CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761194Orig1s000

PRODUCT QUALITY REVIEW(S)

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

LABELS AND LABELING ASSESSMENT

Date of Assessment:	August 4, 2021
Assessor:	Vicky Borders-Hemphill, PharmD Labeling Assessor Office of Biotechnology Products (OBP)
Through:	Fabiola Gomez, PhD, Product Quality Assessor OBP/Division of Biotechnology Review and Research 3
Application:	BLA 761194
Applicant:	Genzyme Corporation
Submission Date:	July 17, 2020
Product:	avalglucosidase alfa-ngpt
Dosage form(s):	For injection
Strength and Container-Closure:	100 mg per vial
Purpose of assessment:	The Applicant submitted a biologics license application for Agency assessment
Recommendations:	The prescribing information (submitted August 4, 2021) and container labels and carton labeling (submitted May 21, 2021) are acceptable from an OBP Labeling perspective.

Materials Considered for this Label and Labeling Assessment	
Materials Assessed	**Appendix Section**
Proposed Labels and Labeling	A
Evaluation Tables	B
Acceptable Labels and Labeling	C

n/a = not applicable for this assessment

DISCUSSION

We assessed the proposed labels and labeling for compliance with applicable requirements in the Code of Federal Regulations. Also, we assessed the proposed labels and labeling for consistency with recommended labeling practices (see Appendix B).

CONCLUSION

The prescribing information (submitted August 4, 2021), and container labels and carton labeling (submitted May 21, 2021) are acceptable from an OBP Labeling perspective (see Appendix C).

APPENDICES

Appendix A: Proposed Labeling

Prescribing Information (submitted on September 18, 2020
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Container Labels (submitted on September 18, 2020)

(b) (4)

Carton Labeling (submitted on September 18, 2020)

(b) (4)

Appendix B: Evaluation Tables
Evaluation Tables: Label[1,2] and Labeling[3] Standards

Container[4] Label Evaluation

Proper Name (container label)	**Acceptable**
Regulations: 21 CFR 610.60(a)(1), 21 CFR 201.10(g)(2), 21 CFR 610.62(a), 21 CFR 610.62(b), 21 CFR 610.62(c), 21 CFR 610.60(c), 21 CFR 201.50(b), 21 CFR 201.10(a), 21 CFR 201.10(h)(2)(i)(1)(i)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices (placement of dosage form outside of parenthesis and/or below the proper name)	✓ Yes ☐ No ☐ N/A

Manufacturer name, address, and license number (container label)	**Acceptable**
Regulations: 21 CFR 610.60(a)(2), 21 CFR 201.1(a), 21 CFR 610.60(c), 21 CFR 201.10(h)(2)(i)(1)(iv), 21 CFR 201.100(e)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices (using the qualifying phrase "Manufactured by:")	✓ Yes ☐ No ☐ N/A
Recommended labeling practices (U.S license number for container bearing a partial label[5])	✓ Yes ☐ No ☐ N/A

Lot number or other lot identification (container label)	**Acceptable**
Regulations: 21 CFR 610.60(a)(3), 21 CFR 610.60(c), 21 CFR 201.18, 21 CFR 201.100(b)(6), 21 CFR 201.10(h)(2)(i)(1)(iii)	✓ Yes ☐ No ☐ 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.
[5] Per 21 CFR 610.60(c) *Partial Label.* If the container is capable of bearing only a partial label, the container shall show as a minimum the name (expressed either as the proper or common name), the lot number or other lot identification and the name of the manufacturer; in addition, for multiple dose containers, the recommended individual dose. Containers bearing partial labels shall be placed in a package which bears all the items required for a package label."

Expiration date (container label)	**Acceptable**
Regulations: 21 CFR 610.60(a)(4), 21 CFR 201.17	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: USP General Chapters <7> Labeling, Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 lines 178-184, which, when finalized, will represent FDA's current thinking on topic	✓ Yes ☐ No ☐ N/A

Beyond Use Date (Multiple-dose containers) (container label)	**Acceptable**
Recommended labeling practices: USP General Chapters: <659> Packaging and Storage Requirements and <7> Labeling	☐ Yes ☐ No ☒ N/A

Product Strength (container label)	**Acceptable**
Regulations: 21 CFR 201.10(d)(1), 21 CFR 201.100(b)(4)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices (expression of strength for injectable drugs) references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 line 176, which, when finalized, will represent FDA's current thinking on topic *USP General Chapters: <7> Labeling*	✓ Yes ☐ No ☐ N/A

Multiple-dose containers (container label)	**Acceptable**
Regulations: 21 CFR 610.60(a)(5), 21 CFR 201.55 *(recommended individual dose)*	☐ Yes ☐ No ☒ N/A

Statement: "Rx only" (container label)	**Acceptable**
Regulations: 21 CFR 610.60(a)(6), 21 CFR 201.100(b)(1)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices (prominence of Rx Only statement) reference: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 line 147, which, when finalized, will represent FDA's current thinking on topic	✓ Yes ☐ No ☐ N/A

Medication Guide (container label)	**Acceptable**
Regulations: 21 CFR 610.60(a)(7), 21 CFR 208.24(d)	☐ Yes ☐ No ☒ N/A

No Package for container (container label)	**Acceptable**
Regulation: 21 CFR 610.60(b)	☐ Yes ☐ No ☒ N/A

No container label (container label)	**Acceptable**
Regulation: 21 CFR 610.60(d)	☐ Yes ☐ No ☒ N/A

Ferrule and cap overseal *(for vials only)*	**Acceptable**
Recommended labeling practices references: United States Pharmacopeia (USP) General Chapters: <7> Labeling (Ferrules and Cap Overseals)	✓ Yes ☐ No ☐ N/A

Comment/Recommendation: Confirm there is no text on the ferrule and cap overseal of the vials. *Applicant's response: There is no text on the ferrule or cap overseal*

Visual inspection	**Acceptable**
Regulation: 21 CFR 610.60(e)	✓ Yes ☐ No ☐ N/A

Comment/Recommendation: Confirm that sufficient area of the container remains uncovered for its full length or circumference to allow for visual inspection when the label is affixed to the container and indicate where the visual area of inspection is located *Applicant's response: When the label is affixed to the container there is a viewable area of ~20mm.*

Route of administration (container label)	**Acceptable**
Regulations: 21 CFR 201.5(f), 21 CFR 201.100(b)(3), 21 CFR 201.100(d)(1)	✓ Yes ☐ No ☐ N/A

Recommended labeling practices (route of administration statement to appear after the strength statement on the principal display panel)	✓ Yes ☐ No ☐ N/A

NDC numbers **(container label)**	**Acceptable**
Regulations: 21 CFR 201.2, 21 CFR 207.35	✓ Yes ☐ No ☐ N/A

Preparation instructions (container label)	**Acceptable**
Regulation: 21 CFR 201.5(g)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (lines 426-430), which, when finalized, will represent FDA's current thinking on topic	☐ Yes ☐ No ☒ N/A

Package type term (container label)	**Acceptable**
Recommended labeling practices: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018) *USP chapter <659> Packaging and Storage Requirements*	✓ Yes ☐ No ☐ N/A

Misleading statements (container label)	**Acceptable**
Regulation: 21 CFR 201.6	☐ Yes ☐ No ☒ N/A

Prominence of required label statements (container label)	**Acceptable**
Regulation: 21 CFR 201.15	✓ Yes ☐ No ☐ N/A

Spanish-language (Drugs) (container label)	**Acceptable**
Regulation: 21 CFR 201.16	☐ Yes ☐ No ☒ N/A

FD&C Yellow No. 5 and/or FD&C Yellow No. 6 (container label)	**Acceptable**
Regulation: 21 CFR 201.20	☐ Yes ☐ No ☒ N/A

Bar code label requirements (container label)	**Acceptable**
Regulations: 21 CFR 201.25, 21 CFR 610.67	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: 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), which, when finalized, will represent FDA's current thinking on topic*	✓ Yes ☐ No ☐ N/A

Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) (container label)	**Acceptable**
Regulations: 21 CFR 610.68, 21 CFR 201.26	☐ Yes ☐ No ☒ N/A

Net quantity (container label)	**Acceptable**
Regulation: 21 CFR 201.51	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: Draft Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors (line 461- 463) which, when finalized, will represent FDA's current thinking on topic *Allowable Excess Volume and Labeled Vial Fill Size in Injectable Drug and Biological Products Guidance for Industry, June 2015 (line 68, 93-99)* *USP General Chapters <1151> Pharmaceutical Dosage Forms (Excess volume in injections).*	✓ Yes ☐ No ☐ N/A

Statement of Dosage (container label)	**Acceptable**
Regulations: 21 CFR 610.60(a)(5), 21 CFR 610.60(c), 21 CFR 201.55, 21 CFR 201.100(b)(2)	☐ Yes ☐ No ☒ N/A

Inactive ingredients (container label)	**Acceptable**
Regulation: 21 CFR 201.100	☐ Yes ☐ No ☒ N/A
Recommended labeling practices reference: USP General Chapters <1091> Labeling of Inactive Ingredients and USP General Chapters <7> Labeling	☐ Yes ☐ No ☒ N/A

Storage requirements (container label)	**Acceptable**
Recommended labeling practices references: USP General Chapters <7> Labeling, USP General Chapters <659> Packaging and Storage Requirements	☐ Yes ☐ No ☒ N/A

Dispensing container (container label)	**Acceptable**
Regulation: 21 CFR 201.100(b)(7)	☐ Yes ☐ No ☒ N/A

Package[6] Labeling Evaluation

Proper name (package labeling)	**Acceptable**
Regulations: 21 CFR 610.61(a), 21 CFR 201.50(b), 21 CFR 201.10(g)(2)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices (placement of dosage form outside of parenthesis and/or below the proper name)	✓ Yes ☐ No ☐ N/A

Manufacturer name, address, and license number (package labeling)	**Acceptable**
Regulations: 21 CFR 610.61(b), 21 CFR 201.1(a), 21 CFR 201.1(i), 21 CFR 201.100(e)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices (using the qualifying phrase "Manufactured by:")	✓ Yes ☐ No ☐ N/A

[6] 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.

Lot number or other lot identification (package labeling)	**Acceptable**
Regulation: 21 CFR 610.61(c), 21 CFR 201.18	✓ Yes ☐ No ☐ N/A

Expiration date (package labeling)	**Acceptable**
Regulations: 21 CFR 610.61(d), 21 CFR 201.17	✓ Yes ☐ No ☐ N/A

Beyond Use Date (Multiple-dose containers) (package labeling)	**Acceptable**
Recommended labeling practices: USP General Chapters: <659> Packaging and Storage Requirements and <7> Labeling	☐ Yes ☐ No ☒ N/A

Preservative (package labeling)	**Acceptable**
Regulation: 21 CFR 610.61(e)	✓ Yes ☐ No ☐ N/A

Comment/Recommendation: Per 21 CFR 610.61(e), The following items shall appear on the label affixed to each package containing a product: The preservative used and its concentration, or if no preservative is used and the absence of a preservative is a safety factor, the words "No Preservative". Ensure that the statement "No Preservative" appears on the carton labeling *The Applicant revised as requested*

Number of containers (package labeling)	**Acceptable**
Regulation: 21 CFR 610.61(f)	✓ Yes ☐ No ☐ N/A

Product Strength (package labeling)	**Acceptable**
Regulations: 21 CFR 610.61(g), 21 CFR 201.10(d)(1), 21 CFR 201.100(b)(4)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize	✓ Yes ☐ No ☐ N/A

Medication Errors, April 2013 (line 176), which, when finalized, will represent FDA's current thinking on topic. USP General Chapters: <7> Labeling	

Storage temperature/requirements (package labeling)	**Acceptable**
Regulation: 21 CFR 610.61(h)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices reference: USP General Chapters: <7> Labeling, USP General Chapters <659> Packaging and Storage Requirements	✓ Yes ☐ No ☐ N/A

Handling: "Do Not Shake", "Do not Freeze" or equivalent (package labeling)	**Acceptable**
Regulation: 21 CFR 610.61(i)	✓ Yes ☐ No ☐ N/A

Multiple dose containers (recommended individual dose) (package labeling)	**Acceptable**
Regulation: 21 CFR 610.61(j)	☐ Yes ☐ No ☒ N/A

Route of administration (package labeling)	**Acceptable**
Regulations: 21 CFR 610.61(k), 21 CFR 201.5(f), 21 CFR 201.100(d)(1)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices (route of administration statement to appear after the strength statement on the principal display panel)	✓ Yes ☐ No ☐ N/A

Known sensitizing substances (package labeling)	**Acceptable**
Regulations: 21 CFR 610.61(l), 21 CFR 801.437 (User labeling for devices that contain natural rubber)	☐ Yes ☐ No ☒ N/A

Inactive ingredients (package labeling)	**Acceptable**
Regulations: 21 CFR 610.61, 21 CFR 201.100	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: USP General Chapters <1091> Labeling of Inactive Ingredients, USP General Chapters <7> Labeling	✓ Yes ☐ No ☐ N/A

Comment/Recommendation: Revise the inactive ingredient list to appear in alphabetical order as follows: Each single-dose vial contains 100 mg of avalglucosidase alfa-xxxx, glycine (200 mg), L-Histidine (10.7 mg), L-Histidine HCl monohydrate (6.5 mg), mannitol (200 mg), and polysorbate 80 (1 mg). *The Applicant revised as requested*

Source of the product (package labeling)	**Acceptable**
Regulation: 21 CFR 610.61(p)	☐ Yes ☐ No ☒ N/A

Minimum potency of product (package labeling)	**Acceptable**
Regulation: 21 CFR 610.61(r)	✓ Yes ☐ No ☐ N/A

Comment/Recommendation: Per 21 CFR 610.61, add the words "No U.S. standard of potency" to the carton labeling *The Applicant revised as requested*

Rx only (package labeling)	**Acceptable**
Regulations: 21 CFR 610.61(s), 21 CFR 201.100(b)(1)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (line 147-149), which, when finalized, will represent FDA's current thinking on topic	✓ Yes ☐ No ☐ N/A

Divided manufacturing (package labeling)	**Acceptable**
Regulation: 21 CFR 610.63 (Divided manufacturing responsibility to be shown)	☐ Yes ☐ No ☒ N/A

Distributor (package labeling)	**Acceptable**
Regulation: 21 CFR 610.64, 21 CFR 201.1(h)(5)	☐ Yes ☐ No ☒ N/A

Bar code (package labeling)	**Acceptable**
Regulations: 21 CFR 610.67, 21 CFR 201.25	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: *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)*	✓ Yes ☐ No ☐ N/A

Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) (package labeling)	**Acceptable**
Regulations: 21 CFR 610.68, 21 CFR 201.26	☐ Yes ☐ No ☒ N/A

NDC numbers (package labeling)	**Acceptable**
Regulations: 21 CFR 201.2, 21 CFR 207.35	✓ Yes ☐ No ☐ N/A

Preparation instructions (package labeling)	**Acceptable**
Regulation: 21 CFR 201.5(g) and 21 CFR 610.61(i)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (lines 426-430), which, when finalized, will represent FDA's current thinking on topic *USP General Chapters <7> Labeling*	✓ Yes ☐ No ☐ N/A

Comment/Recommendation: Consider including reconstitution instructions and storage conditions for the reconstituted product: "After reconstitution with 10 mL of Sterile Water for Injection, USP the resultant concentration is 100 mg/10 mL (10 mg/mL). If not used immediately, the reconstituted product can be stored up to xx hours when refrigerated at 2°C

to 8°C (36° to 46°F)" *The Applicant revised as requested and included refrigerated storage condition for up to 24 hours which was confirmed acceptable by OPMA.*

This may allow for the removal of "See prescribing information (b) (4) *The Applicant revised as requested*

Package type term (package labeling)	**Acceptable**
Recommended labeling practices: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018) *USP chapter <659> Packaging and Storage Requirements*	✓ Yes ☐ No ☐ N/A

Misleading statements (package labeling)	**Acceptable**
Regulation: 21 CFR 201.6	☐ Yes ☐ No ☒ N/A

Prominence of required label statements (package labeling)	**Acceptable**
Regulation: 21 CFR 201.15	✓ Yes ☐ No ☐ N/A

Spanish-language (Drugs) (package labeling)	**Acceptable**
Regulation: 21 CFR 201.16	☐ Yes ☐ No ☒ N/A

FD&C Yellow No. 5 and/or FD&C Yellow No. 6 (package labeling)	**Acceptable**
Regulation: 21 CFR 201.20	☐ Yes ☐ No ☒ N/A

Phenylalanine as a component of aspartame (package labeling)	**Acceptable**
Regulation: 21 CFR 201.21(c)	☐ Yes ☐ No ☒ N/A

Sulfites; required warning statements (package labeling)	**Acceptable**
Regulation: 21 CFR 201.22(b)	☐ Yes ☐ No ☒ N/A

Net quantity (package labeling)	**Acceptable**
Regulation: 21 CFR 201.51	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: Draft Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors (line 461- 463) which, when finalized, will represent FDA's current thinking on topic *Allowable Excess Volume and Labeled Vial Fill Size in Injectable Drug and Biological Products Guidance for Industry, June 2015 (line 68, 93-99)* *USP General Chapters <1151> Pharmaceutical Dosage Forms (Excess volume in injections).*	✓ Yes ☐ No ☐ N/A

Statement of Dosage (package labeling)	**Acceptable**
Regulations: 21 CFR 201.55, 21 CFR 201.100(b)(2)	✓ Yes ☐ No ☐ N/A

Comment/Recommendation: Consider revising the Statement of dosage from (b) (4) to read as follows: "Dosage: See Prescribing Information" *The Applicant revised as requested*

Dispensing container (package labeling)	**Acceptable**
Regulation: 21 CFR 201.100(b)(7)	☐ Yes ☐ No ☒ N/A

Medication Guide (package labeling)	**Acceptable**
Regulations: 21 CFR 610.60(a)(7), 21 CFR 208.24(d)	☐ Yes ☐ No ☒ N/A

Prescribing Information Evaluation

PRESCRIBING INFORMATION

Highlights of Prescribing Information	
PRODUCT TITLE	**Acceptable**
Regulation: 21 CFR 201.57(a)(2)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices reference: Draft Guidance for Industry on Product Title and Initial U.S. Approval in the Highlights of Prescribing Information for Human Prescription Drug and Biological Products - Content and Format (January 2018), which, when finalized, will represent FDA's current thinking on topic	✓ Yes ☐ No ☐ N/A

Highlights of Prescribing Information	
DOSAGE AND ADMINISTRATION	**Acceptable**
Recommended labeling practices reference: USP nomenclature for diluents and intravenous solutions	☐ Yes ☐ No ☒ N/A

Highlights of Prescribing Information	
DOSAGE FORMS AND STRENGTHS	**Acceptable**
Regulations: 21 CFR 201.57(a)(8), 21 CFR 201.10, 21 CFR 201.100	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018) *USP chapter <659> Packaging and Storage Requirements* *USP General Chapters: <7> Labeling*	✓ Yes ☐ No ☐ N/A

Full Prescribing Information	
2 DOSAGE AND ADMINISTRATION	**Acceptable**
Regulation: 21 CFR 201.57(c)(3)(iv)] *Confirm appropriateness of specific direction on dilution, preparation, and administration of the dosage form and storage conditions for stability of the reconstituted or diluted drug; ensure verbatim statement for parenterals: "Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit."*	✓ Yes ☐ No ☐ N/A

Recommended labeling practices reference: USP nomenclature for diluents and intravenous solutions and storage instructions for reconstituted and diluted products; confirm the appropriateness of infusion bags, infusion sets (e.g., tubing, infusion aids, or filter membranes) incompatibilities with these components	✓ Yes ☐ No ☐ N/A

Comment/Recommendation:
Deleted the (b) (4) from labeling as currently FDA recommends against stating (b) (4) which may pose confusion during preparation *The Applicant revised as requested*

(b) (4)

Full Prescribing Information	
3 DOSAGE FORMS AND STRENGTHS	**Acceptable**
Regulation: 21 CFR 201.57(c)(4)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018) *USP chapter <659> Packaging and Storage Requirements* *USP General Chapters: <7> Labeling*	✓ Yes ☐ No ☐ N/A

Full Prescribing Information	
11 DESCRIPTION	**Acceptable**
Regulations: 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)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: USP General Chapters <1091>, USP General Chapters <7>	✓ Yes ☐ No ☐ N/A

Comment/Recommendation:
Added the MW of the drug substance *The Applicant revised as requested* Added the dosage form *The Applicant revised as requested* Added the route of administration *The Applicant revised as requested* Added reconstitution information consistent with more recently approved FDA labeling *The Applicant revised as requested*

Full Prescribing Information	
15 & 16 Hazardous Drug	**Acceptable**
Regulation: 21 CFR 201.57(c)(17)(iv) Section 15: References 1. OSHA Hazardous Drugs. OSHA. *http://www.osha.gov/SLTC/hazardousdrugs/index.html* Section 16: xxxx is a hazardous drug. Follow applicable special handling and disposal procedures.[1]	☐ Yes ☐ No ☒ N/A

Full Prescribing Information	
16 HOW SUPPLIED/ STORAGE AND HANDLING	**Acceptable**
Regulation: 21 CFR 201.57(c)(17)	✓ Yes ☐ No ☐ N/A
Recommended labeling practices: to ensure placement of detailed storage conditions for reconstituted and diluted products	☐ Yes ☐ No ☒ N/A

Comment/Recommendation: Detailed storage conditions for reconstituted and diluted products should be described in the DOSAGE AND ADMINISTRATION section rather than the HOW SUPPLIED/STORAGE AND HANDLING section. In the HOW SUPPLIED/STORAGE AND HANDLING section may include summary statement with a cross-reference to this information in DOSAGE AND ADMINISTRATION section, e.g., "Store reconstituted solutions of DRUG-X at Y temperature *[see Dosage and Administration (2.X)]." The Applicant revised as requested*

Full Prescribing Information	
MANUFACTURER INFORMATION	**Acceptable**
Regulations: 21 CFR 201.100(e), 21 CFR 201.1	✓ Yes ☐ No ☐ N/A
Recommended labeling practices references: 21 CFR 610.61(b) (add the US license number for consistency with the carton labeling), and 21 CFR 610.64 (Name and address of distributor may appear and use a qualifying phrase for consistency with the carton labeling, when applicable)	✓ Yes ☐ No ☐ N/A

Medication Guide Evaluation (N/A)
Patient Information Labeling Evaluation (N/A)
Instructions for Use Evaluation (N/A)

APPENDIX C. Acceptable Labels and Labeling
Prescribing Information (submitted on August 4, 2021
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Carton Labeling (submitted on May 21, 2021)

Vicky Borders-Hemphill

Digitally signed by Vicky Borders-Hemphill
Date: 8/04/2021 12:54:14PM
GUID: 50814c7000007a3d59329f660d8ddf02

Fabiola Gomez

Digitally signed by Fabiola Gomez
Date: 8/04/2021 01:43:55PM
GUID: 57e159c901ffd6075252d6e23734463b

Orphan Drug Designation/Fast Track Designation/Breakthrough Designation Pathway

Recommendation: Approval

BLA/NDA Number: 761194
Assessment Number: 1
Assessment Date: August 3, 2021

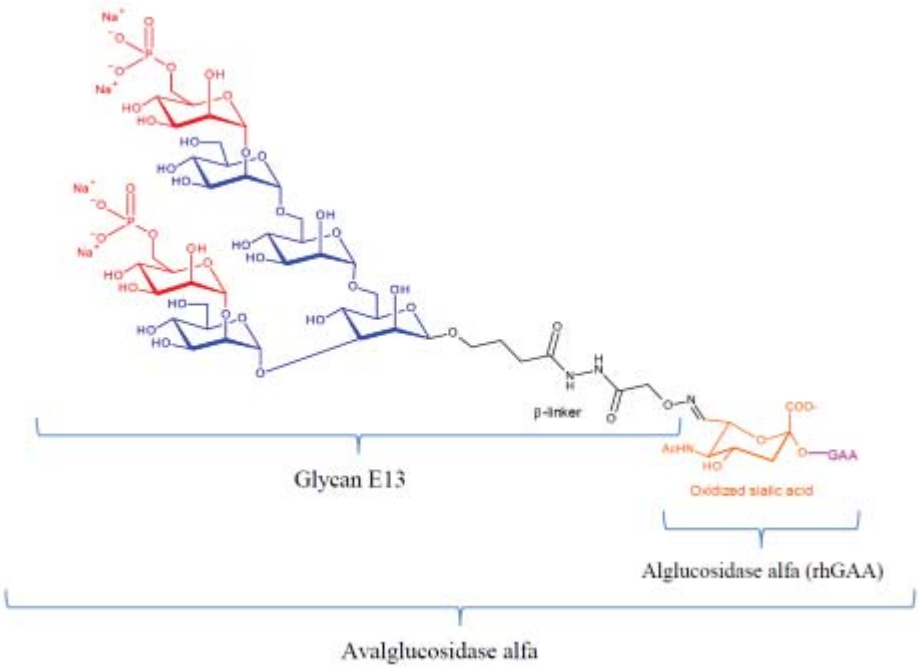
Drug Name/Dosage Form	Nexviazyme (avalglucosidase alfa-ngpt) Injection
Strength/Potency	100 mg/vial powder for injection (10mg/mL after WFI reconstitution)
Route of Administration	Intravenous infusion
Rx/OTC dispensed	Rx
Indication	Avalglucosidase alfa is indicated for the treatment of patients 1 year of afe and older with late-onset Pompe disease (lysosomal acid alpha-glucosidase [GAA] deficiency.
Applicant/Sponsor	Genzyme Corporation

Product Overview

Avalglucosidase alfa-ngpt is a modified form of human α-glucosidase (alglucosidase alfa) conjugated with multiple copies of synthetic glycan E13, a synthetic bis-mannose-6-phosphate-tetra-mannose glycan (bisM6P), linked to sialic acid residues in the enzyme. (b) (4) The structure was confirmed by NMR experiments and Mass Spectrometry. (b) (4)

One molecule of avalglucosidase alfa-ngpt contains approximately seven glycan E13 conjugated to the oxidized sialic acid residues in alglucosidase alfa. For clarity purposes, Figure 1 shows the structure of avalglucosidase alfa-ngpt with only one glycan E13 conjugated to alglucosidase alfa (rhGAA).

Figure 1



Source: Structure section submitted under Module 3.2.S.1.2
Glycan E13 contains 2 bisM6P in red, 4 mannose molecules in blue, and the linker in black, attached to the sialic acid (orange) on the alglucosidase alfa protein (pink). The glycan is conjugated to alglucosidase alfa through an aminoxy nitrogen to carbon double bond (oxime), with the nitrogen (N) coming from the glycan linker and the carbon from the carbon at position 7 (C7) of oxidized sialic acid of alglucosidase alfa.

Recombinant human alglucosidase alfa is expressed, secreted, and purified from CHO cells as a fully glycosylated 110 kDa molecule. Avalglucosidase alfa-ngpt is produced by conjugation of oxidized alglucosidase alfa and glycan E13. The manufacturing process (b) (4)

Avalglucosidase alfa-ngpt drug product is supplied in a single-use (b) (4) glass vial containing 100 mg lyophilized product per vial. Reconstituted drug product contains 10 mg/mL avalglucosidase alfa in 10 mM L-histidine/L-histidine hydrochloride monohydrate, 2% (w/v) glycine, 2% (w/v) mannitol, and 0.01% (w/v) polysorbate 80 at pH 6.2.

Quality Assessment Team

Discipline	Assessor	Branch/Division
Drug Substance – Biologic	Fabiola Gomez	CDER/OPQ/OBP/DBRR III
Drug Substance – Small molecule	Sharon Kelly	CDER/OPQ/ONDP/DNDAPI/NDB2
Drug Product	Fabiola Gomez	CDER/OPQ/OBP/DBRR III
Immunogenicity	Joao Pedras-Vasconcelos	CDER/OPQ/OBP/DBRR III
Labeling	CAPT Vicky Borders-Hemphill	CDER/OPQ/OBP
Facility	Michael Shanks	CDER/OPQ/OPMA/DBM
Microbiology	Reyes Caudau-Chacon	CDER/OPQ/OPMA/DBM
Team Lead	Susan Kirshner (Product Quality - Biologic) Donna Christner (Product Quality – Small molecule) Virginia Carroll (Microbiology and Facilities) Immunogenicity (Zhenzhen Liu)	CDER/OPQ/OBP/DBRR III CDER/OPQ/ONDP/DNDAPI/NDB2 CDER/OPQ/OPMA/DBM CDER/OPQ/OBP/DBRR III
Application Team Lead	Susan Kirshner	CDER/OPQ/OBP/DBRR III
OPQ RBPM	Melinda Bauerlien	CDER/OPQ/OPRO/DRBPMI/RBPMB2

Multidisciplinary Assessment Team

Discipline	Assessor	Office/Division
RPM	Jenny Doan	CDER/OND/ORO/DRO-RDPURM
Cross-disciplinary Team Lead	Linda Jeng	CDER/OND/ORDPURM/DRDMG
Medical Officer	Ann Punoose	CDER/OND/ORDPURM/DRDMG
Pharmacology/Toxicology	Miyun Tsai-Turton	CDER/OND/ORDPURM/DPTRDPURM
Clinical Pharmacology	Katarzyana (Kate) Drozda (genomics) Ruojing Li (pharmacometrics)	CDER/OTS/OCP/DTPM CDER/OTS/OCP/DPM
Statistics	Wonyul Lee	CDER/OTS/OB/DBIV

1. Names:

a. Proprietary Name:	Nexviazyme
b. Trade Name:	Nexviazyme
c. Non-Proprietary Name/USAN:	Avalglucosidase alfa-ngpt
e. Common Name:	neoGAA
f. INN Name:	Avalglucosidase alfa
h. OBP systematic name:	RPROT P10253 (LYAG_HUMAN) [GZ402666]

2. Pharmacological Category: Enzyme Replacement Therapy

Submissions Assessed

Submission(s) Assessed	Document Date	Review Completed by
SNT 761194/0002 0 BLA Original Application	September 18, 2020	OBP
SNT 761194/0005 (response to 11/03/20 IR#1)	November 06, 2020	OPMA
SNT 761194/0006 (response to 11/12/20 IR#2)	November 13, 2020	OPMA
SNT 761194/0011 (response to 12/02/20 IR#3)	January 04, 2021	OPMA
SNT 761194/0012 (response to 12/02/20 IR#3 item 6)	January 06, 2021	OPMA
SNT 761194/0014 (response to 01/13/20 IR#4)	January 21, 2021	ONDP
SNT 761194/0015 (response to 12/04/20 IR#5)	January 22, 2021	OBP
SNT 761194/0016 (site inspection proposal)	January 25, 2021	OPMA/OBP
SNT 761194/0018 (response to 01/19/21 IR#6 and 01/25/21 IR#7)	February 5, 2021	OPMA/OBP
SNT 761194/0020 (response to 02/16/21 IR#8)	February 17, 2021	OPMA
SNT 761194/0022 (response to 02/22/21 IR#9)	February 24, 2021	OPMA
SNT 761194/0024 (response to 02/16/21 IR#8 items 1 and 2)	February 26, 2021	OPMA
SNT 761194/0026 (response to 02/17/21 IR#10 Lumizyme vs Myozyme)	March 3, 2021	OBP
SNT 761194/0033 (response to 04/19/21 IR#11)	April 26, 2021	OBP
SNT 761194/0035 (response to 04/19/21 IR#11 item 7)	April 30, 2021	OBP
SNT 761194/0038 (response to 06/01/21 IR#13)	June 4, 2021	OBP
SNT 761194/0039 (response to 05/27/21 IR#12 Lumizyme vs Myozyme)	June 09, 2021	OBP
SNT 761194/0040 (response to 06/08/21 IR#13)	June 15, 2021	OBP
SNT 761194/0041 (response to 06/08/21 IR#13 item 6)	June 21, 2021	OBP
SNT 761194/0042 (response to 05/27/21 IR#12 Lumizyme vs Myozyme)	June 23, 2021	OBP
SNT 761194/0043 (response to 06/23/21 IR#14)	June 29, 2021	OBP
SNT 761194/0045 (response to 07/01/21 IR#15)	July 07, 2021	OBP

Quality Assessment 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)	V		(b) (4)	3	Adequate	Letter of Authorization provided in Seq 002
	V			3	Adequate	Letter of Authorization provided in Seq 002 Reviewed in (b) (4) (March 29, 2019) was found adequate.
	III			3	Adequate	Letter of Authorization provided in Seq 002

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

2. Action codes for Status column: 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 assessed.

B. Other documents

Document	Application Number	Description
IND	109569	Parent IND

3. **Consults**

Discipline/Topic	Date Requested	Status	Recommendation	Assessor
Inspection of immunogenicity site – Office of Study Integrity and Surveillance (OSIS) consult in Office of Translational Science (OTS).	February 4th, 2021	Waived	Due to the public health emergency, OSIS was unable to perform either an on-site or virtual inspection of the Sanofi US/Genzyme Biomarker and Clinical Bioanalysis Boston facility in Framingham, MA, the primary bioanalytical site involved in validation and testing of immunogenicity assays and	Nicola Fenty-Stewart (CDER/OTS/OSIS) Joao Pedras-Vasconcelos (CDER/OPQ/OBP/DBRR III)

			clinical study samples. This bioanalytical facility also developed and validated the ADA assays that supported approval of BLA 125141 for Lumizyme and BLA125291 for Myozyme, in addition to other Genzyme-licensed ERTs. Therefore, the lack of a bioanalytical inspection during the current review cycle is in not considered a potential approvability issue.	

4. Environmental Assessment of Claim of Categorical Exclusion
   A categorical exclusion is claimed from the requirement to prepare an environmental assessment in accordance with 21 CFR 25.31(c). ***The claim of categorical exemption is accepted.***

Executive Summary

I. Recommendations

A. Recommendation and Conclusion on Approvability

Recommendation: **Approve**

The Office of Pharmaceutical Quality (OPQ), CDER, recommends approval of STN 761194 for NEXVIAZYME (avalglucosidase alfa-ngpt) manufactured by Genzyme Corporation. The data submitted in this application are adequate to support the conclusion that the manufacture of NEXVIAZYME is well-controlled and leads to a product that is safe, 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: Genzyme Flanders NV, Geel Belgium (FEI: 3003623839)
  - Drug Product: Genzyme Ireland Limited, Waterford, Ireland (FEI: 3003809840)

- Fill size and dosage form
  - 100 mg lyophilized product in a (b) (4) mL single-use vial for intravenous injection

- Dating period
  - Drug Product: 48 months at 5±3°C
  - Drug Substance: (b) (4) months at (b) (4) °C
  - Stability:
    - Results of on-going stability should be submitted throughout the dating period, as they become available, including the results of stability studies from the first three production lots.

- Exempt from lot release: Nexviazyme is exempted from lot release per FR 95-29960.

D. Benefit/Risk Considerations

Pompe disease (PD) is an autosomal recessive, lysosomal storage disease that results in deficient activity of alpha glucosidase (GAA), the enzyme that degrades glycogen in lysosomes. Enzyme deficiency leads to myopathy, respiratory weakness, physical disability, and premature death. Enzyme replacement therapy (ERT) with recombinant alpha glucosidase (alglucosidase alfa) is the only approved therapy. However, the improvements seen with alglucosidase alfa are not sustained. Therefore, the treatment and cure of Pompe disease continue to represent unmet needs.

The data submitted in the application support the conclusion that the manufacture of avalglucosidase alfa-ngpt, is well controlled and leads to a product that is safe, pure and potent. The process is under adequate microbial control and sterility assurance of the drug product has been demonstrated. The product is free from endogenous and adventitious infectious agents, and meets the standards recommended by the FDA. The conditions used in the manufacturing process were adequately validated, and the product was consistently manufactured from multiple production runs.
The stability data are sufficient to support an expiration dating period of (b) (4) months for avalglucosidase alfa-ngpt drug substance when stored at (b) (4) C and an expiration dating period of 48 months for lyophilized avalglucosidase alfa-ngpt drug product when stored at 5 ± 3°C.

The Applicant has provided sufficient CMC information to assure the identity, strength, purity, potency, and safety of lyophilized avalglucosidase alfa-ngpt drug product.

The Office of Biotechnology Products Immunogenicity team has no bioanalytical assay related approvability issues for avalglucosidase alfa-ngpt and considers that supporting immunogenicity data are acceptable pending concurrence from Clinical and Clinical Pharmacology teams.

The label/labeling is satisfactory from the CMC perspective.

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

The following PMC was proposed by OPMA and accepted by the Applicant:

PMC 4026-2

- Description: Provide bioburden test method qualification reports for drug substance in-process samples using two additional batches.

(b) (4)

- PMC Schedule Milestones:
  - Final Report Submission: 08/31/2021

The following PMC was proposed by OBP and accepted by the Applicant:

PMC 4026-3

- Description: Provide M6P content specification for drug substance and drug product.

(b) (4)

(b) (4)

- PMC Schedule Milestones:
  - Final Report Submission: 06/30/2022

II. Summary of Quality Assessments

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 1 is a summary of product-related critical quality attributes (CQA), intrinsic to the molecule, that are relevant to both DS and DP. The table includes the identification of the various attributes along with their risk management.

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

CQA (type)	Risk	Origin	Control Strategy	Other
Enzyme activity (Potency)	Efficacy	Intrinsic to the molecule	(b) (4)	
Receptor binding/uptake and Total M6P/bisM6P content (Potency)	Efficacy	Intrinsic to the molecule and Manufacturing process		(b) (4)
Identity	Safety and Efficacy	Intrinsic to the molecule		
Sialic acid (product related variants)	Efficacy	Intrinsic to the molecule		
Sialic acid oxidation (product related variants)	Efficacy	Intrinsic to the molecule and Manufacturing process		
Processed GAA (b) (4) Evaluation of full length (110 kDa) alglucosidase alfa (product related variants)	Efficacy	Intrinsic to the molecule and manufacturing process		

(b) (4)	Efficacy, Safety, Immunogenicity	Intrinsic to the molecule and relative abundance can change during manufacturing process and storage	(b) (4)	(b) (4)
	Efficacy	Intrinsic to the molecule and relative abundance can change during manufacturing process and storage		
	Efficacy, Safety, Immunogenicity	Intrinsic to the molecule and relative abundance can change during manufacturing process and storage		

B. Drug Substance Avalglucosidase alfa-ngpt Quality Summary

Table 2 provides a summary of the identification, risk, and lifecycle knowledge management for drug substance CQAs that derive from the drug substance manufacturing process and general drug substance attributes, including process-related impurities.

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

CQA (type)	Risk	Origin	Control Strategy	Other
Enzyme concentration (Strength)	Efficacy	Manufacturing process, formulation	(b) (4)	
Appearance, color, clarity (General)	Safety	Manufacturing process, formulation		
pH (General)	Safety	Manufacturing process, formulation		
Host Cell Protein content (Process-related impurities)	Safety, Immunogenicity	Cell culture		(b) (4)
Host Cell DNA content (Process-related impurities)	Safety	Cell culture		
Endotoxin (Contaminant)	Safety	Raw materials and contamination during manufacturing		

Bioburden (Contaminant)	Safety	Raw materials and contamination during manufacturing	(b) (4)	
Mycoplasma (Contaminant)	Safety	Raw materials and contamination during manufacturing		
Adventitious viruses (Contaminant)	Safety	Raw materials and contamination during manufacturing		

- **Description:** Avalglucosidase alfa is a modified form of human α-glucosidase (alglucosidase alfa) conjugated with multiple copies of synthetic glycan E13, a synthetic bis-mannose-6-phosphate-tetra-mannose glycan (bisM6P) linked to oxidized sialic acid residues in the enzyme to increase bisM6P levels. One molecule of avalglucosidase alfa contains approximately seven glycan E13 moieties conjugated to the oxidized sialic acid residues in alglucosidase alfa. The molecular weight of the fully glycosylated protein is 118 KDa.

- **Mechanism of Action (MoA):** Binding to mannose-6-phosphate receptors on the cell surface occurs via the mannose-6-phosphate containing glycans on the avalglucosidase alfa molecule, after which the molecule is internalized and transported to the lysosomes. The enzyme then undergoes proteolytic processing and cleaves accumulated glycogen into glucose.

- **Potency Assay**: In vitro enzyme activity assay that measures the rate at which the synthetic substrate p-nitrophenyl-α-D-glucopyranoside (p-NP-α-Glu) is hydrolyzed by avalglucosidase alfa into p-nitrophenol (p-NP), a chromophore with an absorbance maximum of 400 nm. The assay uses a synthetic substrate that is commonly used for assessing α-glucosidase activity.

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

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

(b) (4)

- **Manufacturing process summary:** (b) (4)

(b) (4)

- **Container closure:** (b) (4) .
- **Dating period and storage conditions:** (b) (4)

C. Drug Product Nexviazyme 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	Other
Enzyme concentration (Strength)	Efficacy	Manufacturing process, formulation	(b) (4)	
Appearance, color, clarity (General)	Safety	Manufacturing process, formulation		
pH (General)	Safety	Manufacturing process, formulation		
Osmolality (General)	Patient discomfort	Manufacturing process, formulation		
Visible Particles (General)	Safety	DP manufacturing process, CCS, and product		Recommended infusion through an in-line filter in the label
Subvisible particles (General)	Safety	DP manufacturing process, CCS, and product		Recommended infusion through an in-line filter in the label
Reconstitution time (General)	Powder characteristic	Formulation		
Endotoxin (Contaminant)	Safety	Raw materials and contamination during manufacturing		
Bioburden (Contaminant)	Safety	Raw materials and contamination during manufacturing		
Container closure system (Contaminant)	Safety (Failure in closure integrity may lead to contamination through a loss of sterility) or evaporation/leakage (impacting concentration or content)	May be impacted by storage conditions		
Polysorbate 80 content (Composition)	Safety and Efficacy	Formulation component		(b) (4)

- **Potency and Strength:** 100 mg avalglucosidase alfa-ngpt (b) (4) The vials are filled to a target fill volume (b) (4) mL to allow for a delivery volume of 10 mL. This is consistent with

USP<1511>. The potency of avalglucosidase alfa-ngpt is determined using the same enzymatic activity assay as for DS.

- **Summary of Product Design:** The vials are filled to a target fill volume (b) (4) mL to allow for a delivery volume of 10 mL. This is consistent with USP<1511>. (b) (4)
- **List of Excipients:** 10 mM L-histidine/L-histidine hydrochloride monohydrate, 2% (w/v) glycine, 2% (w/v) mannitol, and 0.01% (w/v) polysorbate 80 at pH 6.2.
- **Reference Materials:** Same reference standard is used for DS and DP.
- **Manufacturing process summary:** (b) (4)

  (b) (4)
- **Container closure:** (b) (4)
- **Dating period and storage conditions:** (b) (4)
- **List of co-package components, if applicable:** None.

D. Novel Approaches/Precedents: None.

E. Any Special Product Quality Labeling Recommendations: Avalglucosidase alfa-ngpt is filled in clear glass vials as sterile lyophilized product that needs to be reconstituted with sterile water for injection (WiFi) and administered as an intravenous infusion. If immediate use is not possible, the reconstituted solution can be stored up to 24 hours at 2°C to 8°C.

The reconstituted product is withdrawn from vials using a disposable sterile syringe and diluted into an intravenous infusion bag containing 5% Dextrose Injection, USP to a final concentration between 0.5 to 4 mg/mL followed by infusion through an intravenous line. The use of an in-line is recommended in the label. The diluted solution can be stored at 2°C to 8°C for up to 24 hours.

F. Establishment Information

Overall Recommendation:					
DRUG SUBSTANCE					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Master Cell Bank (MCB) and Working Cell Bank (WCB) Manufacturing, testing and storage facilities.	Genzyme Corporation 45 New York Avenue Framingham, MA 01701	1220423/968278916	No evaluation Necessary	N/A	No evaluation Necessary
Additional testing facilities used for MCB/WCB characterization and adventitious agents testing	Genzyme 153 2ND Ave Waltham, MA US 02451	3002525139/078456891	No evaluation Necessary	N/A	No evaluation Necessary
Additional testing facilities used for MCB/WCB characterization and adventitious agents testing	(b) (4)	(b) (4)	No evaluation Necessary	N/A	No evaluation Necessary
Additional testing facilities used for MCB/WCB characterization and adventitious agents testing	Sanofi Corporation-Waltham QC-Molecular Biology (QCMB) 153 2nd Avenue , Waltham, MA, USA, 2451	3002525139/078456891	No evaluation Necessary	N/A	No evaluation Necessary
Additional testing facilities used for MCB/WCB characterization and adventitious agents testing; Analytical testing (b) (4) control samples-specific assay: Mycoplasma	(b) (4)	(b) (4)	Acceptable evaluation of firm's compliance history.	N/A	Approve based on history.
Drug substance manufacturing process ((b) (4) Additional MCB and WCB Storage location; Analytical testing, certification and release of drug substance; Analytical testing (b) (4) control samples; DP Quality	Genzyme Flanders bvba Cipalstraat 8 , Geel, NA, Belgium, 2440	3003623839/372153895	Adequate 704(a)(4) Evaluation	N/A	Approve based on 704(a)(4)

Control (QC) Testing					
Analytical testing (b) (4) control samples-specific assay: in vitro viral assay for viral contaminants	(b) (4)	(b) (4)	Acceptable evaluation of firm's compliance history.	N/A	Approve based on history.
DRUG PRODUCT					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Fill / Finish; Labelling, Secondary Packaging, and Release; DP Quality Control (QC) Testing	Genzyme Ireland Limited IDA Industrial Park Old Kilmeaden Road Waterford, Ireland	3003809840/985127419	Acceptable evaluation of firm's compliance history.	N/A	Approve based on history.
Back-up sterility testing site	(b) (4)	(b) (4)	Acceptable evaluation of firm's compliance history.	N/A	Approve based on history.
Labeling, Secondary packaging, and Finished product releasee	Genzyme Corporation 11 Forbes Road Northborough, MA 01532	3009389940/050424395	Acceptable evaluation of firm's compliance history.	N/A	Approve based on history.

G. Facilities

DS manufacturing facility: A review of Genzyme Flanders NV, Geel Belgium (FEI: 3003623839) DS manufacturing documents was performed using the FDA's authority under Section 704(a)(4) of the FD&C Act in advance or in lieu of an inspection. It was determined that a pre-license inspection was not needed and based on the 704(a)(4) review a recommendation for approval was made for this facility.

- **DP manufacturing facility:** An inspection of Genzyme Ireland Limited, Waterford, Ireland (FEI: 3003809840) for DP manufacturing operations was waived based on the history of the facility.

All other proposed manufacturing and testing facilities are acceptable based on their current GMP compliance status and recent relevant inspectional coverage.

Final facility recommendation: Acceptable/ Approval

H. Lifecycle Knowledge Management

a. Drug Substance:

i. Protocols approved:

1. Post-approval Annual Stability Protocol
2. Protocol for (b) (4)

3. Concurrent Release Protocol for [redacted] (b) (4)
4. Concurrent Release Protocol for [redacted] (b) (4)
5. Concurrent Release Protocol for [redacted] (b) (4)

ii. Outstanding assessment issues/residual risk: Refer to PMC 4026-2 and PMC 4026-3.

iii. Future inspection points to consider: None.

b. Drug Product

i. Protocols approved:

1. Shipping Protocol *WAT-GEN-002837*
2. Post-approval Annual Stability Protocol

ii. Outstanding assessment issues/residual risk: Refer to PMC 4026-2 and PMC 4026-3.

iii. Future inspection points to consider: None.

351(a) BLA IMMUNOGENICITY ASSESSMENT

Application Type	BLA 351(a) priority
Application Number	761194
Submit Date	01: July 17, 2020 (non-clinical) 02: September 18, 2020 (Clinical CMC)
Received Date	Same as submit dates
PDUFA Goal Date	May 18, 2021
Division/Office	DRDMG - Division of Rare Diseases and Medical Genetics
Review Completion Date	April 2nd, 2021
Product Code Name	GZ40266
Proposed Proper Name[1]	Avalglucosidase alpha
Proposed Proprietary Name[1]	Nexviazyme
OBP systematic name	RPROT P10253 (LYAG_HUMAN) LYSOSOMAL ALPHA-GLUCOSIDASE [GZ402666]
Pharmacologic Class	Enzyme Replacement Therapy
Applicant	Sanofi Pasteur/Genzyme
Applicant Proposed Indication(s)	Long-term enzyme replacement therapy for the treatment of patients with Pompe disease (acid α-glucosidase deficiency)
Recommended Regulatory Action	Approval

Immunogenicity Assessors

Primary Assessor(s)	Joao Pedras-Vasconcelos, PhD
Secondary Assessor (s)	Zhenzhen Liu, PhD, Team Leader
Tertiary Assessor (s)	Susan Kirshner, Review Chief

[1] The proposed proper and proprietary names are conditionally accepted until such time that the application is approved.

Table of Contents

1. Summary Basis of Recommendation/Executive Summary

1.1 Immunogenicity Executive Summary and Recommendation

Avalglucosidase alfa (GZ402666, neoGAA) is a second-generation recombinant human acid alpha-glucosidase (rhGAA) developed by Sanofi/Genzyme as an Enzyme Replacement Therapy (ERT) for the treatment of Pompe

disease (PD) [redacted] (b) (4) Based on the integrated analysis of product and process related risk factors, as well as clinical study and patient related risk factors summarized in section 2.1 below, neoGAA is considered a high-risk product with regards to immunogenic potential, with anti-drug antibodies having a high likelihood of impacting safety and efficacy to the product in PD patients. This classification is shared with the first generation rhGAA products Myozyme and Lumizyme licensed respectively under BLAs 125141 and 125291. To evaluate immunogenicity of neoGAA, and where applicable the comparator rhGAA, the Applicant followed the recommended bioanalytical tiered strategy for ERT products, developing binding anti-drug antibody assays (BADA), with screening, confirmatory, titer and cross-reactivity assessments, and neutralizing anti-drug antibody (NADA) assays, with separate assessments for inhibition of enzyme activity and inhibition of enzyme uptake. Due to the known risk for hypersensitivity responses during ERT infusion, the Applicant also developed anti-drug IgE (ADIgE) assays to test patient samples suspected of hypersensitivity reactions during product infusion.

To support the clinical program for neoGAA, the Applicant developed eight new drug-specific immunogenicity assays to test subject samples from the late onset Pompe Disease (LOPD) phase 1 TDR12857/LTS 13769 and phase 3 EFC14028/extension studies and infant onset Pompe Disease (IOPD) phase 2 ACT14132/Extension clinical studies. These assays were developed and validated at Sanofi/Genzyme Biomarkers and Clinical Bioanalysis-Boston (BCB, Framingham, MA) which is a bioanalytical facility highly experienced in developing immunogenicity assays for ERTs. The list of immunogenicity assays includes three BADA assays, three NADA assays, one cross reactivity assay, and an ADIgE ImmunoCAP assay to test samples from patients with suspected hypersensitivity reactions. Two early binding anti-drug antibody assays (BADA anti-NeoGAA IgG/IgM antibody ELISA and neoGAA ADA RIP confirmatory assay) and two neutralizing antibody assays (NADA Enzymatic activity inhibition assay and a flow cytometry-based enzyme uptake inhibition assay) were used to test LOPD phase 1 TDR12857 and LTS 13769 study samples through June 2016. To test pivotal Phase 2 IOPD and Phase 3 LOPD EFC1472clinical study samples and Phase 1 LTS13769 post June 2016 samples the Applicant validated a new NeoGAA screening/titer/confirmatory assay, as well as a new neutralizing antibody assay to detect antibodies that inhibit neoGAA uptake into target cells using a cell imaging reader. The early phase neoGAA enzymatic activity inhibition assay and the neoGAA specific IgE ImmunoCAP Assay were utilized throughout clinical development. The assay formats chosen for these various assessments were based on the legacy immunogenicity assays previously developed to test the first generation rhGAA products Myozyme and Lumizyme. Lastly, to test pivotal Phase 3 LOPD and Phase 2 IOPD clinical study samples from patients treated with neoGAA or GAA, the Applicant qualified a new GAA/neoGAA cross-reactivity assay based on magnetic immunodepletion of GAA-cross reactive samples. Depleted samples from Day 25 and Day 49 LOPD study or Day 25 IOPD study were subsequently tested using the validated neoGAA screening/confirmatory/titer assay, and the analogous GAA-specific immunogenicity assay.

In addition, for patients that were treated with the first generation rhGAA product lumizyme, the Applicant also utilized five legacy assays previously developed and validated at BCB for BLA 125291 for Lumizyme, including anti-rhGAA IgG/IgM antibody ELISA and rhGAA ADA RIP confirmatory assay), two neutralizing antibody assays (Enzymatic activity inhibition assay and a flow cytometry-based enzyme uptake inhibition assay), and a rhGAA IgE-ImmunoCAP assay to test patient samples suspected of hypersensitivity reactions. A new binding competition assay for the confirmation of binding antibodies to GAA was validated to test IOPD Phase 2 and LOPD Phase 1 Extension and 3 study samples to replace the RIP confirmatory assay. To test the Phase 3 LOPD clinical study samples from patients that were treated with rhGAA, the Applicant utilized the

same cross-reactivity assay based on magnetic immunodepletion of GAA-cross reactive samples used with the samples from neoGAA treated patients, followed by testing with the GAA specific BADA assay.

The bulk of the immunogenicity assay assessment describe in this memo focused on the tiered BADA, NADA and ADIgE assays used to test the pivotal LOPD Phase 3 EFC14028/Extension studies and IOPD phase 2 ACT14132/Extension studies. The validation data provided in the various reports and summarized in sections 2.2 BADA assays and 2.3 NADA assays support that the assays used to test pivotal study samples for neoGAA-specific treatment-emergent antibody responses (TEAR) are suitable for their intended purpose. Similarly, an assessment of the GAA-specific legacy validation reports and newly developed GAA-specific BADA assays support that the assays remain suitable for their intended purpose.

The available immunogenicity in study performance data discussed in section 2.5 indicate that the validated neo-GAA BADA assays are sensitive and able to detect differences in the magnitude of treatment emergent BADA responses in the LOPD and IOPD study populations and identify when BADA to individual products are cross-reactive to the other product at the two time points tested (study weeks 25 and 49). Similarly, the in-study data indicate that the validated NADA assays can detect differences in the frequency and type the NADA responses between the LOPD and IOPD study populations. Due to changes in assays utilized between the LOPD phase 1 and phase 3 clinical studies, the Applicant also performed bridging studies with ~45 blinded patient samples from Phase 1 TDR12857 study reanalyzed with the validated pivotal study neoGAA BADA ELISA, and a more limited study with 6 NADA+ samples originally assayed using the early phase flow cytometry based cellular uptake inhibition assay reanalyzed with the pivotal cellular imager based cell uptake inhibition assay to justify use of total evaluable data set. In the bridging studies, 45/45 BADA comparability samples and 5 /6 NADA comparability samples from NeoGAA treated patients yielded concordant positive/negative results between the two sets of assays. Given that PD is a rare disease, the high concordance in the bridging study data allow both the Applicant and the Clinical Pharmacology team to pool the totality of the LOPD immunogenicity data to perform the safety and efficacy analysis for this patient population.

Overall, the in-study immunogenicity data support that validated neo-GAA and legacy GAA BADA and NADA assays used in the current application are suitable for their intended purpose. With regards to the ADIgE assays, although validation studies suggest that the IgE ImmunoCAP assays for both products were suitably validated, the in-study performance data were inconclusive as only 1/17 patients suspected of hypersensitivity response tested positive for drug specific IgE, specifically GAA. However, the Applicant also tested suspected hypersensitivity samples for serum tryptase, complement activation and circulating immune complexes using specific commercially available CLIA methods which can complement the usefulness of the ADIgE assays.

Due to current workloads, and public health emergency, OSIS was unable to perform either an on-site or virtual inspection of the Sanofi US/Genzyme Biomarker and Clinical Bioanalysis Boston facility in Framingham, MA, the primary bioanalytical site involved in validation and testing of immunogenicity assays and clinical study samples. This bioanalytical facility also developed and validated the ADA assays that supported approval of BLA 125141 for Lumizyme and BLA125291 for Myozyme, in addition to other Genzyme-licensed ERTs. Therefore, the lack of a bioanalytical inspection during the current review cycle is in not considered a potential approvability issue.

Based on our immunogenicity assay assessment, the OBP Immunogenicity team has no bioanalytical assay related approvability issues for BLA 761194 Avalglucosidase alfa (GZ402666, neoGAA), and considers that supporting immunogenicity data are acceptable pending concurrence from Clinical and Clinical Pharmacology teams.

1.2 Deficiencies and Other Recommended Comments to Applicant

None

2. Review

Document Reviewed	Submission Date
BLA 761194 SN 02	09/18/2020

2.1 Immunogenicity Risk Assessment

Pompe disease (acid alfa-glucosidase deficiency) is a rare genetic disorder (estimated at 1:14,000 births in US) caused by bi-allelic autosomal mutations of the gene coding for acid α-glucosidase (GAA), an enzyme necessary for degradation of lysosomal glycogen. Deficiencies in GAA in patients with Pompe disease result in intralysosomal accumulation of undegraded glycogen, significantly impairing cellular function, particularly in smooth, cardiac, and skeletal muscle cells, leading to progressive losses of motor function. Depending on the degree of genetic deficiency, and expression levels of endogenous enzyme, Pompe disease can be divided into two broad categories:
1) Infantile onset (IOPD), where the disease presents in first months of life leading to severe cardiomyopathy, hypotonia, respiratory failure and if untreated, possible death in first year of life. These patients have low rates of cross-reactive immunological material (CRIM) by Western blot analysis, and typically benefit the most from immune tolerance induction upon administration of enzyme replacement therapy.
2) late onset (LOPD), which appears later in life ranging from 1st year to as late as the 6th decade of life, depending on the degree of enzyme deficiency and has a more heterogenous course of disease. This form is the most common, and patients, who develop this form of the disease later in life, have a high rate of cross-reactive immunological material by Western blot, and tend to have a better prognosis with enzyme replacement therapy.
The proposed therapeutic avalglucosidase alfa (GZ402666, neoGAA) is a synthetically modified version of the approved enzyme replacement therapeutic, alglucosidase alfa (GAA, Myozyme/Lumizyme). Although fully glycosylated due to its production in CHO cells, neoGAA has a higher number of hexamannose structures with two terminal Mannose-6-phosphate (M-6-P) sugars to increase uptake into cells via M-6-P receptor and delivery into the lysosomal compartment. The currently approved ERTs, Myozyme/Lumizyme are considered standard of care, and has led to increased survival and quality of life for patients with IOPD and LOPD.
The current clinical program for neoGAA includes four clinical studies, one in IOPD and three in LOPD, which are listed below:

- Study TDR12857 (NEO1): Phase 1, open-label, ascending-dose neoGAA study in GAA-treatment naïve and GAA-treatment-experienced patients with LOPD. Study includes tiered immunogenicity assessment.
  - ADA sampling: Screen, W1, q4w to W25, W27, W29.
- Study LTS13769 (NEO-EXT): open-label extension of phase 1 Study TDR12857**.** Study continued prior tiered immunogenicity assessment.
  - ADA sampling: study entry, monthly up to W24; quarterly after W24 for first 2 years; every 6 months for years 2-6 of study.
- Study EFC14028 (COMET): Phase 3, randomized, double-blind, active-controlled, noninferiority study in GAA-naive patients with LOPD treated with neoGAA or GAA in stage 1 of study. Includes a stage 2 open label extension with switch to neoGAA after W49. This study includes tiered immunogenicity assessment.
  - ADA sampling: patients randomized to neoGAA: Baseline, D8, W5, W9, W13, W17, W21, W25, W29, W33, W37, W41, W45, W49.
  - ADA sampling: patients randomized to GAA: Baseline, W49.

  Open label stage
  - ADA sampling: patients maintained or switched to neoGAA: W52, W53, W57, W61, W65, W69, W73, W85, W97, W109, W121, W133, W145, every 12 weeks up to End of Study Visit.
- Study ACT14132 (Mini-COMET): Phase 2, open-label, ascending-dose neoGAA study in pediatric patients with IOPD previously treated with GAA who either had a suboptimal response or experienced a clinical decline following initial success. This study includes tiered immunogenicity assessment.
  - Baseline, D8, W5, W9, W13, W17, W21, W25, W28, W37, W49, W61, W73, W85, W97, W109, W121, W133, W145; for patients switched from GAA to neoGAA in extension phase additional sampling at W29, W33, W41, W45, and W49

The applicant Sanofi/Genzyme submitted an Integrated Summary of Immunogenicity (ISI) in section 5.3.5.3 as well as a summary of bioanalytical methods in section 2.7.2. The Applicant included an immunogenicity risk assessment table in the ISI Section 2, which is summarized in assessor table 2.1.1 below:

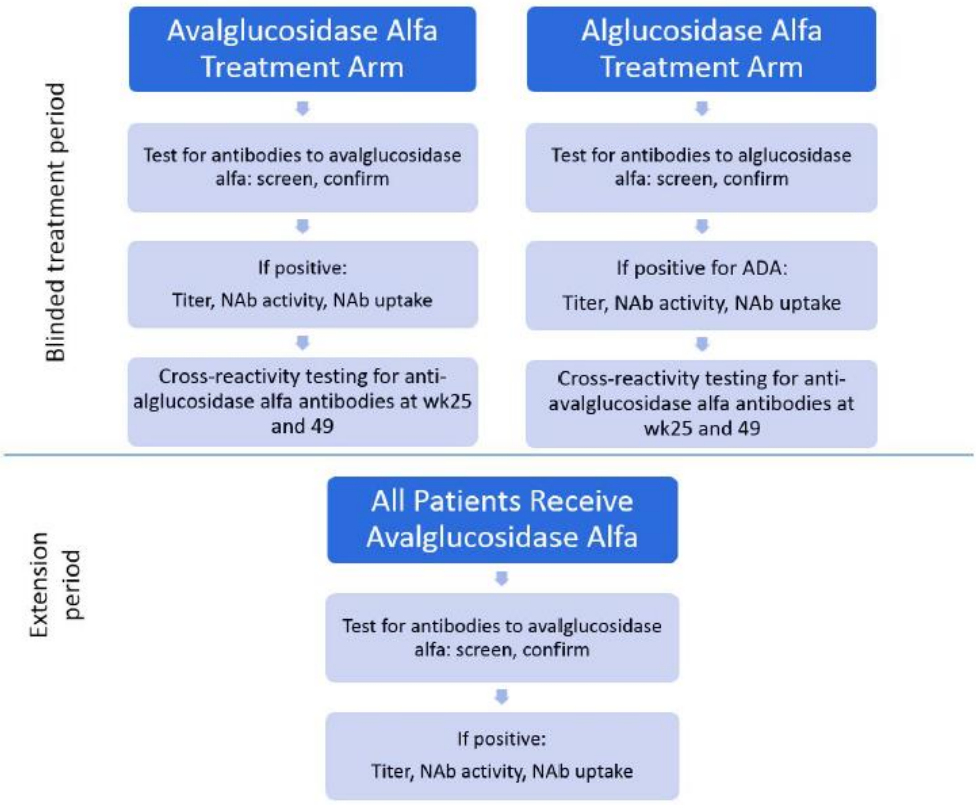
Table 2.1.1: Immunogenicity risk assessment table for Avalglucosidase alfa (neoGAA)

Immunogenicity Risk Factors (RF)		**NeoGAA**	**Assessor comment**
Product Related RF	Degree of foreignness (DF)	Medium: Chemical aminoxyl linker and bisM6P improves delivery to and uptake by cardiac and skeletal muscles, but increases DF	*The Applicant's immunogenicity risk assessment for neoGAA considers all the essential risk factors highlighted in the 2014 FDA guidance "Immunogenicity assessment of Therapeutic proteins".*
	Similarity to endogenous GAA	Partial, depends on genetic mutations in individual patients	
	Endogenous GAA Expression level	IOPD-<1% WT GAA levels (CRIM negative)	

		LOPD 2-40% WT GAA levels (CRIM variable)	*As with the predecessor GAA drug product, neoGAA is classified as a high-risk product in terms of immunogenicity risk assessment.* *The mode of action of both NeoGAA and GAA involves cellular uptake into muscle cells via the M-6P receptor and subsequent delivery into the lysosome where glycogen accumulates.* *This MOA requires development of two different types of ADA assays for assessment of neutralizing anti-drug antibodies (NADA)- an in vitro enzyme inhibition assay and an inhibition of cellular uptake as first stated in the 2015 FDA Guidance "Scientific Considerations in Demonstrating Bioasimilarity to a Reference Product".* *In addition, as patients with LOPD and some patients with IOPD are CRIM positive, cross-reactivity testing to endogenous GAA is also performed for both patient groups.* *The Applicant followed OBP recommendations and provided a list of neoGAA DP lots used in the four clinical trials in Table 3 of the ISI. Only one lot of neoGAA was*
	Drug target	Intracellular (lysosomes)	
	Primary sequence	Human, divergence from WT sequence depends on extent of genetic mutation in individual patients	
	Glycosylation pattern	Mammalian, with chemical modifications of bisM6P	
	Immunological MOA	None; MOA is enzymatic and dependent on intracellular localization	
Process Related RF	Cellular Substrate	CHO-DHFR cells (mammalian)	
	Impurities	Process Control Strategy	
	Aggregation	Product Control strategy	
Clinical Study related RF	Dosing regimen	Chronic dosing (weekly infusions lifelong)	
	Dose	LOPD- 20 mg/kg weekly IOPD - 20 or 40 mg/kg weekly	
	Route of Administration	IV infusion	
	Half-life in vivo	Relatively short ≤1.6hrs	
Patient related RF	Relevant disease factors	Yes, but variable among patients	
	GAA gene mutation	IOPD Frameshift, truncation mutations LOPD missense, leaky splice-site mutations	
	CRIM status	IOPD negative LOPD positive	
	Patient Immune Status	IOPD high levels of inflammation due to tissue damage LOPD typically normal to mid-level inflammation	
	Concomitant medication	IOPD tolerization regimen in a subset of patients LOPD tolerization typically absent	

			used in more than one study (LOPD -LTS13769 and EFC14028). No Lots of GAA were used in more than one study
Overall Immunogenicity Risk assessment classification		**High**	**Based on cumulative product and process related RFs, as well as clinical study and patient related RFs summarized above, neoGAA is considered a high-risk product. This classification is shared with the first generation rhGAA products Myozyme and Lumizyme.**

The tiered approach to immunogenicity testing used by the Applicant in the pivotal/phase III LOPD clinical study EFC14028/Extension which included parallel arms of naïve LOPD patients treated with neoGAA and GAA is summarized in the Figure below:



Assessor comment:
The Applicant followed the recommended tiered approach to immunogenicity testing. This necessitated the development and use of eleven different immunogenicity assays, five for each product. As GAA (myozyme/lumizyme) is approved under BLAs 125141 and 125291 respectably, samples were tested using mostly legacy BADA and NADA assays approved during the review of these BLAs. The focus of the current memo is on the unapproved neoGAA BADA and NADA assays.

2.2 Validation of Binding Anti-Drug Antibody Assays (BADA)

The Applicant developed multiple anti-drug antibody assays to test subject study samples for anti-neoGAA and anti-GAA antibodies . These are listed in table below, along with their respective validation reports, and associated clinical use; *highlighted in yellow are the pivotal study associated ADA assays, which are the focus of the memo*:

Table 4 - ADA and NAb assays used for clinical studies

Assay	Validation Report	Intended Use	Clinical Studies
Avalglucosidase alfa (neoGAA)			
Anti-NeoGAA IgG and IgM Antibody ELISA	ITR-708-0414	ADA screen and titer	TDR12857 and LTS13769 (samples collected through Jun 2016)
NeoGAA ADA RIP confirmatory assay	ITR-636-0513	RIP confirmatory assay	TDR12857 and LTS13769 (samples collected through Jun 2016)
NeoGAA Immunogenicity Screening, Titer, and Confirmatory Assay	DOH1430 (ITR-822-0216)/SPH0499/SPH0501	ADA screen, confirmation and titer	EFC14028, ACT14132, and LTS13769 (samples collected after Jun 2016)
Detection of NeoGAA-Specific Antibodies with No Cross-Reactivity to GAA	ABQ0016	Evaluate for presence of neoGAA specific antibodies	EFC14028
Alglucosidase alfa (Myozyme)			
rhGAA ADA Screening and Titer Assay	ITR-536-0511/SPH0495/SPH0500	ADA to alglucosidase alfa	TDR12857, LTS13769, EFC14028, and ACT14132
rhGAA ADA RIP confirmatory assay	ITR-191-1202	ADA RIP confirmatory assay	TDR12857 and LTS13769 (samples collected through Jun 2016)
A Binding Inhibition Assay for Confirmation of an Immune Response to aglucosidase alfa (Myozyme®/Lumizyme®)	DOH1503	ADA Confirmatory Assay	EFC14028, ACT14132, and LTS13769 (samples collected after Jun 2016)

ADA=anti-drug antibody; GAA= acid alpha-glucosidase; ELISA=enzyme-linked immunosorbent assay; IgG=immunoglobulin G; IgM=immunoglobulin M; RIP=radioimmunoprecipitation assay; NAb=neutralizing antibody; NeoGAA= Avalglucosidase alfa; rhGAA= recombinant human acid alpha-glucosidase.

Assessor comment:
- *The four NeoGAA BADA assays listed in the above table were developed for the current BLA. The assays highlighted in yellow were used to test samples from the LOPD and IOPD pivotal studies.*
- *The GAA confirmatory assay highlighted in yellow was developed for the current application to replace the legacy RIP confirmatory assay used in Phase 1 and was used to test samples from the LOPD and IOPD pivotal studies.*
- *The GAA screening/titer assays are legacy assays approved for use with Myozyme/Lumizyme.*

2.2.1 BADA Method Principle

A) ADA assays for screening, confirmatory and titering assays:
Neo GAA:

The binding ADA assays developed to test the phase 3 LOPD as well as phase 2 IOPD were indirect ELISAs where plates were coated with recombinant human neoGAA (rhneoGAA), blocked with 3% non-fat dry milk/PBS solution, incubated with patient serum or positive control anti-serum, followed by detection with a 1:1 mixture of horse radish peroxidase (HRP) -mouse monoclonal anti-human IgG Fc and anti-IgM Fc secondary antibodies. Detection used TMB enzymatic substrate and OD at 450nm, with reaction stopped with 1N HCl. The positive controls used were three different preparations of purified-rabbit anti-neoGAA polyclonal-conjugated with purified human Ig. The specificity to the drug is provided by the rabbit purified polyclonal portion of the conjugate, while the human IgG provides the right species' specificity to the positive control.

For the Phase 1/2 LOPD samples the same binding ADA sandwich format was used, except that there was a separate radio-immunoprecipitation assay (RIPA) for confirmation rather than a drug competition step in the binding anti-neoGAA antibody assay. The RIPA involved sample incubation with radiolabeled neoGAA, followed by immunoprecipitation with protein A and protein G beads. The antigen-antibody complex is dissociated by boiling, separated by SDS-PAGE and bands visualized by autoradiography. The rabbit-human hybrid positive control preparation used to validate the initial phase 1/2 assays was different from the ones in later validation studies.

GAA (Myozyme/Lumizyme) :
The legacy GAA-specific indirect ELISA was used for testing samples for binding antibodies to GAA (Myozyme/Lumizyme). The assay format is similar to the neoGAA assay except that plates were coated with rhGAA and blocked with 0.1% HSA, and the positive control used was purified pooled human anti-rhGAA anti-serum from GAA-treated LOPD patients and for detection HRP-mouse anti-human IgG Fc is used. A separate GAA confirmation assay using competitive indirect ELISA format was used to test pivotal study samples, that replaces the legacy the RIPA assay confirmatory assay.

B) Cross-reactivity Assays (Detection of NeoGAA-specific antibodies with No Cross-reactivity to GAA):
Cross reactivity to GAA on anti-neoGAA ADA positive samples was tested as separate characterization assay and was not part of binding ADA assay.
NeoGAA testing: To test for cross reactivity in phase 3 LOPD and Phase 2 IOPD samples, confirmed ADA+ samples at week 25 and week 49 of study from neoGAA treated patients were preincubated with biotin-GAA+ streptavidin-magnetic beads using four separate rounds, and resulting bead-GAA-ADA complexes are removed after each round using a magnet. The resulting 1/20 diluted supernatants are pooled further diluted in 1/5 for a final dilution of 1/100 and retested in both the NeoGAA and GAA ADA indirect ELISAS. Cross-reactive samples will retest negative by both ELISAS. Non-cross-reactive samples will test positive in neoGAA ADA assay but negative in GAA ADA assay.
GAA testing: Similar preincubation step is performed with biotin-GAA+ streptavidin-magnetic beads on samples from GAA treated patients in week 25 of study only, followed by retesting of supernatants in both ELISAS. The GAA version of the assay was qualified at the same time as the neoGAA version in 2017, and thus is not considered legacy GAA assay.

2.2.2 BADA Assay Validation Exercises

The studies summarized in table 2.2.2.1 (ADA assays) included cut point analysis, precision, relative sensitivity, selectivity, specificity, hook effect, robustness testing, sample stability (4°C, freeze/thaw, and bench top), and establishment of assay control ranges.

Table 2.2.2.1: Validation Summaries and Assessor Analysis for BADA assays used in Phase 2 IOPD and Phase 3 LOPD studies (Validation Reports DOH1430/ITR-822-0216/ABQ0016 (NeoGAA) and ITR-536-0511/Ad1/DOH1503 (GAA)

Validation Parameter	Validation Reports: DOH1430/ITR-822-0216/ABQ0016 (NeoGAA)	Validation Reports: ITR-536-0511/Ad1/DOH1503 (GAA)	Assessor Comment
Contract Research Org	Sanofi Biomarkers and Clinical Bioanalysis-Boston (BCB) Framingham, MA Validation for screening/confirmatory/titer assays (**DOH1430/ITR-822-0216)** and qualification of cross-reactivity assay (**ABQ0016)** were performed in 2017	Sanofi BCB Validations for screening/titer assay (**ITR-536-0511/Ad1)** were performed in 2012 and validation of confirmatory assay (**DOH1503)** and qualification of cross reactivity assay (**ABQ0016)** were performed in 2017	*Due to the COVID-19 public health emergency travel restrictions and current workloads, OSIS is unable to perform an inspection of the facility within the required time frame.* *This bioanalytical facility also developed and validated the ADA assays that supported approval of BLA 125141 for Lumizyme and BLA125291 for Myozyme, in addition to other Genzyme-licensed ERTs. Therefore, the lack of a bioanalytical inspection during the current review cycle is in not considered a potential approvability issue.*
Assay principle	Screening/confirmatory/titer (SCT) assay: Indirect ELISA Coating rhneoGAA + detection with commercial HRP-Mouse anti-human IgG/IgM (Southern Biotech) +TMB and A450nm Cross-reactivity Assay (XRA): Immunoprecipitation with biotin rhGAA coated beads, and re-testing of supernatant in screening assay for remaining reactivity to neoGAA using above ELISA.	Screening/titer assay (ST): Indirect ELISA Coating rhGAA (lot 19037-61) + detection with commercial HRP-Mouse anti-human IgG(Southern Biotech) +TMB and A450nm *This is the legacy ELISA assay approved for ADA testing for Lumizyme under BLA 125141 and Myozyme under BLA125291.* Confirmatory assay (CfA): Competitive indirect ELISA ADA+ samples pre incubated ± 10mg/ml rhGAA followed by above	*The choice of indirect ELISA format based on HRP-TMB as opposed to an ECL bridging assay is due to the legacy ADA assay formats approved for use with Myozyme/Lumizyme (GAA) commercial products. This indirect HRP- dependent format typically leads to poorer assay sensitivities than ECL-based systems.* *The cross-reactivity assay is intended to characterize non-GAA cross-reactive antibodies and indirectly identify cross-reactive*

	This competitive ELISA assay is designed to detect non-GAA cross-reactive antibodies, by first removing GAA cross-reactive antibodies by immunoprecipitation with GAA-coated magnetic beads.	indirect ELISA *This competitive ELISA assay was developed for the current BLA to replace the legacy RIP confirmation assay.* Cross-reactivity Assay (XRA): Immunoprecipitation with biotin rhGAA coated beads, and re-testing of supernatant in screening assay for remaining reactivity to neoGAA using above S/T ELISA. *This assay has the same sample pretreatment as the neoGAA assay and was qualified at same time.*	*samples. Because the only difference between products is the higher levels of bisM-6-P, most samples are expected to be cross-reactive. This assay is only used to test samples at specific time points (e.g Weeks 25 and 49 of Phase 3 LOPD study)* *GAA utilized separate confirmation assay in addition to the approved screening/titering assay.* *The XR assay has two separate arms- the neoGAA ELISA and the GAA ELISA. The qualification of the XR assay involved performing both GAA and neo-GAA ADA assays after magnetic depletion of GAA-specific antibodies.*
Sample Pretreatment	S/T assay: None Confirmatory tier: 10mg/ml neoGAA XR Assay: Biotin-rhGAA-SA magnetic beads	S/T assay: None Confirmatory assay: 10mg/ml GAA XR Assay: Biotin-rhGAA-SA magnetic beads	*For initial tier testing no sample pre-treatment is required due to short half-life of both drug products. Sample pre-treatment with specific product is individually required for confirmatory assay and for cross-reactivity assay.*
Positive control (PC)	SCT PC: Purified rabbit anti NeoGAA polyclonal conjugated to purified human IgG diluted in 10% NHS *This type of hybrid rabbit/human PC can result in lower sensitivity estimates than if a single affinity purified anti-neoGAA anti-sera were used for system suitability*	PC: prepared from anti-GAA ADA+ pooled sera from LOPD patients treated with rhGAA *This positive control was developed following approval of BLA125141 for Myozyme and resulted from a PMC.* *Two additional controls were developed during initial validation-* • *Purified rabbit anti rhGAA polyclonal conjugated to purified human IgG diluted in 10% NHS* • *Purified mouse anti rhGAA polyclonal conjugated to purified*	*The use of conjugated rabbit polyclonal- human Ig hybrid as a system suitability positive control is not ideal and typically leads to lower sensitivities, which is what is observed. The data for anti-neoGAA assays supports the use of the current positive control. As a late cycle communication, the Applicant will be recommended to develop a human anti-neoGAA system suitability control for use in phase 4 studies, possibly one derived from pooling of confirmed ADA+ LOPD samples for use as a system suitability control. This*

		human IgG.	*avoids use of a PMC like the one which led to the development of the current anti-GAA human IgG positive control.*
PC for XR assay Assay Performance Control (APC)	XRPC: pooled patient sera spiked with anti-GAA antibody and a low level of neoGAA-specific antibody XRAPC: pNHS spiked with anti-GAA and anti-neoGAA antibodies *XR assay required development of separate controls compared to S/C/T assay.*	These are the same PC and APC used for the neoGAA XR assay. *XR assay required development of separate controls from those used S/T assay*	*The same PC was used during validation of each arm of the XR assay (neoGAA and GAA ELISAS)*
PC Dose Curve and Hook Effect	S/C/T Assay: 101ng/ml-12,900ng/ml (triplicate 12x two-fold serial dilutions, NC 1/100); no hook effect observed	5.4 ng/ml-11,000ng/ml (triplicate 12x two-fold serial dilutions, NC 1/100) ; no hook effect detected	*Dose response ranges are impacted by the rabbit-human conjugate nature of the neoGAA PC, leading to lower sensitivity estimates*
LLPC (lower limit positive control)	1/286 dilution of PC (~1.61ug/ml) Determined from interpolation of dose response curve data; 1% failure rate	Not available	*The LLPC used for neoGAA assay was based on interpolation of dose response data*
LPC	1/256 dilution of PC (~1.80 ug/ml)	Determined from interpolation of dose response curve data; 1% failure rate 1/32	*The LPC used for GAA assay was based on interpolation of dose response data*
HPC	1/32 dilution of PC (~14.4 ug/ml)	MPC 1/16 dilution of PC HPC 1/8 dilution of PC	*Legacy GAA assay included an MPC during validation only*
Matrix and NC	Pooled NHS 1/100	Pooled NHS 1/100	*Commonly practice in industry*
MRD	1:100	1:100	*Commonly used MRD for this type of assay*
NC system suitability range	0.05 OD-SCP	0.05 OD-SCP	*System suitability ranges for NC and PCs were established using the mean +/- 2.5 SD of all the respective control values from 79 assays over the course of the validation exercise. They are acceptable.*
LLPC system suitability range	SCP-0.24 OD	Not available	
LPC system suitability range	0.19-0.44 OD	0.08-0.16 OD	
HPC system suitability range	0.90-2.05 OD	0.85-1.29 OD	

Screening cut- point (SCP) Floating CP: Mean NC response × normalization factor Two-three analysts over three days at 6 runs/day/analyst	Based on 60 treatment naïve LOPD samples. Performed outlier exclusion based on 360 data points 3XIQR of logN transformed S/N data. Data not normally distributed, used non parametric 95th percentile; 5% FP, SCP Factor =1.43 *SCP was appropriately calculated using suitable LOPD disease matrix*	Based on 60 NHS and on 108 PD baseline samples (51 LOPD and 57 IOPD). Performed outlier exclusion based on Tukeys test of data points logN transformed OD data. Data not normally distributed, used non parametric 95th percentile; 5% FP, NHS SCP=0.08 Patient SCP =0.06 OD *When legacy assay was validated it used a fixed SCP approach which was commonly used at the time, rather than currently recommended floating SCP.*	*SCP was determined in the appropriate disease matrix (LOPD) for neoGAA ELISA. This matrix can serve as surrogate for IOPD disease matrix, as this population is rarer, and is harder to have sufficient subject numbers for cut point assessment.* *Legacy GAA screening/titer assay used fixed cut point approach and used NHS and sera from both LOPD and IOPD patient baseline samples during assay validation.* *Overall, the approach used to determine the SCP is acceptable because the relevant sources of variability such as inter-analyst, inter-run, intra-run, and inter-subject variability are pooled and incorporated into the SCP calculations. Use of 5% FPR for SCP determination is acceptable and consistent with the FDA 2019 assay guidance.*
Confirmatory cut-point (CCP) Two analysts over three days	Based on the same 60 treatment naïve LOPD samples preincubated ±10 ug/ml neoGAA, using non parametric 99th percentile, 1% FP. Similar outlier analysis as for SCP identified 52 analytical outliers and 9 biological outliers across the 360 data points. CCP = 24.8% *CCP was appropriately calculated using suitable LOPD disease matrix*	Based on 60 treatment naïve LOPD samples preincubated ± 10 ug/ml GAA, using non parametric 99th percentile, 1% FP. 6 analytical outliers removed *CCP:10.8%* *CCP was appropriately calculated using suitable LOPD disease matrix*	*Both NeoGAA and GAA confirmatory assays used LOPD disease matrix.* *The difference in CCPs is in part due to the different PCs. The GAA PC is affinity purified pooled human IgG from treated LOPD patients with confirmed positive samples.*
Titer Cut Point (TCP) Two analysts over three days	Based on same 60 treatment naïve LOPD samples, using	Based on 60 NHS and on 108 LOPD baseline samples, using non	*TCP was determined in the appropriate disease matrix*

	nonparametric 99th percentile, 1%FP TCP factor: 1.80 *TCP was appropriately calculated using suitable LOPD disease matrix*	parametric 99th percentile, 1%FP NHS TCP=0.09 OD LOPD TCP =0.07 OD *Similar to SCP, original titer assay used a fixed CP approach which was commonly used at time of assay validation.*	*(LOPD) for neoGAA ELISA.* *Legacy GAA screening/titer assay used fixed cut point approach and used both NHS and LOPD patient baseline samples during assay validation.*
Cross Reactivity (XRCP) Single analyst	Based on 30 treatment naïve LOPD samples, using robust parametric 99.9th percentile, 1%FP XRCPF: 1.267 *The LOPD sera used were different from those used in earlier validation*	Based on same 30 treatment naïve LOPD samples as neoGAA assay, using non parametric 99.9th percentile, 1%FP XRCPF: 1.261 *The LOPD sera used were the same from those used for neoGAA validation*	*The LOPD sera used were different from those used in earlier validation.* *The qualification report included full set of statistical evaluations exploring both parametric and non-parametric statistical approaches for both the neoGAA and GAA assays.*
Assay Drug tolerance	NHS spiked with LLPC (1.61 ug/ml) and neoGAA 20, 10, 1, 0.1, 0.01ug/ml. Drug tolerance was ≤1ug/ml. *Drug Tolerance acceptable as no onboard neoGAA is expected in samples due to short half-life of drug (<2hrs).*	Not assessed for screening/titering assay or confirmatory assay due to short-half-life of GAA (<2.4hrs) *Justification for lack of drug tolerance testing in legacy assays was accepted at time of original immunogenicity assay assessment.*	*Drug tolerance demonstrated in neoGAA (≤1.0 ug/ml) is acceptable, as the half-life of the drug <2 hrs.* *Lack of testing for legacy GAA assays is acceptable as the sampling is done prior to infusion, so the drug is not expected to be present in any patient samples.*
Sensitivity	Determined by interpolation of dose response curves 251 ng/ml *Although sensitivity is lower than the guidance recommended 100 ng/ml, this is due to the use of hybrid rabbit-human PC which leads to poorer sensitivity estimates compared to hyperimmunized animal*	Determined by interpolation of dose response curves 12,148.3 ng/ml *The sensitivity estimate is well below current recommendations of 100 ng/ml. Despite the poor sensitivity estimate this is a more representative PC of the anti-GAA antibody responses present in the treatment population. This*	*The choice of hybrid positive control for NeoGAA assays can lead to lower sensitivity estimates compared to typical hyperimmunized animal PC. Although lower than recommended 100ng/ml, given the product class, the sensitivity is acceptable for the current BLA.* *Life cycle management advice for development of better a neoGAA PC for*

	serum due to uncoupling of rabbit Ig from human Ig. The hybrid antibody was chosen due to the indirect ELISA format	*pooled human anti-rhGAA has relatively low affinity for GAA likely because it is from chronically treated PD patients which will have undergone a high degree of tolerization to the product.* *The rabbit/human hybrid anti-GAA PC (592.6 ng/ml) and Mouse/human hybrid anti-GAA PC (209.6 ng/ml) used during the original validation had better sensitivity estimates, likely because they were from hyperimmunized animals.*	*phase 4 testing will be recommended part of late cycle communication.*
Repeatability/Intra-assay variability %CV≤20% for all PC	NC %CV 12.5% LLPC %CV 4.5-5.0% LPC %CV 3.6%-4.9% HPC %CV 2.9-4.9%	Screening/titer assay LPC/S2 %CV 0-3.8%% MPC/S3 %CV 2.5-6.7%% HPC/S4 %CV 1-8.2% Confirmatory LPC %CV 2.7-3.9% HPC %CV 0.7-0.9%	*repeatability for both sets of assays is<15% acceptable*
Intermediate Precision (IP)/inter-assay variability %CV≤20% for all PC (2-3 analysts over three days)	NC %CV ≤ 12.5% LLPC %CV ≤17.6% LPC %CV ≤12.9% HPC %CV ≤11.7%	Screening/Titer Assay LPC/S2 %CV 6.9-11.8% MPC/S3 %CV 3.0-11.6% HPC/S4 %CV 3-13.2% Confirmatory Assay LPC %CV 3.1-4.7% HPC %CV 1.4-1.7%	*IP for both assays was <20% and is acceptable*
Intermediate Precision (IP) for XR assay single analyst over 3 days (2 plates per day)	XRPC not-depleted 4.6% XRPC GAA-depleted 8.6 % XRAPC not depleted 4.0% XRAPC GAA depleted 7.0% *GAA depletion did not impact the spiked neoGAA antibodies which remain detectable by neoGAA assay*	XRPC not depleted 4.2% XRPC GAA-depleted <XRCP XRAPC not depleted 4.0% XRAPC GAA depleted <XRCP *GAA depletion led to decrease of signal to below*	*XR assay IP <10% and is acceptable for both GAA and neoGAA assays*

		XRCP for GAA assay as expected	
Selectivity (Matrix)	• 10/10 pretreatment LOPD sera spiked with LLPC 1.61/ug/ml were positive and with %CV<20% • 9/10 unspiked samples were negative and showed a CV <20%. The last sample had a CV>22% so it was not used. *Selectivity in LOPD matrix was successfully demonstrated*	three NHS were tested at LPC ± interfering compounds (hemoglobin, bilirubin and lipid). Three NHS sera tested positive. *For results of interfering compounds see bottom of table* *Selectivity was only tested in NHS for GAA assay*	*Selectivity in LOPD patient matrix was successfully demonstrated for neoGAA assay. The lack of testing in IOPD patient matrix is acceptable given the lack of available samples.* *Legacy GAA assay tested only selectivity of interfering compounds in NHS.*
Selectivity for XR assay Five sera from rhGAA-treated PD patients, previously characterized as positive for anti-GAA antibodies, were spiked with neoGAA-specific hybrid positive control antibody Five baseline sera from same individuals.	• 5/5 non-depleted sera from rhGAA treated patients tested positive for anti-neoGAA antibodies • 5/5 GAA depleted sera spiked with anti-neoGAA LPC tested positive for neoGAA antibodies • 5/5 baseline PD sera were negative with or without GAA depletion *Selectivity for XR assay was demonstrated by successful depletion of GAA specific antibodies in rhGAA treated individuals.* *Anti-NeoGAA PC spiked samples remained positive post depletion*	• 5/5 non-depleted sera from rhGAA treated patients tested positive for anti-GAA antibodies • 0/5 GAA-depleted sera tested positive for anti-GAA antibodies • 5/5 baseline PD sera were negative with without GAA depletion *Selectivity for XR assay was demonstrated by successful depletion of GAA specific antibodies in rhGAA treated individuals.*	*These selectivity experiments are specific to the XR assay and were performed concurrently for both neoGAA and GAA.*
Specificity Positive controls against other ERTs	• anti-Aldurazyme(rhIDU) rabbit-hIgG hybrid PC: negative • anti- acid sphingomyelinase (rhASM) rabbit- hIgG hybrid PC: negative • anti-neoGAA LPC and HPC: both positive	• Screening/Titer assay: • Anti-alpha-galactosidase (rhGAL) rabbit-human IgG PC: negative • Anti-beta-glucocerebrosidase (rhGCR) rabbit-hIgG PC: negative • anti-GAA LPC and HPC: both positive	*Specificity was suitability tested and demonstrated for both sets of assays. Because neoGAA and GAA differ only in bisM-6-P content, anti-sera to either product are expected to have high rate of cross-reactivity to the other ERT product.*

	Specificity for neoGAA was suitably demonstrated for the assay	Confirmatory Assay: anti- rhASM PC negative anti-GAA LPC and HPC both positive *Specificity for GAA was suitably demonstrated for the screening and confirmatory assays*	
Robustness testing: Sample incubation time (SIT) Conjugate incubation time (CIT) Substrate incubation time (SuIT)	LLPC, LPC and HPC tested positive using different incubation times indicated assay can be run with 60-70 min for SIT and CIT and 15-16 min SuIT. *Robustness testing acceptable*	LPC and HPC and five validation samples tested positive using different block incubation times (90-120 min), SIT (50-70min), CIT (50-70min), and SuIT (13-17min) *Robustness testing in legacy assay was acceptable*	*Assay robustness was suitably demonstrated for both neoGAA and GAA assays.*
Stability	LPC and HPC tested positive and were stable for: • up to 72hr at 4°C • 24hr at RT • 5X freeze/thaw cycles *neoGAA PC remained stable under all conditions tested*	LPC and HPC tested positive and were stable for: • up to 120hr at 4°C • 2hr at RT • 5X freeze/thaw cycles HPC stable for 24 months at -60°C *GAA PC remained stable under all conditions tested*	*Short-term stability of PC preparations for both assays was suitably demonstrated.*
Lipemia	• LLPC spiked into single lot of lipemic serum was positive • Unspiked lipemic serum control was negative *No impact observed. The use of single lot of lipemic serum is acceptable as PD sera are not characterized by high levels of lipemia.*	• Three LPC samples spiked ± 10mg/ml lipid were positive • Unspiked lipemic serum control was negative *No impact observed. The legacy assay tested experimentally generated lipemic sera rather than naturally occurring lipemic sera.*	*No impact of lipemia was observed in either assay, using both types of matrices (natural lipemic vs experimentally generated lipemic sera)*
Bilirubin/Icteric	• LLPC spiked into one lot of icteric serum was positive • Unspiked icteric serum was negative	• Three LPC samples spiked ± 0.6 mg/ml bilirubin were equally positive • Unspiked icteric serum was negative	*No impact of bilirubin was observed in either assay, using both types of matrices (natural vs experimentally generated icteric sera).*

	No impact observed. Use of single lot of icteric serum to test impact of bilirubin is acceptable as PD sera are not characterized by high levels of bilirubin	*No impact observed. The legacy assay validation tested experimentally created icteric sera rather than naturally occurring icteric sera*	
Hemolysis	• LLPC spiked into one lot of hemolytic serum was positive • Unspiked hemolytic serum was negative *No impact observed. Use of single lot of hemolytic serum to test impact of hemolysis in GAA is acceptable as PD is not characterized by high levels of hemolysis.*	• Three LPC samples spiked ± 10mg/ml hemoglobin were equally positive • Unspiked hemolytic serum was negative *No impact observed. The legacy assay validation tested experimentally created hemolytic sera rather than naturally occurring hemolytic sera.*	*No impact of hemoglobin was observed in either assay, using both types of matrices (natural vs experimentally generated hemolytic sera).*
ADA Assay Assessment	**NeoGAA specific assays are suitable for Intended purpose**	**Legacy assays determined "Suitable for Intended" purpose during original validation assessment. Legacy and newer assays are suitable for current application as well.**	**Although the assays were validated at different times, both the legacy GAA ADA assays and the current neoGAA ADA assays are suitable for intended purpose.**

Additional Assessor comments:

PD is a rare disease indication, resulting in small number of total study participants across all four clinical studies (LOPD n=124, IOPD n=24). Typically, immunogenicity data derived from different ADA assays should not be pooled due to differences in essential assay validation parameters. However, the Applicant tested samples obtained after June 2016 in the phase 1 LOPD extension study LTS13769 with the assays used to test the Phase 3 EFC14028/EXT study. The applicant performed bridging studies to support the pooling LOPD immunogenicity data from Phase 1 TDR12857 study with the data from Phase 1 LTS 13769 and Phase 3 EFC14028/EXT studies allowing for subsequent cumulative analysis on total BADA evaluable patient population. Two sets of bridging studies were performed:

a) *To compare performance of the BADA assay using the two confirmation methods (RIP verses drug competition), the Applicant tested 23 blinded patient samples with historical RIP confirmatory results (positive or negative) in the new drug competition confirmatory assay. In this study, 95% (22 of 23) of the sample replicates resulted in concordant RIP positive/ negative results. The data met all comparability criteria and thus support the inclusion of phase 1 confirmed positive samples in the overall immunogenicity data set for LOPD (see section 2.5).*
b) *The Applicant re-analyzed 41 blinded patient samples from Phase 1 TDR12857 study with legacy RIP confirmatory results (positive or negative) and 4 RIP internal controls (total of 45 samples) using the validated pivotal study neoGAA BADA ELISA. The original phase 1 binding antibody assay had used a*

fixed cut point approach while the pivotal study assay used the currently recommended floating cut point. When samples were retested with the validated ELISA, the bridging study showed concurrence in 45 out of 45 samples using a screening floating cut point for validated neo-GAA BADA assay. In addition, 38 out of 39 positive samples showed concurrence with phase 1 RIP confirmatory assay. This high concurrence rate (>95%) further supports the inclusion of phase 1 confirmed positive samples in the overall immunogenicity data set for LOPD (see section 2.5).

2.3 ADIgE Assay Validation Exercises

2.3.1 ADIgE Method Principle

Anti-Drug IgE ImmunoCAP assays
Because of known risk of hypersensitivity and anaphylactic responses with GAA in PD patients the applicant developed an anti-neoGAA IgE assay based on the same ImmunoCAP100 Fluoro Immuno Assay system from Phadia Laboratory Systems that was used for GAA hypersensitivity testing. NeoGAA or GAA are covalently coupled to ImmunoCAP cellulose sponges and incubated with the product specific IgE in the patient sample. After washing away non-specific IgE, β-D-galactosidase anti-human IgE are added to form a complex. Following incubation, unbound enzyme-anti-IgE is washed away and the bound complex is then incubated with a 4-Methylumbelliferyl (MLD)-developing agent. After stopping the reaction with NaOH, the fluorescence of the eluate is measured in a Phadia1000 instrument (ThermoFisher). The higher the response units (RU), the higher the levels of enzyme specific IgE is present in the sample. To evaluate the test results, the responses for the patient samples are transformed to concentrations with the use of a human IgE calibration curve based on 2nd human IgE International Reference Preparation 75/502. Results are reported international kIA/L.

2.3.2 ADIgE Assay Validation Exercises

The studies summarized in table 2.2.2.2 (anti-drug IgE assays) included cut point analysis, precision, relative sensitivity, selectivity, specificity, hook effect, robustness testing, sample stability (4ºC, freeze/thaw, and bench top), and establishment of assay control ranges.

Table 2.2.2.2: Validation Summary and Assessor Analysis for anti-drug IgE assays used in Phase 2 IOPD and Phase 3 LOPD studies (Validation Reports ITE-612-0113 (NeoGAA) and ATR-577-0312/Ad1 (GAA))

Validation Parameter	Validation Report: ITE-612-0113 Anti-neoGAA IgE	Validation Report: ATR-577-0312/Ad1 Anti-GAA IgE	Assessor Comment
Contract Research Org	Sanofi BCB Framingham, MA Validation for ImmunoCAP100 anti-neoGAA IgE were	Sanofi BCB Framingham, MA Validation for ImmunoCAP100 anti-GAA IgE were performed in 2012	*See comment in table 2.2.2.1.*

	performed in 2013		
Assay principle	FIA Capture: NeoGAA-ImmunoCAP Detection b-Gal anti-human IgE+ MLF detection reagent+ NaOH stop reaction. RU read in Phadia100 instrument (48 singlet samples in 2.5hr)	FIA Capture: GAA-ImmunoCAP Detection b-Gal anti-human IgE+ MLF detection reagent +NaOH stop reaction RU read in Phadia100 instrument	*The ImmunoCAP100 platform is typically run in CLIA certified laboratories performing allergen testing*
Sample Pretreatment (Acid dissociation)	Not required	Not required	*Testing is performed on samples taken from patients that experience hypersensitivity*
Validation Sample control (VSC)	Rabbit IgG anti-neoGAA coupled human IgE hybrid antibody designated as validation samples (VS) VS1/HPC 40kUA/L (96 ng/mL) VS2/MPC 10kUA/L (24ng/mL) VS3/LPC 1.0 kUA/L (2.4ng/mL) *This hybrid control is like what is used for binding ADA assays*	Rabbit IgG anti-GAA coupled human IgE hybrid antibody designated as validation samples (VS) VS1/HPC 75kUA/L (180 ng/mL) VS2/MPC 7.3kUA/L (17.5 ng/mL) VS3/LPC 0.15 kUA/L (0.35 ng/ml)	*These types of controls (VSC and IgE PC) are specific to the IgE FIA format.*
VSC Dose Curve and Hook effect	0.88 -73.8 kUA/L IgE No hook effect observed (8 two-fold dilutions)	0.64 -82.4 kUA/L IgE No hook effect observed (8 two-fold dilutions)	
IgE Dose Curve and Hook Effect	0.22-3.9 kUA/L IgE (5 dilutions based on 2nd human IgE International Reference Preparation 75/502) *No hook effect observed*	0.35-100 kUA/L IgE (5 dilutions based on 2nd human IgE International Reference Preparation 75/502) *No hook effect observed*	*Although the Applicant used the same hIgE international Reference Preparation for both assays, the ranges in the dose curves are different likely because of experience. The assays were validated at different times, and when the neoGAA was validated, they opted for a narrower dose curve.*
IgE PC	IgE C2/LPC 0.6kUA/L (1.44 ng/ml) IgE C1/HPC 1.8kUA/L IgE (4.32 ng/ml)	IgE C2/LPC 0.4kUA/L (0.96 ng/ml) IgE C1/HPC 0.8 kUA/L (1.92 ng/ml)	*As the assays were validated at different times different preps of IgE PC were utilized*
Matrix and NC	NHS with < 200kU/L of IgE	NHS with < 400kU/L of IgE	*The Applicant pre-screened commercial lots of NHS for IgE levels to reduce chances of false positives due to high levels of IgE.*

MRD	Neat serum	Neat serum	*Given the low levels of IgE in serum (5-0.05 ng/ml) testing neat samples is typical for antigen specific IgE testing.*
Platform Cut Point (PCP) Based on Phadia ImmunoCAP platform (0.35kUA/L/172-175 RU)	Applicant tested 50 NHS (range 10-85 RU) and 25 LOPD (range 11-25 RU) treatment naïve samples to confirm that IgE levels were below assigned assay CP 0.35kUA/L (172-175 RU) based on assay CP for Phadia ImmunoCAP platform allergy testing *As the ImmunoCAP100 platform is CLIA approved, no formal statistical CP assessment was performed, only a verification to confirm that various NHS and LOPD sera were below PCP*	Applicant tested 70 NHS (range 9-70 RU). Data found to be not normally distributed; opted for use of PCP 0.35kUA/L (172-175 RU) based on PCP for Phadia ImmunoCAP allergy testing. *As the ImmunoCAP100 platform is CLIA approved, no formal statistical CP assessment was performed, only a verification to confirm that various NHS were below PCP.*	*The use of platform specific assay cutpoint is common for CLIA laboratory tests such as those used in ImmunoCAP allergen testing.* *The neoGAA data shows that values for NHS and LOPD sera fall below the PCP.* *The GAA assay only examined NHS, but as the samples were far below the PCP, this is acceptable.*
Assay product tolerance	VS1 and VS3 ± neoGAA (0.6, 0.24, 0.98, 3.91 15.63, 62.5, 250 and 1000 ng/ml) VS1/HPC 15.6 ng/ml VS3/LPC 0.98ng/ml *While the level of drug tolerance is low, neoGAA has a half-life < 2hrs, and no product is expected in tested patient samples*	VS1/HPC ± GAA (3.91 15.63, 31.25, 62.5, and 250 ug/ml) and at and VS3/LPC ± GAA (0.015, 0.031, 0.61, 0.122, 0.244, 0.488, 0.976, 1.95, 3.91, 7.81 ug/ml) VS1/HPC 0.122 ug/ml VS3/LPC 0.244ng/ml *While the level of drug tolerance is low, GAA has a half-life < 2hrs, and no product is expected in tested patient samples*	*Only patients that have suspected hypersensitivity reactions during infusion will be tested for product-specific IgE on samples obtained within 24hrs of suspect hypersensitivity reaction.* *Drug tolerance is unlikely to be an issue for either product given the half-life <2.5hrs and samples tested for product specific IgE are unlikely to have any onboard drug.*
Assay anti-product IgG tolerance	VS1 and VS3 ± rabbit anti-neoGAA (0, 5, 20, 50 and 100ug/ml) Both VS1 and VS3 were recoverable at all concentrations of ADA IgG tested	VS1 and VS3 ± rabbit anti-GAA (0, 5, 20, 50 and 100ug/ml) VS1 showed interference at 100 ug/ml rabbit anti-GAA, but VS3 was recoverable at all concentrations of	*Both assays are largely tolerant to presence of anti-product rabbit IgG. No interference of human anti-product IgG was tested due to it being unavailable during validation.*

	The presence of rabbit ADA did not interfere with detection of hybrid PC	rabbit anti-GAA IgG tested *The presence of rabbit ADA at higher concentration interfered slightly with detection of hybrid PC (recovery < 80%)*	
Sensitivity	Interpolated from VSC dilution curve 0.409 kUA/L (982 pg/ml) *Sensitivity is <1ng/ml and is acceptable*	Interpolated from VSC dilution curve 0.35 kUA/L (855 pg/ml) *Sensitivity is <1ng/ml and is acceptable*	*Sensitivity for both neoGAA and GAA IgE FIA assays <1ng/ml and are acceptable*
Repeatability/Intra-assay variability (%CV≤15%)	NC% 5.7-14.2% C1 %CV 0.4-3.9% C2 %CV 2.0-4.4% VS1 %CV 0.2-2.3% VS2 %CV 0.2-3.0% VS3 %CV 0.6-3.5% *Repeatability is acceptable for two instruments (≤5% positive controls)*	NC% 6.8-7.4% C1 %CV 0.4-3.9% C2 %CV 2.0-4.4% VS1 %CV 0.8-8.2% VS2 %CV 0.2-3.0% VS3 %CV 0.1-4.3% *Repeatability is acceptable for two instruments (≤10% positive controls)*	*Both neoGAA IgE and GAA IgE FIAs show acceptable repeatability.*
Intermediate Precision (IP)/inter-assay variability (%CV≤20%)	NC% 0-4.6% C1 %CV 5.6-6.1% C2 %CV 7.7-9.5% VS1 %CV 9.1-9.4% VS2 %CV 8.7-8.8% VS3 %CV 7.8-8.2% *IP is acceptable for two instruments (≤10% positive controls)*	NC% 0-4.6% C1 %CV 3.4-3.8% C2 %CV 3.3-5.7% VS1 %CV 6.8-9.8% VS2 %CV 4.0-5.8% VS3 %CV 0-9.4% *IP is acceptable for two instruments (≤10% positive controls)*	*Both neoGAA IgE and GAA IgE FIAs show acceptable IP.*
Selectivity	Tested IgE C1 and anti neoGAA VS1 ± bilirubin, lipid and hemoglobin in NHS *Did not perform traditional selectivity using different sera*	Tested IgE C1 and anti neoGAA VS1 ± bilirubin, lipid and hemoglobin in NHS *Did not perform traditional selectivity in different sera*	*Data show that bilirubin, lipid and hemoglobin do not impact IgE detection.*
Specificity Positive controls against other ERTs: Aldurazyme (rhIDU) acid sphingomyelinase (rhASM) beta-glucocerobrosidase (rhGCR)	acid sphingomyelinase (rhASM) rabbit-human IgE PC: negative Anti-beta-glucocerobrosidase (rhGCR) rabbit-hIgE PC: negative	acid sphingomyelinase (rhASM) rabbit-human IgE PC: negative Anti-beta-glucocerobrosidase (rhGCR) rabbit-hIgE PC: negative	*Specificity for anti-neoGAA and anti-GAA IgE hybrid controls were suitably demonstrated. As there are no product specific human IgE controls the testing is acceptable.*

	VS1/HPC positive *Assay can discriminate between anti-neoGAA IgE and others anti-ERT IgE hybrid positive controls*	VS1/HPC positive *Assay can discriminate between anti-GAA IgE and others anti-ERT IgE hybrid positive controls*	
Robustness	Using NC, C1, C2, VS1, VS2 and VS3 • Tested two Phadia instruments • Two lots of ImmunoCAP reagents *%CV for various samples <5%. Results are acceptable.*	Using NC, C1, C2, VS1, VS2 and VS3 • Tested two Phadia instruments • Two lots of ImmunoCAP reagents *%CV for various samples <15%. Results are acceptable.*	*Robustness testing performed for each product-specific FIA are acceptable*
Stability (hIgE samples)	STB1: 75kUA/L (180ng/ml) STB2: 7.3 kUA/L (17.5 ng/ml STB1 and STB2 stable for: • 5X freeze-thaw • 7 days at 2-4°C • 4hs at room temp	STB1: 25kUA/L (60ng/ml) STB2: 4 kUA/L (9.6 ng/ml STB1 and STB2 stable for: • 5X freeze-thaw • 7 days at 2-4°C • 4hs at room temp	*Short term IgE sample stability testing performed for each product-specific FIA are acceptable*
Lipemia	C1 and VS2 spiked ± 10mg/ml lipid were positive *No impact observed of spiked lipemia.*	C1 and VS2 spiked ± 10mg/ml lipid were positive *No impact observed of spiked lipemia.*	*No impact of lipemia was observed in either FIA.*
Bilirubin/Icteric	C1 and VS2 spiked ± 0.6 mg/ml bilirubin were equally positive *No impact observed of spiking bilirubin.*	C1 and VS2 spiked ± 0.6 mg/ml bilirubin were equally positive *No impact observed of spiking bilirubin.*	*No impact of bilirubin was observed in either FIA.*
Hemolysis	C1 and VS2 spiked ± 10mg/ml hemoglobin were equally positive *No impact observed of spiked hemoglobin.*	C1 and VS2 spiked ± 10mg/ml hemoglobin were equally positive *No impact observed of spiked hemoglobin.*	*No impact of hemoglobin was observed in either FIA.*
ADA Assay Assessment	**Suitable for Intended purpose**	**Suitable for Intended purpose**	**Although validated at different times, the anti-neoGAA and anti-**

			GAA assays are suitable for intended purpose.

Additional Assessor comments:
The anti-drug IgE assays are only used to test samples from patients that experienced infusion-associated reactions or hypersensitivity treatment-emergent adverse events. The sampling for IgE testing can come from the day the event is experienced or within 24 hours of the event (see section 2.5).

2.4 Validation of Neutralizing Anti-Drug Antibody Assays (NADA)

As with BADA assays, the Applicant developed multiple neutralizing anti-drug antibody (NADA) assays to test subject study samples for neutralizing anti-neoGAA and anti-GAA antibodies. These are listed in table below, along with their respective validation reports, and associated clinical use; *highlighted in yellow are the pivotal studies associated NADA assays*:

Assay	Validation Report	Intended Use	Clinical Studies
Avalglucosidase alfa (neoGAA)			
Neutralizing Antibodies that Inhibit NeoGAA Enzymatic Activity	ITR-644-0713/ITR-861-1016 VR	Detection of NAb that inhibits catalytic activity	TDR12857, LTS13769, EFC14028, and ACT14132
Detection of Neutralizing Antibodies that Inhibit NeoGAA cellular uptake by Flow Cytometry	ITR-681-1113	Detection of NAb that inhibits uptake of NeoGAA into cells	TDR12857 and LTS13769 (samples collected through Jun 2016)
Detection of Antibodies Inhibiting Uptake of NeoGAA by Cellular Imaging	DOH1386 (ITR-819-1215)	Detection of NAb that inhibits NeoGAA uptake into cells	EFC14028, ACT14132 and LTS13769 (samples collected after Jun 2016)
Alglucosidase alfa (Myozyme)			
Neutralizing Antibodies that Inhibit rhGAA Enzymatic Activity	ITR-590-0612	Detection of NAb that inhibits rhGAA uptake into cells	EFC14028 and ACT14132
Detection of Neutralizing Antibodies that Inhibit rhGAA Cellular Uptake by Flow Cytometry	ITR-587-0512/NAQ0003	Detection of NAb that inhibits rhGAA catalytic activity	EFC14028 and ACT14132

ADA=anti-drug antibody; GAA= acid alpha-glucosidase; ELISA=enzyme-linked immunosorbent assay; IgG=immunoglobulin G; IgM=immunoglobulin M; RIP=radioimmunoprecipitation assay; NAb=neutralizing antibody; NeoGAA= Avalglucosidase alfa; rhGAA= recombinant human acid alpha-glucosidase.

The Applicant developed two different types of neutralizing antibody assays to test samples from subjects that received neoGAA and/or GAA during the clinical program: an in vitro enzyme inhibition assay and a cellular uptake inhibition assay. These are discussed separately below.

2.4.1 In Vitro enzyme inhibition Method Principle

a) In vitro enzyme inhibition assay:
NeoGAA (1.9ug/ml in NHS) or GAA (1.9 ug/ml in NHS) are incubated with 5 mM 4-MU-α-D-Glucoside (4-MU-DG) synthetic substrate in presence or absence of rabbit PC antiserum specific for each product, or 1/10 dilution of patient samples. Fluorescence signals directly proportional to enzymatic activity are produced following cleavage of 4-MU portion of synthetic substrate, unless neutralizing antibody capable of inhibiting enzymatic activity is present. Enzyme activity in samples and positive controls are compared to that in the

negative control (neoGAA or GAA enzyme in pNHS) and percent inhibition of enzymatic activity compared to the negative control is calculated.

2.4.2 In Vitro enzyme inhibition Validation Exercises

The studies summarized in tables 2.3.2.1 (Enzyme inhibition NADA assays) included cut point analysis, precision, relative sensitivity, selectivity, specificity, hook effect, robustness testing, sample stability (4ºC, freeze/thaw, and bench top), and establishment of assay control ranges.

Table 2.3.2.1: Validation Summary and Assessor Analysis for In vitro Enzyme inhibition NADA assay(s) used in Pivotal Studies (Validation Reports ITR-861-1016-VR (neoGAA) and ITR-590-0612 (GAA))

Validation Parameter	Validation Report: ITR-861-1016-VR (neoGAA)	Validation Report: ITR-590-0612	Assessor Comment
Contract Research Org	Sanofi BCB Framingham, MA Validation for Enzyme activity inhibition assay was performed in 2017	Sanofi BCB Framingham, MA Validation for Enzyme activity inhibition assay was performed in 2012	*See comment in table 2.2.2.1.*
Assay principle	neoGAA+4-MU-DG+ NC→4MU + DG+ fluorescence (RFU) neoGAA+4-MU-DG+ Nab PC→ reduced RFU *This assay uses the same format as legacy GAA assay.*	neoGAA+4-MU-DG+ NC→4MU + DG+ fluorescence (RFU) neoGAA+4-MU-DG+ Nab PC→ reduced RFU *This is the legacy assay validated and currently performed for Myozyme / Lumizyme NADA testing.*	*Assay format was developed initially for GAA and subsequently adapted to neoGAA. Readout for both assays is %inhibition in test sample compared to NC (full enzyme activity).*
Sample Pretreatment (Acid dissociation)	None required	None required	*No sample pre-treatment required due to short half-life of both drug products.*
Positive control (PC)	In house pooled rabbit anti-neoGAA affinity purified antiserum.	In house pooled rabbit anti-GAA affinity purified anti-serum	*Due to the assay formats there is no need for hybrid rabbit/human positive controls for either assay.*
PC Dose Curve and Hook Effect	PC diluted in NHS at 1.6 - 200 ug/ml (8X 2-fold serial dilution) *Neutralization (Value <NACP) detected at 200, 100 and 50 ug/ml. No hook effect detected.*	PC diluted in NHS at 1.6 - 208 ug/ml (8X 2-fold serial dilution) *Neutralization (Value <NACP) detected at 208, 104 and 52 ug/ml. No hook effect detected.*	
LPC	PC diluted 1/80	PC diluted 1/80	
HPC	PC diluted to a titer of 1/10	PC diluted to a titer of 1/10	

Matrix and NC	NHS	NHS	
MRD	1/10	1/10	
NC system suitability range	168844 RFU to 222964 RFU	184400 RFU to 250403 RFU	*System suitability ranges for NC and PCs were established using the mean +/- 3 SD of all respective control values from all assays over the course of the validation exercise. They are acceptable*
LPC system suitability range	LPC as 61.3% to 87. 7% Maximal Signal	LPC as 68.1% to 90.3% Maximal Signal	
HPC system suitability range	54.0% to 64.2% Maximal Signal	43.1% to 62.2% Maximal Signal	
NAb assay cut- point (NACP) Normalized CP: mean S/N-2.33*SD	Based on 60 treatment naïve LOPD samples, using parametric 99% CI, 1% FP. NACP = <94.3%% enzyme activity *NACP was appropriately calculated using suitable LOPD disease matrix.*	Based on 25 treatment naïve LOPD samples, using parametric 99% CI, 1% FP. NACP = <93.1 %% enzyme activity *NACP was appropriately calculated using suitable LOPD disease matrix*	*Both assays set their NACP based on sufficient number of LOPD treatment naïve samples. Use of 1% FP for NAb assay CP determination is a common industry practice and acceptable*
Assay Drug tolerance	NHS spiked with LPC and neoGAA at 10, 1, 0.1, 0.01ug/ml. Drug tolerance was ≤1ug/ml. *Drug Tolerance acceptable as no onboard neoGAA is expected in samples due to short half-life of drug (<2hrs).*	Not assessed for NAb assay due to short-half-life of GAA (<2.4hrs) *Justification for lack of drug tolerance testing in legacy assays was accepted at time of original immunogenicity assay assessment.*	*Drug tolerance demonstrated in neoGAA (≤1.0 ug/ml) is acceptable, as the half-life of the drug <2 hrs.* *Lack of testing for legacy GAA assay is acceptable as the sampling is done prior to infusion, so GAA is not expected to be present in any patient samples.*
Sensitivity	Based on statistical interpolation from PC dilution curve 38.7ug/ml	Based on statistical interpolation from PC dilution curve 46.1ug/ml	*Only a small proportion of either PC will be neutralizing for enzymatic activity by neoGAA or GAA therefore the low sensitivity estimate is not surprising.*
Repeatability/Intra-assay variability	LPC and HPC %CV 1.0-5.9% *Repeatability for the two analysts was <10% and is acceptable*	LPC and HPC 0.5-4.4% CV *Repeatability for the three analysts was <10% and is acceptable*	*Repeatability is acceptable for both sets of assays.*
Intermediate Precision (IP)/inter-assay variability	LPC and HPC %CV 1.4-7.7% *IP for the two analysts was <10% and is acceptable*	LPC and HPC 1.3-5.5% CV *IP for the three analysts was <10% and is acceptable*	*IP is acceptable for both sets of assays.*

Selectivity	10/10 LPC-spiked LOPD sera inhibited enzyme activity *Selectivity suitably assessed, unlike for ADA assays, which only tested impact of interfering compounds*	three NHS were tested at LPC ± interfering compounds (hemoglobin, bilirubin and lipid). Three NHS sera tested positive. *Selectivity testing for legacy GAA assay was limited to impact of interfering compounds when added to three NHS. For results of interfering compounds see bottom of table.*	*Selectivity in LOPD patient matrix was successfully demonstrated for neoGAA assay. The lack of testing in IOPD patient matrix is acceptable given the lack of available samples.* *Legacy GAA assay tested only selectivity for interfering compounds in NHS.*
Specificity Positive controls against other ERTs:	Anti-alpha-galactosidase (rhGAL) rabbit PC: negative Anti-acid sphingomyelinase (rhASM) PC negative Anti-neoGAA HPC and LPC: both positive *Assay specificity for neoGAA activity inhibition was suitably demonstrated.*	Anti-alpha-galactosidase (rhGAL) rabbit PC: negative Anti-Aldurazyme (rhIDU) rabbit PC: negative Anti-neoGAA HPC and LPC: both positive *Specificity for GAA activity inhibition was suitably demonstrated*	*Specificity of enzyme activity inhibition was suitability tested and demonstrated for both sets of assays.*
Robustness testing: Sample-Enzyme incubation (SEI) Immune complex - Substrate Incubation (ICSI)	LPC and HPC tested using different incubation times indicated assay can be run with 50-60 min for SEI and 105-135 min ICSI. *Robustness testing acceptable*	LPC and HPC tested using different incubation times indicated assay can be run with 50-70 min for SEI and 105-135 min ICSI. *Robustness testing acceptable*	*Robustness testing acceptable for both sets of assays.*
Stability	LPC and HPC were stable: • up to 6 days at 2-8°C • 24hr at RT • 5X freeze/thaw cycles *Short term stability demonstrated for PC*	LPC and HPC were stable: • up to 72hr at 2-8°C • 4hr at RT • 5X freeze/thaw cycles *Short term stability demonstrated for PC*	*Standard short-term stability demonstrated for rabbit anti-neo-GAA and anti-GAA PCs used in the specific assays.*
Lipemia	• LPC spiked into single lot of lipemic serum was positive for inhibition of enzyme activity • Unspiked lipemic serum was negative for enzyme activity inhibition	• Three LPC samples spiked ± 8mg/ml lipid were positive for enzyme activity inhibition • Unspiked lipemic serum was negative for enzyme activity inhibition	*No impact of lipemia was observed in either assay, using both types of matrices (natural lipemic vs experimentally generated lipemic sera)*

	No impact observed. The use of single lot of lipemic serum is acceptable as PD sera are not characterized by high levels of lipemia.	*No impact observed. The legacy assay tested experimentally generated lipemic sera rather than naturally occurring lipemic sera.*	
Icteric	• LPC spiked into one lot of icteric serum was positive • Unspiked icteric serum was negative for enzyme activity inhibition *No impact observed. Use of single lot of icteric serum to test impact of bilirubin is acceptable as PD sera are not characterized by high levels of bilirubin*	• Three LPC samples spiked ± 0.5 mg/ml bilirubin were equally positive for enzyme activity inhibition • Unspiked icteric serum was negative for enzyme activity inhibition *No impact observed. The legacy assay validation tested experimentally created icteric sera rather than naturally occurring icteric sera*	*No impact of bilirubin was observed in either assay, using both types of matrices (natural vs experimentally generated icteric sera).*
Hemolysis	LPC spiked into one lot of hemolytic serum was positive Unspiked hemolytic serum was negative *No impact observed. Use of single lot of hemolytic serum to test impact of hemolysis in GAA is acceptable as PD is not characterized by high levels of hemolysis.*	Three LPC samples spiked ± 8mg/ml hemoglobin were equally positive *No impact observed. The legacy assay validation tested experimentally created hemolytic sera rather than naturally occurring hemolytic sera.*	*No impact of hemoglobin was observed in either assay, using both types of matrices (natural vs experimentally generated hemolytic sera).*
NADA Assay Assessment	**Suitable for Intended purpose**	**Legacy NADA assay remains suitable Not suitable for Intended purpose**	**Although validated at different times, the anti-neoGAA and anti-GAA assays are suitable for intended purpose.**

Additional Assessor comments:

As there was not change in the enzyme inhibition assay used to test samples from Phase 1 LOPD study TDR12857/LTS13769 and phase 3 EFC14028 study, there was no need for a method-specific bridging study to support use the total evaluable NADA data set (see section 2.5).

2.4.3 Cellular Uptake Inhibition Method Principle

a) Cellular uptake inhibition assay:

For the neoGAA pivotal study assay, Human Foreskin Fibroblast Cell (HFF 9F0693) are plated in 96 wells at 16,000-24,000 cells/well and incubated with NeoGAA conjugated to Alexa Fluor 594 dye (neoGAA-AF594) with or without Nab containing test serum (PC, patient serum or NHS NC). Cells are also stained with Hoecht dye to allow for nuclei counting. Following washing of the assay plates to remove labeled drug not taken up by cells, total AF594 fluorescent signal (representing internalized NeoGAA) in a set region of each well (representing 4 x 4X image areas) is quantified using the BioTek Cytation 5 Cell Imaging Reader (CIR). Within the same region of each well, the number of Hoechst- positive events (nuclei) are also counted for cell enumeration. Total AF594 signal from background wells is then subtracted from signal from the test wells, and this is divided by the number of cells to yield an RFU/cell. Presence of NAb inhibiting uptake of cellular enzyme is determined by a decrease in fluorescence in test sample wells as compared to that from cells incubated with enzyme alone (RFU max). A minimum of 3000 cells per sample will be imaged for analysis.
For the early and pivotal GAA and early phase neoGAA studies, the cellular uptake inhibition assay followed the same format, but instead of using a CIR, the assay utilized flowcytometry MFI as a readout for cellular uptake, with >2000 gated events acquired by a BD Biosciences FACSCanto II Flow Cytometer. In addition, the GAA assay use rhGAA-conjugated to Oregon Green dye 488 (rhGAA-OG488), rather than AF594 fluorescence dye used in the neoGAA assay.

2.4.4 Cellular Uptake Inhibition Validation Exercises

The studies summarized in table 2.4.2.1 (Enzyme uptake inhibition NADA assays) included cut point analysis, precision, relative sensitivity, selectivity, specificity, hook effect, robustness testing, sample stability (4ºC, freeze/thaw, and bench top), and establishment of assay control ranges.

Table 2.3.4.1: Validation Summary and Assessor Analysis for In vitro Enzyme uptake inhibition NADA assay(s) used in Phase 3 Safety (Validation Reports DOH1386/ITR-819-1215-VR (neoGAA) and ITR-587-0512/Ad1 (GAA))

Validation Parameter	Validation Report: DOH1386/ITR-819-1215-VR (neoGAA)	Validation Report: ITR-587-0512/Ad1	Assessor Comment
Contract Research Org	Sanofi BCB Framingham, MA Validation for Enzyme activity inhibition assay (**DOH1386/ITR-819-1215-VR)** was performed in 2017.	Sanofi BCB Framingham, MA The original Validation for Enzyme activity inhibition assay (**ITR-587-0512)** was performed in 2012 while addendum revalidation (**ITR-587-0512 Ad1)** was performed in 2019.	*See comment in table 2.2.2.1.*
Assay principle	HFF 9F0693 cells +neoGAA-AF594 →fluorescence (RFU) in BioTek Cytation 5 CIR HFF 9F0693 cells +neoGAA-AF594 + NAb PC→ reduced RFU in BioTek Cytation 5 CIR	Original validation and addendum: HFF 9F0693 cells +rhGAA-OG488→OG488+ cell MFI and cell counts by flowcytometry HFF 9F0693 cells	*Assay format was initially developed for GAA and subsequently adapted to neoGAA.* *Readouts differ slightly given the different instruments used: CIR for neoGAA assay and flow*

	Readout for neoGAA assay: % Uptake = [((RFU test-bkg)/# of cells) / ((RFU max-bkg)/# of cells)] x100 *This assay has the same format as legacy GAA assay except it uses BioTek Cytation 5 CIR instead of flow cytometer as a readout instrument. This assay was used to test pivotal study samples. Phase 1 assay still used flowcytometry as a readout.*	+rhGAA-OG488 + NAb PC→ reduced OG488+ cells MFI and count by flowcytometry Readout for GAA assay: % Uptake = [(MFI sample-background)/ (MFI GAA-OG only-background)] x 100 *This is the legacy assay validated and currently performed for Myozyme / Lumizyme NADA testing.*	*cytometer for GAA assay.*
Sample Pretreatment (Acid dissociation)	None required	None required	*No sample pre-treatment required due to short half-life of both drug products.*
Positive control (PC)	In house pooled rabbit anti-neoGAA affinity purified IgG antiserum.	Original validation: In house pooled rabbit anti-GAA affinity purified IgG anti-serum Addendum 1: Commercial Humanized anti-GAA mAb 3F1 PC (Yurogen Biosystems)	*Due to the assay formats there is no need for hybrid rabbit/human positive controls for either assay. For the GAA assay, Genzyme changed PC controls and revalidated the assay in 2019.*
PC Dose Curve and Hook Effect	Rabbit PC diluted in NHS at 0.39 -200 ug/ml (8X 2-fold serial dilution) *Neutralization (Value <NACP) detected at 200, 100 and 50 ug/ml for rabbit PC. No hook effect detected.*	Original validation: Rabbit PC diluted in NHS at 0.78 -100 ug/ml (8X 2-fold serial dilution) *Neutralization (Value <NACP) detected at 100, 50, 25 and 12.5ug/ml rabbit PC* Addendum: 3F1 PC diluted in NHS at 1.56 - 100 ug/ml (8X 2-fold serial dilution) *Neutralization (Value <NACP) detected at 100, 50 and 25 ug/ml 3F1 mAb PC* *Hook effect was not observed with either PC.*	

LPC	Rabbit PC diluted in NHS to result in ~60-70% uptake (30-40%inhibition)	Original Validation Rabbit PC diluted in NHS to result in ~60% uptake (40% inhibition) Addendum: Rabbit 3F1 PC to result in ~ 60% uptake (40% inhibition)	
HPC	Rabbit PC diluted in NHS to result in ~40-50% uptake (50-60% inhibition)	Original Validation Rabbit PC diluted in NHS to result in ~30% uptake (70% inhibition) Addendum: Rabbit 3F1 Rabbit 3F1 PC to result in 30% uptake (70% inhibition)	
Matrix and NC	Pooled NHS	Pooled NHS	
MRD	1/10	1/10	
NC system suitability range	NHS ≥87.8% enzyme uptake LOPD ≥94.2%% enzyme uptake	Original validation Negative control: % uptake ≥81.5% Cells only (No rhGAA-OG): MFI ≤5.0% of rhGAA-OG only Addendum: NHS Negative control: % uptake ≥73.7% Cells only (No rhGAA-OG): MFI ≤5.0% of rhGAA-OG only	*System suitability ranges for NC and PCs were established using the mean +/- 2.5 SD of all respective control values from all assays over the course of the validation exercise. They are acceptable.* *System suitability ranges differ between neoGAA and GAA assays because of different readout instruments- CIR vs flow cytometer, respectively.*
LPC system suitability range	LPC as 61.3% to 87. 7% of Enzyme Uptake	Original Validation: 50.6%-68.4% enzyme uptake Addendum: <73.7% enzyme uptake; % uptake>HPC	
HPC system suitability range	54.0% to 64.2% of Enzyme Uptake	Original Validation: 20.9%-35.4% enzyme uptake Addendum 16.8%-37.8% enzyme uptake	
NAb assay cut- point (NACP)	Based on 87 NHS samples using parametric 99% CI, 1% FP and 60 treatment- naïve	Original validation: Based on 58 NHS and 20 treatment naïve LOPD	*For both sets of assays, NACP was established in*

Normalized CP: mean MFI-2.33*SD and/or mean MFI-3.09*SD	LOPD samples, using parametric 99.9% CI, 0.1% FP. Performed outlier exclusion based on 480 data points. Data were normally distributed. NACP: NHS ≤87.4% enzyme uptake LOPD ≤94.3%% enzyme uptake *The NACP was appropriately calculated using suitable LOPD disease matrix, in addition to NHS matrix. The Applicant switched from a 99% CI for the NHS to a 99.9% CI when they assess the LOPD cut point. This more stringent NACP was also used for the LOPD sera in the initial validation of the legacy GAA Assay.*	samples, using parametric 99.9%CI, 0.1% FP. Performed outlier exclusion on 696 data points for NHS and 240 LOPD Data points, Data were normally distributed. Opted to use the NACP from NHS due to larger sample size after demonstrating no ANOVA statistical difference in NACP between NHS and LOPD sera. Addendum: Based on 30 treatment naïve LOPD samples, using parametric 99th CI, 1% FP. Outlier exclusion performed on 180 data points. Data was normally distributed. Original validation NACP: NHS <81.5% enzyme uptake *chosen for testing phase 1 samples* LOPD <84.5% enzyme uptake Addendum NACP = LOPD <73.7 %% enzyme uptake *In the original 2012 validation, Genzyme used 99.9% CI, 0.1% FP and the more conservative NHS cut point. Subsequently, for the 2019 addendum validation, they utilized 99% CI, 1% FP based on LOPD sera. This CP was used to test pivotal study samples and is an improvement over the original approach as it is more conservative.*	*treatment naïve LOPD samples.* *The Applicant initially validated the use of 99.9% CI and 0.1% FP for both neoGAA and GAA assays, even though they were validated at different times. However, due to the different readout systems (neoGAA CIR vs GAA flowcytometry), they opted to use the more conservative 99%CI when the GAA assay was reassessed for the current pivotal studies, as flowcytometry has greater variability than CIR system. This is acceptable*

Assay Drug tolerance	NHS spiked with LPC and neoGAA at 10, 1, 0.1, 0.01ug/ml. Drug tolerance was ≤1ug/ml. *Drug Tolerance acceptable as no onboard neoGAA is expected in samples due to short half-life of drug (<2hrs).*	Original validation and addendum: Not assessed for NAb assay due to short-half-life of GAA (<2.4hrs) *Justification for lack of drug tolerance testing in legacy assays was accepted at time of original immunogenicity assay assessment.*	*Drug tolerance demonstrated in neoGAA (≤1.0 ug/ml) is acceptable, as the half-life of the drug <2 hrs.* *Lack of testing for legacy GAA assay is acceptable as the sampling is done prior to infusion, so GAA is not expected to be present in any patient samples.*
Sensitivity	Based on statistical interpolation from PC dilution curve 64.4ug/ml	Based on statistical interpolation from PC dilution curve Original validation Rabbit polyclonal 18.4 ug/ml Addendum: 3F1 mAb 86.0 ug/ml *In the addendum, Genzyme opted to switch to a rabbit mAb over the affinity purified rabbit polyclonal for ease supply. This is acceptable despite the lower sensitivity estimates.*	*Only a small proportion of either PC will be neutralizing for enzyme uptake by neoGAA or GAA therefore the low sensitivity estimate is not surprising.*
Repeatability/Intra-assay variability	LPC %CV 0.8-5.3 % HPC %CV 0.7-9.7% *Repeatability for the two analysts was <10% and is acceptable*	*Original validation* *LPC/VS3 %CV 1.6-5.9%* *MPC/VS2 %CV 1.4-5.8%* *HPC/VS1 %CV 0.8-11.1%* *Repeatability for the three analysts was <12% and is acceptable*	*Repeatability for both neoGAA cell imaging assay and GAA flowcytometry assays are acceptable*
Intermediate Precision (IP)/inter-assay variability	LPC %CV 1.3-8.4% HPC %CV 3.3-8.9% *IP for the two analysts was <10% and is acceptable*	*Original validation* *LPC/VS3 %CV 2.1-18%* *MPC/VS2 %CV 3.0-11.4%* *HPC/VS1 %CV 2.2-9.9%*	*IP for both neoGAA cell imaging assay and GAA flowcytometry assays are acceptable.*

		IP for the three analysts was <20% and is acceptable	
Selectivity	10/10 LPC-spiked LOPD sera inhibited enzyme uptake *Selectivity suitably assessed, unlike for ADA assays, which only tested impact of interfering compounds.*	Original validation: Three levels of anti-GCAA PC (HPC/VS1, MPC/VS2, LPC/VS3) ± interfering compounds (hemoglobin, bilirubin and lipid). *For results of interfering compounds see bottom of table.* *Selectivity was only tested in pooled NHS and not LOPD sera for GAA assay*	*Selectivity in LOPD patient matrix was successfully demonstrated for neoGAA assay. The lack of testing in IOPD patient matrix is acceptable given the lack of available samples.* *Legacy GAA assay tested only selectivity in pooled NHS and not in individual LOPD sera.*
Specificity Part 1: labelled neoGAA or labelled GAA ± Positive controls against other ERTs: Part 2: labelled ERTS ± Positive controls against neoGAA or GAA	Part 1: rhneoGAA-AF488 ± Anti-Aldurazyme (rhIDU) rabbit HPC: negative Anti-acid sphingomyelinase (rhASM) HPC negative Anti-neoGAA HPC: positive *Part 2:* Not performed. *neoGAA assay validation only tested specificity using Part 1 set up. Part 2 is more important for flow cytometry based -assay assays as it demonstrates that the anti-neoGAA PC does not interfere with all M6P mediated uptake but is specific for neoGAA.*	Original validation: Part 1: rhGAA-OG488± Anti-alpha-galactosidase (rhGAL) rabbit HPC: no enzyme uptake inhibition Anti-Aldurazyme (rhIDU) rabbit HPC: no enzyme uptake inhibition Anti-neoGAA HPC/VS1: inhibited enzyme uptake Part 2: rhGAL-OG488 + anti-GAA HPC: no enzyme uptake inhibition rhIDU-AF488 + anti-GAA HPC: no enzyme uptake inhibition rhGAA-OG488 + anti-GAA HPC: inhibited enzyme uptake Addendum: specificity testing not reported *Specificity for GAA was performed only in original validation study not in addendum. For original validation specificity was suitably demonstrated in both parts 1 and 2.*	*Specificity testing was more thorough for legacy GAA flow cytometry assay than for neoGAA cell imager assay due to the different readouts. Both assays show suitable specificity for respective products.*

Robustness testing:	LPC and HPC tested using different incubation times for Sample-Enzyme incubation (SEI), Immune complex - target cell Incubation (ICTC), and Hoechst dye incubation time (HDIT) Data indicated GAA assay can be run with 45-75 min for SEI and 2.5-3.5 hr SESI, and 10-20 min for HDIT. *Robustness testing performed for neoGAA assay is acceptable.*	Original validation: Three PC levels (HPC/VS1, MPC/VS2, LPC/VS3) tested using different incubation times for: Sample-Enzyme incubation (SEI), Immune complex-target cell Incubation (ICTC), Cell trypsinization time (CTT), Cell Storage at 2-8°C before flow cytometry. Cell sample warming time before flow cytometry acquisition Data indicated assay can be run with 45-75 min SEI, 2.5-3.5hr for SET and 1-5 min CTT. GAA-OG488+ Cells can also be stored at 2-8°C for ≤ 120 min and warmed to RT 10-20 min before flow cytometry before Addendum: not reported *Robustness testing was only performed in original validation and not for the validation addendum. This is acceptable as the only change to the method was the addition of the anti-GAA rabbit mAb 3F1 as a PC.*	*Robustness testing performed for neoGAA assay differs slightly from that performed for the legacy GAA assay due to different readouts. This is acceptable.*
Stability (short-term)	LPC and HPC shown to be Stable up to 7 days r at 2-8°C 24hr at RT 5X freeze/thaw cycles *Short term stability demonstrated for PC.*	Original validation Three PC levels (HPC/VS1, MPC/VS2, LPC/VS3): PC samples were stable up to 7 days r at 2-8°C 4hr at RT 5X freeze/thaw cycles *Short term stability demonstrated for rabbit PC in original validation.*	*Standard short-term stability demonstrated for rabbit anti-neo-GAA and anti-GAA PCs used in the specific assays.*

		Addendum: Short term stability studies not reported *As new 3F1 PC is commercially sourced Genzyme did not perform short term stability testing and will use the manufacturers recommended shelf-life, this is acceptable.*	
Lipemia	LPC spiked into pooled NHS containing low, medium and high lipemia showed inhibition of enzyme uptake, and thus no interference. Unspiked lipemic sera did not show interference of neoGAA uptake. *Genzyme tested three undisclosed concentrations of lipids and no impact was observed. Although the tested concentrations should have been listed, the testing is acceptable as PD sera are not characterized by high levels of lipemia.*	Original validation: Three PC levels (HPC/VS1, MPC/VS2, LPC/VS3) spiked ± ± 8mg/ml lipid showed no interference with rhGAA uptake Addendum: not reported *No lipemic impact observed in original validation.*	*No impact of lipemia was observed in either assay, using both types of matrices (natural lipemic vs experimentally generated lipemic sera).*
Icteric	LPC spiked into pooled NHS containing low, medium and high bilirubin showed inhibition of neoGAA uptake, and thus no interference. However low and high bilirubin containing sera not spiked with LPC showed interference with enzyme uptake *Bilirubin in the sera was demonstrated to interfere with GAA uptake in the absence of PC, leading to false positive signal. The SOP was modified to state that icteric sera samples will be noted, and positive result interpreted with caution.*	Original validation: Three PC levels (HPC/VS1, MPC/VS2, LPC/VS3) ± 0.005-0.6 mg/ml bilirubin showed no significant interference at any tested concentration. Addendum: not tested *Bilirubin tested at ≤0.6mg/ml did not interfere with rhGAA uptake in original validation.*	*An impact of bilirubin was observed in the neoGAA assay, leading to false positive results in absence of PC. The resulting SOP was modified to require that icteric sera be flagged, with a caution if a positive result is noted.* *The GAA assay did not show any impact for bilirubin, likely due to the differences in readouts (use of flow cytometer versus CIR)*

Hemolysis	LPC spiked into pooled NHS containing low, medium and high levels of hemolysis showed no interference of enzyme uptake in neoGAA assay. Unspiked hemolytic sera did not show inhibition of neoGAA uptake. *Genzyme tested three undisclosed spiked concentrations of hemoglobin and no impact was observed unlike the GAA assay. Although the tested concentrations should have been listed, the testing is acceptable as PD sera are not characterized by high levels of*	Original validation Three PC levels (HPC/VS1, MPC/VS2, LPC/VS3) spiked ± 0.078-10 mg/ml of hemoglobin. ≥0.625 mg/ml of hemoglobin addition lead to %recoveries <80-120% Addendum: not tested *Hemoglobin ≥0.625 mg/ml shown to have significant impact on flowcytometric MFI readout in original validation. The SOP was modified to note samples that are hemolyzed, and a negative result interpreted with caution. .*	*Hemoglobin ≥0.625 mg/ml was observed to interfere with enzyme uptake in legacy GAA flowcytometry assay, using experimentally generated hemolytic sera. The resulting SOP was modified to require that hemolytic sera be flagged, with a caution if a negative result is noted.* *This effect was not observed in neoGAA assay likely due to the different readout systems (CIR versus flowcytometry).*
NADA Assay Assessment	**Suitable for Intended purpose**	**Legacy NADA assay remains suitable for Intended purpose post-addendum validation with new 3F1 mAb PC.**	**Although validated at different times, the anti-neoGAA and anti-GAA cellular uptake inhibition NADA assays are suitable for intended purpose.**

Additional Assessor Comments:
The Applicant performed a study to bridge the phase 1 neo-GAA cellular uptake inhibition flowcytometry assay, with the phase 3 cellular uptake inhibition assay using the cellular imager to justify use of total evaluable data set. In the study, 5 out of 6 comparability samples from NeoGAA treated patients (n=6) yielded concordant positive/negative results between the two assays. The one sample with discrepant results between the two assays (negative by flow cytometry/ positive by imager) had % uptake results very close to the cut point in both assays suggesting it was a very weak, borderline positive sample. These data support the inclusion of the phase 1 NADA data in the total evaluable population (see 2.5).

2.5 Facility Inspection Summary

Assessor comment:
Due to current workloads and public health emergency, OSIS was unable to perform either an on-site or virtual inspection of the Sanofi US/Genzyme Biomarker and Clinical Bioanalysis Boston facility in Framingham, MA, the primary bioanalytical site involved in validation of immunogenicity assays and testing of clinical study samples. This bioanalytical facility also developed and validated the ADA assays that supported approval of BLA 125141 for Lumizyme and BLA125291 for Myozyme, in addition to other Genzyme-licensed ERTs. Therefore, the lack of a bioanalytical inspection during the current review cycle is in not considered a potential CR issue.

2.6 Assessment of Assay performance in Clinical Studies

Assessor comment:
For a detailed analysis on the clinical impact of BADA, NADA, ADIgE on safety and efficacy of Avalglucosidase Alfa refer to Clin Pharm review by Katarzyna Drozda and Jack Wang. The discussion below focuses on in study immunogenicity data as indicators of individual assay performance.

The Applicant table 2.5.1 below summarizes the immunogenicity data set from the LOPD (phase 1 TDR12857/LTS 13769 and phase 3 EFC14028) and IOPD (phase 2 ACT14132/Extension) clinical studies and includes all evaluable patients. With exception of one pediatric LOPD patient, all other pediatric patients listed are in the IOPD population.

Table 2.5.1 Summary of Immunogenicity Data across all studies-(LOPD phase 1 and 3 and IOPD phase 2) BADA and NADA evaluable population

	Avalglucosidase Alfa				Alglucosidase Alfa	
	Treatment-naïve patients (N=61)	Treatment-experienced patients (N=73)			In Primary Analysis Period (N=54)	
	Adult 20 mg/kg Q2W	Adult 20 mg/kg Q2W	Pediatric 20 mg/kg Q2W	Pediatric 40 mg/kg Q2W	Adult 20 mg/kg Q2W	Pediatric 20 mg/kg Q2W to 40 mg/kg QW
N	61[a]	55	6	10	48	6
Antidrug Antibodies						
ADA at baseline	2 (3.3)	40 (72.7)	1 (16.7)	1 (10.0)	2 (4.2)	3 (50.0)
Treatment-emergent ADA[b]	58 (95.1)	27 (49.1)	1 (16.7)	5 (50.0)	46 (95.8)	3 (50.0)
Neutralizing Antibodies						
Both NAb types	13 (21.3)	2 (3.6)	0	0	ND	ND
Inhibition enzyme activity, only	4 (6.6)	8 (14.5)	0	0	4 (8.3)	2 (33.3)
Inhibition of enzyme uptake, only	10 (16.4)	8 (14.5)	0	0	19 (39.6)	0

N (%); [a] Includes n=1 pediatric patient; ND= Not determined; [b] Treatment emergent=treatment induced + treatment boosted;

The analyst table 2.5.2 below summarizes the anti-neoGAA peak titer ranges from LOPD (phase 1 TDR12857/LTS 13769 and phase 3 EFC14028) and IOPD (phase 2 ACT14132/Extension) clinical studies and includes all evaluable patients.

Table 2.5.2: Anti-Avalglucosidase Alfa (neoGAA) BADA Response across all clinical studies- total evaluable population (LOPD phase 1 and 3 and IOPD phase 2) of treatment naïve and GAA-treatment experienced subjects

Treatment	Avalglucosidase Alfa	
Treatment-Emergent anti-neoGAA antibody levels (peak titer ranges)	Treatment Naïve patients (n=61)	GAA-treatment experienced Patients (n=73)
Low (100-800)	14 (23.0%)	6 (20%)
Medium (1600-6400)	29 (47.5%)	2 (6.7%)
High (≥12,800)	13 (21.3%)	0

Pre-existing BADA	2 (3.3%)	42 (57.5%)

Assessor comment:

Suitability of BADA assays

Based on table 2.5.1 above, the in-study immunogenicity data from evaluable treatment-naïve patients in the safety database indicate that the BADA assays for both neoGAA and GAA detect similar rates of treatment-emergent BADA to the individual products in adult patients. Specifically, ~95% neoGAA treated patients and ~96% of GAA treated patients in the comparative arms were confirmed for BADA to their respective product. In addition, the anti-neoGAA BADA assays detect differences between treatment naïve and GAA-treatment experienced adult patients, with ~95% of the former developing treatment-emergent anti-neoGAA BADA and the latter 49%. These data support the hypothesis that LOPD patients previously treated with GAA undergo a degree of tolerization in vivo possibly due to the prior repeated exposure to the first-generation product. Of note the treatment-experienced patients also had higher rates of pre-existing cross-reactive antibodies at baseline, than treatment naïve patients (57.5% versus 3.2%, respectively). As highlighted in table 2.5.2, neoGAA BADA assays also detected a difference in anti-neoGAA BADA titers between these two groups, with 21.3 % treatment naïve patients showing BADA peak titers > 12,800 compared to none for the GAA-treatment-experienced patients.

These in-study data indicate the BADA assays are sensitive and able to detect differences in the magnitude of BADA responses between treatment naïve and GAA-treatment experienced adult patients and between the LOPD study population, which included 1 pediatric treatment-naïve patient, and IOPD study population and support that the various validated immunogenicity assays are suitable for their intended purpose.

Suitability of NADA assays

Based on table 2.5.1 above, the in-study NADA data show that that enzyme uptake inhibition assays specific to neoGAA and to GAA can detect responses at higher rates in treatment-naïve adult patients than the corresponding enzyme activity inhibition assays (16.4% versus 6.5% for neoGAA assays; 39.6% versus 8.3% for GAA assays). These data suggest that only a small portion of the NADA response is capable of inhibiting enzyme activity, while a greater proportion is capable of interfering with enzyme uptake into target cells.

The assays can also detect differential NADA responses in treatment naïve compared to treatment-experienced adult patients- the former had higher rates of both type of NADAs compared to the latter (21.3% versus 3.6%). However, treatment-experienced adult patients had slightly higher overall rates of either NADA type compared to the former (16/55 or 29.1% versus 14/61 or 23%). Pediatric populations had low detectable levels of either NADA type.

These in study data indicate that the validated NADA assays can detect differences in the frequency and type of NADA responses between the LOPD study populations, which included one pediatric patient, and the IOPD pediatric study population and support that the various validated assays are suitable for their intended purpose.

Suitability of Cross-Reactivity Assays:

Cross-reactivity for BADA binding to neoGAA and GAA was assessed at Week 25 and Week 49 of Phase 3 EFC14028 study in samples from both drug treatment arms. Following depletion with GAA-magnetic beads patients who were positive for neoGAA BADA were tested for BADA binding to GAA, and patients who were positive for GAA ADA were tested for binding to neoGAA. A similar analysis was performed at Week 25 samples of phase 2 ACT14132 study as these neoGAA-treated patients were all rhGAA-treatment experienced.

In study EFC14028 at Week 25, 37/51 patients (72.5%) were negative in both ELISA assays post magnetic depletion indicating cross-reactive BADAs for both drugs and while a minority of LOPD patients (6/43, 11.8%) remained positive only for the neoGAA, indicating development of a unique neo-GAA-specific ADA response. There were 5 patients who became BADA-negative and 3 patients were inconclusive. At Week 49, 3/51 (5.9%) patients were positive for neoGAA-specific BADA while 6 patients became BADA-negative and 5 patients were inconclusive. The 37 patients that tested positive for cross reactive antibodies to both products at Week 25 remained cross-reactive antibody positive at Week 49.
In Study ACT14132, the data were more limited- there were 5 IOPD GAA-treatment experienced patients that developed cross-reactive BADA to neoGAA at Week 25, and 5 additional patients that tested only positive for GAA and negative for neo-GAA-specific BADA at this timepoint.
These in-study data indicate that the qualified cross-reactivity assays can detect differences in the quality of the BADA responses in the LOPD and IOPD study populations and support that the two assays are suitable for their intended purpose.

Suitability of ADIgE assays
The ADIgE assays are only used to test samples from patients that experienced infusion-associated reactions (IARS) or hypersensitivity treatment-emergent adverse events (HTEAEs). The sampling for IgE testing is recommended to be obtained the day the AE is experienced or within 24-48 hours of the event. Based on the safety data summarized below, both IARs and HTEAES were associated with increasing antibody titers to either product.

Treatment-Emergent ADA levels (peak titer ranges)	Infusion associated reactions (IAR)	Hypersensitivity treatment emergent adverse events (HTEAE)
Low (100-800)	7%	14%
Medium (1600-6400)	17%	28%
High (≥12,800)	54%	31%

The Applicant tested 17 patients that received neoGAA and 10 patients that received GAA across all clinical studies using ADIgE Phadia1000 assays. Only one GAA-treated patient from study EFC14028 showed detectable levels of specific IgE against GAA at 0.49-0.55 kUA/L. These data suggest that product specific IgE is not easily detectable in samples from patients that experienced IARs or HTEAES, and that the developed product specific IgE assays may be of limited usefulness for diagnostic purposes. However, the Applicant also tested for serum tryptase, complement activation and circulating immune complexes using specific commercially available CLIA methods which can complement the usefulness of the ADIgE assays.

Anti-drug IgE testing	Number of patients tested (positives)	
Study	neoGAA	GAA
LOPD TDR 12857/LTS13769	3 (no positives)	2 (no positives)
LOPD EFC14028/Ext	11 (no positives)	8 (1 positive at 0.49-0.55 kUA/L)
IOPD ACR14132/Ext	3 (no positives)	0 (no positives)

2.6.1 Executive Summary

The validation reports submitted for the five immunogenicity assays developed to test pivotal IOPD and LOPD pivotal study samples for neoGAA immunoreactivity along with the associated in study performance data support the suitability of these assays for their intended purpose. Similarly, the study reports submitted for the four legacy immunogenicity assays and two novel assays used to test for GAA immunoreactivity along with in study performance data support both that the new assays are suitable for intended purpose while legacy assays also remain suitable for intended purpose. From immunogenicity bioanalytical perspective there are no current approvability issues for BLA 761194.

2.7 Information Requests Sent During Review

None

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