CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761079Orig1s000

PRODUCT QUALITY REVIEW(S)

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

LABELS AND LABELING REVIEW

Date:	May 21, 2018
Reviewer:	Vicky Borders-Hemphill, PharmD Labeling Review Specialist Office of Biotechnology Products (OBP)
Through:	Ying-Xin Fan, PhD, Product Quality Reviewer OBP/Division of Biotechnology Review and Research IV
Application:	BLA 761079
Applicant:	BioMarin Pharmaceutical Inc.
Submission Date(s):	June 30, 2017
Product:	Palynziq (pegvaliase)
Dosage form(s):	Injection
Strength and Container-Closure:	2.5 mg/0.5 mL, 10 mg/0.5 mL, and 20 mg/mL in single-dose prefilled syringes (1 mL long (b) (4) glass prefilled syringe with a stainless steel staked needle).
Indication, dose, route, and frequency of administration:	A PEGylated phenylalanine-metabolizing enzyme indicated to reduce blood phenylalanine in adult patients with phenylketonuria who have uncontrolled blood phenylalanine levels greater than 600 micromol/L on existing management: The proposed recommended starting dose is 2.5 mg subcutaneously once per week for 4 weeks. Escalate the dosage in a step-wise manner based on tolerability to reach a target maintenance dosage of 20 mg subcutaneously once daily. If a minimum of 20% blood phenylalanine reduction is not achieved after 24 weeks, the dosage may be increased to 40 mg subcutaneously once daily
Background and Summary Description:	The Applicant submitted BLA 761079 Palynziq (pegvaliase) proposed to reduce blood phenylalanine in adult patients with phenylketonuria.
Recommendations:	The container labels and carton labeling (submitted on February 23, 2018), prescribing information (submitted on May 18, 2018, and medication guide and instructions for use (submitted on May 15, 2018) for Palynziq (pegvaliase) injection, 2.5 mg/0.5 mL, 10 mg/0.5 mL, and 20 mg/mL single-dose prefilled syringes for subcutaneous use are acceptable from a quality perspective.

Materials Considered for this Label and Labeling Review	
Materials Reviewed	**Appendix Section**
Proposed Labels and Labeling	A
Other	B (n/a)
Relevant Code of Federal Regulations, United States Pharmacopeia (USP) standards, and CDER Labeling Best Practices	C
Acceptable Labels and Labeling	D

n/a = not applicable for this review

DISCUSSION and CONCLUSION

We evaluated the proposed labels for compliance to the applicable requirements in the Code of Federal Regulations and United States Pharmacopeia (USP) standards (see Appendix C).

The container labels and carton labeling (submitted on February 23, 2018), prescribing information (submitted on May 18, 2018, and medication guide and instructions for use (submitted on May 15, 2018), for Palynziq (pegvaliase) injection, 2.5 mg/0.5 mL, 10 mg/0.5 mL, and 20 mg/mL single-dose prefilled syringes for subcutaneous use were reviewed and found to comply with the pertinent regulations (21 CFR 610.60 through 21 CFR 610.67; 21 CFR 201.2 through 21 CFR 201.25; 21 CFR 201.50 through 21 CFR 201.57; 21 CFR 201.100), and USP standards.

The labels and labeling submitted on February 23, 2018 and March 22, 2018 are acceptable (see Appendix D) from a quality perspective.

APPENDICES

Appendix A: Proposed Labeling

Prescribing Information (submitted October 4, 2017
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Medication Guide (submitted October 4, 2017
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Instructions for Use (submitted October 4, 2017
\\cdsesub1\evsprod\bla761079\0013\m1\us\114-labeling\1141-draft-labeling\11412-annotated-draft-labeling-text\annotated-draft-instructions-for-use.pdf)

7 Page(s) of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

Appendix B: Other (n/a)
Appendix C: Evaluation Tables (Label[1,2] and Labeling[3] Standards)

Container[4] Label Evaluation

Syringe

Regulations, Guidance and CDER Best Labeling Practices	Conforms
Proper Name (21 CFR 610.60, 21 CFR 201.50, 21 CFR 201.10) *for container of a product capable of bearing a full label* **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Manufacturer name, address, and license number (21 CFR 610.60) *for container of a product capable of bearing a full label* **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Lot number or other lot identification (21 CFR 610.60, 21 CFR 201.18, 21 CFR 201.100) **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Expiration date (21 CFR 610.60, 21 CFR 201.17) **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Multiple dose containers (recommended individual dose) 21 CFR 610.60 **Comment/Recommendation:** single dose prefilled syringe	☐ No ☐ Yes ☒ N/A
Statement: "Rx only" 21 CFR 610.60 21 CFR 201.100 **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Medication Guide 21 CFR 610.60 21 CFR 208.24 **Comment/Recommendation:** *considered a partial label the MG statement may be placed on the carton labeling per 21 CFR 610.60 (a) (7).*	☐ No ☐ Yes ☒ N/A
No Package for container 21 CFR 610.60	☐ No ☐ Yes ☒ N/A

[1] Per 21 CFR 1.3 (b) *Label* means any display of written, printed, or graphic matter on the immediate container of any article, or any such matter affixed to any consumer commodity or affixed to or appearing upon a package containing any consumer commodity.
[2] Per CFR 600.3(dd) *Label* means any written, printed, or graphic matter on the container or package or any such matter clearly visible through the immediate carton, receptacle, or wrapper.
[3] Per 21 CFR 1.3(a) *Labeling* includes all written, printed, or graphic matter accompanying an article at any time while such article is in interstate commerce or held for sale after shipment or delivery in interstate commerce.
[4] Per 21 CFR 600.3(bb) *Container* (referred to also as "final container") is the immediate unit, bottle, vial, ampule, tube, or other receptacle containing the product as distributed for sale, barter, or exchange.

Comment/Recommendation: syringe provided in tray and carton

Partial label 21 CFR 610.60 21 CFR 201.10	☐ No ☒ Yes ☐ N/A

Comment/Recommendation:
Ensure that the license number appears underneath the manufacturer statement. We consider this to be a partial label, thus the words "(b) (4)" may be omitted. Revise to read as follows:
"BioMarin Pharmaceutical Inc.
US License No. 1649"
The applicant revised as requested

If space permits, consider adding the dosage form "Injection" underneath the proper name as follows:
Palynziq
(pegvaliase)
Injection

The applicant responded: Given the space limitations on the syringe labels, BioMarin is unable to fit the dosage form "Injection" on the syringe label without compromising readability. BioMarin re-ordered the content on the syringe label in the order FDA has requested with the exception of including the dosage form "Injection" as follows:
Palynziq
(pegvaliase-pqpz)
Strength
A white background was added around the proprietary name (Palynziq) and proper name (pegvaliase-pqpz) to improve readability of these elements on the syringe label.

We find the Applicant's response acceptable.

No container label 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
Ferrule and cap overseal	☐ No ☐ Yes ☒ N/A
Visual inspection 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
NDC numbers 21 CFR 201.2 21 CFR 207.35	☐ No ☐ Yes ☒ N/A

Comment/Recommendation: *Not required for partial labels per 21 CFR 610.60(c).*

Route of administration 21 CFR 201.5 21 CFR 201.100	☐ No ☐ Yes ☒ N/A

Comment/Recommendation: *considered a partial label*

Preparation instructions 21 CFR 201.5	☐ No ☐ Yes ☒ N/A
Package type term 21 CFR 201.5	☐ No ☐ Yes ☒ N/A
Comment/Recommendation: *considered a partial label*	
Drugs **Misleading statements** 21 CFR 201.6	☐ No ☒ Yes ☐ N/A
Strength 21 CFR 201.10 21CFR 201.100	☐ No ☒ Yes ☐ N/A
Drugs **Prominence of required label statements** 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
Spanish-language (Drugs) 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
FD&C Yellow No. 5 and/or FD&C Yellow No. 6 21 CFR 201.20	☐ No ☐ Yes ☒ N/A
Phenylalanine as a component of aspartame 21 CFR 201.21	☐ No ☐ Yes ☒ N/A
Sulfites; required warning statements 21 CFR 201.22	☐ No ☐ Yes ☒ N/A
Bar code label requirements 21 CFR 201.25 21CFR 610.67	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: Ensure the linear bar code appears on the syringe label. Ensure there is adequate white space around the linear bar code to facilitate scanning. See *Guidance for Industry: Bar Code Label Requirements Questions and Answers, August 2011*, *Draft Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013* *(lines 511-512), lines 780-786)* *The Applicant responded: BioMarin has applied a linear bar code that contains the NDC number to the Palynziq syringe labels. As described in BioMarin's clarification email to the Agency sent on 12-Feb-2018, BioMarin is not able to include human readable numbers without impacting readability or clear window for visual inspection. FDA agreed to this approach per response received 16-Feb-2018.* *We find the Applicant's response acceptable.*	

Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
Net quantity 21 CFR 201.51 **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Usual dosage statement 21 CFR 201.55 21 CFR 201.100 **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Inactive ingredients 21 CFR 201.100 **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Storage requirements **Comment/Recommendation:** *considered a partial label*	☐ No ☐ Yes ☒ N/A
Dispensing container 21 CFR 201.100	☐ No ☐ Yes ☒ N/A

Package Label[5] Evaluation

Tray

Regulations, Guidance and CDER Best Labeling Practices	Conforms
Proper name (21 CFR 610.61, 21 CFR 201.50, 21 CFR 201.10)	☐ No ☒ Yes ☐ N/A

Comment/Recommendation: Note the proper name does not include the dosage form; historical CDER biological labeling practices places the dosage form below the proper name. To accommodate this, relocate the Rx only statement to appear in the upper right corner of the principal display panel on the tray lidding labeling. Revise using the following example:
Palynziq
(pegvaliase)
Injection
2.5 mg/0.5 mL
For Subcutaneous Use Only

The Applicant revised as requested

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

Manufacturer name, address, and license number 21CFR 610.61	☐ No ☒ Yes ☐ N/A
Lot number or other lot identification 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Expiration date 21 CFR 610.61 21 CFR 201.17	☐ No ☒ Yes ☐ N/A
Preservative 21 CFR 610.61	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: Ensure "No preservative" appears on the tray lidding labeling per 21 CFR 610.61 (e). *The Applicant revised as requested*	
Number of containers 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Strength/volume 21 CFR 610.61 21 CFR 201.10 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Storage temperature 21 CFR 610.61	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: Ensure the recommended storage temperature and handling appears on the tray lidding labeling per 21 CFR 610.61(h) and 21 CFR 610.61(i). Add the following: "**Refrigerate at 36°F to 46°F (2°C to 8°C) in the original carton to protect from light. Do not freeze or shake.**" *The Applicant revised as requested* Remove the statement [redacted] (b) (4) " as this will be recorded on the carton labeling. *The Applicant revised as requested* To accommodate the addition of important storage information on the tray lidding labeling, consider relocating the package type term and discard statement to appear on the principal display panel after the route of administration statement as follows: "For Subcutaneous Use Only Single-dose prefilled syringe. Discard after use" *The Applicant revised as requested*	
Handling: "Shake Well", "Do not Freeze" or equivalent (21 CFR 610.61)	☒ No ☐ Yes ☐ N/A

Comment/Recommendation: see above	
Multiple dose containers (recommended individual dose) 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Route of administration 21CFR 610.61 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Known sensitizing substances 21CFR 610.61	☐ No ☐ Yes ☒ N/A
Inactive ingredients 21 CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Comment/Recommendation: Space considerations, list of inactive ingredients appears on the carton labeling.	
Source of the product 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Minimum potency of product 21 CFR 610.61	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: to appear on carton labeling	
Rx only 21CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Divided manufacturing 21 CFR 610.63	☐ No ☐ Yes ☒ N/A
Distributor 21 CFR 610.64	☐ No ☐ Yes ☒ N/A
Bar code 21 CFR 610.67 21 CFR 201.25	☐ No ☒ Yes ☐ N/A
Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
NDC numbers 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A
Preparation instructions 21 CFR 201.5	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: see above for storage/handling recommendations	
Package type term	☐ No

21 CFR 201.5	☒ Yes ☐ N/A
Drugs **Misleading statements** 21 CFR 201.6	☐ No ☐ Yes ☒ N/A
Drugs **Prominence of required label statements** 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
Net quantity 21 CFR 201.51	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: Ensure the net quantity appears on the PDP less prominent and away from the strength. Add the following statement, for example: "1 x 20 mg/mL prefilled syringe". *The Applicant revised as requested*	
Usual dosage statement 21 CFR 201.55 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Dispensing container 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
Medication Guide 21 CFR 610.60 21 CFR 208.24	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: Ensure the Medication Guide statement ("ATTENTION: Dispense the enclosed Medication Guide to each patient") appears. *The Applicant responded: As described in BioMarin's clarification email to FDA sent on 12-Feb-2018, the Medication Guide is enclosed in the carton, and not inside the tray. Since updated storage conditions require that the product be stored in the carton, this statement is not included on the tray cover when the pharmacist is required to dispense the product at the carton level. BioMarin intends to include a printed Medication Guide inside the carton to accompany each pack presentation. Inclusion of the Medication Guide statement on the tray cover would reduce overall readability on this labeling component. BioMarin increased the prominence (increased font size and applied bolding) of the Medication Guide statement on the carton as suggested by the Agency in email correspondence received 12-Feb-2018.* *We find the Applicant's response acceptable.*	

Carton

Regulations, Guidance and CDER Best Labeling Practices	Conforms
Proper name (21 CFR 610.61, 21 CFR 201.50, 21 CFR 201.10) **Comment/Recommendation:** Note the proper name does not include the dosage form; historical CDER biological labeling practices places the dosage form below the proper name. To accommodate this, relocate the Rx only statement to appear in the upper right corner of the principal display panel on the tray lidding labeling. Revise using the following example: Palynziq (pegvaliase) Injection 2.5 mg/0.5 mL For Subcutaneous Use Only *The Applicant revised as requested*	☐ No ☒ Yes ☐ N/A
Manufacturer name, address, and license number 21CFR 610.61	☐ No ☒ Yes ☐ N/A
Lot number or other lot identification 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Expiration date 21 CFR 610.61 21 CFR 201.17	☐ No ☒ Yes ☐ N/A
Preservative 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Number of containers 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Strength/volume 21 CFR 610.61 21 CFR 201.10 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Storage temperature 21 CFR 610.61 **Comment/Recommendation:** Ensure the recommended handling appears on the carton labeling per 21 CFR 610.61(i). Revise the storage statement to include the following: "Store in refrigerator at 36°F to 46°F (2°C to 8°C) in the original carton to protect from light. Do not freeze or shake. If needed, Palynziq may be stored at room temperature up to 77°F (25°C) for up to 30 days in the sealed tray. Once stored at room temperature, do not place back in the refrigerator. Discard after 30 days. Write date removed from refrigeration __/__/__."	☒ No ☐ Yes ☐ N/A

The Applicant revised as requested. FDA review team requested that the temperature range for the optional room temperature storage conditions be added for improved patient comprehension in the Medguide. The applicant proposed to make the same revision to carton labeling at next printing. We agree.

Delete the statement "[redacted] (b) (4)" as it is not necessary since the required storage temperature range is provided.

The Applicant revised as requested

Handling: "Shake Well", "Do not Freeze" or equivalent (21 CFR 610.61)	☒ No ☐ Yes ☐ N/A
Multiple dose containers (recommended individual dose) 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Route of administration 21CFR 610.61 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Known sensitizing substances 21CFR 610.61	☐ No ☒ Yes ☐ N/A
Inactive ingredients 21 CFR 610.61 21 CFR 201.100	☒ No ☐ Yes ☐ N/A

Comment/Recommendation: Add the quantity of all inactive ingredients, except ingredients added to adjust the pH or to make the drug isotonic may be declared by name and a statement of their effect; and if the vehicle is water for injection it need not be named per 21 CFR 201.100(b)(5) as follows: "Each single dose prefilled syringe delivers x mg pegvaliase in x mL which contains sodium chloride (xx mg), trans-cinnamic acid (xx mg), tromethamine (xx mg), …." Ensure that the inactive ingredients appear in alphabetical order per USP <1091> Labeling of Inactive Ingredients.

The Applicant revised as requested

Source of the product 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Minimum potency of product 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Rx only 21CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A

Comment/Recommendation: Relocate the Rx only statement to appear in the upper right corner of the principal display panel.

The Applicant revised as requested

Divided manufacturing	☐ No

21 CFR 610.63	☐ Yes ☒ N/A
Distributor 21 CFR 610.64	☐ No ☐ Yes ☒ N/A
Bar code 21 CFR 610.67 21 CFR 201.25	☐ No ☒ Yes ☐ N/A
Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
NDC numbers 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A
Preparation instructions 21 CFR 201.5 **Comment/Recommendation:** see storage and handling recommendation above	☒ No ☐ Yes ☐ N/A
Package type term 21 CFR 201.5 **Comment/Recommendation:** Remove the statement "(b) (4)". The appropriate package type term is "single-dose" and has already been included. *The Applicant revised as requested*	☐ No ☒ Yes ☐ N/A
Drugs **Misleading statements** 21 CFR 201.6	☐ No ☐ Yes ☒ N/A
Drugs **Prominence of required label statements** 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
Net quantity 21 CFR 201.51	☐ No ☒ Yes ☐ N/A
Usual dosage statement 21 CFR 201.55 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Dispensing container 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
Medication Guide 21 CFR 610.60 21 CFR 208.24	☐ No ☒ Yes ☐ N/A

Prescribing Information and Patient Labeling Evaluation

Regulations	Conforms
PRESCRIBING INFORMATION	
Highlights of prescribing information	
PRODUCT TITLE 21 CFR 201.57(a)(2)	☐ No ☒ Yes ☐ N/A
DOSAGE AND ADMINISTRATION 21 CFR 201.57(a)(7)	☐ No ☒ Yes ☐ N/A
DOSAGE FORMS AND STRENGTHS 21 CFR 201.57(a)(8)	☐ No ☒ Yes ☐ N/A
Full Prescribing Information	
2 DOSAGE AND ADMINISTRATION 21 CFR 201.57(c)(3)	☐ No ☒ Yes ☐ N/A
3 DOSAGE FORMS AND STRENGTHS 21 CFR 201.57(c)(4) **Comment/Recommendation:** We added the identifying characteristics per 21 CFR 201.57(c)(4) *The applicant revised as requested*	☒ No ☐ Yes ☐ N/A
6.2 IMMUNOGENICITY Draft Guidance for Industry: Labeling for Biosimilar Products **Comment/Recommendation:** We include the following standard statement or appropriate modification at the beginning of the Immunogenicity subsection preceding the immunogenicity data. *The applicant revised as requested*	☒ No ☐ Yes ☐ N/A
11 DESCRIPTION (21 CFR 201.57(c)(12), 21 CFR 610.61 (m), 21 CFR 610.61(o), 21 CFR 610.61 (p), 21 CFR 610.61 (q)) **Comment/Recommendation:** We deleted (b) (4) since the first paragraph discusses the drug substance *The applicant revised as requested* Per USP Chapter <1091> list all inactive ingredients in alphabetical order followed by their quantitative information that is deliverable per prefilled syringe using the metric system of weight in parenthesis (x mg) except for those inactive ingredients added to adjust pH or tonicity or water for injection *The applicant revised as requested* We added the information about the type and calculated amount of antibiotics added during manufacture per 21 CFR 610.61 (m): "During the manufacturing process, fermentation is carried out in a nutrient medium containing the antibiotic kanamycin, 50 mcg/mL. Kanamycin is not detectable in the final product"	☒ No ☐ Yes ☐ N/A

The applicant revised as requested

16 HOW SUPPLIED/ STORAGE AND HANDLING
21 CFR 201.57(c)(17)

☒ No
☐ Yes
☐ N/A

Comment/Recommendation: We added identifying characteristics per 21 CFR 201.57(c)(17).
The applicant revised as requested

Your proposed carton labeling states "Do not freeze", the Med Guide includes "or shake" but this is not stated here. Confirm the addition of the statement here for consistency
The applicant revised as requested

We added "protect from light" in the first paragraph of this subsection as special handling and storage conditions per 21 CFR 201.57(c)(17)(iv).
The applicant revised as requested

We deleted the statement "(b) (4)" as it is not necessary since the required storage temperature range is provided.
The applicant revised as requested

FDA review team requested that the temperature range for the optional room temperature storage conditions be added for improved patient comprehension in the Medguide. The applicant proposed to make the same revision to carton labeling at next printing. The applicant may present the optional storage temperature as "up to 77° (25°C)" since the prescribing information is for healthcare professional or as "68°F to 77°F (20°C to 25°C)".

MANUFACTURER INFORMATION
For BLAs: 21 CFR 610.61, 21 CFR 610.64

☐ No
☒ Yes
☐ N/A

MEDICATION GUIDE and INSTRUCTIONS FOR USE

TITLE (NAMES AND DOSAGE FORM)

☒ No
☐ Yes
☐ N/A

Comment/Recommendation: We added the dosage form to the IFU for consistency with other labeling
The applicant revised as requested

STORAGE AND HANDLING

☒ No
☐ Yes
☐ N/A

Comment/Recommendation: We added the handling information to the IFU. Your proposed carton labeling states "Do not freeze", the Med Guide includes "or shake" but it is not stated here. Confirm the addition of the statement here for consistency.
The applicant revised as requested
We added "protect from light" per 21 CFR 201.57(c)(17)(iv).

The applicant revised as requested

We deleted the statement "(b) (4)" as it is not necessary since the required storage temperature range is provided.

The applicant revised as requested	
INGREDIENTS	☐ No ☒ Yes ☐ N/A
MANUFACTURER INFORMATION For BLAs: 21 CFR 610.61, 21 CFR 610.64 **Comment/Recommendation:** To comply with 21 CFR 610.61(b) the licensed manufacturer must appear at the bottom of the medication guide. We added the U.S. License No per 21 CFR 610.61(b) *The applicant revised as requested*	☒ No ☐ Yes ☐ N/A

APPENDIX D. Acceptable Labels and Labeling

- Prescribing Information (submitted on May 18, 2018)
- Medication Guide (submitted May 15, 2018 **\\cdsesub1\evsprod\bla761079\0076\m1\us\114-labeling\1141-draft-labeling\11412-annotated-draft-labeling-text\annotated-draft-medication-guide.pdf**)
- Instructions for Use (submitted May 15, 2018 **\\cdsesub1\evsprod\bla761079\0076\m1\us\114-labeling\1141-draft-labeling\11412-annotated-draft-labeling-text\annotated-draft-instructions-for-use.pdf**)

Container Labels (submitted February 23, 2018)

(b) (4)

7 Page(s) of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

Vicky Borders-Hemphill

Digitally signed by Vicky Borders-Hemphill
Date: 5/21/2018 10:23:21AM
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Ying Xin Fan

Digitally signed by Ying Xin Fan
Date: 5/21/2018 10:32:39AM
GUID: 508da6dc000266e7f77d37bc2e4a5a87

U.S. FOOD & DRUG ADMINISTRATION

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

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Primary Reviewer: Lindsey Brown, Ph.D.

Secondary Reviewer: Reyes Candau-Chacon, Ph.D.

Date: 9/12/2017

To: Administrative File **STN:** 761079/0

Applicant: BioMarin, Inc.

Submission Reviewed: BLA to support the manufacture of pegvaliase for injection.

US License Number: 1649

Facility: Cook Pharmica LLC, Bloomington, IN **(FEI: 3005949964)**

Product: Pegvaliase (Palynziq-pqpz)

Indication: Pegvaliase is indicated to reduce blood phenylalanine levels in adult patients with Phenylketonuria (PKU) who have uncontrolled blood Phe levels > 600 µmol/L on existing management.

Dosage Form: 5 mg/mL, 20 mg/mL, PFS for subcutaneous injection

PDUFA Date: 5/28/2018

Recommendation for Approvability: BLA 761079 is recommended for approval from a sterility assurance and microbiology product quality perspective.

The following will be submitted as a post-marketing commitment:

Implementation of [redacted] (b) (4)

The following DMFs was reviewed in support of BLA 761079

DMF	Status	Panorama Date
DMF (b) (4)	Adequate	09/25/2017
DMF	Adequate	02/15/2018
DMF	Adequate	01/19/2017

Review Summary

BioMarin submitted BLA 761079 to license pegvaliase to reduce blood phenylalanine levels in adult patients with phenylketonuria (PKU) who have uncontrolled blood Phe levels > 600 µmol/L on existing management. The application was submitted in eCTD format. This review assesses BMN 190 drug product section of the BLA from a sterility assurance and microbiological quality perspective.

Drug Product Quality Microbiology Information Reviewed

Sequence	Date	Description
0001	6/30/2017	Original Submission
0006	8/14/2017	Response to 74-day letter
0008	8/21/2017	Information Request (IR)
0010	9/22/2017	Response to IR
0012	9/29/2017	Response to IR
0017	10/20/2017	Response to IR
0031	12/07/2017	Response to IR
0035	12/15/2017	Response to IR
0041	1/5/2018	Response to IR
0044	1/23/2018	Response to IR
0045	1/31/2018	Response to IR
0049	2/15/2018	Response to IR

0057	3/19/2018	Response to IR
0062	4/4/2018	Response to IR
0065	4/10/2018	Response to IR
0068	4/18/2018	Response to IR

3.2.P.1 Composition of the Drug Product

Table 3.2.P.1.2.1: Nominal Composition of pegvaliase Drug Product

Component	Quality Standard	Function	Composition[1]			
			2.5 mg[2]	10 mg[2]	20 mg[2]	Concentration
Pegvaliase	In-house Standard[3]	Active Ingredient	2.5 mg	10 mg	20 mg	5 mg/mL (0.08 mM) [4,6] or 20 mg/mL (0.32 mM)[5,6]
Tromethamine	USP/Ph. Eur	(b) (4)	(b) (4)			
Tromethamine –HCl	Manufacturer's CoA[7]					
Sodium Chloride	USP/Ph. Eur	Tonicity Agent				
Trans-cinnamic acid	Not Applicable[8]	(b) (4)	0.07 mg	0.07 mg	0.15 mg	0.15 mg/mL (1.0 mM)
Water for Injection (qs)	USP/Ph. Eur	Diluent	0.50 mL	0.50 mL	1.00 mL	Not Applicable

USP: United States Pharmacopeia; Ph. Eur: European Pharmacopeia

[1] The composition provided is for the delivered volume of pegvaliase DP and excludes syringe overfill amounts.

[2] Protein concentrations refer to the protein portion. (b) (4)

[3] Qualified according to procedure described in Section 3.2.S.5 Reference Standards or Materials (pegvaliase, BioMarin Pharmaceutical Inc.).

Description is Satisfactory

3.2.P.2 Pharmaceutical Development

3.2.P.2.4 Container Closure System

The primary container for pegvaliase drug product (DP) is a single-use 1 mL long (b) (4) (b) (4) syringe with a stainless steel staked needle with a rigid needle shield. The syringe is stoppered with a (b) (4) rubber stopper with a (b) (4) on the product contact surface. Additional information regarding the container closure system is provided in 3.2.P.7. Shipping validation data is located in 3.2.P.3.5.

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Lindsey Brown

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Date: 5/16/2018 09:59:53AM
GUID: 57d6b6e00198444ec5ee4152ac22a902

Reyes Candau-Chacon

Digitally signed by Reyes Candau-Chacon
Date: 5/21/2018 09:11:34AM
GUID: 508da7160002977f7ca389c8f849b707

First Approval for Indication/Priority Review (with Extension for Major Amendment)

Recommendation: Approval

BLA Number: 761079
Review Number: 1
Review Date: April 23, 2018

Drug Name/Dosage Form	PALYNZIQ (pegvaliase-pqpz), injection
Strength/Potency	2.5 mg/ 0.5 mL, 10 mg/0.5 mL, 20 mg/mL
Route of Administration	Subcutaneous
Rx/OTC dispensed	Rx
Indication	to reduce blood phenylalanine in adult patients with phenylketonuria who have uncontrolled blood phenylalanine levels > 600 µmol/L
Applicant/Sponsor	BioMarin Pharmaceutical Inc

Product Overview

Pegvaliase-pqpz is a phenylalanine-metabolizing enzyme that is composed of recombinant phenylalanine ammonia lyase (rAvPAL) derived from *Anabaena variabilis,* which is conjugated to N-hydroxysuccinimide (NHS)-methosypolyethylene glycol (PEG). rAvPAL is manufactured in *Escherichia coli*. To manufacture pegvaliase, an average of nine 20kD PEG are conjugated to each monomer of rAvPAL. The drug product is clear to slightly opalescent, colorless to pale yellow, sterile, preservative-free solution, intended for subcutaneous injection, which is supplied as single-dose prefilled syringe with three strengths: 2.5mg/0.5mL, 10mg/0.5mL, and 20mg/mL. This is the first enzyme replacement therapy for the treatment of phenylketonuria (PKU). The initial dose is 2.5mg weekly; however, the dose may be gradually increased over time to the maintenance dose (20mg daily). Consider increasing to a maximum dose of 40 mg daily in patients that have not achieved a response with 20mg daily. Treatment should be individualized so the patient receives the lowest effective and tolerated dose.

Quality Review Team

Discipline	Reviewer	Branch/Division
Drug Substance	Ying-Xin Fan	OBP/DBRR-IV
Drug Product	Ying-Xin Fan	OBP/DBRR-IV
Immunogenicity	Frederick Mills	OBP/DBRR-IV
Labeling	Vicky Borders-Hemphill	OBP
Facility	Peter Qiu / Ephrem Hunde	OPF/DIA
Microbiology -DS	Reyes Candau-Chacon / Maxwell Van Tassell	OPF/DMA
Microbiology -DP	Reyes Candau-Chacon / Lindsey Brown	OPF/DMA
Regulatory Business Project Manager	Oumou Barry	OPRO
Application Team Lead	Joslyn Brunelle	OBP/DBRR-IV

Multi-disciplinary Review Team:

Discipline	Reviewer	Office/Division
RPM	Benjamin Vali	OND/ODEIII/DGIEP
Cross-disciplinary Team Lead	Patroula Smpokou	OND/ODEIII/DGIEP
Medical Officer	Patroula Smpokou / Irena Lavine	OND/ODEIII/DGIEP
Pharm/Tox	David Joseph / Fang Cai	OND/ODEIII/DGIEP
Clinical Pharmacology	Jie Wang / Christine Hon	OTS/OCP/DCPIII
Statistics	Yeh-Fong Chen / Weiya Zhang	OTS/OB/DBIII

a. Names:

i. Proprietary Name: PALYNZIQ
ii. Trade Name: PALYNZIQ
iii. Non-Proprietary/USAN: pegvaliase-pqpz
iv. CAS name: 1585984-95-7
v. Common name: BMN 165
vi. INN Name: pegvaliase-pqpz
vii. Compendial Name: N/A
viii. OBP Systematic name: CONJ: RPROT Q3M5Z3 (PAL_ANAVT) PHENYLALANINE AMMONIA-LYASE; PEG [RAVPALPEG]

b. Pharmacologic category: phenylalanine-metabolizing enzyme

Submissions Reviewed:

Submission(s) Reviewed	Document Date
0001, original BLA submission	June 30, 2017
0006, IR dated August 2, 2017 (DMA)	August 14, 2017
0007, IR dated August 14, 2017 (OBP) 0008, IR dated August 14, 2017 (DMA)	August 21, 2017
0010, IR dated September 19, 2017 (DMA)	September 22, 2017
0011, IR dated September 19, 2017 (OBP)	September 27, 2017
0012, IR dated August 14, 2017 (DMA)	September 29, 2017
0013, IR dated September 12, 2017 (74-day letter)	October 4, 2017
0014, IR dated September 19, 2017 (OBP)	October 10, 2017
0017, IR dated September 12, 2017 (74-day letter) IR dated October 11, 2017 (DMA)	October 20, 2017
0021, IR dated August 2, 2017 (DMA)	October 26, 2017
0023, IR dated August 2, 2017 (DMA)	October 31, 2017
0028, IR dated August 2, 2017 (DMA)	November 15, 2017
0030, IR dated November 16, 2017 (OBP, DMA)	November 30, 2017
0031, IR dated November 16, 2017 (OBP, DMA)	December 07, 2017
0035, IR dated August 2, 2017 (DMA-media fills) 0036, IR dated November 15, 2017 (immuno)	December 15, 2017 (triggered clock extension)
0039, IR dated November 30, 2017 (CDRH-OC)	December 22, 2017
0041, IR dated November 16, 2017 (DMA)	January 5, 2018
0042, IR dated November 16, 2017 (OBP)	January 12, 2018
0044, IR dated January 12, 2018 (DMA)	January 23, 2018
0045, IR dated November 16, 2017; IR dated January 24, 2018	January 31, 2018

0049, IR dated February 1, 2018 (DMA and eCTD updates from IRs dated 9-19-2017, 11-16-17, 2-1-18)	February 15, 2018
0053, IR dated November 16, 2017 and PAI followup	March 1, 2018
0057, IR dated March 13, 2018 (OBP)	March 20, 2018
0059, IR dated March 15, 2018 (CDRH-OC)	March 22, 2018
0060, IR dated March 23, 2018 (OBP)	March 23, 2018
0062, IR dated March 30, 2018 (DMA)	April 4, 2018
0065, IR dated April 04, 2018 (OBP) 0067, Clarification sent April 9 (OBP)	April 10, 2018 (OBP) April 13, 2018 (immunogenicity PMR)
0068, IR dated April 17, 2018 (DMA)	April 18, 2018
0069, IR dated April 18, 2018 (OBP)	April 20, 2018
0070, PMR/PMC correspondence	April 20, 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	Code[1]	Status[2]	Comments
(b) (4)				1	Adequate	
				3	Adequate	
				3	N/A	
				3	Adequate	
				3	Adequate	
				3	Adequate	
(b) (4)		Cook Pharmica LLC	Sterile filling operation	3	N/A	

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

Document	Application Number	Description
IND	76269	Original IND submitted 2007

3. Consults:

Discipline/Topic	Date Requested	Status	Recommendation	Reviewer
CDRH/ODE	July 2017	Complete	Approval	Rong Guo
CDRH/OC	July 2017	Complete	Approval	Paola Barnett

Executive Summary

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

Recommendation:
The Office of Pharmaceutical Quality (OPQ), CDER, recommends approval of BLA 761079 for pegvaliase-pqpz manufactured by BioMarin Pharmaceutical Inc. The data submitted in this application are adequate to support the conclusion that the manufacture of pegvaliase is well-controlled and leads to 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.

C. Approval Action Letter Language:

- Manufacturing location:
  - Drug Substance: BioMarin Pharmaceutical Inc., 46 Galli Drive, Novato, CA 94949
  - Drug Product: Cook Pharmica, LLC., 1300 South Patterson Drive, Bloomington, IN 47403
- Fill size and dosage form:

  Prefilled Syringe: 2.5 mg/0.5 mL, 10 mg/0.5 ml, and 20 mg/ ml
- Dating period:
  - Drug Product:
    - 24 months at 5 ± 3 °C
    - If removed from refrigeration, then may be stored at room temperature (b) (4) - 25 ºC) for ≤ 1 month. Note 24-month cumulative hold time is inclusive of 1 month storage at room temperature.
  - Pegvaliase (rAvPAL-PEG) Drug Substance:
    - (b) (4)
    - [redacted]
    - We have approved the stability protocol in your license application for the purpose of extending the expiration dating of your drug substance under 21 CFR 601.12.
  - rAvPAL Intermediate:
    - (b) (4)
    - [redacted]
    - Results of on-going stability should be submitted throughout the dating period, as they become available
- Exempt from lot release

- Yes; Rationale, for exemption- specified product (exempt according to 21 CFR 601.2a)

C. **Benefit/Risk Considerations:**

Pegvaliase-pqpz substitutes for the deficient phenylalanine hydroxylase (PAH) enzyme activity and reduces blood phenylalanine concentrations in the body. PAH catalyzes phenylalanine (Phe) to tyrosine in the presence of cofactor tetrahydrobiopterin (BH4).

Phenylketonuria (PKU) is a rare disease with an estimated prevalence of 1 in 15,000 in the United States; it is an autosomal recessive genetic disorder caused by mutations in the PAH gene that lead to the inability to break down the amino acid phenylalanine. PKU is controlled by medical nutritional therapy in infants and children; however, the Phe-restricted diet is not sustainable in the adult population. Kuvan (sapropterin dihydrochloride) is currently the only FDA approved therapy for PKU, but the patient population is limited to tetrahydrobiopterin-(BH4-) responsive PKU and is to be used in conjunction with a Phe-restricted diet. There is an unmet medical need for new therapies/treatments for adult PKU. Increased Phe levels in the body lead to neurocognitive, neuropsychiatric, and executive functioning deficits.

Pegvaliase (PEGylated Phenylalanine lyase (PAL) enzyme) substitutes for the deficient PAH by breaking Phe to trans-cinnamic acid (t-CA) and ammonia, which are metabolized and excreted in the urine. PAH enzyme replacement therapy has not been successfully developed due to instability of the enzyme in plasma and difficulty delivering intact active enzyme to the liver. Orphan Drug Designation was granted March 1995 and Fast Track designation granted November 2011 for Pegvaliase. The BLA was initially granted a priority review (with PDUFA goal date of February 28, 2018), but a major amendment related to drug product sterility assurance submitted on December 15, 2017 led to an extended PDUFA goal date of May 28, 2018.

Biomarin's control strategy is sufficient to ensure product quality. The drug substance and drug product manufacturing processes are well controlled and can reproducibly produce pure and potent product. The drug substance manufacturing process consists of (b) (4) Due to changes (b) (4) Biomarin re-validated (b) (4) during the extended review cycle. The drug product manufacturing process consists of (b) (4) (b) (4) and provides microbial control and sterility assurance of the drug product. The analytical methods for release and stability testing are validated for specificity, precision, repeatability, linearity, accuracy, and robustness. The manufacturing facilities for drug substance (Biomarin, Novato, CA) and drug product (Cook, Bloomington, IN) are recommended for approval.

Overall product quality, immunogenicity assays, and labelling (reviewed by OBP), microbiology (reviewed by DMA), facilities (reviewed by DIA), and device (reviewed by CDRH) are located as separate documents in Panorama.

Formation of anti-drug antibodies (ADA) and neutralizing antibodies (Nabs) have been monitored by multiple validated assays, including IgM and IgG for both anti-PEG and anti-PAL, and total antibodies (Tab). These ADA and Nab assays are affected to varying degrees by on-board pegvaliase; however, the assays provide useful clinical information because all subjects

treated with pegvaliase developed high levels and titers of ADA and Nab, which were shown to impact efficacy. Due to the robust immune response, an additional assay was included to assess whether immune responses transition to IgG4 after prolonged treatment with pegvaliase. Due to hypersensitivity events, assays were included to assess IgE levels. Additional assays for Immune complexes (both IgG and IgM) were included to assess long term safety concerns. Overall, there are no issues regarding validation of immunogenicity assays that preclude approval. However, there are postmarketing requirements to revise the following assays: anti-PEG IgG ADA, anti-PEG IgM ADA, and IgE ImmunoCAP.

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

Product Quality Postmarketing commitments

1. 3349-11: Re-develop the enzyme kinetic assay (Km and kcat) so that the substrate concentration is held essentially constant ((b) (4)) during the measurements of initial velocities. Re-validate the assay and re-evaluate the acceptance criteria for drug substance (DS) and drug product (DP) release and stability testing based on the re-validated assay.

   Study completion 12/2018, Final report submission 1/2019

2. 3349-12: Perform a study to evaluate the impact of the removal of kanamycin (b) (4) during the pegvaliase fermentation process. If the data support removal of kanamycin, then submit a plan for the removal of kanamycin from the pegvaliase manufacturing process.

   Final report submission 6/2019

3. 3349-13: Evaluate product quality before and after shipping of formulated bulk drug (b) (4) . The shipping study should demonstrate worst-case conditions regarding distance, duration, background temperature, and vibration.

   Study completion 12/2018, Final report submission 3/2019

4. 3349-14: Evaluate product quality before and after shipping of prefilled syringes (PFS). The shipping study should demonstrate worst-case conditions regarding distance, duration, background temperature, and vibration.

   Study completion 7/2018, Final report submission 9/2018

Product Quality-Microbiology Postmarketing commitments

1. 3349-15: Implement (b) (4) and complete the process qualification report.

Study completion 10/2018, Final report submission 12/2018

Immunogenicity assays- Postmarketing requirements

1. 3349-6: Revise the anti-PEG IgM anti-drug antibody assay in order to improve the drug tolerance and re-validate the assay.

   Study completion 10/2019, Final report submission 12/2019

2. 3349-7: Revise the anti-PEG IgG anti-drug antibody assay in order to improve the drug tolerance and re-validate the assay.

   Study completion 10/2019, Final report submission 12/2019

3. 3349-8: Re-evaluate anti-PEG IgM and IgG antidrug antibody (ADA) in clinical samples from the phase 3 studies 165-301 and 165-302 using the anti-PEG IgM and IgG ADA assays with improved drug tolerance. Re-assess the impact of anti-PEG IgM and IgG ADA on pharmacokinetics (PK), efficacy, and safety.

   Study completion 6/2021, Final report submission 12/2021

4. 3349-9: Evaluate anti-PEG IgM and IgG ADA in samples from the observational study (according to 3349-1) using the anti-PEG IgM and IgG ADA assays with improved drug tolerance. Assess the impact of anti-PEG IgM and IgG ADA on efficacy and safety. An interim report will be submitted every two years during the conduct of the study.

   Study completion 12/2029, Final report submission 6/2030

5. 3349-10: Evaluate the sensitivity of the anti-pegvaliase IgE ImmunoCAP assay to detect anti-PEG IgE antibodies and to make modifications to the method as needed. Test samples from treated patients with the current or modified assay in the prospective study (according to 3349-1) who experience anaphylaxis episodes in order to more comprehensively examine the underlying mechanism of the anaphylaxis. An interim report will be submitted every two years during the conduct of the study.

   Study completion: 12/2019
   Final report submission: 6/2030

II. Summary of Quality Assessments:

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
(b) (4)			(b) (4)
Pegvaliase (rAvPAL-PEG) DS			
(b) (4)	Efficacy (bioavailability) and safety (immunogenicity).	Manufacturing (b) (4)) process	
Lysine site occupancy	Efficacy (bioavailability) and safety (immunogenicity).	Manufacturing (b) (4) process	
Molecular Size Variants (aggregates)	Efficacy (bioavailability) and safety (immunogenicity).	(b) (4)	
Product -related impurities by RP-HPLC	Efficacy, safety	Manufacturing (b) (4) process	
Free PAL	Efficacy (bioavailability) and safety (immunogenicity).	Manufacturing (b) (4) process	
Free PEG	Efficacy (bioavailability) and safety (immunogenicity).	Manufacturing (b) (4) process	

			(b) (4)
Specific Activity (potency)	Linked to Efficacy	Intrinsic to product	
Kinetic parameters (K_m, k_{cat}) (potency)	Linked to Efficacy	Intrinsic to product	
Molecular Mass	Efficacy (bioavailability) and safety (immunogenicity).	Intrinsic to product	
Trans-cinnamic acid (t-CA) content	Stability	Formulation	

B. Drug Substance pegvaliase 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
(b) (4)			

(b) (4) (b) (4)

Pegvaliase (rAvPAL-PEG) Drug Substance		
Endotoxin	Safety and purity	Endotoxin can be introduced by raw materials and throughout the manufacturing process
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
(b) (4)	Safety	(b) (4)
	(b) (4)	
	Stability	
Protein concentration	Efficacy (dosing)	Manufacturing process/formulation

			(b) (4)
pH	Stability	Formulation	
Osmolality	Stability	Formulation	
Appearance	General	Formulation	

- **Description:**
  Pegvaliase-pqpz is a phenylalanine-metabolizing enzyme that is composed of recombinant phenylalanine ammonia lyase (rAvPAL) conjugated to N-hydroxysuccinimide (NHS)-methosypolyethylene glycol (PEG). rAvPAL is manufactured in *Escherichia coli* bacteria transformed with a plasmid containing the phenylalanine ammonia lyase (PAL) gene derived from *Anabaena variabilis*. rAvPAL is a homotetratmeric protein with a molecular weight of 62kD per monomer. To produce pegvaliase, an average of nine (9) 20kD PEG are covalently bound (or conjugated) to each monomer of rAvPAL. The total molecular weight of pegvaliase-pqpz (rAvPAL-PEG) is approximately 1000kD.

- **Mechanism of Action (MoA):**
  Pegvaliase converts phenylalanine to ammonia and trans-cinnamic acid (t-CA). It substitutes for the deficient phenylalanine hydroxylase (PAH) enzyme activity and reduces blood phenylalanine concentrations in adult patients with phenylketonuria.

- **Potency Assay**:
  The potency assay measures the rate of conversion of phenylalanine, the physiological substrate of rAvPAL, to trans-cinnamic acid (t-CA). The absorbance at 290 nm of Phenylalanine is negligible, but the absorbance of t-CA at 290 nm is significant, allowing for quantitation of t-CA generated during the reaction. Activity results are calculated in units/mL. One unit is defined as the generation of 1 µmol of tCA per minute at 30 °C, pH 7.5.
  In addition, the enzyme kinetic parameters (K_m and k_{cat}) are also measured using Michaelis–Menten method.

- **Reference Materials:**
  (b) (4)

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

(b) (4)

There are no animal derived materials used in the manufacturing process.

- **Manufacturing process summary**:
  The process consists of (b) (4) .

(b) (4)

(b) (4)

(b) (4)

(b) (4)

- **Container closure**:

(b) (4)

Based on extractable studies that were conducted under worst case conditions, sponsor concluded that risk posed by leachables on product quality is low.

- **Dating period and storage conditions**:

(b) (4)

C. Drug Product PALYNZIQ 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
Sterility	Safety risk to patients (infection) Efficacy (degradation or modification of the product by microorganisms or their byproducts)	Contaminant could be introduced throughout DP manufacturing.	(b) (4)
Container closure integrity	Safety Failure in closure integrity may lead to contamination (loss of sterility) of DP or evaporation/leakage (impacting concentration or content)	Might be impacted by storage	

			(b) (4)
Endotoxin	Safety, purity, and potential immunogenic reactions	Contaminants could be introduced throughout DP manufacturing or through a container closure integrity failure.	
Protein concentration	Efficacy	Formulation	
Identity (dot blot)	Safety, Efficacy	Intrinsic to molecule	
Subvisible particles	Immunogenicity, safety	Manufacturing process and container closure system	
Leachables/extractables	Safety	Manufacturing equipment and container closure system	
(b) (4)	Safety	Container closure system	
(b) (4)	Safety	Container closure system	
pH	Safety	Formulation	
Osmolality	Safety	Formulation	
Injectable volume	Efficacy/Dosing	Manufacturing process	
Device testing: Injectability, Rigid needle shield removal force, and Needle safety device override	Safety	Device performance	
Appearance	General	Formulation	

- **Potency and Strength**:
  2.5 mg/0.5 mL expressed as amount of rAvPAL protein, conjugated to 7.25 mg 20kD PEG
  10 mg/0.5 mL expressed as amount of rAvPAL protein, conjugated to 29 mg 20kD PEG
  20 mg/mL expressed as amount of rAvPAL protein, conjugated to 58 mg 20kD PEG

- **Summary of Product Design**:
  Subcutaneous Injection,
  clear to slightly opalescent, colorless to pale yellow, sterile, preservative free solution

- **List of Excipients**:
  Tromethamine, Tromethamine-HCl, Sodium chloride, trans-cinnamic acid (t-CA)

- **Reference Materials**:
  Same reference material used for Drug Substance and Drug Product

- **Manufacturing process summary**:

(b) (4)

- **Container closure**:

Prefilled syringe with needle safety device consisting of the following components:

- 1 mL (b) (4) glass syringe ((b) (4)) with (b) (4) stainless steel needle
- Rigid needle shield (inner core of rubber and outer shell of (b) (4) plastic) manufactured by (b) (4)
- Plunger stopper ((b) (4) rubber with (b) (4) coating) from (b) (4)
- Plunger rod (b) (4) from (b) (4)
- Needle safety device (b) (4)) from (b) (4)

Refer to CDRH memo in Panorama for specific details regarding syringe.

Leachable study, (b) (4) spiking studies were conducted.

- **Dating period and storage conditions**:
  24 months at 2-8°C
  The data included in the BLA support storage for up to 30 days at room temperature once removed from refrigerator, as described in the labeling.

- List of co-package components, if applicable: none

D. Biopharmaceutics Considerations: none

E. Novel Approaches/Precedents: None

F. Any Special Product Quality Labeling Recommendations:

Store in refrigerator in its original carton to protect from light. Do not freeze or shake. If needed, store in original carton at room temperature for up to 30 days. Once stored at room temperature, do not return the product to the refrigerator. The shelf-life expires after storage at room temperature for 30 days or after the expiration date on the product carton, whichever is earlier.

G. Establishment Information:

Overall Recommendation: Approval					
DRUG SUBSTANCE					
Function	Site Information	FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Biomarin 46 Galli Dr. Novato, CA	Storage of MCB/WCB Manufacture, packaging, and storage of rAvPAL intermediate and FBDS QC testing of rAvPAL intermediate and FBDS (in-process, release, and stability)	3004079983		Ten 483 observations VAI	Approval
(b) (4)	(b) (4)			N/A	Approval

(b) (4)					
	(b) (4)			N/A	Approval
DRUG PRODUCT					
Function	Site Information	FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Cook	DP Manufacture, Labeling, Packaging In process testing (endotoxin, bioburden) and Release testing (sterility)	3005949964		N/A	Approval
Biomarin 46 Galli Dr. Novato, CA	DP release and stability testing Design control (device)	3004079983		VAI	Approval

H. **Facilities**:
The comprehensive surveillance and pre-approval inspection of DS manufacturing site was conducted on December 4-14, 2017 by ORA (Ashar Parikh and Jolanna Norton) and OBP (Ying-Xin Fan and Joslyn Brunelle). A 10 item 483 was issued. The firm's response to the 483 observations was deemed adequate and the inspection was classified as VAI.
The inspection of DP manufacturing site was waived; it is acceptable based on inspection and compliance history.
For additional information see the Facilities Review by Division of Inspectional Assessment.

I. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols approved:
1. (b) (4) reuse protocols: PVR-115581 (b) (4) and PVR-115739 (b) (4)
2. qualification of future reference standard protocol
3. reprocessing protocols: PVR-101525 and PVR-102369
4. post-approval stability protocol

ii. Outstanding review issues/residual risk: refer to PMCs
iii. Future inspection points to consider: none

b. Drug Product

i. Protocols approved:
    1. Reprocessing protocol PVP-119437
    2. post-approval stability protocol
ii. Outstanding review issues/residual risk: refer to PMC
iii. Future inspection points to consider: none

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]				
	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			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	
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}				

Joslyn Brunelle

Digitally signed by Joslyn Brunelle
Date: 5/17/2018 02:05:33PM
GUID: 508da6da000265eafdde3cc7507da20b

Joel Welch

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GUID: 508da717000297bcbfce0919f8c09594

Thuy Thanh Nguyen

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GUID: 5256cce70002b21dd4c17f3ef09b075c

From: Frederick C. Mills, PhD., Staff Scientist, DBRR- IV, OBP, OPQ, CDER, FDA

To: Joslyn Brunelle, Ph.D., Team Lead, DBRR-IV, OBP, OPQ

Subject: Immunogenicity assay validation for BLA 761079, Pegvaliase (Pegylated phenylalanine lyase) for treatment of PKU deficiency

Sponsor: Biomarin

Date: March 13, 2018
Revised: April 27, 2018
Revised: April 30, 2018
Revised: May 16, 2018

High Level Summary of Review

An overriding concern for the pegvaliase immunogenicity assays is the potential for interference from high levels of pegvaliase (also known as rAvPAL-PEG or BMN165) product. During the clinical trials, trough BMN165 concentrations could range from 0.075 to 132 μg/ml. Review of the immunogenicity validation reports indicates that the expected BMN165 trough concentrations have the potential to affect most of the assays, especially at low ADA levels.

Administration of BMN165 has been found to be highly immunogenic, with resulting effects on efficacy, as well as formation of immune complexes. Formation of ADA and NAbs have been monitored by the validated assays. Although these assays are affected to varying degrees by on-board BMN165, they nonetheless detect very high incidence levels and titers and provide useful clinical information. The validated Circulating Immune Complex (CIC) assays that are in place are not thought to be affected by on-board BMN165 and have provided important insight into hypersensitivity events, as well as raising potential concerns about long term safety effects that may arise form persistent CICs. Taking these considerations together, there are no issues regarding validation of immunogenicity assays that preclude approval.

Biomarin has agreed to PMRs to improve the drug tolerance of their anti-PEG IgM and IgG assays and re-analyze Phase 3 samples with the improved assays. Biomarin has also agreed to a PMR to evaluate the sensitivity of the anti-pegvaliase IgE ImmunoCAP assay to detect anti-PEG IgE antibodies, and to make modifications to the method as needed. This assay will be used to evaluate anti-PEG IgE antibodies in samples from the long term prospective post-marketing study to be completed in 2030.

PMRs for Improvement of Drug Tolerance anti-PEG IgG and IgM assays

OBP and Clinical Pharmacology have negotiated with Biomarin to reach agreement on PMRs to improve the drug tolerance of the anti-PEG IgG and IgM assays. Below I have reproduced text that describes negotiations leading to the current status of the agreement.

The following text asking the company to provide PMRs was sent to the Biomarin on April 4, 2018 as part of an information request.

AGENCY QUESTION #19
During your clinical studies, you observed high incidence and titers of anti-PEG IgG and anti-PEG IgM ADA. There was an apparent decline in these ADA upon prolonged administration. However, this decline is difficult to interpret, due to the poor drug tolerance of the anti-PEG IgG assay (validation report bmn165-13-049) and anti-PEG IgM assay (validation report bmn165-13-051). Persistence of anti-PEG ADA is likely to contribute to a long-term safety risk due to accumulation of immune complexes. Therefore, in order to adequately assess this risk, improve the drug tolerance of your anti-PEG IgG and anti-PEG IgM assays as a postmarketing commitment. Ideas to improve drug tolerance could include acid dissociation of antibody-product complexes (as discussed in Assay Development and Validation for immunogenicity testing of Therapeutic Protein Product-FDA Draft Guidance for Industry,2016) or other methodologies that may reduce the concentration of on-board product in test samples.

Biomarin responded to the Agency with the following email on April 5, 2018

Good Morning Oumou and Ben,
For the comment# 19 in the information request sent yesterday, pertaining to the assessment of the drug tolerance of our anti-PEG IgG and anti-PEG IgM assays, can the Agency confirm whether they have reviewed the BioMarin report BAS-GR-17-032 titled "Investigation of Pegvaliase Anti-Drug Antibody Titer Assay Drug Tolerance" submitted in the original BLA under M5.3.1.4, to specifically investigate drug tolerance, and in which BioMarin evaluated some of the methods the Agency has recommend as part of this IR?

The data in the BAS-GR-17-032 report suggests that the effects of drug interference on titer assay results were relatively low and that the Anti-Drug Antibody assays investigated are valid, fit for purpose, and produce titer data that is reliable for assessment of the impact of pegvaliase immunogenicity on safety and efficacy. Please let me know if there is any additional clarification we need to provide as part of the IR response.

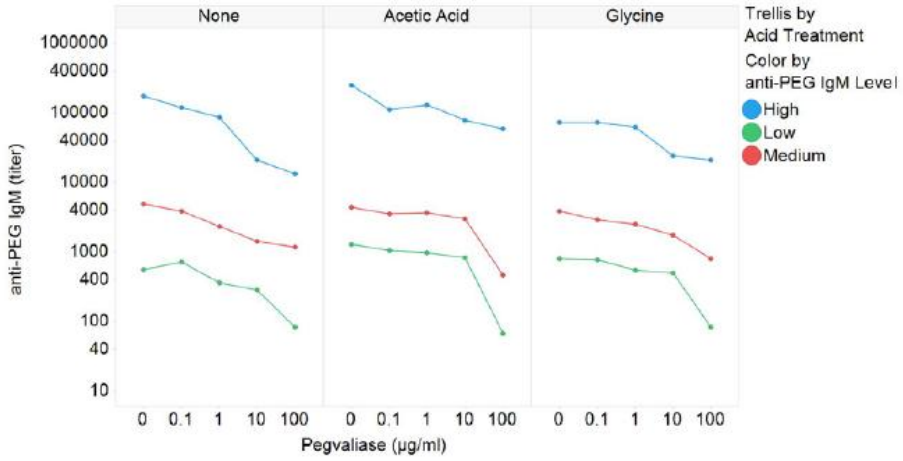
Regards,
Rishabh

Examination of report BAS-GR-17-032 showed that the Sponsor had attempted acid dissociation pre-treatment, as well as pre-treatment with glycine on samples prior to performing the anti-PEG IgG and IgM assays. To assess the impact of free pegvaliase on ADA titer assay results, patient samples with high or low levels of each analyte and < 75 ng/mL pegvaliase (<LLOQ in the

pegvaliase ELISA) from study 165-301 were identified and combined to generate sample pools with varying levels of each analyte and no detectable pegvaliase. These pooled study samples were then aliquoted and pegvalise was added from 0 to 100 µg/mL (approximate maximum trough pegvaliase concentration). Samples were then assayed in ADA titer assays, with increasing concentrations of added pegvaliase.

Shown below are the results from these studies for the anti-PEG IgM assay. For added pegvaliase concentrations < 10 µg/ml, the pretreatments did improve the titers several fold.

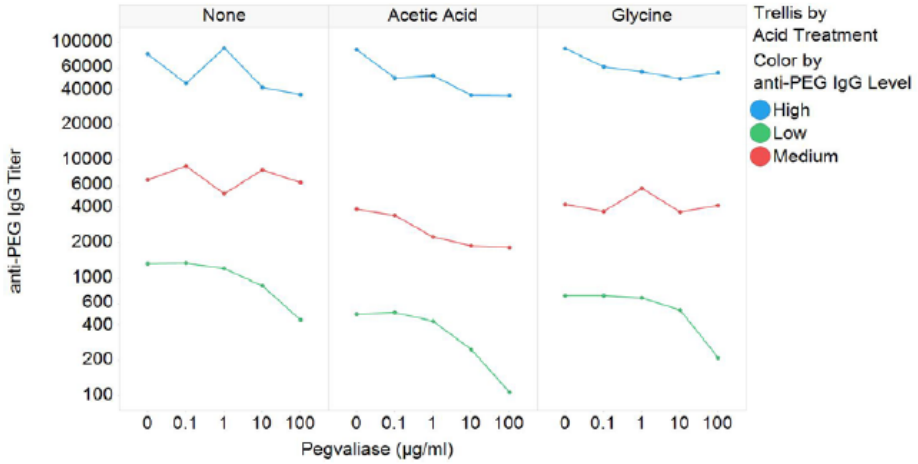
Figure 8.6.1: Drug Tolerance of the anti-PEG IgM Titer Assay



Samples from study 165-301 with high (blue), medium (red) or low (green) titers were assayed in the anti-PEG IgM titer method with or without acid pre-treatment (horizontal trellis), after addition of up to 100 µg/mL pegvaliase. TAb assay titer results (y axis) vs pegvaliase concentration (x axis) are shown.

However, as seen below for the results from pre-treatment studies for the anti-PEG IgG assay, the pre-treatments actually made the results worse.

Figure 8.7.1: Drug Tolerance of the anti-PEG IgG Titer Assay



Samples from study 165-301 with high (blue), medium (red) or low (green) titers were assayed in the anti-PEG IgG titer method with or without acid pre-treatment (horizontal trellis), after addition of up to 100 µg/mL pegvaliase. Anti-PEG IgG assay titer results (y axis) vs pegvaliase concentration (x axis) are shown.

After extensive discussion of these data between myself, Dr. Christine Hon, and her Clinical Pharmacology colleague Dr. Theingi Thway, who has industry experience with immunogenicity assays, a decision was reached that the drug tolerance PMRs were still necessary. The following clarification was provided to Biomarin on April 9, 2018.

In regards to your anti-PEG IgM and IgG anti-drug antibody (ADA) assays and report BAS-GR-17-032 titled "Investigation of Pegvaliase Anti-Drug Antibody Titer Assay Drug Tolerance", the Agency has the following comments:

1, ***For the anti-PEG IgM assay, your data show that acid dissociation improved the assay several-fold in terms of the titer values. Thus, acid dissociation should be incorporated into the assay. Improving the anti-PEG IgM titer detection is important because it allows better characterization of the association between anti-PEG IgM titer and the incidence of adverse events.***

2.***For the anti-PEG IgG assay, acid dissociation reduced the sensitivity of the assay. We recognize that improving the assay may be challenging, but it may be possible if there is capture of on-board product with anti-pegvaliase antibody columns prior to assay. Improving the anti-PEG IgG assay is important because we observed a clear reciprocal relationship between anti-PEG IgG incidence and mean pegvaliase trough concentration during the randomized discontinuation period (i.e., 165-302 Part 2) followed by the reintroduction of pegvaliase in 165-302 Part 3. Our analysis also shows that median anti-PEG IgG titers was low, and anti-PEG IgG antibodies likely disappeared as a result of prolonged treatment and increasing on-board drug, suggesting that the decreasing incidence over time was an artifact of drug interference.***

3. ***Most importantly, anti-PEG antibodies are capable of binding to the large PEG group forming ADA-PEG complexes that can in turn aggregate into immune complexes, which represents an important safety issue that should be re-evaluated using improved anti-PEG assays that have better drug tolerance***

On April 20, 2018, Biomarin communicated agreement with PMR 6, 7, 8, 9. The following represents the final negotiated language for the PMR's related to immunogenicity assays:

PMR 6
Revise the anti-PEG IgM anti-drug antibody assay in order to improve the drug tolerance and re-validate the assay.
Study Completion: 10/2019
Final Report Submission: 12/2019

PMR 7
Revise the anti-PEG IgG anti-drug antibody assay in order to improve the drug tolerance and re-validate the assay.
Study Completion: 10/2019
Final Report Submission: 12/2019

PMR 8
Re-evaluate anti-PEG IgM and IgG antidrug antibody (ADA) in clinical samples from the phase 3 studies 165-301 and 165-302 using the anti-PEG IgM and IgG ADA assays with improved drug tolerance. Re-assess the impact of anti-PEG IgM and IgG ADA on pharmacokinetics (PK), efficacy, and safety.
Study Completion: 06/2021
Final Report Submission: 12/2021

PMR 9
Evaluate anti-PEG IgM and IgG ADA in samples from the observational study (according to PMR 1) using the anti-PEG IgM and IgG ADA assays with improved drug tolerance. Assess the impact of anti-PEG IgM and IgG ADA on efficacy and safety. An interim report will be submitted every two years during the conduct of the study.
Study Completion: 12/2029
Final Report Submission:06/2030

The ImmunoCap IgE assay used in Phase 3 studies only detects IgE Abs to whole pegvaliase, or to the PAL moiety of pegvaliase. There is a high rate of hypersensitivity in pegvaliase-treated patients , including a 9% rate of anaphylaxis, and patient safety requires understanding the immunological mechanisms underlying these hypersensitive reactions. In particular, the review team is concerned that raising anti-PEG IgE in patients treated with pegvaliase would create a hypersensitivity risk resulting from either environmental exposure to PEG or administration of other pegylated products. Conversely, if patients have baseline anti-PEG IgE, this could increase their risk of hypersensitivity when receiving pegvaliase. Thus physicians' awareness of anti-PEG IgE status and the role of anti-PEG antibodies in patients' hypersensitivity reactions is important for ensuring adequate patient-centric clinical management, including treatments to moderate hypersensitivity for confirmed anti-PEG IgE patients

Therefore, on May 4, 2018 the FDA provided Biomarin with the following request via email: "As your ImmunoCap IgE assay only detects IgE antibodies to whole pegvaliase or to the PAL moiety of pegvaliase, you should modify your existing assay to be able to detect anti-PEG IgE antibodies, or develop a new assay for this purpose. With this modified or new IgE assay, you should test samples from treated patients in the PMR1 prospective study who experience anaphylaxis episodes in order to more comprehensively examine the underlying mechanism of the anaphylaxis. We encourage you to discuss ideas for modifying your assay or developing a new assay with the Agency."

On May 10, 2018 Biomarin agreed to PMR 10 for an anti-PEG IgE assay, and the following represents the final negotiated wording and dates.

PMR 10

Evaluate the sensitivity of the anti-pegvaliase IgE ImmunoCAP assay to detect anti-PEG IgE antibodies, and to make modifications to the method as needed. Test samples from treated patients with the current or modified assay in the prospective study (according to PMR 1) who experience anaphylaxis episodes in order to more comprehensively examine the underlying mechanism of the anaphylaxis. An interim report will be submitted every two years during the conduct of the study

Study Completion: **10/2019**
Final Report Submission: **06/2030**

Table of Contents

Executive Summary

This BLA contains qualification reports for 6 immunogenicity assays that were used only in Phase 1 and 2 studies. Because the results from the Phase 1 and 2 studies were not used as part of the decision-making process for approval of this application, these assays were not reviewed.

Thirteen assays were used for immunogenicity assessments in Phase 3 studies, and important aspects of these assays are briefly summarized below, with detailed discussions following in this review in the section entitled (Immunogenicity Assays, Phase 3 Assays, refer to page 20).

Important terms to note are:

ECLA-ElectroChemiLuminescence Assays, or simply , ECL

This technology, used in assays 1-5, and 8 below, has become standard in the industry. The method in brief uses immobilization of antigen (product) in plates whose wells can conduct an electric current. Serum or controls are incubated in the wells, allowing binding of specific antibody (ADA) to the product. In the next step is incubation with a detection antibody, which is typically an antibody specific for human IgG or IgM conjugated with a "sulfotag" that can mediate electron transfer. This detection reagent binds to the product-antibody complexes in the wells. The final step is incubation of a ruthenium-containing luminescent reagent with the complexes in the wells, followed by passage of electric current through the wells. The electric current leads to electron transfer through the ruthenium in the luminescent reagent, resulting in release of light from the product-ADA-detection antibody-luminescent reagent complexes. The released light can be detected with high sensitivity by a photomultiplier. The ECL methodology typically shows low background and high sensitivity relative to ELISA assays.

LPC-Low Positive Control
HPC-High Positive Control
LQC-Low Quality Control
HQC-High Quality Control

Reviewer comments

An overall consideration affecting interpretation of results from the immunogencity assays is potential for interference from high levels of BMN165 product, even at the pre-dose trough, when samples are taken for immunogenicity measurements. As per communication from Dr. Christine Hon, Clinical Phamacology reviewer for this BLA, the trough BMN165 concentrations can range from 75 to 132000 ng/ml or 0.075 to 132 μg/ml. As can be seen from the summaries below, BMN165 concentrations in the high trough ranges have the potential to affect most of the assays, especially at low ADA levels corresponding to the LPCs.

1. ECLA to Detect anti-PAL IgG Ab in Human Serum

The sensitivity of this assay is 122 ng/ml, well within FDA guidance. The assay has a Drug Tolerance of 100 ng /ml BMN165 for the LPC, and 2500 ng/ml for the HPC, meaning that at 100 ng/ ml BMN165 for the LPC and 2500 ng/ ml BMN165 for the HPC, there is still a signal within acceptance criteria. These levels of Drug Tolerance mean that this assay might show interference across the whole range of BMN165 trough concentrations.

2. ECLA to Detect anti-PAL IgM Ab in Human Serum

The sensitivity of this assay was found to be 4 µg/ml on the basis of the minimum mass of the positive control that would give a signal above the cut point. However, the Sponsor used Surface Plasmon Resonance (SPR) to determine that the positive control antibody is only ~ 1% BMN165 reactive species, and on the basis calculates an acceptable sensitivity of 42 ng/ ml. For Drug Tolerance HQC and LQC samples tolerated up to 100 µg/mL BMN165, so the assay would be affected in the high ranges of BMN165 trough concentrations.

3. ECLA to Detect anti-PEG IgG Ab in Human Serum

The sensitivity of this assay is acceptable at 205 ng/ml. However, the Drug Tolerance is only 1 ng/ml for the LPC, meaning the assay may be inhibited across the whole expected range of BMN165 trough concentrations.

4. ECLA to Detect anti-PEG IgM Ab in Human Serum

As was also the case for the anti-PAL IgG assay, the sensitivity of this assay was low, 1079 ng/ml based on the minimum mass of total positive control antibody that would give a signal above the cutpoint. SPR analysis then showed that the PC contained an ~ 1/8 fraction of BMN165 reactive species, so the Sponsor on this basis calculated a sensitivity of 167 ng/ ml on basis of specific Ab, as measured by (Surface Plasmon Resonance) SPR. This assay shows a poor Drug Tolerance of 10 ng/ml for the LPC, so substantial interference across the entire range of BMN165 trough concentrations is expected.

5. ECLA to Detect anti-BMN165 TAbs in Human Serum

This assay is intended to assess ADA that react to any part of the BMN165 molecule, and thus the designation TAb, for Total Antibody. The Sponsor performed SPR analysis on the control antibody for this assay, and on the basis of the fraction of specific reactivates contained in the control, calculated sensitivities of 147 ng/ ml for anti-PEG reactivity and 1450 ng/ml for anti-PAL reactivity. Thus this assay is expected to primarily detect anti-PEG antibodies with acceptable sensitivity, while showing a poor sensitivity for the enzyme (phenylalanine ammonium lyase -PAL) moiety, indicating that the assay is informative primarily for detecting anti-PEG antibodies.

Drug tolerance was determined to be 100 ng/ml for the LPC and 100 µg/ml for HPC, so this assay may show drug interference across the entire range of expected BMN 165 trough concentrations.

6. Detection of anti-BMN165 NAbs in Human Serum

The validated sensitivity of the NAb assay is low calculated as 1785 ng/ml based on a cutpoint factor using plates with duplicate normal serum samples (Mean Signal Cutpoint) , or 1490 ng/ml based on a cutpoint factor using plates with singlicate normal serums (Individual Response cutpoint). The Individual Response cutpoint corresponding to 1490 ng/ ml sensitivity was used in analysis of clinical samples. As discussed in FDA 2016 Guidance for Immunogenicity Assay Methods, sensitivity as determined on basis of a NAb positive control is recognized as

potentially troublesome because it is often hard to obtain a high affinity NAb antibody control. The Drug Tolerance is 25 µg/ml (for LPC) and 100 µg/ml (for HPC). Overall, the Sponsor has performed a thorough Nab validation that includes screening, confirmatory, and titer Nab assays I find this assay is suitable for its intended purpose in the sense that Nabs were detected for most patients.

7. Immunodepletion of IgG and IgM Abs in Serum

This is a supporting method to deplete IgG and IgM antibodies that potentially interfere with antigen-specific IgE detection in human serum samples, while retaining IgE antibodies. This method was validated to support analysis of drug-specific IgE concentrations in human serum samples.

Reviewer comment

This method is highly efficient at depleting IgG in serum samples (mean 92%) which is important, since IgG is the most abundant antibody in serum, with expected concentration ranges of 7-16 mg/ ml in adults (http://emedicine.medscape.com/article/2157901-overview). The method is only (37.3 %) efficient for removing IgM, but adult serum concentrations are only in the range 0.4-2.3 mg/ml, so the less efficient depletion of IgM is expected to bring the concentration of remaining IgM into the same range as the post-depletion concentration of IgG. Given these considerations, the depletion method is likely to improve detection of IgE by substantially reducing potentially interfering antibodies. Moreover, in precision validation runs, recovery of ~ 300 lU/L IgE (720 ng/ml), was very high, with a mean of 99.1% for 99% CV value of 83.2%).

8. Anti-BMN165 IgG4 Assay

The IgG4 assay precision is acceptable, by and large showing relatively low %CVs, well within industry experience for ADA assays, and consistent with the 2016 FDA guidance for immunogenicity methods. The sensitivity of the assay is high at 18.3 ng/ml. The highest neat concentration of BMN 165 at which the LQC (Low Quality Control) sample screened positive was 1 µg/ml. All LQC-C samples spiked with BMN 165 screened positive. The highest concentration of BMN 165 at which the HQC-C sample screened positive was 500 µg/mL. Thus, at low levels of patient serum anti-BMN IgG4, signals will be reduced or lost due to drug interference.

9. RAST to Detect anti-BMN165 and anti-PAL IgE in Human Serum

The RAST method uses standard, older methodology whereby antigen (allergen) is immobilized on paper discs, which are incubated with serum or controls, and IgE bound to the allergen is detected using a chemical detection reagent. The sensitivity of this method is 0.34 kU/L IgE, or 0.85 ng/ml IgE on the basis of rye grass calibrator, since there was no anti-BMN165 IgE control used. This sensitivity is well within the expected range of specific IgE concentrations (ng range). Drug tolerance was not evaluated in this method validation. In Phase 2 trial, 40 subjects were tested with the RAST assay, and two IgE positive samples were detected.

10. ImmunoCAP to Detect anti-BMN165 IgE in human serum

For the ImmunoCAP, which has been developed more recently than the RAST assay, antigen (allergen) is immobilized on a solid phase matrix in a capsule. After binding of patient IgE or controls to the matrix, an anti-human IgE binds to the antigen-IgE complexes, and then detected with a fluorescent reagent.

Lacking a true anti-BMN165 IgE positive control, the method was calibrated with a hybrid rabbit anti-PAL IgG conjugated to human IgE, yielding a sensitivity of 0. 1 KU/L=0.35 ng/ml specific IgE. For Drug Tolerance evaluation, the HQC and LQC remained positive in the presence of up to 250 µg/mL BMN165. However, there was reduction of signal by greater than 40% in the presence of ≥25µg/mL of BMN165. Because BMN165 serum trough concentrations can be as high as 132 µg/ml, IgE results should thus be interpreted in the context of the measured BMN165 concentration at the time of antibody sampling. For 26 patients from clinical trials who experienced anaphylaxis, samples from 25 patients were tested with the ImmunoCAP assay. One of these patients screened positive in the assay, but there was insufficient sample remaining to confirm the result

11. ImmunoCAP to Detect anti-PAL IgE Ab in Human Serum

This assay is essentially the same as the ImmunoCAP anti-BMN165 IgE assay, except that it is validated to detect the PAL moiety of BMN165. The assay has a high sensitivity of 0.1 kU/L, corresponding to 0.24 ng/ ml IgE. As with the ImmunoCAP anti-PMN165 IgE assay, high BMN165 trough concentrations may also largely inhibit the IgE signal for the ImmunoCAP anti-PAL BMN165 IgE assay. .

The following two assays are designed to detect Circulating Immune Complexes (CIC) that likely result from the high dosing concentrations of BMN165 complexed with the high titer antibodies raised in most patients. These CICs can then react with complement components to initiate the complement cascade. In the MicroVue assay, which is a commercial kit, Complement components C1q or C3d are immobilized in plate wells, and serum samples or controls are incubated in the wells, allowing CICs to bind to these components. Following this step, IgG-containing CICs are reacted with anti-human IgG, followed by a detection reagent to allow quantitation of the IgG-CICs. The IgM CIC assay is modified from the MicroVue assay, so that an anti-IgM is used to detect IgM-containing CICs. In addition, only C3d coated plates were used for the IgM-CIC assay. These assays are not susceptible to BMN165 drug interference, since they do not rely on a BMN165 specific reactivity, so Drug Tolerance was not assessed.

12. MicroVue assay to Detect IgG C1q-CIC and C3d-CIC

This was performed by the (b) (4), using kits from MicroVue. The assay was run with a high, medium, and low control, and showed precision within industry practice. The C1q-CIC Normal Range was determined to be < 7.2 µg Eq/ml IgG, while the C3d-CIC Normal Range was ≤ 36 µg Eq/mL IgG. Samples above these ranges were scored as significant CICs.

13. Modified Quidel ELISA to detect IgM-C3d Immune Complexes

The reference range for normal IgM CIC was determined to be 0-7.6 µg Eq/mL IgM. The accuracy of the method was shown to be adequate from 1-60 µg Eq/mL IgM, which corresponds to the expected range of IgM IC. The assay is adequately linear in the linearity phase of the curve and the sponsor has an appropriate sample dilution protocol in place to ensure that the signals from patient samples fall within this validated linear response. The sponsor has an appropriate sample dilution protocol in place to ensure that the signals from patient samples fall within this validated response range.

Clinical trial and clinical immunogenicity overview

BioMarin has developed pegvaliase, a genetically modified phenylalanine ammonia lyase (PAL) enzyme (the PAL sequence is derived from the cyanobacterium *Anabaena variabilis*). Biomarin states that (b) (4) (b) (4). Pegvalise is a novel treatment for phenylketonuria (PKU), a rare autosomal recessive genetic disorder characterized by an inability of the body to break down phenylalanine, and is caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). PAL is used as an alternative to PAH enzyme replacement therapy. This therapy directly addresses the pathophysiology of PKU by substituting for the deficient PAH enzyme and effectively reducing the blood Phe level.

Reviewer comment:

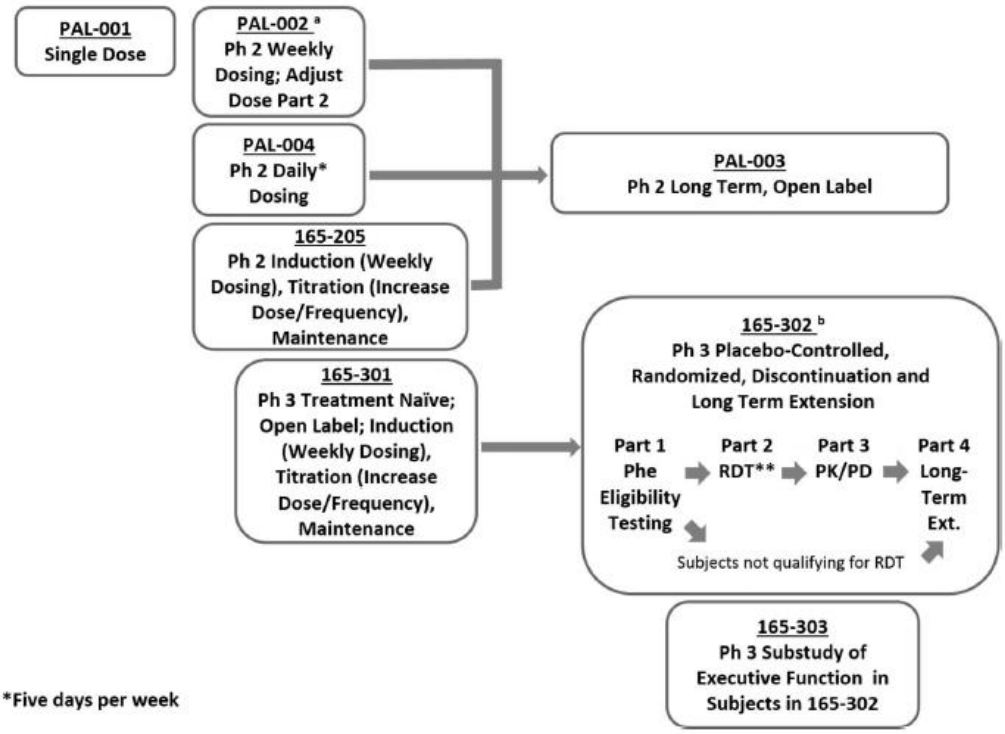
During pre-BLA review and discussions, I observed via a BLAST search that there is some homology between PAL and mammalian histidine lyase (HAL). This was discussed with the sponsor, and they were asked to assess cross-reactivity, or provide adequate justification for not doing so. Their justification is on page 13 of Summary of BioPharmaceutical Studies \\cdsesub1\evsprod\BLA761079\0001\m2\27-clin-sum\summary-biopharm.pdf and excerpted below.

The rAvPAL enzyme shares approximately 33% amino acid identity with endogenous human histidine ammonia lyase (HAL) over approximately 80% of the protein sequence, raising a potential concern that cross-reactive anti-HAL antibodies could develop in subjects treated with pegvaliase. However, binding of potential cross-reactive anti-HAL antibodies to HAL *in vivo* would be improbable due to the exclusively cytosolic (intracellular) location of HAL *in vivo*. We therefore reasoned that while development of cross-reactive anti-HAL antibodies as a result of pegvaliase treatment is theoretically possible, such antibodies in the blood would be unlikely to have any negative effects because they would not be able to bind HAL or other exclusively intracellular proteins. Based on this rationale, anti-HAL antibody assays have not been developed.

Reviewer comment

This rationale appears reasonable because potential antibodies that were cross-reactive with HAL would need to taken up intra-cellularly to see the HAL target, and would likely (1) be degraded by intracellular enzymes and/or (2) be dissociated from the HAL target due to the low pH in the endosomal/lysosomal compartments.

The clinical trials to support BLA 761079 are shown schematically in the following figure



Ext., extension; OLE, open-label extension (Part 4 of 165-302); Ph, Phase; Phe, Phenylalanine; PD, pharmacodynamics; PK, pharmacokinetics; RDT, randomized discontinuation trial (Part 2 of 165-302).

[a] Eleven subjects entered PAL-002 from PAL-001.

[b] Twelve subjects entered 165-302 from a Phase 2 study, PAL-003 or 165-205.

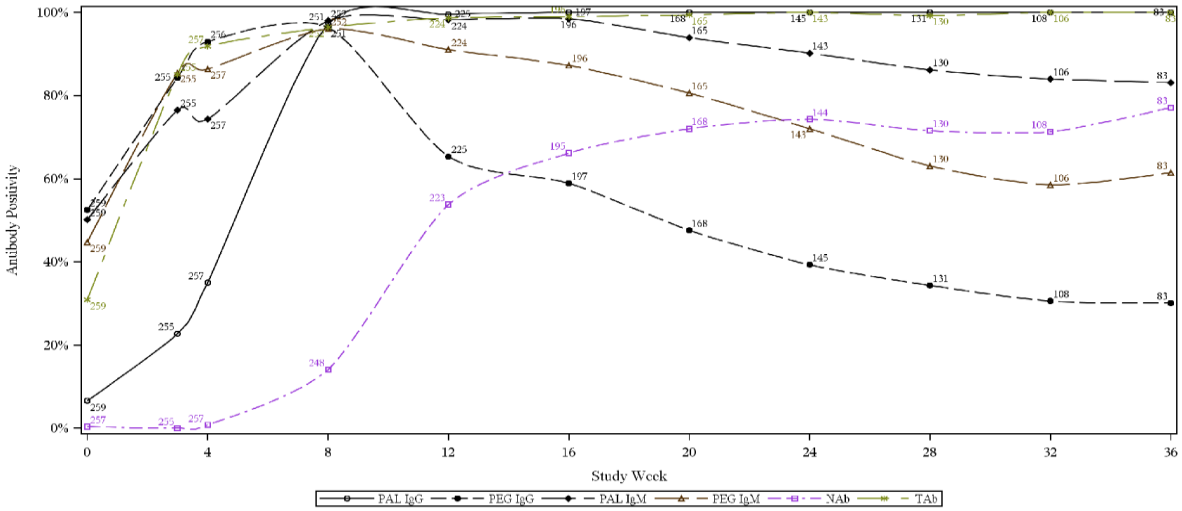
Source: ISE Table 2.7.3.1.1.

The decisions for approvability of BLA 761079 have been based on the data from the Phase 3 trials, and only the immunogenicity assays used in Phase 3 are described in this review.
The following immunogenicity tests were performed on samples from the Phase 3 trials 165-301 and 165-302:

1. Assessment of positivity and titers for total anti-drug antibodies (TAb)
2. IgM and IgG antibody isotypes anti-PAL and anti-PEG
3. NAb assays for positivity and titers
4. pegvaliase IgG4
5. PAL IgE and pegvaliase IgE were tested in Phase 3 studies in the event of a hypersensitivity reaction visit using the same validated RAST assays from Phase 2
6. pegvaliase IgE was assessed in all Phase 3 subjects that experienced anaphylaxis based on any of the 3 NIAID/FAAN criteria using a separate ImmunoCAP IgE assay after depletion of IgM and IgG antibodies.
7. Circulating Immune Complexes (CIC) containing IgG, and binding to complement components C1q or C3d, as well as CICs containing IgM and binding to C3d.

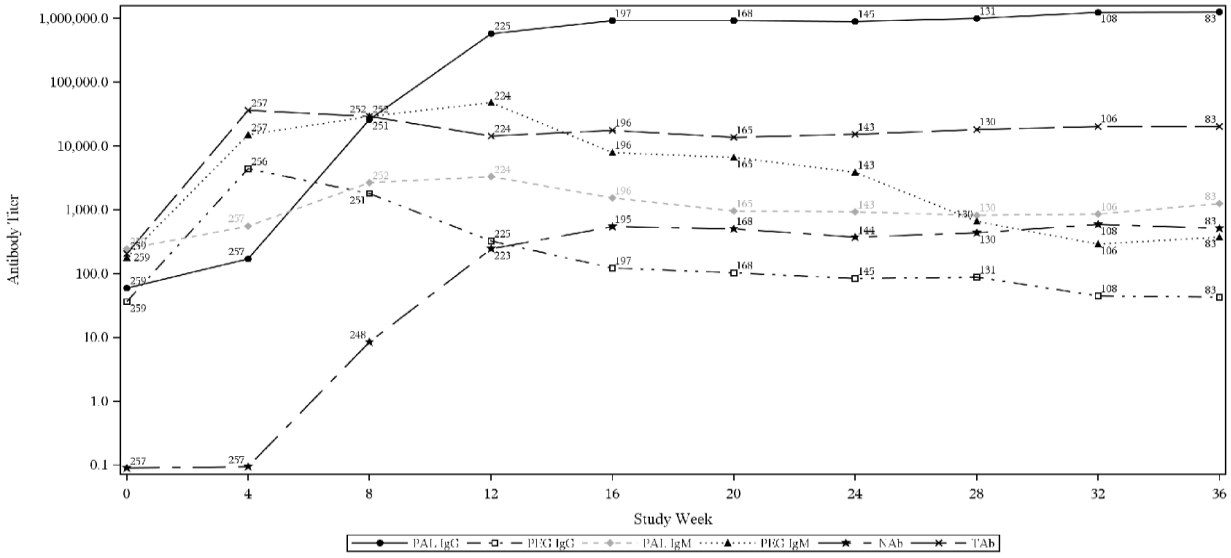
Relevant to the CIC assays, levels of complement components C3 and C4 were also measured, as depletion of these components is indicative of CIC formation. These assays were reviewed by Clinical Pharmacology.

Shown in the following figure (Figure 12.2.1.1.1, 165-301 CSR) is the incidence of antibody positivity for the Phase 3 population.

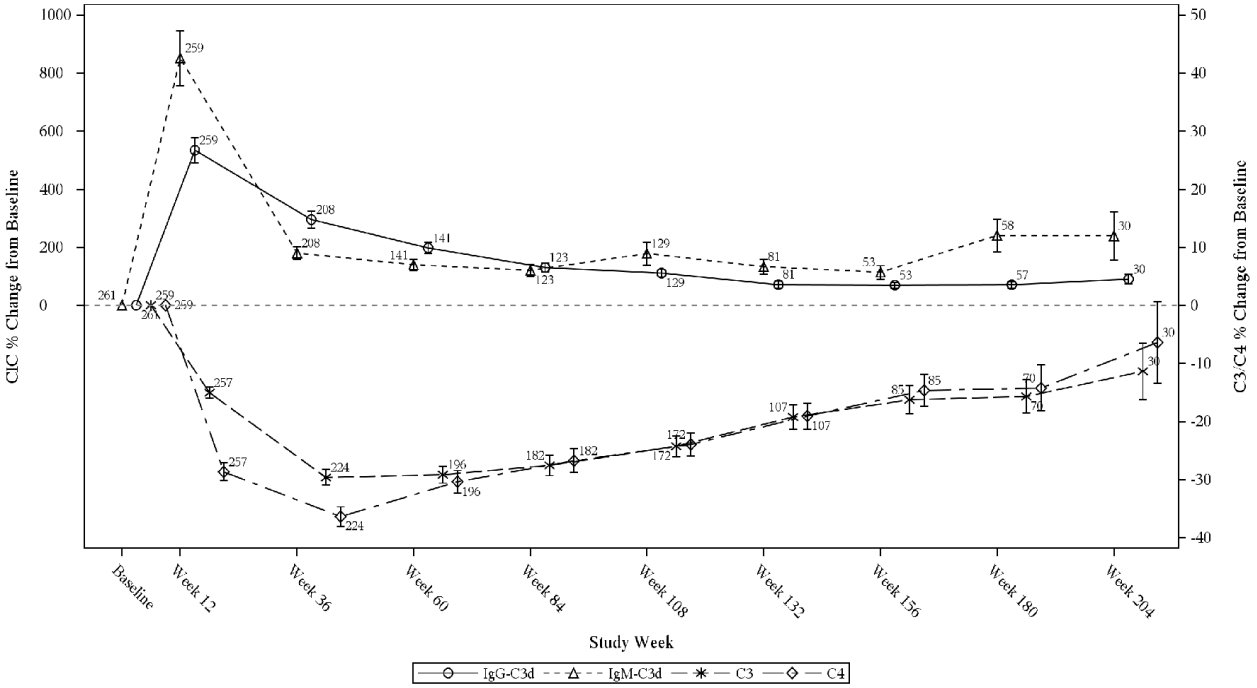


Notably, essentially all patients develop both anti-PEG and anti-PAL IgG and IgM, as would be expected for what is a foreign protein given at high doses (20 mg/kg or 40 mg/kg) daily. Neutralizing antibodies also develop in almost 80% of the patients. The incidence of ADA (except for NAbs) appear to decline with time, although this observation may be at least partially explained by the moderate or poor drug tolerance of the ADA assays, so that high concentration of BMN165 that are reached upon prolonged treatment could interfere with the ADA assays, leading to an artifactual diminished incidence.

ADA titers also become very high during treatment as seen below (Figure 14.3.6.3.2, 165-301 CSR)



The high titers, together with the daily high dosing, created a concern about the potential for immune complex formation, which in fact does occur, as seen in the following figure, provided by Dr. Christine Hon, Clinical Pharmacology reviewer



CICs increase early in treatment and then decline. This decline is not an artifact of high serum BMN165 concentrations, since the CIC assays are not obviously susceptible to drug interference. As expected, there is a reciprocal decrease in complement components C3 and C4, which slowly rebalances in the direction of baseline levels. Notably, however, the CICs and C3/C4 levels never fully return to baseline, indicating long term persistence of immune complexes, with potential safety implications.

There were appreciable levels of hypersensitive reactions, especially early in treatment. In Phase 2 trials, 68 of 285 patients were tested for anti-PAL IgE antibodies, which recognize the recombinant PAL protein, and anti-pegvaliase IgE antibodies during routine study visits (not at times of anaphylaxis episodes) or during hypersensitivity visits. Of those 68 patients, 5 (7%) tested positive at least once for anti-PAL IgE antibodies but none of these patients tested positive for anti-pegvaliase IgE antibodies. For Phase 3 trials 26 patients from clinical trials who experienced anaphylaxis, samples from 25 patients were tested with the ImmunoCAP assay. One of these patients screened positive in the assay, but there was insufficient sample remaining to confirm the result.

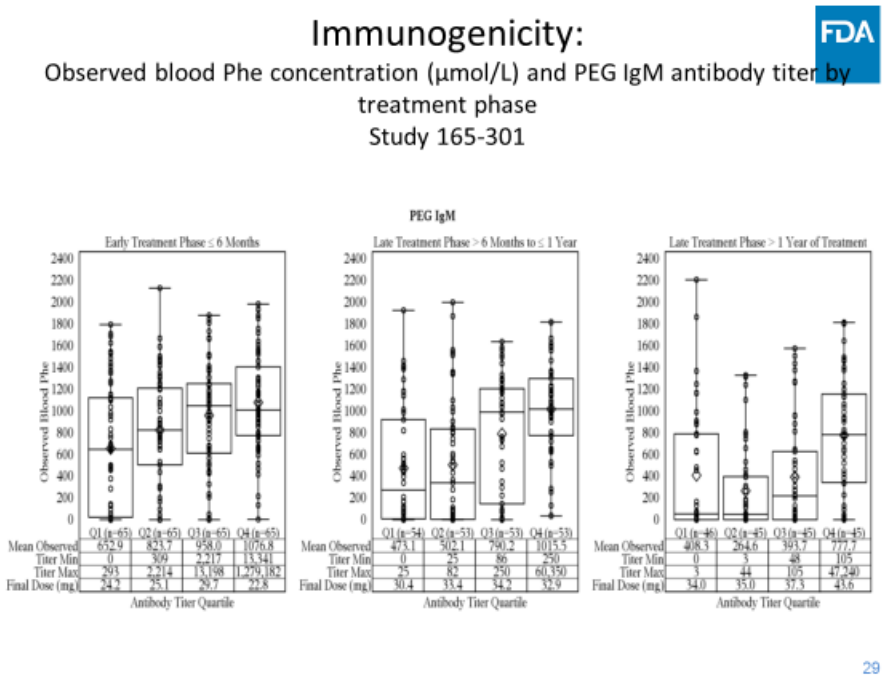
Considering these findings, the Sponsor states

"The observed increases in CIC levels in conjunction with C3/C4 complement reduction and paucity of IgE positivity, suggests that the mechanism of hypersensitivity reactions in the pegvaliase clinical studies was Type III IC-mediated hypersensitivity"

Reviewer comment: I find this explanation to be reasonable, given the a priori concerns regarding high titers and high dosing levels, together with the documented formations of CICs.

However, the Package Insert , as finalized, also calls attention to the incidence of IgE, as noted above. This is appropriate because Type I IgE mediated hypersensitivity has a different clinical management than Type III complement mediated hypersensitivity.

There was an impact of ADA formation on efficacy; i.e. there were increased Phe levels arising from diminished BMN165 activity (or clearance) that correlated with higher ADA.
Shown below is a figure provided by Dr. Christine Hon, showing correlation between PEG IgM antibody titers and Phe. Similar results were seen for other ADA.



<u>Reviewer comment</u>: Overall, administration of BMN165 has been found to be highly immunogenic, with resulting effects on efficacy, as well as formation of immune complexes. Formation of antibodies (ADA and Nabs) have been monitored by a range of validated assays. Although these assays are affected to varying degrees by on-board BMN165, they nonetheless detect very high incidence levels and titers, and thus provide useful information. The CIC assays that are in place are not thought to be affected by on-board BMN165, and also have provided important insight into hypersensitivity events, as well as raising potential concerns about long term safety effects that may arise from persistent CICs.

Review of Immunogenicity assays

Phase 1,2 Assays

Results obtained using the following 6 assays for samples obtained from Phase 1, 2 trials were not used in the decision on the approvability of the BLA, and therefore the validation of these assays was not reviewed.

Application 761079 - Sequence 0001 - ELISA to Detect PEG IgG Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bard-qr-09-001\bard-qr-09-001.pdf

Application 761079 - Sequence 0001 - ELISA to Detect PEG IgM Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bas-qr-09-002\bas-qr-09-002.pdf

Application 761079 - Sequence 0001 - Detection of anti-BMN165 NAb in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bas-vr-06-002\bas-vr-06-002.pdf

Application 761079 - Sequence 0001 - Detection of anti-BMN165 IgG Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bas-vr-07-001\bas-vr-07-001.pdf

Application 761079 - Sequence 0001 - Detection of anti-BMN165 IgM Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bas-vr-06-003\bas-vr-06-003.pdf

Application 761079 - Sequence 0001 - Assay to Detect anti-rAvPAL IgM Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bas-vr-10-010\bas-vr-10-010.pdf

Review of Immunogenicity Assays used for Phase 3 Studies

ECLA to Detect anti-PAL IgG Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bmn165-13-048\bmn165-13-048.pdf
Phase 3
Sensitivity 122 ng/mL
Validated by
(b) (4)

Precision / PC Acceptance Criteria
In each of thirty-four (34) analysis runs (performed over seven days by two analysts), four replicates of positive control samples at two concentrations (250 ng/mL and 2500 ng/mL in 100% serum) were analyzed. Two pooled negative controls were also analyzed in replicates of four (PNC) and eight (CC-PNC), where CC-PNC=Confirmatory Cutpoint Plate Negative Control
Normalized signal = mean response of HPC or LPC / mean response of the CC-PNC

Inter- assay precision CVs
A 30% CV was stipulated in the validation plan
HPC 32.1% CV
Sponsor rationale- All samples with high screening signals (that show % signal inhibition greater than or equal to the confirmatory cut point) will be diluted to produce a titer value, which will be reported.
Reviewer comment: Because all high signal samples will be subjected to further analysis, the rationale is adequate, if the subsequent titer evaluation is adequate.
LPC 26.9% CV
Sponsor rationale-CV meet validation specification
Reviewer comment: the LPC CV met the validation requirement, thus this aspect of validation is adequate.
CC-PNC 35.0%CV
Sponsor rationale- The imprecision of the RLU signal that results from negative samples has no impact on the integrity of the reported results, as all negative samples with both replicate assay signals below the screening cut point will be reported 'negative'.
Reviewer comment
Essentially all patients are detected as positive for anti-PAL IgG, so I agree with the Sponsor's view that imprecision of the negative control does not impact the reported results.

Intra- assay precision CVs
A 25% CV was stipulated in the validation plan
HPC Range 1.46-56.6%, with five out of 34 tabulated runs having % CV > 25%; i.e. % CVs of 29.1, 49.9, 49.6, 25.6, and 56.6.
Reviewer comments

The HPC results were generally within the specified %CV, but the high values in 5 runs suggest occasional uncontrolled assay performance. Again, as per the sponsor's rationale of HPC inter-assay variability, if high signal samples will be subjected to further titer analysis, the intra-assay NPC variability may not be a major concern, assuming the subsequent titer evaluation performed on all high signal samples is adequate.

LPC % CV range was 3.51-55.5%, with one out of 34 run showing a high %CV value; i.e. 55.5%
Reviewer comment
The LPC % CV values indicate satisfactory control of the assay in this range, with a single outlier in 33 runs.

The acceptance criterion for CC-PNC and PNC variability was 25% CV. The mean intra-assay precision of the raw signal for the CC-PNC was 21.0%. The mean intra-assay precision of the raw signal for the PNC was 19.5%. These mean values met the acceptance criterion.
However, the ranges of % CVs were:
CC-PNC %CV values of 11.1-34.8%, with 11 out of 34 runs having high variability; i.e. 33.8, 26.2, 26.6, 30.6, 27.5, 27, 26.2, 33.1, 34.8, 31.3, 26.9, and 46.1%
PNC %CV values of 5.52-39.3%, with 9 out of 34 runs having high variability; i.e. 35.4, 52.2, 26.7, 26.7, 25.1, 26.3, 29.2, 32.3, and 39.3%.
Reviewer comments
The variability of the CC-PNC and PNC is consistent with the high inter-assay variability seen for these parameters, and as stated above for inter-assay variability, could be troublesome as it would impact the initial screening classification of negative vs. positive samples.

The mean normalized signals of the positive controls and the raw signals for the CC-PNC and PNC were used to determine the acceptance criteria for all runs. The lower limits for the HPC and LPC samples were calculated to be 4.66 and 1.25, respectively, using the following formula:
Lower limit = mean normalized signal $-(t_{0.01,df} \times SD)$
where $t_{0.01,df}$ is determined from the t-distribution corresponding to a 1% failure rate and SD is the standard deviation.
The upper limits for the CC-PNC and PNC were calculated to be 632 and 665, respectively, based on the following formula:
Upper limit = mean signal + (t0.01,df x SD)
Reviewer comments
The limits for the normalized lower limits for HPC and LPC are low compared to the observed ranges of values in the 34 tabulated runs, while the upper limits for CC-PNC and PNC are high relative to the actual observed values in the runs. These generous limits did not affect the assay performance in the validation.

Screening Cut Point
One hundred (100) individual lots of normal human serum were evaluated for screening cut point analysis. The lots were analyzed in replicates of two on three separate days by two analysts for a total of three assays. The screening cut point factor was determined to be 2.43. This constant will be applied during sample analysis to

calculate the cut point on a run-by-run basis using the following formula:
Cut point = X * CC-PNCPlate
The sponsor notes that in cases where the cut point factor established during validation does not appear to be representative of study samples, the screening cut point may need to be re-established using pre-dose samples and/or control matrix from the study.

Reviewer comments
The normal distribution and outlier exclusion analysis for the SCP is Appendix 2, as a report from Kevin Larimore, statistician, pp. 98-113, and provides appropriate assessment.
In the 1/15/2017 Information Request, the sponsor was asked to clarify if they have confirmed the SCP with pre-treatment samples.
Agency Question 1 (11/15/2017)
Regarding anti-PAL IgG assay validation (bmn165-13-048):
a. Clarify whether you have confirmed the Screening Cut Point (SCP) for this assay with pre-treatment patient samples.
BIOMARIN RESPONSE 1 (12/15/2017)
In assay method validation BMN165-13-048, the multiplicative screening cut point factor (SCPF) relative to the pooled negative control (PNC) on each plate was determined to be 2.43 X PNC, using a panel of normal serum samples from healthy donors. Applying the same cut point analysis to pre-treatment baseline samples from 261 subjects with PKU in study 165-301 resulted in a very similar SCPF of 2.49 X PNC.
Reviewer comment
The Sponsor has shown that the pre-treatment sample SCPF is comparable to the normal sample SCPF.

Confirmatory Cut Point
One hundred (100) individual lots of normal human serum were evaluated for confirmatory cut point analysis. All the individual lots were analyzed with and without a spike of 204 µg/mL rAvPAL. All lots were analyzed in replicates of two on three separate days by two analysts for a total of three assays. The confirmatory cut point data was evaluated by a BioMarin scientist. The confirmatory cut point was determined to be 56.6%.

Immunocompetition sample results will be determined using the following formula:
% Signal inhibition = [1 – (Mean signal spiked with drug / Mean signal spiked with Diluent Buffer)] × 100
Reviewer comments
The confirmatory cutpoint is adequate, as it does not require excessive inhibition.

Free Drug Interference
Free drug interference was evaluated by measuring the impact of 0.00, 0.100, 1.00, 10.0, 100, and 1000 µg/mL BMN-165 on the detection of anti-rAvPAL IgG positive controls (250 ng/mL and 2500 ng/mL in 100% serum). The concentration of free drug that would reduce a positive signal to a negative (produces a signal below the cut point) can be determined by calculating a cut point for Plate 1.
The cut point value for Plate 1 was calculated to be 996 [CC-PNCRUN (410)

* cut point factor X (2.43)].
At 250 ng/mL of anti-rAvPAL IgG antibodies, addition of 0.100 µg/mL of free drug resulted in a negative signal, indicating interference. At 2500 ng/mL of anti-rAvPAL IgG antibodies, none of the concentrations of free drug tested resulted in a negative signal (validation Table 4).
Reviewer comments
I have examined the data tabulated in Table 4, and find the sponsor's statements regarding drug interference to be accurate.
For the HPC (2500 ng/ ml anti-PAL antibody) at the highest BMN165 concentration of 1000 µg/ ml, the assay signal was 8.5% of the uninhibited signal, but approximately 4 x greater than the negative control signal. Therefore, by this criterion, the drug tolerance at high antibody levels is 1000 µg/ ml.
For the LPC (250 ng/ ml anti-PAL antibody) at a BMN165 concentration of 100 µg/ ml, the assay signal was 40% of the uninhibited signal, and approximately 2 x greater than the negative control signal. Therefore, by this criterion, the drug tolerance at low antibody levels is 100 µg/ ml. The implications of these drug tolerance values for accuracy of patient antibody assessment were discussed with the Clinical Pharmacology reviewer. The clinical immunogenicity data for anit-PAL does not seem to be significantly impacted in the presence of steady-state pegvaliase levels, so the inhibition of the LPC by pegvaliase does not appear to translate into a concern for interpreting patient data.

Sensitivity
For sensitivity evaluation, the concentration of antibody that produced an assay response equal to the plate specific cut point was calculated for each curve using a 4-parameter logistic curve fit interpolation with 1/y2 weighting. The sensitivity results were evaluated using the screening cut point factor of 2.43. The mean assay sensitivity was determined to be 122 ng/mL (validation Table 5).
Reviewer comment
The validated assay sensitivity is adequate, consistent the FDA's 2016 Guidance for Development of Immunogenicity Assay Testing.

An acceptance range for the titer quality control was also determined using the log3 (interpolated titer) data from the sensitivity assessment using the titer cut point factor of 3.46. The acceptance range was determined to be 3.72 to 5.72 based on the following formula (Table 6):
Acceptance range = mean log3 (interpolated titer) ± 1
The inter-assay precision for the Titer Quality Control (TQC) over 30 runs of analysis was 6.02% (Table 6). The intra-assay precision for the Titer Quality Control over 6 assay days was 5.28% (Table 6).
Reviewer comments
The titer cutpoint factor is 3.46, which is close to the screening cutpoint factor of 2.43. Inspection of the tabulated titer validation data confirms that the titer cutpoint is in fact at low signal values allowing lower asymptotes of the titer curves will intersect at these low values, and thus producing accurate titer values. Thus the titer cutpoint is acceptable.

Recovery

The LPC (250 ng/ml) was spiked into 16 individual lots of normal human serum to determine the percent difference (% Difference = [(Mean SignalIndividual / Mean SignalControl) – 1] x 100) from controls prepared in the PNC. A blank of each individual lot was also analyzed for informational purposes. Fourteen out of the sixteen blank samples screened negative when compared to the plate cut point of 656 (Table 7). All sixteen lots were determined to be positive and had percent differences within ± 25.0% (Table 7). The recovery assessment met the following acceptance criteria: at least 80% of the normal individuals must have screening signal responses above the cut point.

Reviewer comments

Examining Table 7, I find that 13 out of 16 individual serum samples had unspiked values approximately two fold lower than the plate 657 plate cutpoint, and essentially the same as the mean CC-PNCvalue for this plate (270). One sample had a value slightly below the cutpoint (570), and two samples had values slightly above the cutpoint (769 and 706). These findings point to the importance of interpreting patient antibody results in the context of baseline serum sample values.

To assess the effect of hemolyzed matrix, the LPC was spiked into normal human serum that had been supplemented with either 2.5% or 5% hemolysate. Spiked samples were evaluated against the LPC . No effect was observed in hemolyzed samples since both samples. Similarly, to assess the effect of lipemic matrix, the LPC was spiked into lipemic human serum

Reviewer comment

The LPC values were essentially the same in hemolyzed or lipemic sera as the PPC values in non-hemolyzed or non lipemic sera, indicating hemolysis and increased lipid are not a concern for interpreting patient antibody results.

Room Temperature Stability

Positive control samples at two concentrations (250 ng/mL and 2500 ng/mL in 100% serum) were stored at ambient temperature for four hours and twenty-four hours prior to processing. After storage, the samples were diluted, processed, and analyzed in replicates of four (Plate 58). Freshly prepared positive control samples were used as the control samples. The percent differences, as calculated by %Difference = ([Mean Responsestressed / Mean Responsefresh] - 1) x 100, for the HPC and LPC stored for four hours were 2.03% and 18.8%, respectively (Table 9). The percent differences for the HPC and LPC stored for twenty-four hours were -4.35% and 17.7%, respectively (Table 10). The results indicate that the anti-rAvPAL IgG antibody is stable in human serum for up to twenty-four hours at ambient temperature since the percent differences between the stability samples and the control samples were within ± 25.0%.

Reviewer comments

I have examined Tables 9 and 10, and find that the sponsor's statements are accurate; i.e. there are no appreciable difference for the assay values among fresh LPC and NPC , and controls stored 4 and 24 hours at RT. Thus storage at RT is validated up to 24 hours.

Freeze/Thaw Stability

LPC and HPC were subjected to six freeze (-70°C) and thaw (ambient temperature) cycles. At the end of the sixth freeze/thaw cycle, the samples were diluted, processed, and analyzed in

replicates of four (Plate 58). The percent differences for the HPC and LPC were 5.17% and 13.7%, respectively (Table 11). The results indicate that the anti-rPAL IgG antibody is stable in human serum for up to six freeze/thaw cycles before analysis since the percent differences between the stability samples and the control samples were within ± 25.0%

Reviewer comments

I have examined Table 11, and find that the sponsor's statements are accurate; i.e. there are no appreciable difference for the assay values among fresh LPC and HPC, and controls after 6 freez/thaw samples. Thus freeze/thaw of the positive controls is at RT is validated up to 6 cycles..

Long Term Stability

Long term storage stability is not routinely assessed since a GMP reference calibrator is rarely available for anti-drug antibodies. As such, long term stability of the reference material (positive control) as formulated bulk is not available for comparison.

Reviewer comment

The positive control is stored at -70 ^{0}C, which is not expected to impact its activity. The impact of freeze-thawing is expected to be more important, and adequate freeze-thaw stability has been demonstrated. Therefore, I find that the overall stability data is adequate.

Robustness

To evaluate assay robustness, three assay plates with multiple incubation times were analyzed on two days by two separate analysts. Plate 1 (Runs 30 and 43) tested the minimum incubation times, Plate 2 (Runs 41 and 54) tested the standard incubation times, and Plate 3 (Runs 42 and 55), tested the maximum incubation times. If plates run under two or more specific sets of incubation times pass plate acceptance criteria, then the assay is considered robust with respect to incubation time.

Results for the robustness assessment (screening assay) were presented in Table 12. Plate 1 (Runs 30 and 43) and Plate 2 (Runs 41 and 54) met the assay acceptance criteria on both days, therefore, the robustness parameters for Plates 1-2 (minimum and standard incubation times) were found to be acceptable. The robustness plate acceptance controls for Plate 3 (Runs 42 and 55) showed a high 36.6% CV for the CC-PNC but met the criteria on day 2 (Run 55). Note that in addition to the robustness plate acceptance controls (filenames: HP2, LP2, N4 and N3), another set of plate acceptance controls were analyzed on the robustness plates (filenames: HP1, LP1, N2, and N1). These plate acceptance controls met the assay acceptance criteria on Run 42 while the robustness plate acceptance controls failed the %CV criteria for the CC-PNC (with no identifiable outlier). Therefore, the robustness parameters for Plate 3 (Run 42) were also found to be acceptable.

Reviewer comments

The Sponsor argues that the robustness parameters for Plate 3 (Run 42) were also acceptable because another set of plate acceptance controls were analyzed on the robustness plates, and these met the acceptance criteria. I find this argument acceptable because it essentially involves only a 3 out of 6 values for negative control one plate, with these values appearing to be outliers.

Results for the robustness assessment (confirmatory assay) are presented in Table 13 of the validation report. Plate 1 (Runs 30 and 43) and Plate 3 (Runs 42 and 55) met the acceptance criteria on day 2 (runs 43 and 55) but not on day 1 (Runs 30 and 42) while, plate 2 met the acceptance criteria on day 2 (Run 41) but not on day 2 (Run 54). These Sponsor states that the performance of the confirmatory assay during sample testing will be monitored with an immunocompetition control (LP control screened and confirmed) on every confirmatory assay plate. If the conditions responsible for the sporadic confirmation failures observed here occur during sample testing, the plates will be rejected as a result of immunocompetition control %SI < CCP, and the runs will be repeated, thus eliminating any impact on the study results.

Reviewer comments

As with the evaluation of screening robustness, these results point to some uncontrolled variability in the assay. However, the sponsor has described an appropriate approach of rejecting results from assays that do not meet the acceptance criteria, and repeating the runs.

ECLA to Detect anti-PAL IgM Ab in Human Serum

\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bmn165-13-050\bmn165-13-050.pdf

Phase 3

The MRD is 1/10, consistent with FDA guidance

Important definitions

Cut Point Control Normal Pooled Serum (CCNPS)

Low Normal Pooled Serum (LNPS) -prepared by selecting normal human serum samples from males and females with very low anti-PAL IgM activity. The LNPS was used for validation.

The HQC (High Quality Control) was prepared by pooling normal human serum samples with high activity in the assay.

The LQC (Low Quality Control) is a normal human pooled serum lot selected for low activity in the assay.

Titer cut point

Because PAL is a foreign bacterial protein, immune tolerance to PAL is not expected, and it is possible that natural anti-PAL IgM antibodies are pre-existing in many drug-naïve individuals. Data from screening normal sera suggest there is a range of pre-existing anti-PAL IgM activity in normal drug-naïve individuals, with no clear delineation of positive from negative sample results. Therefore, it cannot be assumed that serum samples from normal drug-naïve individuals are negative for anti-PAL IgM, and drug-naïve individual samples cannot be used to establish a screening cut point as described by Shankar et al. With this understanding, the assay strategy and method were modified in order to improve the ability to detect changes in anti-PAL IgM potency during Pegvaliase treatment in all subjects. Following the new strategy, all samples were presumed positive for anti-PAL IgM, and will be directly run in a titer method to measure the relative level of anti-PAL IgM in each sample.

A multiplicative titer cut point factor (TCPF) was established relative to the mean RLU signal of the CC on each plate, such that the titer cut point is lower than the lowest normal individual RLU signal observed in validation. The TCPF will be applied to each plate to establish a plate-specific (floating) titer cut point. The titer cut point factor is 0.1 x CC RLU.

Reviewer comment

This low titer cut point control factor is acceptable, because at1/10 of the cut point control, the titer curves should cross the cut point in their low asymptote regions, allowing accurate titer determinations.

Precision

HQC Intra-assay %CV = 7.5, HQC Inter-assay %CV = 18.5, LQC Intra-assay %CV = 1.9, LQC Inter-assay %CV = 5.1.

100.0% of QC titer results were within 0.5x to 2x of the mean titer.

Reviewer comments

The intra-assay and inter-assay precision were within industry practice. The 2 fold range greater than or less than the mean titer is adequate for semi-qualitiative assessment of the strength of patient ADA responses, and is thus suitable for the intended purpose

PC Acceptance Criteria

HQC and LQC titer results from all validation plates were compiled, excluding robustness plates, rejected plates, and samples subjected to stability conditions. For each QC level (HQC and LQC), the mean of titer measurements, and the 99.0% confidence interval around each mean using the t statistic were calculated. The HQC range was 882.6 to 2706 with a mean of 1794 and the LQC range was 117.9 to 156.9, with a mean of 137.4.

Reviewer comments

The LQC range is within ~ 20% of the mean and relatively narrow, indicating good control of the assay at low titers. However, the HQC range is ~50% on either side of the mean, suggesting some difficulty in quantitation of high titers.

Free Drug Interference

BMN165 was added to HQC and LQC samples at 0 µg/mL, 1.00 µg/mL, 10.0 µg/mL, or 100 µg/mL (anticipated BMN165 Cmax in plasma). Each sample was assayed using the titer method in two independent dilution series. Four serial dilutions from each titer series was assayed. HQC and LQC samples tolerated up to 100 µg/mL BMN165.

Reviewer comments

The trough pegvaliase concentrations in Study 165-302 Part 4 ranged from 75 to 132000 ng/ml. Thus, the signal and incidence rate of anti-PAL IgM antibodies is expected be reduced in many treated patients due to the modest drug tolerance of this assay.

Sensitivity

The limit of detection of the assay is 4.00 µg/mL purified human anti-PAL IgM PC, based on the total protein concentration of the positive control. This control was generated by purifying total IgM from pegvaliase-treated subjects, and therefore contains both PAL specific and non-specific human IgM. To further characterize assay sensitivity, the specific anti-PAL concentration of the positive control was determined by surface plasmon resonance, using a more highly purified anti-PAL antibody as a calibrator. The results of this analysis suggest an anti-PAL IgM assay LOD of 42 ng/mL.

Reviewer comments

The validated sensitivity of 4 µg/mL for the anti-PAL IgM assay calculated on the basis of total mass for anti-PAL IgM positive control is approximately 10 fold higher than the target sensitivity suggested in the 2016 FDA Draft Guidance for the Development of Immunogenicity Testing Methods. However, given that the sponsor's SPR analysis indicates only ~1 % of the control antibody is actually reactive, on this basis the sensitivity may be calculated to be an acceptable 42ng/ml. Moreover, acceptable performance of the assay is supported by the fact that anti-PAL IgM antibodies are detected in the majority of BMN165 treated patients.

Selectivity

Assay selectivity, the ability of the assay to detect anti-PAL IgM antibodies in the presence of other matrix components, was assessed using normal individual sera un-spiked and spiked with 10.0 µg/mL human anti-PAL IgM PC and titers were measured in the absence and presence

of hemoglobin, and also in lipemic samples. This study found that 80.0% of normal individuals met the acceptance criteria. 66.7% of hemolytic individuals met the acceptance criteria. 66.7% of lipemic individuals met the acceptance criteria. Based on these results, hemolysis and/or lipemia may interfere with screening testing, and the results of testing of hemolytic and lipemic samples should be interpreted with caution or excluded.

Reviewer comment

I agree with the Sponsor that the selectivity results indicate results from hemolytic or lipemic samples should be interpreted with caution or excluded. Information on hemolysis and hyperlipemia captured in clinical study reports, allowing correlation with assay results

Specificity

Specificity is the ability to detect anti-PAL IgM antibodies without interference from other IgM antibodies that are not specific for rAvPAL. The titer results of Low normal Pooled Serum (LNPS) were compared with the titer results of LNPS spiked with nonspecific human myeloma IgM. The titer values were 37.35 to 39.13 without added IgM vs 37.37 to 41.37 with added IgM.

Reviewer comment

The titer is essentially unaffected by added IgM, indicating good specificity by this criterion.

IgG interference

In a related validation exercise, the effect of anti-PAL IgG on the anti-PAL IgM was assessed. Rabbit anti-PAL IgG was added to HQC and LQC samples at 1.00 µg/mL, 10.0 µg/mL, 50.0 µg/mL, and 100 µg/mL. Two independent serial titer dilution series were performed for each sample. Examination of table 15.12 shows essentially no change in titer at 10.0 µg/mL anti-PAL IgG, and the signal was reduced by 1/3 at 50.0 µg/ml, while at 100.0 µg/ml,, the signal was only ¼ the uninhibited signal, while the table shows little effect on the LPC even at 100 µg/ml.

Reviewer comments

When interpreting titer results for the IgM PAL assay care should be observed in assessing samples that have high IgM anti-PAL levels and also high IgG levels, as the IgG can reduce the IgM titer.

Incubation Time Robustness

Incubation Time Robustness[1]			
Assay Incubation Step	**Minimum**	**Standard**	**Maximum**
rAvPAL Coat	16 hr. (+ 10 min.)	18 hr. (± 10 min.)	20 hr. (- 10 min.)
Block	60 min. (+1 min.)	90 min. (± 1 min.)	120 min. (- 1 min.)
Sample Incubation	90 min. (+1 min.)	105 min. (± 1 min.)	120 min. (- 1 min.)
Detection	50 min. (+1 min.)	60 min. (± 1 min.)	70 min. (- 1 min.)

[1] A small window of time is included at each incubation time for analyst convenience.

All HQC titer results and 81.8% of LQC titer results within run 137 met the acceptance criteria. Therefore, the validated incubation times are: Coating: 16 to 20 hours; Blocking: 60 to 120 min; Sample: 90 to 120 min; Detection: 50 to 70 min.

Reviewer comments

I have examined the tabulated incubation time data in Table 15.13, and find that there is little variation for the HQC and LQC titer values across the incubation times. Therefore, I agree with the validated incubation times.

Stability

Room Temperature Stability

Three aliquots of each QC were thawed and left at RT for at least 24 hours (including thaw time), or left at 2 to 8°C for 48 or 96 hours prior to being assayed.

Freeze/Thaw Stability

HQC and LQC samples were frozen at -60 to -80°C for at least 12 hours after preparation. Tubes were numbered so as to distinguish the number of (F/T) cycles that they underwent. Three sets of stability QCs were removed and thawed at room temperature for four (4) hours (+/- 10 minutes). Samples were then returned to -60 to -80°C for ≥ 12 hours. The process was repeated as needed to generate up to 8 F/T cycles.

Diluted Sample Stability

Three independent titer dilution series of HQC and LQC samples were performed in AD and stored for a total of 48, 72, and 96 hours at 2 to 8°C prior to running in the assay.

Stability Results

Thawed HQC and LQC samples were stable up to 96 hours when stored at 2 to 8°C, however, thawed HQC samples were not stable for up to 24 hours when stored at RT. QC samples were stable for up to 8 F/T cycles. Diluted QC sample titration curves were stable for up to 96 hours at 2 to 8°C. Therefore, thawed samples will be stored at 2 to 8°C for up to 96 hours or returned to -60 to -80°C storage. Frozen samples are stable up to 8 F/T cycles. In addition, diluted sample titration curves are stable up to 96 hours when stored at 2 to 8°C.

Reviewer comment

Acceptable stability is demonstrated for the important storage conditions and times relevant to routine assay performance.

ECLA to Detect anti-PEG IgG Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bmn165-13-049\bmn165-13-049.pdf
Phase 3
Sensitivity 205 ng/mL
Precision / PC Acceptance Criteria
Validation involved thirty-six analysis runs (performed over seven days by two analysts), with analysis of four replicates each of LPC (3500 ng/mL polyclonal monkey anti-PEG IgG) and HPC (800 ng/mL of polyclonal rabbit anti-PEG IgG) in 100% serum.
Reviewer comments
Note that the LPC and HPC are from different sources (monkey vs. rabbit) so that the HPC is actually at a lower concentration that the LPC. A priori, it would appear that the LPC concentration (3500 ng/ ml) is much higher that the sensitivity of the assay (205 ng/ ml). However, inspection of the tabulated data for the LPC and HPC places the raw signal values for the LPC near the raw signal values corresponding to the screening cut point. Therefore, on this basis the LPC is acceptable as a control for routine analysis.

The Pooled negative Control (PNC) was analyzed in replicates of four and the Confirmatory Cutpoint (CC-PNC) was analyzed in replicates of eight.
Normalized positive control signals were calculated using the following formula:
(Normalized signal = mean response of HPC or LPC / mean response of the CC-PNC)

Precision, defined by the percent coefficient of variation (%CV = [(standard deviation / mean) x 100]), was determined from the normalized results of the positive controls and from the raw signal of the PNC and CC-PNC. The inter-assay precision for the positive control samples at two concentrations of anti-PEG IgG antibodies over thirty-six (36) runs of analysis was ≤ 22.2% (Table 2), and therefore met the Sponsor requested acceptance criteria of inter-assay precision ≤ 25.0%.

The inter-assay precision value for the CC-PNC were 42.6% and for the PNC 38.3%, with both these values being greater than the target of 30.0% stated in the validation Plan. The sponsor provides the rationale that the RLU signal results from negative samples have no impact on the integrity of the reported results, as all negative with both replicate assay signals below the screening cut point will be reported 'negative'.
Reviewer comment
The rationale is only adequate if the assay signal in the range of the screening cut point has adequate precision. This variability is troublesome as it would impact the initial screening classification of negative vs. positive samples. However, the %CV of the PNC and CC-PNC replicates on each plate should not exceed 25.0% after exclusion of outliers. The latter criterion provides adequate assurance of plate-specific cut points based on the average CC-PNC signal on each plate.

Mean intra-assay precision of the raw signals for the positive control samples at two concentrations of anti-PEG IgG antibodies over thirty-six (36) runs of analysis was ≤ 13.1%

(Table 2). The mean intra-assay precision of the raw signal for the CC-PNC was 17.4%. The mean intra-assay precision of the raw signal for the PNC was 16.2%. Therefore, the HPC, LPC, and PNC samples met the following criteria: the mean intra-assay precision should not exceed 25.0% as requested by the Sponsor.

Reviewer comment

The intra-assay precision met target values and is therefore acceptable.

The mean normalized signals of the positive controls and the raw signals for the CC-PNC and PNC were used to determine the acceptance criteria for all runs. The lower limits for the normalized signal of the HPC and LPC samples were calculated to be 4.46 and 2.64, respectively (Table 2), using the following formula:

Lower limit = mean normalized signal – (t0.01,df x SD)

where t0.01,df is determined from the t-distribution corresponding to a 1% failure rate and SD is the standard deviation. The upper limits for the raw signal of the CC-PNC and PNC were calculated to be 2745 and 3110, respectively (Table 2), based on the following formula:

Upper limit = mean signal + (t0.01,df x SD)

Reviewer comments

The limits for the normalized lower limits for HPC and LPC are low compared to the observed ranges of values in the 34 tabulated runs, while the upper limits for CC-PNC and PNC are high relative to the actual observed values in the runs. These limits are acceptable because they did not affect the assay performance in the validation, and anti-PEG IgG antibodies were detected in almost all patients.

Screening Cut Point

Serum samples from one hundred normal individuals were evaluated for screening cut point analysis. The samples were analyzed in replicates of two on three separate days by two analysts for a total of three assays. The screening cut point data was evaluated by BioMarin. The data were found to not be normally distributed, but the Log10 distribution of the data was normal, so the serum sample values were log10 transformed to find the screening cut point. The screening cut point factor was determined to be 3.82, as per mean +1.645 SD, corresponding to an ~ 5% false positive rate. This constant was applied during sample analysis to calculate the cut point on a run-by-run basis using the following formula:

Cut point = X * CC-PNCPlate

In cases where the cut point factor established during validation does not appear to be representative of study samples, the screening cut point may need to be re-established using pre-dose samples and/or control matrix from the study.

A titer cut point constant (XEPT) was also determined by BioMarin using the cut point data obtained above. The titer cut point constant was determined to be 7.30 as per mean+ 2.33 xSD, corresponding to an ~ 1% false positive rate.

Reviewer comments

The screening and titer cutpoints were determined in accordance with advice from the FDA 2016 Guidance for Immunogenicity Assay Development, and are acceptable.

The screening cut point is derived at a 5% false positive rate, as recommended. The XEPT is derived at a 1% false positive rate, which is appropriate for what is essentially a confirmatory

assay. The XEPT is ~2 x the SCPT (7.3 vs. 3.82), placing the titer cut point above the 5% false positives, as is necessary for a confirmatory assay. Inspection of the tabulated data before log_{10} transformation also shows an ~ 2 fold difference between the value of the SCPT and the XEPT, placing the XEPT near the raw signal values for the LPC.

*The sponsor states that "*In cases where the cut point factor established during validation does not appear to be representative of study samples, the screening cut point may need to be re-established using predose samples and/or control matrix from the study", *this assessment of the need for a new SCP based on pre-treatment samples should have been performed prior to analysis of patient study samples. In the 11/15/2017 IR, the Sponsor was asked to clarify if they have now determined if there is a need for a pre-treatment sample SCP, and if this is the case, has a pre-treatment sample SCP been developed?*

AGENCY QUESTION 2 (11/15/2017)
Regarding anti-PEG IgG assay validation (bmn165-13-049):
A. For the screening assay you state that "In cases where the cut point factor established during validation does not appear to be representative of study samples, the screening cut point may need to be re-established using predose samples and/or control matrix from the study." Clarify if you have determined that there is a need for a pre-treatment sample SCP. If this is the case, clarify if a pre-treatment sample SCP been developed.

Biomarin Response (12/15/2017)
We have determined that the cut point calculated in validation BMN165-13-049 is appropriate for analysis of samples from subjects with PKU in study 165-301. The anti-PEG IgG assay screening cut point factor (SCPF) relative to the pooled negative control (PNC) on each assay plate determined in validation using a panel of normal serum samples from healthy donors is 3.82 X PNC. Performing the same cut point analysis using 260 pretreatment baseline samples from subjects in study 165-301 resulted in a SCPF value of 5.64 X PNC. Using the more conservative (lower) cut point determined in validation for study sample analysis results in a lower false-negative and higher false-positive rate. However, there is no negative impact to the final study data, as false-positive samples are identified in the second-tier confirmation test. The use of a higher cut point based on pre-treatment baseline results is not preferred as it could increase the rate of false-negative results. Importantly, treatment-induced increases over pre-treatment anti-PEG IgG titers were detected by week 8 in the majority of subjects in study 165-301 (see IIR Section 4.2.1), supporting the appropriateness of the validated cut point for monitoring of anti-PEG IgG responses in subjects with PKU.
Reviewer comment
The Sponsor has made an appropriate decision to accept a potentially increased level of false positives in clinical samples by using the normal serum sample SCPF, rather than a considerably higher SCPF that would result from using pre-treatment patient samples, and might well result in the loss of true positives. This approach ensures that true positives will be captured at screening, with false positives eliminated in the confirmatory step.

Confirmatory Cut Point

Serum samples from one hundred normal individuals also evaluated for confirmatory cut point analysis. All the individual lots were analyzed with and without a spike of 2.00 mg/mL m-PEG. All lots were analyzed in replicates of two on three separate days by two analysts for a total of three assays. Immunocompetition sample results are determined using the following formula:

% Signal inhibition = [1 – (Mean signal spiked with drug / Mean signal spiked with Diluent Buffer)] × 100

Reviewer comment

The following discussion is most relevant to this section on the Confirmatory Cutpoint, but is found in the section on Robustness.

*(Patient)*Sample analysis was initiated following the completion of this validation. Fourteen confirmatory analysis runs were analyzed by the same analysts who performed this validation. Of the fourteen confirmatory sample analysis runs, only eleven plates met the %signal inhibition acceptance criteria for the confirmatory controls initially determined from the validation confirmatory cut point assessment data (confirmatory cut point determined by BioMarin to be 88.7%). No significant changes occurred between this validation and the subsequent sample analysis. After review of the confirmatory cut point assessment data from the validation and discussion with the Sponsor, it was determined that the analytical variability obtained while performing the confirmatory cut point assessment was very high. The confirmatory controls analyzed on three plates during the validation met the confirmatory cut point acceptance criteria but were very close to the cut point

In order to capture the true analytical variability of the method the confirmatory controls analyzed in the fourteen confirmatory sample analysis runs were included in the confirmatory cut point data assessed by the Sponsor and the confirmatory cut point was determined to be 75.0%.

Reviewer comments

The 75% CCP appears to be appropriate for the assay, as it set to be beyond the experimental assay variability seen in actual patient assay runs. However the Sponsor will need to confirm the CCP in pre-treatment samples, and the 11/15/2017 IR included a question to that effect.

AGENCY QUESTION 2 (11/15/2017)

B) Clarify whether you have confirmed the Confirmatory Cut Point (CCP) for this assay with pre-treatment patient samples.

Biomarin response (12/15/2017)

Following a tiered screen, confirm, titer sample analysis strategy, only study samples that screened positive were tested in the confirmation assay. As a result, the confirmation assay was not performed on all pre-treatment samples from study 165-301, and a study-specific confirmation cut point could not be calculated. However, analysis of pre-treatment baseline results suggests the confirmation cut point determined in validation is appropriate for analysis of samples from subjects with PKU in study 165-301. Of 260 pre-treatment baseline samples analyzed, 132 (50.8%) were confirmed positive for anti-PEG IgG, leaving 128 negative samples. Of the 128 negative samples, 10 were false-positives in the screening assay (i.e. screened positive, confirmed negative), resulting in a false-positive rate of 7.8%, close to the 5% false-positive rate targeted by the validation cut points. These data suggest the

confirmation cut point is appropriately set to distinguish true positive from false-positive samples collected from subjects with PKU.

Reviewer comment

The Sponsor has provided an appropriate rationale that confirmation cutpoint is appropriate for PKU subjects.

Free Drug Interference

Free drug interference was evaluated by measuring the impact of 0.00, 0.100, 1.00, 10.0, 100, and 1000 ng/mL BMN-165 on the detection of cynomolgus anti-PEG IgG LPC (3500 ng/mL in 100% serum). The concentration of free drug that would reduce a positive signal to a negative (produces a signal below the cut point) can be determined by calculating a cut point for Plate 1. The cut point value for Plate 1 was calculated to be 4534 [CC-PNCRUN (1187) * cut point factor X (3.82)]. At 3500 ng/mL of cynomolgus anti-PEG IgG antibodies, addition of 10.0 ng/mL of free drug resulted in a negative signal, indicating interference

Reviewer comments

I have examined the data tabulated in Table 4, and find the Sponsor's statements regarding drug interference to be accurate. For the LPC at a BMN165 concentration of 1 μg/ ml, the assay signal was 73% of the uninhibited signal, while at 10 μg/ ml the LPC signal was only 21% of the uninhibited signal. Therefore, by this criterion, the drug tolerance at low antibody levels is 1 ng/ ml. The implications of these drug tolerance values for accuracy of patient antibody assessment will be discussed with the Clinical Pharmacology reviewer, and these discussion of led to the development of a PMR for improvement of the drug tolerance for this assay.

Sensitivity

Sensitivity was performed on 30 plates by analyzing rabbit anti-PEG IgG positive control titer curves prepared in pooled normal human serum. The concentration of antibody that produced an assay response equal to the plate specific cut point was calculated for each curve using a 4-parameter logistic curve fit interpolation. The sensitivity results were evaluated using the screening cut point factor of 3.82. The mean assay sensitivity was determined to be 205 ng/mL (Table 5).

Reviewer comment

The validated assay sensitivity of 205 ng/ ml is adequate, consistent the FDA's 2016 Guidance for Development of Immunogenicity Assay Testing.

An acceptance range for the titer quality control was also determined using the log3 (interpolated titer) data from the sensitivity assessment using the titer cut point factor of 7.30. The acceptance range was determined to be 4.59 to 6.59 based on the following formula (Table 6):
Acceptance range = mean log3 (interpolated titer) ± 1. The inter-assay precision for the Titer Quality Control (TQC) over 30 runs of analysis was 8.80% (Table 6). The intra-assay precision for the Titer Quality Control (TQC) over 6 assay days was 4.33% (Table 6).

Reviewer comment

In order to allow assessment of the appropriateness of the titer cutpoint, the Sponsor will need to provide the concentration of the TQC.

In the 11/15/2017 IR, the Sponsor was asked to provide the concentration of the Titer Quality Control

AGENCY QUESTION 2 (11/15/2017)

C) *Provide the concentration of the Titer Quality Control (TQC)*

BIOMARIN RESPONSE 2C

The TQC sample used in the anti-PEG IgG assay validation BMN165-13-049 is equivalent to the HQC sample, and was prepared with 800 ng/mL PEG-B47 rabbit monoclonal anti-PEG IgG in pooled normal human serum.

Reviewer comment

The Sponsor has provided the concentration of the TQC, as requested.

Recovery

Low positive control samples (3500 ng/mL in 100% serum) were spiked into sixteen (16) individual lots of normal human serum to determine the percent difference (% Difference = [(Mean SignalIndividual / Mean SignalControl) – 1] x 100) from controls prepared in the PNC. A blank of each individual lot was also analyzed for informational purposes. Fifteen (15) out of the sixteen (16) lots were determined to be positive and nine (9) out of the sixteen (16) lots had percent differences within ± 25.0% (Table 7). The recovery assessment met the following acceptance criteria: at least 80% of the normal individuals must have screening signal responses above the cut point

Reviewer comment

Although this assessment met the criterion that at least 80% of the normal individuals must have screening signal responses above the cut point, the fact that nine (9) out of the sixteen (16) lots had percent differences within ± 25.0% points to either excessive intrinsic variability in the assay, or variation in normal individuals that cause wide variation.

To assess the effect of hemolyzed matrix, low positive control samples (3500 ng/mL in 100% serum) were spiked into normal human serum that had been supplemented with either 2.5% or 5% hemolysate. Spiked samples were evaluated against the PNC sample supplemented with 2.5% or 5% hemolysate. No effect was observed in hemolyzed samples since both samples were determined to be positive (Table 8).

To assess the effect of lipemic matrix, the low positive control sample (3500 ng/mL in 100% serum) was spiked into lipemic human serum. The spiked sample was evaluated against the PNC sample prepared in lipemic serum. No effect was observed in the lipemic sample since this sample was determined to be positive Table 8).

Reviewer comments

This assessment demonstrates no important effect of hemolysis or lipid on the assay, subject to the caveat that the LPC concentration is very high.

Room Temperature Stability

Monkey LPC (3500 ng/mL) and rabbit HPC (800 ng/ml) were store at RT for 4 hours and 24 hours prior to processing. After storage, the samples were diluted, processed, and analyzed in replicates of four. Freshly prepared positive control samples were used as the control samples. The percent differences, as calculated by

%Difference = ([$\text{MeanResponse}_{\text{stressed}}$ / $\text{Mean Response}_{\text{fresh}}$] - 1) x 100,

for the HPC and LPC stored for four (4) hours were -9.93% and -24.9%.
However, the percent differences for the HPC and LPC stored for twenty-four (24) hours were -3.70% and -40.2%, respectively.

Reviewer comment

The sponsor has demonstrated RT stability of the LPC and HPC for up to 4 hours

Freeze/Thaw Stability

LPC and HPC were subjected to six freeze (-70°C) and thaw (ambient temperature) cycles. At the end of the sixth freeze/thaw cycle, the samples were diluted, processed, and analyzed in replicates of four. The percent differences for the HPC and LPC were -5.25% and -23.0%, respectively. The results indicate that the anti-PEG IgG antibody is stable in human serum for up to six freeze/thaw cycles before analysis since the percent differences between the stability samples and the control samples were within ± 25.0%.

Reviewer comments

Adequate freeze/thaw stability has been demonstrated for the LPC and HPC up to 6 cycles.

Robustness

To evaluate assay time robustness, assay plates with short (Plate 1), standard (Plate 2), and long (Plate 3) incubation times were analyzed on two days by two separate analysts. Plates 1-3 met the assay acceptance criteria on both days (Table 12), therefore, the robustness parameters for Plates 1-3were acceptable. Plates 1-3 met the % signal inhibition acceptance criteria (greater than or equal to the confirmatory cut point of 75.0%) on both days (Table 13). Therefore, robustness parameters were acceptable on Plates 1-3.

Reviewer comments

I have examined the data in Tables 12 and 13, and find that the sponsor's statements that the acceptance criteria were met are accurate. Therefore, assay robustness is demonstrated.

ECLA to Detect anti-PEG IgM Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bmn165-13-051\bmn165-13-051.pdf
Phase 3
Sensitivity 1.028 µg/mL
This assay validation was performed by Biomarin. A semi-quantitative direct format ECLA method to measure anti-PEG IgM in human serum was developed and validated to support Phase 3 studies. Ninety-six well streptavidin-coated MSD plates were coated with biotin-conjugated methoxy PEG, washed, and blocked. Samples were diluted in buffer (1:20 MRD) and incubated on the plate to capture anti-PEG antibodies. Captured anti-PEG IgM was detected with a ruthenium-labelled polyclonal rabbit anti-human IgM Fc fragment-specific antibody. After washing, plates were read on an MSD Sector Imager reader. Samples were serially diluted in buffer until the samples were no longer reactive above the established cut point. The interpolated dilution factor at which the signal of the dilution curve crossed the titer cut point was reported as the sample titer.

Confirmation of the QC Acceptance Ranges
(from Amendment 3)
The purpose of this assessment was to demonstrate consistency of confirmation values obtained without serial dilution of the HTQC and LTQC within and between assay runs, and to establish lower limits for %SI results from HTQC and LTQC samples to be used as system suitability acceptance limits for the confirmation assay during sample testing.
The precision of confirmation %SI results were assessed for the HTQC and the LTQC using the t-statistic. The two-tailed 95th% range was determined to be 93.5% to 99.4% and 62.5% to 83.6% for the HTQC and LTQC, respectively. Thus acceptance limits set at94.0% for the HTQC and 64.3% for the LTQC
Reviewer comments
These acceptance limits are acceptable, in both cases requiring substantial assay inhibition, which is the intent for a confirmatory assay.

Cut Point Determinations
A total of 100 drug naïve healthy human individual serum samples were selected to determine the screening cut point. Fifty were male and fifty were female of various ages and races.

This study used a total of 30 plates for the analysis. All 30 plates for this analysis were accepted based on the CC (Confirmatory Control) mean raw signal (RLU) with a %CV≤ 25.0% for 8 CC measurements on each plate. Excluding samples with %CV >25.0 for duplicate measurements exactly 24/600 or 4% of the screening data and 69/600 or 11.5% of the confirmatory data were omitted from further analysis. Results were then analyzed by plotting screening raw signal values (RLU) against confirmation %SI (% Spiked Inhibition) values to identify suspected pre-existing positive samples for exclusion. A bivariate plot was used to define suspect positives (high screening, high % of SI), false positives (high screening, low %SI), and negatives (low screening values). Twenty individual samples of the 100 tested (20%) were designated as

declared positives because at least 3 of the 6 values obtained for each fell within the declared positive quadrant

Cut Point Determination

Because the distribution of aggregate screening data from all 6 runs was not normal (Shapiro-Wilk test $p > 0.05$), the data distributions from each run were analyzed independently to assess normality and identify outliers by box plot analysis within each run. Four of the 6 runs were clearly normal by the Shapiro-Wilk test without outlier removal, a 5th run passed the Shapiro-Wilk test but with a value close to the 0.05 threshold for non-normality, and a 6th run required removal of an apparent biological outlier. Screening and titer cut points were then determined with Log_{10} (RLU) data comprised of 455 data points collected over six runs.

For the Confirmatory Cutpoint, neither the total %SI nor the Log_{10}(s/u) data were normally distributed as determined by the Shapiro-Wilk test (p-value < 0.0001). The %SI distribution had the least number of outliers as identified by box plot analysis and the most normal symmetry by inspection, and was therefore selected as the data set for establishment of the CCP. For Shapiro-Wilk analysis of % SI for individual runs, five of the six runs had normal distributions with p values < 0.05 without exclusion of any outliers, and on run required removal of an outlier. The confirmatory cut point was then determined with %SI data comprised of 411 data points collected over six runs.

Data Normalization and Floating Cutpoint Justification

Taking into account the test of means and homogeneity of variance, a fixed cut point could be used. However, to better compensate for potential run-to-run variation during sample testing, floating cut points were determined as described in the validation protocol and (Shankar, 2008, J.Pharm.Biomed.Anal.).

Calculation of Screening and Titer Cut Points and Factors

A log_{10} transformed SCP (Screening Cut Point) was calculated by taking the mean of the included screen data and adding it to the pooled SD multiplied by 1.645 (95_{th} percentile Confidence Level). An initial SCP calculated from log_{10} (RLU) transformed data was untransformed to the original units by taking the antilog of the SCP value before the screening cut point factor was calculated. The CC (RLU) means were used to calculate a grand mean CC (mean of the mean RLU values). The SCPF was then calculated by dividing the untransformed SCP by the grand mean CC. Since the data was first log transformed to obtain the SCPF, each mean plate CC needs to be multiplied by the SCPF to derive the plate-specific SCP.

A very similar procedure was used to calculate a log transformed TCP (Titer Cut Point) , but instead adding the mean of the screening data to its SD multiplied by 2.33 to provide a 99_{th} percentile confidence limit.

Thus the assay SCP for the validation runs was 24,471 RLU and the SCPF is 10.4. For the TCP validation, while the TCP was 48,704 RLU and the TCPF is 20.7.

Reviewer comments
The sponsor has thoroughly analyzed the distributions of data for screening for the titer CP, and demonstrated that a log transformation is necessary to approximate normality. Thus it is appropriate to employ the overall procedure of deriving log values corresponding to the 95th percentile confidence limit for the SCP and the 99th percentile confidence limit for the TCP, with associated antilog SCPF and TCPF values that are used as multiplicative factors to calculate plate-specific cutpoints. In intuitive terms, the TCP is approximately two fold higher than the SCP which is reasonable since the titration assay is essentially a confirmatory assay, requiring a more conservative (and thus higher cutpoint).

Calculation of the Confirmatory Cut Point
The confirmatory cut point was calculated based on a 90th percentile upper Confidence Limit (CL) because of the presence of pre-existing antibodies; i.e. the sponsor states that a Cut Point at the 99th CL would generate an inordinately high cut point. The grand mean of the %SI data was added to the SD multiplied by 1.282 to determine the CCP. The calculated CCP was found to be 82.3%. However, to be inclusive of the positive control and declared positive samples the CCP was realigned to 71.1%. The CCP is a fixed cut point and will be used for all data requiring a confirmatory step.
Reviewer comments
The Sponsor has performed analyses demonstrating approximate normal distribution of %SI values, and has found only modest differences in mean and variance between plates for the %SI, so using a fixed Confirmatory Cutpoint is appropriate. In addition, requiring only a 71.1 % inhibition for confirmation is an appropriately conservative solution, given the sponsor's demonstrated high level of pre-existing anti-PEG reactivity.

Precision
Three sets of cynomologous LQC were run on six screen plates by two analysts over three days. Intra- and inter-assay precision expressed as %CV were determined.
Five sets of TQC serial dilutions (series of at least 6 dilutions at 1:2 dilution steps each), with at least one dilution falling below the titer cut point were ran on six plates, by two analysts over three days. To assess the surrogate rabbit monoclonal anti-PEG IgM LQC, three sets of this control were run on 6 confirmation plates run by 2 analysts over three days. Intra- and inter-assay precision expressed as %CV were determined.

The target acceptance criteria specify that inter- and intra-assay %CV must be $\leq$ 25.0%. All precision %CVs passed the acceptance criteria. The Rabbit HQC intra-assay CV was 6.4%; the HQC inter-assay CV was 6.8%; the Cyno LQC intra-assay CV was 20.1%; the cyno LQC inter-assay CV was 20.1%; the Rabbit LQC intra-assay CV was 10.4%; the rabbit LQC inter-assay CV was 23.6%; the Log10 (TQC titer) intra-assay CV was 2.0%; the Log10 (TQC titer) inter-assay CV was 7.7%.
Reviewer comments
The anti-PEG IgM assay precision is acceptable, with all CVs within the $\leq$ 25% criterion, which is consistent with the 2016 FDA Guidance for Immunogenicity Testing.

Titer precision (from Amendment 3)

Five sets of LTQC and HTQC serial dilutions with at least one dilution in each set falling below the TCP were run on each of 7 plates (LTQC) or 6 plates (HTQC) by two analysts over six days (LTQC). Acceptable intra- and inter-assay precision is demonstrated by the percentage of individual titer results being within +/- 1 two-fold serial dilution of the mean LTQC or HTQC sample titer values.
Five sets of serial dilutions from each of three selected low, medium, and high reactive human serum individual samples, with at least one dilution falling below the TCP, were run on each of at least 6 plates, were run by at least two analysts over eight days. Intra- and inter-assay precision was demonstrated by the percentage of individual titer results being within +/- 1 two-fold serial dilution of the mean sample titer value.

Titers were run in duplicate for comparison with single (singlicate) determinations.
The target acceptance criteria were:
HTQC, LTQC, Individuals: $\geq$ 80% of titer results must be
within +/- 1 two-fold serial dilution of mean sample titer value.
Singlicate titer assessment: $\geq$ 80% of singlicate titer results must be within +/- 1 two-fold serial dilution of their corresponding duplicate titer value.

Data from HTQC, LTQC and both the low and medium reactive anti-PEG IgM individuals passed the acceptance criteria. For the high anti-PEG IgM reactive individual 25% of the singlicate titer results fell outside a single two-fold serial dilution, but could be accomodated by allowing an acceptance criterion of +/- 1 x a 2.5 fold dilution, rather than a 2-fold dilution criterion. The sponsor argues that this imprecision at high titers is acceptable because titers in many subjects increase by a factor of 100x over baseline titers within 2 months of the start of dosing, and meaningful increases in antibody titer are likely to be on the order of a 10-fold increase in titer value (based on preliminary study data), which can easily be appreciated in the context of the observed titer imprecision.
Reviewer comments
I agree with the sponsor that the imprecision of titer determinations at high titers for most patients will not affect a qualitative understanding of their titer increases, although this imprecision was communicated to the clinical review team.

Specificity
Specificity was evaluated in the confirmation assay by testing normal pooled serum spiked with both cyno LQC (17 µg/mL) and non-specific purified human IgM at one of five concentrations (1 mg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL, or 0 µg/mL), and evaluating the results of screening and confirmation testing. For the specificity test, normalized signal values were used (RLU/CC). All samples had RLU/CC values within the acceptance. All human IgM-spiked samples screened and confirmed positive, and there was no apparent trend in assay signals with increasing concentrations of non-specific IgM.
Reviewer comments
Examination of the sponsor's tabulated data indicates no effect of non-specific IgM on the cyno LPC control signal for screening and confirmatory tests. Thus, the LPC signal is demonstrated to specific for BMN165/Pegvaliase.

To characterize drug interference, BMN165 was spiked at five concentrations (10, 1, 0.1, 0.01, and 0.001 ug/mL) into NPS containing cyno anti-PEG IgM at the LQC concentration, as well as spiked into blank NPS. For drug tolerance, normalized signal values were used. Samples spiked with up to the 10 ng/mL BMN165 fell within the acceptance ranges and therefore, passed. Samples with greater than 10 ng/mL BMN165 did not fall within the qualified range.

Reviewer comments

The normalized RLU signal at 10 ng/ml BMN165 is still approximately 60% of the non-BMN165 signal, while the normalized RLU value at 100 ng/ml BMN165 is only ~1/3 the non-BMN165 signal. Thus, it is accurate to say that 10 ng/ml BMN165 is the upper limit of BMN165 drug tolerance. However, this level of drug tolerance appears very low, and its impact on assessment of patient anti-PEG IgM levels was discussed extensively with the Clinical Pharmacology team, leading to development of a PMR to improve the drug tolerance.

Selectivity

10 individual serum samples both un-spiked and spiked at the LQC concentration were prepared. A total of 4 hemolyzed serum samples, including low and high levels of hemolysis, and a total of 4 individual lipemic serum samples including low and high levels of lipemia were obtained. Un-spiked aliquots of hemolytic and lipemic sera were prepared, as well as aliquots spiked at the LQC concentration of PC. All samples were assayed according to the confirmation method of the draft method SOP at least once.

All samples spiked with 17 µg/mL PC ran above the assay screening cut point. Zero of 10 un-spiked normal serum samples ran above the assay screening cut point. Nine of 10 anti-PEG IgM-spiked individual sera confirmed positive. Three of four (75%) IgM-spiked hemolytic sera confirmed positive, two of three (66%) IgM-spiked lipemic sera confirmed positive. One sample out of 4 lipemic samples was removed from analysis as the %CV > 25.0%.

Reviewer comments

This small data set indicates that the assay may generally provide information in the presence of hemolysis or lipidemia, but that hemolyzed or lipidemic samples from some individuals may make it difficult to interpret the results. Thus, results from hemolyzed or lipidemic samples should be treated with caution. Information on hemolysis and hyperlipemia captured in clinical study reports, allowing correlation with assay results

Robustness

Three sets each of LQC and HQC were loaded in duplicate per plate in the assay for the minimum and maximum incubation times specified below. If either the minimum or maximum time did not meet acceptance criteria, the standard time would have been assayed. A small window of ± 1 minute was set around each incubation time for analyst convenience. Each condition was tested by three analysts on two plates over three days by the screen method of the draft SOP.

Table 10.8.1: Incubation Windows and Standard Times

	Incubation Times		
Assay Incubation Step	**Minimum**	**Standard**	**Maximum**
Block	50 (± 1) min.	60 (± 1) min.	70 (± 1) min.
Coat	120 (± 1) min.	130 (± 1) min.	140 (± 1) min.
Sample Pre-Incubation	60 (± 1) min.	70 (± 1) min.	80 (± 1) min.
Sample Incubation (MSD Plate)	50 (± 1) min.	60 (± 1) min.	70 (± 1) min.
Detection Incubation	50 (± 1) min.	60 (± 1) min.	70 (± 1) min.
Plate Readout	Upon addition of buffer	Upon addition of buffer	10 (± 1) min.

The criterion for acceptable robustness at a given incubation time was that two out of three analysis runs for that incubation time met the mean RLU and % CV acceptance criteria.

The sponsor states that all QC samples on the 3 minimum incubation time plates met the QC acceptance criteria (%CV ≤25.0%, and mean RLU values were within QC acceptance ranges in except for one of three LQC replicates within one of the 3 runs was below the acceptance range, and one of the three LQC replicates had a %CV greater than 25%. Run 70 had one LQC with a %CV greater than 25%.

All QC samples on the 3 maximum incubation time plates met the acceptance criteria except for all LQC replicates from run 64 that were above the acceptance range. This caused one of the three maximum incubation time plates (run 64) to fail the acceptance criteria. As the minimum and maximum incubation times passed, standard incubation time plates were not analyzed.

Reviewer comments

I have examined the sponsor's tabulated data for assessment of robustness at minimum and maximum incubation times, and find that their claim that adequate robustness has been demonstrated is accurate in the sense that at least two out of three runs at each incubation time met acceptance criteria. However, the failures of LQC in one of the three runs at each incubation time point to some difficulty in controlling assay performance at low RLU signal levels.

Stability

Stability of the HQC and LQC controls (PC antibodies in NPS) was examined under conditions expected to mimic those of study sample handling and storage, including overnight thawing of samples, room temperature incubation, and multiple freeze-thaw cycles. In addition, the stability of the diluted QCs in assay diluent was investigated in 24, 48, 72, and 96 hour incubations at 2 to 8 °C.

Benchtop and Overnight Thaw Stability

Three sets of QCs (HQC, LQC) were thawed at room temperature for each condition. The samples were incubated on the bench top at RT for a total of 4 and 24 hours (+/- 10 minutes). In

addition, three sets of frozen QCs were incubated at 2 to 8 ^{0}C for 24 hours prior to being assayed. Samples were then assessed in the screening assay.

Freeze/Thaw Stability
F/T stability was determined by testing at least 12 sets (three sets per F/T cycle) of HQC, LQC frozen at -60 to -80°C for at least 12 hours after preparation.

Sample Dilution Stability
Three sets of HQC and LQC were diluted in assay buffer and stored for a total of 24, 48, 72, and 96 hours at 2 to 8 ^{0}C prior to running the assay.

For the 24 hour at 2 to 8 ^{0}C incubation , two of three LQC replicates were above the qualified range. Because only 1 of 3 replicates met the acceptance criteria for this condition, samples should not be considered stable if left at 2 to 8°C for 24 hours. However, samples were stable for up to 24 hours at room temperature. All HQC and LQC replicates for all times tested for diluted sample stability were in the qualified ranges, and therefore samples may be diluted in assay buffer for up to 96 hours prior to transferring samples to the assay plate. All HQC and LQC replicates were in the qualified ranges when stressed through four freeze/thaw cycles, therefore samples may be assessed with up to four freeze/thaws.
Reviewer comments
The sponsor has demonstrated stability of the LQC and HQC in human serum for 4 hours in the cold and 24 hours at RT. Stability to 4 freeze/thaw cycle was also demonstrated. Finally, the LQC and HQC diluted in assay buffer were shown to be stable for as long as 96 hours at room temperature. These assessments cover a reasonable range of conditions that might be encountered in actual assay practice, and thus adequate stability has been demonstrated for the LQC and HQC.

Sensitivity(from Amendment 3)
Data from serial dilutions of a cyno anti-PEG IgM (50 µg/mL) performed in the original assay validation BMN165-13-051 were used to determine the assay LOD. A single serial 2-fold dilution series was run according to the titer assay method on each of six plates run by three analysts over three days (total of 6 serial dilutions on 6 plates) as directed by the Validation Protocol. According to the Assay Validation Protocol, the sensitivity (LOD) of the assay was to be reported as the mean of the cyno anti-PEG IgM antibody concentrations in neat serum detectable at the assay titer cut point.
The mean assay LOD was 1.028 µg/mL cyno anti-PEG IgM in neat serum. The LOD values in this experiment ranged from 0.787 to 1.629 µg/mL cyno anti-PEG IgM in neat serum.
The LOD was determined using the total protein concentration of a heterogeneous positive control in neat serum. However, analysis of antigen-specific ADA concentrations in the control reagent suggest an LOD of 167 ng/mL anti- PEG specific IgM (*Quantitation of Specific anti-PEG and anti-PAL Antibody Concentrations in Buffer solutions by Surface Plasmon Resonance to Characterize Pegvaliase ADA Assay Sensitivity-Appendix*).

Reviewer comment

This current validated sensitivity of 1.028 µg/mL *for the anti-PEG IGM assay is higher than the target sensitivity suggested in the 2016 FDA Draft Guidance for the Development of Immunogenicity Testing Methods. However, given that the sponsor's SPR analysis indicates only ~12 % of the control antibody is actually reactive, on this basis the sensitivity may be calculated to be an acceptable 167 ng/ml. Moreover, acceptable performance of the assay is supported by the fact that anti-PEG antibodies are detected in the majority of BMN165 treated patients.*

ECLA to Detect anti-BMN165 TAbs in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bmn165-13-052\3.pdf
Phase 3
Sensitivity 2.54 µg/mL
Original validation performed July 2014 by Biomarin
Amendment 1 completed May 2017 by Biomarin
Reviewer comment:
The validation reviewed below is from Amendment 1, May 23, 2017, as this contains all current validation data

This is a semi-quantitative direct format ECLA method to measure total anti-pegvaliase IgG, IgM, and IgA antibodies in human serum. Ninety-six well Mesoscale Discovery (MSD) plates were coated with a rabbit anti-PEG monoclonal IgG capture antibody, washed, blocked, washed, coated with pegvaliase, and finally washed before addition of serum samples diluted in buffer (1:50 MRD) to capture anti-TAb antibodies. Plates were incubated with a ruthenium-conjugated rabbit polyclonal antibody as a pan-isotype detection reagent that reacts with human IgG, IgM, and IgA. MSD Read Buffer T (surfactant-free) was added to all wells, and the plates were read on the MSD Sector Imager instrument.

Quality Controls
HQC and TQC: commercially-available drug-naïve individual human serum containing anti-BMN165 antibodies . The sponsor states that because the supply of affinity cynomologus monkey anti-BMN165 control antibody is limited, the HQC and TQC samples were prepared from commercially available individual serum samples with relatively high titers of pre-existing anti-BMN165 antibody. During characterization, immunodepletion using protein A/G and BMN165 confirmed that the HQC/TQC samples contain anti-BMN165 antibodies

AGENCY QUESTION 3 (11/15/2017)
B) The High Quality Control (HQC) and Titer Quality Control (TQC) are simply naïve human serum samples shown to have an appreciable titer in the assay, so it impossible to adequately evaluate the appropriateness of these controls. Because these are from BMN165 naïve individuals, it is quite possible that the reactivity is entirely to PEG. Therefore, provide any available information on the concentrations of anti-PEG and anti-PAL ADA in these controls.
BIOMARIN RESPONSE 3B (12/15/2017)
Characterization of the antigen specificity of antibodies in the High Quality Control (HQC)/Titer Quality Control (TQC) reactive human serum by surface plasmon resonance detected significant reactivity to both PEG and PAL. This demonstrates that quality control samples prepared with this serum are appropriate for monitoring the performance of the anti-BMN165 (PEG-PAL) total antibody assay by SPL analysis. The HQC/TQC normal pooled serum sample contains 59.5 µg/ml anti-PEG antibody, and
35.2 µg/ml anti-PAL antibody.
Reviewer comment.

The naïve serum used for the HQC and TQC has similar amounts of anti-PEG and anti-PAL antibody, and thus I agree that it is an appropriate control for the Total antibody assay (Tab), which needs to detect both reactivities.

LQC: 8.00 µg/mL affinity purified cynomolgus monkey anti-BMN165 in NHPS
NHPS (Normal Human Patient Serum)
LOD control: 50.0 µg/ml purified cynomolgus monkey anti- BMN165 in NHPS (for sensitivity assessment)
Reviewer comments
As discussed below in the section on LOD/Sensitivity, characterization of antigen-specific anti-PEG and anti-PAL ADA concentrations in the monkey LPC by surface plasmon resonance suggest these specific ADAs comprise only a fraction of the total protein. When the specific ADA concentrations in the monkey positive control are used instead of the total protein concentration, the limits of detection are 147 ng/mL anti-PEG ADA and 1,430 ng/mL anti-PAL ADA. Since the sensitivity is 2.45 µg/ ml on a total antibody mass basis, these numbers indicate that the monkey antibody contains 6% anti-PEG ADA and 58% anti-PAL antibody. Continuing this calculation, since the LPC is a control in the assay at 8 µg/ ml, then nominal anti-PEG ADA concentration would be 480 ng/ml and the nominal anti-PAL concentration would be 4.7 µg/ ml. A LPC concentration should be near the sensitivity of the assay, so this LPC concentration appears to be inappropriately high. The Sponsor will be asked to provide justification that the assay is able to routinely detect reactivities near the sensitivity level (see Information request and response below).

Furthermore, the sponsor has not described the levels of anti-PEG and anti-PAL ADA in HQC and TQC, which are simply naïve human serum samples shown to have an appreciable titer in the assay, so it impossible to adequately evaluate the appropriateness of these controls. Because these are from BMN165 naïve individuals, it is quite possible that the reactivity is entirely to PEG. The sponsor will be asked to provide any available information on the concentrations of anti-PEG and anti-PAL ADA in these controls (see information request and response below).

AGENCY QUESTION 3
Regarding anti-BMN165 TAb assay (bmn165-13-052):
a. For this assay, characterization of antigen-specific anti-PEG and anti-PAL ADA concentrations in the monkey LPC by surface plasmon resonance suggest these specific ADAs comprise only a fraction of the total protein. When the specific ADA concentrations in the monkey positive control are used instead of the total protein concentration, the limits of detection are 147 ng/mL anti-PEG ADA and 1,430 ng/mL anti-PAL ADA. Since the sensitivity is 2.45 µg/ ml on a total antibody mass basis, these numbers indicate that the monkey antibody contains 6% anti-PEG ADA and 58% anti- PAL antibody. Continuing this calculation, since the LPC is used as a control in the assay at 8 µg/ ml, the nominal anti-PEG ADA concentration would be 480 ng/ml and the nominal anti-PAL concentration would be 4.7 mg/ ml. A LPC concentration should be near the sensitivity of the assay, but

this LPC concentration appears to be inappropriately high. Provide justification that this assay is able to routinely detect patient ADA near the sensitivity level.
b. The High Quality Control (HQC) and Titer Quality Control (TQC) are simply naïve human serum samples shown to have an appreciable titer in the assay, so it impossible to adequately evaluate the appropriateness of these controls. Because these are from BMN165 naïve individuals, it is quite possible that the reactivity is entirely to PEG. Therefore, provide any available information on the concentrations of anti-PEG and anti-PAL ADA in these controls.

BIOMARIN RESPONSE 3A

The LQC concentration was selected to target an approximate 1% screen negative rate, as suggested in FDA guidance for industry[1] and Shankar *et al.*[2]. LPC assay results collected during assay validation BMN165-13-052 as part of precision and QC acceptance range assessments suggest that the LPC is set at an appropriate level to achieve >1% screen negative rate: 1 of 36 LPC samples assayed as part of the precision assessment screened negative (2.8%), and 1 of 65 LPC samples assayed as part of the QC acceptance range assessment screened negative (1.5%). These data support that the LPC concentration was appropriately selected to monitor the performance of the assay near the limit of detection (LOD). The LPC samples prepared at 8 µg/mL total protein contain 4.5 ug/mL anti-PAL ADA (8 µg/ml x 0.563) and 840 ng/mL anti-PEG ADA (8 µg/mL x 0.105). These values represent concentrations approximately 3 fold greater than the LOD for each analyte.

Reviewer comment

This justification is adequate in the context of the assay's poor sensitivity of 2.54 µg/ml, and by the criterion of false negative rate, consistent with FDA recommendations.

Cut Points and Cut Point Factors

One hundred normal drug-naïve serum samples (equal male and female) were screened. A 'cut point run' consisted of 5 plates containing the full set of 100 samples For CP run, each analyst screened and confirmed 100 individual samples over 5 plates. The CC (Confirmatory Control) was assayed in 8 wells total per plate. The LQC was screened and confirmed on each plate. A total of 6 cut point runs were performed by a total of 3 analysts over 5 days. Likely due to previous exposure to PEG, there were a high level of positive samples, which were identified as samples with both high screen RLU signals and high % signal inhibition in the confirmatory assay relative to the population of normal individuals in the same cut point run, using LQC and NQC for reference. After exclusion of outliers there were 57 remaining samples, and the Log10(RLU) and %SI distributions (for confirmatory analysis) the data were normally distributed on all 6 cut point runs.

The screening and titer cut point factor is 1.83 x CC RLU. The confirmation cut point is 61.6% SI. The overall false-positive rate across 5 cut point runs (25 plates) was 12.8%.

The titer cut point was set at the same level as the screening cut point, for an approximate 5% false-positive screen rate. These calculated cut points applied to the cut point data set, yielded a calculated false-positive rate was 12.8%. The sponsor states that while this rate is higher than the nominal target of 5%, it is reasonable given the relatively frequency of pre-existing positive individuals in the normal population. Moreover, a higher false-positive rate is more conservative, reducing the chance of false-negative assay results during sample testing. In addition using a confirmation step to exclude false-positives with signal inhibition below the validated

confirmation cut point mitigates the risk of having a high false-positive rate during sample testing, as false-positive results can be identified and reported negative.

Reviewer comments

I find that the sponsor's development of cutpoints is optimal, given the high level of reactive samples (presumably to PEG) in normal individuals. The overall approach of using a conservative screening CP, with false positive then excluded by confirmation and titer analysis, will capture all potentially positive samples, with true reactivity demonstrated by subsequence confirmatory steps.

Precision

For intra- and inter-assay precision, 6 sets of QCs (HQC, LQC, and NQC) were assayed in duplicate wells on each of 6 plates by 2 analysts over 3 days. To evaluate precision of the confirmation method, 6 LQC samples were confirmed on each of 6 plates, and 1 NQC sample was confirmed on each of 6 plates for comparison.

To evaluate intra- and inter-assay precision of the titer method, 5 sets of TQC serial dilutions were performed according to the method SOP-106418 (BAS-02-236). The TQC replicates were assayed on a total of 6 plates, by 2 analysts, over 3 days.

Due to statistically significant observed variability between runs in the raw sign (RLU) for the QCs, normalized QC/ CC results were used to evaluate assay precision and for calculation of QC acceptance ranges.

HQC and LQC precision was acceptable with intra- and inter-assay %CV < 25.0%

LQC intra-assay %CV = 20.7%, LQC inter-assay %CV = 21.7% CV

HQC intra-assay %CV = 7.4% inter-assay %CV = 8.5%).

NQC intra-assay %CV = 15.2%, inter-assay %CV = 18.3%.

TQC Log3(Titer) intra-assay %CV = 1.9% inter-assay %CV = 4.1%.

LQC %SI intra-assay %CV = 4.6%, inter-assay% CV = 6.2%.

100.0% of singlicate titer values were within ± one 3-fold dilution of the corresponding duplicate value, meeting the acceptance criteria for singlicate titer measurement.

Reviewer comments

When using normalized QC/CC values, the sponsor has demonstrated % CVs well within the 25% acceptance criterion, indicating adequate run-to-run control over assay performance.

QC Acceptance Ranges

The QC results from all accepted validation plates were used to calculate acceptance ranges to demonstrate system suitability during sample testing. As discussed under the section on Precision, QC results demonstrated significant plate-to-plate variation that was reduced by normalization (QC RLU/ CC RLU), normalized results were used to calculate the acceptance ranges. Acceptance ranges for HQC and LQC screening assay controls were determined based on the 99% confidence interval (CI), as defined by the t statistic.

The normalized HQC RLU/ CC RLU 99% CI was 9.87 to 56.4,

The normalized LQC 99% CI was 1.41 to 4.42.

Because the lower limit of the LQC range was below the screening cut point, the range was set to the SCP (1.83)

An upper limit of the NQC signal was determined using a one-sided t distribution.
The calculated upper limit of NQC/CC signal was 1.48.
The TQC acceptance range was calculated using the 99% CI of Log3(Titer) values, and then converted to titer units by taking the antilog of the Log3(Titer) acceptance range limits.
The TQC titer acceptance range was 779 to 5564.

Reviewer comments

These calculated acceptance ranges appear broad, but the assay nonetheless gave an average titer >10,000, indicating that the does not produce erroneously low titers.

LOD/Sensitivity

To assess sensitivity, cynomologous monkey PC samples at 50.0 µg/mL were serially diluted 1:3 in NHPS 7 times to generate an 8-point dilution series. Each sample from the dilution series was then diluted 1:50 in Diluent 2 and assayed with the titer method. The mean PC concentration of each titer dilution series was interpolated at the SCP. The mean interpolated concentration was 2.54 µg/mL, Additional characterization of antigen-specific anti-PEG and anti-PAL ADA concentrations in the LPC by surface plasmon resonance suggest these specific ADA are only a fraction of the total protein. When the specific ADA concentrations in the monkey positive control are used instead of the total protein concentration, the limits of detection are 147 ng/mL anti-PEG ADA and 1,430 ng/mL anti-PAL ADA.

Reviewer comments

The validated sensitivity of the anti-TAb assay , considering the specific ADA concentrations as per SPR analysis, is consistent with 2016 FDA Draft Guidance for Development of Immunogencitiy Assays for the 1467 ng/ ml anti-PEG ADA, but still high for the 1.430 µg/ ml anti-PAL ADA level . However, since the assay detects anti-TAb ADA in all patients, with an average titer > 10,000 (Integrated Summary of Immunogenicity), the assay sensitivity is suitable for at least qualitative detection of patient antibody positivity.

Selectivity

The assay performance in normal individual sera met the selectivity acceptance criteria, as 100.0% of individuals screened and confirmed positive on 2 of 2 assay plates. One hundred percent of spiked hemolytic sera (4 of 4) had screening RLU signals > SCP. However, 2 of 4 spiked hemolytic samples had %SI results < CCP on 1 of 2 runs, and three of 4 hemolytic individuals had screening results outside the acceptance range on 1 of 2 assays, suggesting that hemolysis may interfere with screening and confirmation. One hundred percent of spiked lipemic individual samples had screening RLU results > SCP. However, 3 of 4 spiked lipemic samples had %SI results < CCP on at least 1 of 2 runs (male low lipemic 2 of 2 runs, male and female high lipemic 1 of 2 runs), suggesting that lipemia may interfere with the confirmation assay. The sponsor states that the results suggest interference with both screening and confirmation testing from hemolytic and lipemic sera. For this reason, visually hemolytic or lipemic samples should be noted, and testing of these samples should be avoided, or the results interpreted with caution.

Reviewer comments

I agree with the sponsor's caution regarding hemolytic and lipdemic samples, since there is substantial assay interference from both of these contaminants. Information on hemolysis and hyperlipemia captured in clinical study reports, allowing correlation with assay results

Specificity

Normal human pooled serum were aliquoted un-spiked and spiked with purified non-specific human IgG, IgM or IgA (separate samples) at 50.0 µg/mL. The un-spiked and spiked samples were assayed using the confirmation method. The results met the acceptance criteria: 100.0% of samples spiked with non-specific IgG, IgM, or IgA generated results with screening RLU < SCP and confirmation %SI < CCP.

Reviewer comments

Examination of the sponsor's tabulated data confirms that there is a little or no effect on assay signal resulting from non-specific human immunoglobulins, demonstrating that these species, which are present in patient sera, will not affect the assay.

Drug Tolerance

BMN165 was added to NHPS, NHPS containing PC at [LQC] (8 µg/mL), or HQC samples at 0 ng/mL, 1.00 ng/mL, 10.0 ng/mL, 100 ng/mL, 1.00 µg/mL, 10.0 µg/mL, or 100 µg/mL. All samples were assayed per the screening procedure of the method SOP BAS-02-236.

The samples were assayed once by one analyst. HQC samples spiked with 0 to 100 µg/mL BMN165 remained within the acceptance range, but increasing drug concentrations dramatically reduced HQC signals. LQC samples spiked with ≥ 1 µg/mL BMN165 screened negative. The highest drug concentration in neat serum that did not interfere with LQC detection was 100 ng/mL. The sponsor states that the lowest concentration of drug in neat serum that did not interfere with detection of the LQC was 100 ng/mL. Circulating levels of drug in subject sera are expected to exceed 100 ng/mL, and could interfere with detection of low levels of ADA in some samples. ADA activity in HQC samples could be detected in the presence of up to 100 µg/mL, although signals were reduced with increasing drug concentrations.

Reviewer comments

The implications the anti-TAb assay's marginal tolerance to on-board drug was discussed with the Clinical Pharmacology reviewer Christine Hon, and the anti-Tab data is largely interpretable, since a minor proportion of patients had trough on-board drug concentrations in the 100 µg/mL range.

Robustness

Three sets of QCs (HQC, LQC and NQC) were assayed, each loaded in duplicate wells per condition for the minimum and maximum incubation times specified below. Each condition was tested on 2 plates by 1 analyst on 1 day.

Incubation Time Robustness[1]			
Assay Incubation Step	**Minimum**	**Standard**	**Maximum**
Capture Antibody Coat	16 hr. (+ 10 min.)	18 hr. (± 10 min.)	24 hr. (- 10 min.)
Block	110 min. (+1 min.)	120 min. (± 1 min.)	130 min. (- 1 min.)
Drug Coat	110 min. (+1 min.)	120 min. (± 1 min.)	130 min. (- 1 min.)
Sample Pre-Incubation	50 min. (+1 min.)	60 min. (± 1 min.)	70 min. (- 1 min.)
Sample Incubation	110 min. (+1 min.)	120 min. (± 1 min.)	130 min. (- 1 min.)
Detection	50 min. (+1 min.)	60 min. (± 1 min.)	70 min. (- 1 min.)

[1] A small window of time is included at each incubation time for analyst convenience.

For both minimum and maximum incubation times, 100% of QC results fell within the

acceptance ranges.

Reviewer comment

Satisfactory robustness to incubation times is demonstrated, since the QC assay signal appear unaffected with within what would appear to be expected ranges of incubation times for the different assay steps.

Stability

Stability of the LQC, HQC and NQC in NHPS was validated as 24 hrs at RT and up to 6 F/T cycles. Additionally, samples were stable after dilution or serial dilution in diluent and storage for up to 96 hrs. at 2 to 8°C before testing. Initially the expiration of the positive control was set as one year from preparation date. Based on acceptable performance in this validation, the reagent expiration will be extended to 2 years from the date of preparation.

Reviewer comments

I have reviewed the sponsor's stability report and find that their statements are accurate. One hundred % of the QC results met the acceptance criteria for storage at RT for up to 24 hrs., and up to 6 F/T cycles. One hundred % of the QC samples that were diluted and stored for 96 hours at 2 to 8°C met the acceptance criteria, including screening and titer assay samples. Thus, stability of the QCs is demonstrated for important storage conditions.

Detection of anti-BMN165 NAbs in Human Serum

\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bmn165-13-053\bmn165-13-053.pdf

Phase 3

The sensitivity was determined to be 1785 ng/mL using a cutpoint based on runs with replicates of two for each of 50 serum normal serum samples (Mean Signal Cutpoint), and 1490 ng/ml using a cutpoint based using runs with singlicates for each of 50 normal serum samples (Individual Response Cutpoint).

This assay was validated by (b) (4). The assay detects neutralizing rAvPAL-PEG antibodies that inhibit the enzymatic activity of rAvPAL to convert a fixed concentration of the substrate L-phenylalanine (Phe) to *trans*-cinnamic acid (*t*-CA) and ammonia. The assay format is constructed into two portions. The first utilizes a ligand binding assay structure in which rAvPAL-PEG is bound to an anti-polyethylene glycol (PEG) capture antibody coated onto a plate. Neutralizing antibodies specific to rAvPAL-PEG present in the serum samples bind to the immobilized rAvPAL-PEG and inhibit the catalyzed conversion of the substrate (Phe). The substrate buffer is then transferred to the second portion of the assay in which the concentration of the conversion product, *trans*-cinnamic acid, is measured using LC/MS/MS.

Precision/PC acceptance criteria

In each of seven analysis runs, two replicates of positive control samples at two concentrations (5000 ng/mL (LQC) and 20,000 ng/mL (HQC) in 100% human serum) were analyzed (total of 14 results). A pooled negative control (PNC) was also analyzed in replicates of two. The normalized concentrations of the positive controls were calculated as per the following formula: (Normalized concentration = concentration of HPC or LPC / concentration of the PNC)

The inter-assay precision for the LQC and HQC was ≤ 7.56%,while the inter-assay precision for the PNC was 6.59%. The acceptance ranges for the HPC and LPC, and PNC samples were determined to be 0.388 – 0.524 , 0.510 – 0.812, and 6.48 – 9.70 respectively using the following formula:

Acceptance Range = mean normalized concentration ± ($t_{0.01,df}$ x SD)

where $t_{0.01,df}$ is determined from the t-distribution corresponding to a 1% failure rate.

A titer quality control (TQC) sample was also analyzed in seven analysis runs (total of 13 results). The log3 (interpolated titer) of the estimated sensitivity was determined for each run. The overall precision of the TQC sample was 3.74%. The acceptance range for the TQC sample was determined to be 7.47 – 9.39 based on the following formula:

TQC Acceptance Range = mean log3 (interpolated titer) ± ($t_{0.01,df}$ x SD)

Reviewer comment

The PC acceptance criteria appear to have adequate statistical justification, and are relatively tight for a cell based Nab assay, as these assays often have high variability.

Screening Cut Point

Fifty individual normal human serum samples were either analyzed using plates with replicates of two on two separate days by two analysts for a total of three assays (Mean Signal Cutpoint) or using plates with singlicates of the normal serum samples on two separate days by two analysts for a total of three assays (Individual Response Cutpoint). A lower limit (LL) of the 95% confidence interval for the non-specific binding of the individual negative controls (INCs) was determined for each plate; i.e.

LL = Mean response of the INC on the plate - (1.645 × SD of the INC)

SD =Standard Deviation, INC =Individual Normal Controls

Reviewer comment

I have examined the sponsor's graphical presentations of log10 INC values for the 3 runs (Figures 1, 2, and 3) and the data distributions appear roughly normal, with this impression confirmed by the Sponsor's Shapiro-Wilkes tests. Thus, the use of the formula above based upon SD is appropriate.

For the Mean Signal Cutpoint determination, a screening cut point factor (X) was then determined as

Screening cut point factor (X) = LL / $Mean_{PNC}$ response,

which ranged from 0.874 to 0.987, with a Mean Signal cutpoint factor averaged across all the plates being 0.918.

Similarly, for the Individual Response Cutpoint determination, cutpoint factors on the individual plates ranged 0.832 to 0.990, yielding an overall average of 0.898 for the Individual Response cutpoint)

The overall cutpoint factors X in actual runs were applied as

Screening cut point = X * PNCPlate

Reviewer comments

The sponsor's assessment of normality and derivation of screening cut point factor is consistent with FDA guidance and is acceptable. However, the appropriateness of the SCP should be confirmed on pre-treatment patient samples.

AGENCY QUESTION 4 (11/15/2017 IR)

Regarding NAb assay (bmn165-13-053):

a. Confirm the Screening Cut Point (SCP) for this assay with pre-treatment patient samples.

b. A titer cut point requiring a 99% confidence interval is appropriate, as the titer assay is a kind of confirmatory assay. However, provide assurance that for samples containing high, medium, and low levels of Nab activity, the cut point does not cross the titration curves well above their asymptotes, as this would create artificially low titer values.

BIOMARIN RESPONSE 4A (12/15/2017

We have confirmed that the screening cut point factor (SCPF) calculated with pre-treatment patient sample results is comparable to the SCPF determined in validation BMN165-13-053. The NAb assay uses a multiplicative SCPF relative to the pooled negative control (PNC)

sample on each assay plate. In assay method validation BMN165-13-053, the SCPF was determined to be 0.898 X PNC, using a panel of normal serum samples from healthy donors. Applying the same cut point analysis to the 262 pre-treatment baseline samples from 165-301 study subjects with PKU resulted in a very similar SCPF of 0.876 X PNC. The use of the cut point determined in validation for study sample analysis is appropriate, as it is very close to the value obtained using pre-treatment baseline samples, but slightly higher. In contrast to many ADA assay where positive samples generate high assay signals and negative samples generate low signals, NAb positive samples generate low NAb assay signals while Nab-negative samples generate high NAb assay signals. Therefore, because the validated cut point is slightly higher, it is more conservative than the value determined using pre-treatment baseline samples, resulting in a lower false negative rate. The increase in false-positive rate associated with a slightly more conservative cut point has no effect on the validity of the final study results, as false-positive samples are identified in the second tier confirmation test and correctly reported as negative.

Reviewer response

It is appropriate to use a normal serum SCPF, which give a higher false negative rate than a pre-treatment derived SCPF, since this conservative approach is more likely to capture true positives, which can then be confirmed in the second tier of analysis.

Titer Cut Point

The Titer Cut Point was determined in a similar fashion to the Screening Cut Point, but with the requirement that

LL = Mean response of the INC on the plate - (2.33 × SD of the INC)

in order to provide a 99% confidence interval.

So again

Titer cut point factor = LL / $Mean_{PNC}$ response

and

Titer cut point = titer cut point factor * PNC_{Plate}

Reviewer comments

A titer cut point requiring a 99% confidence interval is appropriate, as the titer assay is a kind of confirmatory assay. However, the sponsor should provide assurance that for samples containing high, medium, and low levels of Nab activity, the cut point does not cross the titration curves well above their asymptotes, as this would create artificially low titer values.

AGENCY QUESTION 4 (11/15/2017 IR)

Regarding NAb assay (bmn165-13-053):

b. A titer cut point requiring a 99% confidence interval is appropriate, as the titer assay is a kind of confirmatory assay. However, provide assurance that for samples containing high, medium, and low levels of Nab activity, the cut point does not cross the titration curves well above their asymptotes, as this would create artificially low titer values.

BIOMARIN RESPONSE 4B (12/15/2017)

The suitability of the NAb assay titer cut point was confirmed by analyzing raw data from NAb assay titer analysis of samples in study 165-301 with low, medium, or high NAb titers (titer range 2 to 39,366; Figure 4.3). The curve of increasing raw assay signals from serially

diluted study samples plateaued at the next dilution step after crossing the titer cut point, confirming that the cut point is appropriately set for study sample NAb titer analysis.

Reviewer comment

The Sponsor has confirmed, as requested, that the titer curves cross the NAb titer cut point near the asymptotes of the curves. Thus, this response is adequate.

Confirmatory Cut Point

Fifty individual lots of normal human serum were evaluated for confirmatory cut point analysis. All the individual lots were analyzed with and without a spike of 1.00 mg/mL rAvPAL-PEG Final Bulk. All lots were analyzed in replicates of two on two separate days by two analysts for a total of three assays. The confirmatory assay cut point was calculated from the ratios of unspiked /spiked values. The $\log_{10}$ of the ratio values followed a normal distribution and outliers were excluded. The value 3.09 in the calculation, which corresponds to the 99.9th percentile of the normal distribution, targets a 0.1% false positive rate. On this basis, the confirmatory cut point was determined to be 18.5%. Summarizing the calculation:

Step 1
Mean Ratio- (3.09 x Standard Deviation of Ratios) =0.815

Confirmatory Cut Point= 100 x (1-Step1) =18.5%

Reviewer comments

Targeting a 99.9% confidence interval is appropriate for a confirmatory assay. The assay requires only a modest 18.5% change in the unspiked /spiked ratios, and is thus likely to capture all true positives. Therefore, the confirmatory cut point is acceptable.

Drug Interference

Free drug interference was evaluated by measuring the impact of 0.00, 10.0, 25.0, 50.0, 75.0, 100, and 150 µg/mL rAvPAL-PEG (PEGPAL; BMN-165) on the detection of anti-rAvPAL-PEG neutralizing antibody positive controls (5000 ng/mL and 20,000 ng/mL in 100% human serum). By calculating a cut point for ligand-binding Plate 1, the concentration of free drug which increases the signal above the screening cut point, thus the concentration of free drug that would change a positive signal to a negative, can be determined. The screening cut point value for ligand-binding Plate 1 was calculated to be 7.78 [PNCRUN (8.48) * screening cut point factor x (0.918)]. At 5000 ng/mL of anti-rAvPAL-PEG neutralizing antibodies, addition of 50.0 µg/mL of free drug resulted in a negative signal, indicating interference. At 20,000 ng/mL of anti-rAvPAL-PEG neutralizing antibodies, addition of 150 µg/mL of free drug resulted in a negative signal, indicating interference.

Reviewer comment

The validated drug tolerance of 50-150 µg/ml appears high, but because of the high daily dosing of BMN-165, steady state serum concentrations may be in this range. Clinical pharmacology confirms that there may be some trough samples that are high and in this range.

PC sensitivity

Sensitivity was performed on six plates by analyzing a total of 30 positive control titer curves

prepared in pooled normal human serum. The results were evaluated using both the Mean Signal Cutpoint factor of 0.918, or the Individual Response cutpoint factor of 0.898 The concentration of antibody that produced a response equal to the plates specific cut point was calculated for each positive control curve. Using the Mean Signal Cutpoint, the sensitivity was using the 1785 ng/mL, while the sensitivity using the Individual Response cutpoint was 1490 ng/ml

Reviewer comment

The NAb assay sensitivities of 1785 ng/mL or 1490 ng/ml are based on a Nab positive control antibody, which may have low binding affinity. This is known issue in Nab assay development, and the fact that the Nab assay detects positives in a substantial number of patients indicates that it is suitable for its intended purpose. According to the Summary of Biopharmaceutical Studies summary, the Sponsor used the Indivdual Response Cutpoint, corresponding to 1490 ng/ ml sensitivity. Because this cutpoint is lower than the Mean Signal Cutpoint, it is more likely to capture low positive signals, and is therefore better suited for the intended purpose.

Robustness (Ligand binding)

For robustness, assay performance was evaluated at the following minimum and maximum incubation times:

Assay Incubation Step	Minimum	Maximum
Block	30 min.	60 min.
Coat	16 hr.	20.5 hr.
rAvPAL-PEG Capture	90 min.	120 min.
Reaction Incubation	17.5 hr.	20.5 hr.
Drug Coat	120 min.	120 min.

Reviewer comments

The sponsor provided tables of values across plates and between runs for minimum and maximum incubation times, and the HQC, LQC, and NQC values are closely spaced, indicating the assay can be performed within these ranges of incubation times.

Stability of the HQC and LQC

Room Temperature

Freshly prepared HQC and LQC versus QCs stored at RT for 25.5 hours were compared. There were small differences for the means of quadruplicate values for HQC between the two conditions (4.35% difference), and similarly, for the LQC (3.13% difference)

Freeze / Thaw

For freshly prepared HQC and LQC versus QCs taken through 4 freeze/thaw cycles, there were decreases in the means of quadruplicate values for HQC between the two conditions of -2.9 %, and for the LQC, -14. %.

Reviewer comments

These data show relatively small changes upon 25.5 hour RT storage, and after four freeze-thaw cycles, support RT storage $\leq$ 25.5% hours, and four freeze thaw cycles of the QCs.

Selectivity and Linearity (LC/MS)

Reviewer comments
The sponsor evaluated the LC/MS trans-cinnamic acid method, and found that there were essentially no interfering peaks that would affect detection. No carryover was observed between a run with 15 μM trans-cinnamic acid and subsequent blank run. Linearity was evaluated in the range 0.25-15 μM trans-cinnamic acid, and the correlation coefficients for the calibration curves had R values 0.9984, confirming a very highly linear response. Thus, the LC/MS method is suitable for the intended purpose of quantitative detection of trans-cinnamic acid.

Precision and Accuracy (LC/MS)
Six replicates of QC samples were analyzed in one run at five concentrations of trans-cinnamic acid (0.250, 0.750, 4.00, 11.0, and 15.0 μM). For the low, mid, and high QC levels, the precision had to have a % CV $\leq$ 15% with accuracy within ± 15% of the nominal value, while for the LLOQ QC, the requirements were % CV % $\leq$ 20% with accuracy within ± 20% of the nominal value. The intra-run precision for the QC samples at the low, mid, and high QC levels was $\leq$ 3.14%. The intra-run precision for the QC samples at the LLOQ QC level was $\leq$ 4.44%.

Accuracy was defined as
percent relative error (%RE) =(mean observed concentration - nominal concentration) / nominal concentration × 100].
Accuracy for the QCs ranged from -0.909% to 1.33%, while accuracy for the Q LLOQ QC level was 0.800%.

An upper limit of quantitation (ULOQ) of the assay was defined as the highest calibration standard concentration. Samples were at a concentration of 15.0 μM *trans*-cinnamic acid and were analyzed in replicates of six. The precision (%CV) and accuracy (%RE) results were 2.50% and 6.00%, respectively
Reviewer comments
The precision and accuracy of the LC/MS method appear tight, as expected for what is an analytical chemistry method. Thus, the precision and accuracy are suitable for the assay's intended purpose.

Anti-BMN165 IgG4 Assay

\\cdsesub1\evsprod\BLA761079\0022\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\bmn-165-16-055

Phase 3 assay

This is an ECL assay developed and validated by Biomarin in 2017, with assays of clinical samples performed by (b) (4) In this method, ECL plates were coated with a rabbit anti-PEG monoclonal IgG antibody, followed by binding of pegvaliase to the anti-PEG IgG. Serum samples or controls are then incubated in the wells to capture anti-pegvaliase antibodies. Finally, these antibodies are detected with a purified ruthenium-conjugated mouse monoclonal detection antibody specific for human IgG4, via an ECL signal.

The IgG4 method includes three different steps – screening, confirmation, and titer. In the screening step, the method described above is followed to determine whether anti-BMN 165 antibodies are present in the sample. If a sample screens positive, it is tested in the confirmation step for drug specificity, by incubating the sample in the absence or presence of 500 µg/mL unlabeled BMN 165, which inhibits binding of specific antibodies to the BMN 165 coated on the assay plate, resulting in a decrease in signal relative to the signal obtained in the absence of unlabeled drug. The relative reduction in signal is expressed as %Signal Inhibition. Samples that are confirmed to be positive for BMN 165-specific antibodies are then tested in the titer step, in which samples are serially diluted 1:3 and assayed to identify the dilution factor at which the titer curve crosses the cut point signal.

Controls

System suitability controls [High Quality Control/Titer Quality Control (HQC/TQC), Low Quality Control (screening and confirmation LQC-S and LQC-C, respectively), Negative Quality Control (NQC), Cut point Control (CC-unspiked Normal Human Pooled Serum)] were evaluated in a pre-study validation for use during sample testing to accept plate results.

Different NHPS lots were used to prepare the CC (used to normalize results between plates) and NQC samples.

The HQC/TQC (High Quality Control-also uised as Titer Quality Control) was prepared by pooling serum study samples from 165-302 subjects with PKU that had high reactivity (above 10000 RLU) in the assay during development.

The LQC-C (Low Quality Control for Confirmation) was prepared from a pool of study samples, also from 165-302, that had intermediate reactivity (between 4588 and 9432 RLU) in the assay during development followed by a 1500-fold dilution into NHPS for low assay activity.

The LQC-S (Low Quality Control for Suitability) was prepared from a 3000-fold dilution of the intermediate pool at a level of reactivity where it statistically fails (screen negative) 1% of the time.

The LOD PC was used to determine the LOD during validation, but will not be run

on study sample testing plates. The LOD PC was prepared using IgG4 derived from human myeloma plasma chemically conjugated to purified rabbit anti-PEG IgG monoclonal antibody. The LOD PC was used to determine the LOD during validation, but was not run on study sample testing plates

MRD

The MRD was 1/20, and was used for controls, as well as patient samples.

Reviewer comment

The MRD is within the range recommended in the 2016 FDA Guidance (Draft Guidance for Industry Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products, FDA, Draft Guidance April 2016) and is acceptable.

Cutpoints

Forty eight serum samples were obtained from normal drug-naïve adult volunteers (approximately equal numbers male and female). For each cut point run, each of 3 analysts screened and confirmed 48 individual samples over 3 plates. A total of 6 cut point runs were performed by 3 analysts over 2 days. The cut point and CPF determinations were designed to statistically establish an approximately 5% or greater FPR for the SCP, and a 1% FPR for the CCP and TCP. After exclusion of outliers, the screening and confirmation data were normally distributed in each of the 6 cut point runs

CCP (Confirmatory Cutpoint)

To determine the CCP for drug specificity, by incubating the LQC-C in the presence of 500 µg/mL unlabeled BMN 165, which inhibits binding of the LQC-C to the BMN 165 coated on the assay plate, resulting in a decrease in signal relative to the signal obtained in the absence of unlabeled drug. The relative reduction in signal is expressed as %Signal Inhibition.

Grand Mean (%SI) + (pooled variance * 2.33 ("t statistic at the 99th percentile")

CCP=27.3% SI (Signal Inhibition), which was used as a fixed cutpoint

A FPR (False Positive Rate) was then calculated as =2.2%

SCP (Screening Cutpoint)

A Grand Mean was calculated for individual serum values (excluding outliers) when these values had been normalized to the CC (Cut point Control) , and a pooled SD across all the cutpoint runs was also calculated.

The SCP was then=Grand Mean +pooled SD * 1.645 (t-statistic at the 95th percentile), where SCP=1.26. This SCP was initially derived as a fixed cutpoint that was based on ratios relative to the CC, and was then applied as a floating cutpoint to subsequent runs because a CC was included on subsequent plates.

A FPR (False Positive Rate) was then calculated as =5.9%

TCP (Titer Cut Point) see Table 15.4B

Because the titer assay is the 3rd tier assay, and is a confirmatory assay, the Screening Cutpoint validation data was used to calculate a more stringent cutpoint to be used for titer determinations. Again using the Grand Mean calculated for individual serum values (excluding outliers) when

these values had been normalized to the CC (Cut point Control) ,and a pooled SD calculated across all the Cutpoint runs;i.e.
TCP was then=Grand Mean +pooled SD * 2.33 (t-statistic at the 99th percentile)
yielding a TCP of 1.36, which again was initially determined as a fixed cutpoint, and then used as a floating cut point factor (TCPF)
Reviewer comment
The derivation of the cutpoints is consistent with the 2016 FDA immunogenicity method guidance.

Precision
Screening method
At least 2 sets of QCs (HQC, LQC-C, and NQC) were assayed in duplicate wells on each of 39 plates by 3 analysts over 9 days. Subsequently, 3 sets of LQC-S in duplicate were assayed on each of 8 plates by 2 analysts over 3 days.
Confirmation method
At least 3 LQC-C samples were confirmed on each plate. To evaluate intra- and inter-assay precision of Hamilton Star apparatus automated dilutions, at least 3 sets of QCs (HQC, LQCs, and NQC) were assayed in duplicate wells on each 6 plates by 2 analysts over 3 days.
Titer Method
To evaluate intra- and inter-assay precision of the titer method, at least 3 sets of TQC serial dilutions were performed according to the draft method SOP-114205, with the signals from at least two dilutions above and at least one dilution below the titer cut point. To evaluate intra- and inter-assay precision of the titer method using the Hamilton Star, 3 sets of TQC serial dilutions were performed with the signals from at least two dilutions above and at least one dilution below the titer cut point. The TQC serial dilutions were assayed on a total of 6 plates (Runs 24, 26, and 35-38), by 2 analysts over 3 days.

Acceptance Criteria

HQC, LQCs %CVs	%CV≤ 25.0.
NQC	results reported
TQC, Log3 (titer)	%CV results reported.
Confirmation assay LQC-C	%SI results reported
Singlicate titer assessment	≥ 80.0% of singlicate titer results must be within +/- 1 three-fold serial dilution of their corresponding duplicate titer value

Results
CC-normalized screening results of the NQC, LQC-C, LQC-S, and HQC were acceptable with intra- and inter-assay %CV ≤ 19.9

NQC intra- and interassay	%CV ≤ 5.4
LQC-C intra- and inter-assay	%CV ≤ 12.8
LQC-S	%CV ≤ 8.4
HQC intra- and inter-assay	%CV ≤ 19.9
Confirmation LQC-C %SI intra- and inter-assay	%CV ≤ 9.1

Interpolated TQC titer %CV ≤ 12.5

Singlicate titers 96.7% (29/30) values within ± one 3-fold dilution of the corresponding duplicate value

The Hamilton Star automated dilution results were somewhat better than those from manual dilutions; i.e.
CC-normalized screening results of the NQC, LQC-C, LQC-S, and HQC were acceptable with
intra- and inter-assay %CV ≤ 11.1
NQC intra- and interassay %CV ≤ 3.7
LQC-C intra- and inter-assay %CV ≤ 3.9
LQC-S intra- and inter-assay %CV ≤ 11.1
HQC intra- and inter-assay %CV ≤ 5.7
Singlicate titers 100% (29/30) values within ± one 3-fold dilution of the corresponding duplicate value

Reviewer comments
The IgG4 assay precision is acceptable, by and large showing relatively low %CVs, well within industry experience for ADA assays, and consistent with the 2016 FDA guidance for immunogenicity methods. It is noteworthy that because the Hamilton Star results show higher precision than manual methods, the Sponsor may wish to employ this automated methodology in future method development.

QC Acceptance Ranges
The QC results from the 39 accepted manually prepared validation plates used for precision calculations were used to calculate HQC, LQC-C and NQC acceptance ranges to demonstrate system suitability during sample testing. The QC results from the 8 manually prepared validation plates used for precision calculations were used to calculate LQC-S acceptance ranges to demonstrate system suitability during sample testing.

Table 15.12: Calculation of QC Acceptance Ranges

	HQC	LQC-C	NQC	LQC-S
Mean RLU/CC	2252	3.32	0.956	1.74
SD	448	0.424	0.052	0.147
N	85	86	85	24
t stat	2.64	2.63	2.37	2.81
upper 99%	3434	4.44	1.08	2.15
lower 99%	1070	2.20	NA	1.33

A) The upper and lower 99% confidence intervals for the means of all accepted normalized HQC, LQCs, and NQC measurements in this validation were calculated as mean RLU/CC ± t stat*SD, where the t statistic associated with a = 0.01 and degrees of freedom for each N.

Reviewer comments

The acceptance ranges for normalized RLU/CC values are based on 99% confidence intervals, and are therefore are rather broad, with more than two-fold differences between the low and high limits for HQC, LQC-C, and LQC-S. To provide improved assay control, I recommend that in future studies the Sponsor narrow these ranges, perhaps using 95% confidence intervals.

Limit of Detection/Sensitivity

To prepare LOD control samples, surrogate IgG4 conjugate PC was added to NHPS at 10000 ng/mL. LOD control samples were serially diluted 1:3 in NHPS at least 7 times to generate an 8-point dilution series. Each sample from the dilution series was then diluted 1:20 in Blocker Casein in PBS and assayed with the titer method. At least 3 dilution series were prepared and assayed per plate. A total of 6 plates were analyzed by 2 analysts over 3 days. The mean interpolated concentration was 18.3 ng/mL, ranging from 9.09 to 27.8 ng/mL.

Reviewer comment

The sensitivity of the assay is high at 18.3 ng/ml, and well within the recommendations of the FDA 2016 Guidance for Immunogenicity Assays.

Selectivity

This phase of the validation used normal individual serum samples that did not contain pre-existing anti-BMN 165 total antibodies based on data from the cut point analysis and assay development. Ten individual drug-naïve serum samples (equal number of normal males and normal females), 4 visually lipemic serum samples (high and low lipemic levels, and 50/50 male and female), and 4 hemolytic serum samples were prepared un-spiked and spiked with reactive serum at the LQC-S and LQC-C reactivity levels

The sponsor found that 100% of un-spiked individual screened negative on 2 of 2 LQC-S selectivity runs. Although two unspiked individuals screened positive in 2 of 2 LQC-C selectivity runs these individuals were negative in the confirmatory assay. All (4/4) spiked hemolytic individuals screened positive in both LQC-S and LQC-C selectivity runs, but in addition, two unspiked hemolytic samples screened positive. Similarly, all (4/4) spiked lipemic samples were positive in both LQC-S and LQC-C selectivity runs, but one unspiked lipemic sample also screened positive.

Reviewer comments

For the selectivity assessment in normal serum, appropriately none of the unspiked samples confirmed positive. However, some unspiked lipemic and hemolytic samples also screened positive, indicting the importance of confirming screening positive signals for hemolytic or lipemic samples.

Specificity

To characterize the specificity of the assay, free PEG was added at increasing concentrations to pooled serum at the LQC-C, LQC-S and NQC levels and assayed with the screening step. The results met the acceptance criteria: 100.0% of samples spiked with free PEG screened positive (screening RLU > SCP). The highest neat concentration of free PEG at which the LQC-C screened positive was 1000 ng/mL. The highest neat concentration of free

PEG at which the LQC-S screened positive was 1000 ng/mL.

Reviewer comment

The IgG4 assay is not affected by free PEG at 1 µg/ml , which is relevant to drug tolerance, since this is a pegylated product.

Drug tolerance

	NHPS	LQC-C (low reactive serum)	LQC-S (low reactive serum)
BMN 165 Neat Concentration	500000 ng/mL	500000 ng/mL	500000 ng/mL
	250000 ng/mL	250000 ng/mL	250000 ng/mL
	100000 ng/mL	100000 ng/mL	100000 ng/mL
	10000 ng/mL	10000 ng/mL	10000 ng/mL
	1000 ng/mL	1000 ng/mL	1000 ng/mL
	100 ng/mL	100 ng/mL	100 ng/mL
	0 ng/mL	0 ng/mL	0 ng/mL

The highest neat concentration of BMN 165 at which the LQC-S sample screened positive was 1 µg/ml. All LQC-C samples spiked with BMN 165 screened positive. The highest concentration of BMN 165 at which the LQC-C sample screened positive was 500 µg/mL.

Reviewer comments

As per communication from Christine Hon, Clinical Pharmacology reviewer for this BLA, in Study 165-302 the BMN165 trough concentration varied widely from 0.075 to 132 µg/ml. Thus at low trough BMN165 concentrations, the IgG4 assay will not be inhibited. However, at high (132 µg/ml) trough concentrations, low IgG4 ADA levels will not be detected, but high IgG4 ADA levels will still be detectable.

Given these drug tolerance findings, an IgG4 ADA result for a given serum sample will need to be considered carefully in the context of the BMN165 concentration in that sample. This observation was communicated to the Clinical Pharmacology reviewer.

Robustness

Three sets of QCs (HQC, LQCs and NQC) were assayed, each loaded in duplicate wells per condition for the Minimum and Maximum incubation times specified in the table below. Each condition was tested on 2 plates by 1 analyst on 1 day. The assay performance met the robustness acceptance criteria with 100% of QC signals falling within the acceptance ranges.

Reviewer comment

Adequate robustness as a function of incubation time has been demonstrated.

Stability

Bench top stability

HQC, LQCs, and NQC samples were prepared and aliquoted for one time use. At least 3 aliquots of each QC level were thawed and left at RT and 2-8C for at least 24 hours and RT for 24 hours

Freeze thaw

HQC, LQCs, and NQC samples were frozen at -60 to -80°C for at least 12 hours after preparation. Three sets of stability QCs were removed and thawed at room temperature for four (4) hours (+/- 10 minutes). Samples were then returned to -60 to -80°C for ≥ 12 hours. The process was repeated as needed to generate up to 8 F/T cycles.

Diluted sample stability

Three sets of HQC, LQC-C, and NQC were diluted in Assay Diluent to the MRD and stored for a total of 24, 48, and 96 hours at 2 to 8°C prior to running in the assay. Three sets of TQC serial dilutions were performed according to the draft method, and stored for a total of 24, 48 and 96 hours at 2 to 8°C prior to running in the assay. The maximum time (96 hr.) stability test was run once by 1 analyst, using the screening protocol. Each plate also contained freshly-thawed QC samples for evaluating system suitability for stability plates.

Results

All the QC results (100%) met the acceptance criteria for storage at RT for up to 24 hours at 2-8°C for up to 24 hours and up to 8 F/T cycles. One hundred % of the HQC and LQC-C samples and 2/3 of the NQC samples that were diluted and stored for 96 hours at 2 to 8°C met the acceptance criteria, including screening and titer assay samples. LQC-S 96 hour benchtop stability was not performed as the titer at and below the LQC-S level was shown to be stable for that same time period. During sample testing, samples may be considered stable for up to 24 hours at RT and 2 to 8°C, up to 8 F/T cycles, and samples may be pre-diluted and stored at 2 to 8°C for up to 96 hours before testing.

Reviewer comments

The Sponsor has demonstrated stability across the important range of conditions necessary to routinely conduct the IgG4 assay; i.e. room temperature or bench top conditions, 8 freeze-thaw cycles, and for the QCs stored up to 96 hours at 2 to 8°C.

Immunodepletion of IgG and IgM Abs in Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\21120-5589\21120-5589.pdf
This is a method to deplete IgG and IgM antibodies that potentially interfere with antigen-specific IgE detection in human serum samples, while retaining IgE antibodies, and was validated to support analysis of drug-specific IgE concentrations in human serum samples.

Table 1. Validation Results Summary

Performance Characteristic	Acceptance Criteria	Validation Results
Intra-Assay and Inter-Assay Precision	**For intra-assay precision:** The mean % IgG depletion and % IgM depletion must have a CV of ≤ 20% between the respective replicates within each run. Additionally, the mean % IgE recovery must have a CV of ≤ 20%. **For inter-assay precision:** The CVs for % IgG depletion, % IgM depletion, and % IgE recovery from all runs must be ≤ 20%.	**The intra-assay precision ranges for the quality control was determined to be:** % IgG depletion: 0.45 to 0.74% % IgM depletion: 4.98 to 7.68% % IgE recovery: 1.02 to 7.01% **The inter-assay precision for the quality control was determined to be:** % IgG depletion: 1.47% % IgM depletion: 9.40% % IgE recovery: 6.90%
QC Acceptance	The QC acceptance limits will be reported.	The lower 99th CL was determined as the acceptance limit for the quality control and was determined to be: % IgG depletion: 92.0% % IgM depletion: 37.3% % IgE recovery: 83.2%
Selectivity	At least 80% of normal individual sera must have≥ 90% IgG depletion, ≥ 20% IgM depletion, and ≥ 90% IgE recovery.	100% of normal individual serum samples had % IgG ≥ 90% and % IgM depletion ≥ 20%. 50% of samples had ≥ 90% IgE recovery. The mean IgE recovery across 10 individuals was 84%. 90% of individual samples tested (9 of 10) had IgE recovery > 50%.

Table 6: Concentration of IgE (kU/L)				
Run Date	**1/30/2017**	**1/30/2017**	**2/6/2017**	**2/6/2017**
Replicate	**Run#1**	**Run#2**	**Run#3**	**Run#4**
1	299	267	297	251
2	274	318	299	274
3	279	289	ND[1]	284
4	265	302	293	267
5	273	315	293	273
Non-depleted Control	303	292	280	274

[1]Instrument error; sample could not be pipetted on the ImmunoCAP 1000.

Table 7: % IgE Recovery Intra- and Inter Assay Precision					
Run Date	**1/30/2017**	**1/30/2017**	**2/6/2017**	**2/6/2017**	
Replicate	**Run#1**	**Run#2**	**Run#3**	**Run#4**	
1	98.7	91.4	106.1	91.6	
2	90.4	108.9	106.8	100.0	
3	92.1	99.0	NC	103.6	
4	87.5	103.4	104.6	97.4	
5	90.1	107.9	104.6	99.6	
Mean	91.7	102.1	105.5	98.5	99.1
SD	4.21	7.16	1.07	4.44	6.84
%CV	4.59	7.01	1.02	4.50	6.90
Intra-Assay Precision					Inter-Assay Precision

NC: Not Calculated

Reviewer comments

This method is highly efficient at depleting IgG in serum samples (mean 92%) which is important, since IgG is the most abundant antibody in serum, with expected concentration ranges of 7-16 mg/ ml in adults (http://emedicine.medscape.com/article/2157901-overview). The method is only (37.3 %) efficient for removing IgM, but adult serum concentrations are only in the range 0.4-2.3 mg/ml, so the less efficient depletion of IgM is expected to bring the concentration of remaining IgM into the same range as the post-depletion concentration of IgG. Given these considerations, the depletion method is likely to improve detection of IgE by substantially reducing potentially interfering antibodies. Moreover, in precision validation runs, recovery of ~ 300 lU/L IgE (720 ng/ml), was very high, with a mean of 99.1% for 99% CV value of 83.2%).

Selectivity

Table 11: Selectivity - % IgE Recovery			
Sample #	Non-Depleted (kU/L)	Depleted (kU/L)	%IgE Recovery
BRH867908	11.7	12.4	106.0
BRH867917	26.0	28.0	107.7
BRH867920	264	225	85.2
BRH867922	163	115	70.6
BRH867928	857	892	104.1
BRH867929	6.23	4.51	72.4
BRH867930	76.3	23.9	31.3
BRH867931	4.08	2.57	63.0
BRH867932	279	288	103.2
BRH867933	133	125	94.0

Table 12: Selectivity - % IgE Recovery in Pre-Validation			
Sample #	Non-Depleted (kU/L)	Depleted (kU/L)	%IgE Recovery
BRH867908	12.5	13.2	105.6
BRH867929	7.85	6.16	78.5
BRH867930	87.7	20.6	23.5
BRH867933	153	183	119.6

Reviewer comments

Most (9/10) samples gave good IgE recovery, supporting the view that the IgG/IgM depletion method in most cases allows adequate detection of IgE by the subsequently employed ImmunoCAP specific IgE assay. However, one sample (BRH867930) had most of the IgE depleted; i.e., 31.3% recovery in validation, and 23.5% recovery in pre-validation. This finding together with the BMN165 drug inhibition at low IgE levels (discussed below in the section for ImmunoCAP to Detect anti-BMN165 IgE Ab in Human Serum) creates a concern that occasionally samples may lose most of their IgE during the IgG/IgM depletion, and that the resulting low IgE levels may be subject to further loss of signal resulting from BMN165 assay inhibition.

RAST to Detect anti-BMN165 and anti-PAL IgE in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\ps-2556-1vr\ps-2556-1vr.pdf
Phase 2, 3
The RAST limit of detection, based upon calibration with standard sera from atopic patients reactive to a specific allergen such as rye grass, is 0.35 KU/L. This high sensitivity is in the range of serum IgE levels.

Specificity
The specificity of the discs was assessed by preparing discs with biotinylated PAL, PAL-PEG, or an unrelated biologic CTX, and incubated with anti-PAL or anti-PEG, followed by detection of bound antibody using alkaline phosphatase conjugated anti-rabbit IgG, and the signal was then provided as OD generated by an alkaline-phosphatase detection reagent. The specific anti-PAL or anti-PAL-PEG signal was 2 to 3 fold higher than the non-specific signal.
Reviewer comments
This demonstrates the expected specificity for a RAST assay.

Precision
To calibrate the assay in order to determine correlation of assay signal with KU/L of a standard antigen (allergen), (b) (4) used rye grass allergen discs incubated with allergic patient sera followed by detection of bound IgE with radiolabeled rabbit IgG specific for human IgE, and obtained a %CV =12.8%. Because there was no positive control IgE specific for pegvaliase, precision of PAL and PAL-PEG discs was first evaluated with surrogate IgG antibodies by incubating with either radiolabeled anti-PAL IgG, or radiolabeled anti-PAL-PEG IgG as detection reagents. By this method, the PAL disc % CV was 19.88 %, and for the PAL-PEG disc, 10.49%. To further assess PAL and PEG-PAL disc precision, discs were incubated with alkaline-phosphatase conjugated IgG anti-PAL or IgG anti-PEG-PAL, followed by detection of bound IgG with an alkaline phosphatase detection reagent. The %CVs for precision using these surrogate reagents was 2.329% for PAL discs, and 4.539% for PAL-PEG discs.
Reviewer comments
The precision evaluations all met a pre-set target of CV < 20%, which is a common acceptance criterion for precision evaluations. Thus, the assays have adequate precision. The sponsor needs to clarify if a rye grass standard was used as a system suitability control in all runs of patient samples.
AGENCY QUESTION 5
Regarding the RAST IgE assay (ps-2556-1vr):
a. Clarify if a rye grass standard was used as a system suitability control in all runs of patient samples.
BIOMARIN RESPONSE 5
On all runs of patient samples, system suitability for the RAST IgE assay was assessed using ragweed antigen-coupled RAST discs matched with serum containing high or low levels of anti-ragweed IgE as positive controls, or 5% normal goat serum as a negative control. The standard calibrator curve used to quantify IgE concentrations on each run was calculated using perennial rye grass-coupled RAST discs matched with anti-perennial rye grass IgE calibrator samples.

Reviewer comment
As requested, the Sponsor has confirmed that the rye grass standard is used as a system suitability control, and thus this response is adequate.

Cutpoints
Serum samples from 50 normal donors were incubated with PAL or PAL-PEG discs, followed by detection of bound IgE with radiolabeled rabbit IgG with human IgE specificity. The following values were obtained:

	PAL dics	PAL-PEG discs
Mean	0.23 KU/L	0.11 KU/L
SD	0.23 KU/L	0.04 KU/L
1.65 x SD	0.61 KU/L	0.176 KU/L

Reviewer comments
These cutpoints are consistent with the high sensitivity required for IgE assays. It is notable that throughout the validation, the PAL-PEG discs performed better than the PAL discs in terms of precision and background, potentially because of better binding of the larger biotinylated PAL-PEG molecule, as opposed to the smaller, non-pegylated PAL protein.

Sensitivity
Control serum from rye grass allergic patients was diluted to a nominal 0.1 U/L concentration, and was found to give a radiolabeled signal two-fold higher than a blank sample.
Reviewer comments
This result represents a lower limit of detection (LLOD) for the system. However (b) (4) *consistent with the cutpoint evaluations, used > 0.35 KU/L as a criterion for positive samples, as shown in Table 13 below.*

Effect of high total IgE
(b) (4) performed further evaluation of assay specificity using samples from 5 atopic donors having total IgE levels ranging from 669 to 4214, well above the 100 KU/ L IgE threshold for atopy. With PAL discs, all 5 donors had signals above the standard 0.35 KU/L cutpoint, and 2 out of 5 had positive signals with PAL-PEG discs. Inhibition studies were performed adding 0, 1, 5, or 10 µg /disc PAL or PAL-PEG protein. None of the atopic samples were inhibited by either protein. Given these results, (b) (4) recommended measuring total IgE from pegvaliase-treated patient samples, performing inhibition studies for confirmation, with the confirmation algorithm summarized in the following table:

Table 13: How to determine a positive for the confirmation assay.

Uninhibited Result (in kU/L)	Positive Inhibition (>85%)	Negative Inhibition (<15%)	Partial Inhibition (15-85%)	Difference = Uninhib - Inhib	Final Result To Report	Interpretative Comment
>0.35	Yes			>0.35	Positive	Specific IgE detected
>0.35		Yes		NA	Negative	No significant specific IgE detected
>0.35			Yes	>0.35	Positive	Specific IgE detected
>0.35			Yes	<0.35	Negative	No significant specific IgE detected

Reviewer comments

These recommendations are consistent with standard practice for confirmatory immunogenicity assays.

ImmunoCAP to Detect anti-BMN165 IgE Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\21120-4767\21120-4767.pdf
Phase 3
Validation complete
Sensitivity 0.1 kU/l corresponding to 0.25 ng/ml IgE
This assay was validated by (b) (4) in 2016. ImmunoCAP is a proprietary test developed and marketed by Phadia. A solid phase is used for allergen immobilization, binding of IgE, and detection. The ImmunoCAP solid phase consists of a cellulose derivative enclosed in a capsule. The allergen is covalently coupled to the solid phase, and then the capsule is incubated with patient serum or controls. After washing away non-specific IgE, β-galactosidase-labeled mouse monoclonal antibodies against human IgE are added to form a complex. After incubation, unbound enzyme-anti-IgE is washed away and the bound complex is then incubated with the ImmunoCAP® Development Solution containing 4-methylumbelliferyl-β-D-galactoside, a β-galactosidase fluorogenic substrate. After stopping, the IgE-anti-IgE complexes are eluted from the capsule, and the fluorescence of the eluate is measured in a fluorimeter to quantitate allergen specific IgE. ImmunoCAP is regarded as superior to RAST, since the capsule has a high allergen binding capacity relative to the RAST paper discs, and can be washed more effectively. The accepted sensitivity is 0.1 KU/L or 0.1 kAU/L, where A represents allergen-specific antibodies.

Positive Controls
Because there was no anti-PAL human IgE control available, a surrogate positive control (PC) antibody, from ThermoFisher Scientific/Phadia was validated. This surrogate PC, which is comprised of affinity purified PAL-specific rabbit polyclonal IgG antibody conjugated with human myeloma IgE, was used as high and low positive controls during clinical study sample testing.

The QCs are as follows:
HQC 1.7 kAU/L
LQC 0.4 kAU/L
NQC < 0.1 kAU/L
Reviewer comment
The LQC is appropriately set near the 0.1 kAU/L limit of detection.

Sensitivity
Limit of Detection (LOD)
(b) (4) analyzed 50 drug naïve human serum samples in singlicate in one ImmunoCAP® 1000 run. The LOD, which was calculated as the mean response units (RU) + 2 standard deviations (SD), was determined to be 18 RU. For this run, 18 RU fell below the reportable range (<0.10 kUA/L) since for this run the assay sensitivity of 0.1 kUA/L corresponded to a RU value 19.

Lower Limit of Quantitation
The LLOQ was evaluated by spiking positive control into Normal Human Serum Pool at a target concentration of the HQC (1.7 kUA/L), and then nine 1.5-fold serial dilutions were assayed in

triplicate and evaluated over three independent runs. LLOQ was defined as the lowest concentration of analyte, (in kUA/L) with a CV ≤ 25.0%. At the lowest concentration (91.04 ng/mL, all 9 replicates were close together (25-30 RU) , as seen by a % CV of 6.92%. This dilution would actually correspond to a kU/L value less than 0.1 kU/L. However, LLOQ for ImmunoCAP has been determined to be 0.10 kUA/L by the manufacturer Phadia. Thus, even though RU values for this assay were precise below 0.10 kUA/L, the lower limit of acceptable precision was somewhat conservatively set at 0.10 kUA/L.

Reviewer comments

The sensitivity has been shown to be precise, even below the designated sensitivity of 01. kAU/L, and is thus totally appropriate for a specific IgE assay.

Screening cutpoint

Sera from 50 drug-naïve healthy donors (25 male and 25 female donors) were assayed across two runs. Normal distributions of the data were not established even after exclusion of 4 outliers each from both runs, so an empirical 95th percentile was used to determine the screening cut-point for both runs, resulting in an average screening cut-point of 15 RU, which, if converted into kUA/L, would be <0.10 kUA/L

Reviewer comments

I have examined the tabulated data for the screening CP and the (b) (4) *statements are accurate. This CP is appropriate because it is very low and in agreement with the 0.1 kU/L lower limit of quantitation, and the ImmunoCAP manufacturer's determination of sensitivity*

Confirmatory cutpoint

The confirmation cut-point was established with fifty replicates of serum samples from 50 healthy donors spiked with positive control at the level of the LQC (0.40 kUA/L). Additionally, 10 replicates of serum samples from 10 healthy donors were spiked with positive control at the level of HQC (1.7 kUA/L). HQC and LQC-spiked samples were analyzed in duplicate across one run, where 500 µg/mL of either BMN165 (inhibited) or PBS-AF (uninhibited) was spiked into a respective replicate. Even though inhibition could be obtained with lower BMN165 concentrations, (b) (4) used 500 µg/mL out of concern for potential interference from serum anti-BMN165, which could bind to BMN165 and prevent inhibition, leading to false negatives. The %SI data from HQC-spiked samples inhibited with BMN165 was not normally distributed based on the Shapiro-Wilk test of normality even after the removal of one identified outlier, while %SI data from LQC-spiked samples inhibited with BMN165 was normally distributed based on the Shapiro-Wilk test of normality after the removal of one outlier.

(b) (4) calculated cutpoints based on a lower limit of the 99th confidence interval. Spiking with and without BMN165 at the level of the HQC (1.7 kUA/L) and LQC (0.40 kUA/L) produced confirmation cut-points of 80.15% and 66.88%, respectively. (b) (4) and Biomarin chose to use the more conservative 66.88% inhibition CP in order to minimize false negatives.

As an aside, (b) (4) investigated the sample from one individual that screened positive and confirmed negative. This individual was found to have very high total IgE, which may have interfered with the confirmatory assay.

Reviewer comments

(b) (4) *has performed extensive analysis using inhibition of high and low positive controls, and performed appropriate statistical analysis to obtain 80.15% and 66.88% cutpoints based upon inhibition of the HQC and LQC , respectively. They have then conservatively decided to use the 66.88% CP in confirming positive samples. I agree with this approach because it will minimize false negatives and is consistent with the typical inhibitions seen in confirmatory assays.*
It is noteworthy that high total IgE may interfere with the assay, and this should be measured in subjects who are being tested for BMN165 specific IgE.

Precision
The intra-assay precision for the HQC ranged from 4.59 to 12.37% across the 6 runs, while the inter-assay precision for the HQC was 12.27%. The intra-assay precision for the LQC ranged from 7.89 to 15.02% across the 6 runs, while the inter-assay precision for the LQC was 12.65%. The intra-assay precision acceptance criterion for the LQC was a CV of ≤15.0%. All but one of the runs met this criterion, and this run had an intra-precision very close to 15.0% (i.e. 15.02%) which, based on scientific judgement, was considered acceptable.
Reviewer comments
The precision of the assay is acceptable because it is relatively tight, with %CV at or below 15%, well below the 25% acceptance criterion often used for immunogenicity assay precision.

Acceptance Ranges for HQC and LQC
Precision data was used to calculate the ±2SD and ±3SD control ranges for the HQC and LQC. The control ranges were established as the mean kUA/L ± 2SD and the mean kUA/L ± 3SD for each positive QC. Additionally, all NQC data collected were <0.10 kUA/L. The established control ranges are reported in Table 12 and will be used for sample testing as system suitability criteria. Furthermore, NQC values must be <0.10 kUA/L, 1 of the 2 positive controls should be within ±2SD and the 2nd control within ±3SD, and when confirmation testing is performed, the LQC-C must generate %SI values ≥66.88% or signals <0.10 kUA/L for inhibition with BMN165.

Table 12: QC Acceptance Ranges

	HQC (kUA/L)	LQC (kUA/L)
Mean (n = 18)	1.69	0.39
Upper 2SD	2.11	0.49
Lower 2SD	1.28	0.29
Upper 3SD	2.32	0.54
Lower 3SD	1.07	0.24

Reviewer comments
From the data in table 12, allowing one of two HQC or LQC replicates to be as much as 3 SD from the established mean appears to allow an excessively broad controls for system suitability. In order to support confidence in the reproducibility of this IgE method, the sponsor should provide the ranges of HQC and LQC from the actual tests used to assess patient samples.

AGENCY QUESTION 6
Regarding the ImmunoCAP anti-BMN165 IgE assay (21120-4767):
a. From the data for the suitability of the High Quality Control (HQC) and Low Quality Control (LQC) in Table 12 of the validation report, allowing one of two

HQC or LQC replicates to be as much as 3 Standard Deviations from the established mean appears to allow an excessively broad range of controls for system suitability. In order to support confidence in the reproducibility of this IgE method, provide the ranges of HQC and LQC from the actual tests used to assess patient samples.

BIOMARIN RESPONSE 6A

To support confidence in the reproducibility of the ImmunoCAP anti-BMN165 IgE method, system suitability control sample data from validation and sample testing phases were compiled (Table 6.1). Inter-assay precision of HQC and LQC results were good both in validation (HQC: 12.3% CV, LQC: 12.7% CV; Table 6.2) and across clinical sample testing runs (HQC: 6.8% CV, LQC: 7.4% CV). These results demonstrate acceptable assay reproducibility.

Reviewer comments

The actual ranges of HQC and LQC were ≤ 12.7% CV, indicating good reproducibility during the testing of clinical samples.

b. In order to support potential further studies, you should assess the long-term storage stability and freeze/thaw stability of the original anti-BMN165 hybrid IgE-IgG control antibody.

BIOMARIN RESPONSE 6B

The long term stability of the anti-pegvalise IgE surrogate positive control was evaluated during assay development with an accelerated stability study. The accelerated stability analysis demonstrated that the control was stable for at least 8 weeks at +30 ^{0}C (Table 6.3), corresponding to storage stability of 2 years at 2 to 8 ^{0}C. Furthermore, the surrogate positive control was used to prepare quality control samples in normal serum for the anti-PAL IgE ImmunoCAP assay performed at (b) (4) lab; these samples were run in the assay over a period of 19 months. Analysis of the QC sample assay results over time demonstrates consistent results over 19 months (Figure 6.1), demonstrating long term frozen stability of the surrogate positive control in human serum for at least 19 months. We acknowledge your request to evaluate freeze/thaw stability of the surrogate positive control for future studies, and highlight that the assay supporting the BLA submission involved single-use aliquots of the control antibody that were not subject to repeated freeze/thaw cycles.

Reviewer comments

The actual storage practice during analysis of the clinical samples does not appear to have impacted results, and Biomarin has acknowledged that freeze/thaw stability validation may be important for further studies.

c. Provide an adequate rationale for why anti-PAL IgG, instead of anti-BMN165 IgG, was used in this IgG interference study. The use of anti-BMN165 IgG would have been more appropriate, since this validation is for detection of IgE with reactivity to the entire BMN165 molecule.

BIOMARIN RESPONSE 6C

The reagent used for assessment of IgG interference in the anti-BMN165 ImmunoCAP assay method validation contains both anti-PEG and anti-PAL reactivity, and is therefore appropriate for assessment of interference from anti-BMN165 IgG. The reagent was

generated by immunizing rabbits with pegvaliase, followed by enrichment of anti-PAL antibodies in the resulting pooled serum by liquid chromatography using PAL-coupled sepharose. The reagent was referred to as 'anti-PAL IgG', but also contained anti-PEG activity, despite enrichment of anti-PAL activity

Reviewer comments

The Sponsor has clarified that the reagent actually has reactivity to PEG and PAL, and was thus appropriate use in this assay validation.

Stability of the HQC and LQC

Two replicates of each QC level were assayed after storage for 6 and 24 hours at room temperature and at 2-8°C. QCs were prepared as single use aliquots and therefore freeze/thaw stability was not assessed in this validation. One set of validation QCs (HQC, LQC, and NQC) was run with the stability QCs and all passed system suitability criteria established during this validation. For each stability condition tested, the screening assay kUA/L from each QC sample was within the QC acceptance range established during validation. Additionally, all QCs had intra-duplicate CVs of ≤ 20.0%.

Reviewer comments

I have examined the tabulated stability data and find that it does show high precision, with %CVs ranging from 4.19 to 11.16%, and only modest changes from QCs prior to stability testing.

In the 11/15/2017 IR from the Agency, Biomarin was asked to address the stability of the HQC and LQC;i.e.

B) In order to support potential further studies, you should assess the long-term storage stability and freeze/thaw stability of the original anti-BMN165 hybrid IgE-IgG control antibody.

Biomarin provided the following response:

"The long term stability of the anti-pegvalise IgE surrogate positive control was evaluated during assay development with an accelerated stability study. The accelerated stability analysis demonstrated that the control was stable for at least 8 weeks at +30 ^{0}C (Table 6.3), corresponding to storage stability of 2 years at 2 to 8 ^{0}C. Furthermore, the surrogate positive control was used to prepare quality control samples in normal serum for the anti-PAL IgE ImmunoCAP assay performed at (b) (4) lab; these samples were run in the assay over a period of 19 months. Analysis of the QC sample assay results over time demonstrates consistent results over 19 months (Figure 6.1), demonstrating long term frozen stability of the surrogate positive control in human serum for at least 19 months. We acknowledge your request to evaluate freeze/thaw stability of the surrogate positive control for future studies, and highlight that the assay supporting the BLA submission involved single-use aliquots of the control antibody that were not subject to repeated freeze/thaw cycles."

Reviewer comments

Biomarin has provided adequate assurance that long-term stability has been evaluated, and that only single-use aliquots were used, obviating the need for freeze-thaw studies.

Selectivity

For selectivity determination, 10 normal, 2 lipemic, and 2 hemolyzed sera were assayed. To each of these samples, 0 ng/mL (unspiked) and 850 ng/mL (LQC-spike) of anti-PAL PC was added to evaluate selectivity of the assay.

Reviewer comments

The two hemolytic samples were modestly reduced to ~74% of the mean of the 10 normal samples, and the lipemic sample signal was as high or higher than the normal mean. Thus, this small data set indicates that hemolysis and lipid content do not seriously affect the assay signal.

Specificity of the BMN165-specific ImmunoCAP

Five serum samples that contained moderate to highly elevated levels of total human IgE were assayed. All serum samples with varying levels of total human IgE screened lower than the screening cut-point of 0.10 kUA/L suggesting that elevated levels of total IgE in samples do not interfere with the detection of BMN165-specific antibodies.

Reviewer comments

The Sponsor's analysis supports the view that high levels of total IgE, as might be seen in atopic individuals, do not generate false positive signals.

PAL-specific IgG interference on the detection of the HQC and LQC

Purified rabbit polyclonal anti-PAL IgG antibody reagent was used to assess potential interference associated with anti-BMN165 IgG antibodies in study samples on the detection of BMN165-specific IgE. Human serum spiked with PC near the level of the HQC (1.7 kUA/L) and LQC (0.40 kUA/L) were assayed in the presence of increasing rabbit anti-PAL IgG concentrations (0, 1, 10, 50, and 100 µg/mL) diluted in PBS-AF. At 100 µg/ml anti-BMN165 IgG the HQC signal was reduced to 65% of the untreated signal, while the LQC was reduced to 60% of the untreated signal. The inhibition of the HQC and LQC in the confirmatory assay was unaffected.

Reviewer comments

These data indicate that patient samples with high anti-PAL IgG levels may be susceptible to loss or at least impaired quantitation of anti-BMN165 IgE, and point to the utility of employing the IgG/IgM depletion method developed by (b) (4) *for the sponsor and used in the testing of samples. The IgG/IgM depletion method is discussed on page 66 of this review.*

In addition, the Sponsor should explain why anti-PAL IgG, instead of anti-BMN165 IgG, was used in this interference study. The use of anti-BMN165 IgG would have been more appropriate ,since this validation is for detection of IgE with reactivity to the entire BMN165 molecule. BMN165 has the PEG conjugate, which has high immunogenicity.

In their December 15, 2017 response to the Agency's 11/15/2017 IR, Biomarin addressed this issue;

" The reagent used for assessment of IgG interference in the anti-BMN165 ImmunoCAP assay method validation contains both anti-PEG and anti-PAL reactivity, and is therefore appropriate for assessment of interference from anti-BMN165 IgG. The reagent was generated by immunizing rabbits with pegvaliase, followed by enrichment of anti-PAL antibodies in the resulting pooled serum by liquid chromatography using PAL-coupled sepharose. The reagent was referred to as 'anti-PAL IgG', but also contained anti-PEG

activity, despite enrichment of anti-PAL activity."

Reviewer comments

The Sponsor has provided an adequate rationale for the use of this reagent, since it should have both anti-PAL and anti-PEG reactivity

Drug Tolerance

Drug tolerance was evaluated using normal human pooled serum spiked with PAL IgE LQC (0.40 kUA/L) and HQC (1.7 kUA/L). Increasing concentrations of BMN165 drug (0, 0.125, 0.25, 2.5, 25, 250, and 500 µg/mL) were added to each level of PC-spiked samples. The HQC were inhibited as follows:

BMN165 concentration µg/ ml	HQC inhibition	LQC inhibition
0	0	0
0.125	10.03	0
0.25	5.6	3.9
2.5	33.63	27.27
25	65.49	66.23
250	85.84	Below LOD
500	88.79	Below LOD

Reviewer comments

As per communication from Christine Hon, Clinical Pharmacology reviewer for this BLA, in Study 165-302 the BMN165 trough concentration varied widely from 75 to 132000 ng/ml (132 µg/ml). Considering the BMN165 inhibition shown going from 25 µg/ml to 250 µg/ml, high BMN165 trough concentrations may largely inhibit the IgE signal, and IgE results should thus be interpreted in the context of the measured BMN165 concentration at the time of antibody sampling. This concern was communicated to the Clinical Pharmacology reviewer, and it was determined on-board BMN165 was unlike to have affected the samples that were tested for IgE.

ImmunoCAP to Detect anti-PAL IgE Ab in Human Serum
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\21120-4768\21120-4768.pdf

Validated by (b) (4) 5/16/2016
Phase 3
Sensitivity 0.1 kU/L, corresponding to a high sensitivity of 0.24 ng/ml IgE
This assay is essentially the same as the ImmunoCAP anti-PMN165 IgE assay, except that it is validated to detect the PAL moiety of BMN165. As with the ImmunoCAP anti-PMN165 IgE assay, high BMN165 trough concentrations may also largely inhibit the IgE signal for the ImmunoCAP anti-PAL BMN165 IgE assay

Sensitivity
0.10 kUA/L
Reviewer comment
This sensitivity corresponds to a high sensitivity of 0.24 ng/ ml IgE, and is therefore acceptable.

Screening cutpoint
Clinical study samples generating results ≥0.10 kUA/L in the screening assay will be considered presumptive positive.

Confirmatory cutpoint
A false negative rate of ≤ 1% was targeted for the CCP. Samples generating %SI ≥ the CCP of 89.16% for PAL in the confirmation assay will be deemed positive for PAL-specific IgE antibodies. Additionally, samples that screen positive and confirm BLQ (Below Limit of Quantitation) in confirmation testing will also be deemed positive.

Precision

The intra-assay precision ranges for the various controls were determined to be:
HQC (kUA/L): 0.40 to 13.01%
LQC (kUA/L): 2.5 to 12.94%
LQC-C (RU): 7.14 to 31.17% (Refer to the intra-assay precision data)
LQC-C (%SI): 0.44 to 3.61%
NQC (RU): 0 to 24.75% (Refer to the intra-assay precision data)

The inter-assay precision ranges for the various controls were determined to be:
HQC (kUA/L): 7.94%
LQC (kUA/L): 7.26%
LQC-C (RU): 19.12%
LQC-C (%SI): 1.93%

Acceptance Ranges for HQC and LQC

The acceptance ranges for each positive QC level was determined to be:

HQC (kUA/L): 1.30 – 1.79[1], 1.18 – 1.91[2]

LQC (kUA/L): 0.33 - 0.44[1], 0.30 - 0.47[2]

[1] ± 2SD Range
[2] ± 3SD Range

Stability of the HQC and LQC

The stability of QC samples at each level (HQC, LQC, and NQC) under each of the following conditions: room temperature (6 and 24 hours) and 2-8°C (6 and 24 hours) had intra-duplicate CV ≤20.0% and fell within the acceptance ranges established within this validation study. Thus, the PC antibody in normal human serum pool is stable up to 24 hours both at room temperature and 2-8°C. Levey-Jennings trending analysis will be used to monitor long term frozen storage stability of the QCs. Data supporting stability of the QCs for the longest time period observed, and if relevant, suggesting loss of stability will be reported in an amendment to this validation report.

Selectivity

50% of the lipemic and hemolytic samples spiked at the LQC level had signals within the QC acceptance range. Hemolytic samples spiked at the LQC level had signals within the QC acceptance range. Hemolytic and lipemic samples will be noted.

Specificity

All serum samples (5) containing moderate to significantly elevated levels of total IgE had screening results lower than the screening cut-point of 0.10 kUA/L.

PAL-specific IgG interference

All samples containing either high or low concentrations of PC screened positive in the presence of up to 50 µg/mL of antibody. However, reduced signals were observed for both the low and intermediate concentrations of PC where the concentration (in kUA/L) was reduced by ≥55.0% in the presence of ≥50 µg/mL PAL-specific IgG.

BMN165 drug tolerance

All samples containing either high or low concentrations of PC screened positive in the presence of up to 250 µg/mL BMN165. However, a trend was observed where the signal

from the high and low concentrations of PC was reduced to greater than 40% in the presence of ≥25µg/mL of BMN165.

Reviewer comment

The assay signal may be reduced by on-board BMN165. This concern was discussed with Clinical Pharmacology reviewer Christine Hon, and it was determined that the results for patients who were tested for IgE likely were not affected by on-board drug.

MicroVue to Detect IgG C1q-CIC and C3d-CIC

\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\ \cvl072409.pdf

These assays were performed by the [redacted](b) (4), using kits from MicroVue.

Method summary

1. Controls and serum or plasma specimens are added to the C1q or C3 -coated microtiter wells and incubated. Immune Complexes (ICs) that can bind to C1q or C3 complex with C1q or C3-coated wells

Confirmation -sample diluted in high salt, which inhibits binding of CIC to C1q and then added to wells and incubated.

(wash)

2.HRP-conjugated goat anti-human IgG added to wells. HRP-conjugate binds to ICs bound to the C1q-coated wells

(wash)

3. Enzyme substrate added to wells. Bound HRP-conjugated antibody reacts with the chromogenic substrate to give a green color. After incubation, reaction is stopped and OD is read.

Normal range (established by the [redacted](b) (4) from 25 normal human serum samples)

Normal range defined as mean + 2 SD.

C1q-CIC Normal Range < 8.2 µg Eq/mL , does not confirm according to the assay confirmation protocol

C3d-CIC Normal Range: <36 µg Eq/mL , does not confirm according to the assay confirmation protocol

Cynomolgous monkey

Analysis on EDTA-Plasma samples from 44 healthy monkeys.

C1q-CIC Normal Range < 5.4 µg Eq/mL , does not confirm according to the confirmation protocol

C3d-CIC Normal Range: < 7.9 µg Eq/mL , does not confirm according to the confirmation review

Reviewer comment

It was critical to establish these normal ranges, which provide the equivalent of a cutpoint.

Precision

Intra-assay

Three controls were run a minimum of 17 times within one assay.

C1q-CIC	positive control	mean 21.5 µg Eq/mL, %CV 4.6 %
	negative control	mean 5.4 µg Eq/mL, %CV 8.3 %
C3d-CIC	positive control	mean 80.0 µg Eq/mL, %CV 3.1 %
	negative control	mean 9.9 µg Eq/mL, %CV 5.3%

Inter-assay

Values for a high, medium and low control were collected over at least a four-month period, over multiple independent assays.

C1q-CIC	High PC	mean 48.3 µg Eq/mL, %CV 11.2%
	Low PC	mean 4.6 µg Eq/mL, %CV 17.2%
	negative control	
C3d-CIC	High PC	mean 103.9 µg Eq/mL, %CV 4.4 %
	Low PC	mean 9.4 µg Eq/mL, %CV 21%
	negative control	

Reviewer comments

The precision of the assay standard measurements appear acceptable assuming a ≤ 25% CV acceptance criterion. However, no precision acceptance criteria are provided in this report. The low positive controls in the inter-assay precision data appear to be set below the mean of the normal human ranges, but much higher than the assay sensitivities. Moreover the validation report states that for the intra-assay precision, three controls were run, but results are only shown for positive and negative controls. Similarly, for the inter-assay precision the report states that values for a high, medium, and low control were collected, but results are only shown for high and low controls, with the low control values being similar to the negative control values in the intra-assay precision. The reports states that data analysis is included in Appendix A, but this appendix is not attached to the report.

The sponsor is being asked to correct these numerous omissions in the IC assay validation report (see IR below).

AGENCY QUESTION 7 (11/15/2017 IR)

Regarding the MicroVue assay to Detect C1q-CIC and C3d-CIC (cvl072409):

a. Clarify whether your acceptance criterion for precision is < 25% CV. If this is the case, the precision values of the assay standard measurements are acceptable. However, no precision acceptance criteria are provided in this report.

BIOMARIN RESPONSE 7A (12/15/2017)

The acceptance criterion for precision is < 25% CV. The acceptance criteria has been amended to the assay method validation report (CL072409, amendment 4).

b. The low positive controls in the inter-assay precision data appear to be set below the mean of the normal human ranges, but much higher than the assay sensitivities. Provide adequate justification for the low positive controls.

B) The low positive controls in the inter-assay precision data appear to be set below the mean of the normal human ranges, but much higher than the assay sensitivities. Provide adequate justification for the low positive controls.

BIOMARIN RESPONSE 7B

The IgG C3d-CIC assay low positive control appropriately monitors the assay at a CIC level that is both the average concentration expected in normal sera and the low end of concentrations induced by pegvaliase treatment. The IgG C3d-CIC assay was implemented to

measure circulating immune complexes as a biomarker that could potentially be associated with adverse events. The low control normal serum sample at 9.9 ugEq/mL monitors the assay between the mean and median CIC levels found in pre-treatment sera from study 165-301 (Figure 7.1). This level is appropriate, as CIC levels below the median of normal samples are not expected to be associated with adverse events. Eight weeks after initiation of pegvaliase treatment, only ~2% of 276 samples had CIC levels below the low control level (9.9 ugEq/mL, Figure 7.1). Therefore, the level of the low control is appropriate to monitor detection of CIC levels induced by pegvaliase that could potentially be associated with adverse events.

Reviewer comments

The Sponsor has provided a risk-based justification for the concentration of the low control, which is set so as to control reproducibility of the assay slightly below the level of normal CIC, noting that only above these normal levels would there be a safety concern. This justification is adequate, given that the function of the assay to monitor for a potential safety risk to the patients that would arise at levels CIC levels higher than normal.

c. The validation report states that for the intra-assay and inter-assay precision, three controls were run, but results are only shown for positive and negative controls. Provide results for all three controls.

BIOMARIN RESPONSE 7C

Utilization of three controls for precision studies is standard language used in validation templates that was included in error. Quality control samples at two levels were used to monitor assay performance in this validation. The report has been amended accordingly (CL072409, amendment 4).

d. The report states that data analysis is included in Appendix A, but this appendix is not attached to the report. Provide Appendix A.

BIOMARIN RESPONSE 7D

Appendix A was not correctly labelled in the original report. This oversight has been corrected in CL072409, amendment 4.

Reviewer comment

The responses for 7c and 7d are adequate-the requested information is now in the BLA.

Linearity

A sample of known value of 22.8 µg Eq/mL was designated as the high sample, and a dilution at 8.4 µg Eq/mL was designated as the low sample. These samples were then mixed as follows for linearity assessment:

High – 100% High Samples
Medium 1 – 75% High Sample: 25% Low Sample
Medium 2 – 50% High Sample: 50% Low Sample
Medium 3 – 25% High Sample: 25% Low Sample
Low – 100% Low Sample.

The report states that linearity assessment gave a slope of 1.044, with an intercept of 0, with an error of 4.4%.

Confirmation

If independent confirmation of a positive result is required, or if a positive result is inconsistent with the clinical interpretation, the positive specimen may be assayed using a confirmation test. A negative result cannot be confirmed. The confirmation method utilizes a specimen diluent (the Confirmation Diluent), which contains a high concentration of sodium chloride.
To confirm a positive result, an aliquot of the specimen must be diluted (1 :50 or 1 :200) in the Confirmation Diluent and a second aliquot diluted similarly in the (b) (4) Specimen Diluent. Both samples are then assayed according to the usual CIC-C1q assay procedures. See *REAGENT PREPARATION,* and *INTERPRETATION OF RESULTS* sections for details.

Confirmation Diluent **Part A9511** 2 each, 1 o ml
Contains PBS, 2.5% stabilizers, 1.2 M NaCl, 0.035% ProClin 300
Confirmation Test Calculation**.**
To confirm a positive result, the immune complex concentration [ClC] determined if the sample diluted in Confirmation Diluent is divided by the immune complex concentration measured in the sample diluted in (b) (4) Specimen Diluent to generate a ratio:
ratio= [CIC] in Confirmation Diluent

[CIC] in (b) (4) Specimen Diluent

Confirmation Results
If the ratio is less than 0.7, the positive CIC result is confirmed. In other words, greater than 30% reduction confirms a positive result
Reviewer comments
The attached brochures from MicroVue provide graphical plots of linearity and the associated R^2 statistic for the goodness of fit for the C1q and C3 assays, and the linearity looks very good.

Sensitivity
The minimum limit of detection (LOD) will be determined. The zero standard was measured over four months and independent assays by two different technologists.
C1q-CIC
Repeats: ..... 20 Assays
Mean: ..... 0.0856 OD
SD: ..... 0.013 OD
Resulting value ..... 0.4 µg Eq/mL
C3d-CIC
Repeats: ..... 20 Assays
Mean: ..... 0.073 OD
SD: ..... 0.005 OD

Resulting value ..... 0.3 µg Eq/mL
C1q-CIC LLOQ (from the MicroVue kit brochure) ..... 1.0 µg Eq/mL
C3d-CIC LLOQ (from the MicroVue kit brochure)..... 4.0 µg Eq/mL

Reviewer comments

The sensitivity (LLOQ) values from the MicroVue brochures are most relevant for considering the lower limits of the ability of the assay to detect ICs, and lower but consistent with the normal ranges of C1q and C3.

Specificity

The sponsor's validation report states that the manufacturer's testing of specificity was accepted for the validation. The Microvue C1q brochure states that 106 samples from normal, asymptomatic subjects were assessed, and the overall specific of the assay was 94%. Similarly, the Microvue C3 brochure states that 51 control sera were tested and only three were positive (repeatedly greater that 15 mg Eq/ml, yielding a 94% specificity.

Reviewer comments

These results are consistent with expectations for the cutpoint of a screening assay (~5% false positive rate). However they do not demonstrate specificity in the sense of the assays being specific for C1q or C3, as opposed to some other proteins.

Modified Quidel ELISA to detect IgM-C3d Immune Compexes
\\cdsesub1\evsprod\bla761079\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\im-val-028l-001\ im-val-028l-001.pdf

Assay description

The MicroVue CIC C3 (Raji Cell Replacement Enzyme Immunoassay) was redesigned to measure IgM CICs by replacing the anti-IgG HRP conjugate with anti-IgM HRP conjugate., and using heat-aggregated IgM as positive controls, instead of heat-aggregated IgG, as in the original Microvue assay. Results are expressed as μg of heat-aggregated IgM spiked into heat-treated serum (HIGM) equivalents per mL (μg Eq/mL). For internal quality control, a low and high dilution of the heat-aggregated IgM in serum will be tested on each run, to monitor variability of the control.

Accuracy

Accuracy was determined by testing serial dilutions of heat aggregated IgM spiked serum and analyzing them in triplicate. The mean, standard deviation and %CV of triplicate values was calculated. Percent error was calculated using the formula:
(Absolute difference (Expected conc. - calculated conc.)/expected conc.) X 100

The concentration of each individual sample point was interpolated using the average curve OD values and expected concentration (mcg HIGM Equivalents per milliliter). The %CV was then calculated based on the average and standard deviation of the three triplicate concentrations for each standard dilution. The % error was acceptable between concentrations of 60 and 0.74 μg Eq/mL. However, while the %CV was < 25% for the first four concentrations (60, 20, 6.7 and 2.22 μg Eq/mL, with % CVs ranging from 0.21 to9.88%), %CV was > 25% at a concentration of 0.74 μg Eq/mL and below. Taking this data into account, the assay was deemed accurate down to 1 μg Eq/mL, the LLOQ of the assay.

Reviewer comment

The method shows acceptable accuracy within the expected range of IgM IC, and is therefore adequate.

Precision

An acceptable CV for this assay was determined to be 25%.

Intra-Assay precision

For 10 replicates of a low-level dilution of the standard control HIGM (6.7 μg Eq/mL) and 10 replicates of a higher concentration of HIGM (20 μg Eq/mL), the %CV of the low QC was 11.3% and that of the high QC was 9.96% (both less than 25%);

Inter-Assay precision

Inter-assay (between run) precision was examined by testing the low and high controls over 45 runs, analyzed over a period of 2.5 weeks by 3 operators. The low QC had a CV of 8.53% and the high QC had a %CV of 7.31% %.

Reviewer comment

The validation of precision is adequate, showing relatively high precision for both intra-assay and inter-assay

Reportable range

The reportable range was determined by calculating the %CV of 3 replicate values for each standard. In addition, each standard value was adjusted for dilution and compared with the top standard. The reportable range was defined as the concentrations between which the triplicate values had a CV of ≤ 25% and the adjusted concentration of the standard was within 25% of the top standard. For 2.22 µg Eq/ml the % CV was 9.88% while for 0.74 µg/ Eq/ml the % CV was 27%, indicating the lower limit of the reportable range was between these concentrations. This was consistent with the accuracy determination discussed above, in which acceptable accuracy was determined between 60 and 1 µg Eq/ml.

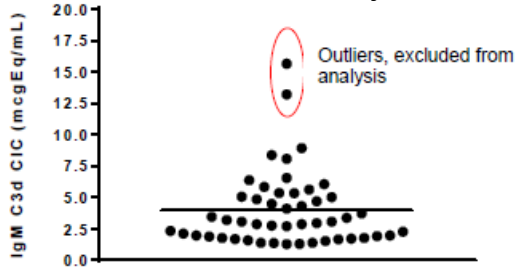
Reviewer comment

The reportable range is consistent with the expected range of sample concentrations.

Normal Range

Eighty nine (89) serum samples were analyzed for IgM C3d-CIC. Forty samples had a concentration that was below the reportable range of 1 mcg Eq/mL and were therefore considered BLOQ (Below Level Of Quantitation). A reference range was calculated using the remaining 49 samples

that had values within the analytical measurement range of the assay



As shown above, 40/89 of the normal samples tested were BLOQ for IgM C3d-CIC. The data collected for the remaining 49 samples was graphed as shown above. Two of the samples (concentrations of 13.21 and 15.65 µg Eq/mL) were obvious outliers and were excluded from the final analysis of the reference range. Upon calculating the mean +/- 2 SD of the 47 samples that had values within the linear range of the assay, the reference range was determined to be 0-7.6 µg Eq/mL

Reviewer comment

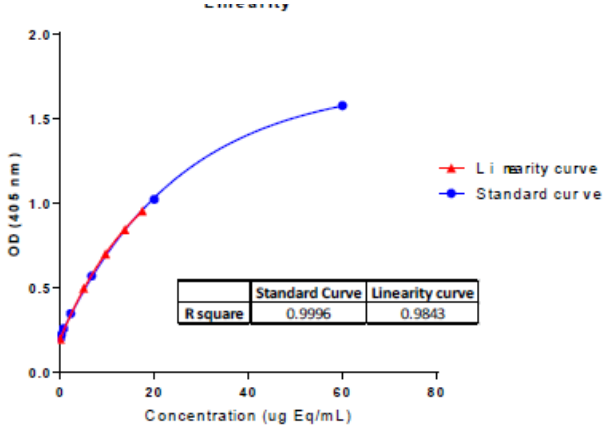
This normal range was determined in an appropriate statistical fashion, and is consistent with the normal range observed for IgG ICs.

Linearity

Samples from the following dilution scheme were assayed in duplicate.

Sample	OD	Average OD	Interpolated Concentration (mcg Eq/mL)
High only	0.945	0.955	17.454
	0.964		
3 high + 1 low	0.840	0.845	13.734
	0.849		
2 high + 2 low	0.701	0.701	9.661
	0.701		
1 high + 3 low	0.499	0.497	5.004
	0.494		
Low only	0.198	0.196	0
	0.193		

And yielded the following response curve



The R2 value for the standard curve (4PL analysis) was 0.9996. The R2 value for the linearity curve was 0.9843, meeting the linearity acceptance criteria of $R2 \geq 0.90$. Patient/study samples will be run at two dilutions—1:100 and 1:1000, to ensure that one of these dilutions will fall within the standard curve, so that the concentration may be interpolated

Reviewer comments

As judged by the high R2 value of 0.9843, the assay is adequately linear in the linearity phase of the curve and the sponsor has an appropriate sample dilution protocol in place to ensure that the signals from patient samples fall with this validated linear response.

Sensitivity

Twelve replicates of the standard material were assayed at an expected concentration of 1.0 µg Eq/mL of the HIGM in AB serum. The %CV was 24.70%, approaching the criteria of ≤ 25%. Therefore, the LLOQ of the assay is 1.0 µg Eq/mL. Study samples that fall below this concentration will be reported as 'BLOQ

Reviewer comments

The sponsor has examined the precision of the assay at the lower limit of quantitation, and found the precision is very close to a %CV of 25%, confirming that measurements should not be trusted at lower signals, and thus defining 1 μg/ ml as the sensitivity of the assay.

Selectivity

Selectivity was defined by taking five normal serum samples (previously determined to have low OD values) and spiking in a known concentration of the HIGM (6.7 μg Eq/ml). The normal serum samples were assessed before and after spiking in 6.7 μg Eq/mL of the HIGM control. The concentration (before and after) was interpolated, and the difference in HIGM μg Eq/mL between the spiked and unspiked samples calculated. Percent error was calculated to examine the difference between the interpolated values and the expected concentration (6.7 μg Eq/mL). Each sample showed less than 25% error between the experimental concentration and the expected concentration suggesting that the assay shows selectivity for IgM CIC.

Reviewer comments

This analysis shows that the assay can detect a difference between normal samples and those with IgM ICs

Sample stability (freeze thaw)

Aliquots of high and low level samples were thawed and aliquoted into lots as follows: lot 1) refreeze immediately, lot 2) Thawed and refrozen once before the assay, lot 3) Thawed and refrozen twice, lot 4) Thawed and frozen three times and lot 5) Thawed and frozen four times. All samples were assayed in duplicate at 1:100 on the same run and the values compared. All values were within a % difference of 20, except for a single value from the high QC, which was clearly an outlier.

Reviewer comment

These data support freeze-thaw of assay samples up to four times. In addition, I note that the assay protocol states that freeze thawing should be avoided, which should further minimize risk.

Frederick Mills

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Joslyn Brunelle

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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

To: Administrative File, **STN 761079**
From: Maxwell Van Tassell, Ph.D., DMA Branch IV
Through: Reyes Candau-Chacon, Ph.D., Acting Quality Assessment Lead, DMA Branch IV
Subject: New 351(a) Biologics License Application (BLA)
US License: 1649
Applicant: BioMarin Pharmaceutical Inc.
Product: Palynziq (pegvaliase-pqpz)
Indication: Adult patients with Phenylketonuria (PKU) who have uncontrolled blood Phe levels > 600 µmol/L on existing management.
Dosage: Solution for SC injection; 5mg/mL, 20 mg/mL
Facilities: BioMarin Pharmaceutical Inc., Novato, CA; (FEI # 1000121235)
Receipt Date: 06/30/2017
Action Date: 05/25/2018

Recommendation for Approvability: The drug substance part of STN 761079 was reviewed from a product quality microbiology perspective and is recommended for approval.

Review Summary

BioMarin Pharmaceutical, Inc. has submitted 351(a) BLA 761079 to obtain licensure of pegvaliase. Pegvaliase is a recombinant *Anabaena variabilis* phenylalanine ammonia lyase (rAvPAL) that is PEGylated and facilitates degradation of excess blood L-phenylalanine in patients with phenylketonuria.

BLA 761079 was submitted in eCTD on June 30, 2017. Module 3.2 was provided with two 3.2.S sections, for the rAvPAL intermediate and formulated bulk drug substance (FBDS) manufacturing processes separately. This review contains the assessment of the drug substance portion of the BLA from a microbiological quality perspective. For review of drug product microbiological aspects of the application, please see the review by Lindsey Brown, Ph.D.

Drug Substance Quality Microbiology Information Reviewed

Sequence number	Date	Description
eCTD 0001	06/30/2017	Original 351(k) BLA submission
eCTD 0007	08/21/2017	Amendment to provide additional information prior to filing
eCTD 0017	10/20/2017	Amendment in response to an information request
eCTD 0030	11/30/2017	Amendment in partial response to an information request
eCTD 0031	12/07/2017	Amendment in partial response to an information request
eCTD 0041	01/05/2018	Amendment in partial response to an information request
eCTD 0042	01/12/2018	Amendment in partial response to an information request
eCTD 0044	01/23/2018	Amendment in response to an information request
eCTD 0045	01/31/2018	Amendment in response to an information request
eCTD 0049	02/15/2018	Amendment in partial response to an information request
eCTD 0053	03/01/2018	Amendment in partial response to an information request
eCTD 0057	03/20/2018	Amendment in response to an information request

Review Assessment

3.2.S DRUG SUBSTANCE [rAvPAL Intermediate, BioMarin Pharmaceutical Inc.]

3.2.S.1 GENERAL INFORMATION

Pegvaliase is a recombinant phenylalanine ammonia lyase from *Anabaena variabilis* that is expressed in genetically engineered *E. coli* and PEGylated with N-hydroxysuccinimide-methoxypolyethylene-glycol (NHS-PEG). The rAvPAL intermediate is an approximately 62 kDa monomer that spontaneously forms a homotetramer, while the PEGylated active drug substance has a molecular weight range of approximately (b) (4) Pegvaliase converts L-phenylalanine (L-Phe) to *trans*–cinnamic acid and ammonia.

3.2.S.2 MANUFACTURE

3.2.S.2.1 Manufacturer(s)

The following facilities are used for the manufacture, testing, and storage of rAvPAL intermediate, as adapted from Table 3.2.S.2.1.1 from the submission:

Table 3.2.S.2.1.1: Drug Substance Manufacturers

Facility Name and Address	Facility Establishment Identifier	Responsibility
BioMarin Pharmaceutical Inc. Novato Campus 46 Galli Drive Novato, CA 94949 USA	3004079983	• Storage of the MCB and WCB • Manufacture, packaging, and storage of rAvPAL intermediate • Quality control testing of rAvPAL intermediate (in-process, release, and stability)
(b) (4)		(b) (4)

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SATISFACTORY

Bioburden
A specification of ≤ (b) (4) CFU/100 mL was established to assure acceptable microbial control of a liquid bulk.

Reviewer Comment
Historical data indicate that < (b) (4) CFU/100 mL is consistently achieved.

SATISFACTORY

3.2.S.6 CONTAINER CLOSURE SYSTEM

The FBDS container closure system is a (b) (4) A certificate of analysis for a representative container is provided.

Reviewer Note:
The extractables and leachables study should be reviewed by OBP.

SATISFACTORY

3.2.S.7 STABILITY

Endotoxin and bioburden testing were not incorporated into stability studies.

Reviewer's Comments:
Endotoxin and bioburden testing are not required for stability testing of drug substance. The stability program and data provided should be further assessed by OBP.

cGMP Status
Refer to Panorama for cGMP status of the relevant facilities.

Conclusion

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

II. Information and data in this submission not related to microbial control of the drug substance should be reviewed by the appropriate division.

III. A pre-license inspection was conducted at BioMarin Pharmaceutical Inc., California, USA, concurrently with a surveillance inspection, from December 4th-8th and December 11th-15th, 2017 by ORA (Ashar Parikh and Jolanna Norton) and OBP (Joslyn Brunelle and Ying-Xin Fan). A ten-item Form FDA 483 was issued. Refer to Panorama for compliance status of the facilities.

Maxwell Van Tassell

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Reyes Candau-Chacon

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DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

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

Date: March 12, 2018
To: Administrative File, STN 761079/0
From: Ephrem Hunde, Ph.D., Chemical Engineer, CDER/OPQ/OPF/DIA
Endorsement: Zhihao Peter Qiu, Ph.D., Branch 1 Chief, CDER/OPQ/OPF/DIA
Subject: New Biologic License Application (BLA)
US License: 1649
Applicant: BioMarin Pharmaceuticals, Inc.
Mfg Facility: Drug Substance: BioMarin Pharmaceutical Inc., Novato, CA. (FEI: 3004079983)
Drug Product: Cook Pharmica LLC, Bloomington. (FEI 3005949964)
Product: pegvaliase (PEGylated recombinant phenylalanine ammonia lyase), solution for injection
Dosage: 5 mg/mL and 20 mg/mL strength
Indication: to reduce blood phenylalanine levels in adult patients with Phenylketonuria (PKU) who have uncontrolled blood Phe levels > 600 µmol/L on existing management.
Due Date: May 28, 2018.

RECOMMENDATION: This submission is recommended for approval from a facilities assessment perspective.

SUMMARY

BLA 761079 was submitted by BioMarin Pharmaceutical Inc., which provided information and data to support the manufacture of pegvaliase, solution for injection.

Pegvaliase active substance is PEGylated recombinant phenylalanine ammonia lyase (rAvPAL) protein derived from the cyanobacterium Anabaena variabilis (ATCC29413) which is expressed in E. coli and produced by a fermentation process in (b) (4) L scale. Pegvaliase drug product (DP) is supplied as a sterile, preservative-free solution contained in single use, glass prefilled syringe (PFS) with staked needle for subcutaneous injection at a concentration of 5 mg/mL and 20 mg/mL. The container closure system consists of a 1 mL-long, stainless steel staked needle syringe ((b) (4)), a (b) (4) plunger stopper and a rigid needle shield.

The subject BLA proposes commercial manufacturing of pegvaliase DS at the Biomarin Pharmaceutical Inc. facility in Novato, California (FEI 3004079983). The DP is filled into PFS at Cook Pharmica LLC facility in Bloomington, Indiana (FEI 3005949964).

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3.2.A.1 FACILITIES AND EQUIPMENT (COOK Pharmica Facility)

The COOK facility is adequately described in this submission, and was recently reviewed for BLA 761070 and documented in the facility review memo dated 7/11/2017.

CONCLUSION

Adequate descriptions were provided for the facilities proposed for pegvaliase DS and DP manufacture and testing. The subject BLA is recommended for approval from a facilities assessment perspective.

Ephrem Hunde, Ph.D.
Chemical Engineer
OPF Division of Inspectional Assessment
Branch 1 _____

Zhihao (Peter) Qiu, Ph.D.
Supervisory Consumer Safety Officer
OPF Division of Inspectional Assessment
Branch 1 Chief _____

Ephrem Hunde

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Zhihao Peter Qiu

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