

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**761081Orig1s000**

**PRODUCT QUALITY REVIEW(S)**

**Recommendation: Approval**

**BLA Number: 761081**  
**Review Number: Second round**  
**Review Date: January 11, 2019**

Drug Name/Dosage Form	Trazimera (trastuzumab-qyyp)/ for injection, Biosimilar to Herceptin
Strength/Potency	420 mg/vial
Route of Administration	Intravenous infusion
Rx/OTC dispensed	Rx
Indication	Treatment of HER2-overexpressing breast cancer, gastric cancer
Applicant/Sponsor	Pfizer, Inc.

The BLA was initially submitted on June 22, 2017. A Complete Response (CR) letter for product quality deficiencies was issued on April 20, 2018 (see OBP Executive Summary for initial BLA 761081 review cycle, dated 4/20/2018). On September 28, 2018, Pfizer submitted responses to address the deficiencies identified in the CR letter.

**Product Overview**

*Note: There is no change in this section from the Executive Summary filed on 4/20/2018 in Panorama.*

TRAZIMERA (trastuzumab-qyyp) is a proposed biosimilar to US-Herceptin (trastuzumab). It is a humanized IgG1k monoclonal antibody produced in CHO cells. Trastuzumab targets and binds human epidermal growth factor receptor 2 (HER2) to inhibit HER2 receptor dimerization and subsequent downstream signaling and to activate antibody-dependent cellular cytotoxicity (ADCC) activity through concomitant binding to Fcγ receptors on immune effector cells.

The PF-05280014 drug substance is produced in genetically engineered CHO cells. Trastuzumab-qyyp drug product, TRAZIMERA, is manufactured to the same concentration and presentation as U.S.-licensed Herceptin at 420 mg/vial; however, TRAZIMERA has a different formulation consisting of (b) (4) mM histidine, (b) (4) sucrose, (b) (4) polysorbate 20, pH 6.0. TRAZIMERA 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. TRAZIMERA is proposed as a treatment for HER2-overexpressing breast cancer and gastric cancer.

**Quality Review Team**

Discipline	Reviewer	Branch/Division
Drug Substance and Drug Product	Lymarie Maldonado-Baez	OPQ/OBP/DBRR I
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CMC RPBM	Anh-Thy Ly	OPQ/OPRO
Application Team Lead	Jennifer Swisher	OPQ/OBP/DBRR I

**Multidisciplinary Review Team:**

<b>Discipline</b>	<b>Reviewer</b>	<b>Office/Division</b>
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Clinical Pharmacology	Christy John/ Salaheldin Hamed (TL)	OTS/OCP/DCPV
CMC Statistics	Hui Zhang /Shenghui Tang (TL)	OTS/OB/DBV
Clinical Statistics	Yu-Yi Hsu/Meiyu Shen (TL)	OTS/OB/DBIV

**Names:**

- Proprietary Name: TRAZIMERA
- Non-Proprietary/USAN/INN: Trastuzumab-qyyp
- CAS Registry number: 180288-69-1
- Company/Laboratory code: PF-05280014
- OBP systematic name: MAB HUMANIZED (IGG1) ANTI P04626 (ERBB2\_HUMAN) [PF-05280014]
- Other Names: None

**Submissions Reviewed in BLA Resubmission Review Cycle:**

<b>Submission(s) Reviewed /sequence number</b>	<b>Document Date</b>
STN 761081/46	9/28/2018
STN 761081/47 (response to IR #1)	10/26/2018
STN 761081/49 (response to IR #2)	12/18/2018
STN 761081/52 (response to IR#3)	01/03/2018
STN 761081/54 (response to IR#4)	01/25/2019
STN 761081/56 (response to IR#5)	02/01/2019
STN 761081/57 (response to IR#6)	02/04/2019

**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 4/20/2018.*

**3. Consults:** None

## Executive Summary

### I. Recommendations:

#### A. Recommendation and Conclusion on Approvability:

The Office of Biotechnology Products, OPQ, CDER, recommends approval of STN 761081 for TRAZIMERA (trastuzumab-qyyp) manufactured by Pfizer, Inc. The data submitted in this application are adequate to support the conclusion that the manufacture of TRAZIMERA 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 original BLA submission was considered adequate to support the conclusion that the biological product, trastuzumab-qyyp, is highly similar to U.S.-licensed Herceptin.

#### B. Approval Action Letter Language:

- Manufacturing Location:
  - Drug Substance:
    - Manufacturing: (b) (4)
  - Drug Product:
    - Manufacturing, fill, labeling, and primary and secondary packaging: Pfizer, Inc., Puurs, Belgium
    - Secondary packaging and labeling: (b) (4)
  - Bacteriostatic Water for Injection (BWFI):
    - Manufacturing, fill, labeling, and primary and secondary packaging: Pfizer, Inc., Puurs, Belgium
    - Secondary packaging and labeling: (b) (4)
- Fill size and dosage forms:
  - Trastuzumab-qyyp: 420 mg/vial for injection, multi-dose vial
  - BWFI: 20 mL/vial
- Dating period:
  - Drug Product: 48 months when stored at 2-8°C; may be stored at 30°C for a single period up to 3 months, not to exceed the expiration date.
  - Drug Substance: (b) (4) months when stored at (b) (4) to (b) (4)°C in (b) (4). Drug substance (b) (4) the (b) (4) month expiry.
  - Packaged Component:
    - BWFI: 48 months when stored at 2-30°C
- Exempt from lot release:
  - Yes. TRAZIMERA is a specified product exempted according to 21 CFR 601.2a.

#### C. Benefit/Risk Considerations:

TRAZIMERA (trastuzumab-qyyp) is proposed as a biosimilar to U.S.-Herceptin (trastuzumab) for treatment of HER2 overexpressing breast cancer and gastric cancer. The analytical similarity assessment provided in the original submission supports the conclusion that PF-05280014 is highly similar to U.S.-licensed Herceptin. No new analytical similarity data were submitted in the September 28, 2018 BLA resubmission.

The deficiencies related to manufacturing and controls described in the Complete Response letter dated April 20, 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 manufacture, release, and stability testing are sufficient to assure a consistent and safe product.

The commercial manufacture of trastuzumab-qyyp drug substance at (b) (4) and of drug product at Pfizer Manufacturing (Puurs, Belgium) is recommended for approval by DIA, OPF, as a prelicense inspection of the drug substance manufacturing site was performed during the original submission review cycle ( (b) (4) ) and the facility was classified as VAI. The prelicense inspection was waived at the drug product manufacturing site based on facility profile evaluation.

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:**

1. To develop and implement a peptide mapping method for release and stability testing of PF-05280014 drug substance and drug product that can adequately assess levels of isomerized Asp102. The method final validation report and the release and stability acceptance criteria should be submitted as a Prior Approval Supplement.
2. Re-evaluate PF-05280014 drug substance lot release and stability specifications for potency by the Fc $\gamma$ RIIIa reporter gene assay and for the CEX-HPLC assay to quantify acidic, main, and basic species after 30 additional drug substance lots have been manufactured at the commercial scale. Submit the corresponding data, analysis, and statistical plans used to evaluate the specifications, and any proposed changes to the specifications as a Prior Approval Supplement.
3. Re-evaluate PF-05280014 drug product lot release and stability specifications for potency by the Fc $\gamma$ RIIIa reporter gene assay and for the CEX-HPLC assay to quantify acidic, main, and basic species after 30 additional drug product lots have been manufactured at the commercial scale. Submit the corresponding data, analysis, and statistical plans used to evaluate the specifications, and any proposed changes to the specifications as a Prior Approval Supplement.

## **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 OBP Executive Summary regarding the BLA original submission (dated 4/20/2018 in Panorama).

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

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

- Protein Content (CR Items #2 and 4e):** In order to ensure that the labeled amount of drug product can be withdrawn from the vial using the current fill strategy, the sponsor clarified the data as well as the drug product fill weight strategy. (b) (4)  
 provides assurance that the labeled amount of drug can be withdrawn from the vial. The sponsor has also added a test of "extractable content" to the drug product release specification with an acceptance criterion of "No less than 420 mg" to ensure that all drug product lots will meet the label claim.
- Potency and Purity (CR Items #4-5 and Additional Comment #1):** Assays with appropriate acceptance criteria were implemented to maintain adequate control of Fc $\gamma$ RIII binding and ADCC at drug substance and drug product release and during stability, levels of high mannose in drug substance, and extractable content and polysorbate 20 levels in drug product release and stability testing. As the HCP antisera used in the assay described in the original BLA submission did not supply adequate coverage, the sponsor has re-immunized with proteins from the host cell line and created a reagent that provides acceptable coverage of the spectrum of host cell proteins (b) (4) As agreed upon in a post-marketing commitment, a peptide mapping assay will be implemented to measure and control isomerization of Asp 102 on the heavy chain, as this modification in the CDR is known to affect potency. Additionally, specifications controlling drug substance and drug product color, allowable charge variation, and size-related variants were tightened to assure acceptable control of manufacture. Overall, these assays and their acceptance criteria, in combination with the rest of the drug substance and drug product release and stability specifications, assure adequate product quality throughout product manufacture and shelf-life and help maintain the product quality profile that established similarity.

## C. Drug Substance, TRAZIMERA, Quality Summary

### Drug Substance CQA Identification, Risk, and Lifecycle Knowledge Management

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

- Potency Assay (CR item #4b and additional item #4):** In addition to the cell-based bioassay that measures growth inhibition of HER2 expressing cancer cells and as mentioned

above, the sponsor has validated and implemented an FcyRIIIa-based Reporter Gene Assay (RGA), which will be used as a surrogate for monitoring ADCC activity of TRAZIMERA. The stability-indicating nature of this assay has been verified and it has been added to the control strategy for release and stability testing of the commercial drug substance and drug product.

- **Critical starting materials or intermediates (CR item #1):** A two-tiered cell banking system was implemented to ensure a continued source of PF-05280014. No animal derived materials were used in the manufacture of the cell banks. The cell lines were appropriately tested to ensure product safety from adventitious and endogenous agents. (b) (4)

[Redacted]

- **Manufacturing process (CR item #8):** (b) (4)

[Redacted]

**Additional item #2:** The previously proposed (b) (4) rejection limit (b) (4), which is inadequate to ensure process consistency and furthermore unsubstantiated by full-scale data or data from appropriate models. In response to the CR letter and following discussion and agreement at the Type 1 meeting with the Agency, the sponsor has updated the limit to (b) (4).

- **Dating period and storage conditions:** Drug substance is (b) (4). While the (b) (4) month dating period of the drug substance has not changed, updated information was provided to support that the drug substance may be (b) (4).

[Redacted]

#### **D. Drug Product, TRAZIMERA, Quality Summary:**

##### **Drug Product CQA Identification, Risk, and Lifecycle Management**

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

- **Method Transfer (CR Item #7):** Data were supplied to validate full and adequate method transfer of reduced and non-reduced CGE assays to control size variants in the drug product from

Pfizer St. Louis to Grange Castle, the site of drug product release and drug substance and drug product stability testing for this method.

- **Drug Product Shipping Validation (CR Item #3):** Additional simulation data and data from shipment of another similar lyophilized biological product were provided to support validation of commercial drug product shipping and provide assurance that PF-05280014 product quality will be maintained during shipping and distribution. Additional simulation studies were performed in order to demonstrate adequate protection of product quality during simultaneous combinations of worst-case conditions of mechanical stress and temperature, and additional data were provided from the passive pallet shippers demonstrating their ability to hold temperature in the actual shipping lanes during winter and summer conditions.
- **Microbial Control (CR Items #9-14):** Data were provided demonstrating successful (b) (4)  
[Redacted text block]
- **Dating period and storage conditions (CR Item #6):** Data were provided to support acceptable stability during storage at 30°C for a single period up to 3 months for unopened PF-05280014 or storage at 2-8°C for up to 28 days for PF-05280014 reconstituted in BWFI, within the long-term storage of 48 months at 2-8°C.

**E. Novel Approaches/ Precedents:**

None.

- F. Any Special Product Quality Labeling Recommendations:** Store unopened TRAZIMERA vials at 2-8°C. Unopened vials may be stored up to 30°C for a single period up to 3 months. Reconstitute each 420 mg vial with 20 mL BWFI for a multiple-dose solution. Store reconstituted drug product at 2-8°C for up to 28 days. Alternatively, the drug product can be reconstituted with sterile WFI without preservative but must be used immediately.

After dilution of reconstituted drug product in 0.9% sodium chloride injection, the infusion solution may be stored at 2-8°C for up to 24 hours prior to use. Do not freeze. The intravenous infusion must be completed over 30-90 minutes, according to the label.

- G. Establishment Information:** There was no change to this information in the resubmission or to conclusions regarding the suitability of the manufacturing establishments for commercial production and control of PF-05280014 from those communicated in the OBP Executive Summary regarding the BLA original submission (dated 4/20/2018 in Panorama).

**H. Facilities:** No approvability issues.

**I. Lifecycle Knowledge Management:**

**a. Drug Substance:**

**i. Protocols approved:**

1. Qualification of future WCB (3.2.S.2.3)
2. Requalification of the MCB and WCB (3.2.S.2.3)
3. At-scale Chromatography (b) (4) Study (3.2.S.2.5)
4. At-scale (b) (4) Study (3.2.S.2.5)
5. Requalification of the Primary and Working Reference Standards (3.2.S.5)
6. Ongoing Stability Studies (3.2.S.7.2)
7. Post-Approval Annual Stability Protocol (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



Jennifer  
Swisher

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Rachel  
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# **BLA STN 761081**

**Trazimera, a proposed biosimilar to US-Herceptin**

**Manufacturer  
Pfizer**

**OBP, Product Reviewer  
Lymarie Maldonado-Báez  
LC/TL Reviewer  
Jennifer Swisher**

## Summary of Quality Assessments

### I. Primary Reviewer Summary Recommendation

This memo contains the review of the information provided by Pfizer in response to the Complete Response letter issued on April 20, 2018. The BLA is not approved due to a number of product quality deficiencies related to PF-05280014 manufacture and control of that were resolved in Pfizer's resubmission of the BLA. It was determined during the original review cycle that PF-05280014 is highly similar to US-Herceptin notwithstanding minor differences in clinically inactive components. Pfizer resubmitted the BLA on September 28, 2018.

We recommend approval of BLA 761081 for trastuzumab-qyyp (Trazimera) sponsored by Pfizer, Inc. Trastuzumab-qyyp drug substance is manufactured by (b) (4). The data submitted in this application are adequate to support the conclusion that the manufacture of trastuzumab-qyyp is well controlled and leads to a product that is pure and potent. The product does not contain detectable endogenous and adventitious infections agents and is sufficient to meet the parameters recommended by FDA. The conditions used in manufacturing have been sufficiently validated and a consistent product has been manufactured from multiple production runs utilizing the intended commercial manufacturing process. It is recommended that this product be approved for human use under the conditions specified in the Prescribing Information.

We recommend an expiration dating period of (b) (4) months for trastuzumab-qyyp drug substance when stored at (b) (4)C to (b) (4)C. The drug substance (b) (4)

(b) (4) We recommend an expiration dating period of 48 months for trastuzumab-qyyp drug product when stored at 2-8°C. Unopened vials may be stored up to 30°C for a single period up to 3 months, and reconstituted PF-05280014 may be stored for up to 28 days at 2-8°C.

### II. List of Deficiencies to be Communicated

There are no deficiencies to be communicated to Pfizer.

### III. List of Post-Marketing Commitment

We recommend that the following post-marketing commitments will be communicated to Pfizer:

1. To develop and implement a peptide mapping method for release and stability testing of PF-05280014 drug substance and drug product that can adequately assess levels of isomerized Asp102. The method final validation report and the release and stability acceptance criteria should be submitted as a Prior Approval Supplement.

2. To re-evaluate PF-05280014 drug substance lot release and stability specifications for potency by the Fc $\gamma$ RIIIa reporter gene assay and for the CEX-HPLC assay to quantify acidic, main, and basic species after 30 additional drug substance lots have been manufactured at the commercial scale. Submit the corresponding data, analysis, and statistical plans used to evaluate the specifications, and any proposed changes to the specifications as a Prior Approval Supplement.
3. To re-evaluate PF-05280014 drug product lot release and stability specifications for potency by the Fc $\gamma$ RIIIa reporter gene assay and for the CEX-HPLC assay to quantify acidic, main, and basic species after 30 additional drug product lots have been manufactured at the commercial scale. Submit the corresponding data, analysis, and statistical plans used to evaluate the specifications, and any proposed changes to the specifications as a Prior Approval Supplement.

## Review

### 1.11.1. Product Quality Information

#### FDA Comment 1

**Reference is made to the information and data provided to the Agency concerning the stability of the PF-05280014 Working Cell Bank (WCB) on January 22, 2018 and February 9, 2018. Although the likely root causes for the instability have been identified and corrective actions were implemented in late 2017, the information and data do not support the suitability of the current WCB for commercial production.**

**Reference is also made to your response received on February 21, 2018 to Question 1 of the Agency's Information Request (IR) dated February 13, 2018 concerning the PF-05280014 Master Cell Bank (MCB). Because the MCB has undergone extensive transfers since its inception, the information and data provided by the February 21, 2018 response are insufficient to account for the potential impact on MCB stability from these transfers.**

**To support a well-controlled and consistent commercial production of PF-05280014, you will need to provide adequate data and information to confirm the stability of the MCB. Once the stability of the MCB is confirmed, you should qualify a new WCB or validate use of the MCB for commercial production.**

#### Response to Comment 1:

Reference is made to the sponsor's BDP Type 1 Briefing Document received on June 25th, 2018 to Pfizer's Proposal to the FDA Comment 1. The sponsor provided data to demonstrate the stability of the MCB and suitability for use in the preparation of the WCB (additional information that was included in response to IR dated March 15th, 2018). The sponsor concluded that the results of the viability studies confirmed that the stability of the MCB was not impacted by previous inventory transfers (documentation was provided but not reproduced here). The data included in the BDP Type 1 Briefing Document, as well as the data submitted in the IR response, were reviewed and recommendations were made to the sponsor based on the data provided. The Agency agreed that the data appear adequate to assess the stability of the MCB (refer to BDP Type 1 Meeting Minutes, July 20th, 2018).

The sponsor updated the WCB W38320 qualification protocol to include (b) (4)

[REDACTED]. Following the Agency's recommendation (refer to BPD Type I Meeting Minutes, July 20th, 2018), the sponsor adjusted the (b) (4) rejection limit to (b) (4). All these qualification elements were included in *Section 3.2.S.2.3 Control of Materials – Protocol for Preparation, Qualification, and Storage of Renewal Working Cell Banks*.

3.2.S.2.3 Control of Materials – Protocol for Preparation, Qualification, and Storage of Renewal Working Cell Banks

The process steps required for WCB preparation and the culture performance parameters and targets are described in the tables below.

**Table 3.2.S.2.3-1. Process Steps for Preparation of Renewal WCBs**

Process Step	Description
(b) (4)	

(b) (4)	
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## FDA COMMENT 2

References are made to your response received on September 22, 2017 to Comment 1 of the Agency's IR dated September 1, 2017, and your response received on February 16, 2018 to Comment 1a of the Agency's IR dated February 9, 2018. The Agency does not agree that the data from the Extractable Content experiment support that the lower limit of the fill weight range used during drug product (DP) manufacturing will result in vials filled with sufficient DP to consistently meet the label claim of 420 mg. The recovery data in the calculations used to support the filling range is based on the average recovery of (b) (4), and not the worst-case recovery of (b) (4). Based on the calculations, a DP vial filled at the lower limit of the fill weight range of (b) (4) recovery would be (b) (4) which would not meet the label claim of 420 mg. Tighten the lower fill weight rejection limit and provide data to support that DP vials filled at the lower fill weight limit can consistently deliver 420 mg of PF-05280014.

## Response to Comment 2

**Reviewer comment:** Regarding the fill weight control strategy, the Agency communicated to the sponsor in the BPD Type 1 Meeting Minutes issued by the Agency on 20 July 2018 that the approach in the initial BLA and the control fill limits of (b) (4) with a target fill weight of (b) (4) are acceptable. Therefore, the sponsor will not take further action concerning the lower fill rejection limit.

### **FDA COMMENT 3**

**The information and data provided to support the commercial DP shipping validation do not provide sufficient assurance that the quality of the DP is maintained during commercial shipping and distribution. We have the following comments:**

**a. The mechanical performance studies, which include an independent evaluation of vibration and physical shock, are not sufficiently representative of potential stresses induced during routine distribution conditions, where additional factors may contribute stress (e.g., temperature and pressure changes) and multiple stresses could occur concurrently. Therefore, these studies cannot on their own be used in lieu of performing real-time DP shipping validation studies. Provide shipping validation data for the DP from real-time shipping studies or from appropriate simulation studies that are sufficiently representative of the commercial shipping conditions. The data should include an assessment of product quality of pre- and post-shipping DP samples. Include a detailed description of how the study was performed and if performed using simulated studies, provide a justification for how the simulated studies are sufficiently representative of the commercial shipping conditions.**

#### **Response to Comment 3(a)**

To address the Agency's Comment 3(a), the sponsor designed a shipping qualification strategy addressing the impact of thermal and mechanical stresses of shipping on DP quality using PF-05280014 DP samples. The thermal stress studies were conducted under various payloads, in summer and winter conditions. The effect on product quality of these stress conditions was assessed through simulated transportation studies using simulated shipping conditions to evaluate container closure protection on DP quality and physical damage.

(b) (4)

**Reviewer comment:** *As recommended by the Agency, the sponsor conducted simulated shipping validation studies assessing the impact of hazardous stress shipping and distribution conditions on PF-05280014 DP quality. These studies were conducted using commercial DP samples individually packed*

*or co-packed with BWIF diluent. Additional studies using a surrogate lyophilized product were conducted to evaluate the ability of the passive shipper to maintain optimal storage temperature; these studies are reviewed as part of the response to comment 3(c).*

*The results of the simulated shipping stress product studies, which were conducted per ASTM D4169-14 distribution cycle (DC) 12, Assurance level II, showed that all tested samples met acceptance criteria, indicating no impact of the simulated shipping conditions on DP quality. Additionally, the results of the simulated transportation study confirmed that product quality was not compromised during shipment and container closure integrity was maintained. The transportation study reported that product cartons and cases suffered physical damage. However, these damages did not affect the usability of the PF-05280014, and DP quality attributes were not affected. Therefore, the results of the simulated studies demonstrate that expected shipping stress hazards do not compromise the quality of PF-05280014 DP; these results are deemed acceptable.*

**b. It is not appropriate to leverage stability data to support the allowable shipping temperature range of (b) (4), because DP is subjected to additional stresses during shipping, which could potentially impact product stability. To support shipment outside of the validated 2-8°C range, real-time shipping studies or sufficiently representative simulated studies, as described above, should be performed to support the allowable shipping temperature range.**

### **Response to Comment 3(b)**

The sponsor stated that the intended transit and storage temperature range is 2 to 8°C and that the shipping validation range of (b) (4) was established to allow for changes in temperature that may be experienced during handling-related procedures. The sponsor conducted a supplemental simulated transportation study to evaluate the impact of mechanical and thermal stresses during shipment on product quality and product packaging protection. The results of the study were reviewed and discussed as part of the sponsor's response to Comment 3(a).

**Reviewer comment:** *The results of the simulated transportation study demonstrated that the proposed shipping validation rate of (b) (4) does not compromise the quality of PF-05280014 DP under real-time shipping conditions. The pre- and post-shipment DP samples met the release acceptance criteria for all product quality attributes; this results support comparability of the products.*

**c. The ability of the passive pallet shipper to maintain the storage temperature of 2-8°C in the actual shipping lanes has not been demonstrated. Provide the shipping validation summary report of the passive pallet shipper in the actual shipping lanes during winter and summer conditions in the BLA resubmission.**

### **Response to Comment 3(c)**

The sponsor provided validation reports for the passive pallet shipper transporter during winter and summer conditions. (b) (4)

(b) (4)

(b) (4)

**Reviewer comment:** *The sponsor provided a validation summary report of the passive pallet shipper in representative shipping lanes instead of using actual shipping lanes. However, the performance qualification studies were representative of worst case scenarios under summer and winter conditions. Therefore, the use of the representative lanes to demonstrate the ability of the passive pallet shipper to maintain the storage temperature is considered adequate. The validation results support the capacity of the PF-05280014 passive pallet shipper to conduct shipping of the product at the appropriate temperature; this is acceptable.*

#### **FDA COMMENT 4**

**Implement the following specifications for the control of PF-05280014:**

**a. Antibody-dependent cell-mediated cytotoxicity (ADCC) is one of the potential mechanisms of action for trastuzumab. In your response received on February 05, 2018 to Comment 6c of the Agency's IR dated January 17, 2018, a cell-based FcγRIIIa reporter gene assay was provided as a surrogate assay to assess ADCC activity in the reference material qualifications. Implement this test, or another validated assay, with appropriate acceptance criterion to control for ADCC activity for DS and DP release and stability. The acceptance criteria should be based on product understanding, current process understanding, and clinical experience.**

#### **Response to Comment 4(a)**

The sponsor implemented the cell-based FcγRIIIa reporter gene assay (FcγRIIIa RGA) presented to the Agency on the IR dated January 17, 2018, as the assay to determine the ADCC activity for PF-05280014 DS and DP release and stability. The ADCC test results for relative potency (%) for eleven DS and thirteen DP lots, and the calculated lower and upper tolerance bounds are presented in the following tables.

**Table 1. PF-05280014 Drug Substance ADCC Test Results**

<b>Batch Number</b>	<b>ADCC Activity (% Relative Potency)</b>
11P122L601	103
11P122L001	107
D11L122E003	80
88200	96
88201	83
88000	84
88001	102
88002	119
88003	103
88005	106
88006	102
<b>Lower Tolerance Bound [95/99]</b>	<b>47</b>
<b>Upper Tolerance Bound [95/99]</b>	<b>150</b>
<b>Lower 3SD</b>	<b>63</b>
<b>Upper 3SD</b>	<b>134</b>

**Table 3. PF-05280014 Drug Product ADCC Test Results**

<b>Lot Number</b>	<b>Presentation (mg PF-05280014 protein per vial)</b>	<b>ADCC Activity (% Relative Potency)</b>
Z00515	150	89
Z02359	150	98
Z04072	150	105
A08735	150	121
L25296	150	112
M04478	150	93
M10096	150	114
Z00514	420	96
Z03694	420	104
Z03695	420	84
M55891	420	99
M52674	420	88
M55890	420	90
<b>Lower Tolerance Bound [95/99]</b>	<b>54</b>	
<b>Upper Tolerance Bound [95/99]</b>	<b>145</b>	
<b>Lower 3SD</b>	<b>66</b>	
<b>Upper 3SD</b>	<b>133</b>	

Additionally, the sponsor conducted ADCC activity tests on stability retain samples: three DS batches stored at (b) (4) and three DP lots stored at 5 °C ± 3°C for 69 or 74 months (see results in the table below).

**Table 2. PF-05280014 Stability Retain Sample Test Results, ADCC Activity**

Quality Attribute	ADCC Activity (%)		
	88000	88002	88001
	79 M, -20 °C	79 M, -20 °C	79 M, -40 °C
ADCC Activity	82	93	85

**Table 4. PF-05280014 Drug Product Stability Retain Sample Test Results, ADCC Activity**

Quality Attribute	ADCC Activity at 5 °C ± 3 °C (%)		
	Z03694	Z03695	Z00514
	69 M	69 M	74 M
ADCC Activity	117	101	107

The sponsor is proposing to set the commercial acceptance criterion for ADCC activity of PF-05280014 DS and DP at release and stability to be (b) (4) which was the range established during product development.

A validation report of the cell-based FcγRIIIa RGA test method was provided in the submission. The method was co-validated at the Pfizer sites in Andover, MA and Grange Castle, Ireland and was transferred to (b) (4)

**Reviewer comment:** The results of the ADCC validation studies were reviewed here, including the lower and tolerance calculated limits for PF-05280014 DS and DP release testing. The ADCC activity acceptance criterion proposed by the sponsor (b) (4) is tighter than the calculated tolerance bound ranges for DS and DP, which are similar and range from 47-133 %. However, the proposed ADCC activity acceptance criterion of (b) (4) relative potency is wider than the range obtain for DP samples on stability (80 – 121%). However, given the variability of the assay (CV = 12%), this proposed specification is acceptable at this time, as it more likely reflects method rather than manufacturing variability. A proposed PMC to re-assess the acceptance criteria for this assay for DS and DP release and stability will be sent to the sponsor. In addition, the sponsor was requested to update the protocol to qualify new WCBs with tighter acceptance criteria (see review of response to Complete Response Comment 1, above), as more stringent criteria are required to ensure consistent product quality over time once new WCBs are implemented. The sponsor agreed to and incorporated these updates.

The validation results for the cell-based FcγRIIIa RGA test method demonstrated that the method is deemed validated, that is suitable for its intended use and was successfully transferred to the Grange Castle, Ireland, and (b) (4)

It was noted that the sponsor did not update several sections of the BLA to reflect the commitment to implement the FcγRIIIa reporter gene assay in release and stability testing. An IR was sent to the sponsor on January 18<sup>th</sup>, 2019 requesting the update of BLA Sections 3.2.S.4.1, 3.2.P.5.1, 3.2.S.7 and 3.2.P.8, Section 3.2.S.4.2 to include the description of the method and to include in Section 3.2.S.4.5 the justification of the proposed specification. The sponsor updated the BLA Sections mentioned above with the requested information. The description of the FcγRIIIa RGA method and the justification of the proposed specification were previously reviewed as part of method similarity study submitted by the sponsor during the review of the original BLA submission. Therefore, the method is not new to the BLA and will not be reviewed in this document.

**b. As noted in the Agency’s IR on November 7, 2017, isomerization of Asp102 (isoAsp102) is shown to impact the potency of trastuzumab. The isoAsp102 is enriched in the B0 peak in CEX-HPLC and the B0 peak of PF-05280114 increases under mild thermal stressed conditions (Section 3.2.S.3.1); therefore, a control strategy for isoAsp102 should be in place to ensure product safety, quality and potency. Given that your current potency assay is not sensitive enough to detect changes in isoAsp102 and it is not appropriate to control isoAsp102 using CEX-HPLC (Refer to IR dated January 4, 2018), an assay should be developed and implemented to control for the level of isoAsp102 for DS and DP release and stability, with appropriate acceptance criterion.**

**Response to Comment 4(b)**

The current control strategy for Basic Species includes (b) (4)

(described in sections *3.2.S.2.6 Manufacturing Process Development – Control Strategy* and *3.2.P.2.3 Manufacturing Process Development– Control Strategy*). The sponsor conducted three studies to demonstrate the capability of the CEX-HPLC method to control isoAsp102 levels. The studies compared the basic species levels detected by CEX-HPLC to isoAsp102 levels identified by LC/MS/MS peptide mapping. The overall design and results of the studies are summarized in the following table.

**Table 6. Studies to Assess Capability of CEX-HPLC to Detect Changes in IsoAsp102 Level**

Study	Description	Goal	Summary of Results
1- Mild thermal stress, multiple lots	Multiple reconstituted DP incubated at 30 °C for 1 month	Evaluate ability of CEX-HPLC to detect changes in isoAsp102 level upon mild thermal stress	For 6 samples (4 PF-05280014 and 2 licensed products), IsoAsp102 increase upon 30°C 1 mo detected by both CEX-HPLC and LC/MS/MS Peptide Mapping at similar magnitudes
2-Time course of mild thermal stress	A reconstituted DP incubated at 30 °C for 1,2, 3, 4 wks	Represents effects of brief exposures to higher temperatures that are possible during normal manufacture, shipping and handling	Trend and magnitude of increase in isoAsp102 detected by CEX-HPLC are comparable to that detected by LC/MS/MS Peptide Mapping, including small changes at earlier time points
3-Spike recovery	A sample with elevated isoAsp102 (induced by mild thermal stress) was spiked into a representative reconstituted DP sample at varying levels	Direct evaluation of the sensitivity of Basic Species by CEX-HPLC to small changes in isoAsp102	Basic Species by CEX-HPLC is capable of detecting 1-2% changes in isoAsp102 levels in multiple tests with different columns

Study 1 was designed to using the same conditions that were used to determine the impact of isoAsp102 on potency, which is described in *Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics*. The objective of this study was to demonstrate the ability of CEX-HPLC to detect changes in isoASP102 levels induced by mild thermal stress (30°C). The results of six different lots showed that increases in basic species levels under mild thermal conditions can be detected by CEX-

HPLC. Additional studies using LC/MS/MS-peptide mapping confirmed the increase in basic species levels correlate with increase in isoASP102 levels. The results of the study and representative CEX-HPLC chromatograms of unstressed and stressed samples were provided in the submission and reviewed here.

In the second study, the sponsor evaluated the sensitivity of CEX-HPLC in detecting thermally induced changes in the levels of isoASP102 that could be caused by brief exposures to high temperatures (after the DP has been reconstituted). A representative DP was reconstituted and exposed to 30°C for a month, followed by the evaluation of basic species levels and isoASP102 levels by CEX-HPLC and LC/MS/MS, respectively. The results of the one-month assessment are summarized in the table below.

**Table 8. Increasing IsoAsp102 and Basic Species Over One Month**

Time (days)	% Basic Species (CEX-HPLC)	% IsoAsp102 (LC/MS/MS Peptide Mapping)
Control at 5 °C for 31 days	18.9	4.9
0	18.7	4.2
3	20.4	5.7
7	22.2	7.3
14	25.3	11.2
21	27.5	14.6
31	30.0	18.8

The spike recovery studies focused on the ability of CEX-HPLC to detect minor changes in the levels of iso Asp102. A sample with elevated iso ASP102 levels was spiked at different levels into a representative reconstituted sample of DP. Tests were conducted using two different HPLC columns and two analysts, and results were compared to LC/MS/MS-peptide mapping results for confirmation. The results of the study showed that CEX-HPLC could detect small increases in iso Asp102 levels, as low as 1-2% difference (see table below).

**Table 9. Levels of Basic Species in Samples Containing Varying Levels of IsoAsp102**

Sample Level #	Predicted IsoAsp102 %	LC/MS/MS Peptide Mapping	CEX-HPLC, Column 1, Analyst 1			CEX-HPLC, Column 2, Analyst 2		
		IsoAsp102 (%)	% Basic, Test 1	% Basic, Test 2	% Basic, Test 3	% Basic, Test 4	% Basic, Test 5	% Basic, Test 6
1	3.9	3.9	18.8	18.7	18.6	18.5	18.6	18.5
2	5.6	5.5	19.9	19.7	19.8	19.6	19.6	19.5
3	7.1	6.8	20.9	20.8	20.6	20.5	20.6	20.5
4	8.7	8.4	21.8	21.7	21.6	21.6	21.5	21.6
5	10.2	9.9	22.7	22.8	22.8	22.6	22.4	22.4
6	11.7	11.6	23.8	23.7	23.6	23.4	23.6	23.4
7	13.3	13.3	24.9	24.8	24.7	24.7	24.6	24.5
8	14.8	14.9	25.9	26.0	25.8	25.6	25.6	25.5
9	17.9	18.1	28.1	28.0	27.9	27.7	27.7	27.7
10	20.9	21.2	29.9	30.3	29.9	30.0	29.9	29.8
11	22.8	22.8	31.5	31.6	31.4	31.2	31.1	31.1

**Table 10. Comparison of Change Detected<sup>a</sup> in Basic Species by CEX-HPLC with Change Detected in IsoAsp102 Levels by Orthogonal LC/MS/MS Peptide Mapping**

Sample Level #	LC/MS/MS Peptide Mapping (Change from Previous Level)		CEX-HPLC, Column 1, Analyst 1 (Change from Previous Level)			CEX-HPLC, Column 2, Analyst 2 (Change from Previous Level)		
	Predicted IsoAsp102 (% Change)	IsoAsp102 (% Change)	Basic Species (% Change), Test 1	Basic Species (% Change), Test 2	Basic Species (% Change), Test 3	Basic Species (% Change), Test 4	Basic Species (% Change), Test 5	Basic Species (% Change), Test 6
1	NA	NA	NA	NA	NA	NA	NA	NA
2	1.7	1.6	1.1	1.0	1.2	1.1	1.0	1.0
3	1.5	1.3	1.0	1.1	0.9	0.9	0.9	1.0
4	1.6	1.6	0.8	0.9	1.0	1.1	0.9	1.1
5	1.5	1.5	0.9	1.1	1.1	0.9	1.0	0.8
6	1.5	1.7	1.1	1.0	0.8	0.9	1.1	0.9
7	1.6	1.7	1.2	1.0	1.1	1.2	1.0	1.2
8	1.5	1.6	1.0	1.2	1.1	1.0	1.1	0.9
9	3.1	3.2	2.2	2.0	2.1	2.0	2.1	2.2
10	3.0	3.1	1.8	2.3	2.0	2.3	2.2	2.2
11	1.9	1.6	1.6	1.3	1.5	1.3	1.2	1.3

a. To demonstrate capability to detect small changes, % iso Asp or % Basic Species experimental value is compared to the corresponding experimental value of the previous sample level. For example, within a given test the experimental value from Sample Level 1 is subtracted from the experimental value from the same test for Sample Level 2 and reported as “Change from Previous Level” for Sample Level 2.

To further support the use of CEX-HPLC as a surrogate for monitoring isoAsp102, the sponsor compared the levels of isoAsp102 in PF-05280014 and in licensed product samples to show that the levels are consistently low and that isoAsp102 levels do not represent a concern for safety or efficacy of the DP. In summary, the results of LC/MS/MS-peptide mapping analysis of 11 lots of PF-05280014 DS and DP, and 6 lots each of trastuzumab-US and trastuzumab-EU, revealed that the levels of isoAsp102 in all these lots were similar and consistently low (3.3-4.9% for PF-05280014 materials, 5.0-5.4% for trastuzumab-US and trastuzumab-EU; data were provided by the sponsor but not reproduced here).

**Review comment:** During the BPD Type 1 Meeting (minutes issued by the Agency on July 20<sup>th</sup>, 2018), the Agency stated: “it may be possible to control iso Asp102 levels by CEX-HPLC; however, sufficient data should be provided in the resubmission to demonstrate the CEX-HPLC method is capable of detecting small changes in iso Asp102 levels under conditions that the product may encounter during typical manufacturing and storage. Based on the results of the additional analyses, if a new method is to be implemented to control iso Asp102 levels, the new method may be implemented as part of a post-marketing commitment”. In response, the sponsor proposed to provide additional information in the resubmission to clarify and justify the control strategy for iso Asp102.

The sponsor provided the results of three independent studies assessing the capability of CEX-HPLC to detect changes in iso Asp102 levels under stress conditions that could stimulate an increase in iso Asp102 levels. The analysis showed that changes in basic species levels detected by CEX-HPLC are comparable in magnitude to changes in iso Asp102 levels reported by LC/MS/MS under mild thermal stressed. The time course study evaluating the impact of mild thermal stress (over one month of

*storage at 30°C) demonstrated that both methods were sensitive enough to detect small changes with similar magnitude at the earlier time points. Therefore, the sponsor argues that the data presented here support the CEX-HPLC determination of Basic Species content as a surrogate for monitoring iso Asp102. Although, LC/MS/MS peptide-mapping and CEX-HPLC can successfully detect parallel changes in iso Asp102 and Basic Species levels, respectively, the contribution of iso Asp102 to the % Basic Species cannot be extrapolated from CEX-HPLC analysis alone, unless a direct correlation is established between levels of Basic Species and isoAsp102 content. However, the trend and the magnitude of Basic Species changes were similar to that of isoAs102 levels reported by the LC/MS/MS-peptide mapping method.*

*The comparison between commercial trastuzumab DP and PF-05280014 DP demonstrated that both drug products exhibit similar iso Asp102 levels. It was also observed that the levels were consistently low independent of the age of the product. Therefore, based on this evidence the sponsor argues that the levels of iso Asp102 do not represent a concern for the safety and efficacy of PF-05280014 DP. However, this observation does not override the requirement of implementing a strategy to control and monitor iso Asp102 levels during the life cycle of the product to prevent any impact on product safety due to exposure to unexpected stress conditions during handling, shipping, and storage.*

*The sponsor insisted on continuing use of CEX-HPLC as the assay to ensure adequate control of isomerized Asp102 in DP. We do not agree with the sponsor because CEX-HPLC is not sufficiently sensitive to control for this critical quality attribute (iso Asp102) due to the number and variability of other modifications that can result in an increase in basic species. Therefore, an IR was sent to the sponsor on December 21<sup>st</sup>, 2018, recommending the implementation of the LC/MS/MS peptide-mapping assay that is currently proposed for characterization for use in control of iso Asp102 in DS and DP release and stability, as it appears to represent the sponsor's only suitable assay for this purpose. On January 3<sup>d</sup>, 2018 the Agency received the IR response, stating that the sponsor agrees to implement the assay as a PMC; this is acceptable. A PMC agreement will be communicated to the sponsor.*

**c. As part of the Agency's current considerations regarding the control of effector function, it is understood from publicly available literature that high mannose species contribute to the total level of afucosylated variants. Given the impact of afucosylated variants on potency, we recommend that the total level of high mannose be included as part of the control strategy for the PF-08250114 DS. Implement an appropriate control for high mannose into the DS release specification. The acceptance criteria should be based on the current process understanding and clinical experience.**

#### **Response to Comment 4(c)**

The sponsor accepted the Agency's recommendation and agreed to implement release acceptance criterion for the Man5 species of  $\leq$  (b) (4) for PF-05280014 drug substance. The levels of Man5 in PF-05280014 DS are assessed by hydrophilic interaction liquid chromatography (HILIC) for every batch of DS at release. The release test results for Man5 for eleven DS lots and the calculated upper tolerance bound are presented in the following table.

**Table 13. PF-05280014 Release Test Results, Man5**

Batch Number	Man5 (%)
11P122L601	0.5
11P122L001	0.3
D11L122E003	0.7
88200	0.5
88201	0.4
88000	0.6
88001	0.7
88002	0.5
88003	0.8
88005	0.9
88006	0.9
<b>Upper Tolerance Bound [95/99]</b>	(b) (4)

The release test results for Man5 range from 0.3 to 0.9%. The upper tolerance bound (95/99) was calculated taking into consideration the process variability (batch-to-batch) and the analytical variability (within-batch). The sponsor has implemented the commercial acceptance criterion for Man5 at release of  $\leq$  (b) (4) based on the DS batch release data, including clinical batch results.

**Reviewer comment:** The sponsor implemented a control strategy for high mannose into the control strategy for the PF-08250114 DS as requested by the Agency. The justification for the acceptance criterion for the release specification for Man5 for PF-05280014 drug substance is deemed acceptable.

**d. Polysorbate 20 is a critical excipient that can impact product quality and stability. Add testing and quantitative acceptance criteria for Polysorbate 20 to the PF-05280014 DP release specification.**

**Response to Comment 4(d)**

The sponsor proposed to set the release acceptance criterion of (b) (4) mg/mL for Polysorbate 20 to the PF-05280014 DP commercial release specification. The acceptance limit was determined based on formulation robustness data, the control strategy implemented during excipient preparation, and stability data. Polysorbate 20 is classified as a non-critical quality attribute for PF-05280014 DS and DP as determined by the results of the formulation robustness studies presented and reviewed in the original BLA-submission. Additionally, Polysorbate 20 was assessed as not relevant to the similarity as the formulation of PF-05280014 differs from the licensed product, making it a product-specific quality attribute.

Seven 150 mg and six 420 mg PF-05280014 DP lots were assessed for Polysorbate 20 levels (results presented in the table below). The release test results showed that the levels of Polysorbate 20 at release ranged from 0.09 to 0.10 mg/mL and met the proposed commercial DP release acceptance criterion of (b) (4) mg/mL.

**Table 15. PF-05280014 Test Results, Polysorbate 20**

Lot Number	Presentation (mg PF-05280014 protein per vial)	PS20 (mg/mL)
Z00515	150	0.10
Z02359	150	0.09
Z04072	150	0.09
A08735	150	0.09
L25296	150	0.09
M04478	150	0.09
M10096	150	0.09
Z00514	420	0.09
Z03694	420	0.09
Z03695	420	0.09
M55891	420	0.09
M52674	420	0.09
M55890	420	0.09

**Reviewer comment:** *The studies conducted to establish the control strategy for Polysorbate 20, the commercial release acceptance criterion, and the criticality of Polysorbate are acceptable. The release testing results and the formulation robustness studies support the Polysorbate 20 limit proposed for PF-05280014 DP. The acceptance criterion for Polysorbate 20 release specification is acceptable.*

**e. Add the test of “extractable content” to the DP release specification with the acceptance criterion of “No less than 420 mg” to ensure that DP lots will meet the label claim.**

**Response to Comment 4(e)**

In the BDP Type 1 Briefing Document (June 25<sup>th</sup>, 2018) the sponsor provided a summary of the experimental work that was conducted to clarify their position on the implementation of an extractable content DP release specification with acceptance criterion of “no less than 420 mg”. It was stated that Pfizer has a robust control strategy to ensure that the label claim content of 420 mg will be met. In brief, the control strategy includes (b) (4)

[Redacted]

**Reviewer comment:** *Regarding adding the test of “extractable content” to the DP release specification and acceptance criterion of “no less than 420 mg”, the Agency communicated to the sponsor (refer to the BDP Type 1 Meeting Minutes issued by the Agency on July 20th, 2018), that based on the reviewed information provided in the BDP Type 1 Briefing Document (June 25th, 2018)*

*the Agency agreed that the extractable volume is not needed at release of the PF-05280014 DP. Therefore, the sponsor will not take further action concerning the implementation of an extractable content specification for PF-05280014 DP. The data showed that the label claim for protein content will be met.*

**FDA COMMENT 5**

**The justifications for specifications provided in Sections 3.2.S.4.5 and 3.2.P.5.6 are not sufficient to support some of the release and end of shelf life acceptance criteria for the PF-05280014 DS and DP. The acceptance criteria for release and end of shelf life should be sufficiently narrow to allow for adequate control of DS and subsequent DP and should be based on your clinical and manufacturing experience. For some product quality attributes that change during storage, it is not appropriate to have the same acceptance criteria for DS and DP release and end of shelf life. The specifications should be set such that materials released at the limit of the acceptance criteria will not result in out of specification results during storage. For some attributes, the DS release and/or end of shelf life specifications should be tighter than those for DP release and/or end of shelf, to avoid DP lots going out of specification. Based on these concepts, the following acceptance criteria should be tightened:**

- a. Appearance (Color) for DS release and end of shelf life**
- b. Acidic and Basic species by CEX-HPLC for DS and DP release and end of shelf life**

**Response to Comment 5(a)**

Appearance (Color) for DS release and end of shelf life

The sponsor is proposing a commercial DS acceptance criterion for appearance (coloration) to be “no more intensely colored than level (b) (4) of the brownish-yellow color standard” for both release and stability. The proposed specification is based on the appearance (coloration) release test results previously submitted in the original BLA in *Section 3.2.S.4.5 Justification of Specification*. The release test results ranged from ≤ level 6 to ≤ level 4 intensity of the color standard used for testing. During stability testing for DS stored at (b) (4) the upper limit of coloration for the PF-05280014 DS lots was either equal or not more intensely colored than level 5. Because no change in coloration was observed on stability, the sponsor stated that a separate acceptance criterion for coloration on stability is not necessary. The tightened acceptance criterion for appearance (color) is indicated in the following table.

**Table 1. PF-05280014 Drug Substance Commercial Release and Stability Acceptance Criterion for Appearance (Coloration)**

<b>Quality Attribute</b>	<b>Original Acceptance Criteria</b>	<b>Tightened Acceptance Criterion</b>
Appearance (Coloration)	Not more intensely colored than level (b) (4) of the brownish-yellow color standard	Not more intensely colored than level (b) (4) of the brownish-yellow color standard

**Reviewer comment:** *The release results support newly proposed acceptance criterion for appearance (coloration); this is deemed acceptable. Based on the release and stability data provided in the submission support, the implementation of the same acceptance criterion for release and stability is considered adequate. The Section 3.2.S.4.1 Specification has been updated to include the new specification.*

**Response to Comment 5(b)**

Acidic and Basic species by CEX-HPLC for DS and DP release and end of shelf life

Based on the results of stability testing for drug substance acidic, main, and basic species presented in the original BLA submission, the sponsor proposed to use the same acceptance criteria for stability and release specifications. The updated DS commercial acceptance criteria for charge heterogeneity determined by CEX-HPLC are summarized below.

**Drug Substance**

Acidic Species: The sponsor proposes to maintain the current PF-05280014 DS acceptance criterion for acidic species ( (b) (4) because the range between the lower and upper tolerance bounds and the specification range are comparable.

Main Species: The sponsor proposes to tighten the commercial PF-05280014 DS acceptance criterion for main species to (b) (4) based on the calculated lower tolerance bound limit for the (b) (4) storage conditions (b) (4) respectively).

Basic Species: The calculated lower tolerance bound for the (b) (4) storage conditions (b) (4) respectively) and the calculated upper tolerance bounds for both (b) (4) storage conditions (b) (4) respectively) for the combined release and stability data. Based on this information the sponsor proposes to tighten the commercial DS acceptance criteria for basic species to (b) (4)

The proposed commercial acceptance criteria for charge heterogeneity for PF-05280014 drug substance at release and stability are presented are listed in the following table.

**Table 2. Commercial Drug Substance Release and Stability Acceptance Criteria for Charge Heterogeneity (Acidic, Main, and Basic Species)**

Quality Attribute		Original Acceptance Criteria	Tightened Acceptance Criteria
Charge Heterogeneity	Acidic Species	(b) (4)	Remains unchanged
	Main Species		(b) (4)
	Basic Species		



calculated upper tolerance bound (specifically for acidic and basic species). The values reported for acidic species in DP lot release and stability were not higher than (b) (4) which is approximately (b) (4) lower than the set upper limit (b) (4)

Overall, the data presented in the submission is not sufficient to support the proposed DS and DP release and stability specification for acidic and basic species by CEX-HPLC. The proposed acceptance criteria for main and basic species were tightened considering statistical and experimental data, however, the proposed values do not reflect the sponsor’s clinical and commercial manufacturing experience. Therefore, an IR was communicated to the sponsor on January 18<sup>th</sup>, 2019, requesting a reanalysis of the DS and DP release and stability data in order to implement charged variants acceptance criteria that reflects the sponsor’s manufacturing experience.

The sponsor agreed to tighten the acceptance criteria for DS and DP release and stability for main, acidic and basic species and conducted a reanalysis of the data without including development lots 11P122L601, 11P122L001, and D11L122E003 (IR response received on January 24<sup>th</sup>, 2018). The sponsor proposed new acceptance criteria for all three specifications taking into consideration the statistical reanalysis of the current data. The revised acceptance criteria for DS and DP charge heterogeneity is presented in Table 4. While the revised acceptance criteria represent a tighter range for each one of the species based on statistical analysis, it is not fully reflective of the sponsor’s clinical and commercial manufacturing experience. However, given the method and manufacturing variability in development thus far, the revised acceptance criteria are adequate for control of PF-05280014. However, we will request a re-evaluation of the acceptance criteria for this method following the manufacture of 30 additional lots each of DS and DP as a PMC from the sponsor.

**Table 4. Commercial Drug Product Release and Stability Acceptance Criteria for Charge Heterogeneity**

Quality Attribute		Original Acceptance Criteria	Tightened Acceptance Criteria
Charge Heterogeneity	Acidic Species		(b) (4)
	Main Species		
	Basic Species		

**FDA COMMENT 6**

**The additional storages for PF-05280014, as specified in the proposed label, include storage at 30°C for a single period up to 3 months for unopened PF-05280014 or storage at 2-8°C for up to 28 days for PF-05280014 reconstituted in BWFI, within the long-term storage of 48 months at 2-8°C. The information and data provided in the submission are insufficient to support these additional storage conditions. For example, product quality attributes including ADCC (or FcγRIIIa binding) and the levels of iso Asp102 were not assessed to demonstrate that there is no impact on these attributes during these storage conditions. Provide additional information and data to support the proposed additional storage conditions.**

## Response to Comment 6

The sponsor assessed the quality of aged DP vials that were stored for a total of 69 months since the date of manufacture, consisting of 6 months of storage at 30°C followed by 63 months of storage at 2-8°C (more information about the samples was provided on *Section 3.2.P.8.1 Stability Summary and Conclusion (420 mg)*). The samples were removed from storage, reconstituted with BWFI diluent and held for an additional 30 days at 2-8°C. After the 30-day post-reconstitution hold, the samples were tested by SE-HPLC, CEX-HPLC, CGE (reducing and non-reducing), appearance, pH, and cell-based assay. The results of the study are presented in the tables below:

**Table 1. Stability of PF-05280014 Drug Product After 6 Months of Storage at 30 °C, followed by 63 Months of Storage at 2-8 °C, Followed by 30 Days of Post- Reconstitution Storage at 2-8 °C**

Analytical Method	Quality Attribute	Commercial Acceptance Criteria	T0 (Release) Result	Stability Result
SE-HPLC	Monomer (%)	(b) (4)	99.3	98.7
	HMMS (%)		0.4	1.0
CEX-HPLC	Acidic Species (%)		25.2	24.3
	Main Species (%)		58.8	55.9
	Basic Species (%)		16.0	19.8
pH	pH		5.8	5.9
CGE (reducing)	Heavy + Light Chain (%)		99.7	99.7
	Fragment (%)		NMT 0.3	0.3
CGE (non-reducing)	Intact IgG (%)		96.3	94.4

Analytical Method	Quality Attribute	Commercial Acceptance Criteria	T0 (Release) Result	Stability Result
Appearance	Coloration	(b) (4)	≤ Y7	≤ BY7
	Clarity (NTU)		3	3
	Visible Particulates		EFVP	EFVP
Cell-based Assay	Relative Potency (%)		87	103

EFVP = essentially free of visible particulates

**Table 2. Characterization of PF-05280014 Drug Product After 6 Months of Storage at 30 °C, Followed by 63 Months of Storage at 2-8 °C, Followed by 30 Days of Post-Reconstitution Storage at 2-8 °C**

Analytical Method	Quality Attribute	Commercial Acceptance Criteria	Representative DP Result Range (2-8 °C)	Stability Result
FcγRIIIa RGA	ADCC (%)	(b) (4)	84-121 <sup>a</sup>	105
LC/MS/MS Peptide Mapping	IsoAsp102 (%)		3.5-4.4 <sup>b</sup>	4.9

a. Result from DP sample to support ADCC specification proposal. See Response to Product Quality Comment 4a.

b. Response to Product Quality Comment 4b, Table 11.

The stability results for ADCC and iso Asp102 levels were comparable to the representative DP results. However, the iso Asp levels observed in the stability sample were slightly higher than in the levels observed for the representative DP (stored at 2-8 °C; (4.9 % compared to 3.5-4.4 %). The sponsor argues that the results are acceptable because the observed value is at a low level and in trend considering the additional storage at 30 °C, and the level is comparable to those observed in licensed product (5.0-5.4 %).

**Reviewer comment:** *The sponsor provided additional data demonstrating that most quality attributes of the DP are not affected during the newly proposed storage conditions. While the levels of iso Asp102 in the stability sample were slightly higher than the reference product, the levels are still acceptable. LC/MS/MS method is being implemented as the release and stability control strategy for iso Asp102 (see IR response dated January 3<sup>rd</sup>, 2019). This is acceptable.*

#### **FDA COMMENT 7**

**Method transfer data were provided for the reduced and non-reduced CGE assays from Pfizer St. Louis to Grange Castle; however, no information and data (e.g., from method validation or method transfer) were provided to support that these assays are validated at the Pfizer St. Louis site. Because Grange Castle is the only DP release testing site for these assays (Section 3.2.P.3.1), the method validation for these assays used for commercial DP lot release and stability testing is considered incomplete. Provide information and data to support the method validation of these assays at Pfizer St. Louis.**

**Reviewer comment:** *The sponsor's response to Comment 7 of the CR letter was considered insufficient. Therefore, an IR (IR #047 Comment 7, 10/24/2018) was sent requesting method transfer/validation reports for the reduced- and non-reduced capillary gel electrophoresis methods at the Pfizer Global Research and Development, Chesterfield, MO. In response (10/25/2018), the sponsor provided the method transfer reports VAL000001465 (Method Qualification Report for Reduced CGE) and VAL000001470 (Method Qualification Report for Non-Reduced CGE), documenting the validation of this method in PGRD, Chesterfield, MO. These validation reports were not included in the response to CR Comment 7 or the original BLA submission.*

*After reviewing the validation reports it was concluded that there were no exceptional conditions during the validation of these methods. The data demonstrate the method successful validation of reduced- and non-reduced CGE at the Pfizer St. Louis site (Chesterfield, MO). The data is considered adequate to support the validation of the reduced- and non-reduced capillary gel electrophoresis methods for commercial DP lot release and stability testing.*

#### **FDA COMMENT 8**

**Reference is made to your response received on January 22, 2018 to Comment 1d of the Agency's information request dated December 26, 2017. We disagree with your assessment that the (b) (4) is a low risk**

operation. As stated in the Agency's IR, (b) (4) represents higher risk for process performance consistency and, subsequently, product quality. Data from commercial scale manufacturing experience and/or process validation that has undergone the (b) (4) operation should be provided to support this process and process controls. Provide information and data to support the proposed (b) (4) operations at scale or remove the description of (b) (4) operations for the (b) (4) from Section 3.2.S.2.2 of the BLA.

### Response to Comment 8

The sponsor decided to remove the option to (b) (4) The description of this operation was removed from *Section 3.2.S.2.2 Description of Manufacturing Process and Process Controls – Cell Culture and Harvest*.

**Reviewer comment:** *The sponsor's decision to remove the option to (b) (4) is acceptable.*

### FDA ADDITIONAL COMMENTS:

#### FDA ADDITIONAL COMMENT 1

The proposed acceptance criteria for the control of size variants, including HMMS by SE-HPLC, Intact IgG by non-reducing CGE, HC+LC and fragments by reducing CGE, for DS and DP lot release and stability are too broad and are not justified based on your manufacturing experience. Provide additional information (e.g., clinical experience) and data (e.g., structure function characterization results) to support the proposed acceptance criteria, or tighten these acceptance criteria to ensure that size variants of PF-05280014 DS and DP are properly controlled.

#### Response to Additional Comment 1

The sponsor acknowledged the Agency's request to provide additional information and data to support the proposed acceptance criteria for the control of size variants or to tighten the acceptance criteria, including all the quality attributes mentioned in Additional Comment 1. The sponsor's proposed acceptance criteria for the individual quality attributes are described below:

(b) (4)

## FDA Additional Comment 2

In Section 3.2.S.2.2, the proposed (b) (4) rejection limit of (b) (4) (b) (4) is not appropriate and does not reflect your current experience for developmental, clinical, and commercial productions. The information and data provided in response to the Agency's IR (Comment 2b; dated December 26, 2017) were based on a small-scale study (b) (4) and therefore are insufficient to support the proposed rejection limit. Tighten the rejection limit for (b) (4) based on your current manufacturing experience, or provide data generated from the commercial scale process to support the proposed rejection limit.

## Response to Additional Comment 2

As previously discussed and presented by the sponsor, according to the BPD Type 1 Meeting Minutes issued by the Agency on 20 July 2018, the Agency confirmed that Pfizer's proposal to adjust (b) (4) rejection limit to (b) (4) appears reasonable. Therefore, the sponsor proposes to tighten the (b) (4) rejection limit (b) (4). The information and data justifying the new (b) (4) rejection limit was presented and reviewed as part of the response to the Agency's CR letter Comment 1. Pfizer also updated *Section 3.2.S.2.2 Description of Manufacturing Process and Process Controls – Cell Culture and Harvest*, *Section 3.2.S.2.4 Control of Critical Steps and Intermediates – In-Process Monitoring and Control*, and *Section 3.2.S.2.6 Manufacturing Process Development – Summary of Process Parameters* to include the new (b) (4) rejection limit.

**Reviewer comment:** The sponsor introduced a new approach to monitor (b) (4) and as recommended by the Agency, the sponsor adjusted the (b) (4) rejection limit to (b) (4). The information and data provided in support of the new (b) (4) rejection limit is acceptable.

## FDA Additional Comment 3

Insufficient information was provided to support the process parameters and acceptable ranges for the (b) (4) (Section 3.2.S.2.2). Provide data from process development studies to support these ranges or tighten the operation ranges based on current manufacturing experience and/or process validation studies.

## Response to Additional Comment 3

(b) (4)

#### **FDA Additional Comment 4**

**Reference is made to your response received on February 21, 2018 to Comment 6c of the Agency's IR dated January 17, 2018. While the validation report (VAL100054746) for the FcγRIIIa reporter gene assay included an assessment of most of method validation parameters expected for a potency assay, the stability indicating capacity of this method was not evaluated. We are recommending that ADCC activity of PF-05280014 be controlled for the DS and DP at release and on stability (see CR Comment 4a above under "Product Quality"). If you choose to use your FcγRIIIa reporter gene assay as the means to control for ADCC activity, the stability indicating capability of this assay should be assessed (e.g., by using stressed/forced degraded samples under appropriate conditions). Provide detailed information and data to demonstrate that this method is suitable for its intended use.**

#### **Response to Additional Comment 4**

The sponsor agreed with the recommendation of the Agency to implement the FcγRIIIa reporter gene assay (RGA) to control ADCC activity for PF-05280014 drug substance (DS) and drug product (DP) release and stability. Analytical procedure description and validation information were provided in support of this method as a release and stability test in the Response to Product Quality Comment 4a. Additionally, Pfizer evaluated the stability-indicating capability of FcγRIIIa (RGA) using heat stressed PF-05280014 samples (results are shown below). The stability studies revealed a time-dependent increase of acidic species, high molecular weight species, and low molecular weight species on heat-stressed samples.

**Table 1. FcγRIIIa RGA Activity and CEX, SEC Results of Heat Stressed PF- 05280014 DP Samples**

Treatment at 65 °C	FcγRIIIa RGA Activity (%) <sup>a</sup>	CEX (%)			SEC (%)		
		Acidic	Main	Basic	HMMS	Monomer	LMMS
0 Day	103	23.0	58.4	18.6	0.4	99.5	0.1
1 Day	78	23.0	40.3	36.7	3.7	95.9	0.5
4 Days	Not Parallel <sup>b</sup>	47.7	19.1	33.1	15.0	81.3	3.7
6 Days	Not Parallel <sup>b</sup>	67.3	8.5	24.2	22.6	72.2	5.2

- a. Mean of three assay plates
- b. Significant decrease in upper asymptote of the dose response curve

**Reviewer comment:** *The results of the stability indicating studies demonstrate that FcγRIIIa reporter gene assay is sensitive to heat stress-induced structural changes and is stability-indicating; this is deemed acceptable.*

**FDA Additional Comment 5**

Reference is made to your response on January 22, 2018 to Comment 8 of the Agency’s IR dated December 26, 2017. The overall coverage of the host cell proteins (HCP) recognized by the antibodies in the HCP ELISA (b) (4) is poor; therefore, the data and information provided in the IR response is not sufficient to support the use of this assay (b) (4). Provide additional information and data to support the coverage of HCP specific for PF-05280014 producing cell line by the anti-HCP antibodies employed in the HCP ELISA.

**Response to Additional Comment 5**



**Reviewer comment:** *The sponsor provided the information and data requested in support of the coverage of HCP specific for PF-05280014 producing cell line by the anti-HCP antibodies employed in the HCP ELISA.* (b) (4)

#### **FDA Additional Comment 6**

**Reference is made to your response on February 05, 2018 to Comment 3b of the Agency's IR dated January 17, 2018. You have agreed to include selectivity criteria to the system suitability criteria for both cation exchange and size exclusion HPLC methods. Provide the updated method description for these methods in the re-submission (e.g., in Section 3.2.S.4.2).**

#### **Response to Additional Comment 6**

The sponsor included selectivity criteria to the system suitability criteria for CEX and SE-HPLC methods. *Section 3.2.S.4.2 Analytical Procedures – Cation Exchange HPLC and Section 3.2.S.4.2 Analytical Procedures – Size Exclusion HPLC* were updated to include a resolution (USP half-height) value of 1.8 between HMMS and Monomer for SE-HPLC and a resolution (USP half-height) value of 2.2 between acidic and main species, respectively.

**Reviewer comment:** *The sponsor complied with the Agency's recommendations and provided adequate data; this is acceptable.*

#### **Additional Information:**

##### Post-Approval Stability Protocol

*On January 28<sup>th</sup>, 2019, the Agency recommended the update of the DP post-approval stability protocol to include additional timepoints during the first year and at the 18-month timepoint. The addition of*

*these timepoints will allow the sponsor to detect any issues that may be apparent on stability at earlier timepoints than at one-year post manufacture, and other issues that may arise later in the life of the product (12 month and 18-month timepoints).*

*Pfizer agreed to add additional three-, six-, nine- and eighteen-month timepoints to the DP post-approval stability protocol; this is acceptable.*

*The sponsor was reminded that evaluation of DS stored under (b) (4) are expected to be more informative in assessing whether small unintentional changes made to the manufacturing process and container closure system will result in a change in the stability profile of the product. It was therefore recommended to Pfizer to monitor the DS stored in both intended container closure systems (b) (4) refer to Section 3.2.S.7.2) to add a (b) (4)*

*The sponsor agreed to monitor the DS stored in the (b) (4) at the recommended temperatures. However, the sponsor decided to keep evaluating the DS stability at (b) (4) as part of the annual stability protocol. The sponsor updated Section 3.2.S.7.2 to reflect the addition of the (b) (4) as recommended. This is acceptable.*



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Maldonado-Baez

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Jennifer  
Swisher

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Date: 2/04/2019 12:09:03PM  
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Center for Drug Evaluation and Research  
Office of Pharmaceutical Quality  
Office of Process and Facilities  
Division of Microbiology Assessment  
WO Building 22  
10903 New Hampshire Ave.  
Silver Spring, MD 20993

## **PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION**

**Reviewer: Virginia Carroll, PhD**  
**Quality Assessment Lead: Reyes Candau-Chacon, Ph.D.**

BLA: 761081/0  
Applicant: Pfizer Inc.  
US License Number: 2060  
Submission Reviewed: Original 351(k) BLA resubmission-46  
Product: TRAZIMERA, PF-05280014, a proposed biosimilar to US approved Herceptin  
Indication: Adjuvant breast cancer, metastatic breast cancer, metastatic gastric cancer  
Dosage Form: Multidose vial contains 420 mg lyophilized product, packaged with 20 mL bacteriostatic water for injection (BWFI), for intravenous infusion  
Manufacturing Sites: Pfizer Manufacturing Belgium NV, Puurs, Belgium (FEI 1000654629) (drug product and BWFI)  
FDA Receipt Date: 9/28/2018  
Action Date: 3/28/2019

### **Conclusion and Approvability Recommendation**

The BLA, as amended, was reviewed from a sterility assurance and quality microbiology perspective and is recommended for approval.

### **Product Quality Microbiology Assessment: Drug Product**

#### **Drug Product Quality Microbiology Information Reviewed**

<b>Sequence number</b>	<b>Date</b>	<b>Description</b>
0045	9/28/2018	Response to CRL
0049	12/18/2018	IR Response

*Reviewer's Comment: Information to support (b) (4) has been transferred from DMF (b) (4) to the BLA. An updated letter of authorization dated July 26, 2018 is provided in section 1.4. Because the information now in the BLA (listed below) is identical to the cross-referenced DMF, a current review of the transferred information is not necessary.*

*See previous review (b) (4) dated March 9, 2018 for the following validation information now located in 3.2.P.3.5 of the BLA:*



## **Drug Product**

*Reviewer's Comment: The responses to comments 3a and 3b are deferred to OBP.*

### **FDA Comment 3c**

*The information and data provided to support the commercial DP shipping validation do not provide sufficient assurance that the quality of the DP is maintained during commercial shipping and distribution. We have the following comments:*

- c. The ability of the passive pallet shipper to maintain the storage temperature of 2-8°C in the actual shipping lanes has not been demonstrated. Provide the shipping validation summary report of the passive pallet shipper in the actual shipping lanes during winter and summer conditions in the BLA resubmission.*

Review of Pfizer Response 3c:



(b) (4)

SATISFACTORY

**FDA Comment 9**

***Media fill simulations used to validate (b) (4) for PF-05280014 drug product are not included in the BLA. Provide summary data from three media fills performed on fill line (b) (4) in the BLA resubmission.***

Review of Pfizer Response 9:

(b) (4)

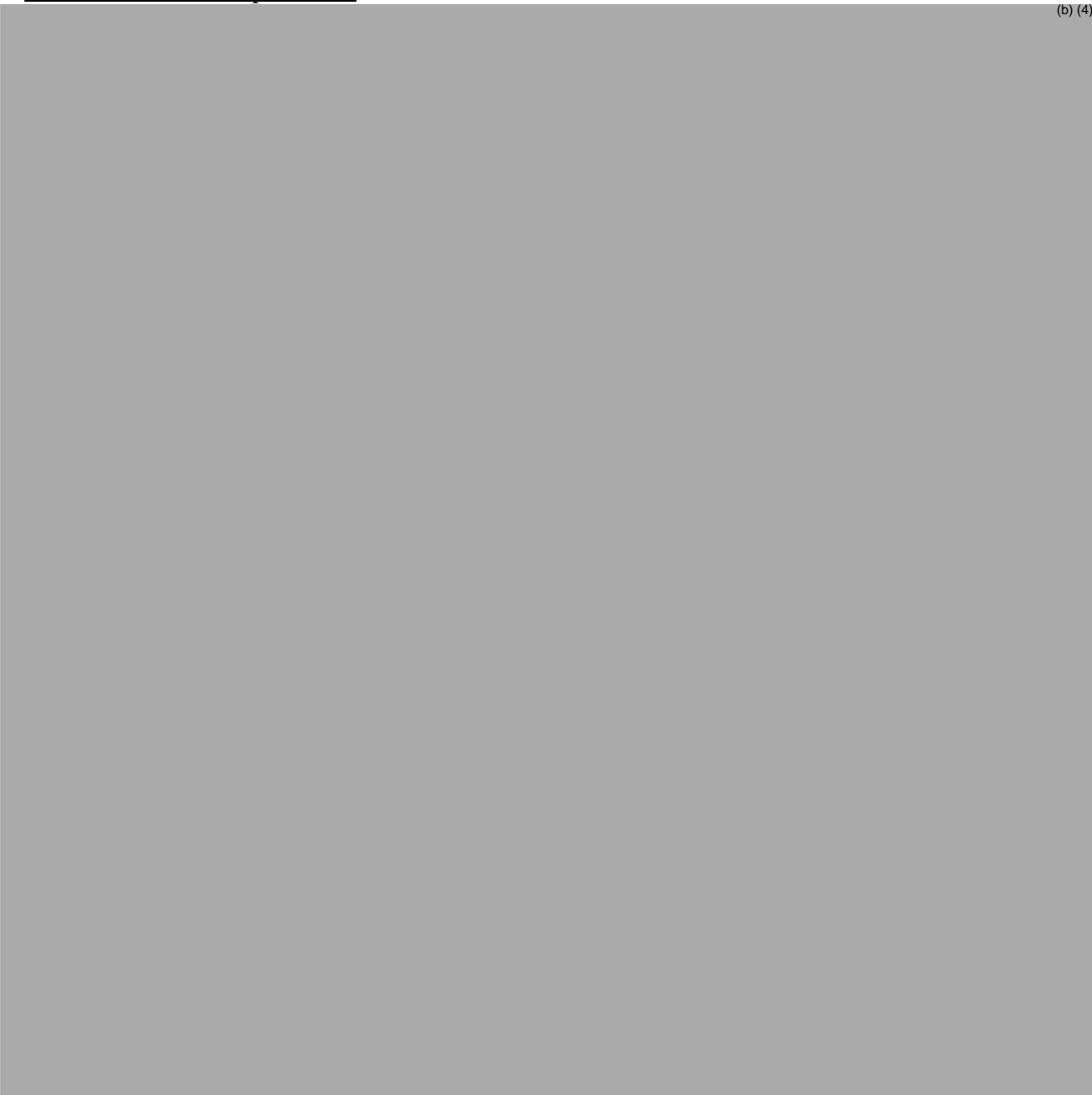
SATISFACTORY

**FDA Comment 10**

***The maximum hold time of PF-05280014 drug product (b) (4) proposed in the BLA is not supported by relevant microbiology data. Provide summary microbial data from hold time studies conducted in the holding vessels used for PF-05280014 in the BLA resubmission.***

Review of Pfizer Response 10:

(b) (4)



*Reviewer's Comment: Hold times were updated in Table 3.2.P.3.3-5. Clarification on the separate hold times (b) (4) was provided. The hold time in the manufacturing vessel (b) (4) is less than (b) (4) therefore no additional microbial data is requested. The proposed cumulative hold time in the holding vessel was sufficiently challenged with three runs, including two PV lots and one media fill. Microbial control was demonstrated over the proposed cumulative hold time.*

SATISFACTORY

**FDA Comment 11**

***Capping process parameters for PF-05280014 drug product and BWFI diluent vials do not specify maximum differential forces. Excessive capping differential forces may damage vials and compromise product sterility. For each capper, specify a maximum differential force and provide summary results which demonstrate container closure integrity after capping at a maximum differential force in the BLA resubmission.***

Review of Pfizer Response 11:

A worst-case parameter for capping is described as maximum combined spring force instead of maximum differential force. The maximum combined spring forces for drug product and BWFI diluent vials have been added to the respective section 3.2.P.3.3 and summarized below:

	Freeze Dry Capper 1 (DP)	Freeze Dry Capper 2 (DP)	(b) (4) (BWFI)
<b>Minimum Spring Force</b>	(b) (4)	(b) (4)	(b) (4)
<b>Maximum Spring Force</b>	(b) (4)	(b) (4)	(b) (4)

Container closure integrity of the vials capped under both minimum and maximum combined spring forces for each capper was previously provided in the amendment dated March 5, 2018 (sequence 0040) in section 3.2.P.2.5. At least (b) (4) vials were tested for each condition by a validated dye ingress method and none showed dye ingress.

*Reviewer's Comment: Maximum capping forces were added to the BLA as requested. Refer to the drug product quality microbiology review memo for original BLA 761081 dated March 9, 2018 for details on the dye ingress test method and complete CCI results of the capping validation studies. Product and diluent vials were demonstrated to be integral under worst-case conditions (minimum and maximum forces) on each capper.*

SATISFACTORY

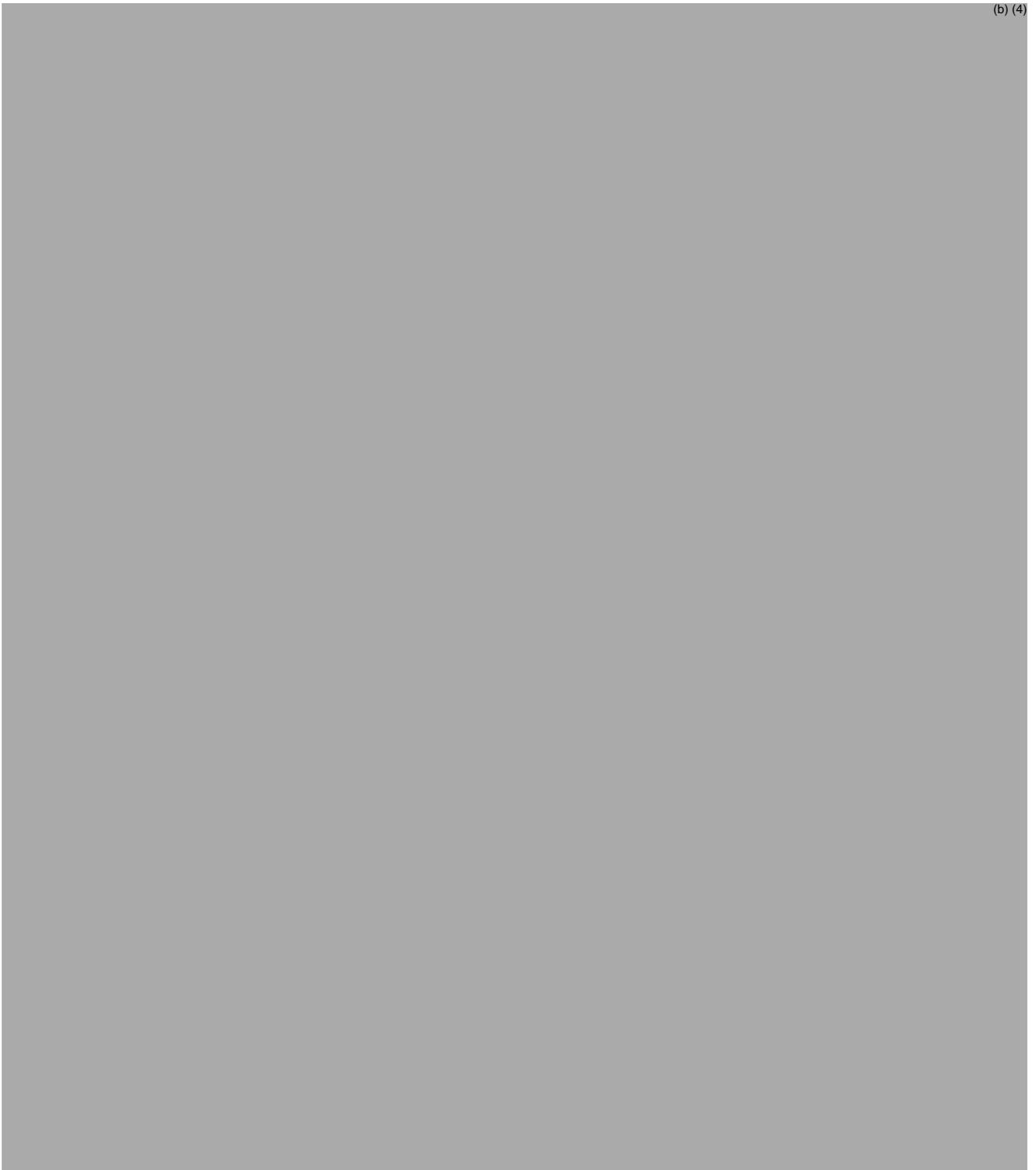
**FDA Comment 12**

***Vial washing parameters and washing validation summary data are not included in the BLA or in DMF (b) (4). Provide validation summary data and information from three runs performed on each vial washing machine for (b) (4) in the BLA resubmission. Specify vial washing parameters for PF-05280014 drug product and BWFI diluent vials.***

Review of Pfizer Response 12:

Vial washing parameters are summarized in the amended DMF (b) (4) and in Table 1 of the response, copied below:

(b) (4)



SATISFACTORY

(b) (4)

SATISFACTORY

**FDA Comment 13**

***Bioburden action limits for PF-05280014 drug product have not been provided for the step [REDACTED] (b) (4). Establish bioburden action limits for this step and provide those limits in the BLA resubmission.***

**Review of Pfizer Response 13:**

A bioburden action limit of [REDACTED] (b) (4) was established for the step [REDACTED] (b) (4). The control limit was added to section 3.2.P.3.4.

*Reviewer's Comment: The bioburden results for the three PV lots manufactured in 2017-2018 [REDACTED] (b) (4) met the new bioburden limit. Refer to FDA Comment 10.*

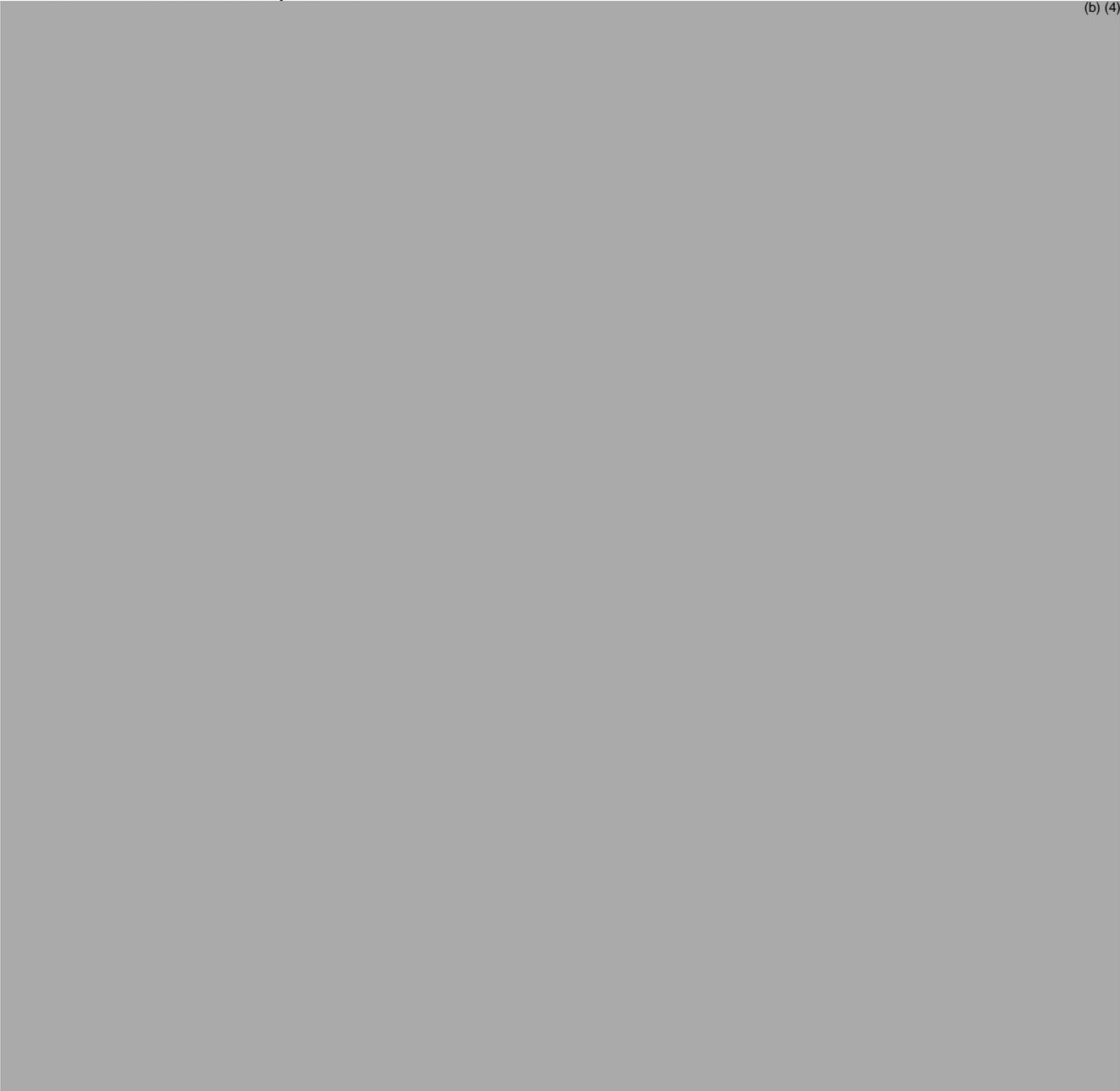
SATISFACTORY

**FDA Comment 14**

*The results of the low endotoxin recovery (LER) study performed with the gel clot method are summarized in section 3.2.P.5.3; however, raw data were not provided. Provide the LER study report in the BLA resubmission which describes the method performed and includes raw data (+/- gel clot) to support the reported percent recoveries. Describe the calculations performed to quantify endotoxin. Clarify the type of endotoxin standard used in the LER studies (CSE or RSE) and update section 3.2.P.5.3 accordingly.*

Review of Pfizer Response 14:

(b) (4)



SATISFACTORY

**FDA Additional Comment 7**

***Submit product-specific information related to sterilization and depyrogenation validation of containers, closures, and equipment in section 3.2.P.3.5 of the BLA rather than in a Drug Master File.***

Review of Pfizer Response:

Information related to sterilization and depyrogenation of containers, closures and equipment has been added to section 3.2.P.3.5, *Vial Washing Sterilization and Depyrogenation and Sterilization and Depyrogenation of Closures and Equipment* for drug product and BWFI.

*Reviewer's Comment: The same sterilization/depyrogenation information added to the BLA is cross-referenced to DMF (b) (4) and was previously reviewed and found adequate. Refer to microbiologist's review #2 dated November 17, 2017 and (b) (4)*

SATISFACTORY

**FDA Additional Comment 8**

***The (b) (4) should be included in section 3.2.P.3.4 of the BLA as an acceptance criterion rather than as a control limit.***

Review of Pfizer Response:

The (b) (4) is listed in 3.2.P.3.4 with an acceptance criterion of "pass". (b) (4)

SATISFACTORY

**CGMP Status**

The assessment of manufacturing facilities is documented in panorama.

**Conclusion**

- I. The BLA, as amended, was reviewed from a sterility assurance and quality microbiology perspective and is recommended for approval.

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

III. No inspection follow-up items were identified.

**DP Quality Microbiology Information Requests Sent and Date**

**December 4, 2018 (sequence 0049)**

1. Provide the investigation report for the positive unit found in media fill (b) (4) on the (b) (4), including the identification of the contaminating microorganism.
2. Hold times should be defined and evaluated for microbial control separately when separated by a (b) (4). Respond to the following:
  - a. Specify the maximum hold time and temperature of the following steps: (b) (4)  
(b) (4)
  - b. Provide the actual hold times for the three drug product process validation lots manufactured with the (b) (4) (b) (4) for the two separate hold steps described above.



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Carroll

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Candau-Chacon

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**Recommendation: Complete Response**

**BLA/NDA Number: 761081**  
**Review Number: First Round**  
**Review Date: April 20, 2018**

Drug Name/Dosage Form	Trazimera (PF-05280014) Biosimilar to Herceptin
Strength/Potency	420mg/vial
Route of Administration	Intravenous
Rx/OTC dispensed	Rx
Indication	Treatment of HER-2 overexpressing breast cancer, gastric cancer
Applicant/Sponsor	Pfizer Inc.

**Product Overview**

TRAZIMERA (trastuzumab-qyyp) is a proposed biosimilar to US-Herceptin (trastuzumab). It is a humanized IgG1k monoclonal antibody produced in CHO cells. Trastuzumab targets and binds human epidermal growth factor receptor 2 (HER2) to inhibit HER2 receptor dimerization and subsequent downstream signaling and to activate antibody-dependent cellular cytotoxicity (ADCC) activity through concomitant binding to Fcγ receptors on immune effector cells.

The PF-05280014 drug substance is produced in genetically engineered CHO cells. Trastuzumab-qyyp drug product (DP), TRAZIMERA, is manufactured to the same strength and presentation as US-Herceptin at 420 mg/vial; however, TRAZIMERA has a different formulation of (b) (4) mM histidine, (b) (4) sucrose, (b) (4) polysorbate 20, pH 6.0. TRAZIMERA 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. TRAZIMERA is proposed as a treatment for HER2-overexpressing breast cancer and gastric cancer.

**Quality Review Team**

Discipline	Reviewer	Branch/Division
Drug Substance	Cishan (Kevin) Li	OPQ/OBP/DBRR1
Drug Product	Cishan (Kevin) Li	OPQ/OBP/DBRR1
Immunogenicity	Cishan (Kevin) Li	OPQ/OBP/DBRR1
OBP Labeling	Vicky Borders Hemphill	OPQ/OBP
Facility	Zhong Li/ Zhihao (Peter) Qiu	OPQ/OPF/DIA
Microbiology	Maria Jose Lopez-Barragan (DS) Virginia Carroll (DP)	OPQ/OPF/DMA
Microbiology Team Lead	Reyes Candau-Chacon	OPQ/OPF/DMA
CMC statistics	Yu-yi Hsu, Meiyu Shen/Yi Tsong (TL)	OTS/OB/DBV
CMC RBPM	Keith Olin	OPQ/OPRO
Application Team Lead	Rachel Novak	OPQ/OBP/DBRR1
OBP Tertiary Reviewer	Qing (Joanna) Zhou	OPQ/OBP/DBRR1

**Multidisciplinary Review Team:**

Discipline	Reviewer	Office/Division
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RPM	Clara Lee	OND/OHOP/DOP-1
Cross-disciplinary Team Lead	Laleh Amiri Kordestani	OND/OHOP/DOP-1
Medical Officer	Sara Horton	OND/OHOP/DOP-1
Pharm/Tox	Claudia Miller/Todd Palmby (TL)	OND/OHOP/DOP-1
Clinical Pharmacology	Christy John/Sarah Schrieber (TL)	OTS/OCP/DCPV
Clinical Statistics	Hui Zhang/Jason Schroeder (TL)	OTS/OB/DBIV

**1. Names:**

- a. Proprietary Name: TRAZIMERA
- b. Trade Name: TRAZIMERA
- c. Non-Proprietary Name: trastuzumab – qyyp

**Communications with Sponsor (OBP)**

Communication/Document	Date
Information Request	
IR #1 Analytical similarity testing sites	07/27/2017
IR #2 Recoverable protein content	09/01/2017
IR #3 Analytical similarity	11/07/2017
IR #4 DS Process	12/26/2017
IR #5 Analytical similarity – Label strength and follow up on charge variants	01/04/2018
IR #6 DS Process, Method and Stability	01/17/2018
IR #7 Stability of WCB	02/05/2018
IR #8 Tiered ranking for HER2 binding	02/09/2018
IR #9: Filling with check and compatibility data	02/09/2018
IR #10 Stability of MCB	02/13/2018
IR #11 DP process	02/22/2018
IR #12 Comment on WCB Stability	03/15/2018
IR #13 Remove RM qualification protocol, (b) (4) protocols, and comparability protocol	04/17/2018

**Submissions reviewed (OBP):**

Submission	Date Received	Review Completed (Yes/No)
761081/0 (Original submission)	06/22/2017	Yes
761081/3 (Response to IR #1, analytical testing sites for similarity)	08/08/2017	Yes
761081/12 (Response to IR #2, extractable content)	09/22/2017	Yes
761081/18 & 26 (Response to IR #3, similarity)	11/22/2017 01/31/2018	Yes

761081/23 (Response to IR #4, DS process description)	01/22/2018	Yes
761081/22 & 30 (Response to IR #5, Label strength and charge variants)	01/16/2018 02/13/2018	Yes
761081/27 (Response to IR #6, DS process, characterization, method, stability)	02/05/2018 02/21/2018	Yes
761081/28 (Response to IR #7, WCB stability)	02/09/2018	Yes
761081/36 (Response to IR #8, HER2 binding tier reassignment)	02/21/2018	Yes
761081/31 (Response to IR #9, filling weight check and compatibility data)	02/16/2018	Yes
761081/37 (Response to IR #10, MCB Stability)	02/21/2018	Yes
761081/41 (Response to IR #11, DP Process IR)	03/01/2018	Yes
761081/41 (Response to IR #12, Comment on WCB deficiency)	03/26/2018 04/04/2018	No
761081/44 (Response to IR#13, removal of protocols)	4/19/2018	Yes

**Submissions Reviewed (DMA):**

Submission(s) Reviewed	Document Date
0016 (16)	11/13/2017
0019 (19)	12/04/2017
0020 (20)	12/12/2017
0025 (25)	01/29/2018
0038 (38)	02/28/2018
0039 (39)	03/01/2018
0040 (40)	03/05/2018

**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 <sup>1</sup>	Status <sup>2</sup>	Date Review Completed	Comments
(b) (4)	V	Pfizer	(b) (4)	1	Deficient	3/14/2018	See CR items 9, 10 and 12. Information pertaining to CR items 9

							and 10 provided in amendment dated 4/17/2018.
(b) (4)	II	(b) (4)	3	Adequate			
	III		3	Adequate			
	III		3	Adequate			
	III		3	Adequate			

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

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

B. Other documents: IND, Referenced Listed Drug (RLD), or sister application. **None**

3. Consults: **None**

### **Executive Summary**

#### **I. Recommendations:**

##### **A. Recommendation and Conclusion on Approvability:**

###### **a. Recommendation:**

The Office of Biotechnology Products (OBP), OPQ, CDER, has completed review of STN 761081 for TRAZIMERA (trastuzumab-qyyp) manufactured by Pfizer Inc. The data submitted in this application are not sufficient to support a conclusion that the manufacture of TRAZIMERA 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 that a Complete Response letter be issued to Pfizer to outline the deficiencies noted below and the information and data that will be required to support approval.

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

###### **b. Complete Response Issues (OBP and DMA):**

Note: The information provided in amendments dated March 26, 2018, April 10, 2018, and April 17, 2018 were not reviewed for this action.

1. Reference is made to the information and data provided to the Agency concerning the stability of the PF-05280014 Working Cell Bank (WCB) on January 22, 2018 and February 9, 2018. Although the likely root causes for the instability have been identified and corrective actions were implemented in late 2017, the information and data do not support the suitability of the current WCB for commercial production.

Reference is also made to your response received on February 21, 2018 to Question 1 of the Agency's Information Request (IR) dated February 13, 2018 concerning the PF-05280014 Master Cell Bank (MCB). Because the MCB has undergone extensive transfers since its inception, the information and data provided by the February 21, 2018 response are insufficient to account for the potential impact on MCB stability from these transfers.

To support a well-controlled and consistent commercial production of PF-05280014, you will need to provide adequate data and information to confirm the stability of the MCB. Once the stability of the MCB is confirmed, you should qualify a new WCB or validate use of the MCB for commercial production.

2. References are made to your response received on September 22, 2017 to Comment 1 of the Agency's IR dated September 1, 2017, and your response received on February 16, 2018 to Comment 1a of the Agency's IR dated February 9, 2018. The Agency does not agree that the data from the Extractable Content experiment support that the lower limit of the fill weight range used during drug product (DP) manufacturing will result in vials filled with sufficient DP to consistently meet the label claim of 420 mg. The recovery data in the calculations used to support the filling range are based on the average recovery of (b) (4) and not the worst-case recovery of (b) (4). Based on the calculations, a DP vial filled at the lower limit of the fill weight range of (b) (4) recovery would be (b) (4) which would not meet the label claim of 420 mg. Tighten the lower fill weight rejection limit and provide data to support that DP vials filled at the lower fill weight limit can consistently deliver 420 mg of PF-05280014.
3. The information and data provided to support the commercial DP shipping validation do not provide sufficient assurance that the quality of the DP is maintained during commercial shipping and distribution. We have the following comments:
  - a. The mechanical performance studies, which include an independent evaluation of vibration and physical shock, are not sufficiently representative of potential stresses induced during routine distribution conditions, where additional factors may contribute stress (e.g., temperature and pressure changes) and multiple stresses could occur concurrently. Therefore, these studies cannot on their own be used in lieu of performing real-time DP shipping validation studies. Provide shipping validation data for the DP from real-time shipping studies or from appropriate simulation studies that are sufficiently representative of the commercial shipping conditions. The data should include an assessment of product quality of pre- and post-shipping DP samples. Include a detailed description of how the study was performed and if performed using simulated studies, provide a justification for how the simulated studies are sufficiently representative of the commercial shipping conditions.
  - b. It is not appropriate to leverage stability data to support the allowable shipping temperature range of (b) (4) because DP is subjected to additional stresses during shipping, which could potentially impact product stability. To support shipment

outside of the validated 2-8°C range, real-time shipping studies or sufficiently representative simulated studies, as described above, should be performed to support the allowable shipping temperature range.

- c. The ability of the passive pallet shipper to maintain the storage temperature of 2-8°C in the actual shipping lanes has not been demonstrated. Provide the shipping validation summary report of the passive pallet shipper in the actual shipping lanes during winter and summer conditions in the BLA resubmission.
4. Implement the following specifications for the control of PF-05280114:
    - a. Antibody-dependent cell-mediated cytotoxicity (ADCC) is one of the potential mechanisms of action for trastuzumab. In your response received on February 05, 2018 to Comment 6c of the Agency's IR dated January 17, 2018, a cell-based FcγRIIIa reporter gene assay was provided as a surrogate assay to assess ADCC activity in the reference material qualifications. Implement this test, or another validated assay, with appropriate acceptance criteria to control for ADCC activity for DS and DP release and stability. The acceptance criteria should be based on product understanding, current process understanding, and clinical experience.
    - b. As noted in the Agency's IR on November 7, 2017, isomerization of Asp102 (iso Asp102) is shown to impact the potency of trastuzumab. The iso Asp102 is enriched in the B0 peak in CEX-HPLC and the B0 peak of PF-05280114 increases under mild thermal stressed conditions (Section 3.2.S.3.1); therefore, a control strategy for iso Asp102 should be in place to ensure product safety, quality and potency. Given that your current potency assay is not sensitive enough to detect changes in iso Asp102 and it is not appropriate to control iso Asp102 using CEX-HPLC (Refer to IR dated January 4, 2018), an assay should be developed and implemented to control for the level of iso Asp102 for DS and DP release and stability, with appropriate acceptance criterion.
    - c. As part of the Agency's current considerations regarding the control of effector function, it is understood from publicly available literature that high mannose species contribute to the total level of afucosylated variants. Given the impact of afucosylated variants on potency, we recommend that the total level of high mannose be included as part of the control strategy for the PF-08250114 DS. Implement an appropriate control for high mannose into the DS release specification. The acceptance criteria should be based on the current process understanding and clinical experience.
    - d. Polysorbate 20 is a critical excipient that can impact product quality and stability. Add testing and quantitative acceptance criteria for Polysorbate 20 to the PF-05280014 DP release specification.
    - e. Add the test of "extractable content" to the DP release specification with the acceptance criterion of "No less than 420 mg" to ensure that DP lots will meet the label claim.
  5. The justifications for specifications provided in Sections 3.2.S.4.5 and 3.2.P.5.6 are not sufficient to support some of the release and end of shelf life acceptance criteria for the PF-05280014 drug substance (DS) and DP. The acceptance criteria for release and end of shelf life should be sufficiently narrow to allow for adequate control of DS and subsequent DP and should be based on your clinical and manufacturing experience. For some product quality attributes that change during storage, it is not appropriate to have the same acceptance

criteria for DS and DP release and end of shelf life. The specifications should be set such that materials released at the limit of the acceptance criteria will not result in out of specification results during storage. For some attributes, the DS release and/or end of shelf life specifications should be tighter than those for DP release and/or end of shelf, to avoid DP lots going out of specification. Based on these concepts, the following acceptance criteria should be tightened:

- a. Appearance (Color) for DS release and end of shelf life
  - b. Acidic and Basic species by CEX-HPLC for DS and DP release and end of shelf life
6. The additional storages for PF-05280014, as specified in the proposed label, include storage at 30°C for a single period up to 3 months for unopened PF-05280014 or storage at 2-8°C for up to 28 days for PF-05280014 reconstituted in BWFI, within the long-term storage of 48 months at 2-8°C. The information and data provided in the submission are insufficient to support these additional storage conditions. For example, product quality attributes including ADCC (or FcγRIIIa binding) and the levels of iso Asp102 were not assessed to demonstrate that there is no impact on these attributes during these storage conditions. Provide additional information and data to support the proposed additional storage conditions.
  7. Method transfer data were provided for the reduced and non-reduced CGE assays from Pfizer St. Louis to Grange Castle; however, no information and data (e.g., from method validation or method transfer) were provided to support that these assays are validated at the Pfizer St. Louis site. Because Grange Castle is the only DP release testing site for these assays (Section 3.2.P.3.1), the method validation for these assays used for commercial DP lot release and stability testing is considered incomplete. Provide information and data to support the method validation of these assays at Pfizer St. Louis.
  8. Reference is made to your response received on January 22, 2018 to Comment 1d of the Agency's information request dated December 26, 2017. We disagree with your assessment that the (b) (4) is a low risk operation. As stated in the Agency's IR, (b) (4) represents higher risk for process performance consistency and, subsequently, product quality. Data from commercial scale manufacturing experience and/or process validation that has undergone the (b) (4) operation should be provided to support this process and process controls. Provide information and data to support the proposed (b) (4) operations at scale, or remove the description of (b) (4) operations for the (b) (4) from Section 3.2.S.2.2 of the BLA.
  9. Media fill simulations used to validate (b) (4) for PF-05280014 drug product are not included in the BLA. Provide summary data from three media fills performed on fill line (b) (4) in the BLA resubmission.
  10. The maximum hold time of PF-05280014 drug product (b) (4) proposed in the BLA is not supported by relevant microbiology data. Provide summary microbial data from hold time studies conducted in the holding vessels used for PF-05280014 in the BLA resubmission.

11. Capping process parameters for PF-05280014 drug product and BWFI diluent vials do not specify maximum differential forces. Excessive capping differential forces may damage vials and compromise product sterility. For each capper, specify a maximum differential force and provide summary results which demonstrate container closure integrity after capping at a maximum differential force in the BLA resubmission.
12. Vial washing parameters and washing validation summary data are not included in the BLA or in DMF (b) (4). Provide validation summary data and information from three runs performed on each vial washing machine for (b) (4) in the BLA resubmission. Specify vial washing parameters for PF-05280014 drug product and BWFI diluent vials.
13. Bioburden action limits for PF-05280014 drug product have not been provided for the step (b) (4). Establish bioburden action limits for this step and provide those limits in the BLA resubmission.
14. The results of the low endotoxin recovery (LER) study performed with the gel clot method are summarized in section 3.2.P.5.3; however, raw data were not provided. Provide the LER study report in the BLA resubmission which describes the method performed and includes raw data (+/- gel clot) to support the reported percent recoveries. Describe the calculations performed to quantify endotoxin. Clarify the type of endotoxin standard used in the LER studies (CSE or RSE) and update section 3.2.P.5.3 accordingly.

#### **Additional Comments (OBP and DMA)**

1. The proposed acceptance criteria for the control of size variants, including HMMS by SE-HPLC, Intact IgG by non-reducing CGE, HC+LC and fragments by reducing CGE, for DS and DP lot release and stability are too broad and are not justified based on your manufacturing experience. Provide additional information (e.g., clinical experience) and data (e.g., structure function characterization results) to support the proposed acceptance criteria, or tighten these acceptance criteria to ensure that size variants of PF-05280014 DS and DP are properly controlled.
2. In Section 3.2.S.2.2, the proposed (b) (4) rejection limit of (b) (4) (b) (4) is not appropriate and does not reflect your current experience for developmental, clinical, and commercial productions. The information and data provided in response to the Agency's IR (Comment 2b; dated December 26, 2017) were based on a small-scale study (b) (4) and therefore are insufficient to support the proposed rejection limit. Tighten the rejection limit for (b) (4) based on your current manufacturing experience, or provide data generated from the commercial scale process to support the proposed rejection limit.
3. Insufficient information was provided to support the process parameters and acceptable ranges for the (b) (4) (Section 3.2.S.2.2). Provide data from process development studies to support these ranges or tighten the operation ranges based on current manufacturing experience and/or process validation studies.

4. Reference is made to your response received on February 21, 2018 to Comment 6c of the Agency's IR dated January 17, 2018. While the validation report (VAL100054746) for the FcγRIIIa reporter gene assay included an assessment of the majority of method validation parameters expected for a potency assay, the stability indicating capacity of this method was not evaluated. We are recommending that ADCC activity of PF-05280014 be controlled for the DS and DP at release and on stability (see CR Comment 4a above under "Product Quality"). If you choose to use your FcγRIIIa reporter gene assay as the means to control for ADCC activity, the stability indicating capability of this assay should be assessed (e.g., by using stressed/forced degraded samples under appropriate conditions). Provide detailed information and data to demonstrate that this method is suitable for its intended use.
5. Reference is made to your response on January 22, 2018 to Comment 8 of the Agency's IR dated December 26, 2017. The overall coverage of the host cell proteins (HCP) recognized by the antibodies in the HCP ELISA (b) (4) is poor; therefore, the data and information provided in the IR response is not sufficient to support the use of this assay (b) (4). Provide additional information and data to support the coverage of HCP specific for PF-05280014 producing cell line by the anti-HCP antibodies employed in the HCP ELISA.
6. Reference is made to your response on February 05, 2018 to Comment 3b of the Agency's IR dated January 17, 2018. You have agreed to include selectivity criteria to the system suitability criteria for both cation exchange and size exclusion HPLC methods. Provide the updated method description for these methods in the re-submission (e.g., in Section 3.2.S.4.2).
7. Submit product-specific information related to sterilization and depyrogenation validation of containers, closures, and equipment in section 3.2.P.3.5 of the BLA rather than in a Drug Master File.
8. The (b) (4) should be included in section 3.2.P.3.4 of the BLA as an acceptance criterion rather than as a control limit.

**c. Manufacture Information:**

- Manufacturing location:
  - Drug Substance: (b) (4)
  - Drug Product: Pfizer Manufacturing Belgium NV (Puurs, Belgium)
- Fill size and dosage form: 420mg multi-dose vial for injection
- Dating period:
  - Drug Product: 48 months when stored at 2 to 8°C
  - Drug Substance: (b) (4)
  - For packaged products: Bacteriostatic Water for Injection (BWFI)
    - BWFI: 24 months when stored at 2 to 8°C
  - Stability Option:

- For stability protocols:
  - The stability protocol(s) in the license application is acceptable for the purpose of extending the expiration dating of the drug substance and drug product under 21 CFR 601.12. However, the application will not be approved at this time due to the issues listed above.
- Exempt from lot release
  - Yes
  - Rationale: specified product in accordance with 21 CFR 601.2a.

**B. Benefit/Risk Considerations:**

TRAZIMERA (trastuzumab-qyyp) is proposed as a biosimilar to US-Herceptin (trastuzumab) for treatment of HER2 overexpressing breast cancer and gastric 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 ADCC activity, has also been identified as a potential mechanism of action of trastuzumab.

With respect to analytical similarity, TRAZIMERA was evaluated and compared to US- Herceptin and EU-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 TRAZIMERA is highly similar to US-Herceptin. The amino acid sequences of TRAZIMERA and US-Herceptin are identical, and a comparison of the secondary and tertiary structures and the impurity profiles of TRAZIMERA and US-Herceptin support the conclusion that the two products are highly similar.

Inhibition of proliferation of HER2 expressing cells and ADCC activity, which reflect the presumed primary mechanisms of action of US-Herceptin, were determined to be equivalent. HER2 binding was found to be similar between TRAZIMERA and US-Herceptin. Some tests indicate small differences in low abundance glycan forms [e.g., sialic acid, high mannose] exist and are likely an intrinsic property of TRAZIMERA due to the manufacturing process. High mannose and sialic acid containing glycans can impact PK; however, the residual uncertainties related to the differences in total mannose forms and sialic acid were addressed by the ADCC similarity and by the PK similarity between TRAZIMERA and US-Herceptin as concluded by the clinical review team.

Subtle differences in charge related variants were detected; however, these differences are not expected to impact the biological activity of TRAZIMERA. Further, the data submitted by the applicant support the conclusion that TRAZIMERA 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, TRAZIMERA is highly similar to US-Herceptin, notwithstanding minor differences in clinically inactive components.

PF-05280014 meets the statutory “same strength” requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.

The overall control strategy for TRAZIMERA 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 several issues with the DS and DP control strategy, although the major issue and reason for the CR action has to do with the instability of the Working Cell Bank (WCB) with additional concerns about the stability of the Master Cell Bank (MCB).

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

The technical assessments for OBP (including analytical similarity, drug substance, drug product quality and immunogenicity assays), DMA (including drug substance and drug product quality microbiology), and DIA facility are located as separate documents in Panorama.

**C. Recommendation on Phase 4 (Post-Marketing) Commitments, Requirements, Agreements, and/or Risk Management Steps, if approvable: Not approvable**

**II. Summary of Quality Assessments:**

**A. Analytical Similarity Assessment**

**Table 1. Summary of Analytical Similarity Assessment**

Quality Attribute	Analytical Procedure	Statistical Tier
Potency	<ul style="list-style-type: none"> <li>Inhibition of cell growth assay</li> <li>Primary NK cell ADCC assay</li> </ul>	1 1
Potency	<ul style="list-style-type: none"> <li>Binding to HER2 target antigen (SPR)</li> <li>Binding to cell surface HER2 (flow cytometry)</li> <li>HER2 and HER3 phosphorylation (Western blot)</li> <li>PBMC ADCC assay</li> <li>Apoptosis assay</li> </ul>	2 3 3 3 3
N-linked glycan Profile	<ul style="list-style-type: none"> <li>Total afucosylation (HILIC)</li> <li>Terminal galactosylation (HILIC)</li> <li>High Mannose</li> <li>HILIC (fluorescence and MS detection)</li> <li>N-linked glycan profile by exoglycosidase analysis</li> <li>Sialic acid analysis</li> </ul>	2 2 2 3 3 3
Charge heterogeneity	<ul style="list-style-type: none"> <li>CEX-HPLC</li> <li>MS</li> <li>CEX-HPLC (with and without CPB)</li> </ul>	2 3 3

Size Variants/Aggregates	• SE-HPLC	2
	• CGE (reducing)	2
Protein Concentration (after reconstitution)	• UV spectroscopy at 280 nm	2
Primary Structure	• Amino acid sequence (de novo sequencing)	3
	• Molecular mass (nanoESI MS)	3
	• Molecular size (Reducing CGE)	3
	• Primary structure (LC/MS subunit analysis)	3
	• Primary structure (LC/MS peptide mapping (Trypsin))	3
	• Isoform Identification (LC/MS subunit analysis)	3
Higher Order Structure	• Near and far UV Circular Dichroism (CD)	3
	• Intrinsic Fluorescence (IF)	3
	• Fourier transform infrared spectroscopy (FTIR)	3
	• Peptide Mapping (disulfide bond characterization)	3
	• Differential scanning calorimetry (DSC)	3
	• Ellman's reagent (free cysteines)	3
Fc-receptor binding	• FcγRIIIa reporter gene assay	2
	• Binding to FcγRIIIa by SPR	2
	• Binding to FcRn by SPR	3
	• FcγRI (SPR)	3
	• FcγRIIa (SPR)	3
	• FcγRIIb (SPR)	3
	• FcγRIIIb (SPR)	3
	• CDC assay bioassay	3
	• C1q binding assay (immunoassay)	3

PF-05280114 was found to have the same primary, secondary and tertiary structure as US-Herceptin and EU-Herceptin by the methods listed in Table 1, above. Secondary structure ( $\alpha$ -helix,  $\beta$ -sheet and random coil structures) was analyzed through far UV CD spectroscopy and FTIR results, and information on tertiary structure was provided by the near UV CD spectroscopy and IF results. All four methods yielded overlapping spectra between PF-05280114 and US-Herceptin. DSC was used to measure the melting temperatures of the protein subunits. Quantitative analysis and representative thermograms also indicate structural similarity among the three products (i.e., PF-08250114, US-Herceptin and EU-Herceptin).

Analysis by CEX-HPLC showed a similar number of peaks, retention times, and % acidic species among PF-05280114, US-Herceptin, and EU-Herceptin. Differences were noted in the charge profile by CEX-HPLC when comparing PF-05280114 to US-Herceptin and EU-Herceptin. PF-05280114 had increased levels of basic species, with concomitant decreases in the neutral peak, compared to those of US-Herceptin and EU-Herceptin. Both attributes fell slightly outside the quality range (QR) established based on data from US-Herceptin and EU-Herceptin. Further analysis by digesting the products with carboxypeptidase demonstrated a similar charge profile and indicates that the increase in basic species was due to increased C-terminal lysine present on PF-05280114. C-terminal lysine has been shown to be cleaved upon administration at physiological pH. No impact on potency determined using bioassays was observed. Therefore, this difference does not preclude a demonstration of highly similar between PF-05280114 and US-Herceptin or establishment of the analytical component of the three-way scientific bridge.

Levels of sialylation and galactosylation were similar among all three products. PF-05280114 lots had slightly less afucosylated and high mannose species compared to those of US-Herceptin and EU-Herceptin; however, the results for PF-05280114 lots were within the QR for these glycan species. Therefore, these differences do not preclude a demonstration of highly similar between PF-05280114 and US-Herceptin or establishment of the analytical component of the three-way scientific bridge.

CGE (non-reduced and reduced) was used to evaluate purity of intact IgG, non-glycosylated heavy chain (NGHC), and the sum of light and heavy chains (H+L). The levels of fragment in the PF-05280114 lots were low and similar to those of US-Herceptin and EU-Herceptin. The total Heavy Chain (HC) and Light Chain (LC) levels were also similar under reducing conditions for all three products. Four PF-05280114 lots were outside of the QR for intact IgG by non-reduced CGE, demonstrating only 64% of PF-05280114 lots were within the QR. One of the lots that was outside the QR was an early development lot, and is not representative of the clinical or commercial material. However, no impact on biological activity determined using bioassays was noted for the PF-05280114 lots. Therefore, these differences do not preclude a demonstration that the products are highly similar or establishment of the analytical component of the three-way scientific bridge.

No differences were observed in biological activity between PF-05280114 and US-Herceptin using any of the biological assays. Such assays included inhibition of proliferation of the HER2 overexpressing cell line (SKBR3) proliferation, antibody-dependent cellular cytotoxicity (ADCC) with Natural Killer (NK) cells, a FcγRIIIa Reporter Gene Assay (RGA) and various HER2 and Fc Receptor binding assays.

Thus, based on the extensive comparison of the functional, physicochemical, protein and higher order structure attributes, TRAZIMERA is highly similar to US-Herceptin, notwithstanding minor differences in clinically inactive components. In addition, the analytical component of the three-way scientific bridge has been adequately established.

**B. CQA Identification, Risk and Lifecycle Knowledge Management**

**Table 1:** Active Pharmaceutical Ingredient 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.		N/A

		<p>Impacted by aggregation, fragmentation, and potentially deamidation.</p> <p>Minimal change is expected during storage through expiry.</p>	(b) (4)	
ADCC activity (potency)	Efficacy	<p>Intrinsic to the molecule.</p> <p>Impacted by glycosylation, aggregation and fragmentation and potentially deamidation</p> <p>Minimal change is expected during storage through expiry.</p>		The sponsor will be asked to add the testing of this attribute to the DS and DP release and stability specifications in the CR letter. An indirect measure by FcγRIIIa binding can be used to control ADCC activity.
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	<p>Manufacturing process and exposure to heat.</p> <p>Minimal change is expected during storage under recommended conditions through expiry.</p>		N/A
Fragments (LMW species)	Efficacy and PK	Manufacturing process and exposure to heat and light stress.		N/A

		Minimal increase in fragments is expected during storage under recommended conditions.	(b) (4)	
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.		The sponsor will be asked to add this attribute to the DS release specifications in the CR letter.
Heavy Chain Asp102 isomerization	Efficacy	Manufacturing process including hold, storage, exposure to heat.		Insufficient controls are currently in place to adequately monitor this attribute for PF-05280114 during manufacturing. The sponsor will be requested to add testing for this attribute during routine DS and DP release and stability testing.
Osmolality	Safety, Efficacy (control of degradation through formulation)	Formulation		N/A

pH	Safety and Efficacy	Formulation	(b) (4)	N/A
Protein Content	Efficacy	Manufacturing process		N/A
Polysorbate 20	Safety and efficacy (control of degradation)	Formulation		The sponsor will be asked to add this attribute to DP release specifications in the CR letter.

**C. Drug Substance [TRAZIMERA] Quality Summary**

COA Identification, Risk, and Lifecycle Knowledge Management

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

COA	Risk	Origin	Control Strategy	Other notes
Appearance	Safety	Controlled by the manufacturing process	(b) (4)	The sponsor will be asked to tighten this criterion in the CR letter.
Host Cell Proteins (Process-related impurity)	Safety and Immunogenicity	Production cell line		The current HCP assay has low coverage (b) (4). The sponsor will be asked to provide additional data to support the use of this assay in the CR letter.
Host Cell DNA (Process-related impurity)	Safety	Production cell line		N/A

			(b) (4)	
(b) (4)	Safety and Immunogenicity	Process related impurity (b) (4)		N/A
(Process-related impurity)				
(b) (4)	Safety, immunogenicity	(b) (4)		N/A
(Process-related impurity)				
Viruses (Contaminant)	Safety	Contamination during manufacture, most likely during cell culture operations		N/A

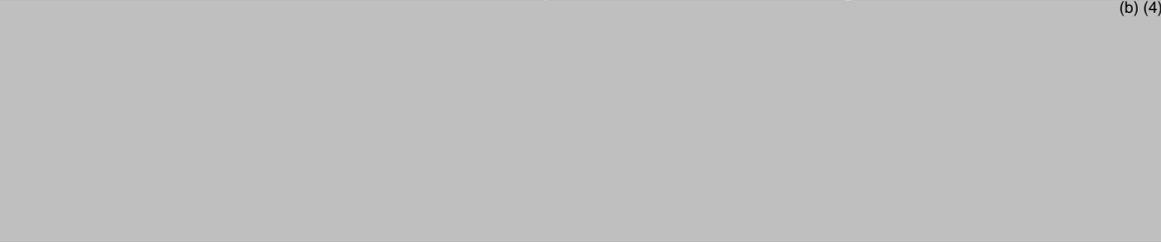
			(b) (4)	
Mycoplasma (Contaminant)	Safety	Mycoplasma would most likely be introduced during cell culture operations.		N/A
Leachables (Process-related impurity)	Safety	Manufacturing components and the DS container closure system		N/A
Bioburden	Safety, purity, and efficacy (degradation or modification of the product by contaminating microorganisms)	Bioburden can be introduced by raw materials and throughout the manufacturing process		N/A
Endotoxin	Safety and purity	Endotoxin can be introduced by raw materials and throughout the manufacturing process		N/A

- **Description:** PF-05280014 is an IgG1 kappa monoclonal antibody with two identical HCs and two identical LCs, covalently linked with four inter-chain disulfide bonds. It contains one Asn linked glycosylation site at Asn30 in the Fc region. The theoretical

molecular masses are 148057.4 Da. The theoretical isoelectric point (pI) is 8.6 and the experimental extinction coefficient at 280 nm is 1.46 (mg/mL)<sup>-1</sup> cm<sup>-1</sup>.

- **Mechanism of Action (MoA):** Multiple mechanisms of action have been proposed for trastuzumab. These 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 potential 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 Assay:** The potency assay is a cell based bioassay which measures the level of growth inhibition of HER2 expressing cancer cells (SKBR3) induced by PF-05280014. The SKBR3 cells are incubated with different dilutions of PF-05280014 for 5 days at 37°C. Cell proliferation is detected using a commercially available CellTiter Glo kit to measure the presence of ATP in the live cells. Relative potency is calculated against the PF-05280114 reference standard by parallel line analysis using PLA software.

The sponsor also provided validation data for an FcγRIIIa Reporter Gene Assay (RGA), which can be used as a surrogate for monitoring ADCC activity of TRAZIMERA. This assay is not currently implemented into the control strategy for release and stability testing of the commercial DS and DP. However, a comment will be conveyed in the CR letter for the sponsor to add a potency test to control ADCC activity of the commercial product, such as the FcγRIIIa Reporter Gene Assay.

- **Reference Materials:** For commercial operation, a two-tiered system was established (b) (4)  

- **Critical starting materials or intermediates:** The PF-05280014 Master Cell Bank (MCB, MCB (b) (4)) was developed (b) (4)  
 The Working Cell Bank (WCB, WCB (b) (4)) was created by the expansion of the MCB. This two-tiered cell banking system was implemented to ensure continued source of product. No animal-derived materials were used in the manufacture of the both cell banks. The cell lines were appropriately tested to ensure product safety from adventitious and endogenous agents.



(b) (4)

- **Manufacturing process summary:** PF-05280114 DS is manufactured at

(b) (4)



(b) (4)

- **Container closure:** The DS is filled in

(b) (4)



- **Dating period and storage conditions:** The DS can be stored

(b) (4)



#### **D. Drug Product [TRAZIMERA] Quality Summary:**

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

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

CQA (Type)	Risk	Origin	Control Strategy	Other
Color and clarity of solution (general)	Safety and Efficacy	Formulation, contamination, or degradation	(b) (4)	N/A
Lyophilizate appearance	Safety and Efficacy (stability)	Manufacturing process		N/A
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		Sponsor will be asked to add this testing to the DP release specification. See CR letter comments.
Deliverable Content (general)	Efficacy/Dosing	Manufacturing process		Sponsor will be asked to tighten the fill control limit, provide more information to support the filling control strategy and implement testing for deliverable content in the DP release specifications. See CR comments.
Leachables (process-related impurities)	Safety	Manufacturing equipment and container closure		N/A

			(b) (4)	
Sterility (Contaminant)	Safety risk to patients (infection) Efficacy (degradation or modification of the product by microorganisms or their byproducts)	Contaminants could be introduced throughout DP manufacturing		Deficient sterilization process validation to support sterility assurance of the finished DP. See CR items for media fills, hold time studies, and bioburden action limits (b) (4)
Endotoxin (Contaminant)	Safety, purity, and immunogenicity	Contaminants could be introduced throughout DP manufacturing process and through raw materials		The low endotoxin recovery (LER) study report was not provided. See CR comment.
Container Closure Integrity (Sterility Assurance)	Safety (Failure in closure integrity may lead to contamination through a loss of sterility) or evaporation/leakage (impacting concentration or content)	May be impacted by storage conditions		N/A

- Potency and Strength:** TRAZIMERA is supplied at 420 mg/vial. Potency is defined as the percent activity relative to the current PF-05280014 reference standard. The potency assays are the same as described for the DS.
- Summary of Product Design:** TRAZIMERA is supplied as a sterile, multi-dose, preservative-free lyophilized powder for IV infusion in a 30-mL vial. TRAZIMERA is formulated in (b) (4) mM histidine, (b) (4) sucrose, (b) (4) polysorbate 20 at pH 6.0. Reconstitution with 20 mL diluent yields a solution containing 21 mg/mL TRAZIMERA. The deliverable amount is 420 mg. TRAZIMERA is supplied with a 20-mL vial of BWFI, USP. (b) (4)
- List of Excipients:** L-Histidine, Polysorbate 20, and sucrose. All excipients are compendial.

- **Reference Materials:** See DS section above.
- **Manufacturing process summary:** PF-0528114 drug substance is (b) (4)

[Redacted]

[Redacted] (b) (4)

- **Container closure:** PF-05280014 is packaged in 30 mL (b) (4) clear glass vials with (b) (4) stoppers and crimp seals with flip-off caps.
- **Dating period and storage conditions:** The sponsor proposed (b) (4) months of shelf life when stored at  $5 \pm 3$  °C, protected from light. Additional storage conditions have been proposed including 2-8°C for no more than 28 days for the reconstituted drug product in the BWFI and 30°C for a period of up to 3 months, within the (b) (4) month expiry period for unopened vials of TRAZIMERA; however, additional data will need to be provided to support these storage conditions (See CR comments).
- **List of co-package components, if applicable:** TRAZIMERA is co-packaged with sterile BWFI.

**E. Drug Product [BWFI diluent] Quality Summary:**

Table 4 provides a summary of the identification, risk, and lifecycle knowledge management for drug product diluent COAs that derive from the drug product manufacturing process and general drug product attributes.

**Table 4:** Drug Product diluent CQA Identification, Risk, and Lifecycle Management

COA (type)	Risk	Origin	Control Strategy <sup>(b) (4)</sup>	Other
Sterility (Contaminant)	Safety risk to patients (infection) Efficacy (degradation or modification of the product by microorganisms or their byproducts)	Contaminants could be introduced throughout DP manufacturing or through a container closure integrity failure.		N/A
Endotoxin (Contaminant)	Safety and purity Can increase immunogenicity risk	Contaminants could be introduced throughout DP manufacturing or through a container closure integrity failure.		N/A
Container Closure Integrity (Sterility Assurance)	Safety Failure in closure integrity may lead to contamination (loss of sterility) of DP or evaporation/leakage (impacting concentration or content)	Failure in closure integrity may lead to contamination (loss of sterility) of DP or evaporation/leakage (impacting concentration or content)		N/A
Identity - Benzyl Alcohol	Safety risk to patients (infection) Efficacy (degradation or modification of the product by microorganisms or their byproducts after reconstitution)	Contaminants could be introduced after reconstitution of DP and storage for multiple uses or through a container closure integrity failure.		N/A
Antimicrobial Agents – Content (Benzyl Alcohol)	Safety risk to patients (infection) Efficacy (degradation or modification of the product by microorganisms or their byproducts after reconstitution)	Contaminants could be introduced after reconstitution of DP and storage for multiple uses or through a container closure integrity failure.		N/A

- Manufacturing process summary:** The manufacturing process of BWFI involves (b) (4)
- Container closure:** The BWFI diluent is packaged in 20 mL Type 1 clear glass vials with a (b) (4) stopper and (b) (4) seals with flip-off caps.
- Dating period and storage conditions:** The BWFI diluent can be stored for up to 24 months at 2-8°C when packaged with TRAZIMERA drug product or at 28-32°C when stored alone.

**F. Novel Approaches/Precedents:** None

**G. Any Special Product Quality Labeling Recommendations:** Store unopened TRAZIMERA vials at 2-8°C. Unopened vials may be stored up to 30°C for a single period of up to 3 months. Reconstitute each 420 mg vial with 20 mL BWFI for a multiple-dose solution. Store reconstituted DP at 2-8°C for up to 28 days. Alternatively, the DP can be reconstituted with sterile WFI without preservative but must be used immediately. The acceptability of these storage conditions is pending on response to the CR.

After dilution of reconstituted DP in 0.9% sodium chloride injection, the infusion solution may be stored at 2-8°C for up to 24 hours prior to use. Do not freeze. The intravenous infusion will be completed over 30-90 minutes, according to the label.

**H. Establishment Information:**

Overall Recommendation: Approve					
<b>DRUG SUBSTANCE</b>					
Function	Site Information	FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Drug substance manufacture; in-process control testing and release testing; bulk DS and cell bank storage	<span style="background-color: #cccccc;">(b) (4)</span>	<span style="background-color: #cccccc;">(b) (4)</span>	Inspect Facility DMA lead and OBP participation	Pre-license Inspection <span style="background-color: #cccccc;">(b) (4)</span> VAI	Approve facility
Cell bank storage; Drug substance release testing and stability testing	Pfizer Ireland Pharmaceuticals, Grange Castle Business Park Clondalkin, Dublin 22, Ireland	3004145594	Waive inspection	Waived	Approve facility

Cell bank manufacture and storage	Wyeth BioPharma, Division of Wyeth Pharmaceuticals Inc., One Burtt Road, Andover, MA 01810 USA	1222181	Inspect Facility OBP participation	Pre-license Inspection 10/10 – 12/2017 NAI	Approve facility
<b>DRUG PRODUCT</b>					
<b>Function</b>	<b>Site Information</b>	<b>FEI Number</b>	<b>Preliminary Assessment</b>	<b>Inspectional Observations</b>	<b>Final Recommendation</b>
Drug product manufacture, primary packaging, and secondary packaging and labeling; release testing	Pfizer Manufacturing Belgium NV Rijksweg 12 2870 Puurs Belgium	1000654629	Waive inspection	Waived	Approve facility
Drug product release testing and stability testing	Pfizer Ireland Pharmaceuticals Grange Castle Business Park Clondalkin, Dublin 22 Ireland	3004145594	Waive inspection	Waived	Approve facility

I. **Facilities:** No approvability issues

J. **Lifecycle Knowledge Management:**

a. **Drug Substance:**

- i. **Protocols approved:** None
- ii. **Outstanding review issues/residual risk:** See CR comment Section
- iii. **Future inspection points to consider:** None

b. **Drug Product**

- i. **Protocols approved:** None
- ii. **Outstanding review issues/residual risk:** See CR comment Section
- iii. **Future inspection points to consider:** None

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

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



Zihao Peter  
Qiu

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**BLA STN 761081/1**  
**Product [Trazimera, a proposed biosimilar to US-Herceptin]**  
**Manufacturer [Pfizer]**

**OBP, Product Reviewer**  
**Kevin (Cishan) Li**  
**OPF/DMA, DS/DP Microbiology Reviewer**  
**Maria Jose Lopez-Barragan**  
**OPF/DMA, DP Microbiology Reviewer**  
**Virginia Carroll**  
**OPF/DIA, Facilities Reviewer**  
**Zhong Li**  
**OPRO RBPM**  
**Keith Olin**  
**ATL**  
**Rachel Novak**

## OPQ CMC BLA Review Data Sheet

1. **BLA#:** STN 761081
2. **REVIEW DATE:** April 20, 2018
3. **PRIMARY REVIEW TEAM:**  
**Medical Officer:** Sara Horton and Laleh Amiri Kordestani (TL and CDTL)  
**Pharm/Tox:** Claudia Miller and Todd Palmby (TL)  
**RPM:** Clara Lee  
**Product Quality Team:** Kevin (Cishan) Li and Rachel Novak (ATL)  
**CMC Microbiology:** Maria Jose Lopez-Barragan, Virginia Carroll  
**Facilities:** Zhong Li  
**Clinical Pharmacology:** Christy John and Sarah Schrieber (TL)  
**CMC Statistics:** Yu-yi Hsu, Meiyu Shen and Yi Tsong (TL)  
**Clinical Statistics:** Hui Zhang and Jason Schroeder (TL)  
**OBP Labeling:** Vicky Borders-Hemphill  
**RBPM:** Keith Olin  
**RPM:** Clara Lee
4. **MAJOR 21<sup>st</sup> Century Review DEADLINES**  
**Filing Meeting:** August 4, 2017  
**Mid-Cycle Meeting:** November 30, 2017  
**Wrap-Up Meeting:** March 6, 2018  
**Primary Review Due:** March 9, 2018  
**Secondary Review Due:** March 16, 2018  
**CDTL Memo Due:** April 10, 2018  
**BsUFA Action Date:** April 22, 2018
5. **COMMUNICATIONS WITH APPLICANT AND OND:**

Communication/Document	Date
Information Request	
IR #1 Analytical similarity testing sites	07/27/2017
IR #2 Recoverable protein content	09/01/2017
IR #3 Analytical similarity	11/07/2017
IR #4 DS Process	12/26/2017
IR #5 Analytical similarity – Label strength and follow up on charge variants	01/04/2018
IR #6 DS Process, Method and Stability	01/17/2018
IR #7 Stability of WCB	02/05/2018
IR #8 Tiered ranking for HER2 binding	02/09/2018
IR #9: Filling with check and compatibility data	02/09/2018
IR #10 Stability of MCB	02/13/2018

IR #11 DP process	02/22/2018
IR #12 Comment on WCB deficiency	03/15/2018
IR #13 Remove RM qualification protocol, (b) (4) protocols, and comparability protocol	04/17/2018

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

Submission	Date Received	Review Completed (Yes/No)
761081/0 (Original submission)	06/22/2017	Yes
761081/3 (Response to IR #1, analytical testing sites for similarity)	08/08/2017	Yes
761081/12 (Response to IR #2, extractable content)	09/22/2017	Yes
761081/18 & 26 (Response to IR #3, similarity)	11/22/2017 01/31/2018	Yes
761081/23 (Response to IR #4, DS process description)	01/22/2018	Yes
761081/22 & 30 (Response to IR #5, Label strength and charge variants)	01/16/2018 02/13/2018	Yes
761081/27 (Response to IR #6, DS process, characterization, method, stability)	02/05/2018 02/21/2018	Yes
761081/28 (Response to IR #7, WCB stability)	02/09/2018	Yes
761081/36 (Response to IR #8, HER2 binding tier reassignment)	02/21/2018	Yes
761081/31 (Response to IR #9, filling weight check and compatibility data)	02/16/2018	Yes
761081/37 (Response to IR #10, MCB Stability)	02/21/2018	Yes
761081/39 (Response to IR #11, DP process IR)	03/01/2018	Yes
761081/41&42 (Response to IR #12, Comment on WCB deficiency, including additional MCB stability data)	03/26/2018 04/10/2018	No
761081 (Response to IR #13, protocols removal)	04/18/2018 (by email)	The sponsor removed the requested protocols

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

a. Proprietary Name: Trazimera

- b. Trade Name: Trazimera
- c. Non-Proprietary Name/USAN: trastuzumab – qyyp
- d. CAS Name: 180288-69-1
- e. Common Name: PF-05280014
- f. INN Name: trastuzumab
- g. Compendial Name
- h. OBP systematic name: MAB HUMANLIZED (IgG1k) ANTI P04626 (HER2\_HUMAN) [PF-05280014]
- i. Other names: N/A

- 8. **PHARMACOLOGICAL CATEGORY:** HER2/neu receptor antagonist
- 9. **DOSAGE FORM:** for injection
- 10. **STRENGTH/POTENCY:** 420 mg/vial
- 11. **ROUTE OF ADMINISTRATION:** intravenous infusion
- 12. **REFERENCED MASTER FILES:**

DMF #	HOLDER	ITEM REFERENCED	Letter of Cross-Reference	COMMENTS (STATUS)
(b) (4)			Yes	No review required. Sufficient information in the BLA.
			Yes	No review required. Sufficient information in the BLA.
			Yes	No review required. Sufficient information in the BLA.
			Yes	No review required. Sufficient information in the BLA.
(b) (4)	Pfizer Manufacturing Belgium NV	Drug Product Manufacturing Facilities and Equipment	Yes	No review required. Sufficient information in the BLA.

13. **INSPECTIONAL ACTIVITIES**

A PLI of the (b) (4) for the drug substance manufacturing was conducted from (b) (4) by Maria J. Lopez-Barragan (Lead Inspector), Qing (Joanna) Zhou (OBP) and Kevin (Cishan) Li (OBP). A two-item FDA Form 483 was issued to (b) (4) at the end of the inspection which included inadequate SOPs and an inadequate cleaning validation protocol for equipment used in the

manufacture of PF-05280014 drug substance. In addition, four recommendations were conveyed to the firm at the end of the inspection. Following the response provided by the applicant, the inspection outcome is classified as VAI.

A PLI of the Wyeth Biopharma (Andover, MA: FEI 1222181) for the analytical similarity testing was conducted from October 10-12, 2017 by Sean R Marcsisin (ORA, Lead Investigator) and Kristen Nickens. No inspectional observations were noted and no 483 letter was issued.

The PLI inspection for the drug product manufacturing facility (Belgium, FEI 1000654629) was waived based on a facility profile evaluation, which was found to be acceptable.

14. **CONSULTS REQUESTED BY OBP: NONE**

15. **QUALITY BY DESIGN ELEMENTS**

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

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

16. **PRECEDENTS: None**

## **SUMMARY OF QUALITY ASSESSMENTS**

### **I. Primary Reviewer Summary Recommendation**

Sufficient analytical data are provided to support that PF-05280014 is highly similar to US-Herceptin and to support the establishment of the three-way analytical bridges among PF-05280014, US-Herceptin, and EU-Herceptin. However, OBP recommends a Complete Response action for this 351 (k) application due to several deficiencies, the most notable is regarding the instability of current Working Cell Bank (WCB) and the residual uncertainty of the stability of the Master Cell Bank (MCB). Because the WCB is not considered stable, the current manufacturing process is not considered well controlled to ensure a consistent production of safe, pure and potent product.

**II. List Of Deficiencies To Be Communicated:** Outstanding issues have been communicated in the April 20, 2018 Complete Response Letter.

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

**IV. Review Of Common Technical Document-Quality Module 1**

**Environmental Assessment or Claim of Categorical Exclusion**

A categorical exclusion from the requirement of an environment assessment was requested by Pfizer under 21 CFR Part 25.31(b).

*Reviewer comment: The claim of a categorical exclusion is appropriate.*

**V. Primary Container Labeling Review**

The OBP review of the DP labeling was performed separately by Vicky Borders-Hemphill (see review in Panorama).

**VI. Review Of Common Technical Document-Quality Module 3.2**

The review of Module 3.2. is provided below.

**VII. Review of Immunogenicity Assays – Module 5.3.1.4**

The review of the immunogenicity assay validations is included following the DP review below.

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## DESCRIPTION OF DRUG SUBSTANCE AND DRUG PRODUCT

*Note: Reviewer's notes and comments are emphasized in italics and all comments to the sponsor are summarized at the end of this review. The proposed Pfizer biosimilar product is referred to as "PF-05280014", the US-licensed product is referred to as "US-Herceptin", and the EU-approved product is referred to as "EU-Herceptin" throughout the review. Unless otherwise indicated, figures and tables in the review memo are directly excerpted from the submission.*

*In addition, the US licensed-Herceptin multiple-dose vial label claim was recently changed from 440 mg to 420 mg in April 2017 per current Herceptin label. The 440 mg presentation is equivalent to the 420 mg for the reference products. The current PF-05280014 presentation was developed to match the extractable protein content of 420 mg. The sponsor updated the BLA Module 3 section to reflect the change in response to Agency's IR (03/01/2018). For ease of review, all multi-dose vial strength was changed to 420 mg whenever possible. However, some of the figures and tables in this review still refer to the presentation as 440 mg presentation.*

*Multiple IRs were sent throughout the review. The responses are incorporated in the final review. Only responses that require detailed assessment or are inadequate are discussed in details in the review.*

## ABBREVIATIONS

---

<b>Abbreviation</b>	<b>Definition</b>
AR	acceptable range
AEX	anion exchange chromatography
ADA	anti-drug-antibodies
ADCC	antibody-dependent cell-mediated cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
BWFI	bacteriostatic water for injection
BLA	Biologics License Application
BPD	biosimilar product development
CGE	Capillary gel electrophoresis
CEX	cation exchange chromatography
CMC	Chemistry, Manufacturing and Control
CDC	complement-dependent cytotoxicity
CCI	container closure integrity
CMA	critical material attributes
CQA	critical quality attribute
CPP	critical process parameter
CPD	cumulative population doublings
DF	diafiltration
DO	dissolved oxygen
DMA	division of microbiological assessment
DP	drug product
DS	drug substance

EOP	end of product
EU	European Union
E&L	Extractables and leachables
Fab	fragment antigen-binding
Fc	fragment crystallizable
FcRn	fragment crystallizable receptor neonatal
HC	heavy chain
HHL	Heavy-heavy-light chains fragments of a mAb
HCP	host cell protein
HER2	human epidermal growth factor receptor 2
HM	high mannose
HMMS	high molecular mass species
IgG	immunoglobulin
IPC	in-process controls
IV	intravenous
LC	light chain
L	light chain fragment of a mAb
LIVCA	limit of in vitro cell age
LRV	log <sub>10</sub> reduction value
LMMS	low molecular mass species
MCB	master cell bank
MoA	mechanism of action
mAb	monoclonal antibody
MMV	mouse minute virus
NK	natural killer
NAb	neutralizing antibody
NOR	normal operation range
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PI3K	phosphoinositide 3-kinase
PLI	pre-licensure inspection
PK	pharmacokinetics
PTM	post translational modification
PP	process parameter
PPQ	process performance qualification
PHS	Public Health Service
QA	quality attributes
QbD	Quality-by-Design
RM	reference materials
RGA	reporter gene assay
SWFI	sterile water for injection
SPR	surface plasmon resonance
TCV	temperature-controlled vehicle
TOC	total organic contaminants
UF	Ultrafiltration
V/F	valine/phenylalanine

WFI	water for injection
WCB	working cell bank
xMuLV	Xenotropic Murine Leukemia virus

### 3.2. S. DRUG SUBSTANCE – PF-05280014 Drug Substance

#### 3.2. S.1.1 Nomenclature

- INN for Reference Product: Trastuzumab
- INN for PF-05280014: To be determined
- Chemical Name (IUPAC): Not applicable
- Internal Company or Laboratory code: PF-05280014 or trastuzumab-Pfizer
- CAS Number: 180288-69-1

#### 3.2. S.1.2 Structure

PF-05280014 is an IgG1 kappa monoclonal antibody (mAb) directed against the HER2 antigen. It contains two identical HCs and two identical LCs, covalently linked with four inter-chain disulfide bonds. It contains one Asn linked glycosylation site at Asn300 in the Fc region, which is occupied with nonsialylated, core-fucosylated, complex type biantennary N-linked glycans with zero to one terminal galactose residues, abbreviated as G0F and G1F, respectively. The theoretical molecular masses are 148057.4 Da (G0F/G0F), 148219.6 (G0F/G1F) and 148381.7 (G1F/G1F). The theoretical isoelectric point (pI) is 8.6. The experimental absorption coefficient at 280 nm is  $1.46 \text{ (mg/mL)}^{-1} \text{ cm}^{-1}$ . The protein sequences are shown in the figure below.

**Figure 2.3.S.1-1. PF-05280014 Primary Structure (Amino Acid Sequence)**

**Light (L) Chain**

```

1 DIQMTQSPSSLSASVGRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS 60
                                     |
61 RFSGSRSGTDFTLTISSLPEDFATYYCQOHYTPPTFGQGTKVEIKRTVAAPSVFIFPP 120
                                     |
121 SDEQLKSGTASVVCLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLT 180
                                     |
181 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                               214
                                     |
                                     H Chain
    
```

**Heavy (H) Chain**

```

1 EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY 60
                                     |
61 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSS 120
                                     |
121 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS 180
                                     |
181 GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG 240
                                     |
                                     L Chain ←
                                     → H Chain
241 PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN 300
                                     |
301 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE 360
                                     |
361 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRW 420
                                     |
421 QQGNVFSCSVMHEALHNHYTQKSLSLSPG (K)                               450
    
```

PF-05280014 amino acid sequence for the individual L and H chains; residues are numbered sequentially starting with the respective N termini. The predicted intra- and inter-chain disulfide bonds are illustrated with connecting lines. The putative complementarity-determining regions are underlined, the N-linked glycosylation consensus sequence appears in bold italics, and the essentially absent C-terminal K is shown in parentheses.

**3.2. S.1.3 General Properties**

PF-05280014 is a proposed biosimilar to US-licensed Herceptin (trastuzumab). Trastuzumab is a monoclonal antibody that binds to HER2 and is licensed for the treatment of HER2 positive breast cancer. IgG1The mechanisms of action of trastuzumab includes 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.

**3.2. S.2 Manufacture**

### 3.2.S.2.1 Manufacturer(s)

The following table describes the corresponding manufacturing, testing and storage of the PF-05280014 DS and cell banks.

**Table 3.2.S.2.1-1. Sites and Responsibilities for Manufacture and Testing of PF-05280014 Drug Substance**

Site	Responsibility
Wyeth BioPharma Division of Wyeth* Pharmaceuticals Inc. One Burtt Road Andover, MA 01810 USA	Cell bank manufacture and storage
(b) (4)	Drug substance manufacture Drug substance in-process control testing and release testing (except Potency Assay) Drug substance and cell bank storage
Pfizer Ireland Pharmaceuticals Grange Castle Business Park Clondalkin, Dublin 22 Ireland	Cell bank storage Drug substance release testing and stability testing
(b) (4)	Release and stability testing (Potency Assay only)

\* Wyeth is a wholly owned subsidiary of Pfizer Inc.

An IR (IR #6 Comment 2a) was sent for the sponsor to clarify which release and stability tests are performed at which sites. In response (02/05/2018), the sponsor provided the above table to clarify release/stability tests performed at each testing site for PF-05280014.

**Reviewer comment:** Potency (cell inhibition) assay is performed at both PPD and Pfizer Grange Castle sites for DS/DP testing. See review in S.4.2 regarding method transfer, bridging and validation among sites.

### 3.2.S.2.2 Description of Manufacturing Process and Process Controls

(b) (4)

### **3.2. P.8 Stability**

#### **3.2.P.8.1 Stability Summary and Conclusion**

Primary stability studies were conducted using the three clinical DP batches to establish the proposed DP shelf life. Additional stability studies were conducted using the three PV batches. The studied conditions include long term (2-8°C), accelerated (28-32°C/70-80% relative humidity), thermal stress (-20, 40 and 50°C), thermal cycling (between 2-8°C and 28-32°C) and photostability conditions. Up to 48 months long term stability data are available. The proposed shelf life is 48 months at 2-8°C. The sponsor is requesting that the DP may also be stored at  $\leq 30^{\circ}\text{C}$  for a single period up to 3 months within the original expiration date.

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

The sponsor committed to enroll a minimum of one DP lot into the commercial stability program at 2-8°C each year that DP is manufactured. The commercial stability protocol is provided in the table below.

**Table 3.2.P.8.2-1 Post-Approval Commercial Stability Protocol for PF-05280014  
440 mg Drug Product Stored at 5 ± 3 °C**

Analytical Procedure/ Quality Attribute		Test Interval (months)
Appearance (Before reconstitution)		0, 12, 24, 36, 48, 60
Appearance (Clarity)		0, 12, 24, 36, 48, 60
Appearance (Coloration)		0, 12, 24, 36, 48, 60
Appearance (Visible Particulates)		0, 12, 24, 36, 48, 60
Moisture Content		0, 12, 24, 36, 48, 60
Reconstitution Time		0, 12, 24, 36, 48, 60
pH		0, 12, 24, 36, 48, 60
UV Spectroscopy	Protein Concentration	0, 12, 24, 36, 48, 60
Particulate Matter	Particles ≥ 10 µm	0, 12, 24, 36, 48, 60
	Particles ≥ 25 µm	
CEX-HPLC	Acidic Species	0, 12, 24, 36, 48, 60
	Main Species	
	Basic Species	
SE-HPLC	Monomer	0, 12, 24, 36, 48, 60
	HMMS	
CGE (reducing)	Heavy Chain + Light Chain	0, 12, 24, 36, 48, 60
	Fragments	
CGE (non-reducing)	Intact IgG	0, 12, 24, 36, 48, 60
Cell-based Assay	Potency/Biological Activity	0, 12, 24, 36, 48, 60
Container Closure Integrity Test		12, 24, 36, 48
Sterility		0, 60
Endotoxin		0, 60

Abbreviations: CEX-HPLC = Cation exchange high performance liquid chromatography; SE-HPLC = Size exclusion high performance liquid chromatography; CGE = Capillary gel electrophoresis; HMMS = High Molecular Mass Species; IgG = Immunoglobulin G

**Reviewer comment:** *The sponsor will be asked in the CR letter to add assays to monitor ADCC activity and Asp102 isomerization for PF-05280014 DP on stability. In addition, the sponsor should monitor the stability of PF-05280014 at 30°C for up to 3 months if this claim will be part of the storage conditions for the package insert. This will also be communicated in the CR letter.*

### 3.2.P.8.3 Stability Data

A summary of the stability data is provided below:

- Under long term storage conditions (2-8°C), no changes were noted up to 48 months.
- Under accelerated storage conditions (28-32°C), an increase of ~1% HMMS, an increase of ~5% in basic species, a decrease of ~2% in acidic species and ~2% in main species, were observed by the 12-month time point. No other trends were noted up to 12 months.
- Under thermal stressed conditions (-20, 40 and 50°C), no changes were noted at one month at -20°C; an increase of ~0.6% in HMMS, an increase of ~2% in basic species, a

decrease of ~2% in acidic species were observed at one month at 40°C; an increase of ~1 % in HMMS, an increase of ~5% in basic species, a decrease of ~2% in acidic species and ~3% in %main species were observed at one month at 50°C.

- Under photostability condition (ICH Q1B, Option 2), an increase of ~5% in basic species and acidic species, and a decrease of ~9% in main species were observed for naked vials. No changes were noted for vials protected in commercial packaging.
- Under thermo-cycling conditions (see table below for detailed description below), no degradation was noted at 2-8°C; an increase of 0.8% HMMS, an increase of 3.6% in basic species, and a decrease of 1.6% in intact IgG were observed in the worst case during the 6 month study period at 30°C.

**Table 3.2.P.8.1-7. PF-05280014 Drug Product Thermal Cycling Study Conditions**

Study Type	Study Conditions	Study Objective
Thermal Cycling 1	Drug product is stored at 5 ± 3 °C for 48 months then stored at 30 ± 2 °C/75 ± 5% RH for 6 months.	To provide label claim support of storage of up to 6 month at 30 ± 2 °C/ 75 ± 5% RH in addition to long term storage at 5 ± 3 °C.
Thermal Cycling 2	Drug product is stored at 30 ± 2 °C/75 ± 5% RH for 6 months then stored at 5 ± 3 °C for 42 months.	
Thermal Cycling 3	Drug product is stored at 5 ± 3 °C for 30 months then stored at 30 ± 2 °C/75 ± 5% RH for 6 months.	
Thermal Cycling 4	Drug product is stored at 5 ± 3 °C for 42 months then stored at 30 ± 2 °C/75 ± 5% RH for 6 months.	
Thermal Cycling 5	Drug product is stored at -20 ± 5 °C for 1 month and then stored at 5 ± 3 °C for the duration of the study.	To provide temporary excursion support below the intended storage condition. Study is designed to support situations when product is frozen for a short duration and then stored at the recommended storage temperature.
Thermal Cycling 6	Drug product is stored is cycled through 3 cycles of 3 days at -5 ± 2 °C and 3 days at 30 ± 2 °C/75 ± 5% RH. Drug product is then stored at 5 ± 3 °C for the duration of the study.	To provide temporary excursion support when product is cycled below the intended storage condition (but not frozen) and above the intended storage condition for short durations.

**Reviewer comment:** *There is minimal degradation observed for DP under long term storage condition. The data support the proposed 48 month shelf life.*

*The primary degradation pathways under accelerated conditions (30°C) include increases in basic species and HMMS and the decrease of intact IgG. An additional storage for the unopened DP vials at 30°C for up to 3 months is proposed in the label. While the stability data provided show the changes in quality attributes tested are minor, the stability testing does not include an evaluation of ADCC and iso Asp102, which might be impacted by this storage condition. The sponsor will be asked to provide additional information and data to support this storage condition. This will be communicated in the CR letter. .*

**In Use Stability:** Upon reconstitution with BWFI, PF-05280014 vial can be stored at 2-8°C up to 28 days. To validate the storage time after reconstitution, the three PV batches DP were stored at long term storage condition for 0/12/24/36/48/59 months. At each time point samples were reconstituted with BWFI, stored at 2-8°C for 28 days and tested for in use stability (1/13/25/37/49/60 months). Up to 13 months stability data were provided. Very small increase in basic species (~ 2%) was noted during the 28 day period. No other changes on product quality were noted.

**Reviewer comment:** *The data provided support the storage period of 28 days at 2-8°C after reconstitution from a quality prospective for the quality attributes assessed; however, no assessment of ADCC activity and Asp102 isomerization was performed, which might be impacted by this storage condition. The sponsor will be asked to provide additional information and data to support this storage condition. This will be communicated in the CR letter.*

The proposed shelf life is 48 months at 2-8°C. The sponsor is requesting that the DP may also be stored at ≤30°C for a single period up to 3 months within the original expiration date.

**Reviewer Comment:** *the proposed shelf life supported by real time stability data. The expiry date is acceptable.*

### 3.2.R.3 Analytical Similarity

**Reviewer Comment:** *In April 2017, the US-Herceptin multiple-dose vial labeling was revised from a stated content of 440 mg to 420 mg. PF-05280014 was developed to be the same strength as US-Herceptin 420 mg multiple-dose vial. Based on the data summarized in this section and manufacturing information/data provided in Sections 3.2.P.2 and 3.2.P.3, PF-05280014 has the same strength (420 mg) as US-licensed Herceptin. Of note, some of the figures and tables excerpt from the Applicant submission and copied in this review still refer to both PF-05280014 and US-Herceptin presentations as 440 mg presentation.*

### General Information and Overview

PF-05280014 is developed as a biosimilar to US-Herceptin. The PF-05280014 drug product (DP) is developed as a lyophilized powder in a dosage strength of 420 mg, the same as US-Herceptin. Reconstitution with 20 mL of either Sterile Water for Injection (SWFI) or the supplied Bacteriostatic Water Injection (BWFI) yields a solution of 21 mg/ml DP in a formulation of (b) (4) mM histidine buffer, (b) (4) mg/mL sucrose, and (b) (4) mg/mL polysorbate 20, pH 6.0. The DP is supplied in a 30 ml glass vial sealed with a stopper and an aluminum seal with flip-off cap.

Trastuzumab is an IgG1 mAb that binds to HER2 antigen on breast cancer cells and subsequently inhibits cell growth. The binding of the antibody to effector cells also mediates antibody dependent cellular cytotoxicity (ADCC). US-Herceptin has been approved for the treatment of HER2-overexpressing adjuvant breast cancer, metastatic breast cancer and metastatic gastric cancer.

To support a demonstration of no clinically meaningful differences between PF-05280014 and US-Herceptin, Pfizer conducted four clinical studies (B3271001, B3271006, B3271002, and

B3271004). Studies B3271002 and B3271004 evaluated the safety and efficacy of PF-05280014 in comparison to EU-Herceptin. The clinical studies are described in detail in the clinical review for this application.

Pfizer performed extensive analytical characterization studies using PF-05280014, US-Herceptin and EU-Herceptin to establish high similarity between PF-05280014 and US-Herceptin and to establish the analytical portion of the scientific bridge between PF-05280014, US-Herceptin and EU-Herceptin to be able to leverage clinical data generated from studies using EU-Herceptin. Information regarding methods used in the analytical similarity assessment can be found in 3.2.S.4.2 (Analytical Procedures) and 3.2.R.3.1.1 (Description of Characterization Methods). The release methods were all validated (see Section S.4.3) and other characterization methods were shown to be suitable for their intended use. Additional technical reports for the function assays (binding to HER2 target antigen by SPR, primary NK cell ADCC assay, FcγRIIIa RGA, binding to Fcγ receptors and FcRn by SPR) were provided in 3.2.R.3.8 Technical Reports.

**Reviewer Comment:** For a review of the validation of methods used for release, see Section 3.2.S.4 or 3.2.P.5. Adequate descriptions of the characterization methods were provided.

### **Risk Ranking and Statistical Analysis of Quality Attributes**

Quality attributes (QAs) related to similarity were ranked according to their potential impact on activity, PK/PD, safety and immunogenicity. Attributes that have the highest level of risk (relevant to the clinical mechanism(s) of action of trastuzumab) are assigned to Tier 1. Attributes having moderate risk are assigned as Tier 2 and the lowest are Tier 3.

The statistical analysis of the QAs applies varying rigor depending on which tier is assigned. Equivalence testing was used for the assessment of attributes assigned to Tier 1. Quality range (Mean ± 3 SD) was used to evaluate attributes assigned to Tier 2. Attributes assigned to Tier 3 were evaluated in table or graphical format for visual comparisons. The statistical methods used in the equivalence test and quality range analysis were provided in Section 3.2.R.3.1.1.2.4 and are not shown here.

The tiered rankings for PF-05280014 quality attributes are summarized by the reviewer in the table below.

<b>Quality Attribute</b>	<b>Analytical Procedure</b>	<b>Statistical Tier</b>
Potency	Inhibition of cell growth assay Primary NK cell ADCC assay	1
Potency	Binding to HER2 target antigen by SPR FcγRIIIa reporter gene assay Binding to FcγRIIIa by SPR Binding to FcRn by SPR	2
N-linked glycan (total afucosylation and terminal galactosylation were used for statistical	N-linked glycan profile by HILIC	2

tiers)		
Acidic and basic species	Charge heterogeneity by CEX-HPLC	2
Monomer	Purity assessment by SE-HPLC	2
High molecular mass species (HMMS)	Purity assessment by SE-HPLC	2
Protein Concentration (after reconstitution)	Protein concentration	2
Identity	Amino acid sequence (de novo sequencing) Molecular mass (nanoESI MS) Molecular size (Reducing CGE) Primary structure (LC/MS subunit analysis) Primary structure (LC/MS peptide mapping (Trypsin)) Isoform Identification (LC/MS subunit analysis) Higher order structure	3
Potency / Biological activity	Binding to cell surface HER2 target antigen by flow cytometry HER2 and HER3 phosphorylation by Western blot PBMC ADCC assay Apoptosis assay Binding to Fc $\gamma$ RI, Fc $\gamma$ RIIa, Fc $\gamma$ RIIb and Fc $\gamma$ RIIIb SPR CDC assay C1q binding assay	3
N-linked glycan profile	N-linked glycan profile by HILIC (fluorescence and MS detection) N-linked glycan profile by exoglycosidase analysis Sialic acid analysis	3
Charge Heterogeneity (acidic, main, and basic species)	Charge heterogeneity characterization by MS Charge heterogeneity characterization by CEX-HPLC (with and without CPB) CEX-HPLC as part of comparative forced degradation	3
HC + LC	Purity assessment by CGE (reducing)	3
Fragments	Purity assessment by CGE (reducing)	3
Comparative forced degradation -Fragments	Purity assessment by CGE (reducing)	3
Comparative forced degradation - Monomer	Purity assessment by SE-HPLC	3
Comparative forced degradation - High Molecular	Purity assessment by SE-HPLC	3
Comparative forced	Protein concentration	3

degradation - Protein Concentration (after reconstitution)		
Comparative forced degradation – Sub-visible Particles	Sub-visible particles (elevated temperature study)	3

**Reviewer Comment:** *The proposed QA rankings are largely consistent with the comments provided by the Agency at the IND 110427 BPD Type 2 meeting held on 09/01/2015. The HC+LC purity and fragments by reducing CGE are ranked as Tier 3, rather than Tier 2; however, Tier 2 analysis is performed by the reviewer. The purity by non-reducing CGE was not ranked originally but the sponsor provided Tier 2 analysis in response to Agency’s IR #3 Comment 3 (11/22/2017). High mannose forms of the N-linked glycans were not characterized; however, based on the data provided, Tier 2 analysis was performed by the reviewer. Refer to review below regarding the similarity assessment of these QAs.*

*The equivalence tests for Tier 1 were reviewed by CMC STAT reviewer Yu-Yi Hsu.*

### **Lots Enrolled In Similarity Assessment – US-Herceptin and EU-Herceptin**

Sixty-four US-Herceptin lots (420 mg presentation) and 74 EU-Herceptin lots (150 mg presentation) were included in the similarity assessment; however, not all lots were used for every assay. The expiry date of the lots ranged from 10/2012 to 06/2019. At the time of testing, individual lots had 12 to 44 months shelf life remaining. There are no apparent degradation trends observed with the age of the product (data provided in Section 3.2.R.3.1.2 Lots Enrolled in Similarity Assessment and not included here).

It was noted by the Applicant during the analysis of US-Herceptin and EU-Herceptin lots that lots with expiry between January 2017 and June 2019 have a shift in the glycan profile including total afucosylation, terminal galactosylation and G0 species. Throughout the submission, the Applicant has designated lots prior to the glycan shift as “pre-glycan shift” lots and the ones after are called “post-glycan shift” lots. Data analysis shows that the “post-glycan shift” lots have lower ADCC and FcγRIIIa binding activity compared to the “pre-glycan shift” lots. Similar trends were observed in FcγRIIIa 158V and 158F binding by SPR. In the analytical similarity assessment below, the Applicant provided data for all US-Herceptin and EU-Herceptin “pre- and post-glycan shift” lots tested. For the Tier 1 NK cell ADCC assay and for Tier 2 assays for terminal galactosylation, total afucosylation, FcγRIIIa RGA and FcγRIIIa SPR binding tests, the statistical analysis conducted by the Applicant excluded the “post-glycan shift” lots data for assessment of statistical equivalence and quality ranges, respectively.

**Reviewer comment:** *The assessment of statistical equivalence for NK-ADCC was performed by CMC STAT reviewer Yu-yi Hsu that included all reference product lots. The data establish equivalence among PF-05280014, EU-Herceptin and US-Herceptin for the NK ADCC assay.*

*While Pfizer provided raw data for all US-Herceptin and EU-Herceptin lots for all Tier 2 QAs that might be affected by the glycosylation differences, Pfizer states that the current*

*manufacturing process was developed against the "pre-glycan shift" product profiles and therefore used only the "pre-glycan shift" lots for the quality range analysis for these QAs in the review below. This is acceptable because the quality ranges established based on the smaller subset of US-Herceptin and EU-Herceptin lots (11-64 "pre-glycan shift" lots, depending on the assay) were always more stringent than the quality ranges established based on a combination of those and ~10 post-glycan shift lots in the assays analyzed. From the reviewer's perspective, the assessment of analytical similarity of PF-05280014 to US-Herceptin will be based on the information provided for all reference product lots.*

The clinical lots for US-Herceptin, EU-Herceptin and PF-05280014 were provided in Section 2.7.1 and summarized in the table below.

## Appendix 1. Lot Number of Study Drugs Administered in Clinical Studies

Description of Study	Test Samples	Lot Number(s) <sup>a</sup>
Study B3271001	PF-05280014	12-000813(Z00515)
	Trastuzumab-EU	12-000381(H0773B01)
	Trastuzumab-US	991942
Study B3271002	PF-05280014	14-000547 (A08735) 15-004815 (L25296) 12-003164 (Z04027) 12-002983 (Z02359)
	Trastuzumab-EU	12-005681(H4135B02), 12-005682 (H4142B02), 12-005413(H4111B04), 12-005680 (H4134B03), 13-109694 (H4198B01), 12-000381(H0773B01) 12-005411(H4112B02), 12-005412 (H4101B03), 13-106741 (H4112B02), 12-005410 (H4115B01), 13-106742 (H4133B02), 12-005679 (H4115B01) 13-106739 (H4115B01), 12-005678 (H4112B02), 15-001001(H4514H02), 15-002057(N1023H04), 15-006290( H4594H04), 13-111302(H4296B01) 14-003788(H4433B01), 15-000964 (H4498H01), 15-004359 (H4543H01), 15-007546 (N1037H01), 12-005409 (H4079B07), 12-005677 (H4079B07) 13-106736 (H4079B07), 13-109689 (H4208B02), 15-004079 (N1029H05), 14-004267 (H4459B01), 15-004546 (N1030H03), 14-001466 (H4384B01) 15-003457 (H4435B01), 16-000738 (N1041H02)
Study B3271004	PF-05280014	14-000547 (A08735) 12-003164 (Z04072)
	Trastuzumab-EU	13-111300 (H4268B06) 12-000656 (H0775B01) 13-111301 (H4268B06) 13-109687 (H4259B01)
Study B3271006	PF-05280014	12-003164 (Z04072) 12-002983(Z02359) 12-000813(Z00515)
	Trastuzumab-US	13-111451(554763) 13-111452 (554761) 13-111453 (566304)

Abbreviations: EU = European Union; US = United States.

a. Drug product lot numbers. The nominal protein concentration after reconstitution is 21 mg/mL.

**Reviewer Comment:** The number and the age ranges of the reference US-Herceptin and EU-Herceptin lots selected for similarity assessment are adequate. Trastuzumab is relatively stable in the final presentation (lyophilized form) and shows no degradation trends within shelf life under normal storage conditions. Of note, EU-Herceptin lots spanning the entire range of expiry dates were used in the comparative Study B3271002.

**Lots Enrolled In Similarity Assessment – PF-05280014:**

Eight DS batches and 13 DP lots were produced at the commercial scale and included in the similarity assessment. In addition, three development DS lots (D11L122E003/11P122L601/11P122L001) and clinical reference material 124281pg9, derived from development DS D11L122E003, were also included. The lot genealogy of all Pfizer DS/DP lots is summarized by the reviewer in the table below.

Drug Substance		Drug Product		
Batch Number	Manufacturing Scale (L)	Drug Product Lot Number	Dose Presentation (mg)	Drug Product Lot Use
11P122L601	(b) (4)			
11P122L001				
D11L122E003				
88200		Y09020	150	Engineering Batch
		Y09741	420	Engineering Batch
88201				
88000		Z00515	150	Stability, Nonclinical study 13GR047, Clinical Studies B3271001 and B3271006
		Z00514	420	Stability, Clinical Inventory
88001		Z02359	150	Stability, Clinical Study B3271006
		Z03695	420	Stability, Clinical Inventory
88002		Z04072	150	Stability, Clinical Studies B3271004 and B3271006
		Z03694	420	Stability, Clinical Inventory
		A08735	150	Clinical Studies B3271001, B3271002 and B3271004

88003	(b) (4)	L25296	150	Process Validation, Stability, Clinical Study B3271002
		M55891	420	Process Validation, Stability, Clinical Inventory
88005				
88003 & 88005		M04478	150	Process Validation, Stability, Clinical Inventory
		M52674	420	Process Validation, Stability, Clinical Inventory
88006		M10096	150	Process Validation, Stability, Clinical Inventory
		M55890	420	Process Validation, Stability, Clinical Inventory

	Lots used in the statistical analysis, D11L122E03 lot was used only for cell inhibition due to sample limitation.
	Additional lots used in similarity comparison but not in statistical analysis.
	Not applicable

For lot selection, PF-05280014 lots used for Tier 1 and Tier 2 evaluations were based on the following criteria: independent DP lots of the 420 mg presentation (to correspond to US-Herceptin presentation), DS lots without a corresponding clinical DP lot and only one DP lot was selected if there multiple DP lots were derived from the same DS lot.

A total of 11 independent DS/DP lots were identified. One DS lot D11L122E003 was only used for the statistical analysis of cell inhibition assay (Tier 1), basic and acidic species (Tier 2), monomer and HMMS (Tier 2) due to material limitation. The detailed lot information was provided in the table below.

**Table 3.2.R.3.1.2-1. Independent Pfizer Lots Selected for Statistical Analysis**

Statistical Tier	Methodology/Quality Attribute	Total Number of Independent Pfizer Lots	Pfizer Lots Selected
1	Inhibition of Cell Growth Bioassay	11	D11L122E003, 11P122L601, 11P122L001, 88201, 88200, 88005, Z00514, Z03694, Z03695, M55890, M55891
1	Binding to target (HER2)	10	11P122L601, 11P122L001, 88201, 88200, 88005, Z00514, Z03694, Z03695, M55890, M55891
1	Primary NK cell ADCC assay	10	11P122L601, 11P122L001, 88201, 88200, 88005, Z00514, Z03694, Z03695, M55890, M55891
2	FcγRIIIa reporter gene assay	10	11P122L601, 11P122L001, 88201, 88200, 88005, Z00514, Z03694, Z03695, M55890, M55891
2	FcγRIIIa (158F and 158V) binding (SPR)	6	88200, 88003, Z00514, Z03694, Z03695, M55890
2	FcRn binding (SPR)	6	88200, 130986-8-PRM, Z00514, Z03694, Z03695, M55890
2	Protein Concentration	5	Z00514, Z03694, Z03695, M55890, M55891
2	Total Afucosylation	6	D11L122E003, 11P122L601, 11P122L001, 88201, 88200, 88005, 88001, 88002, 88003, 88006
2	Terminal Galactosylation	6	D11L122E003, 11P122L601, 11P122L001, 88201, 88200, 88005, 88001, 88002, 88003, 88006
2	Basic Species	11	D11L122E003, 11P122L601, 11P122L001, 88201, 88200, 88005, Z00514, Z03694, Z03695, M55890, M55891

Statistical Tier	Methodology/Quality Attribute	Total Number of Independent Pfizer Lots	Pfizer Lots Selected
2	Acidic Species	11	D11L122E003, 11P122L601, 11P122L001, 88201, 88200, 88005, Z00514, Z03694, Z03695, M55890, M55891
2	Monomer	11	D11L122E003, 11P122L601, 11P122L001, 88201, 88200, 88005, Z00514, Z03694, Z03695, M55890, M55891
2	HMMS	11	D11L122E003, 11P122L601, 11P122L001, 88201, 88200, 88005, Z00514, Z03694, Z03695, M55890, M55891

**Reviewer Comment:** Data (CEX/ Reducing CGE/ SE-HPLC/ Potency/ A280) from four additional (b) (4) DP lots (b) (4) were provided in the Section 3.2.R.3.5 Appendix- Raw Data; however, the (b) (4) was not used in the similarity assessment and are not reviewed here.

Only the PF-05280014 150 mg presentation was used in the clinical studies, while the 11 independent lots utilized only data from the 420 mg presentation. Comparability data between PF-50280014 150 mg and 420 mg presentations were provided and reviewed in 3.2.P.2.3. The data demonstrate that both presentations are comparable and therefore, the use of the 420 mg presentation in the analytical similarity assessment is acceptable.

The selection of independent lots and the selection criteria are reasonable. The selected lots covered differences in manufacturing scale and sites and included materials used for development, clinical studies and process validation studies. For some Tier 2 QAs, only 5-6 independent lots were chosen. See review on individual QA regarding the adequacy of the similarity assessment.

*It was unclear if multiple reference standards were used to analyze samples in the analytical similarity assessment. An IR (IR #3 Comment 2) was sent on 11/07/2017 requesting the sponsor to clarify what reference standards were used in the analytical similarity assessment. Pfizer responded on 11/22/2017 stating that only one reference material (124281pg9) was used for the assessment of relative activity in all assays.*

### **Sample handling**

The sponsor provided data to support the suitability of the handling procedure for the reference product in Section 3.2.R.3.6 Appendix – Sampling Handling. Reference trastuzumab materials were stored at 2-8°C as instructed. Lots were reconstituted in water, aliquoted frozen and stored at -60°C to -90°C until further testing for similarity. Three reference product lots (one EU and two US) were subjected to the following studies to assess the impact of freezing and long term storage on product quality:

1. Freeze/Thaw (F/T) study for 3 cycles: frozen at -80°C and thawed for 1-3 cycles
2. Long term (3 years) storage at -60 to -90°C after reconstitution

Samples were analyzed before and after treatment by CEX, SEC, reducing and non-reducing CGE, HILIC, Met oxidation, bioassay, LC/MS-subunit analysis and Near UV CD. The data were provided in Section.3.2.R.3.6 – Sampling Handling and not copied here. A 0.5% decrease in fragments by reducing CGE was observed in the EU lot (H0750B01) between the initial and all subsequent timepoints in the long term stability study. A 0.8% decrease in fragment was observed in one US lot (849245) between initial and the 10 month timepoints before the study was discontinued due to limited quantity of the samples. No other notable changes were observed.

**Reviewer Comment:** *No degradation was detected in the reconstituted samples after storage at -60 to -90°C for 3 years. The data supported the use of reconstituted reference product stored at the specified storage condition for the similarity assessment.*

### **Assessment for Individual QAs**

**Note:** *An IR (IR #8, 02/21/2018) was sent requesting reassigning HER2 binding from Tier 1 to Tier 2 to reflect the current thinking at the Agency. In response (02/21/2018), the sponsor downgraded Her 2 binding from Tier 1 to Tier 2 and performed the statistical analysis.*

**Note:** *For the similarity assessment of Tier 1 and Tier 2 attributes, the sponsor provided figures for all PF-05280014 lots (24 lots) as well as the sub-set of 11 independent lots. Although the data from the 24 lots is supportive of the overall conclusion, the statistical analysis, and determination of similarity for Tier 1 and Tier 2 attributes, were based on analysis of the independent lots.*

### **Tier 1 QAs: Inhibition of Cell Growth and NK ADCC assay**

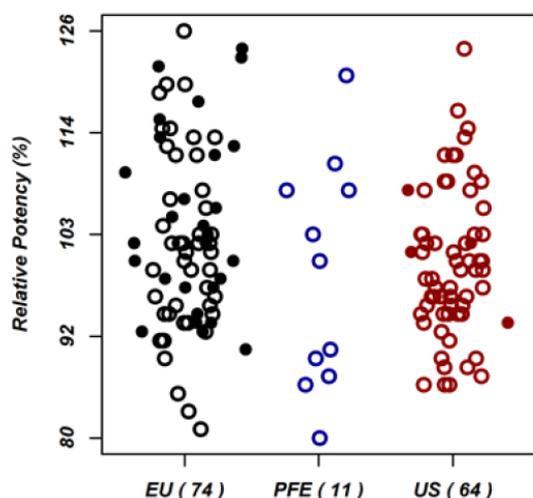
Inhibition of cell growth: The inhibition of cell growth is assessed using human breast cancer cell line SKBR3. The cells are incubated with different dilutions of trastuzumab for 5 days at

37°C. Cell proliferation is detected using a commercially available kit, CellTiter Glo, to measure the presence of ATP in the live cells. The results are reported as percent potency relative to RM 124281pg9.

**Reviewer comment:** *The use of commercial reagents in the cell growth assay can introduce variability. The same reference standard material was used when performing this assay for the analytical similarity assessment; therefore, any variability in commercial reagents would be captured.*

The dose response curves of representative PF-05280014, US-Herceptin and EU-Herceptin materials are all comparable to RM (data not copied here). PF-05280014 materials have potency values range of 80%-121%, comparable to the ranges of US-Herceptin (86%-124%) and EU-Herceptin (81%-126%). The potency results from PF-05280014 (11 lots), EU-Herceptin (74 lots) and US-trastuzumab (64 lots) are summarized in the figure below.

**Figure 3.2.R.3.2.2-3. Inhibition of Cell Growth Activity of Trastuzumab-EU, PF-05280014 Independent Lots, and Trastuzumab-US Lots**

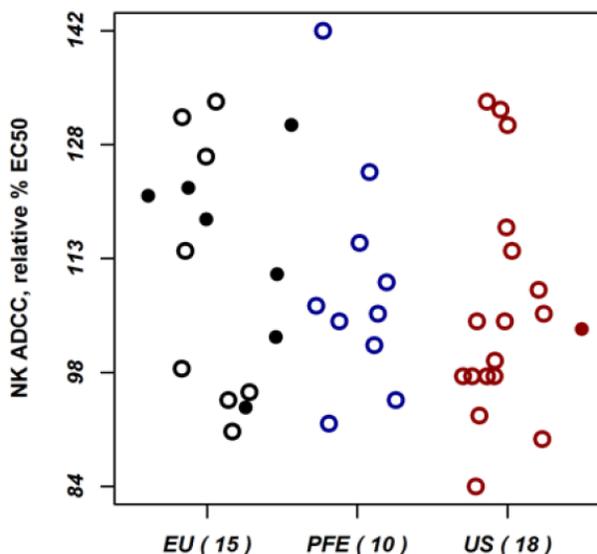


**The solid circles represent the lots used in the clinical studies.**

**Reviewer Comment:** *Tier 1 evaluations for cell growth inhibition showed statistical equivalence among PF-05280014, US-Herceptin and EU-Herceptin, which supports a determination of high similarity between PF-05280014 and US-Herceptin.*

ADCC assay: The ADCC activity of trastuzumab was assessed using primary NK cells (isolated from one healthy donor with FcγRIIIa 158 V/V isotype) as effector cells. The profiles are comparable among representative PF-05280014 lots, US-Herceptin and EU-Herceptin lots (provided in Figure 3.2.R.3.2.2-5 and not copied here). The Tier 1 statistical analysis (equivalence testing) includes 10 independent PF-05280014 lots, 18 US-Herceptin lots and 15 EU-Herceptin lots.

**Figure 3.2.R.3.2.2-7. Primary NK ADCC Activity of Trastuzumab-EU, PF-05280014 Independent Lots, and Trastuzumab-US Lots**



**Reviewer Comment:** Unlike the cell inhibition assay which has been tested since 2010, ADCC was not included in similarity assessment until late 2015; therefore, less US-Herceptin and EU-Herceptin lots were tested for ADCC than for the cell inhibition assay. The selected reference product lots contain low, medium and high levels of total afucosylation and G0 glycans that cover the full ranges of total afucosylation (US, 5.8% -11.6%; EU 6.1%-11.0%) and G0 (US, 3.6%-4.5%; EU, 3.6-4.8%).

The ADCC activity was calculated as the relative EC<sub>50</sub> value against RM 124281pg9. RM 124281pg9 has the lowest level of afucosylated glycoforms (7.0%) compared to other PF-05280014 lots (7.7%-9.4%) (data summarized by the reviewer). This difference accounts for the >100% relative potency values observed in both PF-05280014 and reference product samples. The values of PF-05280014 materials range from 92% to 142%, slightly outside of the upper ranges of EU-Herceptin (91%-133%) and US-Herceptin (84%-133%); however, there was only one PF-05280014 sample outside of the range. The differences may be due to the inherent variability of the cell based assay.

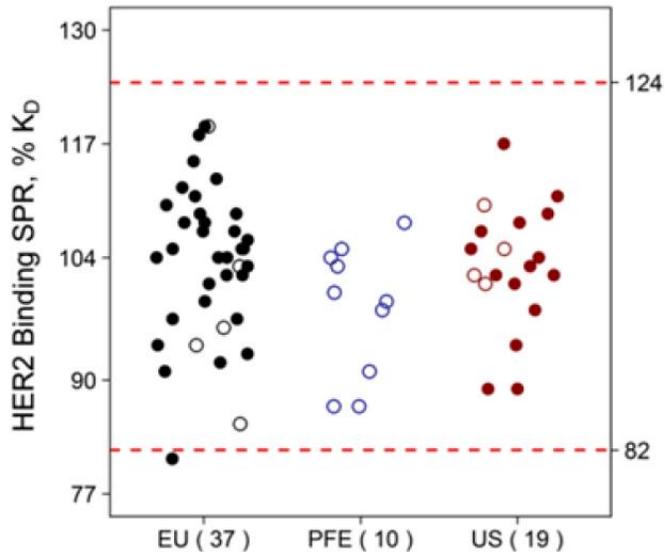
Tier 1 evaluations for ADCC showed statistical equivalence among PF-05280014, US-Herceptin and EU-Herceptin, which supports a determination of high similarity between PF-05280014 and US-Herceptin.

### **Tier 2 QAs:**

HER2 binding by SPR: The binding to HER2 by trastuzumab was determined using SPR. The kinetic information,  $k_a$ ,  $k_d$  and %relative  $K_D$  values of all samples were provided in the 3.2.R.3.5 Appendix Table 3.2.R.3.5-12 and not copied here. The representative overlaid SPR sensorgrams in Figure 3.2.R.3.2.2-5 demonstrated similar binding profiles for all three materials (not copied

here). The % relative  $K_D$  from PF-05280014 (10 lots), US-Herceptin (19 lots) and EU-Herceptin (37) were summarized in the figure below.

**Figure 1. Statistical Quality Range for HER2 Binding Comparison of PF-05280014, Trastuzumab-US, and Trastuzumab-EU**



The solid circles represent the lots used in the clinical studies.

Only a subset of US-Herceptin and EU-Herceptin lots were analyzed by SPR. The selection is based on material availability and those lots analyzed by other bioassays. All clinical lots from EU-Herceptin were tested. The quality range of 82-124% was established based on mean  $\pm$  3SD from US-Herceptin lots.

**Reviewer Comment:** *The selection of reference product lots cover all lots used in clinical studies and is acceptable. The representative overlay SPR sensorgrams from PF-05280014 US-Herceptin and EU-Herceptin are comparable. All the PF-05280014 lots are within the quality range of the US-Herceptin (82-124%) or EU-Herceptin (76%-130%). All EU-Herceptin lots are also within the quality range of US-Herceptin. The data suggest that PF-05280014, US-Herceptin and EU-Herceptin are similar in their ability to bind HER2.*

**Fc $\gamma$ RIIIa RGA:** This reporter gene assay is an orthogonal quantitative method to measure ADCC activity. Representative dose response curves from multiple lots of the three materials show they have similar profiles (data provided in Figure 3.2.R.3.2.2-11 and not copied here).

The majority of lots used in the similarity assessment in the NK cell ADCC assay were tested by the RGA assay. The Tier 2 statistical analysis table below is prepared by the reviewer.

	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	129.4% (10)	91.1% (28)	101.7% (22)
Range ( $\pm$ 3SD)	95.5% -163.3%	12.7% - 169.3%	14.4% - 189%
Range ( $\pm$ 2SD)	106.8% - 152.0%		

**Reviewer Comment:** All of the PF-05280014 lots are within the quality range of the US-Herceptin (12.7-169.3%) or EU-Herceptin (14.4-189%). All EU-Herceptin lots are also within the quality range of US-Herceptin. The data suggest that PF-05280014, US-Herceptin and EU-Herceptin are similar for RGA activity.

**Binding of FcγRIIIa by SPR:** The binding of trastuzumab products to both 158V and 158F allotypes were assessed using SPR. Representative dose response curves from multiple lots of the three materials show that they have similar profiles to the RM 124281pg9 at 1000 nM level for both FcγRIIIa 158V and 158F allotypes (data provided in Figure 3.2.R.3.2.2-14 and not copied here).

Among the 13 PF-05280014 lots tested, only 6 are independent lots. Independent lots from D11L122E003, 11P122L601, 11P122L001, 88201, 88003, M55891 are not tested. The Tier 2 statistical analysis tables below are prepared by the reviewer.

FcγRIIIa 158V Binding	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	112.3% (6)	107.4% (12)	102.3% (11)
Range (± 3SD)	87.1% -137.5%	58.5% -156.3%	56.7% - 147.9%

FcγRIIIa 158F Binding	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	107.3% (6)	104.7% (12)	105.1% (11)
Range (± 3SD)	89.4% -125.2%	55.8% -153.6%	57.7% - 152.5%

**Reviewer Comment:** All PF-05280014 lots are within the quality range of the US-Herceptin or EU-Herceptin. All EU-Herceptin lots are also within the quality range of US-Herceptin Lots. The data suggest that PF-05280014, US-Herceptin and EU-Herceptin are similar in binding of FcγRIIIa. While the sample size is limited for PF-05280014, this is acceptable as this test is an orthogonal method for ADCC activity and the similarity in ADCC among PF-05280014, US-Herceptin and EU-Herceptin has been established in both NK ADCC and RGA assays.

**FcRn Binding by SPR:** FcRn binding can affect the *in vivo* half-life of monoclonal antibodies. The FcRn binding was measured by SPR for PF-05280014, US-Herceptin and EU-Herceptin. Representative sensorgrams show similar binding profiles to RM 124281pg9 among all three materials (provided in Figure 3.2.R.3.2.2-21 and not copied here). The  $k_a$ ,  $k_d$ ,  $K_D$  and %  $K_D$  values for all samples were provided in Section 3.2.R.3.5 Appendix Table 3.2.R.3.5-12 and not copied here. The summary data were provided in the figure below.

Six independent PF-05280014 lots, 11 US-Herceptin and 11 EU-Herceptin lots are included in the Tier 2 similarity assessment. The analysis is summarized by the reviewer in the table below.

FcRn Binding	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	97.5% (6)	100.0% (11)	94.6% (11)
Range (± 3SD)	75.6% -119.4%	75.7% -124.3%	75.1% - 114.1%

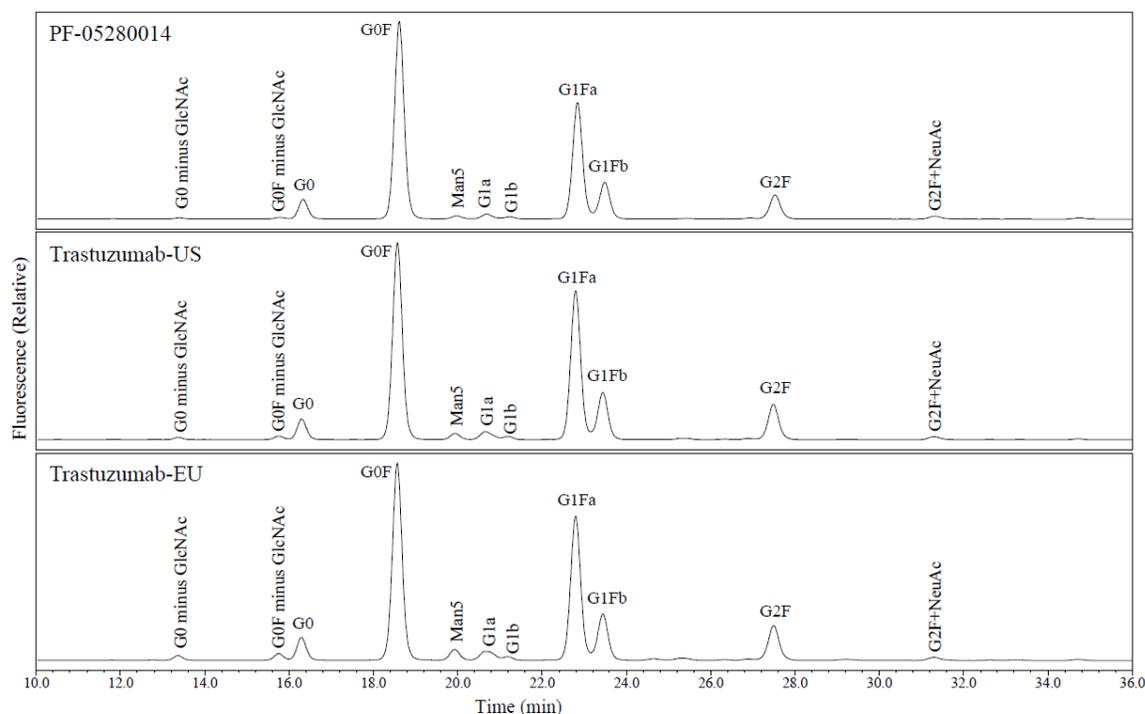
Range ( $\pm$ 2SD)	82.9% - 112.1%		
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**Reviewer Comment:** All PF-05280014 lots are within the quality range of the US-Herceptin (76%-124%) or EU-Herceptin (75%-114%) and all EU-Herceptin lots are also within the quality range of US-Herceptin. The data suggest that PF-05280014, US-Herceptin and EU-Herceptin are similar regarding binding to FcRn.

N-Glycan Structure: Trastuzumab has one Asn linked glycosylation in the Fc region that could impact ADCC activity. The glycosylation of trastuzumab products was analyzed by HILIC/MS (Tier 2), enzymatic mapping for composition and linkages (Tier 3), and sialic acid content analysis (Tier 3).

HILIC/MS: This method provides the identification and quantification of individual glycans. Representative chromatograms for the 2-AB labeled glycans from PF-05280014, US-Herceptin and EU-Herceptin are shown in the figure below.

**Figure 3.2.R.3.2.3-1. Representative 2-AB HILIC N-Linked Glycan Mapping Profiles of PF-05280014, Trastuzumab-US, and Trastuzumab-EU**



The identities of individual glycans were confirmed to be consistent with the predicted (theoretical) masses (Table 3.2.R.3.2.3-1 of Section 3.2.R.3.2.3) and not copied here. The major glycans are G0F, G1F and G2F for all three materials.

Quantitative values of individual glycoforms from 11 independent PF-05280014 lots, 52 US-Herceptin lots and 64 EU-Herceptin lots were provided in Section 3.2.R.3.5 Appendix Table 3.5.R.3.5-16 and not copied here. All samples are analyzed side by side with RM124281pg9. The ranges for the glycoforms are summarized in the table below.

**Table 3.2.R.3.2.3-1. Summary of Ranges for Total Afucosylation and Terminal Galactosylation and Individual N-linked Glycoforms in Trastuzumab-US, PF-05280014 and Trastuzumab-EU by HILIC**

Glycan Species	PF-05280014 (n=11)	Trastuzumab-US (n=52)	Trastuzumab-EU (n=64)
Total Afucosylated Species (%)	7.1 – 9.1	5.8 – 11.6	6.1 – 11.0
Terminal Galactosylated Species (%)	41.0 – 52.2	37.2 – 53.1	32.1 – 53.1
G0 minus GlcNAc	0.1 – 0.3	0.4 – 1.4	0.4 – 1.4
G0F minus GlcNAc	0.2 – 0.5	0.6 – 1.9	0.7 – 4.2
G0	4.3 – 6.0	3.6 – 4.5	3.6 – 4.8
G0F	40.1 – 50.2	38.7 – 52.6	37.0 – 54.9
Man5	0.3 – 0.9	0.9 – 4.2	1.0 – 2.5
G1(a+b)*	1.7 – 2.4	0.4 – 2.8	0.0*** – 3.4
G1F minus GlcNAc*	ND	0.0 – 2.7	0.0 – 3.1
G1Fa	25.4 – 31.2	23.0 – 31.7	19.7 – 31.4
G1Fb	8.5 – 10.4	8.1 – 10.3	6.9 – 10.6
G2**	0.1 – 0.3	ND	ND
G1F + NeuAc minus GlcNAc	ND	0.1 – 0.7	0.4 – 1.0
G1F + NeuAc	0.1 – 0.3	0.2 – 0.5	0.0 – 0.5
G2F	5.1 – 7.9	4.1 – 8.2	3.2 – 8.5
G2F + NeuAc	0.8 – 1.1	0.4 – 0.8	0.3 – 0.9
G2F + 2NeuAc	0.3 – 0.5	0.1 – 0.6	0.1 – 0.3

ND = not detected

\* In some trastuzumab-US and trastuzumab-EU lots, the G1(a+b) glycoforms were present as the major glycoforms whilst G1F minus GlcNAc glycoform was detected either at very low level or not present in the chromatographic profile. Conversely, there were some trastuzumab-US and trastuzumab-EU lots that had G1F minus GlcNAc glycoform as the main species, with G1(a+b) glycoforms detected either at very low levels or not present in the chromatographic profile.

\*\* The G2 glycoform is typically detected either below the level of quantitation or not present in the chromatographic profile of trastuzumab-US and trastuzumab-EU lots.

\*\*\* Data reported were generated throughout the product development timeframe, including prior to and after the establishment of the QL.

**Reviewer Comment:** *The overall profiles and ranges of major glycoforms are similar among PF-05280014, US-Herceptin and EU-Herceptin. Minor differences exist among PF-05280014, US-Herceptin and EU-Herceptin: PF-05280014 has less Man5 (0.3%-0.9%) compared to US-Herceptin (0.9%-4.2%) and EU trastuzumab (1.0%-2.5%); PF-05280014 has trace amount of G2 (0.1%-0.3%) while the other two have none; trace level of G1F-GlcNAc (0.0%-3.1%) and G1F+NeuAc-GlcAc (0.1-1.0%) are present in US-Herceptin and EU-Herceptin materials but not in PF-05280014; the amount of G0-GlcNAc and G0F-GlcNAc (0.1%-0.3% and 0.2%-0.5%, respectively) in PF-05280014 are also lower than that of the US-Herceptin materials (0.4%-1.4% and 0.6%-1.9%) and EU-Herceptin materials (0.4%-1.4% and 0.7%-4.2%). These differences are minor and do not preclude a demonstration that PF-05280014 and US-Herceptin are highly similar.*

*Assessment of HM species was included in the calculation of total afucosylated species. It is recommended that HM glycoforms be assessed as a Tier 2 quality attribute for trastuzumab because they are known to affect PK and ADCC and FcγRIIIa binding; however, the sponsor did not do this assessment. Data was provided such that the reviewer was able to assess the quality*

ranges for US-Herceptin and EU-Herceptin (see table below) to assess HM species for PF-05280014.

The overall galactosylation levels are comparable among PF-05280014 (41.0%-52.2%), US-Herceptin (22.4%-53.1%) and EU-Herceptin (21.5%-53.1%%).

The % total afucosylation and % galactosylation values for PF-05280014 (11 lots), EU-Herceptin (74 lots) and US-Herceptin (64 lots) were provided. The Tier 2 statistical analysis tables below are prepared independently by the reviewer. The following analysis on HM species and afucosylated species without HM is prepared by the reviewer based on the raw data provided in Section 3.2.R.3.5. Appendix.

% Total afucosylation	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	8.4% (11)	7.6% (64)	7.7% (74)
Range ( $\pm$ 3SD)	6.3% -10.4%	3.2% -12.0%	3.8% - 11.7%

% Galactosylation	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	43.9% (11)	42.3% (64)	40.9% (74)
Range ( $\pm$ 3SD)	34.5% -53.4%	15.6% -69.1%	18.2% - 63.6%

% High Mannose	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	0.6% (11)	1.7% (52)	1.5% (64)
Range ( $\pm$ 3SD)	0% -1.2%	0% -4.7%	0.3% - 2.7%

% Total afucosylation (HM excluded)	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	7.8% (11)	6.2% (52)	6.5% (64)
Range ( $\pm$ 3SD)	5.9% -9.7%	4.1% -8.3%	3.5% - 9.5%

**Reviewer Comment:** For total afucosylation (HM included), All PF-05280014 lots (7.1-9.1%) are within the quality range of the US-Herceptin (3.2-12.0%) or EU-Herceptin (3.8%-11.7%). All EU-Herceptin lots are also within the quality range of US-Herceptin.

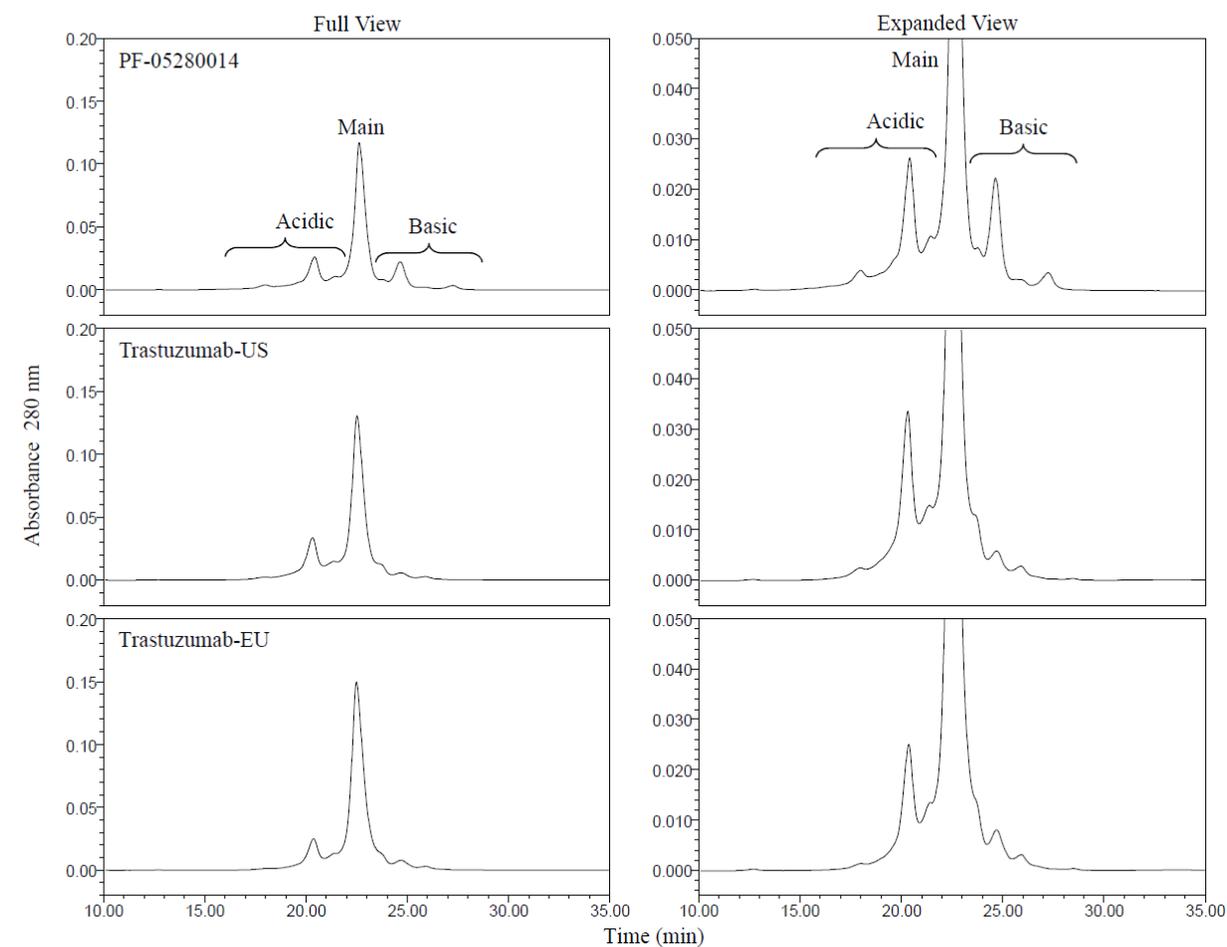
For analysis of HM (Man 5) species, data were only available from the pre-shift subset to derive the quality ranges. All PF-05280014 lots (0.3-0.9%) are within the quality range of the US-Herceptin (0.0%-4.7%) or EU-Herceptin (0.3%-2.7%). For total afucosylated species excluding HM, Two PF-05280014 lots (18% of total lots) and two EU lots (3% of total lots) are outside of the quality range of the US-Herceptin (4.1%-8.3%). The slight lower HM level and slightly highly level afucosylated species (HM excluded) in PF-05280014 have minimal impact, because there are no differences observed in all three Tier 1 potency assays. These minor differences do not preclude a demonstration that PF-05280014 and US-Herceptin are highly similar.

For galactosylation, all PF-05280014 lots (41.0-52.2%) are within the quality range of the US-Herceptin (15.6-69.1%) or EU-Herceptin (18.2-63.6%). The data suggest that PF-05280014 is similar to US-Herceptin and EU-Herceptin with respect to this attribute.

Charge Heterogeneity by CEX-HPLC: The charge variants were evaluated by CEX-HPLC. Individual species from the acidic, main and basic peaks were identified in the characterization report (3.2.R.3.2.4. Charge Heterogeneity- Characterization).

The representative overlaid chromatograms for PF-05280014, US-Herceptin and EU-Herceptin were shown in the figure below.

**Figure 3.2.R.3.2.4-1. Representative CEX-HPLC Profiles of PF-05280014, Trastuzumab-US, and Trastuzumab-EU**



The number of peaks and retention times are comparable among all three materials; however, PF-05280014 has increased levels of basic species compared to US-Herceptin and EU-Herceptin. The %acidic and %basic peaks were summarized in the figure below for PF-05280014 (24 lots), US-Herceptin (64 lots) and EU-Herceptin (74 lots).

The % acidic species are similar among PF-05280014 (23.2-28.2%), US-Herceptin (18.9%-27.6%) and EU-Herceptin (18.1-28.6%). PF-05280014 product has more % basic species (10.5%-18.2%) compared to US-Herceptin (8.7% - 14.4%) and EU-Herceptin (7.2%-11.9%).

The Tier 2 statistical analysis tables below are prepared by the reviewer.

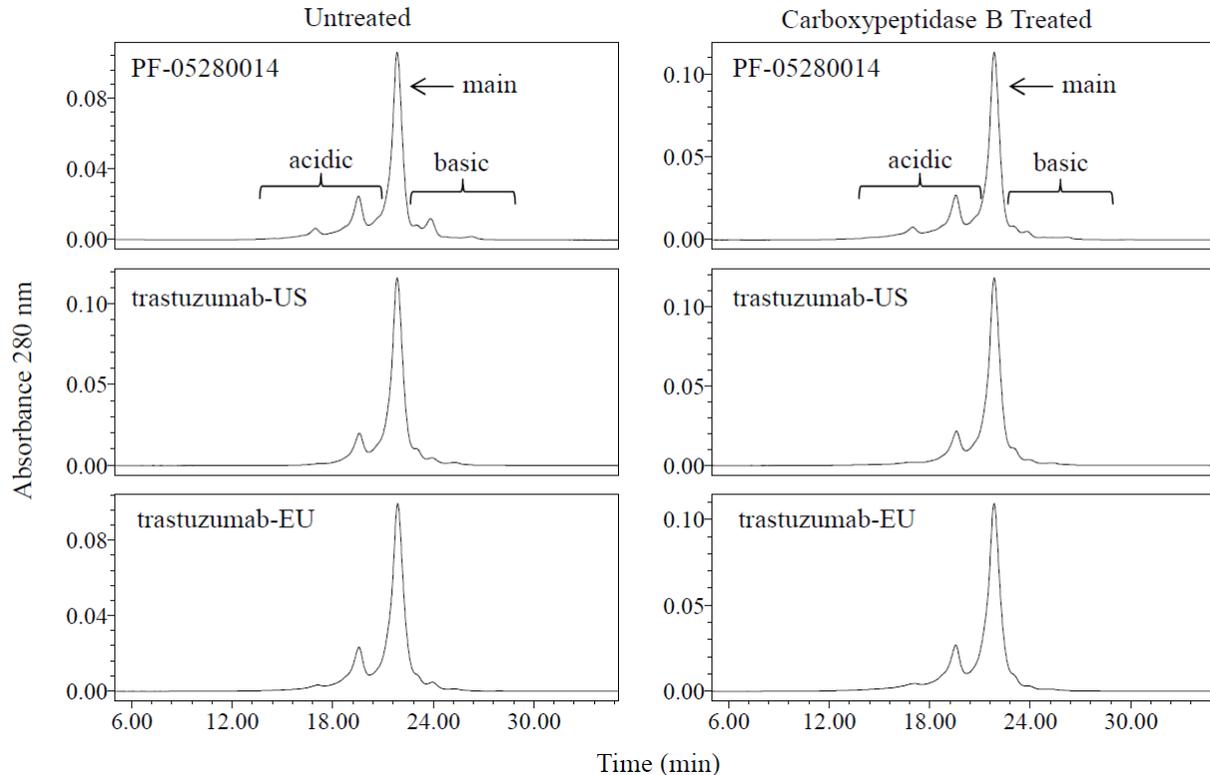
Acidic Species	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	24.9% (11)	24.7% (64)	22.4% (74)
Range ( $\pm$ 3SD)	19.7% -30.2%	18.4% -31.0%	14.0% - 30.8%

Basic Species	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	15.2% (11)	10.3% (64)	9.8% (74)
Range ( $\pm$ 3SD)	7.6% -22.8%	6.7% -13.9%	6.5% - 13.1%

Main Peak	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	59.9% (11)	65.0% (64)	67.8% (74)
Range ( $\pm$ 3SD)	55.1% -64.7%	59.9% -70.1%	61.2% - 74.4%

The increase in basic species observed in PF-05280014 could be due to increased C-terminal lysine. To assess the impact of C-terminal lysine of the HC on the quantitation of basic and main species, PF-05280014 (9 lots), US-Herceptin (3 lots) and EU-Herceptin (9 lots) are subjected to Carboxypeptidase B (CbP) treatment to remove the remaining C-terminal lysine residues. Representative profiles before and after treatment were shown in the figure below.

**Figure 3.2.R.3.2.4-2. CEX-HPLC Profiles of PF-05280014, Trastuzumab-US, and Trastuzumab-EU Untreated and Carboxypeptidase B Treated**



After digestion, the levels of basic species in PF-05280014 (7.8%-10.1%) are comparable to that of US-Herceptin (7.8%-8.3%) or EU-Herceptin (8.1%-10.1%). The remaining basic species were fractionated by HPLC into 3 fractions (basic fractions B0-B3). The collected fractions were

characterized by SEC/ESI MS and LC/MS/MS-peptide mapping (Lys-C/trypsin) for isoform identification. The basic species contain Asp102 isomerization, amidated proline and signal peptide sequence extensions on the H chain in all three materials, with trace amount of HM glycoforms present only in US-Herceptin and EU-Herceptin (data provided in Table 3.2.R.3.2.4-1 of 3.2.R.3.2.4. Charge Heterogeneity – Characterization and not copied here).

**Reviewer Comment:** *For acidic species, all PF-05280014 lots (23.2-28.2%) are within the quality range of the US-Herceptin (18.4%-31.0%) or EU-Herceptin (14.0%-30.8%). One EU lot fell outside the US quality range, but still meets the criteria for similarity (> 90% values fall within the quality range).*

*The levels of basic species are higher in PF-05280014 (10.5%-17.8%) than that of US-Herceptin (8.7%-14.4%) or EU-Herceptin (7.2%-11.9%). Consequently, the levels of main peak are lower in PF-05280014(57.9%-63.8%) than that of US-Herceptin (62.1%-72.0%) or EU-Herceptin (62.2%-71.7%). The Tier 2 statistical analysis shows that PF-05280014 falls outside the quality range for the basic and main peaks. Additional studies provided demonstrate that the increase in basic species is due to increased levels of C-terminal lysine. C-terminal lysine does not affect the function of trastuzumab as no differences were noted in potency in the Tier 3 analysis. In addition, C-terminal lysine are known to be cleaved rapidly in vivo upon administration; therefore, the increase in basic species is not thought to have a clinical relevance. The identity of the remaining basic species, after CbP digestion, are similar among PF-0528114, US-Herceptin and EU-Herceptin. Overall, differences in charge species between PF-05280014 and US-Herceptin and EU-Herceptin do not preclude a determination that PF-05280014 and US-Herceptin are highly similar or the establishment of the three-way analytical bridge.*

Characterization of charge variants in CEX-HPLC: The individual CEX peaks fractions for PF-05280014, US-Herceptin and EU-Herceptin were collected individually into 10 fractions (acidic fractions, A1-A5, main, basic fractions, B1-B3). The chromatographic overlays with assigned peaks fractions were shown in the figure below.

**Figure 3.2.R.3.2.4-1. CEX-HPLC Individual Charge Isoform Fraction Assignments for PF-05280014, Trastuzumab-US, and Trastuzumab-EU**

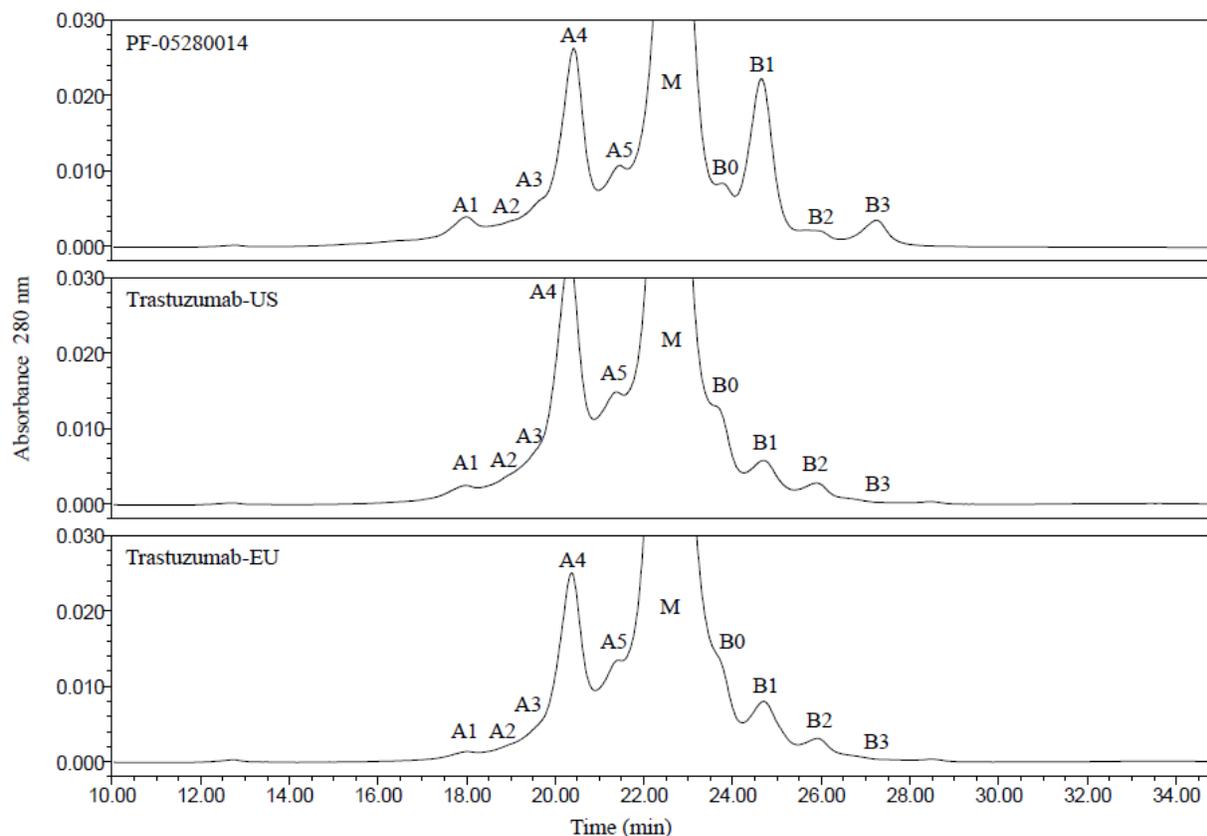


Figure 3.2.R.3.2.4-1 the various acidic (A), main (M), and basic (B) individual fractions isolated for in-depth characterization by MS are noted on the chromatograms.

The collected fractions were characterized by SEC/ESI MS and LC/MS/MS-peptide mapping (Lys-C/trypsin) for isoform identification. There are no differences in charge isoforms identified in peaks A1- B0 among all three materials. In peak B1, US-Herceptin and EU-Herceptin contain additional HM glycoforms and glycated species compared to PF-05280014. In peak B2, PF-05280014 contains additional 1 HC with a signal peptide sequence extension, as well as 2 HCs terminated with amidated proline. In peak B3, the C-terminal lysine isoforms at both HCs are detected in both PF-05280014 and EU-Herceptin; HM glycoforms are also detected in EU-Herceptin.

In all three materials, the A1-A5 fractions contain Asn30 deamidation at one light chain and various levels of sialylated glycoforms. The A1, A3, and A5 fractions contain mono-sialylated glycoforms while the A2 and A4 fractions contain di-sialylated glycoforms. The A1 and A3 fractions also contained Asn55 deamidation on one H chain. The A5 fraction also contains Asp102 isomerization at one HC. The A1, A2, A3, and A5 fractions also contained glycated species. B0 fraction contains Asn102 isomerization at one HC. B1 fraction contains C-terminal lysine at one H chain (predominant) along with C-terminal amidated proline at the HC. B2 fraction contains Asp102 succinimide at one HC as the predominant form.

**Reviewer Comment:** *The presence or lack of small/trace amount of certain charge isoforms in PF-05280014 as mentioned above in comparison to US-Herceptin and EU-Herceptin does not preclude a determination that PF-05280014 and US-Herceptin are highly similar.*

Isomerization at Asp102 at one HC is known to decrease trastuzumab potency (Harris et al., 2001). The B0 fraction is identified as containing isomerized Asp102 at one HC. The potency (cell inhibition) results of B0 fraction from all three materials are all comparable (99%-104%) to the RM. The findings are inconsistent with the results reported by Harris and an IR (IR #3 Comment 1, 11/07/2017) was sent. In the responses (11/22/2017, 01/31/2018, 02/13/2018), the sponsor stated the current bioassay could not detect the impact of Iso Asp102 on bioactivity. The sponsor initially proposed to use % B0 peak in CEX assay as a surrogate to control Iso Asp102 but was not accepted by the Agency as both A5 and B0 contained Iso Asp102 species. Subsequently, the sponsor provided an alternative characterization method (LC/MS/MS) to quantitate this species. The suitability of the methods was evaluated by analyzing samples of different levels of Iso Asp 102. The results were shown in the table below.

**Table 1. Isomerized Asp102 Levels in Prepared Samples**

Sample	Target <sup>a</sup> Isomerized Asp102 (%)	Observed Isomerized Asp102 (%)	Recovery (%)
PF-05280014 RM	NA	3.6	NA
25/75 Enriched/RM	9.9	8.7	88
50/50 Enriched/RM	16.2	15.6	96
Iso Asp102 Enriched	NA	28.7	NA

a. The observed levels for the PF-05280014 RM and the isomerized Asp102 enriched material were used to calculate the target levels for the 25/75 and 50/50 samples. The mix samples were prepared by adding a proportional amount of each material together. The observed values were obtained from the LC/MS/MS-peptide mapping analysis.

The sponsor then determined the level of Iso Asp102 in PF-05280014 (6 lots), US-Herceptin (6 lots) and EU-Herceptin (6 lots). The expiry dates for the materials are 09/2016-06/2019 (PF-05280014), 03/2018-06/2019 (US-Herceptin) and 01/2018-10/2018. The Iso Asp levels are 3.6-4.4% (PF-05280014), 5.0-5.4% (US-Herceptin), and 5.1-5.4% (EU-Herceptin).

**Reviewer Comment:** *The LC/MS/MS method is suitable for the quantitation of Iso Asp102 in trastuzumab. The data show the levels of Iso Asp102 in PF-05280014 samples are slightly lower than that of US-Herceptin and EU-Herceptin. All three materials are low (< 5.4%) consistently at all ages of the materials. In addition, there is minimal increase (<4%) in %basic species (a worst-case surrogate for Iso Asp102 assuming all increase in % basic species is due to Iso Asp102 formation) during DS/DP long term storage condition. This is consistent with the frozen nature of DS and the lyo nature of the DP.*

Purity by SEC-HPLC: SEC was used to separate and quantitate HMMS, monomer and LMMS. The representative SEC chromatograms for all three samples were provided in Section 3.2.R.3.2.5 Product Purity and not copied here.

Results for the monomer and HMMS from 11 PF-05280014 lots, 64 US-Herceptin lots and 74 EU-Herceptin lots were compared in the figure below.

The Tier 2 statistical analysis tables below are prepared by the reviewer.

Monomer	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	99.5% (11)	99.5% (64)	99.5% (74)
Range ( $\pm 3SD$ )*	99.1% -100%	98.9% -100%	99.1% - 100%

\*: The upper limit of the range is set at 100%

HMMS	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	0.3% (11)	0.5% (64)	0.5% (74)
Range ( $\pm 3SD$ )*	0.1% -0.5%	0.0% -1.0%	0.2% - 0.8%

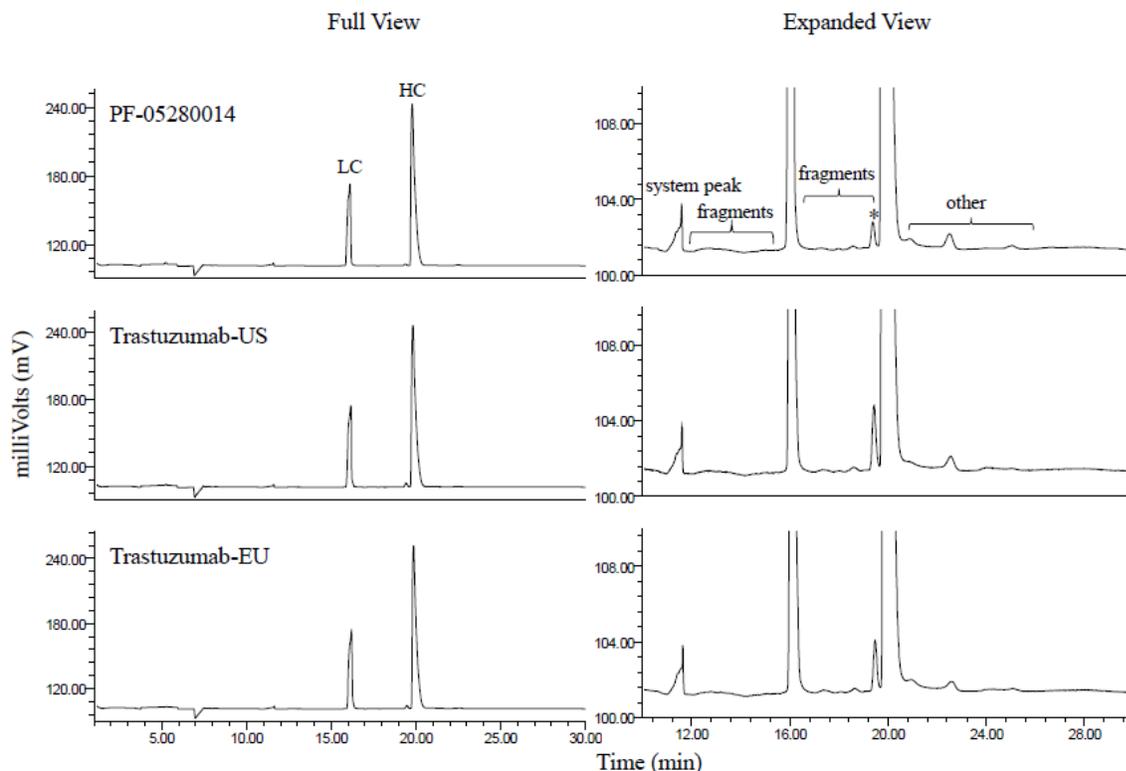
\*: The lower limit of the range is set at 0%

**Reviewer Comment:** For monomer, all PF-05280014 lots (99.3-99.7%) are within the quality range of the US-Herceptin (98.9%- 100%) or EU-Herceptin (99.1%-100%). 100% EU-Herceptin lots are also within the quality range of US-Herceptin. The data suggest that PF-05280014, US-Herceptin and EU-Herceptin are similar with respect to this attribute.

For HMMS, all PF-05280014 lots (0.2-0.4%) are within the quality range of the US-Herceptin (0%-1.0%) or EU-Herceptin (0.2%-0.8%). 100% EU-Herceptin lots are also within the quality range of US-Herceptin. The data suggest that PF-05280014, US-Herceptin, and EU-Herceptin are similar with respect to this attribute.

Purity by reducing CGE: Representative electropherograms for all three materials were provided in the figure below.

**Figure 3.2.R.3.2.6-5. CGE (Reducing) Profiles of PF-05280014, Trastuzumab-US and Trastuzumab-EU**



There are no new peaks identified in PF-05280014. A small peak prior to the main HC peak is identified as the aglycosylated species and is present in all three materials.

Purity and fragments results from 11 PF-05280014 lots, 59 US-Herceptin lots and 74 EU-Herceptin lots were compared in the figures below.

The Tier 2 statistical analysis tables below are prepared by the reviewer.

Purity	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	99.4% (11)	99.3% (59)	99.3% (74)
Range ( $\pm$ 3SD)*	98.0% -100%	98.5% -100%	98.3% - 100%

\*: The upper limit of the range is set at 100%

Fragments	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	0.3% (11)	0.7% (59)	0.6% (74)
Range ( $\pm$ 3SD)*	0.0% -1.2%	0.0% -1.4%	0.0% - 1.0%

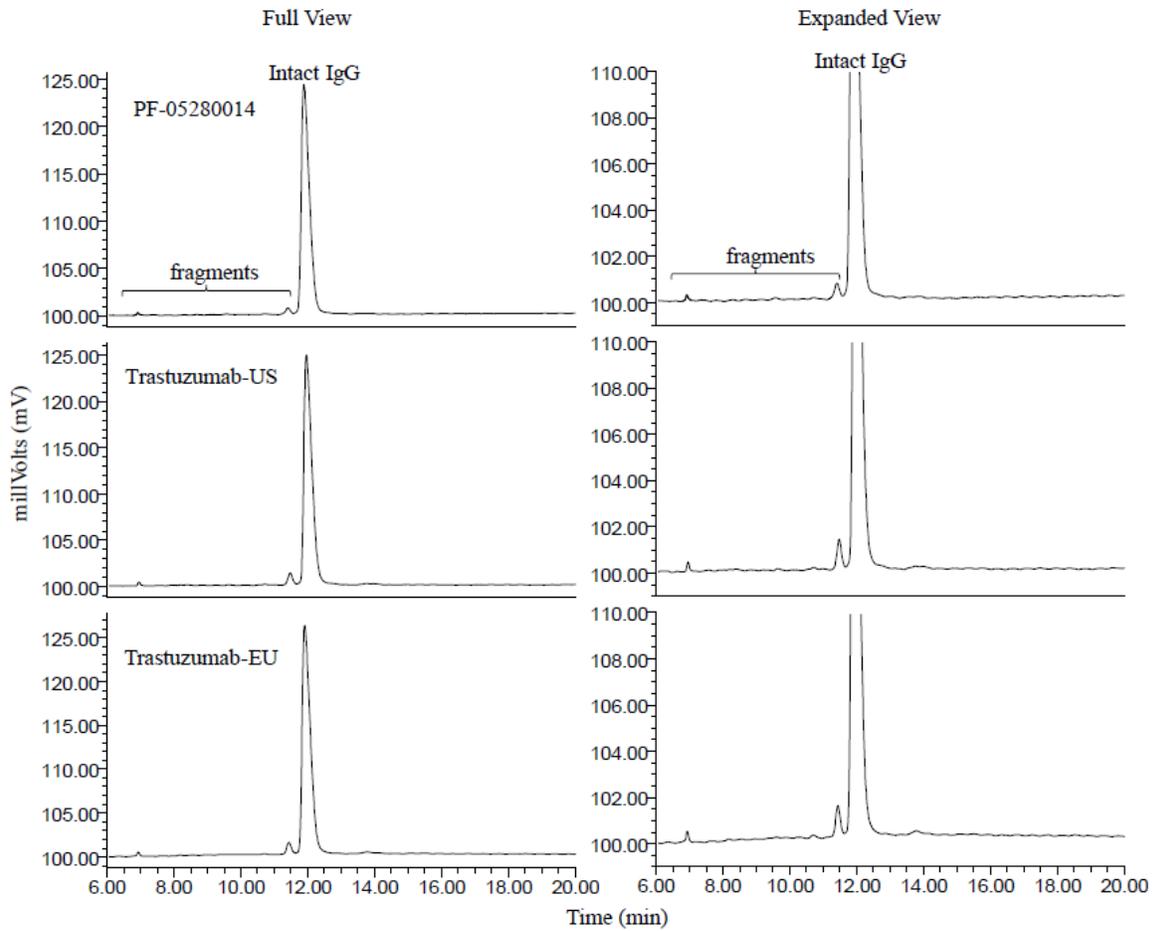
\*: The lower limit of the range is set at 0%

**Reviewer comment:** Purity and fragments by reducing CGE were classified as Tier 3 quality attributes by the sponsor; however, a Tier 2 quality range analysis was more appropriate and the analysis was performed by the reviewer. For both QAs, all PF-05280014 lots (purity, 98.5-100%; fragments, 0-1.0%) are within the quality range of the US-Herceptin or EU-Herceptin. all

*EU-Herceptin lots are also within the quality range of US-Herceptin. The data suggest that PF-05280014, US-Herceptin and EU-Herceptin have similar purity in this assay.*

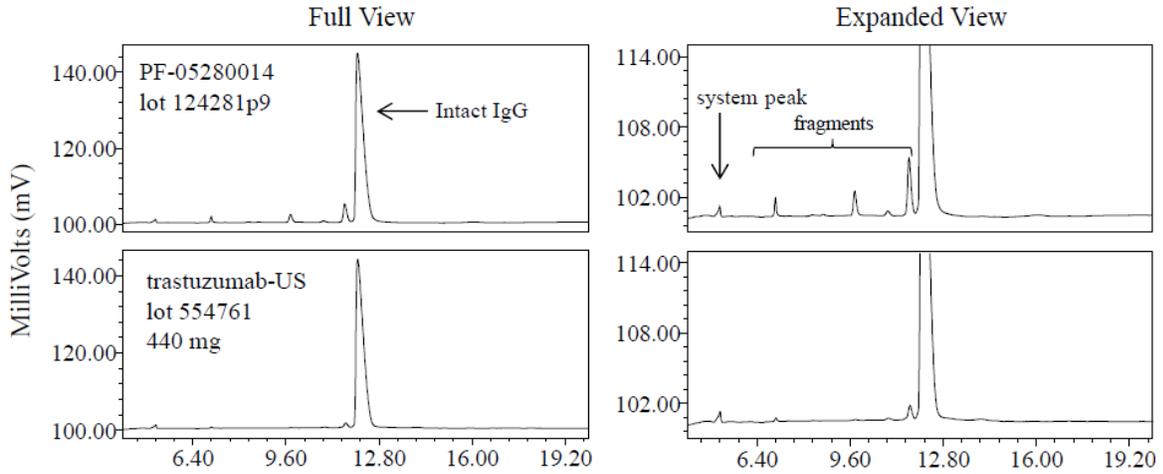
Purity by Non-Reducing CGE: Representative profiles for all three materials were provided in the figures below.

**Figure 3.2.R.3.2.5-8. CGE (Non-Reducing) Profiles of PF-05280014, Trastuzumab-US and Trastuzumab-EU**



The peak patterns are similar among all three materials; however, results copied from Section 3.2.R.3.5. Appendix show the presence of additional fragments in PF-05280014 (See figure below) not observed in US-Herceptin.

**Figure 3.2.R.3.5-252. CGE (Non-reducing) Profile of PF-05280014 and Trastuzumab-US Lots Pre-glycan Shift**



**Reviewer comment:** The additional fragments at 7 mins and 9.6 mins are most likely HHL and L fragments based on peak patterns of reducing and non-reducing CGEs.

An IR (IR#3 Comment 3) was sent on 11/07/2017 requesting data for % intact IgG for individual lots used in analytical similarity assessment. In response (11/27/2017), the sponsor provided tabulated data for intact IgG by non-reducing CGE for PF-05280014, US-Herceptin and EU-Herceptin materials. The results were shown in the figure below.

**Figure 1. Individual Results for Intact IgG of Trastuzumab-EU, PF-05280014 and Trastuzumab-US Determined by CGE (Non-Reducing)**

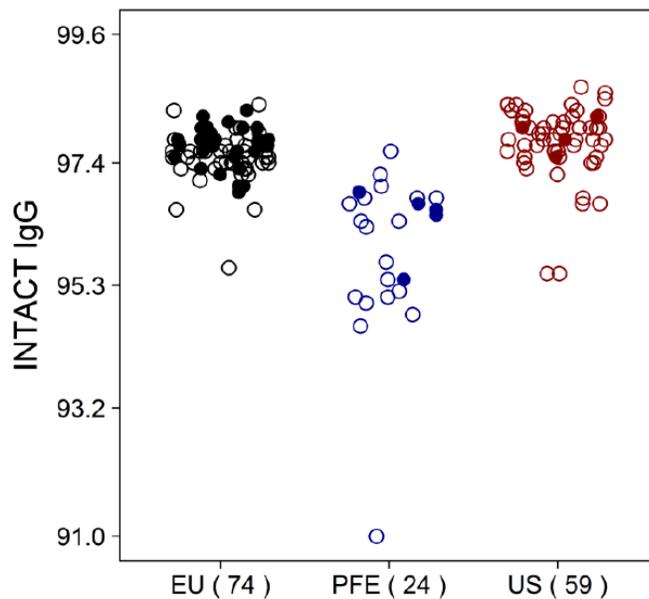


Figure 1. The solid circles indicate lots used in the clinic.

The Tier 2 statistical analysis tables below are prepared by the reviewer.

Purity	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	95.9% (11)	97.8% (59)	97.6% (74)
Range ( $\pm$ 3SD)*	90.1% -100%	96.0% -100%	96.3% - 100%

\*: The upper limit of the range is set at 100%

**Reviewer comment:** Four out of eleven PF-05280014 lots (91.9%-97.6%) are outside the quality range of the US-Herceptin (96.0%-100%) and EU-Herceptin (96.3%-100%). One EU lot and two US lots are also outside the quality range of US-Herceptin. Given the number of US-Herceptin lots and the minimal variability between lots (see figure above), it is not unexpected, considering the current statistical assumption, that a small percentage of data could fall outside of the quality range. One PF-05280014 early development lot (D11L122E003) has significant lower purity (91.0%). All subsequent lots have intact IgG > 94.6%. It is not unexpected for development lots to be less pure than the final commercial product due to improvements made to the manufacturing process as more experience is gained. The lower purity is likely because of the presence of HHL and L fragments in PF-05280014, because the purity from reducing CGE is 99.3% and the sizes of fragments on non-reducing CGE correspond to the size of HHL and L fragments. The HHL and L fragments are caused by incomplete oxidation of the cysteines, which may induce the formation of HMMS. HMMS are monitored by SEC during manufacturing and storage. The levels of HMMS, as well as purity by SEC and reducing CGE, are similar among all three materials in release and stability testing. No significant differences in degradation rates were observed among all three materials in long term and forced degradation studies. Such differences do not preclude the conclusion that PF-05280014 and US-Herceptin

are highly similar, or the establishment of the analytical component of the three-way scientific bridge.

**Protein Concentration:** The similarity in protein concentration was assessed among PF-05280014 (5 lots), US-Herceptin (64 lots) and EU-Herceptin (74 lots). The results were summarized in the figure below.

The Tier 2 statistical analysis table below is prepared by the reviewer.

Concentration (mg/mL)	PF-05280014	US-Herceptin	EU-Herceptin
Mean (lots)	21.7 (5)	21.3 (64)	21.1 (74)
Range ( $\pm$ 3SD)	21.1-22.7	19.3 -23.3	19.1-23.1

**Reviewer Comment:** Only 5 independent 420 mg PF-05280014 DP lots are available up to date. All PF-05280014 lots (21.4-22.1 mg/mL) are within the quality range of the US-Herceptin (19.3-23.3 mg/mL) or EU-Herceptin (19.1-23.1 mg/mL). For EU-Herceptin lots, 98% were within the quality range of US-Herceptin. The data suggest that the protein concentration in PF-05280014, US-Herceptin and EU-Herceptin are similar after reconstitution.

### Tier 3 QAs: Biological activities

**Binding to HER2 by Flow Cytometry:** The binding to HER2 on the SKBR3 cells by trastuzumab was also determined using an orthogonal qualitative flow cytometry method. Representative dose-response curves from multiple PF-05280014, US-Herceptin and EU-Herceptin lots were provided in Figure 3.2.R.3.2.2-9 and now shown here.

**Reviewer Comment:** The flow cytometry data show the binding profiles of PF-05280014 (2 lots), US-Herceptin (4 lots) and EU-Herceptin (4 lots) are similar.

**HER2 and HER3 Phosphorylation by Western:** Trastuzumab is known to induce the phosphorylation of HER2 and inhibit the phosphorylation of HER3. The target sites are HER2-Tyr1248 and HER2-Tyr1221/1222. The effect of trastuzumab on both receptors was assessed by western blot using HER2 expressing cancer cell BT474. The results were provided in Figure 3.2.R.3.2.2-10 and not copied here. The induction of phosphorylation at HER2-Tyr1248 and the inhibition of HER3 phosphorylation at HER3-Tyr1289 were observed at 4  $\mu$ g/mL trastuzumab level in all three materials. No major differences were detected between 4 and 40  $\mu$ g/mL trastuzumab in all three materials. The extent of activation at HER2-Tyr1248 was comparable at 4  $\mu$ g/mL level among all three materials (PF-05280014, 2.8 fold; US, 2.8 fold; EU, 2.6 fold). The extent of HER3 inhibition was also comparable at 4  $\mu$ g/mL level (PF-05280014, 2.8 fold; US, 2.8 fold; EU 3.9 fold).

**Reviewer Comment:** The data suggest PF-05280014, US-Herceptin, and EU-Herceptin are similar in the activation/inhibition of HER2/HER3 phosphorylation.

**Apoptosis:** Representative PF-05280014, US-Herceptin, and EU-Herceptin samples were measured in an apoptosis assay for the activation of caspase 3/7. The negative control is set as

100% apoptosis. Very low levels of apoptosis (~150%) were observed in all three materials, compared to positive control (500% to 1600%).

Cell Cycle Analysis: The effect of trastuzumab on cell cycle analysis was performed using BT474 cells, where treatment of BT474 cells with 10 µg/mL of trastuzumab causes a decrease in the S-phase of the cell-cycle compared to the untreated cells. PF-05280014 (38% decrease), US-Herceptin (42% decrease), and EU-Herceptin (34% decrease) demonstrated a similar level of reduction in S-phase.

**Reviewer Comment:** Apoptosis and cell cycle impact are not part of MOA for trastuzumab. PF-05280014, US-Herceptin, and EU-Herceptin show similar apoptosis activity and effects on the cell cycle in these assays.

PBMC ADCC activity: PBMC ADCC assay was tested as an additional method for assessing ADCC to the NK ADCC assay. US-Herceptin and EU-Herceptin lots with high, medium and low afucosylated and G0 N-linked glycan levels were selected. Representative dose dependent curves from all three materials were provided in Figure 3.2.R.3.2.2-9 and not shown here. The relative %EC<sub>50</sub> values for all materials were provided in the table below.

**Table 3.2.R.3.2.2-5. PF-05280014, Trastuzumab-US, and Trastuzumab-EU Lots Tested in PBMC ADCC Assay**

	Lot #	PBMC ADCC (Relative % EC50)	Total Afucosylation (%)	G0 (%)
PF-05280014	124281pg9 (RM)	100	7.1	4.3
	Z00514	104	9.0	5.6
	L25296	117	8.7	5.4
	M55890	105	8.8	5.3
Trastuzumab-US	849245	121	11.6	4.5
	991942	107	6.9	3.7
	626308	102	7.8	4.0
Trastuzumab-EU	H3134B01	89	6.1	3.6
	H0775B01	113	7.7	4.1
	H4384B01	123	11	4.5

1. Sample results are the mean of three assay plates. PF-05280014 124281pg9 (RM) is assigned 100%.

2. Glycan values for PF-05280014 drug product lots were taken from the corresponding ingoing drug substance batches.

**Reviewer Comment:** The results from PBMC ADCC assay are in line with the results from the NK ADCC assay, which showed all three materials have similar ADCC activity.

FcγRIIa binding by SPR: The ability of trastuzumab to induce ADCP was evaluated by FcγRIIa (131H or 131R) binding by SPR. Representative SPR sensorgrams show that the profiles for all three materials are similar to the RM 124281pg9. The binding affinity to 131R is too low ( $K_D > 2500$  nM) to report reliable %  $K_D$  values. The %  $K_D$  values for 131H were provided in the table below.

**Table 3.2.R.3.2.2-11. Summary of Descriptive Statistics for FcγRIIa 131H SPR Binding Activity of Trastuzumab-EU, PF-05280014, and Trastuzumab-US**

Region	N	Relative K <sub>D</sub> (%)				
		Mean	SD	CV (%)	Min	Max
EU	6	99	4.3	4.3	92	104
Pfizer	5	100	3.4	3.4	96	105
US	6	99	2.3	2.3	95	101

N = sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

**Reviewer Comment:** *The affinity values of PF-05280014 (96%-105%), US-Herceptin (95%-101%) and EU-Herceptin (92%-104%) are similar.*

FcγRI, FcγRIIb, FcγRIIIb binding by SPR: The affinities of trastuzumab to other Fcγ receptors were also determined by SPR. Representative SPR sensorgrams demonstrated comparable profiles for all three materials to RM 124181pg9 for the binding of FcγRI, FcγRIIb, FcγRIIIb (data provided in Figure 3.2.R.3.2.2-19 and not copied here). The % K<sub>D</sub> values for FcγRI binding were summarized in the table below. The % K<sub>D</sub> values for the other receptors can't be determined due to low binding affinities (K<sub>D</sub> > 2500 nM).

**Table 3.2.R.3.2.2-12. Summary of Descriptive Statistics for FcγRI SPR Binding Activity of Trastuzumab-EU, PF-05280014, and Trastuzumab-US**

Region	N	Relative K <sub>p</sub> (%)				
		Mean	SD	CV (%)	Min	Max
EU	6	95	3.1	3.3	91	99
Pfizer	5	106	1.9	1.8	104	108
US	6	101	2.7	2.7	96	103

N = sample size, SD = standard deviation, CV = coefficient of variation, Min = minimum, Max = maximum

**Reviewer Comment:** *The % K<sub>D</sub> values of PF-05280014 (104%-108%) are slightly higher than that of US-Herceptin (96%-103%) and EU-Herceptin (91%-99%). The k<sub>a</sub> and k<sub>d</sub> values are also similar among all three materials (data from 3.2.R.3.5 Appendix Table 3.2.R.3.5-12). These differences are minor and may related to method variability and/or the small size (N=5 or 6) tested and do not preclude a finding of high similarity between PF-05280014 and US-Herceptin.*

CDC-Assay: The assessment of CDC activity of trastuzumab was performed using the breast cancer cell line SKBR3 with human complement. No apparent CDC activity was detected in all three materials.

C1q binding: The binding of trastuzumab to C1q protein was assessed using a C1q binding immunoassay. The binding profiles are similar between PF-05280014, US-Herceptin, EU-Herceptin samples and the RM 124281pg9.

**Reviewer Comment:** *CDC is not part of MOA for trastuzumab. The lack of CDC activity and similar binding profiles for C1q suggest PF-05280014, US-Herceptin and EU-Herceptin are similar regarding these attributes.*

### Tier 3 QAs: Physicochemical Characterization

Composition and linkages of glycans: The composition of oligosaccharides was obtained by sequencing with a series of highly specific exoglycosidases. The data confirmed that all

trastuzumab glycans are complex type glycans. The exoglycosidase digestion scheme was provided in Figure 3.2.R.3.2.3-1 of Section 3.2.R.3.2.3 –linked Glycan Structure-Composition and Linkages and not copied here.

Sialic Acid Analysis: The identity and quantity of sialic acid species found in all three materials were determined using DMB labeling method. The summary data were shown in the table below.

**Table 3.2.R.3.2.3-1. Sialic Acid Forms and Relative Abundance in PF-05280014, Trastuzumab-US, and Trastuzumab-EU**

Sialic Acid Form	PF-05280014	Trastuzumab-US	Trastuzumab-EU
Neu5Ac (%)	97.2	92.9	94.1
Neu5Gc (%)	2.8	7.1	5.9

N-acetylneuraminic acid (Neu5Ac) was observed as the predominant sialic acid form with low levels of N-glycolylneuraminic acid (Neu5Gc) in PF-05280014, trastuzumab-US and trastuzumab-EU materials.

**Reviewer Comment:** *The total amount of sialic acid containing glycoforms are low and are similar among PF-05280014 (1.2%-1.7%), US-Herceptin (1.1%-1.9%) or EU-Herceptin (1.2%-2.1%) (Data summarized by the reviewer based on HILIC data). The percentage of potentially immunogenic Neu5GC is significantly lower in PF-05280014. The difference does not preclude a determination of highly similar between PF-05280014 and US-Herceptin, considering the absolute amount of Neu5GC containing glycoforms (<0.2%) in total glycoforms. The difference does not preclude the establishment of the analytical component of the three-way scientific bridge.*

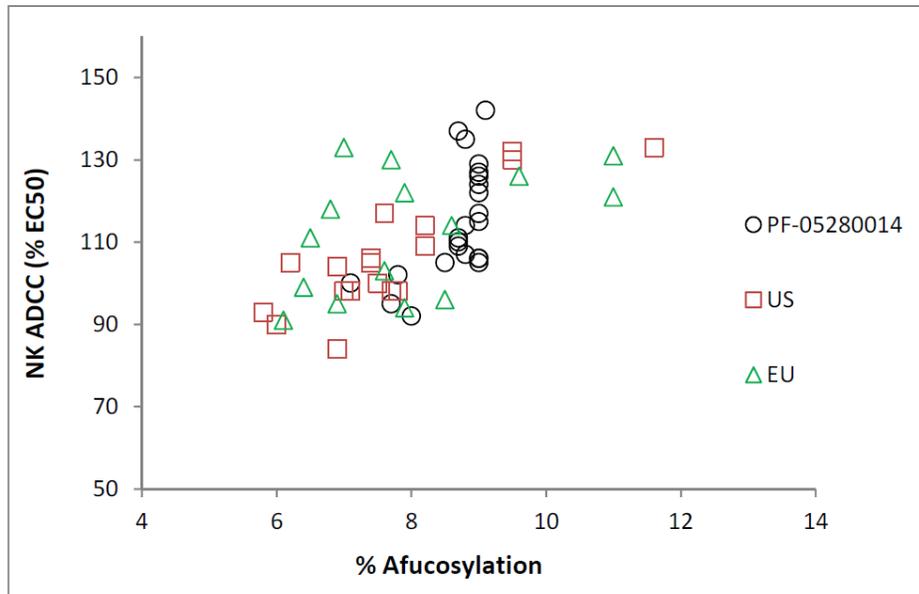
To further characterize the structure-function relationship of different glycoforms on the FcγRIIIa binding and ADCC activity of trastuzumab, the sponsor provided the following assessments:

- The impact of afucosylated N-linked glycans on NK cell ADCC activity
- The impact of afucosylated glycans on FcγRIIIa binding activity
- Response of ADCC activity to varied levels of afucosylated N-linked glycans in the primary NK cell ADCC assay

The ADCC data were plotted against the corresponding levels of afucosylated and G0 glycoforms in PF-05280014, US-Herceptin, and EU-Herceptin. The results were shown in the figure below.

**Figure 3.2.R.3.2.3-1. Impact of Total Afucosylated N-Linked Glycan Species and G0 Glycan Species to NK Cell ADCC Activity**

A.



B.

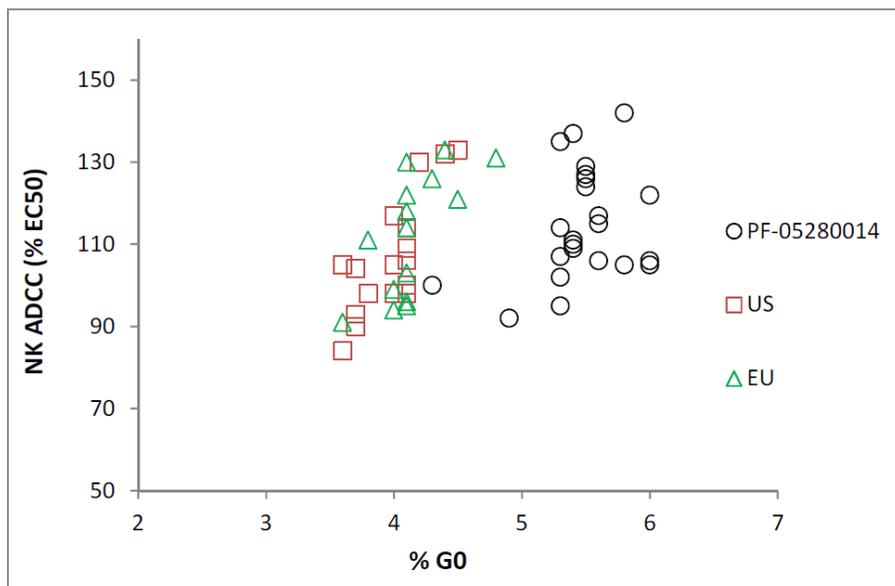
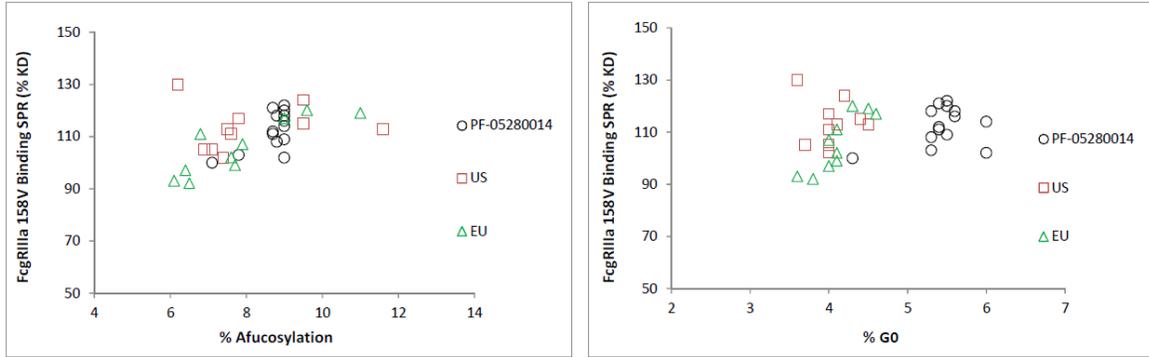


Figure 3.2.R.3.2.3-1. NK cell ADCC activities from PF-05280014 drug substance and drug product, trastuzumab-US, and trastuzumab-EU lots were plotted against their corresponding levels of afucosylation (Panel A) and G0 glycan (Panel B). Total afucosylation and G0 N-linked glycan values for PF-05280014 drug product lots were taken from the values of their corresponding ingoing drug substance batches.

The FcγIIIa binding data were plotted against the corresponding levels of afucosylated and G0 glycoforms in PF-05280014, US-Herceptin, and EU-Herceptin samples. The results were shown in the figure below.

**Figure 3.2.R.3.2.3-2. Impact of Total Afucosylated N-Linked Glycan Species and G0 Glycan Species on FcγRIIIa Binding SPR Activity**

**A. Binding to FcγRIIIa 158V**



**B. Binding to FcγRIIIa 158F**

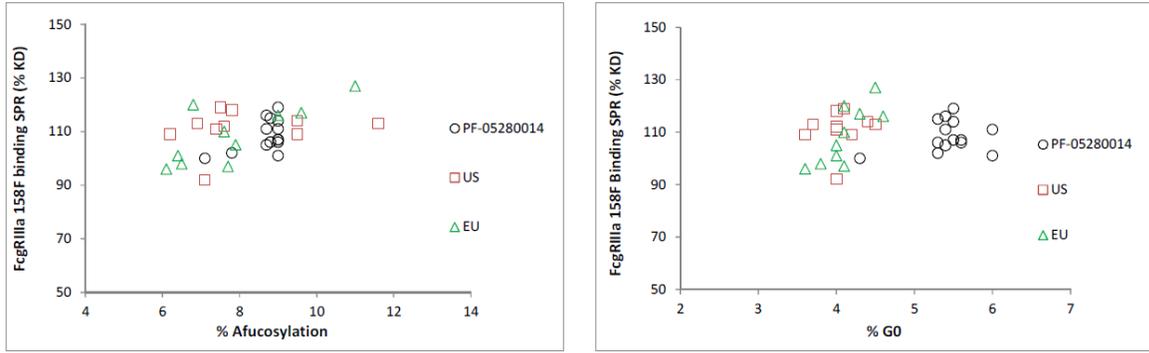


Figure 3.2.R.3.2.3-2. FcγRIIIa binding activities from PF-05280014 drug substance and drug product, trastuzumab-US, and trastuzumab-EU lots were plotted against their corresponding levels of afucosylation and G0 N-linked glycan. Panel A - binding to FcγRIIIa 158V, Panel B - binding to FcγRIIIa 158F. Total afucosylation and G0 glycan values for PF-05280014 drug product lots were taken from the values of their corresponding ingoing drug substance batches.

In addition, the afucosylated glycoforms from PF-05280014 were enriched using FcγRIIIa affinity chromatography. The enriched species (50% afucosylation and 30% G0) were then mixed with the flow through (contains <6% afucosylated species) to generate PF-05280014 samples with 6%-12% afucosylated and 3% to 7% G0 glycoforms. The NK ADCC values of

those samples were determined and shown in the figure below.

**Figure 3.2.R.3.2.3-3. NK Cell ADCC Activities for PF-05280014 with Different Levels of Afucosylation and G0 Glycan**

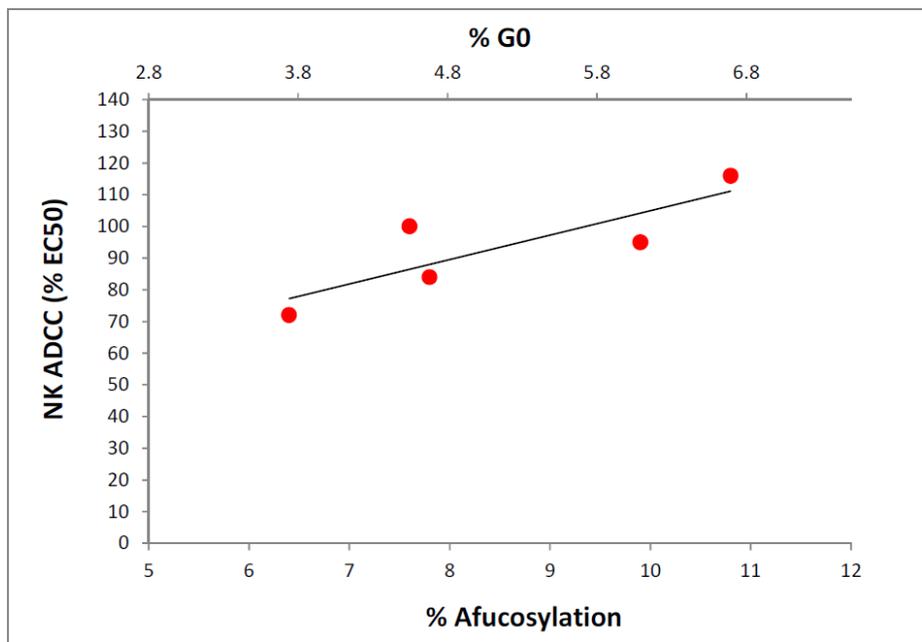


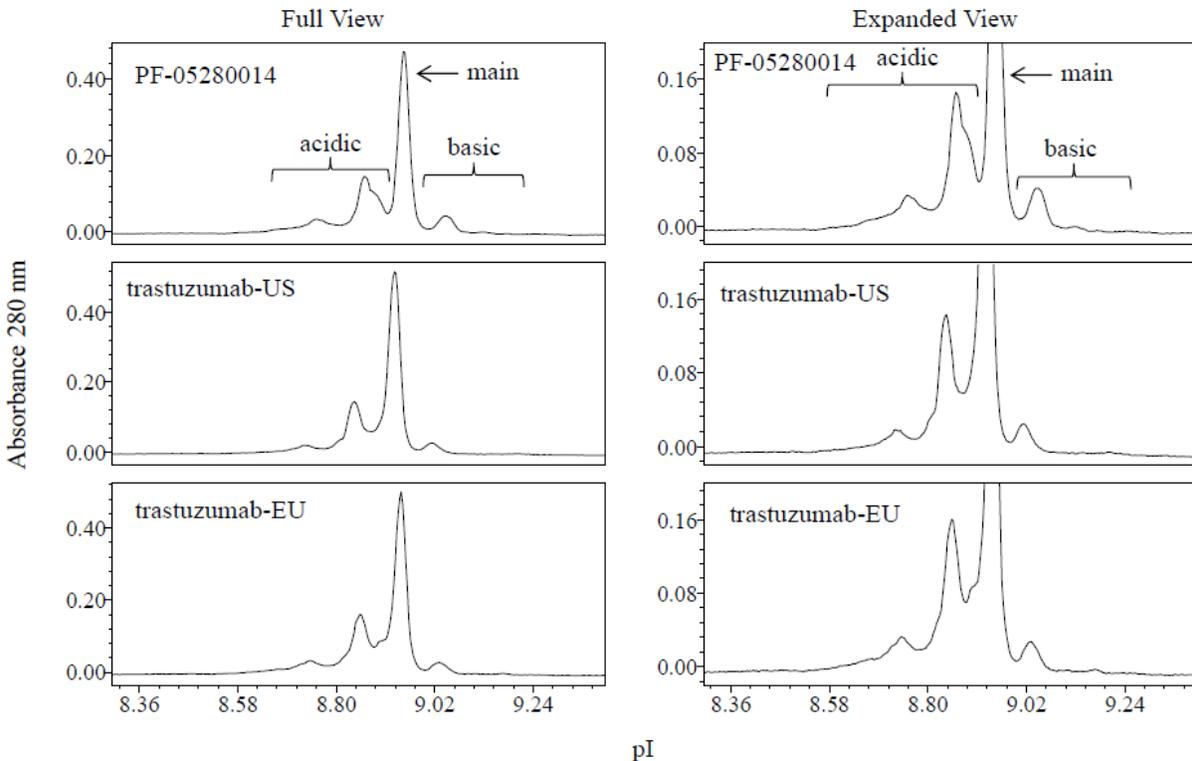
Figure 3.2.R.3.2.3-3. PF-05280014 samples with different levels of total afucosylation and G0 glycan were prepared from Fc $\gamma$ R3a affinity column and tested in the primary NK cell ADCC assay. The range of total afucosylation of N-linked glycans and G0 glycan species is relevant to the combined range observed in PF-05280014 and trastuzumab licensed product (6 to 12% afucosylation, 3 to 7% G0 glycan).

Based on these data and the data from the similarity assessment of the NK ADCC and Fc $\gamma$ R3a binding, the sponsor concluded that small differences in the ranges of 6% to 12% afucosylation and 3% to 7% G0 glycan in trastuzumab, commonly seen among PF-05280014, US-Herceptin, and EU-Herceptin, does not significantly affect ADCC activity.

**Reviewer Comment:** *The use of G0 glycan as a quantitative marker for total afucosylation is not appropriate as the relative percentages of G0 in total afucosylation vary widely (37%-69%); however, the application also included a comparison of total afucosylation and that attribute was found to be similar among all three materials elsewhere in the review. In addition, no impact on ADCC activity was observed in the Tier 1 assessment.*

Charge variants analysis by cIEF: A cIEF method was used as orthogonal way to measure charge heterogeneity. The representative electrograms of PF-05280014, US-Herceptin and EU-Herceptin, as well as the quantitation were shown below.

**Figure 3.2.R.3.2.4-5. iCE Electropherograms of PF-05280014, Trastuzumab-US, and Trastuzumab-EU**



**Table 3.2.R.3.2.4-3. Distribution of Acidic, Main, and Basic Species by iCE of PF-05280014, Trastuzumab-US, and Trastuzumab-EU**

Sample	Acidic (%)	Main (%)	Basic (%)
PF-05280014	39.8	51.9	8.3
Trastuzumab-US	32.5	61.5	6.1
Trastuzumab-EU	36.4	57.9	5.7

**Reviewer Comment:** The pIs of the main peak and the peak patterns are largely similar among all three materials. PF-05280014 has higher level of acidic species (39.8%) compared to US-Herceptin (32.5%) and EU-Herceptin (36.4%). A minor shoulder peak at around pI 8.85 was detected only in PF-05280014 and a minor shoulder at ~pI 8.90 was detected only in EU product. The differences in % basic peaks among the three materials are smaller than the CEX results. It bears noting that the charge separation mechanisms are different between the cIEF and CEX methods. The observed differences are relatively minor and do not preclude a finding of high similarity between PF-05280014 and US-Herceptin or the establishment of the three-way analytical bridge.

**Tier 3 QAs: Primary sequence, post translational modifications, purity and impurities**

Amino acid sequence: The amino acid sequence of PF-05280014 was verified against EU-Herceptin, US-Herceptin by LC/MS subunit analysis and Edman sequencing coupled with LC/MS/MS-peptide mapping with specialized bioinformatics (*de novo* sequencing techniques).

In the LC/MS subunit analysis, PF-05280014 is cleaved at the hinge region by the site-specific protease IdeS, yielding one Fab2 and two single chain Fc fragments. The fragments are then reduced and separated in RP-UPLC and the molecular weight of those fragments is determined by MS. The masses of all PF-05280014 subunits match the exact and theoretical values for US-Herceptin and EU-Herceptin. The summary table was provided in Section 3.2.R.3.2.1 and not shown here.

The amino acid sequence of PF-05280014 was also verified using *de novo* sequencing techniques. The techniques can distinguish transposed residues and isomeric residues such as Leu and Ile. PF-05280014, US-Herceptin and EU-Herceptin samples are reduced, alkylated, digested with multiple endoproteinasases and analyzed. The LC variable regions (1-107) and HC (1-120) variable regions were confirmed in all three samples. The full sequences were consistent among all three samples with 100% sequence coverage.

**Reviewer comment:** *The raw data for de novo sequencing were not provided in the submission. This is acceptable because the sequence of PF-05280014 is confirmed by the other methods.*

**Molecular Mass:** The molecular masses for the intact glycoforms of PF-05280014, US-Herceptin and EU-Herceptin were determined by nanoESI MS. The masses of intact molecules from all three materials are consistent and in agreement with the theoretical values. The results were summarized in Figure 3.2.R.3.2.1-4 and Table 3.2.R.3.2.1-2 and not copied here. The major glycoforms are G0F/G0F (148,059 Da), G0F/G1F (148,220 Da) and G1F/G1F (148,382) and their relative abundances are similar in all three materials. The US-Herceptin has slightly higher level of the Man5/Man5 glycoform and EU-Herceptin has slightly higher level of the G0F/G0F minus GlcNAc glycoform.

**Reviewer comment:** *The molecular mass is similar between PF-05280014, US-Herceptin and EU-Herceptin. See review below regarding the similarity assessment of the individual glycoforms.*

**LC/MS-Subunit Analysis:** The subunit analysis was also used for the product isoform identification. The results were provided in Section 3.2.R.3.2.1 and not copied here. Single chain Fc domain analysis shows G0F and G1F glycoforms without C-terminal lysine are the main species in all three products; however, qualitatively PF-05280014 has higher C-terminal lysine species compared to US-Herceptin and EU-Herceptin based on Figure 3.2.R.3.2.1.6 (not copied here). The glycosylation profiles are consistent with the nanoESI MS and the HILIC/MS results (Section 3.2.R.3.2.3).

The data on Fd' domain show the masses for all three samples agree with the theoretical mass (25,367.5 Da) with trace levels of glycated isoform detected in all three materials. The data on the LC also shows that the masses from all three samples agree with the theoretical mass (23,428.5 Da) with trace (< 4% of maximum peak height, PF-05280014) or minor (4-40% of maximum peak height) levels of glycated isoform detected in all three materials.

**Reviewer Comment:** *The data suggest that PF-05280014, US-Herceptin and EU-Herceptin have similar single chain Fc, the LC and the Fd' domains. Glycation in mAb is known to induce the*

*formation of HMMS, which is monitored by SEC. The lower level of glycation in PF-05280014 does not negatively affect the product quality based on SEC data provided in the similarity assessment and does not preclude a determination of high similarity between PF-05280014 and US-Herceptin.*

LC/UV- Peptide mapping (trypsin): The method was used to provide in-depth analysis of the primary structure and posttranslational modifications of PF-05280014, US-Herceptin and EU-Herceptin. The chromatographic overlays of all three materials were provided in the figures below and the corresponding mass values were provided in Table 3.2.R.3.2.1-6. All peptides match theoretical values. The sequence coverages are 97.7% for LC and 98.7% for HC. The results show that the majority of the C-terminal lysine at the HC was removed in all three materials. Low levels of oxidation at H21 was detected in all three materials. Although deamidation sites in both LC and HC were observed, those modifications may be because of the elevated pH used during sample preparation.

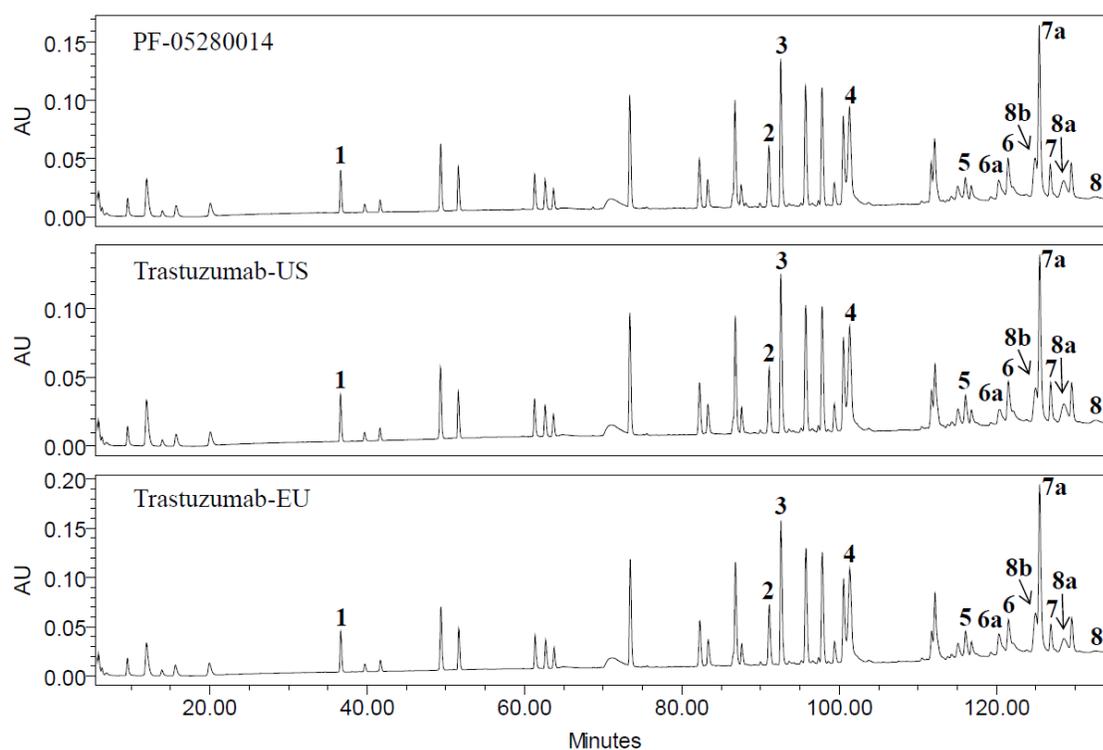
Additionally, chromatographic profiles of multiple lots of PF-05280014 (n=8), US-Herceptin (n=7) and EU-Herceptin (n=28) were compared side by side and the data were provided in Section 3.2.R.3.5.2 (not copied here). The results demonstrate that all materials have similar profiles.

***Reviewer Comment:*** *The peptide mapping chromatograms for PF-05280014, US-Herceptin and EU-Herceptin are similar.*

Purity by Reducing SDS-PAGE: Reducing SDS-PAGE was performed as an orthogonal size variant method to reducing CGE for PF-05280014, US-Herceptin and EU-Herceptin. One lot from each of the three materials were analyzed. The gel image was provided in Figure 3.2.R.3.2.6-10 of Section 3.2.R.3.2.5 Product Purity and not copied here. Similar band patterns (predominantly H and L chains with some minor bands) were observed in all samples. The identity of the protein bands was confirmed by Western using anti-human IgG antibody (figure not shown here).

Disulfide Bond by Peptide Mapping: Trastuzumab is a typical IgG1 antibody with 16 disulfide bonds. The disulfide bonds were determined by LC/MS non-reduced peptide mapping (Lys-C). The chromatographic profiles of PF-05280014, US-Herceptin and EU-Herceptin were provided in the figure below. The profiles are superimposable. No mispaired disulfide bonds were detected in all samples. All predicted disulfide bonds peptides were detected in all three products.

Figure 3.2.R.3.2.6-1. LC/MS-Non-Reduced Peptide Mapping of PF-05280014, Trastuzumab-US, and Trastuzumab-EU



**Reviewer Comment:** The PF-05280014, US-Herceptin and EU-Herceptin samples have the same disulfide bond linkages as the predicted structure.

**Sulfhydryl analysis:** Sulfhydryl analysis using Ellman's reagent was performed to quantitative the level of free thiol in the three products. The results were shown in the table below.

Table 3.2.R.3.2.6-2. Summary of Sulfhydryl Analysis for Representative PF-05280014, Trastuzumab-US, and Trastuzumab-EU Lots

Sample	N	Mean mol SH/ mol mAB	SD	CV (%)
PF-05280014	3	0.8	0.023	16
Trastuzumab-US	3	0.5	0.002	3
Trastuzumab-EU	3	0.4	0.002	3

N = replicates, SD = standard deviation, CV = coefficient of variation

**Reviewer Comment:** PF-05280014 product has higher level of free thiol (0.8 mol/ mol) compared to the US-Herceptin (0.5 mol/ mol) and EU-Herceptin (0.4 mol/ mol). The slightly higher level of free thiol in PF-05280014 correlates with the presence of small amount of HHL and L fragments detected by non-reducing CGE. Higher level of free thiol may contribute to aggregation formation, which is monitored by SEC during release and storage. This small difference does not preclude a determination of highly similar between PF-05280014 and US-Herceptin or the establishment of the analytical component of the three-way scientific bridge.

### **Tier 3 QAs: Higher Order Structures (Far UV, FTIR, Near-UV CD and Intrinsic Fluorescence)**

Far UV CD: Far UV (200-250 nm) CD was used to determine the secondary structure of PF-05280014, US-Herceptin and EU-Herceptin. The representative CD spectra for all three materials were provided and not copied here. The profiles are superimposable.

FTIR: FTIR was used to further assess the secondary structures. The FTIR spectra (1710  $\text{cm}^{-1}$  to 1590  $\text{cm}^{-1}$ ) were recorded in triplicates for PF-05280014, US-Herceptin and EU-Herceptin. Representative averaged spectra were provided and not copied here. The profiles are superimposable.

Near UV CD: Near UV (250 nm to 350 nm) CD was used to determine the tertiary structure of the PF-05280014, US-Herceptin and EU-Herceptin. Representative averaged spectra were provided and not copied here. The profiles are superimposable.

Intrinsic Fluorescence: Intrinsic fluorescence of Trp residues in a protein is sensitive to the microenvironment of the molecule therefore can be used to monitor differences in tertiary structure. The emission spectra were recorded in triplicates for PF-05280014, US-Herceptin and EU-Herceptin. Representative averaged spectra were provided and not copied here. The profiles are superimposable.

Thermal Stability by DSC: DSC was used to characterize the thermal induced transitions between the folded and unfolded structures of trastuzumab. The unfolding thermograms for all three materials were provided and not copied here. The profiles are superimposable.

**Reviewer Comment:** *The  $T_m1$  (~71.2°C) and  $T_m2$  (~83.6°C) values are similar among all three products.*

**Review comment:** *All biophysical studies data presented here suggest that the overall higher order structures of PF-05280014, US-Herceptin and EU-Herceptin are similar.*

### **Comparative Forced Degradation Study:**

PF-05280014, US-Herceptin and EU-Herceptin were subjected to various forced degradation studies. The conditions studies include elevated temperature storage, photo-oxidation, deamidation by phosphate buffer incubation and oxidation by peracetic acid.

**Note:** *The formulation of PF-05280014 is different from US-Herceptin and EU-Herceptin. Pfizer chose sucrose in the final formulation over trehalose used in the US-Herceptin (b) (4)*

*(b) (4)*. See 3.2.P.2.2 for detailed information. This difference does not preclude a finding of high similarity between PF-05280014 and US-Herceptin.

Elevated temperature: Lyophilized PF-05280014, US-Herceptin and EU-Herceptin products were held at 50°C for three months. Three studies were:

1. Initial studies: 3 lots of US-Herceptin and EU-Herceptin were put on stability study to assess comparability of the stability profiles.
2. Continuation Study: Four lots of PF-05280014 420 mg and 150 mg were put on stability to assess comparability of presentations on stability.
3. Final study: For PF-05280014, three DP lots and three aged DP lots for both 420 mg and 150 presentations were put on stability to assess comparability of presentations and comparability between fresh and aged materials.

**Reviewer comment:** Refer to Section 3.2.P.2.3 regarding the comparability assessment between PF-05280014 150 mg and 420 mg presentations.

Appearance (before and after reconstitution), protein concentration, HP-SEC, charge heterogeneity, reducing and non-reducing CGE, potency and subvisible particles were analyzed at the 0 and three month time points.

The detailed results from the elevated temperature studies were provided in 3.2.R.3.3 Comparative Forced Degradation Study and not copied here. The results are summarized as below:

- US-Herceptin (initial study): Between the 0 and 3 month time points, notable increases in basic species (14.5%-16.1% by CEX), HMMS (5.4%-6.1%) were observed; decreases in acidic species (0.9-1.6% by CEX), main species (13.5-14.6% by CEX), monomer (5.4%-6.1% by SEC), HC/LC (1.1%-1.7% by reducing CGE) and intact IgG (2.1%-4.6% by non-reducing CGE). No other trends were observed.
- EU-Herceptin (initial study): Between the 0 and 3 month time points, notable increases in basic species (12.0%-13.8% by CEX), HMMS (5.1%-5.5%) were observed; decreases in acidic species (0.4-1.9% by CEX), main species (11.6-14.0% by CEX), monomer (5.2%-5.6% by SEC), HC/LC (1.3%-1.8% by reducing CGE) and intact IgG (0.7%-3.3% by non-reducing CGE). No other trends were observed.
- PF-05280014 (initial and continuation studies): Between the 0 and 3 month time points, notable increases in basic species (12.6%-15.2% by CEX), HMMS (2.7%-3.7%) were observed; decreases in acidic species (3.3-4.0% by CEX), main species (9.2-11.5% by CEX), monomer (2.6%-3.6% by SEC), HC/LC (0.3%-1.7% by reducing CGE) and intact IgG (1.9%-3.0% by non-reducing CGE) were observed. No clear trends in potency are observed. No other trends were noticed.
- PF-05280014 (Final study): Between the 0 and 3 month time points, notable increases in basic species (11.9%-13.8% by CEX), HMMS (2.4%-3.2%) were observed; decreases in acidic species (2.6%-4.4% by CEX), main species (7.9%-10.0% by CEX), monomer (2.4%-3.3% by SEC), HC/LC (0.6%-1.7% by reducing CGE) and intact IgG (0.2%-2.4% by non-reducing CGE) were observed. One outlier (Lot Z02359, 150 mg) in which the intact IgG increased from 94.4% to 95.6%. No clear trends in potency are observed in PF-05280014. No notable differences were observed corresponding to product age.

**Reviewer Comment:** All PF-05280014 lots have similar degradation rates at the elevated temperature therefore the data can be pooled together for analysis. No new degradation species were observed in the SEC/CE-SDS/CEX profiles of PF-05280014 materials compared to US-

*Herceptin and EU-Herceptin. The primary degradation pathways are changes in charge variants, HMMS, and fragments for all three products and these pathways are similar among PF-05280014, US-Herceptin and EU-Herceptin. PF-05280014 has slightly more %acidic species (2.6%-4.4%) compared to that of US-Herceptin (0.9%-1.6%) and EU-Herceptin (0.4%-1.9%). PF-05280014 also has less HMMS (2.4%-3.2%) compared to that of US-Herceptin (5.4%-6.1%) and EU-Herceptin (5.1%-5.5%). Smaller changes in potency (cell inhibition) were observed for PF-05280014 samples upon exposure to elevated temperature, in contrast to the decrease observed in US-Herceptin (7%-26%) and EU-Herceptin (10%-19%) samples. The data provided in this study assess stability up to 3 months; however, data from additional PF-05280014 characterization does show a significant drop in potency at 6 months at 50°C. Although there are minor differences in the rates of change between PF-05280014, US-Herceptin and EU-Herceptin, the pathways are similar. These differences do not preclude the conclusion that the degradation profiles among PF-05280014, US-Herceptin and EU-Herceptin are similar.*

Statistical comparison of the degradation rates among PF-05280014, US-Herceptin, and EU-Herceptin are performed. The general linear regression model was applied to all data. The commonly-accepted 0.05 level of statistical significance was used to determine the existence of any statistically significant effects.

No significant differences in degradation rates observed between the (b) (4) 150 mg and 420 mg PF-05280014 presentations (See table below).

**Table 3.2.R.3.3-13. Statistical Comparison of PF-05280014 (b) (4) 150 mg, and 440 mg Drug Product Lots Under Elevated Temperature Forced Degradation Conditions**

Attribute	(b) (4)	Regression slope of 150 mg lots	Regression slope of 440 mg lots	Statistically Significant Differences (p-value)
% Acidic Species		-1.1	-1.1	None
% Basic Species		3.7	3.8	None
% Main Species		-2.6	-2.7	None
% HMMS		0.9	0.9	None
% Heavy Chain+ Light Chain <sup>b</sup>		-0.3	-0.5	(b) (4) 150 mg & 440 mg (0.00)

a. Regression slope is presented as percent change per month during storage at 50 °C.

b. Heavy chain + light chain as measured by CGF (reducing)

**Review comment:** (b) (4)  
 (b) (4) The degradation trends are similar between PF-05280014 150 mg and 420 mg presentations.

Statistical analysis was also performed between PF-05280014 PV DP lots and ICH DP lots (> 48 months aged lots). The results were shown in the table below.

**Reviewer comment:** While there are statistically significant ( $p < 0.05$ ) differences in % acidic species, % HMMS and % HC/LC, the differences are very small and the degradation trends remain similar. This difference does not preclude a finding of high similarity between PF-

05280014 and US-Herceptin or the establishment of the analytical component of the three-way scientific bridge.

**Table 3.2.R.3.3-14. Statistical Comparison of PF-05280014 Drug Product Lots Under Elevated Temperature Forced Degradation Conditions**

Attribute	Regression slope of ICH Lots <sup>a</sup>	Regression slope of PV Lots	P-value
% Acidic Species	-1.0	-1.2	0.04
% Basic Species	3.6	3.7	0.24
% Main Species	-2.6	-2.6	0.97
% HMMS	0.8	1.0	0.01
% Heavy Chain+ Light Chain <sup>b</sup>	-0.4	-0.4	0.03

a. Regression slope is presented as percent change per month during storage at 50 °C.

b. Heavy chain + light chain as measured by CGE (Reducing)

**Review comment:** The data suggest the degradation trends under elevated temperature are similar between PF-05280014 clinical lots (ICH) and PPQ lots.

The sponsor then pooled all new and aged PF-05280014 data (6 lots of 150 mg or 420 mg presentation) and compared against US-Herceptin (3 lots) and EU-Herceptin products (3 lots). The results were shown in the tables below.

**Table 3.2.R.3.3-15. Statistical Comparison of PF-05280014 Drug Product, 150 mg to Trastuzumab-EU 150 mg Under Elevated Temperature Forced Degradation Conditions**

Attribute	Regression slope of trastuzumab-EU lots <sup>a</sup>	Regression slope of PF-05280014 150 mg lots	P-value
% Acidic Species	-0.3	-1.1	0.00
% Basic Species	4.2	3.7	0.03
% Main Species	-4.0	-2.6	0.00
% HMMS	1.7	0.9	<0.0001
% Heavy Chain+ Light Chain <sup>b</sup>	-0.5	-0.3	0.04

a. Regression slope is presented as percent change per month during storage at 50 °C.

b. Heavy chain + light chain as measured by CGE (Reducing)

**Table 3.2.R.3.3-16. Statistical Comparison of PF-05280014 Drug Product, 440 mg to Trastuzumab-US Under Elevated Temperature Forced Degradation Conditions**

Attribute	Regression slope of trastuzumab-US lots <sup>a</sup>	Regression slope of PF-05280014 440 mg lots	P-value
% Acidic Species	-0.3	-1.1	0.00
% Basic Species	4.8	3.8	0.00
% Main Species	-4.5	-2.7	<0.0001
% HMMS	1.9	0.9	<0.0001
% Heavy Chain+ Light Chain <sup>b</sup>	-0.5	-0.5	0.97

a. Regression slope is presented as percent change per month during storage at 50 °C.

b. Heavy chain + light chain as measured by CGE (reducing)

**Reviewer Comment:** *The trends identified in the statistical analysis are similar to the ones commented above. The increase rate of degradation in % acidic species does not preclude a determination of highly similar between PF-05280014 and US-Herceptin because the acidic species does not affect potency. The degradation profiles and pathways are similar among all three materials despite minor differences in degradation rate.*

**Light Exposure:** Representative PF-05280014 DP (one 150 mg lot and one 420 mg lot), US-Herceptin DP (1 lot), EU-Herceptin DP (1 lot) were reconstituted with sterile water for injection. The reconstituted samples were then placed in a light chamber with 5.1 klux of light for 14 days at 25°C and then analyzed for protein concentration, charge heterogeneity by CEX, size variants by SEC, purity by CGE (non-reducing), potency (cell inhibition) and PTMs by LC/MS-peptide mapping. The detailed results were provided in 3.2.R.3.3 and not shown here. The results are summarized as below:

For PF-05280014, US-Herceptin and EU-Herceptin, the following changes were noted during the study period:

- Notable increases in acidic species (35%), HMMS, fragments, basic species, LMMS other peaks by (by non-reducing CGE) were observed; notable decreases in main species by CEX and intact IgG and monomer were observed.
- The potency dropped for both US-Herceptin (19%) and EU-Herceptin (27%), but the changes in PF-05280014 are not clear (The potency dropped from 101% to 78% for the 150 mg presentation but increased from 89% to 110% for the 420 mg presentation).
- Increased levels of M255 (H21) and M431 (H41) oxidation were detected in all products at days 14 by LC/MS- peptide mapping based on the chromatographic profiles. The levels of oxidation were not specified.
- No other trends were noted.

**Reviewer Comment:** *Primary degradation pathways under the light stress are charge variants, aggregation, fragmentation and Met oxidation for all three materials. The primary aggregates formed are dimers. The degradation patterns are similar between the 150 mg and 420 mg PF-05280014 presentations. The degradation profiles are also similar among all three materials. PF-05280014 has higher levels of other species but lower level of fragments compared to the reference products measured by non-reducing CGE. The rates of degradation for other QAs are similar among all materials. The differences in trends in potency may be due to method variability associated with the assay; however, data from this study is unclear. Other stability studies have shown similar changes in potency between the three products. The differences do not preclude a finding of high similarity between PF-05280014 and US-Herceptin or the establishment of the analytical component of the three-way scientific bridge.*

**Deamidation:** Trastuzumab has several primary deamidation sites at the CDR region (LC-Asn30, HC-Asn55 and HC-Asp102) according to report by Harris et al. 2001. The deamidation profiles of all three materials under alkaline pH were also evaluated. PF-05280014 DP (one 150 mg lot and one 420 mg lot), US-Herceptin (1 lot), EU-Herceptin (1 lot) were reconstituted with sterile water for injection. The reconstituted samples were then dialyzed into 100 mM sodium phosphate buffer, pH 8.0 and incubated for 14 days at 25°C. The samples were then analyzed for

protein concentration, charge heterogeneity by CEX, size variants by SEC, purity by CGE (non-reducing), potency (cell inhibition) and PTMs by LC/MS-peptide mapping. The SEC and non-reducing CGE data for deamidated samples were not provided initially and an IR was sent (IR #4 Comment 4, 11/07/2017). In response to 11/22/2017), the sponsor provided the requested data. The detailed results from the forced deamidation study were provided in 3.2.R.3.3 and not shown here. The results are summarized below:

- US-Herceptin: Between the 0 and 14 day time points, acidic species increased to 94%, fragments (by non-reducing CGE) increased ~7%. No changes in HMMS, potency and protein concentration were noted.
- EU-Herceptin: Between the 0 and 14 day time points, acidic species increased to 93%, fragments (by non-reducing CGE) increased ~8%. The potency changed from 115% to 84%. No changes in HMMS and protein concentration were noted.
- PF-05280014 (150 mg and 420 mg presentations): Between 0 and 14 day time points, acidic species increased to 91%, fragments (by non-reducing CGE) increased ~8%. The potency changed from 108% to 80%. No changes in HMMS and protein concentration were noted.

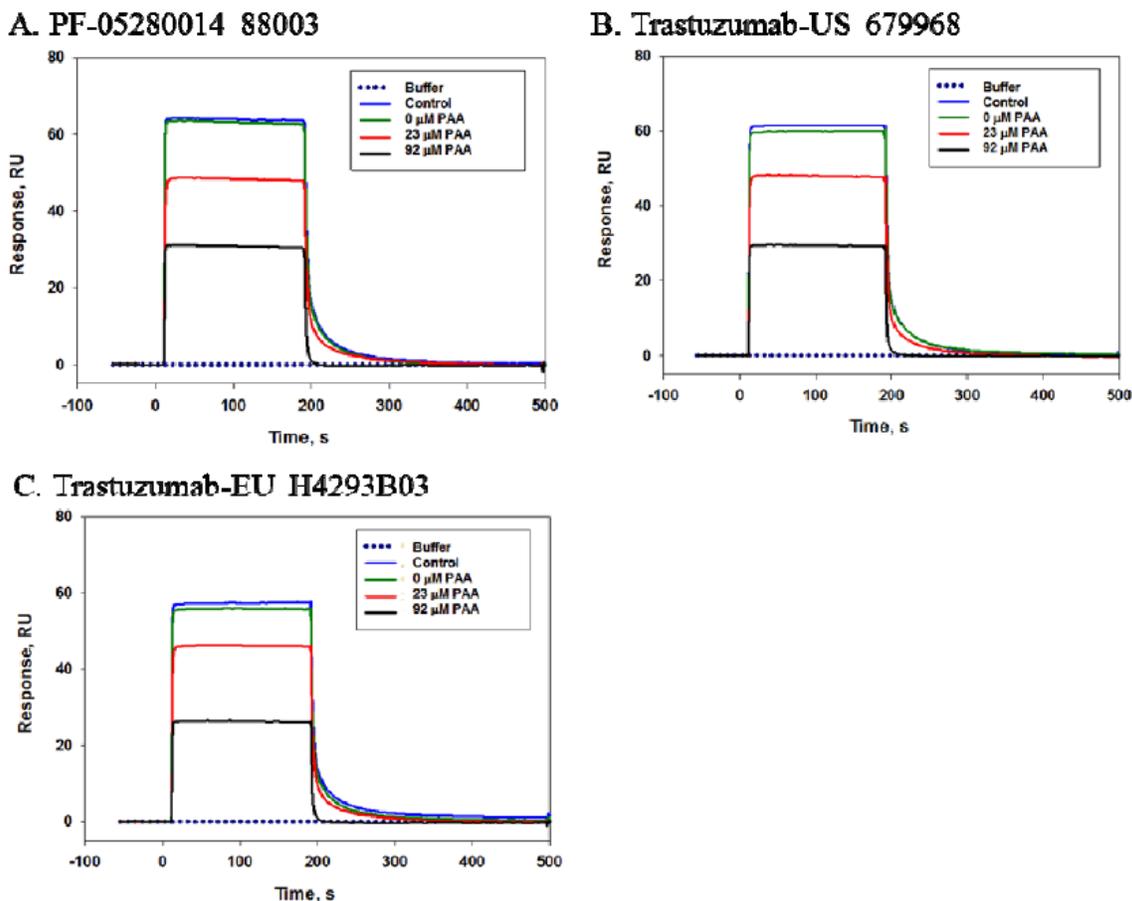
**Reviewer Comment:** *Similar degradation pattern occurred in all materials. The deamidation sites are the same among all three materials and consistent with the information reported by Harris et al.2001. The high HMMS level observed in both T0 and T14 samples (~10%) in all three materials may be an assay artifact because of the deamidation buffer pH (8.0) is close to the pI of the molecule (pI 8.6).*

Peracetic Acid Oxidation: The potential oxidation sites for all three materials were assessed using forced oxidation study. Chemical oxidation of trastuzumab was induced using peracetic acid (PAA). PF-05280014 DP (one 420 mg lot), US-Herceptin (1 lot), EU-Herceptin (1 lot) were incubated with increase concentration of peracetic acid (0, 23 and 92  $\mu$ M). The treated samples were then analyzed by LC/MS-peptide mapping (trypsin), limited Lys-C peptide mapping, bioassay (cell inhibition) and FcRn binding.

Major changes in HC Met107, Met255, Met361 and Met431, as well as minor oxidation at HC Met83 and LC Met4 were observed. The HC-Met255 is the most susceptible residue in all materials, consistent with the published literature (Harris 2005). The extent of Met255 oxidation under different concentration of PAA is similar among all three materials (data provided in Table 3.2.R.3.3-26 and not copied here). The LC-MS chromatograms were provided in Section 3.2.R.3.3 and not copied here.

Oxidation at HC-Met107 (CDR region) was detected and the modification has no impact on potency. The oxidation at HC-Met255 significantly decreased FcRn binding measured by SPR (see figure below).

**Figure 3.2.R.3.3-34. Binding of PF-05280014, Trastuzumab-US and Trastuzumab-EU to FcRn by SPR, Peracetic Acid Incubation**



**Reviewer Comment:** Oxidation at HC-Met255 is known to affect FcRn binding affinity for monoclonal antibody products. The levels of oxidation at Met255 for PF-05280014, US-Herceptin and EU-Herceptin are PAA concentration dependent and are similar among all three materials. The degradation profiles under peracetic acid treatment are similar among PF-05280014, US-Herceptin and EU-Herceptin.

#### **Summary of Similarity Conclusions:**

The applicant used EU-Herceptin in the clinical studies and therefore a three-way scientific bridge must be established to justify the relevance of the clinical data generated using EU-Herceptin to support the demonstration of biosimilarity. Therefore, the applicant provided similarity studies that included PF-05280014, US-Herceptin and EU-Herceptin. The similarity assessment amongst PF-05280014, US-Herceptin and EU-Herceptin involved a range of orthogonal physicochemical and functional assays, as well as forced degradation studies. Overall, based on the analytical data, including the statistical analyses, the quality attributes including primary structure, secondary and tertiary structure, potency, charge and size variants, glycoforms, binding assays and stability profiles, the data support that PF-05280014 and US-Herceptin are highly similar. For quality attributes where differences were noted, such as basic species and main species by CEX, and intact IgG by non-reducing CGE, these differences do not

preclude a determination that PF-02580014 is highly similar to US-Herceptin. Moreover, the analytical data establish a three-way analytical bridge between PF-05280014, US-Herceptin, and EU-Herceptin.

### **3.2.A.2 Adventitious Agents Safety Evaluation**

#### **Adventitious Agents and Safety Evaluation**

*Reviewer comment: See review in Sections 3.2.S.2.3 and 3.2.S.2.4 regarding the assessment of viral safety on raw materials, cell banks and unprocessed bulk.*

#### **Validation of Viral Clearance**



#### **5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies**

**Immunogenicity Assays:** Two binding ELISA Electrochemiluminescence (ECL) based methods are used to determine anti-trastuzumab or anti-PF-05280014 ADAs (screening, confirmatory and titer). Purified anti-trastuzumab antibodies were used the primary positive controls (PC).

Confirmed ADA positive samples were analyzed using alternative anti-PF-05280014 PC for cross-reactivity assessment. The original methods were validated using serum from healthy volunteers (report # B3277001 and B3277002) and used to support the single dose PK study B3271001. The methods were later validated with sample pretreatment steps and using HER2+ serum (report # B3277009 and B3277010). The validated methods were used to support the efficacy, safety, PK and immunogenicity studies B3271002 and B3271004. All methods were validated at (b) (4)

Two competitive ligand binding (CLB) methods are used to determine neutralizing antibody (Nab) against trastuzumab or PF-05280014. Purified anti-trastuzumab antibodies were used the primary positive controls. Confirmed Nab samples were analyzed using additional anti-PF-05280014 antibodies as an alternative PC for cross-reactivity assessment. Original methods were validated (report # 134959 and 135245) using healthy human serum to support the single dose PK study B3271001. The methods were later validated with sample pre-treatment steps and using HER2-positive serum (report # B3277007 and B3277008). The validated methods were used to support the efficacy, safety, PK and immunogenicity studies B3271002 and B3271004. The original methods were validated at Pfizer internal bioanalytical lab (Andover, MA), while the revised methods were validated at (b) (4)

**Review comment:** *The validations using healthy volunteers' serum were exploratory and not assessed in this review. Only method description and validations for ECL/CLB validated with serum from representative patient population are summarized below.*

#### **Screening/Confirmatory/Titer ADA assay validation**

Diluted control and test samples are pretreated with a conjugated anti-HerB2 mAb to prevent HER-2 interference. After that test samples are diluted at the minimal required dilution (MRD) of 1:10. The samples are then incubated with Biotin labeled and sulfo-tag labeled trastuzumab (or PF-05280014) to allow the formation of ADA-labeled drugs complex. The resulted samples were then bound to a streptavidin plate and an ECL signal will be generated when the ADA forms a bridge between the biotin- and Sulfo-tagged drugs. All samples, negative control, positive controls are evaluated in duplicate for screening. The mean relative luminescent unit values from the duplicates were reported.

**Review comment:** *The two ECL methods for anti-trastuzumab and anti PF-05280014 antibodies are largely identical with the exception of the drugs used and the values (such as cutoff values) established in respectively validation studies.*

#### **Controls and critical reagents:**

- Assay diluent: 1% BSA in PBST
- Negative control: Pooled normal human serum (PNHS) in assay diluent.
- Positive control: Positive Controls: Purified rabbit anti-trastuzumab IgG and mouse anti-PF-05280014 antibody (secondary, for cross reactivity only)
- Her2+ patient serum
- ECL reagents: Biotin labeled and Sulfo-Tag trastuzumab or PF-05280014
- Pre-treatment reagents: Anti-HER2 mAb and Streptavidin coated nanoparticles
- EU-Herceptin, PF-05280014 and HER2

- Her-2

#### System suitability

- Negative control: The mean RLU of the negative control must be less than 125.661 (for anti-trastuzumab ADA assay); the % CV must be  $\leq 25\%$  after outlier removal; outlier should be removed based on Dixon Test and if more than one well is an outlier, the plate fails.
- High positive control: The log<sub>10</sub> titer must be within 2.98-3.78 using a 1.22 Cut Point Factor (for anti-PF-05280014 ADA assay); the % CV must be  $\leq 25\%$  for dilutions greater than or equal to the Plate Cut point; The %CV for unspiked and spiked positive controls must be  $\leq 25\%$ .

#### **Screening Assay Cut Point (Anti-trastuzumab ADA)**

Forty individual HER+ patient serum samples were diluted at MRD (1:10) and ran  $\geq 3$  times over three days with  $\geq 2$  analysts (N = 116). Measured RLU values were back calculated into titer against the calibration curve and negative controls. The summary data were provided in Table 2 and Appendix 5 and not copied here. Four samples were excluded from cut point calculation based on box-plot outlier analysis. The normality of the data was verified by Shapiro-Wilk analysis and the parametric approach was used for cut point calculation. The cut point factor (CPF) of 1.22 was determined at 5% false positive rate. The cut point was defined as the mean value of NCs times the CPF. A total of 5 out of 116 data points were above CP and considered false positive (4.3% false positive rate).

*Reviewer comment: The statistical summary tables (Page 142 and 144 of B3277009) provided in Appendix 5 is barely legible. However, this will not affect the assessment here.*

#### **Screening Assay Cut Point (Anti-PF-05280014 ADA)**

Forty individual HER2+ patient serum samples were diluted at MRD (1:10) and ran 3 times over three days with  $\geq 2$  analysts (N = 116). Measured RLU values were back calculated into titer against the calibration curve and negative controls. The statistical data were provided in Appendix 5 and not copied here. Five samples were excluded from cut point calculation based on box-plot outlier analysis. The normality of the data was verified by Shapiro-Wilk analysis for Day 1 and Day 3. Data from Day 2 failed the Shapiro-Wilk test ( $P < 0.05$  for null hypothesis) and the 95% of the response were used for the cut point determination. The cut point factor (CPF) of 1.19 was calculated in the same way as that of the anti-trastuzumab ADA assay. The cut point was defined as the mean value of NCs times the CPF. A total of 6 out of 116 data point were above the CP and considered false positive (5.2% false positive rate).

*Reviewer comment: Sufficient data were provided to support the statistical approach used for the screening cut point determination for both assays. The actual false positive rate is  $\sim 5\%$ , in line with the theoretical value. Sample mean values correlate with negative control mean values, suggesting that the negative control is appropriate for use as a normalization factor for the floating cut-point approach.*

#### **Confirmatory cut-point (Anti-trastuzumab ADA)**

Confirmatory assay was performed by measuring the trastuzumab induced inhibition in the ECL method. The cut-point values were established from around ~30 HER+ patient serum samples analyzed independently for three times (Day 1, Day 2 and Day 3, N = ~90). Percent inhibition values were analyzed by for outliers and data distribution normality: 5 data points were excluded from calculation because of high %CV (>25%), alongside with 2 outliers (based on box plot analysis); the normal distribution of outlier-excluded data was demonstrated using Shapiro-Wilk W test (P values reported at Appendix 5 and not reported here. The cut-point at the 0.1% (mean+3.09xSD) false positive rate is 37.1% inhibition.

#### **Confirmatory cut-point (Anti-PF-05280014 ADA)**

Two validation activities were performed for the confirmatory cut point (CP). The CP from the original validation resulted in a higher than expected ~7% confirmed positive rate in baseline samples. Additional validation (Addendum 1) was performed using ~36 HER2+ patient samples analyzed independently for three times (Day 1, Day 2 and Day 3). Percent inhibition values were analyzed by statistical methods to assess for outliers and data distribution normality: 14 outliers are excluded from calculation based on box plot analysis; the normal distribution of outlier-excluded data was rejected using Shapiro-Wilk W test (P values reported at Appendix 5 and not reported here). The cut point at the 0.1% false positive rate (mean+3.09xSD) is 33.9% inhibition. The new cut point will be retroactively applied to the samples already analyzed.

***Reviewer comment:** The original cut point was 16.7%. The 0.1% false positive rate is not acceptable for the confirmatory cut point calculation because it may underestimate the number of patient samples with low levels of binding ADAs. An IR will be sent.*

#### **Titration Assay Cut Points**

No cut points for titers were determined. The same CPFs for screening (1.05 for anti-trastuzumab and 1.19 for anti-PF-05280014) were used the titer assay. The end point titer (EPT) of the sample is the reciprocal of the dilution of the sample at which the response is equal to the plate cut point value.

**Cross Reactivity Assessment:** Samples have > 1.00 titer values in the original anti-trastuzumab or anti PF-05280014 ADA assay were tested for cross-reactivity of using the alternative mouse anti-PF-05280014 antibody positive control. For anti-trastuzumab ADA assay cross activity, the % CV for EPT is 3.2% and the assay sensitivity is 4.64 ng/mL (mean at assay cut point + 1.645\*SD); for anti-PF-05280014 assay, the % CV for EPT is 5.6% and the assay sensitivity is 3.47 ng/mL.

***Reviewer Comment:** The reactivity, sensitivity and variability of both anti PF-05280014 and anti-trastuzumab positive controls are comparable.*

**Sensitivity:** The assay sensitivity was determined by the concentration at which the positive control products a response equal to the screening assay cut point. It is reported as the 95% confidence interval value from all validation runs. The sensitivity is calculated using the formula below.

*PC concentration at ACP = [Highest starting Primary PC con in the assay]/Interpolated Dilution at ACP x MRD*

*Relative Assay Sensitivity = mean PC concentration at ACP + (1.645\*SD)*

The relative sensitivity is 6.36 ng/ml or 6.22 ng/ml (outlier removed) for the anti-trastuzumab ADA assay. The relative sensitivity is 4.82 ng/ml or 4.77 ng/ml (outlier removed) for the anti-PF-05280014 ADA assay.

**Reviewer Comment:** *The sensitivity for both assays is acceptable and both assays are suitable for the analysis of clinical samples after the washout period.*

**Low positive Control:** A LPC concentration of 300 ng/ml was selected for both assays.

**Reviewer Comment:** *The LPC was not determined statistically and no justification for the selection of the LPC is provided. The LPC is too high for the binding ADA assay.*

**Precision:** Intra-run and inter-run precision (%CV) for PC (EPT) and NC were evaluated in all validation runs. The worse %CV value for PC (EPT) is 5.6% and for NC is 7.7% for Anti-trastuzumab ADA assay. The worse %CV value for PC (EPT) is 6.7% and for NC is 7.8% for Anti-PF-05280014 ADA assay.

**Drug Interference:** The HPC (1000 ng/mL) can tolerate up to 20 µg/mL trastuzumab and the LPC (300 ng/mL) can tolerate up to 5 µg/mL in the anti-trastuzumab ADA assay. The HPC and LPC can tolerate up to 80 µg/mL in the anti-PF-05280014 ADA assay.

**Reviewer Comment:** *The drug tolerance levels in the anti-trastuzumab ADA assay is much lower than the drug trough level observed in patients (0-206 ug/mL with a mean concentration of 34.56 ug/mL). The results suggest that the anti-trastuzumab ADA assay may be significantly underestimating the ADA rate for EU-Herceptin in patient samples. The drug tolerance level in the anti-PF-05280014 ADA assay is above the mean trough level (34.59 ug/mL) but below the highest level of PF-05280014 observed in patients (131 ug/mL). Although the drug tolerance for the anti-PF-05280014 is less than optimal, the assay is more accurate than the anti-trastuzumab assay. Currently the overall ADA rates are low and comparable between both EU-Herceptin and PF-05280014, suggesting that the risk of immunogenicity of PF-05280014 is at least as low as, if not lower than the reference product. This is acceptable.*

**Target (HER2) Interference:** The HPC (1000 ng/mL) and LPC (300 ng/mL) can tolerate up to 2000 ng/mL HER2 in both anti-trastuzumab and anti-PF-05280014 ADA assay.

**Target/Drug complex (HER2 plus trastuzumab or PF-5280014) Interference:** The HPC (1000 ng/mL) and LPC (300 ng/mL) can tolerate up to 2000 ng/mL HER2 and 50 µg/mL trastuzumab in the anti-trastuzumab ADA assay. The HPC (1000 ng/mL) can tolerate 2000 ng/mL HER-2 and 80 µg/mL PF-05280014, the LPC (300 ng/mL) can tolerate 2000 ng/mL HER-2 and 20 µg/mL PF-05280014, in the anti-PF-05280014 ADA assay.

**Matrix Selectivity (Recovery) in PC:** The recovery levels are 94.1-116.0% for 100% (10 out of 10) HER-2+ patients serum samples spiked with either HPC or LPC in both anti-trastuzumab or anti-PF-05280014 assays.

**Stability:** The stability of HPC and LPC samples in both ADA assays is assessed by comparing the titer of those samples between treated and untreated conditions: no significant changes in titer were observed for samples stored at room temperature for 24 hours; no significant changes in titer were observed for samples with  $\geq 9$  freezer/thaw cycles at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$  respectively.

Long term storage stability of the PC samples in matrix at  $-70^{\circ}\text{C}$  was not assessed. The sponsor will monitor positive control performance during sample analysis.

*Reviewer Comment: The data provided suggest that clinical serum samples are likely to be stable for up to 9 freeze thaw cycles at  $-20^{\circ}\text{C}/-70^{\circ}\text{C}$  and 24 hours at room temperature. The long term stability of PC is controlled by monitoring the PC plate acceptance range during routine analysis.*

**Prozone effects:** The prozone effects of high concentration positive controls are not assessed.

#### **Neutralization Antibody Assay:**

A competitive binding assay using ECL method was developed to quantitate the anti-trastuzumab Nab in HER2+ patient samples. Briefly, all samples are pretreated with acid and then incubated with Biotin-trastuzumab, followed by immobilization on a streptavidin coated plate. The bounded anti-trastuzumab antibodies is then released from the plate and incubated with Ru-trastuzumab overnight. The resulted samples were incubated with a HER-2 coated plate. The plate is then washed and the developed using ECL method. The presence of Nab competes with the binding of Ru-trastuzumab to Her-2, therefore the generated signal is reversely proportional to the amount of Nab.

The anti PF-05280014 Nab assay is almost the same as mentioned above except that PF-05280014 was used in place for trastuzumab for the preparation of capture and detection reagents.

#### **Controls and Critical Assay Reagents:**

- Negative control: Pooled normal human serum
- Positive control:
  - Primary: affinity purified human anti-trastuzumab mAb
  - Cross reactivity: affinity purified mouse anti-PF-05280014 mAb
- Her2+ patient serum
- ECL reagents: biotinylated and ruthenylated trastuzumab or PF-05280014
- EU-Herceptin, PF-05280014 and HER2

#### **System suitability**

- Negative control:

- The mean response of the negative control must be 5060 to 17700 for the anti-PF-05280014 Nab assay; the mean response must be between 2780 to 17400 for Anti-trastuzumab Nab assay.
- The % CV must be  $\leq 25\%$  after outlier removal; outlier should be removed based on Dixon Test.
- Positive control:
  - The PC end point titer must be within 2.20-2.86 for anti-trastuzumab Nab assay; the titer must be within 2.26-2.96 for anti-PF-05280014 Nab assay.
  - The % CV from the sample duplicates must be  $\leq 25\%$ .

**Validation of Anti-trastuzumab Nab Assay:** These two assays were validated in study reports B3277007 (anti-trastuzumab) and B3277008 (anti-PF-05280014).

**Cut Point:** Around 42 individual HER2+ patient serum samples were analyzed 3 times over three days with 2 analysts. The average RU values for individual samples were log10 transformed and subjected to statistical analysis for outlier and normality assessment. The statistical data were provided in Appendix 8 and not copied here.

- For anti-trastuzumab assay, seven samples were excluded from cut point calculation based on box-plot outlier analysis. The data from run 1 and 3 were normally distributed while the data from run 2 was not normally distributed based on the Shapiro-Wilk Test. The cut point factor is calculated to be 0.75 assuming 0.1% false positive rate. The cut point was defined as the mean value of NCs times 0.75.
- For anti-PF-05280014 assay, six samples were excluded from cut point calculation based on box-plot outlier analysis. The data from run 1 and 3 were normally distributed while the data from run 2 was not normally distributed based on the Shapiro-Wilk Test. The cut point factor is calculated to be 0.76 assuming 0.1% false positive rate. The cut point was defined as the mean value of NCs times 0.76.

**Reviewer Comment:** *The 0.1% false positive rate is too tight for samples with confirmed ADA response. The sponsor will be asked to revise it to 1%.*

**PC Precision (inter and intra run):** The worst %CV of the EPT is 5.8% among all inter and intra runs in both assay validations.

**Mean NC RU/Mean PC RU at maximum Concentration Precision:** 18.1% for the anti-trastuzumab Nab assay and 12.8% for the anti-PF-05280014 assay.

**PC EPT acceptable range:** The acceptable positive EPT range is 2.20 to 2.86 (mean  $\pm$  3SD) for the anti-trastuzumab Nab assay and 2.26 to 2.96 for the anti-PF-05280014 Nab assay.

**Drug Tolerance:** HPC (2500 ng/mL) can tolerate up to 100  $\mu$ g/mL trastuzumab while LPC (500 ng/mL) can tolerate up to 25  $\mu$ g/mL trastuzumab for the anti-trastuzumab Nab assay, while both HPC (2500 ng/mL) and LPC (500 ng/mL) can tolerate up to 100  $\mu$ g/mL PF-05280014 for the anti-PF-05280014 Nab assay.

**Review comment:** *The drug tolerance level of the LPC for the anti-trastuzumab Nab assay is much lower than the drug trough level observed. The drug tolerance level for the anti-PF-05280014 Nab assay is above the mean drug trough level (~ 35 ug/mL) but below the highest trough level of 131 ug/mL). As described for the screening assay, because the levels of NABs were low (0%) and similar between both EU-Herceptin and PF-05280014, it is unlikely that PF-05280014 is more immunogenic than EU-Herceptin. This is acceptable.*

**Target (HER2) Interference:** The HPC (2500 ng/mL) and LPC (500 ng/mL) can tolerate up to 2000 ng/mL HER2 in both assays.

**Target/Drug complex (HER2 plus trastuzumab or PF-5280014) Interference:** The HPC (2500 ng/mL) and LPC (500 ng/mL) can tolerate up to 2000 ng/mL HER2 and 80 µg/mL trastuzumab in the anti-trastuzumab Nab assay; the HPC (2500 ng/mL) and LPC (500 ng/mL) can tolerate up to 2000 ng/mL HER2 and 80 µg/mL PF-05280014 in the anti-PF-05280014 Nab assay.

**Matrix Selectivity (Recovery) in PC:** For anti-trastuzumab Nab assay, 11 HER2+ patients serum samples spiked with HPC has a recovery of 75-125%. 8 out of the 11 samples spiked with LPC have recovery between 75-125%; for anti-PF-05280014 Nab assay, all 11 samples spiked with HPC or LPC have recovery between 75-125%.

Relative sensitivity of PC: The relative sensitivity is 324.7 ng/mL for anti-trastuzumab Nab assay and 262.4 ng/mL for anti-PF-05280014 Nab assay.

Dilution Linearity in PC: The linearity was observed in 500 ng/mL, 1000 ng/mL, and 2500 ng/mL PC samples in both assays.

**Stability of the Positive Controls:** The stability of HPC and LPC samples in both assays is assessed by comparing the titer of those samples between treated and untreated conditions: no significant changes in titer were observed for samples stored at room temperature for 21 hours; no significant changes in titer were observed for samples with ≥ 5 freezer/thaw cycles at -20°C or -70°C respectively.

Long term storage stability of the PC samples in matrix at -70°C was not assessed. The sponsor will monitor positive control performance during sample analysis.

**Reviewer Comment:** *The long term stability of PC is controlled by monitoring the PC plate acceptance range during routine analysis.*

**Stability of Labeled Trastuzumab or PF-05280014:** The labeled trastuzumab or PF-05280014 is stable with at least three freeze/thaw cycles at -70°C.

**PC (Mouse anti-PF-05280014 antibody) Cross reactivity for trastuzumab or PF-05280014:** The PC precision at EPT is 1.0% and the relative sensitivity is 1110 ng/mL for the anti-trastuzumab Nab assay; the PC precision at EPT is 0.6% and the relative sensitivity is 1317 ng/mL for the anti-PF-05280014 Nab assay.

**Robustness:** The assay is robust (titer difference < 0.30) with different lots of 300 mM acetic acid, HER-2, BSA and two analysts in both assays.

***Overall assessment of immunogenicity assay:*** Although there are deficiencies identified in ADA and Nab immunogenicity assays with respect to drug tolerance, those deficiencies would not change the comparative immunogenicity assessment considering the clinical data and the low risk of immunogenicity for trastuzumab products in the intended treatment populations.



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**PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION**

**To:** Administrative File, STN 761081/0  
**From:** Maria Jose Lopez Barragan, PhD., Reviewer, CDER/OPQ/OPF/DMA/BIV  
**Through:** Reyes Candau-Chacon, PhD., Acting Quality Assessment Lead, CDER/OPQ/OPF/DMA/BIV  
**Subject:** Biosimilar Biologic License Application (BLA)  
**US License:** 2001  
**Applicant:** Pfizer Inc.  
**Facilities:** [REDACTED] (b) (4)  
FEI: [REDACTED] (b) (4) (Drug Substance Manufacturer)  
**Product:** TRAZIMERA™ (PF-05280014, aka PF8-01, a proposed biosimilar to Herceptin®)  
**Dosage:** Lyophilized cake or powder (440 mg/multidose vial) for intravenous infusion  
**Indication:** Treatment of patients with HER2-positive metastatic breast cancer and HER2-positive gastric cancer  
**Goal date:** 4/22/2018

**Recommendation for approvability:** The drug substance part of the BLA was reviewed from a microbial control and microbiology product quality perspective and it is recommended for approval.

**SUMMARY**

Pfizer Inc. has submitted BLA 761081 to obtain approval of Trazimera™ (PF-05280014), a proposed biosimilar to U.S. licensed Herceptin® (trastuzumab). PF-05280014 is a humanized immunoglobulin 1 (IgG1) monoclonal antibody directed against the human epidermal growth factor receptor 2 (HER2/neu receptor). PF-05280014 has been developed for the same indications as the reference product (treatment of patients with HER2-positive metastatic breast cancer and HER2-positive gastric cancer). BLA 761081 was submitted in eCTD format on 6/22/2017. This review contains the assessment of PF-05280014 drug substance from a microbiology quality perspective. Microbiology quality and sterility assurance of PF-05280014 drug product were reviewed by Dr. Virginia Carroll.

### Drug Substance Microbiology Quality Information Reviewed

Description	eCTD Sequence	Date
Original BLA	0001	6/22/2017
Amendment (response to Information Request, Q6)	0016	11/13/2017
Amendment (response to Information Request)	0019	12/4/2017

## 3.2.S DRUG SUBSTANCE

### S.1 GENERAL INFORMATION

PF-05280014 is a recombinant humanized immunoglobulin 1 (IgG1) kappa monoclonal antibody directed against the human epidermal growth factor receptor 2 (HER2/neu receptor). PF-05280014 has been developed as a proposed biosimilar to the U.S. licensed Herceptin® (trastuzumab) for intravenous infusion and treatment of the same indications as the reference product. PF-05280014 is produced in a recombinant Chinese Hamster Ovary (CHO) cell line.

*The description is satisfactory*

### S.2 MANUFACTURE

#### S.2.1 MANUFACTURE(S)

Table 3.2.S.2.1-1 below (reproduced from the submission) summarizes the facilities involved in the manufacture and in-process, release, and stability testing of PF-05280014 drug substance:

**Table 3.2.S.2.1-1. Sites and Responsibilities for Manufacture and Testing of PF-05280014 Drug Substance**

Site	Responsibility
Wyeth BioPharma Division of Wyeth* Pharmaceuticals Inc. One Burtt Road Andover, MA 01810 USA	Cell bank manufacture and storage
(b) (4)	Drug substance manufacture Drug substance in-process control testing and release testing Drug substance and cell bank storage
Pfizer Ireland Pharmaceuticals Grange Castle Business Park Clondalkin, Dublin 22 Ireland	Cell bank storage Drug substance release testing and stability testing

\* Wyeth is a wholly owned subsidiary of Pfizer Inc.

*Reviewer's comment: a pre-license inspection (PLI) was conducted at the PF-05280014 drug substance manufacturing facility (b) (4)*

*(b) (4) from (b) (4) (see under FACTS assignment (b) (4))*

*Satisfactory*

## **S.2.2 DESCRIPTION OF MANUFACTURING PROCESS AND PROCESS CONTROLS**

### **OVERVIEW OF MANUFACTURING PROCESS**

(b) (4)





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Lopez-Barragan

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Reyes  
Candau-Chacon

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Center for Drug Evaluation and Research  
Office of Pharmaceutical Quality  
Office of Process and Facilities  
Division of Microbiology Assessment  
WO Building 22  
10903 New Hampshire Ave.  
Silver Spring, MD 20993

## **PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION**

**Reviewer: Virginia Carroll, Ph.D.**  
**Quality Assessment Lead: Reyes Candau-Chacon, Ph.D.**

BLA: 761081  
Applicant: Pfizer Inc.  
US License Number: 2060  
Submission Reviewed: 351(k) BLA  
Product: TRAZIMERA, PF-05280014, a proposed biosimilar to US approved Herceptin  
Indication: Adjuvant breast cancer, metastatic breast cancer, metastatic gastric cancer  
Dosage Form: Multidose vial contains 420 mg lyophilized product, packaged with 20 mL bacteriostatic water for injection (BWFI), for intravenous infusion  
Manufacturing Sites: Pfizer Manufacturing Belgium NV, Puurs, Belgium (FEI 1000654629) (DP and BWFI)  
FDA Receipt Date: 6/22/2017  
Action Date: 4/20/2018

### **Conclusion and Approvability Recommendation**

The drug product part of the BLA was reviewed from a sterility assurance and quality microbiology perspective and is not recommended for approval.

#### **The following deficiencies should be communicated to the sponsor:**

1. Drug Master File (b) (4) is inadequate to support (b) (4) of PF-05280014 drug product.
2. Media fill simulations to validate (b) (4) for PF-05280014 drug product are not included in the BLA. Provide summary data for three media fills performed on fill line (b) (4) in the BLA resubmission.

3. The maximum hold times of PF-05280014 drug product (b) (4) proposed in the BLA are not supported by relevant microbiology data. Provide summary microbial data from hold time studies conducted in the holding vessels used for PF-05280014 in the BLA resubmission. Identify the holding vessels used in the studies and describe the (b) (4), if applicable. Alternatively, (b) (4) of PF-05280014 drug product (b) (4) [REDACTED]
4. Maximum differential forces for capping of PF-05280014 drug product and BWFI diluent vials are not included in the capping process parameters. This is not acceptable because excessive capping differential forces may compromise product sterility due to cracked vials. Specify a maximum differential force for capping of drug product and BWFI diluent vials in the BLA resubmission.
5. Integrity of the container closure has not been demonstrated after worst-case capping conditions with the maximum differential force for the PF-05280014 drug product and BWFI diluent vial cappers. This is not acceptable because excessive capping differential forces may compromise product sterility due to cracked vials. Provide summary results which demonstrate container closure integrity after capping at a maximum differential force for each capper in the BLA resubmission.
6. Vial washing parameters and washing validation summary data are not included in the BLA. Provide validation summary data and information from three runs performed on each vial washing machine for (b) (4) in the BLA resubmission. Specify vial washing parameters for PF-05280014 drug product and BWFI diluent vials.

### **Additional Comments**

1. Provide product-specific information related to sterilization and depyrogenation validation of containers, closures, and equipment in section 3.2.P.3.5 of the BLA rather than in a Drug Master File.
2. Establish bioburden action limits (b) (4) and provide those limits in the BLA resubmission.
3. (b) (4) should be addressed in section 3.2.P.3.4 of the BLA as an acceptance criteria rather than as a control limit.
4. Include shipping validation summary report of the passive pallet shipper in the actual shipping lanes in the BLA resubmission.
5. The results of the low endotoxin recovery (LER) study performed with the gel clot method are summarized in section 3.2.P.5.3, however, raw data were not provided. Provide the LER study report in the future BLA submission which describes the method

performed and includes raw data (+/- gel clot) to support the reported percent recoveries. Describe the calculations performed to quantify endotoxin. Clarify the type of endotoxin standard used in the LER studies (CSE or RSE) and update section 3.2.P.5.3 accordingly.

## **Product Quality Microbiology Assessment: Drug Product**

*Reviewer's Comment: Additional information to support (b) (4) validation is provided in DMF (b) (4). See separate DMF (b) (4) review #3.*

### **Drug Product Quality Microbiology Information Reviewed**

Sequence number	Date	Description
0001	6/22/2017	Original BLA
0016	11/13/2017	Response to IR
0020	12/12/2017	Response to IR
0025	1/29/2018	Response to IR
0038	2/28/2018	Response to IR
0039	3/1/2018	Response to IR
0040	3/5/2018	Response to IR

## **Module 3.2**

### **P.1 Description and Composition of the Drug Product (420 mg)**

PF-05280014 DP is a sterile lyophilized product in a dosage strength of 420 mg. Reconstitution with 20 mL of either Sterile Water for Injection (SWFI) or supplied Bacteriostatic Water for Injection (BWFI) yields a solution containing 21 mg/mL PF-05280014 at a pH of approximately 6. Lyophilized product reconstituted with SWFI contains no preservative and is for single use only. Lyophilized product reconstituted with BWFI contains benzyl alcohol as a preservative and is intended as a multi-use product that can be used for up to 28 days post-reconstitution. The solution is further diluted with sterile 0.9% sodium chloride for intravenous infusion.

**Table 3.2.P.1-1. Composition of PF-05280014 Drug Product, 420 mg**

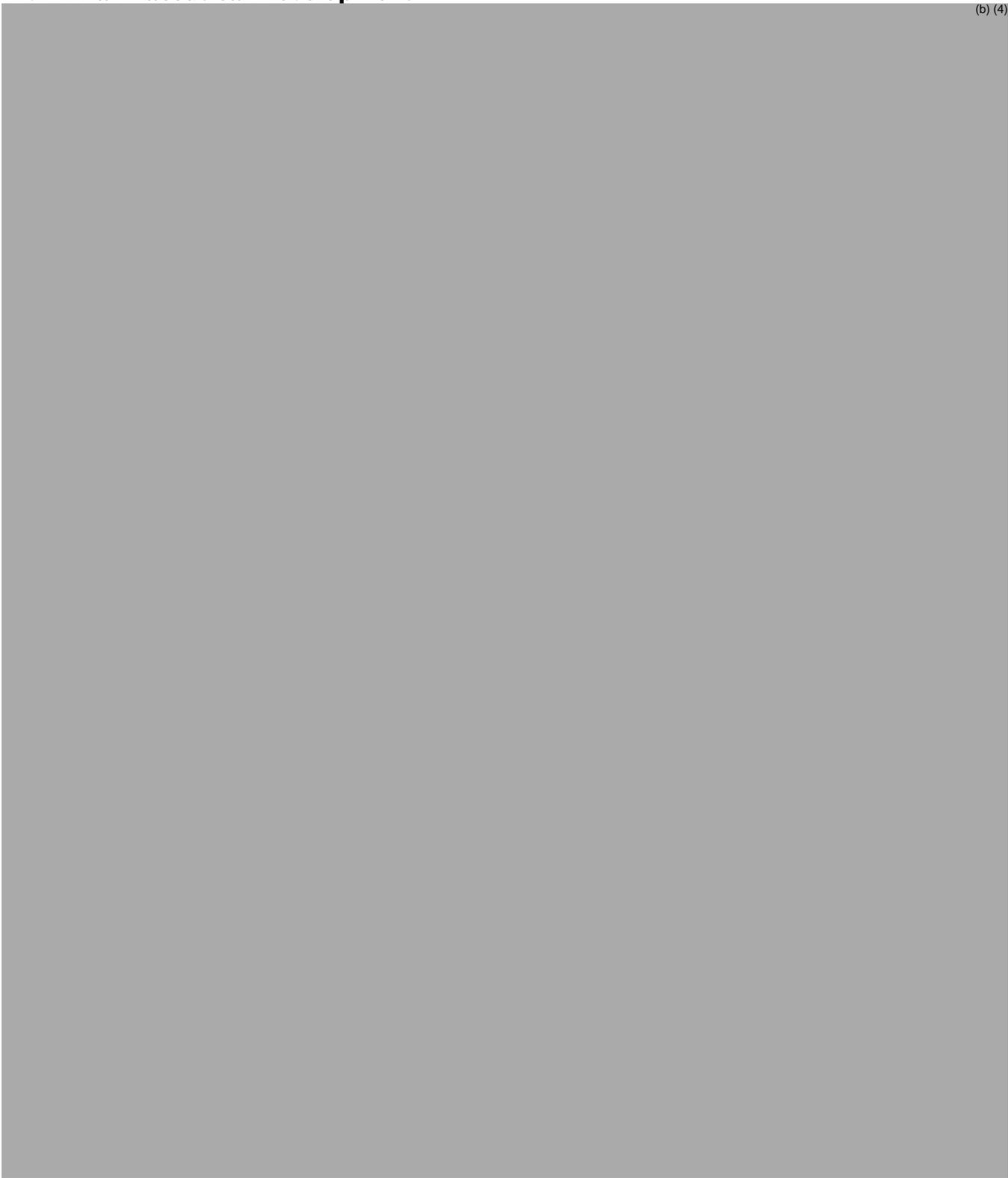
Name of Ingredients	Reference to Standard	Function	Unit Formula (mg/vial)
PF-05280014	In-house specification	Active ingredient	420
L-histidine	Ph. Eur., USP, JP	(b) (4)	7.9
L-histidine hydrochloride monohydrate	Ph. Eur., JP		9.5
Polysorbate 20	Ph. Eur., NF, JPE		1.7
Sucrose	Ph. Eur., NF, JP		386

To ensure that the nominal dose can be delivered, there is an overfill of 4.8%.

*Reviewer's Comment: As requested by OBP, the BLA was corrected to reflect the 420 mg rather than 440 mg presentation in an amendment (0039).*

## P.2 Pharmaceutical Development

(b) (4)





Virginia  
Carroll

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