CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761068Orig1s000

PRODUCT QUALITY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

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

Date: 2/23/2017
To: Administrative File, STN 761068
From: Thuy T. Nguyen, Facility Reviewer, CDER/OPQ/OPF/DIA
Endorsement: Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA
Subject: Original BLA
US License: None
Applicant: Ultragenyx Pharmaceutical Inc.
Mfg. Facility: Drug Substance and Drug Product: Kyowa Hakko Kirin Co., Ltd. Takasaki Plant FEI 3007588904

Product: CRYSVITA
Dosage: 10 mg/mL; 20 mg/mL; 30 mg/mL
Indication: For the treatment of X-linked Hypophosphatemia
Due Date: 3/18/2018

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

SUMMARY

The subject BLA proposes manufacture of Crysvita (burosumab) drug substance (DS) and drug product (DP) at Kyowa Hakko Kirin Co., Ltd. Takasaki Plant (FEI 3007588904). Please refer to the table below for facilities perform testing for DS and DP.

ASSESSMENT

DRUG SUBSTANCE AND DRUG PRODUCT FACILITIES

- **3.2.S.2.1 DS and DP Manufacturers.**

  The sites proposed for Crysvita DS and DP manufacture, cell banking operations, and testing are presented below in **Table 1**.

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

Site Name/Location	FEI/ Profile	Responsibilities	Facility Status
Kyowa Hakko Kirin Co., Ltd 100-1, Hagiwara-machi Takasaki-shi, Gunma, Japan	3007588904 CBI/SVS	Manufacturing Crysvita of DS, DP, release and stability testing MCB and WCB Storage	PLI completed 12/21/2017
(b) (4)		For Drug Substance: (b) (4) testing	Acceptable
		For Drug Product: drug product vial labeling and secondary packaging; finished drug product lot release	No Further Evaluation
		For Drug Product: drug product vial labeling and secondary packaging; finished drug product lot release	No Further Evaluation
		For Drug Product: drug product vial labeling and secondary packaging	No Further Evaluation

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

- **Current Prior Approval Inspection Decisions**
  - Kyowa Hakko Kirin Co., Ltd., Takasaki, Japan: DS and DP Crysvita manufacturing and testing site.
    - Profiles CBI and SVS. A pre-license inspection was completed by CDER/OPF and CDER/OBP from 12/11-21/2017 in support of this BLA 761068 for Crysvita. The inspection conducted in accordance with CPGM 7346.832 and ICH Q7. A comprehensive review of the facilities, utilities, equipment, processes and procedures was conducted to evaluate product quality, compliance to commitments in the BLA and compliance to cGMPs. A 9-item Form FDA-483, Inspectional Observations was issued at the conclusion of this inspection. The firm adequately addressed the FDA-483. Kyowa Hakko Kirin Co., Ltd., Takasaki, Japan is

recommended for approval for manufacture of Crysvita DS and DP manufacturing and testing under BLA 761068.

Reviewer Comment 2: *A pre-licensing inspection was conducted at Kyowa Hakko Kirin and a 9-items FDA-483 was issued at the conclusion of the inspection. The observations were for the following:*

- *Batch release review procedures by the quality unit are deficient. Specifically, QA currently does not review QC test records before approving the Certificates of Analysis for drug substance ((b) (4) burosumab) and drug product (burosumab)*
- *Deviations are not adequately investigated*
- *Change control procedure are inadequate*
- *Inadequate visual inspection program*
- (b) (4)
- *Shipping procedure for Burosumab drug product does not specify the limit for allowable transport time between your facility and the packaging site of the drug product into passive containers prior to shipping to the Secondary Packaging site*
- *SOPs do not provide clear instructions on how cell culture media should be prepared for (b) (4) cell lines*
- *No written procedures are implemented for the investigation of invalid assays*
- *critical steps in the pertinent procedure performed by QC analysts are not verified by another qualified analyst until the completion of the analytical procedure*

CDER/DIA had a T-con with the facility management and followed with additional information requests. The inspection review was conducted by CDER/DIA with the input of CDER/OBP and the inspection was classified as acceptable. The compliance statuses of facilities associated with the manufacture of Crysvita DS and DP are adequate.

3.2.S.2.2. Overview of Crysvita DS Manufacturing Operations Conducted at Kyowa Hakko Kirin Co., Ltd. (KHK) .

(b) (4)

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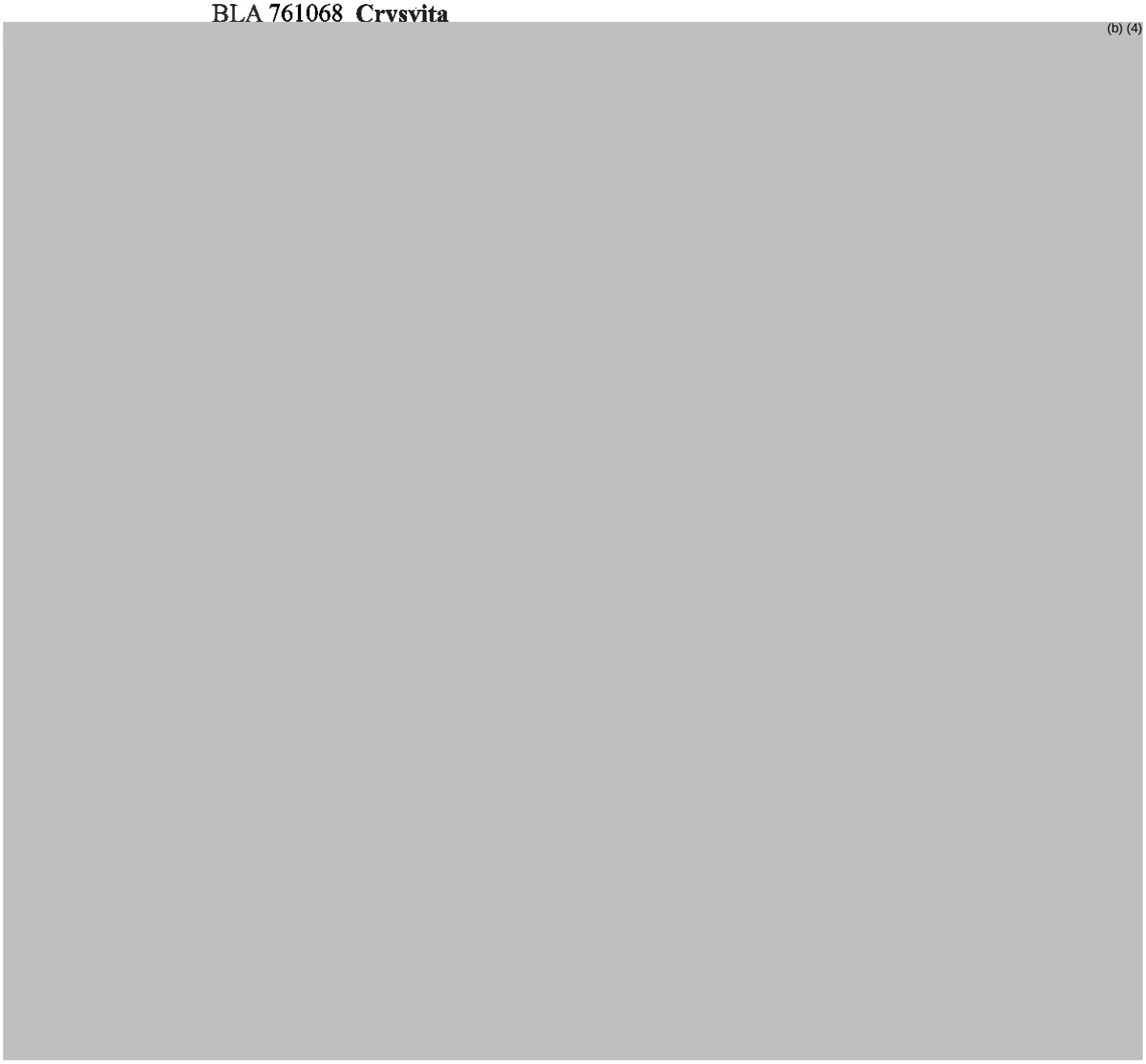
(b) (4)



Reviewer Comment 4: *Facility product contact equipment and cleaning procedures are adequately described and verified during inspection.*

Detailed description of plant Utilities was not provided in the appendixes but was verified during inspection as adequate.

Reviewer Comment 5: *Plant utilities were verified during inspection.*

DRUG PRODUCT FACILITIES

- **3.2.P.3.1. DP Manufacturers.**

The sites proposed for Crysvita DP manufacture, testing, packaging, and labeling are presented in the DS section.

3.2.A.1.2.4. Overview of Crysvita DP Manufacturing Operations Conducted at KHK.

(b) (4)

- **3.2A.1.2 Drug Product Manufacturing Facilities and Equipment**

Burosumab Drug Product (DP) is manufactured at the (b) (4) facility, located in KHK's Takasaki Plant in Japan. The (b) (4) facility is a multi-product facility and manufactures DPs for injection for early to late phase clinical development, as well as for commercial production. (b) (4)

(b) (4)

Table 3.2.A.1.2.1.1: A List of All Products Manufactured at (b) (4) Facility

(b) (4)

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(b) (4)

Reviewer Comment 12: *Contamination precaution were adequately described and verified during inspection.*

Detailed description of plant Utilities was not provided in the appendixes but was verified during inspection as adequate.

Reviewer Comment 13: *Plant utilities were verified during inspection.*

CONCLUSION

Adequate descriptions were provided for the Crysvita DS and DP manufacture at the **Kyowa Hakko Kirin Co., Ltd.** All proposed manufacturing and testing sites are recommended for approval from a facilities assessment standpoint.

Thuy T. Nguyen -S

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Thuy T. Nguyen, M.P.H.
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OPF Division of Inspectional Assessment
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Zhihao Peter Qiu, Ph.D.
Branch Chief
OPF Division of Inspectional Assessment
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Food and Drug Administration
Center for Drug Evaluation and Research
10903 New Hampshire Avenue,
Building 22,
Silver Spring, MD 20993

Date: February 08, 2018
To: Administrative File, BLA 761068\0
From: Lakshmi Rani Narasimhan, Ph.D., CDER/OPQ/OPF/DMA
Endorsement: Patricia F. Hughes, Ph.D., Acting Branch Chief, CDER/ OPQ/OPF/DMA
Subject: Biological License Application (BLA)
US License: 1773
Applicant: Ultragenyx Pharmaceutical Inc.
Facility: Kyowa Hakko Kirin Co., Ltd. Takasaki Plant, 100-1, Hagiwara-machi, Takasaki, Gunma, Japan (FEI# 713636520)
Product: CRYSVITA (burosumab) UX023/KRN23
Dosage: Sterile, solution for subcutaneous injection in a single use vial (10mg/mL, 20mg/mL and 30mg/mL)
Indication: For the treatment of X-linked Hypophosphatemia
Due Date: April 17, 2018

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

SUMMARY: This addendum addresses the assessment of revalidation data for the dye immersion container closure integrity (CCI) test method submitted in sequence number 0041. Amendments submitted in sequence number 0042 and 0044 in response to information requests were also reviewed. The dye ingress CCI revalidation information for the drug product container closure system was reviewed and found adequate.

INTRODUCTION:

As commitment to the Agency, Applicant performed the revalidation study for the dye immersion container closure integrity (CCI) test method for the drug product container closure system. The revalidation study included pressure /vacuum challenge during testing and the sensitivity of the test in terms of leak detection was ≤ 20 µm breach size. Information and data from this study was submitted in sequence number 0041 dated January 31, 2018. Validation Report for vial integrity test was provided in section 3.2.R and the section 3.2.P.5.3 was updated with revalidated CCI test information.

ASSESSMENTS:

The Process 4 DP lot YU16206 (30mg/mL) vials was used for the revalidation of dye immersion challenge study. The positive controls (defective vials) were prepared by inserting capillary tubes through the rubber stopper using a needle and vials with the following breach sizes, 5µm, 10µm, and 20µm (international diameter) were used for the study. Vials without any defect served as

negative control. The positive controls were immersed in 1g/L of methylene blue dye solution, placed in vacuum desiccator and subjected to reduced pressure of −80 kPa for minimum of 30 minutes, followed by exposure to atmospheric pressure for minimum of 30 minutes. This process was repeated 6 times before inspecting the vials for dye ingress. Test samples were visually inspected for evidence of dye penetration by comparing to vials with no defects which are not exposed to test conditions. Results in Table 3, reproduced below indicated that all negative controls were negative for dye ingress and all vials with breach size of 20µm showed the presence of dye. Acceptance criteria of absence of blue color for negative controls and presence of blue color for positive controls and 100% detection of positive controls with 20µm were met.

Table 3 Test results of Dye Immersion

Test	Sample		Test results (Number of vials)	
	Defect (Present/Absent)	Defect diameter (µm)	Immersion observed	No Immersion observed
1	Present	5	1	9
	Absent	–	0	10
2	Present	10	7	3
	Absent	–	0	10
3	Present	20	10	0
	Absent	–	0	10

Reviewer's comments: The revalidated dye immersion CCI test method is capable of detecting ≤ 20 µm breach sizes with burosumab drug product vials. The challenge conditions used for requalifying the CCI test method for burosumab drug product vials are acceptable.

FDA question (February 01, 2018): Update section 3.2.P.5.2 with revalidated CCI test method and indicate that a defective vial with a breach size of 20 µm will be included as system suitability control during container closure integrity testing of stability samples.
Applicant's response in amendment (sequence # 0042) dated February 05, 2018: Section 3.2.P.5.2 was updated that a vial with a defect size of 20 µm will be included as system suitability control for dye ingress CCI testing.
Reviewer's comments: Description of revalidated dye ingress CCI test method is not provided.
FDA question (February 06, 2018):

1. *Revise Table 3.2.P.5.2.1 in section 3.2.P.5.2 to indicate the correct revalidated dye ingress CCI test method used for testing stability samples.*
2. *Description of revalidated dye ingress CCI test method is not provided in section 3.2.P.5.2.2.2 "Container Closure Integrity". Update section 3.2.P.5.2.2.2 with the description of revalidated dye ingress CCI test method used for CCI testing of stability samples. Description should include the critical parameters (concentration of dye, pressure challenge and time of exposure of sample and control units to the challenge and dye) used for the CCI testing.*

Applicant's response in amendment (sequence # 0044) dated February 07, 2018:

1. Table 3.2.P.5.2.1 in section 3.2.P.5.2 was updated to reflect that the revalidated dye ingress CCI test method is used for testing stability samples.
2. Section 3.2.P.5.2.2.2 "Container Closure Integrity" was revised with description of the revalidated dye ingress CCI test method.

Satisfactory

Lakshmi Rani Narasimhan

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Patricia Hughes Troost

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Recommendation: Pending Completion of Reviews by DMA and DIA

NOTICE: The Product Quality and Labeling Aspects of BLA 761068 are completed and there are no outstanding issues precluding approval. The reviews from DIA and DMA are still pending. This review will be addended and with a final OPQ recommendation once those reviews are complete.

BLA/NDA Number: 761068
Review Number: 01
Review Date: 1/22/2018

Drug Name/Dosage Form	Crysvita (burosumab) / Solution for Injection
Strength/Potency	10 mg/ 1 mL , 20 mg/ 1 mL, 30 mg/ 1 mL
Route of Administration	Subcutaneous injection
Rx/OTC dispensed	Rx
Indication	X-linked hypophosphatemia in adult and pediatric patients 1 year of age and older
Applicant/Sponsor	Ultragenix Pharmaceutical Inc.
US agent, if applicable	n/a

Product Overview

Crysvita (burosumab) is a human IgG1(κ) antibody that specifically binds to fibroblast growth factor (FGF) 23 to inhibit the interaction between FGF23 and the Klotho-FGF receptor complex. Burosumab is produced in a recombinant CHO cell line and has an approximate molecular mass of 147 kilodaltons. Crysvita is a sterile, clear to slightly opalescent, colorless to pale brownish-yellow solution. Crysvita is supplied in a sterile, single-use, ready-to-use, preservative-free vial. Crysvita vials are produced with 10, 20, or 30 mg/ 1 mL burosumab formulated in: L-histidine (1.552 mg), D-sorbitol (45.91 mg), Polysorbate 80 (0.500 mg), L-methionine (1.492 mg), Hydrochloric acid (b) (4)% (as required), and Water for Injection, USP. The Crysvita solution has a pH of 6.25.

Discipline	Reviewer	Branch/Division
Drug Substance	Chikako Torigoe	OBP/DBRRII
Drug Product	Bruce Huang	OBP/DBRRII
Immunogenicity	Bruce Huang	OBP/DBRRII
Labeling	Vicky Hemphill-Borders	OBP
Facility	Thuy Thanh Nguyen	OPF/DIA
Microbiology – DS	Scott Nichols	OPF/DMA
Microbiology – DP	Lakshmi Narasimhan	OPF/DMA
QAL Microbiology - DP	Reyes Candauchacon	OPF/DMA
Application Team Lead	William Hallett	OBP/DBRRII

Multidisciplinary Review Team:

Discipline	Reviewer	Office/Division
RPM	Samantha Bell	DBRUP
Cross-disciplinary Team Lead	Theresa Kehoe	DBRUP
Medical Officer	Stephen Voss	DBRUP
Pharm/Tox	Gemma Kuijpers	DBRUP
Clinical Pharmacology	Lin Zhou, Ada Zhuang	OCP/DCPIII
Statistics	Jia Guo	OB/DBIII

1. Names:

a. Proprietary Name: Crysvita
b. Trade Name: Crysvita
c. Non-Proprietary/USAN: Burosumab
d. CAS name: 1610833-03-8
e. Common name: N/A
f. INN Name: Burosumab
g. Compendial Name: N/A
h. OBP systematic name: MAB HUMAN (IGG1) ANTI Q9ZZV9 (FGF23_HUMAN)[KRN23]
i. Other Names: KRN23, UX023

2. Pharmacologic category: Therapeutic recombinant human monoclonal antibody

Submissions Reviewed:

Submission(s) Reviewed	Document Date
STN 761068/0	8/17/2017
STN 761068/4	9/28/2017
STN 761068/6	10/06/2017
STN 761068/8	10/19/2017
STN 761068/13	11/14/2017
STN 761068/18	11/29/2017
STN 761068/20	12/04/2017
STN 761068/26	12/14/2017
STN 761068/28	12/19/2017
STN 761068/34	1/11/2018

Quality Review Data Sheet

1. Legal Basis for Submission: 351(a)

2. Related/Supporting Documents:

A. DMFs:

DMF #	DMF Type	DMF Holder	Item referenced	Letter of Cross-Reference	Comments
(b) (4)				YES	Not reviewed. Sufficient Leachables and Extractables data and primary stability program in BLA. DMF previously reviewed and no revision since last review.
				YES	

B. Other documents: IND, Referenced Listed Drug (RLD), or sister application.
IND 076488 for the treatment of X-linked Hypophosphatemia

Executive Summary

I. Recommendations: Pending final review recommendation from OPF

A. Recommendation and Conclusion on Approvability:

Recommendation:
The Office of Biotechnology Products, OPQ, CDER, recommends approval of STN 761068 for Crysvita manufactured by Ultragenix Pharmaceuticals Inc. The sponsor provided an adequate control strategy to produce a product that is pure and potent. It is recommended that this product be approved for human use under conditions specified in the package insert.

B. Approval Action Letter Language:

- Manufacturing location:
  - Drug Substance: Kyowa Hakko Kirin Co., Takahashi Plant, 100-1, Hagiwara-machi, Takasaki-shi, Gunma, Japan
  - Drug Product: Kyowa Hakko Kirin Co., Takahashi Plant, 100-1, Hagiwara-machi, Takasaki-shi, Gunma, Japan
- Fill size and dosage form: 10 mg/ 1 mL, 20 mg/ 1 mL, or 30 mg/ 1 mL single-dose vial
- Dating period:
  - Drug Product: 36 months: 2-8°C
  - Drug Substance: (b) (4) months: (b) (4) °C
  - Stability:
    - We have approved the stability protocols in your license application for the purpose of extending the expiration dating of your drug substance and drug product under 21 CFR 601.12.
- Exempt from lot release
  - Crysvita is exempted from lot release because it is a specified product per 601.2(a)

D. Benefit/Risk Considerations:

Burosumab is an inhibitor of FGF-23 indicated for the treatment of X-linked hypophosphatemia. The data submitted in this application support the conclusion that the manufacture of burosumab is well controlled and yields a consistently high quality product. The conditions used in manufacturing have been sufficiently validated, and a consistent product is prepared from the multiple production runs presented. From a product quality perspective, this product is approvable for human use.

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

There are two Post Marketing Commitments recommended by the CMC review team.

1. Conduct studies to further characterize the burosumab master cell bank (MCB) and to confirm the monoclonality of the MCB.

2. Conduct studies to evaluate effector functions (i.e., antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity) of burosumab. The final PMC report should be submitted based on the outcome of the studies per 21 CFR 601.12.

II. Summary of Quality Assessments:

Table 1 below is a summary of critical quality attributes and the associated control strategies for attributes that are relevant to both Drug Substance and Drug Product. For additional information, see the technical document on primary reviews of the Drug Substance Quality and Drug Product Quality by OBP/DBRR-II and the Drug Substance Microbiology and the Drug Product Microbiology by OPF/DMA.

A. CQA Identification, Risk and Lifecycle Knowledge Management

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

CQA (type)	Risk	Origin	Control Strategy	Other
Biological Potency by Cell Based Assay	Efficacy	Intrinsic to the molecule, impacted by oxidation, glycation, deamidation, aggregation, and fragmentation	(b) (4)	N/A
Protein Concentration	Efficacy and Safety	Manufacturing Process		N/A
Identity	Safety and Efficacy	Intrinsic to molecule		N/A
High Molecular Weight (HMW) species/Aggregates	Efficacy, Pharmacokinetics, and Safety/Immunogenicity	Manufacturing process (b) (4)		N/A
Fragmentation (%IgG monomer)	Efficacy, Pharmacokinetics, and Immunogenicity	Affected by manufacturing and storage conditions. (b) (4)		N/A
Fragmentation (%light chain and %heavy chain)	Efficacy, Pharmacokinetics, and Immunogenicity	Affected by manufacturing and storage conditions. (b) (4)		N/A
Charge Variant Profile (deamidation, C-terminal and N-terminal variants, and oxidation)	Efficacy, Pharmacokinetics, and Immunogenicity	(b) (4) conditions and degradation during manufacture and storage		N/A

Glycosylation variants (non-glycosylated, galactosylated, non-fucosylated, sialylated, high mannose species)	Efficacy, Pharmacokinetics, and Immunogenicity	(b) (4) conditions and degradation during manufacture and storage	(b) (4)	N/A

B. Drug Substance Burosumab Quality Summary

CQA Identification, Risk, and Lifecycle Knowledge Management

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

CQA (type)	Risk	Origin	Control Strategy	Other
Appearance	Safety	Controlled by the manufacturing process	(b) (4)	N/A
Host Cell Proteins (process-related impurity)	Safety and Immunogenicity	Production cell line		Process Validation studies show minimal host cell protein level observed (limit (b) (4) ng/mg)
Host Cell DNA (process-related impurity)	Safety	Production cell line		N/A
(b) (4) (process-related impurity)	Safety and Immunogenicity	Process-related impurity (b) (4)		N/A
(b) (4)	Safety	Process-related impurity (media component)		N/A
	Safety	Process-related impurity (media component)		N/A
cDNA Sequence	Safety	Inherent to the product		N/A
Viruses (contaminant)	Safety	Contamination during manufacture, mostly likely during cell culture operations		N/A
Mycoplasma (contaminant)	Safety	Mycoplasma would most likely be introduced during cell culture operations		N/A
Leachables (Process-related impurity)	Safety	Process-related impurities potentially from manufacture and		N/A

		the DS container closure system (CCS)	(b) (4)

- Description:
  Burosumab is a recombinant human IgG1κ monoclonal antibody that binds human FGF-23. Burosumab consists of 4 polypeptide chains (2 identical heavy chains, 446/447 amino acids each, and 2 identical light chains, 213 amino acids each) and has a molecular weight of 147 kDa. The burosumab expression construct is expressed in a CHO cell line. Amino acid sequencing and tryptic peptide mapping with mass spectrometry analysis confirms the correct amino acid sequence. Burosumab drug substance is formulated (b) (4)

- Mechanism of Action (MoA):
  Burosumab binds specifically to FGF-23 and blocks its interaction with the Klotho-FGF receptor complex. FGF-23 is a naturally occurring cytokine known to be responsible for phosphate and vitamin D metabolism. FGF-23 is primarily produced by osteocytes. In people with X-linked hypophosphatemia, a mutation in the PHEX gene results in excess levels of circulating FGF-23 leading to increased urinary phosphate excretion, reduced vitamin D synthesis, and subsequent hypophosphatemia resulting in defective bone mineralization and impacts to other tissues including muscle. Burosumab binds to FGF-23 and inhibits the ability of FGF-23 to bind to fibroblast growth factor receptor 1 (FGFR1) and the obligate co-receptor Klotho. This inhibition restores tubular reabsorption of phosphate from the kidney and increases production of vitamin D, which enhances intestinal absorption of calcium and phosphate. These combined actions improve serum phosphorus levels and bone mineralization.

- Potency Assay:
  The potency assay is a cell-based assay that measures neutralization of soluble FGF-23. In the assay, burosumab binds to FGF-23 preventing binding to the Klotho and FGF23 receptors expressed on HEK#18 cells, inhibiting Egr-1 expression and thus Egr-1-promoter-driven luciferase reporter activity. The luminescence intensity after addition of Steady-Glo® substrate is dose-dependent and reported as a relative percentage of the activity of the reference standard.

- Reference Materials:
  A well characterized primary reference standard was derived from a clinical batch. The sponsor has a two-tiered reference standard system with appropriate protocols in place that will ensure sufficient inventory of working reference standard for routine release and stability testing while minimizing drift (b) (4) .

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

(b) (4)

- Dating period and storage conditions:
  The sponsor conducted real-time, accelerated, and stressed stability studies on 5 intended-for-commercial lots, and 5 clinical lots to support a proposed dating period of (b) (4) months when stored at (b) (4) °C.

C. Drug Product Crysvita Quality Summary:

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

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

CQA (type)	Risk	Origin	Control Strategy
Color and Degree of Opalescence	Safety and Efficacy	Formulation, Contamination, or Degradation	Controlled through the manufacturing/formulation process, and tested for drug product lot release and stability testing
Polysorbate 80 Concentration	Safety	Formulation	Controlled through the formulation process, and tested for drug product release
Methionine Concentration	Safety	Formulation	Controlled through the formulation process, and tested for drug product release
Osmolality	Safety, Efficacy (control of degradation through formulation)	Formulation	Controlled at drug product lot release
pH	Safety and Efficacy	Formulation	Controlled at drug substance and drug product lot release and stability

Particulate Matter	Safety / Immunogenicity	Manufacturing process and container closure system	Controlled through the manufacturing process and preparation of the container closure system. Controlled by drug product release and stability testing.
Extractable Volume	Efficacy / Dosing	Manufacturing process	Controlled through the filling process (b) (4)
Leachables (process-related impurities)	Safety	Manufacturing equipment and CCS	Extractables study conducted on drug product container closure system

- Potency and Strength:
  Burosumab is supplied in a single use 5mL vials in three strengths: 10 mg/mL, 20 mg/mL, and 30 mg/mL. Potency of burosumab is determined with an Egr-1 reporter gene assay that measures the inhibition of FGF23-induced signaling by burosumab as a percent of reference. The potency assay is the same as described in the drug substance section of this review.

- Summary of Product Design:
  Burosumab is supplied as a sterile, single-use, ready-to-use, preservative-free solution for subcutaneous injection in a vial. The drug product formulation consists of L-histidine (10 mmol/L), D-sorbitol (252 mmol/L), Polysorbate 80 (0.5 mg/mL), L-methionine (10 mmol/L), Hydrochloric acid, (b) (4)% (as needed), and Water for Injection at pH 6.25. The extractable volume is 1.0 mL

- List of Excipients:
  Excipients include L-histidine (10 mmol/L), D-sorbitol (252 mmol/L), Polysorbate 80 (0.5 mg/mL), L-methionine (10 mmol/L).

- Reference Materials:
  The same reference material is used for drug substance and drug product.

- Manufacturing process summary:
  The drug product manufacturing process consists of (b) (4) All accepted vials are shipped to a labeling and packaging facility for labeling with approved labels before storage at 2-8°C.

  The control strategy includes in-process testing of critical and non-critical parameters and release testing of drug product. Critical parameters selected for routine monitoring relate to (b) (4)

- Container closure:

The container closure system for burosumab drug product consists of a 5 mL vial, a rubber stopper, and an aluminum sealing cap with a (b) (4) top. Appropriate compatibility studies were performed for the container closure system.

- Dating period and storage conditions:
  The dating period for burosumab drug product is 36 months at 2-8°C, protected from light.

D. Novel Approaches/Precedents: None

E. Any Special Product Quality Labeling Recommendations:

Store in a refrigerator at 2°C to 8°C (36°F to 46°F).
Store in product carton until time of use.
Protect from light.
Do not freeze.
Do not shake.

F. Establishment Information:

There are PENDING inspection or facility issues. The Division of Inspectional Assessment recommends approval of the BLA. The manufacturing and testing facilities, their responsibilities in burosumab production, and inspectional outcomes are summarized in the table below.

Overall Recommendation: PENDING			
DRUG SUBSTANCE			
Function	**Site Information**	**DUNS/FEI Number**	**Facility Status**
- Bulk drug substance manufacturing, storage - In-process testing - Master/Working Cell Bank storage (b) (4) manufacturing - QC testing for release and stability - Bulk DP lot release	Kyowa Hakko Kirin Co. Ltd. Takasaki Plant 100-1, Hagiwara-machi, Takasaki-shi, Gunma, 370-0013, Japan	DUNS: 713636520 FEI: 3007588904	PENDING
(b) (4)		(b) (4)	No Further Evaluation
- DP vial labeling - DP secondary packaging			No Further Evaluation

- Finished DP lot release		(b) (4)	
- DP vial labeling - DP secondary packaging - Finished DP lot release		(b) (4)	No Further Evaluation
- DP vial labeling - DP secondary packaging			No Further Evaluation

G. Facilities:

Burosumab master and working cell bank storage, drug substance manufacturing, and drug product manufacturing occurs at the Kyowa Hakko Kirin in Takasaki-shi, Gunma, Japan (FEI: 3007588904).

A Pre-License Inspection was performed at (b) (4). A nine item Form FDA 483 was issued. The firm is PENDING.

H. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols approved:
- annual stability protocol
- qualification of new working reference standard
- concurrent validation of (b) (4)

ii. Outstanding review issues/residual risk:
-n/a

iii. Future inspection points to consider:
- Follow up on 483 observations
- Evaluate trending of release and in-process tests results

b. Drug Product

i. Protocols approved:
- annual stability protocol

ii. Outstanding review issues/residual risk:
See Post-Marketing Commitments in Section IB

iii. Future inspection points to consider:
- Follow up on 483 observation
- Evaluate trending of release and in-process tests results

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

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

Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Biotechnology Products
Division of Biotechnology Review and Research II

BLA761068 KRN23 (BUROSUMAB) DRUG PRODUCT REVIEW MEMORANDUM

DATE: January 17, 2018
BLA: 761068
PRIMARY REVIEW: Bruce Huang; Product Quality Reviewer
THROUGH: William Hallett; Biologist (Team Leader)
PRODUCT: KRN23 (burosumab, solution for SC injection)
RPM: Samantha Bell
CLINICAL DIVISION: CDER/ODEIII/DBRUP
INDICATION: X-linked hypophosphatemia
SPONSOR: Ultragenyx Pharmaceutical, Inc.
SUBMISSION DATE: September 17, 2017
PDUFA GOAL DATE: April 17, 2018

DRUG PRODUCT REVIEW

Section 3.2.P - Drug Product: Review of KRN23 (burosumab) DP

3.2.P.1 - Description and Composition of the Drug Product (burosumab, Solution for Injection)

Burosumab DP for SC administration has the following characteristics:

- Sterile, single-use, ready-to-use, preservative-free
- Clear to slightly opalescent, colorless to pale brownish-yellow
- Three DP concentrations (same volume / vial capacity for each):

Table 3.2.P.1.1.1: Burosumab Drug Product Strengths

	Strength		
	10 mg/1 mL	**20 mg/1 mL**	**30 mg/1 mL**
Burosumab concentration	10 mg/mL	20 mg/mL	30 mg/mL
Labeled volume	1 mL	1 mL	1 mL
Vial capacity	5 mL	5 mL	5 mL

The constituents of different DP strengths / vial are summarized in Table 3.2.P.1.2.1 below:

Table 3.2.P.1.2.1: Burosumab Drug Product Composition

Component	Function	Quality Standard	Concentration	Quantity per Vial		
				10 mg/1 mL	20 mg/1 mL	30 mg/1 mL
Burosumab	API	In-house[a]	10, 20, or 30 mg/mL	10 mg	20 mg	30 mg
L-histidine	(b) (4)	USP/Ph.Eur./JP	10 mmol/L	1.552 mg	1.552 mg	1.552 mg
D-sorbitol		NF/Ph.Eur./JP	252 mmol/L	45.91 mg	45.91 mg	45.91 mg
Polysorbate 80		NF/Ph.Eur./JP	0.5 mg/mL	0.500 mg	0.500 mg	0.500 mg
L-methionine		USP/Ph.Eur./JP	10 mmol/L	1.492 mg	1.492 mg	1.492 mg
Hydrochloric acid, (b) (4)%		NF/Ph.Eur./JP	Adjust to pH 6.25	As required	As required	As required
Water for injection		USP/Ph.Eur./JP	q.s. to each labeled volume	q.s. to 1.000 mL[b]	q.s. to 1.000 mL[b]	q.s. to 1.000 mL[b]

API: Active Pharmaceutical Ingredient
q.s.: quantum sufficit
a: Meets the Drug Substance specification (see Section 3.2.S.4.1)
b: The additional volume is filled per vial to ensure that labeled volume (1 mL) is available to the user.

Reviewer comment: *The depiction of DP composition provides function, quality standard, and concentration and is therefore adequate.*

3.2.P.2 – Pharmaceutical Development

3.2.P.2.1 - Components of the Drug Product (burosumab, Solution for Injection)

(b) (4)

Also, the excipients are the same irrespective of the three different API concentrations corresponding to the three DP strengths. No novel, or human- / animal-origin excipients are used for DP formulation.

Reviewer comment: *The description of DP components is adequate.*

3.2.P.2.2 – Formulation Development (burosumab, Solution for Injection)

3.2.P.2.2.1 Formulation Development

(b) (4)

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Reviewer comment: *The specifications for the Vial and Stopper (Packaging components contacting the DP) are in stipulated to be compliant with the relevant USP, Ph.Eur., and JP requirements. This is acceptable. Secondary packaging of light-shielding paper-board cartons are acceptably appropriate. Labeling review is deferred to the OBP Labeling reviewer.*

3.2.P.8 – Stability

3.2.P.8.1: Stability Summary and Conclusions – A program of stability studies was conducted in compliance with ICH Q1A and Q5C. Burosumab DP lots used for stability studies are summarized in the Table below:

Table 3.2.P.8.1.1.1.1: Summary of burosumab DP Lots Used in the Stability Program

Process and Manufacturing Facility	Lot Number	Strength	Date of Manufacture	Stability Program
Process 3 (b) (4)	YU011	10 mg/1 mL	Oct. 4, 2012	Long-term
	YU015		Feb.20, 2015	Long-term Accelerated (25±2°C)
	YU016		Feb.24, 2015	Long-term Accelerated (25±2°C) Stress condition (40±2°C and photostability)
	YU017		Feb.26, 2015	Long-term Accelerated (25±2°C)
	YU009	30 mg/1 mL	Apr. 17, 2012	Long-term
	YU012		Oct. 2, 2013	
	YU014		Oct.2, 2014	Long-term Accelerated (25±2°C)
	YU018		Mar.09, 2015	
	YU019		Mar.11, 2015	Long-term Accelerated (25±2°C) Stress condition (40±2°C and photostability)
	YU020		Mar.16, 2015	Long-term Accelerated (25±2°C) Freeze/Thaw
Process 4 (b) (4)	YU15X01	10 mg/1 mL	Oct.20, 2015	Long-term Accelerated (25±2°C) Stress condition (40±2°C and photostability)
	YU16201		Feb.9, 2016	Long-term Accelerated (25±2°C)
	YU16204		Feb.22, 2016	
	YU16205		Feb.24, 2016	
	YU16701	20 mg/1 mL	Jul.26, 2016	Long-term Accelerated (25±2°C) Stress condition (40±2°C)
	YU15X02	30 mg/1 mL	Oct.22, 2015	Long-term Accelerated (25±2°C) Stress condition (40±2°C and photostability)
	YU16202		Feb.9, 2016	Long-term Accelerated (25±2°C)
	YU16203		Feb.9, 2016	
	YU16206		Feb.29, 2016	

DP produced by Process 3 and 4 were studied to confirm comparability of stability between the material produced using the different processes. The container closure system used for stability testing was identical to the trials and proposed commercial specifications. The three different

strengths were each tested in the Stability Program, though only one representative lot of the 20 mg/1 ml strength was tested, invoking the bracketing concept – four lots each of the Process 4 (b) (4) 10 mg/1 ml and 30 mg/1 ml strength were used for stability testing. All assayed analytical attributes of the the three strengths were found to be similar after long-term storage (up to 36 months at 2-8°C) by the following testing:

Table 3.2.P.8.1.1.3.2.1: Long-term Stability Protocol for DP

Test Item		Storage Condition and Periods (Months)							
			Long-term at 2-8°C						
		Initial	3	6	9	12	18	24	36[a]
Appearance	USP<631> Ph.Eur.2.2.1, 2.2.2	X	X	X	X	X	X	X	X
	JP<6.06> USP<1> Ph.Eur.2.9.20	X	X[b]	X[c]	X[d]	X	X[e]	X	X
pH	JP<2.54> USP<791>	X	X	X	X	X	X	X	X
	Ph.Eur.2.2.3[f]	X	X	X	X	X	X	X	X
Particulate matter in injections	USP <788> Ph. Eur.2.9.19	X	X[g]	X[h]	X[i]	X	X[j]	X	X
Protein concentration	JP<2.24> USP<857><1057> Ph.Eur.2.2.25, 2.5.33	X	X	X	X	X	X	X	X
Purity	SE-HPLC	X	X	X	X	X	X	X	X
	CE-SDS (R)	X	X	X	X	X	X	X	X
	CE-SDS (NR)	X	X	X	X	X	X	X	X
	CEX-HPLC	X	X	X	X	X	X	X	X
	HI-HPLC	X	X	X	X	X	X	X	X
Potency	Egr-1 reporter gene assay	X	X	X	X	X	X	X	X
Contaminants Sterility	USP <71> Ph.Eur.2.6.1	X	NT	X[k]	X[l]	X[m]	X[n]	X[o]	X[p]
Container closure integrity	USP<381> Ph.Eur.3.2.9	X	NT	X[q]	X[r]	X	X[s]	X	X

X: Tested, or planned to be tested.
NT: Not tested or not planned to be tested.

Figures 3.2.P.8.1.2.1.1-19 (not reproduced here) show data illustrating stability of DP lots stored at 2-8°C (some lots stored for up to 36 months) as analyzed by:

- pH
- Protein concentration
- Main peak content: (SE-HPLC)
- HMWS, LMWS: (SE-HPLC)
- Main peaks (HC+LC), HMWS, LMWS, MMWS, DSHC: (CE-SDS(R))
- Glycosylation: (CE-SDS(R))
- Main peak, HMWS, LMWS: (CE-SDS(NR))
- Acidic peak, main peak, basic peak content (CEX-HPLC)
- Fc Pre Peaks content: (HI-HPLC)

All the assays used to test the DP lots produced using Process 3, as compared to Process 4, demonstrated similar stability trends, including the testing of the various lots of differing strengths. Some changes in assay results were observed over time, but were minor, and well within the allowable ranges, not resulting in, or trending towards out-of-specification readings, even for lots tested out to 36 months:

- Slight main peak decrease (<1%), slight increase in LMWS (SE-HPLC)

- Slight main peak (HC+LC) decrease, slight increase (<1%) in LMWS, MMWS, DSHC (CE-SDS(R))
- Slight increase in LMWS (<0.2%) (CE-SDS(NR))
- Increase in acidic peaks (~5%), decrease in basic peak (5%) (CEX-HPLC)

Reviewer comment: *The stability results presented by the Sponsor for different lots of burosumab DP stored at long-term storage (up to 36 months at 2-8°C) are supportive of stability by the burosumab DP for up to 36 months at the long-term condition. The trends that were observed in the analysis of DP at up to 36 month are expected for monoclonal antibodies and are well within specifications.*

Burosumab testing at accelerated conditions consisted of storage up to 6 months at 25±2°C (DP lots used are indicated in Table 3.2.P.8.1.1.1.1, above). Attributes for testing under accelerated conditions include:

- Appearance: USP<631><1>, JP<6.06>, Ph.Eur.2.2.1, 2.2.2, 2.9.20
- pH: USP<791>, JP<2.54>, Ph.Eur.2.2.3
- Particulate matter: USP<788>, Ph.Eur.2.9.19
- Protein concentration: USP<857><1057>, JP<2.24>, Ph.Eur.2.2.25, 2.5.33
- Purity: SE-HPLC, CE-SDS(R), CE-SDS(NR), CEX-HPLC, HI-HPLC
- Potency: Egr-1 reporter gene assay

Results of accelerated condition testing are reported in Figures 3.2.P.8.1.2.2.1-20 (not reproduced here). No changes were found in appearance, pH, protein concentration, or particulates. Observed changes in DP lots stored over time at 25±2°C included:

- Purity (SE-HPLC): decrease of Main Peak and increase of LMWS (main peak out-of-spec at 3 months)
- Purity (CE-SDS(R)): decrease of Main Peak (HC+LC) to out-of-spec at 6 months, increase of LMWS, MMWS, DSHC, HMWS to differing degrees
- Purity (CE-SDS(NR)): decrease of Main Peak content to out-of-spec at 3 months, increase of LMWS to out-of-spec at 6 months
- Purity (CEX-HPLC): decrease of Main Peak content to out-of-spec at ~3-6 months, increase of Acidic Peak Content to out-of-spec at ~6 months
- Potency (Egr-1 reporter assay): decrease of potency in several lots to out-of-spec by 3 months

Changes at the accelerated condition were comparable between DP produced by Process 3 and Process 4, in both 10 mg/1 ml and 30 mg/1 ml strengths.

Reviewer comment: *The observed losses of purity and potency in the burosumab DP after storage at accelerated conditions of 25±2°C are expected; comparable patterns of degradation were seen between DP of 10 and 30 mg/1 ml, produced by the two processes. The similarity is indicative of comparability between DP produced by the two different processes that were used for DP production. This is acceptable.*

Burosumab testing at stress conditions consisted of storage up to 4 weeks at 40±2°C (DP lots used are indicated in Table 3.2.P.8.1.1.1.1, above). Attributes for testing under accelerated conditions include:

- Appearance: USP<631><1>, JP<6.06>, Ph.Eur.2.2.1, 2.2.2, 2.9.20

- pH: USP<791>, JP<2.54>, Ph.Eur.2.2.3
- Particulate matter: USP<788>, Ph.Eur.2.9.19
- Protein concentration: USP<857><1057>, JP<2.24>, Ph.Eur.2.2.25, 2.5.33
- Purity: SE-HPLC, CE-SDS(R), CE-SDS(NR), CEX-HPLC, HI-HPLC
- Potency: Egr-1 reporter gene assay

Results of stress conditions testing are reported in Figures 3.2.P.8.1.2.3.1.1-20 (not reproduced here). No changes were observed in appearance, pH, protein concentration, or particulates. Observed changes in DP lots stored over time at 40±2°C included:

- Purity (SE-HPLC): decrease of Main Peak and increase of LMWS (main peak out-of-spec past 1-2 weeks)
- Purity (CE-SDS(R)): decrease of Main Peak (HC+LC) to out-of-spec in 2weeks, increase of LMWS, MMWS, DSHC, HMWS to differing degrees, some exceeding acceptance criteria by 4 weeks.
- Purity (CE-SDS(NR)): decrease of Main Peak content to below acceptance criteria in ~3 weeks, increase of LMWS to above acceptance criteria by 3 weeks
- Purity (CEX-HPLC): decrease of Main Peak content to below acceptance criteria in ~2-3 weeks, increase of Acidic Peak Content to above acceptance criteria in ~2-4 weeks

Changes at the stress conditions were comparable between DP produced by Process 3 and Process 4, in both 10 mg/1 ml and 30 mg/1 ml strengths.

3.2.P.8.1.2.3.2 – Photostability: Burosumab stability was evaluated by storage in direct light conditions. Data is shown graphically in Figures 3.2.P.8.1.2.3.2.1-20. No changes due to direct light exposure were observed for appearance, protein concentration, or particulates. However, direct light exposure (but not shaded conditions) lead to the following changes:

- pH: slight decrease as compared to dark control or shaded samples (remained compliant to AC)
- Purity (SE-HPLC) of direct light samples compared to dark control or shaded:
    - decreased Main peak (30 mg/1 ml samples to a greater extent; dropped below level of AC)
    - increased HMWS (30 mg/1 ml samples to a greater extent; exceeded AC)
    - increased LMWS (10 mg/1 ml and 30 mg/1 ml to roughly equal extent)
- Purity (CE-SDS(R)) of direct light samples compared to dark control or shaded:
    - decreased Main peak (HC+LC) (30 mg/1 ml samples to a greater extent; dropped below level of AC)
    - increased HMWS (10 mg/1 ml and 30 mg/1 ml to roughly equal extent)
- Purity (CE-SDS(NR)) of direct light samples compared to dark control or shaded:
    - decreased Main peak (10 mg/1 ml and 30 mg/1 ml samples to roughly equal extent; dropped below level of AC)
    - increased HMWS (30 mg/1 ml samples to a greater extent)
    - increased LMWS (10 mg/1 ml and 30 mg/1 ml to roughly equal extent; exceeded the AC)
- Purity (CEX-HPLC) of direct light samples compared to dark control or shaded:
    - decreased Main peak (30 mg/1 ml samples to a greater extent; fell below the AC)
    - increased Acidic peak (30 mg/1 ml samples to a slightly greater extent; exceeded the AC)

- Fc Pre Peaks Content (degradation product) (HI-HPLC): increased (30 mg/1 ml samples to a greater extent)
- Potency (Egr-1 reporter assay): decreased potency (30 mg/1 ml samples to a greater extent; 30 mg/1 ml Process 4 batch dropped below level of AC)

Reviewer comment: *Stress conditions for photostability testing were consistent with the ICH Q1B guidelines, and involved conditions of 2-8°C, 1.2e6 lux*h, and 200 W*h/m^2. It appears clear that several crucial aspects of the burosumab DP are sensitive to light exposure, to varying degrees, and that protection from light during transport and storage is important.*

3.2.P.8.1.2.3.3 – Freeze/Thaw stability: In the 3.2.P.8.3 Stability Data section, Tables 3.2.P.8.3.2.5.1-2 (not reproduced here) outline the results of three freeze/thaw cycles for one lot each of burosumab 10 mg/1 ml and 30 mg/1 ml DP (both produced by Process 4) regarding stability study analytical assay results. No significant changes in the product quality were observed between the control samples and samples subjected to three F/T cycles, beyond the previously noted increases in subvisible particles, none of which exceeded the allowable amount.

Reviewer comment: *The data presented are indicative of acceptable freeze/thaw stability in the burosumab DP through 3 freeze thaw cycles.*

3.2.P.8.1.4 – Labeled Storage Condition and Shelf Life: The Stability data collected for burosumab DP, manufactured by the Process 4 at the (b) (4) commercial site, indicates sufficient stability characteristics for long-term storage at 2°-8°C for up to 36 months, with adequate container closure comparability, and protection from light by the packaging carton. The Sponsor concludes that burosumab DP of 10, 20, and 30 mg/1 ml should be labeled with the statement: "Store at 2°-8°C with protection from light."

Reviewer comment: *The Sponsor's proposed directions for storage condition and shelf life for burosumab DP are compatible and consistent with the experimental stability evidence presented.*

3.2.P.8.2: Post-approval Stability Protocol and Commitment
3.2.P.8.2.2 – DP Post-approval Stability Studies
A minimum of one lot per year of each of the three DP strengths at the 2-8°C long-term storage condition will be placed on long-term post-approval stability studies. The test items shown below will be assayed on the specified schedule by the indicated acceptance criteria (see Table below):

Test Items		Acceptance Criteria	Storage Condition and Periods (Months)							
			Initial[a]	Long-term at 2-8°C						
				3	6	9	12	18	24	36
Appearance	USP<631> Ph. Eur.2.2.1, 2.2.2	(b) (4)	X	X	X	X	X	X	X	X
	JP<6.06> USP <1> Ph.Eur.2.9.20		X	X	X	X	X	X	X	X
pH	JP<2.54> USP<791>		X	X	X	X	X	X	X	X
	Ph. Eur.2.2.3		X	X	X	X	X	X	X	X
Particulate matter in injections	USP <788> Ph. Eur.2.9.19		X	X	X	X	X	X	X	X
Protein concentration	JP<2.24> USP<857> <1057> Ph. Eur.2.2.25, 2.5.33		X	X	X	X	X	X	X	X
Purity	SE-HPLC		X	X	X	X	X	X	X	X
	CE-SDS (R)		X	X	X	X	X	X	X	X
	CE-SDS (NR)		X	X	X	X	X	X	X	X
	CEX-HPLC		X	X	X	X	X	X	X	X
Potency	Egr-1 reporter gene assay		X	X	X	X	X	X	X	X
Container closure integrity	USP<381> Ph.Eur.3.2.9		X	–	–	–	X	–	X	X

The acceptance criteria and analytical procedures used for stability testing are described in Sections 3.2.P.5.1 and 3.2.P.5.2, respectively. Acceptance criteria are the same as that of Section 3.2.P.8.3.
a: The results of release testing except for container closure integrity will be used as initial data.
X: Scheduled, –: Not scheduled

HI-HPLC is also excluded from both DP and DS stability protocols because oxidized and deamidated species are product-related substances, not impurities, and furthermore it was shown in DS analysis that oxidized and deamidated species have "Low" risk for negative impact on burosumab efficacy, PK, and safety (see Section 3.2.S.3.2).

Reviewer comment: *The Sponsor's protocol for stability testing is consistent with the recommendations conveyed by the Agency, and the reasoning for not testing sterility or performing HI-HPLC analysis through the stability protocol is reasonable.*

IMMUNOGENICITY REVIEW

BLA761068 Burosumab Immunogenicity Assay Validations

Review of Bioanalytical Method Validation Report: Validation of an Electrochemiluminescent Assay for the Detection of Anti-KRN23 Antibodies in Human Serum (Section 5.3.1.4 Document number: (b) (4) 17-139-081-REP)

The KRN23 ADA detection assay is a drug-tolerant electrochemiluminescent method employing the Meso Scale Discovery platform using a bead-based acid dissociation treatment of samples, with ADA capture by biotinylated KRN23, and detection by ruthenium-labeled KRN23. Validation was performed at (b) (4) The assay utilizes an initial acid dissociation treatment step to separate potentially drug-bound ADA. This step helps to reduce the possibility of assay interference resulting from the presence of residual KRN23 drug in the patient serum samples. Detection of ADA is achieved using Meso Scale Discovery (MSD) apparatus. The initial steps of the assay involve the preparation of KRN23-labelled magnetic beads, which are constructed from bead-bound streptavidin binding to biotinylated KRN23.

Serum samples to be tested are first treated by acidification to dissociate potential antibody-drug complexes, then neutralized. The neutralized samples, or controls, are subsequently mixed with the labeled beads. After incubation, the samples are acidified again, and the beads are immobilized by magnetic field, so that the anti-KRN23 antibodies can be recovered in supernatant (this is the extraction process). Evaluation of anti-KRN23 antibody presence in the supernatant is then performed by Meso Scale Discovery Sector Imager (Screening Assay). For Confirmatory Assay, an identical procedure is utilized, but with the addition of 20 µg/ml of KRN23; the binding inhibition magnitude is based on the reduction of raw assay signal. A summary of the Assay Validation is shown in Table 4, below:

Table 4. Validation Run Summary

Paramater Evaluated	Result	Comments
Cut Point (Screening)	1.14 normRLU	N/A
Cut Point (Confirmatory)	16.3%	N/A
Drug Tolerance	HPC = 80 µg/mL MPC = 26.7 µg/mL LPC = 13.3 µg/mL	Drug Tolerance values are the mean of 3 runs
Sensitivity (Screening)	54.0 ng/mL	Interpolated at the Cut Point
Sensitivity (Confirmatory)	25.7 ng/mL	Interpolated at the Cut Point
InterAssay	CVs ranged 14.9 - 20.6%	N/A
IntraAssay	CVs ranged 4.0 - 6.4%	N/A
Prozone	No prozone effect was seen	N/A
Specificity	High recoveries	Spike samples recovered above 130% of the HPC.
Selectivity/Matrix Interference	Met acceptance criteria	N/A
Titration Linearity	1:32 at the HPC 1:8 at the MPC 1:2 at the LPC	Linearity values are the mean of 3 runs
Robustness	CVs ranged 14.9 - 20.6%	Used variability in InterAssay runs
Freeze/Thaw Stability	Stable to 6 F/T	N/A
Short Term Stability RT (before extraction)	24 hrs and 13 min	N/A
Short Term Stability 2-8C (before extraction)	48 hrs and 6 min	N/A
Short Term Stability RT (after extraction)	24 hrs	N/A
Short Term Stability 2-8C (after extraction)	48 hrs	N/A
1 Month Stability -80C (after extraction)	41 Days	N/A

Immunogenicity Assay Method Validation

Minimum Required Dilution (MRD): No MRD is assigned, because assay samples of ADA are obtained by the complete extraction from 150 µl neat samples. All assessments involving concentration were evaluated based on concentration in the neat sample.

Review comment: The use of an MRD does not apply due to the extraction of Ig's from the serum samples. This approach is acceptable.

Screening Cut Point: SCP was established by assay of 60 serum samples obtained from normal drug-naïve adults. Four separate extractions were performed on different days by ≥2 analysts over ≥3 runs. JMP software was utilized for outlier removal:

- Technical outliers: readings falling 1.5*inter-quartile range below first quartile or above third quartile of sample data
- Biological outliers: determined by Tukey-Kramer All Pairs Test

The remaining data was evaluated for normality by Shapiro Wilks test, equal means by ANOVA, and unequal variances by Levene's test. The ANOVA F-test value of 0.0745 indicated means and variances between runs was not significant.
The normalized screening parametric (floating) cut point was based on the 95th percentile of the data, resulting in a value of 1.14 normRLU (see Figure 1 in the submission).

Review comment: The method for determination of the SCP is appropriately performed, using an adequate number of samples. This is acceptable.

Confirmatory Cut Point: Samples used for establishing the SCP were also run concurrently with excess KRN23 (20 µg/ml) added after extraction, on the same plates as non-spiked samples. The following formula was used for calculating the percentage of change due to the addition of the DP spike (% Inhibition):

% Inhibition = 1- (Signal of Spiked Sample / Signal of Non-Spiked Sample) x 100

The CCP was calculated by the following formula, for 1% false positive rate:

CCP = Mean % Inhibition + 2.33*SD of % Inhibition

The CCP was found to be 16.3% (see Figure 4, below):

Review comment: The competitive inhibition method for determination of the CCP is appropriately performed, using a sufficient number of samples, and adequate false positive rate.

Titration Cut Point:
Titration assay runs will use the SCP for analysis. However, if plateau effect is encountered, parametric alternative titer floating cut point will be determined using the same data, but calculated using the 99.9th quantile (0.1% false positive rate, ie. Mean+3.09SD), as described in USP<1106>. Plate-specific titer cut point values will be determined by multiplying the titer cut point correction factor by the mean NC value of each plate.

Review comment: The method described for determination of titration cut point is acceptably consistent with the recommendations of USP<1106>.

Specificity:
Spiking assay reactions with human IgG at equivalent, or 5x concentrations as the SPC, in the presence of SPC, in pooled normal human serum, resulted in increased signal (142% recovery) as compared to SPC assays without the spiked human IgG (see Table 6 in the submission). The increase in signal with addition of IgG was attributed to differences in extraction between fresh samples and control extracts. It was noted that the most extreme addition of 10,000 ng/ml of IgG actually resulted in a lower recovery (130.3%) than the addition of 2,000 ng/ml IgG.

Review comment: The results indicating elevated sample signals when in the presence of IgG, do not signify interference with the assay, which would have been demonstrated by loss of signal in the presence of IgG. The specificity of the assay is acceptable.

Assay Sensitivity:
The 2000 ng/ml SPC was diluted in neutralization buffer, and extractions were performed, for comparison against undiluted SPC and matrix. The assay was performed in 3 runs by 2 analysts, producing sensitivity curves with mean interpolated sensitivity of 54.0 ng/ml (representative curve shown in Figure 5, and sensitivity interpolations shown in Table 7, in the submission). The confirmatory assay had mean interpolated sensitivity of 25.7 ng/ml (representative curve shown in Figure 6, and sensitivity interpolations shown in Table 8, in the submission). [Acceptance criteria: Sensitivity ≤100 ng/ml; CV ≤25%].

Review comment: The 54.0 ng/ml sensitivity interpolation for the screening assay is found almost at the very bottom of the curve, occurring between the third and fourth dilution points shown on the graph. This indicates the assay is sufficiently sensitive to low positive responses. The 25.7 ng/ml sensitivity interpolation for the confirmatory assay is also found almost at the very bottom of the curve, which does affect the variability, and thus results in the relatively high CV of 34%, which is beyond the Acceptance Criteria of ≤25%. However, higher CVs are expected near the bottom of the assay, so these results do not indicate the assay is performing poorly; therefore, the confirmatory assay's sensitivity is acceptable.

Selectivity / Matrix Interference:
2000 and 100 ng/ml (HPC and LPC, respectively) of SPC was spiked into 10 normal human serum samples, prior to extraction. Samples were also analyzed as non-spiked controls (0 ng/ml SPC). Samples were run in duplicate, over 2 runs by 2 analysts (see Table 9, in the submission). One sample ran abnormally high in one non-spiked state, attributed to technical error. [Acceptance criteria: ≥80% of non-spiked samples read below the SCP; ≥80% of spiked samples read at or above the SCP 1.14, CV≤25%].

Review comment: The results demonstrate acceptable selectivity of the assay, in all spiked samples reading above the SCP of 1.14.

Drug Tolerance:
The HPC, MPC, and LPC (2000, 500, and 100 ng/ml of SPC, respectively) were assayed in the presence of dilutional concentrations of KRN23, starting with 80 µg/ml, down to 0 µg/ml (see Table 10, in the submission).

The HPC (2000 ng/ml SPC) was found to have mean tolerance of at least 80 µg/ml KRN23 (all three replicates reading above the 1.14RLU SCP), the MPC (500 ng/ml SPC) was found to have mean tolerance of 26.7 µg/ml KRN23, and LPC (100 ng/ml SPC) was found to have mean tolerance of 13.3 µg/ml KRN23.

Review comment: The methodology for determination of the tolerance is acceptable. The validation for the previously-submitted (outdated) immunogenicity assay had tolerances of 3.75, 0.94, and 0.47 µg/ml for the HPC, MPC, and LPC, respectively. The presently-reviewed assay marks an improvement in the ability to detect anti-burosumab antibody in the presence of burosumab.

Prozone (Hook) Effect:
Spiking high levels of the SPC into pooled normal human serum (20 and 100 µg/ml, or 10- and 50-times concentrations of the HPC) for analysis, did not result in decrease to the resulting signal below the SCP (see Table 11 in the submission).

Review comment: The Sponsor has acceptably demonstrated that the ADA-detection assay does not have prozone effect.

Titration Assay Linearity:

The HPC, MPC, and LPC were serially diluted by 2-fold, and measured by the assay. Back-calculating the dilutions of the HPC, MPC, and LPC (2000, 500, and 100 ng/ml of SPC,

respectively), demonstrate that the last dilutional reading above the 1.14 ACP for each of the PCs represent:

1:32 HPC = 62.5 ng/ml of SPC
1:8 MPC = 62.5 ng/ml of SPC
1:2 LPC = 50.0 ng/ml of SPC

The titered control sampled elicited proportional assay responses.

Review comment: The Sponsor has acceptably demonstrated that the ADA-detection assay possesses linearity over the range of the positive controls.

Intra-assay Precision:
Over eight replicates of HPC, MPC, LPC, and NC, run by a single analyst, the readings met the acceptance criteria of CV≤20%.

Review comment: The Sponsor has acceptably demonstrated that the ADA-detection assay possesses intra-assay precision.

Inter-assay Precision:
The HPC, MPC, LPC, and NC were assayed in ≥duplicate over 46 independent runs, by 3 analysts, over 15 days. The acceptance criteria for normalized RLU CV ≤25% were met:

Review comment: The assay has been demonstrated by the Sponsor to possess adequate Inter-assay precision. Interestingly, the Sponsor also argues that this same data, obtained through 46 independent runs, by 3 analysts, over 15 days, is also indicative of Robustness. This approach is commonly used by sponsor and is generally supported; dedicated robustness experiments do not provide meaningfully different information than the inherent variability of 46 different assessments; therefore, this approach of not performing dedicated robustness assessments is acceptable.

Short-term Stability:
Immunoassays with HPC, LPC, and NC stored in neat serum were performed after storage at RT for 24 hours 13 minutes, 2-8°C for 48 hours 6 minutes prior to extraction, and compared to performance against HPC, LPC, and NC samples prepared fresh. The samples subjected to RT and refrigeration performed equivalently (close to 100% recovery) in comparison to freshly-prepared samples.

Review comment: The Sponsor adequately demonstrated short-term stability inherent to their ADA immunoassay.

Freeze/thaw Stability:
Immunoassays were performed using HPC, LPC, and NC samples subjected to multiple cycles of -80°C±15°C freezing and thawing. The assay tolerates up to 6 cycles of freeze/thawing by meeting the following criteria.

- NC generates signal below 1.14 ACP
- HPC and LPC generate signals ≥1.14 ACP
- The assay signals should follow the HPC > LPC trend

Review comment: The Sponsor has adequately demonstrated freeze/thaw stability over 6 cycles, which is most likely acceptable for regular day-to-day performance of the ADA immunoassay.

Short-term Stability of Extracted Samples:
Immunoassays were performed using HPC, LPC, and NC samples extracted, then stored at RT for 24 hours, 2-8°C for 48 hours, and -80°C for 41 days.

Some replicates of the 24 hours RT HPC samples slightly under-recovered as compared to fresh samples, but 24 hour RT LPC and NC samples were acceptable. All samples stored at 2-8°C for 48 hours had acceptable recovery.

Review comment: Short-term stability is demonstrated by having similar performance of the assay when the reagents are prepared fresh, left at room temperature for 24 hours, or at 2-8°C for 24 hours. The short term stability is acceptable.

Longer-term Stability of Extracted Samples:
Storage of extracted HPC, LPC, and NC samples for 41 days at -80°C did not result in any significant loss of signal as compared to fresh samples (the fresh samples values were obtained from the mean of control values recorded during validation, see Table 18 in the submission).

Review comment: The stability of the samples stored at longer-term conditions is acceptable.

Review of Bioanalytical Method Validation Report: Validation of an Electrochemiluminescent-Based Ligand Binding Assay for the Detection of Anti-KRN23 Neutralizing Antibodies in Human Serum ((b) (4) Study number: P14-33402)

Summary of Validation:

Validation parameter	Result	
Cut point	0.761	
Sensitivity (LLRD)	133 ng/mL (267 ng/mL)	
Hook effect	Not observed	
Selectivity	SAVs were less than the screening cut point in all individuals spiked with the positive control. SAVs were greater than the screening cut point in all individuals.	
Drug interference	Positive control (ng/mL)	KRN23 (ng/mL) that indicated SAV less than the screening cut point
	0	None
	500	0, 4000, and 8000
	1000	0, 500, 1000, 2000, 4000, 8000, 16000, and 32000
Antigen interference	Positive control (ng/mL)	Recombinant human FGF23 (ng/mL) that indicated SAV less than the screening cut point
	0	800
	500	0, 50, 100, 200, 400, and 800
Intra-assay precision	Precision of SAV of samples with positive control: 2.7% and 4.4%	
	Precision of ECL response of samples without positive control: 4.8%	
Inter-assay precision	Precision of SAV of samples with positive control: 10.0% and 14.3%	
	Precision of ECL response of samples without positive control: 19.2%	

The bioanalytical method is a competitive assay that measures the extent of rhFGF23 and ruthenylated KRN23 binding inhibition by anti- KRN23 NAb.

In the assay, antibody samples are diluted 10-fold, bound to biotinylated KRN23 (in the presence of anti-FGF23, to block the biotinylated KRN23 from binding endogenous FGF32), then added to streptavidin-coated plates to capture the ADA complexes. Subsequent acid dissociation releases the plate-bound anti-KRN23 antibodies, which is then bound to ruthenylated KRN23. The samples are then incubated in plates coated with rhFGF23, and signal is measured from the ruthenylated KRN23. The presence of anti-KRN NAb leads to low signal, while absence of anti-KRN NAb results in relatively higher signal.

Screening Cut Point Determination

Fifty-four individual samples were analyzed by 2 analysts on six different days, over three rounds, with removal of outliers by box-plot analysis, and confirmation of normality (to significance level of 0.05) by Shapiro-Wilk test. The SCP was calculated by the mean and SD values of the six independent assays (data from 54 subjects contained in Table 8 of Method Validation Report, not reproduced here):

- Mean = 0.953
- SD = 0.083
- SCP = mean-2.33*SD = 0.761

Review comment: The SCP was acceptably calculated using a sufficient number of samples, and an appropriate method (using a 1% False-positive rate).

Sensitivity

Samples were spiked with PC at 2x dilutions from 3200 to 25 ng/ml, and analyzed 6 different days by 2 analysts (see Table 9 of Method Validation Report, not reproduced here). The lower limit of reliable detection (LLRD) threshold was calculated from the screening data set:

- LLRD threshold = mean-3.29*SD =0.682

The ECL assay responses corresponding to the SCP were calculated as:

- Mean ECL response of drug control*SCP (see Table 9, Sensitivity)

The ECL assay responses corresponding to the LLRD threshold were calculated as:

- Mean ECL response of drug control*LLRD threshold (see Table 9, Sensitivity)

The lowest positive control concentrations in each run generating data less than the corresponding screening cut point and LLRD threshold were determined to be the sensitivity and LLRD for each of the six runs. The overall sensitivity and LLRD for the assay validation were calculated as the means of the sensitivities and LLRDs generated for each run.

- Sensitivity = 133 ng/ml
- LLRD = 267 ng/ml

Review comment: The Sensitivity and LLRD were acceptably calculated using an appropriate mathematical method.

Hook effect

Samples were spiked with PC at 10,000, 50,000, and 250,000 ng/ml and analyzed for false-negative signals at high concentration (prozone). As shown in Table 10 (not reproduced here), there were no false-negative results recorded for any of the high PC concentrations tested.

Review comment: The assay for hook effect was appropriately performed, and the results are acceptable.

Selectivity

Ten total samples were analyzed with or without a spike of 400 ng/ml positive control. All spiked samples elicited assay values lower than their corresponding spiked assay values, indicative of the expected neutralizing activity by the control antibody (see Table 11, not reproduced here).

Review comment: The assay for selectivity acceptably demonstrated the appropriate analytical response.

Drug Interference

Assays containing 0, 500, and 1000 ng/ml control antibody were analyzed in the presence of a range of KRN23 concentrations from 0-32000 ng/ml (see Table 12, not reproduced here). All assays containing 0 ng/ml control antibody generally read above the cut point, indicating no neutralizing activity present in the wells. The Sponsor noted abnormal results with the 500 ng/ml control antibody, because of a seeming lack of correlational relationship with drug concentration: the 500 ng/ml control antibody assays with 0, 4000 and 8000 ng KRN23 concentrations resulted in assay values below the cut point, while those 500 ng/ml PC assays with 500, 1000, 2000, 16000, and 32000 ng/ml KRN23 concentrations resulted in assay values above the cut point. However, with 1000 ng/ml control antibody, all assay values were found to report below the cut

point, irregardless of KRN23 concentration. The Sponsor's conclusion is that even in the presence of up to 32000 ng/ml KRN23, a concentration of 1000 ng/ml control antibody results in readings below the cut point (indicative of positive detection).

Review comment: The assay for drug interference in the NAb assay found a tolerance of 32000 ng/ml, which is in the same general range as the tolerance of the binding antibody assay (see above). This is acceptable.

Antigen Interference

Assays with 0 or 500 ng/ml control antibody were performed with concentrations of rhFGF23 from 0-800 ng/ml. In all cases, the assays returned values below the screening cut point, indicative of positive detection, and thus indicating tolerance of FGF23 up to at least 800 ng/ml.

Review comment: The assay for antigen interference in the NAb assay found a tolerance of up to 800 ng/ml FGF23. This level of interference is normal and is considered acceptable.

Intra-assay Precision

Over six assay replicates using 0, 200, and 400 ng/ml positive control, the %CVs were found to be all below 5%.

Review comment: The assay appears to possess an acceptable level of Intra-assay precision.

Inter-assay Precision

Eighteen replicates of the assay were performed over six days, using positive control concentrations of 0, 200, and 400 ng/ml. The %CVs were found to be all below 20%.

Review comment: The assay appears to possess an acceptable level of Inter-assay precision.

William Hallett

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