (b) (4) the DS release specification for protein concentration was updated by increasing the lower limit of acceptable concentration from (b) (4) mg/mL to (b) (4) mg/mL.

CR Item #5: Tightened DS (b) (4) acceptance criteria in order to ensure manufacturability of DP with acceptable (b) (4).

Additional Comment #5: Adequate validation data were supplied to ensure that the (b) (4) demonstrate adequate performance when used as in-process tests on the appropriate drug substance manufacturing intermediates.

C. Drug Substance, trastuzumab-pkrb, Quality Summary

Drug Substance CQA Identification, Risk, and Lifecycle Knowledge Management

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

CR Item #4: A revised (b) (4) to ensure adequate control of Asp102 isomerization, a CQA known to affect potency that is monitored by IEC-HPLC, through DS and DP shelf life.

- **Reference Standards (RS):** A two-tiered approach was intended for the commercial RS; primary RS RF0650-02 was derived from a drug substance batch produced using the manufacturing process and formulation intended for commercialization. The primary RS (PRS) is stored at ≤ (b) (4) °C. However, no information regarding a WRS was provided in the BLA original submission and the protocols for qualification of future WRS were deficient following numerous IR responses. Additional Comment #4 recommended the development of a two-tier reference standard system in order to support the product lifecycle. Information regarding WRS RF-C1N01, manufactured from commercial batch 17200A001, was provided to Section 3.2.S.5; the WRS is intended for use in all release and stability testing as well as method validation and annual qualification of the PRS going forward. WRS RF-C1N01 is very closely matched in CQAs to the PRS and is appropriate for this purpose. In addition, Celltrion provided additional detail regarding their WRS trending program.

- **Manufacturing process summary:** Trastuzumab-pkrb DS is manufactured at Celltrion Inc., Incheon, Republic of Korea. (b) (4)

The trastuzumab-pkrb drug substance manufacturing process development is based on minimal process characterization and process validation studies. There was concern regarding the criticality ranking of numerous process parameters given the lack of experience with values outside of the proposed ranges and the company was advised that the adequacy of their control strategy would be a review issue (Additional Comment #2). However, information provided in the resubmission as well as during the pre-license inspection led to the determination that their quality risk management system was adequate to assure acceptable product quality under the current manufacturing process and control strategy as described in the BLA.

- **Dating period and storage conditions:** In response to Additional Comment #6, updated information was provided regarding DS storage at (b) (4) °C supporting adequate DS stability for a dating period of (b) (4) months.

D. Drug Product, Herzuma, Quality Summary:

Drug Product CQA Identification, Risk, and Lifecycle Management

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

Additional Comment #3: An adequate adjustment of the control strategy- including updating the criticality of appropriate parameters- for the lyophilization process following discussions about the additional process characterization studies provided near the end of the original review cycle.

Additional Comment #6d: Validation data from shipment of unlabeled and labeled DP and companion diluent (BWFI) to ensure a lack of impact on product quality from the shipment process.

Additional Comment #6e: Data that demonstrates the in-use stability of polysorbate 20 to confirm the adequacy of their raw material control strategy.

Additional Comment #7: Description and validation of the new (b) (4) step for BWFI, (b) (4), as well as release and stability data to support the suitability of BWFI produced by this process.

- **Dating period and storage conditions:**
  - In response to Additional Comment #6, updated information was provided regarding DP storage at 5±3°C supporting adequate stability for a shelf life of 60 months.
  - In response to Additional Comment #7, updated information was provided regarding (b) (4) BWFI storage at 5±3°C supporting adequate stability for a shelf life of 60 months.

E. Novel Approaches/ Precedents:

None.

F. Any Special Product Quality Labeling Recommendations:

None.

G. Establishment Information:

Overall Recommendation:				
DRUG SUBSTANCE				
Function	**Site Information**	**DUNS/FEI Number**	**Preliminary Assessment**	**Final Recommendation**
DS manufacturing, release and stability testing,	Celltrion, Inc. 23, Academy-ro (b) (4)	3005241015	Approve	Approve

MCB and WCB Storage	Yeonsu-gu Incheon, S. Korea			
Viral testing of unprocessed bulk; Testing of the MCB, WCB and EPCB	(b) (4)		n/a	Approve based on facility profile
Sterility testing of EPCB			n/a	Approve based on facility profile
Mycoplasma testing of unprocessed bulk; Testing of the MCB, WCB, and EPCB			n/a	Approve based on facility profile
Production of the MCB and WCB; MCB and WCB testing			n/a	Approve based on facility profile
Storage of the MCB and WCB; MCB and WCB testing			n/a	Approve based on facility profile
MCB and WCB testing			n/a	Approve based on facility profile
DRUG PRODUCT				
Function	**Site Information**	**DUNS/FEI Number**	**Preliminary Assessment**	**Final Recommendation**
DP manufacturing, release and stability testing	Celltrion, Inc. 23, Academy-ro (b) (4) Yeonsu-gu Incheon, S. Korea	3005241015	Approve	Approve

DS - drug substance, DP - drug product, MCB - Master Cell Bank, WCB - Working Cell Bank, UPB – Unprocessed bulk

H. Facilities:

The subject BLA proposes manufacture of Herzuma drug substance and drug product at Celltrion, Inc. (FEI 3005241015). A post-approval inspection for BLA 125544 (Inflectra) 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 and was the basis for CR item #1. The OAI status was cleared by OMQ after a for-cause inspection conducted in July 2018.

A PLI of Celltrion was conducted from August 20-28, 2018 by OPQ/OFP/DIA and OPQ/OBP. This inspection covered DS and DP manufacture as well as the GMP laboratories and the similarity assessment data. There were no issues with the similarity data, but a seven-item 483 was issued upon completion of the inspection. OPF/DIA review of the inspection deemed the sponsor's responses adequate and recommended approval of the facility for BLA 761091. The compliance status of the DS and DP manufacturing facility is acceptable.

I. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols approved:

1. Requalification of the MCB and WCB (3.2.S.2.3.2)
2. At-scale (b) (4) Study (3.2.S.2.5.6)
3. At-scale (b) (4) Study (3.2.S.2.5.7)
4. Requalification of the Primary and Working Reference Standards (3.2.S.5)
5. Ongoing Stability Study (3.2.S.7.2)

ii. Outstanding review issues/residual risk: None.

iii. Future inspection points to consider: None.

b. Drug Product

i. Protocols approved:

1. Ongoing Stability Studies (3.2.P.8.2)
2. Post-Approval Annual Stability Protocol (3.2.P.8.2)

ii. Outstanding review issues/residual risk: None

iii. Future inspection points to consider: None

Rachel Novak

Digitally signed by Rachel Novak
Date: 12/11/2018 11:53:10AM
GUID: 52e163f70002ba9e745b4e39384c76d7

Jennifer Swisher

Digitally signed by Jennifer Swisher
Date: 12/11/2018 11:06:07AM
GUID: 508da6d7000262dc015dcdc5f6541612

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: 10/24/2018
To: Administrative File, BLA 761091-ORIG-1-RESUB-42
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: Herzuma
Dosage: 420 mg/vial (b) (4)
Indication: Adjuvant Breast Cancer, Metastatic Breast Cancer, Metastatic Gastric Cancer
Due Date: 12/1/2018

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

SUMMARY

The subject BLA proposes manufacture of Herzuma drug substance (DS) and drug product (DP) at Celltrion, Inc. (FEI 3005241015). A post-approval for BLA 125544 (Inflectra) 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-license inspection was conducted during this review cycle to cover BLA 761091ORIG-1-RESUB-42 and BLA 761088-ORIG-1-RESUB-34. The inspection was classified as VAI. The firm adequately addressed 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.

_____
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

BLA 761091

Trastzumab-(b) (4)

Celltrion

OBP CMC Review Data Sheet

1. BLA#: BLA 761091

2. Review Date: February 21, 2018

3. Primary Review Team:
   - Medical Officer: Jennifer Gao and Sanjeeve Balasubramaniam (OND/OHOP/DOP-1)
   - Pharm/Tox: Wei Chen and Todd Palmby (OND/OHOP/DOP-1)
   - Product Quality Team
     - Drug Substance quality reviewer: Riley Myers (OPQ/OBP/DBRRI)
     - Drug Product quality reviewer: Shadia Zaman (OPQ/OBP/DBRRI)
     - Immunogenicity assay reviewer: Shadia Zaman (OPQ/OBP/DBRRI)
     - Analytical similarity reviewer: Riley Myers (OPQ/OBP/DBRRI)
     - Drug Substance Microbiology: Scott Nichols (OPQ/OPF/DMAIV)
     - Drug Product Microbiology: Candace Gomez-Broughton (OPQ/OPF/DMAIV)
     - Microbiology QAL: Reyes Candau-Chacon (OPQ/OPF/DMAIV)
     - Application Team Lead: Jennifer Swisher (OPQ/OBP/DBRRI)
   - Facilities: Thuy Thanh Nguyen and Peter Qiu (OPQ/OPF/DIA)
   - Clinical Pharmacology: Christy John and Sarah Schrieber (OTS/OCP/DCPV)
   - Statistics
     - CMC Statistics: Erik Bloomquist and Shenghui Tang (OTS/OB/DBV)
     - Clinical Statistics: Chao Wang and Meiyu Shen (OTS/OB/DBIV)
   - OBP Labeling: Vicky Borders Hemphill (OPQ/OBP)
   - RBPM: Keith Olin (OPQ/OPRO)
   - RPM (clinical): Leyish Minie (OND/OHOP/DOP-1)

4. Major GRMP Deadlines:

   Filing Meeting: 7/18/2017
   Mid-cycle meeting: 10/30/2017
   Wrap-up meeting: 10/26/2018
   Primary review due: 2/21/2018
   Secondary review due: 2/28/2018
   BsUFA action date: 3/30/2018

5. Communications with Sponsor and OND:

Communication/Document:	Date:
Information Request #1	8/31/2017
Information Request #2	9/26/2017
Information Request #3	12/7/2017
Information Request #4	12/22/17
Information Request #5	1/16/2018
Information Request #6	2/5/2018
Information Request #7	2/12/2018
Information Request #8	2/15/2018
Information Request #9	2/17/2018

6. Submission Reviewed:

Submission:	Date Received:	Review Completed (yes or no)
STN 761091 /2	5/30/2017	Yes
STN 761091/9 (response to IR #1)	9/11/2017	Yes
STN 761091 /15 (response to IR #2)	10/16/2017	Yes
STN 761091 /15 (response to IR #2)	10/23/2017	Yes
STN 761091 /22 (response to IR #2)	12/18/2017	Yes
STN 761091 /23 (response to IR#3)	12/21/2017	Yes
STN 761091 /25 (response to IR #4)	1/11/2017	Yes
STN 761091 /27 (response to IR #2)	1/22/2018	Yes
STN 761091 /29 (response to IR #5)	1/29/2018	Yes
STN 761091 /31 (response to IR #6)	2/09/2018	Yes
STN 761091 /34 (response to IR#7)	2/16/2018	Yes
STN 761091 /35 (response to IR #8)	2/20/2018	Yes
STN 761091 /36 (response to IR #9)	2/20/2018	Yes

7. Drug Product Name/Code/Type:
   Proprietary Name: Herzuma
   Non-Proprietary Name/USAN: trastuzumab-(b) (4)
   CAS Registry number: 180288-69-1
   Company/Laboratory code: CT-P6
   OBP systematic name: MAB HUMANIZED (IGG1) ANTI P04626 (ERBB2_HUMAN) [CT-P6]
   Other Names: None

8. Pharmacological Category: HER2/neu receptor antagonist

9. Dosage Form: for injection

10. Strength/Potency: 420 mg per vial

11. Route of Administration: Intravenous

12. Referenced Drug Master Files (DMF): None

13. Inspectional Activities: No prelicensure inspection conducted due to facility OAI status.

14. Consults Requested by OBP: None

15. Quality by Design Elements:
The following was submitted in the identification of QbD elements (check any that apply):

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

	Expanded Change Protocol

16. Precedents: None

17. Administrative:

Signature Block

Discipline	**Reviewer**	**Branch/Division**	**Signature**
Drug substance and analytical similarity	Riley Myers	Division of Biotechnology Review and Research I	See Panorama
CMC TL	Jennifer Swisher	Division of Biotechnology Review and Research I	See Panorama
Division Director	Kathleen Clouse	Division of Biotechnology Review and Research I	See Panorama

Summary of Quality Assessments

I. Primary Reviewer Summary Recommendation

CT-P6 appears to be highly similar to US-license Herceptin. However, during review of BLA 761091, the Office of Compliance sent a Warning Letter to Celltrion that lists observations made during inspection of their manufacturing facility, including the location of CT-P6 manufacture. Due to the status of Celltrion's manufacturing facility, a pre-license inspection for CT-P6 was not conducted. There are several components to a quality review that require a pre-license inspection, which are noted in this review and remain unresolved. Therefore, CMC recommends that Celltrion be sent a Complete Response letter for BLA 761091.

This memo contains the reviews for Drug Substance manufacture and the Analytical Similarity Assessment between CT-P6 and US-license Herceptin.

II. List of Deficiencies to be Communicated (Draft)

1. (b) (4)

(b) (4)

2. In the response to an IR received 2/16/2018, Celltrion's proposed acceptance criterion for (b) (4) is unacceptably broad based on your manufacturing experience. To ensure appropriate (b) (4) of the DP, tighten your acceptance criterion for DS release to (b) (4) (b) (4)

3. Per the "Guidance for Industry: Allowable Excess Volume and Labeled Vial Fill Size in Injectable Drug and Biological Products" (https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm389069.pdf), "In the case of drug products requiring reconstitution, the product should be designed to meet the label claim and acceptable overfill, and allow for correct dosing". The DP release specification of (b) (4) mg/vial must be adjusted to ensure that the recoverable protein content at the lower limit of the acceptance criterion will consistently meet the label claim of 420 mg. Adjust the (b) (4) control strategy to meet the updated specification.

4. As committed in the response to an IR received 2/20/2018, Celltrion will perform process characterization studies in order to establish acceptable ranges for lyophilization process parameters such as (b) (4). The criticality of these process parameters must be justified based on characterization of their impact on CT-P6 product quality attributes.

   Additional comments that are not approvability issues:

5. We cannot fully assess the adequacy of Celltrion's control strategy for CT-P6 in the absence of a pre-license manufacturing inspection. In the responses received on 10/16/2017 and 1/29/2018 to our IRs regarding the acceptable ranges for numerous process parameters that have the potential to impact product quality, the limited process characterization and process validation data may not fully justify the criticality ranking of these parameters. Their acceptability will be a review issue once the entire control strategy is updated and available for review.

6. FDA recommends that Celltrion develop a two-tiered reference material system to support the product lifecycle. An appropriately characterized primary reference material that is representative of production and clinical materials can be used to calibrate or qualify a working reference material and contributes to mitigating the risk of drift in quality attributes over time.

Use of a working or secondary reference material calibrated against a single primary reference material for routine release and stability testing of commercial lots provides additional assurance that commercially manufactured product is representative of the clinical trial material.

7. In response to an IR received 2/20/2018, additional validation data for (b) (4) assays were provided to demonstrate assay performance for in-process tests. These studies were conducted with (b) (4), but additional data demonstrated (b) (4) Revalidate the (b) (4) assays using actual (b) (4).

8. In the Complete response submission, provide updated data from the following studies that are ongoing:
   a. The commercial scale (b) (4) study for (b) (4) (in section 3.2.S.2.5),
   b. The commercial (b) (4) lifetime study (in section 3.2.S.2.5),
   c. The ongoing CT-P6 DS and DP stability studies (in sections 3.2.S.7 and 3.2.P.8),
   d. The shipping validation of the finished CT-P6 drug product (in section 3.2.P.3.5),
   e. The stability study on three in-use PS20 lots for up to 12 months, committed in your 2/20/2018 response (in Section 3.2.P.4).

III. List of Post-Marketing Commitments/Requirements: None, this BLA will not be approved.

IV. Review of Common Technical Document- Quality Module 1
A. Environmental Assessment of 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." While approval of this application may increase the use of the active moiety, a claim of categorical exclusion is acceptable based on 21 CFR 25.31(c).

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

The review of Module 3.2.S, 3.2.A, and 3.2.R is provided below. Module 3.2.P is a separate review is uploaded in Panorama by Dr. Shadia Zaman.

VII. Review of Immunogenicity Assays- Module 5.3.1.4

Review of the immunogenicity assays was performed by Dr. Shadia Zaman and is a separate review is uploaded in Panorama.

TABLE OF CONTENTS

Description of Drug Substance

Trastuzumab is a humanized IgG1 monoclonal antibody targeting the extracellular domain of the human epidermal growth factor receptor 2 (HER2). CT-P6 has been developed as a biosimilar product to US-licensed Herceptin. CT-P6 is a glycoprotein with one N-linked glycosylation site at residue Asn300 in the CH2 domain of each heavy chain that predominantly contains G0F and G1F structures. CT-P6 consists of two identical heavy and light chains that are covalently linked through inter-chain disulfide bonds. The heavy chains lack C-terminal lysine.

S. Drug Substance

3.2.S.1.2 Structure

CT-P6 has a theoretical mass of 145.2 kDa for the intact antibody, and the light and heavy chains have masses of 23.4 kDa and 49.2 kDa, respectively. The experimentally determined extinction coefficient of CT-P6 is 1.46 L g-1 cm-1.

The amino acid sequence for the heavy and light chain of CT-P6 is below:

	Heavy Chain
1	EVQLVESGGG LVQPGGSLRL S**C**AASGFNIK DTYIHWVRQA PGKGLEWVAR
51	IYPTNGYTRY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYY**C**SRWG
101	GDGFYAMDYW GQGTLVTVSS *ASTKGPSVFP LAPSSKSTSG GTAALG**C**LVK*
151	*DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT*
201	*YI**C**NVNHKPS NTKVDKKVEP KS**C**DKTHT**C**P P**C**PAPELLGG PSVFLFPPKP*
251	*KDTLMISRTP EVT**C**VVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQY**N**[1]*
301	*STYRVVSVLT VLHQDWLNGK EYK**C**KVSNKA LPAPIEKTIS KAKGQPREPQ*
351	*VYTLPPSREE MTKNQVSLT**C** LVKGFYPSDI AVEWESNGQP ENNYKTTPPV*
401	*LDSDGSFFLY SKLTVDKSRW QQGNVFS**C**SV MHEALHNHYT QKSLSLSPGK*[2]
	Light Chain
1	DIQMTQSPSS LSASVGDRVT IT**C**RASQDVN TAVAWYQQKP GKAPKLLIYS
51	ASFLYSGVPS RFSGSRSGTD FTLTISSLQP EDFATYY**C**QQ HYTTPPTFGQ
101	GTKVEIKRTV *AAPSVFIFPP SDEQLKSGTA SVV**C**LLNNFY PREAKVQWKV*
151	*DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYA**C**EVTHQG*
201	*LSSPVTKSFN RGE**C***

[1]Glycosylation site is Asn300 of the heavy chain.
[2] C-terminal lysine is nearly 100 % cleaved from the heavy chain.
Note: The amino acid sequences were defined according to both the patented protein and gene structure. The sequences have been confirmed for CT-P6 and Herceptin® by CELLTRION using MS/MS analysis. The letters shown denote the one-letter code for 20 common amino acids. Variable regions for both heavy and light chains are indicated in normal font; italicized letters designate the constant regions for both chains. Cysteine residues are in bold font; all cysteine residues are involved in disulfide bonds.

Figure 3.2.S.1.2-1: Amino Acid Sequence of CT-P6 (Trastuzumab)

3.2.S.1.3 General Properties

Trastuzumab targets HER2 and acts through multiple mechanisms of action. HER2 is a ligand-independent human epidermal growth factor receptor that preferentially dimerizes with other epidermal growth factors or ErbB family of receptor tyrosine kinases. Dimerization of the receptors results in the autophosphorylation of tyrosine residues within the cytoplasmic domain. This leads to activation of numerous signaling pathways including the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cascades that are involved in epithelial cell proliferation and transformation. In addition to dimerization, HER2 also undergoes proteolytic cleavage that results in an extracellular domain fragment released into the serum of breast cancer patients and a NH2-terminally truncated membrane-associated fragment with increased kinase activity. This increased kinase activity is associated with a greater transforming efficiency of the truncated protein.

The binding of trastuzumab to HER2 prevents HER2 signaling and in turn reduces tumor cell growth. Binding of trastuzumab to HER2 also prevents the proteolytic cleavage of its extracellular domain and subsequent activation of its intracellular kinase domain, preventing HER2 from stimulating the signaling cascade that causes tumor cells to grow. The Fc portion of trastuzumab binds Fc receptors (FcR), inducing tumor cell death via antibody dependent cell-mediated cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP), and effector cell deletion via activating FcγR.

3.2.S.2 Manufacture
3.2.S.2.1 Manufacturer(s)

CT-P6 manufacturing and testing sites are listed in Table 3.2.S.2.1-1:

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Trastuzumab quality attributes and associated analytical methods were classified into one of three statistical tiers based on criticality ranking of likelihood of occurring and severity of clinical impact (Table 3.2.R.2-5, not included in review). Tier 1 assays were analyzed via an equivalence test in which the equivalence margin (EM) was defined as ±1.5*Standard Deviation (SD) based on reference product. US-licensed Herceptin and CT-P6 products were determined equivalent if the 90% confidence interval (CI) of mean difference was within the corresponding EM. Tier 2 assays were analyzed via the quality range approach in which the range of the values obtained from reference product variation expressed as X times the US-licensed Herceptin SD (±XSD). The multiplier X was determined for each Tier 2 attribute based on the variability of each assay and a risk assessment that is summarized in Table 3.2.R.2-6 (not included in review). High similarity was considered to have been demonstrated if 90% of the CT-P6 lots were within the QR of US-licensed Herceptin. Tier 3 attributes and associated assays are qualitative or of low criticality, so a statistical analysis is not applied to these assays.

Reviewer comment: *Except for IEC-HPLC, which is reviewed in 3.2.R.3.2.5.2, the attributes assigned to each tier and the multipliers for each Tier 2 attribute are acceptable. The multipliers for Tier 2 attributes and their justifications are not discussed for each attribute below, but this can be located in Table 3.2.R.2-7 (not included in review).*

3.2.R Regional Information
3.2.R.1 Executive Summary—Analytical Similarity Assessment

Reviewer comment: *This section contains a summary of the 2-way Analytical Similarity Assessment between CT-P6 and US-licensed Herceptin that is reviewed in detail in 3.2.R.2-7.*

3.2.R.2 Introduction—Analytical Similarity Assessment

Celltrion conducted a 2-way analytical similarity assessment that evaluated physicochemical and biological quality attributes of the 420 mg presentation of trastuzumab to support that the CT-P6 DP is high similar to US-licensed Herceptin. US-licensed Herceptin 440 mg DP is reconstituted with 20 mL of bacteriostatic water for injection (BWFI) containing 1.1% benzyl alcohol to yield 21 mg/mL trastuzumab. For the Analytical Similarity Assessment, 420 mg CT-P6 and US-licensed Herceptin were reconstituted with sterile water for injection (SWFI). Reference standard RF0650-02 was used for similarity assessments, assay qualifications and validations, as well as the release of the clinical DS and DP lots of CT-P6.

Reviewer comment: (b) (4)

(b) (4)

The analytical similarity assessment included 12 lots each of US-licensed Herceptin and CT-P6 as summarized in Table 3.2.R.2-2 (not included in review). The 12 lots of CT-P6 are the only 12 lots manufactured to date, all of which were manufactured by the proposed commercial processes, Clinical DS process and DP Process B. The age of US-licensed Herceptin lots were 10-40 months. Five CT-P6

lots were >27 months of age and the remaining seven lots were one month old. The number of lots to be included in the analytical similarity assessment was determined through assay variation of six lots of US-licensed Herceptin in the two Tier 1 assays, in vitro bioactivity and ADCC, and the sample size for the equivalence test was calculated to obtain a statistical power of 90% (Table 3.2.R.2-1, not included in review).

CT-P6 DP lot 13A3C005 was produced by pooling two DS batches, 13200A02 and 13200A03. These DS batches were also used to produce DP lots 13A3C002 and 13A3C003, respectively. Therefore, CT-P6 DP lot 13A3C005 was not included in the analytical similarity assessment. Lot 16A3C07 was also manufactured using pooled DS batches 14200A001, 14200A005 and 14200A009. However, because these DS batches were not included in the manufacture of any other DP lots used in the analytical similarity studies, the data from lot 16A3C07 are included in the statistical analysis.

Trastuzumab quality attributes and associated analytical methods were classified into one of three statistical tiers based on criticality ranking of likelihood of occurring and severity of clinical impact (Table 3.2.R.2-5, not included in review). Tier 1 assays were analyzed via an equivalence test in which the equivalence margin (EM) was defined as ±1.5*Standard Deviation (SD) based on reference product. US-licensed Herceptin and CT-P6 products were determined equivalent if the 90% confidence interval (CI) of mean difference was within the corresponding EM. Tier 2 assays were analyzed via the quality range approach in which the range of the values obtained from reference product variation expressed as X times the US-licensed Herceptin SD (±XSD). The multiplier X was determined for each Tier 2 method based on the variability of each assay and a risk assessment that is summarized in Table 3.2.R.2-6 (not included in review). High similarity was considered to have been demonstrated if 90% of the CT-P6 lots were within the QR of US-licensed Herceptin. Tier 3 methods are qualitative or of low criticality, so a statistical analysis is not applied to these assays.

Reviewer comment: *Except for IEC-HPLC, which is reviewed in 3.2.R.3.2.5.2, the attributes assigned to each tier and the multipliers for each Tier 2 attribute are acceptable. The multipliers for Tier 2 methods and their justifications are located in Table 3.2.R.2-7 (not included in review); the multiplier of 3 (mean ± 3 SD) was used for all attributes except HER2 binding affinity by ELISA, cell-based HER2 binding affinity (CELISA), SPR assays for FcγRIIIa-V and FcγRIIIa-F binding, and the ADCC reporter assay, for which mean ± 2 SD was used to set the QR due to the criticality of those attributes.*

3.2.R.3 2-Way Analytical Similarity Assessment
3.2.R.3.1 Executive Summary of 2-Way Similarity Study

A summary of the statistical analysis results is in Table 3.2.R.3-3 below. A summary of the data from each assay was provided in Table 3.2.R.3-2 (not included in review).

Table 3.2.R.3-3: Summary of Statistical Analysis Results

Type	Analytical Test Items	Measurements	CT-P6 vs US-licensed Herceptin®
Tier 1 Analysis[1]			
Biological Assays	*In vitro* Bioactivity	Relative *In Vitro* Bioactivity (%) by EC_{50}	Within EM
	ADCC (PBMC)	Relative ADCC Activity (%) At 0.5 ng/mL At 1.3 ng/mL At 3.2 ng/mL	Within EM
Tier 2 Analysis[2]			
Biological Assays	HER2 binding affinity (ELISA)	Relative HER2 Binding Affinity (%) by EC_{50}	100%[3]
	Cell-based CD20 Binding Affinity (CELISA)	Relative Cell-based Binding Affinity (%) by EC_{50}	100%[3]
	FcγRIIIa-V Binding Affinity (SPR)	% Relative binding affinity (K_D)	100%[3]
	FcγRIIIa-F Binding Affinity (SPR)	% Relative binding affinity (K_D)	100%[3]
	FcγRIIIb Binding Affinity (SPR)	% Relative binding affinity (K_D)	100%[4]
	FcγRIIa Binding Affinity (SPR)	% Relative binding affinity (K_D)	91%[4]
	FcγRIIb Binding Affinity (SPR)	% Relative binding affinity (K_D)	100%[4]
	FcRn binding Affinity (SPR)	% Relative binding affinity (K_D)	100%[4]
	ADCC Reporter Assay	% Relative potency (EC_{50})	100%[3]

Type	Analytical Test Items	Measurements	CT-P6 vs US-licensed Herceptin®
Physicochemical Assays	Molar Absorptivity	Molar absorptivity	100%
		Extinction coefficient	100%
	Peptide Mapping by LC-MS	% Deamidation at HC Asn55	91%
		% Deamidation at HC Asn318	100%
		% Deamidation at HC Asn387	18%
		% Deamidation at LC Asn30	64%
		% Isomerization at HC Asp102	100%
	Free Thiol Analysis	Average free SH/IgG (µM/µM)	91%
	DSC	Tm1	100%
		Tm2	91%
		Tm3	91%
	Protein Concentration	mg/mL	91%
	SEC-HPLC	Monomer (%)	100%
		HMW (%)	100%
		LMW (%)	91%
	SEC-MALS	Monomer (%, UV)	100%
		HMW (%, UV)	100%
		LMW (%, UV)	100%
		Monomer (%, MALS)	100%
		HMW (%, MALS)	100%
		LMW (%, MALS)	100%
		Monomer (MW, kDa)	100%
	AUC	Monomer (s-value)	100%
		Monomer (%)	91%
		Monomer(MW, kDa)	100%
		Dimer (s-value)	100%
		Dimer (%)	91%
		Dimer (MW, kDa)	100%
	Non-reduced CE-SDS	% Intact IgG	82%
	Reduced CE-SDS	% Non-glycosylated heavy chain	82%
		% H + L	82%
	IEC-HPLC	% Peak 1+Peak 2+Peak 3+Peak4	100%
		% Peak 6	100%
	Grouped Oligosaccharide Profiling	% Afucosylation (G0+G1+G2)	100%
		% High Mannose (Man5+Man6+Man8)	100%
		% Total Afucosylation (G0+G1+G2+Man5+Man6+Man8)	100%
	N-linked Glycan Analysis	% Afucosylation (G0+G1+G2)	100%
		% High Mannose (Man5)	100%
		% Total Afucosylation (G0+G1+G2+Man5)	100%

Type	Analytical Test Items	Measurements	CT-P6 vs US-licensed Herceptin®
Tier 3 Analysis			
Physicochemical Assays	Amino Acid Analysis	Molar ratio	Highly similar
	Peptide Mapping (HPLC)	Peak profile	Highly similar
	Peptide Mapping by LC-MS	% oxidation at HC Met255	Highly similar
		% N-terminal pyroglutamate at HC Glu01	Highly similar
		% C-terminal unclipped lysine at HC Lys450	Highly similar
		% C-terminal proline amidation at HC Pro448	C-terminal proline amidation of CT-P6 is higher than Herceptin®
	Intact Mass (LC-MS)	Intact IgG mass	Highly similar
	N-terminal Sequencing	Sequence identity	Highly similar
	C-terminal Sequencing	Sequence identity	Highly similar
	Peptide Mapping (LC-MS)	Peak profile	Highly similar
	Disulfide Bond	Disulfide bond positions	Highly similar
	FTIR	Structure of protein by spectroscopy	Highly similar
	CD	α-helical, β-sheet and unordered conformations	Highly similar
	Antibody Array	Epitope exposure	Highly similar
	SEC-MALS	HMW (MW, kDa)	Highly similar
	Residual Host Cell Protein	Host cell protein	Highly similar
	Residual Host Cell DNA	Host cell DNA	Highly similar
	Residual rProtein A	rProtein A	Highly similar
	MFI	Sub-visible Particles	Highly similar
	IEF	pI values of charge variants	Highly similar
	IEC-HPLC	% Peak 5	Highly similar
		% Peak 7	Highly similar
	Oligosaccharide Profiling	% Galactosylation (G1F-GN+G1F+G2F)	Highly similar
		% Sialylation ((G1F-GN+NANA)+(G1F+NANA)+(G2F+NANA)+(G2F+2NANA))	CT-P6 contains higher level of sialylated glycan compared to Herceptin®
	Sialic acid	NGNA (mole/mole protein)	Highly similar
		NANA (mole/mole protein)	CT-P6 contains higher level of NANA compared to Herceptin®
Biological Assays	C1q Binding Affinity	% Relative binding affinity (EC_{50})	Highly similar
	FcγRI Binding Affinity (SPR)	% Relative binding affinity (K_D)	Highly similar

[1] EM was determined as $1.5\sigma_R$ of US-licensed Herceptin® 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 Standard Deviation (SD). High similarity was considered to have been demonstrated if 90% of data points were within the QR of US-licensed Herceptin® lots.
[3] X=2.
[4] X=3.

3.2.R.3.2 Physicochemical Similarity Assessment
3.2.R.3.2.1 Primary Structure

The amino acid content of CT-P6 and US-licensed Herceptin was evaluated as a Tier 3 assay. Due to acid hydrolysis, asparagine and glutamine are converted to their respective acidic counterparts (aspartic acid and glutamic acid) and could not be measured directly. Methionine is oxidized to methionine sulfoxide and tryptophan is destroyed by acid hydrolysis so these amino acids were not measured. The measured concentrations of serine, threonine and tyrosine were lower than the expected concentrations because these residues are subject to hydrolytic breakdown. Cysteine is oxidized during derivatization and shows poor recovery and precision after acid hydrolysis due to the formation of side products such as cysteine. All other amino acids were highly similar between CT-P6 and US-licensed Herceptin.

Molar absorptivity and the extinction coefficient were evaluated as Tier 2 methods with the QR determined using a multiplier of 3 (mean ± 3SD). US-licensed Herceptin showed an average molar absorptivity of 212,833 $L \cdot mol^{-1} \cdot cm^{-1}$ and an average extinction coefficient of 1.47 $L \cdot g^{-1} \cdot cm^{-1}$. CT-P6 showed an average molar absorptivity of 211,833 $L \cdot mol^{-1} \cdot cm^{-1}$ and an average extinction coefficient of 1.46 $L \cdot g^{-1} \cdot cm^{-1}$. All CT-P6 lots were within the QR for molar absorptivity and the extinction coefficient.

Peptide mapping was performed by HPLC as a Tier 3 method and in combination with LC/MS as a Tier 2 method. The chromatograms generated by HPLC are similar between CT-P6 and US-licensed Herceptin. By peptide mapping with LC/MS, deamidation and isomerization were evaluated as a Tier 2 method and oxidation as well as N- and C-terminal variants were evaluated as a Tier 3 method. Isomerization and deamidation at HC Asn55 and Asn318 of all lots of CT-P6 was within the Herceptin QR. However, only 18% and 64% of CT-P6 lots were within the QR for deamidation at HC Asn387 and LC Asn30, respectively.

Reviewer comment: *The CT-P6 lots that are outside of QR for HC Asn387 and LC Asn30 were below the lower bound of the QR, which indicates that there is less deamidation at these sites for CT-P6 as compared to US-licensed Herceptin. This difference does not preclude a determination of highly similar because lower deamidation does not negative impact product quality and does not appear to be clinically meaningful.*

Additionally, it is acceptable to evaluate oxidation as well as N- and C-terminal variants using Tier 3 method because characterization studies (3.2.S.3) demonstrated that these variants do not have a significant impact on the function of CT-P6 and US-licensed Herceptin.

Intact mass and N-terminal and C-terminal sequencing were evaluated as Tier 3 methods. The mass of each species was 148065.5-148072.2 Da for G0F-G0F, 148226.7-148233.0 Da for G0F-G1F, 148387.5-148393.2 Da for G1F-G1F or G0F-G2F, and 148545.3-148556.8 Da for G1F-G2F in US-licensed Herceptin and CT-P6 lots. The detected N- and C-terminal sequences of the CT-P6 light chain and heavy chains matched the expected sequences of US-licensed Herceptin. There was also similar variability between CT-P6 and US-licensed Herceptin with pyroglutamic acid detected in <3% of the N-terminus and lysine detected in <2% if the C- terminus in both products.

3.2.R.3.2.2 Higher Order Structure

Disulfide Bond positions was evaluated as a Tier 3 method. Eight disulfide bond linked peptides were identified in CT-P6 and they were identical to US-licensed Herceptin. Free thiol (SH) was evaluated as a Tier 2 method using Ellman's assay to determine the moles of free SH groups per mole IgG. US-licensed Herceptin contained 0.29-0.34 moles/IgG and CT-P6 contained 0.25-0.35 moles/IgG. All but one of the CT-P6 lots were within the QR. CT-P6 and US-licensed Herceptin were also evaluated by FTIR, near UV, and far UV CD as Tier 3 method. All spectra were comparable between CT-P6 and US-licensed Herceptin, supporting that secondary and tertiary structure of CT-P6 is similar to US-licensed Herceptin. The endothermic transitions of CT-P6 and US-licensed Herceptin were evaluated by DSC as a Tier 3 method, which were 71.1°C, 81.1°C and 82.9°C for both products. 11 CT-P6 lots were within the QR. CT-P6 lot 13A3C001 had the same temperature for transitions 2 and transition 3 as the upper limit of the quality range of US-licensed Herceptin and was considered to be outside the QR. CT-P6 and US-licensed Herceptin were also evaluated by a Protein Conformational Array (PCA) ELISA as a Tier 3 method that consists of 34 pools of polyclonal antibodies, each raised against a short linear segment of the mAb peptide sequence and is intended to detect differences in structure (epitope exposure). All lots of both products showed consistent in epitope exposure, supporting that they are structurally similar.

Reviewer comment: *Each CT-P6 lot that was considered outside of the QR for free thiol and thermal transitions was equal to the lower and upper bound, respectively, of the QR. Therefore, these lots were considered outside the QR for statistical analysis. However, given that these lots were equal to the QR bound and that the differences between the lots within the QR and the lots on the bound of the QR are minimal, these lots do not preclude a determination of "highly similar."*

3.2.R.3.2.3 Content

Protein concentration was evaluated as a Tier 2 method. The mean protein concentration was 21.7 mg/mL with an SD of 0.5 for US-licensed Herceptin and 21.3 mg/mL with an SD of 0.6 for CT-P6. CT-P6 lot 13A3C001 had a protein concentration with the same value as the lower limit of the QR, but all other CT-P6 lots were within the QR.

Reviewer comment: *Although US-licensed Herceptin is labeled as 440 mg, the recoverable protein content is 420 mg. As a result, the label for US-licensed Herceptin was changed to 420 mg. Celltrion originally labeled CT-P6 as 440 mg but have revised their label to reflect the recoverable protein content, which is also 420 mg. Their filling strategy and justification of specification with regard to recoverable protein content is found in the Drug Product review for this BLA.*

3.2.R.3.2.4 Purity/Impurities

Impurities in trastuzumab include aggregates, fragments, HCP, DNA, protein A, and sub-visible particles, each of which are evaluated by multiple methods as Tier 2 methods. SEC-HPLC detects monomer, HMW, and LMW content. The range for US-licensed Herceptin monomer as (b) (4)% and CT-P6 was (b) (4)%, HWM content for US-licensed Herceptin was (b) (4)% and CT-P6 was (b) (4)%, and LWM content for US-licensed Herceptin was (b) (4)% and CT-P6 was (b) (4)%. All CT-P6 lots were within the QR for all three QAs except one lot was on the upper limit of the QR for LWM content. By SEC-MALS, all CT-P6 lots were within the QR for all attributes. AUC resolves antibody monomers and dimers. One CT-P6 lot contained a higher %monomer than the upper bound of the QR and this lot also contained lower %dimer than the lower bound of the QR, both of which do not negatively impact product quality. All other CT-P6 lots were within the monomer and dimer QRs by AUC.

CE-SDS was used to evaluate intact IgG, non-glycosylated heavy chain (NGHC), and the sum of light and heavy chains (H+L) as Tier 2 methods. The same two CT-P6 lots were outside of the QR for each attribute, meaning only 82% of CT-P6 lots were within the QR.

Reviewer comment: *The two CT-P6 lots outside the QR were among the first lots manufactured in April 2013 and the difference between these lots and the CT-P6 lots within the QR is ~1%. Additionally, orthogonal methods SEC-HPLC, SEC-MALS, and AUC show that LWM and HWM content of CT-P6 is within their respective QRs for each method. Therefore, the differences seen by CE-SDS do not preclude a determination of "highly similar". In an IR sent on December 7, 2017, requesting a justification for the differences in CT-P6 seen by CE-SDS and SEC-HPLC. In their response on December 21, 2017, Celltrion stated that H2L1, H+L, and non-glycosylated heavy chain are low molecular weight variants that are not well resolved from the main peak by SEC-HPLC and SEC-MALS. Data were also provided that support that levels of H2L1, H+L, and non-glycosylated heavy chain that are higher than the differences observed between the two CT-P6 lots outside the QR and US-licensed Herceptin do not impact the function of CT-P6. Therefore, these differences do not preclude a determination of "highly similar".*

HCP in DP was evaluated using a CT-P6 process-specific ELISA. The HCP content in all lots of CT-P6 and US-licensed Herceptin were below the limit of detection (LOD). Celltrion also developed a non-immunological method to compare the HCP content. Using peptide mapping in conjunction with MS/MS, HCP was below the limit of detection in all of the CT-P6 and US-licensed Herceptin lots. DNA content was evaluated using qPCR and all CT-P6 and US-licensed Herceptin lots were below the limit of detection (LOD). Residual Protein A was evaluated by ELISA as a and all CT-P6 and US-licensed Herceptin lots were below the limit of detection (LOD). Sub-visible particles were evaluated by Micro-flow imaging (MFI). The relative size and number of sub-visible particles were similar between CT-P6 and US-licensed Herceptin.

Reviewer comment: *CT-P6 lot 13A3C003 contained the largest number of sub-visible particles of all CT-P6 and US-licensed Herceptin lots studied. However, lot 13A3C003 was among the earliest CT-P6 lots manufactured and there was no consistent trend or increase of sub-visible particles on stability. Sub-visible particles in all other CT-P6 lots were consistent with US-licensed Herceptin.*

3.2.R.3.2.5 Charge Variants

Charge variants of CT-P6 and US-licensed Herceptin were evaluated by IEF as a Tier 3 method because IEF is a qualitative assay. Both products showed four major bands and four minor bands. All bands were similar between CT-P6 and US-licensed Herceptin except bands 5 and 6. These bands were not consistently detected in all lots because of their low intensity. Charge variants are evaluated quantitively by IEC-HPLC where %Peaks 1-4 and 6 are Tier 2 methods and %Peaks 5 and 7 are Tier 3 methods. All lots of CT-P6 were within the QR for %Peaks 1-4 and 6.

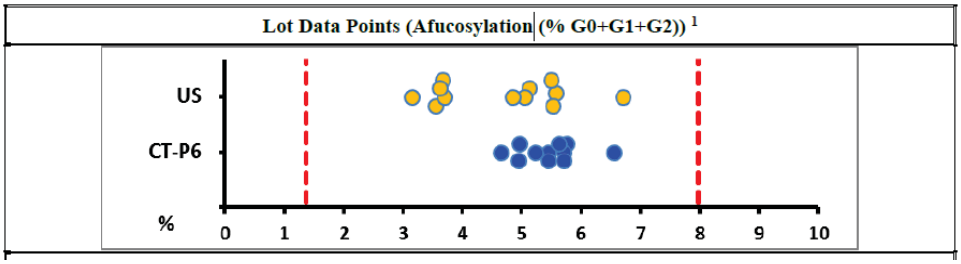
Reviewer comment: *Per the "OBP Standard Language Handbook for Biosimilars," charge variants should be Tier 2 methods. When a ±2 or ±3SD QR is applied to %Peaks 5 and 7 as if they were Tier 2 methods, all CT-P6 lots were within the QR for Peak 5. However, all CT-P6 lots were outside the upper bound of the QR for Peak 7. Peak 7 captures C-terminal lysine variants of trastuzumab, and Celltrion provided additional characterization studies in 3.2.R.5 that show that when fractionated, C-terminal lysine variants in Peak 7 do not significantly impact potency of CT-P6. C-terminal lysine variants also do*

not impact the function of antibodies injected i.v. because proteases in the serum rapidly cleave C-terminal lysine. Taken together, the differences in Peak 7 between CT-P6 and US-licensed Herceptin do not preclude a determination of "highly similar".

3.2.R.3.2.6 Glycosylation

Oligosaccharide Profiling and N-linked glycan analysis were used to evaluate afucosylation, high mannose, and total afucosylation (G0+G1+G2+Man5+Man6+Man8) as Tier 2 methods and galactosylation and sialylation as Tier 3 methods. All CT-P6 lots were within the QR for afucosylation, high mannose, and total afucosylation. US-licensed Herceptin lots showed a mean galactosylation of 33.93±10.45, whereas CT-P6 lots showed 45.69±1.09. US-licensed Herceptin lots showed a mean sialylation of 1.13±0.41 and CT-P6 lots showed 2.71±0.34. The levels of N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid (NGNA) were evaluated as Tier 3 methods. US-licensed Herceptin lots showed a mean NGNA of 0.004±0.000 mol sialic acid/mol protein and a mean NANA of 0.044±0.009. CT-P6 lots showed a mean NGNA of 0.003±0.000 mol sialic acid/mol protein and a mean NANA of 0.130±0.004.

Reviewer comment: *A portion of the US-licensed Herceptin lots used in the Analytical Similarity Assessment have lower afucosylation (Figure 3.2.R.3-29 below). All CT-P6 lots were within the QR and more consistent with the predominant population of US-licensed Herceptin lots, which have higher afucosylation values.*

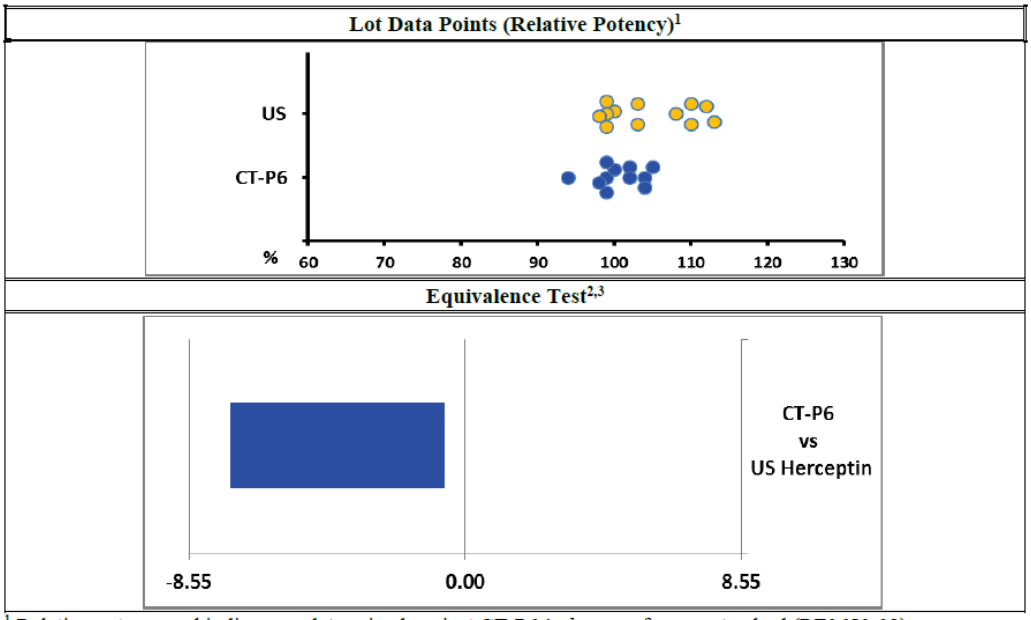


Although there are differences in galactosylation and sialylation between CT-P6 and US-licensed Herceptin, published studies show that galactosylation does not affect ADCC (Boyd et al Mol Immunol 1995; Hodoniczky et al Biotechnol Prog 2005; Raju Curr Opin Immunol 2008) and only impacts C1q binding and CDC (Prang et al British Journal of Cancer 2005), which is not a mechanism of action of trastuzumab. Likewise, published studies suggest that sialylation does not directly impact biological activity of antibodies (Kaneko et al Science 2006; Scallon et al Mol Immunol 2007; Boyd et al Mol Immunol 1995; Lin et al PNAS 2015). Additionally, the difference in sialylation between CT-P6 and US-licensed Herceptin is 1.58%. Differences in sialylation may impact PK; however a difference of this magnitude would not be expected to cause biologically meaningful change. Therefore, the minor differences in galactosylation and sialylation between CT-P6 and US-licensed Herceptin do not preclude a determination of "highly similar".

3.2.R.3.3 Biological Similarity Assessment

HER2 binding affinity was evaluated by anti-HER2 and cell-based ELISAs as Tier 2 methods. The relative binding affinity of samples was determined from the comparison of the mean EC_{50} (effective concentration yielding a 50% response) of the reference standard to the mean EC_{50} of sample. All CT-P6 lots were within the QRs for both ELISAs.

In vitro bioactivity assay was performed using HER2 over-expressing human breast cancer cell line BT-474 to evaluate the anti-proliferation activity of US-licensed Herceptin and CT-P6 as a Tier 1 method. The inhibition of BT-474 proliferation is measured by CCK-8 colorimetric reagent after a five day incubation. US-licensed Herceptin showed a mean activity of 104.5±5.7% and CT-P6 showed 100.5±3.2%. Given that the in vitro bioactivity assay is a Tier 1 assay, the results are analyzed via an equivalence test. As shown in Figure 3.2.R.3-38 below CT-P6 is within the equivalence margin established by US-licensed Herceptin.



[1] Relative potency or binding was determined against CT-P6 *in-house* reference standard (RF0650-02)
[2] EM for Equivalence Acceptance Criterion was determined as 1.5σR of US-licensed Herceptin® data accounting for lot-to-lot variability.
[3] Results are presented as 90% CI of mean difference between 2 products.

Figure 3.2.R.3-38: Scatter Plot and Equivalence Test Result for *In Vitro* Bioactivity Results

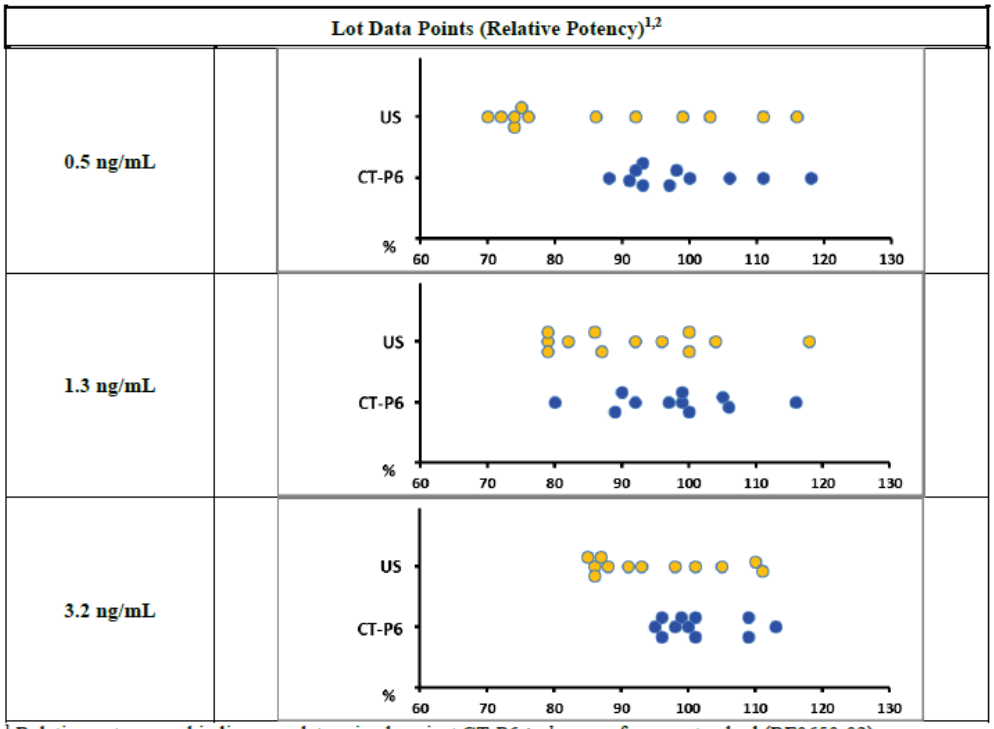
C1q binding was evaluated by ELISA as a Tier 3 method because CDC is not a mechanism of action of trastuzumab. US-licensed Herceptin showed a mean C1q binding of 102±7% and CT-P6 showed 104±7%.

Reviewer comment: *As discussed above, CT-P6 had higher levels of galactosylation, which may impact CDC activity, compared to US-licensed Herceptin. However, the C1q binding ELISA supports that the differences in galactosylation between the two products do not have a functional impact and do not preclude a determination of "highly similar".*

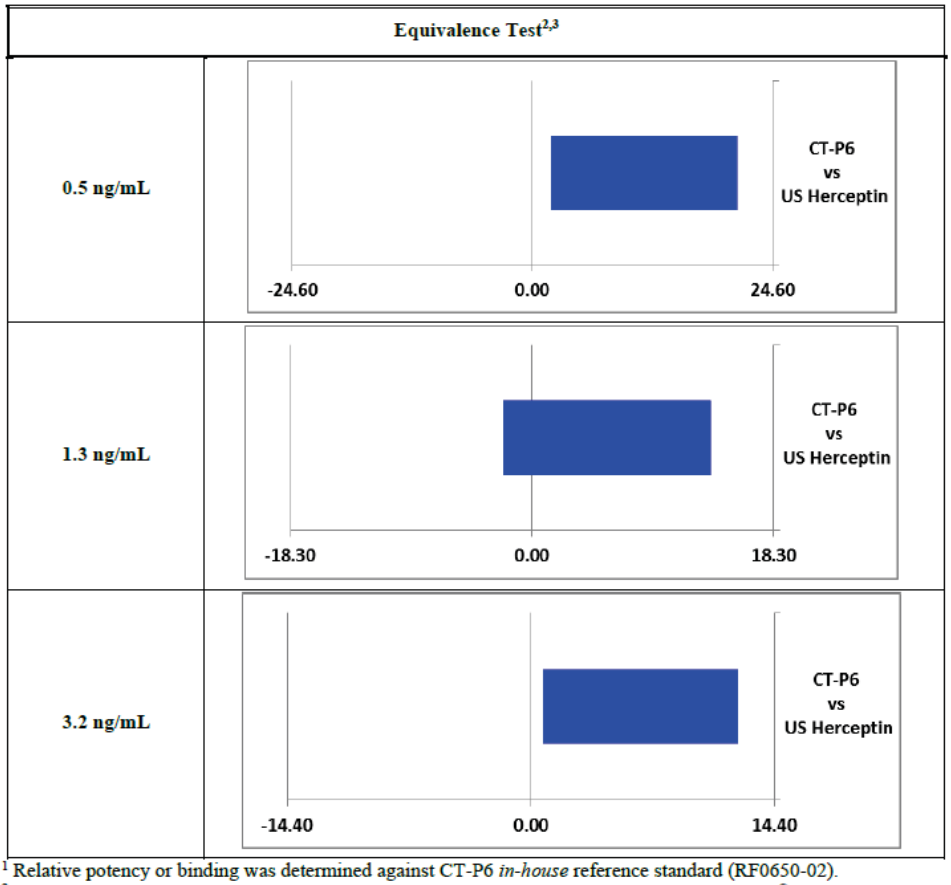
FcγRIIIa-V, FcγRIIIa-F, FcγRIIIb, FcγRIIa, FcγRIIb, and FcRn binding were evaluated by SPR as Tier 2 methods. All CT-P6 lots were within the QRs for FcγRIIIa-V, FcγRIIIa-F, FcγRIIIb, FcγRIIb, and FcRn binding. CT-P6 lot 16A3C07 showed a binding affinity for FcγRIIa that was equal to the upper bound of the QR, so 11 of the 12 CT-P6s lots were within the QR for this attribute. FcγRI binding was also evaluated by SPR as Tier 3 method. US-licensed Herceptin showed a mean FcγRI binding of 97±2% and CT-P6 showed 98±3%.

Reviewer comment: *As discussed above, there are differences in the afucosylation levels in five of the US-licensed Herceptin lots included in the analytical similarity assessment. Afucosylation directly impacts FcγRIIIa binding; the US-licensed Herceptin lots that have higher afucosylation levels shown increased FcγRIIIa binding affinity. Because the level of afucosylation in CT-P6 lots is similar to the US-licensed Herceptin lots that have higher afucosylation levels, FcγRIIIa binding by CT-P6 is also consistent with these US-licensed Herceptin lots.*

The ADCC activity was evaluated using PBMCs as a Tier 1 method and using a reporter cell line as a Tier 2 method. In the PBMC assay, the target cells were HER2 overexpressing breast cancer cell line, SK-BR-3, and PBMCs from health donors were the effector cells. The assay was performed at three concentrations of CT-P6 and US-licensed Herceptin, 0.5 ng/mL, 1.3 ng/mL and 3.2 ng/mL of antibody using effector to target cell ratio of 16:1. The relative ADCC activity of sample was determined from the comparison of the % cytotoxicity of sample to that of the reference standard at each concentration. As shown in Figures 3.2.R.3-58 below CT-P6 is within the equivalence margin established by US-licensed Herceptin at all three concentrations.



[1] Relative potency or binding was determined against CT-P6 *in-house* reference standard (RF0650-02).
[2] EM for Equivalence Acceptance Criterion was determined as 1.5σR of US-licensed Herceptin® data accounting for lot-to-lot variability.
[3] Results are presented as 90% CI of mean difference between 2 products.

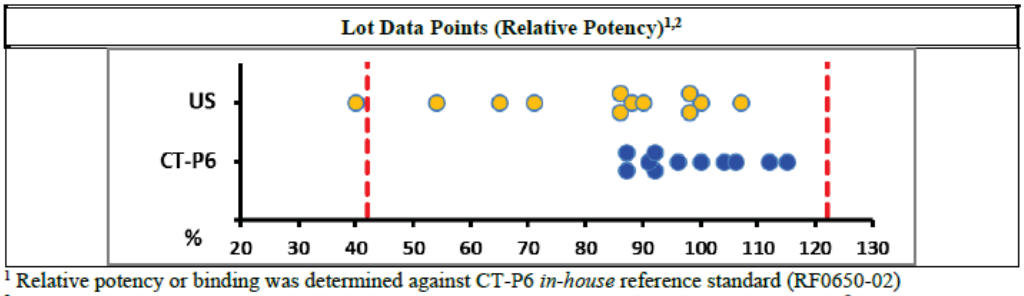


[1] Relative potency or binding was determined against CT-P6 *in-house* reference standard (RF0650-02).
[2] EM for Equivalence Acceptance Criterion was determined as 1.5σR of US-licensed Herceptin® data accounting for lot-to-lot variability.
[3] Results are presented as 90% CI of mean difference between 2 products.

Figure 3.2.R.3-58: Scatter Plot and Equivalence Test Result of ADCC Assay (PBMC) Results (Cont'd)

The ADCC reporter assay was performed as an orthogonal evaluation of FcγRIIIa-specific ADCC activity using Jurkat cells expressing the FcγRIIIa V158 and an NFAT response element driving expression of firefly luciferase as effector cells at an effector to target cell ratio of 2:1. All CT-P6 lots were within the QR.

Reviewer comment: *This assay is intended to assess FcγRIIIa-specific ADCC activity, which is impacted by levels of afucosylation. The lots of US-licensed Herceptin that have less afucosylation show lower FcγRIIIa-specific ADCC activity. All CT-P6 lots are within the QR and the results of the ADCC test correlate with the results of ADCC test for US-licensed Herceptin lots with higher afucosylation levels.*



[1] Relative potency or binding was determined against CT-P6 *in-house* reference standard (RF0650-02)
[2] The dotted red line represents the limits based on mean ± 2SD range of US-licensed Herceptin® lots

Figure 3.2.R.3-60: Scatter Plot of ADCC Activity (Reporter Assay) Results

The data provided in the analytical similarity assessment supports that CT-P6 is "highly similar" to US-licensed Herceptin. There are minor differences in intact antibody and charge heterogeneity, but these differences do not preclude a determination of "highly similar".

3.2.R.4 Additional Studies to Investigate Mechanisms of Actions

In addition to the analytical similarity assessment Celltrion conducted additional studies to evaluate potential mechanisms of actions of trastuzumab including down-regulation of HER2 expression, inhibition of downstream signaling pathways, induction of cell cycle arrest, apoptosis, potentiation of chemotherapy effects, suppression of vascular endothelial growth factor (VEGF), antibody-dependent cell-mediated phagocytosis (ADCP), and ADCC in gastric cancer.

Reviewer comment: *Qualification data for each assay is provided in Table 3.2.R.4-12 (not included in review). Each assay reviewed below is appropriately qualified for its purpose.*

Inhibition of HER2 Extracellular Domain Cleavage

Trastuzumab blocks cleavage of the extracellular domain of HER2, which prevents formation of the constitutively active membrane-bound 95-kDa HER2 protein called p95 HER2 (Nahta et al., 2012). HER2 shedding is also activated by 4-aminophenylmercuric acetate (APMA), a well-known matrix metalloprotease activator, in HER2-overexpressing breast cancer cells (Molina et al., 2001). APMA and 1.39, 0.23, or 0.039 µg/mL of US-licensed Herceptin or CT-P6 was incubated with BT-474 cells for two hours and HER2 in the culture supernatant was then measured by ELISA. The OD values were then used to calculate the blockage of HER2 extracellular domain cleavage relative to the CT-P6 reference standard. The mean relative blockage for US-licensed Herceptin was 102±3%, 99±2%, and 99±3% for each of the antibody concentrations, respectively, and CT-P6 showed 100±3%, 99±2%, and 99±2%.

Downregulation of HER2 Expression

In vitro assays have shown that Herceptin treatment down-regulates HER2 expression, which results in inhibition of downstream signaling pathways involved in cell survival, cell proliferation and metastasis (Klapper et al., 2000). HER2 downregulation by CT-P6 and US-licensed Herceptin was evaluated using a cell-based HER2 binding affinity ELISA with SK-BR-3 cells. CT-P6 and US-licensed Herceptin was incubated with SK-BR-3 cells for 72 hours at 5 ng/mL, 150 ng/mL and 2,000 ng/mL, cells were then fixed, and HER2 expression was identified with anti-human ErbB2. Human IgG1 was used as a negative control. The OD values were then used to calculate HER2 downregulation relative to the CT-P6 reference standard. The mean relative HER2 downregulation for US-licensed Herceptin was 101±3%,

100±2%, and 99±2% for each of the antibody concentrations, respectively, and values obtained using CT-P6 were 99±3%, 100±3%, and 99±3%.

Inhibition of Downstream Signaling Pathways

Trastuzumab binding correlates with a down-regulation of Akt signaling, which leads to an increase in the cyclin dependent kinase 2 (CDK2) inhibitor p27 (Lane et al., 2000; Lee et al., 2001; Yakes et al., 2002). The capacity of CT-P6 and US-licensed Herceptin to inhibit HER3 phosphorylation that occurs after receptor dimerization and PI3K/Akt signaling was evaluated by incubating 0.063, 0.25, and 1.0 µg/mL of each product with BT-474 cells for 24 hours and then Akt and HER3 phosphorylation was measured in BT-474 cell lysates using PathScan total Akt1/Phospho-Akt1 sandwich ELISA and PathScan total HER3/Phospho-HER3 sandwich ELISA. The OD values were then used to calculate phospho-Akt1 and phospho-HER3 inhibition relative to the CT-P6 reference standard. The mean relative phospho-Akt1 inhibition for US-licensed Herceptin was 100±3%, 96±5%, and 102±6% for each of the antibody concentrations, respectively, and CT-P6 showed 97±3%, 97±4%, and 104±5%. The mean relative phospho-HER3 inhibition for US-licensed Herceptin was 106±10%, 104±9%, and 102±6% for each of the antibody concentrations, respectively, and CT-P6 showed 106±7%, 106±9%, and 106±10%.

Induction of Cell Cycle Arrest

Trastuzumab-induced expression of p27kip1, a cyclin-dependent kinase inhibitor 1B, results in cell cycle arrest at G1 phase (Le et al., 2008). The capacity of CT-P6 and US-licensed Herceptin to induce cell cycle arrest was evaluated by incubating 133 ng/mL of each product with SK-BR-3 cells for 48 hours. Paclitaxel was added to each well and incubated for one additional day. Cell cycle arrest was assessed by flow cytometry with propidium iodide. Cell cycle arrest was determined as follows: Arrested G1 phase population (%) = (G1 population of US-licensed Herceptin®+Paclitaxel or CT-P6+Paclitaxel) – (G1 population of only Paclitaxel). The mean relative cell cycle arrest for US-licensed Herceptin was 101±11% and CT-P6 showed 100±12%.

Suppression of VEGF Secretion

HER2-overexpressing human breast cancer cells secrete VEGF at high levels, which leads to angiogenesis at the site of the tumor, and trastuzumab suppresses VEGF secretion. To evaluate the capacity of CT-P6 and US-licensed Herceptin to suppress VEGF secretion, BT-474 cells were incubated with 0.1, 0.35, and 1.225 µg/mL of each product for two hours and then VEGF production was measured using cell supernatant by ELISA. The OD values were then used to calculate VEGF suppression relative to the CT-P6 reference standard. The mean relative VEGF suppression for US-licensed Herceptin was 100±7%, 101±12%, and 97±8% for each of the antibody concentrations, respectively, and CT-P6 showed 99±5%, 102±6%, and 94±8%.

Potentiation of Chemotherapy Effects (Anti-proliferation in the Presence of Paclitaxel)

Trastuzumab is used alone or in combination with chemotherapeutic agent such as paclitaxel and docetaxel against breast cancer. The capacity of CT-P6 and US-licensed Herceptin to inhibit cell proliferation in the presence of paclitaxel was evaluated by incubating either product with BT-474 cells in the presence of paclitaxel for four days. The inhibition of cell proliferation was measured using a CCK-8 proliferation kit. The OD values were then used to calculate inhibition of cell proliferation relative

to the CT-P6 reference standard. The mean for US-licensed Herceptin was 104±6% and CT-P6 showed 103±9%.

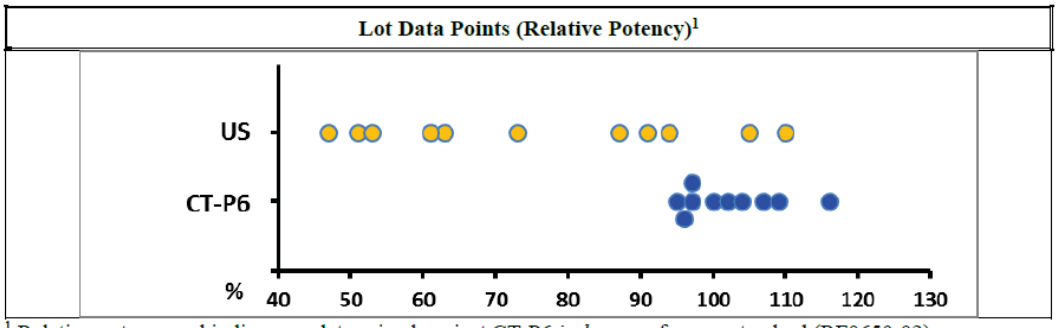
Antibody Dependent Cellular Phagocytosis

Reports have shown that therapeutic antibodies induce Fc-mediated ADCP of tumor cells, including breast cancer cells (Richards et al., 2008; Nagashima et al., 2011; Matlawska-Wasowska et al., 2013; Watanabe et al., 1999; Lazar et al., 2006; Karagiannis et al., 2009). Monocytes were isolated from human PBMCs using Pan Monocyte isolation kit and then incubated with CM-CSF for 10-14 days. SK-BR-3 cells were stained with PKH67 green fluorescent cell linker kit and incubated with 9.0, 3.6, or 1.4 ng/mL CT-P6 or US-licensed Herceptin for 30 minutes. Monocyte-derived macrophages were then added to the cells and incubated for three hours at an effector to target ratio of 1:1. After incubation cells were stained with CD11b-APC for FACS analysis. Phagocytosis was determined by dividing the %CD11b+PKH67+ cells by %CD11b+PKH67+ cells plus PKH67+ cells. Results were then normalized to the CT-P6 reference standard. The mean relative phagocytosis for US-licensed Herceptin was 99±6%, 97±7%, and 94±7% for each of the antibody concentrations, respectively, and CT-P6 showed 100±5%, 99±5%, and 100±7%.

ADCC in Gastric Cancer

US-licensed Herceptin is approved for clinical use to treat HER2-expressing advanced gastric cancer. To support that the analytical and clinical data can be extrapolated to support use of CT-P6 to the gastric cancer, an in vitro assay using HER2-expressing NCI-N87 cells was used. NCI-N87 cells were incubated with CT-P6 or US-licensed Herceptin and engineered Jurkat cells expressing the FcγRIIIa and an NFAT response element driving expression of firefly luciferase as effector cells at an effector to target ratio of 2:1 for 18-24 hours. The ADCC activity was measured by a Bio-glo luciferase assay. The relative luminescence unit (RLU) values were then used to calculate ADCC activity relative to the CT-P6 reference standard. The mean for US-licensed Herceptin was 76±23% and CT-P6 showed 102±7%.

Reviewer comment: *There is significant variability between US-licensed Herceptin lots in the gastric cancer ADCC assay as shown in Figure 3.2.R.4-18 below:*



[1] Relative potency or binding was determined against CT-P6 *in-house* reference standard (RF0650-02)

Given that this assay depends on FcγRIIIa binding and that this is directly impacted by the level of afucosylation, the differences in values obtained with US-licensed Herceptin lots are likely to be due to the differences in afucosylation. This agrees with other data regarding FcγRIIIa binding and afucosylation values, although this assay appears to be more sensitive to those differences.

Complement-Dependent Cytotoxicity (CDC)

CDC is not a mechanism by which trastuzumab exerts its therapeutic effect. However, per FDA feedback at a Type 3 meetings in March 2017, CDC activity for 10, 100, and 1000 ng of CT-P6 and US-licensed Herceptin was evaluated using one lot each of the two products by assessing propidium iodide staining of BT-474 cells lysis via FACS. Human IgG1 was used as a negative control. CT-P6, US-licensed Herceptin, and human IgG1 showed similar relative PI+ population, supporting that the CDC activity is similar between CT-P6 and US-licensed Herceptin.

Reviewer comment: *The data contained within the characterization of additional MOA studies support that CT-P6 is "highly similar" to US-licensed Herceptin. There were differences between the two products in ADCC activity in the in vitro gastric cancer model, but these differences appear to be primarily due to lot-to-lot variability in afucosylation levels with US-licensed Herceptin. These differences do not preclude a determination of "highly similar".*

3.2.R.5 Additional Characterization Studies for Product-Related Variants and Impurities in Herceptin and CT-P6

This section contains additional characterization of the product-related variants and impurities in US-licensed Herceptin and CT-P6 identified by IEC-HPLC and by glycosylation analysis. Forced degradation studies were also provided to assess the similarity between US-licensed Herceptin and CT-P6 under forced degradation conditions and to confirm the analytical methods that are stability-indicating. US-licensed Herceptin lot 3001168 and CT-P6 lot 15A3C01 were included in the additional characterization studies.

3.2.R.5.1 IEC-HPLC Peak Characterization

Each of the seven trastuzumab peaks resolved by IEC-HPLC was fractionated to >79% purity and characterized by SEC-HPLC, IEF, and peptide mapping. SEC-HPLC showed that the variants captured by each peak were >99% monomer and <1% HWM and LWM content for US-licensed Herceptin and CT-P6. By IEF, US-licensed Herceptin and CT-P6 showed similar banding patterns for all seven peaks. Peptide mapping showed that Peaks 1-4 capture deamidated variants, Peak 5 captures native trastuzumab, Peak 6 captures isomerized trastuzumab, and Peak 7 captures C-terminal lysine variants as shown in Figure 3.2.R.5-3 below. There were minor differences between CT-P6 and US-licensed Herceptin in the distribution of charge variants within the seven peaks. However, with the exception of Peak 7, which was reviewed in 3.2.R.3, the overall charge heterogeneity is similar between CT-P6 and US-licensed Herceptin.

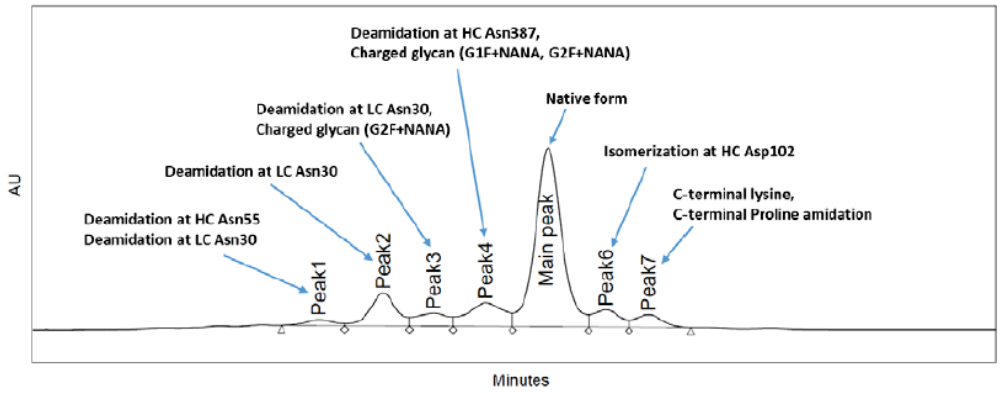


Figure 3.2.R.5-3: Peak Assignments for CT-P6 Drug Product [440mg] (Lot No. 15A3C01)

Each peak was also evaluated for HER2 and C1q binding by ELISA, FcγRIIIa-V and FcRn binding by SPR, and with the in vitro bioactivity (anti-proliferation) assay relative to the unfractionated DP. Each peak for US-licensed Herceptin and CT-P6 showed similar binding to HER2, C1q, FcγRIIIa-V, and FcRn, supporting that charge variants do not impact those functions of trastuzumab. Peaks 1-3 for both products showed a minor reduction in HER2, FcγRIIIa-V, and FcRn binding. However, these differences were within the variability of the assays. The US-licensed Herceptin and CT-P6 anti-proliferation activity for the variants in Peaks 4, 5, and 7 were similar to both unfractionated products. Peaks 1-3 for both products showed a minor reduction in potency, but this was within the variability of the assay. However, as reviewed in 3.2.S.3, isomerized trastuzumab captured by Peak 6 does not show parallelism anti-proliferation assay with the CT-P6 reference material, demonstrating significantly reduced potency for CT-P6 and US-licensed Herceptin.

3.2.R.5.2 Impact of Glycosylation on Biological Activities

The analytical similarity assessment showed that there are differences between CT-P6 and US-licensed Herceptin in the levels of galactosylation and sialylation. As a result, Celltrion conducted a study focused on aglycosylation, agalactosylation, amannosylation, and asialylation to assess the impact of glycan variability on the biological activity of US-licensed Herceptin and CT-P6.

Non-glycosylation

To evaluate the impact of glycan moieties in the Fc region, samples of CT-P6 and US-licensed Herceptin that contain various amounts of fully aglycosylated product were prepared by spiking the samples with PNGase F treated antibody. The samples for each product were assessed by FcγRIIIa-V, FcRn, and HER2 binding affinity as well as the in vitro bioactivity (anti-proliferation) assay. The results and the relative amount of aglycosylated product in each sample for both products are shown in Table 3.2.R.5-12 below.

Table 3.2.R.5-12: Biological Activities of US-licensed Herceptin® (3001168) and CT-P6 Drug Product [440mg] (15A3C01) with Different Levels of Non-glycosylated Molecules

Sample ID	US-licensed Herceptin® (3001168)						CT-P6 Drug Product (15A3C01)					
Level [1]	Control	P1	P2	P3	P4	P5	Control	P1	P2	P3	P4	P5
Non-Glycosylated Heavy Chain (%)	0.60	2.64	4.68	8.64	19.24	38.25	0.58	2.55	4.58	8.54	18.53	37.53
FcγRIIIa-V Binding Affinity [2]	100	99	99	95	87	65	100	100	98	98	85	65
FcRn Binding Affinity [2]	100	102	100	99	96	91	100	96	97	95	94	88
HER2 Binding Affinity [3]	100	104	108	108	111	100	100	103	101	105	106	102
In vitro bioactivity [4]	100	97	91	96	90	91	100	99	90	97	91	92

[1] P1-P5 refer to the samples with various level of non-glycosylated product, produced by spiking samples with fully aglycosylated mAb resulting from PNGase F treatment.
[2] Relative binding affinity is calculated as %Rel of sample / %Rel of Control sample * 100; %Rel is calculated as K_D of CT-P6 *in-house* reference standard / K_D of sample * 100.
[3] Relative binding affinity is calculated as %Rel of sample / %Rel of Control sample * 100; %Rel is calculated as EC_{50} of sample / EC_{50} of CT-P6 *in-house* reference standard * 100.
[4] Relative potency is calculated using PLA software (version 3.0)

The relationship between aglycosylated product and biological activity was determined by the Pearson correlation coefficient through linear regression analyses. The Pearson correlation coefficient was considered significant if the p-value from the linear regression analyses was ≤ 0.05. The data show that non-glycosylation of CT-P6 and US-licensed Herceptin had an adverse impact on FcγRIIIa-V binding. Non-glycosylation had a significant, but moderate adverse impact on FcRn binding with an R-square value of 0.7877. Non-glycosylation did not significantly impact in vitro bioactivity or HER2 binding.

Galactosylated Glycans

In a similar study design, the impact of terminal galactose on biological activity of CT-P6 and US-licensed Herceptin was evaluated using samples of both products that contain different relative amounts of highly agalactosylated product. The samples for each product were assessed by FcγRIIIa-V, FcRn, and HER2 binding affinity as well as the in vitro bioactivity (anti-proliferation) assay. The results and the relative amount of agalactosylated product in each sample for both products are shown in Table 3.2.R.5-22 below. Agalactosylation did not significently impact any attribute for CT-P6 and US-licensed Herceptin.

Table 3.2.R.5-22: Biological Activities of US-licensed Herceptin® (3001168) and CT-P6 Drug Product [440mg] (15A3C01) with Different Levels of galactosylated Glycans

Sample ID	US-licensed Herceptin® (3001168)					CT-P6 Drug Product (15A3C01)				
Level [1]	Control	G1	G2	G3	G4	Control	G1	G2	G3	G4
Moles Galactose/Mole Heavy Chain	0.256	0.196	0.147	0.110	0.066	0.657	0.516	0.403	0.308	0.215
FcγRIIIa-V Binding Affinity [2]	100	104	101	97	99	100	99	97	95	91
FcRn Binding Affinity [2]	100	98	97	96	94	100	99	94	92	93
HER2 Binding Affinity [3]	100	106	106	103	104	100	104	105	104	99
In vitro bioactivity [4]	100	97	96	95	97	100	91	95	87	90

[1] G1-G4 refer to the samples with various levels of galactosylated glycan produced by spiking samples with fully agalactosylated mAb resulting from galactosidase treatment.
[2] Relative binding affinity is calculated as %Rel of sample / %Rel of Control sample * 100; %Rel is calculated as K_D of CT-P6 *in-house* reference standard / K_D of sample * 100.
[3] Relative binding affinity is calculated as %Rel of sample / %Rel of Control sample * 100; %Rel is calculated as EC_{50} of sample / EC_{50} of CT-P6 *in-house* reference standard * 100.
[4] Relative potency is calculated using PLA software (version 3.0).

Reviewer comment: *These data support that the differences in galactosylation between CT-P6 and US-licensed Herceptin observed in the analytical similarity assessment do not preclude a determination of "highly similar" (reviewed in 3.2.R.3.2.6).*

High Mannose Glycans

The impact of high mannose glycans on biological activity of CT-P6 and US-licensed Herceptin was evaluated using samples of both products that contain different relative amounts of mannosidase-treated antibody. The samples for each product were assessed by FcγRIIIa-V, FcRn, and HER2 binding affinity as well as the in vitro bioactivity (anti-proliferation) assay. The results for both products are shown in Table 3.2.R.5-24 below. Amannosylation did not impact biological activity of CT-P6 and US-licensed Herceptin.

Table 3.2.R.5-24: Biological Activities of US-licensed Herceptin® (3001168) and CT-P6 Drug Product [440mg] (15A3C01) Following Mannosidase Treatment

Sample ID	US-licensed Herceptin® (3001168)		CT-P6 Drug Product (15A3C01)	
Level	Control	Mannosidase treated	Control	Mannosidase treated
High Mannose (%)	2.62	0.41	2.33	0.43
FcγRIIIa-V Binding Affinity [1]	100	97	100	100
FcRn Binding Affinity [1]	100	103	100	103
HER2 Binding Affinity [2]	100	100	100	96
In vitro bioactivity [3]	100	99	100	102

[1] Relative binding affinity is calculated as %Rel of sample / %Rel of Control sample * 100; %Rel is calculated as K_D of CT-P6 *in-house* reference standard / K_D of sample * 100.
[2] Relative binding affinity is calculated as %Rel of sample / %Rel of Control sample * 100; %Rel is calculated as EC_{50} of sample / EC_{50} of CT-P6 *in-house* reference standard * 100.
[3] Relative potency is calculated using PLA software (version 3.0)

Reviewer comment: *High mannose glycans are generally afucosylated, which is thought to be the primary reason for their high affinity for FcγRIIIa and increased ADCC. Removal of high mannose would be expected to affect PK but not necessarily ADCC activity. Literature supports that mannosylation at these levels does not significantly impact the function of trastuzumab and high mannose species are controlled through CT-P6 DS release testing. Therefore, it is acceptable to not designate mannosylation as a CQA.*

Sialic Acids

The impact of terminal sialic acid on biological activity of CT-P6 and US-licensed Herceptin was evaluated using samples of both products that contain different relative amounts of neuraminidase-treated antibody. The samples for each product were assessed by FcγRIIIa-V, FcRn, and HER2 binding affinity as well as the in vitro bioactivity (anti-proliferation) assay. The results for both products are shown in Table 3.2.R.5-26 below. The levels of terminal sialic acid did not impact biological activity of CT-P6 and US-licensed Herceptin.

Table 3.2.R.5-26: Biological Activities of US-licensed Herceptin® (3001168) and CT-P6 Drug Product [440mg] (15A3C01) Following Neuraminidase Treatment

Sample ID	US-licensed Herceptin® (3001168)		CT-P6 Drug Product (15A3C01)	
Level	Control	Neuraminidase treated	Control	Neuraminidase treated
Sialic acid (%)	0.64	0.16	2.86	0.23
FcγRIIIa-V Binding Affinity [1]	100	101	100	102
FcRn Binding Affinity [1]	100	100	100	98
HER2 Binding Affinity [2]	100	99	100	102
In vitro bioactivity [3]	100	92	100	97

[1] Relative binding affinity is calculated as %Rel of sample / %Rel of Control sample * 100; %Rel is calculated as K_D of CT-P6 *in-house* reference standard / K_D of sample * 100.
[2] Relative binding affinity is calculated as %Rel of sample / %Rel of Control sample * 100; %Rel is calculated as EC_{50} of sample / EC_{50} of CT-P6 *in-house* reference standard * 100.
[3] Relative potency is calculated using PLA software (version 3.0)

Afucosylated Glycans

To study the impact of afucosylation on the ADCC activity and FcγRIIIa-V binding of CT-P6, cells were cultured in small-scale bioreactor with a fucose analog to reduce fucosylation. The harvested antibody was purified via the CT-P6 downstream purification process. Samples of CT-P6 with various levels of afucosylated glycans were generated by mixing afucosylated CT-P6 with the CT-P6 reference standard. The results and the relative amount of afucosylated product in each sample for both products are shown in Table 3.2.R.5-29 below.

Table 3.2.R.5-29: FcγRIIIa-V Binding Affinity and ADCC Reporter Assay Results for CT-P6 [440mg] Samples with Varying Levels of Afucosylated Glycans

Sample ID	Afucosylation level (%)[1]	FcγRIIIa-V Binding Affinity (%, K_D)	ADCC Reporter Assay (%, EC50)
Afucose_Control	4.64	100	100
Afucose_SP1	8.56	122	125
Afucose_SP2	12.16	141	142
Afucose_SP3	18.9	162	162
Afucose_SP4	24.43	178	176
Afucose_SP5	31.91	205	214
Afucose_SP6	41.64	223	244
Afucose_SP7	53.30	270	261

[1] G0+G1+G2 by NP-UPLC.

Pearson correlation coefficient and linear regression analyses were used to determine the relationship between afucosylation and biological activity of CT-P6. The data generated during the analytical similarity assessment was used to determine the relationship between afucosylation and biological activity US-licensed Herceptin. The data show that there is a significant relationship between the levels of afucosylation and FcγRIIIa-V binding (R2 0.97939), between the levels of afucosylation and ADCC

activity (R2 0.94913), and between FcγRIIIa-V binding and ADCC activity (R2 0.9490), all with a p-value <0.0001.

Reviewer comment: *These additional characterization data support that the differences in the glycan profile between CT-P6 and US-licensed Herceptin are not meaningful and that CT-P6 is highly similar to US-licensed Herceptin. These data are also in agreement with the data in 3.2.S.3 that afucosylation significantly impacts ADCC activity of trastuzumab.*

3.2.R.5.3 Forced Degradation Study

A forced degradation study was performed to evaluate the similarity of the degradation profiles between CT-P6 and US-licensed Herceptin under oxidizing conditions, UV exposure conditions, high temperature, and low and high pH. Table 3.2.R.5-42 shows the exposure conditions and the assays used to evaluate CT-P6 and US-licensed Herceptin quality attributes.

Table 3.2.R.5-42: Sample Treatment Conditions and Test Methods Used For Forced Degradation Study

Sample Treatment Condition			
Stressed Type	**Description**	**Incubation Time**	**Sample Name**
Control	N/A		Control
Hydrogen Peroxide	0.01% H_2O_2, 2 – 8°C	7 hrs	H1
		3 days	H2
UV Light	25 w/m², 25°C, 60% RH	1 day	U1
		3 days	U2
High Temperature	50°C	1 week	T1
		2 weeks	T2
Low pH condition	pH 2.8 , 25°C	3 days	A1
		7 days	A2
High pH condition	pH 11.0 , 2 – 8°C	1 day	B1
		3 days	B2

Test Method	
Test Item	**Purpose**
IEC-HPLC	Comparison of charge variant distribution
SEC-HPLC	Comparison of aggregate content and monomeric purity
Peptide Mapping (LC/MS)	Comparison of peptide map profiles and detection of amino acid modification
CE-SDS (non-reduced, reduced)	Determination of intact IgG level and fragments
Iso Electric Focusing (IEF)	Determination of isoelectric point
Oligosaccharide profiling (NP-UPLC)	Determination of glycosylation pattern
FcRn Binding Affinity (SPR)	Evaluation of the binding affinity to FcRn, related to antibody pharmacokinetic half-life
FcγRIIIa-V Binding Affinity (SPR)	Evaluation of the binding affinity to FcγRIIIa-V, related to trigger antibody dependent cell-mediated cytotoxicity
HER2 Binding Affinity (ELISA)	Evaluation of the HER2 binding affinity
In vitro bioactivity (Anti-proliferation)	Evaluation of anti-proliferation effect

N/A: Not applicable

Exposure to H2O2 had a significant impact on oxidation at HC Met255 for CT-P6 and US-licensed Herceptin. There was also a small reduction on intact antibody for both products (98 to 94%).

Oxidation had a greater impact on US-licensed Herceptin (100 to 83%) compared to CT-P6 (100 to 90%). The degradation profiles between CT-P6 and US-licensed Herceptin were similar after exposure to oxidizing conditions.

Reviewer comment: *As noted in 3.2.S.3, although CT-P6 contains 12 oxidation sides, only HC Met255 was evaluated because it was assessed that this residue is the most susceptible to oxidation. In their October 16 IR response, Celltrion reevaluated all CT-P6 oxidation sites in detail and this risk assessment supports that oxidation is not a CQA.*

Exposure to UV light stress had a significant impact on charge heterogeneity, HWM and LWM content, oxidation at HC Met255, and potency as well as FcRn, FcγRIIIa-V, and HER2 binding. The degradation profiles were similar between CT-P6 and US-licensed Herceptin. After three days after exposure, CT-P6 showed higher, but not significant HMW and LMW content compared to US-licensed Herceptin.

After exposure to high temperature, charge heterogeneity, deamidation, isomerization, intact antibody by CE-SDS, HER2 binding, and potency were significantly impacted for both products. The degradation profiles between CT-P6 and US-licensed Herceptin were similar after exposure to high temperature.

Under acid stress conditions, charge heterogeneity, intact antibody, HMW and LWM content, and potency as well as FcRn, FcγRIIIa-V, and HER2 binding were significantly impacted for both products. Under base stress conditions, charge heterogeneity, HMW content, and HER2 binding were significantly impacted for both products. Under both conditions, the degradation profiles between CT-P6 and US-licensed Herceptin were similar.

Reviewer comment: *The degradation profiles for CT-P6 and US-licensed Herceptin were similar under various stress conditions. The data support that CT-P6 is "highly similar" to US-licensed Herceptin.*

The data also show that under high temperatures and base stress, deamidation increases in CT-P6 and US-licensed Herceptin, which suggests that deamidation may be a CQA for CT-P6. Celltrion was sent an IR on September 26, requesting data and justification to support that deamidation is not a CQA. As reviewed in 3.2.S.3, Celltrion was instructed to designate deamidation of LC Asn30 a CQA.

3.2.R.6 Analytical Method Description

Reviewer comment: *This section contains a description of each method used in the analytical similarity assessment that was reviewed in 3.2.R.2-3.2.R.5 and 3.2.S.3.*

3.2.R.7 Analytical Method Qualification

Qualification information was provided for each assay included in the analytical similarity assessment that was not validated and provided in 3.2.S.4.3. The assays in this section are amino acid analysis, peptide mapping (LC-MS), intact mass (LC-MS), N- and C-terminal sequencing, disulfide bonds, free thiol analysis, FTIR, CD, DSC, SEC-MALS, sialic acid analysis, N-linked glycan analysis, cell-based HER2 binding, C1q binding, FcγRIIIa-F binding, FcγRIIIb binding, FcγRIIa binding, FcγRIIb binding, FcγRI binding, FcRn binding, ADCC with PBMCs, and the ADCC reporter assay.

Reviewer comment: *All assays included in this section are qualified and suitable for their intended purpose. Intermediate precision for peptide mapping by LC-MS was ≤54% RSD. However, the data generated by this assay suggest that there is low lot-to-lot variability and orthogonal methods such as HPLC support that the peptide sequence of CT-P6 is highly similar to US-licensed Herceptin. Additionally, SEC-MALS shows 22-31% RSD for concentration-dependent measurement, repeatability, and intermediate precision for dimers, which indicates SEC-MALS is not suitable for resolution of trastuzumab dimers. SEC-HPLC showed lower assay variability and supports that the HWM content of CT-P6 is highly similar to US-licensed Herceptin. Therefore, the limitations of these assays do not impact the overall conclusion of the analytical similarity assessment.*

3.2.R.8 Appendices

This section contains a table of contents for the appendices contains within 3.2.R.

Executed Batch Records

The batch records were evaluated as part of the BLA review.

Reviewer comment: *The batch records were acceptable.*

Riley C Myers

Digitally signed by Riley C Myers
Date: 2/21/2018 03:40:41PM
GUID: 57f6809300502f3978f97f5b5a09d128

Jennifer Swisher

Digitally signed by Jennifer Swisher
Date: 2/21/2018 04:02:37PM
GUID: 508da6d7000262dc015dcdc5f6541612

Kathleen Clouse Strebel

Digitally signed by Kathleen Clouse Strebel
Date: 2/21/2018 08:43:25PM
GUID: 508da6d70002630c9a2555c796176955

BLA 761091
Trastuzumab-(b) (4)
Celltrion, Inc.

Summary of Quality Assessments

I. Primary Reviewer Summary Recommendation

This memo contains the reviews for Drug Product manufacture and Immunogenicity. Trastuzumab-(b) (4) drug product is manufactured and supplied as a 420 mg/vial presentation, which is the same as U.S.-licensed Herceptin. The drug product is a sterile, lyophilized powder for intravenous infusion and is supplied as a multi-dose vial. The formulation is identical except for an increase in α,α-trehalose dihydrate (from 381 to 839 mg/vial), which is (b) (4) During review of this application, it was determined that the drug product release specification must be adjusted to ensure that the recoverable protein content meets the minimum label claim of 420 mg, which would require adjustment of the (b) (4) control strategy. However, during the review cycle, the drug product manufacture facility was under Official Action Indicated (OAI) status associated with a Warning Letter (WL 320-18-28) issued to Celltrion. Therefore, adjustment of the recoverable protein content control strategy will be a deficiency that will be communicated in the Complete Response letter. CMC recommends that Celltrion be sent a Complete Response letter for BLA 761091.

II. List of Deficiencies to be Communicated for Drug Product

1. Per the "Guidance for Industry: Allowable Excess Volume and Labeled Vial Fill Size in Injectable Drug and Biological Products" (https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm389069.pdf), "In the case of drug products requiring reconstitution, the product should be designed to meet the label claim and acceptable overfill, and allow for correct dosing". The DP release specification of (b) (4) mg/vial must be adjusted to ensure that the recoverable protein content at the lower limit of the acceptance criterion will consistently meet the label claim of 420 mg. Adjust the (b) (4) control strategy to meet the updated specification.

2. As committed in the response to an IR received 2/20/2018, Celltrion will perform process characterization studies in order to establish acceptable ranges for lyophilization process parameters such as (b) (4). The criticality of these process parameters must be justified based on characterization of their impact on CT-P6 product quality attributes.

   Additional comments that are not approvability issues:

3. We cannot fully assess the adequacy of Celltrion's control strategy for CT-P6 in the absence of a pre-license manufacturing inspection. In the responses received on 10/16/2017 and 1/29/2018 to our IRs regarding the acceptable ranges for numerous process parameters that have the potential to impact product quality, the limited process characterization and process validation data may not fully justify the criticality ranking of these parameters. Their acceptability will be a review issue once the entire control strategy is updated and available for review.

4. In the Complete response submission, provide updated data from the following studies that are ongoing:

a. The commercial scale (b) (4) study for (b) (4) (in section 3.2.S.2.5),
b. The commercial (b) (4) lifetime study (in section 3.2.S.2.5),
c. The ongoing CT-P6 DS and DP stability studies (in sections 3.2.S.7 and 3.2.P.8),
d. The shipping validation of the finished CT-P6 drug product (in section 3.2.P.3.5),
e. The stability study on three in-use PS20 lots for up to 12 months, committed in your 2/20/2018 response (in Section 3.2.P.4).

Contents

3.2.P: Drug Product

3.2.P. herzuma - 420 mg Lyophilized Powder in a Multi-Dose Vial for Reconstitution

3.2.P.1 Description and Composition of the Drug Product [420 mg]

The 420 mg presentation of CT-P6 DP is formulated for IV administration as a multi-dose vial containing 420 mg API as a lyophilized, sterile powder. The powder is reconstituted with 20 mL of bacteriostatic water for injection (BWFI) to yield 21 mg/mL DS at pH 6.0. A (b) (4)% overfill is applied during manufacturing to ensure the labeled dose of 420 mg can be withdrawn from each vial. The composition of the DP is listed in Table 3.2.P.1-1.

Table 3.2.P.1-1: Composition of the CT-P6 Drug Product [420 mg]

Ingredient	Quantity/Vial	Function	Grade
CT-P6	420 mg	Active ingredient	In-house
α,α-Trehalose, Dihydrate	839.0 mg	(b) (4)	USP/NF/Ph. Eur.
L-Histidine HCl	9.5 mg		Ph. Eur.
L-Histidine	6.1 mg		USP/Ph. Eur.
Polysorbate 20	1.7 mg		NF/Ph. Eur.

NF: National Formulary, Ph. Eur.: European Pharmacopoeia, USP: United States Pharmacopoeia

Reviewer comment: *The US Herceptin label claim was recently changed from 440 mg to 420 mg. Therefore, CELLTRION submitted their development and manufacturing data for the 440 mg DP presentation, which was developed to match the measured content in the US Herceptin vial, as a 420 mg DP presentation. In response to IR sent on January 9, 2018, the sponsor has updated Module 3 to reflect the change from 440 mg/vial presentation to 420 mg/vial presentation. The Sections that were updated were 3.2.P.1, 3.2.P.2.2, 3.2.P.2.3, 3.2.P.3.2, 3.2.P.3.3, 3.2.P.3.4, 3.2.P.3.5, 3.2.P.5.1, 3.2.P.5.6, and 3.2.P.8.1.*

3.2.P.2 Pharmaceutical Development [420 mg]

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(b) (4)

3.2.P.8 Stability [420 mg]

3.2.P.8.1 Stability Summary and Conclusion

3.2.P.8.1.1 Introduction

Stability studies were conducted using 6 lots of CT-P6 DP. Three of these lots were manufactured by Process A at CELLTRION (b) (4) and three of the lots were manufactured by the proposed commercial process, Process B, at (b) (4). All 6 DP lots were manufactured using CT-P6 DS derived from the Clinical Process (Process B for DS).

Stability studies for CT-P6 DP have been conducted for the following conditions:

- long-term storage condition of 5±3°C
- accelerated stability condition of 25±2°C/60±5% RH
- stress stability condition of 40±2°C/75±5% RH
- 'in-use' study of DP reconstituted with BWFI at 5±3°C
- in-use stability of reconstituted and diluted DP and its compatibility with infusion bags at 30±2°C/75±5% RH
- photostability study
- forced degradation study to characterize and understand the processes and pathways associated with CT-P6 degradation (presented in Section 3.2.R.5.3).

Stability studies have also been conducted with 3 lots of US-licensed Herceptin at the accelerated condition of 25±2°C/60±5% RH and stress condition of 40±2°C/75±5% RH for similarity assessment.

Reviewer comment: *The sponsor has provided stability data on 6 DP lots at the long-term storage condition of 5±3°C, accelerated stability condition of 25±2°C/60±5% RH, and stress stability condition of 40±2°C/75±5% RH. Three of these lots were manufactured using Process A and three lots were manufactured using Process B which is the commercial process. According to ICH Q5C, "stability information should be provided for three batches of final container product representative of that which will be used at manufacturing scale." The stability data submitted by the sponsor is acceptable.*

3.2.P.8.1.2 Information on Lots included in Stability Studies

CT-P6 DP lots used for stability studies is presented in Table 3.2.P.8.1-1 and US-licensed Herceptin lots used in stability studies is presented in Table 3.2.P.8.1-2.

Table 3.2.P.8.1-1: Overview of CT-P6 Drug Product [440mg] Lots Employed in Stability Studies

Storage Condition	Lot No.	Date of Initiation of Stability Study	Manufacture Site of Drug Product (b) (4)	Date of Manufacture	Drug Substance Batch Used	Drug Product Manufacturing Process
Long-term Condition at 5±3°C	13A3C002	20 Apr 2013		20 Apr 2013	13200A02	Process A
	13A3C003	25 Apr 2013		25 Apr 2013	13200A03	Process A
	13A3C004	29 Apr 2013		29 Apr 2013	E13200A01	Process A
	15A3C01	02 Jan 2016		02 Jan 2016	14200A007	Process B
	16A3C01	06 Jan 2016		06 Jan 2016	14200A008	Process B
	16A3C02	10 Jan 2016		10 Jan 2016	14200A003	Process B
Accelerated Condition at 25±2°C / 60±5% RH	13A3C002	23 May 2013		20 Apr 2013	13200A02	Process A
	13A3C003	23 May 2013		25 Apr 2013	13200A03	Process A
	13A3C004	23 May 2013		29 Apr 2013	E13200A01	Process A
	15A3C01	05 Apr 2016		02 Jan 2016	14200A007	Process B
	16A3C01	06 Apr 2016		06 Jan 2016	14200A008	Process B
	16A3C02	11 Apr 2016		10 Jan 2016	14200A003	Process B
Stress Condition at 40±2°C / 75±5% RH	13A3C002	23 May 2013		20 Apr 2013	13200A02	Process A
	13A3C003	23 May 2013		25 Apr 2013	13200A03	Process A
	13A3C004	23 May 2013		29 Apr 2013	E13200A01	Process A
	15A3C01	05 Apr 2016		02 Jan 2016	14200A007	Process B
	16A3C01	06 Apr 2016		06 Jan 2016	14200A008	Process B
	16A3C02	11 Apr 2016		10 Jan 2016	14200A003	Process B
In-use Stability at 5±3°C	13A3C002	02 Oct 2013		20 Apr 2013	13200A02	Process A
Infusion Stability at 30±2°C / 75±5% RH	13A3C002	31 Oct 2016		20 Apr 2013	13200A02	Process A
Confirmatory Photostability in 1,200,000 Lux hr Vis. and 200 Whr/m^2 UV light	13A3C002	24 Oct 2016		20 Apr 2013	13200A02	Process A
at 25±2°C / 60±5% RH						

Table 3.2.P.8.1-2: Lots of US-licensed Herceptin® Employed in Accelerated and Stress Stability Studies

Lot No.	Dosage per Vial	Expiration Date	Source	Age at Stability Study Initiation
529850	440 mg	Nov 2016	US	10 Months
548420	440 mg	Dec 2016	US	9 Months
554761	440 mg	Nov 2016	US	10 Months

3.2.P.8.1.3 Specification for Stability Studies

The specification for stability studies is presented in Table 3.2.P.8.1-3. The specification and test methods are identical to those for CT-P6 DP QC release, except for identity test by IEC-HPLC, which was not included for stability testing because it is not considered to be a stability-indicating parameter.

Peptide mapping, CE-SDS (non-reduced/reduced), HER2 binding affinity by ELISA, FcγRIIIa binding affinity (V type) by SPR, and methylene blue dye penetration for container/closure integrity were also assessed as part of the stability studies; however, these tests have not been included in the proposed end-of-shelf-life specification and annual stability program post approval.

Reviewer comment: *The specification for stability studies is acceptable. The sponsor has conducted additional tests as part of the stability studies for information purpose only. These tests are peptide mapping, CE-SDS (non-reduced/reduced), HER2 binding affinity by ELISA, FcγRIIIa binding affinity (V type) by SPR, and methylene blue dye penetration. These tests will not be included in the end-of-shelf-life specification for CT-P6 DP. This is acceptable, except for methylene blue dye penetration test which should be included in the specification because it provides data on long-term storage in the container closure system.*

Table 3.2.P.8.1-3: End-of-Shelf-Life Specification for CT-P6 Drug Product [440mg]

Test Item	Acceptance Criterion
General	
Color and Physical State of Lyophilizate	White to pale yellow lyophilized powder
Clarity upon Reconstitution	Clear to slightly opalescent solution
Color upon Reconstitution	Colorless to pale yellow
Visible Particles upon Reconstitution	Free of visible particles
Reconstitution Time	≤ (b) (4) (min:sec)
Residual Moisture	≤ (b) (4) %
pH	6.0 (b) (4)
Osmolality	(b) (4) mOsm/kg
Sub-visible Particles	≥ 10 µm: ≤ (b) (4) /vial ≥ 25 µm: ≤ vial
Extractable Volume	≥ (b) (4) mL
Safety	
Endotoxin	≤ (b) (4) EU/mL
Sterility	No growth
Identity	
IEF	All bands of the test sample are ≤ (b) (4) pI unit of the corresponding bands of the reference material. No new band.
Purity/Impurity	
IEC-HPLC	Peak 1+Peak 2+Peak 3+Peak 4: (b) (4) % Peak 5 ≥ (b) (4) % Peak 6 ≤ % Peak 7 ≤ %
SEC-HPLC	Monomer ≥ (b) (4) %
Quantity	
Protein Content (UV_{280})	(b) (4) mg/vial
Potency	
In vitro Bioactivity	(b) (4) U/mg

[1] Acceptance Value
[2] Reference Value

3.2.P.8.1.4 Stability Study Protocols and Current Status

Stability tests are the same as those for QC release of DP and are described in the relevant sections in Section 3.2.S.4.2 and Section 3.2.P.5.2.

3.2.P.8.1.4.1 Long-term (Real-Time/Real-Temperature) Stability Study (5±3°C)

The stability testing schedule to determine the shelf-life for long-term storage of CT-P6 DP at 5±3°C is shown in Table 3.2.P.8.1-4.

Table 3.2.P.8.1-4: Long-Term Stability Study (5±3°C) Protocol

Test Item	Time Point (Month)												
	0	3	6	9	12	18	24	30	36	45	48	60	72
Color and Physical State of Lyophilizate	T	T	T	T	T	T	T	T	T	T	T	T	T
Clarity upon Reconstitution	T	T	T	T	T	T	T	T	T	T	T	T	T
Color upon Reconstitution	T	T	T	T	T	T	T	T	T	T	T	T	T
Visible Particles upon Reconstitution	T	T	T	T	T	T	T	T	T	T	T	T	T
Reconstitution Time	T	T	T	T	T	T	T	T	T	T	T	T	T
pH	T	T	T	T	T	T	T	T	T	T	T	T	T
Osmolality	T	T	T	T	T	T	T	T	T	T	T	T	T
Sub-visible Particles	T[1]	T[1]	T	T	T	T	T	T	T	T	T	T	T
Residual Moisture	T	T	T	T	T	T	T	T	T	T	T	T	T
Extractable Volume	T	T	T	T	T	T	T	T	T	T	T	T	T
IEF	T	T	T	T	T	T	T	T	T	T	T	T	T
Peptide Mapping	T	T	T	T	T	T	T	T	T	T	T	T	T
CE-SDS (Non-reduced)	T	T	T	T	T	T	T	T	T	T	T	T	T
CE-SDS (Reduced)	T	T	T	T	T	T	T	T	T	T	T	T	T
IEC-HPLC	T	T	T	T	T	T	T	T	T	T	T	T	T
SEC-HPLC	T	T	T	T	T	T	T	T	T	T	T	T	T
Protein Content (UV_{280})	T	T	T	T	T	T	T	T	T	T	T	T	T
In vitro Bioactivity	T	T	T	T	T	T	T	T	T	T	T	T	T
HER2 Binding Affinity	T	T	T	T	T	T	T	T	T	T	T	T	T
FcγRIIIa Binding Affinity (V type)	T	T	T	T	T	T	T	T	T	T	T	T	T
Sterility	T	-	T	-	T	-	T	T	T	T	T	T	T
Endotoxin	T	-	T	-	T	T	T	T	T	T	T	T	T
Methylene Blue Dye Penetration	-	-	-	-	T	-	T	-	T	-	T	T	T

[1] The sub-visible particles of <10 μm were tested only for CT-P6 drug product (Process B).
T: Testing planned, –: No testing

Reviewer comment: *Stability testing data is available for long-term storage at 5±3°C for 48 months for 3 lots of DP manufactured using Process A and for 18 months for 3 lots of DP manufactured using Process B.*

3.2.P.8.1.4.2 Accelerated Stability Study (25±2°C/60±5% RH)

Accelerated stability studies at 25±2°C/60±5% RH have been conducted on 6 lots of CT-P6 DP (3 Process A and 3 Process B DP lots) and 3 lots of US-licensed Herceptin. The study time points were 0, 1, 2, 3, and 6 months and the stability indicating tests are the same tests as those for the long-term storage condition of 5±3°C which are listed in Table 3.2.P.8.1-4 (above). Only the methylene blue dye penetration study has not been included in the stability protocol for 25±2°C/60±5% RH. Stability studies for US-licensed Herceptin for similarity assessment only included IEF, peptide mapping, CE-SDS (reduced and non-reduced), IE-HPLC, SEC-HPLC, protein content, in vitro bioactivity, HER2 binding

activity, and FcγRIIIa binding activity. All accelerated stability studies have been completed by the sponsor.

Reviewer comment: *Accelerated stability studies have been conducted with 6 lots of CT-P6 DP and 3 lots of US-licensed Herceptin. Accelerated study protocol was only for 6 months and the study has been completed by the sponsor. The accelerated stability study protocol is consistent with the guidelines in ICH Q1A R2 Stability Testing of New Drug Substances and Products and is therefore, acceptable.*

3.2.P.8.1.4.3 Stress Stability Study (40±2°C/75±5% RH)

Stress stability studies at 40±2°C/75±5% RH were conducted with 6 lots of CT-P6 DP (3 Process A and 3 Process B DP lots). The testing time points were 0, 1, 2, and 3 months and the stability indicating tests are the same tests as those for the long-term storage condition of 5±3°C which are listed in Table 3.2.P.8.1-4 (above), except for sterility, endotoxin, and methylene blue dye penetration. Stability studies for US-licensed Herceptin for similarity assessment included IEF, peptide mapping, CE-SDS (reduced and non-reduced), IE-HPLC, SEC-HPLC, protein content, in vitro bioactivity, HER2 binding activity, and FcγRIIIa binding activity. All stress stability studies have been completed by the sponsor.

Reviewer comment: *Stress stability studies were conducted with 6 lots of CT-P6 DP and 3 lots of US-licensed Herceptin. The study protocol was only for 3 months and the study has been completed by the sponsor.*

3.2.P.8.1.4.4 In-Use (Reconstituted CT-P6 Drug Product) Stability Study (5±3°C)

One lot of CT-P6 DP (Lot No. 13A3C002 manufactured using Process A) was tested for stability after reconstitution with 20 mL of BWFI containing 1.1% benzyl alcohol prior to storage at 5±3°C. The stability indicating tests were clarity upon reconstitution, color upon reconstitution, visible particles upon reconstitution, pH, (b) (4), sub-visible particles, IEF, peptide mapping, CE-SDS (non-reduced and reduced), IEC-HPLC, SEC-HPLC, protein content, in vitro bioactivity, HER2 binding affinity, FcγRIIIa binding affinity (V type), antimicrobial preservative, sterility, and endotoxin, which are listed in Table 3.2.P.8.1-10 of the submission. The testing schedule was 0, 14, and 28 days. The testing time point for antimicrobial preservative was at 0 days. However, the sponsor states that no testing was done at this time point and that the testing for antimicrobial preservative was covered for up to 28 days as per USP <51> Antimicrobial Effectiveness Testing.

Reviewer comment: *Stability testing of reconstituted CT-P6 DP was done with Lot No. 13A3C002 which is manufactured using Process A. Process A is not the commercial DP manufacturing process. Is this acceptable?*

The sponsor states that testing for antimicrobial preservative is scheduled for on day 0; however, the testing was not done at that time point. Furthermore, the sponsor states that the testing for antimicrobial preservative was covered for up to 28 days as per USP <51> Antimicrobial Effectiveness Testing. This statement is not clear because the 2 statements seem to be conflicting with each other.

An update (on December 22, 2017) was provided for In-Use (Reconstituted CT-P6 Drug Product) Stability Study (5±3°C) where potential microbial contamination during reconstitution was examined in

a microbiological study. The study was performed with DP lot 15A3C001, which is manufactured using DP Process B. This study will be reviewed by DMA.

3.2.P.8.1.4.5 In-Use (Dilution for Infusion) Stability Study (30±2°C/75±5% RH)

One lot of CT-P6 DP (Lot No. 13A3C002 manufactured using Process A) was tested for stability after dilution in either polyethylene (PE) or polyvinyl chloride (PVC) infusion bag containing 0.9% sodium chloride. The drug concentrations tested were for a dose of 2 mg/kg and 8 mg/kg administered to a 60 kg patient. To represent these doses, 120 mg and 480 mg CT-P6 were diluted in 250 mL PE or PVC infusion bag resulting in a final dose concentration of 0.47 mg/mL and 1.76 mg/mL, respectively. The stability indicating tests were clarity upon reconstitution, color upon reconstitution, visible particles upon reconstitution, pH, (b) (4), sub-visible particles, IEF, peptide mapping, CE-SDS (non-reduced and reduced), IEC-HPLC, SEC-HPLC, protein content, in vitro bioactivity, HER2 binding affinity, and FcγRIIIa binding affinity (V type), which are listed in Table 3.2.P.8.1-12 of the submission. The testing time points were 0, 24, and 48 hours. This stability study has been completed by the sponsor.

Reviewer comment: *The sponsor has conducted in-use stability studies for dilution in either PE or PVC infusion bag. The range of stability indicating tests and testing schedule appear acceptable. However, these stability studies were only conducted with one DP lot (Lot No. 13A3C002) which was manufactured using Process A.*

The sponsor states that final dose concentration of the 8 mg/kg DP dose after dilution in infusion bag was approximately 1.76 mg/mL. The dose after dilution should be 1.92 mg/mL.

An update (on December 22, 2017) was provided for In-Use (Dilution for Infusion) Stability Study (30±2°C/75±5% RH) where potential microbial contamination during reconstitution and dilution was examined in a microbiological study. The study was performed with DP lot 15A3C001, which is manufactured using DP Process B. This study will be reviewed by DMA.

3.2.P.8.1.4.6 Confirmatory Photostability Study (1,200,000 Lux hr Vis. and 200 Whr/m2 UV under 25±2°C/60±5% RH)

Photostability of CT-P6 DP was assessed using Lot No. 13A3C002 which was manufactured using Process A. DP stored in primary package (nude vials) were subjected to illumination of 1,200,000 Lux hr of visible light (Lux hr Vis.) and near UV energy of 200 Whr/m^2 under 25±2°C/60±5% RH. The stability indicating tests were color and physical state of lyophilizate, clarity upon reconstitution, color upon reconstitution, visible particles upon reconstitution, pH, (b) (4), sub-visible particles, residual moisture, IEF, peptide mapping, CE-SDS (non-reduced and reduced), IEC-HPLC, SEC-HPLC, protein content, in vitro bioactivity, HER2 binding affinity, and FcγRIIIa binding affinity (V type), which are listed in Table 3.2.P.8.1-14 of the submission. The testing schedule is 0 and 70 hours. A 70 hour dark control is included in the study. This photostability study has been completed by the sponsor.

Reviewer comment:
Photostability study is conducted using one lot of CT-P6 DP manufactured using Process A, which is not the commercial process. In addition, the studies were done using DP in 50 m (b) (4) glass vials with the (b) (4) ubber stoppers and 20 mm flip-off seal which is the immediate container closure system for the DP.

According to ICH Q1B Photostability Testing of New Drug Substances and Products, photostability studies on DP should be conducted in a sequential manner starting with testing the fully exposed product, then progressing as necessary to the product in the immediate pack (container with direct contact with the DP) and then in the marketing pack. Therefore, photostability studies for CT-P6 DP appear to be incomplete.

In terms of the number of batches used for photostability studies, ICH Q1A R2 states that photostability testing should be conducted on at least one primary batch of the DP if appropriate. ICH Q1B states that "Normally, only one batch of drug product is tested during the development phase, and then the photostability characteristics should be confirmed on a single batch selected as described in the parent guideline if the product is clearly photostable or photolabile." The studies submitted in this BLA is the only photostability study conducted by the sponsor. This may be acceptable and will depend on the review of the photostability raw data.

3.2.P.8.1.5 Stability Conclusions

3.2.P.8.1.5.1 Long-term (Real-Time/Real-Temperature) Stability

Long-term stability studies were conducted at 5±3°C using three DP Process A lots (13A3C002, 13A3C003, 13A3C004) and three DP Process B lots (15A3C01, 16A3C01, 16A3C02) manufactured at (b) (4). Data are available for Process A lots for 48 months and for Process B lots for 18 months. The study results demonstrate the all the DP lots met the stability indicating tests' acceptance criteria. Additional characterization tests that were conducted for information purposes also show that the DP does not change much over the study periods. The only test result that showed a changing trend was reconstitution time (acceptance criterion ≤ (b) (4) (min:sec)) which gradually increased over time. The following is the reconstitution time data for the DP lots:

- Lot No. 13A3C002, Process A: Reconstitution time increases from 1:03 minutes at 0 months to 2:31 minutes at 48 months.
- Lot No. 13A3C003, Process A: Reconstitution time increases from 1:21 minutes at 0 months to 2:20 minutes at 48 months.
- Lot No. 13A3C004, Process A: Reconstitution time increases from 1:26 minutes at 0 months to 2:25 minutes at 48 months.
- Lot No. 15A3C01, Process B: Reconstitution time increases from 1:30 minutes at 0 months to 2:50 minutes at 18 months.
- Lot No. 16A3C01, Process B: Reconstitution time increases from 1:06 minutes at 0 months to 2:54 minutes at 18 months.
- Lot No. 16A3C02, Process B: Reconstitution time increases from 1:19 minutes at 0 months to 2:10 minutes at 18 months.

Trend analysis (Section 3.2.P.8.3.7) for charge variants by IEC-HPLC, CE-SDS (reduced and non-reduced), monomer content by SEC-HPLC, in vitro bioactivity, and FcγRIIIa binding affinity showed no adverse trend.

Reviewer comment: *The 6 DP lots that were placed on long-term stability study at 5±3°C met the acceptance criteria for the stability indicating tests for the time period of 18 to 48 months. The stability studies will continue for another 72 months. The only test result that showed a changing trend was an increase in reconstitution time. The reason for this increase is not clear. The results for residual moisture do not show any consistent trend that would explain the increase in reconstitution time.*

3.2.P.8.1.5.2 Accelerated Stability

Accelerated stability studies at 25±2°C/60±5% RH for 6 months were conducted with 6 lots of CT-P6 DP and 3 lots of US-licensed Herceptin. All the test results met the acceptance criteria. The following tests showed a changing trend:

- Lot No. 13A3C002, Process A: HER2 binding affinity data showed a decrease in binding affinity over the course of 6 months. HER2 binding affinity decreased from 110.56% to 88.52% over the 6 month period. FcγRIIIa binding affinity increased from 100% to 106% over the 6 month period. Both HER2 binding affinity and FcγRIIIa binding affinity data are provided as additional tests for information purposes, therefore, an acceptance criteria have not been set for these tests.
- Lot No. 13A3C003, Process A: HER2 binding affinity decreased from 104.58% to 96.66%. FcγRIIIa binding affinity increased from 91% to 98%.
- Lot No. 13A3C004, Process A: HER2 binding affinity decreased from 119.06% to 90.48%. FcγRIIIa binding affinity increased from 97% to 102%.
- Lot No. 15A3C01, Process B: HER2 binding affinity decreased from 96.69% to 92.42%. FcγRIIIa binding affinity decreased from 105% to 96%.
- Lot No. 16A3C01, Process B: No consistent trend was seen for HER2 binding affinity. FcγRIIIa binding affinity decreased from 103% to 96%.
- Lot No. 16A3C02, Process B: No consistent trend was seen for HER2 binding affinity. FcγRIIIa binding affinity decreased from 107% to 100%.
- Color upon reconstitution was pale yellow for the 3 Process A DP lots and colorless for the 3 Process B DP lots. Both sets of test results meet the acceptance criterion.
- Trend analysis for HER2 binding affinity (Section 3.2.P.8.3.7) shows there is a consistent decrease in binding affinity from 0 months to 6 months. This decrease is more pronounced for Process A lots. HER2 binding affinity results for Process B lots do not show this trend.
- Similarity assessment for HER2 binding affinity with US-licensed Herceptin shows that the Process B lots have a similar HER2 binding profile to US-licensed Herceptin. The Process A lots show a larger decrease in binding affinity and therefore, shows a slightly different binding affinity trend compared to US-licensed Herceptin.
- For FcγRIIIa binding affinity, a specific trend in binding affinity for all the different DP lots is not seen. However, differences are seen for the 3 sets of DP lots. Process A DP lots show an increase in binding affinity over time. Process B DP lots show a decrease in binding over time. For US-licensed Herceptin, there is no consistent trend in binding affinity. However, binding affinity for US-licensed Herceptin is slightly lower than CT-P6 DP lots. Because FcγRIIIa binding affinity is provided as an additional test for information purposes, an acceptance criterion has not been established for this test.

3.2.P.8.1.5.3 Stress Stability

Stress stability studies at 40±2°C/75±5% RH for 3 months were conducted with 6 lots of CT-P6 DP and 3 lots of US-licensed Herceptin. All the test results met the acceptance criteria. The following tests showed a changing trend:

- Lot No. 13A3C002, Process A: HER2 binding affinity data showed a decrease in binding affinity over the course of 6 months. HER2 binding affinity decreased from 110.56% to 96.46% over the 3 month period. HER2 binding affinity data are provided as additional tests for information purposes, therefore, an acceptance criteria have not been set for these tests.
- Lot No. 13A3C003, Process A: HER2 binding affinity decreased from 104.58% to 96.35%.

- Lot No. 13A3C004, Process A: HER2 binding affinity decreased from 119.06% to 94.12%.
- Lot No. 15A3C01, Process B: HER2 binding affinity increased from 96.69% to 106.94%. FcγRIIIa binding affinity decreased from 105% to 98%.
- Lot No. 16A3C01, Process B: HER2 binding affinity increased from 92.35% to 97.77%. FcγRIIIa binding affinity decreased from 103% to 96%.
- Lot No. 16A3C02, Process B: HER2 binding affinity increased from 93.19% to 95.32%. FcγRIIIa binding affinity decreased from 107% to 99%.
- Trend analysis shows that HER2 binding affinity decreases for Process A lots. Process B lots show a slight increase in binding affinity over the 3 month time period. US-licensed Herceptin shows a trend towards increase in binding affinity. Therefore, Process B lots are similar to US-licensed Herceptin but Process A lots appear to be slightly different. HER2 binding affinity is provided as additional data and an acceptance criterion has not been set for it, so, a quantitative similarity assessment is not possible at this time.
- Trend analysis for FcγRIIIa binding shows that it increased for Process A lots, slightly decreased for Process B lots and decreased for US-licensed Herceptin lots. FcγRIIIa binding affinity is provided as additional data and an acceptance criterion has not been set for it, so, a quantitative similarity assessment is not possible at this time.

Reviewer comment: *The stress stability study period of 3 months is too short to see any consistent changing trends in CT-P6 DP. The ICH Q1A R2 guideline recommends conducting the accelerated stability study for 6 months. No recommended period is provided for stress stability studies.*

3.2.P.8.1.5.4 Additional Evaluation of Sub-visible Particles (<10 µm)

Sub-visible particles of sizes 1≤, 2≤, 5≤, and 10≤, <100 µm were characterized by mciro-flow imaging (MFI) (Section 3.2.R.3.2.4.8). The data are shown in Table 3.2.R.3-29 and Figure 3.2.R.3-24 and demonstrated that there is variability in the number of particles in each size range for CT-P6 and US-licensed Herceptin lots. The data for CT-P6 and US-licensed Herceptin® lots overlap with no consistent pattern of more or fewer particle levels in either of the two products. Thus, the data support that CT-P6 drug product is similar to US-licensed Herceptin® in the number of sub-visible particles in 1≤, 2≤, 5≤, and 10≤, <100 µm size ranges.

Reviewer comment: *The particle number decreases with increasing size range for both CT-P6 and US-licensed Herceptin DP lots.*

3.2.P.8.1.5.5 In-Use Stability (Reconstituted CT-P6 Drug Product [440 mg])

Review of in-use stability data shows that all the stability indicating test results meet the acceptance criteria (Table 3.2.P.8.3-25). Anti-microbial effectiveness was confirmed on Day 0 for bacteria and yeast and molds (Table 3.2.P.8.3-25).

3.2.P.8.1.5.6 Dilution of Infusion Stability (Reconstituted and Diluted CT-P6 Drug Product [440 mg], 250 mL bag, 2 mg/kg and 8 mg/kg)

Review of in-use in infusion bag (PVC and PE) shows that all the stability indicating test results met the acceptance criteria (Table 3.2.P.8.3-25, Table 3.2.P.8.3-26, Table 3.2.P.8.3-27, and Table 3.2.P.8.3-28). Microbial effectiveness was not done for this in-use stability study.

3.2.P.8.1.5.7 Confirmatory Photostability

Photostability studies shows that all the stability indicating test results met the acceptance criteria.

3.2.P.8.1.6 Proposed Shelf-Life and Storage Claim

CELLTRION is proposing a shelf life of 48 months when stored at 5±3°C. The proposed shelf-life of CT-P6 DP reconstituted with BWFI and stored at 5±3°C is 28 days. The proposed shelf-life of reconstituted and diluted CT-P6 DP in 250 mL PE and PVC is 24 hours at 5±3°C.

Reviewer comment: *The proposed shelf-life for CT-P6 DP and reconstituted in-use DP are based on data obtained from stability studies using Process A DP. The proposed shelf-life for DP is not acceptable.* (b) (4) *Process B DP manufacturing process, for which only 18 months of stability data is available. This is a major change and we cannot ascertain that the stability data for Process A DP will be representative for Process B DP. Therefore, the current proposed shelf life for DP stored at long-term storage condition of 5±3°C is* (b) (4) *months. The proposed shelf-life of reconstituted and diluted CT-P6 DP in 250 mL PE and PVC is acceptable.*

3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment

CELLTRION will continue to test all lots until the 72 month time point according to the protocol shown in Table 3.2.P.8.2-1. The data will be reported to FDA in the annual report.

Post-approval Annual Stability Commitment:

- A minimum of one production lot (if produced during that year) will be added to the long-term stability program each year and tested in accordance with the post-approval protocol presented in Table 3.2.P.8.2-1.

- Any out of specification (OOS) results will be investigated and if deemed to have the potential impact to the safety and efficacy of the product, it will be reported and discussed with FDA.

Table 3.2.P.8.2-1: Drug Product [420mg] Long-Term Commercial Stability Protocol (5±3°C)

Test Item	Time Point (Month)										
	0	3	6	9	12	18	24	36	48	60	72
Color and Physical State of Lyophilizate	T	T	T	T	T	T	T	T	T	T	T
Clarity upon Reconstitution	T	T	T	T	T	T	T	T	T	T	T
Color upon Reconstitution	T	T	T	T	T	T	T	T	T	T	T
Visible Particles upon Reconstitution	T	T	T	T	T	T	T	T	T	T	T
Reconstitution Time	T	T	T	T	T	T	T	T	T	T	T
pH	T	T	T	T	T	T	T	T	T	T	T
Osmolality	T	T	T	T	T	T	T	T	T	T	T
Sub-visible Particles	T	T	T	T	T	T	T	T	T	T	T
Residual Moisture	T	T	T	T	T	T	T	T	T	T	T
Extractable Volume	T	T	T	T	T	T	T	T	T	T	T
IEF	T	T	T	T	T	T	T	T	T	T	T
CE-SDS (Non-reduced)	T	T	T	T	T	T	T	T	T	T	T
IEC-HPLC	T	T	T	T	T	T	T	T	T	T	T
SEC-HPLC	T	T	T	T	T	T	T	T	T	T	T
Protein Content (UV_{280})	T	T	T	T	T	T	T	T	T	T	T
Polysorbate 20	T	T	T	T	T	T	T	T	T	T	T

Test Item	Time Point (Month)										
	0	3	6	9	12	18	24	36	48	60	72
In vitro Bioactivity	T	T	T	T	T	T	T	T	T	T	T
Endotoxin	T	-	-	-	-	-	T	-	T	-	T
Methylene Blue Dye Penetration	T	-	-	-	T	-	T	T	T	T	T

T: Testing planned, –: No testing

Table 3.2.P.8.2-2: End-of-shelf-life specification for Drug Product [420mg]

Test Item	Acceptance Criterion
General	
Color and Physical State of Lyophilizate	White to pale yellow lyophilized powder
Clarity upon Reconstitution	Clear to slightly opalescent solution
Color upon Reconstitution	Colorless to pale yellow
Visible Particles upon Reconstitution	Free of visible particles
Reconstitution Time	≤ (b) (4) (min:sec)
Residual Moisture	≤ (b) (4) %
pH	6.0± (b) (4)
Osmolality	(b) (4) mOsm/kg
Sub-visible Particles	≥ 10 µm: ≤ (b) (4) /vial ≥ 25 µm: ≤ vial
Extractable Volume	≥ (b) (4) mL
Safety	
Endotoxin	≤ (b) (4) EU/mL
Identity	
IEF	All bands of the test sample are ≤ (b) (4) pI unit of the corresponding bands of the reference material. No new band.
Purity/Impurity	
Non-reduced CE-SDS	H2L2 ≥ (b) (4) %
IEC-HPLC	Peak 1+Peak 2+Peak 3+Peak 4: (b) (4) % Peak 5 ≥ (b) (4) % Peak 6 ≤ % Peak 7 ≤ %
SEC-HPLC	Monomer ≥ (b) (4) %
Quantity	
Protein Content (UV_{280})	(b) (4) mg/vial
Polysorbate 20	(b) (4) %

Test Item	Acceptance Criterion
Potency	
In vitro Bioactivity	(b) (4) U/mg
Container Closure Integrity	
Methylene Blue Dye Penetration	No indication of blue color

[1] Acceptance Value
[2] Reference Value

Reviewer comment: *An IR was sent to the sponsor on February 15, 2018 to add stability specifications to the post-approval annual stability study protocols. In addition, either sterility testing or container closure integrity testing should be performed on an annual basis and should be added to Table 3.2.P.8.2-1. In response to the IR, the sponsor has added Table 3.2.P.8.2-2 End-of-shelf-life specification for Drug Product [420 mg] and added methylene blue due penetration test for container closure integrity testing.*

In response to the IR sent on February 15, 2018, the long-term stability protocol has been updated to include testing for intact H2L2 by nonreduced CE-SDS and polysorbate 20.

3.2.P.8.3 Stability Data [420 mg]

Analytical method validation for additional stability test parameters ELISA (HER2 Binding Affinity), and method qualification for methylene blue dye penetration test, sub-visible particles (<10 µm) and anti-microbial effectiveness test are described in this section.

3.2.P. herzuma - Diluent in a vial for reconstitution

3.2.P.1 Description and Composition of the Drug Product [Diluent]

The diluent is bacteriostatic water for injection (BWFI). It is a sterile, clear and colorless liquid solution containing 1.1% benzyl alcohol in sterile water for injection. The diluent is supplied in a 20-mL (b) (4) vial closed with a 20 mm (b) (4) rubber stopper and an aluminum flip-off seal. A (b) (4)% overfill is applied to ensure that the labeled volume can be withdrawn from the vial. The diluent composition is provided in Table 3.2.P.1-1.

Table 3.2.P.1-1: Composition of the CT-P6 Diluent

Ingredient	Quantity/Vial[1]	Function	Grade
Benzyl Alcohol	0.22 g	Preservative	Ph. Eur./NF
Water for Injection	QS to 20 mL	Solvent	USP/Ph. Eur.

NF: National Formulary; Ph. Eur.: European Pharmacopoeia; USP: United States Pharmacopoeia; QS: Quantum Satis
[1] The amount of each component per vial is the nominal value which does not consider the overfill.

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(b) (4)

3.2.P.8 Stability [Diluent]

3.2.P.8.1 Stability Summary and Conclusion

The proposed shelf-life for CT-P6 diluent is 48 months at 5±3°C. Stability studies have been conducted at long-term storage condition of 5±3°C, accelerated stability condition of 25±2°C / 60±5% RH, and photostability condition of 1,200,000 Lux hours vis. and 200 Whr / m^2 UV at 25±2°C / 60±5% RH. Long-term stability protocol (Table 3.2.P.8-2) is for 72 months and is ongoing with three lots of CT-P6 diluent (Lot# 12I0001, 12I0002, and 12I0003). Accelerated stability study (protocol in Table 3.2.P.8-3) of 6 months is complete with three lots (Lot# 12I0001, 12I0002, and 12I0003). The tests for long-term stability study and accelerated stability study is the same as those for CT-P6 diluent release tests. One lot of CT-P6 diluent (lot# 12I0001) was placed on confirmatory photostability study, which has been completed (protocol in Table 3.2.P.8-4). Photostability tests were clarity, color, visible particles, pH, sub-visible particles, benzyl alcohol identification, assay of benzyl alcohol, anti-microbial agent, calcium, carbon dioxide, and sulfate. The long-term stability results show that there was no significant change in the quality during storage at 5±3°C for 48 months.

The proposed commercial end-of-shelf-life specification for real-time/real-temperature stability testing of diluent is shown in Table 3.2.P.8-5. The tests are the same as the release tests, with the addition of test for anti-microbial preservative effectiveness which constitute tests for bacteria at 24 hours, 7, 14, and 28 days and tests for yeast and molds at 7, 14, and 28 days.

Table 3.2.P.8-5: Proposed Commercial End-of-Shelf-Life Specification for Real-Time/Real-Temperature (5±3°C) Stability Testing of Diluent

Test Item	Acceptance Criterion		
Clarity	Clear		
Color	Colorless		
Visible Particles	Free of visible particles		
Uniformity of Dosage Unit	Stage I (n = 10): AV [1] ≤ (b) (4) Stage II (n = 30): AV [1] ≤ (b) (4) and No unit is outside of ± (b) (4)% of M [2]		
Extractable Volume	≥ (b) (4) mL		
Identification (HPLC)	%RSD between retention time of benzyl alcohol peak and mean retention time of reference peak should be ≤ (b) (4)%		
Assay of Benzyl Alcohol (HPLC)	(b) (4)%		
Sub-visible Particles	≥ 10 µm: ≤ (b) (4)/vial ≥ 25 µm: ≤ vial		
pH	(b) (4)		
Calcium	No turbidity is produced		
Carbon dioxide	The mixture remains clear		
Sulfate	No turbidity is produced		
Anti-microbial Agent [3]	Type of organism	Interval	Log_{10} Reduction
	Bacteria	24 hours	Not less than (b) (4) from the initial sample
		7 days	Not less than from the initial sample
		14 days	Not less than from the initial sample
		28 days	Not be increased from the 7 and 14 day counts
	Yeast and Molds	7 days	Not be increased from the initial sample count
		14 days	Not less than (b) (4) from the initial sample count
		28 days	Not be increased from the 14 day count
Endotoxin	< (b) (4) EU/mL		
Sterility	No growth		

[1] AV: Acceptance value
[2] M: Reference value
[3] Antimicrobial preservative effectiveness is only monitored for stability study (Section 3.2.P.5.1 [Diluent]).

3.2.P.8.2 Post-Approval Stability Commitment

A minimum of one production batch (if produced during that year) will be added to the long-term stability program each year and tested according to the post-approval protocol presented in Table 3.2.P.8-9. Any out of specification (OOS) results will be investigated and if deemed to have the potential to impact the safety and efficacy of the product, will be reported and discussed with FDA.

Table 3.2.P.8-9: Long-Term Commercial Stability Protocol (5±3°C)

Test Item	Time Point (Month)										
	0	3	6	9	12	18	24	36	48	60	72
Clarity, Color, Visible Particles	T	T	T	T	T	T	T	T	T	T	T
pH	T	T	T	T	T	T	T	T	T	T	T
Sub-visible Particles	T	T	T	T	T	T	T	T	T	T	T
Uniformity of Dosage Unit	T	-	-	-	T	-	T	T	T	T	T
Extractable Volume	T	-	-	-	T	-	T	T	T	T	T
Identification (HPLC)	T	T	T	T	T	T	T	T	T	T	T
Assay of Benzyl Alcohol (HPLC)	T	T	T	T	T	T	T	T	T	T	T
Bacterial Endotoxin	T	-	-	-	-	-	T	-	T	-	T
Sterility	T	-	-	-	-	-	T	-	T	-	T
Calcium	T	T	T	T	T	T	T	T	T	T	T
Carbon Dioxide	T	T	T	T	T	T	T	T	T	T	T
Sulfate	T	T	T	T	T	T	T	T	T	T	T

Reviewer comment: *Test for antimicrobial effectiveness is not included in the long-term commercial stability protocol.*

3.2.P.8.3 Stability Data

Reviewer comment:
Long-term storage stability study at 5±3°C: All three lots of CT-P6 diluent met the proposed commercial specification during the time period of 48 months.
Accelerated stability study at 25±2°C / 60±5% RH: All three lots of CT-P6 diluent met the proposed commercial specification during the time period of 6 months
Photostability study: One lot of CT-P6 diluent placed on photostability study met the acceptance criteria for all tests.

Immunogenicity

The sponsor developed and validated an electrochemiluminescent (ECL) assay for screening and confirmatory analyses of anti-drug antibodies (ADAs) and a cell-based ADCC assay for neutralizing activity analysis of ADAs. These assays were validated using serum from healthy volunteers and breast cancer patients. The assays were initially developed by (b) (4) and was later transferred to (b) (4) performed the validation for both ADA and neutralizing antibody (NAb) and analyzed the clinical samples from study CT-P6 3.2 for immunogenicity assessment.

Validation of an Electrochemiluminescent method for the detection of anti-CT-P6 antibodies in human serum (Report RBNW2)

Anti-Drug Antibody (ADA) Assay

Anti-CT-P6 antibodies in human serum were detected using a qualitative electrochemiluminescent (ECL) assay. The serum samples are incubated with biotinylated CT-P6 (BT-CT-P6) and sulfo-tagged CT-P6 (Ru-CT-P6) and then added to MSD-Streptavidin (MSD-SA) plate. Any anti-drug antibodies (ADAs) in the serum samples will be bound by BT-CT-P6 and captured on the MSD-SA plate. Anti-CT-P6 antibodies are detected using tripropylamine-containing read buffer that causes the ruthenium in the complex to produce a chemiluminescent signal, which was measured in relative light units (RLU) using the Meso-Scale Discovery (MSD) SECTOR Imager 6000 Plate Reader.

Assay Controls

Negative Control

The negative control was prepared by pooling 48 individual healthy human serum drug naïve samples with no detectable ADA.

Positive Control

The positive control was human monoclonal anti-trastuzumab antibody from (b) (4) and was used prior to the expiration date. The antibody is a human IgG1 antibody selected from the HuCLA phage display library that were expressed in human cell line and purified by affinity chromatography on Protein A (CoA from (b) (4) Matrix-based positive controls were prepared by spiking anti-trastuzumab antibodies into the negative control pool at 3.51 ng/mL (LPC), 40 ng/mL (old LPC), 200 ng/mL (MPC), and 2000 ng/mL (HPC).

Screening cut-point for healthy human serum samples

The screening cut-point was determined from analysis of 50 drug-naïve healthy human serum samples. The samples were analyzed in three independent assays performed by three analysts. The data was evaluated for outliers using Tukey's Outlier Filter. After removal of outliers, the data was tested for normality using Shapiro-Wilk test. Both the non-transformed data and ln-transformed data analyses indicated that the data distribution was non-normal. Therefore, a non-parametric approach was taken using non-transformed data to determine the screening cut-point. Next, the medians across assay panels were tested for equality using Friedman's test which indicated that there was a statistically significant difference between the medians across the three panels. Test for homogeneity of variances was not performed because %CV for each panel was less than 15%. Based on these analyses, a floating cut-point was calculated and assay cut-point was determined at 95th percentile of the pooled data. The normalization cut-point factor was calculated as NCF (normalization correction factor) = CPV (cut-point value) – NC (negative control). The CPV and NCF at the 95th, 99th, and 99,9th percentile are provided in Table 1.

Table 1
Assay Cut Points Using Mean Uninhibited Values

Mean NC Response	Estimates for Different Upper Percentiles					
	95th		99th		99.9th	
	CPV	NCF	CPV	NCF	CPV	NCF
157.0	176.0	19.0	189.5	32.5	209.5	52.5

Reviewer comment: *Determination of floating screening cut-point in healthy human serum is acceptable.*

Screening cut-point for breast cancer serum samples

The screening cut-point was determined from analysis of 50 drug-naïve breast cancer serum samples. The samples were analyzed in three independent assays performed by four analysts. The analyses described above for determination of the screening cut-point in healthy human serum samples were done to determine the screening cut-point in breast cancer serum samples and it was determined that a floating screening cut-point would be appropriate. The CPV and NCF at the 95th, 99th, and 99,9th percentile are provided in Table 1.

Table 1
Assay Cut Points Using Mean Uninhibited Values

Mean NC Response	Estimates for Different Upper Percentiles					
	95th		99th		99.9th	
	CPV	NCF	CPV	NCF	CPV	NCF
145.5	177.5	32.0	185.5	40.0	215.0	69.5

Reviewer comment: *Determination of floating screening cut-point in breast cancer serum is acceptable.*

Confirmatory cut-point in healthy human serum

The confirmatory cut-point was determined from 50 normal human serum samples that were tested naïve (uninhibited) and in the presence of excess (100 µg/mL) drug (inhibited). The samples were analyzed in three independent assays performed by three analysts. The data was evaluated for outliers using Tukey's Outlier Filter. After removal of outliers, the non-transformed data was tested for normality using Shapiro-Wilk test and the test indicated that the data distribution was normal. Equality of means was evaluated across assay panels using ANOVA, which indicated that there was no statistically significant difference between the means across the three panels. Therefore, a fixed cut-point was calculated. The confirmatory cut-point (CCP) was calculated as CCP = mean – z x SD, where z is equal to 1.645, 2.33 and 3.09 for 95%, 99% and 99.9% cut-points, respectively. Percent inhibition cut-point was determined as (1 – CCP) x 100. The Confirmatory Percent Inhibition Cut-Points associated with each of the prediction intervals are provided in Table 1. The Confirmatory Percent Inhibition Cut-Points associated with the 99% upper prediction interval was applied to each of the samples tested within this study.

Table 1
Confirmatory Cut Points

Mean Ratio	Mean Inhibition	Confirmatory Cut Point Estimates for Different Upper Prediction Intervals					
		95%		99%		99.9%	
		Ratio	%Inhibition	Ratio	%Inhibition	Ratio	%Inhibition
0.986	1.4%	0.910	9.0%	0.878	12.2%	0.843	15.7%

Reviewer comment: *Determination of fixed confirmatory cut-point in healthy human serum is acceptable.*

Confirmatory cut-point in breast cancer human serum

The confirmatory cut-point was determined from analysis of 50 breast cancer serum samples. The samples were analyzed in three independent assays performed by four analysts. The analyses described above for determination of the confirmatory cut-point in healthy human serum samples were done to determine the confirmatory cut-point in breast cancer serum samples and it was determined that a fixed confirmatory cut-point would be appropriate. The Confirmatory Percent Inhibition Cut-Points associated with each of the prediction intervals are provided in Table 1. The Confirmatory

Percent Inhibition Cut-Points associated with the 99% upper prediction interval was applied to each of the samples tested within this study.

Table 1
Confirmatory Cut Points

Mean Ratio	Mean Inhibition	Confirmatory Cut Point Estimates for Different Upper Prediction Intervals					
		95%		99%		99.9%	
		Ratio	%Inhibition	Ratio	%Inhibition	Ratio	%Inhibition
0.980	2.0%	0.916	8.4%	0.889	11.1%	0.860	14.0%

Reviewer comment: *Determination of fixed confirmatory cut-point in breast cancer serum is acceptable.*

Precision

Intra-assay precision was evaluated by analyzing multiple determinations (n=6) of the negative control and the positive control pools prepared at 3.51 ng/mL (LPC), 200 ng/mL (MPC), and 2000 ng/mL (HPC) within a single run. For all the controls, %CV was less than 5% and met the pre-determined acceptance criterion (%CV < 30%). Inter-assay precision was evaluated by analyzing the same controls across all acceptable validation runs (after sensitivity and LPC determination), which were performed by multiple analysts. For all the controls, %CV was ≤16.1% and met the pre-determined acceptance criterion (%CV < 30%).

Reviewer comment: *The assay validation shows that the assay can be performed with high intra- and inter-assay precision.*

Sensitivity and LPC

Assay sensitivity was determined from two-fold serial dilution of surrogate control (100 ng/mL) diluted to 12 dilutions to span the assay cut-point. Assays were performed by two different analysts. Assay sensitivity was reported as the mean of the lowest concentration of ADA that consistently produced a positive response greater than or equal to the assay cut-point and was determined to be 0.433 ng/mL. The screening LPC was calculated to be 1.04 ng/mL (mean concentration sensitivity + $t_{0.01}$ x SD), which is the concentration level with 1% rejection rate.

Potential LPC concentrations were analyzed in a confirmatory assay starting with 1.04 ng/mL. Surrogate controls at 1.04, 1.56, 2.34, 3.51, 5.27, 7.90, 11.8, 17.8, 26.7, and 40.0 ng/mL were analyzed uninhibited and inhibited. The lowest LPC concentration giving a response higher than 1.5 fold the cut-point and confirming positive was 3.51 ng/mL. Therefore, 3.51 ng/mL was set at the LPC for all subsequent validation runs.

Reviewer comment: *The assay sensitivity (0.433 ng/mL) was appropriately determined. The LPC that will be used for all validation runs is 3.51 ng/mL and is acceptable.*

Stability

Freeze/thaw (F/T) stability was evaluated using controls (LPC and HPC). Control samples were subjected to six F/T cycles (frozen at -80±10°C and thawed at room temperature) and compared to freshly prepared controls. All the samples met the pre-determined acceptance criterion of percent difference of the stability samples versus freshly-prepared controls are within ±30.0% (Table 6).

Control stability in thawed matrix (TM) was evaluated by allowing controls (LPC and HPC) to thaw and remain at room temperature for 24 hours prior to analysis versus freshly prepared controls. The thawed matrix stability data met the pre-determined acceptance criterion of percent difference of the stability samples versus freshly-prepared controls are within ±30.0% (Table 6).

Effect of Hemolysis
Blank (HS) and low- and high-level controls (HEMPC) prepared in hemolyzed matrix containing 5% fully lysed whole blood were evaluated. Samples were acceptable if the product mean relative to their prepared concentrations were HPC> LPC> cut-point> blank. The data in Table 7 shows that hemolysis had no effect on the detection of anti-CT-P6 antibodies.

Effect of Lipemia
Blank (LIP) and low- and high-level controls (LIPQC) prepared in lipemic matrix were evaluated. Samples were acceptable if the product mean relative to their prepared concentrations were HPC> LPC> cut-point> blank. The data in Table 7 shows that lipemia had no effect on the detection of anti-CT-P6 antibodies.

Reviewer comment for hemolysis and lipemia: *Even though the assay met the pre-determined acceptance criteria for effect of hemolysis and lipemia, these data would have been more informative if they had provided us the recovery values. However, their evaluation is acceptable because this is a qualitative assay and the LPC is detectable above the cut-point indicating that ADAs present at LPC concentrations will be detected in human serum samples.*

Matrix Interference and Selectivity
Ten individual healthy human serum lots were prepared at blank, LPC, MPC, and HPC concentrations. The pre-determined acceptance criterion was ≥80% of the spiked matrix samples relative to their prepared concentrations must be HPC>LPC>cut-point>blank and the results of ≥80% of the blank samples must be < cut-point. All the samples met this acceptance criterion (Table 8).

Ten individual breast cancer serum lots were prepared at blank, LPC, MPC, and HPC concentrations. The pre-determined acceptance criterion was the same as that for healthy human serum (see above). All the samples except for one met this acceptance criterion (Table 9). The results for one individual demonstrated unacceptable selectivity. The sponsor stated that if there is drug present in a sample, it will interfere with the detection of ADA and may result in data such as that observed for this specific sample. No further investigation of this specific sample was deemed necessary as the overall experiment passed the acceptance criteria.

Reviewer comment: *The evaluation of matrix interference in both healthy human serum and breast cancer serum met the pre-determined acceptance criterion. However, one of the breast cancer serum lots had interfering factors that prevented accurate determination of ADA at LPC and MPC concentrations. The nature of the interfering factor was not investigated.*

Drug Tolerance
LPC, MPC, and HPC were incubated with 15 concentrations of drug ranging from 0.1 to 800 µg/mL CT-P6 at room temperature for one hour prior to analysis. The drug tolerance of the assay was 2 µg/mL at the LPC level, 50 µg/mL at the MPC level and 100 µg/mL at the HPC level. The drug tolerance was 25 µg/mL in the presence of 100 ng/mL ADA.

Reviewer comment: *The C_{trough} level of CT-P6 was determined to be 16.80 – 18.92 µg/mL for early breast cancer patients in clinical study CT-P6 3.2 (Section 2.5 Clinical Overview). Therefore, the assay is capable of detecting low concentrations of ADA in the presence of C_{trough} level of drug. The ADA assay is acceptable for detection of antibodies against CT-P6.*

Antigenic Equivalence Determination (located in 5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies) (Report RBNW6)

Reviewer comment: *The determination of antigenic equivalence between CT-P6 and US-licensed Herceptin was performed separately from the ADA assay validation. Therefore, inter-assay precision and drug tolerance were repeated during this validation.*

Validation of an Electrochemiluminescent (ECL) Method for the Detection of Anti-CT-P6 Antibodies in Human Serum

Inter-Assay Precision
Inter-assay precision was determined from 7 assay runs that included HPC, MPC, LPC, and NC run in duplicate (Table 1). The results demonstrated that %CV was 25.8% for HPC, 25.1% for MPC, 17.6% for LPC, and 14.9% for NC. The mean result of the controls relative to their prepared concentrations was HPC>MPC>LPC>NC.

Reviewer comment: *The inter-assay precision is acceptable.*

Antigenic Equivalence
The antigenic equivalence experiment tests the ability of each drug to comparably inhibit PC response. The HPC was spiked in NC pool and evaluated in the confirmatory assay using each of three inhibition drugs (CT-P6, US-Herceptin, and EU-Herceptin) at concentrations of 100, 33.3, 11.1, 3.7, 1.2, 0.41, and 0.0 µg/mL. Three inhibition curves were generated per plate. The results demonstrated that %CV between duplicates of all samples above the cut point was ≤30% and the %CV of the inhibition resulting from each drug was less than 10% at all levels (Table 3A and 3B).

Reviewer comment: *The evaluation of antigenic equivalence is acceptable and demonstrates that CT-P6 is similar to Herceptin in inhibiting detection of the PC in the assay.*

In-study Screening Assay Cut Point Evaluation for CT-P6 Clinical Studies Using Pre-dose Human Breast Cancer Serum

Screening assay cut point was determined using pre-dose samples collected from subjects in study CT-P6 3.2 and was applied for the detection of anti-CT-P6 antibodies in breast cancer human serum. The data was evaluated for outliers using Tukey's outlier filter approach. After exclusion of outliers, the non-transformed data as tested for normality using Shapiro-Wilk test. The results demonstrated that the data was skewed and therefore, the normality hypothesis was rejected. The data was ln transformed (after exclusion of outliers) and tested for normality using Shapiro-Wilk test. The ln transformed data was not normally distributed. As a result, the non-parametric approach was utilized using the non-transformed data to determine the screening cut point. The cut point value (CPV) and the normalization correction factor (NCF = CPV – NC, where NC is the average response associated with the negative control tested within the experiment) are provided in Table 4. The assay cut point (ACP) was the NC+NCF associated with the upper 95th percentile was applied to each of the runs.

Table 4.
Assay Cut Points Using Mean Uninhibited Values

Mean NC Response	Estimates for Different Upper Percentiles					
	95th		99th		99.9th	
	CPV	NCF	CPV	NCF	CPV	NCF
184.2	201.5	17.3	222.0	37.8	236.5	52.3

Reviewer comment: *The calculation of the assay screening cut point is acceptable.*

Drug Tolerance in Breast Cancer Serum

Drug tolerance was tested by combining 2X concentrations (0.2, 0.5, 1.0, 2.0, 4.0, 10.0, 20.0, 50.0, 100, 200, 400, 800, 1200, 1400, 1600 µg/mL) of samples containing each drug (CT-P6 and US-Herceptin) at 1:1 ratio with 2X concentrations of positive control (PC) equal to 4000, 400, 200, and 7.02 ng/mL resulting in final PC concentrations of 2000, 200, 100, and 3.51 ng/mL. The ADA limit of detection in the presence of 25.0 µg/mL of CT-P6 and US-Herceptin was 100 ng/mL.

Reviewer comment: *For CT-P6, 100 ng/mL of the PC, in the presence of 25 µg/mL of CT-P6, produced signal that was above the assay cutpoint (Table 2A). For US-Herceptin, 100 ng/mL of the PC was also detectable in the presence of 25 µg/mL of US-Herceptin (Table 2B). Therefore, drug tolerance was determined to be equivalent for CT-P6 and US-Herceptin using this ADA assay.*

Validation of a Cell-Based Method for the Detection of Neutralizing Antibodies to CT-P6 in Human Serum – VSDCBA 62 Validation Report – Project Code RDYK2

The method validation summary is shown in the following table:

Bioanalytical Method Validation Summary

Species/Matrix	Human Serum
Matrix Population	Healthy and Breast Cancer Human Serum
Analysis Method	Luminescence using Bright-Glo®
Data Capture of RLU	PerkinElmer, Wallac Envision 2104 Multi-label Reader
Additional Data Analysis and Calculations	Microsoft® Excel 2003 and 2010, Assist LIMS
Negative Control (NC)	NC pool + Cells
Drug Control (DC)	CT-P6 + NC pool + Cells
Positive Controls (PC)	CT-P6+ anti-trastuzumab antibody + NC pool + Cells
Sample Volume (µL)	10-µL aliquot for screening + 75-µL aliquot for confirmatory
Sample Storage Temperature	-80 °C ± 10 °C
DC/NC Ratio	2.33
Cell Passage Limit (SK-BR-3 Cells)	Passages 6 to 24
Cell Passage Limit (Jurkat Cells)	Passages 6 to 20
Minimum Required Dilution	Controls and Samples: 1:20 in assay medium
Screening Assay Cut Point (HHS)	≤ 0.729 (upper bound 95.0% limit of prediction interval)
Screening Assay Cut Point (BCS)	≤ 0.578 (upper bound 95.0% limit of prediction interval)
Confirmatory Assay Cut Point (HHS and BCS)	≥ 55.3% (upper bound 99.0% limit of prediction interval)
Relative Assay Sensitivity	1500 ng/mL
Matrix Interference/Selectivity (HHS)	
Unspiked	Ten of ten individuals were negative.
LPC	Nine of ten individuals were positive.
MPC	Ten of ten individuals were positive.
HPC	Ten of ten individuals were positive.

Matrix Interference/Selectivity (BCS)				
Unspiked	Ten of ten individuals were negative.			
LPC	Ten of ten individuals were positive.			
MPC	Ten of ten individuals were positive.			
HPC	Ten of ten individuals were positive.			
Drug Tolerance				
LPC	Neutralizing antibodies can be detected in samples in the presence of up to 1000 ng/mL excess CT-P6.			
MPC	Neutralizing antibodies can be detected in samples in the presence of up to 3000 ng/mL excess CT-P6.			
HPC	Neutralizing antibodies can be detected in samples in the presence of up to 5000 ng/mL excess CT-P6.			
Homogeneity	100% of the LPC samples met acceptance criteria			
Precision Across PCs		HPC	MPC	LPC
	Concentration	10,500 ng/mL	7000 ng/mL	4500 ng/mL
	Inter-assay (%CV) (Somru antibody)	15.9%	9.25%	28.9%
	Inter-assay (%CV) (AbD Serotec antibody)	22.6%	20.1%	17.6%
	Intra-assay (%CV)	6.82%	4.81%	3.07%
Drug Comparison	The LPC can be detected in all drugs tested.			
Thawed Matrix Stability	24.5 hours at room temperature and 24 hours at 2 to 8 °C			
Freeze/Thaw Stability	Seven cycles thawed at room temperature.			
Hemolysis Interference				
1% Hemolysis	Six of six unspiked and spiked samples met acceptance criteria at all levels.			
5% Hemolysis	Six of six unspiked and spiked samples met acceptance criteria at all levels.			
10% Hemolysis	Six of six unspiked and spiked samples met acceptance criteria at all levels.			
Lipemia Interference				
Unspiked	Six of six individuals were negative.			
LPC	Two of six individuals were positive.			
HPC	Six of six individuals were positive.			
Titration	Sample 1	Sample 2		
HHS	Dil 80	Dil 80		
BCS	Dil 80	Dil 160		

Neutralizing (NAb) Assay

Neutralizing antibodies against CT-P6 were measured using an antibody-dependent cell cytotoxicity (ADCC) assay. In this assay, human breast cancer SK-BR-3 cells are plated. The cells are then incubated with CT-P6, samples, and controls. During this incubation period, CT-P6 binds to target antigens on the cell surface of SK-BR-3. After this incubation period, effector cells are added. The Fc

effector portion of CT-P6 binds to FcγRIIIa receptors on effector cells leading to pathway activation of ADCC. For this assay, the effector cells are engineered Jurkat cell line that stably expresses the FcγRIIIa receptor, V158 (high affinity) variant, and an NFAT response element driving expression of firefly luciferase. The luciferase activity of the effector cells is measured. If neutralizing antibodies are present in the serum samples, the capacity of CT-P6 to kill the target cells will be prevented. Samples producing screening assay values (SAV) at or below the screening assay cut-point are deemed "confirmation required" for the presence of NAbs, while samples producing SAVs above the screening assay cut-point are deemed "negative." Samples found "confirmation required" in the screening assay are then analyzed in a confirmatory assay. Confirmed positive samples will then be serially diluted and the highest titer at or below the screening assay cut-point will be reported.

The minimum required dilution (MRD) for healthy human serum is 1:20 in assay medium. The limit of detection for this assay is 1500 ng/mL of commercially available anti-trastuzumab neutralizing antibodies neat healthy human serum. Lipemic samples may interfere with the detection of neutralizing antibodies in this assay.

Positive Controls

During the assay cut-point and reliable assay sensitivity runs, the LPC was 2,000 ng/mL, the MPC was 4,000 ng/mL and the HPC was 6,250 ng/mL. The LPC was adjusted to 4,500 ng/mL, MPC to 7,000 ng/mL and HPC to 10,500 ng/mL based on relative assay sensitivity data (relative assay sensitivity: 1500 ng/ml). Positive control antibody was obtained from [redacted] (b) (4) and then from [redacted] (b) (4) due to backorder at [redacted] (b) (4).

Screening Assay Cut Point Determination

The screening cut-point is the level of response at or below which a sample is defined to be "confirmation required" for the presence of ADAs and above which it is defined to be "negative." The healthy human serum (HHS) screening assay cut-point was determined statistically from the analysis of 50 individual female healthy human serum samples. The breast cancer serum (BCS) screening assay cut-point was determined statistically from the analysis of 50 individual female breast cancer serum samples. Samples from each serum type were analyzed in at least three assays by at least two analysts on at least two days.

The HHS and BCS populations were determined to be statistically different; therefore, two separate cut-points were generated. The HHS fixed assay cut point was determined to be 0.729 (upper bound 95.0% limit of prediction interval) which gave a false positive percentage of 6.80%. The BCS fixed assay cut point was determined to be 0.578 (upper bound 95.0% limit of prediction interval) which gave a false positive percentage of 5.10%.

Screening Assay Cut-Point for Healthy Human Serum

The screening assay cut-point was set using the Screening Assay Value (SAV = Mean RLU of Sample/Mean RLU of the Drug Control).

The HHS screening assay cut-point was determined from the analysis of 50 individual healthy human serum samples in at least three assays by at least two analysts. The data was evaluated for outliers using Tukey's Outlier Filter and showed no outliers. The data was tested for normality using Shapiro-Wilk test, which indicated that the data was normally distributed. Because the SAV values were normalized (to the Drug Control) and a fixed cut-point was going to be used, the data was only evaluated to assess whether the variances were equal across the panels using Levene's test, which

indicated that there was no statistical evidence of a difference in the variances across panels. The screening assay cut-point was determined using a lower prediction interval and calculated as CPV = mean – z x SD, where z is equal to 1.645, 2.33, and 3.09 for 95%, 99%, and 99.9% cut-point, respectively. The CPV associated with the lower prediction interval is provided in Table 1.

Table 1
Assay Cut Points Using Screening Assay Values

Total Number of Values	Mean SAV	95% CPV	99% CPV	99.9% CPV
148	1.060	0.729	0.591	0.438

The CPV associated with the 95% prediction interval was applied to each of the panels performed within the study. The cut-points listed were applied to the values within this experiment. The resulting false positive evaluation is provided in Table 4. The false positive at the 95% prediction interval was 6.8%, indicating the determination of the screening cut-point was acceptable.

Table 4
False-Positive Evaluation

		Excluding Outliers			Including Outliers		
Cut Point	Cut Point Value	Sample Count	Positive Count	% Positive	Sample Count	Positive Count	% Positive
95% CPV	0.729	148	10	6.8%	148	10	6.8%
99% CPV	0.591	148	2	1.4%	148	2	1.4%
99.9% CPV	0.438	148	0	0.0%	148	0	0.0%

Reviewer comment: *The determination of the screening cut-point for HHS samples in the NAb assay is acceptable.*

Screening Assay Cut-Point for Breast Cancer Serum Samples

The breast cancer screening assay cut-point was determined from the analysis of 50 individual female breast cancer serum samples. The samples were run in at least three assays by at least two analysts.

Two samples were excluded because %CV for replicate measurements exceeded 30%. One sample was excluded as a biological outlier. Two samples were excluded because they were determined to have drugs in the pharmacokinetic assay.

The data was evaluated for outliers using Tukey's Outlier Filter. After removal of outliers, the data was tested for normality using Shapiro-Wilk test. Both the non-transformed SAV data and ln-transformed SAV data analyses indicated that the data was not normally distributed. Therefore, a non-parametric approach was taken using non-transformed SAV values to determine the screening cut-point. Next, the pooled data was evaluated for outliers using Tukey's Outlier Filter and one result that was determined to be an outlier was excluded.

For the analysis of homogeneity of means and variances, the sponsor states that because the SAV values are normalized and a fixed cut-point will be used, the data was only evaluated to assess whether the variances were equal across the panels. Levene's test for homogeneity of variances

indicated that there was no statistical evidence of a difference in the variances across the panels. Because the responses have already been normalized to the DC (drug control), a fixed screening cut-point using a non-parametric method will be determined on the SAV values. The cut-point value (CPV) was determined using percentiles of the pooled data and is shown in Table 1 for the 95th, 99th and 99.9th percentiles. The CPV associated with the lower 95th percentile was applied to each of the panels performed within this study.

Table 1
Lower Assay Cut Points Using SAV Values

95th Percentile CPV	99th Percentile CPV	99.9th Percentile CPV
0.578	0.500	0.491

Reviewer comment: *The determination of the fixed cut-point SAV is acceptable. The sponsor did not test for homogeneity of means because they used data that was normalized to the DC (drug control). This is acceptable. The sponsor developed a screening NAb assay and a confirmatory NAb assay, which is more than what is required for the NAb assay. The final determination whether this cut-point is acceptable will be based on analysis of the clinical samples and assessing if the number tested positive/total number of samples tested is 2 – 11%.*

The false positive evaluation is shown in Table 7. Using the 95th percentile CPV, after excluding outliers, 5.1% of the samples tested within this experiment would be considered positive.

Table 7
Breast Cancer Serum Sample False-Positive Evaluation
Using Breast Cancer Serum Sample Cut Point

		Excluding Outliers			Including Outliers		
Cut Point	CPV Value	Sample Count	Positive Count	% Positive	Sample Count	Positive Count	% Positive
95th CPV	0.578	138	7	5.1%	139	8	5.8%
99th CPV	0.500	138	1	0.7%	139	2	1.4%
99.9th CPV	0.491	138	1	0.7%	139	2	1.4%

The false positive rate was 26.1% using the 95th percentile CPV determined from healthy human serum is shown in Table 9.

Table 9
Breast Cancer Serum Sample False-Positive Evaluation
Using Healthy Human Serum Sample Cut Point

		Excluding Outliers			Including Outliers		
Cut Point	CPV Value	Sample Count	Positive Count	% Positive	Sample Count	Positive Count	% Positive
95% CPV	0.729	138	36	26.1%	139	37	26.6%
99% CPV	0.591	138	9	6.5%	139	10	7.2%
99.9% CPV	0.438	138	0	0.0%	139	1	0.7%

Reviewer comment: *These results show that the fixed screening cut-point determined for breast cancer serum samples for the NAb assay was appropriately determined.*

Confirmatory Assay Cut Point Determination
In order to confirm the reduction in signal observed in samples is due to antibodies and not due to non-specific matrix components, samples were immunodepleted using protein A/G/L. The confirmatory cut-point is the % depletion below which a sample is defined as "negative" or at or above which it is defined as "positive" for the presence of ADAs. The healthy human serum (HHS) confirmatory assay cut-point was determined statistically from the analysis of 50 individual female healthy human serum samples. The breast cancer serum (BCS) confirmatory assay cut-point was determined statistically from the analysis of 50 individual female breast cancer serum samples. Samples from each serum type were analyzed in at least three assays by at least two analysts on at least two days.

It was found that there was a statistically significant difference between the HHS and BCS populations; however, the differences between the means and medians were only ~3%. Therefore, the confirmatory assay cut-point was determined on the combined HHS and BCS matrix samples. The confirmatory assay cut point was determined to be 55.3 (upper bound 99.0% limit of prediction interval) which gave a false positive percentage of 0.70% (HHS) and 1.5% (BCS).

RDYK2 Confirmatory Cut-Point Analysis Report
For the confirmatory cut-point, the ratio of the response of the uninhibited sample versus the response of the inhibited sample is calculated and the percent inhibition is calculated (100 x (1 – ratio)).

Matrix Comparison: After removing outliers by matrix, the data was evaluated for differences between analysts and the results demonstrated that there was no statistical difference between analysts (p-value = 0.766). Next, the data was evaluated to determine if the means between the two matrices were equivalent and the results demonstrated that there was a statistically significant differences between the means (p-value = 0.004) and medians (p-value = 0.006) of the two matrices. However, the difference between the means and medians was only ~3%; therefore, the confirmatory cut-point was determined on the combined HHS and BCS matrix samples.

The combined HHS and BCS data was evaluated for outliers using Tukey's Outlier Filter and outliers were excluded. The data was then tested for normality using the Shapiro-Wilk test which indicated that the data was normally distributed. Therefore, further analyses were performed on the non-transformed data. The confirmatory cut-point (CCP) was determined as mean – z x SD, where z is equal to 1.645, 2.33 and 3.09 for 95%, 99%, and 99.9% cut-points, respectively. The corresponding % inhibition was determined as (1-CCP) x 100 and the data are shown in Table 1.

Table 1
Confirmatory Cut Points

			Confirmatory Cut Point Estimate for Different Prediction Intervals					
			95%		99%		99.9%	
Mean Ratio	Mean Inhibition	RMSE	Ratio	% Inhibition	Ratio	% Inhibition	Ratio	% Inhibition
0.719	28.149%	0.1164	0.527	47.3%	0.447	55.3%	0.359	64.1%

The false positive evaluation by matrix type is shown in Table 7. Using the 99% prediction interval, two BCS samples (1.5%) and one HHS sample (0.7%) tested within this experiment would be considered positive, indicating that the confirmatory cut-point represents both matrices.

Table 7
False-Positive Evaluation by Matrix

			Excluding Outliers			Including Outliers		
Matrix	Cut Point	Cut Point Value	Sample Count	Positive Count	% Positive	Sample Count	Positive Count	% Positive
BCS	95% CCP	47.3%	133	13	9.8%	137	14	10.2%
BCS	99% CCP	55.3%	133	2	1.5%	137	3	2.2%
BCS	99.9% CCP	64.1%	133	0	0.0%	137	0	0.0%
HHS	95% CCP	47.3%	144	6	4.2%	145	6	4.1%
HHS	99% CCP	55.3%	144	1	0.7%	145	1	0.7%
HHS	99.9% CCP	64.1%	144	0	0.0%	145	0	0.0%

Reviewer comment: *The sponsor pooled the HHS and BCS samples to determine the confirmatory cut-point even though the two populations were determined to be statistically different because there was only ~3% difference between the means and median between the two sample populations. This is acceptable because when they applied the cut-point to determine the false positive rates, the number of samples that would test positive in the BCS and HHS population were within the acceptable range. Therefore, the determination of confirmatory cut-point for the NAb assay for HHS and BCS populations is acceptable.*

Relative Assay Sensitivity

To determine relative assay sensitivity, anti-trastuzumab antibody was spiked into the NC pool. Initial set of concentrations for the antibody curve were 12,500, 6,250, 3,125, 1,563, 781, 391, 195, 97.7, and 48.8 ng/mL. The samples were analyzed in three assays by two analysts on at least two days. A total of six antibody curves were generated and the SAV was calculated for each sample. For these analyses, the assay sensitivity changed between 1563 ng/mL and 3125 ng/mL and because the sensitivity of 3125 ng/mL differed from developmental data, the sponsor ran the experiments with new concentrations that included more concentrations between 3125 ng/mL and 1563 ng/mL. The new concentrations were 8,000, 4,000, 3,000, 2,800, 2,400, 2,000, 1,500, 750, 375, and 187.5 ng/mL anti-trastuzumab antibody. To determine the assay sensitivity, the average of all six curves was taken and the sensitivity was determined to be 1,500 ng/mL.

LPC

Prior to assay sensitivity determination, the LPC was set at 2,000 ng/mL. Based on the results of the assay sensitivity, the LPC was adjusted to 3x assay sensitivity or 4,500 ng/mL.

Reviewer comment for assay sensitivity and LPC
The determination of the assay sensitivity and LPC is acceptable. FDA does not provide guidance for assay sensitivity. However, USP recommends NAb assays to have a sensitivity of 100 – 2000 ng/mL. It seems that the sponsor was trying to meet this requirement and repeated the analysis with a different set of positive control antibodies to meet this criterion. This is acceptable because this is a NAb assay using a cell-based system and it is expected to have lower sensitivity compared to the ADA assay or ligand-binding assays.

Matrix Interference/Selectivity
The ability to detect anti-trastuzumab NAb in the presence of other constituents in the sample was determined by spiking anti-trastuzumab antibodies at concentrations of LPC (4,500 ng/mL), MPC (7,000 ng/mL), and HPC (10,500 ng/mL) into ten samples of either HHS or BCS that screened negative during the assay cut-point determination and analyzed in the screening and confirmatory assays. Ten of ten individual unspiked samples had a final result of negative, for both HHS and BCS. When spiked at the low PC, the HHS had a final result of positive in nine of ten samples, and the BCS had a final result of positive in ten of ten samples. When spiked at the mid and high PC, the HHS and BCS had a final result of positive in ten of ten samples. Therefore, tests at the four different concentrations of anti-trastuzumab antibodies met the pre-determined acceptance criterion.

Drug Tolerance
CT-P6 was prepared at 20,000, 10,000, 6,000, 2,000, 680, 450, 300, and 150 ng/mL in the NC pool. Anti-trastuzumab antibody samples were prepared in the NC pool at 2XLPC, 2XMPC, and 2XHPC. Each PC sample was diluted 1:1 with each CT-P6 sample, incubated, and analyzed. The LPC spiked sample (4,500 ng/mL) could be detected in the presence of up to 1,000 ng/mL of excess CT-P6. The MPC spiked sample (7,000 ng/mL) could be detected in the presence of up to 3,000 ng/mL excess CTP6, and the HPC spiked at (10,500 ng/mL) could be detected in the presence of up to 5,000 ng/mL excess CT-P6.

Reviewer comment: *None of the positive controls are detectable in the presence of 10 µg/mL of CT-P6 (Table 11, pg 392 of doc 16.1.14 Other Analytical and Validation Reports). Given that the trough levels of CT-P6 was determined to be 16.80 – 18.92 µg/mL for early breast cancer patients in clinical study CT-P6 3.2, the drug tolerance of the NAb assay is low indicating that low concentrations of NAb might be missed using this assay.*

Plate Homogeneity
Plate homogeneity determination with one plate of LPC at n = 25 demonstrated that 100% of SAVs ≤ 0.729 (assay cut-point) and met the pre-determined acceptance criterion of 90% of LPC sample SAVs had to be at or below the assay cut-point.

Precision
Intra-assay precision from six of six replicates of LPC, MPC, and HPC were 3.07%, 4.81%, and 6.82%, respectively and met the pre-determined acceptance criterion of %CV ≤ 30%.

For inter-assay precision, the pre-determined acceptance criterion was %CV ≤ 30%. For the (b) (4) antibody, the %CV for inter-assay precision sample SAVs for the LPC, MPC, and HPC were

28.9%, 9.25%, 15.9%, respectively, and met the pre-determined acceptance criterion. For the (b) (4) antibody, the %CV for inter-assay precision sample SAVs for the LPC, MPC, and HPC were 17.6%, 20.1%, 22.6%, respectively, and met the pre-determined acceptance criterion.

Cell Line Stability
Cell line stability was evaluated based on the DC/NC values from the validation runs. The data are presented in Table 44 and shows that SK-BR-3 cells were tested within passages of 8 – 11 and Jurkat cells were tested within passages of 8 – 12. The acceptable passages for analysis for SK-BR-3 cell line is passage 6 – 24 and for Jurkat cell line is passage 6 – 20.

Reviewer comment: *The acceptance criterion for DC/NC is not provided here.*

Drug Comparison
Samples were spiked with eight different PC concentrations (12,000 to 187.5 ng/mL anti-trastuzumab) in the presence of 200 ng/mL CT-P6 or 200 ng/mL Herceptin. Samples were analyzed three times at n = 1 per plate in healthy human serum by at least two analysts on at least two different days for a total of three curves. To be acceptable, the LPC level (4,500 ng/mL) had to be positive when analyzed with both CT-P6 and Herceptin. Samples spiked at the LPC level were "confirmation required" with SAVs at or below the screening assay cut point when analyzed in the presence of 200 ng/mL CT-P6 or 200 ng/mL Herceptin and therefore, met the acceptance criterion.

Titration
During sample analysis for the clinical study, "positive" confirmatory assay samples will be two fold serially diluted in order to determine its titer. In order to validate this testing process, two individual HHS and two BCS samples were spiked with 16,000 ng/mL anti-trastuzumab antibody. The prepared samples were two fold serially diluted in 100% pooled normal human serum and processed in the screening assay. To be acceptable, dilutions less than or equal to the screening assay cut-point must have %CV ≤ 30%. The highest dilution with results at or below the screening assay cut-point was reported. For the HHS, the highest dilution resulting in a SAV at or below the cut point for both samples was Dil 80. For the BCS, the highest dilution resulting in a SAV at or below the cut point for each sample was Dil 80 and Dil 160, respectively.

Stability
Freeze/Thaw (F/T) stability was evaluated by analyzing six replicates of LPC and HPC subjected to seven F/T cycles and analyzing in the screening assay. All six runs of LPC and HPC produced SAVs which were ≤ assay cut-point (0.729) and met the pre-determined acceptance criteria of 5/6 samples ≤ assay cut-point.

Analyte stability in thawed matrix was evaluated by analyzing six replicates each of LPC and HPC that remained at room temperature for approximately 24.5 hours or at 2 - 8°C for approximately 24 hours. All the samples met the pre-determined acceptance criterion (same as F/T).

Interference

Hemolysis
Interference from hemolysis was examined by analyzing six replicates of pooled healthy human serum samples that were spiked with hemolysed blood at 1%, 5%, and 10% and then spiked with either LPC or HPC. Each sample, spiked and unspiked, was analyzed in the screening and confirmatory assays.

The acceptance criteria was 5/6 unspiked samples at each hemolysis had to have overall result of negative for each PC level, 5/6 samples at each hemolysis level had to have an overall result of positive. The results demonstrated that the acceptance criterion was met.

Lipemia

To evaluate the effect of lipemia on recovery of neutralizing antibodies, six human serum samples > 300 mg/dL triglycerides were analyzed. The samples were spiked with LPC and HPC. One replicate of each sample, spiked and unspiked, was analyzed in the screening and confirmatory assays. The acceptance criteria was 5/6 unspiked samples at each triglyceride level range had to have an overall result of negative and for each PC level; 5/6 spiked samples at each triglyceride level range had to have an overall result of positive. The results showed that all unspiked samples had an overall result of negative. Two of six samples spiked at the LPC level had an overall result of positive. Six of six samples spiked at the HPC level had an overall result of positive. These results indicate that there is interference on the detection of NAbs from lipemic samples.

Assay Acceptance Criteria

All acceptable validation runs were used to determine the final DC/NC ratio for plate acceptance. The acceptance criteria was established upon consideration of the following: the mean DC/NC ratio of all acceptable runs – (1-3X SD) = lower limit of acceptance. The best fit DC/NC ratio for plate acceptance was determined to be the lowest ratio obtained, which was 2.33. The LPC, MPC, and HPC SAVs must be at or below the assay cut point for acceptance.

Reviewer comment: *The NAb assay has low assay sensitivity (1,500 ng/mL). However, the assay is a cell-based ADCC assay, which is expected to have low sensitivity. Overall, the validation of the NAb assay is acceptable.*

Shadia Zaman

Digitally signed by Shadia Zaman
Date: 2/21/2018 03:13:45PM
GUID: 583dce940076eea0edb730e401622d6d

Jennifer Swisher

Digitally signed by Jennifer Swisher
Date: 2/21/2018 03:15:54PM
GUID: 508da6d7000262dc015dcdc5f6541612

Kathleen Clouse Strebel

Digitally signed by Kathleen Clouse Strebel
Date: 2/21/2018 08:49:37PM
GUID: 508da6d70002630c9a2555c796176955

U.S. FOOD & DRUG ADMINISTRATION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

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

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Date:	February 21, 2018
To:	Administrative File, 761091/0
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:	1996
Applicant:	CELLTRION, Inc.
Facilities:	CELLTRION, Inc. (b) (4) Yeonsu-gu, Incheon, 22014, Republic of Korea (FEI: 3005241015)
Product:	HERZUMA, CT-P6 (anti-HER2 monoclonal antibody)
Dosage:	Lyophilized powder for injection: (b) (4) 420 mg/vial (multiple use)
Indication:	Adjuvant Breast Cancer, Metastatic Breast Cancer, Metastatic Gastric Cancer
Action Date:	March 30, 2018

Conclusion and Approvability Recommendation:

The drug substance portion of BLA 761091, 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	5/30/2017	Original BLA
0008	9/11/2017	Response to Information Request dated 31 August 2017
0030	2/9/2018	Response to Information Request dated 5 February 2018

Review Narrative

S DRUG SUBSTANCE
S.1 General Information

CT-P6 215 is a humanized monoclonal antibody (IgG1 subclass) that binds the extracellular domain of human epidermal growth factor receptor 2 (HER2). CT-P6 is heterologously expressed in Chinese Hamster Ovary (CHO) cells that secrete the antibody into the growth medium. The DP is a lyophilized powder in (b) (4) multiple-use vials for reconstitution with diluent, which is co-packaged.

S.2 Manufacture
S.2.1 Manufacturer(s)

23 Pages have been Withheld in Full as b4 (CCI/TS) immediately following this page

THE DESCRIPTION OF THE CONTAINER CLOSURE SYSTEM IS SATISFACTORY.

S.7 Stability
S.7.1 Stability Summary and Conclusions & S.7.3 Stability Data

Reviewer Comment:
In the original submission, bioburden and endotoxin were included as part of the post-approval stability protocol for long-term (-40°C), intermediate (5°C), and accelerated (25°C, 5% RH) studies. An FDA information request dated 5 February 2018 advised that these microbiological stability samples were not required for US licensure. The applicant removed these samples from the protocol.

cGMP Status

Refer to Panorama for cGMP status of the relevant facilities.

Conclusion

I. The drug substance section of BLA 761091 was reviewed from a product quality microbiology perspective and is recommended for approval.

II. Product quality aspects other than microbiology should be reviewed by OBP.

III. The pre-license inspection of the DS manufacturing facility, Celltrion (b) (4) was not performed because the facility was deemed OAI following a routine CGMP surveillance inspection conducted by the ORA district office.

Information Requests

FDA Information Request sent on 31 August 2017 (refer to sequence 0008)

Section 3.2.S.2.2 – Description of Manufacturing Process and Process Control

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

STATISTICAL REVIEW AND EVALUATION

BLA NO.	761091
DATE RECEIVED BY THE CENTER	07/18/2017
DRUG NAME	CT-P6
DOSAGE FORM	(b) (4) Vial
INDICATION	Adjuvant Breast Cancer, Metastatic Breast Cancer, Metastatic Gastric cancer
SPONSOR	Celltrion
REVIEW FINISHED	2/14/2018
STATISTICAL REVIEWER	Chao Wang, Ph.D.
SECONDARY REVIEWER	Meiyu Shen, Ph.D.
PROJECT MANAGER	Leyish Minie, RN, MSN

Chao Wang, Ph.D., Mathematical Statistician, CDER/OTS/OB/DBVI
Meiyu Shen, PhD., Lead Mathematical Statistician, CDER/OTS/OB/DBVI

Concur: _____
Yi Tsong, Ph.D., Division Director, CDER/OTS/OB/DBVI

CC List:
Lillian Patrician, CDER/OTS/OB
Leyish Minie, CDER/OND/OHOP/DOPI

Contents

1 Executive summary and recommendation

The CMC statistics reviewer in the Office of Biostatistics analyzed the data and reviewed Celltrion analysis for two Tier 1 quality attributes, in vitro bioactivity and ADCC. The assays for ADCC were performed at 3 concentrations (0.5 ng/mL, 1.3 ng/mL and 3.2 ng/mL). Both attributes are relative values and all assays were tested against CT-P6 in-house reference standard RF0650-02. Celltrion used 12 US-licensed Herceptin drug product batches and no apparent trend related to age was found. Celltrion used 11 CT-P6 DP batches, all of which were produced from independent drug substance lots.

Based on FDA independent analysis, both QAs passed the statistical equivalence test and support a demonstration that CT-P6 is highly similar to US-licensed Herceptin.

2 Statistical evaluation of evidence

2.1 Introduction

On July 18, 2017, Celltrion submitted to the U.S. Food and Drug Administration (FDA) a Biologic License Application (BLA) under Section Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). This BLA includes an analytical similarity assessment of two Tier 1 quality attributes (QA), namely, in vitro bioactivity and ADCC between CT-P6 and US-licensed Herceptin.

Celltrion performed assay for the two QAs in the following way.

- In vitro bioactivity: The sponsor performed the in vitro bioactivity assay to measure the anti-proliferation activity of US-licensed Herceptin and CT-P6 drug product to HER2 over-expressing human breast cancer cell line, BT-474. The inhibition of BT-474 proliferation was measured by CCK-8 colorimetric reagent after 5 days incubation. The OD values for the reference standard and samples were fitted using parallel line assay. The relative potency of samples was determined using 4-PL logistic curve analysis in PLA software.
- ADCC: The sponsor determined the ability to induce ADCC activity of HER2 overexpressing breast cancer cell line, SK-BR-3 target cells (T) using PBMCs from healthy donor as effector cells (E) using US-licensed Herceptin and CT-P6 drug product. The assays were performed at 3 concentrations (0.5 ng/mL, 1.3 ng/mL and 3.2 ng/mL) of antibody using E:T ratio of 16:1. The relative ADCC activity of sample was determined from the comparison of the percentage cytotoxicity of sample to the percentage cytotoxicity of reference standard at each concentration.

Both QAs are relative values and all assays were tested against CT-P6 in-house reference standard RF0650-02.

2.2 Evaluation of data quality

2.2.1 Data for US-licensed Herceptin

Celltrion used 12 US-licensed Herceptin drug product (DP) (440 mg) batches for Tier 1 analytical similarity assessment. The DP batch ID, age, and measured values for both Tier 1 QAs are given in Table 1. The measure values are also illustrated in Figure 1, which shows no apparent trend related to age.

DP batch ID	Age	In vitro bioactivity	ADCC (0.5ng/mL)	ADCC (1.3ng/mL)	ADCC (3.2ng/mL)
635219	40	99	92	100	105
548420	38	103	74	79	86
586305	33	100	99	100	101
579938	30	99	103	118	110
626308	27	103	116	92	111
626323	23	112	111	104	98
3044031	19	99	75	82	86
671194	18	108	86	96	91
3001143	17	110	72	86	85
3014299	14	110	70	79	87
3022456	12	113	76	79	93
3022461	10	98	74	87	88

Table 1: Information about US-licensed Herceptin batches and measured batch values for Tier 1 QAs.

2.2.2 Data for CT-P6

Celltrion used 11 CT-P6 DP (440 mg) batches for Tier 1 analytical similarity assessment. The DS batch ID, DP batch ID, age, and measured values for both Tier 1 QAs are given in Table 2, from which we see that all DP lots were produced from independent drug substance (DS) lots. We also note that the DS/DP lots were produced in two different production processes and OBP reviewer determined that the two processes are comparable so all data were used in evaluating the two Tier 1 QAs.

DS batch ID	DP batch ID	Age	In vitro bioactivity	ADCC (0.5ng/mL)	ADCC (1.3ng/mL)	ADCC (3.2ng/mL)
13200A04	13A3C001	34	102	97	106	109
13200A02	13A3C002	34	99	118	92	109
13200A03	13A3C003	34	99	93	89	96
El3200A01	13A3C004	34	105	91	80	101
14200A007	15A3C01	1	102	111	105	99
14200A008	16A3C01	1	100	106	90	95
14200A003	16A3C02	1	99	98	100	113
14200A006	16A3C03	1	104	92	99	98
l4200A002	16A3C05	1	98	93	116	101
14200A004	16A3C06	1	94	100	99	96
14200A001 14200A005 14200A009	16A3C07	1	104	88	97	100

Table 2: Information about CT-P6 batches and measured batch values for Tier 1 QAs.

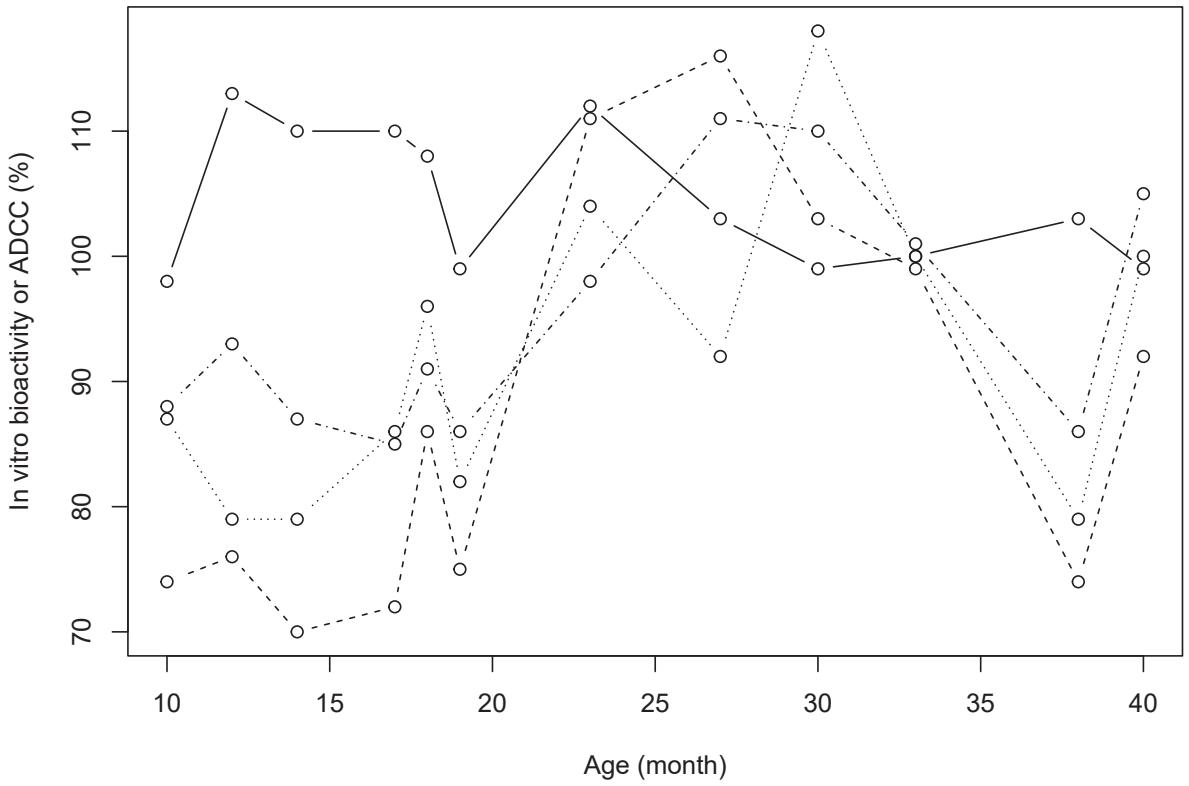

Figure 1: Plot of Tier 1 QA measurements for US-Herceptin against age at analysis (in vitro bioactivity, solid line; ADCC (0.5 ng/ml), dashed line; ADCC (1.3 ng/ml), dotted line; ADCC (3.2 ng/ml), dotdash line).

2.3 Sponsor's analysis

Celltrion followed the FDA recommendation which was given in INDs. The sponsor's analysis result is the same as FDA independent analysis reported below.

2.4 Method for equivalence testing recommended by FDA

FDA currently recommends an average equivalence test in testing equivalence for Tier 1 QAs between a proposed biosimilar product (B) and the reference product (R). To test the equivalence in mean, the null and alternative hypotheses are formulated as follows:

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

and

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

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

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

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

and

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

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

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

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

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

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

and

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

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

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

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

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

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

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

2.5 The FDA Statistical reviewer's analysis

Here we report FDA independent analysis of two QAs.

2.5.1 In vivo bioactivity

For in vitro bioactivity, the data are illustrated in Figure 2 and the equivalence test result is summarized in Table 3, which shows that in vitro bioactivity passed the equivalence test.

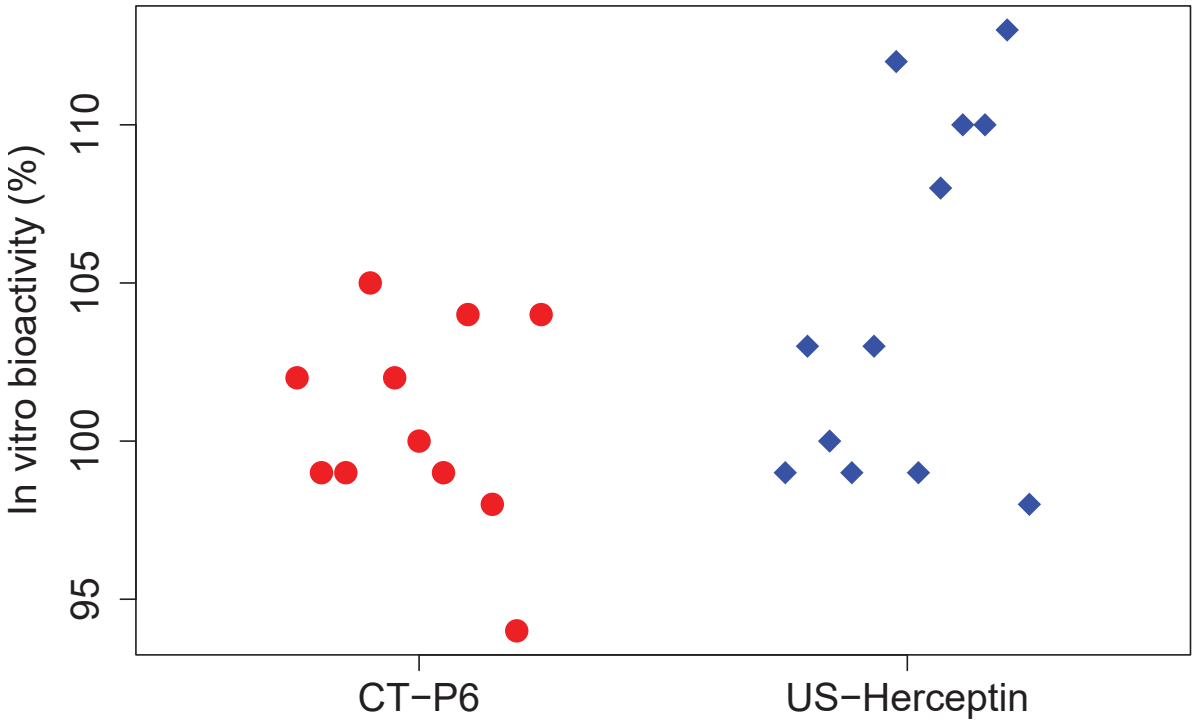

Figure 2: Scatter plot for in vitro bioactivity.

Product	# of Lots	Range (%)	Mean (%)	Std. Dev. (%)	Mean Diff. (%) (90% CI)	Margin (%)	Pass ET?
CT-P6	11	(94-105)	101	3.2	-3.95 (-7.28, -0.63)	(-8.57,8.57)	Yes
US-Herceptin	12	(98-113)	104	5.7			

Table 3: Summary of equivalence test for in vitro bioactivity (%) between CT-P6 and US-Herceptin.

2.5.2 ADCC

Since Celltrion performed the ADCC assay at three concentrations (0.5 ng/mL, 1.3 ng/mL and 3.2 ng/mL), we performed the equivalence test for the three concentrations separately. The data for 0.5 ng/mL, 1.3ng/mL, and 3.2 ng/mL concentrations are illustrated in Figure 3, 4, and 5 respectively. The equivalence test results are summarized in Table 4, 5, and 6 respectively. ADCC passed the equivalence test at all concentrations.

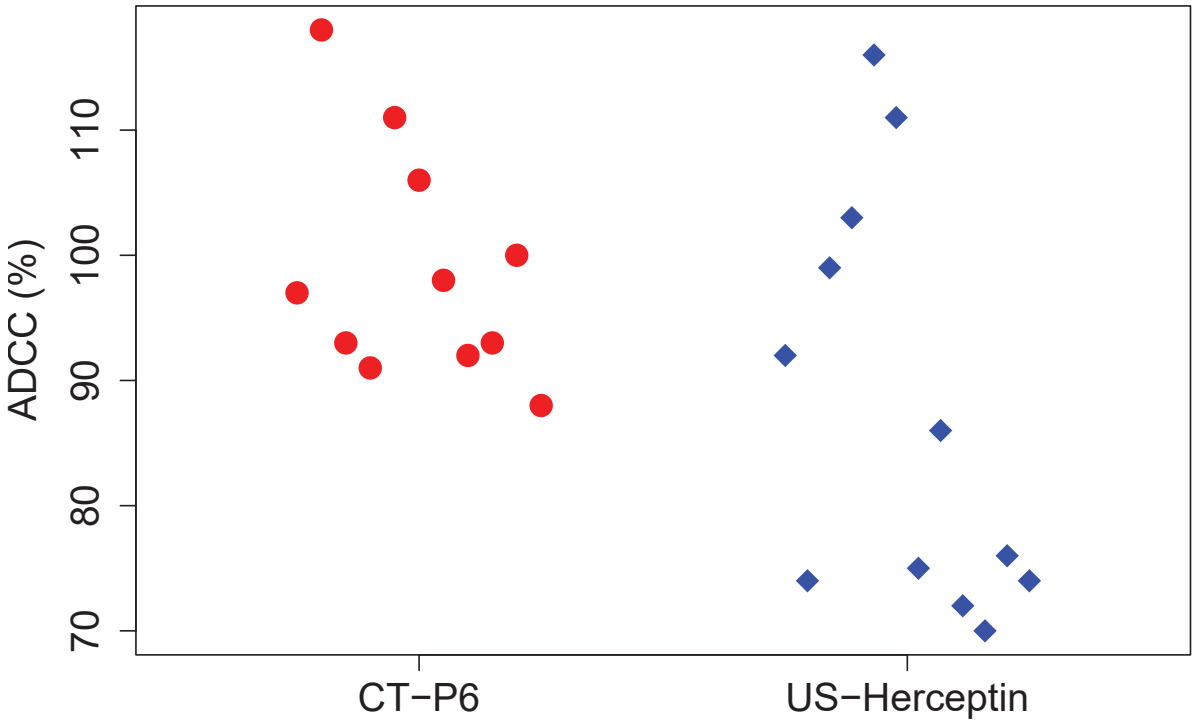

Figure 3: Scatter plot for ADCC measured at the concentration 0.5 ng/mL.

Product	# of Lots	Range (%)	Mean (%)	Std. Dev. (%)	Mean Diff. (%) (90% CI)	Margin (%)	Pass ET?
CT-P6	11	(88-118)	99	9.3	11.48 (1.93, 21.04)	(-24.6,24.6)	Yes
US-Herceptin	12	(70-116)	87	16.4			

Table 4: Summary of equivalence test for ADCC (%) between CT-P6 and US-Herceptin measured at the concentration 0.5 ng/mL.

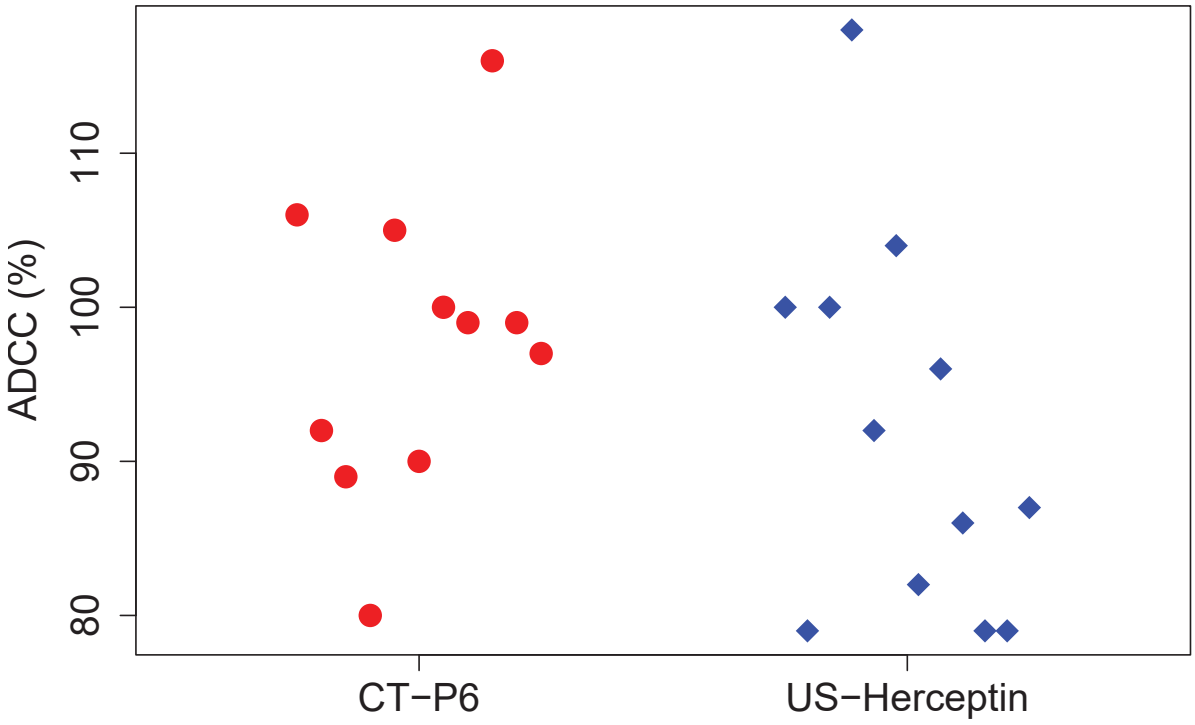

Figure 4: Scatter plot for ADCC measured at the concentration 1.3 ng/mL.

Product	# of Lots	Range (%)	Mean (%)	Std. Dev. (%)	Mean Diff. (%) (90% CI)	Margin (%)	Pass ET?
CT-P6	11	(80-116)	98	9.7	5.71 (-2.17, 13.6)	(-18.27,18.27)	Yes
US-Herceptin	12	(79-118)	92	12.2			

Table 5: Summary of equivalence test for ADCC (%) between CT-P6 and US-Herceptin measured at the concentration 1.3 ng/mL.

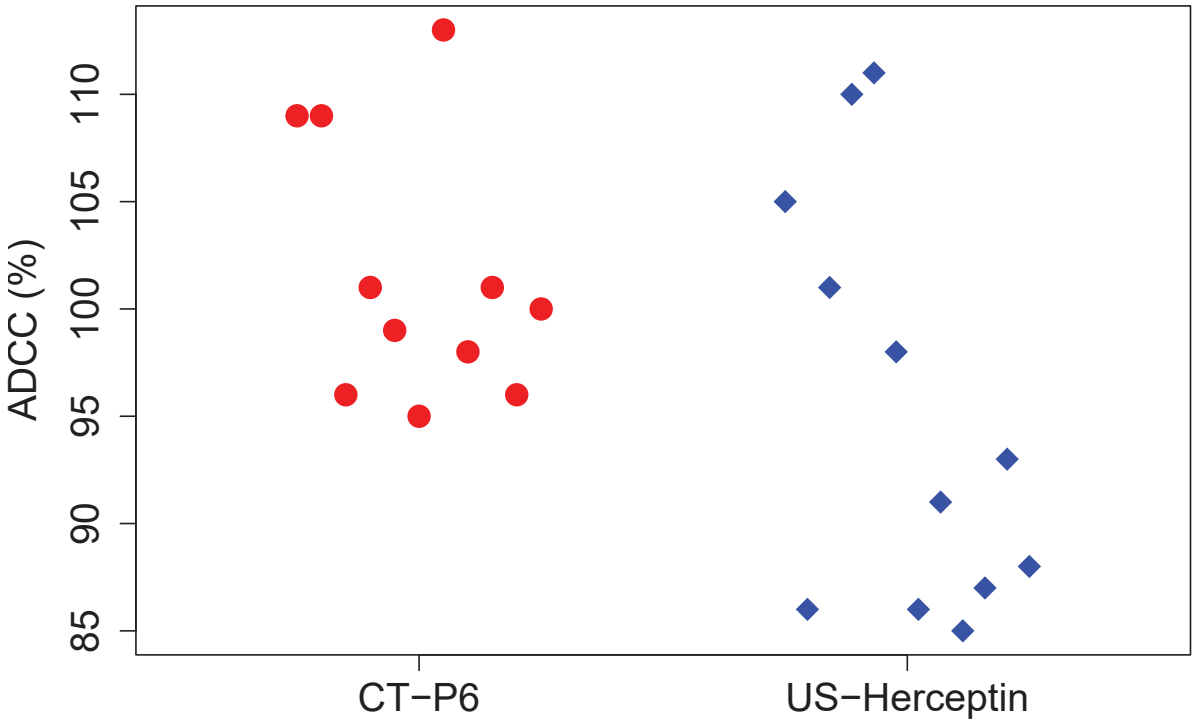

Figure 5: Scatter plot for ADCC measured at the concentration 3.2 ng/mL.

Product	# of Lots	Range (%)	Mean (%)	Std. Dev. (%)	Mean Diff. (%) (90% CI)	Margin (%)	Pass ET?
CT-P6	11	(95-113)	102	6.1	6.46 (0.7, 12.22)	(-14.45,14.45)	Yes
US-Herceptin	12	(85-111)	95	9.6			

Table 6: Summary of equivalence test for ADCC (%) between CT-P6 and US-Herceptin measured at the concentration 3.2 ng/mL.

2.6 Recommendation and conclusion

The statistical equivalence analyses of in vitro bioactivity and ADCC shown above support a demonstration that CT-P6 is highly similar to US-licensed Herceptin.

Reference

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

Tsong, Yi, Xiaoyu Dong, and Meiyu Shen. 2017. "Development of Statistical Methods for Analytical Similarity Assessment." *Journal of Biopharmaceutical Statistics* 27 (2). Taylor & Francis: 197–205.

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/s/

CHAO WANG
02/20/2018

MEIYU SHEN
02/20/2018

YI TSONG
02/20/2018

Recommendation: Complete Response

BLA Number: 761091
Review Number: First round
Review Date: February 8, 2018

Drug Name/Dosage Form	Herzuma (trastuzumab-(b) (4) for injection
Strength/Potency	420 mg/vial
Route of Administration	Intravenous infusion
Rx/OTC dispensed	Rx
Indication	Treatment of HER2-overexpressing breast cancer
Applicant/Sponsor	Celltrion, Inc.

Product Overview

Herzuma (trastuzumab-(b) (4) is a proposed biosimilar to US-Herceptin (trastuzumab). It is a humanized IgG1κ monoclonal antibody produced in CHO cells. Trastuzumab targets human epidermal growth factor receptor 2 (HER2) and when bound to HER2 on HER2-expressing cells, trastuzumab 1) inhibits HER2 receptor dimerization and downstream signaling, 2) increases destruction of the endocytic portion of the HER2 receptor 3) inhibits HER2 extracellular domain shedding, and 4) activates cell-mediated immune defenses such as ADCC activity through concomitant binding to Fcγ receptors on immune effector cells.

Trastuzumab-(b) (4) is produced in genetically engineered CHO (b) (4) cells. Trastuzumab-(b) (4) drug product, Herzuma, is manufactured to the same concentration and presentation as U.S.-licensed Herceptin at 420 mg/vial; the formulation is identical except for an increase in α,α-trehalose dihydrate (from 381 to 839 mg/vial), which is (b) (4) Herzuma drug product is supplied at 420 mg/vial as a sterile, lyophilized powder for intravenous infusion; the 420 mg presentation is a multi-dose vial. Herzuma is proposed as a treatment for HER2-overexpressing breast cancer.

Quality Review Team

Discipline	Reviewer	Branch/Division
Drug Substance	Riley Myers	OPQ/OBP/DBRR I
Drug Product	Shadia Zaman	OPQ/OBP/DBRR I
Drug Substance Microbiology Drug Product Microbiology	Scott Nichols Candace Gomez-Broughton	OPQ/OPF/DMA IV OPQ/OPF/DMA IV
Facility	Thuy Thanh Nguyen	OPQ/OPF/DIA
Immunogenicity assay	Shadia Zaman	OPQ/OBP/DBRR I
Analytical Similarity	Riley Myers	OPQ/OBP/DBRR I
Labeling	Vicky Borders Hemphill	OPQ/OBP
Product quality Team Lead Microbiology QAL Facility Branch Chief	Jennifer Swisher Reyes Candau-Chacon Peter Qiu	OPQ/OBP/DBRR I OPQ/OPF/DMA IV OPQ/OPF/DIA
CMC RPBM	Keith Olin	OPQ/OPRO
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Multidisciplinary Review Team:

Discipline	Reviewer	Office/Division

RPM	Leyish Minie	OND/OHOP/DOP-1
Cross-disciplinary Team Lead	Sanjeeve Balasubramaniam	OND/OHOP/DOP-1
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CMC Statistics Clinical Statistics	Erik Bloomquist /Shenghui Tang (TL) Chao Wang/Meiyu Shen (TL)	OTS/OB/DBV OTS/OB/DBIV

Names:

- Proprietary Name: Herzuma
- Non-Proprietary/USAN/INN: Trastuzumab-(b) (4)
- CAS Registry number: 180288-69-1
- Company/Laboratory code: CT-P6
- OBP systematic name: MAB HUMANIZED (IGG1) ANTI P04626 (ERBB2_HUMAN) [CT-P6]
- Other Names: None

Submissions Reviewed:

Submission(s) Reviewed /sequence number	**Document Date**
STN 761091 /2	5/30/2017
STN 761091/9 (response to IR #1)	9/11/2017
STN 761091 /15 (response to IR #2) STN 761091 /15 (response to IR #2) STN 761091 /22 (response to IR #2)	10/16/2017 10/23/2017 12/18/2017
STN 761091 /23 (response to IR#3)	12/21/2017
STN 761091 /24 (Stability Update)	12/22/2017
STN 761091 /25 (response to IR #4)	1/11/2017
STN 761091 /27 (response to IR #2)	1/22/2018
STN 761091 /29 (response to IR #5)	1/29/2018
STN 761091 /31 (response to IR #6)	2/09/2018
STN 761091 /34 (response to IR#7)	2/16/2018
STN 761091 /35 (response to IR #8)	2/20/2018
STN 761091 /36 (response to IR #9)	2/21/2018

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	Not reviewed. Sufficient information related to

(b) (4)	III	(b) (4)	3	N/A	N/A	compatibility with the product is in the BLA.
	III		3	N/A	N/A	
	III		3	N/A	N/A	
	V		3	N/A	N/A	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 119650

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 761091 for HERZUMA (trastuzumab-(b) (4) manufactured by Celltrion, Inc. The data submitted in this application are not sufficient to support a conclusion that the manufacture of HERZUMA 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 HERZUMA 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.

Pending the pre-license inspection that may include an on-site assessment of similarity data, the analytical similarity assessment is adequate to support the conclusion that the biological product, trastuzumab-(b) (4) is highly similar to U.S.-licensed Herceptin.

B. Summary of Complete Response (CR) Issues:

Product Quality CR comments (provided by OBP):

1. Per the "Guidance for Industry: Allowable Excess Volume and Labeled Vial Fill Size in Injectable Drug and Biological Products" (https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm389069.pdf), "In the case of drug products requiring reconstitution, the product should be designed to meet the label claim and acceptable overfill, and allow for correct dosing". The DP release specification of (b) (4) mg/vial must be adjusted to ensure that the recoverable protein content at the lower limit of the acceptance criterion will consistently meet the label claim of 420 mg. Adjust the (b) (4) control strategy to meet the updated specification.
2. The proposed (b) (4) a critical quality attribute (CQA) known to adversely affect potency. Current DS and drug product (DP) lot release and stability specifications for (b) (4) by IEC-HPLC are (b) (4) respectively; therefore, the proposed (b) (4) Revise the (b) (4) that is validated by Celltrion's manufacturing experience. Alternatively, tighten the DS lot release and stability

specifications for (b) (4) using IEC-HPLC to ensure acceptable product quality upon DP manufacture at a worst-case scenario (e.g., using DS released at the upper/lower limit and stored till the end of shelf life) in support (b) (4).

3. As committed in the response to an IR received 2/20/2018, Celltrion will perform process characterization studies in order to establish acceptable ranges for lyophilization process parameters such as (b) (4). The criticality of these process parameters must be justified based on characterization of their impact on CT-P6 product quality attributes.
4. In the response to an IR received 2/16/2018, Celltrion's proposed acceptance criterion for (b) (4) is unacceptably broad based on your manufacturing experience. To ensure appropriate (b) (4) of the DP, tighten your acceptance criterion for DS release to (b) (4)

Facility CR comment (provided by OPF/DIA):

5. 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.

C. Summary of Non-CR Issues (provided by OBP and OPF/DMA):

1. We cannot fully assess the adequacy of Celltrion's control strategy for CT-P6 in the absence of a pre-license manufacturing inspection. In the responses received on 10/16/2017 and 1/29/2018 to our IRs regarding the acceptable ranges for numerous process parameters that have the potential to impact product quality, the limited process characterization and process validation data may not fully justify the criticality ranking of these parameters. Their acceptability will be a review issue once the entire control strategy is updated and available for review.
2. FDA recommends that Celltrion develop a two-tiered reference material system to support the product lifecycle. An appropriately characterized primary reference material that is representative of production and clinical materials can be used to calibrate or qualify a working reference material and contributes to mitigating the risk of drift in quality attributes over time. Use of a working or secondary reference material calibrated against a single primary reference material for routine release and stability testing of commercial lots provides additional assurance that commercially manufactured product is representative of the clinical trial material.
3. In response to an IR received 2/20/2018, additional validation data for (b) (4)
4. In the Complete Response submission, provide updated data from the following studies that are ongoing:
    a. The commercial scale (b) (4) study for (b) (4) (in section 3.2.S.2.5),
    b. The commercial (b) (4) lifetime study (in section 3.2.S.2.5),

c. The ongoing CT-P6 DS and DP stability studies (in sections 3.2.S.7 and 3.2.P.8),
d. The shipping validation of the finished CT-P6 drug product (in section 3.2.P.3.5),
e. The stability study on three in-use PS20 lots for up to 12 months, committed in your 2/20/2018 response (in Section 3.2.P.4).

5. (b) (4) Feasibility of (b) (4) for BWFI has not been determined. In the Complete response submission include feasibility studies for (b) (4). Update Section 3.2.P.2 to include a description of the studies as well as the results. If the feasibility studies support the use of (b) (4), the manufacturing process for BWFI should be revised accordingly.

D. Benefit/Risk Considerations:

Herzuma (trastuzumab-(b) (4) is proposed as a biosimilar to US-Herceptin (trastuzumab) for treatment of HER2 overexpressing breast cancer. Multiple mechanisms of action have been proposed for trastuzumab, which targets human epidermal growth factor receptor 2 (HER2.) The mechanisms of action include inhibition of HER2 signaling through the receptor and subsequent cell proliferation, adhesion, and differentiation through inhibition of HER2 receptor dimerization, increased destruction of the endocytic portion of the HER2 receptor, and inhibition of extracellular domain shedding. Activation of cell-mediated immune defenses, such as antibody-dependent cellular cytotoxicity (ADCC) activity, has also been identified as a mechanism of action of trastuzumab; it appears that natural killer (NK) cells are important mediators of ADCC activity in the context of trastuzumab-treated breast cancer.

With respect to analytical similarity, Herzuma was evaluated and compared to US-Herceptin using a battery of biochemical, biophysical, and functional assays, including assays that addressed each major potential mechanism of action (see Section II A, Table 1, Analytical Similarity, below). The analytical data submitted support the conclusion that Herzuma is highly similar to US-Herceptin. The amino acid sequences of Herzuma and US-Herceptin are identical. A comparison of the secondary and tertiary structures and the impurity profiles of Herzuma and US-Herceptin support the conclusion that the two products are highly similar. HER2 binding, inhibition of proliferation, and ADCC activity, which reflect the presumed primary mechanisms of action of US-Herceptin, were determined to be equivalent. Some tests indicate that small shifts in low abundance glycan forms [e.g., sialic acid, high mannose, and non-glycosylated heavy chain (NGHC)] exist and are likely an intrinsic property of Herzuma due to the manufacturing process. High mannose and sialic acid containing glycans can impact PK, while NGHC is associated with loss of effector function through reduced FcγRIIIa binding and reduced ADCC activity. However, ADCC activity was equivalent and FcγRIIIa binding was similar between Herzuma and US-Herceptin. The residual uncertainties related to the increase in total mannose forms and NGHC and decrease in sialic acid were addressed by the ADCC similarity and by the PK similarity between Herzuma and US-Herceptin as concluded by the clinical review team. Additional subtle differences in size and charge related variants were detected; however, these variants generally remain within the quality range criteria. Further, the data submitted by the applicant support the conclusion that Herzuma and US-Herceptin can function through the same mechanisms of action for the indications for which Herceptin is currently approved, to the extent that the mechanisms of action are known or can reasonably be determined. Thus, based

on the extensive comparison of the functional, physicochemical, protein and higher order structure attributes, Herzuma is highly similar to US-Herceptin, notwithstanding minor differences in clinically inactive components. CT-P6 meets the statutory "same strength" requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.

The overall control strategy for Herzuma manufacture incorporates control over raw materials, facilities and equipment, the manufacturing process, adventitious agents, release of Drug Substance (DS) and Drug Product (DP), and stability of these materials. There were numerous issues with the DS and DP control strategy: Some were able to be resolved during the review cycle through communication with the applicant and a significant number of corrections to the BLA and updates and additions to the control strategy. However, OBP will not be able to adequately assess some issues regarding the adequacy of the control strategy until a pre-license inspection of the facility can be conducted. The current proposed manufacturing control strategy, in-process controls, process monitoring tests, release, and stability testing as presented in the BLA may not be sufficient to ensure process consistency and DS and DP that have appropriate quality and are free of adventitious agents.

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 1-4 in section I B). The drug substance manufacturing process is robust for inactivation and removal of adventitious agents.

The commercial manufacture of trastuzumab-(b) (4) 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 17 in section I B).

The technical assessments for OBP analytical similarity and drug substance, drug product quality and immunogenicity assay, DMA microbiological drug substance and drug product, and DIA facility 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 Herzuma (trastuzumab-(b) (4) CT-P6) is highly similar to the U.S.-licensed Herceptin reference product, Celltrion performed an analytical similarity assessment using 12 lots each of CT-P6 drug product and U.S.-licensed Herceptin. Trastuzumab molecular attributes are collectively assigned to the correct tiers and attribute similarity was evaluated using appropriate statistical methods (Table 1). Inhibition of proliferation and ADCC activities were assigned into tier 1 similarity assessment based on primary mechanisms of action of trastuzumab. 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-P6 meets the statutory "same strength" requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.

Pending an inspection that includes an assessment of similarity data integrity, the data provided support the conclusion that CT-P6 is highly similar to U.S.-licensed Herceptin.

Table 1: Summary of Similarity Assessment

Quality Attribute	Methods	Tier
Primary structure	• Amino acid analysis (RP-HPLC) • Molar absorptivity (UV spectroscopy at 280 nm) • Peptide mass fingerprinting by liquid chromatography (LC) with electrospray (ESI) mass spectrometry (MS) detection • Peptide mapping by HPLC • Intact molecular mass (LC-MS) • N-terminal and C-terminal sequencing	• Tier 3 • Tier 3 • Tier 3 • Tier 3 • Tier 3 • Tier 3
Protein content	• Concentration [ultraviolet (UV) spectroscopy at 280 nm]	• Tier 2
Higher order structure	• Near and far UV circular dichroism (CD) • Fourier transform infrared spectroscopy (FTIR) • LC-ESI-MS (disulfide bond characterization) • Differential scanning calorimetry (DSC) • Ellman's reagent (free cysteines) • Antibody array	• Tier 3/2 • Tier 3 • Tier 3 • Tier 3 • Tier 3 • Tier 3
Size Variants/Aggregates	• Size exclusion high performance liquid chromatography (SEC-HPLC) with UV detection • SEC with multi-angle light scattering (MALS) • Analytical ultracentrifugation sedimentation velocity (AUC) • Capillary Electrophoresis-Sodium Dodecyl Sulfate (CE-SDS, Reduced and Non-Reduced)	• Tier 2 • Tier 3 • Tier 3 • Tier 2
Charge and hydrophobic variants	• Isoelectric focusing (IEF) • Ion exchange HPLC (IEC-HPLC) • Peptide mapping (oxidation, deamidation, isomerization)	• Tier 3 • Tier 2 • Tier 2
Glycosylation	• Site-specific glycan analysis by LC-MS peptide mapping • Oligosaccharide profiling by 2-AB labeling, HILIC-UPLC • CE-SDS reduced (Non-glycosylated heavy chain)	• Tier 2 • Tier 2 • Tier 3
Potency	• HER2 binding assay by ELISA • Inhibition of proliferation bioassay • Antibody-dependent cellular cytotoxicity (ADCC) bioassay (PBMC as effector cells) • ADCC reporter assay	• Tier 2 • Tier 1 • Tier 1 • Tier 2
Fc-receptor binding and function	• FcγRIIIa V type binding affinity ((b) (4) • FcγRIIIb binding affinity ((b) (4) • FcγRIIa binding affinity ((b) (4) • FcγRIIb binding affinity ((b) (4) • FcγRIa binding affinity ((b) (4) • FcRn binding affinity ((b) (4) • Cellular dependent cytotoxicity (CDC) bioassay • C1q binding assay (ELISA)	• Tier 2 • Tier 2 • Tier 2 • Tier 2 • Tier 3 • Tier 2 • Tier 3 • Tier 3

Trastuzumab-(b) (4) was found to have the same primary and secondary structure as US-licensed Herceptin by the methods listed in Table 1, above. The secondary and tertiary structures of the two products were evaluated for the proper folding that is critical for the effective function and serum half-life of proteins. Secondary structure (α-helix, β-sheet and random coil structures) was provided by the far UV CD spectroscopy and FTIR results, and information on tertiary structure was provided by the near UV CD and IF results. All four methods yielded overlapping spectra between Herzuma and US-Herceptin. DSC was used to measure the melting temperatures of the protein subunits.

Quantitative analysis and representative thermograms demonstrate structural similarity among the three products.

Small amounts of aggregation are present in Herzuma and US-Herceptin. By SEC-HPLC, the difference in average level of aggregates between US-Herceptin and Herzuma as quantified by the applicant's SEC-HPLC assay was 0.09%, which was well within a quality range established based on the US-Herceptin data (mean ± 3sd) and may have been due to differences in the age of the lots at the time of analysis. From a quality standpoint, these differences are negligible and are not expected to have any clinical impact.

CE-SDS was used to evaluate intact IgG, non-glycosylated heavy chain (NGHC), and the sum of light and heavy chains (H+L). Two CT-P6 lots were outside of the QR for both attributes, meaning only 82% of CT-P6 lots were within the QR. However, the levels of fragment in both cases were low and the difference between these lots and the CT-P6 lots within the quality range is ~1%; the absolute difference for each impurity peak was less than 0.1% for all species except H2L1 (where there were 0.16% more fragments in CT-P6, although this fragment was shown to retain nearly full potency in characterization.) These differences do not preclude a demonstration that the products are highly similar and are not expected to have clinical impact.

Charge variants of CT-P6 and US-licensed Herceptin were evaluated by IEF, where products showed four major bands and four minor bands, which were all similar between CT-P6 and US-licensed Herceptin except bands 5 and 6, which were not consistently detected in all lots due to low intensity. Charge variants are evaluated quantitively by IEC-HPLC where all peaks were within the quality range set by the reference product.

All CT-P6 lots were within the quality range for afucosylation and high mannose, which are the most important glycans for Herceptin function. While small differences in sialylation and a moderate difference in galactosylation were found, they were not expected to affect potency or PK. While galactosylation can affect complement-dependent cytotoxicity, this is not a mechanism of action for trastuzumab.

No differences were observed between CT-P6 and US-licensed Herceptin in any biological activities, either as assayed by binding assays such as FcγRIIIa binding or in cell-based assays. Such assays included inhibition of BT-474 cell proliferation, antibody-dependent cellular cytotoxicity (ADCC) with PBMC, or an ADCC reporter assay using HER2 over-expressing human breast cancer cell line, SK-BR-3 as target cell and engineered Jurkat cell stably expressing FcγRIIIa upstream of an NFAT-driven reporter gene.

Thus, based on the extensive comparison of the functional, physicochemical, protein and higher order structure attributes, Herzuma is highly similar to US-Herceptin, notwithstanding minor differences in clinically inactive components.

B. CQA Identification, Risk and Lifecycle Knowledge Management

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

Table 2: Drug Substance (DS) and Drug Product (DP) CQA Identification, Risk and Lifecycle Knowledge Management

CQA	Risk	Origin	Control Strategy	Other notes
HER2 binding (potency)	Efficacy	Intrinsic to the molecule. Impacted by aggregation, fragmentation, and potentially deamidation. Minimal change is expected during storage through expiry.	(b) (4)	N/A
Inhibition of proliferation (potency)	Efficacy	Intrinsic to the molecule. Impacted by aggregation, fragmentation, and potentially deamidation. Minimal change is expected during storage through expiry.		N/A
ADCC activity (potency)	Efficacy	Intrinsic to the molecule. Impacted by glycosylation, aggregation and fragmentation and potentially deamidation Minimal change is expected during storage through expiry.		No CDC activity was detected.
Identity	Safety and Efficacy	Intrinsic to the molecule		N/A
High Molecular Weight (HMW) species/Aggregates (product-related impurities)	Efficacy (HER2 binding and ADCC), Safety/Immunogenicity and potentially PK	Manufacturing process and exposure to heat. Minimal change is expected during storage under recommended		N/A

		conditions through expiry.	(b) (4)	
Fragments (LMW species)	Efficacy and PK	Manufacturing process and exposure to heat and light stress. Minimal increase in fragments is expected during storage under recommended conditions.		N/A
Glycosylation (afucosylated species)	Efficacy (ADCC activity/FcγRIIIa binding)	Cell culture process No change is expected during storage.		N/A
Glycosylation (high mannose)	PK and efficacy (afucosylated)	Cell culture process No change is expected during storage.		N/A

			(b) (4)	
Light chain Asn30 deamidation	Efficacy	Manufacturing process and exposure to heat. Minimal change is expected during storage under recommended conditions.		N/A
Heavy Chain Asp102 isomerization	Efficacy	Manufacturing process including hold, storage, exposure to heat.		(b) (4)
Osmolality	Safety, Efficacy (control of degradation through formulation)	Formulation		N/A
pH	Safety and Efficacy	Formulation		N/A
Protein Content	Efficacy	Manufacturing process		N/A
Polysorbate 20	Safety and efficacy (control of degradation)	Formulation		N/A

C. Drug Substance, trastuzumab-(b) (4) Quality Summary

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	Risk	Origin	Control Strategy	Other notes
Appearance	Safety	Controlled by the manufacturing process	(b) (4)	N/A
Host Cell Proteins (Process-related impurity)	Safety and Immunogenicity	Production cell line		(b) (4)
Host Cell DNA (Process-related impurity)	Safety	Production cell line		N/A
Residual Protein A (Process-related impurity)	Safety and Immunogenicity	Process related impurity (b) (4)		(b) (4)

(b) (4) (Process-related impurity)	Safety, immunogenicity	(b) (4)	(b) (4)	Evaluated by pharm/tox review team also.
(b) (4) (Process-related impurity)	Safety, immunogenicity	(b) (4)		N/A
Viruses (Contaminant)	Safety	Contamination during manufacture, most likely during cell culture operations		N/A
Mycoplasma (Contaminant)	Safety	Mycoplasma would most likely be introduced during cell culture operations.		N/A
Leachables (Process-related impurity)	Safety	Manufacturing components and the DS container closure system		N/A

Endotoxin (contaminant)	Safety and Purity	Endotoxin can be introduced through raw materials and throughout the manufacturing process	(b) (4)	N/A
Bioburden (contaminant)	Safety, Purity and Efficacy (degradation or modification of the product by contaminating microorganisms)	Bioburden can be introduced through raw materials and throughout the manufacturing process		N/A

- **Description:** Trastuzumab-(b) (4) is a recombinant, humanized IgG1κ monoclonal antibody and consists of two heavy chains that are each composed of 450 amino acids and two light chains that are each composed of 214 amino acids. Each heavy chain contains an N-linked glycan site at asparagine 300 (Asn300). The molecular weight of trastuzumab-(b) (4) without C-terminal lysine is 148,055 Da. The theoretical extinction coefficient was calculated to be 1.48 (mg/mL) -1 cm-1, and it was determined experimentally to be 1.44 (mg/mL) -1 cm-1. The theoretical value has been used during development and will continue to be used to determine the trastuzumab-(b) (4) protein concentration for commercial use.

- **Mechanisms of Action (MoA):** Multiple mechanisms of action have been proposed for trastuzumab, which binds to HER2. The mechanisms of action include inhibition of HER2 signaling through the receptor and subsequent cell proliferation, adhesion, and differentiation through inhibition of HER2 receptor dimerization, increased destruction of the endocytic portion of the HER2 receptor, and inhibition of extracellular domain shedding. Activation of cell-mediated immune defenses, such as ADCC activity, through concomitant binding to HER2 and to Fcγ receptors on immune effector cells has also been identified as a mechanism of action of trastuzumab. It appears that NK cells are important mediators of ADCC activity in the context of trastuzumab-treated breast cancer. Complement-dependent cytotoxicity (CDC) is not considered to be a major mechanism of action for trastuzumab.

- **Potency Assays:** The in vitro bioactivity is determined using an anti-proliferation assay. Inhibition of proliferation is determined using BT474 cells, a ductal carcinoma cell line derived from a human mammary gland, which over-express HER2 antigen on the surface. Anti-proliferation activity is determined by measuring BT474 metabolic activity using a colorimetric method to indicate cell viability. Relative potency is calculated by parallel line analysis using PLA software.

  FcγRIIIa binding is a surrogate assay for ADCC activity. A surface plasmon resonance (SPR) method is used to evaluate the binding affinity of CT-P6 Fc region to the FcγRIIIa receptor (V type). Samples are injected over the sensor chip containing the immobilized FcγRIIIa and binding affinity is evaluated. A series of curves are generated from which

both association and dissociation kinetics can be derived and the result is reported as the relative binding affinity to FcγRIIIa ligand of the sample using the dissociation constant of the reference material versus that of the sample.

- **Reference Standards (RS):** A two-tiered approach is intended for the commercial RS; primary RS RF0650-02 was derived from a drug substance batch produced using the manufacturing process and formulation intended for commercialization. The primary RSs are stored at ≤ -60°C. The protocols for stability of the RSs and for qualification of future RSs are deficient and IR responses have been inadequate. Celltrion will be asked by IR to remove the protocol to qualify new reference standards and instructed to propose testing that with acceptance criteria that are sufficiently tight to prevent drift, as well as a trending program. A non-CR comment (#2, section I.C., above) reminds them of the need to address this in the Complete Response submission.

- **Critical starting materials or intermediates:** The trastuzumab- (b) (4) Master Cell Bank (MCB, CTC-06M-247) was developed (b) (4) The Working Cell Bank (WCB, MCB, CTC-06W-247) was created by the expansion of the MCB. This two-tiered cell banking system was implemented to ensure continued source of product. Non-animal derived materials were used in the manufacture of the WCB. The cell lines were appropriately tested to ensure product safety from adventitious and endogenous agents. Viability of both the MCB and WCB is monitored as part of a stability program.

- **Manufacturing process summary:** Trastuzumab- (b) (4) DS is manufactured at Celltrion Inc., Incheon, Republic of Korea. (b) (4)

(b) (4)

The trastuzumab-(b) (4) drug substance manufacturing process development is based on minimal process characterization and process validation studies; however, justifications for several process parameters and/or their proposed ranges carry some risk (see details in Table 2). The overall adequacy of the control strategy cannot be assessed in the absence of a pre-license inspection, which could not take place during this review cycle and is necessary to finalize the review.

- **Container closure:** CT-P6 drug substance is stored in (b) (4)
- **Dating period and storage conditions:** The dating period for the DS will be (b) (4) months when stored at (b) (4) °C (long term).

D. Drug Product, Herzuma, 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)	Risk	Origin	Control Strategy	Other
Sterility (DP and BWFI) (contaminant)	Safety (infection) and Efficacy (degradation or modification of the product by contaminating microorganisms)	Contamination introduced throughout DP manufacturing process or due to failure of container closure integrity	(b) (4)	N/A
Container Closure Integrity (DP and BWFI) (contaminant)	Safety (contamination)	Manufacturing failure or impact of storage conditions		N/A
Endotoxin (DP and BWFI) (contaminant)	Safety and Purity	Contamination introduced throughout DP manufacturing process or due to failure of container closure integrity		N/A

			(b) (4)	
Color and clarity of solution (general)	Safety and Efficacy	Formulation, contamination, or degradation		N/A
Lyophilizate appearance	Safety and Efficacy (stability)	Manufacturing process		See CR item #5 under Section I.B., above.
Moisture content	Efficacy (stability)	Manufacturing Process		N/A
Particulate Matter (visible and subvisible) (Product or Process Related Impurities)	Safety/ Immunogenicity	Manufacturing process and container closure system		N/A
Polysorbate 20 concentration	Safety and Efficacy (control over degradation)	Manufacturing process		N/A
Deliverable Content (general)	Efficacy/Dosing	Manufacturing process		See CR item #4 under Section I.B., above
Leachables (process-related impurities)	Safety	Manufacturing equipment and container closure		N/A

- **Potency and Strength:** Herzuma is supplied at 420 mg/vial. Potency is defined as the percent activity relative to the current trastuzumab-(b) (4) RS. The potency assays are the same as described in the DS section of this memo.

- **Summary of Product Design:** Herzuma is supplied as a sterile, multi-dose, preservative-free lyophilized powder for IV infusion in a 50 mL vial. Herzuma is

formulated in 839.0 mg α,α-trehalose dihydrate, 9.5 mg L-histicine HCl, 6.1 mg L-histidine, and 1.7 mg polysorbate 20. Reconstitution with 20 mL diluent yields a solution containing 21 mg/mL trastuzumab-(b) (4) at a pH of approximately 6. The deliverable amount is 420 mg. Herzuma is supplied with a 20-mL vial of bacteriostatic WFI (BWFI), USP. BWFI is (b) (4). Herzuma reconstituted with BWFI can be stored for up to 28 days at 2-8°C.

- **List of Excipients:** α,α-trehalose dihydrate, L-histicine HCl, L-histidine, and polysorbate 20. All excipients are compendial.

- **Reference Standards:** Same as the CT-P6 drug substance RSs.

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

  The BWFI manufacturing process includes the following steps: (b) (4)

  Herzuma drug product manufacturing process development is based on minimal process characterization and process validation studies. The control strategy is inadequate (see details in Table 4 and CR items #4 and 5 in Section I B above.).

- **Container closure:** 50-mL (b) (4) glass vial (b) (4) with a (b) (4) rubber stopper and a 20 mm aluminum flip-off cap. Each DP vial is packaged in an outer carton box (b) (4)

- The primary container closure system for BWFI is the same as that of Herzuma, with the exception of size (20 mL).

- **Dating period and storage conditions:** Celltrion proposed (b) (4) months of shelf life when stored at 5 ± 3 °C, (b) (4) Drug product reconstituted with BWFI can be stored at 2-8°C for no more than 28 days. The proposed storage time is supported by Anti-Microbial Effectiveness Test (AET) data.

E. Novel Approaches/Precedents: None

F. Any Special Product Quality Labeling Recommendations:

Labeling review was not finalized 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 (b) (4) Yeonsu-gu Incheon, S. Korea	3005241015	Unacceptable due to OAI status	Withhold
Viral testing of unprocessed bulk; Testing of the MCB, WCB and EPCB	(b) (4)		n/a	Approve based on facility profile
Sterility testing of EPCB			n/a	Approve based on facility profile
Mycoplasma testing of unprocessed bulk; Testing of the MCB, WCB, and EPCB			n/a	Approve based on facility profile
Production of the MCB and WCB; MCB and WCB testing			n/a	Approve based on facility profile
Storage of the MCB and WCB; MCB and WCB testing			n/a	Approve based on facility profile
MCB and WCB testing			n/a	Approve based on facility profile
DRUG PRODUCT				
Function	**Site Information**	**DUNS/FEI Number**	**Preliminary Assessment**	**Final Recommendation**
DP manufacturing, release and stability testing	Celltrion, Inc. 23, Academy-ro (b) (4) Yeonsu-gu Incheon, S. Korea	3005241015	Unacceptable due to OAI status	Withhold

DS - drug substance, DP - drug product, MCB - Master Cell Bank, WCB - Working Cell Bank, UPB – Unprocessed bulk
* See section H. Facilities.

H. Facilities:

The subject BLA proposes manufacture of Herzuma drug substance and drug product at Celltrion, Inc. (FEI 3005241015). A post-approval inspection for BLA 125544 (Inflectra) 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 G), which are not inspected within scope of this BLA. The Celltrion facility is recommended for Withhold from a facilities assessment standpoint (see CR comment 5 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.

Kathleen Clouse Strebel

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Zhihao Peter Qiu

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Reyes Candau-Chacon

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U.S. FOOD & DRUG ADMINISTRATION

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

Date: January 19, 2018
STN: 761091/0
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. (b) (4) Incheon, Republic of Korea
FEI: 3005241015
Product: Herzuma® (CT-P6)
Dosage: Lyophilized powder (420 mg/vial)
Indication: Treatment for adjuvant breast cancer, metastatic breast cancer, and metastatic gastric cancer
Action Date: May 30, 2018

Recommendation: The drug product section of the BLA, as amended, is recommended for approval from a microbiology product quality perspective.

Introduction
Celltrion Inc. has submitted a 351 (k) biological license application (BLA) for the approval of Herzuma ® (CT-P6). CT-P6 was developed as a biosimilar to the US-licensed Herceptin®. CT-P6 is a humanized immunoglobulin G1 (IgG1) monoclonal that is produced in a CHO cell line. The drug is packaged along with bacteriostatic water for injection (BWFI) to be used as a diluent.

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

Amendments Reviewed
- Sequence 0034 submitted 20 Feb 2018
- Sequence 0035 submitted 21 Feb 2018

Assessment
1.14 Labeling
Preparation for Administration
Reconstitution
Each vial of lyophilized Herzuma is to be reconstituted with 20 mL of bacteriostatic water for injection (BWFI) which contains 1.1% benzyl alcohol as a preservative to yield a solution containing 21 mg/mL of drug product. The DP can also be reconstituted with sterile water for injection (SWFI) with preservative for patients with hypersensitivity to benzyl alcohol. The reconstituted drug product can be stored at 2-8°C no more than 28 days. Drug product reconstituted with SWFI must be used immediately and any unused portion must be discarded.

Dilution
The reconstituted drug product is diluted by withdrawing a predetermined amount and adding it to an infusion bag containing 250 mL of 0.9% sodium chloride injection. The prepared solution for infusion can be stored for no more than 24 hours prior to use. The maximum infusion time is 90 minutes.

Reviewer comment: Storage times for reconstituted and diluted drug product are supported by microbial data presented later in this review.

P Drug Product

P.1 Description and Composition of the Drug Product

CT-P6 drug product (DP) is a lyophilized, sterile powder that is formulated for intravenous (IV) administration as a multi-dose vial. The drug product is reconstituted with 20 mL of BWFI to yield a dosage of 21 mg/mL. The components of the DP are listed below:

- CT-P6 (420 mg)
- α, α-Trehalose, Dihydrate (839.0 mg)
- L-Histidine HCl (9.5 mg)
- L-Histidine (6.1 mg)
- Polysorbate 20 (1.7 mg)

P.2 Pharmaceutical Development

P.2.4 Container Closure System

The drug product (DP) is filled into 50-mL (b) (4) glass vials with (b) (4) rubber stoppers and 20 mm flip-off seals. The vials are packaged in an outer carton box.

P.2.5 Microbiological Attributes

CT-P6 is a sterile product and therefore must be (b) (4) conform to sterility requirements. Media fill studies were completed to demonstrate sterile manufacturing at the Celltrion facility. In addition, container closure integrity was determined using the dye ingress and vacuum decay tests. Sterility tests were performed to confirm the CCIT for DP on stability.

Microbial Control

(b) (4)

Media fill studies were performed to confirm sterile manufacturing. The facility did the studies by filling vials with tryptic soy broth (TSB). The vials were incubated for 7 days at 20-25°C and an additional 7 days at 30-25°C. The vials were visually inspected and results show not microbial growth. Media fill studies are discussed in Section P.3.5.

P.2.5.1.4 Rabbit Pyrogen Test

Rabbit Pyrogen Test was completed per USP <151> using 3 lots of CT-P6 DP manufactured using the commercial process. The tests were complete in March 2017 in by (b) (4). Lots 16A3C02, 16A3C02, and 16A3C03 were used for the tests. During the study the baseline temperatures of the rabbits were determined before administering the samples. Rabbits were injected with 10 mL/kg of DP after which temperatures were recorded in 30-minute intervals. None of the rabbits displayed a temperature rise during the observation period. Therefore, the results met the acceptance criteria. Results are summarized in Table 3.2.P.2.5-2 which has been copied below.

Table 3.2.P.2.5-2: Results of Rabbit Pyrogen Test for CT-P6 Drug Product

Sample (Lot No.)	Animal No.	Baseline Temperature (°C)	Highest Temperature (°C)	Temperature Rise (°C)	Pass/Fail
16A3C01	48742	39.4	39.6	0.2	Pass
	48894	39.3	39.7	0.4	Pass
	48896	39.0	39.3	0.3	Pass
16A3C02	48898	38.9	39.3	0.4	Pass
	48899	39.3	39.5	0.2	Pass
	48900	39.3	39.2	0.0	Pass
16A3C03	48737	39.4	39.6	0.2	Pass
	48738	39.3	39.5	0.2	Pass
	48739	39.5	39.4	0.0	Pass

Note: A negative value is reported as zero (0.0) temperature rise.

Reviewer comment: Requirements of 21 CFR 610.13 (b) have been met.

P.2.5.1.5 Container Closure Integrity for CT-P6 Drug Product

As previously mentioned, container closure integrity was confirmed using both the dye penetration and vacuum decay tests.

The vacuum decay test is used as an in-process test during the capping process. During this test a total of 60 capped vials are taken from the start, middle, and end of the capping process (20 vials from each time point). The vacuum decay test is completed using a Bonfiglioli Vial Leak Tester. Vials are placed inside of the test chamber then vacuum was applied. The chamber is allowed to stabilize and the change in pressure was measured over time. "Dummy" vials made of stainless steel were used to verify the suitability of the leak tester. A passing result requires a change of pressure of less than 2.82 mbar. The test can detect a 5.21 µm breach. The test is described in more detail in section P.8.3.8.2.

The dye penetration test will be performed as part of the annual stability studies to confirm CCI. Vials are immersed in 0.1% methylene blue dye solution at 127 mmHg for 30 minutes. Atmospheric pressure is restored and the vials are left in the dye solution for an additional 30 minutes. The samples are removed from the solution and rinsed. The vials are compared to negative controls vials that had not been immersed in the dye solution. Positive control vials were prepared by breaching them with 34-gauge needles and immersing them in the dye solution. Test vials were compared to both positive and negative controls and samples passed if there was no indication of dye solution. Sensitivity of the assay was confirmed by showing that the test can detect breaches of 6.011 µm or larger for the 50-mL vials. These studies are described further in Section 3.2.P.8.3.8.2 in the BLA.

SATISFACTORY

P.3 Manufacture

P.3.1 Manufacturer (s)

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(b) (4)

CONCLUSION

I. The drug product section of the BLA as amended is recommended for approval from a microbiology product quality perspective.
II. CMC product specific information and data is reviewed by the OBP reviewer.
III. Inspectional issues will be discussed in a separate review.

Information Requested During the Review Cycle

1. Provide a description of the (b) (4) in section 3.2.P.5.2.

2. According to study reports (b) (4) .

3. Provide summary results from shipping validation studies in section 3.2.P.3.5.

4. Container closure integrity testing should be performed in lieu of sterility testing for stability samples every 12 months (annually) until expiry for drug product using a validated container closure integrity test. Describe a CCIT to be used on stability samples. The method should be validated for use on stability samples and the validation should demonstrate that the assay is sensitive enough to detect breaches that could allow microbial ingress (≤ 20 microns).

5. Sterile drug products should be manufactured (b) (4) Please comment.

Reyes Candau-Chacon

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Candace Gomez-Broughton

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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 761091
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: Herzuma
Dosage: 420 mg/vial (b) (4)
Indication: Adjuvant Breast Cancer, Metastatic Breast Cancer, Metastatic Gastric Cancer
Due Date: 1/8/2018

RECOMMENDATION: This submission is recommended for **Withhold** from a facility review perspective.
CR lauguage: 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 Herzuma drug substance (DS) and drug product (DP) at Celltrion, Inc. (FEI 3005241015). A post-approval for BLA 125544 (Inflectra) 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-licensing 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 Herzuma DS and DP manufacture, cell banking operations, and testing are presented below in **Table 1**.

Table 1: Proposed Sites for Herzuma DS and DP Manufacture, Cell Banking and Testing Operations

Site Name/Location	FEI/ Profile	Responsibilities	Facility Status
Celltrion, Inc. 23, Academy-ro (b) (4) Yeonsu-gu Incheon, S. Korea	3005241015 CBI/SVS	Manufacturing Herzuma of DS, DP, release and stability testing MCB and WCB Storage	Unacceptable due to OAI status
(b) (4)		Production of the MCB and WCB; Testing of the MCB and WCB	No Further Evaluation
		Testing of the MCB and WCB	No Further Evaluation
		Testing of the MCB, WCB, EPCB and CT-P6 unprocessed bulk	No Further Evaluation
		Sterility testing of EPCB	No Further Evaluation
		Testing of the EPCB and CT-P6 unprocessed bulk	No Further Evaluation
		MCB and WCB storage	No Further

[redacted] (b) (4)		Evaluation

Reviewer Comment 1: *The facilities for manufacture of DS and DP are adequately described.*

- **Current Prior Approval Inspection Decisions**
  - Celltrion, Inc. Incheon, Republic of Korea DS and DP Herzuma 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 schedule 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 Herzuma due to Celltrion' s current compliance status.*

3.2.S.2.2. Overview of Herzuma (CT-P6) DS Manufacturing Operations Conducted at Celltrion, Inc..

CT-P6 drug substance is produced in a CHO cell line [redacted] (b) (4)

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(b) (4)

CONCLUSION

Adequate descriptions were provided for the Herzuma DS and DP at the Celltrion facility but unable to verify by inspection. A post-approval for BLA 125544 (Inflectra) and GMP 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-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