CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761278Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Application Information

Application type	BLA
Application number(s)	761278
Priority or standard	Priority
Submit date(s)	6/17/2022
Received date(s)	6/17/2022
PDUFA goal date	2/17/2023
Division/office	Division of Rare Diseases and Medical Genetics (DRDMG)
Review completion date	See electronic stamp date
Established/proper name	Velmanase alfa-tycv
(Proposed) proprietary name	Lamzede
Pharmacologic class	Lysosomal alpha-mannosidase
Other product name(s)	CHF-LMZYMAA1
Applicant	Chiesi Farmaceutici S.p.A.
Dosage form(s)/formulation(s)	10 mg of velmanase alfa-tycv as a lyophilized powder in a single-dose vial for reconstitution
Dosing regimen	1 mg/kg (actual body weight) administered once every week as an intravenous infusion
Applicant-proposed indication(s)/ population(s)	Treatment of patients with a confirmed diagnosis of alpha-mannosidosis
SNOMED CT code for proposed indication disease term(s)[1]	65524005 |Mannosidosis (disorder)|
Regulatory action	Approval
Approved dosage (if applicable)	1 mg/kg (actual body weight) administered once every week as an intravenous infusion
Approved indication(s)/ population(s) (if applicable)	Treatment of non-central nervous system manifestations of alpha-mannosidosis in adult and pediatric patients.
SNOMED CT code for approved indication disease term(s)[1]	65524005 |Mannosidosis (disorder)|

[1] For internal tracking purposes only.
Abbreviations: PDUFA, Prescription Drug User Fee Act; SNOMED CT, Systematized Nomenclature of Medicine Clinical Terms

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Glossary

3MSCT	3-minute stair climb test
6MWT	6-minute walk test
ADA	anti-drug antibody
ADR	adverse drug reaction
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
AM	alpha-mannosidosis
ANCOVA	analysis of covariance
AR	adverse reaction
ARF	acute renal failure
AUC	area under the concentration-time curve
CI	confidence interval
BLA	biologics license application
C_{max}	maximum plasma concentration
CMC	chemistry, manufacturing, and controls
CNS	central nervous system
CSF	cerebral spinal fluid
(b) (4)	(b) (4)
EOP2	end-of-phase 2
E-R	exposure-response
ERT	enzyme replacement therapy
EU	European Union
FDA	Food and Drug Administration
FMQ	Food and Drug Administration Medical Dictionary for Regulatory Activities query
FVC	forced vital capacity
GFR	glomerular filtration rate
HSP	Henoch-Schonlein Purpura
IAR	infusion-associated reaction
IgE	immunoglobulin E
IgG	immunoglobulin G
IND	investigational new drug
IR	information request
IRR	infusion-related reaction
ISS	integrated summary of safety
IV	intravenous
KO	knockout
LLN	lower limit of normal
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
MOA	mechanism of action

MRHD	maximum recommended human dose
NADA	neutralizing antibody
NOAEL	no observed adverse effect level
OPQ	Office of Pharmaceutical Quality
PD	pharmacodynamic
PI	Prescribing Information
PK	pharmacokinetic
PMC	postmarketing commitment
PSUR	periodic safety update report
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SE	surrogate endpoint
(b) (4)	(b) (4)
SMQ	standardized Medical Dictionary for Regulatory Activities queries
SOC	system organ class
TADA	titering anti-drug antibody
TEAE	treatment-emergent adverse event

I. Executive Summary

1. Summary of Regulatory Action

Chiesi (Applicant) submitted this biologics license application (BLA) for velmanase alfa-tycv (tradename Lamzede), seeking approval of this product as an enzyme replacement therapy (ERT) for alpha-mannosidosis (AM), a rare autosomal recessive lysosomal disorder. Patients with AM have an enzyme deficiency leading to oligosaccharide accumulation in various tissues. AM is a multisystemic condition involving central nervous system (CNS), musculoskeletal, immune systems, among others, with highly variable clinical manifestations, severity, and progression. Velmanase alfa provides an exogenous source of alpha-mannosidase that catabolizes the oligosaccharides that accumulate in the lysosomes. Currently, there is no approved therapy for AM in the United States.

Substantial evidence of effectiveness for velmanase alfa in AM patients was established with one adequate and well-controlled trial with confirmatory evidence. This trial in 25 adult and pediatric patients with AM showed large and clinically meaningful improvement in lung function, and favorable improvements in a 3-minute stair climbing test and a 6-minute walking test. Confirmatory evidence provided strong mechanistic support, including the well-established etiology of the disease, the mechanism of action of velmanase alfa, evidence the product reaches its target tissue, and pharmacodynamic biomarker data showing statistically significant reductions in serum oligosaccharide concentration. The team determined that in the setting of a very rare disease with unmet medical need, regulatory flexibility is warranted in accepting the residual uncertainty associated with the lack of statistical significance on the clinical endpoints, and, that this was balanced by the strength of the design and conduct of the trial, the consistent improvements observed across the three key clinical endpoints, the magnitude of the improvement in lung function, and the robust confirmatory evidence, which together supported a conclusion of substantial evidence of effectiveness.

The safety profile for velmanase alfa is acceptable for its intended use. Clinically important adverse reactions will be addressed via labeling. These include a boxed warning for hypersensitivity reactions, including anaphylaxis, and a warning and precaution for embryo-fetal toxicity based on the observation of skeletal and visceral malformations in studies of pregnant rats and rabbits. All the identified risks for velmanase alfa can be adequately mitigated through labeling and routine pharmacovigilance.

The Applicant will be required to conduct a post-market assessment of the potential effects of increased alpha-mannosidase exposure (and increased MAN2B1 expression) on tumor formation. In addition, the Applicant committed to post-market studies for chemistry, manufacturing, and controls; animal toxicology; and clinical pharmacology.

Each scientific discipline and the clinical team recommend approval of velmanase alfa for the treatment of non-central nervous system manifestations of alpha-mannosidosis in adult and pediatric patients. The CDTL, division director, and signatory authority concur with this recommendation.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of condition	• Alpha-mannosidosis (AM) is a very rare autosomal recessive inborn error of oligosaccharide catabolism caused by biallelic pathogenic variants in the MAN2B1 gene leading to deficiency of alpha-mannosidase, a lysosomal enzyme. The enzyme deficiency leads to progressive accumulation of mannose-rich N-linked oligosaccharides in various organs and tissues. • The prevalence of AM is 1:500,000 (NORD 2022) people in the general population and can affect men and women equally and potentially affect individuals of any ethnic group. • AM symptoms, progression and severity vary widely from one patient to another, even among patients who share the same genetic variant(Malm 2018). Residual enzymatic activity does not predict clinical phenotype or progression. • AM is typically categorized into the following 3 subtypes: – Severe Form (Type 3) – Presents in early childhood with intellectual decline, skeletal abnormalities, recurrent infections, central nervous system deterioration, and death by end of the first decade. – Moderate Form (Type 2) – Typically diagnosed before 10 years of age, and includes skeletal abnormalities, myopathy, and progression with ataxia appearing by 2nd or 3rd decade and death in early adulthood most commonly from pneumonia (Hennermann et al. 2022). – Mild Form (Type 1) – This later onset form typically presents after the first decade and includes progressive myopathy and ataxia, which usually results in loss of ability to independently ambulate and wheelchair dependence. Death occurs within the fourth to fifth decade most commonly from pneumonia (Hennermann et al. 2022).	AM is a serious disease and extremely rare that can lead to death in early childhood from recurrent bacterial infections and CNS deterioration (in the early onset severe form) and to progressive and severe motor impairment (i.e., myopathy, ataxia) in later onset forms. Disease manifestation (symptoms and severity) and progression are highly variable.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• Patient and Caregiver Persepective: – Caregivers report that the rarity of disease can lead to a protracted diagnostic pathway(Verrecchia et al. 2021). – Caregivers experience high levels of stress and anxiety from their caregiving responsibilities (Adam et al. 2019).	
Current treatment options	• In the US, there are no approved therapies for the treatment of AM. • The current standard of care is symptomatic treatment addressing the medical complications of the disease. • Hematopoietic stem cell transplant (HSCT) has been investigated for the preservation of neurocognitive function and the prevention of early death in younger AM patients who have not yet experienced neurocognitive decline. The results have been variable, and the specific values for transplantation-related mortality rates are not available (Ceccarini et al. 2018; Naumchik et al. 2020). HSCT is not considered standard of care at this time.	There is a clear unmet need for therapeutic options for AM.
Benefit	• Velmanase is proposed for the treatment of patients with AM of all ages. • The primary support for efficacy is from Trial rhLAMAN-05, an adequate and well-controlled phase 3, multicenter, double-blind, placebo-controlled study in 25 treatment naïve patients with AM aged 6 to 35 years, randomized 2:1 to velmanase 1 mg/kg IV or placebo once a week for 52 weeks. • The trial demonstrated results favoring velmanase in the following clinical endpoints: the 3-minute stair climb test (3MSCT, a primary endpoint), and the 6-minute walk test (6MWT) and FVC percent of predicted normal value (FVC%), both key secondary endpoints. The trial also demonstrated a statistically significant reduction in serum oligosaccharides (a biomarker that was a primary endpoint). Reduction in serum levels of oligosaccharides supports the proposed mechanism of cellular uptake of velmanase into the lysosome to exert its ERT effect. However, this biomarker is not a validated surrogate endpoint and therefore could not provide the primary support for efficacy in this trial. The consistent results for the clinical endpoints of 3MSCT, 6MWT and FVC% favoring velmanase, however, are thought to represent a clinically meaningful treatment benefit for AM patients. • Trial rhLAMAN-08 was an uncontrolled, open label, safety and pharmacokinetic (PK) study in 5 patients with AM less than 6 years of	The clinically meaningful improvements in 3MSCT, 6MWT and FVC% in Trial rhLAMAN-05, favoring velmanase, supported by the statistically significant reduction in serum oligosaccharides, provided evidence of efficacy of velmanase for the treatment of non-CNS manifestations of AM. This conclusion was informed by the following key factors: consistently favorable numerical improvement in several clinical endpoints in the setting of the considerable clinical heterogeneity and slow progression of AM, and very small sample size (these elements posed significant noise biasing towards the null and also likely contributed to the underpowering of the trial to detect a statistically significant drug effect on the clinical endpoints) and the a priori likelihood of a treatment effect, based on the well-understood underlying pathophysiology of AM (i.e., caused by a single enzyme deficiency) and a highly targeted therapy (i.e., enzyme replacement, with a general mechanism of action supported by nonclinical data),

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	age administered velmanase 1 mg/kg IV for 24 months. This study demonstrated a reduction in serum oligosaccharides from baseline that was similar in magnitude to the reduction from baseline observed in the velmanase treatment group in Trial rhLAMAN-05. Individual results for the 3MSCT suggest either stabilization or improvement from baseline in performance over time. Although not sufficient alone to establish efficacy in patients <6 years, the data from this study, in combination with the data from Trial rhLAMAN-05, are sufficient to establish efficacy in this age group using an efficacy extrapolation approach. Extrapolation of efficacy from Trial rhLAMAN-05 is justified because of 1) the disease similarity between patients < 6 years and older pediatric patients and adults (in terms of both the underlying pathophysiology and clinical manifestations) and 2) the similar pharmacologic activity of and 3) therapeutic response to velmanase (as demonstrated by a similar magnitude in % change from baseline in serum oligosaccharides between the two age groups). • A body of confirmatory evidence to confirm the treatment benefit demonstrated in Trial rhLAMAN-05 includes the following: o A well-understood underlying disease pathophysiology (i.e., caused by a single enzyme deficiency) and a clear, targeted mechanism of action of velmanase (i.e., enzyme replacement), and evidence the drug reaches its intended tissues. o Biomarker data: Evidence from Trial rhLAMAN-05 and additional clinical Trials (rhLAMAN-03, rhLAMAN-04 and rhLAMAN-08) demonstrated a reduction in serum oligosaccharides with velmanase treatment. Serum oligosaccharide levels are a physiologically relevant, disease-specific pharmacodynamic biomarker that reflects the proposed mechanism of cellular uptake of velmanase into the lysosome to exert its ERT effect. o Exposure-response data: When all available data across clinical studies were analyzed, there was a trend of dose-response on serum oligosaccharides concentrations; a higher dose of velmanase was associated with greater reduction in serum oligosaccharide concentrations. Additionally, nonclinical data to support the general mechanism of action of velmanase included the following: • Patient-derived cells were capable of internalizing velmanase	and evidence from the clinical biomarker supporting that the treatment reaches its target tissue. Efficacy in patients <6 years old primarily relies on extrapolation of efficacy data from patients >6 years old (Trial rhLAMAN-05), and is supported by results of Trial rhLAMAN-08. The extrapolation is scientifically justified by the same underlying disease pathophysiology in patients of all ages, the same mechanism of action of ERT across all ages, and the observation of a similar reduction of serum oligosaccharides in patients of all ages. Substantial evidence of effectiveness was established with the results of the single adequate and well-controlled clinical trial (rh-LAMAN-05) taken together with adequate confirmatory evidence. There are multiple lines of evidence that contribute to an adequate body of confirmatory evidence, which included strong mechanistic support, additional clinical Trials (rhLAMAN-03, -04, and -08) providing data on a physiologically relevant biomarker (serum oligosaccharides), and exposure-response data. Because the effect of velmanase was only demonstrated on non-CNS manifestations of AM (in both humans and animals), (b) (4) a labeled indication specifying treatment of the “non-central nervous system manifestations of AM” is warranted.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• Nonclinical model demonstrating that velmanase is internalized into target tissue. The Applicant did not evaluate the oligosaccharide biomarker in these studies, but nonclinical studies showed an apparent qualitative reduction in tissue vacuolation at approximately 16x the clinical dose. • Intravenous administration of rhLAMAN in *MAN2B1* KO mice was observed to distribute to the liver, spleen, kidney, and heart, and was associated with an apparent reduction in vacuole size in the liver and kidney.	
Risk and risk management	• Trial rhLAMAN-05 provided the data for the main safety assessment. There were no deaths or premature study discontinuations. The median exposure (in treatment arm) in pediatric patients was 365 days (range 357 to 380 days) and in adult patients 371 days (range 361 to 372 days). A total of 5 patients experienced serious adverse events (SAEs) in the velmanase arm. Of these, 1 SAE, acute renal failure, was assessed as possibly related to velmanase. No SAEs occurred in placebo arm. Overall, the velmanase group experienced a higher rate of treatment emergent adverse events (TEAEs) than placebo group (100% versus 90%, respectively). • rhLAMAN-10 Integrated Analysis consisted of an integrated analysis that pooled the cumulative database for all previous velmanase trials and included 33 unique patients, 19 of which were pediatric patients aged 6 to 18 years with median exposure of 1170 days (range of 357 to 1881 days) and 14 adult patients with median exposure of 776 days (range of 373 to 1175 days). Trial rhLAMAN-08 (which included 5 pediatric patients aged 3 to 5 years old with median exposure of 108 weeks and range 105 to 170 weeks) provided additional data to support the main safety assessment. The safety findings of velmanase in these studies were generally consistent with those observed in rhLAMAN-05. Additional SAEs potentially related to velmanase exposure were reported in Trial rhLAMAN-10 (seizures) and Trial rhLAMAN-08 (Henoch-Schoenlein Purpura). • The key identified risks from the above studies include hypersensitivity events (including anaphylaxis) and infusion-associated reactions (IARs). Based on the analysis of the integrated summary of safety (ISS), which included all patients from rhLAMAN-10 Integrated Analysis (33 patients) and rhLAMAN-08 (5 patients) for a total of 38 unique patients, 50% (19 of 38 patients) experienced symptoms of hypersensitivity reactions. Of	The safety database was adequate for a safety assessment of velmanase for the proposed indication, patient population, dosage regimen, and duration. Identified risks can be adequately managed through labeling, including: • Risks of hypersensitivity, including anaphylaxis and IARs, are known risks with ERT. These risks with velmanase are consistent with ERTs approved for other indications and can be adequately mitigated through labeling including: – A boxed warning for hypersensivity including anaphylaxis – A warning/precaution for hypersensitivity events and IARs – Dosage and Administration recommendation for pre-treatment with antihistamines, antipyretics, and/or corticosteroids • A warning/precaution of potential for embryofetal risk • The occurrence of malignant histiocytoma seen in rats will be described in subsection 13.1 of the label along with a statement that animal carcinogenicity studies have not been conducted.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	these 19 patients, 14 (74%) were pediatric patients. In total, 5 patients experienced events clinically concerning for anaphylaxis: 2 patients (1 pediatric and 1 adult) met full criteria for anaphylaxis, and an additional 3 patients (all pediatric) were assessed to have severe hypersensitivity reactions that required medical treatment. All patients had at least 3 months of exposure to velmanase prior to anaphylaxis or severe hypersensitivity reactions. A total of 19 patients (50%) experienced IARs varying from mild to moderate severity. Of these 19 patients, 5 (13% of all patients) required pretreatment in the clinical trials. Four of the 5 patients who experienced events concerning for anaphylaxis developed anti-drug antibodies (ADAs). • In nonclinical studies, a malignant histiosarcoma of the ovary was observed in rats treated with velmanase. • Embryofetal malformations were observed in rats and rabbits. An increase in the incidence of skeletal and visceral embryofetal malformations that exceeded facility historical control ranges was observed in rats and rabbits. • There was lack of data to support safety multiples for fertility and the pre-and postnatal study.	Because of lack of certain nonclinical information, postmarketing requirement will be issued for an assessment of the potential effects of increased alpha-mannosidase exposure (and increased MAN2B1 expression) on tumor formation.

Abbreviations: CNS, central nervous system, ERT, enzyme replacement therapy; FVC, forced vital capacity; IV, intravenous; PD, pharmacodynamics; PK, pharmacokinetics

2.2. Conclusions Regarding Benefit-Risk

Alpha- mannosidosis is a very rare autosomal recessive lysosomal disorder caused by biallelic pathogenic variants in the *MAN2B1* gene that encodes the lysosomal enzyme alpha-mannosidase. Alpha mannosidase normally catalyzes the degradation of accumulated mannose-containing oligosaccharides in various tissues. In AM, genetic variants lead to deficiency of the alpha-mannosidase enzyme. The effect of this deficiency is prevention of the degradation of glycoproteins, which results in the accumulation of mannose-rich oligosaccharides in lysosomes of various tissues, resulting in the impairment of cellular function. Eventually, lysosomal apoptosis or cell decomposition leads to release of circulating mannose-rich substrates into the extracellular space and consequently, increased levels of oligosaccharides in tissues and fluids, including serum and urine.

Velmanase alfa (velmanase) is a new molecular entity recombinant human alpha-mannosidase that functions as an ERT for the endogenous enzyme that is deficient in AM. Velmanase is internalized by cells and transported to the lysosomes where it functions as the endogenous enzyme. Velmanase (1 mg/kg intravenous [IV] once weekly) is approved for the treatment of non-CNS manifestations of AM in the European Union, Brazil, Israel, Saudi Arabia, and Ukraine.

The Applicant (Chiesi) submitted this biologics license application (BLA) for velmanase for the treatment of patients with AM. The proposed dosing regimen is 1 mg/kg administered IV once every week. To support the application, the Applicant submitted the results of Trial rhLAMAN-05, a multicenter, double-blind, placebo-controlled study in 25 treatment-naïve patients aged 6-35 years with AM randomized 2:1 to velmanase 1 mg/kg IV or placebo once a week for 52 weeks. The primary efficacy endpoints were change from baseline to Week 52 in 1) serum oligosaccharide concentration and 2) the 3-minute stair climb (3MSCT). The 3MSCT is a clinically relevant outcome measure in patients with AM and has previously been used as a clinical endpoint to support regulatory approvals. The serum oligosaccharide biomarker is a physiologically relevant pharmacodynamic (PD) biomarker that, mechanistically, could reflect tissue oligosaccharides, but it is not currently considered a validated surrogate endpoint. Key secondary efficacy endpoints included change from baseline in forced vital capacity (FVC) percent of predicted normal value (FVC%) and change from baseline in the 6-minute walk test (6MWT). Both clinical endpoints have been used to support drug approvals. In Trial rhLAMAN-05, compared to placebo, velmanase led to statistically significant lower concentrations of serum oligosaccharides at Week 52. Additionally, the treatment differences of the Week 52 changes from baseline in the 3MSCT, FVC%, and 6MWT all favored velmanase, compared to placebo. The review team concludes the consistent clinically meaningful improvements in all three clinical endpoints (3MSCT, 6MWT and FVC%) in Trial rhLAMAN-05, supported by the statistically significant reduction in serum oligosaccharides, were sufficient to provide evidence of efficacy of velmanase for the treatment of patients with AM. The following key considerations in AM, together, support a conclusion that velmanase is effective in the treatment

of the non-CNS manifestations of AM and that the trial findings are unlikely to be spurious or due to chance:

- The high quality of the clinical evidence to establish effectiveness, which included a randomized, double-blind, placebo-controlled trial with robust quality of trial conduct (e.g., completeness of follow up).
- The consistency of key clinical endpoints showing improvement favoring velmanase in the setting of significant noise in AM (e.g., heterogenous AM disease manifestations, variable AM disease progression) biasing towards the null in a placebo-controlled superiority trial.
- The presence of multiple factors that likely contributed to the lack of statistical significance observed for the clinical endpoints (e.g., the slow, variable progression of the clinical AM disease manifestations measured by these clinical endpoints and the very small sample size).
- Robust evidence of a drug PD effect evidenced by the statistically significant reduction in serum oligosaccharides, a pharmacologically relevant biomarker, with velmanase compared to placebo.
- The a priori likelihood of a treatment effect, based on the well-understood underlying pathophysiology of AM (i.e., caused by a single enzyme deficiency), a highly targeted therapy (i.e., enzyme replacement, with a general mechanism of action supported by nonclinical data), and evidence to support velmanase reaches its target tissue.

The Applicant submitted a body of confirmatory evidence in the BLA, which the review team determined was adequate to confirm the treatment benefit demonstrated in Trial rhLAMAN-05 (as detailed in the section on Adequacy of the Confirmatory Evidence, Section 6.3.3).

In sum, although there is residual uncertainty regarding clinical benefit with velmanase, the review team concluded that the degree of uncertainty is small and acceptable considering the unique clinical circumstance of AM as a very rare, serious disease with an unmet therapeutic need. Under such circumstance, the team considered the harmful consequences of false positive (Type 1 error) and false negative (Type 2 error) conclusions, in addition to the amount of evidence that could practically be acquired given the rarity of the disease and the fact that velmanase is already approved for AM in multiple regions of the world. This overall landscape warrants the team's use of regulatory flexibility in applying evidentiary approval standards to conclude substantial evidence of effectiveness for velmanase, accepting a higher Type 1 error rate to minimize the risk of a Type 2 error (FDA draft guidance *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biologic Products* (December 2019)). The team concluded, taken together, the findings from the single adequate and well-controlled clinical trial (Trial rhLAMAN-05) and the adequate body of confirmatory evidence constitute substantial evidence of effectiveness of velmanase. Since the effect of velmanase was only demonstrated on non-CNS manifestations of AM (in both humans and animals), (b) (4) a labeled indication specifying treatment of the "non-CNS manifestations of AM" is warranted.

The safety profile of velmanase was generally consistent with the known safety of other ERTs in adult and pediatric patients. In the safety analysis, which was primarily based on the controlled data in rhLAMAN-05, there were no deaths or trial discontinuations. There were 5 serious adverse events (SAEs) reported in the velmanase group in Trial rhLAMAN-05, one of which was possibly treatment related: acute renal failure. Data from rhLAMAN-10 Integrated Analysis,

an integrated analysis of 33 patients pooling the cumulative databases for the early phase velmanase studies and the long-term extension studies for Trial rhLAMAN-05, and Trial rhLAMAN-08 (a single-arm, open label, safety and pharmacokinetic (PK) study of 24 months duration in 5 patients with AM less than 6 years of age) provided additional safety data from patients enrolled in velmanase trials. The main safety concern identified in these clinical trials is a risk for hypersensitivity reactions (including anaphylaxis) and infusion-associated reactions. These hypersensitivity risks can be adequately addressed with labeling, which will include a boxed warning. A safety signal that arose from animal studies is a potential risk of malignancy based on the observation of a malignant ovarian histiocytoma in one high dose (30 mg/kg) rat. Since this type of tumor is an extremely rare occurrence in the studied rats and occurred with a high dose, and since dysregulation of MAN2B1 has been observed in numerous tumors (Lin et al. 2022) (lending biologic plausibility for a drug relatedness), a PMR for an assessment of the potential effects of increased alpha-mannosidase exposure (and increased MAN2B1 expression) on tumor formation will be required. Another safety concern that arose from animal studies is a potential for embryofetal risk based on an increased incidence of embryofetal malformations in rats and rabbits. A warning/precaution will be added to labeling to reflect this potential risk. Overall, the identified safety concerns can be adequately mitigated through labeling.

The establishment of efficacy in patients <6 years of age relied primarily on extrapolating the efficacy findings from Trial rhLMAN-05, which enrolled patient 6 years and older, to younger pediatric patients. The Applicant conducted a trial in pediatric patients less than 6 years of age (Trial rhLAMAN-08), which was an uncontrolled, open label, safety and PK study with descriptive analyses of the efficacy endpoints. The fact that the underlying pathophysiology and characteristics of disease manifestation are generally the same in affected individuals (irrespective of age), and treatment response to ERT is expected to be the same between younger and older pediatric and adult patients with AM, combined with the available PK and PD data in patients less than 6 years of age (from Trial rhLAMAN-08 and from additional data obtained from the worldwide post-marketing use of velmanase) supported such extrapolation of efficacy. Trial rhLAMAN-08 provided safety data in children less than 6 years old.

Overall, in the context of AM as a very rare, serious disease with significant morbidity and mortality with an unmet therapeutic need, the review team has determined that the benefit-risk profile is favorable for the proposed IV weekly dosing regimen for the treatment of non-CNS manifestations of AM in patients of all ages. This determination considered the discussion and input from the FDA Medical Policy and Program Review Council. Velmanase will provide the first approved treatment for patients with AM in the U.S.

II. Interdisciplinary Assessment

3. Introduction

Clinical Condition

Alpha-mannosidosis (AM) is an autosomal recessive, very rare, lysosomal disorder caused by biallelic pathogenic variants in the *MAN2B1* gene that encodes the lysosomal enzyme alpha-mannosidase, which catalyzes the degradation of accumulated mannose-containing oligosaccharides in various tissues. Over 130 disease-causing genetic variants have been reported in the literature (Malm D and Nilssen 2001). These genetic variants lead to deficiency of alpha-mannosidase activity, with lysosomal activity levels in fibroblasts of patients with AM reported to be less than 1.3% of that in controls (Malm and Nilssen 2008). The effect of this enzyme deficiency is accumulation of mannose-rich oligosaccharides in lysosomes of various tissues, resulting in severe impairment of cellular function in these tissues. Eventually, lysosomal apoptosis or cell decomposition is hypothesized to lead to the release of circulating mannose-rich substrates into the extracellular space and consequently, increased levels of oligosaccharides in serum and urine (Borgwardt et al. 2015; Thomas 2019). The diagnosis of AM is made by measuring acid alpha-mannosidase activity in leukocytes or other nucleated cells (e.g., fibroblasts) and the identification of 2 disease-causing genetic variants in *MAN2B1.*

AM has an estimated global prevalence of 1:500,000(NORD 2022). However, this is thought to be underestimated given its phenotypic variability. AM is heterogeneous in clinical presentation (presence and severity of symptoms) and in the rate of clinical progression (Adam et al. 2019; Verrecchia et al. 2021). There is no clear correlation between genotype or residual enzymatic activity with clinical phenotypes (Hennermann et al. 2022). AM encompasses a continuum of clinical findings from mild to severe. Although clinical phenotypes may not be clearly distinguishable due to the broad heterogeneity of the disease (Hennermann et al. 2022), three major subtypes have been suggested(Malm D and Nilssen 2001). The severe infantile form (type 3) includes skeletal abnormalities, progressive central nervous system (CNS) involvement, myopathy, and recurrent infections. Death typically occurs in the first decade of life from recurrent bacterial infections and CNS deterioration(Malm D and Nilssen 2001). The moderate form (type 2) is typically diagnosed before 10 years of age and includes skeletal abnormalities, myopathy, and slower progression with ataxia appearing by the 2nd or 3rd decade of life. Types 2 and 3 typically include coarse facial features and may have ophthalmological abnormalities (including strabismus, clouded corneas, hyperopia, and/or myopia). The mild form (Type 1) typically presents after the first decade of life, does not have skeletal abnormalities, and has very slow progression of myopathy. Across the subtypes, manifestations may include a variable combination of intellectual disability, psychiatric symptoms, hearing loss, and ophthalmologic issues(Malm 2018). Patients with milder forms can survive to later adulthood. Myopathy and ataxia are progressive over time, resulting in the majority of adult patients not being ambulatory.

In the U.S., there are no approved therapies for the treatment of AM. The current standard of care is symptomatic treatment addressing the medical complications of the disease. Hematopoietic stem cell transplant (HSCT) has been investigated for the preservation of neurocognitive function and the prevention of early death in younger AM patients who have not

yet experienced neurocognitive decline. The results have been variable, and the specific values for transplantation-related mortality rates are not available (Ceccarini et al. 2018; Naumchik et al. 2020). HSCT is not considered standard of care at this time.

Product and Mechanism of Action

Velmanase alfa (velmanase) is a new molecular entity, a recombinant human alpha-mannosidase, that functions as an enzyme replacement therapy (ERT) for the endogenous enzyme that is deficient in AM. Velmanase is internalized by cells and transported to the lysosomes where it functions as the endogenous enzyme to catabolize asparagine-linked glycans of glycoproteins and terminal, non-reducing alpha-D-mannose residues in alpha-D-mannosides. Velmanase is administered by intravenous (IV) infusion. The recommended dose is 1 mg/kg IV administered once every week. Velmanase is approved for non-CNS manifestations in patients with AM in the European Union, Brazil, Israel, Saudi Arabia, and Ukraine.

Key Regulatory History

Velmanase received orphan designation in February 2006, rare pediatric designation in December 2018, and Fast Track Designation in December 2019.

The development program of velmanase for the treatment of AM was conducted under investigational new drug (IND) 113186. The first discussion between the Applicant and the Food and Drug Administration (FDA) was a pre-IND meeting on February 14, 2012, during which the Agency provided advice on general chemistry requirements, stated that further toxicity studies would be required to support a clinical trial, and specified that formal process validation would be required at the time of biologics license application (BLA) submission. The IND was opened on August 23, 2019, with the submission of Protocol CLI-LMZYMAA2-01 "A Multicenter, Randomized, Double-Blind, Placebo Controlled, Parallel Group, phase 3 Study to Evaluate the Efficacy and Safety of Velmanase Alfa in Patients with Alpha-Mannosidosis (SHAMAN Study)," which the Applicant planned to conduct in the US to serve as the pivotal trial to support a BLA. A Study May Proceed letter was issued on September 22, 2019.

The Applicant informed the FDA at a Type B pre-BLA teleconference (T-CON) held on June 29, 2021, that the SHAMAN study was stopped in October 2020 because of COVID-19 pandemic-related issues. No subjects had been screened or enrolled in the SHAMAN study. The Applicant proposed to use rhLAMAN-05, a completed phase 3 multi-center European trial that was used to support the European Medicines Agency (EMA) approval of velmanase in 2018, in support of a BLA under the accelerated approval pathway. RhLAMAN-05 was a randomized, double-blind, placebo-controlled 52-week trial in 25 patients aged 6 to 35 years old with AM that evaluated as primary endpoints, change in serum oligosaccharides and change in the 3-minute stair climb test (3MSCT). The FDA advised the Applicant to request a type C surrogate endpoint (SE) meeting to further discuss the evidence to support the use of change in serum oligosaccharides as a SE and to discuss the most appropriate regulatory pathway (i.e., accelerated approval versus traditional approval).

A Type C SE T-CON meeting occurred on January 31, 2022. At this meeting, the Applicant proposed to submit the BLA under the traditional approval pathway and, in case of failure to agree on serum oligosaccharides as a validated surrogate endpoint during the BLA review, proposed that serum oligosaccharides be considered a surrogate endpoint reasonably likely to

predict clinical benefit to support an accelerated approval pathway. The FDA acknowledged that there appeared to be a reasonable scientific basis for the use of serum oligosaccharides as a surrogate endpoint in the velmanase development program and that submission of the BLA under the traditional approval pathway appeared reasonable. However, because this assessment was based on a review of the limited, mostly summary-level data provided at the time of the meeting, the Agency stated that the final determination of whether serum oligosaccharides are an acceptable surrogate endpoint will depend on a detailed review of all evidence in the BLA.

BLA 761278 was submitted on June 17, 2022, with the current PDUFA date of February 17, 2023. This application met the criteria for a priority review because velmanase treats a serious condition for which there is currently no approved treatment, and if approved, would provide a significant improvement in effectiveness in treatment of AM.

Refer to Section 12 for a detailed regulatory history.

3.1. Review Issue List

The review team identified the key review issues listed in Section 3.1.1 and 3.1.2 below relevant to the evaluation of benefit and risk, respectively. A detailed assessment of these benefit and risk issues can be found in Sections 6.3 and 7.7, respectively.

3.1.1. Key Efficacy Review Issues

3.1.1.1. Establishment of Efficacy in Trial rhLAMAN-05

The review team concluded that the results for the clinical endpoints in Trial rhLHAMAN-05 (the primary endpoint of 3MSCT and the key secondary endpoints of 6MWT and FVC%), which consistently favored velmanase and were supported by a statistically significant reduction in serum oligosaccharides with velmanase, were sufficient to establish the efficacy of velmanase in the treatment of AM. For a detailed discussion, refer to Section 6.3.1.

3.1.1.2. Adequacy of the Confirmatory Evidence

The following lines evidence constitute adequate confirmatory evidence of treatment benefit: (1) well-understood underlying disease pathophysiology (i.e., AM is known to be caused by a single enzyme deficiency); (2) clear, targeted mechanism of action (MOA) of velmanase (i.e., replacement of the deficient enzyme); (3) evidence velmanase reaches its target tissue; (4) evidence of drug effect on the pharmacologically relevant biomarker; and (5) evidence of exposure-response (biomarker). For a detailed discussion, refer to Section 6.3.3.

3.1.1.3. Establishment of Efficacy in Patients <6 Years of Age

The single adequate and well-controlled trial, Trial rhLAMAN-05, enrolled AM subjects at least 6 years of age. The establishment of efficacy of velmanase in pediatric patients < 6 years old relies on extrapolation of efficacy seen in AM patients 6 years and older in Trial rhLAMAN-05. The pharmacokinetic (PK) and pharmacodynamic (PD) data from Trial rhLAMAN-08, the

uncontrolled, open label trial in patients less than 6 years of age, as well as PK and PD data from post-marketing global use of velmanase in this age group, provided further support for velmanase's effect in this younger age group. For a detailed discussion, refer to Section 6.3.3.

3.1.2. Key Safety Review Issues

3.1.2.1. Hypersensitivity Reactions (Including Anaphylaxis) and Infusion-Related Reactions

Review of the safety data from the velmanase clinical studies identified hypersensitivity reactions (including anaphylaxis) and infusion-related reactions (IRRs) as key safety concerns with velmanase therapy. Labeling will include a boxed warning for the risk of severe hypersensitivity reactions (including anaphylaxis). For details, refer to Section 7.7.1.

3.1.2.2. Assessment of Safety in Patients <6 Years of Age

Safety data for patients less than 6 years of age are limited, since the only study that included this age group (Trial rhLAMAN-08) was an uncontrolled, open label trial in 5 patients and did not include any patients less than 3 years of age. However, the review team determined that, taken together, the safety data from rhLAMAN05, the safety data from Trial rhLAMAN-08, and the safety data from postmarketing global use, were adequate to inform a safety profile the team finds acceptable in pediatric AM patients down to birth. For details, refer to Section 7.7.2.

3.2. Approach to the Clinical Review

The Applicant submitted data from 8 clinical studies in AM patients to support the efficacy and safety of velmanase. These include 3 early phase trials (rhLMAN02, rhLMAN03, and rhLAMAN04), a single adequate and well controlled pivotal trial (rhLAMAN-05), a pooled long-term safety and efficacy analysis trial (rhLMAN-10), an open label trial in pediatric patients <6 years of age (rhLAMAN-08), and open label extension trials to rhLAMAN-05 (rhLAMAN-07 and rhLAMAN-09). These trials provide clinical data for a total of 38 unique patients - 14 adult subjects and 24 pediatric subjects.

Three of these trials, rhLAMAN-05, rhLAMAN-10 Integrated Analysis, and rhLAMAN-08 (summarized in Table 3 below), form the basis of the benefit-risk assessment for velmanase in patients diagnosed with AM.

Determination of Efficacy

The efficacy of velmanase in adult and pediatric subjects aged 6 years and older was assessed using placebo-controlled data from rhLAMAN-05. Of the 25 subjects in rhLAMAN-05, 15 subjects in the active treatment group and 10 subjects in the placebo group were evaluated in the primary analysis. The two primary efficacy endpoints in rhLAMAN-05, change from baseline in serum oligosaccharides and change from baseline in the 3MSCT, were evaluated in terms of percent change from baseline to Week 52 as specified in the statistical analysis plan (SAP). The

absolute change from baseline to Week 52 for these two endpoints was also evaluated as sensitivity analyses.

The 3MSCT primary endpoint, as well as the key secondary endpoints of 6MWT and FVC%, are clinically meaningful endpoints in the AM population. Furthermore, all three endpoints have been used previously in other drug development programs to support traditional approval. Therefore, the review team considered these clinical functional endpoints appropriate to support traditional approval for velmanase (refer to Section 6.2.1.5 for details). Regarding the serum oligosaccharide primary endpoint, although it is not a validated surrogate endpoint, it is a physiologically relevant, disease-specific biomarker, and the review team considered this endpoint to demonstrate drug effect and suitable to support the results observed for the clinical endpoints.

The efficacy of velmanase in pediatric subjects younger than 6 years of age relied on the extrapolation of efficacy from Trial rhLAMAN-05 that enrolled children 6 and older and adults with AM, with supportive evidence from Trial rhLAMAN-08. Trial rhLAMAN-08 was a single-arm, open-label, safety and PK study that enrolled 5 children aged 3 to <6 years treated with velmanase for 24 months that collected efficacy measurements. (Refer to Section 6.3.3).

Because there was only one adequate and well-controlled trial (rhLAMAN-05), confirmatory evidence of effectiveness, including clinical PK/PD and biomarker data from other clinical trials (rhLAMAN-03, -04 and -08) and nonclinical mechanistic data, was also evaluated (refer to Section 6.3.3 for a discussion of the confirmatory evidence review).

Determination of Safety

Safety data from Trials rhLAMAN-05, rhLAMAN-10 and rhLAMAN-08 were reviewed to determine the safety of velmanase in patients with AM. The safety data from these trials were pooled by the Applicant and presented as the integrated summary of safety (ISS). The full population evaluated for safety included 38 unique patients aged 3 to 35 years.

The analysis of adverse events (AEs), laboratory evaluations, and vital signs was based on descriptive summaries and tables provided by the Applicant and Clinical Data Scientists from the FDA and the team's review of source data. Case report forms and patient narratives were reviewed for anaphylaxis, discontinuations, and withdrawals. Clinical trial data were independently analyzed using JMP and R Software. All safety assessments and conclusions are those of the clinical review team unless otherwise specified.

Table 3. Clinical Trials Submitted in Support of Efficacy and Safety Determinations for Velmanase

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Patients Planned; Actual Randomized	No. of Centers and Countries
rhLAMAN-05	Subjects 5-35 years of age with alpha-mannosidosis	Control Type: Placebo Randomization: Standard randomization (3:2 ratio) Blinding: Double blind Biomarkers: CSF fluid biomarkers (oligosaccharide, Tau, NfL, and GFAP) Innovative design features: None	Drug (established name): velmanase Dose: velmanase 1 mg/kg body weight (N=15) and placebo (N=10) Number treated: 25 Duration (treatment): 12 months	Primary: Change from baseline to week 52 in serum oligosaccharides Change from baseline to week 52 in the 3-minute stair climb test (3MSCT). Secondary: Change from baseline to week 52 in 6-minute walk test (6MWT) Change from baseline to week 52 in FVC percent of predicted normal value (FVC %)	Planned: 25 Actual: 25	Centers: 9 Countries: 5
rhLAMAN-10	subjects with alpha-mannosidosis who previously participated in velmanase trials.	Control Type: NA Randomization: NA Blinding: NA (open-label) Biomarkers: CSF fluid biomarkers (oligosaccharide, Tau, NfL and GFAP)	Drug (established name): CHF-LMZYMAA1 (velmanase) Dose: velmanase 1 mg/kg body weight (N=33) Number treated: 33 Duration (treatment): 12-48 months	Primary: Change from Baseline in oligosaccharides in serum. Change from Baseline in the 3MSCT Secondary: The 6MWT (m and adjusted for age, height and gender and given as percent of predicted value). The best value from 2 performances was used. PFT endpoints: FVC percentage of predicted, FVC (L), FEV1 of predicted), FEV1 (L) and PEF, L/s.	Planned: 20 Actual: 33	Centers: 1 Countries: 5

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Patients Planned; Actual Randomized	No. of Centers and Countries
rhLAMAN-08	Pediatric patients below 6 years of age with alpha-mannosidosis	Control Type: NA Randomization: NA Blinding: NA (open-label) Biomarkers: CSF fluid biomarkers (Tau, NfL and GFAP)	Drug (established name): CHF-LMZYMAA1 (velmanase) Dose: velmanase 1 mg/kg body weight (N=5) Number treated: 5 Duration (treatment): 24 months (40 months for patient # (b) (6) enrolled in (b) (6))	Primary: • Safety and tolerability of velmanase as per: ▪ Adverse events (AEs) including infusion-related reactions (IRRs). ▪ Vital signs. ▪ Clinical laboratory parameters (haematology, biochemistry, and urinalysis). • Detection of ADAs. Secondary: changes from baseline to 24 months except for the (b) (6) patient (# (b) (6)) since the treatment could last 40 months)	Planned: 3 Actual: 5	Centers: 7 Countries: 5

Source: Clinical Study Report and adsl.xpt
Abbreviations: FVC, forced vital capacity; CSF, cerebrospinal fluid; NfL, neurofilament light chain protein; GFAP, Glial fibrillary acidic protein; Tau, Tau protein

4. Patient Experience Data

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
Clinical Outcome Assessment Data Submitted in the Application		
☐	Patient-reported outcome	Section 6.2
☐	Observer-reported outcome	
☒	Clinician-reported outcome	
☒	Performance outcome	
Other Patient Experience Data Submitted in the Application		Section 6.2.1.5
☐	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☒	Observational survey studies Natural history studies	
☐	Patient preference studies	
☒	Other: (please specify)	
☐	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☐	Perspectives shared at patient stakeholder meeting	Published literature (Adam et al. 2019; Verrecchia et al. 2021) discussed in Sections 2.1, and 6.2.
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☒	Observational survey studies	
☒	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

5.1. Nonclinical Assessment of Potential Effectiveness

The Applicant provided data in which cells from healthy individuals and AM patients were shown to internalize the exogenous enzyme, and that internalization was largely dependent on the mannose-6-phosphate receptor. They also evaluated the biodistribution and tissue uptake in animals; however, the methods used were non-quantitative and the model (a transgenic knock-in/knockout (KO) model in which a defective human *MAN2B1* gene was knocked into a murine

MAN2B1 KO) does not appear to replicate human disease. Carbohydrate accumulation was observed; however, it was low relative to those observed in patients (approximately 2-3x those of wildtype controls in most organs except the liver, in which oligosaccharide levels were was approximately 10% higher in the KOs than in wildtype controls). Vacuolation of tissues was shown in the liver, bone, kidney, pancreas, and brain; however, the contents of the vacuoles were not evaluated. Administration of exogenous rhLAMAN led to uptake in the spleen, kidney, liver, lung, thymus, heart, and brain. Uptake was strongest at 1-hour post-dose in all organs but the brain, which showed increased but low accumulation at 24 hours(Stinchi et al. 1999; Roces et al. 2004). Exogenous administration of rhLAMAN was associated with an apparent reduction in oligosaccharide content in the liver, spleen, kidney, and heart, with maximal clearance observed at a dose of 250 mU/g body weight (Roces et al. 2004); however, the method employed was inadequate to quantify the magnitude of the reduction. The authors report that maximal reduction in oligosaccharide content was observed at Day 4 post-dose, and that re-accumulation of the oligosaccharides began by Day 8 post-dose but continued through Day 16.

The effect of rhLAMAN administration on neurocognitive functioning was evaluated in the Tg+KO mouse model. At approximately 16-fold the clinical dose of velmanase, authors state that treatment was associated with a reduction in markers of gliosis (CD68/IBA1 positivity) in the hippocampus of treated versus control animals(Stroobants et al. 2017). The authors suggests that treatment resulted in modest improvements on short-term memory and/or motor and coordination.

In a chronic toxicology study in the Tg+KO mouse, the Applicant evaluated histopathology in only high-dose and control animals. In these animals, qualitative evidence of reduced tissue vacuolation was observed at a dose that was approximately 49-fold higher than the clinical dose. They did not evaluate histological effects at lower doses, nor did they evaluate serum or tissue oligosaccharides; thus, the data are limited and inadequate to support an understanding of the effect of dose (or exposure) on oligosaccharide content and tissue damage. Other data provided by the Applicant suggest that oligosaccharide levels in the liver, spleen, and kidney (Roces et al. 2004) may be transiently reduced in response to treatment. The dose-effect and exposure-effect relationships, however, have not been established; in most cases, the methods used were non-quantitative and the published reports did not provide information about plasma exposures or tissue- or serum-oligosaccharide levels.

Overall, the weight of evidence from the good laboratory practice-compliant toxicology study in the Tg+KO mouse and from several published and Applicant-provided reports provides support for the general concept that in patients with AM, administration of exogenous α-mannosidase may reduce tissue vacuolation. Although a reduction of tissue vacuolation was observed, it did not correlate with an improvement of a functional outcome; therefore, the extent to which treatment reduces oligosaccharide levels, and the extent to which a reduction in oligosaccharide levels elicits the desired, clinically meaningful effect is unclear. The lack of biomarker in the Applicant's animal studies that would have allowed them to assess the reduction in tissue oligosaccharides and correlate those to histological and functional effects, greatly limits the utility of the nonclinical data to support a mechanistic basis for clinical efficacy.

5.2. Clinical Pharmacology/Pharmacokinetics

The pharmacologic activity, pharmacokinetics (PK), and clinical pharmacology of velmanase relevant to the interpretation of benefit and risk are summarized in Table 5.

Table 5. Summary of Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information
	Pharmacologic Activity
Established pharmacologic class (EPC)	Velmanase is a recombinant human lysosomal alpha-mannosidase.
Mechanism of action	Alpha-mannosidosis is a lysosomal storage disease that results from reduced activity of the enzyme alpha-mannosidase, caused by gene variants in the Mannosidase Alpha Class 2B Member 1 gene. Alpha-mannosidase catalyzes the degradation of accumulated mannose-containing oligosaccharides. The deficiency of alpha-mannosidase causes an intra-lysosomal accumulation of mannose-rich oligosaccharides in various tissues. Velmanase provides an exogenous source of alpha-mannosidase. Velmanase is internalized via binding to the mannose-6-phosphate receptor on the cell surface and transported into lysosomes where it is thought to exert enzyme activity.
Pharmacodynamics	Serum oligosaccharide concentrations are elevated in patients with alpha-mannosidosis. In clinical studies, serum oligosaccharide concentrations were quantified by assessment of 2-mannose oligosaccharides. Velmanase treatment resulted in reductions of serum oligosaccharide concentrations in patients with alpha-mannosidosis. Refer to Section 6.2 for the pharmacodynamic results.
Active moieties	Velmanase is a recombinant human lysosomal alpha-mannosidase and is the active moiety.
QT prolongation	Velmanase is a therapeutic protein with an approximate molecular weight of 130 kDa and has a low likelihood of direct ion channel interactions. A thorough QT/QTc study has not been performed to evaluate the QT prolongation potential of velmanase.

<table>
<tr><th>Characteristic</th><th>Drug Information</th></tr>
<tr><td></td><td>General Information</td></tr>
<tr><td>Bioanalysis</td><td>Plasma concentrations of velmanase were determined using a validated ELISA assay with a lower limit of quantitation (LLOQ) of 224 µg/L.</td></tr>
<tr><td>Healthy subjects versus patients</td><td>The PK of velmanase has not been characterized in healthy subjects.</td></tr>
<tr><td>Drug exposure at steady state following the therapeutic dosing regimen (or single dose, if more relevant for the drug)</td><td>The mean ± SD plasma C_{max}, AUC_t, and AUC_{inf} of velmanase at Day 1 and at steady state following intravenous (IV) infusion of 1 mg/kg dose of velmanase once every week in patients with alpha-mannosidosis are summarized in Table 6 and Table 7, respectively.

Table 6. Summary of Pharmacokinetic Parameters for Velmanase at Day 1
<table>
<tr><th>Age Groups</th><th><6 (n=5)</th><th>6 to <18 (n=6-7)</th><th>≥18 (n=9)</th></tr>
<tr><td>AUC_{inf} (hr·µg/mL)</td><td>102.9 (15.6)</td><td>83.3 (33.3)</td><td>111.0 (17.6)</td></tr>
<tr><td>AUC_t (hr·µg/mL)</td><td>88.9 (11.4)</td><td>70.7 (30.8)</td><td>99.8 (15.9)</td></tr>
<tr><td>C_{max} (ug/mL)</td><td>8.0 (1.4)</td><td>6.4 (2.3)</td><td>10.0 (5.2)</td></tr>
</table>
Source of data: Table 18 of Summary of Clinical Pharmacology Studies. C_{max} = maximum plasma concentration; AUC_{inf} = the area under the plasma concentration-time curve from zero to time infinity calculated as AUC_t plus intercept of the slowest disposition slope with the ordinate (cz)/terminal disposition rate constant (λz); AUC_t = the area under the plasma concentration-time curve from zero to time t of last evaluable concentration.

Table 7. Summary of Pharmacokinetic Parameters for Velmanase Alfa at Steady State
<table>
<tr><th>Age Groups</th><th><6 (n=5)</th><th>6 to <18 (n=6-7)</th><th>≥18 (n=9)</th></tr>
<tr><td>AUC_{inf} (hr·µg/mL)</td><td>108.3 (27.1)</td><td>118.9 (201.9)</td><td>178.9 (28.7)</td></tr>
<tr><td>AUC_t (hr·µg/mL)</td><td>75.9 (39.7)</td><td>109.8 (17.8)</td><td>159.8 (24.4)</td></tr>
<tr><td>C_{max} (ug/mL)</td><td>7.0 (2.3)</td><td>6.6 (1.0)</td><td>7.9 (0.9)</td></tr>
</table>
Source of data: Table 18 of Summary of Clinical Pharmacology Studies. C_{max} = maximum plasma concentration; AUC_{inf} = the area under the plasma concentration-time curve from zero to time infinity; AUC_t = the area under the plasma concentration-time curve from zero to time t of the last evaluable concentration.</td></tr>
<tr><td>Range of effective dose(s) or exposure</td><td>The recommended adult and pediatric dosage of velmanase is 1 mg/kg (actual body weight) infused intravenously once every week (QW).</td></tr>
<tr><td>Maximally tolerated dose (MTD) or exposure</td><td>An MTD of velmanase was not determined in alpha-mannosidosis patients.</td></tr>
<tr><td>Dose proportionality</td><td>The dose proportionality of velmanase could not be determined due to limited PK data and small number of patients receiving doses higher or less than 1.0 mg/kg.</td></tr>
<tr><td>Accumulation</td><td>The AUC_{inf} of velmanase at steady state was 5% to 61% higher than the AUC_{inf} after the first dose administration following IV infusion of 1 mg/kg velmanase once every week in patients with alpha-mannosidosis across different age groups.</td></tr>
</table>

Characteristic	Drug Information
	Distribution
Volume of distribution	The mean (SD) volume of distribution of velmanase was 276 (43) mL/kg in adult patients with alpha-mannosidosis.
Plasma protein binding	Plasma protein binding has not been characterized for velmanase.
	Elimination
Clearance	The mean (SD) total body clearance of velmanase is 5.7 (0.9), 8.6 (1.4), and 10.1 (2.9) mL/hr/kg in patients with alpha-mannosidosis ≥18, 6 to 17, and <6 of age, respectively.
Half-life	The mean half-life ranged from 17 to 34 hours in patients with alpha-mannosidosis across the age groups.
Metabolic pathway(s)	The metabolic pathway of velmanase has not been characterized. Velmanase is expected to be metabolized into small peptides via catabolic pathways.
	Intrinsic Factors and Specific Populations
Body weight	Body weight was identified as a significant covariate on clearance of velmanase. Population PK covariate analysis suggested approximately 20% decrease in exposure in patients with body weight ≤18 kg in reference to subjects with a body weight of 60 kg, which is not considered clinically meaningful based on the current understanding of the exposure-response relationships for velmanase, and additional dose adjustments beyond the proposed body weight-based dosing are not necessary.
Age	Based on population PK analysis, after consideration of the body weight effect, age (3-35 years) did not have clinically meaningful effects on the PK of velmanase. However, PK data in patients <3 years of age is currently not available. Refer to Section 6.3.3.
Renal impairment	No dedicated trial of the impact of renal impairment on the PK of velmanase has been conducted. Intact velmanase (molecular weight of approximately 130 kDa) is unlikely to be filtered by kidney or excreted in urine.
Hepatic impairment	No dedicated trial of the impact of hepatic impairment on the PK of velmanase has been conducted. Metabolism by CYP enzymes or secretion into bile is generally not a significant contributor to the elimination of therapeutic proteins such as velmanase.
	Drug Interactions
Inhibition/induction of metabolism	No CYP450-mediated drug-drug interaction studies were conducted for velmanase. As a therapeutic protein, velmanase is unlikely to be involved in CYP450-mediated drug interactions.
Inhibition/induction of transporter systems	No transporter-mediated drug-drug interaction studies were conducted for velmanase. As a therapeutic protein, velmanase is unlikely to be involved in transporter-mediated drug interactions
	Immunogenicity
Bioanalysis	The immunogenicity of velmanase was assessed in six clinical studies in patients with alpha-mannosidosis. An ELISA with Protein G coating was used for detection of anti-velmanase antibodies (antidrug antibodies or ADA) in serum. An enzymatic assay was used to detect neutralizing antibodies (NAb) that inhibit velmanase enzyme activity. Limitations of the current immunogenicity assays were identified. See post marketing commitment/requirement (PMC/PMR) recommendations.
Incidence	In Trial rhLAMAN-08 following 104 weeks treatment with velmanase, 4 out of 5 pediatric patients (80%) developed ADA. Three out of 4 ADA-positive patients (75%) developed neutralizing antibodies that inhibit velmanase enzyme activity. In Trial rhLAMAN-10, 33 patients (10 adult, 23 pediatric) received velmanase for up to 209 weeks. Among the 33 patients, 5 patients (1 adult and 4 pediatric) (15%) had ADA before receiving velmanase treatment and for 1 patient the ADA level

Characteristic	Drug Information
	increased after treatment with velmanase. Four other patients (1 adult and 3 pediatric) (12%) developed ADA after treatment with velmanase. ADA-positive samples were tested for neutralizing antibodies that inhibit velmanase enzyme activity during treatment in Trial rhLAMAN-05. Four patients with ADA-positive results also had positive NAb results during treatment with velmanase. However, Nab-positive results of similar magnitude were detected in 4 patients during treatment with placebo. NAbs that inhibit cellular uptake of velmanase have not been characterized.
Clinical impact	Development of ADA was associated with lower plasma concentrations of velmanase. Two pediatric patients who developed ADA had reduced pharmacodynamic responses in reduction of serum oligosaccharides at the time when high ADA levels were observed. Infusion-associated reactions (including anaphylaxis and severe hypersensitivity reactions) occurred in a higher incidence in velmanase-treated patients who developed ADA compared to patients who were ADA-negative (80% versus 20%). In Trial rhLAMAN-5 following treatment with velmanase for up to 52 weeks, 1 out of 5 ADA-positive patients developed severe hypersensitivity and this patient developed the highest ADA level among all the ADA-positive patients in the trial. In Trial rhLAMAN-08 following treatment with velmanase for up to 174 weeks, 2 out of 4 ADA-positive pediatric patients experienced IARs. In Trial rhLAMAN-10, 3 out of 33 patients (9.1%) reported IARs; two of these patients were ADA positive (one of these two patients is described in rhLAMAN-05); one patient was ADA-negative.

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CYP, cytochrome P450; ELISA, enzyme-linked immunosorbent assay; IAR, infusion-associated reaction; IV, intravenous; PK, pharmacokinetics; QW, once weekly; SD, standard deviation

6. Efficacy (Evaluation of Benefit)

6.1. Assessment of Dose and Potential Effectiveness

6.1.1. Proposed Velmanase Dosage Regimen

Velmanase is a recombinant human lysosomal alpha-mannosidase and is proposed as an enzyme replacement therapy for the treatment of patients with alpha-mannosidosis. Velmanase is administered via intravenous infusion. The proposed dosage regimen is 1 mg/kg, based on patient's actual body weight, administered once every week. The proposed dosage regimen was tested in patients 6 years of age and older in Trial rhLAMAN-05 and in pediatric patients less than 6 years of age in Trial rhLAMAN-08. The proposed velmanase dosage regimen is acceptable and is the recommended dosage regimen for pediatric and adult patients with alpha-mannosidosis.

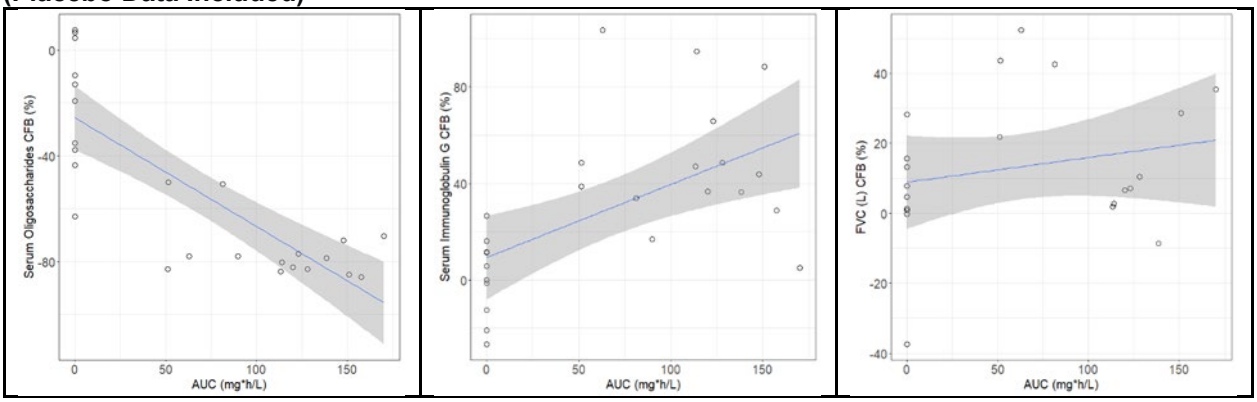
6.1.2. Dose Selection Rationale for Clinical Trials

The dose selection for the pivotal Trial rhLAMAN-05 has considered in vitro data, in vivo study findings in a mouse model with AM KO, and human PK and PD data from phase 1/2 clinical trials. The results from the mouse model indicated that a wide range of dose levels, from 25 to 500 U/kg (0.8 to 16 mg/kg), would reduce oligosaccharide levels in peripheral tissues. The dose-response for reduction in serum oligosaccharides based on the data from phase 1/2 trials overall supported that the 1 mg/kg dose would be adequate to achieve maximal reduction in serum oligosaccharide levels in the general patient population.

6.1.3. Exposure-Response Relationship for Efficacy

Based on PK data from Trials rhLAMAN-02, -03, -04, -05, -08, -09 and -10, a 3-compartment PK model with first order elimination best described the PK of velmanase. Individual PK parameters were used to explore the relationship between velmanase exposure and PD biomarkers and efficacy. With placebo data included, the exposure-response (E-R) analysis showed a significant E-R relationship between velmanase area under the concentration (AUC) time curve and serum oligosaccharides % change from baseline (left panel of Figure 1, $p<0.001$), a significant E-R relationship between velmanase AUC and serum immunoglobulin G (IgG) % change from baseline (middle panel of Figure 1, $p<0.005$), and an E-R relationship between velmanase AUC and forced vital capacity (FVC) (L) % change from baseline (right panel of Figure 1, $p=0.05$). When placebo data were excluded, all the above E-R relationships disappeared suggesting that the 1 mg/kg dose could have reached plateau phase of the E-R curve for the PD biomarkers (e.g., serum oligosaccharide and IgG) and efficacy endpoint (e.g., FVC). Refer to Section 6.3.3 for more information about PK/PD, dose-response, and exposure-response relationship of velmanase.

Figure 1. Exposure-Response Relationships for the Pharmacodynamic Biomarkers (Serum Oligosaccharides and Serum IgG) and Efficacy Endpoint (FVC) of Velmanase, Trial rhLAMAN-05 (Placebo Data Included)



Source: Reviewer's analysis
Note: With placebo data included, significant exposure-response relationships were observed for PD responses in reduction of serum oligosaccharides and increase of serum IgG. Exposure-response relationship was also observed for clinical endpoint FVC.
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; FVC, forced vital capacity

6.1.4. Exposure-Response Relationship for Safety

The currently available safety data were too limited to support a reasonable exposure-response analysis of any specific AE.

6.2. Clinical Studies/Trials Intended to Demonstrate Efficacy

6.2.1. Trial rhLAMAN-05

6.2.1.1. Design and Eligibility Criteria

Design

The pivotal trial, rhLAMAN-05, was a randomized, double-blind, placebo-controlled multi-center, international trial in treatment-naïve patients with AM aged 6 to 35 years. The primary objective of this trial was to demonstrate the efficacy of velmanase (1 mg/kg IV once a week) in terms of improvement in the 3MSCT and reduction in serum oligosaccharide levels over a 52-week period compared to placebo. The key secondary objective was to demonstrate a positive trend of velmanase over placebo in improvement in the 6MWT and FVC% predicted normal value over the 52-week period.

The primary efficacy endpoints were change from baseline to week 52 in serum oligosaccharides and change from baseline to week 52 in 3MSCT. There were a total of seven secondary efficacy endpoints with change from baseline to week 52 in FVC percent of predicted normal value (FVC % predicted) and change from baseline to week 52 in 6MWT stated as "prioritized" (i.e., key) secondary endpoints.

Eligibility Criteria

Inclusion Criteria

- Alpha-mannosidosis confirmed by alpha-mannosidase activity <10% of normal activity in blood leukocytes (historical data)
- Age 5 to 35 years (inclusive) at screening
- Normal echocardiogram

Exclusion Criteria

- No confirmed AM diagnosis
- Patient cannot walk without support
- Presence of known chromosomal abnormality and syndromes (other than AM) affecting psychomotor development
- History of bone marrow transplant
- Cardiovascular, hepatic, pulmonary, or renal disease or other medical conditions that would preclude participation in the trial
- Any other medical condition or serious intercurrent illness, or extenuating circumstance that, in the opinion of the Investigator, would preclude participation in the trial
- Pregnancy
- Psychosis or any psychotic disease (inclusive of disease in remission)
- Planned major surgery that, in the opinion of the Investigator, would preclude participation in the trial
- Participation in other interventional trials testing the investigational medicinal product (including velmanase) within the last 3 months
- Total immunoglobulin E (IgE) >800 international units/ml

Although the diagnosis of AM is confirmed by molecular genetic testing of the *MAN2B1* gene (NORD 2022) this genetic molecular confirmation was not explicitly stated as an inclusion criterion. However, genotype data for pathogenic genetic variants in *MAN2B1* were captured for all patients except for Patient (b) (6) (randomized to placebo).

The above eligibility criteria are acceptable**.**

6.2.1.2. Patient Screening, Enrollment, and Disposition

According to the study report, a total of 26 patients were screened for the trial. There was one screening failure due to having a level of IgE that was incompatible with the inclusion/exclusion criteria. The 25 remaining patients were randomized 3:2 to velmanase (n=15) or placebo (n=10). None of the 25 patients enrolled in the trial discontinued from the study drug or the study.

One patient who discontinued the study drug during an earlier phase Trial (rhLAMAN-03) was later enrolled into the rhLAMAN-05 treatment group as a treatment-experienced patient.

6.2.1.3. Demographics and Baseline Characteristics

The following Table 7 provides the demographics and baseline characteristics for the patients enrolled in Trial rhLAMAN-05. There are no significant differences in these baseline characteristics between treatment or placebo arms. As described in Section 3, Trial rhLAMAN-05 is a European trial. The review team assessed that using this European data to support velmanase approval in the US was acceptable. The etiology of AM, a deficiency of alpha-mannosidase, is the same across all populations worldwide. And the patient ages included in the trial (6 to 35 years old) is representative of the AM population in the US. With regards to the genetic variants present in the Trial rhLAMAN-05 population, there is no significant different in the pathologic genetic variants of patients in placebo versus treatment arm. Additionally, there is no known genotype-phenotype correction, thus minor baseline imbalances are not likely to influence efficacy outcomes.

Table 8. Baseline Demographics and Clinical Characteristics, Trial rhLAMAN-05

Characteristic	Velmanase N=15 n (%)	Placebo N=10 n (%)	Total Population (N=25) n (%)
Sex, n (%)			
Male	9 (60)	5 (50)	14 (56)
Female	6 (40)	5 (50)	11 (44)
Age, years			
Mean (SD)	18.5 (9.0)	19.7 (8.9)	19.0 (8.8)
Median (min, max)	20 (6, 35)	18.5 (6, 35)	20 (6, 35)
Age group (years), n (%)			
≥6 to <18	7 (47)	5 (50)	12 (48)
≥18 to ≤35	8 (53)	5 (50)	13 (52)
Race, n (%)	n (%)	n (%)	n (%)
White	15 (100)	10 (100)	25 (100)
Country of participation, n (%)			
United States	0 (0)	0 (0)	0 (0)
United Kingdom	0 (0)	1 (10)	1 (4)
Poland	3 (20)	0 (0)	3 (12)
Spain	2 (13)	1 (10)	3 (12)
Germany	4 (27)	1 (10)	5 (20)
France	4 (27)	2 (20)	6 (24)
Other	2 (13)	5 (50)	7 (28)
Serum oligosaccharides (umol/L)			
Mean (SD)	6.8 (1.2)	6.6 (1.9)	6.7 (1.5)
Median (min, max)	7.0 (4.9, 8.7)	6.3 (4.4, 10.2)	6.4 (4.4, 10.2)

Characteristic	Velmanase N=15 n (%)	Placebo N=10 n (%)	Total Population (N=25) n (%)
Alphamannosidase activity (nmol/h/mg)			
Mean (SD)	12.4 (3.5)	12.7 (6.2)	12.5 (4.7)
Median (min, max)	11.5 (8.5, 22.2)	11.6 (8.3, 29.0)	11.5 (8.3, 29.0)
Alphamannosidase activity (relative) (%)			
Mean (SD)	4.5 (1.3)	4.6 (2.3)	4.6 (1.7)
Median (min, max)	4.2 (3.1, 8.1)	4.3 (3.0, 10.6)	4.2 (3.0, 10.6)
3MSCT (steps/min)			
Mean (SD)	52.9 (11.2)	55.5 (16.0)	54.0 (13.1)
Median (min, max)	50 (37.7, 83.3)	54.5 (32.0, 78.0)	50.0 (32.0, 83.3)
6MWT (meters)			
Mean (SD)	459.6 (72.3)	465.7 (140.5)	462.0 (102.2)
Median (min, max)	434.0 (335.0, 627.0)	482.5 (219.0, 696.0)	476.0 (219.0, 696.0)
FVC (% predicted)			
Mean (SD)	85.1 (22.8)	85.5 (18.5)	85.3 (20.8)
Median (min, max)	80.0 (50.0, 120.0)	91.5 (41.0, 109.0)	91.0 (41.0, 120.0)
Genetic variant, n (%)			
c.2248C > T	6 (40)	1 (10)	7 (28)
Other[1]	9 (60)	8 (80)	17 (68)
Missing	0	1	1 (4)
Genetic variant type, n (%)			
Deletion	0	1 (10)	1 (4)
Frameshift	2 (13)	2 (20)	4 (16)
Missense	6 (40)	2 (20)	8 (32)
Mixed	5 (33)	4 (40)	9 (36)
Nonsense	2 (13)	0	2 (8)
Missing	0	1 (10)	1 (4)

Source: [Include Applicant source and/or software tools used].
[1] "Other" includes the following variants: c.1026+2T > G, c.1055T > C, c.1333C > T, c.1358C > T, c.2234C > G, c.231G > A, c.2355G > A, c.2398G > T, c.2436+5G > A, c.283G > C, c.338_348dup11, c.383G > A, c.418C > T, c.598C > A, c.685C > T, c.809dupA, c.812_813dupTG; each variant was present in one patient, except c.1055T > C, which was present in two patients
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients with given characteristic; SD, standard deviation; 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; FVC, forced vital capacity

6.2.1.4. Statistical Analysis Plan

Serum oligosaccharide level, 3MSCT, FVC%, and 6MWT were measured at baseline, week 26, and week 52 of the trial. Serum oligosaccharide level was measured using an assay at a central laboratory at the (b) (4). The 3MSCT, FVC%, and 6MWT were administered to all subjects at the (b) (4) site.

The 3MSCT is measured as the number of steps divided by time (minutes). If a subject reaches the top of the stairs in less than three minutes, the number of steps will be divided by the time it took to reach the top of the stairs. According to the SAP, if a subject stops prematurely due to exhaustion or a similar reason, the number of steps taken will be divided by three minutes.

The SAP stated that the primary analysis would be performed on the relative change from baseline to week 52 for the primary efficacy endpoints and the key secondary efficacy endpoints. The analyses for the endpoints of absolute change from baseline were considered sensitivity analyses.

The efficacy endpoints were analyzed using an analysis of covariance (ANCOVA) model including treatment as fixed factor and baseline value and age as continuous covariates. The

model produced estimates of adjusted means for each treatment group and the adjusted difference in means between treatment groups, along with corresponding 95% confidence intervals (CIs) and p-values.

Review Team's Comments

*For the relative change from baseline endpoints, the SAP stated that the ANCOVA models would be performed based on the log-transformed data (baseline and Week 52) to test the treatment effect, but the SAP did not provide any details on how the mean estimates and the associated 95% CI were calculated for the endpoint without transformation. The Applicant's study report (on page 164) indicated that the formula {exp(mean) – 1} *100 was used, where the mean was obtained based on the analysis of the log-transformed data. Because of the statistical issues associated with using this formula (see Section 16.2.1), the review team conducted and reported the analyses based on the relative change from baseline without making any transformation.*

*For the p*rimary analysis of the primary efficacy endpoints, a multiple imputation method would be applied before conducting the primary analysis, in the case of missing data and under an assumption of missing at random. The SAP does not define the method for handling missing data for the key secondary endpoints.

Review Team's Comments

Based on the Applicant's analysis datasets, there were no missing data for the primary endpoints and the 6MWT endpoints. For the FVC endpoints (relative change and absolute change from baseline at 52 weeks), four subjects (one in the placebo group and three in the velmanase group) were listed as having missing values because their baseline values were deemed unreliable due to the poor quality of maneuver during pulmonary function testing. In their response to the review team's information request dated January 09, 2023, the Applicant stated that the missing data were handled using the multiple imputation method specified for the primary endpoints. The review team conducted two additional analyses: (1) treated these four subjects as having no change from baseline and (2) excluded these subjects from the analysis. The review team's analyses produced similar results as the Applicant's analysis.

Sensitivity analyses for each endpoint include (1) evaluating the treatment effect of velmanase at Week 26 and (2) fitting the ANCOVA model, as specified for the primary analysis, on the absolute change from baseline at Week 52. Responder analyses were also utilized for each endpoint, where a responder was defined as:

- A percentage reduction of 70% or more for serum oligosaccharides
- Additional responder analyses using the cutoffs of 80% or more, 90% or more, and 95% or more
- A percentage increase of 10% or more for 3MSCT
- Additional responder analyses using the cutoffs of 0% or more and 15% or more
- A percentage increase of 0% or more, 10% or more, and 15% or more for FVC and 6MWT

Review Team's Comments

The Applicant's responder analyses were not considered in the assessment of efficacy, because the Applicant did not provide a rationale for the clinical meaningfulness of the defined thresholds. Refer to Section 16.2.2 for further details.

The SAP pre-specified an interim analysis using Week 26 data. The interim analysis was not binding, in that the trial could continue even if efficacy had been established based on the pre-specified definition. Demonstration of efficacy was pre-specified in the protocol and SAP as:

- A statistically significant improvement in serum oligosaccharides and 3MSCT, at the significance levels of 0.025 and 0.05 respectively, at the interim (Week 26) analysis, or
- A statistically significant improvement in serum oligosaccharides at the significance level of 0.025 and a trend for improvement in the 3MSCT and either FVC or 6MWT at the final (Week 52) analysis

Review Team's Comments

Trial rhLAMAN-05 was conducted outside of the United States and its protocol and SAP were not submitted to the FDA prior to study initiation or completion. According to the Applicant's study report, the first visit of the first enrolled patient occurred on September 10, 2012, and the last visit of the last enrolled patient occurred on May 2, 2014. This study supported the approval of velmanase (tradename Lamzede) in the European Union (EU) in 2018. The IND for velmanase (IND 113186) was not opened until August 2019.

6.2.1.5. Results of Analyses

Table 8 contains the primary results for the primary and key secondary endpoints at Week 52. The treatment difference of the Week 52 change from baseline in serum oligosaccharides produced a statistically significant p-value of < 0.001 in favor of velmanase. The estimated treatment differences of the Week 52 changes from baseline in 3MSCT, FVC%, and 6MWT favored velmanase, compared to placebo. Based on their pre-specified criteria for demonstration of efficacy ("a statistically significant improvement in serum oligosaccharides at the significance level of 0.025 and a trend for improvement in the 3MSCT and either FVC or 6MWT"), the Applicant concludes that efficacy of velmanase has been established in Trial rhLAMAN-05.

Table 9. Changes From Baseline in Primary and Key Secondary Endpoints at Week 52, Trial rhLAMAN-05

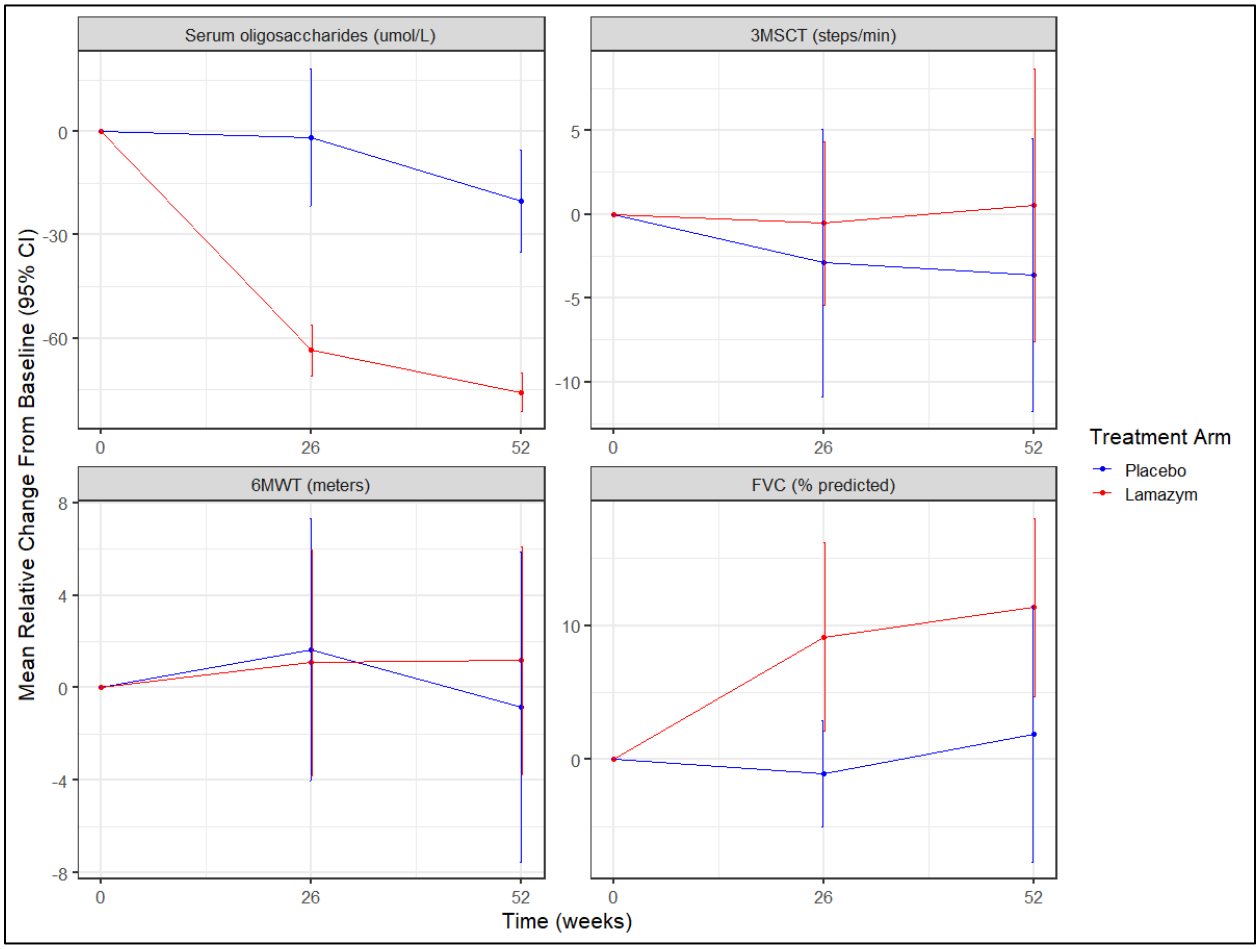
Variable	Velmanase (n=15)	Placebo (n=10)	Adjusted Treatment Difference (95% CI)
Serum oligosaccharides (umol/L)			
Relative change (%) from baseline	-75.8 (-84.5, -67.2)	-20.2 (-30.8, -9.7)*	-55.6 (-69.3, -41.9) P<0.001
Absolute change from baseline	-5.1 (-5.7, -4.6)	-1.6 (-2.3, -0.9)	-3.5 (-4.4, -2.6) p<0.001
3MSCT (steps/min)			
Relative change (%) from baseline	0.2 (-7.9, 8.4)	-3.2 (-13.1, 6.8)	3.4 (-9.5, 16.3) p=0.59

Absolute change from baseline	0.5 (-3.6, 4.5)	-2.2 (-7.1, 2.8)	2.7 (-3.8, 9.1) p=0.41
FVC (% predicted)			
Relative change (%) from baseline	10.5 (2.1, 18.9)	3.1 (-6.7, 12.8)	7.4 (-5.7, 20.5) p=0.25
Absolute change (% predicted) from baseline	7.9 (1.1, 14.7)	2.4 (-5.5, 10.2)	5.5 (-5.0, 16.1) p=0.28
6MWT (meters)			
Relative change (%) from baseline	1.0 (-4.5, 6.6)	-0.6 (-7.4, 6.2)	1.6 (-7.2, 10.4) p=0.71
Absolute change from baseline	3.7 (-20.3, 27.8)	-3.6 (-33.1, 25.9)	7.4 (-30.8, 45.5) p=0.69

Source: The absolute changes from baseline were taken from rhLAMAN-05 Clinical Study Report, Version 2.0, March 23, 2016, Tables 11-3, 11-7, 11-11, 11-15. The statistical review of the changes from baseline produced the same results. The relative changes from baseline were derived by the review team using non-transformed data. Patients with missing data for the FVC endpoint were excluded in the analysis for this endpoint.
*The Applicant reports that spontaneous decreases in serum oligosaccharides in the placebo arm can be explained by natural fluctuations.
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test CI, confidence interval; FVC, forced vital capacity

Figure 2 presents the mean (95% CI) relative change from baseline by treatment arm for the primary and key secondary endpoints for Weeks 26 and 52. The mean relative change favors velmanase for all four endpoints and at both timepoints, except 6MWT at Week 26. However, the 95% CIs between the velmanase and placebo groups overlap for the clinical outcomes at each time point.

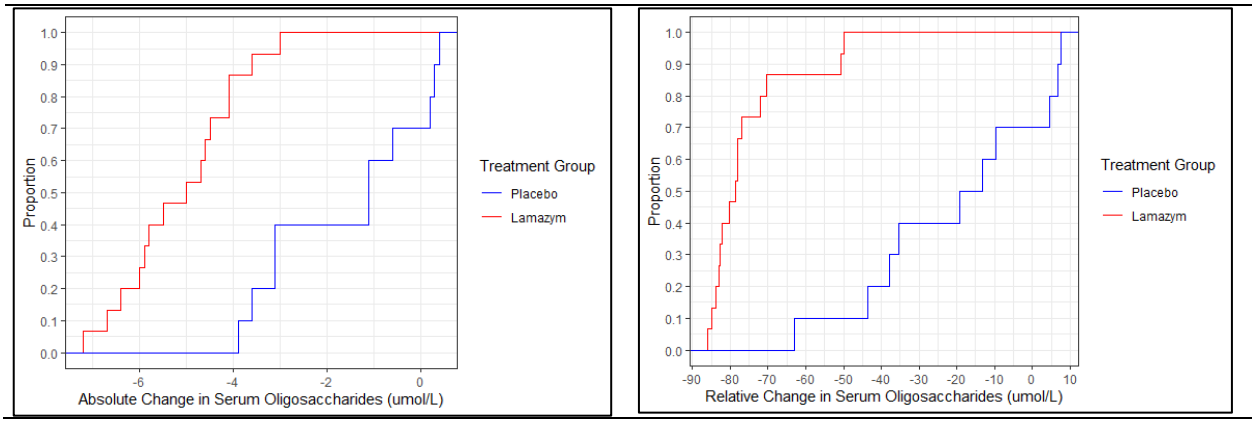
Figure 2. Mean (95% CI) Relative Change From Baseline Over Time, Trial rhLAMAN-05

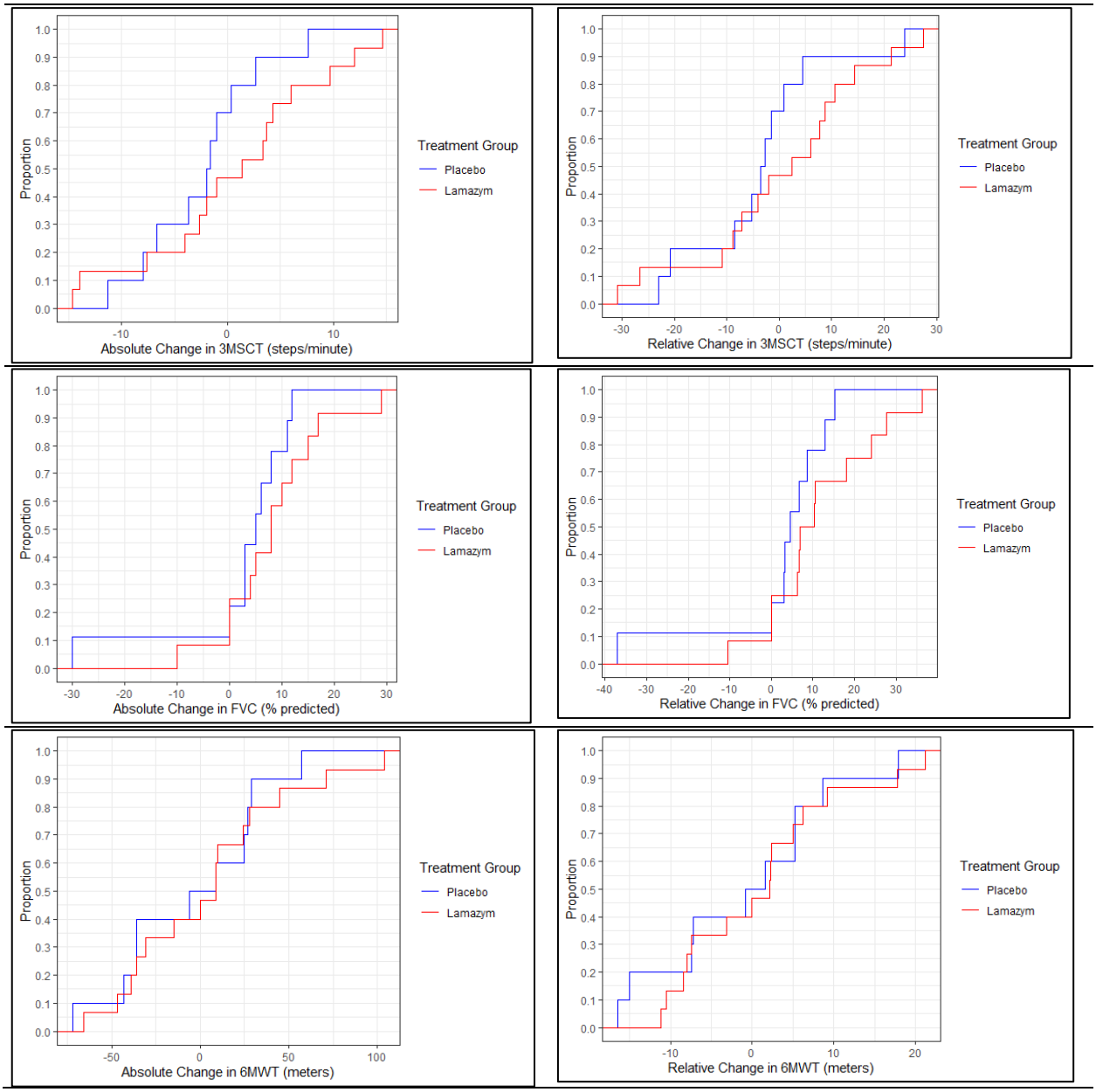


Source: review team's analyses.
Abbreviations: CI, confidence interval

Figure 3 depicts empirical cumulative distribution functions (eCDF) of Week 52 absolute and relative changes from baseline in serum oligosaccharides, 3MSCT, FVC (% predicted), and 6MWT. Each eCDF plot suggests that the velmanase group has a favorable distribution of changes from baseline, compared to the placebo group.

Figure 3. Empirical Cumulative Distribution Functions of Week 52 Changes From Baseline, Trial rhLAMAN-05





Source: review team's analyses.
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; FVC, forced vital capacity

The FVC (% predicted), 3MSCT and 6MWT clinical endpoints detailed above assess clinical outcomes of pulmonary function, ambulation and balance. These clinical outcomes are all highly relevant to patients with AM. A published single arm multicenter longitudinal natural history study (Beck et al. 2013) that included 45 patients with AM (of which 43 patients completed the study) aged 1-42 years old, followed for 24 months, reported baseline deficits in FVC (% predicted) and 6MWT. When compared to published normal values in healthy adults and children, on average adult AM patients' FVC (% predicted) was reduced at 60% of normal and pediatric AM patients' FVC (% predicted) was reduced at 71% of normal. Additionally, over the 24-month observation period, the average FVC (% predicted) decreased from 71% to 61% in pediatric patients but was unchanged in adults.

When compared to published normal values in healthy adults and children, baseline 6MWT in AM pediatric patients is 50-60% of normal and in AM adult patients is 40-45% of normal (Beck et al. 2013). In AM, musculoskeletal malformations and neurological abnormalities are thought to contribute to the reduced 6MWT and reduced pulmonary function(Nir et al. 2020). Sixty-two percent (62%) of the pediatric patients in this natural history study were reported to have abnormal musculoskeletal findings (including contractures, scoliosis, genua valga, hip dysplasia, and feet deformities) and 92% of adult patients were reported to have similar pathological musculoskeletal findings. Pathological neurological findings (including ataxia and dysmetria) were reported in 71% of pediatric patients and 79% of adult patients.

The review team determined that the magnitudes of the changes observed in the FVC (% predicted), 3MSCT and 6MWT clinical endpoints in rhLAMAN-05 were clinically meaningful, as detailed below.

Pulmonary Function

In the pivotal trial (rhLAMAN-05), the estimated treatment effect of velmanase on the placebo-subtracted absolute change from baseline at 52 weeks in FVC% of +5.5% reflects a clinically meaningful improvement for patients. FVC is expressed as a percentage of expected depending on age, size, and sex. FVC% is one of the primary measurements obtained with pulmonary function testing (David 2022). Improved FVC has been interpreted as supportive of Lumizyme (BLA-125291) in improving lung function in Pompe Disease. According to the FDA review of this BLA (May 2010), the change from baseline in FVC% at 52 weeks was a primary endpoint in the pivotal study that had a mean change from baseline of 1.2% in the Lumizyme group and -2.2% in the placebo group; the treatment difference was 3.4% (95% CI: 1.03, 5.77).

Within the literature describing pulmonary fibrosis, small changes of FVC (2-6% absolute change in % predicted) are associated with clinically relevant changes in disease status and changes of 5 to 10% over a 24-week period are highly predictive of mortality during the subsequent 1-year period (Behr 2011). Additionally, since the FDA agreed that a categorical reduction of FVC of ≥ 10% is clinically meaningful and associated with increased mortality(Loveman et al. 2015), an improvement of 10% with treatment could reasonably be expected to provide clinically meaningful benefit. In Trial rhLAMAN-05, 12 patients randomized to velmanase, and 9 patients randomized to placebo had non-missing percent change from baseline in FVC at Week 52. Of those, 6 (50%) in the velmanase group compared to 2 (22%) in the placebo group had an increased percent change from baseline in FVC of 10% or more at Week 52.

In summary, the observed magnitude of improvement in FVC in the pivotal trial is clinically meaningful.

Ambulatory and Balance Function

The improvements in the 3MSCT and 6MWT clinical endpoints favoring velmanase in Trial rhLAMAN-05 are evidence of clinical benefit across the range of symptoms that patients with AM experience. These endpoints are clinically relevant, as patients with AM and their caregivers report that the abilities to ambulate independently and maintain balance are of paramount

importance in their lives (Adam et al. 2019; Verrecchia et al. 2021). The 3MSCT and 6MWT incorporate aspects of ambulation and balance impacted by AM.

- The 3MSCT is a functional measure of a commonly performed activity in daily living of motor function that incorporates multiple systems including musculoskeletal, neurological, and cardiorespiratory systems (Nightingale et al. 2014) and functionally is related to standing balance (Bean et al. 2007). Motor function is impaired in AM secondary to joint abnormalities, metabolic myopathy, and difficulty with fine motor and muscular coordination (Malm D and Nilssen 2001). In approved BLA 125117 (Naglazyme for mucopolysaccharidosis VI, an improvement of 7 stairs/minute on the 3MSCT in the treatment group compared to placebo was considered a clinically meaningful improvement in endurance. In rhLAMAN-05, the estimated treatment effect of velmanase on the placebo-subtracted absolute change from baseline at 52 weeks was +2.7 steps/min.
- The 6MWT evaluates the global and integrated responses of all the systems (cardiovascular, pulmonary, circulation, and neuromuscular) involved during a submaximal level of functional capacity that is reflective of most activities of daily living (American Thoracic Society 2002). Improved 6MWT has been interpreted as supportive of Nexviazyme (BLA-761194) in improving walking distance in patients with Pompe Disease. Heart failure literature reports that a change of 2 m (increasing from approximately 30 m to 32 m is clinically meaningful(Shoemaker et al. 2013). In rhLAMAN-05, the estimated treatment effect of velmanase on the placebo-subtracted absolute change from baseline at 52 weeks was +7.4 meters.

In summary, compared to placebo, AM patients in the velmanase group improved on several clinical endpoints (FVC, 3MSCT, 6MWT) relevant to the disease manifestations and patient experience.

FDA's Post-Hoc Analyses

Given the heterogenous clinical manifestations of patients with alpha-mannosidosis (in severity, affected organ systems, and disease progression), an individual clinical endpoint (3MSCT, FVC%, and 6MWT) may not be able to capture the overall treatment effect of velmanase. To address this limitation, the review team conducted post-hoc analyses for the composite endpoint defined as the maximum percent change from baseline at 52 weeks in 3MSCT, FVC%, and 6MWT. This composite endpoint captures the best outcome of the three clinical endpoints for each subject. As presented in Table 9, the analysis results of the best outcome numerically favor velmanase: the estimated mean treatment difference ranges from 6% to 7% and the p-value for testing the treatment difference ranges from 0.08 to 0.14. In addition, the analysis results of the worst outcome also numerically favor velmanase. Therefore, favorable trends seen for both ends of the clinical response spectrum suggest velmanase improving the best outcome did not have a detrimental effect on the worst outcome.

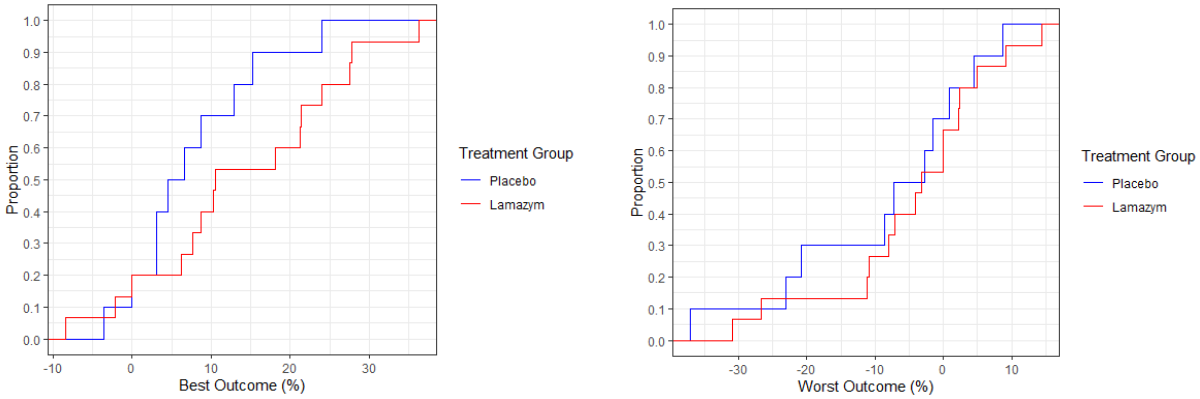
Table 10. Analyses for the Maximum and Minimum Percent Changes at 52 Weeks in 3MSCT, FVC%, and 6MWT, Trial rhLAMAN-05

	Velmanase	Placebo	Treatment Difference (95% CI)
Mean for the best outcome: maximum percent change from baseline at 52 weeks			
Un-adjusted analysis	14.1	7.5	6.6 (-2.3, 15.3); p-value=0.14
ANCOVA adjusted for baseline age as continuous variable	13.8	7.8	6.0 (-2.4, 14.1); p-value=0.16
ANCOVA adjusted for baseline age as categorical variable	14.5	7.5	7.0 (-0.9, 14.7); p-value=0.08
Mean for the worst outcome: minimum percent change from baseline at 52 weeks			
Un-adjusted analysis	-5.3	-8.8	3.5 (-6.2, 13.5); p-value=0.52
ANCOVA adjusted for baseline age as a continuous variable	-5.5	-8.5	3.0 (-6.5, 13.2); p-value=0.56
ANCOVA adjusted for baseline age as categorical variable	-5.1	-8.8	3.7 (-5.9, 13.9); p-value=0.49

Source: review team's analyses.
Mean: least square mean for adjusted analysis. Categorical age: <18 years vs. ≥18 years. The 95% CIs and nominal p-values were obtained using a permutation method.
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; ANCOVA, analysis of covariance; CI, confidence interval; FVC, forced vital capacity

Additionally, the empirical cumulative distribution functions (eCDF) by treatment group for the best and worst outcomes at Week 52, shown in Figure 4, indicate that the group-level distribution of both best and worst outcomes favors the velmanase treatment group over the placebo group.

Figure 4. Empirical Cumulative Distribution Functions of Best and Worst Outcomes (%) at Week 52, Trial rhLAMAN-05



Source: Review team's analysis.

Furthermore, the review team conducted several global tests for the three clinical endpoints, percent changes from baseline at week 52 in 3MSCT, FVC%, and 6MWT. These global tests focus on testing a global null hypothesis of no treatment effect on any of the three endpoints. As presented in Table 10, the p-values range from 0.16 to 0.24 for the global tests adjusted for the categorical age, whereas the p-values range from 0.18 to 0.37 for the global tests adjusted for the continuous age.

Table 11. Nominal P-Values of Global Tests for Three Endpoints: Percent Changes From Baseline at 52 Weeks in 3MSCT, FVC%, And 6MWT, Trial rhLAMAN-05

Global Tests Using Permutation Method	Unadjusted Analysis	Adjusted Analysis (Continuous Age)	Adjusted Analysis (Categorical Age)
NS-Sum	0.25	0.18	0.16
Test-Statistics-Sum	0.27	0.30	0.20
O'Brien's Rank-Sum	0.45	0.37	0.24

Source: Review team's analysis. Categorical age: <18 years vs. ≥18 years.
NS-Sum: performed a permutation test on the sum of the normalized outcomes of the three clinical endpoints.
O'Brien's Rank-Sum: performed a permutation test on the sum of the ranks of the three clinical endpoints.
Test-Statistics-Sum: performed a permutation test on the sum of the t-test statistics for the three clinical endpoints.
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; FVC, forced vital capacity

Subgroup Analyses by Age

The analysis results for the pediatric patients (age <18 years) and adult patients (age ≥18 years) are presented in Section 16.3. While the 95% CIs for these two subgroups overlap for the treatment difference for all endpoints, numerically larger point estimations were observed in the pediatric group for serum oligosaccharides, 3MSCT, FVC, and the best outcome endpoint.

Conclusion

Considering the rarity, considerable clinical heterogeneity, and slowly progressive nature of the disease, and the small sample size and short duration of Trial rhLAMAN-05 relative to disease progression, the review team concludes that the consistency of improved clinical outcomes (3MSCT, 6MWT, FVC%), individually and in composite, supported by the statistically significant reduction in serum oligosaccharides as evidence of drug effect, provided evidence of efficacy of velmanase in AM patients 6 years and older.

6.2.2. rhLAMAN-10 Integrated Analysis

6.2.2.1. Design, Eligibility Criteria, Statistical Analysis Plan

Design

The rhLAMAN-10 Integrated Analysis pooled the cumulative databases for all previous velmanase trials. In total, 33 patients who were still receiving weekly infusions of velmanase according to the AfterCare Program (an open label extension program consisting of patients who had been treated with velmanase in previous trials) were included. This integrated analysis also included efficacy endpoints collected in Trial rhLAMAN-10, a small separate study that included a single efficacy assessment (for details of Trial rhLAMAN-10, refer to Section 16.3).

Key Efficacy Assessments of rhLAMAN-10 Integrated Analysis included the following:

Primary Efficacy Endpoint

- Change from Baseline in serum oligosaccharides
- Change from Baseline in the 3MSCT

Key Secondary Efficacy Endpoints

- 6MWT – The best value from 2 performances was used.
- Pulmonary function testing: FVC (% predicted), FVC (L), forced expiratory volume in the first second (% of predicted), forced expiratory volume in the first second (L), and peak expiratory flow rate (L/s)

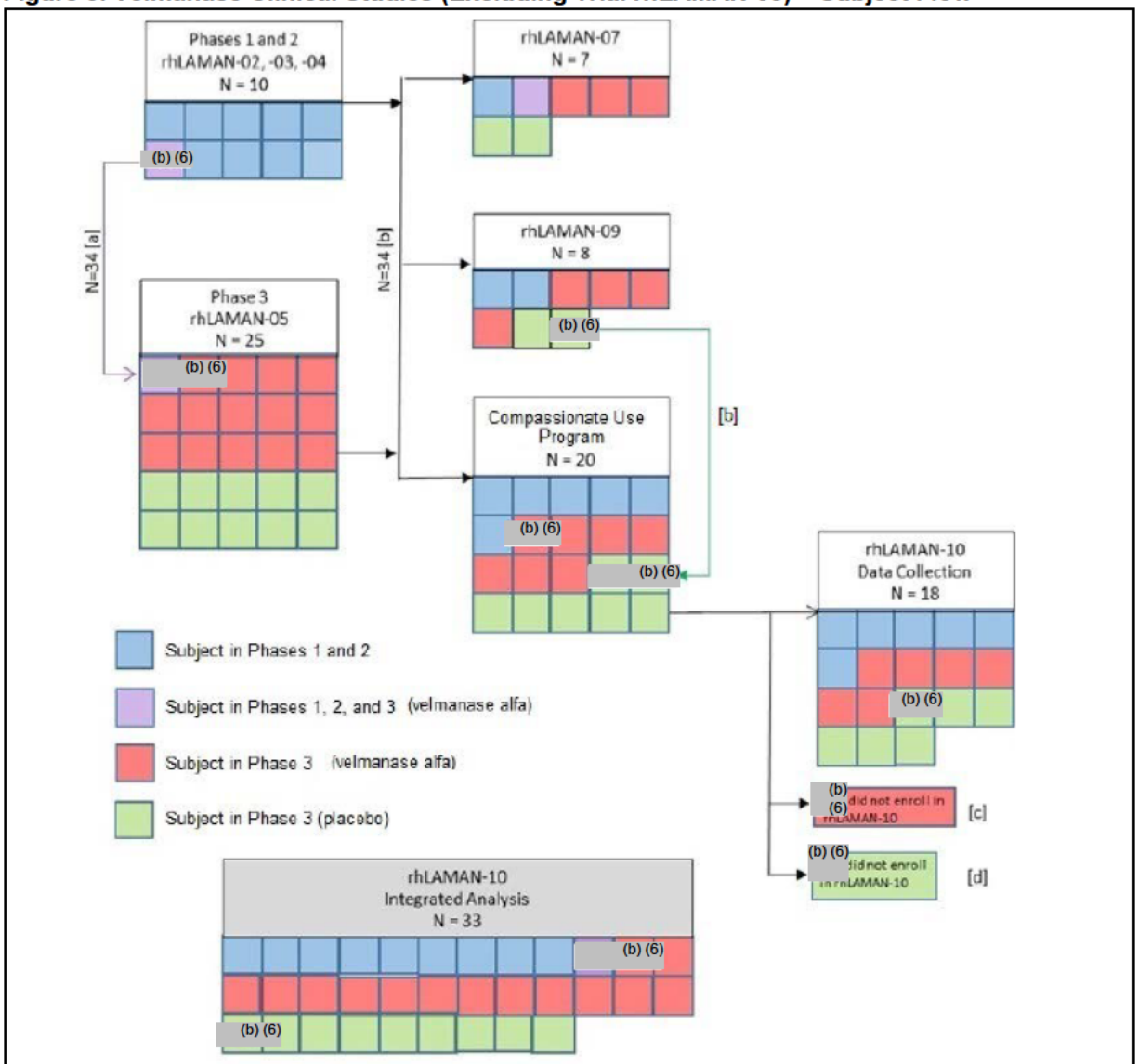
Eligibility Criteria

Other than prior enrollment in a velmanase trial, there was no specific eligibility criteria for the rhLAMAN-10 Integrated Analysis.

Flow Of Patients Into the rhLAMAN-10 Integrated Analysis

- The data from 18 patients (from Trial rhLAMAN-10) were combined with data from 7 patients who were currently in enrolled in Trial rhLAMAN-07, and 8 patients currently enrolled in Trial rhLAMAN-09, for a total of 33 patients (aged 6 to 35 years old). Trials rhLAMAN-07 and rhLAMAN-09 are open label extension studies to rhLAMAN-05. Of note, rhLAMAN-02 (phase 1 trial) was the parental study for 9 of these patients and rhLAMAN-05 was the parental study for 24 patients. All 33 patients in the pooled analysis were still receiving weekly infusion of velmanase according to the AfterCare Program.
- The flow of patients into the rhLAMAN-10 Integrated Analysis is illustrated in the Applicant-supplied Figure 5 below.

Figure 5. Velmanase Clinical Studies (Excluding Trial rhLAMAN-08) – Subject Flow



Abbreviations: CEV: Comprehensive Evaluation Visit; IRR: Infusion-related reaction; N: Number of subjects.

a. Subject (b) (6) 1 subject participated in rhLAMAN-02 and rhLAMAN-03 as Subject (b) (6) and later was included in rhLAMAN-05 as Subject (b) (6) This subject was counted only once.
b. Subject (b) (6) received 4 doses of velmanase alfa in rhLAMAN-09 and then transferred to the Compassionate Use Program before being enrolled in rhLAMAN-10. The CEV for this subject was conducted under rhLAMAN-10.
c. Subject (b) (6) participated in rhLAMAN-05 in the velmanase alfa group. After completing rhLAMAN-05, the subject received velmanase alfa in the Compassionate Use Program but did not participate in rhLAMAN-10. Since the subject received velmanase alfa in rhLAMAN-05 for 12 months, data from this subject have been included in the integrated analysis.
d. Subject (b) (6) participated in rhLAMAN-05 in the placebo group and then entered the Compassionate Use Program. The subject discontinued the treatment shortly after starting the Compassionate Use Program due to IRRs. As this subject had no data collected during the active treatment, the subject was excluded from the integrated analysis.

Source: rhLAMAN-10 Listing 16.2.1.

Source: rhLAMAN-10 Listing 16.2.1

rHLAMAN-10 Integrated Analysis Statistical Analysis Plan

The rhLAMAN-10 Integrated Analysis pools data from patients enrolled in Trial rhLAMAN-10 with patients who were enrolled in Trials rhLAMAN-07 and rhLAMAN-09. At the time of the integrated analysis, all patients had been receiving velmanase 1 mg/kg for at least one year.

The baseline value for all patients is defined as the last non-missing value before the first dose of velmanase in the parental study, either Trial rhLAMAN-02 or rhLAMAN-05. For patients randomized to placebo in Trial rhLAMAN-05, the baseline value is defined as the last non-missing value before switching to velmanase after completion of the trial. The last observation is defined as the last available value at the end of rhLAMAN trials, either Trial rhLAMAN-07, rhLAMAN-09, or rhLAMAN-10. For the 3MSCT and 6MWT, the best of two performances will be utilized in the integrated analysis. [NC1] [KJ2]

The integrated analysis did not utilize any formal statistical testing. Results include 95% CI and p-values, derived from paired t-tests, for descriptive purposes only. The primary endpoints were assessed using graphs and summary tables by timepoint as well as by age group (<18 years, ≥18 years) and/or parental Trials (rhLAMAN-02, rhLAMAN-05).

6.2.2.2. Patient Screening, Enrollment, and Disposition

A total of 33 patients are included in this integrated analysis. A total of 34 patients had been enrolled into the various trials in the velmanase development program. The 34 patients utilized 35 patient identifiers. One patient participated in Trials rhLAMAN-02 and rhLAMAN-03 as Patient (b) (6) The patient discontinued treatment in Trial rhLAMAN-03 due to AE (chills and increase in temperature). This patient was subsequently withdrawn from Trial rhLAMAN-03. This patient was later enrolled in Trial rhLAMAN-05 and assigned patient number (b) (6) This patient will only be counted once in the rhLAMAN-10 Integrated Analysis.

One patient, Patient (b) (6) was randomized to the placebo arm in Trial rhLAMAN-05. The patient was enrolled in the AfterCare program to receive velmanase but discontinued treatment shortly after starting the program. No data were collected from this patient during treatment with velmanase. Therefore, the patient has been excluded from the analysis.

6.2.2.3. Demographics and Baseline Characteristics

The following Table 11 provides the demographics and baseline characteristics for the patients in the rhLAMAN-10 Integrated Analysis.

Table 12. Baseline Demographics and Clinical Characteristics, Safety Population, rhLAMAN-10 Integrated Analysis

Characteristic	Pediatric Population (<18 Years of Age) 19 (58)	Adult Population (≥18 Years of Age) 14 (42)	Total Population 33 (100)
Sex, n (%)			
Male	13 (68)	7 (50)	20 (61)
Female	6 (32)	7 (50)	13 (39)
Age, years			
Mean (SD)	11.6 (3.7)	24.6 (5.3)	17.1 (7.8)
Median (min, max)	12 (6, 17)	22.5 (18, 35)	15 (6, 35)
Race, n (%)			
White	19 (100)	14 (100)	33 (100)

Characteristic	Pediatric Population (<18 Years of Age) 19 (58)	Adult Population (≥18 Years of Age) 14 (42)	Total Population 33 (100)
Country of participation, n (%)			
United States	0 (0)	0 (0)	0 (0)
United Kingdom	0 (0)	1 (7)	1 (3)
Poland	3 (16)	2 (14)	5 (15)
Spain	1 (5)	2 (14)	3 (9)
Germany	1 (5)	4 (29)	5 (15)
France	4 (21)	3 (21)	7 (21)
Other or Missing	10 (53)	2 (14)	12 (36)
Serum oligosaccharides (umol/L)			
Mean (SD)	7.6 (2.5)	5.9 (1.5)	6.9 (2.3)
Median (min, max)	7.7 (4.6, 15.0)	6.3 (2.3, 7.8)	7 (2.3, 15.0)
Alphamanosidase activity (nmol/h/mg)			
Mean (SD)	12.3 (5.2)	12.5 (3.5)	12.4 (4.5)
Median (min, max)	10.5 (7.6, 29.0)	12.1 (8.4, 22.2)	11.5 (7.6, 29.0)
Alphamanosidase activity (relative) (%)			
Mean (SD)	4.5 (1.9)	4.6 (1.3)	4.5 (1.6)
Median (min, max)	3.8 (2.8, 10.6)	4.4 (3.1, 8.1)	4.2 (2.8, 10.6)

Source:
review team's analyses.
NOTE: One patient, Patient (b) (6), was included using baseline data from Trial rhLAMAN-05, rather than treatment-naïve baseline data from Trial rhLAMAN-02.
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients with given characteristic; SD, standard deviation

6.2.2.4. Statistical Analysis Plan

As previously stated in Section 6.2.2.1 the rhLAMAN-10 Integrated Analysis utilizes no formal statistical testing. Refer to Section 6.2.2.1 for further details.

6.2.2.5. Results of Analyses

The efficacy data from rhLAMAN-10 Integrated Analysis suggest continued reductions in serum oligosaccharides and trends of improvement or stabilization from baseline on the 3MSCT, 6MWT, and FVC% over time. Table 12 contains results of the analysis of serum oligosaccharides over time. The results suggest sustained reduction of serum oligosaccharides, on average, over time. While Month 36 shows an increase in the average observed serum oligosaccharides compared to the previous timepoint, Month 24, the results at Month 48 indicate a similar reduction in serum oligosaccharides as observed at Month 24. It should be noted there are considerable missing data beyond Month 12 for the efficacy endpoints in the rhLAMAN-10 Integrated Analysis.

Table 13. Observed Results for the Change From Baseline in Serum Oligosaccharides Over Time, rhLAMAN-10 Integrated Analysis

	Observed		Absolute Change From Baseline (umol/L)		Relative Change From Baseline (%)	
Visit	N	Mean (SD)	Mean (SD)	95% CI	Mean (SD)	95% CI
Baseline	33	6.9 (2.3)	-	-	-	-
Month 6	24	2.6 (1.0)	-5.0 (2.3)	-6.0, -4.0	-64.1 (14.9)	-70.4, -57.8
Month 12	31	1.6 (1.1)	-5.4 (2.9)	-6.5, -4.4	-72.7 (23.5)	-81.4, -64.1
Month 18	11	1.6 (1.6)	-6.7 (3.8)	-9.3, -4.1	-76.0 (31.2)	-97.0, -55.0
Month 24	10	1.5 (0.6)	-5.1 (1.1)	-5.9, -4.3	-77.7 (9.3)	-84.3, -71.1
Month 36	3	6.2 (5.5)	-0.4 (4.2)	-10.8, 10.0	-13.6 (59.2)	-160.6, 133.4
Month 48	9	1.6 (0.9)	-7.4 (2.8)	-9.6, -5.3	-81.8 (11.7)	-90.7, -72.8

Source:
review team's analyses.

Abbreviations: CI, confidence interval; N, number of subjects; SD, standard deviation

Refer to Section 16.3.3 of the review for more details of the results for 3MSCT, 6MWT, and FVC over time.

6.2.3. Trial rhLAMAN-08

6.2.3.1. Design and Eligibility Criteria

Design

Trial rhLAMAN-08 was an open label phase 2 trial conducted in 7 centers in 5 countries in treatment naïve patients confirmed to have AM and aged <6 years of age. The youngest patient enrolled was 3 years old. The main objectives were to evaluate the safety and tolerability of velmanase in pediatric patients with AM aged <6 years old and detect anti-velmanase immunoglobulin antibodies (anti-drug antibodies (ADAs)). A total of 5 patients (aged 3 to 5 years old) enrolled. Velmanase was administered as an IV infusion, 1 mg/kg body weight weekly for 24 months. A single patient residing in (b) (6) remained on treatment in this study for 40 months because he did not have a local option for commercial treatment after 24 months. At the end of the trial, all patients began treatment in a clinical setting, with the exception of the aforementioned patient from (b) (6), who was instead treated in the rhLAMAN-07 clinical trial.

Primary Endpoints

- No primary efficacy analysis was defined.

Key Secondary Efficacy Endpoints

- Serum oligosaccharides measured at baseline and weeks 26, 52, 78, 104, and for a single patient week 166.
- 3MSCT measured at baseline and weeks 26, 52, 78, 104, and for a single patient week 166.
- Walk test measured at baseline and weeks 26, 52, 78, 104, and for a single patient week 166.:
- 6MWT in pediatric patients from 4 years of age (or when applicable according to the judgment of the Investigator)

- 2-minute walk test in pediatric patients below 4 years of age (or when applicable according to the judgment of the Investigator)

Eligibility Criteria

Inclusion Criteria

- Confirmed diagnosis of AM (defined by alpha-mannosidase activity in leukocytes or fibroblasts <10% of normal activity (historical data)).
- Aged <6 years at the time of screening.

Exclusion Criteria

- No confirmed AM diagnosis
- Presence of known chromosomal abnormality and syndromes (other than AM) affecting psychomotor development
- History of bone marrow transplant
- Cardiovascular, hepatic, pulmonary, or renal disease or other medical conditions that would preclude participation in the trial
- Any other medical condition or serious intercurrent illness, or extenuating circumstance that would preclude participation in the study
- Planned major surgery that, in the opinion of the Investigator, would preclude participation in the study
- Participation in other interventional trials testing the investigational medicinal product within the last 3 months.

6.2.3.2. Patient Screening, Enrollment, and Disposition

According to the study report, a total of 6 patients were screened for the trial. There was 1 screening failure due to due exclusion criteria, not met (patient reported to have number ventricular extrasystoles). Enrolled patients received velmanase IV weekly for 24 months. All 5 patients completed the 24-month study and one patient (CCD-LMZYMAA1-08-(b) (6)) completed 40 months. None of the 5 patients enrolled in the trial discontinued the study drug or the study.

6.2.3.3. Demographics and Baseline Characteristics

The following Table 13 provides the demographics and baseline characteristics for the patients enrolled in Trial rhLAMAN-08. This study, like Trials rhLAMAN-05 and rhLAMAN-10 is a European trial. The reviewed team assessed that using this European data to support velmanase approval in the USA was acceptable (refer to Section 6.2.1.3 for further details).

Table 14. Baseline Demographics and Clinical Characteristics, Trial rhLAMAN-08

Characteristic	Total Population (N=5) n (%)
Sex, n (%)	
Male	3 (60)
Female	2 (40)
Age, years	
Mean (SD)	4.0 (0.7)
Median (min, max)	4 (3, 5)
Age (years), n (%)	
3	1 (20)
4	3 (60)
5	1 (20)
Race, n (%)	n (%)
White	5 (100)
Country of participation, n (%)	
Germany	2 (40)
Austria	1 (20)
France	1 (20)
Italy	1 (20)
Weight (kg)	
Mean (SD)	18.9 (2.5)
Median (min, max)	18.6 (16.2, 22.9)
Serum oligosaccharides (umol/L)	
Mean (SD)	11.6 (3.5)
Median (min, max)	10.0 (8.8, 17.5)
Alphamannosidase activity (relative) (%)	
Mean (SD)	2.0 (3.4)
Median (min, max)	0.1 (0, 8.0)
3MSCT (steps/min)	
N	4*
Mean (SD)	138.8 (30.1)
Median (min, max)	143.5 (100, 168)
6MWT (meters)	
N	4
Mean (SD)	295.8 (68.4)
Median (min, max)	278.5 (235, 391)

Source: Review team's analyses
* NOTE: Patient (b) (6) (age 4 years at baseline) does not have baseline 3MSCT or 6MWT data
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; SD, standard deviation

6.2.3.4. Statistical Analysis Plan

Safety was assessed at every visit, which occurred weekly. However, efficacy assessments took place every six months: at baseline and at Weeks 26, 52, 78, and 104. Serum oligosaccharides was measured during efficacy assessments. In addition, cerebral spinal fluid (CSF) oligosaccharides were measured at Weeks 52 and 104 only. The 3MSCT and 6MWT was administered to pediatric subjects from four years of age or when applicable according to physician judgment. Subjects below four years of age were assessed using the 2-minute walk test, rather than the 6MWT. According to the protocol, additional efficacy evaluations included assessments of functional capacity, hearing, immunological profile, CSF biomarkers, brain MRS/MRI, and quality of life questionnaires.

According to the protocol and SAP, data were to be presented as data listings due to the small sample size of the trial. Otherwise, descriptive statistics would be utilized. Efficacy variables would also be presented by time points and individual profiles by time would be created. The trial did not pre-specify hypothesis testing. Missing data would not be imputed. Finally, the SAP did not define specific methods for safety analyses.

6.2.3.5. Results of Analyses

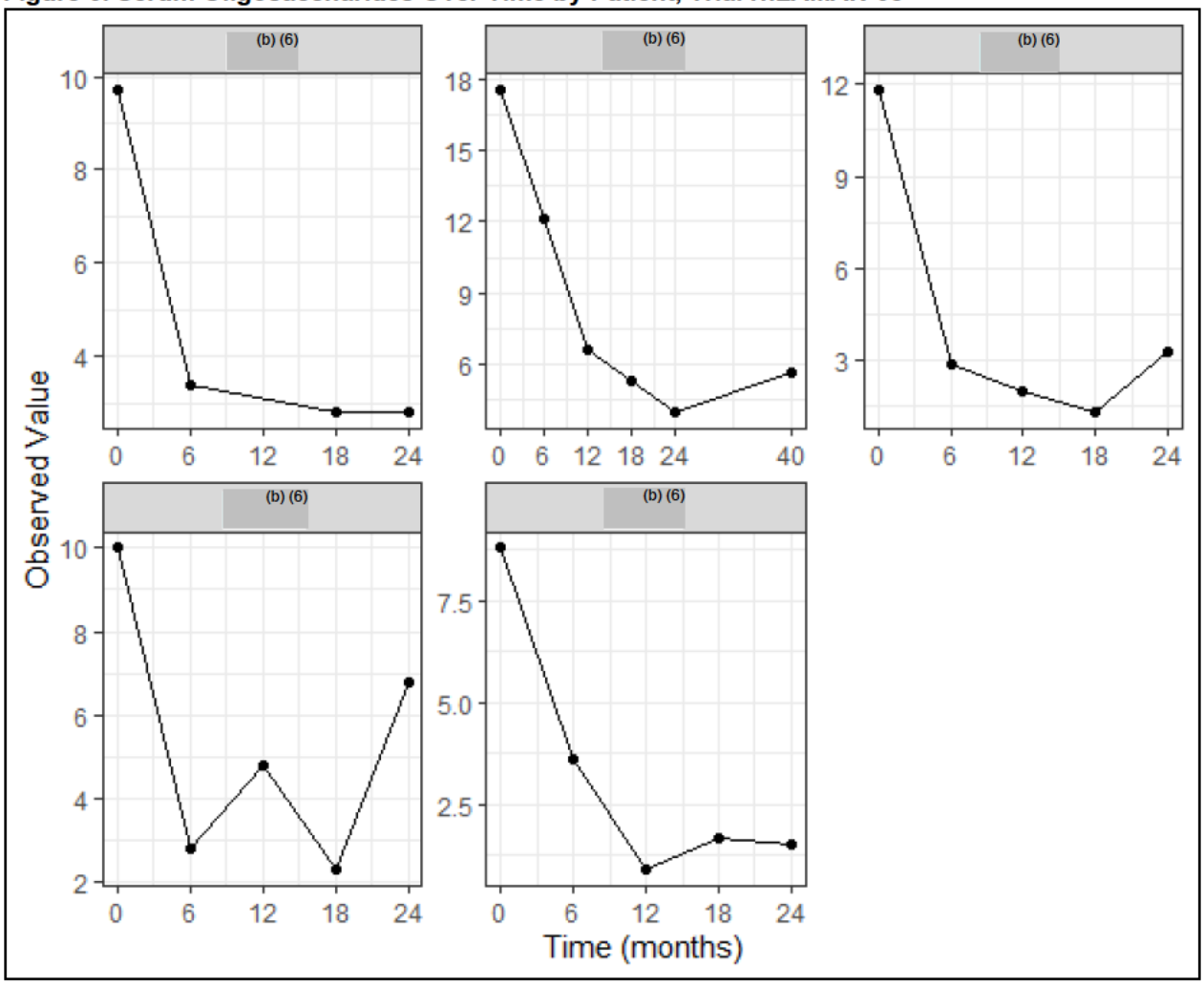
On average, serum oligosaccharides levels declined compared to baseline and the reductions persisted across time (Table 14). The mean percent changes from baseline over time are comparable to the mean percent changes from baseline observed in patients in the rhLAMAN - 10 Integrated Analysis. Individual trajectories (Figure 6) also suggest continued reductions in serum oligosaccharides over time, although the reductions are variable between patients.

Table 15. Observed Results for the Change From Baseline in Serum Oligosaccharides Over Time

Serum Oligosaccharides (umol/L)	Observed		Absolute Change From Baseline (umol/L)		Relative Change From Baseline (%)	
	N	Mean (SD)	Mean (SD)	95% CI	Mean (SD)	95% CI
Baseline	5	11.6 (3.5)	-	-	-	-
Month 6	5	5.0 (4.0)	-6.6 (1.5)	-8.5, -4.7	-60.5 (17.7)	-75.4, -30.9
Month 12	4	3.6 (2.6)	-8.5 (2.5)	-12.4, -4.5	-71.8 (17.6)	-89.8, -52.0
Month 18	5	2.7 (1.6)	-8.9 (2.4)	-11.8, -6.0	-77.5 (7.8)	-89.0, -69.7
Month 24	5	3.7 (2.0)	-7.9 (3.7)	-12.5, -3.3	-67.1 (20.2)	-83.0, -32.0
Month 40	1	5.7 (-)	-11.8 (-)	-	-67.4 (-)	-

Source: Review team's analyses
Abbreviations: CI, confidence interval; SD, standard deviation

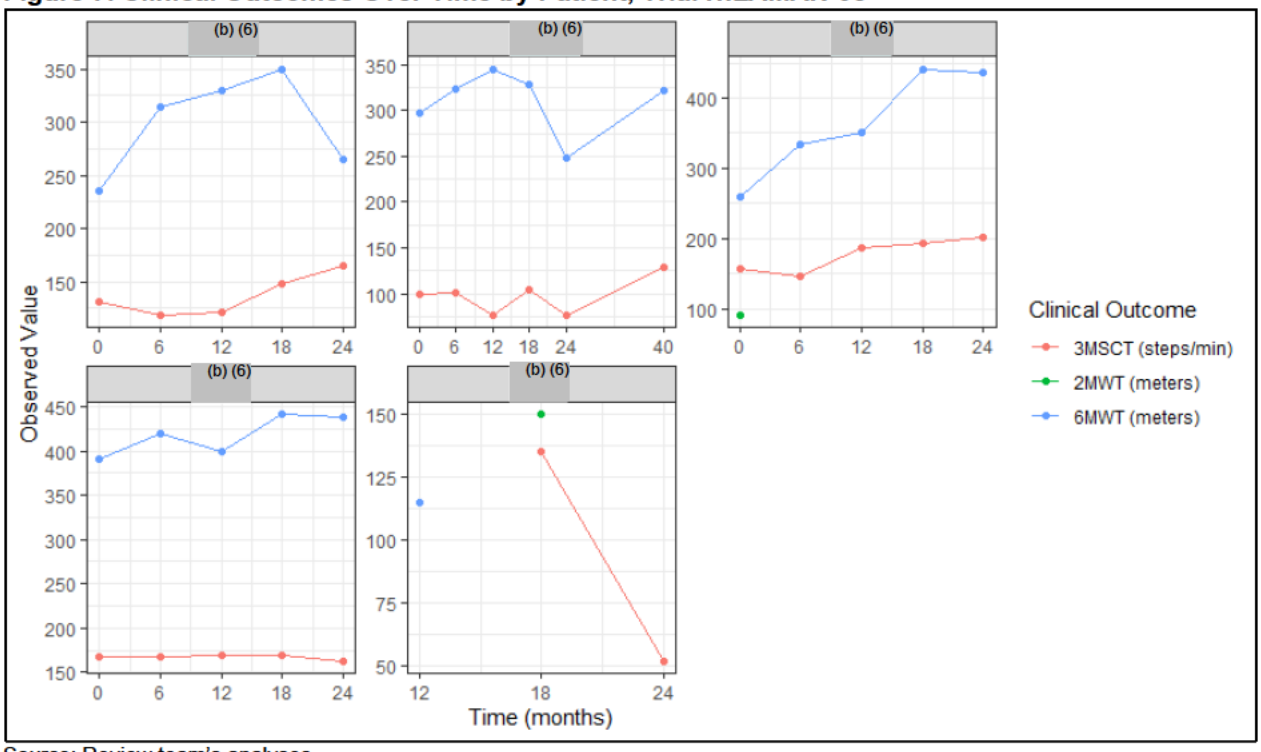
Figure 6. Serum Oligosaccharides Over Time by Patient, Trial rhLAMAN-08



Source: Review team's analyses
NOTE: Patient (b) (6) had an observed value measured at a visit coded as "unscheduled" on analysis day 562. This day is within the Month 18-time window (Days 511-581) and is considered a Month 18 observation. Patient (b) (6) had an observed value at an unscheduled visit on analysis day 779 and does not have an observed value in the Month 24-time window (Days 693-763). The missing Month 24 observation has been imputed as the observed value on analysis day 779.

Individual trajectories of 3MSCT suggest either stabilization or improvement in performance over time (Figure 7). Individual trajectories of 6MWT are more varied, although performance in 6MWT was improved at Month 24 compared to baseline for four of the five subjects. However, due to the limited sample size and the lack of a concurrent control, Trial rhLAMAN-08 cannot provide the primary evidence of efficacy as assessed by the effect of velmanase on performance on the 3MSCT and 6MWT.

Figure 7. Clinical Outcomes Over Time by Patient, Trial rhLAMAN-08



Source: Review team's analyses
Patient (b) (6) had two observed values measured at visits coded as "unscheduled" on analysis days 568 and 569 (6MWT) and 569 and 570 (3MSCT). These days are within the Month 18-time window (Days 511-581) and are considered Month 18 observations. Patient (b) (6)'s Month 18 values were calculated as the mean of the two observations. Patient (b) (6) had two observed values at unscheduled visits on analysis days 771 and 772. Patient (b) (6) does not have an observed value in the Month 24-time window (Days 693-763) for 3MSCT or 6MWT. However, Patient (b) (6) has observed values at unscheduled visits on analysis days 771 and 772. For 3MSCT and 6MWT, the missing Month 24 observation has been imputed as the mean of the observed values on analysis days 771 and 772.
Abbreviations: 2MWT, 2-minute walk test; 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test

6.3. Key Efficacy Review Issues

6.3.1. Establishment of Efficacy in Trial rhLAMAN-05

Issue/Background

The single adequate and well-controlled clinical trial in the velmanase development program, Trial rhLAMAN-05, assessed two primary endpoints, the change from baseline in a biomarker, serum oligosaccharide levels, and the change from baseline in a clinical endpoint, the 3MSCT, as well as two key secondary endpoints, the 6MWT and FVC%. Although the results for the serum oligosaccharide biomarker endpoint were statistically significant, this surrogate biomarker has not been validated for FDA regulatory purposes for AM or any other indication. Although the results for the clinical endpoints (the 3MSCT, the 6MWT and FVC%) were considered clinically meaningful and consistently favored velmanase, they did not reach statistical significance at a p-level of < 0.05. To determine whether the data from Trial rhLAMAN-05 were sufficient to

establish the efficacy of velmanase in the treatment of AM, the review team considered the following (which are discussed in detail in the "Assessment" section below):

- The evidence the Applicant submitted in the BLA to support the use of serum oligosaccharides as a validated surrogate endpoint for velmanase in the treatment of AM
- Whether the degree of uncertainty arising from the lack of statistical significance for the clinical primary and key secondary endpoints is mitigated by other factors and considerations, including the pre-test likelihood of a treatment effect, supportive results from other analyses conducted by FDA, and the unique clinical circumstance of AM as a very rare, serious disease with an unmet therapeutic need.

Assessment

Evidence to Support Serum Oligosaccharides as a Validated Surrogate Endpoint

The Applicant submitted evidence in the BLA to support serum oligosaccharides concentration as a surrogate endpoint that predicts clinical benefit. This evidence included data from the velmanase development program as well as data from published literature. This evidence is described and reviewed in detail in Section 16.1. In summary, the review team determined that the key strengths and key limitations of this evidence are as follows:

Key Strengths

- Oligosaccharides are known to accumulate in tissues where AM causes structural damage and functional impairment (based on published studies and case reports in the literature).
- Because α-mannosidase is functional only at the acidic pH of the lysosome (and not in the serum), a reduction of serum oligosaccharides supports the proposed mechanism of cellular uptake of velmanase into the lysosome to exert its ERT effect (Liao et al. 1996).
- The analyses of correlation between the change in serum oligosaccharides and changes in each of the clinical outcomes in rhLAMAN-10 Integrated Analysis suggest that a decline in serum oligosaccharide may be correlated with an improvement in these clinical outcomes.

Key Limitations

- There is no data on quantitative relationship between serum oligosaccharide levels and tissue oligosaccharide levels in humans or in the animal disease model. Tissue oligosaccharide levels were not assessed in the clinical studies.
- The abovementioned correlation observed between changes in serum oligosaccharides and changes in clinical outcomes was weak, with an absolute value of the correlation co-efficient <0.35. Although this finding may be due to the clinical heterogeneity, small sample size and relatively short duration of evaluation in the study (in relation to the slow progression of the disease), the lack of a demonstrated strong correlation between the biomarker and clinically meaningful outcomes precludes the ability to conclude that the serum oligosaccharide biomarker predicts clinical benefit.

The review team concluded that, although evidence supports that the oligosaccharide biomarker accumulates in tissues and causes disease-specific pathology and serum oligosaccharides

supports velmanase's mechanism of action in the lysosome, there is not sufficient evidence to support a conclusion that serum oligosaccharide level predicts clinical benefit in patients with AM. Therefore, the team determined this biomarker is not a validated surrogate endpoint for the velmanase clinical development program.

Factors and Considerations Regarding Evidence of Efficacy for the Clinical Endpoints

Results of Clinical Endpoints and Analyses in Trial rhLAMAN-05

Trial rhLAMAN-05, the single randomized, placebo-controlled trial, evaluated three key clinical endpoints: 3MSCT, 6MWT, and FVC (%predicted). The absolute and relative changes from baseline did not demonstrate a statistically significant improvement, at a p-level of < 0.05, in any of the three clinical endpoints after 52 weeks of treatment with velmanase. However, the efficacy results for Trial rhLAMAN-05, which can be found in Section 6.2.1.5, show consistently improved performance on all three clinical endpoints with velmanase, compared to placebo, at Week 52. In addition, results of the changes from baseline at Week 26 and Week 52 indicate that, numerically, the treatment effect of velmanase was greater at Week 52 than Week 26. This suggests that the treatment effect of velmanase may increase over time and mitigates the uncertainty of the treatment effect.

The review team also conducted additional analyses to evaluate an overall treatment effect of velmanase, compared to placebo, given AM is a multisystemic disease. FDA's post-hoc analysis of the best outcome, defined as each patient's maximum percent change from baseline at Week 52 of the three clinical outcomes, demonstrated a favorable mean treatment effect for the velmanase group, with a smaller p-value compared to testing each endpoint separately. It also suggests a favorable distribution of best outcomes for the velmanase group, compared to the placebo group. A post-hoc analysis of the worst outcome, defined as each patient's minimum percent change from baseline at Week 52 of the three clinical outcomes, also demonstrated a favorable mean treatment effect for the velmanase group, indicating that improvements on the best outcome did not occur with an adverse effect on the worst outcome. Finally, the review team's post-hoc analyses using global hypothesis testing approaches for the three clinical endpoints also favored the velmanase arm over placebo.

The heterogeneity of the AM disease phenotype, severity, and progression also presents significant noise in the trial population, which decreases the probability of detecting treatment effect, if one exists. In a superiority, placebo-controlled trial, such noise biases towards the null. As such, the consistent findings on several clinical endpoints favoring velmanase in Trial rhLAMAN-05 are unexpected if the disease is left untreated and decreases the uncertainty about velmanase's therapeutic effect.

The results of the change from baseline in serum oligosaccharides in Trial rhLAMAN-05 support the evidence of clinical benefit demonstrated by the clinical endpoints. As summarized above (under "Evidence to Support Serum Oligosaccharides as a Clinically Meaningful Surrogate Endpoint") and as discussed in detail Section 16.1, although the available evidence is not adequate to demonstrate that the serum oligosaccharide biomarker predicts clinical benefit, there is evidence to support that oligosaccharides accumulate in tissues and cause disease-specific pathology, and that a reduction in serum oligosaccharides supports the proposed mechanism of cellular uptake of velmanase into lysosome to exert its ERT effect (since velmanase only functions at the acidic pH of the lysosome). The trial demonstrated a statistically significant reduction in serum oligosaccharides at Week 52 in the velmanase group, compared to the placebo group. This robust evidence of a drug effect on this physiologically relevant, disease specific pharmacodynamic biomarker increases the likelihood that the improvements on the

clinical endpoints observed in rhLAMAN-05 reflect a true treatment benefit and were not spurious.

Potential Contributors to the Observed Lack of Statistical Significance

Given the very limited sample size and slow progression and heterogeneity in the measured clinical outcomes, it would be very difficult for Trial rhLAMAN-05 to produce a statistically significant result for the clinical endpoints. Trial rhLAMAN-05 appears to have been underpowered to demonstrate a statistically significant result, at the conventional $p < 0.05$ statistical significance threshold, on the three clinical endpoints. Assuming the mean and standard deviation of the absolute changes from baseline observed at Week 52 in Trial rhLAMAN-05, it would require approximately 93, 54, and 369 patients to detect a significant treatment effect for 3MSCT, FVC%, and 6MWT, respectively, at a two-sided alpha level of 0.05 (refer to Section 16.2.3 for the absolute mean (SD) changes from baseline for each endpoint). Also, these estimated sample sizes indicate having a new placebo-controlled trial adequately powered for these clinical outcomes for AM is unlikely to be feasible given the rarity of AM and the fact that velmanase is already approved for AM outside the U.S.

Pretest Probability of a Treatment Effect

Another important factor is the strength of the a priori likelihood that velmanase was likely to have an effect on reduction of the pathologic substrate and hence the clinical manifestations.

As detailed in Section 3 (Introduction), AM has a well-understood underlying pathophysiology, as it is characterized by a deficiency of a single enzyme (the lysosomal enzyme alpha-mannosidase, which catalyzes sedation of accumulated mannose-containing oligosaccharides in various tissues). Deficiency of this enzyme leads to the accumulation of mannose-rich oligosaccharides in lysosomes of various tissues, resulting in severe impairment of cellular function. Eventually, lysosomal apoptosis and cell decomposition lead to a release of circulating mannose-rich substrates into the extracellular space and consequently, increased levels of oligosaccharides in the serum.

Velmanase, a recombinant human alpha mannosidase, provides an exogenous source of this enzyme that is deficient in patients with AM. In-vitro data (from cultured cells in healthy humans and AM patients) demonstrate that velmanase is internalized via binding to the mannose-6-phosphate receptor on the cell surface and transported into lysosomes where it exerts the enzyme activity. Animal data from published literature demonstrated a qualitative reduction in vacuolation in the target tissues with velmanase treatment.

Since the underlying pathophysiology of AM is well-understood (i.e., it is caused by a single enzyme deficiency), and since velmanase directly targets this pathophysiology (i.e., by replacing the deficient enzyme), with data to support that it is internalized by cells and reaches its intended target, there is clear biologic plausibility for a treatment effect, which increases the pre-test probability of a treatment effect. Therefore, the improvement in the clinical endpoints observed in rhLAMAN-05 likely reflect a true treatment benefit of velmanase.

Unique Clinical Circumstance/Regulatory Flexibility

Finally, an important factor in the assessment of velmanase's efficacy is the consideration for regulatory flexibility given the unique clinical circumstance of AM. Although there remains a degree of uncertainty regarding the treatment benefit of velmanase in AM arising from the lack of statistical significance for the key clinical endpoints, this degree of uncertainty is small (for the reasons explained above) and is acceptable for a rare, serious disease with no available therapies. In the setting of a rare, serious disease with an unmet that is AM, a higher type 1 error rate is acceptable in order to minimize the risk of a type 2 error. As stated in the FDA draft guidance for industry *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biologic Products* (December 2019), "in certain settings, a somewhat greater risk of false positive conclusions – and therefore less certainty about effectiveness – may be acceptable, when balanced against the risk of rejecting or delaying the marketing of an effective therapy for an unmet medical need. The data supporting effectiveness could, despite the greater risk of error, support a conclusion that there is substantial evidence of effectiveness." Consistent with regulatory requirements, the available data support a conclusion of evidence of effectiveness and the residual uncertainty is clinically appropriate and scientifically sound for the velmanase program in AM for the reasons discussed in this section.

Conclusion

While the possibility of a type 1 error is a concern due to the lack of statistical significance on the key clinical endpoints, the review team has determined that there is sufficient evidence to conclude that velmanase is effective for the treatment of non-CNS manifestations of AM. The review team determined that the uncertainty arising from the lack of statistical significance for the clinical primary endpoint in Trial rhLAMAN-05 was surmounted by several factors as discussed in detail above.

In summary, these factors include (1) the strength of the clinical evidence, including the trial design as a randomized, double-blind, placebo-controlled study and the quality of trial conduct; (2) results from clinical endpoints and various analyses in Trial rhLAMAN-05, with *all* three clinical endpoints showing an improvement favoring velmanase, in the context of high noise biasing towards the null; (3) the following likely contributors to the lack of statistical significance: considerable clinical heterogeneity and slow progression of the disease, and very small sample size; and (4) at least a moderate pre-test likelihood of a treatment effect based on the well-understood underlying pathophysiology of AM (i.e., caused by a single enzyme deficiency), a well-defined MOA and highly targeted therapy (i.e., enzyme replacement, with a general MOA supported by nonclinical data), and evidence indicating velmanase reaches its intended target.

These factors lead to the confidence that the results from Trial rhLAMAN-05 are sufficient to establish the efficacy of velmanase in the treatment of AM, with adequate confirmatory evidence (see below), in the context of the unique clinical circumstance of AM as a very rare, serious disease with an unmet therapeutic need that appropriately warrants regulatory flexibility in establishing substantial evidence of effectiveness.

6.3.2. Adequacy of the Confirmatory Evidence

Issue/Background

Substantial evidence of effectiveness, the regulatory requirement for approval, generally consists of evidence from at least two adequate and well-controlled trials. In certain circumstances, a single adequate and well-controlled trial demonstrating efficacy may be sufficient to generate substantial evidence of effectiveness if there is adequate confirmatory evidence. Such circumstances are described in the FDA draft guidance for industry *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products* (December 2019), which includes the persuasiveness of the single investigation; the robustness of the confirmatory evidence; the seriousness of the disease, particularly when there is an unmet medical need; the size of the patient population; and whether it is ethical and practicable to conduct more than one adequate and well-controlled clinical investigation. These criteria and considerations are all relevant to the velmanase development program for AM, a very rare and serious disease with an unmet need. Because the velmanase BLA has only one adequate and well-controlled trial, we sought to assess whether there is adequate confirmatory evidence to generate substantial evidence of effectiveness.

Assessment

In this BLA, a single adequate and well-controlled clinical trial demonstrating this ERT's efficacy is supported by the following lines of evidence, which together provide confirmatory evidence for the effectiveness of velmanase in AM:

A Well-Understood Disease Pathophysiology, a Clear Mechanism of Action of Velmanase Directly Targeting this Pathophysiology, and Evidence Velmanase Reaches Its Intended Target

The well-understood disease pathophysiology (i.e., the condition is known to be caused by a single enzyme deficiency) and MOA (i.e., a replacement of the deficient enzyme), together with evidence indicating velmanase reaches its intended target, are described in detail in Section 3.

Biomarker Data and Assessment

A. *Serum Oligosaccharides*: Serum oligosaccharide levels are a physiologically relevant, disease-specific pharmacodynamic biomarker that is thought, mechanistically, to reflect tissue levels of this substrate accumulation (for a detailed discussion of the data supporting this point, refer to Section 16.1). Evidence from Trial rhLAMAN-05 demonstrated a statistically significant reduction in serum oligosaccharides with velmanase treatment (for details of these results, refer to Section 6.2.1.5). Furthermore, data from the following additional clinical studies also demonstrated a reduction in serum oligosaccharides from baseline with velmanase treatment:

- In rhLAMAN-03, a phase 2a single center open-label multiple dose trial of the efficacy and long-term safety of the investigational drug in patients with AM, 10 patients (who had previously been enrolled in rhLAMAN-02, a phase 1 trial) were followed for 6 months. In total 9 patients completed this study. The Applicant reports a statically significant decrease in serum oligosaccharides at 6 months (with majority of this decrease observed in the first 3 months) when compared to untreated baseline (established in phase 1 trial, rhLAMAN-02).
- In rhLAMAN-04, a phase 2b multicenter open label trial for long-term efficacy and safety of velmanase in patients with AM, 9 patients (who had previously been enrolled in rhLAMAN-

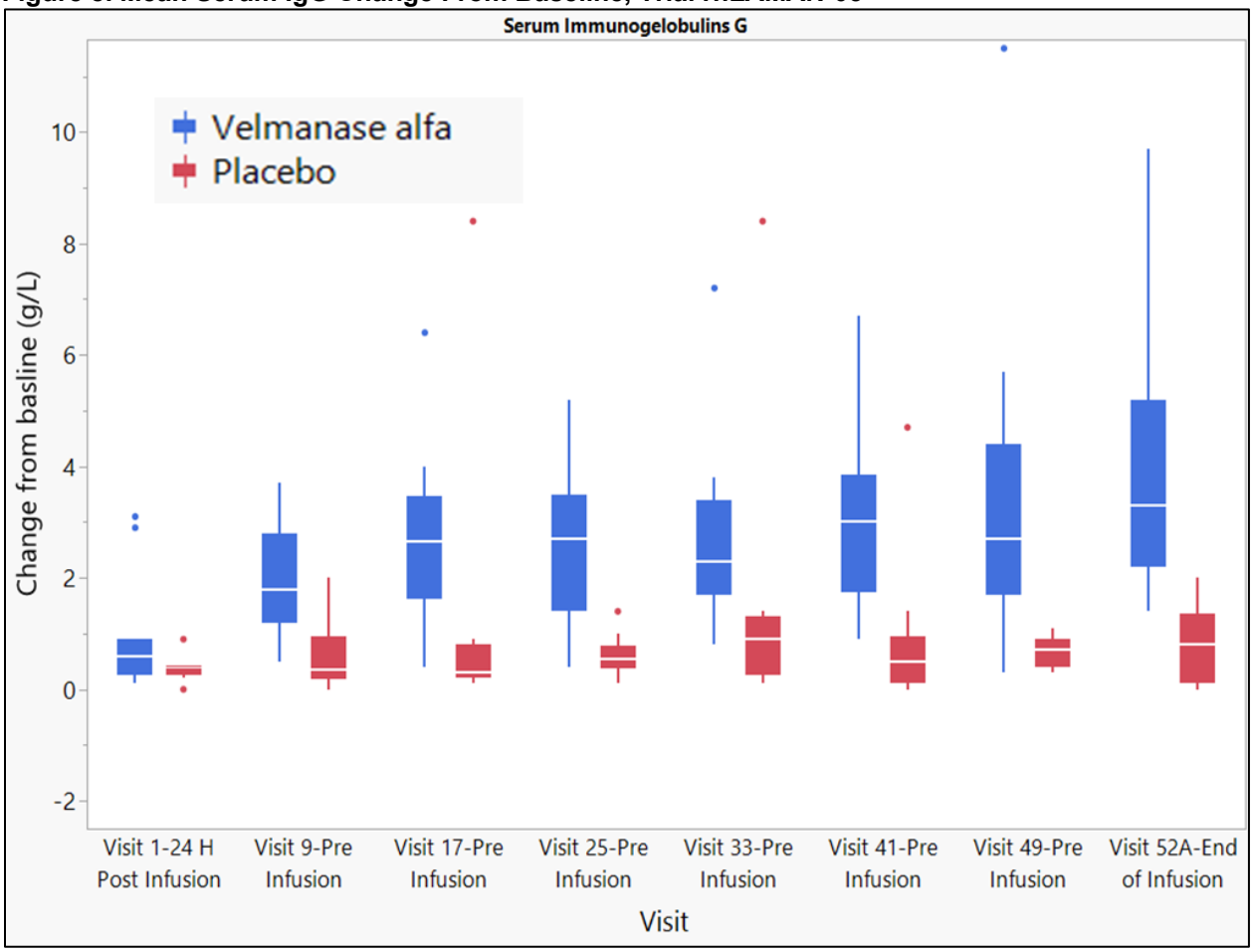
03) were followed for 6 months. The Applicant reports that serum oligosaccharide remained low when compared to baseline ($p<0.001$) (established in phase 1 trial, rhLAMAN-02).

- Additionally, results from Trial rhLAMAN-10 (the integrated analysis of Trials rhLAMAN-03, 04, 05 and their long-term open-label follow up studies) indicated continued reductions in serum oligosaccharides over longer term-follow-up. (For details of these results, refer to Section 6.2.2.5).
- In rhLAMAN-08, an open label phase 2 trial conducted in treatment naïve patients confirmed to have AM and aged <6 years of age, serum oligosaccharides levels declined compared to baseline and the reductions persisted across the 24-month duration of the study. (For details of these results, refer to Section 6.2.3.5).

B. *Serum IgG*: Additional supportive biomarker data include results for serum IgG. Patients with AM may have low serum IgG levels. Treatment with velmanase increased serum IgG levels in patients with AM in Trial rhLAMAN-05 (Figure 8 and Figure 9). The mean (standard deviation (SD)) serum IgG levels at baseline were 9.0 (5.0) and 7.3 (1.6) g/L for the velmanase and the placebo treatment groups, respectively; at Week 52, the mean (SD) serum IgG levels were 12.6 (5.6) and 7.3 (2.1) g/L, respectively. The mean change from baseline to Week 52 was 3.6 (95% CI: 2.8, 4.4) in the velmanase group and 0.1 (95% CI: -0.9, 1.2) in the placebo group, with a statistically significant difference between the two groups ($p<0.001$). All patients in the velmanase treatment group, regardless of baseline IgG levels, had increased serum IgG levels at Week 52 compared to baseline (Figure 9 and refer to Section 14.2.2 for individual patient data). Of note, the increase in serum IgG levels in the velmanase treatment group was not due to IgG ADA because the overall IgG ADA concentrations (<0.1 g/L) accounted for <1% of the total serum IgG concentrations at Week 52 (12.6 g/L)

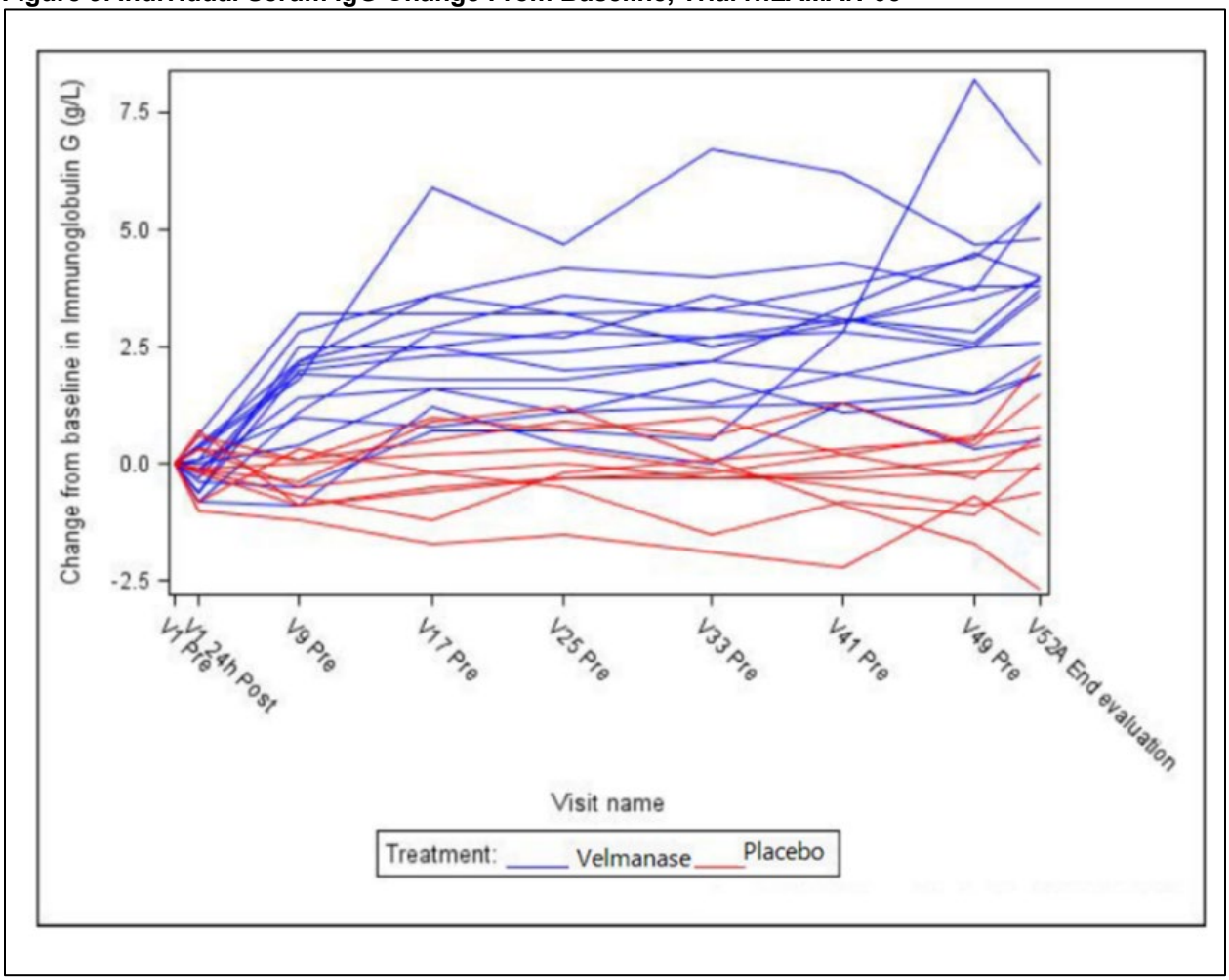
At baseline, three out of ten patients (30%) in the placebo group and five out of fifteen patients (33%) in the velmanase group had serum IgG levels below the normal range. None of the three patients in the placebo group (0%) while three out of the five patients in the velmanase group (60%) achieved serum IgG levels within the normal range by Week 52.

Figure 8. Mean Serum IgG Change From Baseline, Trial rhLAMAN-05



Source: Reviewer's analysis results.
Abbreviations: IgG, immunoglobulin G

Figure 9. Individual Serum IgG Change From Baseline, Trial rhLAMAN-05



Source: Clinical study report – rhLAMAN-05
Abbreviations: IgG, immunoglobulin G

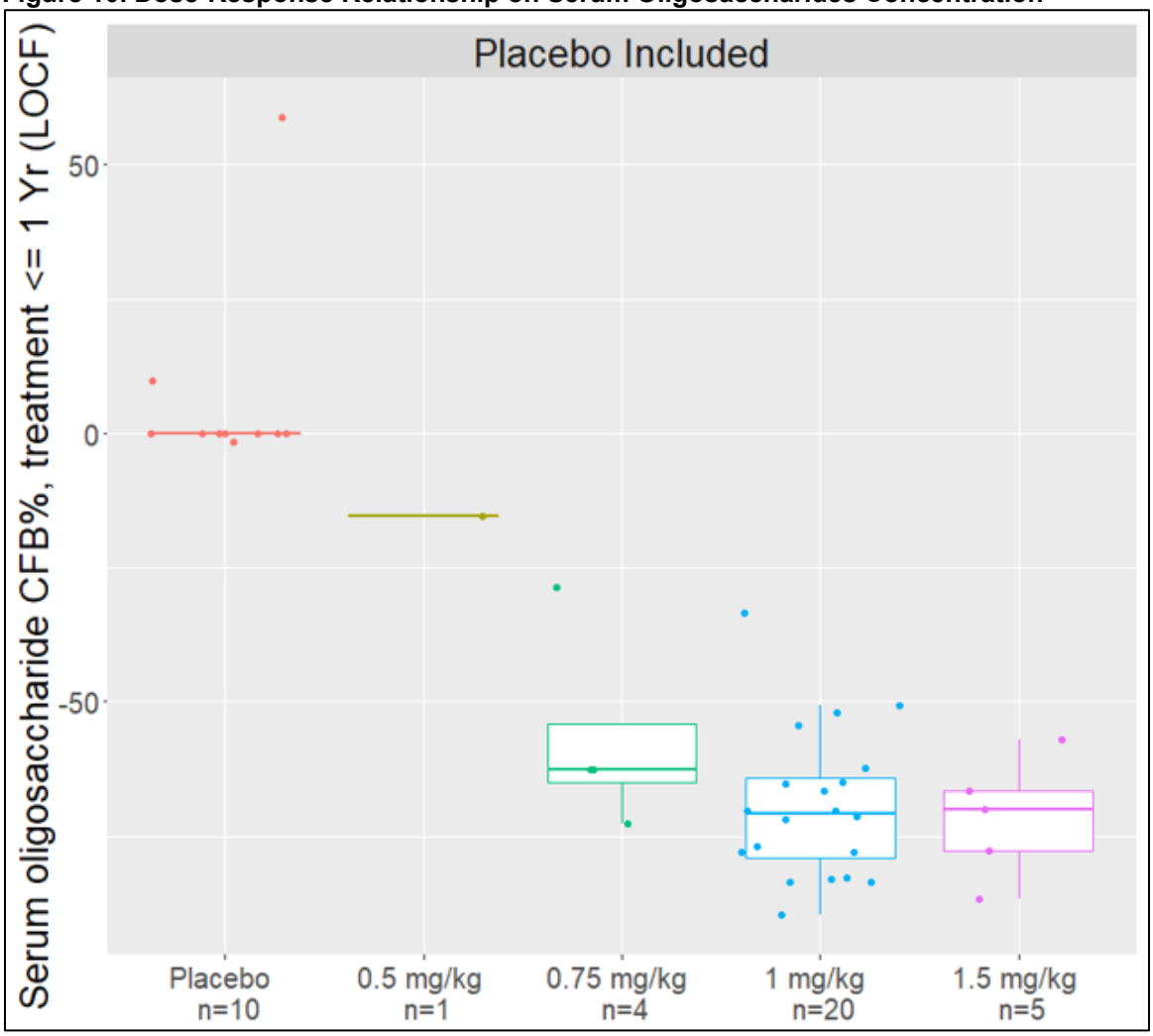
In summary, compared to placebo, velmanase resulted in increased serum IgG concentrations in patients with AM. While the underlying mechanism by which velmanase exerts its effects on serum IgG concentrations is unknown, the observed pharmacological activity on increased serum IgG levels is a distinct measure from that of reduction in serum oligosaccharide. Because reduction in serum oligosaccharide represents a target engagement biomarker (substrate reduction in ERT), the observed pharmacological activity on serum IgG levels is considered a downstream PD response biomarker. Of note, the results of a study by Malm et al (Malm et al. 2000), which compared humoral and cellular immunocompetence in patients with AM to that of healthy subject, showed that patients with AM had decreased production of specific antibodies in response to antigen presentation.

Dose- and Exposure-Response Data and Assessment

When all available data across clinical studies were analyzed, there appeared to be a trend of dose-response on serum oligosaccharides concentrations; a higher dose of velmanase was associated with greater reduction in serum oligosaccharide concentrations (Figure 10). The pharmacokinetics (PK)/PD analysis results also showed a positive E-R relationship between

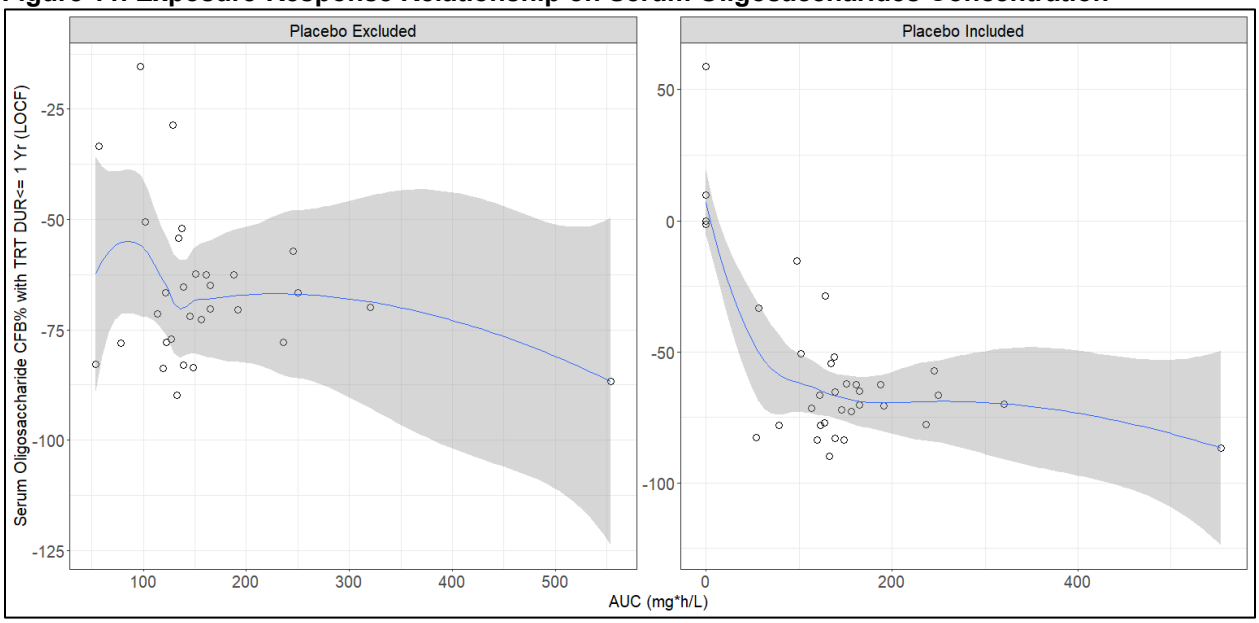
plasma velmanase concentrations and reduction in serum oligosaccharides when subjects in the placebo treatment group were included in the pooled E-R analyses; however, the E-R relationship was weak after removing subjects receiving placebo due to very limited PK/PD data points at the lower doses of 0.5 mg/kg (n=1) and 0.75 mg/kg (n=4) and that the E-R is plateaued at doses greater than 1 mg/kg (Figure 11). The dose-response data overall suggested the 1 mg/kg dose would be adequate to achieve maximal reduction in serum oligosaccharide levels in the general patient population. The sensitivity analysis that excluded data from Trial rhLAMAN 05 also showed a similar trend of E-R relationship (Figure 12). The collective biomarker data on serum oligosaccharide across clinical trials overall showed a positive dose-/E-R relationship, which represent independent analysis results of the biomarker data from the single adequate and well-controlled Trial rhLAMAN-05.

Figure 10. Dose-Response Relationship on Serum Oligosaccharides Concentration



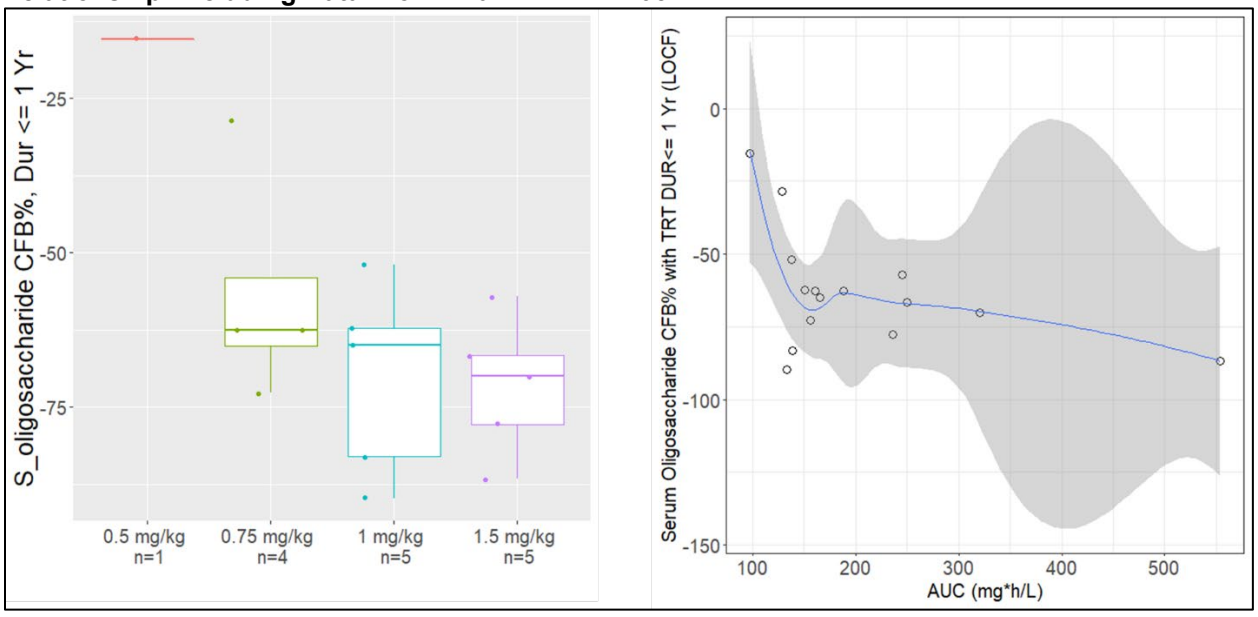
Source: FDA Reviewer's Analysis.
Note: A trend of positive dose-response relationship was observed across velmanase doses ranging from 0.5 mg/kg to 1.5 mg/kg when placebo was included in the analysis. The dose-response relationship overall suggested the 1 mg/kg dose would be adequate to achieve maximal reduction in serum oligosaccharide levels in the general patient population.
Abbreviations: CFB, change from baseline; LOCF, last observation carried forward

Figure 11. Exposure-Response Relationship on Serum Oligosaccharides Concentration



Source: FDA Reviewer's Analysis.
Note: A trend of positive exposure-response relationship was observed when placebo was included in the analysis (right panel); however, the exposure-response relationship was weak when the placebo was excluded in the analysis (left panel).
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; LOCF, last observation carried forward; TRT, treatment

Figure 12. Sensitivity Analysis for Serum Oligosaccharides Dose-/Exposure-Response Relationship Excluding Data From Trial rhLAMAN-05



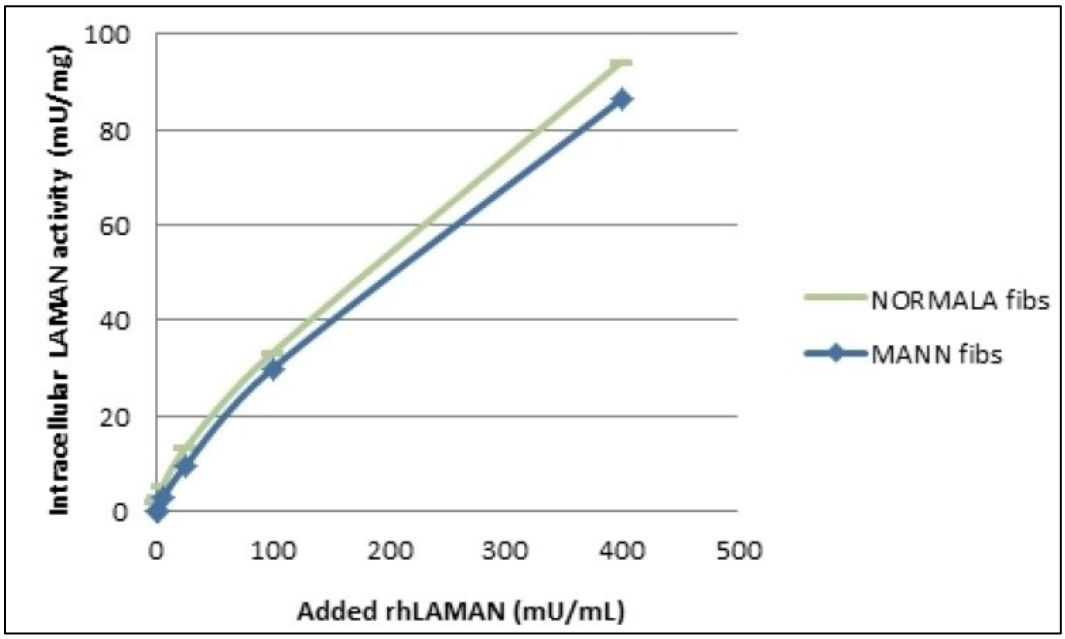
Source: FDA Reviewer's Analysis.
Note: A trend of positive dose-response relationship was observed across velmanase doses ranging from 0.5 mg/kg to 1.5 mg/kg (left panel). A trend of positive exposure-response relationship was also observed when placebo was included in the analysis (right panel).
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; TRT, treatment

Nonclinical Data and Assessment

As detailed below, the nonclinical confirmatory evidence is primarily limited to the observation that, in the 26-week repeat-dose toxicology study in the MAN2B1-deficient mouse (Tg+KO mouse), weekly administration of rhLAMAN at a dose of 1300 U/kg for 26 weeks, led to a reduction in cellular vacuolation in the brain, liver, lymph nodes, and spleen. Neither serum nor urine oligosaccharide content was measured in animals; therefore, nonclinical data to support serum oligosaccharide as a validated biomarker are not available.

Other data included studies to assess cellular uptake, biodistribution in animals, and qualitative effects in tissues, as well as qualitative measures suggestive of functional improvement. In cultured cells from healthy individuals and AM patients, the Applicant demonstrated that exogenous enzyme could be internalized via the mannose-6-phosphate receptor. There was no apparent difference in the extent of uptake in patient-derived cells compared with normal cells (Figure 13) when cultured in the presence of rhLAMAN for 48 hours, but little uptake was observed in cells that lacked the M6P Receptor (data not shown).

Figure 13. Intracellular Uptake of rhLAMAN in Normal Fibroblasts and Fibroblasts Derived From a Patient With Mannosidosis



Source: Excerpted from the Applicant's BLA
Abbreviations: fibs, fibroblasts

In published studies (Stinchi et al. 1999; Roces et al. 2004) conducted in a MAN2B1-deficient mouse model (Tg+KO mice), the levels of carbohydrate accumulation were approximately 2-3x those of wildtype controls in most organs except the liver (in which oligosaccharide levels were approximately 10% higher in the mouse model than in wildtype controls).

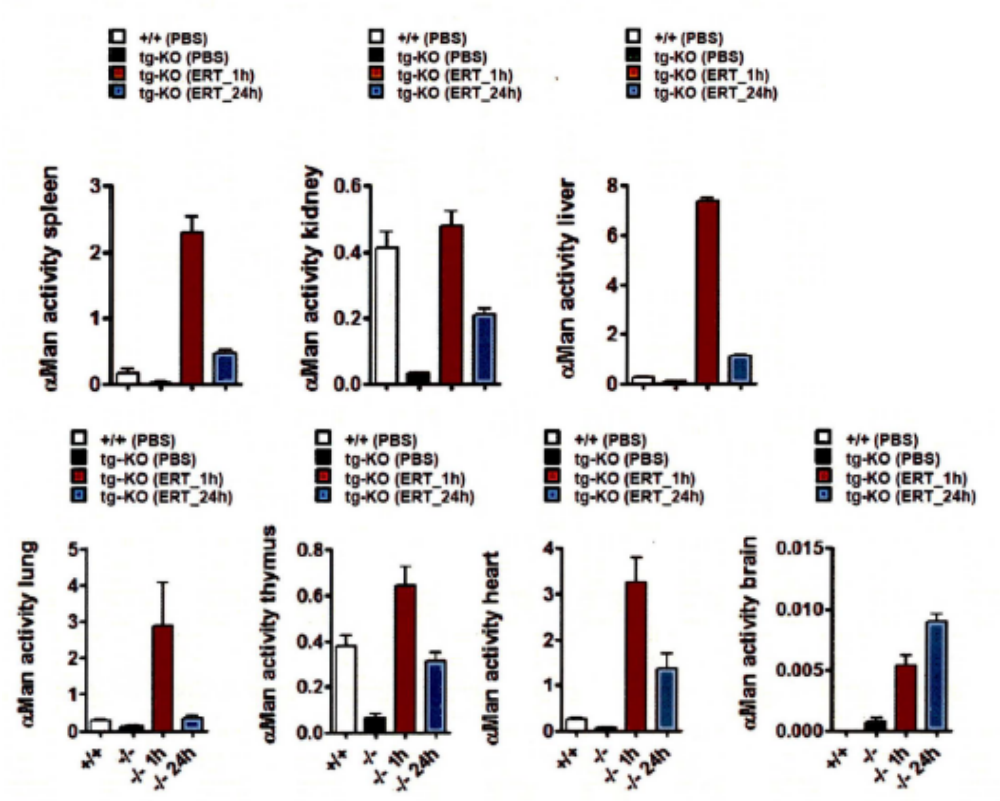
Table 16. Oligosaccharide Content in Tissues of Wildtype and MAN2B1 KO Mice

	Neutral carbohydrate (mg neutral carbohydrate/g wet weight) (± SD)	
Tissue	α-mannosidase$^{+/+}$	α-mannosidase$^{-/-}$
Kidney	0.34 (±0.1)	0.95 (±0.13)
Spleen	0.36 (±0.08)	0.93 (±0.06)
Brain	0.32 (±0.13)	0.71 (±0.08)
Liver	12.22 (±2.91)	13.61 (±2.31)
Testis	0.42 (±0.16)	0.67 (±0.33)

Source: Excerpted from the Applicant's BLA and (Stinchi et al. 1999)
Abbreviations: KO, knockout; SD, standard deviation

Administration of exogenous rhLAMAN led to uptake in the spleen, kidney, liver, lung, thymus, heart, and brain. Uptake was strongest at 1-hour post-dose in all organs but the brain, which showed increased (but low) uptake at 24 hours (Figure 14); Study ERT-36).

Figure 14. Biodistribution of rhLAMAN in KO Mice

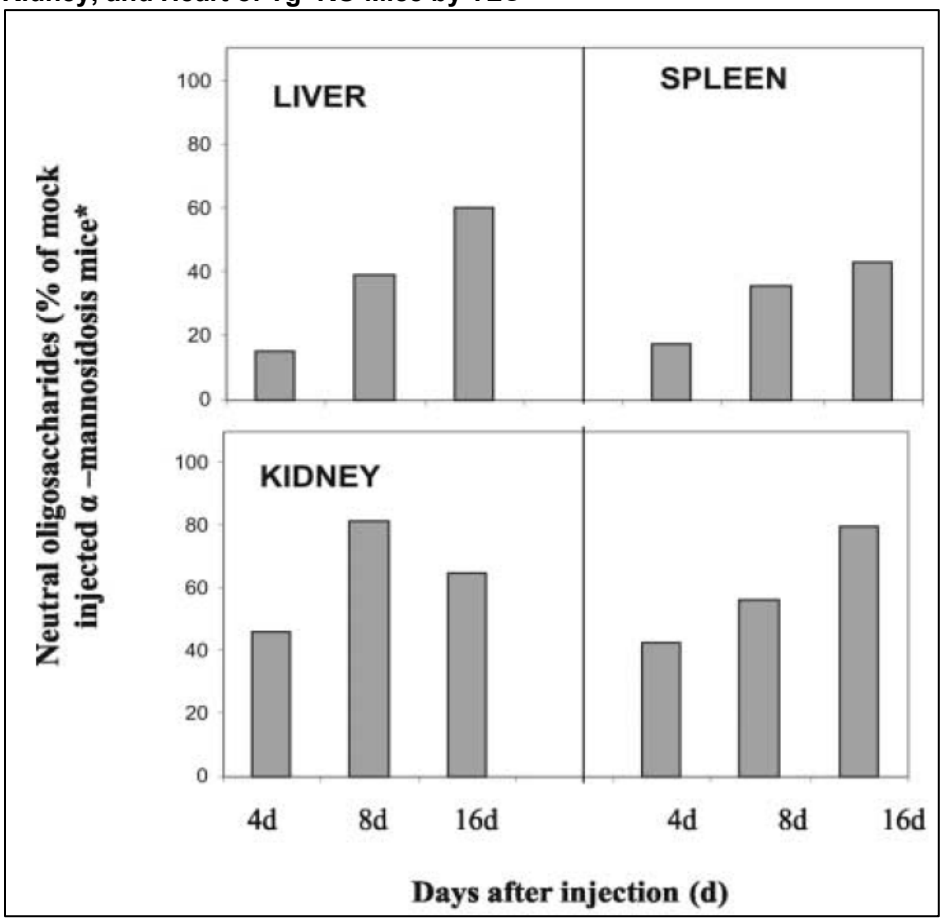


Source: Excerpted from the Applicant's BLA and Report ERT-36
Abbreviations: ERT, enzyme replacement therapy; KO, knockout

Administration of exogenous rhLAMAN in the mouse model led to a reduction of vacuolation. Vacuolation of tissues was shown in the liver, bone, kidney, pancreas, and brain. No functional consequences of the increased mannose content or tissue vacuolation were noted; thus, the Tg+KO mouse is considered a mild disease model (Stinchi et al. 1999).

In another study (Roces et al. 2004), administration of rhLAMAN was associated with an apparent reduction in oligosaccharide content in the liver, spleen, kidney, and heart, with maximal clearance observed at a dose of 250 mU/g body weight. The authors report that maximal reduction in oligosaccharide content was observed at Day 4 post-dose, and that accumulation began by Day 8 post-dose but continued through Day 16 (Figure 15).

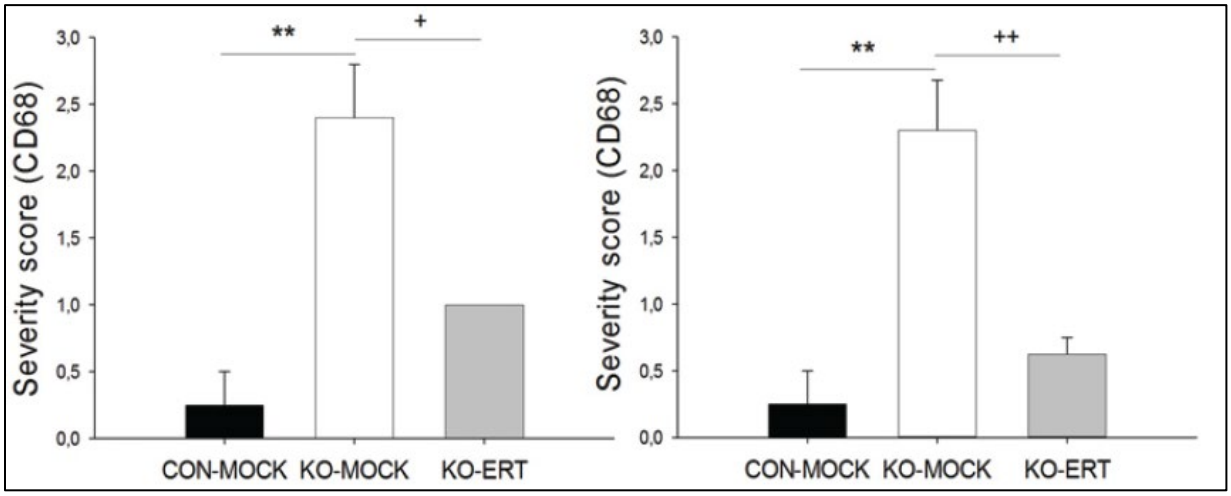
Figure 15. Effect of rhLAMAN Administration on Oligosaccharide Content in the Liver, Spleen, Kidney, and Heart of Tg+KO Mice by TLC



Source: Excerpted from the Applicant's BLA and (Roces et al. 2004)
Abbreviations: KO, knockout; TLC, thin layer chromatography

In a long-term administration model (Stroobants et al. 2017), the authors evaluated the effect of rhLAMAN administration on neurocognitive functioning in the Tg+KO mouse model. Animals received weekly injections at a dose of 500 mU/g body weight for up to 30 weeks. The authors state that treatment was associated with a reduction in markers of gliosis (CD68/IBA1 positivity; (Figure 16) in the hippocampus of treated versus control animals.

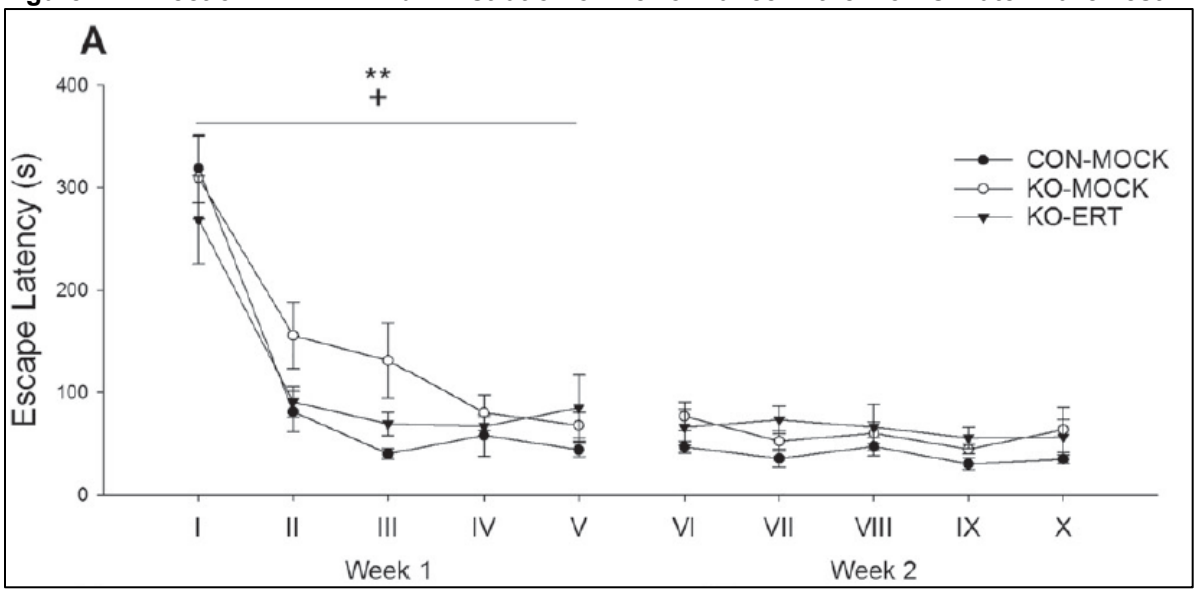
Figure 16. CD68 Staining in Wildtype and Tg+KO Mice Following 30 Weeks of Treatment With rhLAMAN



Source: Excerpted from the Applicant's BLA and (Stroobants et al. 2017)
Abbreviations: ERT, enzyme replacement therapy; KO, knockout

The authors also evaluated correlating neurocognitive effects and found that at 10 weeks of treatment, a small but statistically significant increase in escape latency (considered a measure of learning) in the water maze was observed. The effect was not observed after 30 weeks of treatment. The authors concluded that this likely reflects an effect on short-term memory only.

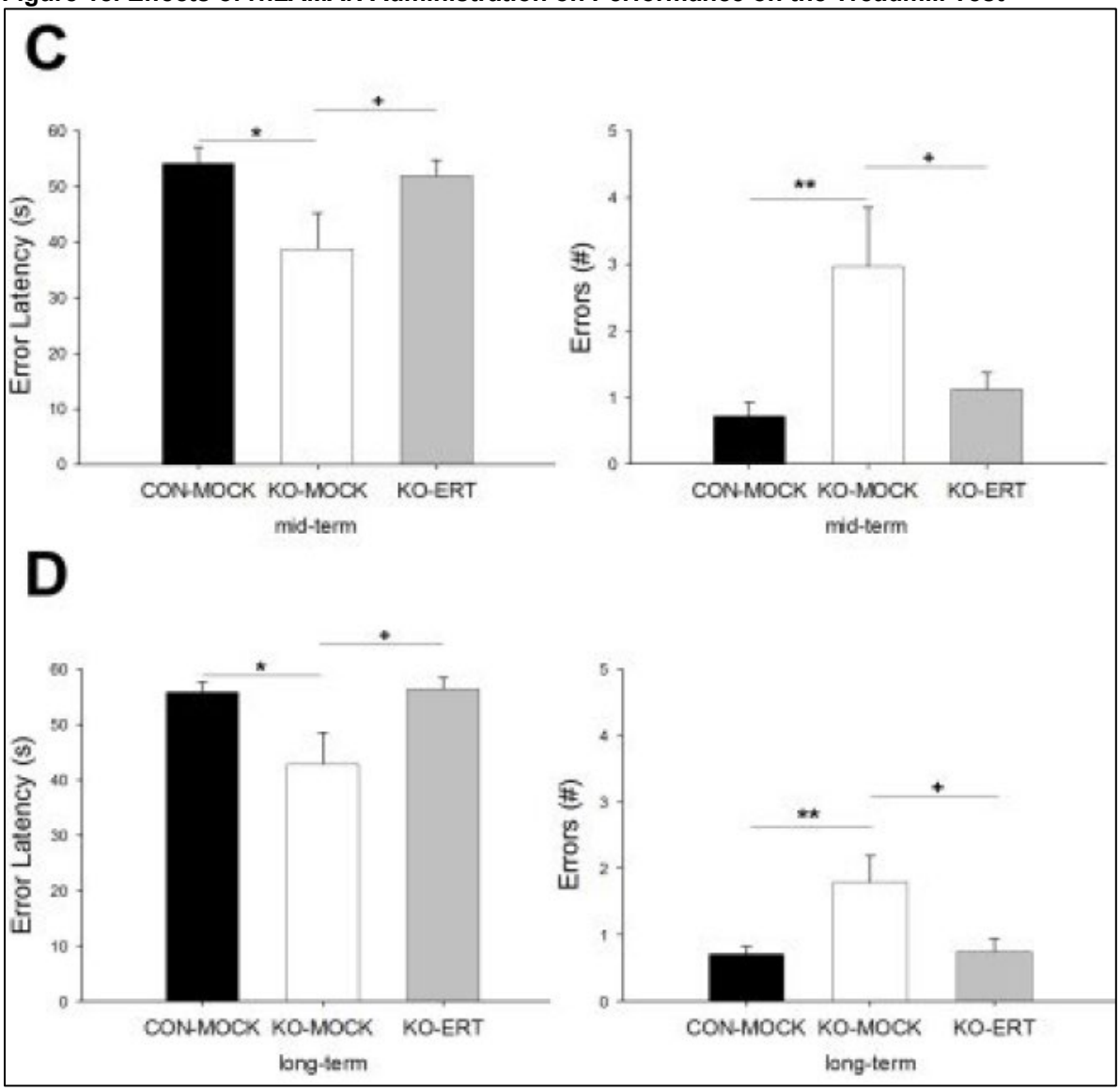
Figure 17. Effect of rhLAMAN Administration on Performance in the Morris Water Maze Test



Source: Excerpted from the Applicant's BLA and (Stroobants et al. 2017)
Abbreviations: ERT, enzyme replacement therapy; KO, knockout

The authors also evaluated the number of errors and the latency to error (also considered a measure of memory) in the treadmill test. For both measures in the treadmill test, a small but statistically significant effect was observed in treated animals after 10 (Panel C) and 30 (Panel D) weeks of treatment.

Figure 18. Effects of rhLAMAN Administration on Performance on the Treadmill Test



Source: Excerpted from the Applicant's BLA and (Stroobants et al. 2017)
Abbreviations: ERT, enzyme replacement therapy; KO, knockout

Although the Tg+KO mouse model used by the Applicant does not fully recapitulate human disease, it does replicate some of the biochemical and histological characteristics observed in humans that lack *MAN2B1*, such as tissue vacuolation and oligosaccharide accumulation. As a result, it could be used to define exposure-effect relationships on tissues and matrices that are not easily evaluable in humans. Published reports suggest that the model exhibits an approximately 1.5-4x increase in oligosaccharide content in different tissues compared with wildtype controls. This increase in oligosaccharide content correlates with histopathological changes, including vacuolation. Mice that lack *MAN2B1* do not appear to exhibit significant cognitive, motor, or immunological deficiencies, or limited lifespans (as seen in humans with AM); therefore, interpretation of the dose-effect relationship depends to a large extent on histopathological and biochemical analyses.

The data provided by the Applicant support the concept that exogenous α-mannosidase can be administered intravenously in Tg+KO mice, distributed into tissues, and internalized by cells. Data characterizing the outcome of treatment, however, including the magnitude, kinetics, and/or duration of oligosaccharide reduction, were largely descriptive, because the data were not collected using quantitative methods.

Several studies were conducted in which the authors attempted to characterize the effect of treatment in Tg+KO mice. The authors evaluated tissues histologically and performed thin layer chromatography to evaluate oligosaccharide levels; however, the assays were non-quantitative. In the absence of a calibration curve or other loading control it is difficult to distinguish inadvertent differences in sample loading from true effects of the drug. In many studies that attempted to quantify oligosaccharide content, data were also presented without information about variability that would enable the reviewer to assess the differences from controls (e.g., standard errors, error bars, etc.). There were also no studies in which the intended clinical biomarker (serum oligosaccharide concentration) was evaluated in animals.

An effect of dose on treatment outcome was also difficult to discern from the data presented. The animal studies that were conducted generally included only a single dose-level and/or key endpoints were only evaluated at single dose levels. In the good laboratory practice-compliant 26-week repeat-dose study in the Tg+KO mouse, for example, tissues were taken from all animals, but histopathology was only evaluated in control and high-dose animals. A decrease in tissue vacuolation was observed; however, the histological severity in control animals was mostly marginal to moderate. While treatment appeared to reduce the incidence and/or severity of vacuolation in several tissues, it is not possible to determine if there was a dose response and/or how the decreased vacuolation related to oligosaccharide levels (which were not evaluated). The decreased vacuolation was not associated with an improvement of a function outcome. In addition, the doses selected in this study were also about 3x higher than those used in other studies (e.g., (Stroobants et al. 2017)) so it is possible that the pharmacology was fully saturated at lower doses and/or that the oligosaccharide reduction was apparent at doses lower than those associated with clear evidence of vacuolation. Although exposure was maintained throughout the study, the lack of histological and biochemical data at lower doses prevented the Applicant from evaluating the exposure-response (E-R) relationship in animals.

Importantly, the Applicant has published widely on the potential of exogenously administered rhLAMAN to improve cognitive and/or neurological function in Tg+KO animals. These publications report a reduction in gliosis that appears to correspond to reductions in markers of neurodegeneration and marginally improved neurological function (e.g., (Stroobants et al. 2017) and (b) (4) Study 24963). The studies in which neurocognitive function was assessed were conducted at doses that are in excess of 15-49x the clinical dose (b) (4) .

There is some evidence for the ability of velmanase to enter the CNS in mice. CNS uptake in Tg+KO mice was found to be time-, as well as dose- and/or concentration-dependent (Zymenex Study ERT 36). No appreciable uptake was noted at doses of <500 mU/kg (15.6 mg/kg), but at doses of 15.6 mg/kg/week, a small but detectable increase in brain exposure was reported (Zymenex Study ERT 36 and Zymenex Study z-2012-09-17). In Study ERT 36, uptake was initially low at 1 hour, but increased by 24 hours post-dose (see the appended full Pharmacology Review). In contrast, exposure in peripheral tissues was highest at 1-hour post-dose and fell precipitously by 24-hours. These data suggest that in the mouse, there is potential for enzyme

uptake into the CNS, but that the efficiency is low and uptake sufficient to show effects on neurodegeneration or neurocognitive performance in the mouse required much higher doses than those that are being administered clinically. The doses administered in the 26-week repeat-dose study in Tg+KO mice, for which histopathology was performed, were 0, 144, 433, and 1300 U/kg, which correspond to doses of 0, 5.4, 16.2, and 49 mg/kg/week. At the high dose of 49 mg/kg/week, there was a reduction in tissue vacuolation compared with concurrent controls; however, in this study, lower doses were not evaluated histologically, so it is not possible to assess the effect of dose on tissue vacuolation.

Overall, the data provided by the Applicant are suggestive of the proposed mechanism, but they are not robust. The lack of biochemical data was the most limiting aspect of the dataset provided by the Applicant. The Applicant has not provided adequate evidence to establish a clear correlation between exposure, effect on tissue or plasma oligosaccharide levels, and histopathological effects, limiting their ability to use nonclinical data to validate its use as a surrogate biomarker in clinical trials. The strongest data provided do support the assumption that in the context of *MAN2B1* deficiency, administration of exogenous rhLAMAN has the potential to reduce cellular vacuolation. The amount of drug needed to achieve vacuole reduction and how the dose correlates to changes in in tissue and serum oligosaccharides in Tg+KO mice, however, is not clear. Nor is it clear from the nonclinical data that serum biomarkers can inform the adequacy of the selected clinical doses or dose regimens.

Conclusion

The multiple different lines of evidence discussed above (i.e., a well-understood disease pathophysiology of a single enzyme deficiency and a clear, targeted MOA of an ERT, mechanistic evidence that velmanase reaches its intended lysosomal target, biomarker data across multiple clinical studies, E-R evidence) provide acceptable confirmatory evidence of the treatment benefit of velmanase demonstrated in Trial rhLAMAN-05.

Given the particular clinical context of AM, there is an acceptable degree of uncertainty supporting the conclusion that there is substantial evidence of effectiveness for velmanase in the treatment of AM, with primary support derived from Trial rhLAMAN-05 together with a body of adequate confirmatory evidence.

6.3.3. Establishment of Efficacy in Patients <6 Years of Age

Issue/Background

The single adequate and well-controlled trial in the velmanase development program, Trial rhLAMAN-05, only included patients 6 years of age and older. Although the development program did include a trial in patients less than 6 years of age (Trial rhLAMAN-08), this was an uncontrolled, open label safety and tolerability trial with no formal statistical analyses of the efficacy endpoints (only descriptive statistics were used). Therefore, the results from Trial rhLAMAN-08 alone were not adequate to establish efficacy. In order to make a determination of efficacy in patients less than 6 years of age, the review team had to determine whether there was adequate scientific justification for extrapolating efficacy from patients 6 years of age and older (i.e., as established in Trial rhLAMAN-05) to the younger population.

Assessment

The etiology of AM is the same across all age groups in terms of underlying disease pathophysiology, with deficiency of alpha-mannosidase enzyme activity resulting in pathological accumulation of oligosaccharides in all age groups. The clinical manifestations of AM are also similar across all age groups. Additionally, the mechanism of velmanase, exogenously supplying the deficient enzyme, is the same across all age groups. Therefore, extrapolation of efficacy of velmanase from patients across age groups is scientifically justified.

Results from Trial rhLAMAN-08, as described in Section 6.2.3, supplement this extrapolation approach by confirming that the velmanase dose is appropriate in patients less than 6 years of age (with a similar exposure based on the PK results) and also with PD results demonstrating that patients less than 6 years of age experienced a decline from baseline in serum oligosaccharide levels similar to that observed in patients 6 years of age and older in Trial rhLAMAN-05. These PK and PD results are summarized below.

PK Results: Exposure of Velmanase Across Age Groups

The PK results of velmanase by age groups (3 to <6 years, 6 to <18 years, and ≥18 years) in patients with AM who received the 1 mg/kg dose in the integrated Trial rhLAMAN-10 and Trial rhLAMAN-08 are shown in Table 16. The PK results showed the following:

- The AUC and maximum plasma concentration (C_{max}) of velmanase in pediatric patients 3 to <6 years of age were generally comparable to these in the older pediatric patient group (6 to <18 years) and adult patients on Day 1.
- C_{max} in pediatric patients 3 to <6 years of age was generally comparable to that in the older pediatric patient group (6 to <18 years) and adult patients at steady state; however, AUC in adult patients were higher than that in the two pediatric age groups at steady state. The reason for the higher AUC in adult patients at steady state was unclear as velmanase is not expected to accumulate following multiple dose administrations. The AUC in pediatric patients 3 to <6 years of age was still comparable to that in pediatric patients 6 to <18 years of age at steady state.

The overall PK results would provide support to an extrapolation of efficacy from older pediatric patients (6 to <18 years of age) to younger pediatric patients (3 to <6 years of age). Of note, PK data in patients <3 years of age is currently not available.

Table 17. Pharmacokinetics of Velmanase in Patients With Alpha-Mannosidosis by Age Groups

	Day 1			Steady Sate		
Age Group	**3 to <6 (n=5)**	**6 to <18 (n=6-7)**	**≥18 (n=9)**	**3 to <6 (n=5)**	**6 to <18 (n=4)**	**≥18 (n=8)**
AUC_{inf} (h·µg/mL) Mean (SD)	102.9 (15.6)	83.3 (33.3)	111.0 (17.6)	108.3 (27.0)	118.9 (20.2)	178.9 (28.7)
C_{max} (µg/mL) Mean (SD)	8.0 (1.4)	6.4 (2.4)	10.0 (5.2)	7.0 (2.3)	6.6 (1.0)	7.9 (0.9)

Source: Table 18, Summary of Clinical Pharmacology Studies.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; SD, standard deviation

PD Results: Reduction in Serum Oligosaccharides Across Age Group

The serum concentrations of oligosaccharides at baseline and at different timepoints following treatment with velmanase in patients with AM are summarized by age groups (3 to <6 years, 6 to <18 years, and ≥18 years) in Table 17. The PD results showed the following:

- At baseline, the mean (SD) serum oligosaccharide levels were 11.6 (3.5), 7.6 (2.5), and 5.9 (1.5) umol/L in age groups of 3 to <6 years, 6 to <18 years, and ≥18 years, respectively, indicating pediatric patients had higher mean serum oligosaccharide levels than adult patients and the younger pediatric patient age group (i.e., 3 to <6 years of age) had the highest mean serum oligosaccharide levels among the three age groups.
- Similar mean (SD) % change from baseline serum oligosaccharide levels at 6 months (i.e., the first time-point of measurement) were observed: -60.5 (17.7), -62.2 (17.5), and -67.2 (9.2), respectively, in age groups of 3 to <6 years, 6 to <18 years, and ≥18 years, indicating similar onset of the PD effect across the age groups.
- The mean (SD) % change from baseline serum oligosaccharide levels at time-points from 6 to 24 months generally indicated the PD effect were well maintained across the age groups.
- The mean (SD) % change from baseline serum oligosaccharide levels at 24 months were -65.8 (23.05), -75.5 (13.4), and -79.2 (6.4), respectively, in age groups of 3 to <6 years, 6 to <18 years, and ≥18 years, indicating potentially greater PD effect in adult patients compared to pediatric patients in long-term treatment. This together with the higher baseline levels in pediatric patients resulted in relatively higher mean (SD) serum oligosaccharide concentrations in younger pediatric patients at 24 months: 3.8 (2.3), 1.6 (0.7), and 1.3 (0.5), respectively, in age groups of 3 to <6 years, 6 to <18 years, and ≥18 years.

Of note, the PD data in pediatric age group of 3 to <6 years were collected with an analytical method (i.e., [redacted] method) with higher sensitivity than the method (i.e., [redacted] method) used in patients of other age groups. The use of different bioanalytical methods may confound the comparison of the PD results for this pediatric age group with the results of other age groups. Refer to Section 14.3 for the assay performance information of these two bioanalytical methods. The Applicant conducted a comparison of serum oligosaccharide concentrations measured by the [redacted] method (used for pediatric patients 3 to <6 years of age in Trial rhLAMAN-08) with the [redacted] method (used for patients in all other clinical studies). The results showed that the [redacted] method generated serum oligosaccharide concentrations 31% to 104% higher than the concentrations measured by the [redacted] method using the same clinical samples across different time-points post velmanase dosing. However, the assay differences (i.e., 31% to 104% higher) would not explain all the observed higher residual serum oligosaccharide concentrations in pediatric patients 3 to <6 years of aga, because the mean serum oligosaccharide concentrations at Month 24 in pediatric patients 3 to <6 years of age were 138% and 192% higher than the concentrations in pediatric patients 6 to <18 years of age and adult patients ≥18 years of age (3.8 versus 1.6µMol/L; and 3.8 versus 1.3µMol/L), respectively.

The Applicant provided PD data from a 7-month-old pediatric patient who received velmanase treatment through a compassionate use program. The patient began receiving velmanase treatment following the diagnosis and continued the treatment for two months while waiting for

hematopoietic stem cell therapy. Significant reduction from baseline in serum oligosaccharides (53%) was observed as early as 2 weeks after treatment initiation. Increment of serum alpha mannosidase activity was also observed. The data from this single pediatric patient supported the pharmacological activity of velmanase in infants with AM.

The overall PD results supported the extrapolation of efficacy from adult and older pediatric patients (6 to <18 years of age) to younger pediatric patients (0 to <6 years of age).

- While the overall PK and PD results support that it is acceptable to extrapolate efficacy from patients 6 years of age and older to patients less than 6 years of age and that the proposed dose of 1 mg/kg would be effective and safe across all age groups, there are remaining uncertainties whether the proposed 1 mg/kg dose would be "optimal" for patients <3 years of age for the considerations listed below:

- PK data in patients <3 years of age are currently not available. Population PK covariate analysis suggested approximately 20% decrease in exposure in patients with body weight ≤18 kg in reference to subjects with a body weight of 60 kg. While 20% difference in exposure is not considered clinically meaningful based on the current understanding of the exposure-response relationships for velmanase, pediatric patients <3 years of age (e.g., infants) may have even lower exposures due to extremely small body size.
- Although significant reductions in serum oligosaccharides levels were observed across all the age groups at the currently proposed dose of 1 mg/kg, as discussed above the residual serum oligosaccharides levels in younger pediatric patients (3 to <6 years of age) appeared to be higher than these in older pediatric and adult patients possibly due to higher serum oligosaccharides levels at baseline in the younger pediatric patients. It is unknown if pediatric patients <3 years of age may or may not have an even higher serum oligosaccharide concentrations at baseline and/or higher residual serum oligosaccharide concentrations following treatment with velmanase at the currently proposed dose of 1 mg/kg.

The collective PK and PD data indicated that a further exploration of a dose higher than 1 mg/kg in pediatric patients <3 years of age would be warranted. See Section 24 for postmarketing commitment (PMC) recommendations.

Table 18. Serum Oligosaccharides (µmol/L) by Timepoint and Age Groups

Age Group			3 to <6 years		6 to <18 years		≥18 years
Timepoint		**n**	**Mean (SD)**	**n**	**Mean (SD)**	**n**	**Mean (SD)**
Baseline	Actual value	5	11.6 (3.5)	19	7.6 (2.5)	14	5.9 (1.5)
Month 6	Actual value	5	5.0 (4.0)	15	2.9 (1.0)	9	2.1 (0.6)
	Change from baseline Absolute		-6.6 (1.5)		-5.4 (2.8)		-4.3 (1.0)
	Change from baseline %		**-60.5 (17.7)**		**-62.2 (17.5)**		**-67.2 (9.2)**
Month 12	Actual value	4	3.6 (2.6)	18	1.6 (1.4)	13	1.7 (0.7)
	Change from baseline Absolute		-8.5 (2.5)		-6.2 (3.3)		-4.3 (1.8)
	Change from baseline %		**-71.8 (17.6)**		**-76.1 (24.1)**		**-68.0 (22.8)**
Month 18	Actual value	4	2.7 (1.8)	10	1.2 (0.8)	1	1.3 (0.5)
	Change from baseline Absolute		-9.4 (2.4)		-7.4 (3.1)		-5.0 (1.0)
	Change from baseline %		**-79.1 (8.0)**		**-85.0 (1.0)**		**-79.2 (6.4)**
Month 24	Actual value	4	3. 8 (2.3)	4	1.6 (0.7)	6	1.3 (0.48)
	Change from baseline Absolute		-7.7 (4.3)		-5.3 (1.5)		-5.0 (1.0)
	Change from baseline **%**		**-65.8 (23.1)**		**-75.5 (13.4)**		**-79.2 (6.4)**

Source: rhLAMAN08- study-report, Table 13, Page 82, rhLAMAN-10-Trial-report, Table 11, Page 18
Abbreviations: SD, standard deviation

Conclusion

The review team determined that extrapolation of efficacy from patients 6 years of age and older (established in Trial rhLAMAN-05) to patients less than 6 years of age is scientifically justified based on the same underlying disease pathophysiology, similarity in clinical manifestations and the same therapeutic MOA across age groups (i.e., enzyme replacement and substrate reduction). The findings from Trial rhLAMAN-08 are supportive of this extrapolation, as they provide evidence that the evaluated dose patients less than 6 years of age results in similar exposure and similar reductions in serum oligosaccharides to patients 6 years of age and older. Therefore, taken together, the results from Trials rhLAMAN-05 and rhLAMAN-08 support a labeled indication for pediatric patients down to birth. However, the overall PK and PD results also indicate that pediatric patients less than 3 years of age may achieve additional benefit from a dose higher than 1 mg/kg.

7. Safety (Risk and Risk Management)

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

Two potential safety risks were identified based on nonclinical data. In the rat pre- and post-natal development study, a finding of histiocytic sarcoma was observed in one high-dose (30 mg/kg) female. Exposures at the 30 mg/kg dose level were approximately 9-fold the AUC at the maximum recommended dose. According to (b) (4), the supplier of rats for that study, the incidence of this tumor in 4- to 6-month-old rats (the presumed age of rats in this study) was zero. The incidence in all control female Wistar rats was one in 4,962. The incidence in all Wistar rats (control + treated) was two in 13,550; therefore, this is an extremely rare tumor and given that it occurred in a high-dose dam, a relationship to treatment cannot be excluded.

Standard carcinogenicity bioassays were not conducted with velmanase; however, a mechanistic relationship between tumorigenesis and increased levels of α-mannosidase levels cannot be excluded. According to the Catalogue of Somatic Mutations in Cancer (COSMIC 2022), dysregulation (mutations and/or overexpression) of *MAN2B1* expression has been observed in numerous tumors, including tumors of the adrenal, breast, CNS, cervix, endometrium, hematopoietic/lymphoid systems, kidney, intestinal tract, liver, lung, esophagus, pancreas, parathyroid, prostate, skin, stomach, thyroid, and urinary tract. Overexpression of *MAN2B1* has also been determined to be a prognostic biomarker in some tumors (Lin et al. 2022).

In an embryofetal toxicity study in Han Wistar rats, administration of rhLAMAN during the period of organogenesis from gestation day 6-17 was associated with induction of major malformations at the high dose of 20 mg/kg/day, which was a non-maternally toxic dose. Because the major malformations were limited to fetuses in high-dose dams, a role of rhLAMAN in induction of these malformations cannot be excluded. There were also numerous minor visceral and skeletal malformations and variations in treated animals at levels that exceeded those observed in concurrent controls. The AUC at 20 mg/kg/day, which was associated with induction of malformations in rats, was approximately 7-fold those observed in humans at the maximum recommended human dose. The no observed adverse effect level (NOAEL) in this study was 10 mg/kg/day, which is approximately 1.6x the clinical AUC at the maximum recommended dose.

In an embryofetal toxicity study in the rabbit, there were no apparent effects on embryofetal survival; however, treatment was associated with induction of numerous variations and malformations, the incidences of which exceeded concurrent controls. A NOAEL was not identified in this study.

There were no other nonclinical safety concerns identified in toxicology studies conducted during the development program. Nonclinical studies supporting the marketing application include a 26-week repeat-dose toxicology study in the Tg+KO mouse; a 13-week repeat-dose toxicology study in cynomolgus monkeys; and reproductive and developmental toxicology studies in rats and rabbits. The NOAELs and associated exposures are provided in Table 18.

Table 19. Summary of NOAELs and Exposure Multiples in Velmanase GLP Toxicity Studies

Toxicity Study	Study Number	Species	NOAEL	Safety Margin Based on AUC*
26-Week	24963	Mouse	1300 U/kg	89x
13-Week	PWM0002	Cynomolgus Monkey	1310 U/kg	79x
EFD	IZU0004	Rat	10 mg/kg	1.6x
EFD	IZU0005	Rabbit	None	None
PPND	IZU0007	Rat	10 mg/kg	9x
FEED	IZU0006	Rat		9x
JAS	495126	Rat	1290 U/kg	163x

Source:
Asterix indicates: Relative to the clinical AUC_{Last} of 143 µg*hr/mL at a dose of 1 mg/kg
Abbreviations: AUC, area under the concentration-time curve; GLP, good laboratory practice; NOAEL, no observed adverse effect level

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

Velmanase is an enzyme replacement therapy and the main identified potential risks with this class of drugs include hypersensitivity reactions (including anaphylaxis), infusion-associated reactions (IARs), and immunogenicity.

7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience

There is no US postmarket experience. For ex-US postmarket experience refer to Section 7.6.3.

7.4. FDA Approach to the Safety Review

Safety data from the following trials were submitted to support the safety review of velmanase:

- Trial rhLAMAN-05 (25 patients, aged 6 to 35 years old), a randomized double-blind placebo (for details refer to Section 6.2.1.1).
- The rhLAMAN-10 Integrated Analysis . pooled the cumulative databases for all previous velmanase trials. This integrated analysis includes a total of 33 patients (aged 6 to 35 years old). Of these 33 patients, 18 were from Trial rhLAMAN-10 (for details refer to Section 16.3.1), 7 from rhLAMAN-07, and 8 from rhLAMAN-09 (for details refer to Section 6.2.2.1).
- Trial rhLAMAN-08 (5 patients, aged 3 to 5 years old), an open label trial in treatment native patients (for details refer to Section 6.2.3.1).

The post-marketing data provided by Applicant (including periodic safety update report (PSUR)-7 and 120-day safety update) were reviewed and discussed in Section 7.3.

The review team primarily assessed data from the randomized, placebo-controlled trial (rhLAMAN-05). This trial provided data to compare the incidences of AEs between velmanase

and placebo. In addition, data from rhLAMAN-10 and rhLAMAN-08 were reviewed to ensure completeness of the safety assessment. The combined safety population from these trials consisted of 38 unique patients (24 pediatric patients, of which 5 were aged <6 years old, and 14 adult patients).

First, the datasets for trials rhLAMAN-05, -10, and -08 were examined for the required and standardized components and for completeness of the data. Then, the review team reviewed the AE verbatim terms and confirmed that the AEs reported by the Applicant accurately described the AEs reported by the investigators.

Once accuracy was confirmed, the frequencies of each AE were assessed and described by occurrence. The review team reviewed all adverse events of special interest (AESI), SAEs, prolonged treatment pauses, and withdrawals.

Each of the above studies will be discussed individually in the below Sections (7.6.1, 7.6.2, and 7.6.3).

The ISS was reviewed specifically for AESIs (for details refer to Section 7.6.2.1).

7.5. Adequacy of the Clinical Safety Database

Given the extreme rarity of AM, the safety database of 38 total unique patients was deemed adequate for a sufficient safety assessment of velmanase for the proposed indication and patient population. The review team did not identify any major data quality or integrity issues that precluded performance of a thorough safety review. No major issues were identified with respect to the coding of AEs.

Table 19 shows the baseline demographic and clinical characteristics of the patient population in Trial rhLAMAN-05. All patients were White race. However, given the extreme rarity of this disease, the lack of racial diversity in this trial is accepted.

Table 20. Baseline Demographic and Clinical Characteristics, Safety Population, Trial rhLAMAN-05

	Patients <18 Years		Patients ≥18 Years		All Patients	
Characteristic	Velmanase N=7	Placebo N=5	Velmanase N=8	Placebo N=5	Velmanase N=15	Placebo N=10
Sex, n (%)						
Female	3 (42.9)	3 (60.0)	3 (37.5)	2 (40.0)	6 (40.0)	5 (50.0)
Male	4 (57.1)	2 (40.0)	5 (62.5)	3 (60.0)	9 (60.0)	5 (50.0)
Age, years						
Mean (SD)	10.6 (4)	12.8 (4.4)	25.5 (5.4)	26.6 (6.2)	18.5 (9)	19.7 (8.9)
Median (min, max)	10 (6, 17)	14 (6, 17)	24 (20, 35)	28 (20, 35)	20 (6, 35)	18.5 (6, 35)
Race, n (%)						
White	7 (100)	5 (100)	8 (100)	5 (100)	15 (100)	10 (100)

Characteristic	Patients <18 Years		Patients ≥18 Years		All Patients	
	Velmanase N=7	Placebo N=5	Velmanase N=8	Placebo N=5	Velmanase N=15	Placebo N=10
Country of participation, n (%)						
Belgium	0	2 (40.0)	0	1 (20.0)	0	3 (30.0)
Germany	2 (28.6)	0	4 (50.0)	1 (20.0)	6 (40.0)	1 (10.0)
Denmark	1 (14.3)	1 (20.0)	2 (25.0)	2 (40.0)	3 (20.0)	3 (30.0)
Spain	0	1 (20.0)	2 (25.0)	0	2 (13.3)	1 (10.0)
France	4 (57.1)	1 (20.0)	0	1 (20.0)	4 (26.7)	2 (20.0)

Source: adsl.xpt; Software: R
Abbreviations: N, number of patients in treatment group; n, number of patients with given characteristic; SD, standard deviation

Table 20 (below) summarizes the velmanase exposure for the rhLAMAN-05 population. Patients treated with velmanase for 365-399 days (10 patients, 66.7%) comprise the largest cohort in the pivotal trial. The other 5 patients treated with velmanase (33.3%) were treated for 300-364 days.

Table 21. Duration of Exposure, Safety Population, Trial rhLAMAN-05

Parameter	Patients <18 Years		Patients ≥18 Years		All Patients	
	Velmanase N=7 n (%)	Placebo N=5 n (%)	Velmanase N=8 n (%)	Placebo N=5 n (%)	Velmanase N=15 n (%)	Placebo N=10 n (%)
Duration of treatment, days						
Mean (SD)	366.4 (14.3)	369.4 (13.3)	364.5 (12.2)	372 (11.1)	365.4 (12.8)	370.7 (11.6)
Median (Q1, Q3)	365 (357, 379.5)	371 (365, 380)	371 (360.8, 372.2)	378 (365, 379)	371 (357, 373)	374.5 (365, 379.8)
Min, Max	345, 382	349, 382	345, 373	356, 382	345, 382	349, 382
Total exposure (person years)	7	5	8	5	15	10
Patients treated, by duration, n (%)						
<300 days	0	0	0	0	0	0
≥300 to <365 days	3 (42.9)	1 (20.0)	2 (25.0)	1 (20.0)	5 (33.3)	2 (20.0)
≥365 to <400 days	4 (57.1)	4 (80.0)	6 (75.0)	4 (80.0)	10 (66.7)	8 (80.0)

Source: adex.xpt and adsl.xpt; Software: R
Duration is 12 months.
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; SD, standard deviation

Table 21 (below) summarizes the baseline demographic and clinical characteristics of the patient population for the rhLAMAN-10 Integrated Analysis. Given the extreme rarity of this disease, the lack of diversity in this study is also accepted.

Table 22. Baseline Demographic and Clinical Characteristics, Safety Population, rhLAMAN-10 Integrated Analysis

	Velmanase		
Characteristic	**<18 years N=19**	**≥18 years N=14**	**All N=33**
Sex, n (%)			
Female	6 (31.6)	7 (50.0)	13 (39.4)
Male	13 (68.4)	7 (50.0)	20 (60.6)
Age, years			
Mean (SD)	11.6 (3.7)	24.6 (5.3)	17.1 (7.8)
Median (min, max)	12 (6, 17)	22.5 (18, 35)	15 (6, 35)
Age group, years, n (%)			
<18	19 (100)	0	19 (57.6)
≥18	0	14 (100)	14 (42.4)
Race, n (%)			
White	19 (100)	14 (100)	33 (100)
Country of participation, n (%)			
Belgium	2 (10.5)	1 (7.1)	3 (9.1)
Germany	2 (10.5)	5 (35.7)	7 (21.2)
Denmark	11 (57.9)	3 (21.4)	14 (42.4)
Spain	1 (5.3)	2 (14.3)	3 (9.1)
France	3 (15.8)	3 (21.4)	6 (18.2)

Source: adsl.xpt; Software: R
Abbreviations: N, number of patients in treatment group; n, number of patients with given characteristic; SD, standard deviation

Table 22 (below) summarizes the velmanase exposure for the rhLAMAN-10 Integrated Analysis population. Patients treated with velmanase for <600 days (7 patients, 21.1%) and ≥800 to <1000 days (7 patients, 21.1%) comprise the largest cohorts in the trial.

Table 23. Duration of Exposure, Safety Population, rhLAMAN-10 Integrated Analysis

	Velmanase		
Parameter	**<18 years N=19 n (%)**	**≥18 years N=14 n (%)**	**All N=33 n (%)**
Duration of treatment, days			
Mean (SD)*	1198.2 (501.9)	736 (229.9)	1002.1 (465.8)
Median (Q1, Q3)	1170 (852.5, 1600.5)	776 (545.5, 831.2)	935 (733, 1562)
Min, Max	357, 1881	373, 1175	357, 1881
Total exposure (person years)	62	28	91
Patients treated, by duration, n (%)			
<600 days	3 (15.8)	4 (28.6)	7 (21.2)
≥600 to <800 days	2 (10.5)	4 (28.6)	6 (18.2)
≥800 to <1000 days	2 (10.5)	5 (35.7)	7 (21.2)
≥1000 to <1200 days	3 (15.8)	1 (7.1)	4 (12.1)
≥1200 to <1400 days	0	0	0
≥1400 to <1600 days	4 (21.1)	0	4 (12.1)
≥1600 days	5 (26.3)	0	5 (15.2)

Source: adex.xpt and adsl.xpt; Software: R Duration is 12 to 48 months.
*This table is not comparing exposure between treatment arm and placebo arm. For Trials rhLAMAN-05, CCD-LMZYMAA1-08, and rhLAMAN-10, "extent of exposure (days)" was calculated using the following formula: Extent of exposure (days) = Date of last study medication intake (i.e., TRTEDT) - Date of first study medication intake (i.e., TRTSDT) + 1. For Trial rhLAMAN-10, the Applicant used the variable ADEX.ASTDY (Analysis Start Relative Day) of last velmanase alfa infusion for analysis of extent of exposure days (ASTDT – TRTSDT +1).
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; SD, standard deviation

Table 23 (below) shows the baseline demographic and clinical characteristics of the patient population in Trial rhLAMANM-08. The patients are 3 to 5 years old.

Table 24. Baseline Demographic and Clinical Characteristics, Safety Population, Trial rhLAMAN-08

Characteristic	**Velmanase N=5**
Sex, n (%)	
Female	2 (40.0)
Male	3 (60.0)
Age, years	
Mean (SD)	4 (0.7)
Median (min, max)	4 (3, 5)
Race, n (%)	
White	4 (80.0)
Missing	1 (20.0)
Country of participation, n (%)	
Austria	1 (20.0)
France	1 (20.0)
Germany	2 (40.0)
Italy	1 (20.0)

Source: adsl.xpt; Software: R
Abbreviations: N, number of patients in treatment group; n, number of patients with given characteristic; SD, standard deviation

Table 24 (below) summarizes the velmanase exposure for the rhLAMAN-08 population. Two patients were treated for <108 weeks, two patients were treated for ≥108 to 116 weeks, and one patient was treated for ≥180 weeks.

Table 25. Duration of Exposure, Safety Population, Trial rhLAMAN-08

Parameter	Velmanase N=5 n (%)
Duration of treatment, weeks	
Mean (SD)	120.6 (27.5)
Median (Q1, Q3)	108.3 (107.3, 112.3)
Min, Max	105.4, 169.6
Total exposure (person years)	12
Patients treated, by duration, n (%)	
<108 weeks	2 (40.0)
≥108 to <116 weeks	2 (40.0)
≥116 to <180 weeks	1 (20.0)
≥180 weeks	0

Source: adex.xpt and adsl.xpt; Software: R
Duration is 24 months (40 months for patient #(b) (6) enrolled in (b) (6)).
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; SD, standard deviation

7.6. Safety Results

Overall Summary

The demonstrated safety profile of velmanase is acceptable at the dose of 1 mg/kg IV administered once every week in patients with AM of all ages. All SAE patient narratives and AESI patient narratives were reviewed. As with most ERTs, hypersensitivity events (including anaphylaxis) and IRRs were identified with velmanase. No data on pregnancy and lactation are available with velmanase.

No patient discontinuations occurred within the 3 safety trials. However, as described in Section 7.6.3 patient discontinuations were reported in post marketing experience.

There were no reported deaths during the 3 trials. However as described in Section 7.6.3, one patient death has been reported in post marketing experience.

The review team concluded that labeling, including a boxed warning for hypersensitivity reactions including anaphylaxis, along with routine pharmacovigilance monitoring, is adequate to mitigate and monitor the identified safety risks.

7.6.1. Safety Results From Controlled Study, Trial rhLAMAN-05

7.6.1.1. Overview of Treatment-Emergent Adverse Events Summary, Trial rhLAMAN-05

Table 25 provides an overview of AEs reported in rhLAMAN-05. There were no permanent discontinuations of treatment in either the velmanase or placebo group. Four patients in the velmanase group experienced a total of 7 AEs leading to dose modification including a reduction in the dose rate or interruption of treatment.

A higher number of patients experienced treatment-emergent AEs (TEAEs) in the velmanase group (100%, 15 of 15 patients) than the placebo group (90%, 9 of 10 patients), with 5 unique patients experiencing a SAE in the velmanase group and 0 patients experiencing SAE in placebo group.

Table 26. Overview of Adverse Events, Safety Population, Trial rhLAMAN-05

	Patients <18 Years			Patients ≥18 Years			All Patients		
Event Category	Velmanase N=7 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n (%)	Placebo N=10 n (%)	Risk Difference (%) (95% CI)
SAE	2 (28.6)	0	28.6 (-4.9, 62.0)	3 (37.5)	0	37.5 (4.0, 71.0) *	5 (33.3)	0	33.3 (9.5, 57.2) *
SAEs with fatal outcome	0	0	0 (0, 0)	0	0	0 (0, 0)	0	0	0 (0, 0)
Life-threatening SAEs	0	0	0 (0, 0)	0	0	0 (0, 0)	0	0	0 (0, 0)
AE leading to permanent discontinuation of study drug	0	0	0 (0, 0)	0	0	0 (0, 0)	0	0	0 (0, 0)
AE leading to dose modification of study drug	1 (14.3)	0	14.3 (-11.6, 40.2)	3 (37.5)	0	37.5 (4.0, 71.0) *	4 (26.7)	0	26.7 (4.3, 49.0) *
Interruption of study drug	1 (14.3)	0	14.3 (-11.6, 40.2)	1 (12.5)	0	12.5 (-10.4, 35.4)	2 (13.3)	0	13.3 (-3.9, 30.5)
Reduction of study drug	1 (14.3)	0	14.3 (-11.6, 40.2)	2 (25.0)	0	25.0 (-5.0, 55.0)	3 (20.0)	0	20.0 (-0.2, 40.2)
Dose delay of study drug	0	0	0 (0, 0)	0	0	0 (0, 0)	0	0	0 (0, 0)
Other	0	0	0 (0, 0)	0	0	0 (0, 0)	0	0	0 (0, 0)
Any AE	7 (100)	4 (80.0)	20.0 (-15.1, 55.1)	8 (100)	5 (100)	0 (0, 0)	15 (100)	9 (90.0)	10.0 (-8.6, 28.6)
Severe and worse	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Moderate	5 (71.4)	2 (40.0)	31.4 (-23.0, 85.9)	6 (75.0)	3 (60.0)	15.0 (-37.4, 67.4)	11 (73.3)	5 (50.0)	23.3 (-14.9, 61.6)
Mild	2 (28.6)	2 (40.0)	-11.4 (-65.9, 43.0)	1 (12.5)	2 (40.0)	-27.5 (-76.2, 21.2)	3 (20.0)	4 (40.0)	-20.0 (-56.5, 16.5)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 12 months.
Risk difference (with 95% confidence interval) is shown between velmanase and placebo.
Severity as assessed by the investigator.
Asterisk (*) indicates rows where the 95% confidence interval excludes zero.
Abbreviations: AE, adverse event; CI, confidence interval; N, number of patients in treatment arm; n, number of patients with at least one event; SAE, serious adverse event

7.6.1.2. Deaths, Trial rhLAMAN-05

No deaths were recorded in rhLAMAN-05.

7.6.1.3. Serious Treatment-Emergent Adverse Events, Trial rhLAMAN-05

Five unique patients experienced a total of 5 SAEs. All 5 of these patients were in the velmanase group (see Table 26 below), there were no SAEs in the placebo group. Two of the SAEs occurred in patients <18 years old and the other three occurred in patients >18 years old. The patient narratives of each SAE were reviewed, and key information from these narratives is summarized below.

Table 27. Patients With Serious Adverse Events by System Organ Class and Preferred Term, Safety Population, Trial rhLAMAN-05

	Patients <18 Years			Patients ≥18 Years			All Patients		
System Organ Class Preferred Term	Velmanase N=7 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n (%)	Placebo N=10 n (%)	Risk Difference (%) (95% CI)
Any SAE	2 (28.6)	0	28.6 (-4.9, 62.0)	3 (37.5)	0	37.5 (4.0, 71.0) *	5 (33.3)	0	33.3 (9.5, 57.2) *
Infections and infestations (SOC)	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Sepsis	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Musculoskeletal and connective tissue disorders (SOC)	2 (28.6)	0	28.6 (-4.9, 62.0)	1 (12.5)	0	12.5 (-10.4, 35.4)	3 (20.0)	0	20.0 (-0.2, 40.2)
Joint swelling	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Knee deformity	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Sjogren`s syndrome	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Renal and urinary disorders (SOC)	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Renal failure acute	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Serious adverse events defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.
Duration is 12 months.
Risk difference (with 95% confidence interval) is shown between velmanase and placebo.
Asterisk (*) indicates rows where the 95% confidence interval excludes zero
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients with adverse event; SOC, system organ class

RHLAMAN-05-(b) (6) – a 35-year-old female patient experienced acute renal failure (ARF) diagnosed with an elevated creatinine of 5.95 mg/dL two days after receiving velmanase 1 mg/kg IV (on (b) (6), 291 days after receiving her 1st dose of velmanase). Velmanase infusion was suspended for 4 weeks. The Applicant also states this patient had concomitant medication of Ibuprofen (1200 mg/day). The Investigator assessed this event as possibly related to study drug.

RHLAMAN-05-(b) (6) – A 23-year-old female patient experienced sepsis on (b) (6), 2 days after second dose of velmanase. The patient had port-a-cath implanted on (b) (6). At time of sepsis the Applicant reports patient had redness and warmth around port-a-cath site. The patient was treated with IV antibiotic therapy and was discharged on (b) (6). Velmanase infusions were paused from (b) (6), until sepsis resolution. The patient restarted velmanase on (b) (6). The investigator assessed this event as not related to velmanase.

RHLAMAN-05-(b) (6) – A 6-year-old female patient developed Sjogren's syndrome 129 days after the first dose of velmanase. The SAE has not resolved and likely is a chronic disease. There have been reported cases of systemic lupus erythematosus (N=3) (Urushihara et al. 2004) and Sjogren syndrome (N=1) (Lipinski et al. 2021) occurring in patient(s) with AM.

CCD-LMZYMAA1-10_(b) (6) – A 22-year-old female patient was hospitalized for an operation due to swollen left ankle. Of note, this patient's medical history was notable for 3 previous surgeries on the left ankle. The Investigator assessed this event as not related to velmanase.

CCD-LMZYMAA1-10_(b) (6) – A 14-year-old female patient was hospitalized for repair of bilateral knee deformities. The investigator assessed this event as not related to velmanase. The Applicant determined that the ARF episode was possibly related to the investigational drug and that the other 4 SAEs are not related to the drug. The review team agrees that this is a reasonable assessment of the SAEs based on the patient narratives.

7.6.1.4. Adverse Events Leading to Treatment Discontinuation, Trial rhLAMAN-05

There were no treatment discontinuations.

Treatment-Emergent Adverse Events, Trial rhLAMAN-05

Table 27 lists the TEAEs that occurred in patients who participated in the trial comparing the risk difference between those who received velmanase and those who received placebo. The treatment group presented a higher risk for several TEAEs than placebo, most notably for acute tonsillitis, eye pruritis, gastroenteritis, hypersensitivity, influenza, syncope, and toothache (each with risk difference of 13.3%). The risk difference (RD) for any AE is greater in pediatric patients than adult patients, with RD of 20% in pediatric patients and 0% in adult patients.

Refer to Section 7.7.1 for a discussion of hypersensitivity events.

Table 28. Patients With Adverse Events, Safety Population, Trial rhLAMAN-05

	Patients <18 Years			Patients ≥18 Years			All Patients		
Preferred Term	Velmanase N=7 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n (%)	Placebo N=10 n (%)	Risk Difference (%) (95% CI)
Any AE	7 (100)	4 (80.0)	20.0 (-15.1, 55.1)	8 (100)	5 (100)	0 (0, 0)	15 (100)	9 (90.0)	10.0 (-8.6, 28.6)
Acute tonsillitis	2 (28.6)	0	28.6 (-4.9, 62.0)	0	0	0 (0, 0)	2 (13.3)	0	13.3 (-3.9, 30.5)
Eye pruritus	0	0	0 (0, 0)	2 (25.0)	0	25.0 (-5.0, 55.0)	2 (13.3)	0	13.3 (-3.9, 30.5)
Gastroenteritis	1 (14.3)	0	14.3 (-11.6, 40.2)	1 (12.5)	0	12.5 (-10.4, 35.4)	2 (13.3)	0	13.3 (-3.9, 30.5)
Hypersensitivity	0	0	0 (0, 0)	2 (25.0)	0	25.0 (-5.0, 55.0)	2 (13.3)	0	13.3 (-3.9, 30.5)
Influenza	1 (14.3)	0	14.3 (-11.6, 40.2)	1 (12.5)	0	12.5 (-10.4, 35.4)	2 (13.3)	0	13.3 (-3.9, 30.5)
Syncope	1 (14.3)	0	14.3 (-11.6, 40.2)	1 (12.5)	0	12.5 (-10.4, 35.4)	2 (13.3)	0	13.3 (-3.9, 30.5)
Toothache	0	0	0 (0, 0)	2 (25.0)	0	25.0 (-5.0, 55.0)	2 (13.3)	0	13.3 (-3.9, 30.5)
Arthralgia	2 (28.6)	0	28.6 (-4.9, 62.0)	1 (12.5)	1 (20.0)	-7.5 (-49.4, 34.4)	3 (20.0)	1 (10.0)	10.0 (-17.5, 37.5)
Acne	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Alanine aminotransferase increased	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Amylase increased	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Arthropod sting	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Asthma	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Blister	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Blood bilirubin decreased	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Bradycardia	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Chills	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Cold sweat	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Conjunctival hyperaemia	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Contusion	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Decreased appetite	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Dyspnoea	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Excoriation	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Eye oedema	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Eyelid oedema	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Food poisoning	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Hallucination	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Hydrocele	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Hyperhidrosis	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Infusion related hypersensitivity reaction	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)

	Patients <18 Years			Patients ≥18 Years			All Patients		
Preferred Term	Velmanase N=7 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n (%)	Placebo N=10 n (%)	Risk Difference (%) (95% CI)
Inguinal hernia	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Insomnia	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Iron deficiency	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Joint swelling	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Knee deformity	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Lip blister	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Loss of consciousness	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Lymphadenopathy	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Mouth cyst	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Nasal congestion	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Ocular hyperaemia	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Oral herpes	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Otitis media	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Paronychia	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Parotitis	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Pharyngotonsillitis	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Pollakiuria	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Post lumbar puncture syndrome	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Psychotic behaviour	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Rash	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Renal failure acute	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Scar pain	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Sepsis	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Sjogren`s syndrome	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Skin abrasion	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Spondylolisthesis	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Tinea versicolour	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Tooth infection	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Urticaria	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Vitiligo	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Back pain	2 (28.6)	0	28.6 (-4.9, 62.0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	2 (13.3)	1 (10.0)	3.3 (-22.0, 28.7)
Ear infection	1 (14.3)	0	14.3 (-11.6, 40.2)	1 (12.5)	1 (20.0)	-7.5 (-49.4, 34.4)	2 (13.3)	1 (10.0)	3.3 (-22.0, 28.7)
Headache	3 (42.9)	2 (40.0)	2.9 (-53.6, 59.3)	2 (25.0)	1 (20.0)	5.0 (-41.1, 51.1)	5 (33.3)	3 (30.0)	3.3 (-33.8, 40.4)
Urinary tract infection	0	0	0 (0, 0)	2 (25.0)	1 (20.0)	5.0 (-41.1, 51.1)	2 (13.3)	1 (10.0)	3.3 (-22.0, 28.7)
Constipation	0	0	0 (0, 0)	1 (12.5)	1 (20.0)	-7.5 (-49.4, 34.4)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)

	Patients <18 Years			Patients ≥18 Years			All Patients		
Preferred Term	Velmanase N=7 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n (%)	Placebo N=10 n (%)	Risk Difference (%) (95% CI)
Dental caries	1 (14.3)	1 (20.0)	-5.7 (-49.3, 37.9)	0	0	0 (0, 0)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Epistaxis	0	1 (20.0)	-20.0 (-55.1, 15.1)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Fatigue	1 (14.3)	1 (20.0)	-5.7 (-49.3, 37.9)	0	0	0 (0, 0)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Nasopharyngitis	5 (71.4)	2 (40.0)	31.4 (-23.0, 85.9)	5 (62.5)	5 (100)	-37.5 (-71.0, -4.0) *	10 (66.7)	7 (70.0)	-3.3 (-40.4, 33.8)
Nausea	1 (14.3)	1 (20.0)	-5.7 (-49.3, 37.9)	0	0	0 (0, 0)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Oedema peripheral	0	0	0 (0, 0)	1 (12.5)	1 (20.0)	-7.5 (-49.4, 34.4)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Pain in extremity	1 (14.3)	0	14.3 (-11.6, 40.2)	0	1 (20.0)	-20.0 (-55.1, 15.1)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Abdominal pain	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Abdominal pain upper	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Agitation	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Aphonia	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Cardiac murmur	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Catheter site pain	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Cough	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Depression	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Ear congestion	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Fall	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Food allergy	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Gastrooesophageal reflux disease	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Gingival swelling	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Haemorrhoids	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Head injury	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Herpes simplex	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Infusion site oedema	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Mouth ulceration	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Muscle spasms	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Myalgia	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Neck pain	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Oral mucosal blistering	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Otitis externa	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Pain in jaw	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Procedural headache	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Pyrexia	4 (57.1)	3 (60.0)	-2.9 (-59.3, 53.6)	2 (25.0)	2 (40.0)	-15.0 (-67.4, 37.4)	6 (40.0)	5 (50.0)	-10.0 (-49.7, 29.7)
Rectal haemorrhage	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)

	Patients <18 Years			Patients ≥18 Years			All Patients		
Preferred Term	Velmanase N=7 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n (%)	Placebo N=10 n (%)	Risk Difference (%) (95% CI)
Seasonal allergy	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Stress	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Sunburn	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Tachycardia	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Tongue injury	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Wound infection	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Wrist fracture	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Dizziness	1 (14.3)	0	14.3 (-11.6, 40.2)	0	2 (40.0)	-40.0 (-82.9, 2.9)	1 (6.7)	2 (20.0)	-13.3 (-41.2, 14.5)
Diarrhoea	0	2 (40.0)	-40.0 (-82.9, 2.9)	2 (25.0)	1 (20.0)	5.0 (-41.1, 51.1)	2 (13.3)	3 (30.0)	-16.7 (-49.9, 16.5)
Ear discomfort	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	2 (20.0)	-20.0 (-44.8, 4.8)
Vomiting	2 (28.6)	2 (40.0)	-11.4 (-65.9, 43.0)	1 (12.5)	2 (40.0)	-27.5 (-76.2, 21.2)	3 (20.0)	4 (40.0)	-20.0 (-56.5, 16.5)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of velmanase An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 12 months.
Coded as MedDRA preferred terms.
Risk difference (with 95% confidence interval) is shown between velmanase and placebo.
Asterisk (*) indicates rows where the 95% confidence interval excludes zero.
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients with adverse event

For details on TEAEs by system organ class (comparing the TEAEs that occurred in patients who participated in trial comparing the risk difference between those who received velmanase and those who received placebo), refer to Section 17.1.

7.6.1.5. Laboratory Findings, Trial rhLAMAN-05

Overall Summary

Routine safety monitoring blood work (hematology, clinical chemistry, creatine kinase) were obtained throughout the trial. The review team concluded, other than the SAE of ARF (discussed in Section 7.6.1.3), there are no clinically meaningful differences between lab values for the treatment and placebo groups.

Hematological Analyses

The review team focused on the following hematological bloodwork obtained during this trial: leukocytes, hemoglobin, and platelets. The mean values and change over time of each mean value were reviewed. When significant outliers were identified, they were reviewed at the individual patient level to assess for clinical significance.

- The mean values for hemoglobin remained normal throughout exposure to velmanase.
- The platelet mean value remained normal throughout exposure to velmanase. Of note a single patient (RHLAMAN-05-(b) (6)) experienced a single abnormally low platelet value of 33x10^9/L). All other platelet values for this patient were within normal range, and no thrombocytopenia related clinical events were observed. The review team concluded that there is no evidence for significant risk of thrombocytopenia with velmanase treatment identified.
- The leukocyte mean value remained normal throughout exposure to velmanase. Outlier leukocyte values (defined as leukocytes <3 x10^9/L) were reviewed at the individual patient level. One patient (RHLAMAN-05-(b) (6)) experienced borderline leukopenia during course of trial. White blood cells ranged from 2.3-8.8 x10^9/L with mean 3.4x10^9/L and median 3.2x10^9/L. This patient was borderline leukopenic at baseline (3.62x10^9/L) prior to receiving velmanase treatment. This patient did not experience any serious infections during the course of trial. The review team concluded that there is no evidence for significant risk of leukopenia with velmanase treatment.

For details regarding these specific outlier laboratory values refer to Table 86 in Section 17.1.

Liver Function

The mean values for alkaline phosphatase and total bilirubin remained within normal during exposure to velmanase. alanine aminotransferase (ALT) mean value was mildly elevated at baseline assessment (prior to velmanase or placebo exposure) in both the treatment and placebo groups. In the treatment group at week 40 and 48 the ALT mean value was mildly elevated at 156 U/L and 158 U/L, respectively. In the placebo group at week 24 and week 40 the ALT mean value was mildly elevated at 161 U/L and 169 U/L respectively. There were no individual outliers with an alkaline aminotransferase value at >2x upper limit of normal. There is no clinically meaningful difference in liver function between treatment and placebo groups identified.

Kidney Function

The mean values for creatinine and glomerular filtration rate (GFR) reported during scheduled study assessments remained normal during exposure to velmanase. One patient (RHLAMAN-05-(b) (6)) experienced an abnormally elevated creatinine level. Refer to Section 7.6.1.3 for details of this SAE of acute renal failure. The kidney function related laboratory values for patient RHLAMAN-05-(b) (6) related to the episode of ARF were not included by Applicant in the rhLAMAN05 dataset. A table listing the single creatinine and GFR outliers is presented in Appendix (See Section 17.1). Of note Table 87 does not contain the creatinine and GFR values related to the episode of ARF because the Applicant did not include these laboratory values in the rhLAMAN-05 dataset. For details of refer to patient narrative in Section 7.6.1.3.

Other Laboratory Trends

Sodium, potassium, calcium, phosphate, creatine kinase, and amylase levels were followed throughout the trial. In the treatment group the mean values for all of these parameters remained within normal limits during the course of velmanase treatment. Any significant outlier value was assessed on the individual patient level. These outliers are listed in Section 17.1, Table 88. No patient experienced persistent electrolyte abnormalities.

7.6.1.6. Vital Signs, Trial rhLAMAN-05

The review team assessed the vital signs of heart rate and systolic and diastolic blood pressures for patients in the treatment group. Oxygen saturation was not routinely measured during velmanase administration. Because abnormalities in respiratory rate and body temperature may be more reflective of hypersensitivity reactions (including anaphylaxis) and IARs, discussion of these abnormalities when clinically significant are included in the AESI narratives in Section 7.7.1.

No clinically meaningful abnormalities or changes in median heart rates or median systolic or diastolic blood pressures were observed over time in pediatric or adult patients.

Therefore, while vital signs should be monitored during infusions, especially due to the risk for hypersensitivity reactions (including anaphylaxis) and IARs, there were no notable changes in vital signs of pulse and blood pressure noted in Trial rhLAMAN-05.

7.6.2. Safety Results From Uncontrolled Trials, rhLAMAN-10 Integrated Analysis and rhLAMAN-08

The only adequate and well controlled trial in the velmanase development program is Trial rhLAMAN-05. TherhLAMAN-10 Integrated Analysis and Trial rhLAMAN-08 provide uncontrolled data that was reviewed to supplement the controlled data provided by review of rhLAMAN-05.

7.6.2.1. Safety Results From rhLAMAN-10 Integrated Analysis and Trial rhLAMAN-08

All the patient narratives reporting SAEs from rhLAMAN-10 Integrated Analysis and Trial rhLAMAN-08 were reviewed. Two SAEs were assessed as possibly related to velmanase exposure, these include seizure and Henoch-Schonlein Purpura (HSP) (also more recently referred to as Immunoglobulin A vasculitis) episodes. The patient narratives of these SAEs are described below.

Seizures

Patient RHLAMAN-10-(b) (6), a 15-year-old male, experienced 2 episodes of seizures. The patient had no history of seizures prior to velmanase. The first seizure was 7 days after velmanase administration. The patient was found unconscious, "breathless", followed by body stiffness with tonic seizures. The patient regained consciousness and breath after a few minutes. The patient was taken by ambulance to the hospital and treated with IV saline and glucose. At the time of evaluation his electroencephalogram was normal, his laboratory values were reported as normal, and electrocardiogram was normal. He was discharged the following day. The investigator reported this event as an event of "loss of conscious" and possibly related to velmanase. The patient then experienced a second seizure on (b) (6) (which was 6 days after a velmanase infusion) which did not require hospitalization and thus was not considered an SAE. In the ex-US post-market experience (detailed below in Section 7.6.3) there were 2 other patients reported to have seizures (with no prior seizure history). There is concern for risk of seizures related to velmanase exposure. This will be addressed in labelling recommendations to include seizures in the label's adverse reaction (AR).

Henoch-Schonlein Purpura:

Patient CCD-LMZYMAA1-08_(b) (6), a 6-year-old male, was diagnosed with HSP that was assessed by Investigator to not be related to velmanase. The patient received a dose of velmanase one day prior to reported onset of symptoms. This patient did not have a documented infection in the 30 days prior to HSP diagnosis. This patient developed high ADA level.

The literature suggests that in AM the elevated serum oligosaccharides may alter glycoprotein structures to be recognized as "non-self" and induce systemic autoimmune disease (Urushihara et al. 2004). Despite the fact that no cases of HSP in AM patients have been published, the review team assessed that is reasonable that this presentation of HSP may be related to patient's underlying disease of AM, but it cannot be excluded that exposure to velmanase, and resultant development of high ADA level is also related to this HSP presentation. This will be addressed in labelling recommendations to include HSP in the label's AR.

For further details of the rhLAMAN-10 Integrated Analysis safety review including TEAEs, SAEs, vital signs, and laboratory findings see Section 17.2.

For further details of the rhLAMAN-08 safety review including TEAEs, SAEs, vital signs, and laboratory findings see Section 17.3.

7.6.2.2. Safety Results, Integrated Summary of Safety, rhLAMAN-10 Integrated Analysis and Trial rhLAMAN-08

The ISS dataset is the pooling of rhLAMAN-10 and rhLAMAN-08 and provides the most complete data regarding (AESI). The individual protocols for Trials rhLAMAN-05, rhLAMAN-10, and rhLAMAN-08 did not contain a separate data collection or analysis of AESI. The ISS definition for AESI included standardized Medical Dictionary for Regulatory Activities queries (SMQs) selections for 'anaphylactic reaction', 'hypersensitivity', and 'oropharyngeal allergic conditions', and a selection of PTs for potential symptoms associated with hypersensitivity (nausea, vomiting, pyrexia, chills, feeling hot, malaise, hyperhidrosis, and hyperthermia) and IRRs.

Refer to Section 7.7.1 for a discussion of the safety review of the ISS and an assessment of the risks of hypersensitivity (including anaphylaxis) and IRR.

7.6.3. Ex-US Postmarket Experience

Velmanase is approved currently in the EU, Brazil, Ukraine, Israel, Mexico, and Kingdom of Saudi Arabia. On March 25, 2018, marketing authorization was granted for velmanase in the EU and PSURs are required by the EU every 6 months.

PSUR-7

The Applicant submitted PSUR-7 to this BLA, which provides cumulative data from approval in the EU until a data lock point on March 21, 2021, and includes safety information on 49 unique patients. IRRs, immunogenicity, and hypersensitivity have been followed by the Applicant as important identified risk factors AESI and loss of consciousness and acute renal failure have been followed by the Applicant as important potential risks. In this PSUR-7, the Applicant reported 9 adverse drug reactions (ADRs) labeled IRRs (using SMQ "anaphylactic reaction"), of which 3 were considered serious by the Applicant. The serious ADRs are described below. The Applicant also reported 3 ADRs assessed as serious related to hypersensitivity (using SMQ "hypersensitivity").

Serious Infusion-Related Reactions:

- A 1-year-old female patient experienced an episode concerning for anaphylaxis with the following PTs: rash, pharyngeal edema, wheezing within minutes of starting velmanase infusion. Velmanase was withdrawn, and the patient recovered from the event the same day. The Applicant stated this event was probably related to velmanase. The Applicant also separately labeled this serious IRR a hypersensitivity event.
- A 16-year-old female patient experienced an event concerning for anaphylaxis which included angioedema, otodynia, erythema, and edema of eyelids and tongue. The event onset was 23 minutes after velmanase infusion was initiated. The drug dose was stopped prematurely, and the patient was treated with dexchlorpheniramine maleate IV, hydrocortisone IV, and desloratadine IV. After approximately 3 hours the event resolved. The Applicant stated this event was "certainly related to velmanase alfa."

- A 22-year-old male patient experienced bilateral elbow swelling, erythema, and urticaria at an unspecified time after velmanase infusion. These events resolved within a day. The Applicant stated this event as possibly related to velmanase.

The Applicant reports there have been no ADRs related to renal failure or loss of consciousness in the PSUR-7 monitoring period.

The Applicant reports that in the safety data for patients under 6 years of age reviewed from March 23, 2018, to March 22, 2021, three patients reported ADRs. One of these ADRs was the serious IRR described above. The other two are described as follows:

- A 5-year-old male patient experienced asthenia for 2 days (at an unspecified time related to velmanase infusion) and spontaneously recovered. The Applicant stated this event was possibly related to velmanase (per World Health Organization – Uppsala Monitoring Center causality assessment method). The Applicant labeled this event as possibly related to velmanase.
- A 4-year-old male patient experienced worsening hearing loss within the first month of therapy with velmanase that has not recovered. Although the Applicant stated this event was possibly related to velmanase, the team acknowledges hearing loss has been reported as part of natural history of AM (Lehalle et al. 2019) and no further action is needed at this time for this AE.

120-Day Safety Update

The Applicant also submitted a 120-day safety update to this BLA which presents post-marketing (i.e., after approval in the non-US countries listed above) data through July 8, 2022. In the 120-day update, the Applicant describes multiple trials that continued post-approval, registries, PSUR-8, and PSUR-9. Relevant safety data that were not previously discussed in PSUR-7 were discussed here. The programs that contained SAEs or discontinuations are described below.

- The Compassionate Use Program is an option to offer velmanase to patients in locations where the product has not been approved and/or reimbursement was not available to the patient. This program was not designed as a registry or trial.
- After-Trial care program (comprised of rhLAMAN-07 and rhLAMAN-09, which are long-term phase 3b studies with continued monitoring of safety and efficacy in patients who previously participated in velmanase studies) continued following patients' post-approval. In total, the Applicant included data from 13 patients from rhLAMAN-07 and 8 patients in rhLAMAN-09 in the 120-day safety report. An overview of the TEAEs from trials rhLAMAN-07 and rhLAMAN-09 are presented below. The SAEs are described in detail.
- *Programma velmanase* -Home therapy program in Italy.
- Etoile Alpha – French Retrospective Registry

Notable Findings From the 120-Day Safety Update Are Described Below

Deaths

The Applicant reports that a 44-year-old male patient enrolled in the Programma Lamzede-Home therapy died on (b) (6), from cardiac arrest. The last dose of velmanase prior to death was on (b) (6) The reporter considered this death not related to velmanase.

Treatment-Emergent Adverse Events (TEAEs)

Trial rhLAMAN-07 - The Applicant reported that a total of 185 TEAEs occurred in 12 of the 13 patients in rhLAMAN-07. Of these 12 patients, 5 patients experienced 14 ADRs of which 2 were labeled serious ADRs by the Applicant. The 2 SAEs were diarrhea resulting in hypokalemia which required hospitalization for hypokalemia treatment and an episode of vomiting requiring hospitalization for IV hydration.

Trial rhLAMAN-09 - The Applicant reported a total of 237 TEAEs occurred in 8 patients in rhLAMAN-09. Of these TEAEs, the Applicant labeled 4 ADRs. All 4 of the ADRs per the Applicant were non-serious, mild, and resolved without any specific treatment. These 4 ADRs were experienced by 2 unique pediatric patients.

PSUR-8 - The Applicant reports 2 serious ADRs (petit mal seizure and angioedema). The angioedema ADR occurred within an AESI of anaphylaxis/'anaphylactic reaction'.

PSUR-9 - The Applicant reports 8 serious ADRs which includes hypersensitivity, petit mal epilepsy, cough, nausea, vomiting (2 events), rash, and oxygen saturation decreased. All but the epilepsy serious ADRs occurred within AESI episodes of anaphylaxis/'anaphylactic reaction'.

Seizure Concern

The review team sent an information request (IR) to the Applicant for further clarification of the seizure episodes reported in the velmanase development program. In addition to the patient narrative discussed in Section 7.6.2.1, 2 unique patients (Case 2021CHF04584 Case 2017CHF01421) with no reported seizure history experienced seizures during velmanase treatment.

Discontinuations

Three patients discontinued velmanase, 2 discontinued while enrolled in the After-Trial Care Program (rhLAMAN-07) and 1 discontinued while enrolled in the Compassionate Use Program. For details of these patient discontinuations see Section 17.4.

7.7. Key Safety Review Issues

7.7.1. Hypersensitivity Reactions (Including Anaphylaxis) and Infusion-Related Reactions

Issue

Data from Trials rhLAMAN-05, rhLAMAN-08, and rhLAMAN-10 indicate that there is a risk of hypersensitivity reactions (including anaphylaxis) and IRRs during treatment with velmanase. Enzyme replacement therapies (ERTs), as a class, typically include a boxed warning in labeling for these risks.

Background

As discussed in Section 7.6.2.1, the Applicant conducted an ISS specifically to assess AESI. In this ISS, the definition for AESI included SMQ selections for 'anaphylactic reaction', 'hypersensitivity', and 'oropharyngeal allergic conditions', and selection of PTs for symptoms of hypersensitivity (nausea, vomiting, pyrexia, chills, feeling hot, malaise, hyperhidrosis, and hyperthermia) which were considered as potential IRRs. The Applicant reported that a total of 25 individual patients (out of the 38 unique patients in the velmanase safety database) experienced a total of 182 AESIs. All 25 of the patient narratives of these AESI were reviewed. Additionally, the review team performed a separate analysis of the ISS database using FDA Medical Queries (FMQs). The pertinent findings are discussed below.

Hypersensitivity (Including Anaphylaxis):

The review team performed an independent analysis of the ISS with FMQs. Among the patients treated with velmanase, 50% (19 of 38) experienced symptoms of hypersensitivity reactions (Hypersensitivity FMQ Broad). Table 28 below demonstrates that the most common clinical manifestations, including erythema and rash.

Table 29. Adverse Events of Special Interest, Hypersensitivity FMQ (Broad), Safety Population, Trials rhLAMAN-08 and rhLAMAN-10

	Velmanase		
Hypersensitivity FMQ (Broad)	<18 years N=24 n (%)	≥18 years N=14 n (%)	All N=38 n (%)
AE grouping related to AESI	14 (58.3)	5 (35.7)	19 (50.0)
Asthma	1 (4.2)	0	1 (2.6)
Conjunctivitis allergic	1 (4.2)	0	1 (2.6)
Erythema	5 (20.8)	1 (7.1)	6 (15.8)
Eye oedema	0	1 (7.1)	1 (2.6)
Eyelid oedema	0	1 (7.1)	1 (2.6)
Henoch-Schonlein purpura	1 (4.2)	0	1 (2.6)
Hypersensitivity	2 (8.3)	2 (14.3)	4 (10.5)
Infusion related hypersensitivity reaction	1 (4.2)	0	1 (2.6)
Infusion-related reaction	2 (8.3)	0	2 (5.3)
Oedema	0	1 (7.1)	1 (2.6)
Rash	3 (12.5)	3 (21.4)	6 (15.8)
Swelling face	3 (12.5)	0	3 (7.9)
Urticaria	1 (4.2)	1 (7.1)	2 (5.3)

	Velmanase		
Hypersensitivity FMQ (Broad)	**<18 years N=24 n (%)**	**≥18 years N=14 n (%)**	**All N=38 n (%)**
Maximum severity			
Death	0	0	0
Life-threatening	0	0	0
Severe	0	0	0
Moderate	3 (12.5)	2 (14.3)	5 (13.2)
Mild	11 (45.8)	3 (21.4)	14 (36.8)
Serious	1 (4.2)	0	1 (2.6)
Deaths	0	0	0
Resulting in discontinuation	0	0	0

Source: ISS adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 24 months (40 months for patient #(b) (6) enrolled in (b) (6)) for Trial rhLAMAN-08. Duration is 12 to 48 months for Trial rhLAMAN-10.
Abbreviations: AE, adverse event; AESI, adverse events of special interest; FMQ, FDA Medical Query; N, number of patients in treatment arm; n, number of patients with adverse event

The review team reviewed the narratives of the 25 patients the Applicant flagged as experiencing AESI. Based on the review team's assessment of the narratives of the 25 patients who had AESI, 2 patients (Patient CCD-LMZYMAA1-08-(b) (6) and Patient CCD-LMZYMAA1-10-(b) (6)) who received velmanase met Sampson's criteria for anaphylaxis (Sampson et al. 2006) and 3 patients were determined to have had potential anaphylaxis. These patient narratives are described in detail below.

Some of these patients received pre-treatment and some did not. Where this information was provided, it is included in the narrative.

Narratives of Patients Assessed as Having Anaphylaxis:

The following narratives of two patients are assessed by the review team as having anaphylaxis.

CCD-LMZYMAA1-08-(b) (6)

- A 5.9-year-old male patient enrolled in Trial rhLAMAN-08
- On day the 374th day of treatment, 65 minutes after the start of drug infusion experienced oxygen desaturation (oxygen saturation decreased to low of 76%). Velmanase infusion was stopped. The patient was treated with supplemental oxygen (inhalation at a flow of 1.5mL/minute), dexchlorpheniramine maleate (5 mg IV) and sodium chloride (15 mL/kg IV). The Applicant reported this event as serious (prolonging hospitalization) and considered related to velmanase infusion.
- On day 463, 75 minutes after the start of study of drug infusion the patient experienced cyanosis. The patient was treated with methylprednisolone acetate 60 mg IV. The Applicant reported this event as related to the drug. Prior to this episode patient had received premedication with dexchlorpheniramine maleate 5 mg weekly IV drip and paracetamol 400 mg IV.
- On day 477, 65 minutes after the start of the study drug infusion, the patient experienced cyanosis. The patient was treated with supplemental nasal oxygen and methylprednisolone

acetate 60 mg IV. The Applicant reported this event as related to the drug. Prior to this episode patient had received premedication with dexchlorpheniramine maleate 5 mg weekly IV drip and paracetamol 400 mg IV.

The review team sent an information request to clarify why these AEs were not assessed as anaphylaxis. The Applicant disagreed with classification of narrative as anaphylaxis. The Agency will classify episode on day 374 as anaphylaxis because it fulfills the Sampson criteria of respiratory compromise (hypoxia treated with supplemental oxygen) and assumed reduced blood pressure (patient received fluid bolus) occurring rapidly after exposure to velmanase. Additionally, patient was treated with dexchlorpheniramine maleate (a drug approved in the US for treatment of hypersensitivity reactions) that may have halted further anaphylaxis manifestations.

The subsequent events on day 463 and 477 in which the patient experienced cyanosis shortly after exposure to velmanase are likely anaphylaxis reactions. However, the patient was treated with methylprednisolone IV, a known treatment for acute allergic reactions and adjunctive treatment for anaphylaxis. Such treatment likely prevented further clinical manifestations of anaphylaxis from occurring.

CCD-LMZYMAA1-10-(b) (6)

- A 30-year-old man enrolled in Trial rhLAMAN-05 and then continued into rhLAMAN-10.
- On the 317th day of treatment, 165 minutes after the start of the drug infusion experienced hypotension (systolic blood pressure decreased to 89 mm Hg) and 30 minutes later experienced eyelid edema. Pre-infusion the patient's blood pressure was recorded as 131/66. The Applicant reported this event as probably related to velmanase.

The review team sent an information request to clarify why the concomitant AEs of hypotension (systolic blood pressure decreased to 89 mm Hg) and 30 minutes later experienced skin-mucosal tissue involvement (eyelid edema), were not assessed as anaphylaxis. The Applicant disagreed in response with classification of narrative as anaphylaxis. The Applicant did not supply sufficient explanation for why the above episode would not qualify as anaphylaxis per the Sampson criteria and thus the review team classified this patient narrative as anaphylaxis.

Narratives of Patients Assessed as Having Severe Hypersensitivity

The following narratives of three patients are assessed by the review team as cases of severe hypersensitivity. The patients were all treated with medical therapy targeted at preventing anaphylaxis.

CCD-LMZYMAA1-10-(b) (6)

- This 8-year-old female who received treatment for approximately 3 months during rhLAMAN-02 and rhLAMAN-03, and for approximately 29 months during rhLAMAN-05 and rhLAMAN-07.
- On Day 7 of rhLAMAN-05, within 2 hours of infusion stop the patient experienced emesis. The patient was treated with paracetamol 500 mg IV, dexchlorpheniramine 5 mg IV, and budesonide 1 puff inhalation, and methylprednisolone 60 mg IV. Of note the patient received

pretreatment with paracetamol PO (total dose unknown). The Applicant reports this was not related to drug infusion. The patient received pretreatment with desloratadine 5 mg orally.

- On day 351, approximately 55 minutes after the start of drug infusion, the patient experienced emesis. The infusion was paused for 70 minutes. The patient was treated with hydroxyzine 25 mg IV, chlorpheniramine maleate 5 mg IV, and methylprednisolone hemisuccinate 60 mg IV. The Applicant reported this was possibly related to the study drug. The patient received pretreatment with paracetamol 500 mg orally.

The review team sent information request to clarify why these concomitant AEs were not assessed as anaphylaxis. The Applicant's response that treatment with inhaled steroid (budesonide) does not qualify as respiratory compromise presentation is reasonable. The Applicant did not provide a clear explanation for the treatment of emesis on day 7 with antihistamine (dexchlorpheniramine) and steroid (methylprednisolone) and on day 351 with (hydroxyzine and chlorpheniramine) and steroid (methylprednisolone). Antihistamines and steroids are known treatments and/or adjunctive treatments for hypersensitivity and/or anaphylaxis. Without these interventions (antihistamine and steroid) it is unclear if these events would have progressed clinically to anaphylaxis as defined by Sampson criteria. For this reason, these events are assessed as severe hypersensitivity reactions.

CCD-LMZYMAA1-08- (b) (6)

- A 4.1-year-old female enrolled in Trial rhLAMAN-08.
- Of note the patient received premedication prophylaxis with cetirizine hydrochloride 5 mg orally weekly from Study Day 1. On 4 occasions the patient experienced urticaria either during infusion or within 30 minutes after end of infusion and was treated with IV steroid treatment. The patient received premedication with cetirizine hydrochloride prior to each of the following events.
- Day 219 – 30 minutes after end of drug infusion experienced urticaria and was treated with prednisolone 100 mg IV
- Day 303 – 73 minutes after the start of drug infusion experienced urticaria and was treated with prednisolone 100 mg IV once
- Day 408 – 100 minutes after the start of drug infusion experienced urticaria and was treated with prednisolone 100 mg IV once
- Day 570 – 50 minutes after the start of drug infusion experienced urticaria and was treated with prednisolone 125 mg IV once

The Applicant did not record oxygen saturations at the time of the above episodes. Recorded systolic blood pressure values were 78 mm Hg and this did not qualify as hypotensive for age.

The above clinical manifestations do not meet anaphylaxis as defined by Sampson criteria but were treated with IV steroid.

The patient received IV steroids with each presentation of urticaria during velmanase infusion. Although urticaria alone does not qualify as anaphylaxis as defined by Sampson criteria, the possibility that episodes would have progressed to anaphylaxis if not treated by steroid cannot be ruled out. For this reason, these events were assessed as severe hypersensitivity.

CCD-LMZYMAA1-10-(b) (6)

- 15-year-old male patient enrolled in Trials rhLAMAN-02, rhLAMAN-03, rhLAMAN-04, and then rhLAMAN-10 for a total of approximately 52 months of treatment.
- On day 344, at approximately 1 hour after start of drug infusion, the patient experienced a "moderate event of hypersensitivity" that per the Applicant was "definitely related to the study drug and to infusion." The infusion was stopped. The patient was treated with cetirizine q 10 mg orally once, methylprednisolone sodium succinate 80 mg IV once, and paracetamol 1,000 mg orally once. The patient had received infusion premedication prophylaxis with cetirizine hydrochloride 10 mg oral.

During the review of this event, the review team asked the Applicant for further details in order to comprehensively assess this event. No further details regarding the patient's physical exam were provided by Applicant and thus it was not possible for the review team to apply Sampson criteria to review of this narrative. The review team assessed this event as severe hypersensitivity because the Investigator reported this event as hypersensitivity, the patient treated with IV steroids (therapy targeted at treating anaphylaxis), drug infusion was stopped, and Applicant labeled this event as definitely related to velmanase.

Infusion-Related Reactions (Excluding Anaphylaxis and Severe Hypersensitivity)

Hypersensitivity is a broad categorization which encompasses anaphylaxis and may include IRRs. Since the term IRR used by the Applicant implies causality, the review team determined that a more appropriate term is infusion-associated reactions (IARs). To characterize risk of IARs, the ISS was assessed with a custom grouped query term named "Infusion-associated reaction (IAR)" that combined the following FMQ terms: hypersensitivity (broad), anaphylactic reaction (broad), and local administration reaction (broad). In this assessment, the events considered to be anaphylaxis or severe hypersensitivity (described in the above patient narratives) were excluded. Table 29 below, lists the patients who experienced IARs after excluding for episodes of anaphylaxis or severe hypersensitivity described above. In summary, 20 patients experienced IARs varying from mild to moderate severity.

Table 30. Infusion-Associated Reaction (Custom Grouped Query), Safety Population, Trials rhLAMAN-08 and rhLAMAN-10

Hypersensitivity FMQ (Broad) or Anaphylactic Reaction FMQ (Broad) or Local Administration Reaction FMQ (Broad)	Velmanase <18 years N=24 n (%)	Velmanase ≥18 years N=14 n (%)	Velmanase All N=38 n (%)
AE grouping related to AESI	15 (62.5)	5 (35.7)	20 (52.6)
Asthma	1 (4.2)	0	1 (2.6)
Conjunctivitis allergic	1 (4.2)	0	1 (2.6)
Erythema	5 (20.8)	1 (7.1)	6 (15.8)
Eye oedema	0	1 (7.1)	1 (2.6)
Eyelid oedema	0	1 (7.1)	1 (2.6)
Henoch-Schonlein purpura	1 (4.2)	0	1 (2.6)
Hypersensitivity	2 (8.3)	2 (14.3)	4 (10.5)
Infusion related hypersensitivity reaction	1 (4.2)	0	1 (2.6)
Infusion-related reaction	2 (8.3)	0	2 (5.3)
Infusion site swelling	1 (4.2)	0	1 (2.6)
Injection site pain	2 (8.3)	0	2 (5.3)
Oedema	0	1 (7.1)	1 (2.6)
Rash	3 (12.5)	3 (21.4)	6 (15.8)
Swelling face	3 (12.5)	0	3 (7.9)
Urticaria	1 (4.2)	1 (7.1)	2 (5.3)
Maximum severity			
Death	0	0	0
Life-threatening	0	0	0
Severe	0	0	0
Moderate	3 (12.5)	2 (14.3)	5 (13.2)
Mild	12 (50.0)	3 (21.4)	15 (39.5)
Serious	1 (4.2)	0	1 (2.6)
Deaths	0	0	0
Resulting in discontinuation	0	0	0

Source: ISS adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 24 months (40 months for patient #(b) (6) enrolled in (b) (6)) for Trial rhLAMAN-08. Duration is 12 to 48 months for Trial rhLAMAN-10.
Relatedness is determined by the investigator.
Abbreviations: AE, adverse event; AESI, adverse events of special interest; FMQ, FDA Medical Query; N, number of patients in treatment arm; n, number of patients with adverse event

The Applicant defined AESI as Standardized MedDRA Queries (SMQs) selections for 'anaphylactic reaction', 'hypersensitivity', and 'oropharyngeal allergic conditions', and selection of PTs for symptoms of hypersensitivity including nausea, vomiting, pyrexia, chills, feeling hot, malaise, hyperhidrosis, and hyperthermia. When an AESI occurred within 24 hours after the end of velmanase infusion, it was defined as an IAR. The 24-hour cutoff is acceptable, as most IARs are expected to occur within the first 24 hours following exposure (Karimian et al. 2017) and this cutoff is consistent with other ERT programs. The Applicant reported that 19 unique patients (3 adult and 16 pediatric patients) experienced IAR(s) including conjunctivitis, cough, pyrexia, chills, hyperthermia, cyanosis, urticaria, vomiting, malaise, hypersensitivity, rash, erythema, eye pruritis, nausea, hyperhidrosis, eyelid edema, and ocular hyperemia. Labeling will reference these 19 patients and the most frequent symptoms of IARs that occurred in >10% of the patients in the ISS (i.e., pyrexia, chills, erythema, vomiting, cough, urticaria, rash and conjunctivitis).

Per FDA guidance for industry *Warnings and Precautions, Contraindications, and Boxed Warning Sections of Labeling for Human Prescription Drug and Biological Products — Content and Format* (October 2011), the warnings and precautions section should include adverse reactions that have implications for prescribing decisions or for patient management. Since IARs may impact both prescribing decisions (such as potential premedication therapies) and patient management during velmanase infusion, IARs will be included in the warning and precautions section of the label.

Immunogenicity

Four of the five patients concerning clinically for anaphylaxis or severe hypersensitivity were ADA positive (patient CCD-LMZYMAA1-10-(b) (6) who had clinical concern for anaphylaxis was not ADA positive). Development of ADA may contribute to development of anaphylaxis or severe hypersensitivity in patients exposed to velmanase. See Section 14.4.4for further details.

Conclusion

Hypersensitivity reactions (including anaphylaxis) and IARs are known risks with ERTs. The labeling will include a boxed warning for the risk of hypersensitivity including anaphylaxis. Additionally, labeling will include further details of symptoms of hypersensitivity and IARs in the warning and precautions section because the management of these reactions will impact prescribing decisions (such as premedication(s) and patient management during velmanase infusion).

7.7.2. Assessment of Safety in Patients <6 Years of Age

Issue/Background

Since the only trial in the velmanase development program to include patients less than 6 years of age (Trial rhLAMAN-08) was an uncontrolled, open label trial, the interpretation of the safety data in this age group was somewhat limited by the absence of a control group. Additionally, Trial rhLAMAN-08 did not have any patients less than 3 years of age.

Assessment/Conclusion

During the review cycle the Applicant provided real world data on 4 pediatric patients (from ex-US postmarketing experience) less than 3 years old (age range included 6 weeks to 28 months old) at initiation of velmanase therapy. These data included AEs from all patients and PD information from some of these patients. No PK, immunogenicity, or lab values were provided. Two of these 4 patients had reports of IRR, hypersensitivity, and/or anaphylaxis. One event was assessed as severe because the patient required hospitalization for anaphylaxis. The safety findings in these patients are similar to that observed in Trial rhLAMAN-08 (see Section 7.6.2).

Although the safety data in patients younger than 6 years old (from Trial rhLAMAN-08 and the real-world setting) is uncontrolled, these direct safety data in this age group are sufficient to support the safety of velmanase in this very rare population. Furthermore, the safety profile demonstrated by these data in patients younger than 6 years old is similar to the safety profile demonstrated in Trial rhLAMAN-05, the adequately controlled trial that supports a favorable

benefit/risk determination, which provides further support to a determination of safety in this age group.

There is a concern that the youngest patients may have a higher risk of immunogenicity and hypersensitivity reactions (based on known safety risks for ERTs as a class); however, these safety concerns will be addressed comprehensively in labeling.

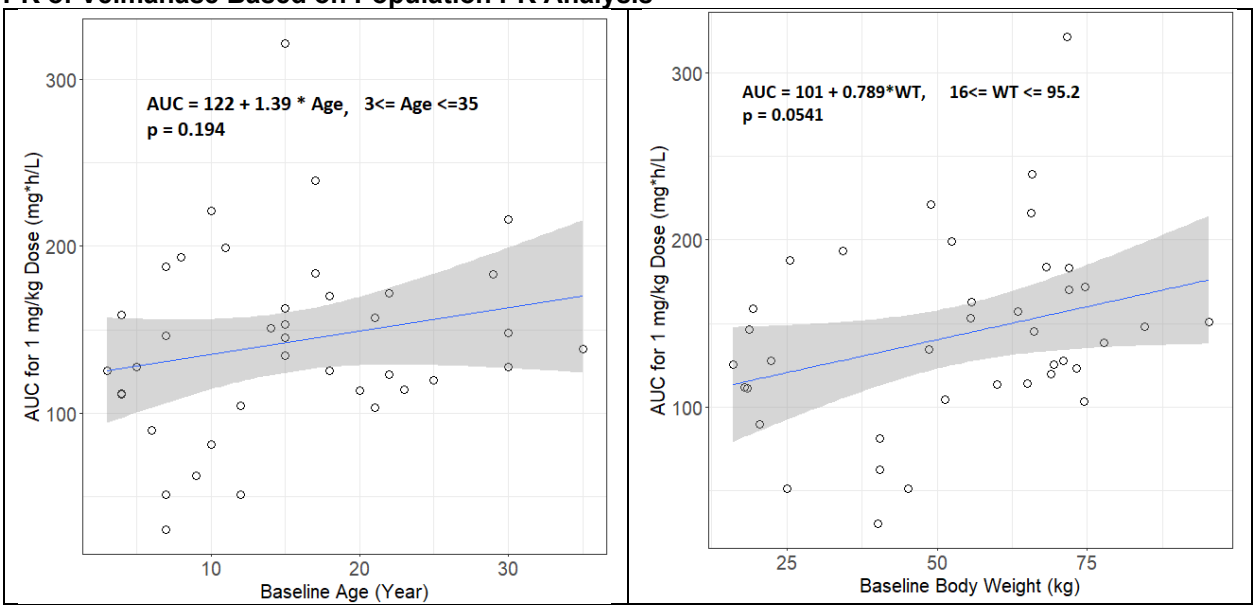
8. Therapeutic Individualization

8.1. Intrinsic Factors

The recommended dosage regimen (i.e., 1 mg/kg administered once every week via IV infusion) for velmanase in patients with AM is based on individual patient's body weight. The current available data does not support a recommendation for further dose adjustment based on other intrinsic factors.

Based on PK data from Trials rhLAMAN-02, -03, -04, -05, -08, -09 and -10, a 3-compartment PK model with 1st order elimination best described the PK of velmanase. Immunogenicity (i.e., development of ADA) and body weight were identified as significant covariates on clearance of velmanase in the population PK analysis. Covariate analysis suggested approximately 20% decrease in exposure in patients with body weight ≤18 kg in reference to subjects with a body weight of 60 kg which is not considered clinically meaningful based on the currently understanding of the exposure-response relationship of velmanase in patients with AM. Covariate analysis also suggested approximately 33% decrease in exposure in ADA-positive patient with median ADA level of 3.1 U/mL in reference to ADA negative subjects; however, dose adjustment based on ADA status has not been evaluated in patients with AM and is generally not a recommended approach for ERT. The effect of age and body weight on PK of velmanase is shown in Figure 19. Refer to Clinical Pharmacology (Section 14.5) for the details of the population PK analysis results.

Figure 19. The Effect of Age (Left Panel; 3-35 Years) and Body Weight (Right Panel; 16-95 Kg) on PK of Velmanase Based on Population PK Analysis



Source: *Reviewer's Analysis.*
Abbreviations: AUC, area under the concentration-time curve; PK, pharmacokinetics; WT, weight

8.1.1. Renal and Hepatic Impairment

The effect of renal impairment or hepatic impairment on the PK velmanase has not been studied in dedicated clinical pharmacology studies. Intact velmanase (molecular weight of approximately 130 kDa) is unlikely to be filtered by kidney or excreted in urine. Metabolism by cytochrome P450 enzymes or secretion into bile is generally not a significant contributor to the elimination of therapeutic proteins such as velmanase.

8.2. Drug Interactions

No specific drug-drug interaction studies were conducted with velmanase.

8.3. Plans for Pediatric Drug Development

Not applicable. Pediatric studies have already been conducted.

8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential

No studies have been conducted in pregnant or lactating females.

9. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review

Human Subjects Protections

As stated by the Applicant, the clinical trials were conducted in substantial conformance with International Council for Harmonization good clinical practice requirements and with applicable country and/or local statutes and regulations regarding ethical committee review, informed consent, and protection of human subjects participating in biomedical research.

Clinical Site Inspections

FDA clinical site inspections were performed at two sites in Europe for trials rhLAMAN-05 and rhLAMAN-08 (one in Denmark and one in Germany) (refer to Section 22 for details).

Financial Disclosure

The Applicant adequately disclosed financial interests/arrangements with clinical investigators as recommended in the guidance for industry *Financial Disclosure by Clinical Investigators* (February 2013) (see Section 25), and by 21 CFR 54.4. None of the investigators of the 2 studies (rhLAMAN-05 and rhLAMAN-08) were employed by the Applicant, and none disclosed financial interests with the Applicant. In conclusion, the likelihood that trial results were biased on financial interests is minimal and should not affect the approvability of the application.

10. Advisory Committee Summary

Not Applicable.

11. Product Quality

Approval With a PMC

The Office of Pharmaceutical Quality (OPQ), CDER, review team has assessed BLA 761278 with respect to chemistry, manufacturing, and controls (CMC) and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports. As such, OPQ recommends approval of this BLA from a quality perspective.

Lamzede (velmanase alfa-tycv) is a recombinant human lysosomal alpha-mannosidase developed as an enzyme replacement therapy for the treatment of pediatric and adult patients with confirmed diagnosis alpha-mannosidosis. (b) (4) Velmanase alfa drug substance is a clear to slightly opalescent solution (b) (4) and lyophilized to manufacture the velmanase alfa drug product.

Velmanase alfa drug product is supplied as 10 mg lyophilized powder (white to off-white powder) in a 10 mL glass vial, which contains 10 mg velmanase alfa, (b) (4), (b) (4), 227.5 mg mannitol, and 10.1 mg glycine. Each vial is reconstituted with 5 mL sterile water for injection to 2 mg/mL velmanase alfa, (b) (4) 45.5 mg/mL mannitol, and 2.02 mg/mL glycine, at a pH of (b) (4) for intravenous infusion. The stability data support an expiration dating period of (b) (4) months for velmanase alfa-tycv drug substance when stored at (b) (4) and an expiration dating period of 36 months for lyophilized velmanase alfa-tycv drug product when stored at 5 ± 3°C.

The CMC PMC and (any other post-approval quality agreements) between OPQ and the Applicant are listed below should be included in the action letter.

- Submit a risk assessment of the extractables identified for the drug substance container closure system and drug product container closure system, including a risk assessment of the threshold of toxicological concern, acceptable daily exposure, and/or permissible daily exposure.
- Submit the leachables study protocol as well as the time zero report.
- Implement a drug product release specification for deliverable volume (e.g., according to United States Pharmacopoeia <697>)
- Develop, validate, and implement a test method with justified numerical acceptance criteria to reliably detect and control for the presence of Chinese hamster ovary lysosomal enzyme alpha-mannosidase in the final velmanase alfa drug substance.
- Implement a cell-based potency assay in drug product release specifications with pre-defined acceptance criteria.
- Develop, validate, and implement a test method with justified numerical acceptance criteria for (b) (4) during drug product release and stability testing. (b) (4) If applicable, justification and data supporting the use of a synthetic substrate, and its relevance to the natural substrate, will be provided. The method validation report for the (b) (4) assay, the revised drug product release and stability specifications, and all supporting studies and data will be provided in a final study report per 21 CFR 601.12.
- Implement (b) (4) prior to vial fill at (b) (4) mg/mL. Include "gross content of protein content per vial" in the drug product release specification to control the total amount of velmanase alfa in the final vial.
- Conduct a worst-case drug product transport qualification study shipping 10 mg vials of velmanase alfa drug product from Chiesi Farmaceutici S.p.A. in Italy to distribution sites in the USA. Perform product quality testing on the final shipped velmanase alfa drug product to support purity and potency after worst-case shipping conditions.
- Develop and validate a titering anti-drug antibody (TADA) assay as recommended the FDA guidance for industry Immunogenicity Testing for Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection (February 2019). This TADA assay will be used to test available confirmed anti-drug antibody positive samples

from Trials rhLAMAN-07, rhLAMAN-08, rhLAMAN-09, and rhLAMAN-10 and subsequent phase 4 studies to complement and replace the current rabbit anti-velmanase alfa reference standard-based semi-quantitative ADA assay. Provide a final validation report detailing the performance of the TADA assay.

- Develop and validate cell-based neutralizing antibody (NADA) assay to test inhibition of velmanase alfa enzyme uptake into target cells. This NADA assay will be used to test available confirmed anti-drug antibody positive samples from clinical Trials rhLAMAN-07, rhLAMAN-08, rhLAMAN-09, and rhLAMAN-10 and subsequent phase 4 studies. Provide a final validation report detailing the performance of the cell-based NADA assay.

11.1. Device or Combination Product Considerations

Not Applicable.

III. Additional Analyses and Information

12. Summary of Regulatory History

The Applicant, Chiesi Farmaceutici S.p.A. (Chiesi), has developed velmanase alfa (non-proprietary proper name velmanase alfa-tycv), also referred to during development by the Chiesi product development code CHF-LMZYMAA1, for the treatment of patients with confirmed diagnosis of alpha-mannosidosis (AM). Velmanase alfa (velmanase) is recombinant human alpha-mannosidase produced by recombinant DNA technology in a Chinese hamster ovary expression system. It is supplied as lyophilized powder reconstituted for intravenous (IV) infusion to be administered once weekly. Velmanase has previously been approved as a therapy for AM in the European Union (EU), Brazil, Israel, Saudi Arabia, and Ukraine.

Velmanase was granted orphan drug designation by the Agency on February 2, 2006.

The first meeting pertaining to velmanase was held under Pre-IND 113186 between the original Sponsor, Zymenex A/S, and the FDA's Division of Gastroenterology and Inborn Error Products (DGIEP). The type B meeting occurred on February 14, 2012, during which the Agency provided feedback on the nonclinical and clinical plans to support submission of an IND. The Agency advised that sufficient chemistry, manufacturing, and controls (CMC) information and chronic toxicity studies were needed and made recommendations on the proposed phase 2b study. Meeting minutes were issued on March 14, 2012.

In response to an April 2, 2014, meeting request, a type B End of Phase 2 (EOP2) meeting was held on June 3, 2014. The Sponsor discussed their plans for filing the biologics license application (BLA), including the clinical and nonclinical aspects of the development program. While the nonclinical program was deemed acceptable, the Agency did not find the proposed phase 3 trial adequate and sufficient to demonstrate efficacy of the study drug. Meeting minutes were issued on June 24, 2014.

A second type B EOP2 meeting was held on June 17, 2014, to discuss CMC and regulatory aspects of the program. The Agency was not able to provide a thorough assessment of the Sponsor's stability plan due to significant gaps in CMC information and recommended submitting a comprehensive CMC package for review with their commercial manufacturing processes and stability plan. Meeting minutes were issued on July 17, 2014.

Transfer of ownership from Sponsor Zymenex A/S to Chiesi Farmaceutici S.p.A. (Chiesi) occurred on March 14, 2016, and the Agency issued an acknowledgement letter for the change of ownership on June 6, 2016.

A type B pre-IND meeting occurred on June 13, 2018, to discuss the adequacy of the data to support IND submission and a proposed clinical development plan supported by a single pivotal study to assess safety and efficacy of velmanase. The Agency recommended Chiesi request an additional meeting to further discuss potential endpoints, data analyses, and feasible trial designs before submitting the proposed study in an IND. The meeting minutes were issued on June 25, 2018.

A Rare Pediatric Disease designation was granted for velmanase on December 11, 2018.

A type C guidance meeting was held on February 26, 2019, as a follow-up to the meeting held on June 13, 2018. Chiesi obtained feedback on its proposed clinical design and statistical approaches that would provide substantial evidence to support a claim of effectiveness for velmanase. The Agency recommended Chiesi complement their existing clinical and biochemical data from their European trials with a single clinical trial assessing one or more clinically meaningful endpoints in a rigorous and well-controlled manner. Meeting minutes were issued on February 27, 2019.

On August 23, 2019, Chiesi submitted an IND containing the phase 3 protocol CLI-LMZYAA2-01 titled "A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group, Phase 3 Study to Evaluate the Efficacy and Safety of Velmanase Alfa in Patients with Alpha-Mannosidosis (SHAMAN)." This trail was to be the first for velmanase conducted under a U.S. IND as other studies had been conducted outside of the U.S. Following an information request outlining potential clinical hold issues with the IND, an informal teleconference was held on September 16, 2019. During the teleconference, the Agency highlighted that the phase 3 pivotal trial design was not adequate and well-controlled to support a potential marketing application and that the endpoints selection and study duration needed modification to ensure informative data were being collected.

Following the September 18, 2019, receipt of written commitments by Chiesi to amend the proposed protocol for CLI-LMZYMAA2-01, a Study May Proceed letter was issued on September 22, 2019. A revised protocol for CLI-LMZYMAA2-01, version 2.0, was received on October 22, 2019.

A request for Fast Track designation for velmanase was requested by Chiesi on October 25, 2019, and the designation was granted by the Agency on December 12, 2019.

In 2020, responsibilities for the IND for velmanase were transferred from DGIEP to the Division of Rare Diseases and Medical Genetics (DRDMG).

In a type B pre-BLA meeting request received April 26, 2021, Chiesi informed the Agency that the SHAMAN study was stopped in October 2020 because of COVID-19 pandemic related issues, and that no subjects had been screened or enrolled in the trial. Chiesi proposed to use a phase 3 randomized, double-blind, placebo-controlled trial completed in Europe (rhLAMAN-05) as the phase 3 study to support accelerated approval in the U.S. The Pre-BLA meeting was granted to discuss Chiesi's proposal and the design of a proposed confirmatory study. In the teleconference meeting held on June 29, 2021, the Agency expressed concerns about the feasibility of the proposed randomized, placebo-controlled trial as a post-approval confirmatory trial and stated that an alternatively proposed externally controlled, single arm, open-label, post-approval trial would not be acceptable due to the inherently high risk of bias. The Agency recommended that further discussion on the most appropriate regulatory pathway for velmanase and suggested Chiesi request a type C surrogate endpoint (SE) meeting to discuss details of the evidentiary basis for their proposed surrogate endpoint(s) along with the most appropriate regulatory pathway: accelerated approval versus traditional approval. Meeting minutes were issued on July 16, 2021.

As per the Agency's advice, Chiesi requested a type C Surrogate Endpoint meeting to discuss serum oligosaccharides as a surrogate endpoint and the appropriate regulatory pathway option for a future BLA submission. The teleconference meeting was held on January 31, 2022. In the meeting, the Agency acknowledged that the use of serum oligosaccharides as surrogate endpoint

was reasonable, but the assessment was based on limited data in the meeting package and that a final determination of whether serum oligosaccharides would be an acceptable surrogate endpoint would depend on a detailed review of all evidence submitted in the BLA. Meeting minutes were issued on February 11, 2022.

On February 7, 2022, Chiesi submitted a proposed proprietary name request for Lamzede, which was found conditionally acceptable on September 13, 2022.

The marketing application for velmanase, BLA 761278, was received on June 17, 2022. BLA 761278 received a Priority Review determination and was filed under Section 351(a) of the Public Health Service Act on August 16, 2022.

Labeling negotiations for the BLA were opened with Chiesi on September 1, 2022. The Mid-Cycle Communication meeting occurred on September 29, 2022, with minutes issued on October 20, 2022. The proposed nonproprietary name and suffix, velmanase-tycv, was found conditionally acceptable on August 23, 2022. The Late Cycle meeting occurred on December 15, 2022, with meeting minutes issued on January 12, 2023. The BLA was reviewed according to the provisions of "the Program" under the Prescription Drug User Fee Act (PDUFA) VI.

13. Pharmacology Toxicology

13.1. Summary Review of Studies Submitted With the Investigational New Drug Application

Refer to the Nonclinical Review

13.2. Individual Reviews of Studies Submitted With the New Drug Application

Refer to the Nonclinical Review

14. Clinical Pharmacology

14.1. In Vitro Studies

In vitro metabolism or drug interaction studies were not conducted for velmanase.

14.2. In Vivo Studies

14.2.1. Individual Study Summary of Pharmacokinetics of Velmanase

Pharmacokinetics (PK) of velmanase were evaluated in patients with AM in Trials rhLAMAN-02, rhLAMAN-03, rhLAMAN-05, rhLAMAN-10 and rhLAMAN-08. The PK results are summarized in this section.

Trial rhLAMAN-02

Trial rhLAMAN-02 was a single-center, open-label, dose escalation study that evaluated the PK of velmanase following a single dose IV infusion in patients with AM. The study enrolled 10 patients with AM: 7 males and 3 females, 7.6 to 17.5 years of age on the date of dosing. Blood samples for PK analysis were collected pre-dose and at approximately 0.17, 0.5, 1, 2, 4, 6, 24, 48, 72, and 168 hours after the end of the first infusion. The PK parameters are summarized in Table 30.

Table 31. Mean (SD) PK Parameters of Velmanase Following Single Dose IV Infusion in Patients With AM, Trial rhLAMAN-02

Dose	C_{max} (µg/L)	t_{max} (h)	AUC_{inf} (h·µg/L)	AUC_t (h·µg/L)	$AUC_{extr.}$ (%)	$t_{½}$ (h)	V_z (L/kg)	CL (L/h/kg)
6.25 U/kg (N=2)	1,405 (275.8)	0.5 (0.0)	12,221 (7,388)	8,411 (5,784)	33.3 (7.0)	8.950 (6.859)	0.145 (0.032)	0.0146 (0.0087)
12.5 U/kg (N=2)	4,020 (2,036)	1.2 (0.7)	54,684 (14,056)	46,935 (13351)	14.5 (2.4)	16.80 (0.283)	0.173 (0.054)	0.0071 (0.0021)
25 U/kg (N=2)	8,315 (3,656)	1.1 (0.1)	101,930 (27,336)	88,742 (30,304)	13.8 (6.6)	21.10 (2.828)	0.227 (0.035)	0.0076 (0.0021)
50 U/kg (N=2)	13,150 (1,485)	2.7 (1.4)	268,538 (44,872)	241,691 (55,152)	10.5 (5.6)	42.05 (20.44)	0.310 (0.105)	0.0053 (0.0008)
100 U/kg (N=2)	26,600 (0.0)	8.2 (1.3)	697,375 (264,080)	667,378 (244,618)	4.1 (1.2)	47.70 (1.131)	0.316 (0.108)	0.0046 (0.0017)

Source: Table 18, Summary of Clinical Pharmacology Studies; Of note, the average velmanase activity 1 U = 31.25 µg
Abbreviations: AM, alpha-mannosidosis; AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum plasma concentration; IV, intravenous; N, number of subjects; PK, pharmacokinetics; SD, standard deviation; $t_{1/2}$, half-life; t_{max}, time to maximum plasma concentration; V_z, volume of distr bution

Trial rhLAMAN-03

Trial rhLAMAN-03 was a single-center, randomized, open-label, multiple-dose study that evaluated the PK of velmanase following multiple dose administrations. Blood samples for PK analysis were collected pre-dose and at approximately 0.17, 0.5, 1, 2, 4, 6, 24, 48, 72, and 168 hours after the end of the at least 14th infusion dose. The study enrolled 10 patients with AM who had previously been enrolled in Trial rhLAMAN-02. The PK parameters are summarized in Table 31.

The PK results of rhLAMAN-02 and rhLAMAN-03 showed that the plasma area under the concentration-time curve (AUC) for velmanase increased more than proportionally with dose, and the half-life increased with increasing dose levels.

Table 32. Mean (SD) PK Parameters of Velmanase Following Multiple Dose IV Infusions in Patients With AM, Trial rhLAMAN-03

Dose	C_{max} (µg/L)	t_{max} (h)	AUC_{inf} (h·µg/L)	AUC_t (h·µg/L)	$AUC_{extr.}$ (%)	$t_{½}$ (h)	V_z (L/kg)	CL (L/h/kg)
25 U/kg	8,857.5	1.7050	159,120	143,925	11.7	24.40	0.172	0.0
(N=4)	(2,700.5)	(0.2199)	(106,004)	(99,419)	(5.4)	(18.29)	(0.023)	(0.0)
50 U/kg	17,260	2.5780	444,046	407,623	9.0	43.72	0.232	0.0
(N=5)	(2,051.3)	(1.2232)	(139,984)	(140,646)	(4.4)	(16.38)	(0.059)	(0.0)

Source: Table 18, Summary of Clinical Pharmacology Studies; Of note, the average velmanase activity 1 U = 31.25 µg
Abbreviations: AM, alpha-mannosidosis; AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum plasma concentration; IV, intravenous; N, number of subjects; PK, pharmacokinetics; SD, standard deviation; $t_{1/2}$, half-life; t_{max}, time to maximum plasma concentration; V_z, volume of distr bution

Trial rhLAMAN-05

Trial rhLAMAN-05 was a multi-center, double-blind, randomized, placebo-controlled study that evaluated efficacy and safety of velmanase in patients with AM. A total of 25 patients with AM were enrolled (14 males and 11 females, 6.0 to 35.0 years of age) and were randomized in a 3:2 ratio to receive weekly IV infusions of 1 mg/kg velmanase (n=15) or placebo (n=10). PK samples for measurement of serum velmanase concentrations were obtained pre-dose and at approximately 0.17, 1, 2, 24, 72, and 168 hours after the end of the first infusion. The PK parameters are summarized in Table 32.

Table 33. Mean (SD) PK Parameters of Velmanase Following the First Dose IV Infusion of 1 mg/kg in Patients With AM, Trial rhLAMAN-05

Age Group	C_{max} (µg/L)	t_{max} (h)	AUC_{inf} (h·µg/L)	AUC_t (h·µg/L)	$t_{½}$ (h)	Other K_e (L/h)
Overall	8,673	1.90	101,150	88,368	14.85	0.051
(N=15)	(4,557)	(0.95)	(28,410)	(27,528)	(4.22)	(0.017)
<12	6,258	1.36	67,983	61,430	10.10	0.072
(N=4)	(1,919)	(0.70)	(16,296)	(15,435)	(2.51)	(0.018)
12≤18	7,230	2.67	143,858	103,450	20.22	0.034
(N=3)	(3,200)	(0.73)	(1,859)	(48,703)	(1.23)	(0.002)
≥18	10,421	1.88	107,057	96,182	15.89	0.045
(N=8)	(5,414)	(1.00)	(13,964)	(12,403)	(2.80)	(0.007)

Source: Table 18, Summary of Clinical Pharmacology Studies
Abbreviations: AM, alpha-mannosidosis; AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum plasma concentration; IV, intravenous; N, number of subjects; PK, pharmacokinetics; SD, standard deviation; $t_{1/2}$, half-life; t_{max}, time to maximum plasma concentration; V_z, volume of distr bution; Ke, elimination rate constant.

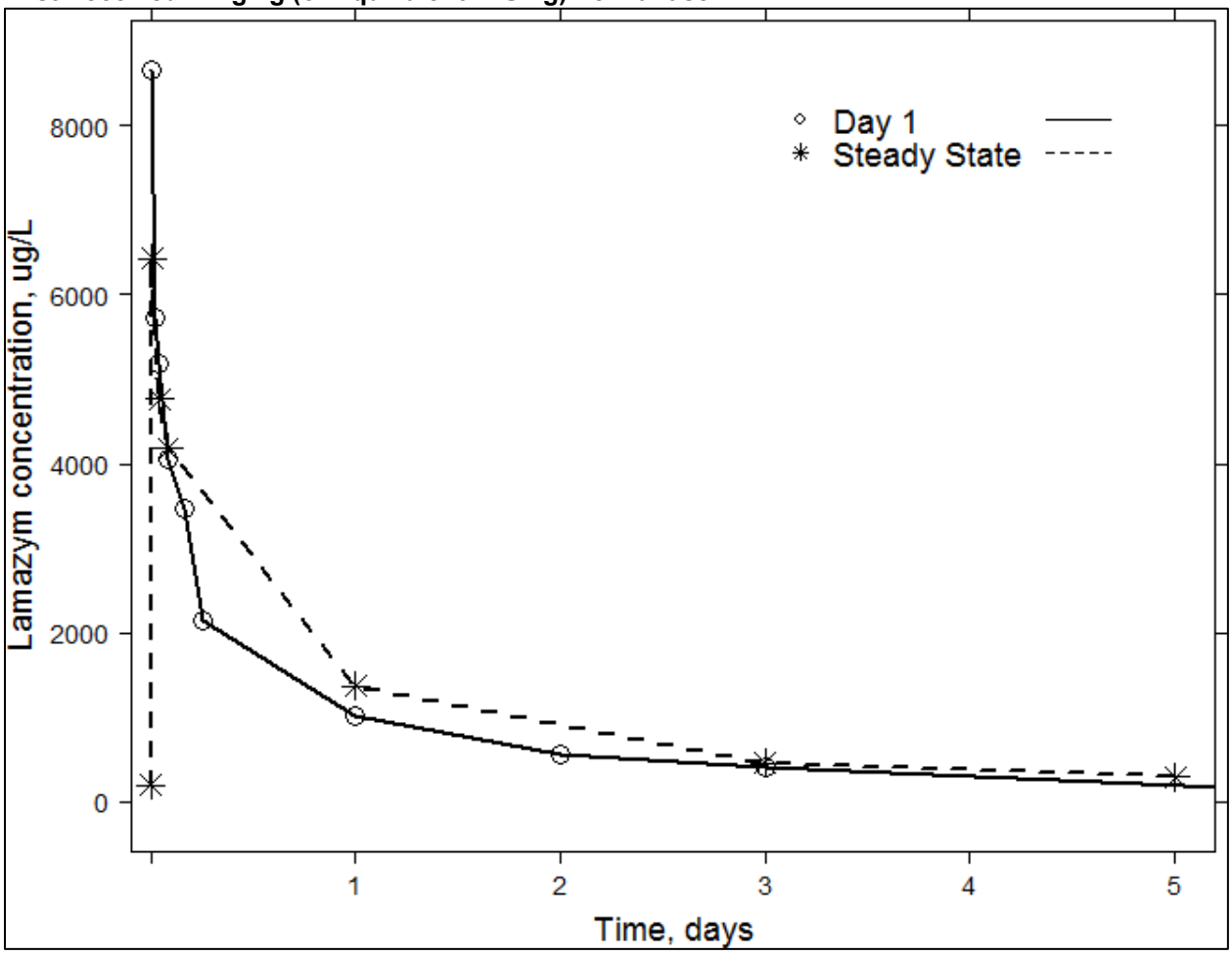
Trial rhLAMAN-10

Trial rhLAMAN-10 was a single-center, open-label study that evaluated the long-term efficacy of velmanase in patients with AM who had previously participated in velmanase clinical studies. The PK analyses included data from subjects entered into Trial rhLAMAN-10 and from the comprehensive evaluation visit from the ongoing Trials rhLAMAN-07 and rhLAMAN-09. At the time of the last PK evaluations in Trials rhLAMAN-07, rhLAMAN-09, and rhLAMAN-10, all subjects had received velmanase at a once-weekly dose of 1 mg/kg for at least 1 year.

The velmanase PK profiles and PK parameters at Day 1 following the first dose administration and at steady are shown in Figure 20 and Table 33, respectively. Day 1 refers to the first velmanase administration and was obtained from rhLAMAN-02 for Subject (b) (6) and from

rhLAMAN-05 for the other 15 subjects. Steady state refers to the last PK assessment (from Trials rhLAMAN-07, rhLAMAN-09, and rhLAMAN-10).

Figure 20. Mean Velmanase Plasma Concentration on Day 1 and at Steady State for Subjects Who First Received 1 mg/kg (or Equivalent in U/kg) Velmanase



Source: Figure 5, Summary of Clinical Pharmacology Studies

Table 34. Mean (SD) PK Parameters of Velmanase Following IV Infusions of 1 mg/kg Velmanase at Day 1 and at Steady State, Trial rhLAMAN-10

PK Parameters		Day 1	Steady State
AUC_t (h·µg/L)	N	16	12
	Mean (SD)	87,052.51 (27,110.75)	143,128.85 (32,730.06)
	Median (Min, Max)	84,924.41 (40,480.54, 134,454.42)	141,186.35 (91,882.00, 202,998.52)
	CV%	31.14	22.87
	Geometric mean	82,771.20	139,614.45
AUC_{inf} (h·µg/L)	N	15	12
	Mean (SD)	99,913.60 (27,792.01)	158,877.64 (38,824.62)
	Median (Min, Max)	97,966.78 (46,911.37, 145,172.35)	155,211.96 (98,340.26, 228,700.71)
	CV%	27.82	24.44
	Geometric mean	95,956.32	154,395.38
AUC_{extr} (%)	N	15	12
	Mean (SD)	10.42 (2.89)	9.54 (2.48)
	Median (Min, Max)	10.28 (6.98, 18.50)	8.91 (6.57, 16.00)
	CV%	27.77	26.04
	Geometric mean	10.10	9.29
C_{max} (µg/L)	N	16	12
	Mean (SD)	8,488.75 (4,463.84)	7,485.00 (1100.33)
	Median (Min, Max)	7,775.00 (4,000.00, 23,200.00)	7,555.00 (5570.00, 9650.00)
	CV%	52.59	14.70
	Geometric mean	7,729.78	7,409.39
t_{max} (h)	N	16	12
	Mean (SD)	1.851 (0.931)	1.781 (0.317)
	Median (Min, Max)	1.750 (0.667, 3.500)	1.658 (1.483, 2.483)
	CV%	50.294	17.777
	Geometric mean	1.714	1.765
CL (L/h/kg)	N	15	12
	Mean (SD)	0.0107 (0.0038)	0.0067 (0.0018)
	Median (Min, Max)	0.0091 (0.0069, 0.0213)	0.0065 (0.0044, 0.0103)
	CV%	35.4021	26.4465
	Geometric mean	0.0102	0.0065
V_z (L/kg)	N	15	12
	Mean (SD)	0.217 (0.037)	0.274 (0.037)
	Median (Min, Max)	0.216 (0.137,0.280)	0.267 (0.209, 0.339)
	CV%	17.130	13.479
	Geometric mean	0.213	0.272

Source: Table 18, Summary of Clinical Pharmacology Studies
Abbreviations: AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum plasma concentration; CV, coefficient of variation; IV, intravenous; N, number of subjects; PK, pharmacokinetics; SD, standard deviation; $t_{1/2}$, half-life; t_{max}, time to maximum plasma concentration; V_z, volume of distr bution

Trial rhLAMAN-08

Trial rhLAMAN-08 was a multi-center, open-label study that evaluated safety and efficacy of velmanase in pediatric patients <6 years of age with AM. The study enrolled 5 patients: 3 males and 2 females, 3.7 to 5.9 years of age at screening. Study subjects received weekly doses of 1 mg/kg velmanase via IV infusion. Blood samples for PK analysis were collected pre-dose and at approximately 0.17, 4, 8, 24, and 46 hours after the end of infusion at the first dose visit and at the 6 months evaluation visit. The PK parameters are summarized in Table 34.

Table 35. Mean (SD) PK Parameters of Velmanase Following IV Infusion of 1 mg/kg in Pediatric Patients With AM, Trial rhLAMAN-08

	C_{max} (µg/L)	t_{max} (h)	AUC_{inf} (h·µg/L)	AUC_t (h·µg/L)	$t_{½}$ (h)	CL (mL/h)
Day 1	8,018.0	2.2	102,878.0	88,854.4	17.4	187.3
N=5	(1,423.42)	(0.98)	(15,635.08)	(11,460.95)	(1.79)	(30.61)
Month 6	7,044.0	1.7	108,320.3	75,865.1	17.4	191.6
N=5	(2,291.89)	(0.08)	(27,053.32)	(39,716.17)	(4.40)	(55.01)

Source: Table 18, Summary of Clinical Pharmacology Studies
Abbreviations: AM, alpha-mannosidosis; AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum plasma concentration; IV, intravenous; N, number of subjects; PK, pharmacokinetics; SD, standard deviation; $t_{1/2}$, half-life; t_{max}, time to maximum plasma concentration

14.2.2. Pharmacodynamics: Effect of Velmanase on Serum IgG in Trial rhLAMAN-5

Individual patients' serum IgG levels at baseline and after treatment with velmanase are provided in Table 35. Three out of ten patients in the placebo group and five out of fifteen patients in the velmanase group had serum IgG levels below the normal range at baseline. None of the three patients in the placebo group, but three out of the five patients in the velmanase group, achieved serum IgG levels within the normal range by Month 12.

Table 36. Individual Patients Serum IgG Levels (g/L) by Visits

			Age at	Serum IgG (g/L)		
Treatment	**Subject No**	**Gender**	**Start**	**Baseline**	**6 Months**	**12 Months**
Velmanase	(b) (6)	F	35	11	13	15
Placebo		M	35	7.1	7.1	7.5
Velmanase		M	30	7.8	11	11.6
Velmanase		M	29	8.9	12.1	12.8
Placebo		**F**	**29**	**5.3**	**5**	**5.9**
Placebo		M	28	6.9	7.6	7.7
Velmanase		M	25	7.1	9.5	9.7
Velmanase		**F**	**23**	**5.8**	**9.4**	**11.3**
Velmanase		F	22	7.3	12	12.1
Placebo		F	21	9.3	10.2	10.8
Velmanase		M	20	12.5	14.3	16.1
Placebo		**M**	**20**	**4.8**	**5.1**	**4.2**
Velmanase		M	20	8.2	11.3	12.5
Velmanase		F	17	10.1	10.5	10.6
Placebo		**F**	**17**	**6.4**	**6.2**	**6.4**
Placebo		F	16	10.1	9.9	7.4
Placebo		F	14	8.3	9.5	10.5
Velmanase		**M**	**14**	**2.6**	**4.2**	**4.9**
Velmanase		**M**	**12**	**3.9**	**5**	**5.8**
Velmanase		**F**	**10**	**5.6**	**6.7**	**7.5**
Velmanase		**M**	**8**	**5.4**	**9.6**	**11**
Placebo		M	11	7.3	7	7.2
Velmanase		M	7	16.5	17.2	22.9
Placebo		M	6	7.2	5.7	5.7
Velmanase		F	6	22	24.7	25.7

Source: Clinical study report of rhLAMAN-05
In bold, subjects had IgG level below the normal range at baseline.
Abbreviations: F, female; M, male; IgG, immunoglobulin G

The PD results indicated that treatment with velmanase increased serum IgG levels in patients with AM. To understand whether the increased serum IgG levels might be associated with any improvement in clinically relevant immune responses, the review team sent an information request (IR) to the Applicant for an analysis evaluating whether treatment with velmanase would have an effect on post-immunization antibody responses using available laboratory data. The Applicant responded that a total of seven patients in rhLAMAN-10 Integrated Analysis and two patients in Trial rhLAMAN-08 received any type of vaccination while in the velmanase clinical development program; however, no information on particular post-vaccination antibody titers is available. For three patients with data on the total IgG level both before and after immunization, there was no remarkable change in IgG levels. Overall, the data were too limited to draw any definitive conclusion regarding the potential effect of velmanase on post-immunization immune response.

14.3. Bioanalytical Method Validation and Performance

14.3.1. PK Assay: Bioanalytical Methods for the Measurement of Velmanase Concentrations in Human Plasma

Bioanalytical methods to determine velmanase concentration in plasma in Trials rhLAMAN-02, rhLAMAN-03, rhLAMAN-05, rhLAMAN-07, rhLAMAN-09, rhLAMAN-10 and rhLAMAN-08 were validated at (b) (4). Summary of life cycle information of assay methods used during development, assay validation parameters, and performance of assays used in clinical trials are provided in Table 36, Table 37, Table 38, and Table 39 below. The method validation was acceptable.

Table 37. Bioanalytical Method Life Cycle Information – Velmanase

Parameter	Method Validation 2010-038-R	rhLAMAN-02 (2010-C-136)	rhLAMAN-03 (2010-C-137)	rhLAMAN-05 (2010-C-139)	rhLAMAN-07 (2014-C-307)	rhLAMAN-09 (2014-C-308)	rhLAMAN-10 (2014-C-299)	rhLAMAN-08 (2016-C-353)
Analyte	Velmanase	Velmanase	Velmanase	Velmanase	Velmanase	Velmanase	Velmanase	Velmanase
Validation type	Full validation	In-study	In-study	In-study	In-study	In-study	In-study	In-study
CTD reference #	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4
Method ID	Draft SOP no. (b) (4) dated 05 October 2010; SOP no. (b) (4) dated 05 January 2011; SOP no. (b) (4) dated 13 January 2011	SOP (b) (4) dated 05 Jan 2011; SOP A (b) (4) dated 13 January 2011	SOP A (b) (4) effective 13 Jan 2011 (b) (4) effective date 13 January 2011	SOP (b) (4) Revision 03, effective date 22 May 2013	SOP (b) (4) Revision 04, effective date 04 February 2015	SOP (b) (4) Revision 04, effective date 04 February 2015	SOP (b) (4) Revision 04, effective date 04 February 2015	SOP, (b) (4) Revision 05, effective date 30 March 2017
Duration of time method is in use	06 October 2010 to 20 October 2010 (2010-038-R) 16 November 2010 to 12 January 2011 (2010-038-V-R-A1)	06 January 2011 to 04 February 2011	12 May 2011 to 14 June 2011	14 May 2013 to 04 June 2013	24 February 2015 to 06 May 2015	12 February 2015 to 22 April 2015	24 February 2015 to 07 July 2015	26 April 2017 to 10 December 2018

Parameter	Method Validation 2010-038-R	rhLAMAN-02 (2010-C-136)	rhLAMAN-03 (2010-C-137)	rhLAMAN-05 (2010-C-139)	rhLAMAN-07 (2014-C-307)	rhLAMAN-09 (2014-C-308)	rhLAMAN-10 (2014-C-299)	rhLAMAN-08 (2016-C-353)
Bioanalytical site	(b) (4)							
Matrix	Human plasma							
Platform	ELISA							
Format	A validated sandwich format using microtiter plates pre-coated with a polyclonal capture antibody against velmanase and an excess of polyclonal antibody-biotin conjugate for detection. The bound antibody-biotin conjugate was then allowed to react with streptavidin- horseradish peroxidase. Bound antibody-enzyme complex was then determined by enzymatic color reaction. Absorbance was measured at 450 nm (reference 540 nm).							
Stock reference, lot number, expiration date	rhLAMAN[a] standard SR-922 PI.1/S019-004 expiry/retest date 26 April 2012	rhLAMAN standard SR-92 PI.1/S019-004 stability ongoing, see expiry date in rhLAMAN-03	rhLAMAN standard SR-922PI.1/S019-004, expiry 26 April 2012rhLAMAN[a] standard SR-922PI.1/S019-004, expiry 26 April 2012rhLAMAN standard SR-922PI.1/S019-004, expiry 26 April 2012 standard SR-922PI.1/S019-004, expiry 26 April 2012	rhLAMAN standard SR-922PI.1/S019-004, expiry 13 November 2013rhLAMAN[a] standard SR-922PI.1/S019-004, expiry 13 November 2013rhLAMAN standard SR-922PI.1/S019-004, expiry 13 November 2013 standard SR-922PI.1/S019-004, expiry 13 November 2013	rhLAMAN Standard SR-922 PL1/S019-004; expiry: November 2015	rhLAMAN Standard SR-922 PL1/S019-004;0 expiry: November 2015	rhLAMAN Standard SR-922 PL1/S019-004; expiry: November 2015	rhLAMAN Standard SR-969PI.2/S0 19-006; expiry: 09 December 2019

Parameter	Method Validation 2010-038-R	rhLAMAN-02 (2010-C-136)	rhLAMAN-03 (2010-C-137)	rhLAMAN-05 (2010-C-139)	rhLAMAN-07 (2014-C-307)	rhLAMAN-09 (2014-C-308)	rhLAMAN-10 (2014-C-299)	rhLAMAN-08 (2016-C-353)
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	0.15 to 10.2 µg/L (curve reading)	LLOQ: 224 µg/L (minimum sample dilution 1:1,000); ULOQ: 7,170 µg/L (at minimum sample dilution 1:1,000) 50,164 µg/L (at dilution 1:7,000)	LLOQ: 224 µg/L (minimum sample dilution 1:1,000); ULOQ: 7,170 µg/L (at minimum sample dilution 1:1,000) 50,164 µg/L (at dilution 1:7,000)	224 µg/L to 50,164 µg/L (at dilutions of 1:1,000 and 1:7,000 respectively, corresponding to curve readings 0.224 µg/L to 7.17 µg/L)	224 µg/L to 50,164 µg/L (at dilutions of 1:1,000 and 1:7,000, respectively, corresponding to curve readings of 0.224 µg/L to 7.17 µg/L)	224 µg/L to 50,164 µg/L (at dilutions of 1:1,000 and 1:7,000, respectively, corresponding to curve readings of 0.224 µg/L to 7.17 µg/L)	224 µg/L to 50,164 µg/L (at dilutions of 1:1,000 and 1:7,000, respectively, corresponding to curve readings of 0.224 µg/L to 7.17 µg/L)	224 µg/L to 50,164 µg/L (at dilutions of 1:1,000 and 1:7,000 respectively, corresponding to curve readings of 0.224 µg/L to 7.17 µg/L)
Matrix study population	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM
Relevant reference and applicable report amendment	2010-038-R; 2010-038-V-R-A1; 2010-038-V-R-A2; 2010-038-V-R-A3; 2010-038-V-R-A4	2010-C-136	2010-C-13	2010-C-139	2014-C-307	2014-C-308	2014-C-299	2016-C-353

Parameter	Method Validation 2010-038-R	rhLAMAN-02 (2010-C-136)	rhLAMAN-03 (2010-C-137)	rhLAMAN-05 (2010-C-139)	rhLAMAN-07 (2014-C-307)	rhLAMAN-09 (2014-C-308)	rhLAMAN-10 (2014-C-299)	rhLAMAN-08 (2016-C-353)
Synopsis of amendment history	2010-038-V-R-A1: amendment to 2010-038-R for the assessment of long-term stability (3 months) and intra- assay drift; 2010-038-V-R-A2: amendment to 2010-038-R to clarify some reported results; 2010-038-V-R-A3: amendment to 2010-038-R for assessment of long-term stability (6 months); 2010-038-V-R-A4 amendment to 2010-038-R for in-study assessment of intra-assay drift; 2010-048-R Assessment stability of coated plates	-	-	2010-C-139 Amendment 1: amendment to 2010-C-139 to present analytical results and repeat analyses (study samples) which were not included in the bioanalytical report	-	-	-	-

Source: Table 5, Summary of Biopharmaceutic Studies and Associated Analytical Methods
[a] rhLAMAN (recombinant human lysosomal alpha-mannosidase) is a former term for velmanase
Abbreviations: AM, alpha-mannosidosis; ELISA, enzyme-linked immunosorbent assay; ID, identification; LSD, lysosomal storage disorder; SOP, standard operating procedure

Table 38. Summary Method Performance Validation of a Method for the Determination of Velmanase in Human Plasma

Method Parameter	Method Information	
Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Analytical Method for Determination of rhLAMAN in Human Plasma Using Enzyme-Linked Immunosorbent Assay (ELISA) Validation Report, Final 17 November 2010	
Method description	Velmanase was determined in human plasma by using an ELISA method. Appropriately diluted plasma samples were incubated in microtiter plates pre- coated with a polyclonal capture antibody raised against velmanase. After this incubation step and subsequent washing, an excess of a detecting polyclonal antibody-biotin conjugate was added and allowed to react with captured velmanase. After this step, excess antibody-biotin was removed by washing, and bound antibody-biotin conjugate was then allowed to react with streptavidin- horseradish peroxidase. Bound antibody-enzyme complex was then determined by means of an enzymatic color reaction. Absorbance was measured at 450 nm (reference 540 nm).	
Materials used for standard calibration curve and concentration	velmanase reference standard SR-922Pl.1/S019-004	
Validated Assay Range	0.148 (anchor), 0.237, 0.380, 0.608, 0.973, 1.56, 2.49, 3.98, 6.38, and 10.2 µg/L (anchor); LLOQ: 224 µg/L (diluted 1:1,000, curve reading 0.224 µg/L); ULOQ: 50,164 µg/L (diluted 1:7,000, curve reading 7.17 µg/L)	
Material used for quality controls (QCs) and concentration	rhLAMAN reference standard SR-922Pl.1/S019-004; QC_{LOW}: 705 µg/L (analyzed diluted 1:1,000, curve reading 0.705 µg/L); QC_{MED}: 5,016 µg/L (analyzed diluted 1:4,000, curve reading 1.25 µg/L); QC_{HIGH}: 25,500 µg/L (analyzed diluted 1:5,000, curve reading 5.10 µg/L)	
Minimum required dilutions (MRDs)	Samples can be diluted up to 1:160,000 (=10,000×16). This gives a measuring range of LLOQ times MRD to ULOQ times maximum dilution (224 µg/L to 1.15 g/L).	
Source and lot of reagents	SuperBlock® Blocking buffer in TBS: Thermo Scientific/Pierce Biotechnology, lot: LD145506; Human plasma pool: ID 20100810, screened negative for hepatitis B and human immunodeficiency virus; rhLAMAN antibody: Zymenex, lot: LAF-09; Biotin-labelled rhLAMAN antibody: Zymenex, lot: BIO-LPA03 and BIO- LPA10; Biotin-labelled rhLAMAN antibody: Zymenex, lot: BIO-LPA03 and BIO- LPA10; Biotin-labelled rhLAMAN antibody: Zymenex, lot: BIO-LPA03 and BIO- LPA10; antibody: Zymenex, lot: BIO-LPA03 and BIO- LPA10; One-Step Ultra™ TMB-ELISA substrate: Thermo Scientific/Pierce Biotechnology, lot: LF1292303, LD1292301, LH1324622; Streptavidin-horseradish peroxidase: MABTECH AB, lot: 40094-9	
Regression model and weighting	The calibration was fitted by StatLIA (Brendan Technologies) using a 5-parameter-logistic-curve algorithm.	
Validation Parameters		
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	10
	Cumulative accuracy (%bias) from LLOQ to ULOQ	95.9% to 105.3%
	Cumulative precision (CV%) from LLOQ to ULOQ	1.4% to 5.7%

Method Parameter	Method Information	
Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Analytical Method for Determination of rhLAMAN in Human Plasma Using Enzyme-Linked Immunosorbent Assay (ELISA) Validation Report, Final 17 November 2010	
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs QC_{LOW}: 705 µg/L (diluted 1:1,000 resulting in curve reading 0.705 µg/L) QC_{MED}: 5,016 µg/L (diluted 1:4,000 resulting in curve reading 1.25 µg/L) QC_{HIGH}: 25,500 µg/L (diluted 1:5,000 resulting in curve reading 5.10 µg/L)	89.8% to 102.8%
	Inter-batch CV% QC_{LOW}: 705 µg/L QC_{MED}: 5,016 µg/L QC_{HIGH}: 25,500 µg/L	 ≤12.2% ≤9.3% ≤12.7%
	Total error QC_{LOW}: 705 µg/L QC_{MED}: 5,016 µg/L QC_{HIGH}: 25,500 µg/L	 ≤13.7% ≤12.1% ≤18.1%
Selectivity and matrix effect	Selectivity includes specificity and matrix effect (hemolysis and lipemic effect)	
Interference and specificity	Samples of control matrix from 10 subjects were analyzed in triplicate. The same 10 control matrix samples were spiked to a concentration near the LLOQ (340 µg/L) and analyzed in triplicate. Accuracy (specificity) of samples spiked to near LLOQ level was between 85.8% and 113.6%, with a mean accuracy of 97.7%.	
Hemolysis effect	One sample of control matrix was spiked with velmanase to the concentration level of QC_{MED}. The sample was divided into 4 aliquots: 1 reference (baseline) sample and 3 aliquots with added hemolyzed red blood cells (approximate hemoglobin concentration of <1, 3, and 6 g/L, respectively). The samples were assayed in triplicate in 1 run. The 3 plasma samples spiked with hemolyzed red blood cells had mean hemoglobin concentrations of 1.3, 3.8, and 7.7 g/L, and showed accuracies compared to non-hemolyzed sample (mean hemoglobin concentration of 0.3 g/L) of 105.9, 94.9, and 85.6%, respectively. Moderate degrees of hemolysis in human plasma samples (up to 7.7 g/L hemoglobin) did not affect the analysis.	
Lipemic effect	Not Performed	
Dilution linearity and hook effect	For the high-dilutional-linearity sample (ULOQ sample spiked at 50,164 µg/L) the accuracy was between 90.4% and 96.7% for all 5 dilutions. Precision was between 0.8% and 3.1% for 4 of the 5 dilutions, and 23.7% for the second-to-last dilution. Since this elevated mean CV% mainly resulted from poor precision for 1 of the 3 replicates, it was not considered relevant. For the medium-dilutional-linearity sample (QC_{MED} spiked at 5,016 µg/L), the accuracy was between 93.0% and 102.1% for all 5 dilutions. Precision was between 1.6% and 6.9%. Samples can be diluted up to 1:160.000 (=10,000×16). This gives a measuring range of LLOQ times MRD to ULOQ times maximum dilution (224 µg/L to 1.15 g/L).	

Method Parameter	Method Information
Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Analytical Method for Determination of rhLAMAN in Human Plasma Using Enzyme-Linked Immunosorbent Assay (ELISA) Validation Report, Final 17 November 2010
Bench-top/process stability	QC_{LOW} and QC_{HIGH} samples were stored at room temperature for approximately 4 and 24 hours and analyzed immediately without any freezing step. One sample (baseline) was analyzed immediately after spiking to confirm that the spiking was valid. Velmanase in human plasma was stable at room temperature for up to 24 hours. At 24 hours, accuracy was 102.3% to 102.6% and precision ≤7.1%.
Freeze-thaw stability	Velmanase in human plasma was stable for up to 5 freeze/thaw cycles at ≤-16°C (accuracy: 98.6% to 99.6%, precision ≤12.2%) and at ≤-70°C (accuracy 83.0% to 93.5%, precision ≤11.8%).
Long-term storage	Velmanase in human plasma was stable for up to 6 months when stored at ≤-16°C and at ≤-70°C.
Parallelism	Not Performed
Carryover	Not Applicable
Method Performance in rhLAMAN-02 (2010-C-136)	
Assay passing rate	100% ISR passing rate
Standard curve performance	Cumulative bias range: 97.4% to 103.0% Cumulative precision: ≤4.1% CV
	Cumulative bias range: 88.9% to 101.9% Cumulative precision: ≤9.4% CV Total error: ≤19.5% (low)
QC performance	ISR was performed in 10% of study samples (14 samples); 100% of the samples met the pre-specified criteria.
	Study sample analysis/stability: 32 days
	100% ISR passing rate
Method reproducibility	Cumulative bias range: 97.4% to 103.0% Cumulative precision: ≤4.1% CV
Study sample analysis/stability	Cumulative bias range: 88.9% to 101.9% Cumulative precision: ≤9.4% CV Total error: ≤19.5% (low)
Standard calibration curve performance during accuracy and precision runs	10 from LLOQ to ULOQ (including 2 anchor points): 0.15 (anchor), 0.24, 0.38, 0.61, 0.97, 1.56, 2.49, 3.98, 6.38, and 10.20 µg/L (anchor)
Method Performance in rhLAMAN-03 (2010-C-137)	
Assay passing rate	100%
Standard curve performance	Cumulative bias range: 96.0 to 104.2%
	Cumulative precision: ≤4.8% CV
QC performance	Cumulative bias range: 95.4 to 99.3%
	Cumulative precision: ≤7.9% CV
	Total error: ≤11.4%
Method reproducibility	ISR was performed in rhLAMAN-02 (2010-C-136); no further ISR has been performed in this study.

Method Parameter	Method Information
Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Analytical Method for Determination of rhLAMAN in Human Plasma Using Enzyme-Linked Immunosorbent Assay (ELISA) Validation Report, Final 17 November 2010
Study sample analysis/stability	43 days
Standard calibration curve performance during accuracy and precision runs	10 from LLOQ to ULOQ (including 2 anchor points).
Method Performance in rhLAMAN-05 (2010-C-139)	
Assay passing rate	100%
Standard curve performance	Cumulative bias range: 98.1 to 102.1%
	Cumulative precision: ≤4.3% CV
0QC performance	Cumulative bias range: 87.6 to 95.5%
	Cumulative precision: ≤7.5% CV
	Total error: ≤19.5%
Method reproducibility	Incurred sample reanalysis was not performed in this study.
Study sample analysis/stability	178 days (longest number of days between collection date and analysis date)
Standard calibration curve performance during accuracy and precision runs	10 from LLOQ to ULOQ (including 2 anchor points): 0.15 (anchor), 0.24, 0.38, 0.61, 0.97, 1.56, 2.49, 3.98, 6.38, and 10.20 µg/L (anchor)
Method Performance in rhLAMAN-07 (2014-C-307)	
Assay passing rate	85.7%
Standard curve performance	Cumulative bias range: 97.4% to 103.5%
	Cumulative precision: ≤5.9% CV
QC performance	Cumulative bias range: 105.9% to 106.6%
	Cumulative precision: ≤7.0% CV
	Total error: ≤13.4%
Method reproducibility	Incurred sample reanalysis was not performed in this study.
Study sample analysis/stability	10 days
Standard calibration curve performance during accuracy and precision runs	10 from LLOQ to ULOQ (including 2 anchor points): 0.15 (anchor), 0.24, 0.38, 0.61, 0.97, 1.56, 2.49, 3.98, 6.38, and 10.20 µg/L (anchor)
Method Performance in rhLAMAN-09 (2014-C-308)	
Assay passing rate	100%
Standard curve performance	Cumulative bias range: 99.4% to 102.6%
	Cumulative precision: ≤4.7% CV
QC performance	Cumulative bias range: 106.4% to 107.3%
	Cumulative precision: ≤8.6% CV

Method Parameter	Method Information
Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Analytical Method for Determination of rhLAMAN in Human Plasma Using Enzyme-Linked Immunosorbent Assay (ELISA) Validation Report, Final 17 November 2010
	Total error: ≤15.9%
Method reproducibility	Incurred sample reanalysis was not performed in this study.
Study sample analysis/stability	11 days
Standard calibration curve performance during accuracy and precision runs	10 from LLOQ to ULOQ (including 2 anchor points): 0.15 (anchor), 0.24, 0.38, 0.61, 0.97, 1.56, 2.49, 3.98, 6.38, and 10.20 µg/L (anchor)
Method Performance in rhLAMAN-10 (2014-C-299)	
Assay passing rate	77%
Standard curve performance	Cumulative bias range: 99.2% to 102.1%
	Cumulative precision: ≤4.2% CV
QC performance	Cumulative bias range: 102.3% to 108.0%
	Cumulative precision: ≤6.8% CV
	Total error: ≤14.8%
Method reproducibility	Incurred sample reanalysis was not performed in this study.
Study sample analysis/stability	28 days
Standard calibration curve performance during accuracy and precision runs	10 from LLOQ to ULOQ (including 2 anchor points): 0.15 (anchor), 0.24, 0.38, 0.61, 0.97, 1.56, 2.49, 3.98, 6.38, and 10.20 µg/L (anchor)
Method Performance in rhLAMAN-08 (2016-C-353)	
Assay passing rate	83%
Standard curve performance	Cumulative bias range: 98.4% to 102.6%
	Cumulative precision: ≤4% CV
QC performance	Cumulative bias range: 99.1% to 112.7%
	Cumulative precision: ≤11.3% CV
	Total error: ≤22.7%
Method reproducibility	Incurred sample reanalysis was not performed in this study.
Study sample analysis/stability	98 days
Standard calibration curve performance during accuracy and precision runs	10 from LLOQ to ULOQ (including 2 anchor points): 0.15 (anchor), 0.24, 0.38, 0.61, 0.97, 1.56, 2.49, 3.98, 6.38, and 10.20 µg/L (anchor)

Source: Table 6, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: CV, coefficient of variation; ID, identification; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification

The PK assay was validated to estimate the threshold level for interference by anti-velmanase antibodies on the accuracy of measurement of different plasma concentrations of velmanase. The results showed that there was no interference at 5% of C_{max} at up to 30.2 U anti-velmanase antibody/mL, and accuracy for detection of 4.3 µg velmanase /mL plasma (43% C_{max}) was satisfactory at up to 11.3 µg anti-velmanase antibody/mL ≡113 U/mL. Since there were not many samples with anti-drug antibody (ADA) concentrations more than 30 U anti-velmanase antibody/mL, ADA interference was not expected to have compromised the precision of PK measurements in the velmanase clinical trials. However, ADA interference in the accuracy of detection of velmanase plasma concentration cannot be precluded for samples with ADA levels over 30 U anti-velmanase antibody/mL.

Table 39. Bioanalytical Method Life Cycle Information for Assessment of Interference of ADAs With the Quantification of Velmanase

Parameter	Method Validation 2021-08-20-V-R
Analyte	Velmanase
Validation Type	Interference of ADAs
Method ID	(b) (4) SOP (b) (4) rhLAMAN dated 25 June 2021
Duration of Time Method is in Use	23 July 2021 to 30 July 2021
Bioanalytical Site	(b) (4)
Matrix	Human plasma
Platform	ELISA
Format	A validated sandwich format using microtiter plates pre-coated with a polyclonal as a capture antibody against velmanase and an excess of polyclonal antibody- biotin conjugate for detection. The bound antibody-biotin conjugate was allowed to react with streptavidin-horseradish peroxidase. Bound antibody-enzyme complex was then determined by means of an enzymatic color reaction. Absorbance was measured at 450 nm (reference 540 nm).
Stock Reference, Lot Number, Expiration Date	rhLAMAN Standard SR-1098SI.1/1060280, expiration date: 20 August 2021
Calibration Range from LLOQ to ULOQ	LLOQ: 224 µg/L ULOQ: 50,164 µg/L
Matrix Study Population	Subjects with the LSD AM
Link to Reports & Applicable Amendments	2021-08-20-V-R

Source: Table 7, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: ADA, anti-drug antibody; AM, alpha-mannosidosis; ELISA, enzyme-linked immunosorbent assay; ID, identification; LLOQ, lower limit of quantification; LSD, lysosomal storage disorder; SOP, standard operating procedure; ULOQ, upper limit of quantification

Table 40. Summary of Method Performance for Assessment of Interference of ADAs With the Quantification of Velmanase

Bioanalytical Method Validation Report Name, Amendments, & Hyperlinks	**Validation of ELISA Assay Interference by Presence of ADAs Against Velmanase in Plasma Samples Spiked With Velmanase (2021-08-20-V-R)**	
Method Description	Velmanase was determined in diluted human plasma using an ELISA method. Diluted plasma samples were incubated in microtiter plates pre-coated with a polyclonal capture antibody raised against velmanase. After this incubation step and subsequent washing, an excess of a detecting polyclonal antibody-biotin conjugate was added and allowed to react with captured velmanase. After this step, excess antibody-biotin was removed by washing, and bound antibody-biotin conjugate was then allowed to react with streptavidin-horseradish peroxidase. Bound antibody-enzyme complex was determined by means of enzymatic color reaction. Absorbance was measured at 450 nm (reference 540 nm).	
Materials used for Standard Calibration Curve & Concentration	rhLAMAN standard SR-1098SI.1/1060280	
Validated Assay Range	0.16, 0.26, 0.42, 0.67, 1.07, 1.71, 2.74, 4.39, 7.02, 11.23 µg/L LLOQ: 224 µg/L ULOQ: 50,164 µg/L	
Material Used for QC and Concentration	rhLAMAN standard SR-1098SI.1/1060280; QC_{LOW}: 776 µg/L (analyzed diluted 1:1,000, curve reading 0.776 µg/L); QC_{MED}: 6,054 µg/L (analyzed diluted 1:4,000, curve reading 1.513 µg/L); QC_{HIGH}: 24,972 µg/L (analyzed diluted 1:5,000, curve reading 4.994 µg/L)	
MRDs	Samples can be diluted up to 1:1,000 according to [(b) (4)] SOP [(b) (4)] rhLAMAN, dated 25 June 2021	
Source & Lot of Reagents	SuperBlock® blocking buffer in TBS: Thermo Scientific/Pierce Biotechnology; Lot: WB322815; Human plasma pool: ID 21897; screened negative for hepatitis B and human immunodeficiency virus; rhLAMAN antibody: CAPRA Science; Lot: 1072.547; Biotin-labelled rhLAMAN antibody: CAPRA Science; Lot: 1072.547.bt; One-Step Ultra™ TMB-ELISA substrate: Thermo Scientific/Pierce Biotechnology; Lot: VL3152682; Streptavidin-horseradish peroxidase: MABTECH AB; Lot: 43649.2-9	
Regression Model and Weighting	The calibration was fitted by StatLIA (Brendan Technologies) using a 5-parameter-logistic-curve algorithm	
Validation Parameters	Method Validation Summary	
Standard Calibration Curve Performance during Accuracy & Precision Runs	Number of standard calibrators from LLOQ to ULOQ	10
	Cumulative accuracy (%bias) from LLOQ to ULOQ	88.1 to 111.3%
	Cumulative precision (CV%) from LLOQ to ULOQ	2.7 to 10.4%

Bioanalytical Method Validation Report Name, Amendments, & Hyperlinks	Validation of ELISA Assay Interference by Presence of ADAs Against Velmanase in Plasma Samples Spiked With Velmanase (2021-08-20-V-R)	
Validation Parameters	**Method Validation Summary**	
Performance of QCs during Accuracy & Precision Runs	Cumulative accuracy (%bias) in 5 QCs QC_{LOW}: 776 µg/L (diluted 1:1,000 resulting in curve reading 0.776 µg/L) QC_{MED}: 6,054 µg/L (diluted 1:4,000 resulting in curve reading 1.513 µg/L) QC_{HIGH}: 24,972 µg/L (diluted 1:5,000 resulting in curve reading 4.994 µg/L)	101.0 to 112.6%
	Inter-batch CV% QC_{LOW}: 776 µg/L QC_{MED}: 6,054 µg/L QC_{HIGH}: 24,972 µg/L	≤7.6% ≤7.4% ≤13.5%
	Total error	NA

Source: Table 8, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: ADA, anti-drug antibody; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; ID, identification; LLOQ, lower limit of quantification; MRD, minimum required dilution; NA, not applicable; QC, quality control; ULOQ, upper limit of quantification

14.3.2. Validation of Bioanalytical Methods for the Measurement of Serum Oligosaccharides

The 2-mannose oligosaccharide was measured in serum, cerebral spinal fluid (CSF), and urine using a high performance liquid chromatography with ultraviolet detection coupled with Orbitrap Velos mass spectrometry. This method was mentioned as [redacted] Analytical Methods. The [redacted] method was used for the assays of samples from Trials rhLAMAN-02, rhLAMAN-03, rhLAMAN-04, rhLAMAN-05, and rhLAMAN-10. A second analytical method was used to detect n-mannose oligosaccharide based on liquid chromatography with tandem mass spectrometry (LC-MS/MS) method; this method was mentioned as [redacted] ([redacted]) method. The [redacted] method was used for the assays of samples from Trial rhLAMAN-08. Summary of life cycle information of assay methods used during development, assay validation parameters, and performance of assays used in clinical trials are provided in Table 40, Table 41, Table 42, Table 43, Table 44, and Table 45. These method validations were acceptable.

Table 41. Bioanalytical Method Life-Cycle Information

Parameter	Analytical Method Validation 2012_04_25	Analytical Method Validation 2013_12_04	Analytical Method Validation 2015_10_07	rhLAMAN-02 rhLAMAN-03	Analytical Report rhLAMAN-04 (2014_09_26)	Analytical Report rhLAMAN-05 (2014_09_19)	Analytical Report 2016_02_12
Analyte	GlcNac(Man)2	GlcNac(Man)2	GlcNac(Man)2	GlcNac(Man)2	GlcNac(Man)2	GlcNac(Man)2	GlcNac(Man)2
Validation type	Analytical method validation	Analytical method validation (complementary to 2012_04_25)	Analytical method validation (serum LLOQ)	In-study	In-study	In-study	In-study
CTD reference #	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4
Method ID	SOP (b) (4)	SOP (b) (4) SOP (b) (4) 23May2012; SOP (b) (4)	SOP (b) (4) : 2013_08_05 2014_02_10; 2014_02_28	SOP (b) (4) Revision 03, effective date 22 May 2013	SOP (b) (4) Revision 04, effective date 04 February 2015	(b) (4) Revision 04, effective date 04 February 2015	SOP (b) (4) Revision 04, effective date 04 February 2015
Duration of time method is in use	From 2011 to 2013	From 2013 to 2015	From 2015	14 May 2013 to 04 June 2013	24 February 2015 to 06 May 2015	12 February 2015 to 22 April 2015	24 February 2015 to 07 July 2015
Bioanalytical site	(b) (4)						
Matrix	Human serum						
Platform	HPLC instrument with UV detection (230nm) for detection and quantification of maltotriose and GlcNac(Man)2. The identified peaks used for quantification were verified using an Orbitrap Velos mass spectrometer.						
Format	NA						
Stock reference, lot number, expiration date	Maltotriose (Sigma-Aldrich M-8378)	Maltotriose (Sigma-Aldrich M-8378)	Maltotriose (Sigma-Aldrich M-8378)	Maltotriose	Maltotriose (Sigma-Aldrich M-8378)	Maltotriose (Sigma-Aldrich M-8378)	Maltotriose (Sigma-Aldrich M-8378)

Parameter	Analytical Method Validation 2012_04_25	Analytical Method Validation 2013_12_04	Analytical Method Validation 2015_10_07	rhLAMAN-02 rhLAMAN-03	Analytical Report rhLAMAN-04 (2014_09_26)	Analytical Report rhLAMAN-05 (2014_09_19)	Analytical Report 2016_02_12
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	Standards: 100 to 7,000 pmol in 200 µL sample; Serum: 100 to 20,000 pmol in 200 µL sample (corresponding to 0.5 to 100 µmol/L)	4 to 200 µmol/L of 2-AB-derivatized maltotriose without the interference from matrix and SPE purification	LLOQ of internal standard: 1.5 µmol/L to 2.0 µmol/L	Pure standards 100 to 7,000 pmol in 200 µL sample; Serum 100 to 20,000 pmol in 200 µL sample (corresponding to 0.5 to 100 µmol/L) (SOP [redacted] (b) (4))	Pure standards 100 to 7;000 pmol in 200 µl sample; Serum 100 to 20,000pmol in 200 µL sample (corresponding to 0.5 to 100 µmol/L) (SOP [redacted] (b) (4))	4 to 200 µmol/L [redacted] (b) (4) 2014_02_28; 2013_12_04)	4 to 200 µmol/L (SOP [redacted] (b) (4) 2015_09_08)
Matrix study population	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM	Subjects with the LSD AM
Relevant reference and applicable report amendment	2012_04_25	2013_12_04	2015_10_07	CSR rhLAMAN-02 Appendix 16.1.10; CSR rhLAMAN-03 Appendix 16.1.10	2014_09_26	2014_09_19	2016_02_12

Source: Table 9 and 10, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: AM, alpha-mannosidosis; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; ID, identification; LSD, lysosomal storage disorder; NA, not applicable; SOP, standard operating procedure; UV, ultraviolet

Table 42. Summary of Method Performance for Serum Oligosaccharide Assessment, HPLC-UV, and ESI-MS Method (b) (4))

Method Parameter	Method Information	
Bioanalytical method validation report name, amendments, and hyperlinks	Quantitative Analysis of 2-Mannose Oligosaccharide in Serum and Cerebrospinal Fluid from Patients with Alpha-Mannosidosis (2013_12_04)	
Method description	Validation using HPLC in combination with UV detection and MS quantification of GlcNac(Man)2 in human serum. Because of the unavailability of oligosaccharides of interest as reference substance, the approach was the quantification of the oligosaccharide test items using maltotriose as surrogate reference standard.	
Materials used for standard calibration curve and concentration	Maltotriose as surrogate reference standard in human serum samples 0, 0.004, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 nmol/µL of 2-AB-derivatized maltotriose without the interference from matrix and SPE purification.	
Validated Assay Range	4-200 µmol/L; LLOQ 0.5 µmol/L.	
Material used for quality controls (QCs) and concentration	Maltotriose (Sigma-Aldrich M-8378)	
Minimum required dilutions (MRDs)	NA	
Source and lot of reagents	Maltotriose (Sigma-Aldrich M-8378)	
Regression model and weighting	r^2=0.9996	
Validation Parameters		
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	10
	The accuracy of the system was investigated by performing triple injections of both serum samples from 4 subjects spiked with maltotriose as internal standard.	CV% <4% for maltotriose CV% ≤20% for GlcNac(Man)2
	The precision of the analytical system was verified by performing 3 repeated injections of 0.1nmol/µL of a 2-AB-derivatized internal standard (maltotriose).	CV% for the 3 injections 0.78%
Performance of QCs during accuracy and precision runs	Accuracy: 1.0 µmol/L internal standard mean CV=40.7%; 1.5 µmol/L internal standard mean CV=14.7%; 2.0 µmol/L internal standard mean CV=12.3%; Intra-sample CV (serum): 1 sample spiked with 3 concentrations of maltotriose internal standard (N=5); Precision: 1.0 µmol/L internal standard mean CV=24.9%; 1.5 µmol/L internal standard mean CV=21.7%; 2.0 µmol/L internal standard mean CV=23.2%	
	Intra-sample CV (no matrix): Samples containing 1.0, 1.5, and 2.0 µmol maltotriose internal standard (N=3) Precision: 1.0 µmol/L internal standard mean CV=0.5%; 1.5 µmol/L internal standard mean CV=0.2%; 2.0 µmol/L internal standard mean CV=1.9%	

Method Parameter	Method Information
Bioanalytical method validation report name, amendments, and hyperlinks	Quantitative Analysis of 2-Mannose Oligosaccharide in Serum and Cerebrospinal Fluid from Patients with Alpha-Mannosidosis (2013_12_04)
Selectivity and matrix effect	The sample preparation procedure was validated by repeating the sample preparation for serum spiked with either 0.01 or 0.1 nmol/µL internal standard (maltotriose). The UV-detected peaks from maltotriose and GlcNac(Man)2 were validated by MS analysis where the m/z of 625 was observed for maltotriose and m/z 666 for GlcNac(Man)2. The analytical method was selective as it was easy to differentiate the analyte and the internal standard from other components. Even without UV-detection it was possible to distinguish the maltotriose from the matrix as they had different m/z-values (625 and 666). The analytical method provided relatively pure sample and it was therefore easy to detect both the matrix and maltotriose. Derivatization efficiency in serum was 35% (% in relation to no matrix).
Interference and specificity	Repeated sample preparation for serum spiked with 0.01 nmol/µL of internal standard: CV% ≤12.4%. Serum spiked with 0.1 nmol/µL of internal standard: CV% ≤21.0%.
Hemolysis effect	NA
Lipemic effect	Not Performed
Dilution linearity and hook effect	Matrix has been spiked with higher amount of the standard in 2012_04_25. Lower amount did not affect the precision or accuracy (CV% <4.3% for maltotriose 0.01 nmol/µL and CV% <12.2% for maltotriose 0.1 nmol/µL).
Bench-top/process stability	The CV% of the mean GlcNac(Man)2 concentration for the 3 sample preparations of sample 004 was <20% and accepted. For Sample 013 the CV% was >20% and not accepted. All values were below 2.8 µmol/L, and more variations exist when interpreting the spectra of lower GlcNac(Man)2 concentrations.
Freeze-thaw stability	A defrosted serum sample was compared with a new serum sample: CV% 1.58%.
Long-term storage	4-month stability of 2 samples stored at -80°C: CV% of the mean GlcNac(Man)2 concentration ≤13.98%; 9-month stability at ≤-70°C: CV% 1.7%, deviation as %initial -1.6%
Parallelism	Not required
Carryover	No carryover was observed by injecting blank samples after the analysis of high-concentration samples or calibration standards.

Source: Table 11, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: CV, coefficient of variation; (b) (4); ESI, electrospray ionization; HPLC, high performance liquid chromatography; ID, identification; LLOQ, lower limit of quantification; MS, mass spectrometry; NA, not applicable; ULOQ, upper limit of quantification; UV, ultraviolet

Table 43. Method Performances, Trials rhLAMAN-04, rhLAMAN-05, and rhLAMAN-07, -09, and -10

Method Performance in rhLAMAN-04 (2014_09_26)	
Standard curve performance	A standard series of maltotriose was analyzed prior to starting a batch of samples. A standard curve was generated from the detected peak areas, to verify the linearity of the system. The standard curve was not used to measure/read any results and was not used for calibration.
QC performance	Triple injection was performed for all subject samples and a mean mannose value was used.
Method reproducibility	Not done
Study sample analysis/stability	Analyzed upon receipt at (b) (4)
Method Performance in rhLAMAN-05 (2014_09_19)	
Standard curve performance	A standard series of maltotriose was analyzed prior to starting a batch of samples. A standard curve was generated from the detected peak areas to verify the linearity of the system. The standard curve was not used to measure/read any results and was not used for calibration.
QC performance	Triple injection was performed for all subject samples and a mean value was used. The CV% for low amount of GlcNac(Man)2 was between 10-20%. The CV% for high amount of GlcNac(Man)2 was ≤5%.
Method reproducibility	Not done
Study sample analysis/stability	328 days for Visit 0, 136 days for Visit 26, 81 days for Visit 52
Standard calibration curve performance during accuracy and precision runs	8 concentrations: 0, 0.004, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1 nmol/µL of 2-AB-derivatized maltotriose for Visit 0 serum (r^2=0.9787); 8 concentrations: 0, 0.004, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1 nmol/µL of 2-AB-derivatized maltotriose for Visit 26a serum (r^2=0.9858); 10 concentrations: 0, 0.004, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0. nmol/µL of 2-AB-derivatized maltotriose for Visit 52a serum (r^2=0.9728 and r^2=0.995)
Method Performance in rhLAMAN-07, -09, and -10 (2016_02_12)	
Standard curve performance	A standard curve was generated from the detected peak areas to verify the linearity of the system. The standard curve was not used to measure/read any results and was not used for calibration. The serum standard curve was accepted if r^2>0.98, based on a minimum of 6 concentration levels.
QC performance	A QC sample (0.1 nmol/µL maltotriose) was analyzed in triplicate, before and after analyzing 4 samples in triplicate, as a quality control of the HPLC system before and after subject samples. The analysis of maltotriose standard was accepted if the relative standard deviation (CV%) of triplicate injections was <10%. The acceptance criteria of CV% ≤20% included peak area of GlcNac(Man)2, calculated GlcNac(Man)2 concentrations, and peak area of the internal standard (maltotriose).
Method reproducibility	Not done
Study sample analysis/stability	15 days of sample storage
Standard calibration curve performance during accuracy and precision runs	10 concentrations: 0, 0.004, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 nmol/µL of 2-AB-derivatized maltotriose

Source: Table 11, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: CV, coefficient of variation; (b) (4); HPLC, high performance liquid chromatography; QC, quality control

Table 44. Bioanalytical Method Life Cycle Information for Serum Oligosaccharide Assessment, LC-MS/MS Method (b) (4)

Parameter	Method Validation 1607/14143-14196	rhLAMAN-08 2001/14520-23A2000160
Analyte	GlcNac(Man)n	GlcNac(Man)n
Validation type	Full validation	In-study
CTD reference #	5.3.1.4	5.3.1.4
Method ID	1607/14143-14196 Section 4	NA
Duration of time method is in use	From 2017	From 06 June 2017 to 22 July 2020
Bioanalytical site	(b) (4)	
Matrix	Serum	
Platform	Acquity UPLC I-Class integrated system coupled to a Xevo TQ-S mass spectrometer	
Format	NA	
Stock reference, lot number, expiration date	Isomaltotriose, batch numbers: LC09753V, expiry: September 2017; LC18169V, expiry: January 2019	Isomaltotriose, batch numbers: LC21549V, expiry: 30 June 2019; LC24442V, expiry: 31 January 2020; LRAB8226, expiry: 31 March 2021
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	0.5 to 25.0 µmol/L of isomaltotriose	0.5 to 25.0 µmol/L of isomaltotriose
Matrix study population	Subjects with the LSD AM	Subjects below 6 years of age with the LSD AM
Relevant reference and applicable report amendment	1607/14143-14196	2001/14520-23A2000160

Source: Table 18, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: AM, alpha-mannosidosis; ID, identification; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LSD, lysosomal storage disorder; NA, not applicable

Table 45. Summary of Method Performance for Serum Oligosaccharide Assessment – LC-MS/MS Method (b) (4)

Method Parameter	Method Information
Bioanalytical method validation report name, amendments, and hyperlinks	Bioanalytical Method Validation for the Quantification of Oligosaccharides by LC-MS/MS in Human Serum Samples, 19 May 2017 (1607/14143-14196)
Method description	An LC-MS/MS method has been validated for the quantification of GlcNac(Man)n in human serum samples. Because of the unavailability of oligosaccharides of interest as reference substance the approach was the quantification of the oligosaccharide test items using isomaltotriose as surrogate reference standard.
Materials used for standard calibration curve and concentration	Isomaltotriose as surrogate reference standard in human serum samples: 0.5 (i.e., LLOQ), 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, and 25.0 µmol/L of isomaltotriose with 10 µmol/L of internal standard.
Validated Assay Range	0.5 to 25 µmol/L

Method Parameter	Method Information	
Bioanalytical method validation report name, amendments, and hyperlinks	Bioanalytical Method Validation for the Quantification of Oligosaccharides by LC-MS/MS in Human Serum Samples, 19 May 2017 (1607/14143-14196)	
Material used for quality controls (QCs) and concentration	Concentrations in 50µL of human serum: 0.5, 1.5, 10, and 20 µmol/L of isomaltotriose with 10 µmol/L of internal standard for QC_{LLOQ}, QC_{LOW}, QC_{MED}, and QC_{HIGH}, respectively	
Minimum required dilutions (MRDs)	NA	
Source and lot of reagents	Isomaltotriose, DP3 Sigma-Aldrich LC09753V, LC18169V, LC18169V Maltotetraose, DP4 Sigma-Aldrich LC11470V	
Regression model and weighting	Range 0.5-25 µmol/L, linear model (r^2=0.9918), $1/x^2$ weighting factor	
Validation Parameters		
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-4.4% to 2.0%
	Cumulative precision (CV%) from LLOQ to ULOQ	≤8.3%
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs: Isomaltotriose 0.5 µmol/L (QC_{LLOQ}), 1.5 µmol/L (QC_{LOW}), 10 µmol/L (QC_{MED}), 20 µmol/L (QC_{HIGH}), 2,500 µmol/L (QC sample dilution), and 100 µmol/L (QC sample dilution)	Intra-assay: -10% to 7.8% Inter-assay: -3.3% to 2.0%
	Inter-batch CV% 0.5 µmol/L (QC_{LLOQ}), 1.5 µmol/L (QC_{LOW}), 10 µmol/L (QC_{MED}), 20 µmol/L (QC_{HIGH}), For each GlcNac(Man)n detected with a signal higher than the LLOQ, CV calculated from the 6 replicates analyzed	Intra-assay: 0% to 12% Inter-assay: 4.5% to 7.9% GlcNac(Man)2: 2.9% GlcNac(Man)3: 6.2%
	Total error	NA
Selectivity and matrix effect	The matrix effect was evaluated for isomaltotriose and internal standard using 6 different individual sources of blank human serum. The matrix effect was evaluated at 2 concentration levels: 1.5 and 10 µmol/L. The CV% on the internal standard normalized matrix factors (corrected with the use of internal standard) was 1.5% at the concentration of 1.5 µmol/L and 3.2% at the concentration of 10 µmol/L.	
Interference and specificity	Blank human serum samples, obtained from different single donors, were individually prepared, processed, and analyzed in singlicate to demonstrate the absence of interference between isomaltotriose, maltotetraose (internal standard), GlcNac(Man)n, and endogenous substances: 6 different sources of blank human serum samples not spiked (neither with isomaltotriose nor internal standard); 6 different sources of blank human serum samples spiked with isomaltotriose at the LLOQ and without internal standard; 6 different sources of blank human serum samples spiked with internal standard and without isomaltotriose (“zero samples”); 6 different sources of blank human serum samples spiked with isomaltotriose at the LLOQ and with internal standard (“LLOQ samples”); A human serum sample prepared in 6 replicates in order to have the retention time of the analytes of interest.	

Method Parameter	Method Information
Bioanalytical method validation report name, amendments, and hyperlinks	Bioanalytical Method Validation for the Quantification of Oligosaccharides by LC-MS/MS in Human Serum Samples, 19 May 2017 (1607/14143-14196)
Hemolysis effect	NA
Lipemic effect	NA
Dilution linearity and hook effect	Up to 2,500 µmol/L (with dilution factor 250) -9.4% to -7.8% (100 µmol/L and 2,500 µmol/L, mean concentration [N =6] within ±15%) 2.0% to 4.9% (100 µmol/L and 2,500 µmol/L CV% [N =6] not exceed 15%)
Bench-top/process stability	Isomaltotriose and internal standard were stable for 25 hours at 10°C (in the auto-sampler) in the validated range. GlcNac(Man)2 and GlcNac(Man)3 were stable for 25 hours at 10°C (in the autosampler). Stability of the other analytes of interest with concentrations close to or below the LLOQ could not be demonstrated accurately. Considering that the chemical structure of these compounds is very similar to GlcNac(Man)2 and -3, identical stability is expected.
Freeze-thaw stability	For 1.5 µmol/L: -4.2% (2 cycles) and -6.3% (3 cycles) For 10 µmol/L: -1.3% (2 cycles) and -4.1% (10 µmol/L, 3 cycles)
Long-term storage	-10.4% for 1, 3 and 9 months (1.5 µmol/L); -16.7% for 6 months (1.5 µmol/L) Ranging between -12.3 and -1.6% (10 µmol/L)
Parallelism	NA
Carryover	Calculated carryover percentage areas for isomaltotriose and for internal standard: <0.4% of the LLOQ; Calculated carryover percentage areas for each GlcNac(Man)n detected: <15% of the LLOQ.

Source: Table 19, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: CV, coefficient of variation; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LLOQ, lower limit of quantification; NA, not applicable; ULOQ, upper limit of quantification

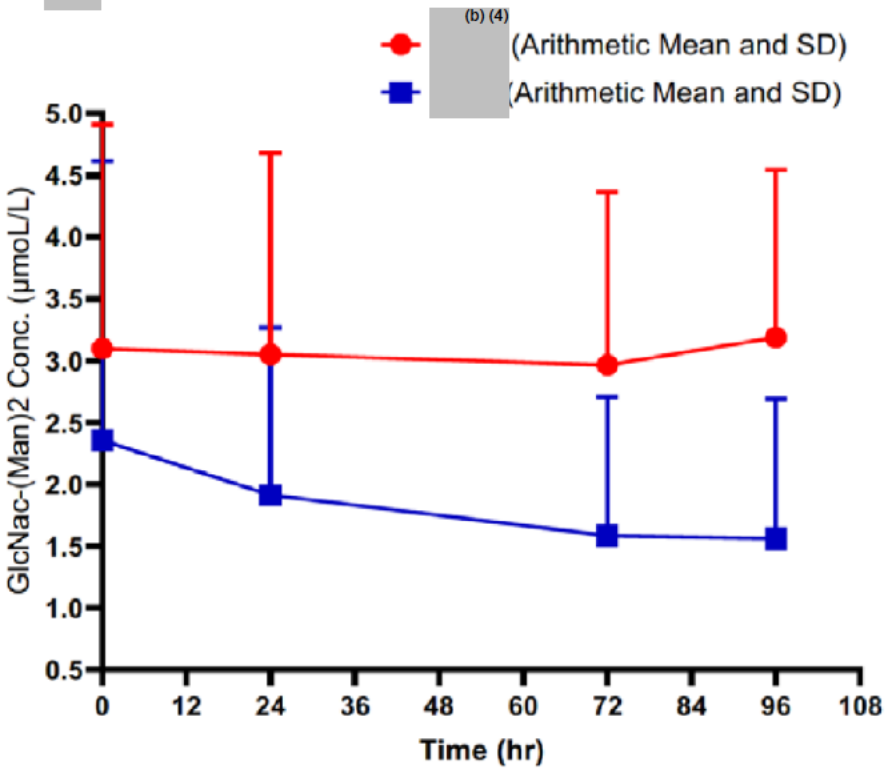
Table 46. Method Performances, Trial rhLAMAN-08

Method Performance in Trial rhLAMAN-08	
Standard curve performance	Cumulative bias range: -3.67 to 4.23% Cumulative precision: <8.01% CV
QC performance	Cumulative bias range: -3.7 to 1.2% Cumulative precision: <8.3% CV
Method reproducibility	NA
Study sample analysis/stability	Longest storage period: 230 days
Standard calibration curve performance during accuracy and precision runs	8 (0.5, 1, 2.5, 5, 10, 15, 20, 25 µmol/L)

Source: Table 18, Summary of Biopharmaceutic Studies and Associated Analytical Methods
Abbreviations: CV, coefficient of variation; NA, not applicable; QC, quality control

Comparison of Methods for Assessment of 2-Mannose Oligosaccharides: The (b) (4) and (b) (4) methods for evaluating oligosaccharides (GlcNac(Man)2) in serum were compared using samples from Trial rhLAMAN-10. A comparison of the mean GlcNac(Man)$_2$ concentrations analyzed at (b) (4) and (b) (4) is presented graphically in Figure 21 and Table 46. The (b) (4) method yielded higher GlcNac(Man)$_2$ concentrations relative to the DTI method.

Figure 21. Arithmetic Mean GlcNac(Man)$_2$ Concentrations of rhLAMAN-10 Samples Analyzed at [(b) (4)] and [(b) (4)]



Source: Figure 1, Summary of Biopharmaceutic Studies and Associated Analytical Methods
SD: Standard Division.

Table 47. Comparison of Mean GlcNac(Man)2 Concentrations (umol/L) Analyzed at [(b) (4)]

Method	Statistics	Time Post-Infusion (hours)			
		0	24	72	96
Concentration per [(b) (4)] Method	N	31	31	31	29
	Mean	3.094	3.045	2.961	3.193
	CV (%)	58.5	53.8	47.0	42.3
Concentration per [(b) (4)] Method	N	31	31	31	29
	Mean	2.358	1.913	1.587	1.562
	CV (%)	95.7	70.9	70.7	72.3
Concentration Difference between [(b) (4)] and DTI Methods	Difference	0.7355	1.132	1.374	1.631
	SE of difference	0.5195	0.3818	0.3212	0.3269
	p-value	0.162039	0.004334	0.000069	0.000006
	t statistic	1.416	2.965	4.278	4.990
	Degree of freedom	60.00	60.00	60.00	56.00

Source: Table 2, Summary of Biopharmaceutic Studies and Associated Analytical Methods
CV: Coefficient of Variation; SE: Standard Error

14.4. Immunogenicity and its Clinical Impact on PK, PD, Efficacy, and Safety

14.4.1. Overall Immunogenicity Assessment Strategies

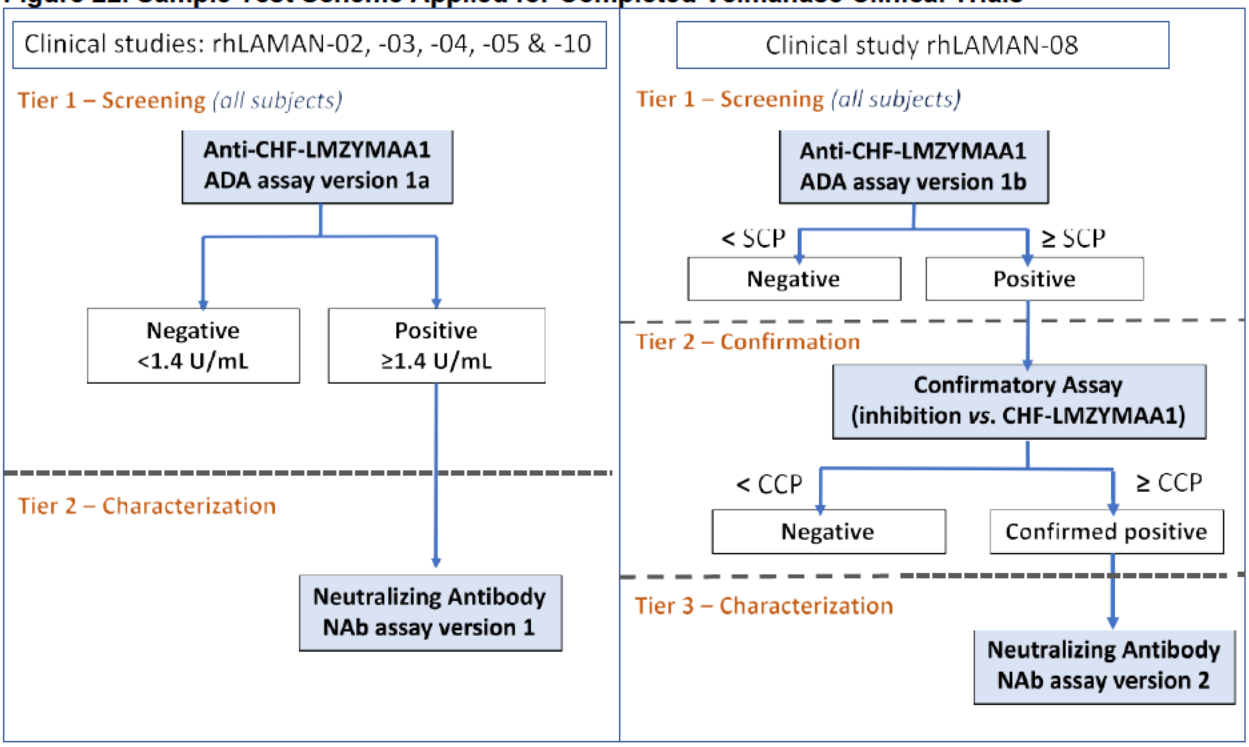
Blood samples for immunogenicity assessment were collected in all velmanase clinical trials. Two approaches were used in the immunogenicity assessment:

- For the earlier clinical trials (rhLAMAN-02, -03, -04, -05) and for the integrated analyses presented in the context of rhLAMAN-10, immunogenicity samples were tested in a two-tier approach and ADA levels were expressed as U/mL relative to a calibration curve for the positive control (rabbit anti-velmanase antibody). ADA positive samples were defined as the ADA level ≥1.4 U/mL; ADA negative samples were defined as the ADA level <1.4 U/mL. ADA positive samples were further evaluated in the neutralizing antibody (NAb) assay for the activity inhibiting enzymatic activity (Figure 22).
- For the later trial, i.e., Trial rhLAMAN-08, a three-tier approach was applied (i.e., screening, confirmatory, and followed by the assessment of NAb (Figure 22)).

Of note, the Applicant did not develop a NAb assay for inhibition of cellular uptake. Also, there was no assessment for cross-reactive immunologic material at baseline in velmanase clinical trials. At the occurrence of clinical adverse events (AEs) suggestive of hypersensitivity reactions, immunogenicity samples were collected for further evaluation the presence of immunoglobulin E (IgE) ADA, however, the immunogenicity assay for IgE ADA was not fully validated.

Refer to OBP's review for an assessment of bioanalytical method validation and performance of the ADA and neutralizing antibody assays.

Figure 22. Sample Test Scheme Applied for Completed Velmanase Clinical Trials



Source: Figure 4, ISI report.
Abbreviations: ADA, anti-drug antibody; CCP, confirmatory cut point; NAb, neutralizing ant body; SCP, screening cut point

14.4.2. Immunogenicity Incidences

The incidence of ADA response in patients with AM are presented in Table 47. Table 48 summarizes the ADA levels for the 10 subjects who had ADA positive results at any time (pre- or on-treatment with placebo or velmanase) and Table 49 summarizes the ADA level for 5 subjects in Trial rhLAMAN-08. Table 50 summarizes individual patient's ADA level by timepoint in Trial rhLAMAN-08.

- In Trial rhLAMAN-05, 24 pediatric and adult patients received treatment with velmanase up to 48 months; 8 out of 24 (37%) patients developed anti-velmanase IgG antibodies (referred to as IgG ADA).
- Six out of ten (60%) pediatric patients (6 to <18 years) and 2 out of 12 (16%) adult patients had IgG ADA. Of note, high baseline ADA+ subjects were observed in the ADA analyses.
- Four out of five (80%) ADA+ patients had NAbs that inhibited the velmanase enzyme activity.
- In Trial rhLAMAN-08, following 24 months treatment of velmanase, four out of five pediatric patients with AM developed IgG ADA. Three out of four (75%) pediatric patients developed NAb that inhibited velmanase enzyme activity.
- Samples from two subjects (Subjects [redacted] (b) (6)) who experienced infusion-related reactions (IRRs) in Trial rhLAMAN-02, rhLAMAN-03 and rhLAMAN-05 were tested for total IgE antibodies.

Table 48. Summary of Anti-Drug Antibodies by Time Point and Age Group, Full Analysis Set

		Parental Trial rhLAMAN-02	Parental Trial rhLAMAN-05			Overall Subjects Included in rhLAMAN-10 Integrated Database		
Parameter	**ADA**	**<18 years**	**<18 years**	**≥18 years**	**Total**	**<18 years**	**≥18 years**	**Total**
Baseline	n	9	10	14	24	19	14	33
	+		5 (50.0)	1 (7.1)	6 (25.0)	5 (26.3)	1 (7.1)	6 (18.2)
	-	9 (100.0)	5 (50.0)	13 (92.9)	18 (75.0)	14 (73.7)	13 (92.9)	27 (81.8)
0-6 months	n	9	7	12	19	16	12	28
	+	1 (11.1)	5 (71.4)	1 (8.3)	6 (31.6)	6 (37.5)	1 (8.3)	7 (25.0)
	-	8 (88.9)	2 (28.6)	11 (91.7)	13 (68.4)	10 (62.5)	11 (91.7)	21 (75.0)
6-12 months	n	9	8	11	19	17	11	28
	+	1 (11.1)	4 (50.0)	1 (9.1)	5 (26.3)	5 (29.4)	1 (9.1)	6 (21.4)
	-	8 (88.9)	4 (50.0)	10 (90.9)	14 (73.7)	12 (70.6)	10 (90.9)	22 (78.6)
12-18 months	n	9	8	8	16	17	8	/5
	+	1 (11.1)	4 (50.0)	-	4 (25.0)	5 (29.4)	-	5 (20.0)
	-	8 (88.9)	4 (50.0)	8 (100.0)	12 (75.0)	12 (70.6)	8 (100.0)	20 (80.0)
18-24 months	n	8	5	3	8	13	3	16
	+	1 (12.5)	2 (40.0)	-	2 (25.0)	3 (23.1)	-	3 (18.8)
	-	7 (87.5)	3 (60.0)	3 (100.0)	6 (75.0)	10 (76.9)	3 (100.0)	13 (81.3)
24-30 months	n	-	4	6	10	4	6	10
	+	-	2 (50.0)	-	2 (20.0)	2 (50.0)		2 (20.0)
	-	-	2 (50.0)	6 (100.0)	8 (80.0)	2 (50.0)	6 (100.0)	8 (80.0)
30-36 months	n	3	-	-	-	3	-	3
	+	-	-	-	-	-	-	-
	-	3 (100.0)	-	-	-	3 (100.0)	-	3 (100.0)
36-42 months	n	3	-	-	-	3	-	3
	+	-	-	-	-	-	-	-
	-	3 (100.0)	-	-	-	3 (100.0)	-	3 (100.0)
42-48 months	n	9	-	-	-	9	-	9
	+	1 (11.1)	-	-	-	1 (11.1)	-	1 (11.1)
	-	8 (88.9)	-	-	-	8 (88.9)	-	8 (88.9)
Overall[a]	n	9	10	14	24	19	14	33
	+	1 (11.1)	6 (60.0)	2 (14.3)	8 (33.3)	7 (36.8)	2 (14.3)	9 (27.3)[a]
	-	8 (88.9)	4 (40.0)	12 (85.7)	16 (66.7)	12 (63.2)	12 (85.7)	24 (72.7)

Source: Table 23, ISI report
ADA+: Value ≥1.4, ADA-: Value <1.4 (b) (6)
[a] "overall" includes Baseline. Subject was ADA positive at Baseline, but not on-treatment (Listing 16.2.25.1). Subject (b) (6) had ADA measurements ≥1.4 U/mL during placebo treatment and the subject was therefore considered as ADA+ in the Listings (Listing 16.2.25.1). However, after their first dose of velmanase the subject only had ADA measurements <1.4 and therefore is considered ADA negative in this table.
Subject (b) (6) who was excluded from the FAS due to no on-treatment data, was also ADA+ at baseline.
Data from Trial rhLAMAN-03 are not summarized for Subject (b) (6) who was also positive for ADA in this earlier study and therefore overall, during a longer treatment exposure than reported in the pooled data.
Abbreviations: ADA, anti-drug antibody; n, number of subjects

Table 49. Summary of ADA Levels for Positive Samples, rhLAMAN-10 Integrated Analysis Population

Subject	Age (years)	Gender	IRR	Baseline ADA (U/mL)	Maximal ADA on Velmanase (U/mL)	Day of Maximal ADA	Final ADA (U/mL)	Day of Final ADA
(b) (6)	8	F	Yes	<1.4[a]	1012	875	1012	875
	15	M	Yes	<1.4	440	358	43.0	1576
	35	F	No	<1.4	2.3	144	<1.4	340
	22	F	No	1.4	NA[b]	NA[b]	<1.4	483
	15	F	No	2.1	2.0	8	<1.4	393
	12	M	No	3.1	3.6	256	2.7	757
	9	M	No	1.8	2.3	310	<1.4	784
	12	M	No	<1.4	NA[b]	NA[b]	<1.4	357
	7	M	No	2.9	4.9	218	<1.4	708
	7	M	No	<1.4	NA[b]	NA[b]	24.0	462

Source: Table 24, ISI report
[a] This value is for Subjec (b) (6) baseline assessment (on (b) (6)); The same subject was treated as Subjec (b) (6) in rhLAMAN-05, when the baseline assessment (on (b) (6)) ADA level was 2.2 U/mL.
[b] NA, Not applicable because Subjects (b) (6) had only a single assessment of ADA, taken at the CEV in rhLAMAN-10 (Final ADA), as these subjects were allocated in the placebo group in rhLAMAN-05 and then treated in the compassionate use program
Abbreviations: ADA, anti-drug antibody; F, female; IRR, infusion-related reaction; M, male; n, number of subjects

Table 50. Summary of ADA Levels, Trial rhLAMAN-08

Subject	Age	Gender	IRR	Baseline ADA (U/mL)	Maximal ADA (U/mL)	Day of Maximal ADA	Final ADA	Day of Final ADA
(b) (6)	4.6	M		Negative	0.77	542	0.603	738
	5.9	M	Y	Negative	174	309	24.8	1187
	3.7	F		Negative	<0.22	648	<0.22	778
	4.1	F	Y	Negative	6.03	743	6.03	743
	4.3	M		Negative	Negative	- 7	Negative	764

Source: Table 27, ISI report
Abbreviations: ADA, anti-drug antibody; F, female; IRR, infusion-related reaction; M, male; n, number of subjects

Table 51. ADA and NAb Results by Time Point for Evaluable Population, Trial rhLAMAN-08

Subject No.	Visit	ADA Positive / Negative	ADA Level (U/mL)	NAb Positive / Negative
(b) (6)	Baseline	Negative		
	6-month evaluation	Negative		
	12-month evaluation	Negative		
	18-month evaluation	Positive	0.73	Negative
	24-month evaluation	Positive	0.60	Negative
	Baseline	Negative		
	6-month evaluation	Positive	96.5	Positive
	12-month evaluation	Positive	167	Positive
	18-month evaluation	Positive	50.4	Positive
	24-month evaluation	Positive	15	Positive
	V133 (31 months)	Positive	5.3	Positive
	40-month evaluation	Positive	24.8	Positive
	Baseline	Negative		
	6-month evaluation	Negative		
	12-month evaluation	Negative		
	18-month evaluation	Negative		
	V93 (21 months)	Positive	<0.22	Positive

Subject No. (b) (6)	Visit	ADA Positive / Negative	ADA Level (U/mL)	NAb Positive / Negative
	Baseline	Negative		
	6-month evaluation	Negative		
	12-month evaluation	Positive	3.64	Negative
	18-month evaluation	Positive	0.78	Negative
	24-month evaluation	Positive	6.03	Positive
	Baseline	Negative		
	6-month evaluation	Negative		
	12-month evaluation	Negative		
	18-month evaluation	Negative		
	24-month evaluation	Negative		

Source: Table 28, ISI report
Abbreviations: ADA, anti-drug antibody; NAb, neutralizing ant body

Reviewer's Comments

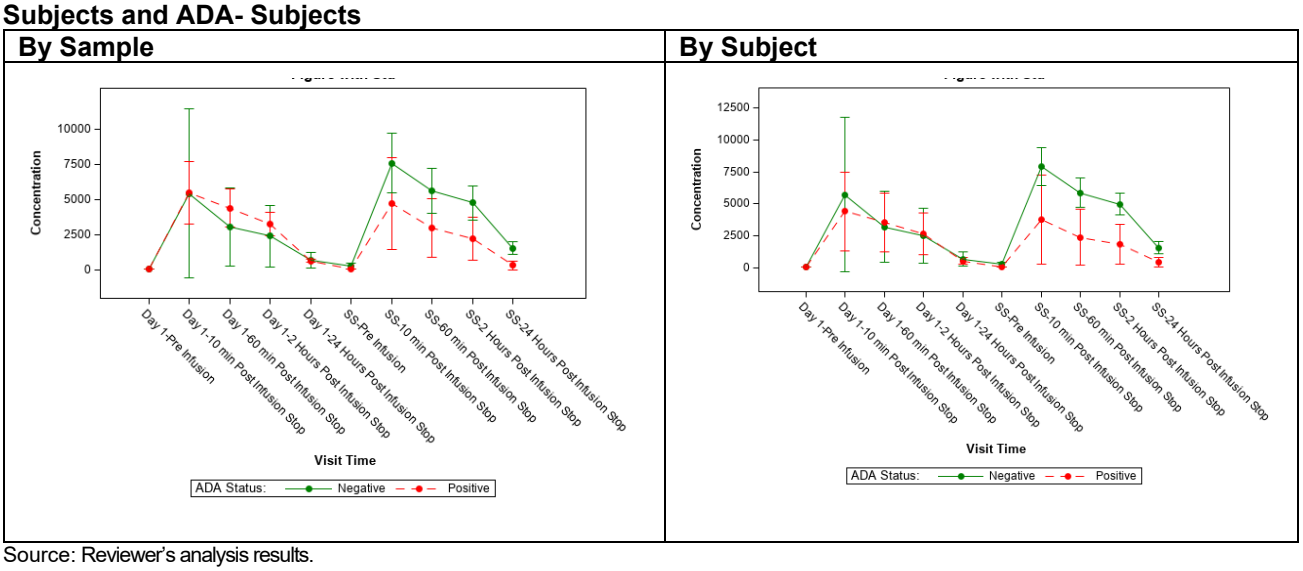
The Applicant did not characterize the potential neutralizing activity of ADA based on their inhibitory effect on cellular uptake of velmanase. As velmanase is a lysosomal enzyme replacement therapy (ERT) that requires cellular internalization to achieve its pharmacological activity, ADA inhibiting the cellular uptake of velmanase may reduce the drug effect and, thus, should be considered as NAb. An IR was sent for the Applicant to provide the status of the development of the cellular uptake NAb assay and to evaluate the immunogenicity samples when the validated cellular uptake NAb assay becomes available. The Applicant responded on July 16, 2022, that a new method to evaluate the inhibition of cellular uptake (i.e., NAb cellular uptake assay) is under development.

14.4.3. Impact of Immunogenicity on PK

Comparison of PK Between ADA+ and ADA- Subjects and Between ADA+ and ADA- Samples

ADA positive subjects (n=8) in the rhLAMAN-10 integrated analyses had a lower geometric mean velmanase alfa plasma concentration at steady-state compared to the ADA negative subjects (N=23) at all post-infusion time points (Figure 23). Similarly, ADA positive samples were also associated with lower velmanase alfa plasma concentrations. At the steady-state 10-minute post-infusion time point, corresponding to the highest velmanase alfa plasma concentrations, the geometric mean velmanase plasma concentration was 2365.3 µg/L in ADA positive subjects compared to 7880.8 µg/L in ADA negative subjects (Table 51).

Figure 23. Comparison of Velmanase PK Between ADA+ and ADA- Samples and Between ADA+ Subjects and ADA- Subjects



Source: Reviewer's analysis results.
Abbreviations: ADA, anti-drug antibody; PK, pharmacokinetics

Table 52. Velmanase Plasma Concentrations at Steady State in Patients with AM by Subject ADA Status

Plasma Concentration (µg/L)	ADA Negative	ADA Positive
Steady State, Pre-Infusion		
N	23	8
Geometric mean (CV%)	213.8 (76.8)	123.8 (37.7)
Mean (SD)	278.5 (213.9)	129.3 (48.8)
Steady State, 10 min Post-Infusion Stop		
N	23	8
Geometric mean (CV%)	7880.8 (19.0)	2365.3 (62.7)
Mean (SD)	8010.4 (1524.4)	4981.8 (3123.1)
Steady State, 60 min Post-Infusion Stop		
N	23	8
Geometric mean (CV%)	5771.1 (21.1)	1846.5 (66.0)
Mean (SD)	5880.4 (1239.4)	3660.5 (2414.4)
Steady State, 2 Hours Post-Infusion Stop		
N	23	8
Geometric mean (CV%)	4958.0 (17.4)	1759.3 (63.3)
Mean (SD)	5026.5 (872.3)	2832.3 (1792.6)
Plasma Concentration (mg/L)	ADA Negative	ADA Positive
Steady State, 24 Hours Post-Infusion Stop		
N	23	8
Geometric mean (CV%)	1556.5 (29.5)	546.3 (70.7)
Mean (SD)	1617.4 (477.0)	802.6 (567.4)
Steady State, 3 Days Post-Infusion Stop		
N	23	8
Geometric mean (CV%)	465.0 (49.7)	248.1 (62.1)
Mean (SD)	534.6 (265.9)	303.6 (188.5)

Plasma Concentration (µg/L)	ADA Negative	ADA Positive
Steady State, 5 Days Post-Infusion Stop		
N	22	7
Geometric mean (CV%)	301.7 (52.0)	177.8 (62.7)
Mean (SD)	352.7 (183.4)	208.9 (131.0)

Source: Table 30, ISI report.
Steady state = Last PK assessment of rhLAMAN-07/rhLAMAN-09/rhLAMAN-10
Cross-reference: rhLAMAN-10 Listing 16.2.33.7
Abbreviations: ADA, anti-drug antibody; CV, coefficient of variation; SD, standard deviation

Impact of ADA on PK: Within Subject Evaluations

In studies rhLAMAN-02 and -03, PK parameters were evaluated following the first velmanase alfa infusion and then at the 3-month evaluation visit following 13 doses of velmanase alfa. Of the two subjects who developed treatment-induced ADA, Subject (b) (6) was withdrawn from treatment after 12 infusions due to an infusion-associated reaction (IAR). Thus, comparative PK data before and after seroconversion are available for only subject (b) (6) with treatment-induced ADA. The velmanase alfa plasma concentrations following the first velmanase alfa administration and following the 13th dose for Subject (b) (6) are shown in Figure 24. Despite the detection of ADA prior to and following the 13th dose, velmanase alfa plasma concentrations were higher compared to those measured following the 1st dose. Subsequent dosing of subject had 440 U/mL ADA level, but PK concentration was not detectable. PK concentration was detectable for the steady-state measurement when the ADA level was 43 U/mL.

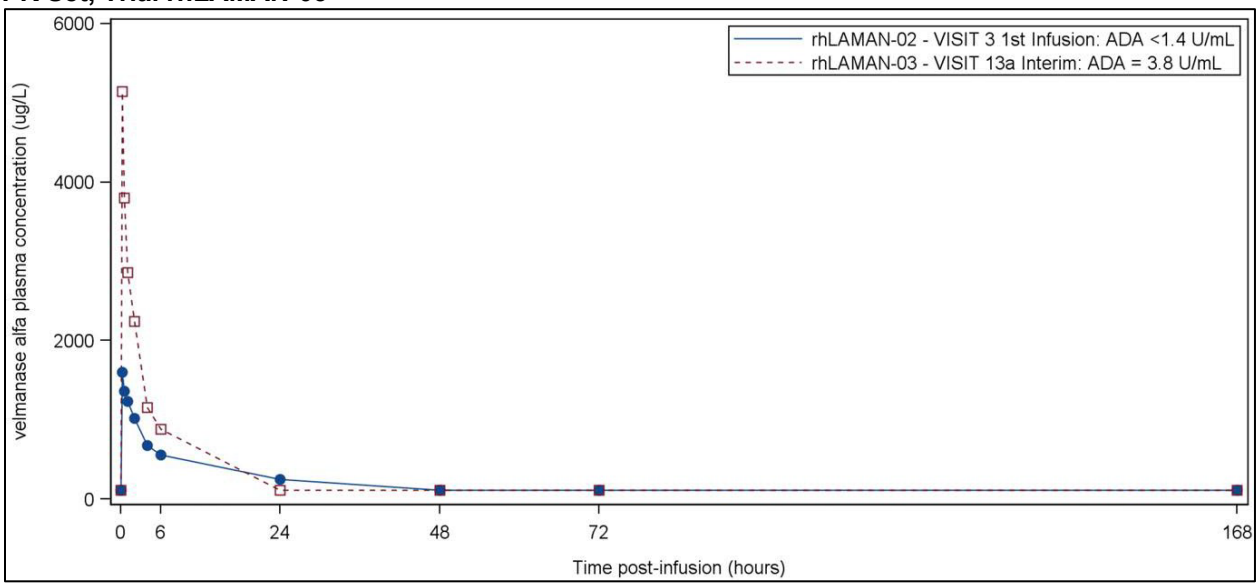
In Trial rhLAMAN-05, Subject (b) (6) had no quantifiable velmanase alfa plasma concentration at any time point in the rhLAMAN-10 PK evaluation (Figure 25). This subject had an ADA level at the last collection time point of 1012 U/mL.

Subject (b) (6) in Trial rhLAMAN-08 showed lower velmanase alfa plasma concentrations at the 6-month evaluation visit and had an ADA level of 96.5 U/mL (Figure 26). Subject (b) (6) showed similar velmanase alfa concentrations at visits 1 and 26a when ADA was negative at both visits (Figure 27).

Reviewer's Comment

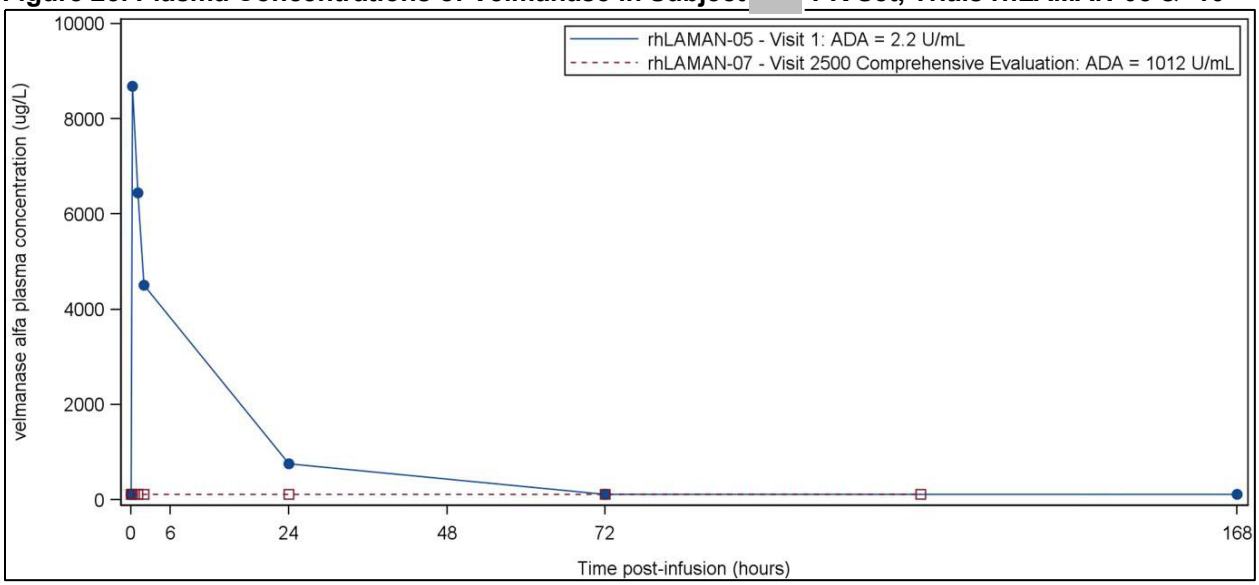
Overall, PK concentrations decreased in ADA+ positive subjects compared to the ADA- subjects. The analysis of the impact of immunogenicity on PK, however, was confounded by potential ADA interference on PK assay. When ADA level is >30 U/mL, PK measurement was affected by ADA interference and caution should be taken while interpreting the results of impact of immunogenicity on PK.

Figure 24. Plasma Concentrations of Velmanase in Subject (b) (6) Before and After Seroconversion, PK Set, Trial rhLAMAN-03



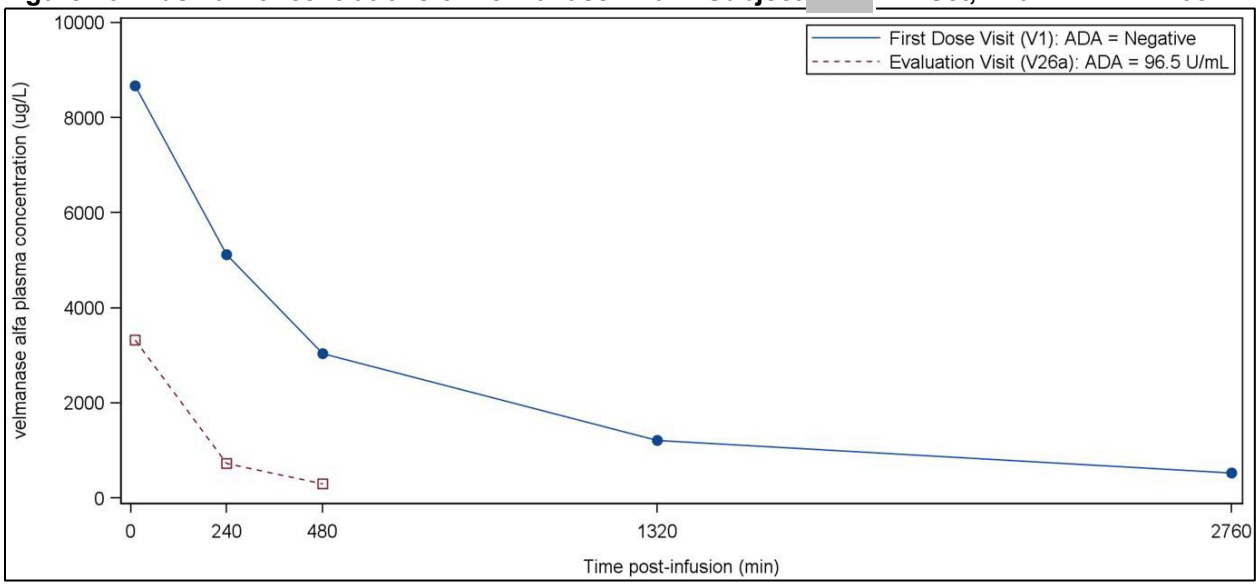
Source: Figure 13, ISI report.
Abbreviations: ADA, anti-drug antibody

Figure 25. Plasma Concentrations of Velmanase in Subject (b) (6) PK Set, Trials rhLAMAN-05 & -10



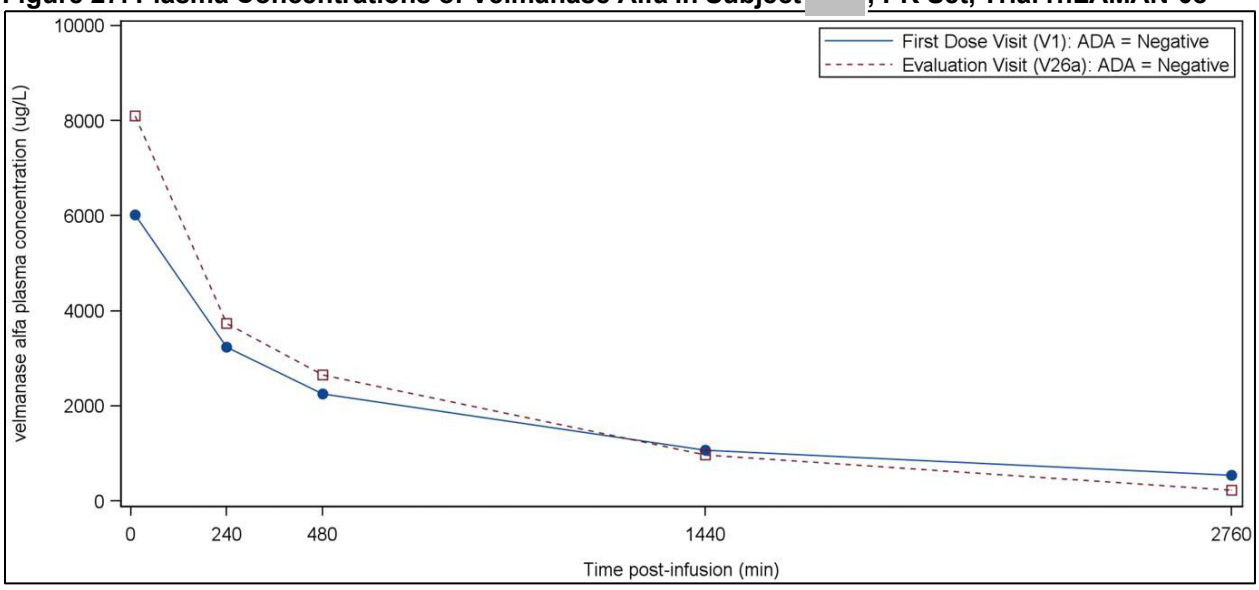
Source: Figure 14, ISI report.
Abbreviations: ADA, anti-drug antibody; PK, pharmacokinetics

Figure 26. Plasma Concentrations of Velmanase Alfa in Subject (b) (6) PK Set, Trial rhLAMAN-08



Source: Figure 15, ISI report.
Abbreviations: ADA, anti-drug antibody; PK, pharmacokinetics

Figure 27. Plasma Concentrations of Velmanase Alfa in Subject (b) (6), PK Set, Trial rhLAMAN-08



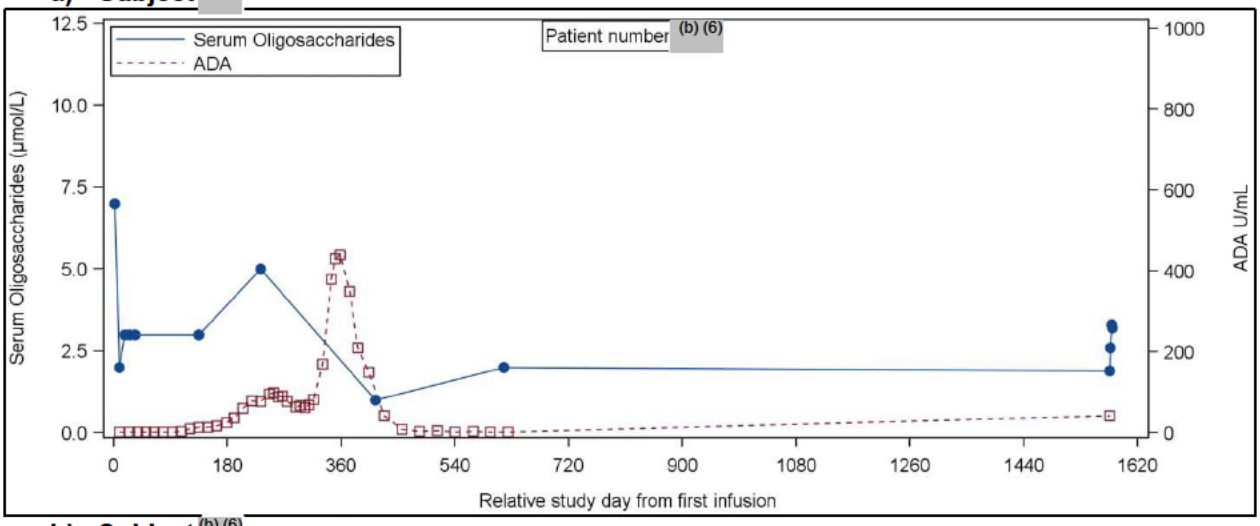
Source: Figure 16, ISI report.
Abbreviations: ADA, anti-drug antibody; PK, pharmacokinetics

14.4.4. Impact of Immunogenicity on PD

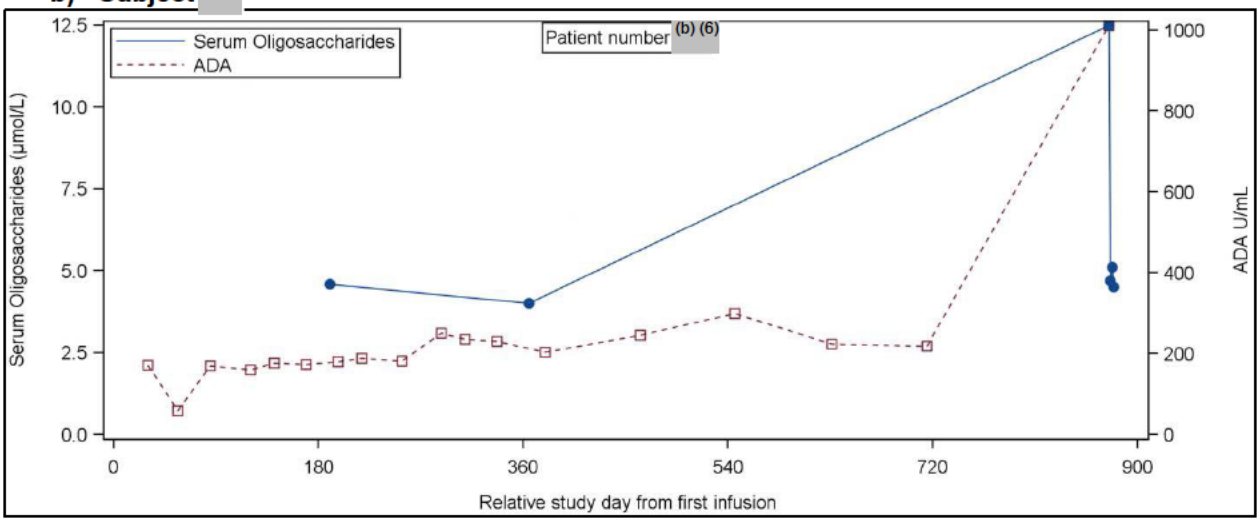
Among 33 patients in the rhLAMAN-10 integrated analysis population and 5 patients in rhLAMAN-08, development of ADA was associated with reduced PD responses in subjects (b) (6) and (b) (6) at the time when high ADA levels were observed (Figure 28).

Figure 28. Serum Oligosaccharide and ADA Levels in Subjects (b) (6) and (b) (6)

a) Subject (b) (6)



b) Subject (b) (6)



Source: Figure 18, ISI report.
Note: The compressed data points on the right-hand side of each plot represent the results for the rhLAMAN-10 Comprehensive Evaluation Visit. ADA results being '<1.4' were imputed to 1.4
Abbreviations: ADA, anti-drug antibody

14.4.5. Impact of Immunogenicity on Safety

Among 5 subjects in studies rhLAMAN-10 and rhLAMAN-08 who were reported with IARs, four subjects ((b) (6) and (b) (6)) were ADA positive (80%) and one subject ((b) (6)) was ADA negative (20%).

In Trial rhLAMAN-05, mild to moderate IARs occurred in a higher incidence in ADA positive patients compared to ADA negative patients. Two subjects who had developed severe hypersensitivity was ADA positive and one of the subjects developed highest levels of ADA. One ADA negative patient developed anaphylactic type reaction.

In Trial rhLAMAN-08, 2 out of 4 ADA positive pediatric patients experienced mild to moderate IARs. Among these 2 patients, one patient experienced an anaphylactic reaction during treatment

and developed a high ADA level, and the other ADA positive patient experienced severe hypersensitivity.

Subject CCD-LMZYMAA1-08- (b) (6)

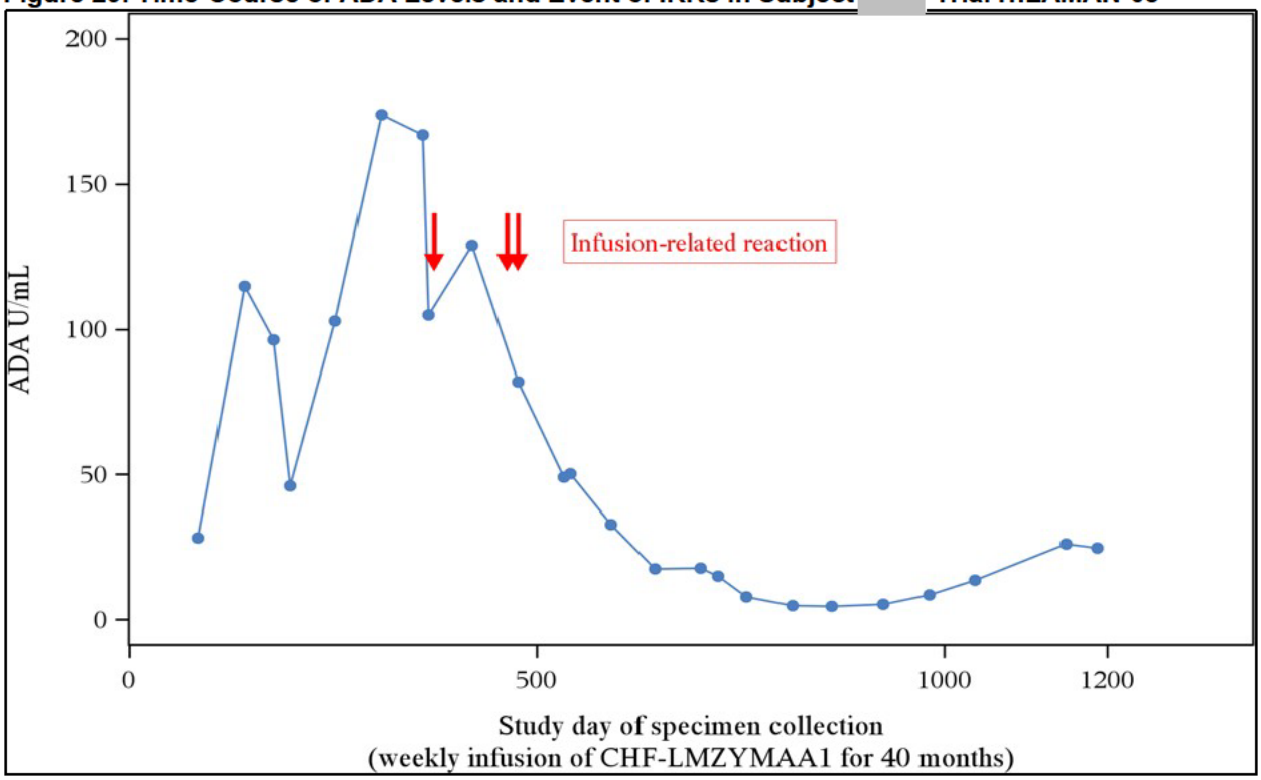
Subject (b) (6) (5.9-year-old male) experienced IARs on 3 occasions that coincided with relatively high ADA levels (Table 52). From Dose Visit 13 (Day 85) through the end of the trial, ADA were found at concentrations ranging from 4.63 to 174 U/mL. Approximately 10 months after the initial ADA detection, the patient had an AE that necessitated an overnight hospital stay at Dose Visit 54. At Days 463 and 477, further IRRs were found, and IRR reactions were noted (Figure 29).

Table 53. IRRs and ADA Levels for Subject (b) (6)

Date of IRR (Study Day)	Description of IRR	ADA Level at Preceding Time Point	Concomitant Medications
(b) (6) (Day 374) (Dose Visit 54)	Anaphylaxis fulfilling the Simpson criteria respiratory compromise (hypoxia treated with supplemental oxygen) and assumed reduced blood pressure (patient received fluid bolus) occurring rapidly after exposure to velmanase chills, hyperthermia	105 U/mL	dexchlorpheniramine maleate
(b) (6) (Day 463) (Dose Visit 67)	Severe hypersensitivity, chills, hyperthermia, cyanosis	129 U/mL	Methylprednisolone IV
(b) (6) (Day 477) (Dose Visit 69)	Severe hypersensitivity, chills, cyanosis	82 U/mL	Methylprednisolone IV

Source: Reviewer's generated table based on Table 41 of ISI report and FDA reviewer's safety analyses.
Abbreviations: ADA, anti-drug antibody; IRR, infusion-related reaction; IV, intravenous

Figure 29. Time-Course of ADA Levels and Event of IRRs in Subject [(b) (6)] Trial rhLAMAN-08



Source: Figure 24, ISI report
Abbreviations: ADA, anti-drug antibody; IRR, infusion-related reaction

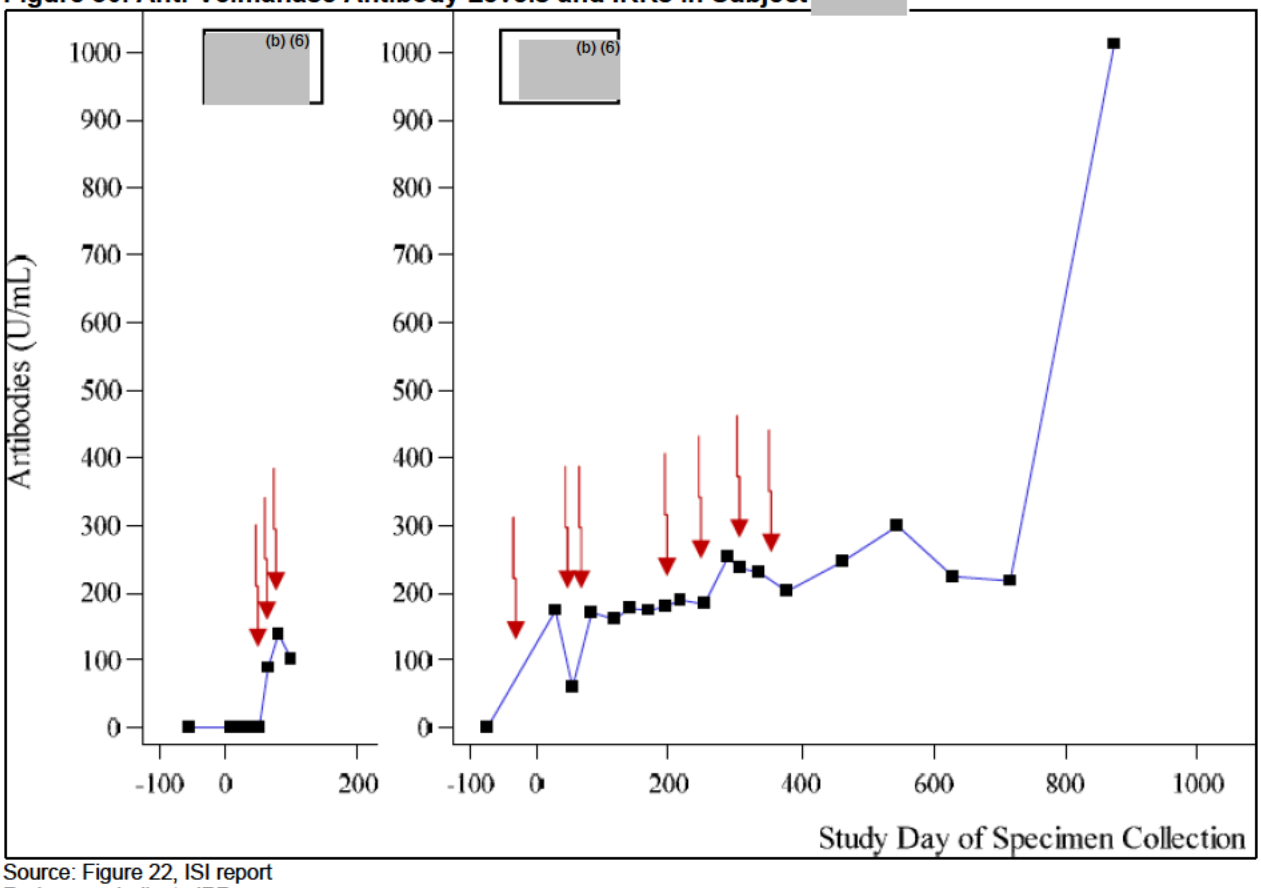
Subject: CCD-LMZYMAA1-10-520 ([(b) (6)])

Subject [(b) (6)] (8-year-old female) was first enrolled in rhLAMAN-02 and rhLAMAN-03 as Subject [(b) (6)] discontinued treatment, and then was enrolled in rhLAMAN-05 and rhLAMAN-07 as Subject [(b) (6)] after 21 months. Velmanase was administered to the subject in Trial rhLAMAN-05 at dose of 1 mg/kg and in earlier studies at dose of 0.8 mg/kg. The subject experienced a total of 14 IRRs and a total of 27 ADA assessments. The ADA levels and some of the major AE events are presented in Figure 30.

The subject was ADA negative at baseline (enrolled as Subject [(b) (6)]) and became ADA positive (1.8 U/L) detected on Day 51. The ADA level increased to 140 U/L on Day 79. On Day 58, 64 minutes into the infusion, there was a report of the first IRR (mild anaphylactoid response).

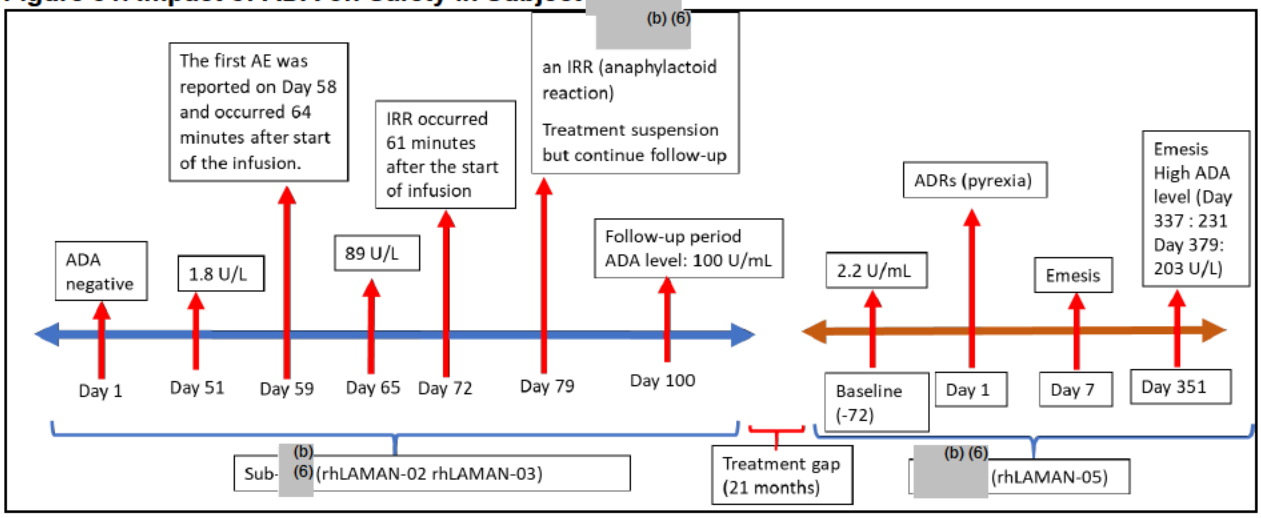
The subject's ADA levels during the Baseline assessment for rhLAMAN-05 (enrolled as Subject [(b) (6)]) was 2.2 U/mL, i.e., ADA positive. The subject had ADA levels from 59.5 U/mL to 299.0 U/mL up to Day 715 and had a peak ADA level of 1012 U/mL on Day 875. The ADA levels and clinical safety findings are presented in Figure 31.

Figure 30. Anti-Velmanase Antibody Levels and IRRs in Subject (b) (6)



Source: Figure 22, ISI report
Red arrows indicate IRR

Figure 31. Impact of ADA on Safety in Subject (b) (6)



Source: Reviewer's generated plot
Abbreviations: ADA, anti-drug antibody; ADR, adverse drug reaction; AE, adverse event; IRR, infusion-related reaction

Subject: CCD-LMZYMAA1-08- (b) (6)

Subject (b) (6) (4.1-year-old female) experienced IARs on 5 occasions. The ADA levels at the preceding sampling time points are presented in Table 53.

Table 54. IRRs and ADA Levels in Subject (b) (6)

Date of IRR (Study Day)	Description of IRR	Intensity	ADA Level at Preceding Time Point
(b) (6) Day 219)	Urticaria	Moderate	Negative
Day 303)	Urticaria	Moderate	Negative
Day 408)	Urticaria	Mild	Negative
(Day 483)	Urticaria	Mild	0.51 U/mL
(Day 570)	Urticaria	Moderate	0.78 U/mL

Source: Table 24, ISI report
Abbreviations: ADA, anti-drug antibody; IRR, infusion-related reaction

14.5. Pharmacometrics Assessment

14.5.1. Applicant's Population Pharmacokinetics Analysis

Objectives

To develop a population pharmacokinetic (PopPK) model for intravenous administered velmanase.

Data

This PopPK analysis evaluated data from 7 phase 1-3 trials (rhLAMAN-02, 03, 05, 07, 08, 09, and 10) as listed in Table 54.

Table 55. Summary of Studies Included in the Population Pharmacokinetics Analysis in Patients With α- Mannosidosis on Weekly IV Dose

Trial	Subject N (Age)	Dose (mg/kg)	PK Sampling
rhLAMAN-02 phase 1, safety, tolerability, PK	10 (7-17 years)	0.2, 0.4, 0.8, 1.6 or 3.2	0.08, 0.5, 1, 2, 4, 6, 24, 48, 72, and 168 hours after the end of infusion
rhLAMAN-03 phase 2a safety and efficacy trial with PK	9 (7-17 years)	0.8, and 1.6	0.08, 0.5, 1, 2, 4, 6, 24, 48, 72, and 192 hours after the end of the 11th infusion dose
rhLAMAN-05 phase 3 safety and efficacy trial	15 active, 10 placebo (6-35 years)	1	0.16, 1, 2, 24, 72, and 168 hours after the end of the first infusion
rhLAMAN-07 phase 3b aftercare treatment and long-term safety trial	7 rolled over from previous trials (9-29 years)	1	0.16, 1, 2, 24, 72, and 120 hours after the end of the infusion, at steady state
rhLAMAN-09 phase 3b aftercare treatment and long-term safety trial	7 rolled over from previous trials (8-31 years)	1	0.16, 1, 2, 24, 72, and 120 hours after the end of the infusion, at steady state
rhLAMAN-10 phase 3a long-term efficacy trial	33 overall (8-32 years)	1	0.16, 1, 2, 24, 72, and 120 hours after the end of the infusion, at steady state

Trial	Subject N (Age)	Dose (mg/kg)	PK Sampling
rhLAMAN-08 PK, efficacy and safety trial in pediatric subjects	5 (3.7–5.9 years)	1	Pre-dose, 10', 4h, 8h, 24h and 46h post-infusion stop after first dose and at steady state

Source: Table 1 of Applicant's PopPK ER Report.
Abbreviations: IV, intravenous; N, number of subjects; PK, pharmacokinetics

Overall PK analysis dataset included 468 measurable plasma PK observations from 39 subjects who were in most cases rolled over from one study to another, so that a total of 78 PK occasions were considered. A summary of the demographics of the PopPK dataset is provided in Table 55 and Table 56.

Table 56. Mean (SD) of Baseline Continuous Covariates, PopPK Dataset

Continuous Demographic Covariates	Children (3-6 years) (N=6)	Children (6-12 years) (N=11)	Adolescents (N=10)	Adults (N=12)	Overall (N=39)
Age (years)					
Mean (SD)	4.33 (1.03)	9.27 (2.10)	16.4 (1.84)	26.1 (4.85)	15.5 (8.68)
Median (CV%)	4.00 (23.8)	9.00 (22.7)	16.0 (11.2)	24.0 (18.6)	15.0 (56.0)
[min, max]	[3.00, 6.00]	[7.00, 13.0]	[14.0, 19.0]	[20.0, 35.0]	[3.00, 35.0]
Body weight (kg)					
Mean (SD)	19.1 (2.16)	41.3 (14.8)	68.1 (12.9)	70.1 (7.75)	53.6 (22.1)
Median (CV%)	18.9 (11.3)	40.5 (35.9)	70.0 (19.0)	70.0 (11.0)	60.0 (41.2)
[min, max]	[16.0, 22.3]	[18.7, 69.0]	[48.6, 95.2]	[60.0, 84.5]	[16.0, 95.2]
Height (cm)					
Mean (SD)	105 (5.80)	137 (17.9)	161 (7.98)	164 (7.82)	146 (23.7)
Median (CV%)	104 (5.5)	140 (13.0)	160 (5.0)	161 (4.8)	153 (16.2)
[min, max]	[98.0, 112]	[113, 172]	[153, 175]	[155, 181]	[98.0, 181]
Body mass index (kg/m^2)					
Mean (SD)	17.2 (0.928)	21.2 (3.15)	26.3 (3.89)	26.1 (2.76)	23.4 (4.55)
Median (CV%)	17.0 (5.4)	21.7 (14.9)	25.8 (14.8)	25.2 (10.6)	23.6 (19.4)
[min, max]	[16.2, 18.6]	[14.6, 25.0]	[20.8, 31.1]	[21.8, 31.6]	[14.6, 31.6]
ADA in ADA-positive subjects (U/mL)					
Mean (SD)	96.5 (NA)	6.12 (8.77)	28.5 (20.5)	NA	21.1 (31.5)
Median (CV%)	96.5 (NA)	2.80 (143.4)	28.5 (72.0)	NA	3.10 (149.1)
[min, max]	[96.5, 96.5]	[1.80, 24.0]	[14.0, 43.0]	NA	[1.80-96.5]

Source: Table 6 of Applicant's PopPK ER Report.
Abbreviations: ADA, anti-drug antibody; CV, coefficient of variation; N, number of subjects; NA, not applicable; PopPK, population pharmacokinetics; SD, standard deviation

Table 57. Baseline Categorical Covariate Information, PopPK Dataset

Categorical Demographic Covariates	Children (3-6 years) (N=6)	Children (6-12 years) (N=11)	Adolescents (N=10)	Adults (N=12)	Overall (N=39)
Sex					
Females	3 (50.0%)	8 (72.7%)	5 (50.0%)	7 (58.3%)	23 (59.0%)
Males	3 (50.0%)	3 (27.3%)	5 (50.0%)	5 (41.7%)	16 (41.0%)
ADA (at baseline)					
Negative	6 (100%)	6 (54.5%)	10 (100%)	12 (100%)	34 (87.2%)
Positive	0 (0%)	5 (45.5%)	0 (0%)	0 (0%)	5 (12.8%)
ADA (post-treatment)					
Negative	4 (80.0%)	11 (84.6%)	13 (86.7%)	11 (100%)	39 (88.6%)
Positive	1 (20.0%)	2 (15.4%)	2 (13.3%)	0 (0%)	5 (11.4%)

Source: Table 7 of Applicant's PopPK ER Report.
Abbreviations: ADA, anti-drug antibody; N, number of subjects; PopPK, population pharmacokinetics; SD, standard deviation

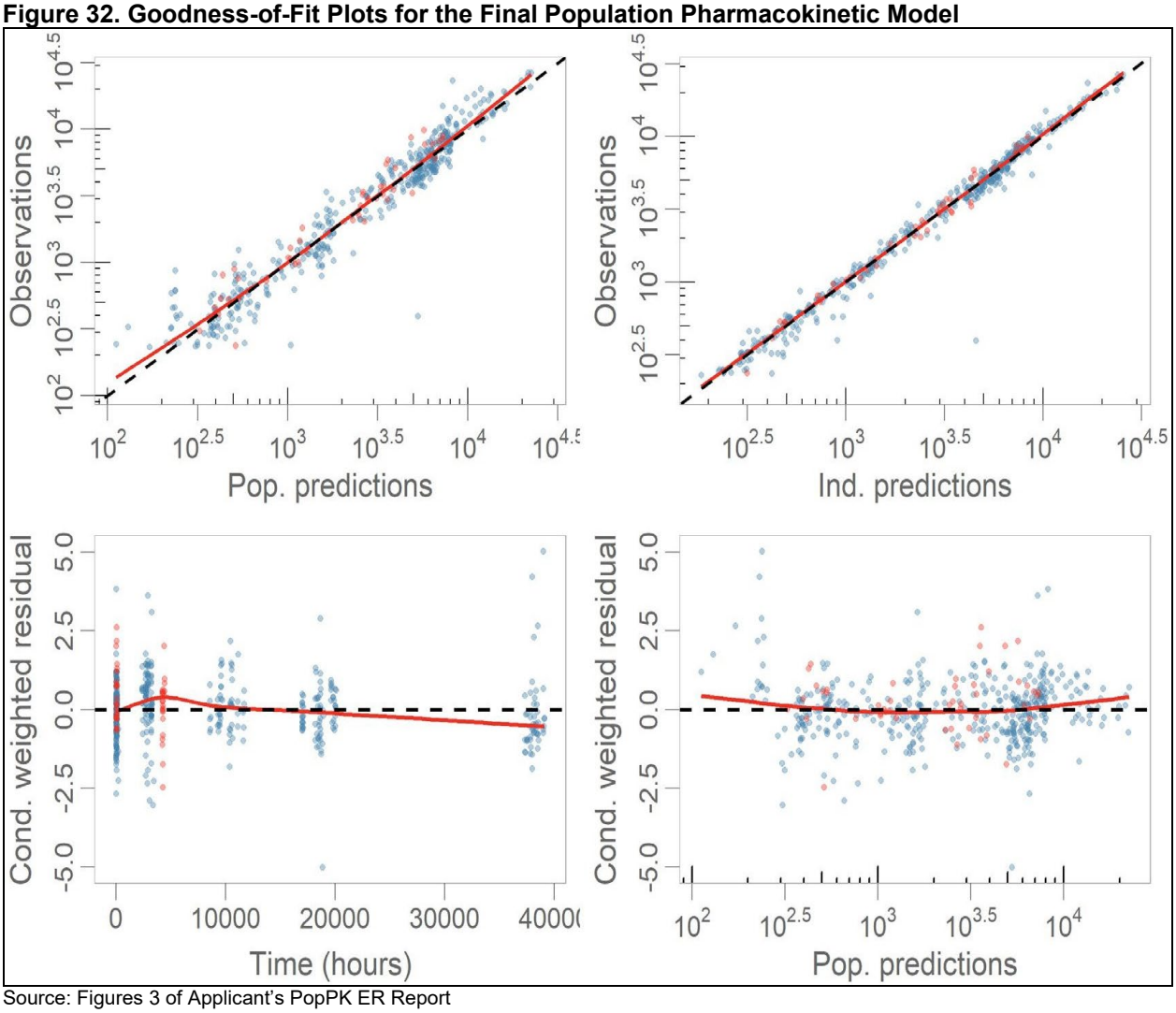
Methods

NONMEM® v7.4.3 (ICON, Hanover, MD, US), a software package for nonlinear mixed-effects analysis, was used for pop PK modeling [7]. R v4.0.2 or above [8] was used for simulations, graphical analyses, model diagnostics, and statistical summaries.

Results

A 3-compartment open PK model with zero-order input and first order elimination from the central compartment was used. The model confirmed the limited clearance and moderate volume of distribution at steady state of velmanase obtained in previous analyses: based on the present assessment, their values were approximately 0.401 L/h and 31 L for studies different from -02 and -03, respectively. The half-lives of the exponential phases were 1, 12, and 104 h, respectively, with minimal contribution of the terminal phase to the total exposure. The PK model included the effect of body weight (allometric coefficients fixed to theoretical values), study and dose on elimination clearance (CL) and volume of the central compartment (V1). The final population model indicated that the systemic exposure at the clinical dose of 1 mg/kg did not show clinically relevant dependency on body weight and age, thus indicating that the clinical dose of 1 mg/kg used from Trial rhLAMAN-05 onwards, and currently used for the marketed product, is appropriate for pediatric, adolescent, and adult subjects. A significant effect of ADA was observed, indicating a relevant increase of CL in subjects developing ADA following velmanase treatment.

Figure 32. Goodness-of-Fit Plots for the Final Population Pharmacokinetic Model



Source: Figures 3 of Applicant's PopPK ER Report
Abbreviations: Ind, individual; Pop, population

The final model included the effects of ADA, trial (02 and 03 versus others), and dose. The effect of ADA and dose were eventually implemented as a log-linear models; a categorical term was instead used for implementing the study effect. Inter-occasion variability was also implemented in the model to account for different behaviors in the different occasions (and different studies). Model parameters are reported in Table 57.

Table 58. Parameters of the Final PopPK Model of Velmanase in Patients

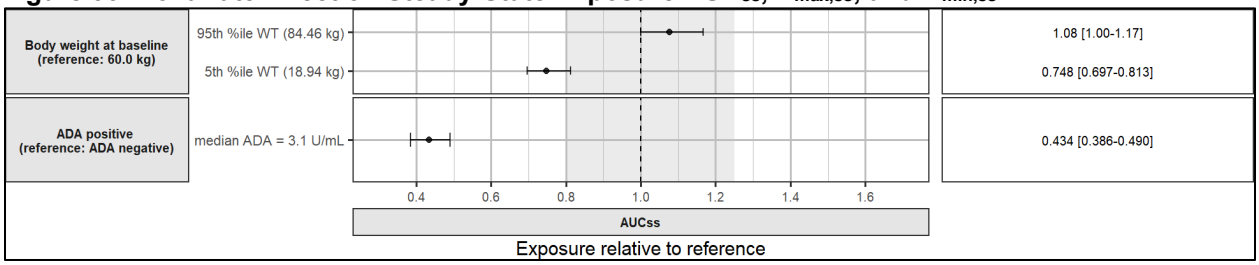
Parameter	Description	Estimate (Shrinkage)	RSE(%)
CL (L/h)		0.269	3.79
V1 (L)		4.04	11.7
Q2 (L/h)		0.221	12.6
V2 (L)		19.7	19.9
Q3 (L/h)		1.35	24.7
V3 (L)		5.00	7.46
WT on CL and Q	$(WT/medianWT)^{\theta}$	0.75 FIX	NA
WT on V	$(WT/medianWT)^{\theta}$	1 FIX	NA

Parameter	Description	Estimate (Shrinkage)	RSE(%)
ADA on CL	θ×log(ADA concentration)	0.479	13.3
Studies other than 2 and 3 on CL	θ	0.132	13.1
Studies other than 2 and 3 on V1	θ	2.01	24.1
Dose on CL	θ×log(dose [mg/kg])	-0.0448	36.7
Dose on V1	θ×log(dose [mg/kg])	-1.06	26.8
IIV on CL	Variance	0.0274 (22%)	34.9
IIV on V1	Variance	0 FIX	NA
IIV on Q2	Variance	0.185 (22.7%)	33.1
IIV on V2	Variance	0.186 (47%)	55.6
IIV on Q3	Variance	0.267 (29.3%)	40.8
IIV on V3	Variance	NA	NA
IOV on CL	Variance	0.022 [a]	28.2
IOV on V1	Variance	0.084[b]	40.2
RUV	Additive variance	0.001 FIX	NA
RUV	Proportional variance	0.161	11.4

Source: Table 9 of Applicant's PopPK Analysis ER Report (run039.ctl)
Abbreviations: ADA, anti-drug antibody; CL, clearance; IIV, interindividual variability; IOV, inter-occasion variability; NA, not applicable; Q, quartile; RSE, relative standard error; RUV, residual unexplained variability; V, volume; WT, weight

Simulations were performed (accounting for uncertainty in fixed-effect parameters) considering 5th and 95th percentiles of the body weight distribution (reference being the systemic exposure of a subject with median body weight). Also, the systemic exposure in subjects positive to ADA was evaluated (considering subjects developing ADA at the median concentration observed in the PK dataset, relative to subjects ADA negative). The results are shown in Figure 33.

Figure 33. Covariate Effect on Steady-State Exposure AUC_{ss}, $C_{max,ss}$, and $C_{min,ss}$



Source: Figure 8 of Applicant's PopPK ER Analysis Report
Abbreviations: ADA, anti-drug antibody; AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; SS, steady state; WT, weight

Reviewer's Comment About Applicant's Population PK Analyses: The Applicant's population PK analysis is acceptable. Of note, the USUBJIDs for each of the 7 studies were not provided in the initial population PK dataset. Multiple information requests were sent to the Applicant to solve this problem.

14.5.2. Applicant's Exposure-Response Analysis

Objectives

To describe the exposure-response (ER) relationships between serum oligosaccharides concentrations and metrics of velmanase systemic exposure.

Data

Response data included 289 serum oligosaccharides concentration observations collected from 40 subjects (Table 58).

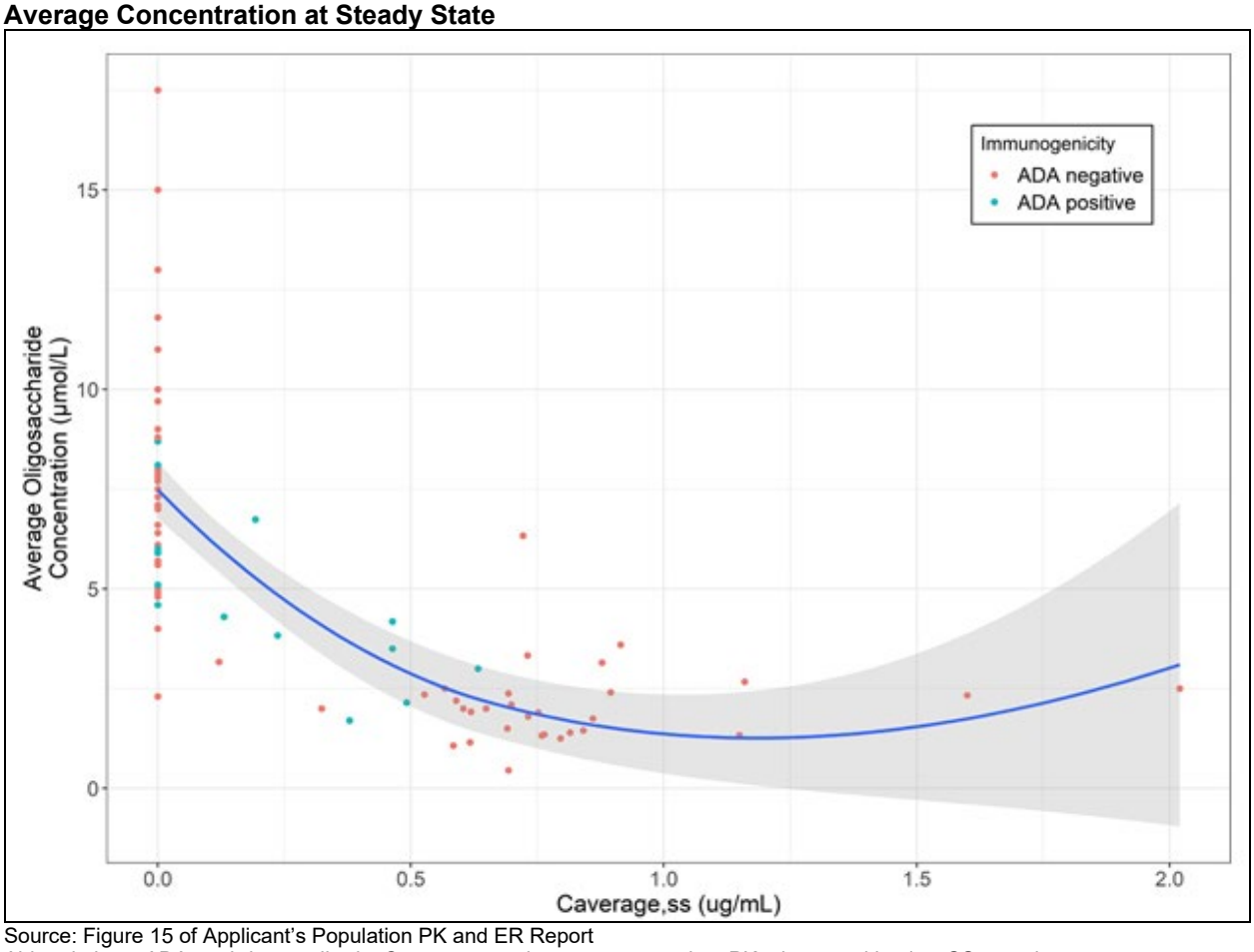
Table 59. Number of Subjects and Serum Oligosaccharides Concentrations, PD Analysis Dataset

Studies	Population	Subjects	Measurable Observations (Number of Excluded Observations)
rhLAMAN-02,	Subjects with α-Mannosidosis (children, adolescents, adults)	10	40 (10)
rhLAMAN-03	Subjects with α-Mannosidosis (children, adolescents, adults)	10	19 (0)
rhLAMAN-04	Subjects with α-Mannosidosis (children, adolescents, adults)	4	8 (0)
rhLAMAN-05	Subjects with α-Mannosidosis (children, adolescents, adults)	25	75 (10)
rhLAMAN-07	Subjects with α-Mannosidosis (children, adolescents, adults)	6	24 (0)
rhLAMAN-09	Subjects with α-Mannosidosis (children, adolescents, adults)	7	28 (0)
rhLAMAN-10*	Subjects with α-Mannosidosis (children, adolescents, adults)	18	70 (0)
rhLAMAN-08	Subjects with α-Mannosidosis (children)	5	25 (0)
Overall		40 1 subject received only placebo	289 (20)

Source: Table 10 of Applicant's PopPK ER Report.steri*: The same subjects were enrolled in Studies rhLAMAN-02 and rhLAMAN-03. These subjects along with subjects enrolled in rhLAMAN-05 were also enrolled in one of the phase 3b and 3a Studies: rhLAMAN-07, rhLAMAN-09 or rhLAMAN-10
Abbreviations: PD, pharmacodynamics

Dose levels higher than 1 mg/kg did not provide additional benefit in terms of further reduction of serum oligosaccharides concentrations and the E-R relationship of serum oligosaccharides endpoints with exposure appeared relatively flat (Figure 34).

Figure 34. Relationship Between Mean Serum Oligosaccharides Concentrations and Velmanase Average Concentration at Steady State



Source: Figure 15 of Applicant's Population PK and ER Report
Abbreviations: ADA, anti-drug antibody; C_{avg}, average plasma concentration; PK, pharmacokinetics; SS, steady state

Parameter estimates of the E_{max} model are listed in Table 59 for the PK/PD relationship between velmanase PK and oligosaccharides concentration.

Table 60. Model Parameter of the Final Exposure-Response Model (E_{max} Model With IIV on Baseline)

Parameter	Estimate ± SEM	Backtransformed Estimate
Baseline	1.9893±0.0565	7.31µM
Maximal effect	1.227±0.213	0.773 (fraction)
EC_{50}	-2.668±0.387	0.0694 µg/mL
IIV on Baseline	0.329±0.837	

Source: Table 12 of Applicant's PopPK ER Report.
Abbreviations: EC_{50}, half maximal effective concentration; IIV, interindividual variability; SEM, standard error of the means

Reviewer's Comment About Applicant's Exposure-Response Analyses:

The Applicant's exposure-response analyses appear acceptable. Of note, serum oligosaccharide concentration has not been validated as a surrogate biomarker for efficacy, so the result of the E-R analyses for the biomarker may not reflect the E-R relationship for clinical endpoints.

14.5.3. FDA Reviewer's Analysis

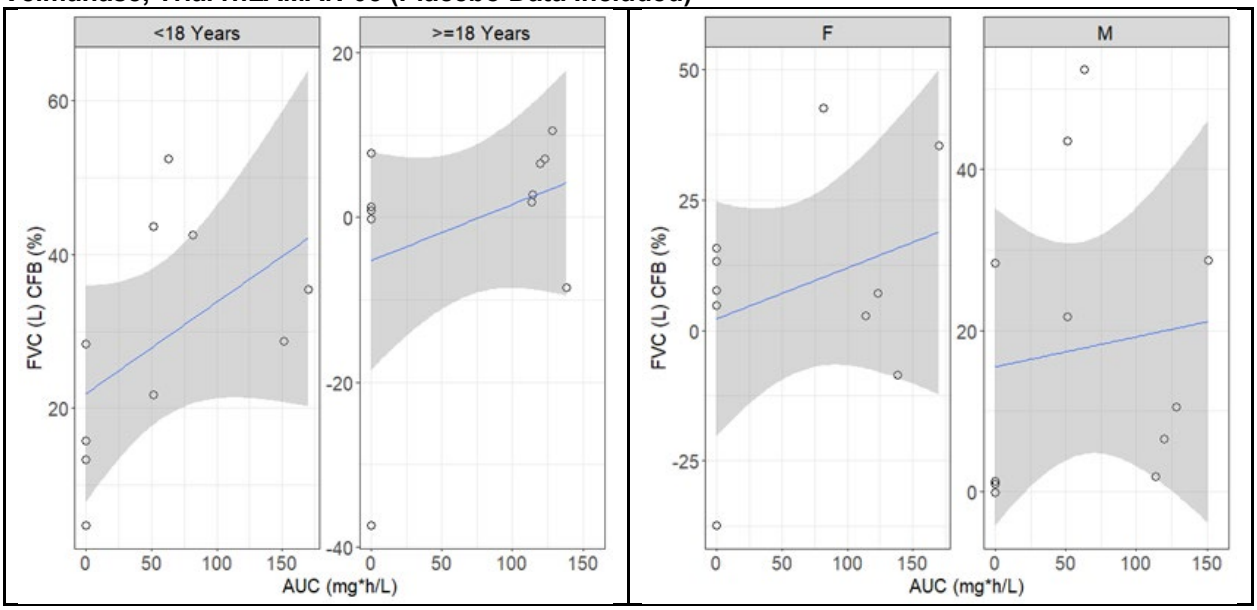
The method of FDA pharmacometrics reviewer's dose-response (D-R) and exposure-response (E-R) analysis and the analysis results are presented in Sections 6.3.2 and 6.3.3.

Multivariate linear regression (using sex and age as covariates) showed no E-R relationship for any efficacy endpoint of Trial rhLAMAN-05 when placebo data were excluded. With placebo data included, the same analysis resulted in the following: 1), a negative E-R relationship between velmanase AUC and serum oligosaccharides %change from baseline ($p<0.001$); 2), a positive E-R relationship between velmanase AUC and immunoglobulin G %change from baseline ($p<0.005$); and 3), a positive E-R relationship between velmanase AUC and forced vital capacity (FVC) (L) %change from baseline ($p=0.05$), with age <18 years and male appeared to be associated with better efficacy (Figure 35).

In Trial rhLAMAN-05, there was a negative correlation between IgG level and oligosaccharides concentration in serum for velmanase arm, while there was no correlation for the placebo arm (Figure 36).

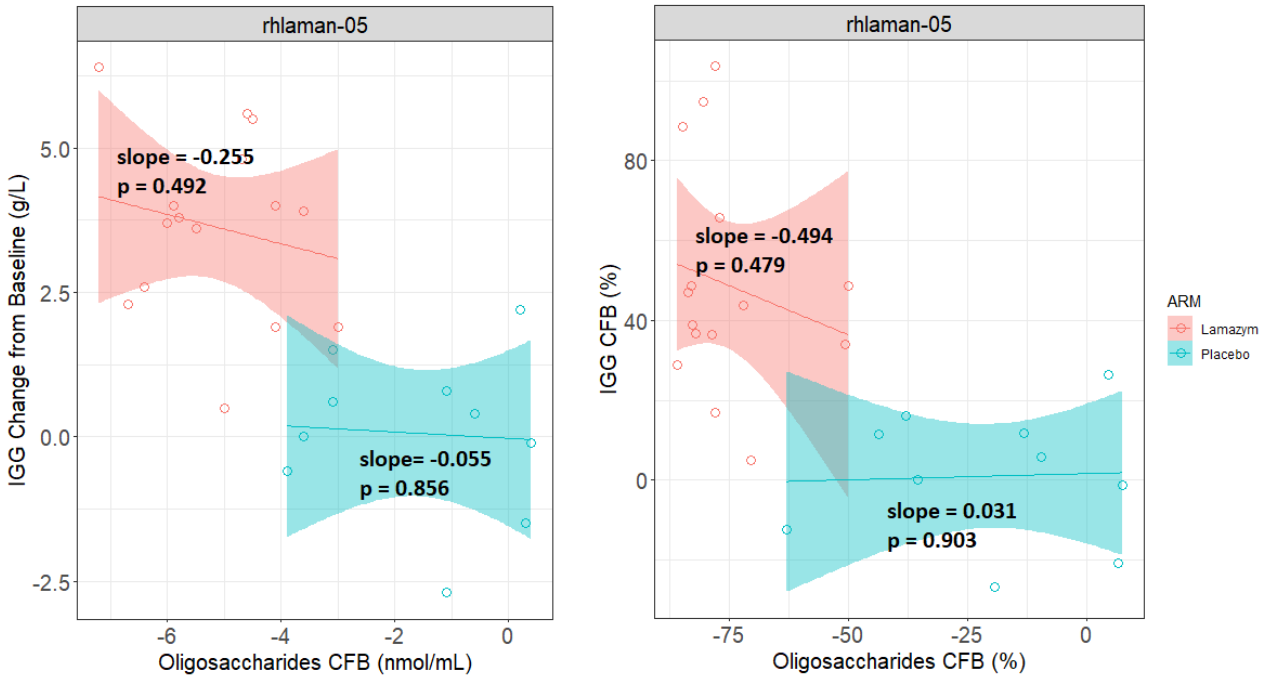
When all available data across clinical studies were analyzed, there appeared to be a trend of dose-response on serum oligosaccharides concentrations; a higher dose of velmanase was associated with greater reduction in serum oligosaccharide concentrations.

Figure 35. Exposure-Response for FVC (L) by Subgroups of Age and Sex for the Efficacy of Velmanase, Trial rhLAMAN-05 (Placebo Data Included)



Source: Reviewer's analysis
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; FVC, forced vital capacity

Figure 36. Correlation Between Serum IgG and Oligosaccharides Levels, Trial rhLAMAN-05



Source: Reviewer's Analysis.
Left Panel: change from baseline; Right Panel: change% from baseline
Abbreviations: IgG, immunoglobulin G

15. Study/Trial Design

No additional analysis or information is needed.

16. Efficacy

16.1. Review of Data on the Serum Oligosaccharide Biomarker

As discussed under Key Efficacy Review Issue 1 (Section 6.3.1), the only primary efficacy endpoint in Trial rhLAMAN-05 that had a statistically significant result was the serum oligosaccharide biomarker endpoint. However, this biomarker has not been established as a validated surrogate endpoint known to predict clinical benefit. Therefore, the review team assessed the available data (from the velmanase development program and from the literature) to determine whether this biomarker may predict clinical benefit.

As requested by the Agency (at the Type B pre-BLA teleconference on June 29, 2021), the Applicant submitted evidence to support the following points listed below to support the use of the change in serum oligosaccharide biomarker as evidence of velmanase's drug effect and potential clinical prognostic value:

The biomarker accumulates in tissues where AM causes structural damage and/or functional impairment (for the specified clinical benefit).

AM is an autosomal recessive disease caused by mutations in the *MAN2B1* gene. There is ubiquitous expression of this gene, with highest expression in kidney, pancreas, and peripheral blood leukocytes (Malm and Nilssen 2008). In AM, deficiency of the lysosomal α-mannosidase enzyme causes a block in the degradation of glycoproteins and thereby a progressive lysosomal accumulation of soluble mannose-rich oligosaccharides (Malm and Nilssen 2008). This accumulation is hypothesized to cause lysosomal swelling, leading to impaired cellular function, and eventual apoptosis or cell decomposition, which leads to release of circulating mannose rich substrates. The multisystemic accumulation of undigested oligosaccharides that leads to increased levels of oligosaccharides in the tissues, serum, and urine (Malm D and Nilssen 2001).

Multiple studies show that the accumulation of oligosaccharides can occur in a large variety of cells and tissues in patients with AM, causing a variety of distinct symptoms and disease manifestations. The following are some examples of case reports and studies that show that the development of AM is associated with accumulation of oligosaccharides in various tissues:

- A 4-year-old patient with AM who on autopsy had greatly increased amounts of mannose-containing component in liver and the reticuloendothelial system cells as well as spleen and lymph node (P.A. Öckerman and Lund 1967; Kjellman et al. 1969).
- A 13-year-old patient with AM who experienced bilateral destructive synovitis of the ankle region. Synovium histopathology demonstrated presence of oligosaccharides (Weiss and Kelly 1983).
- A 19-year-old patient with AM who experienced right hip joint destruction including joint effusion, synovitis, and bone loss (specifically in acetabulum and femur) that required total hip prosthesis. Based on histopathology that revealed macrophages with glycogen breakdown products (such as oligosaccharides) in the bone marrow and synovial fluid and accumulation of oligosaccharides in the synovial cells, the authors concluded that oligosaccharide accumulation can cause inflammation and resorption of bone (Gerards et al. 2004).
- A published multicenter longitudinal natural history study (Beck et al. 2013) that included 45 patients with AM aged 1-42 years old, followed for 24 months, reported that serum oligosaccharides are elevated in AM. The serum is also considered one of the “target” tissues in AM given the leukocyte dysfunction and immunodeficiency seen in the disease. A study of 6 AM patients and 6 healthy controls suggests that patients with AM have decreased ability to produce specific antibodies in response to antigen presentation and that their leukocytes have decreased capacity for intracellular killing (Malm et al. 2000; Malm D and Nilssen 2001).

Evidence also supports that serum oligosaccharide reduction reflects oligosaccharide reduction in tissues. Since α-mannosidase is only functional at the acidic pH of the lysosome and not in the serum, a reduction of serum oligosaccharides supports the proposed mechanism of cellular uptake of velmanase into the lysosome and its ability to restore deficient enzymatic activity in tissues. Therefore, the observed reduction in serum oligosaccharides with velmanase is thought to reflect reduction of accumulated oligosaccharides in white blood cells and other cells of target tissues through the action of velmanase in the lysosomes. Observation of this treatment effect

supports that reduced serum oligosaccharide levels in patients with AM likely reflect reduced oligosaccharide tissue levels.

This biomarker accumulation is toxic to the tissues where it accumulates.

During normal turnover and catabolism, glycoproteins are digested by proteinases and glycosidases within the lysosomes. These enzymes degrade glycoproteins into small fragments that are transported to the cytosol for reuse. In AM, there is a deficiency of this catabolic process, which results in the multi-systemic accumulation of undigested material in the lysosomes. Consequently, the lysosomes swell resulting in severe impairment of cellular functions and eventually the cells undergo apoptosis or decompose (Malm and Nilssen 2008).

As described in the above AM patient case reports, this abnormally increased storage of oligosaccharides in cells (of various tissues) results in disruption of tissue structure and function.

Published data from a MAN2B1 knockout (KO) mouse model of AM demonstrated that mannosidase deficiency led to accumulation of mannose-containing oligosaccharides in tissues (Stinchi et al. 1999; Roces et al. 2004; Stroobants et al. 2017), which was histologically associated with tissue vacuolation in multiple organs.

The degree of biomarker accumulation correlates with the degree of tissue damage and/or organ functional impairment.

Oligosaccharide accumulation appears to be toxic to tissues where it accumulates based on the limited available data, which suggest a qualitative correlation. There is no reported direct quantitative correlation between a given degree of biomarker accumulation and a given degree of tissue and/or specific organ functional impairment. The difficulty in establishing such a quantitative correlation is not unexpected in the context of an ultra-rare disease with heterogeneous manifestations.

The animal data also demonstrated a qualitative relationship between tissue accumulation and tissue damage/functional impairment. However, no quantitative relationship was demonstrated (refer to Section 5.1)

Reduction/ normalization of the biomarker concentration (in appropriate and relevant tissues) is associated with improvement in tissue structure and/or organ function.

As detailed above, in AM patients, evidence of oligosaccharide accumulation in liver, hip, and synovial fluid is associated with abnormal histopathology. However, no evidence is available to demonstrate that reduction or normalization of the serum or tissue oligosaccharides is associated with normalization of this histology in humans. Trial rhLAMAN-05 did not evaluate tissue structure or organ function.

However, in the AM animal model, histological evidence of reduced vacuolation in the liver, heart, and kidney, appeared to correlate with decreased levels of mannose-containing oligosaccharide within the tissue (Roces et al. 2004).

Treatment with the product in the target population leads to consistent and durable reductions of the biomarker in relevant tissues/body fluids.

Treatment with velmanase led to a statistically significant and sustained reduction in serum oligosaccharides compared to placebo (refer to Sections 6.2.1 and 6.2.2). Refer to the subsection

above entitled "The biomarker(s) accumulate(s) in tissues where AM causes structural damage and/or functional impairment (for the specified clinical benefit)" for a discussion of why serum is considered a relevant tissue/body fluid. In the AM animal model, the Applicant qualitatively showed that velmanase reduces accumulation of oligosaccharides in brain tissue (Stroobants et al. 2017). Other publications have shown similar effects on tissue vacuolation and/or qualitative evidence of reduced oligosaccharide levels in tissue extracts following administration of lysosomal enzyme alpha-mannosidase enzyme (Stinchi et al. 1999; Roces et al. 2004).

As evident from nonclinical histopathological evaluation, a reduction in tissue vacuolation occurred following treatment of Tg+KO mice in the 6-month mouse study. Reduced vacuolation in tissues was observed in the brain, liver, spleen, and lymph nodes. However, the Applicant did not correlate reduced vacuolation with serum oligosaccharide content. Refer to Section 5.1 for further details.

The degree of observed biomarker reductions in treated patients are reasonably expected to lead or conclusively lead to clinical benefit in the target population and specify that benefit.

To determine whether reductions in serum oligosaccharides may predict clinical benefit in patients with AM, the review team analyzed the correlation of the change in serum oligosaccharide levels with changes in the following clinical outcomes that were evaluated in Studies rhLAMAN-05 and rhLAMAN-10: the 3MSCT, the 6MWT and FVC (all deemed clinically relevant in the AM population as discussed in Section 6.2.1.5).

Trial rhLAMAN-05

The Pearson's correlation coefficients were computed to assess the relationship between the absolute change from baseline in serum oligosaccharides and the changes from baseline in 3MSCT, 6MWT, and FVC at Week 52 in Trial rhLAMAN-05. The estimated correlation coefficients are variable and modest, ranging from -0.15 to 0.38 with large confidence intervals (Table 60). These inconclusive results may be due to actual low correlation or due to the small sample size, short study duration, and slowly progressive and heterogenous nature of the disease.

Table 61. Estimated Pearson's Correlation Coefficients Between Change in Serum Oligosaccharides and Change in Clinical Outcome at Week 52, by Treatment Arm, Trial rhLAMAN-05

Clinical Outcome	Placebo (95% CI) (n=10)	Velmanase (95% CI) (n=15)	All[1] (95% CI) (n=25)
3MSCT	0.00 (-0.63, 0.63)	0.01 (-0.51, 0.52)	-0.15 (-0.52, 0.26)
6MWT	0.04 (-0.60, 0.65)	0.38 (-0.17, 0.75)	0.05 (-0.35, 0.44)
FVC	0.21 (-0.53, 0.77)	0.27 (-0.36, 0.73)	-0.07 (-0.49, 0.37)

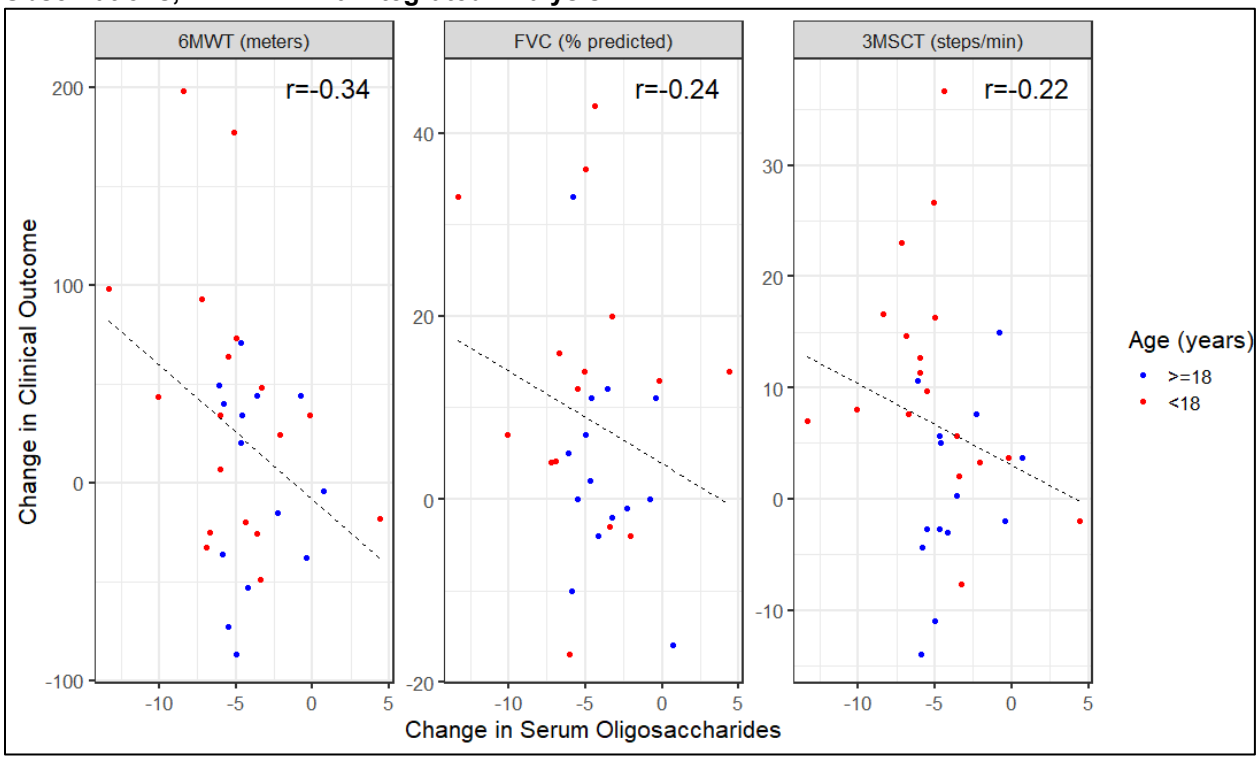
Source: Produced by the review team
All[1]: Estimated correlations using Week 52 changes from baseline from the total patient population
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; CI, confidence interval; FVC, force vital capacity; n, number of subjects

rhLAMAN-10 Integrated Analysis

The estimated correlation coefficient between the baseline serum oligosaccharides and baseline 3MSCT, 6MWT, and FVC were -0.13 (95% confidence interval [CI]: -0.45, 0.22), -0.26 (95% CI: -0.56, 0.09), and -0.38 (95% CI: -0.66, -0.02), respectively.

At the time of the rhLAMAN-10 Integrated Analysis, patients had been treated with velmanase between one and four years, with more than 60% of the patients treated for at least 2 years. This is a longer treatment duration, compared to the one-year duration of Trial rhLAMAN-05. The assessment of correlation between changes from baseline in serum oligosaccharides and changes from baseline in clinical outcomes produced estimated correlations with negative signs for all three clinical outcomes, suggesting that as the decline in serum oligosaccharides increases, the performance on the clinical outcome improves. Nevertheless, the estimated correlations using subjects' last observations are weak, as all three estimated correlations except for 6MWT have a magnitude less than 0.30 (Figure 37) and have 95% confidence intervals that include zero (Table 61). However, the estimated correlation using Month 12 data only and the estimated correlation using subjects' last observations only suggest that the correlation may strengthen over time.

Figure 37. Change in Serum Oligosaccharides Versus Change in Clinical Outcomes Using Last Observations, rhLAMAN-10 Integrated Analysis



Source: Produced by the review team
NOTE: The last observation is defined as the most recent non-missing value for a given variable in the rhLAMAN- 10 Integrated Analysis.
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; FVC, force vital capacity

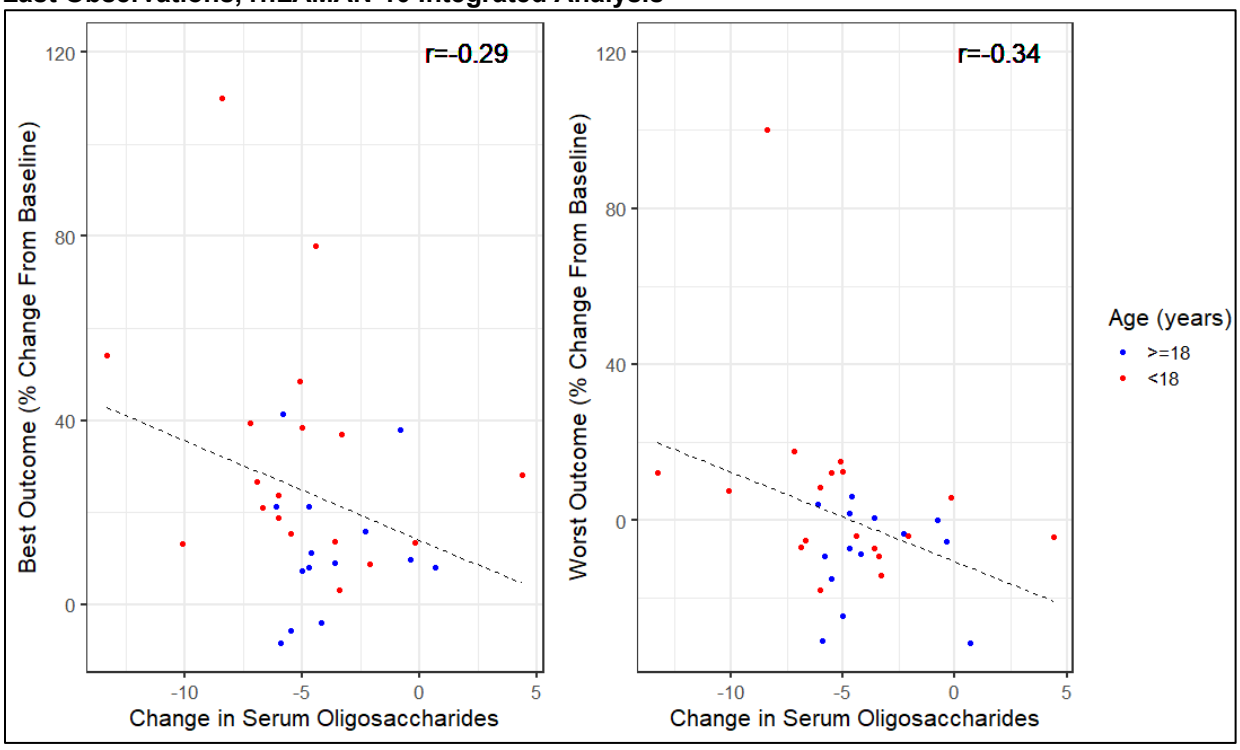
Table 62. Correlation Between Absolute Changes From Baseline in Serum Oligosaccharides and Clinical Outcomes, rhLAMAN-10 Integrated Analysis

Clinical Outcome	Measurement	Month 12	Last Observation
3MSCT	N	31	32
	Correlation (95% CI)	-0.23 (-0.54, 0.13)	-0.22 (-0.53, 0.14)
	Number of patients with an associated change	22	22
6MWT	N	31	32
	Correlation (95% CI)	-0.22 (-0.53, 0.14)	-0.34 (-0.62, 0.00)
	Number of patients with an associated change	22	19
FVC	N	28	27
	Correlation (95% CI)	-0.07 (-0.43, 0.31)	-0.24 (-0.57, 0.15)
	Number of patients with an associated change	23	18

Source: Produced by the review team
Associated change is defined as a change from baseline in serum oligosaccharides ≤0 and change from baseline in clinical outcome ≥0.
Of the 32 patients included in the last observation estimates for 3MSCT and 6MWT, 21 (66%) had last observations measured at 24 months of treatment or later. Of the 27 patients included in the last observation estimates for FVC, 17 (63%) had last observations measured at 24 months of treatment or later.
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; CI, confidence interval; FVC, force vital capacity; n, number of subjects

The estimated correlations between the change from baseline in serum oligosaccharides and the best and worst outcomes using subjects' last observations are also considered weak (Figure 38) with 95% CIs that include zero (Table 62). However, the estimated correlations using Month 12 data only and using last observations also suggest a strengthening of the correlation over time.

Figure 38. Change in Serum Oligosaccharides Versus Change in Best and Worst Outcomes Using Last Observations, rhLAMAN-10 Integrated Analysis



Source: Produced by the review team

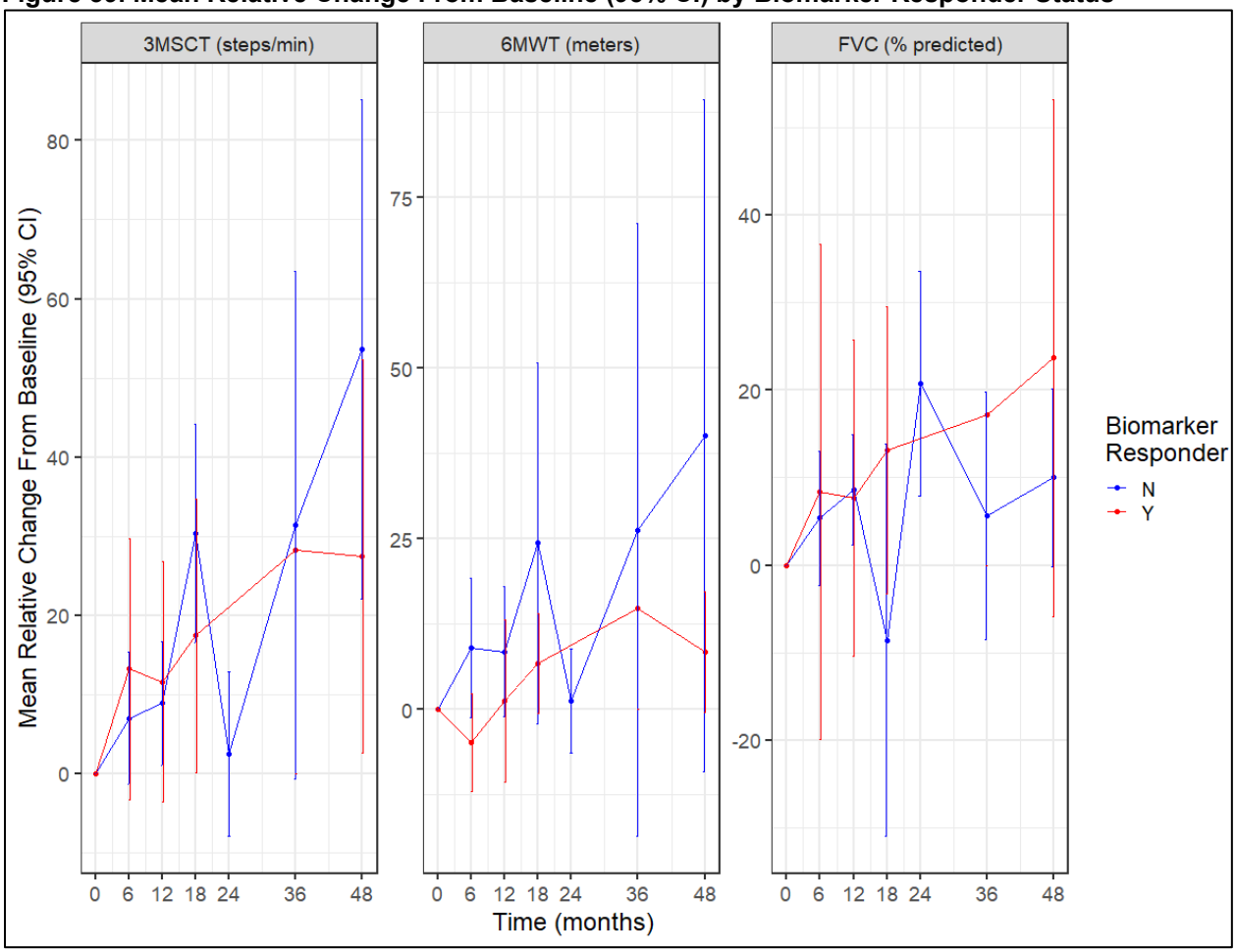
Table 63. Correlation Between Absolute Changes From Baseline in Serum Oligosaccharides and Best/Worst Outcomes, rhLAMAN-10 Integrated Analysis

Clinical Outcome	Measurement	Month 12	Last Observation
Best Outcome	N	31	32
	Correlation (95% CI)	-0.30 (-0.59, 0.07)	-0.29 (-0.58, 0.07)
	Number of patients with an associated change	29	27
Worst Outcome	N	31	32
	Correlation (95% CI)	-0.02 (-0.37, 0.34)	-0.34 (-0.62, 0.01)
	Number of patients with an associated change	16	14

Source: Produced by the review team
Associated change is defined as a change from baseline in serum oligosaccharides ≤0 and change from baseline in clinical outcome ≥0.
Of the 32 patients included in the last observation estimates for 3MSCT and 6MWT, 21 (66%) had last observations measured at 24 months of treatment or later.
Abbreviations: CI, confidence interval; n, number of subjects

The Applicant submitted results of post-hoc responder analyses based on responder definitions for 3MSCT, 6MWT, and FVC. However, these analyses are not considered in the evaluation due to a lack of agreement on the proposed responder thresholds for the clinical outcomes. An assessment of changes in 3MSCT, 6MWT, and FVC in patients in Trial rhLAMAN-10 that achieved a serum oligosaccharide level of 0.5µMol/L did not suggest increased performance on the clinical outcomes, compared to patients who did not achieve a serum oligosaccharide level of 0.5µMol/L, as shown in Figure 39.

Figure 39. Mean Relative Change From Baseline (95% CI) by Biomarker Responder Status



Source: Produced by the review team
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; CI, confidence interval; FVC, force vital capacity

Quantification of the biomarker(s) is based on appropriately validated laboratory assays with performance characteristics that are fit for their intended use.

The review team has assessed the bioanalytical method performance summary tables, individual assay validation reports, and in-study assay performance reports of the two analytical methods used for the assessment of serum oligosaccharide levels in velmanase clinical trials and determined that the two analytical methods were appropriately validated for their intended use.

The Applicant assessed serum oligosaccharide concentrations by detecting 2-Mannose oligosaccharide in serum using a high-pressure liquid chromatography with ultraviolet detection coupled with Orbitrap Velos mass spectrometry; this method was referred to as (b) (4) Analytical Methods. The (b) (4) method was used for analyzing samples from clinical studies rhLAMAN-02, rhLAMAN-03, rhLAMAN-04, rhLAMAN-05, and rhLAMAN-10. The second analytical method was used to detect n-Mannose Oligosaccharide based on LC-MS/MS method; this method was referred to as (b) (4) method. The (b) (4) method was used for analyzing samples from clinical Trial rhLAMAN-08.

Of note, in addition to 2-mannose oligosaccharide, other n-mannose oligosaccharides such as 3-mannose, 4-mannose, 5-mannose, 6-mannose, 7-mannose, 8-mannose, and 9-mannose

oligosaccharides have been found in serum and urine of patients with AM. The levels of 3-mannose, 4-mannose, 5-mannose, and 6-mannose oligosaccharides in samples collected from Trial rhLAMAN-08 were below the lower limit of quantification, making the 2-mannose oligosaccharide the most feasible biomarker to quantify in clinical trials.

Summary of life cycle information of the two bioanalytical methods used during velmanase clinical development and related assay validation parameters can be found in Section 14.3.

Overall assessment of the data to support use of serum oligosaccharide biomarker as a surrogate endpoint (based on the available data to support the above points).

There are substantial limitations in the available evidence described above to support the use of serum oligosaccharide biomarker as a surrogate endpoint. One key limitation is the lack of a demonstrated quantitative relationship between serum oligosaccharide levels and tissue oligosaccharide levels in humans and the absence of any data on serum oligosaccharide levels in the animal disease model (as these were not assessed in the animal studies). The other major limitation in the evidence to support validation of this surrogate endpoint is that the data from the clinical trials (Trial rhLAMAN-05 and rhLAMAN-10 Integrated Analysis) failed to demonstrate a strong correlation between changes in serum oligosaccharide levels and changes in clinical endpoints. Although the sign of the correlation between change in serum oligosaccharides and change in each of the clinical outcomes in rhLAMAN-10 Integrated Analysis (as discussed above) suggest that a decline in serum oligosaccharide may be correlated with an improvement in the clinical outcomes, the lack of a demonstrated strong correlation between the biomarker and clinically meaningful outcomes precludes the ability of the team to conclude that the serum oligosaccharide biomarker predicts clinical benefit.

16.2. Trial rhLAMAN-05

16.2.1. Primary Analysis Method for the Primary and Key Secondary Efficacy Endpoints

For the primary analysis method of primary and key efficacy endpoints (percent changes from baseline at Week 52 in serum oligosaccharides, 3MSCT, FVC%, and 6MWT), the Applicant's statistical analysis plan (SAP) included the following excerpts:

> *An analysis of covariance (ANCOVA) model based on log-transformed data (baseline and Week 52) will be performed to test the treatment effect as relative change from baseline. The model will include treatment (Velmanase and placebo) as fixed factor and baseline value and age as continuous covariates. The adjusted means in each treatment group, the adjusted mean difference between treatments, their 95% CIs and associated p-values will be estimated by the model.*

Although the SAP also stated that "For log-transformed analyses, the anti-log transformation will be applied before presentation," it did not include any details on the "anti-log transformation." The review team was able to reproduce the Applicant's results using the formulas in Table 63.

Table 64. "Anti-Log Transformation" Formulas Used to Obtain the Applicant's Results

	Mean		Difference in Means
	Velmanase	**Placebo**	**{95% CI}**
Results for the log-transformed data*	μ_V	μ_P	$\mu_V - \mu_P$ {LL, UL}
Results for the un-transformed data using anti-log transformation	(exp (μ_V) -1)*100	(exp (μ_P) -1)*100	(exp($\mu_V - \mu_P$) -1)*100 {(exp(LL) -1)*100, (exp(UL) -1)*100}

Source: Produced by the review team.
* For the log-transformed data, μV and μP denote the adjusted means for the velmanase and placebo groups, respectively, and they are obtained from the ANCOVA model including log (Y52/Y0) as the dependent variable and three variables "log(Y0), baseline age, and treatment group" as the covariates, where Y52 and Y0 denote the efficacy outcome at Week 52 and baseline visits, respectively.
Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; LL, lower limit of confidence interval; UL, upper limit of confidence interval

However, the review team questions the interpretation of the Applicant's results based on the anti-log transformation due to the following observation: the estimated treatment difference quantified by (exp ($\mu_V - \mu_P$) -1) *100 can be very different from the difference in the estimated mean parameters quantified by {(exp (μ_V) -1)*100 - (exp (μ_P) -1)*100}. For example, for serum oligosaccharides, the Applicant's estimated mean percent changes from baseline are -77.6% and -24.2% for the velmanase and placebo groups, respectively, resulting a treatment difference of -53.4% (Table 64); on the other hand, the estimated treatment difference is -70.5% which is more than 30% larger in magnitude than the treatment difference of -53.4%.

Therefore, the review team does not consider the Applicant's analysis method acceptable. The review team conducts analyses for the percent change endpoints without any transformation and the results are presented in Table 64. The 95% CIs and p-values for the treatment difference presented in Table 64 are obtained using normal approximation. To examine the robustness of these results, the review team calculates the 95% CI and p-value using a non-parametric method (permutation method) without relying on normal approximation. The non-parametric analysis results do not alter the overall efficacy conclusion. For example, for serum oligosaccharides, the non-parametric analysis results (95% CI: -80, -28; p-value<0.001) showed a statistically significant treatment difference favoring the velmanase group.

Table 65. Applicant's and FDA's Analyses Results

Parameter	**Velmanase**	**Placebo**	**Treatment Difference (95% CI)***
Percent change from baseline in serum oligosaccharides at Week 52			
FDA's analysis: Adjusted mean	-75.8	-20.2	-55.6 (-69.3, -41.9); p<0.001
Applicant's analysis: Adjusted mean	-77.6	-24.2	-70.5 (-78.3, -59.7); p<0.001
Percent change from baseline in 3MSCT at Week 52			
FDA's analysis: Adjusted mean	0.2	-3.2	3.4 (-9.5, -16.3); p =0.58
Applicant's analysis: Adjusted mean	-1.1	-4.0	3.1 (-9.9, 17.8); p =0.64
Percent change from baseline in FVC% at Week 52			
FDA's analysis: Adjusted mean	10.5	3.1	7.4 (-5.7, 20.5); p =0.25
Applicant's analysis: Adjusted mean	9.8	1.6	8.2 (-6.2, 24.4); p =0.27

Parameter	Velmanase	Placebo	Treatment Difference (95% CI)*
Percent change from baseline in 6MWT at Week 52			
FDA's analysis: Adjusted mean	1.0	-0.6	1.6 (-7.2, 10.4); p =0.71
Applicant's analysis: Adjusted mean	0.6	-1.2	1.9 (-6.6, 11.1); p =0.66

Source: Produced by the review team.
*obtained based on normal approximation.
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; CI, confidence interval; FDA, U.S. Food and Drug Administration; FVC, force vital capacity; missing data for FVC% were not imputed.

16.2.2. Applicant's Responder Analyses

The SAP pre-specified responder analyses for each of the primary and secondary efficacy endpoints. The responder analyses have been described in Section 6.2.1.4.

The review of the efficacy of the study did not consider the Applicant's responder analyses. The pre-specified responder thresholds were not agreed upon by the Agency. In addition, the Applicant did not provide a rationale for the clinical meaningfulness of the defined thresholds.

16.2.3. Endpoint Results by Visit

Table 65, Table 66, Table 67, Table 68, Table 69, Table 70, Table 71, Table 72, Table 73, Table 74, Table 75, and Table 76 report the observed, absolute change from baseline, and relative change from baseline in serum oligosaccharides, 3MSCT, FVC, and 6MWT by visit. In addition, the last two tables, Table 77 and Table 78, report results of the best and worst outcome, respectively, at Weeks 26 and 52.

Table 66. Serum Oligosaccharides (µmol/L) by Visit (Observed)

	Velmanase				Placebo			
Visit	N	Mean (SD)	Median	Min, Max	N	Mean (SD)	Median	Min, Max
Baseline	15	6.8 (1.2)	7.0	4.9, 8.7	10	6.6 (1.9)	6.3	4.4, 10.2
Week 26	15	2.4 (1.0)	2.4	1.3, 4.6	10	6.2 (1.8)	6.3	3.3, 9.3
Week 52	15	1.6 (0.8)	1.4	0.8, 4.0	10	5.1 (1.4)	5.0	2.3, 7.3

Source: Produced by the review team.
Abbreviations: N, number of subjects; SD, standard deviation

Table 67. Absolute Change From Baseline in Serum Oligosaccharides (µmol/L) by Visit (Observed)

	Velmanase				Placebo			
Visit	N	Mean (SD)	Median	Min, Max	N	Mean (SD)	Median	Min, Max
Week 26	15	-4.4 (1.4)	-4.5	-6.8, -2.0	10	-0.4 (2.2)	-0.1	-3.7, 2.8
Week 52	15	-5.1 (1.2)	-5.0	-7.2, -3.0	10	-1.6 (1.7)	-1.1	-3.9, 0.4

Source: Produced by the review team.
Abbreviations: N, number of subjects; SD, standard deviation

Table 68. Relative Change From Baseline in Serum Oligosaccharides (%) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	15	-63.6 (14.5)	-66.7	-83.5, -33.3	10	-1.6 (32.2)	-1.6	-46.8, 52.8
Week 52	15	-75.8 (11.2)	-78.7	-85.9, -50.0	10	-20.3 (24.0)	-16.2	-62.9, 7.6

Source: Produced by the review team.
Abbreviations: N, number of subjects; SD, standard deviation

Table 69. 3MSCT (Steps/Minute) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Baseline	15	52.9 (11.2)	50.0	27.7, 83.3	10	55.5 (16.0)	54.5	32.0, 78.0
Week 26	15	52.9 (13.8)	50.3	33.3, 90.0	10	53.8 (17.2)	50.0	32.0, 82.0
Week 52	15	53.5 (15.7)	54.3	31.3, 95.3	10	53.1 (15.6)	54.2	30.3, 71.3

Source: Produced by the review team.
NOTE: 3MSCT (steps/min) were measured down to 0.33 of a step
Abbreviations: 3MSCT, 3-minute stair climb test; N, number of subjects; SD, standard deviation

Table 70. Absolute Change From Baseline in 3MSCT (Steps/Minute) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	15	-0.02 (5.3)	0	-15.3, 6.7	10	-1.7 (5.3)	-2.0	-9.7, 7.7
Week 52	15	0.6 (8.6)	1.3	-14.7, 14.7	10	-2.4 (5.5)	-1.8	-11.3, 7.7

Source: Produced by the review team.
Abbreviations: 3MSCT, 3-minute stair climb test; N, number of subjects; SD, standard deviation

Table 71. Relative Change From Baseline in 3MSCT (%) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	15	-0.5 (9.7)	0.0	-27.9, 10.0	10	-2.9 (12.9)	-2.8	-20.4, 24.0
Week 52	15	0.5 (16.1)	2.5	-30.9, 27.5	10	-3.6 (13.1)	-3.1	-23.1, 24.0

Source: Produced by the review team.
Abbreviations: 3MSCT, 3-minute stair climb test; N, number of subjects; SD, standard deviation

Table 72. FVC (% Predicted) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Baseline	12	81.7 (20.7)	78	50, 119	9	90.4 (10.4)	92	72, 109
Week 26	13	90.4 (18.4)	88	60, 116	8	91 (14.1)	90.5	64, 113
Week 52	14	91.4 (21.8)	85	62, 127	9	92.4 (18.2)	98	51, 114

Source: Produced by the review team.
Abbreviations: FVC, forced vital capacity; N, number of subjects; SD, standard deviation

Table 73. Absolute Change From Baseline in FVC (% Predicted) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	11	5.8 (9.6)	6	-8, 20	8	-0.6 (5.5)	-1	-8, 8
Week 52	12	8.2 (9.9)	8	-10, 29	9	2.0 (12.6)	5	-30, 12

Source: Produced by the review team.
Abbreviations: FVC, forced vital capacity; N, number of subjects; SD, standard deviation

Table 74. Relative Change From Baseline in FVC (%) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	11	9.2 (13.9)	9.4	-8.3, 40.0	8	-1.0 (6.4)	-1.0	-11.1, 8.6
Week 52	12	11.4 (13.1)	8.6	-10.4, 36.2	9	1.9 (15.4)	4.6	-37.0, 15.3

Source: Produced by the review team.
Abbreviations: FVC, forced vital capacity; N, number of subjects; SD, standard deviation

Table 75. 6MWT (Meters) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Baseline	15	459.6 (72.3)	434.0	335, 627	10	465.7 (140.5)	482.5	219, 696
Week 26	15	464.3 (82.7)	441.0	332, 620	10	466.4 (126.2)	466.0	240, 690
Week 52	15	464.0 (82.5)	437.0	375, 690	10	461.1 (138.7)	475	183, 690

Source: Produced by the review team.
Abbreviations: 6MWT, 6-minute walk test; N, number of subjects; SD, standard deviation

Table 76. Absolute Change From Baseline in 6MWT (Meters) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	15	4.7 (42.8)	11.0	-90, 66	10	0.7 (37.6)	3.5	-55, 56
Week 52	15	4.4 (46.1)	9.0	-66, 104	10	-4.6 (40.8)	1.5	-72, 57

Source: Produced by the review team.
Abbreviations: 6MWT, 6-minute walk test; N, number of subjects; SD, standard deviation

Table 77. Relative Change From Baseline in 6MWT (%) by Visit (Observed)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	15	1.1 (9.7)	2.4	-21.3, 15.2	10	1.7 (9.2)	0.8	-11.3, 16.7
Week 52	15	1.2 (9.8)	2.1	-11.1, 21.2	10	-0.8 (10.8)	0.4	-16.4, 17.9

Source: Produced by the review team.
Abbreviations: 6MWT, 6-minute walk test; N, number of subjects; SD, standard deviation

Table 78. Best Outcome (%) by Visit (Derived From Observed Values)

	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	15	10.9 (10.1)	9.4	0, 40.0	10	6.1 (10.0)	6.9	-11.3, 24.0
Week 52	15	13.9 (12.5)	10.6	-8.4, 36.2	10	7.5 (8.1)	5.6	-3.5, 24.0

Source: Produced by the review team.
Abbreviations: N, number of subjects; SD, standard deviation

Table 79. Worst Outcome (%) by Visit (Derived From Observed Values)

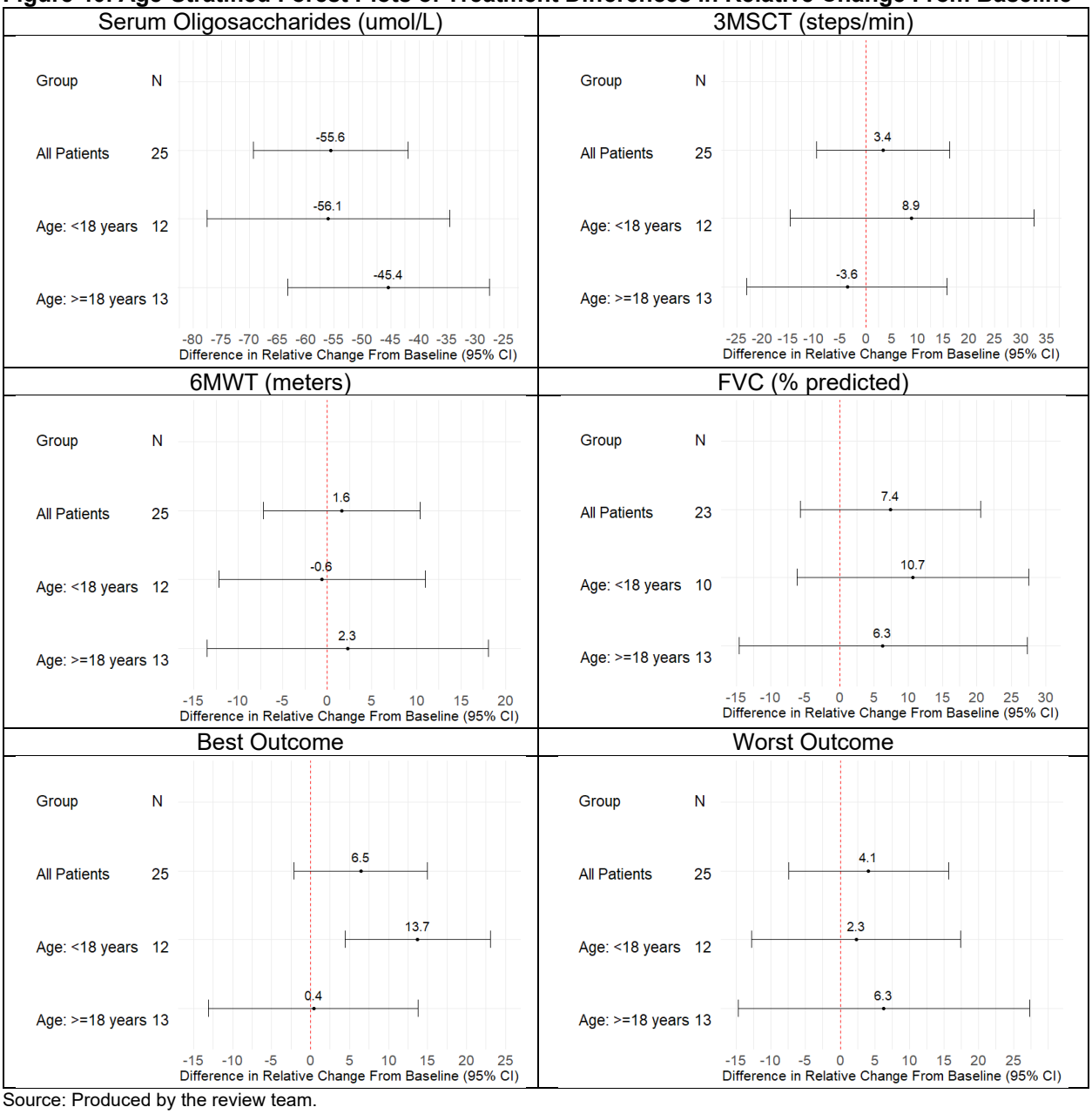
	Velmanase				Placebo			
Visit	**N**	**Mean (SD)**	**Median**	**Min, Max**	**N**	**Mean (SD)**	**Median**	**Min, Max**
Week 26	15	-6.3 (9.1)	-5.0	-27.9, 7.1	10	-8.0 (7.7)	-6.3	-20.4, 4.0
Week 52	15	-4.6 (12.1)	-3.1	-30.9, 14.4	10	-8.7 (14.2)	-5.0	-37.0, 8.7

Source: Produced by the review team.
Abbreviations: N, number of subjects; SD, standard deviation

16.2.4. Subgroup Analyses

Figure 40 presents age-stratified forest plots of the treatment difference in the relative change from baseline for serum oligosaccharides, the three clinical outcomes, and the best and worst outcomes.

Figure 40. Age-Stratified Forest Plots of Treatment Differences in Relative Change From Baseline



Source: Produced by the review team.
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; CI, confidence interval; FVC, force vital capacity

16.2.5. Additional Correlation Analyses

Table 79 presents the Pearson's correlation coefficients between absolute change from baseline in serum oligosaccharides and the changes from baseline in 3MSCT, 6MWT, and FVC at Week 52 in Trial rhLAMAN-05. The estimated correlations for each outcome in each group are variable and modest, with large confidence intervals.

Table 80. Estimated Pearson's Correlation Between Change in Serum Oligosaccharides and Change in Clinical Outcome at Week 52 in Trial rhLAMAN-05 by Treatment Arm

Clinical Outcome	Placebo (95% CI) (n=10)	Velmanase (95% CI) (n=15)	All[1] (95% CI) (n=25)
3MSCT	0.00 (-0.63, 0.63)	0.01 (-0.51, 0.52)	-0.15 (-0.52, 0.26)
6MWT	0.04 (-0.60, 0.65)	0.38 (-0.17, 0.75)	0.05 (-0.35, 0.44)
FVC	0.21 (-0.53, 0.77)	0.27 (-0.36, 0.73)	-0.07 (-0.49, 0.37)

Source: Produced by the review team.
All[1]: Estimated correlations using Week 52 changes from baseline from the total patient population
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; CI, confidence interval; FVC, force vital capacity; n, number of subjects

Table 80 presents the Pearson's correlation coefficients between absolute change from baseline in serum oligosaccharides and the changes from baseline in 3MSCT, 6MWT, and FVC at Week 26 in Trial rhLAMAN-05. As with the Week 52 results, the results are variable and modest.

Table 81. Estimated Pearson's Correlation Between Change in Serum Oligosaccharides and Change in Clinical Outcome at Week 26 in Trial rhLAMAN-05 by Treatment Arm

	Week 26		
Clinical Outcome	Placebo (95% CI) (n=10)	Velmanase (95% CI) (n=15)	All[1] (95% CI) (n=25)
3MSCT	0.14 (-0.53, 0.71)	0.01 (-0.51, 0.52)	-0.07 (-0.45, 0.34)
6MWT	0.37 (-0.34, 0.81)	0.24 (-0.31, 0.67)	0.15 (-0.26, 0.52)
FVC	-0.84 (-0.97, -0.34)	0.21 (-0.45, 0.72)	-0.39 (-0.72, 0.07)

Source: Produced by the review team.
All[1]: Estimated correlations using Week 26 changes from baseline from the total patient population
Abbreviations: 3MSCT, 3-minute stair climb test; 6MWT, 6-minute walk test; CI, confidence interval; FVC, force vital capacity; n, number of subjects

The estimated correlations for all patients at Week 52 are closer to -1 than the estimated correlations at Week 26 for 3MSCT and 6MWT. This may suggest strengthening of the correlation over time. However, the interpretability of the results is limited by the small sample size, short treatment duration, and slowly progressive and heterogeneous nature of the disease.

16.3. Trial rhLAMAN-10 and rhLAMAN-10 Integrated Analysis

16.3.1. Design and Eligibility Criteria

Trial rhLAMAN-10 was a brief open-label phase 3a study, comprised of 1 dose of velmanase (administered on Day 1) followed by efficacy assessments (scheduled to occur pre-dose and post dose at 10 minutes, 60 minutes, 2 hours, 24 hours, 72 hours, 5 days, and 6 days). This study was completed at a single study center in Denmark. A total of 18 patients enrolled and completed this

weeklong study. Eligibility criteria stated that only patients who had participated in the phase 1 trial (rhLAMAN-02), phase 2a trial (rhLAMAN-03), phase 2b trial (rhLAMAN-04), or phase 3 trial (rhLAMAN-05), and were still receiving weekly intravenous infusions of velmanase (according to the AfterCare Programs), could be included. Patients who were participating in other interventional trials with velmanase were excluded (including rhLAMAN-07 and rhLAMAN-09). The efficacy assessments were completed in a standardized format so that the data could be pooled into the rhLAMAN-10 Integrated Analysis.

Enrollment for Trial rhLAMAN-10:

Inclusion Criteria

Patients had participated in the phase 1 Trial rhLAMAN-02 (NCT01268358), phase 2a Trial rhLAMAN-03 (NCT01285700), phase 2b Trial rhLAMAN-04 NCT01681940) or the phase 3 Trial rhLAMAN-05 (NCT01681953) and were still receiving weekly IV infusions of velmanase according to the After-Trial Care Programs.

Exclusion Criteria

Patients who were participating in other interventional trials with velmanase were excluded (including rhLAMAN-07, NCT01908712, and rhLAMAN-09, NCT01908725).

16.3.2. Baseline Demographics and Clinical Characteristics by Parent Study

Table 82. Baseline Demographics and Clinical Characteristics, Safety Population, rhLAMAN-10 Integrated Analysis

Characteristic	Parent Trial rhLAMAN-02 9 (27)	Parent Trial rhLAMAN-05 24 (73)	Total Population 33 (100)
Sex, n (%)			
Male	7 (78)	13 (54)	20 (61)
Female	2 (22)	11 (46)	13 (39)
Age, years			
Mean (SD)	12.4 (3.8)	18.9 (8.3)	17.1 (7.8)
Median (min, max)	15 (7, 17)	19 (6, 35)	15 (6, 35)
Age group (years), n (%)			
≥6 to <18	9 (100)	10 (42)	19 (58)
≥18 to ≤35	0 (0)	14 (58)	14 (42)
Race, n (%)			
White	19 (100)	14 (100)	33 (100)
Country of participation, n (%)			
United States	0 (0)	0 (0)	0 (0)
United Kingdom	0 (0)	1 (4)	1 (3)
Poland	2 (22)	3 (13)	5 (15)
Spain	0 (0)	3 (13)	3 (9)
Germany	0 (0)	5 (21)	5 (15)
France	1 (11)	6 (25)	7 (21)
Other or Missing	6 (67)	6 (25)	12 (36)

Characteristic	Parent Trial rhLAMAN-02 9 (27)	Parent Trial rhLAMAN-05 24 (73)	Total Population 33 (100)
Serum oligosaccharides (umol/L)			
Mean (SD)	9.0 (2.7)	6.1 (1.5)	6.9 (2.3)
Median (min, max)	8 (6.0, 15.0)	6.1 (2.3, 8.7)	7 (2.3, 15.0)
Alphamanosidase activity (nmol/h/mg)			
Mean (SD)	11.7 (3.9)	12.7 (4.7)	12.4 (4.5)
Median (min, max)	10.5 (7.6, 20.1)	11.8 (8.3, 29.0)	11.5 (7.6, 29.0)
Alphamanosidase activity (relative) (%)			
Mean (SD)	4.3 (1.4)	4.6 (1.7)	4.5 (1.6)
Median (min, max)	3.8 (2.8, 7.3)	4.3 (3.0, 10.6)	4.2 (2.8, 10.6)

Source: Produced by the review team
NOTE: One patient, Patient (b) (6), was included using baseline data from Trial rhLAMAN-05, rather than treatment-naïve baseline data from Trial rhLAMAN-02.
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients with given characteristic; SD, standard deviation

16.3.3. Efficacy Results of Clinical Outcomes

Table 82 and Table 83 present the absolute and relative change from baseline in 3MSCT and 6MWT, respectively, over time. With the exception of Month 24, the absolute and relative changes from baseline increase over time. The increase suggests continued improvement on the 3MSCT and 6MWT over time, up to 48 months.

Table 83. Observed Results for the Change From Baseline in 3MSCT Over Time

	Observed		Absolute Change From Baseline (Steps/Minute)		Relative Change From Baseline (%)	
Visit	N	Mean (SD)	Mean (SD)	95% CI	Mean (SD)	95% CI
Baseline	33	53.6 (12.5)	-	-	-	-
Month 6	24	56.6 (14.5)	3.7 (7.9)	0.4, 7.1	8.3 (18.3)	0.6, 16.1
Month 12	31	58.5 (14.9)	4.3 (8.6)	1.1, 7.4	9.3 (19.6)	2.1, 16.5
Month 18	11	62.6 (17.0)	11.6 (9.5)	5.2, 17.9	24.5 (18.8)	11.9, 37.1
Month 24	10	57.3 (18.2)	1.9 (9.3)	-4.8, 8.6	2.5 (16.8)	-9.6, 14.5
Month 36	6	60.7 (19.0)	11.6 (9.3)	1.9, 21.4	30.9 (32.7)	-3.5, 65.2
Month 48	9	56.6 (14.5)	17.1 (9.9)	9.4, 24.7	39.1 (31.3)	15.0, 63.2

Source: Produced by the review team
Abbreviations: 3MSCT, 3-minute stair climb test; CI, confidence interval; N, number of subjects; SD, standard deviation

Table 84. Observed Results for the Change From Baseline in 6MWT Over Time

	Observed		Absolute Change From Baseline (Meters)		Relative Change From Baseline (%)	
Visit	N	Mean (SD)	Mean (SD)	95% CI	Mean (SD)	95% CI
Baseline	33	466.6 (90.1)	-	-	-	-
Month 6	24	474.6 (84.1)	17.6 (62.7)	-8.9, 44.0	6.1 (21.1)	-2.8, 15.0
Month 12	31	492.4 (83.7)	21.9 (65.2)	-2.0, 45.8	7.3 (23.3)	-1.2, 15.9
Month 18	11	499.9 (95.6)	55.5 (66.3)	11.0, 100.0	16.4 (25.7)	-0.9, 33.6
Month 24	10	486.6 (90.7)	5.0 (58.5)	-36.9, 46.9	1.2 (12.3)	-7.6, 10.0
Month 36	6	471.2 (83.5)	59.3 (85.9)	-30.8, 149.5	24.4 (46.1)	-24.0, 72.7
Month 48	9	522.6 (77.1)	69.7 (81.1)	7.4, 132.0	22.5 (35.8)	-5.0, 50.0

Source: Produced by the review team
Abbreviations: 6MWT, 6-minute walk test; CI, confidence interval; N, number of subjects; SD, standard deviation

Table 84 presents the absolute and relative change from baseline in FVC over time. The results at all time points indicate an increase in performance on the FVC from baseline. Unlike the 3MSCT and 6MWT, the absolute and relative changes from baseline do not increase over time but fluctuate. This may be due to decreased sample sizes at Months 18, 24, 36, and 48.

Table 85. Observed Results for the Change From Baseline in FVC Over Time

	Observed		Absolute Change From Baseline (% Predicted)		Relative Change From Baseline (%)	
Visit	N	Mean (SD)	Mean (SD)	95% CI	Mean (SD)	95% CI
Baseline	29	84.9 (18.6)	-	-	-	-
Month 6	22	87.1 (18.6)	3.5 (14.7)	-3.5, 10.4	6.1 (20.3)	-3.4, 15.6
Month 12	30	93.2 (20.8)	6.6 (12.8)	1.6, 11.5	8.5 (16.5)	2.1, 14.9
Month 18	8	84.8 (23.6)	4.4 (13.9)	-7.3, 16.0	5.0 (20.9)	-12.5, 22.5
Month 24	8*	106.1 (18.0)	16.1 (14.8)	2.5, 29.8	20.7 (18.5)	3.6, 37.9
Month 36	6	78.8 (20.0)	5.6 (10.3)	-5.3, 16.4	7.6 (15.2)	-8.4, 23.5
Month 48	7	98.3 (12.4)	13.7 (19.6)	-4.4, 31.9	19.8 (28.4)	-6.5, 46.1

Source: Produced by the review team
* Eight patients had observed FVC at 24 months but only seven of the eight patients had an analyzable absolute and relative change from baseline in FVC at 24 months
Abbreviations: CI, confidence interval; FVC, forced vital capacity; N, number of subjects; SD, standard deviation

It is important to note that although the results suggest that treatment with velmanase leads to consistent changes from baseline over time, a large proportion of patients included in the integrated analysis have missing data for the outcomes at later time points. For the change in serum oligosaccharides, 70%, 91%, and 73% of patients included in the integrated analysis had missing serum oligosaccharide data at 24, 36, and 48 months, respectively. The same proportion of patients had missing 3MSCT and 6MWT measurements at 24 and 48 months. For the change in FVC (% predicted), 79%, 82%, and 79% of patients included in the integrated analysis had missing FVC measurements at 24, 36, and 48 months, respectively. The amount of missing data limits the interpretability of the results.

17. Clinical Safety

17.1. Safety Results, Trial rhLAMAN-05

Table 85 lists the treatment-emergent adverse events (TEAEs) by system organ class and compares the TEAEs that occurred in patients who participated in the trial comparing the risk difference between those who received velmanase and those who received placebo. Table 85 below demonstrates that the treatment group presented a higher risk than placebo for several TEAEs, most notably in the system organ classes of immune system disorders (hypersensitivity) with risk difference (RD) of 20%, skin and subcutaneous tissue disorders rash RD 20%, pruritis 13.3%, infections infestations (bacterial infection) with RD of 13.3%, and nervous system disorders (syncope) with RD 13.3%.

Table 86. Patients With Adverse Events by System Organ Class and FDA Medical Query (Narrow), Safety Population, Trial rhLAMAN-05

	Patients <18 Years			Patients ≥18 Years			All Patients		
System Organ Class FMQ (Narrow)	Velmanase N=7 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n (%)	Placebo N=5 n (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n (%)	Placebo N=10 n (%)	Risk Difference (%) (95% CI)
Cardiac disorders (SOC)									
Arrhythmia	1 (14.3)	0	14.3 (-11.6, 40.2)	0	1 (20.0)	-20.0 (-55.1, 15.1)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Tachycardia	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Gastrointestinal disorders (SOC)									
Constipation	0	0	0 (0, 0)	1 (12.5)	1 (20.0)	-7.5 (-49.4, 34.4)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Nausea	1 (14.3)	1 (20.0)	-5.7 (-49.3, 37.9)	0	0	0 (0, 0)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Dyspepsia	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Diarrhea	0	2 (40.0)	-40.0 (-82.9, 2.9)	2 (25.0)	1 (20.0)	5.0 (-41.1, 51.1)	2 (13.3)	3 (30.0)	-16.7 (-49.9, 16.5)
Abdominal Pain	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	2 (20.0)	-20.0 (-44.8, 4.8)
Vomiting	2 (28.6)	2 (40.0)	-11.4 (-65.9, 43.0)	1 (12.5)	2 (40.0)	-27.5 (-76.2, 21.2)	3 (20.0)	4 (40.0)	-20.0 (-56.5, 16.5)

System Organ Class FMQ (Narrow)	Patients <18 Years Velmanase N=7 n (%)	 Placebo N=5 n (%)	 Risk Difference (%) (95% CI)	Patients ≥18 Years Velmanase N=8 n (%)	 Placebo N=5 n (%)	 Risk Difference (%) (95% CI)	All Patients Velmanase N=15 n (%)	 Placebo N=10 n (%)	 Risk Difference (%) (95% CI)
General disorders and administration site conditions (SOC)									
Decreased Appetite	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Fatigue	1 (14.3)	1 (20.0)	-5.7 (-49.3, 37.9)	0	0	0 (0, 0)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Peripheral Edema	0	0	0 (0, 0)	1 (12.5)	1 (20.0)	-7.5 (-49.4, 34.4)	1 (6.7)	1 (10.0)	-3.3 (-25.8, 19.1)
Fall	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Pyrexia	4 (57.1)	3 (60.0)	-2.9 (-59.3, 53.6)	2 (25.0)	2 (40.0)	-15.0 (-67.4, 37.4)	6 (40.0)	5 (50.0)	-10.0 (-49.7, 29.7)
Dizziness	1 (14.3)	0	14.3 (-11.6, 40.2)	0	2 (40.0)	-40.0 (-82.9, 2.9)	1 (6.7)	2 (20.0)	-13.3 (-41.2, 14.5)
Local Administration Reaction	0	2 (40.0)	-40.0 (-82.9, 2.9)	0	0	0 (0, 0)	0	2 (20.0)	-20.0 (-44.8, 4.8)
Hepatobiliary disorders (SOC)									
Hepatic Injury	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Immune system disorders (SOC)									
Hypersensitivity	1 (14.3)	0	14.3 (-11.6, 40.2)	2 (25.0)	0	25.0 (-5.0, 55.0)	3 (20.0)	0	20.0 (-0.2, 40.2)
Infections and infestations (SOC)									
Bacterial Infection	0	0	0 (0, 0)	5 (62.5)	2 (40.0)	22.5 (-32.0, 77.0)	5 (33.3)	2 (20.0)	13.3 (-21.1, 47.7)
Viral Infection	1 (14.3)	0	14.3 (-11.6, 40.2)	2 (25.0)	1 (20.0)	5.0 (-41.1, 51.1)	3 (20.0)	1 (10.0)	10.0 (-17.5, 37.5)
Fungal Infection	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Nasopharyngitis	5 (71.4)	2 (40.0)	31.4 (-23.0, 85.9)	5 (62.5)	5 (100)	-37.5 (-71.0, -4.0)*	10 (66.7)	7 (70.0)	-3.3 (-40.4, 33.8)
Musculoskeletal and connective tissue disorders (SOC)									
Arthralgia	2 (28.6)	0	28.6 (-4.9, 62.0)	1 (12.5)	1 (20.0)	-7.5 (-49.4, 34.4)	3 (20.0)	1 (10.0)	10.0 (-17.5, 37.5)
Back Pain	2 (28.6)	0	28.6 (-4.9, 62.0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	2 (13.3)	1 (10.0)	3.3 (-22.0, 28.7)
Fracture	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Myalgia	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)

System Organ Class FMQ (Narrow)	Patients <18 Years Velmanase N=7 n (%)	 Placebo N=5 n (%)	 Risk Difference (%) (95% CI)	Patients ≥18 Years Velmanase N=8 n (%)	 Placebo N=5 n (%)	 Risk Difference (%) (95% CI)	All Patients Velmanase N=15 n (%)	 Placebo N=10 n (%)	 Risk Difference (%) (95% CI)
Nervous system disorders (SOC)									
Syncope	1 (14.3)	0	14.3 (-11.6, 40.2)	1 (12.5)	0	12.5 (-10.4, 35.4)	2 (13.3)	0	13.3 (-3.9, 30.5)
Headache	3 (42.9)	2 (40.0)	2.9 (-53.6, 59.3)	2 (25.0)	1 (20.0)	5.0 (-41.1, 51.1)	5 (33.3)	3 (30.0)	3.3 (-33.8, 40.4)
Psychiatric disorders (SOC)									
Insomnia	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Psychosis	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Anxiety	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Depression	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Irritability	0	0	0 (0, 0)	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Renal and urinary disorders (SOC)									
Acute Kidney Injury	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Renal and Urinary Tract Infection	0	0	0 (0, 0)	2 (25.0)	1 (20.0)	5.0 (-41.1, 51.1)	2 (13.3)	1 (10.0)	3.3 (-22.0, 28.7)
Respiratory, thoracic, and mediastinal disorders (SOC)									
Bronchospasm	1 (14.3)	0	14.3 (-11.6, 40.2)	0	0	0 (0, 0)	1 (6.7)	0	6.7 (-6.0, 19.3)
Dyspnea	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)
Cough	0	1 (20.0)	-20.0 (-55.1, 15.1)	0	0	0 (0, 0)	0	1 (10.0)	-10.0 (-28.6, 8.6)
Skin and subcutaneous tissue disorders (SOC)									
Rash	0	0	0 (0, 0)	3 (37.5)	0	37.5 (4.0, 71.0)*	3 (20.0)	0	20.0 (-0.2, 40.2)
Pruritus	0	0	0 (0, 0)	2 (25.0)	0	25.0 (-5.0, 55.0)	2 (13.3)	0	13.3 (-3.9, 30.5)
Urticaria	0	0	0 (0, 0)	1 (12.5)	0	12.5 (-10.4, 35.4)	1 (6.7)	0	6.7 (-6.0, 19.3)

System Organ Class FMQ (Narrow)	Patients <18 Years Velmanase N=7 n (%)	 Placebo N=5 n (%)	 Risk Difference (%) (95% CI)	Patients ≥18 Years Velmanase N=8 n (%)	 Placebo N=5 n (%)	 Risk Difference (%) (95% CI)	All Patients Velmanase N=15 n (%)	 Placebo N=10 n (%)	 Risk Difference (%) (95% CI)
Vascular disorders (SOC)									
Hemorrhage	1 (14.3)	2 (40.0)	-25.7 (-75.9, 24.4)	1 (12.5)	0	12.5 (-10.4, 35.4)	2 (13.3)	2 (20.0)	-6.7 (-36.8, 23.5)

Source: adae.xpt; Software: R
Asterisk (*) indicates rows where the 95% confidence interval excludes zero.
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of Velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 12 months.
Risk difference (with 95% confidence interval) is shown between velmanase and placebo.
Each FMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some FMQs may contain PTs from more than one SOC.
For specific preferred terms under each FMQ, see the table "Adverse Events by System Organ Class, FDA Medical Query (Narrow) and Preferred Term"
Abbreviations: CI, confidence interval; FMQ, FDA medical query; N, number of patients in treatment arm; n, number of patients with adverse event; SOC, system organ class

Table 86 below lists the outlier hematological laboratory values described in Section 7.6.1.5.

Table 87. Patients With One or More Hematology Analyte Values Exceeding Specified Levels, Safety Population, Trial rhLAMAN-05

Laboratory Parameter	Patients <18 Years Velmanase N=7 n/N_{ew} (%)	 Placebo N=5 n/N_{ew} (%)	 Risk Difference (%) (95% CI)	Patients ≥18 Years Velmanase N=8 n/N_w (%)	 Placebo N=5 n/N_w (%)	 Risk Difference (%) (95% CI)	All Patients Velmanase N=15 n/N_w (%)	 Placebo N=10 n/N_w (%)	 Risk Difference (%) (95% CI)
Complete Blood Count									
WBC, low (cells/uL)									
Level 1 (<3500)	2/7 (28.6)	3/5 (60.0)	-31.4 (-85.9, 23.0)	0/8 (0)	2/5 (40.0)	-40.0 (-82.9, 2.9)	2/15 (13.3)	5/10 (50.0)	-36.7 (-72.1, -1.2)
Level 2 (<3000)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	1/5 (20.0)	-20.0 (-55.1, 15.1)	1/15 (6.7)	1/10 (10.0)	-3.3 (-25.8, 19.1)
Level 3 (<1000)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
WBC, high (cells/uL)									
Level 1 (>10800)	0/7 (0)	0/5 (0)	0 (0, 0)	1/8 (12.5)	0/5 (0)	12.5 (-10.4, 35.4)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 2 (>13000)	0/7 (0)	0/5 (0)	0 (0, 0)	1/8 (12.5)	0/5 (0)	12.5 (-10.4, 35.4)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 3 (>15000)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)

Laboratory Parameter	Patients <18 Years			Patients ≥18 Years			All Patients		
	Velmanase N=7 n/N_{ew} (%)	Placebo N=5 n/N_{ew} (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n/N_{w} (%)	Placebo N=5 n/N_{w} (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n/N_{w} (%)	Placebo N=10 n/N_{w} (%)	Risk Difference (%) (95% CI)
Hemoglobin, low (g/dL)									
Level 2 (>1.5 g/dL dec. from baseline)	2/7 (28.6)	0/5 (0)	28.6 (-4.9, 62.0)	1/8 (12.5)	0/5 (0)	12.5 (-10.4, 35.4)	3/15 (20.0)	0/10 (0)	20.0 (-0.2, 40.2)
Level 3 (>2 g/dL dec. from baseline)	0/7 (0)	0/5 (0)	0 (0, 0)	1/8 (12.5)	0/5 (0)	12.5 (-10.4, 35.4)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Hemoglobin, high (g/dL)									
Level 2 (>2 g/dL inc. from baseline)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	1/5 (20.0)	-20.0 (-55.1, 15.1)	0/15 (0)	1/10 (10.0)	-10.0 (-28.6, 8.6)
Level 3 (>3 g/dL inc. from baseline)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Platelets, low (cells/uL)									
Level 1 (<140000)	1/7 (14.3)	1/5 (20.0)	-5.7 (-49.3, 37.9)	1/8 (12.5)	2/5 (40.0)	-27.5 (-76.2, 21.2)	2/15 (13.3)	3/10 (30.0)	-16.7 (-49.9, 16.5)
Level 2 (<125000)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	1/8 (12.5)	2/5 (40.0)	-27.5 (-76.2, 21.2)	2/15 (13.3)	2/10 (20.0)	-6.7 (-36.8, 23.5)
Level 3 (<100000)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	1/5 (20.0)	-20.0 (-55.1, 15.1)	1/15 (6.7)	1/10 (10.0)	-3.3 (-25.8, 19.1)
WBC Differential									
Lymphocytes, low (cells/uL)									
Level 1 (<1000)	0/7 (0)	1/5 (20.0)	-20.0 (-55.1, 15.1)	2/8 (25.0)	1/5 (20.0)	5.0 (-41.1, 51.1)	2/15 (13.3)	2/10 (20.0)	-6.7 (-36.8, 23.5)
Level 2 (<750)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	1/5 (20.0)	-20.0 (-55.1, 15.1)	0/15 (0)	1/10 (10.0)	-10.0 (-28.6, 8.6)
Level 3 (<500)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Lymphocytes, high (cells/uL)									
Level 1 (>4000)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 2 (>10000)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 3 (>20000)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)

Laboratory Parameter	Patients <18 Years			Patients ≥18 Years			All Patients		
	Velmanase N=7 n/N_{ew} (%)	Placebo N=5 n/N_{ew} (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n/N_w (%)	Placebo N=5 n/N_w (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n/N_w (%)	Placebo N=10 n/N_w (%)	Risk Difference (%) (95% CI)
Neutrophils, low (cells/uL)									
Level 1 (<2000)	6/7 (85.7)	5/5 (100)	-14.3 (-40.2, 11.6)	4/8 (50.0)	2/5 (40.0)	10.0 (-45.2, 65.2)	10/15 (66.7)	7/10 (70.0)	-3.3 (-40.4, 33.8)
Level 2 (<1000)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	0/5 (0)	0 (0, 0)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 3 (<500)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Eosinophils, high (cells/uL)									
Level 1 (>650)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	0/5 (0)	0 (0, 0)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 2 (>1500)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 3 (>5000)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)

Source: ad b.xpt; Software: R
Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Duration is 12 months.
Risk difference (with 95% confidence interval) is shown between velmanase and placebo.
Abbreviations: CI, confidence interval; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; PT, prothrombin time; PTT, partial thromboplastin time; ULN, upper limit of normal; WBC, white blood cells

Table 87 below lists the patient with abnormally high creatinine and decreased glomerular filtration rate (GFR) in Trial rhLMANA-05. The creatinine values for patient RHLAMAN-05-(b) (6) during an SAE of acute renal failure were not included by Applicant in the rhLAMAN05 dataset (and are not reflected in Table 87 below) and instead were reported within patient narrative (Refer to Section 7.6.1.3).

Table 88. Patients With One or More Kidney Function Analyte Values Exceeding Specified Levels, Safety Population, Trial rhLAMAN-05

	Patients <18 Years			Patients ≥18 Years			All Patients		
Laboratory Parameter	**Velmanase N=7 n/N_w (%)**	**Placebo N=5 n/N_w (%)**	**Risk Difference (%) (95% CI)**	**Velmanase N=8 n/N_w (%)**	**Placebo N=5 n/N_w (%)**	**Risk Difference (%) (95% CI)**	**Velmanase N=15 n/N_w (%)**	**Placebo N=10 n/N_w (%)**	**Risk Difference (%) (95% CI)**
Creatinine, high (mg/dL)									
Level 1 (≥1.5X baseline)	0/7 (0)	0/5 (0)	0 (0, 0)	1/8 (12.5)	0/5 (0)	12.5 (-10.4, 35.4)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 2 (≥2X baseline)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 3 (≥3X baseline)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
eGFR, low (ml/min/1.73 m^2)									
Level 1 (≥25% decrease)	0/7 (0)	0/5 (0)	0 (0, 0)	1/8 (12.5)	0/5 (0)	12.5 (-10.4, 35.4)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 2 (≥50% decrease)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 3 (≥75% decrease)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)

Source: ad b.xpt; Software: R
Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Duration is 12 months.
Risk difference (with 95% confidence interval) is shown between velmanase and placebo.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data

Table 88 below reports the laboratory trends (sodium, potassium, calcium, creatine kinase, and amylase) discussed in Section 7.6.1.5.

Table 89. Patients With One or More Chemistry Analyte Values With Elevated or Low Values Meeting Specified Levels, Safety Population, Trial rhLAMAN-05

	Patients <18 Years			Patients ≥18 Years			All Patients		
Laboratory Parameter	Velmanase N=7 n/N_w (%)	Placebo N=5 n/N_w (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n/N_w (%)	Placebo N=5 n/N_w (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n/N_w (%)	Placebo N=10 n/N_w (%)	Risk Difference (%) (95% CI)
Sodium, low (mEq/L)									
Level 1 (<132)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	0/5 (0)	0 (0, 0)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 2 (<130)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	0/5 (0)	0 (0, 0)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 3 (<125)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	0/5 (0)	0 (0, 0)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Sodium, high (mEq/L)									
Level 1 (>150)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 2 (>155)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 3 (>160)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Potassium, low (mEq/L)									
Level 1 (<3.6)	1/7 (14.3)	1/5 (20.0)	-5.7 (-49.3, 37.9)	1/8 (12.5)	1/5 (20.0)	-7.5 (-49.4, 34.4)	2/15 (13.3)	2/10 (20.0)	-6.7 (-36.8, 23.5)
Level 2 (<3.4)	0/7 (0)	0/5 (0)	0 (0, 0)	1/8 (12.5)	0/5 (0)	12.5 (-10.4, 35.4)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 3 (<3)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Potassium, high (mEq/L)									
Level 1 (>5.5)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 2 (>6)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 3 (>6.5)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Calcium, low (mg/dL)									
Level 1 (<8.4)	1/7 (14.3)	1/5 (20.0)	-5.7 (-49.3, 37.9)	1/8 (12.5)	1/5 (20.0)	-7.5 (-49.4, 34.4)	2/15 (13.3)	2/10 (20.0)	-6.7 (-36.8, 23.5)
Level 2 (<8)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	0/5 (0)	0 (0, 0)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 3 (<7.5)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	0/5 (0)	0 (0, 0)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Calcium, high (mg/dL)									
Level 1 (>10.5)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 2 (>11)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 3 (>12)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)

Laboratory Parameter	Patients <18 Years			Patients ≥18 Years			All Patients		
	Velmanase N=7 n/N_w (%)	Placebo N=5 n/N_w (%)	Risk Difference (%) (95% CI)	Velmanase N=8 n/N_w (%)	Placebo N=5 n/N_w (%)	Risk Difference (%) (95% CI)	Velmanase N=15 n/N_w (%)	Placebo N=10 n/N_w (%)	Risk Difference (%) (95% CI)
Phosphate, low (mg/dL)									
Level 1 (<2.5)	0/7 (0)	0/5 (0)	0 (0, 0)	3/8 (37.5)	2/5 (40.0)	-2.5 (-57.0, 52.0)	3/15 (20.0)	2/10 (20.0)	0 (-32.0, 32.0)
Level 2 (<2)	0/7 (0)	0/5 (0)	0 (0, 0)	2/8 (25.0)	0/5 (0)	25.0 (-5.0, 55.0)	2/15 (13.3)	0/10 (0)	13.3 (-3.9, 30.5)
Level 3 (<1.4)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
CPK, high (U/L)									
Level 1 (>3X ULN)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 2 (>5X ULN)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Level 3 (>10X ULN)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)
Amylase, high (U/L)									
Level 1 (>1.1X ULN)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	1/5 (20.0)	-20.0 (-55.1, 15.1)	1/15 (6.7)	1/10 (10.0)	-3.3 (-25.8, 19.1)
Level 2 (>1.5X ULN)	1/7 (14.3)	0/5 (0)	14.3 (-11.6, 40.2)	0/8 (0)	0/5 (0)	0 (0, 0)	1/15 (6.7)	0/10 (0)	6.7 (-6.0, 19.3)
Level 3 (>3X ULN)	0/7 (0)	0/5 (0)	0 (0, 0)	0/8 (0)	0/5 (0)	0 (0, 0)	0/15 (0)	0/10 (0)	0 (0, 0)

Source: ad b.xpt; Software: R
Note that glucose values for hyperglycemia do not follow a nested format like the other labs. Level 1 corresponds to the diagnosis of prediabetes and is not inclusive of Level 2 and 3. Level 2 corresponds to the diagnosis of diabetes. Level 3 represents significant hyperglycemia that may indicate need for insulin or increased risk for diabetic ketoacidosis or other complications.
Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Duration is 12 months.
Risk difference (with 95% confidence interval) is shown between velmanase and placebo.
Abbreviations: CI, confidence interval; CPK, creatine phosphokinase; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; ULN, upper limit of normal

17.2. Safety Results, rhLAMAN-10 Integrated Analysis

17.2.1. Overview of Treatment-Emergent Adverse Events Summary, Trial rhLAMAN-10

The rhLAMAN-10 Integrated Analysis is not a clinical study, as it included data captured prior to and including the single assessment conducted in Trial rhLAMAN-10.

Clinically relevant abnormalities are discussed in the patient narratives in previous sections (see Section 7.6.2). For details regarding rhLAMAN-10 trial and integrated analysis design, refer to Section 6.2.2. For the safety review, the integrated analysis portion of rhLAMAN-10 will simply be referred to as -rhLAMAN-10 Integrated Analysis. Because there is no control group within rhLAMAN-10 Integrated Analysis, the review of safety data within it is viewed as supportive to the pivotal trial (rhLAMAN-05).

Table 89 below gives an overview of AEs in the rhLAMAN-10 Integrated Analysis.

Within the rhLAMAN-10 Integrated Analysis, 12 unique patients experienced SAEs. In total, 9 patients experienced an AE that led to a dose modification of the study drug including interruption or reduction of velmanase.

At least one treatment-emergent adverse event (TEAE) was reported in 88% (29 of 33) of patients.

Table 90. Overview of Adverse Events, Safety Population, rhLAMAN-10 Integrated Analysis

	Velmanase		
Event Category	<18 years N=19 n (%)	≥18 years N=14 n (%)	All N=33 n (%)
SAE	7 (36.8)	5 (35.7)	12 (36.4)
SAEs with fatal outcome	0	0	0
Life-threatening SAEs	0	0	0
AE leading to permanent discontinuation of study drug	0	0	0
AE leading to dose modification of study drug	5 (26.3)	4 (28.6)	9 (27.3)
AE leading to interruption of study drug	5 (26.3)	2 (14.3)	7 (21.2)
AE leading to reduction of study drug	2 (10.5)	2 (14.3)	4 (12.1)
AE leading to dose delay of study drug	0	0	0
Other	0	0	0

Any AE	17 (89.5)	12 (85.7)	29 (87.9)
Severe and worse	2 (10.5)	1 (7.1)	3 (9.1)
Moderate	11 (57.9)	9 (64.3)	20 (60.6)
Mild	4 (21.1)	2 (14.3)	6 (18.2)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of Velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 12 to 48 months.
Severity as assessed by the investigator.
Abbreviations: AE, adverse event; N, number of patients in treatment arm; n, number of patients with at least one event; SAE, serious adverse event

17.2.2. Deaths, rhLAMAN-10 Integrated Analysis

No deaths were reported in the rhLAMAN-10 Integrated Analysis.

17.2.3. Serious Treatment-Emergent Adverse Events, rhLAMAN-10 Integrated Analysis

Table 90 (below) lists the SAEs reported in 12 patients (36%) of the patients. The most common SAEs are in system organ class (SOC) of musculoskeletal and connective tissue disorders. Of note five of these SAEs occurred during trial rhLAMAN-05 and were therefore previously described in Section 7.6.1.3. In total, there were 3 SAEs the review team assessed as possibly treatment related. These include acute renal failure (described in Section 7.6.1.3), seizure (described in Section 7.6.2), and malabsorption from injection site. Patient narratives for the latter two of these are below.

Table 91. Patients With Serious Adverse Events by System Organ Class and Preferred Term, Safety Population, rhLAMAN-10 Integrated Analysis

	Velmanase		
System Organ Class Preferred Term	<18 years N=19 n (%)	≥18 years N=14 n (%)	All N=33 n (%)
Any SAE	7 (36.8)	5 (35.7)	12 (36.4)
General disorders and administration site conditions (SOC)	0	1 (7.1)	1 (3.0)
Malabsorption from injection site	0	1 (7.1)	1 (3.0)
Infections and infestations (SOC)	2 (10.5)	1 (7.1)	3 (9.1)
Device related infection	1 (5.3)	0	1 (3.0)
Ear infection	1 (5.3)	0	1 (3.0)
Sepsis	0	1 (7.1)	1 (3.0)
Injury, poisoning and procedural complications (SOC)	1 (5.3)	0	1 (3.0)
Craniocerebral injury	1 (5.3)	0	1 (3.0)
Musculoskeletal and connective tissue disorders (SOC)	3 (15.8)	1 (7.1)	4 (12.1)
Arthritis	1 (5.3)	0	1 (3.0)
Knee deformity	1 (5.3)	0	1 (3.0)
Sjogren`s syndrome	1 (5.3)	0	1 (3.0)
Joint swelling	0	1 (7.1)	1 (3.0)
Nervous system disorders (SOC)	1 (5.3)	1 (7.1)	2 (6.1)
Loss of consciousness	1 (5.3)	0	1 (3.0)
Somnolence	0	1 (7.1)	1 (3.0)

Renal and urinary disorders (SOC)	0	1 (7.1)	1 (3.0)
Renal failure acute	0	1 (7.1)	1 (3.0)
Vascular disorders (SOC)	1 (5.3)	0	1 (3.0)
Syncope	1 (5.3)	0	1 (3.0)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of Velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Serious adverse events defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.
Duration is 12 to 48 months.
Abbreviations: N, number of patients in treatment arm; n, number of patients with adverse event; SOC, system organ class

Patient rhLAMAN-10-(b) (6) experienced 2 episodes of seizures. This patient narrative was detailed previously in Section 7.6.2.1. In context of the 2 other patients reported to have seizures (previously discussed in Section 7.6.2) there is a concern for risk of seizures, and this will be addressed in labelling recommendations to include seizures in ARs section.

Patient rhLAMAN-10-(b) (6) experienced malabsorption from injection site (site dysfunction) on the day of velmanase infusion, and the patient was hospitalized. The infusion site was changed 18 days later, and the event resolved. The Investigator assessed this event as not related to velmanase. No further details are provided by the Applicant. The review team assessed that this event was likely related to the technique used for injection and not likely related to velmanase itself.

17.2.4. Adverse Events and FDA Medical Queries Leading to Treatment Discontinuation, rhLAMAN-10 Integrated Analysis

No permanent discontinuations of drugs reported in the rhLAMAN-10 Integrated Analysis.

17.2.5. Treatment-Emergent Adverse Events, rhLAMAN-10 Integrated Analysis

Table 91 (below) lists the TEAs that occurred in patients who participated in the rhLAMAN-10 Integrated Analysis by SOC. An AE was reported in 29 of the 33 patients (88%). The most common TEAEs are in SOC of infection and infestations with nasopharyngitis in 73% (24 of 33) patients.

Table 92. Patients With Adverse Events by System Organ Class and FDA Medical Query (Narrow), Safety Population, rhLAMAN-10 Integrated Analysis

	Velmanase		
System Organ Class FMQ (Narrow)	<18 years N=19 n (%)	≥18 years N=14 n (%)	All N=33 n (%)
Cardiac disorders (SOC)			
Arrhythmia	1 (5.3)	0	1 (3.0)
Endocrine disorders (SOC)			
Hypoglycemia	0	1 (7.1)	1 (3.0)

System Organ Class FMQ (Narrow)	Velmanase <18 years N=19 n (%)	 ≥18 years N=14 n (%)	 All N=33 n (%)
Gastrointestinal disorders (SOC)			
Vomiting	8 (42.1)	2 (14.3)	10 (30.3)
Diarrhea	6 (31.6)	3 (21.4)	9 (27.3)
Abdominal Pain	5 (26.3)	0	5 (15.2)
Nausea	3 (15.8)	0	3 (9.1)
Dyspepsia	2 (10.5)	0	2 (6.1)
Constipation	0	1 (7.1)	1 (3.0)
General disorders and administration site conditions (SOC)			
Pyrexia	9 (47.4)	3 (21.4)	12 (36.4)
Fatigue	4 (21.1)	1 (7.1)	5 (15.2)
Local Administration Reaction	4 (21.1)	0	4 (12.1)
Dizziness	3 (15.8)	0	3 (9.1)
Fall	1 (5.3)	1 (7.1)	2 (6.1)
Peripheral Edema	1 (5.3)	2 (14.3)	3 (9.1)
Decreased Appetite	0	1 (7.1)	1 (3.0)
Hepatobiliary disorders (SOC)			
Hepatic Injury	1 (5.3)	0	1 (3.0)
Immune system disorders (SOC)			
Hypersensitivity	3 (15.8)	2 (14.3)	5 (15.2)
Infections and infestations (SOC)			
Nasopharyngitis	15 (78.9)	9 (64.3)	24 (72.7)
Viral Infection	7 (36.8)	2 (14.3)	9 (27.3)
Bacterial Infection	5 (26.3)	5 (35.7)	10 (30.3)
Fungal Infection	2 (10.5)	0	2 (6.1)
Purulent Material	1 (5.3)	0	1 (3.0)
Musculoskeletal and connective tissue disorders (SOC)			
Arthralgia	5 (26.3)	2 (14.3)	7 (21.2)
Back Pain	3 (15.8)	2 (14.3)	5 (15.2)
Myalgia	2 (10.5)	0	2 (6.1)
Fracture	1 (5.3)	0	1 (3.0)
Tendinopathy	1 (5.3)	0	1 (3.0)
Nervous system disorders (SOC)			
Headache	10 (52.6)	4 (28.6)	14 (42.4)
Syncope	2 (10.5)	1 (7.1)	3 (9.1)
Confusional State	1 (5.3)	0	1 (3.0)
Seizure	1 (5.3)	1 (7.1)	2 (6.1)
Tremor	1 (5.3)	0	1 (3.0)
Somnolence	0	1 (7.1)	1 (3.0)
Psychiatric disorders (SOC)			
Arthritis	1 (5.3)	0	1 (3.0)
Depression	1 (5.3)	0	1 (3.0)
Insomnia	1 (5.3)	1 (7.1)	2 (6.1)
Psychosis	0	1 (7.1)	1 (3.0)
Study Agent Abuse Potential	0	1 (7.1)	1 (3.0)
Renal and urinary disorders (SOC)			
Renal and Urinary Tract Infection	1 (5.3)	2 (14.3)	3 (9.1)
Acute Kidney Injury	0	1 (7.1)	1 (3.0)
Respiratory, thoracic, and mediastinal disorders (SOC)			
Cough	8 (42.1)	1 (7.1)	9 (27.3)
Bronchospasm	1 (5.3)	0	1 (3.0)
Dyspnea	0	1 (7.1)	1 (3.0)

System Organ Class FMQ (Narrow)	Velmanase <18 years N=19 n (%)	 ≥18 years N=14 n (%)	 All N=33 n (%)
Skin and subcutaneous tissue disorders (SOC)			
Erythema	6 (31.6)	1 (7.1)	7 (21.2)
Pruritus	2 (10.5)	2 (14.3)	4 (12.1)
Rash	2 (10.5)	5 (35.7)	7 (21.2)
Urticaria	0	1 (7.1)	1 (3.0)
Vascular disorders (SOC)			
Hemorrhage	7 (36.8)	1 (7.1)	8 (24.2)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of Velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 12 to 48 months.
Each FMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some FMQs may contain PTs from more than one SOC.
For specific preferred terms under each FMQ, see the table "Adverse Events by System Organ Class, FDA Medical Query (Narrow) and Preferred Term…"
Abbreviations: FMQ, FDA medical query; N, number of patients in treatment arm; n, number of patients with adverse event; SOC, system organ class

17.2.6. Laboratory Findings and Vital Signs Analyses, rhLAMAN-10 Integrated Analysis

Clinically relevant abnormalities are discussed in the patient narratives in previous sections (see Section 7.6.2).

Hematological Analyses

The review team focused on the following hematological results from rhLAMAN-10: leukocytes, hemoglobin, and platelets. The mean values and change over time for leukocytes, hemoglobin, and platelets remained in normal range for adult and pediatric patients during rhLAMAN-10.

Liver Function

The mean values for alanine aminotransferase and total bilirubin remained within normal range during exposure to velmanase. In adult patients, the mean values for alkaline phosphatase were mildly elevated (mean of 69 U/L with range of 51-78 U/L) during exposure to velmanase. No serious adverse events (SAEs) were related to hepatic systems and this mild elevation, and the review team concluded this mildly abnormal lab trend was not clinically significant. In pediatric patients, the mean value for alkaline phosphatase was elevated at 215 U/L with range of mean values from 120 to 231 U/L during exposure to velmanase. The pediatric patients with alkaline phosphatase >300 U/L were reviewed at individual level. In total 6 pediatric patients had alkaline phosphate >300 U/L during trial. Each of these patients prior to velmanase exposure had abnormal elevated alkaline phosphatase values recorded. The pre-exposure baseline values ranged from 264-633 U/L. The review team assessed the elevated alkaline phosphatase values in these pediatric patients was not related to velmanase exposure. Additionally, no SAEs related to hepatic system were reported.

Kidney Function

The mean values for creatinine and GFR remained normal during exposure to velmanase.

Other Laboratory Trends

The mean values for sodium, potassium, calcium, phosphate, creatinine kinase, and amylase remained within normal range during exposure to velmanase. The review team concluded that no further analyses were necessary.

Vital Signs

CCD-LMZYMAA1-10-(b) (6) Patient experienced clinically significant hypotension. Refer to Section 7.7.1 for details of this event in patient narrative.

The review team focused on the following vital signs heart rates, and systolic and diastolic blood pressures for patients in the treatment group. Of note oxygen saturation was not routinely measured during velmanase administration. Because abnormalities in respiratory rate and body temperature would be more reflective of hypersensitivity reactions (including anaphylaxis) and IARs, discussion of these abnormalities when clinically significant are included in narratives in Section 7.7.1.

The findings for median heart rates, means systolic blood pressure, and mean diastolic blood pressure values in both pediatric and adult patients were similar to Trial rhLAMAN-05. One single episode of hypotension, patient CCD-LMZYMAA1-10-(b) (6) occurred in the context of anaphylaxis (refer to Section 7.7.1 for details). Apart from that single episode of hypotension there were no significant findings.

The review team concluded vital signs should be monitored during infusions, especially due to the risk for hypersensitivity reactions (including anaphylaxis) and IARs.

17.3. Safety Results, Trial rhLAMAN-08

17.3.1. Overview of Treatment-Emergent Adverse Events Summary, Trial rhLAMAN-08

There is no control group within rhLAMAN-08, the safety review of this study was viewed as supportive to safety review of pivotal trial (rhLAMAN-05).

Table 92 below gives an over of the AEs that occurred in Trial rhLAMAN-08. All 5 patients in this study were reported to have at least 1 SAE. For details refer to Section 7.6.2.

There was no permanent discontinuation of therapy in either treatment or placebo group. In total 4 patients experienced an AE that led to a dose modification of the study drug including interruption or reduction of velmanase.

Table 93. Overview of Adverse Events, Safety Population, Trial rhLAMAN-08

Event Category	Velmanase N=5 n (%)
SAE	5 (100)
SAEs with fatal outcome	0
Life-threatening SAEs	0
AE leading to permanent discontinuation of study drug	0
AE leading to dose modification of study drug	4 (80.0)
AE leading to interruption of study drug	4 (80.0)
AE leading to reduction of study drug	1 (20.0)
AE leading to dose delay of study drug	0
Other	0
Any AE	5 (100)
Severe and worse	1 (20.0)
Moderate	4 (80.0)
Mild	0

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 24 months (40 months for patient #(b) (6) enrolled in (b) (6)).
Severity as assessed by the investigator.
Abbreviations: AE, adverse event; N, number of patients in treatment arm; n, number of patients with at least one event; SAE, serious adverse event

17.3.2. Deaths, Trial rhLAMAN-08

No patient deaths were reported in Trial rhLAMAN-08.

17.3.3. Serious Treatment-Emergent Adverse Events, Trial rhLAMAN-08

Five of 5 patients in the study were reported to have experienced at least 1 SAE. A total of 17 SAEs occurred in these 5 patients, and patient CCD-LMZYMAA1-08_(b) (6) experienced 10 of these SAE episodes. Table 93 (below) lists these events by preferred terms and SOC. The most common SAEs are in the SOCs of gastrointestinal disorders, infections and infestations, and respiratory, thoracic, and mediastinal disorders. All patient narratives of these SAEs were reviewed. The review team assessed that 3 of these SAEs were possibly related to velmanase exposure. All 3 of these events occurred a single patient (CCD-LMZYMAA1-08_(b) (6) The summary of this patient's narrative is below.

Table 94. Patients With Serious Adverse Events by System Organ Class and Preferred Term, Safety Population, Trial rhLAMAN-08

System Organ Class Preferred Term	Velmanase N=5 n (%)
Any SAE	5 (100)
Blood and lymphatic system disorders (SOC)	1 (20.0)
Lymphadenopathy	1 (20.0)
Gastrointestinal disorders (SOC)	2 (40.0)
Gastritis	1 (20.0)
Vomiting	1 (20.0)
General disorders and administration site conditions (SOC)	1 (20.0)
Chills	1 (20.0)
Hyperthermia	1 (20.0)
Infections and infestations (SOC)	2 (40.0)
Cat scratch disease	1 (20.0)
Nasopharyngitis	1 (20.0)
Pharyngitis streptococcal	1 (20.0)
Tonsillitis	1 (20.0)
Injury, poisoning and procedural complications (SOC)	1 (20.0)
Concussion	1 (20.0)
Product issues (SOC)	1 (20.0)
Device malfunction	1 (20.0)
Respiratory, thoracic, and mediastinal disorders (SOC)	2 (40.0)
Oropharyngeal pain	1 (20.0)
Tonsillar hypertrophy	1 (20.0)
Skin and subcutaneous tissue disorders (SOC)	1 (20.0)
Henoch-Schonlein purpura	1 (20.0)
Vascular disorders (SOC)	1 (20.0)
Vascular fragility	1 (20.0)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Serious adverse events defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.
Duration is 24 months (40 months for patient #(b) (6) enrolled in (b) (6)).
Abbreviations: N, number of patients in treatment arm; n, number of patients with adverse event; SOC, system organ class

Patient CCD-LMZYMAA1-08_(b) (6) experienced an episode of chills and hyperthermia. This event occurred in the context of an anaphylaxis episode (discussed further in Section 7.7.1). This patient was also diagnosed with Henoch-Schonlein Purpura (HSP) which was previously discussed in Section 7.6.2.1. There is concern for risk of HSP related to velmanase exposure. This will be addressed in labelling recommendations to include HSP in the label's AR.

17.3.4. Adverse Events and FDA Medical Queries Leading to Treatment Discontinuation, Trial rhLAMAN-08

No discontinuations were reported in Trial rhLAMAN-08.

17.3.5. Treatment-Emergent Adverse Events, Trial rhLAMAN-08

Table 94 lists the TEAEs that occurred in patients who participated in the trial rhLAMAN-08.

Every patient (5 of 5 patients) experienced at least one TEAEs. The most common TEAEs reported were vomiting (100% of patients), nasopharyngitis (100% of patients), cough (80% of patients), and pyrexia (80%).

Table 95. Patients With Adverse Events by System Organ Class and FDA Medical Query (Narrow), Safety Population, Trial rhLAMAN-08

System Organ Class FMQ (Narrow)	Velmanase N=5 n (%)
Blood and lymphatic system disorders (SOC)	
Anemia	1 (20.0)
Gastrointestinal disorders (SOC)	
Vomiting	5 (100)
Diarrhea	3 (60.0)
General disorders and administration site conditions (SOC)	
Pyrexia	4 (80.0)
Fall	2 (40.0)
Fatigue	1 (20.0)
Infections and infestations (SOC)	
Nasopharyngitis	5 (100)
Bacterial Infection	3 (60.0)
Viral Infection	1 (20.0)
Nervous system disorders (SOC)	
Headache	2 (40.0)
Renal and urinary disorders (SOC)	
Renal and Urinary Tract Infection	1 (20.0)
Respiratory, thoracic, and mediastinal disorders (SOC)	
Cough	4 (80.0)
Respiratory Failure	1 (20.0)
Skin and subcutaneous tissue disorders (SOC)	
Rash	2 (40.0)
Pruritus	1 (20.0)
Urticaria	1 (20.0)
Vascular disorders (SOC)	
Hemorrhage	3 (60.0)

Source: adae.xpt; Software: R
Treatment-emergent adverse events defined as any AE event when onset of the event occurred after first injection of velmanase. An AE which started the same day of the first study drug administration but for which the exact time was missing, was defined as a TEAE.
Duration is 24 months (40 months for patient #(b) (6) enrolled in (b) (6)).
Each FMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some FMQs may contain PTs from more than one SOC.
For specific preferred terms under each FMQ, see the table "Adverse Events by System Organ Class, FDA Medical Query (Narrow) and Preferred Term…"
Abbreviations: FMQ, FDA medical query; N, number of patients in treatment arm; n, number of patients with adverse event; SOC, system organ class

17.3.6. Laboratory Findings and Vital Signs Analyses, Trial rhLAMAN-08

Since there is no control group within rhLAMAN-08, the review of laboratory findings and vital signs was viewed as supportive to the safety review of the controlled trial (rhLAMAN-05).

Clinically relevant abnormalities are discussed in patient narratives in Section 17.3.3.

Hematological Analyses

The review team focused on the following hematological assessments obtained during this trial: leukocytes, hemoglobin, and platelets. The mean values and change over time for leukocytes and platelets is within normal range.

The mean baseline for hemoglobin was low, likely related to anemia of chronic disease. Normal range for hemoglobin in patients aged 1-6 years old is 9.5-14 g/dL and the baseline mean in Trial rhLAMAN-08 was 7.6 g/dL. The mean values over time ranged from 7.2 to 7.9 g/dL. A single patient (CCD-LMZYMAA1-08_ (b) (6) experienced a single significant hemoglobin outlier of 1.49 mmol/L. Of note, the Investigator reported this outlier value in units of mmol/L. The review team assessed the normal range for hemoglobin using units corresponding to the units reported by Investigator. All reported AEs for this patient were reviewed. The patient did not experience any clinically significant episodes related to anemia and all other hemoglobin values for this patient ranged from 7.2 to 8.9 mmol/L. Additionally, the single significant outlier of 1.49mmol/L was not related to clinically significant events in the patient.

Liver Function

The mean values for alkaline phosphatase and total bilirubin remained within normal range for age (Wanjian et al. 2017) during exposure to velmanase. Lab values for alanine aminotransferase were not included in the rhLAMAN-08 dataset.

Kidney Function

The mean values for creatinine and GFR remained normal during exposure to velmanase.

Other Laboratory Trends

The mean values for potassium, calcium, and creatine kinase were normal at baseline and remained normal during exposure to velmanase.

The baseline mean value for sodium was within normal range and over the monitoring period a single mean value was mildly elevated at 147 mmol/L. Outlier values were reviewed on an individual patient level. There were no persistent abnormalities. The review team concluded no further analyses were necessary.

The baseline mean value for amylase was mildly low (28 U/L) and the mean value remained low (range 26 to 32 U/L) during velmanase exposure. Because 4 of the 5 participating patients had baseline low amylase values (range 6 to 35 U/L) prior to velmanase exposure, and their amylase values remained stably low during velmanase exposure, the review team assessed these low amylase values were not related to velmanase exposure.

Vital Signs

The review team focused on the following vital signs heart rates, and systolic and diastolic blood pressures for patients in the treatment group. Of note oxygen saturation was not routinely measured during velmanase administration. Because abnormalities in respiratory rate and body temperature may be more reflective of hypersensitivity reactions (including anaphylaxis) and IARs, discussion of these abnormalities when clinically significant is included in the adverse event of special interest (AESI) narratives in Section 7.7.1.

At baseline, the mean systolic pressure, diastolic pressure, and pulse values were within normal ranges for pediatric age group. No clinically meaningful changes in these vital signs were observed over time.

The review team concluded vital signs should be monitored during infusions, especially due to the risk for hypersensitivity reactions (including anaphylaxis) and IARs.

17.4. Ex-US Postmarket Experience - Discontinuations

The Applicant reports that 2 patients discontinued velmanase while enrolled in the After-Trial Care Program (trial rhLAMAN-07) and 1 patient discontinued while enrolled in the Compassionate Use Program. The details are as follows:

- Patient (b) (6) (USUBJID CCD-LMZYMAA1-10_(b) (6)) participated in the following trials rhLAMAN-03 (Phase 2 trial), rhLAMAN-05, and rhLAMAN-07 (open label). This patient completed Trial rhLAMAN-05 (which is included in safety review). This patient stopped velmanase treatment twice, once in rhLAMAN-03 and once in rhLAMAN-07 due to repeated IRRs.
- Patient (b) (6) completed Trial rhLAMAN-05. The patient was then enrolled in Compassionate Use Program (which is not classified as a Registry or a Trial). After experiencing three IRRs described as "allergic reactions" related to velmanase in the Compassionate Use Program, this patient was permanently discontinued from velmanase treatment.
- Case reference (b) (6) The patient was enrolled in Etoile Alpha from (b) (6), until (b) (6). On (b) (6), the patient experienced a SAE concerning for anaphylaxis within 23 minutes of velmanase infusion start. The patient discontinued treatment with velmanase following these event.

18. Clinical Virology

Insert text here.

19. Clinical Microbiology

Insert text here.

20. Mechanism of Action/Drug Resistance

Insert text here.

21. Other Drug Development Considerations

Not applicable.

22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)

Drs. Hennermann and Lund, two of the clinical investigators within this development program, were inspected in support of BLA 761278 covering two clinical trials, Protocols rhLAMAN-05 and CCD-LMZYMAA1-08. The studies appear to have been conducted adequately, and the data generated by these sites appear acceptable in support of the respective indication.

During the clinical investigator inspections, the source records related to the primary and key efficacy endpoint as noted in Section II of this Clinical Inspection Summary [e.g., 3MSCT, 6MWT, pulmonary function tests, Peabody Developmental Motor Scale, Mullen Scale for Early Learning, Bruininks-Oseretsky Test of Motor Proficiency, hearing evaluations, CSF biomarkers] were reviewed and verified against the Applicant's data line listings for the 25 subjects randomized in Protocols rhLAMAN-05 and 1 subject enrolled in Protocol CCD-LMZYMAA1-08 at the two sites inspected. No discrepancies or issues were noted.

In addition, source records documenting the primary efficacy endpoint of the change from baseline to week 52 in serum and CSF oligosaccharides were neither maintained nor retained at the central site (i.e., Dr. Lund's site) nor available during inspection for data verification. In a 22 Nov 2022 response to an IR, the Applicant submitted to the BLA, certified copies of the source records related to the oligosaccharide serum and CSF levels at Baseline and Week 52 for the 25 randomized subjects. An internal Office of Scientific Investigations review of the certified copies was performed, verifying them against the Applicant's data line listings for the 25 randomized subjects. No discrepancies were noted.

See the Clinical Inspection Summary dated December 14, 2022, for additional details.

23. Labeling: Key Changes and Considerations

This Prescribing Information (PI) review includes a high-level summary of the rationale for major changes incorporated into the finalized PI (see Table 95). The PI was reviewed to ensure that PI meets regulatory/statutory requirements, is consistent (if appropriate) with labeling guidance, conveys clinically meaningful and scientifically accurate information needed for the safe and effective use of the drug, and provides clear and concise information for the healthcare practitioner.

(b) (4)

2 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)

23.1. Approved Labeling Types

Upon approval of this application, the following labeling documents will be FDA-approved:

- US Prescribing Information

24. Postmarketing Requirements and Commitments

24.1. Pharmacology/Toxicology

1. Nonclinical PMR #1: Perform an assessment of the potential effects of increased alpha-mannosidase exposure (and increased MAN2B1 expression) on tumor formation.

   - Interim Report Submission: 02/2024
   - Final Report Submission: 08/2024

2. Nonclinical PMC #1: Perform a bridging pharmacokinetic study in rats to characterize the velmanase alfa-tycv exposure in the reproductive toxicity and pre- and postnatal development studies.

   - Draft protocol submission: 08/2023
   - Final protocol submission: 02/2024
   - Study completion: 08/2024
   - Final report submission: 02/2025

3. Nonclinical PMC #2: Conduct a 26-week repeat-dose pharmacodynamic (PD) study in alpha mannosidase-deficient transgenic-knockout mice to evaluate changes in the M2 biomarker and histopathology in response to treatment with velmanase alfa-tycv.

   - Draft protocol submission: 08/2023
   - Final protocol submission: 02/2024
   - Study Completion: 02/2025
   - Final report submission: 10/2025

   Study Protocol Details: Use a validated bioanalytical method to evaluate changes in the M2 biomarker. Perform histopathology on all animals. Perform expanded neuropathology and use an expert neuropathologist to either read the slides or perform a peer review of the brains and spinal cords taken from all animals on this study. Measure oligosaccharide contents of tissues (particularly neural tissues) in the toxicokinetic cohort animals. Characterize the contents of the vacuoles and perform quantitative micrometry of the dose-effect on vacuolar burden in tissues from the study.

24.2. Clinical Pharmacology

Evaluate the pharmacodynamics of velmanase alfa-tycv in pediatric patients less than 3 years of age with a confirmed diagnosis of alpha-mannosidosis. If the results suggest inadequate pharmacodynamic response at the currently recommended dose of 1 mg/kg, additional clinical studies may be needed to explore doses higher than 1 mg/kg for patients who cannot achieve an optimal pharmacodynamic response at 1 mg/kg.

- Draft protocol submission: 06/2023
- Final protocol submission: 09/2023
- Trial completion: 09/2029

- Interim report: 09/2026
- Final report submission: 09/2030

24.3. OPQ

Develop and validate a titering anti-drug antibody (TADA) assay as recommended in the FDA guidance for industry Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection (January 2019). This TADA assay will be used to test available confirmed anti-drug antibody positive samples from Trials rhLAMAN-07, rhLAMAN-08, rhLAMAN-09, rhLAMAN-10, and on-going clinical studies to complement and replace the current rabbit anti-velmanase alfa reference standard-based semi-quantitative ADA assay. Provide a final validation report detailing the performance of the TADA assay.

- Final report submission: 05/2023

Develop and validate cell-based neutralizing antibody (NADA) assay to test inhibition of velmanase alfa enzyme uptake into cells. This NADA assay will be used to test available confirmed anti-drug antibody positive samples from clinical Trials rhLAMAN-07, rhLAMAN-08, rhLAMAN-09, rhLAMAN-10, and on-going clinical studies. Provide a final validation report detailing the performance of the cell-based NADA assay.

- Final report submission: 05/2023

Submit a risk assessment of the extractables identified for the drug substance container closure system and drug product container closure system, including a risk assessment of the threshold of toxicological concern, acceptable daily exposure, and/or permissible daily exposure.

- Final report submission: 03/2023

Submit a leachables study protocol as well as the time zero report.

- Final report submission: 06/2023

Implement a drug product release specification for deliverable volume (e.g., according to United States Pharmacopoeia <697>).

- Final report submission: 03/2023

Develop, validate, and implement a test method with justified numerical acceptance criteria to reliably detect and control for the presence of Chinese hamster ovary lysosomal enzyme alpha-mannosidase in the final velmanase alfa drug substance.

- Final report submission: 12/2024

Implement a cell-based potency assay in drug product release specifications with pre-defined acceptance criteria.

- Final report submission: 12/2024

Develop, validate, and implement a test method with justified numerical acceptance criteria for [redacted] during drug product release and stability testing. [redacted] If applicable, justification and data supporting the use of a synthetic substrate, and its relevance to the natural substrate, will be provided. The method validation report for the [redacted] assay, the revised drug product release and stability specifications, and all supporting studies and data will be provided in a final study report per 21 CFR 601.12.

- Final report submission: 12/2025

Implement [redacted] prior to vial fill at [redacted] mg/mL. Include "gross content of protein content per vial" in the drug product release specification to control the total amount of velmanase alfa in the final vial.

- Final report submission: 03/2023

Conduct a worst-case drug product transport qualification study shipping 10 mg vials of velmanase alfa drug product from Chiesi Farmaceutici S.p.A. in Italy to distribution sites in the USA. Perform product quality testing on the final shipped velmanase alfa drug product to support purity and potency after worst-case shipping conditions.

- Final report submission: 12/2023

25. Financial Disclosure

Table 97. Covered Clinical Trials: rhLAMAN-05, rhLAMAN-08

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 13		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): N/A		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: Enter text here. Significant payments of other sorts: Enter text here. Proprietary interest in the product tested held by investigator: Enter text here. Significant equity interest held by investigator: Enter text here. Sponsor of covered study: Enter text here.		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): Enter text here.		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

Abbreviation: FDA, Food and Drug Administration

26. References

Literature

Adam, J, R Malone, S Lloyd, J Lee, CJ Hendriksz, and U Ramaswami, 2019, Disease progression of alpha-mannosidosis and impact on patients and carers - A UK natural history survey, Mol Genet Metab Rep, 20:100480.

American Thoracic Society, 2002, Guidelines for the Six-Minute Walk Test, American Journal of Respiratory and Critical Care Medicine, 166(1):111-117.

Bean, JF, DK Kiely, S LaRose, J Alian, and WR Frontera, 2007, Is stair climb power a clinically relevant measure of leg power impairments in at-risk older adults?, Arch Phys Med Rehabil, 88(5):604-609.

Beck, M, KJ Olsen, JE Wraith, J Zeman, JC Michalski, P Saftig, J Fogh, and D Malm, 2013, Natural history of alpha mannosidosis a longitudinal study, Orphanet J Rare Dis, 8:88.

Behr, J, 2011, A small change in FVC but a big change for IPF: defining the minimal clinically important difference, Am J Respir Crit Care Med, 184(12):1329-1330.

Borgwardt, L, HM Stensland, KJ Olsen, F Wibrand, HB Klenow, M Beck, Y Amraoui, L Arash, J Fogh, O Nilssen, CI Dali, and AM Lund, 2015, Alpha-mannosidosis: correlation between phenotype, genotype and mutant MAN2B1 subcellular localisation, Orphanet J Rare Dis, 10:70.

Ceccarini, MR, M Codini, C Conte, F Patria, S Cataldi, M Bertelli, E Albi, and T Beccari, 2018, Alpha-Mannosidosis: Therapeutic Strategies, Int J Mol Sci, 19(5).

COSMIC, 2022, Catalogue of Somatic Mutations in Cancer, Wellcome Sanger Institute, accessed February 2, 2023, https://cancer.sanger.ac.uk/cosmic.

David, A, 2022, Overview of pulmonary function testing in adults, accessed, 2022, https://www.uptodate.com/contents/overview-of-pulmonary-function-testing-in-adults?search=forced%20vital%20capacity&sectionRank=3&usage_type=default&anchor=H17&source=machineLearning&selectedTitle=1~150&display_rank=1#H17.

Gerards, AH, WP Winia, J Westerga, BA Dijkmans, and RM van Soesbergen, 2004, Destructive joint disease in alpha-mannosidosis. A case report and review of the literature, Clin Rheumatol, 23(1):40-42.

Hennermann, JB, EM Raebel, F Dona, ML Jacquemont, G Cefalo, A Ballabeni, and D Malm, 2022, Mortality in patients with alpha-mannosidosis: a review of patients' data and the literature, Orphanet J Rare Dis, 17(1):287.

Karimian, Z, CB Whitley, KD Rudser, and JRJ Utz, 2017, Delayed Infusion Reactions to Enzyme Replacement Therapies, JIMD Rep, 34:63-70.

Kjellman, B, I Gamstorp, A Brun, PA Ockerman, and B Palmgren, 1969, Mannosidosis: a clinical and histopathologic study, J Pediatr, 75(3):366-373.

Lehalle, D, R Colombo, M O'Grady, B Héron, N Houcinat, P Kuentz, S Moutton, A Sorlin, J Thevenon, J Delanne, S Gay, C Racine, A Garde, F Tran Mau-Them, C Philippe, A Vitobello, S Nambot, F Huet, Y Duffourd, F Feillet, C Thauvin-Robinet, S Marlin, and L Faivre, 2019,

Hearing impairment as an early sign of alpha-mannosidosis in children with a mild phenotype: Report of seven new cases, Am J Med Genet A, 179(9):1756-1763.

Liao, YF, A Lal, and KW Moremen, 1996, Cloning, expression, purification, and characterization of the human broad specificity lysosomal acid alpha-mannosidase, J Biol Chem, 271(45):28348-28358.

Lin, X, H Liu, H Zhao, S Xia, Y Li, C Wang, Q Huang, S Wanggou, and X Li, 2022, Immune Infiltration Associated MAN2B1 Is a Novel Prognostic Biomarker for Glioma, Front Oncol, 12:842973.

Lipinski, P, A Rozdzynska-Swiatkowska, K Iwanicka-Pronicka, B Perkowska, P Pokora, and A Tylki-Szymanska, 2021, Long-term outcome of patients with alpha-mannosidosis - A single center study, Mol Genet Metab Rep, 30:100826.

Loveman, E, VR Copley, J Colquitt, DA Scott, A Clegg, J Jones, KM O'Reilly, S Singh, C Bausewein, and A Wells, 2015, The clinical effectiveness and cost-effectiveness of treatments for idiopathic pulmonary fibrosis: a systematic review and economic evaluation, Health Technol Assess, 19(20):i-xxiv, 1-336.

Malm, D, 2018, Alpha-Mannosidosis, accessed 11/07, 2022, https://rarediseases.org/rare-diseases/alpha-mannosidosis/.

Malm D and Nilssen, 2001, Oct 11 [Updated 2019 Jul 18] Alpha-Mannosidosis, GeneReviews® [Internet], Adam MP, E. D., Mirzaa GM, et al., Seattle (WA): University of Washington, Seattle; 1993-2022.

Malm, D, DS Halvorsen, L Tranebjaerg, and H Sjursen, 2000, Immunodeficiency in alpha-mannosidosis: a matched case-control study on immunoglobulins, complement factors, receptor density, phagocytosis and intracellular killing in leucocytes, Eur J Pediatr, 159(9):699-703.

Malm, D and O Nilssen, 2008, Alpha-mannosidosis, Orphanet J Rare Dis, 3:21.

FDA Center for Drug Evaluation and Research *Summary Review of BLA 125291 Lumizyme (alglucosidase alfa)* (May 2010)

Naumchik, BM, A Gupta, H Flanagan-Steet, RA Steet, SS Cathey, PJ Orchard, and TC Lund, 2020, The Role of Hematopoietic Cell Transplant in the Glycoprotein Diseases, Cells, 9(6).

Nightingale, EJ, F Pourkazemi, and CE Hiller, 2014, Systematic review of timed stair tests, J Rehabil Res Dev, 51(3):335-350.

Nir, V, L Bentur, G Tal, M Gur, G Gut, A Ilivitzki, M Zucker-Toledano, M Hanna, Y Toukan, and R Bar-Yoseph, 2020, Comprehensive cardiopulmonary assessment in alpha mannosidosis, Pediatr Pulmonol, 55(9):2348-2353.

NORD, 2022, Alpha-Mannosidosis, National Organization for Rare Disorders, accessed May 16, 2022, https://rarediseases.org/rare-diseases/alpha-mannosidosis/.

P.A. Öckerman and MD Lund, 1967, A GENERALISED STORAGE DISORDER RESEMBLING HURLER'S SYNDROME, The Lancet, 290(7509):239-241.

Roces, DP, R Lullmann-Rauch, J Peng, C Balducci, C Andersson, O Tollersrud, J Fogh, A Orlacchio, T Beccari, P Saftig, and K von Figura, 2004, Efficacy of enzyme replacement therapy in alpha-mannosidosis mice: a preclinical animal study, Hum Mol Genet, 13(18):1979-1988.

Sampson, HA, A Munoz-Furlong, RL Campbell, NF Adkinson, Jr., SA Bock, A Branum, SG Brown, CA Camargo, Jr., R Cydulka, SJ Galli, J Gidudu, RS Gruchalla, AD Harlor, Jr., DL Hepner, LM Lewis, PL Lieberman, DD Metcalfe, R O'Connor, A Muraro, A Rudman, C Schmitt, D Scherrer, FE Simons, S Thomas, JP Wood, and WW Decker, 2006, Second Symposium on the Definition and Management of Anaphylaxis: Summary Report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium, J Allergy Clin Immunol, 117(2):391-397.

Shoemaker, MJ, AB Curtis, E Vangsnes, and MG Dickinson, 2013, Clinically meaningful change estimates for the six-minute walk test and daily activity in individuals with chronic heart failure, Cardiopulm Phys Ther J, 24(3):21-29.

Stinchi, S, R Lullmann-Rauch, D Hartmann, R Coenen, T Beccari, A Orlacchio, K von Figura, and P Saftig, 1999, Targeted disruption of the lysosomal alpha-mannosidase gene results in mice resembling a mild form of human alpha-mannosidosis, Hum Mol Genet, 8(8):1365-1372.

Stroobants, S, M Damme, A Van der Jeugd, B Vermaercke, C Andersson, J Fogh, P Saftig, J Blanz, and R D'Hooge, 2017, Long-term enzyme replacement therapy improves neurocognitive functioning and hippocampal synaptic plasticity in immune-tolerant alpha-mannosidosis mice, Neurobiol Dis, 106:255-268.

Thomas, G, 2019, Disorders of Glycoprotein Degradation: α-Mannosidosis, β-Mannosidosis, Fucosidosis, and Sialidosis, The Online Metabolic and Molecular Bases of Inherited Disease, Valle DL, A. S., Ballabio A, Beaudet AL, Mitchell GA.: McGraw Hill.

Urushihara, M, S Kagami, K Yasutomo, M Ito, S Kondo, A Kitamura, D Malm, H Klenow, O Nilssen, and Y Kuroda, 2004, Sisters with alpha-mannosidosis and systemic lupus erythematosus, Eur J Pediatr, 163(4-5):192-195.

Verrecchia, E, LL Sicignano, MG Massaro, R Rocco, G Silvestri, S Rossi, and R Manna, 2021, Caregivers' and Physicians' Perspectives on Alpha-Mannosidosis: A Report from Italy, Adv Ther, 38(1):1-10.

Wanjian, G, H Jie, G Liang, W Cheng, X Tian, S Jianjiang, and Z Chunni, 2017, Establishment of Reference Interval for Alkaline Phosphatase in Healthy Children of Various Ethnicities, Aged 0-12 Years, Lab Med, 48(2):166-171.

Weiss, SW and WD Kelly, 1983, Bilateral destructive synovitis associated with alpha mannosidase deficiency, Am J Surg Pathol, 7(5):487-494.

Guidances

Draft guidance for industry *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biologic Products* (December 2019)

Guidance for clinical investigators, industry, and FDA staff *Financial Disclosure by Clinical Investigators* (February 2013)

Guidance for industry *Immunogenicity Testing for Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection* (February 2019)

Guidance for industry *Warnings and Precautions, Contraindications, and Boxed Warning Sections of Labeling for Human Prescription Drug and Biological Products — Content and Format* (October 2011)

27. Review Team

Table 98. Reviewers of Integrated Assessment

Role	Names
Regulatory Project Manager	Avinash Kalsi, PharmD
Chief Project Management Staff	Michael G. White, PhD
Nonclinical Team Leader (Acting)	Shawna Weis, PhD
Nonclinical Division Director	Mukesh Summan, PhD, DABT
Office of Clinical Pharmacology Reviewers	Nayeem Hossain, PhD, Clinical Pharmacology Reviewer Hongshan Li, PhD, Pharmacometrics Reviewer
Office of Clinical Pharmacology Team Leaders	Jie (Jack) Wang, PhD, Clinical Pharmacology Team Lead Jiang Liu, PhD, Pharmacometrics Team Lead
Clinical Reviewer	Sarah Fuchs, MD, MSCI
Clinical Team Leader	Jacqueline Karp, MD
Statistical Reviewer	Therri Usher, PhD
Statistical Team Leader	Yan Wang, PhD
Cross-Disciplinary Team Leader	Jacqueline Karp, MD
Division Director (pharm/tox)	Mukesh Summan, PhD
Division Director (OCP)	Michael Pacanowski, PharmD, MPH
Division Director (OB)	Lei Nie , PhD
Division Director (ORO)	Pamela Lucarelli
Division Director (clinical)	Kathleen M. Donohue, MD, MSc
Office Deputy Director	Christine P. Nguyen, MD

Abbreviations: OB, Office of Biostatistics; OCP, Office of Clinical Pharmacology; ORO, Office of Regulatory.

Table 98. Additional Reviewers of Application

Office or Discipline	Names
Division of Pediatrics and Maternal Health (DPMH) Team Leaders	Tamara Johnson, MD, MS, Maternal Health Shetarra Walker, MD, MSCR, Pediatrics
DPMH/Maternal Health Reviewer	Christos Mastroyannis, MD
DPMH/Pediatrics Reviewer	Ethan Hausman, MD
DPMH/Regulatory Project Manager	Denise Johnson-Lyles, RPM George Greeley, Chief Project Management Staff
Division of Rare Diseases and Medical Genetics (DRDMG)	Mona Patel, PharmD, RAC, Associate Director Labeling Yuliya Yasinskaya, MD, Deputy Director for Safety Cheronda Cherry-France, RN, MHA, BSN, Safety Regulatory Project Manager
New Drug Transition Team	Rhonda Hearns-Stewart, MD Salman Hosain, PhD, Clinical Data Scientist Jinzhong (Jin) Liu, PhD, Director, Clinical Data Science (CDS) Staff Monika Deshpande, Medical Editor Ramsha Baig, Medical Editor Katherine Bradley, Medical Editor, Team Leader

Office or Discipline	Names
Office of Pharmaceutical Quality	Ian McWilliams, PhD, Application Team Leader Asha Hewarathna, PhD, CMC Reviewer Hamet Touré, PharmD, Primary Drug Substance Microbiology and Facility Reviewer Michael Shanks, BSc, Facility Team Lead Candace Gomez-Broughton, PhD, Primary Drug Product Microbiology and Facility Reviewer Virginia Carroll, PhD, Microbiology Team Lead Joao Pedras-Vasconcelos, PhD, Immunogenicity Reviewer Scott Dallas, , Labeling Reviewer Melinda Bauerlien, MS, Senior Regulatory Business Project Manger
Office of Prescription Drug Promotion	Carrie Newcomer, Regulatory Reviewer Elvy Varghese, Regulatory Reviewer James Dvorksy, Team Leader
Office of Scientific Investigations	Cheryl A. Grandinetti, PharmD, Clinical Pharmacologist Phillip Kronstein, MD, Team Lead
Office of Surveillance & Epidemiology (OSE)/ Division of Epidemiology (DEPI)	Sally Peprah, PhD, Reviewer Benjamin Booth, PhD, Team Leader
OSE/ Division of Medication Error Prevention and Analysis (DMEPA)	Sali Mahmoud, PharmD, BCPS, Safety Evaluator Ashleigh Lowery, PharmD, Team Leader
OSE/Division of Pharmacovigilance (DPV)	Mohamed A. Mohamoud, PharmD, MPH, BCPS, Safety Evaluator Ivone Kim, MD, Medical Officer Carmen Cheng, MD, Team Leader
OSE/Division of Risk Management (DRM)	Yasmeen Abou-Sayed, PharmD, Team Leader Cristen Lambert, PharmD, Reviewer
OSE/ Safety Regulatory Project Manager (SRPM)	Aleksander Winiarski, PharmD, RPh, Team Leader Commander Su-Lin Sun, RPh, PharmD, GWCPM, SRPM

Abbreviations: DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRISK, Division of Risk Management; OPDP, Office of Prescription Drug Promotion; OPQ, Office of Pharmaceutical Quality; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations

27.1. Reviewer Signatures

Table 99. Signatures of Reviewers

See appended table

Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Tertiary Reviewer	**Signature:** Kathleen Donohue -S Digitally signed by Kathleen Donohue -S Date: 2023.02.10 17:02:13 -05'00'		
Clinical	Kathleen M. Donohue, MD, MSc Director	OND/Division of Rare Diseases and Medical Genetics (DRDMG)	All ☐ Authored ☒ Contributed ☒ Approved

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Jacqueline Karp, MD	OND/DRDMG	2.2, 3.1, 6.3 ☒ Authored 2.1, 3.2, 6, 7, 16 ☒ Contributed All ☒ Approved
Cross-Disciplinary Team Lead	**Signature:** Jacqueline E. Karp -S Digitally signed by Jacqueline E. Karp -S Date: 2023.02.08 17:50:55 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Sarah Fuchs, MD MSCI	OND/DRDMG	☒ Authored 2.1, 3.2, 4, 6.2, 7, 9, 10, 12, 15, 16, 17, 21, 22, 25 ☒ Contributed 6.3 ☐ Approved
Primary Reviewer	**Signature:** Sarah R. Fuchs -S Digitally signed by Sarah R. Fuchs -S Date: 2023.02.09 08:29:06 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Mona Patel, PharmD, RAC	OND/DRDMG	23 ☒ Authored ☐ Contributed ☐ Approved
Associate Director for Labeling	**Signature:** Mona Patel -S Digitally signed by Mona Patel -S Date: 2023.02.09 09:28:45 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Pharmacology/Toxicology	Mukesh Summan, PhD, DABT Director	OND/Division of Pharm/Tox for Rare Diseases, Pediatric, Urologic and Reproductive Medicine/Specialty Medicine (DPT-ORPURM/SM)	5.1, 6.1, 13, 24.1 Pharmacology Review Addendum ☐ Authored ☒ Contributed ☒ Approved
Tertiary Reviewer	**Signature:** Mukesh Summan -S Digitally signed by Mukesh Summan -S Date: 2023.02.13 15:08:34 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Pharmacology/Toxicology	Shawna Weis, PhD Team Leader	OND/Division of Pharm/Tox for Rare Diseases, Pediatric, Urologic and Reproductive Medicine/Specialty Medicine (DPT-ORPURM/SM)	5.1, 6.1, 13, 24.1 Pharmacology Review Addendum ☒ Authored ☐ Contributed ☐ Approved
Secondary Reviewer	**Signature:** Shawna L. Weis -S Digitally signed by Shawna L. Weis -S Date: 2023.02.09 08:40:09 -05'00'		

[1] Include “IA” for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Biometrics	Lei Nie, PhD Director	OB/Division of Biometrics IV (DBIV)	6.2, 6.3, and 16 ☐ Authored ☒ Contributed ☒ Approved
Tertiary Reviewer	**Signature:** Lei Nie -S Digitally signed by Lei Nie -S Date: 2023.02.09 11:11:41 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Biometrics	Yan Wang, PhD Team Leader	OB/DBIV	6.2, 6.3, and 16 ☒ Authored ☒ Contributed ☒ Approved
Secondary Reviewer	**Signature:** Yan Wang -S Digitally signed by Yan Wang -S Date: 2023.02.09 11:48:07 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Biometrics	Therri Usher, PhD	OB/DBIV	6.2, 6.3, and 16 ☒ Authored ☐ Contributed ☐ Approved
Primary Reviewer	**Signature:** Therri A. Usher -S Digitally signed by Therri A. Usher -S Date: 2023.02.09 11:25:05 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Product Quality	Ian McWilliams, PhD Application Team Leader	OPQ/Office of Biotechnology	9 ☒ Authored ☐ Contributed ☒ Approved
Secondary Reviewer	**Signature:** Ian L. Mcwilliams -S Digitally signed by Ian L. Mcwilliams -S Date: 2023.02.08 20:02:30 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Regulatory Project Management	Michael G. White, PhD Chief, Project Management Staff	OND/DRO-RPURM	12, 27 ☐ Authored ☒ Contributed ☒ Approved
Secondary Reviewer	**Signature:** Michael White -S Digitally signed by Michael White -S Date: 2023.02.08 16:46:00 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Regulatory Project Management	Avinash Kalsi, PharmD	OND/DRO-RPURM	12, 27 ☒ Authored ☐ Contributed ☐ Approved
Secondary Reviewer	**Signature:** Avinash K. Kalsi -S Digitally signed by Avinash K. Kalsi -S Date: 2023.02.08 16:20:14 -05'00'		

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Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Michael Pacanowski, PharmD, MPH Director	OTS/OCP/ Division of Translational and Precision Medicine (DTPM)	5.2, 6.1, 6.3, 8.1, 8.2, 14, 24 ☐ Authored ☒ Contributed ☒ Approved
Tertiary Reviewer	**Signature:** Michael Pacanowski -S Digitally signed by Michael Pacanowski -S Date: 2023.02.07 12:00:29 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Jie (Jack) Wang, PhD Team Leader	OTS/OCP/DTPM	5.2, 6.1, 6.3, 8.1, 8.2, 14, 24 ☒ Authored ☒ Contributed ☒ Approved
Secondary Reviewer	**Signature:** Jie Wang -S Digitally signed by Jie Wang -S Date: 2023.02.08 07:37:05 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Nayeem Hossain, PhD	OTS/OCP/DTPM	5.2, 6.1, 6.3, 8.1, 8.2, 14, 24 ☒ Authored ☐ Contributed ☐ Approved
Primary Reviewer	**Signature:** Md Nayeem Hossain -S Digitally signed by Md Nayeem Hossain -S Date: 2023.02.07 21:31:20 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology/Pharmacometrics	Jiang Liu, PhD Team Leader	OTS/OCP/Division of Pharmacometrics (DPM)	5.2, 6.1, 6.3, 8.1, 8.2, 14, 24 ☐ Authored ☒ Contributed ☒ Approved
Secondary Reviewer	**Signature:** Jiang Liu -S Digitally signed by Jiang Liu -S Date: 2023.02.07 12:10:34 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology/Pharmacometrics	Hongshan Li, PhD	OTS/OCP/DPM	5.2, 6.1, 6.3, 8.1, 8.2, 14, 24 ☒ Authored ☐ Contributed ☐ Approved
Primary Reviewer	**Signature:** Hongshan Li -S Digitally signed by Hongshan Li -S Date: 2023.02.07 19:31:21 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

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/s/

CHRISTINE P NGUYEN
02/16/2023 10:26:56 AM

U.S. FOOD & DRUG ADMINISTRATION

PHARMACOLOGY/TOXICOLOGY MEMORANDUM

Date:	February 13, 2023
Subject:	BLA 761278
Author:	Mukesh Summan, Ph.D., D.A.B.T Director, Division of Pharmacology/Toxicology for Rare Diseases, Pediatrics, Urologic and Reproductive Medicine Office of Rare Diseases, Pediatrics, Urologic and Reproductive Medicine Center for Drug Evaluation and Research

Memo/ Summary Statement

BLA 761278 was submitted on June 17, 2022 for marketing authorization of velmanase-alpha-tycv (recombinant human lysosomal alpha mannosidase, rhLAMAN) to treat non-central nervous system manifestations of alpha mannosidosis (AM) in adult and pediatric patients.

Reports of studies submitted in support of this application include primary pharmacology studies, published studies of alpha-mannosidase supplementation in murine disease models; reproductive and developmental toxicology studies in rats and rabbits; repeat-dose toxicology studies in monkeys and an alpha-mannosidosis disease mouse model (Tg+KO mouse); and a juvenile toxicology study in mice.

The primary review was performed by Shawna Weis, PhD (Pharmacology/Toxicology BLA Review, Shawna L. Weis, PhD, February 12, 2023), who concluded that the data were generally adequate to support approval for the proposed indication.

This memo summarizes the data submitted in support of this approval as it relates to the finalized labeling, nonclinical confirmatory evidence (CE) and the nonclinical post-marketing commitments (PMCs).

Alpha-mannosidosis (AM) is a lysosomal storage disease that results from reduced activity of the enzyme alpha-mannosidase, caused by gene variants in Mannosidase Alpha Class 2B Member 1 (MAN2B1). Alpha-mannosidase catalyzes the degradation of accumulated mannose-containing oligosaccharides. The deficiency of alpha-mannosidase (AM) causes an intra-lysosomal accumulation of mannose-rich oligosaccharides in various tissues. Velmanase alfa-tycv provides an exogenous source of alpha-mannosidase. Velmanase alfa-tycv is internalized via binding to the mannose-6-phosphate receptor on the cell surface and transported into lysosomes where it is thought to exert enzyme activity. The applicant has suggested serum oligosaccharides as a potential clinical biomarker. However, serum oligosaccharides were not collected or evaluated in the nonclinical studies submitted in the BLA submission. In addition, serum oligosaccharides were not validated as a biomarker.

To support chronic use of the product the applicant completed toxicology study in the transgenic knock-out mouse model of the disease (Tg +KO, 26-week, GLP) and non-human primate (13-week GLP). In the monkey and mouse animal model, toxicology findings were unremarkable.

In an embryo-fetal development study in the rat, velmanase alfa-tycv was administered during the period of organogenesis from gestation day (GD) 6 to GD 17. Major malformations and variations were observed at exposures that were approximately 7-fold greater than the recommended dose of 1 mg/kg. Treatment-related major malformations included cleft palate, cleft palatine skull, severely bent pelvic girdle, and duplicated sternebrae.

In an embryofetal toxicity study in the rabbit, there were no apparent effects on embryofetal survival; however, treatment was associated with induction of numerous variations and malformations at exposures approximately 2.5-fold the 1 mg/kg clinical dose, the incidences of which exceeded concurrent controls. A NOAEL was not identified in this study.

The frequency of these adverse embryofetal effects in rats and rabbits exceeded facility historical control ranges. Because alpha mannosidase is an autosomal recessive disease, most fetuses carried by patients receiving velmanase-alfa-tycv are expected to be carriers of the maternal pathogenic *MAN2B1*, but will be phenotypically unaffected unless they inherit a pathogenic paternal allele; thus, the findings in this study are considered relevant to the development of a fetus by a patient under treatment with velmanase-alfa-tycv.

In a fertility study in the rat, velmanase alfa-tycv was administered twice weekly by IV injection. There were no effects on fertility; however, because exposures were not measured in this study and because the dose frequency differed from other studies in the rat, exposures could not be accurately estimated. A PMC for a bridging pharmacokinetic study has been requested to support interpretation of data from this study.

Genotoxicity and carcinogenicity studies were not conducted by the applicant. However, in the rat pre- and post-natal development study, a finding of malignant histiocytic sarcoma was observed in one high-dose (30 mg/kg) female. Exposures at the 30 mg/kg dose level were approximately 9-fold the AUC at the maximum recommended dose. Dysregulation of *MAN2B1* expression has been observed in numerous tumors, including tumors of the adrenal, breast, CNS, cervix, endometrium, hematopoietic/lymphoid systems, kidney, intestinal tract, liver, lung, esophagus, pancreas, parathyroid, prostate, skin, stomach, thyroid, and urinary tract. Consequently, a mechanistic relationship between tumorigenesis and increased levels of α-mannosidase levels cannot be excluded. To address this concern a nonclinical PMC was requested to evaluate the role of increased alpha-mannosidase exposure and tumor formation.

The nonclinical confirmatory evidence to support approval of velmanase-alfa-tycv with a single adequate and well control trial, was limited. The applicant submitted studies to support the uptake and distribution of the drug, but these studies lacked data to support the fate of the drug upon cellular uptake nor a demonstration of lysosomal site of action.

To evaluate in vivo efficacy with administration of exogenous rhLAMAN, the applicant created a *MAN2B*-deficient (knockout (KO)) mouse model containing a defective human *MAN2B1* allele (Tg knockin) to tolerize animals to treatment (Tg +KO). The absence of lysosomal alpha-mannosidase enzyme activity was confirmed in brain, liver, and kidney. Using non-quantitative thin layer chromatography method (TLC), the Tg +KO animals exhibited an approximately 2x increase in mannose-containing oligosaccharide content in the kidney, spleen, testes, and brain. Orscein staining of TLC plates was used to assess oligosaccharide accumulation, which is not specific for mannose, nor did the assay employ standard curves or any controls for sample loading. Although histologically, evidence of vacuolation was observed in multiple tissues, the Applicant did not evaluate the composition of the vacuoles. Importantly, the animals did not present with evidence of human disease over the 12-month observation period, suggesting that the model does not recapitulate the clinical course of the disease.

To support efficacy of enzymatic replacement of α-mannosidase in a murine α-mannosidosis model, the applicant cited a publication (Roces et.al., 2004) where the publication authors administered lysosomal α-mannosidase (LAMAN) from bovine kidney, or recombinant human, or mouse α-mannosidase in a mouse α-mannosidase knockout model. LAMAN enzyme levels were measured in lysates of perfused tissues. and were highest in the liver, spleen and heart, but in the brain were about 10% of those present in wild type control tissues, suggesting poor brain penetrance. The authors also evaluated oligosaccharide clearance in tissues. Oligosaccharide reduction was greatest in liver, followed by minimal oligosaccharide reduction in the kidney and spleen and, oligosaccharide content of the brain was unaffected. Reduction of tissue oligosaccharides was not examined to determine a correlation to an improvement to functional outcome in this animal model. Tissue vacuolation was reduced in the liver, spleen and kidney, however the kinetics of vacuole reduction differ in each tissue. In addition, reduction of cellular vacuolation was not correlated to a functional outcome.

In the 26-week repeat-dose toxicology study in the Tg+KO mouse, weekly administration of rhLAMAN at a dose of 1300 U/kg for 26 weeks, led to a reduction of cellular vacuolation in the brain, liver, lymph nodes, and spleen. Reduction of cellular vacuolation was not evaluated or correlated to a functional outcome. Consequently, the clinical meaningfulness of the reduction of vacuolation is unknown and correlation between reduction in vacuolation and reduction in oligosaccharide content is also unknown, because neither serum nor urine oligosaccharide content was measured in animals. Therefore, nonclinical data to support serum oligosaccharide as a validated biomarker are not available.

Conclusion and Recommendation

From a safety perspective there are no nonclinical objections to the approval of velmanase and I concur with the opinion of the nonclinical reviewer, Dr. Weis. However, it should be noted the dataset, including nonclinical confirmatory evidence was weak. PMRs and PMCs have been requested to address the deficiencies described above.

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/s/

MUKESH SUMMAN
02/14/2023 12:12:48 PM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	BLA 761,278
Supporting document/s:	001
Applicant's letter date:	17 June 2022
CDER stamp date:	17 June 2022
Product:	LAMZEDE
Indication:	Alpha-Mannosidosis
Applicant:	Chiesi Farmaceutici S.p.A.
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Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 761,278 are owned by Chiesi Farmaceutici, S.p.A or are data for which Chiesi Farmaceutici, S.p.A has obtained a written right of reference. Any information or data necessary for approval of BLA 761,278 that Chiesi Farmaceutici, S.p.A does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 761,278.

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1 Executive Summary

1.1 Introduction

LAMZEDE is a recombinant human lysosomal alpha mannosidase (LAMAN, MAN2B1) enzyme replacement therapy that was developed by Chiesi Farmaceutici S.p.A. for the treatment of patients with alpha-mannosidosis (AM).

1.2 Brief Discussion of Nonclinical Findings

AM is a rare lysosomal storage disease that occurs in approximately 1:500,000 people, and is caused by a deficiency in the alpha mannosidase gene, *MAN2B1*. Patients with AM may present with cognitive disability, characteristic facial features, hearing loss, and skeletal defects. Other symptoms include motor difficulties, muscle weakness, speech impairments, increased risk of infection, and enlargement of the liver and spleen. Patients may also present with psychiatric symptoms including anxiety, depression, and hallucinations. Although urinary secretion of mannose-containing oligosaccharides is suggestive of AM, it is not conclusive and an evaluation of enzyme activity, usually using peripheral blood leukocytes, is considered definitive.

The range of severity and age of onset of AM varies widely. Early-onset cases present in infancy and patients often do not survive past childhood. In less severe cases, patients may not present with symptoms until adulthood and may survive into their 50s. There are no approved therapies for AM. Bone marrow transplantation (BMT) has been used as a therapeutic option in lysosomal storage disorders, such as Gaucher disease. Relatively few attempts to treat α-mannosidosis with BMT have been reported and the results have been variable.

α-mannosidase is expressed as a single 1011 amino acid protein, which undergoes cleavage into several smaller polypeptides (Hansen, et al., 2004). The intracellular form is transported to the lysosomes by a mannose-6-phosphate (MP6)-dependent mechanism, where it is retained. Extracellular α-mannosidase can also be internalized into cells by a receptor mediated process. The major receptors responsible for the cellular uptake of circulating lysosomal enzymes are mannose 6-phosphate/insulin-like growth factor II receptors (MPR300), which are ubiquitously expressed, and the mannose receptor, which is specific to cells of the monocyte/macrophage lineage (Roces, et al., 2004). Other minor receptors are also thought to contribute to uptake, and uptake via pinocytosis has also been proposed; however, based on the sponsor's data, uptake via pathways other than the MPR300 pathway, appears to be nearly negligible, as cells that are deficient in M6PR have little to no mannosidase uptake when cultured in the presence of exogenous enzyme.

Under normal conditions, lysosomal mannosidase (LAMAN) is a glycohydrolase that cleaves the α–1,2, α–1,3, and α–1,6 mannosidase linkages leading to degradation of N-linked oligosaccharides. In patients with AM, accumulation of oligosaccharides in the lysosomes leads to the formation of aberrant storage vacuoles, which accumulate in tissues and are thought to contribute to functional organ impairment. The proposed pathogenic mechanism is that accumulated oligosaccharides contributes to disease

progression by inhibition of normal cellular function, induction of cellular apoptosis, and end-organ impairment (Thomas, et al., 2019).

The syndromes associated with AM in humans have been observed in a number of species, including cows, cats, and guinea pigs. In these species, disease outcomes are similar; however, no clear biochemical marker has been specifically implicated in the disease pathogenesis, making it difficult to associate severity of disease with a given molecular marker or to show evidence of clinical improvement that correlates with its reduction. The sponsor has relied on serum oligosaccharide levels in humans as a potential clinical biomarker; however, they did not employ this biomarker in any of the nonclinical studies included in the original BLA submission.

The Sponsor proposes that the pharmacodynamic (PD) mechanism of action is that the drug is (1) internalized into cells and tissues following exogenous administration. (2) The enzyme is transferred to the lysosome, where it (3) acts to degrade stored oligosaccharides in the lysosome, thereby (4) reducing the size of the lysosome and preventing (5) cellular dysfunction and end-organ impairment. The application contains data to support some, but not all, of these steps in the proposed process.

The Sponsor provided a number of studies in which cells from healthy individuals and AM patients can internalize the exogenous enzyme in culture (PD Step 1 above). There was no apparent difference in the extent of uptake in patient-derived cells compared with normal cells when cultured in the presence of rhLAMAN for 48 hours. Demonstration of lysosomal activity for a disease that is associated with lysosomal storage is a critical component of the sponsor's pharmacological argument, but the Sponsor did not provide data about the fate of the drug after its uptake (PD Step 2), nor did they provide data to support a lysosomal site of action following internalization into the cell. Published data (Liao, et al., 1996) demonstrate that native MAN2B1 expressed in cells localizes to the lysosome. In addition, according to Module 3, the activity assay used for the drug substance is performed at pH 4-5, which is consistent with lysosomal conditions (Diering and Numata, 2014). The Sponsor did not show that in cultured cells treated with a drug that blocks acidification of the lysosome, it would reduce the ability of the enzyme to reduce stored oligosaccharide content in animals or cells; however, the Applicant has demonstrated an association between treatment and apparent reduction of tissue vacuolation in knockout (KO) animals (PD Step 4). Therefore, taken together, it is reasonable to conclude that the drug exerts activity via the lysosome; however, data showing PD in Step 3 (reduction in oligosaccharide content) is complex and difficult to interpret.

While it is intuitively plausible that reduction in oligosaccharide content corresponds to the decrease in the extent of cellular and tissue vacuolation, the sponsor did not clearly make that case (PD Step 3). Nor did they identify which, if any, oligosaccharide moiety is pathogenic in cultured cells or animals (which is pertinent to the selection of biomarkers). In patients and animals that lack LAMAN activity, there appears to be an array of oligosaccharide species present in the storage vacuoles, so it is unclear whether reduction in total oligosaccharide content, or reduction in one or more of the

subspecies best correlates with the desired clinical outcome(s); thus, a specific biomarker supported by nonclinical mechanistic data has not been determined based on the in vitro and in vivo pharmacology data presented. They attempted to show a reduction in total tissue oligosaccharide species by TLC; however, as discussed below, those experiments are problematic because they are nonquantitative and insufficient information about assay variability is available to determine whether there are differences between treatment groups.

The sponsor also has not clearly demonstrated a mechanistic link between treatment-mediated reduction in oligosaccharide content and reduction in cellular or tissue dysfunction (PD Step 5). This proposed mechanism (Thomas, et al., 2001), which states that organ impairment results from lysosomal accumulation of oligosaccharides and subsequent cellular apoptosis, does not appear to have been completely characterized. Reports of mitochondrial dysfunction in patients with AM has been described in the literature (Brantova, et al. 2009); however, the Applicant did not provide data about the effect of treatment on indices of cellular health/survival in animals or cultured cells.

To assess the potential for in vivo efficacy with administration of exogenous rhLAMAN, the Sponsor created a *MAN2B*-deficient (knockout (KO)) mouse model (Stinchi, et al., 1999). The model was generated through targeted gene disruption by introduction of a translational stop codon in the *Man2b* gene. The absence of lysosomal α-mannosidase enzyme activity was confirmed at pH 4.5 in brain, liver, and kidney. α-mannosidase activity at pH 6.0 was observed, which confirmed the presence of nonlysosomal mannosidases in the endoplasmic reticulum, Golgi, and cytosol. Using a non-quantitative thin layer chromatography method (TLC), the Sponsor concluded that animals exhibited an approximately 2x increase in mannose-containing oligosaccharide content in the kidney, spleen, testes, and brain. Histologically, evidence of vacuolation was observed in multiple tissues. Of note, however, the animals did not present with evidence of human disease over the 12-month observation period, suggesting that the model does not recapitulate the clinical course of the disease.

Several publications were provided to support the effects of enzyme supplementation on manifestations of *MAN2B* deficiency in their KO mouse model. In the publication by Roces, et al., 2004, intravenous administration of different exogenous LAMAN preparations (derived from bovine kidney as well as recombinant human) led to an apparent reduction in oligosaccharide content in multiple organs. Administration of 250 mU/g rhLAMAN was stated to reduce oligosaccharide content in the liver, kidney, and spleen and the reduction persisted for up to 12 days. Using TLC, peak reductions occurred in the liver and kidney by Day 3 but levels began to accumulate by Day 6. In the kidney, levels reached the nadir at Day 6 but began to accumulate by Day 12. The reduction in oligosaccharide levels were accompanied by a histological appearance of reduced tissue vacuolation.

To support the identification of a toxicology species, the Sponsor evaluated uptake by nonhuman cells (mouse, rabbit, rat, monkey, pig, dog) and concluded that uptake by mouse cells was comparable to humans. Uptake by rabbits, monkeys, and rats was

about 60% of that observed for cultured human cells. Very little uptake was observed in cultured dog and pig cells.

Administration of rhLAMAN in the original KO strain was associated with a high rate of mortality that was attributed to anaphylaxis. To tolerize the animals to rhLAMAN, the Applicant knocked in a defective human *MAN2B1* gene, which was found to permit long-term administration of rhLAMAN. The effects of long-term (up to 30 weeks) administration of rhLAMAN were assessed on neurocognitive and histological changes in the brains in these animals. Effects on markers of glial cell activation, hippocampal histology, performance on a treadmill test, and performance in the Morris water maze test (MWMT) were evaluated, as well as effects on other in vitro and ex-vivo endpoints in isolated hippocampal preparations. The dose used was 500 mU/g body weight, (15.6 mg/kg/dose).

Administration of ERT for up to 9 months led to an apparent increase in the levels of α-mannosidase in the brains of KO-ERT mice and an apparent reduction in mannose content, compared with KO-MOCK-treated mice (Roces, et al., 2004; Stroobants, et al., 2017). The authors concluded that these data suggest normalization of lysosomal function sufficient to ameliorate end-organ pathology. The assays that the Applicant used to evaluate mannose levels in these studies (thin layer chromatography, TLC) was an orsinol/sulfuric acid-based reaction that is not specific to mannose. The assays were not validated, lacked concurrent standard curves, and there was no evidence that the authors used loading controls to ensure that similar amounts of extract were loaded across the different test lanes within a single experiment, or across different experiments. The authors quantified the orsinol-stained TLC plates using scanning densitometry but no standards were included, or was there any way to assess the contribution of other saccharide moieties to the signals detected by TLC.

In addition to the lack of saccharide specificity, lack of concurrent standard curves, and lack of loading controls, it is not clear whether the resulting graphical data are derived from single studies or multiple studies (or whether graphical datapoints represent single or multiple animals), and in many cases, the publications did not provide information about the variability of the measurements (e.g., standard deviations in tables, or error bars on graphs) to enable the reader to assess whether meaningful differences existed between treatment groups or timepoints. Thus, while the data appear to align with expectations based on the biochemical activity of the enzyme, there is no quantitative data upon which to draw conclusions about the extent of mannose reduction in tissue or the robustness of the effect, and no way to draw meaningful conclusions about a dose-response relationship.

The authors also did not measure the clinical biomarker in animals and did not evaluate tissue levels in their GLP study in the Tg+KO mouse in which they had obtained correlating tolerability, clinical pathology, and histopathological data in animals treated with the drug for 6 months. In addition, histopathological assessments were conducted only in control and high-dose animals; thus, the dataset lacks an assessment of dose-response on vacuolation.

Key adverse outcomes in patients with AM are cognitive decline and impaired immune function leading to severe, often fatal, infections. To evaluate effects on end-organ function, the authors (Stroobants, et al., 2017) evaluated effects of rhLAMAN administration on neurocognitive endpoints and evidence of effects on gliosis in the brain. By immunohistochemistry, treatment was associated with a statistically-significant decrease in CD68-positivity in the hippocampus of treated Tg+KO animals compared with mock-treated Tg+KO mice. The authors also provided fluorescence micrographs showing an apparent decrease in the lysosomal marker, LAMP-1 hippocampal CA3 cells of KO-ERT mice compared with KO-MOCK-treated animals (Stroobants, et al., 2017).

To assess whether these hippocampal effects were reflective of functional changes in learning and memory, Tg+KO mice were tested in the Morris water maze (MWM) and the treadmill tests. In the MWM test, a modest improvement in escape latency (a measure of learning) was observed in treated animals compared with mock-treated animals. This effect was only reflective of potential to improve short-term working memory; improvement in measures of long-term memory were not observed (Stroobants, et al., 2017). In the treadmill test, more consistent evidence of improved performance was observed after administration for both 10 and 30 weeks. The major measures in this assay were error latency and number of errors per replicate. It is not completely clear whether the assay reflects improved cognition, or if it may also reflect aspects of endurance, as the test conditions differed between animals treated for 10 weeks compared with those treated for 30 weeks.

The Sponsor conducted 26-week GLP-compliant, repeat-dose toxicology studies in monkeys and in Tg+KO mice. In monkeys, there were no effects on any endpoint evaluated. In the Tg+KO diseased mouse model, no toxicologically significant effects were observed on any endpoint evaluated. An apparent reduction in the histopathological severity of vacuolation was observed in multiple tissues. The pathologist was not blinded to treatment, however, and a peer review was not conducted. In general, histopathological evidence of reduced vacuolation was modest, as in control animals, the severity scores were low (minimal to mild), which is consistent with published data suggesting that *MAN2B1* deficiency in mice does not fully replicate human disease (Stinchi, et al., 1999). While many animals were ADA positive by the end of the study, exposure was maintained in some animals. The presence of high circulating drug levels in animals at the higher doses may have inhibited the detection of ADA in animals; thus, the incidence of ADA may be under-estimated. No information was available about the impact of ADA on the drug's activity or kinetics.

In an embryofetal toxicity study in Han Wistar rats, administration of rhLAMAN during the period of organogenesis from GD 6-17 was not associated with reduced maternal or fetal survival at any dose level, but was associated with induction of major malformations at the high dose of 20 mg/kg/day. Because the major malformations were limited to fetuses in high-dose dams, a role of rhLAMAN in induction of these malformations cannot be excluded. There were also numerous minor visceral and

skeletal malformations and variations in treated animals at levels that exceeded those observed in concurrent controls. The AUC at 20 mg/kg/day, which was associated with induction of malformations in rats, was approximately 7-fold those observed in humans at the MRHD. The NOAEL in this study was 10 mg/kg/day, which is approximately 1.7x the clinical AUC at the MHRD.

In an embryofetal toxicity study in the rabbit, there were no apparent effects on embryofetal survival; however, treatment was associated with induction of numerous variations and malformations, the incidences of which exceeded concurrent controls. A NOAEL was not identified in this study.

There were no effects on male or female fertility or sperm quality endpoints when rhLAMAN was administered twice weekly in males or females for 2 weeks prior to pairing and during the first six days of gestation. Toxicokinetic assessments were not performed.

In the pre- and postnatal development study conducted in the Han Wistar rat, there were no effects on developmental milestones, CNS evaluations, or sexual development indices in animals that were exposed during gestation and lactation, and no effects on pre-coital indices or mating outcomes (including fertility assessments) in F1 animals when rhLAMAN was administered twice weekly at doses of up to 30 mg/kg/dose.

A malignant histiocytic sarcoma of the ovary was observed in a rat in the pre- and postnatal development study at exposures of approximately 9-fold those observed in humans at the recommended clinical dose. The role of velmanase alfa-tycv in the induction of this tumor cannot be excluded.

Taken together, based on the data submitted to BLA 761278 appear to partially support the proposed mechanism of action. Drug uptake in cultured cells was demonstrated to depend on the mannose 6-phosphate receptor, and administration in Tg+KO mice was found to be associated with a decrease in the histological appearance of vacuoles, but data to support reduction in oligosaccharide content are limited, and data to support the use of a serum or urinary biomarker were not provided. No quantitative assessment of tissue oligosaccharide content in animals was made, and other data that were supplied in the form of publications are non-quantitative and insufficient information about the degree of variability in the measurements is available. Treatment in Tg+KO animals was found to produce small effects in measures that may predict cognitive and/or motor improvements, but the doses at which these effects were observed were considerably higher than those administered clinically; therefore, the relationship between these outcomes and the outcomes in the intended patient population, appears to be unclear.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical concerns that would preclude approval of LAMZEDE.

Nonclinical has requested several post-marketing commitments to address nonclinical deficiencies that affect labeling.

1. **Section 8.1 Pregnancy**
   Perform a bridging PK study in rats to support labeling for fertility and the pre- and postnatal development study.
2. **Section 13.2 Carcinogenicity**
   Perform an assessment of the potential effects of increased alpha-mannosidase exposure (and increased MAN2B1 expression) on tumor formation.
3. **Section 12.1 Mechanism of Action:**
   Conduct a 26-week repeat-dose pharmacodynamic study in Tg+KO mice using the doses previously used in the 26-week chronic toxicity study of rhLAMAN in Tg+KO mice ((b) (4) Study Report No. 24963). Use a validated bioanalytical method to evaluate changes in the M2 biomarker in response to treatment. Perform histopathology on all animals. Perform expanded neuropathology and use an expert neuropathologist to either read the slides, or have the neuropathologist perform a peer review of the brains and spinal cords taken from all animals on this study. Measure oligosaccharide contents of tissues (particularly neural tissues) in TK animals. Characterize the contents of the vacuoles and perform quantitative micrometry of the dose-effect on vacuolar burden in tissues from the study.

1.3.2 Additional Nonclinical Recommendations

None

(b) (4)

2 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

2 Drug Information

2.1 Drug

Velmanase alfa is a recombinant human lysosomal enzyme alpha-mannosidase (rhLAMAN), which is stably-transfected Chinese Hamster Ovary (CHO) cells. The polypeptide precursor is expressed as a 1011 amino acid protein that includes a 49 residue cleavable N-terminal signal sequence (Figure 1). The mature enzyme contains 962 amino acids and has a theoretical molecular mass of 109 kDa (130 kDa by SDS PAGE).

Figure 1: Alpha-Mannosidase Expressed Precursor and Its Processed Forms

(Excerpted from the Applicant's BLA)

Table 1: Summary of Drug Substance Properties

Property	Specific Attribute
Physical appearance	Clear solution
Chemical formula	$C_{4883}H_{7478}N_{1366}O_{1406}S_{28}$
Extinction coefficient [a]	1.8
pH	7.0 – 8.0
Solubility	Soluble in water based buffer solutions up to at least 10 mg/mL
Number of amino acids	Mature protein: 962 aa (1011 aa excluding 49 aa peptide signal sequence)
Molecular Mass Calculated based on the amino sequence	108,713.0 Da (962 aa mature non-glycosylated protein)
Molecular Mass Based on SDS-PAGE analysis	130 kDa (mature glycosylated monomer)
Isoelectric Point (pI) Experimental value	4.5–6.2 (with glycosylation) ~6.8 (de-glycosylated)

a. Abs 0.1% (=1 g/l) = 1.8 (theoretical)

(Excerpted from the Applicant's BLA)

There are no novel excipients. Adequate safety information for the individual excipients and their levels in LAMZEDE is provided in FDA's inactive ingredient database and/or in the published literature.

Table 2: Composition of the Drug Product

Component	CAS	Amount per Vial	IV Amount Covered*
Velmananse alfa	1492823-75-2	10 mg	--

			(b) (4)
Mannitol	69-65-8	227.5 mg	MDE: (b) (4) mg
Glycine	56-40-6	10.1 mg	(b) (4) mg

-- = active ingredient; *Based on FDA's Inactive Ingredients Database; CAS = Chemical Abstracts Service Unique Identifier; MDE = Maximum Daily Exposure

2.2 Relevant INDs, NDAs, BLAs, and DMFs

- IND 113186
- IND 157117

3 Studies Submitted

3.1 Studies Reviewed

Study Number	Title
Pharmacology	
ERT-23	ERT 23: LAMAZYM Batch Comparison
ERT-36	Biodistribution of LAMAZYM
EXP-14-AZ1479	Cellular uptake of rhLAMAN by normal human fibroblasts in vitro for later HPLC analysis developing a biological assay - first test
z-2012-09-17	Development of an immunotolerant a-mannosidosis mouse model and its treatment with enzyme replacement therapy
z-2014-09-11	In vitro characterization of rhLAMAN
Analytical Method Validation	
062-2009-r	Validation of an Analytical Method for Determination of rhLAMAN in Cynomolgus Monkey Plasma using Enzyme-Linked Immunosorbent Assay (ELISA)
2009-064-r	Validation of an Analytical Method for Determination of rhLAMAN in Rat Plasma using Enzyme-Linked Immunosorbent Assay (ELISA)
2010-026-r	Fit for Purpose Validation of an Analytical Method for Screening and an associated method for determination of Anti-rhLAMAN Antibody Titer in Cynomolgus Monkey Serum using Enzyme-Linked Immuno Sorbent Assay (ELISA)
2010-027-r	Fit for Purpose Validation of an Analytical Method for Screening and an associated method for determination of Anti-rhLAMAN Antibody Titer in Rat Serum using Enzyme-Linked Immuno Sorbent Assay (ELISA)
2010-036-r	Validation of an Analytical Method for Determination of rhLAMAN in Mouse Plasma using Enzyme-Linked Immunosorbent Assay (ELISA)
2010-044-r	Fit for Purpose Validation of an Analytical Method for Screening and an associated method for determination of Anti-rhLAMAN Antibody Titer in Mouse Serum using Enzyme-Linked Immuno Sorbent Assay (ELISA)

2013-075-v-r	Validation Report: Fit for Purpose Methods for Anti-rhLAMAN antibody Screening and Determination of antibody titer in Rabbit Serum Using Enzyme-Linked Immuno Sorbent Assay (ELISA)
2013-076-v-r	Validation Report: Re-establishment of Validated Methods for Anti-rhLAMAN antibody Screening and Determination of antibody titer in Rat Serum Using Enzyme-Linked Immuno Sorbent Assay (ELISA)
2013-077-v-r	Validation of an Analytical Method for Determination of rhLAMAN in Rabbit Plasma Using' Enzyme-Linked Immunosorbent Assay (ELISA)
z-2011-04-12	Validation of method for determination of inhibitory antibodies to rhLAMAN Date Sign/
z-2013-03-06	Production and purification of rabbit polyclonal antibodies against human lysosomal alpha-mannosidase (rhLAMAN)
Absorption	
171-011	Toxicokinetic Report: rhLAMAN
Toxicology	
Izu0001	rhLAMAN: Single Dose Intravenous (Slow Bolus) Tolerability Study in the Rabbit
24963	26-WEEK CHRONIC TOXICITY STUDY OF rhLAMAN BY REPEATED INTRAVENOUS ADMINISTRATION (TWICE WEEKLY) TO TG+/KO MICE
515896	rhLAMAN: Intravenous Maximum Tolerated Dose Study in Cynomolgus Monkeys
Pwm0001	rhLAMAN Feeding Study in Cynomolgus Monkeys
Pwm0002	13 Week Intravenous Toxicity Study with rhLAMAN in Cynomolgus Monkeys
Reproductive & Developmental Toxicology	
Izu0006	rhLAMAN: Intravenous (Slow Bolus) Fertility and Early Embryonic Development Study in the Rat
Izu0002	rhLAMAN: Intravenous (Slow Bolus) Preliminary Embryo-Foetal Development Study in the Rabbit with Toxicokinetic Sampling
Izu0003	rhLAMAN: Intravenous Preliminary Embryo-Foetal Development Study in the Rat
Izu0004	rhLAMAN: Intravenous (Slow Bolus) Embryo-Foetal Development Study in the Rat with Toxicokinetic Sampling
Izu0005	rhLAMAN: Intravenous (Slow Bolus) Embryo-Foetal Development Study in the Rabbit with Toxicokinetic Sampling
Izu0007	rhLAMAN: Intravenous (Slow Bolus) Pre-and Post-Natal Development Study in the Rat
495110	rhLAMAN: Preliminary Juvenile Toxicity Study in Rats
495126	rhLAMAN: Juvenile Toxicity Study in Rats
z-2015-07-01	rhLAMAN: Investigation into the Clinical Symptoms found in a Preliminary Juvenile Toxicity Study in Rats
Other Toxicology Studies	
2010-p-106	Bioanalytical Report (ref: 13 Week Intravenous Toxicity Study with rhLAMAN in Cynomolgus Monkeys)

z-2016-02-04	Evaluation of inhibitory antibodies in cynomolgus monkey and rat serum samples from tox studies PWM0001, (b) (4) 515896, PWM0002 and (b) (4) 495110

3.2 Studies Not Reviewed

z-2017-06-29	Cellular uptake of rhLAMAN by normal human fibroblasts

4 Pharmacology

4.1 Primary Pharmacology

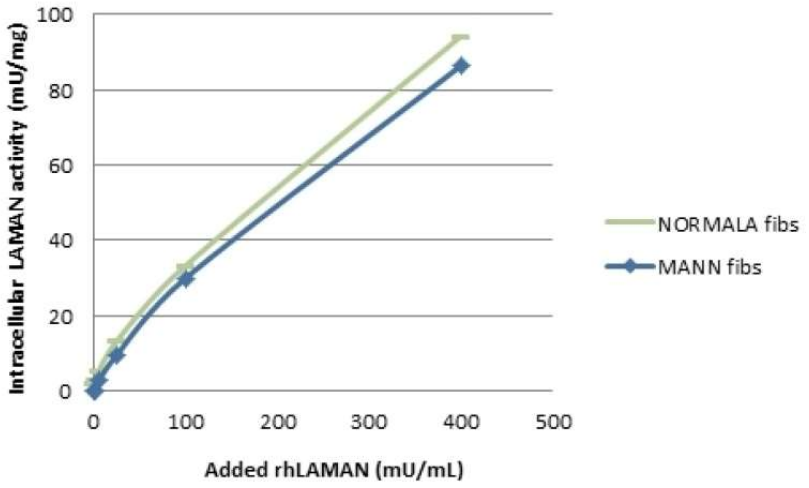
Pharmacology

4.1 Primary Pharmacology

4.2.1 Cellular Uptake of rhLAMAN by Normal Human Fibroblasts in Vitro for Later HPLC Analysis Developing a Biological Assay – First Test (Study EXP-14-AZ1479)

The purpose of this study was to compare uptake of rhLAMAN by normal human fibroblasts and fibroblasts taken from a patient with mannosidosis (purchased from the (b) (4)). Cells were cultured in the presence of rhLAMAN (clinical batch 2N210) at concentrations of 0, 1, 5, 25, 100, or 400 mU/mL for 48 hours before being washed twice and lysed for analysis by HPLC. As shown in Figure 2, uptake in patient-derived fibroblasts was comparable with uptake in normal human fibroblasts. Background levels of α-mannosidase were negligible (~2%) in PBS-treated normal fibroblasts and was stated to be zero from patients with mannosidosis; however, numerical data from this and all experiments in this report, were not provided. As a result, the information about uptake in patient-derived cells is difficult to evaluate.

Figure 2: Intracellular Uptake of rhLAMAN in Normal Fibroblasts and Fibroblasts Derived from a Patient with Mannosidosis



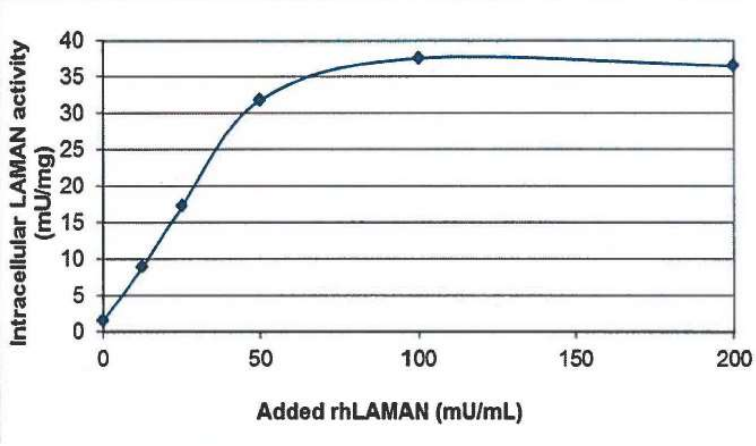
(Excerpted from the Applicant's BLA)

4.2.2 In vitro Characterization of rhLAMAN (Study z-2014-09999-11)

The purpose of this study was to characterize the uptake of rhLAMAN in cultured normal cells (fibroblasts, neuronal cells, HeLa cells, and monocytes/macrophages) and cells derived from patients with α–mannosidosis, and to assess the mechanism of uptake. Uptake in cells derived from other species (mouse rat, dog, pig, monkey, rabbit) was also evaluated.

Peak uptake of rhLAMAN in cells that lacked α–mannosidase deficiency (e.g., α–mannosidase-competent tumor-derived cells such as HeLa and J774 cells) and fibroblasts derived from patients was time- and concentration-dependent. Peak uptake was observed at a concentration of about 40 mU/mL (Figure 3) and could be inhibited by addition of mannose-6-phosphate (data not shown). The Applicant states that when higher enzyme concentrations were used in complete medium and incubation was increased to 4 days, higher levels of uptake were observed; however, the data were not provided. The Sponsor did not provide an estimation of variability (error bars or SEMs) for any of the experiments conducted.

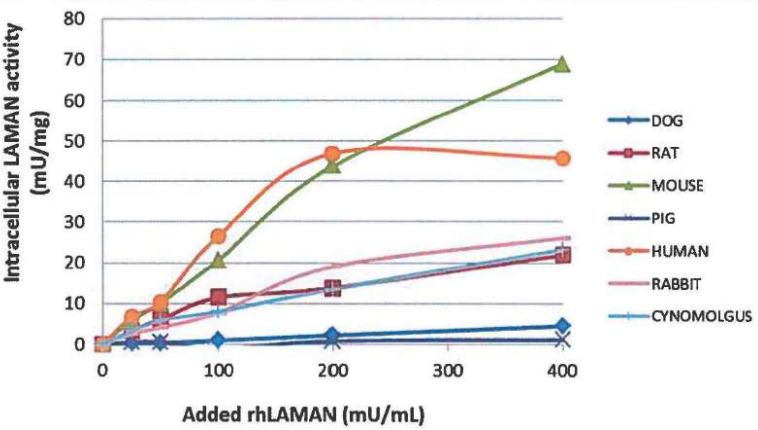
Figure 3: Cellular Uptake of rhLAMAN in Cultured Cells from Patients with α–mannosidosis



(Excerpted from the Applicant's BLA)

Uptake of rhLAMAN was also evaluated using normal fibroblasts of human, mouse, rat, rabbit, cynomolgus monkey, pig, and dog (Figure 4). Uptake was best for mouse and human but lowest for dog and pig. Uptake for rat, rabbit, and monkey were similar.

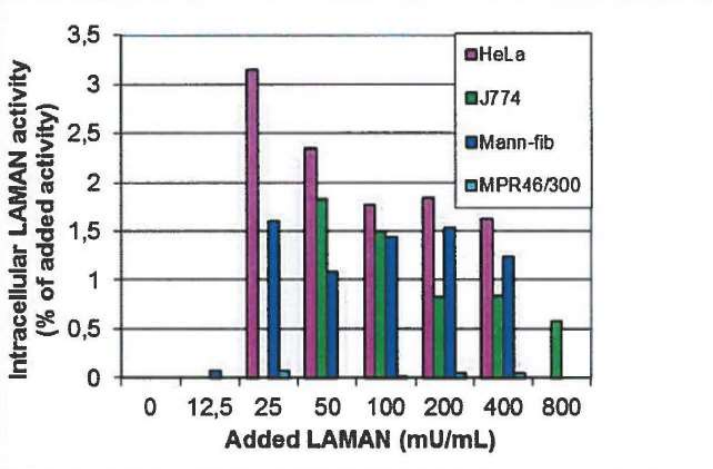
Figure 4: Cross-Species Comparison of rhLAMAN Uptake in Cultured Fibroblasts



(Excerpted from the Applicant's BLA)

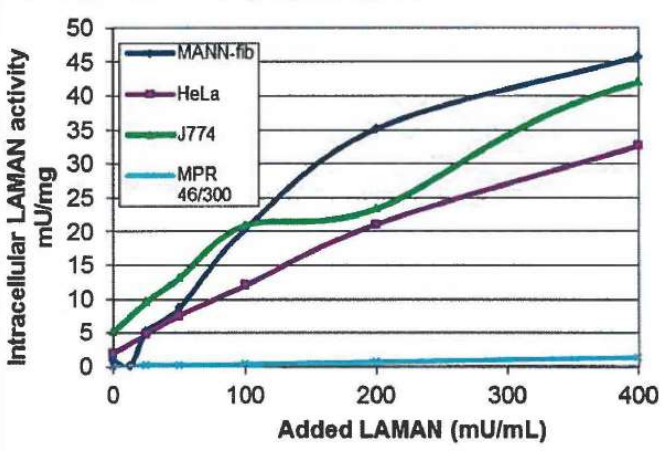
The Applicant also evaluated uptake in cells of different origins (HeLa – cervical cancer; J774 - reticulum cell sarcoma; Mann-Fib - α–mannosidosis; and MPR46/300 – M6PR-deficient). As shown in Figure 5 and Figure 6, relatively little uptake was observed in the absence of the M6P receptor. The report states that peak uptake was about 3-4% (in multiple experiments), and variable but consistent uptake was observed in M6PR-expressing cell lines, including those of α–mannosidosis patients.

Figure 5: Percent Uptake of rhLAMAN in Cells of Different Types



(Excerpted from the Applicant's BLA)

Figure 6: Uptake in Cells of Different Origins



(Excerpted from the Applicant's BLA)

Overall, the experiments in this report suggest that M6PR is responsible for the uptake of rhLAMAN into fibroblasts and that there is relatively little contribution from other pathways; that patient-derived cells can internalize rhLAMAN; and that rhLAMAN uptake can be achieved in animals. The lack of methodological data and the lack of information about assay variability (tables with numerical data and SDs/SEMs, and/or error bars on figures) makes it difficult to interpret the claims that the authors are making.

1.1.1 ERT 23: LAMAZYM Batch Comparison (Study ERT-023)

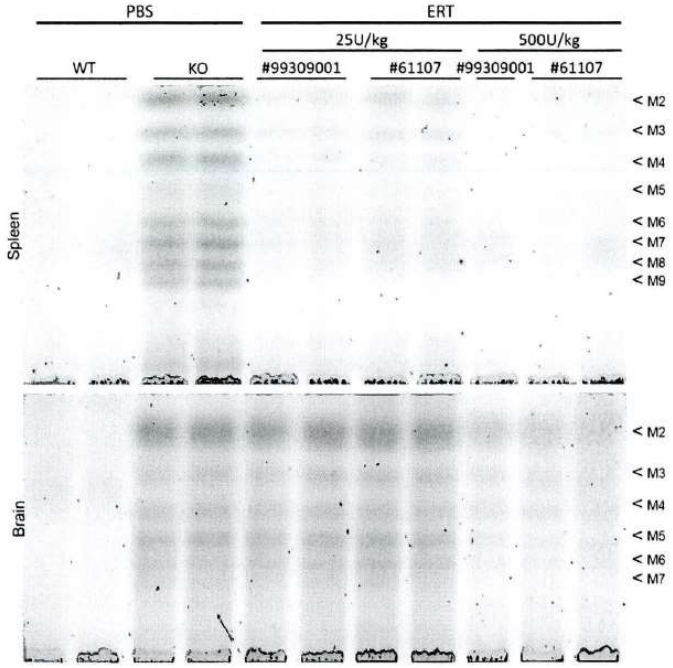
The purpose of this study was to compare the activity of the material used in preclinical studies with the activity of the clinical batches, which were prepared with a different process.

Wildtype and *MAN2B1* knockout animals were injected three times with either the 2-step purified preclinical lot (61107) or the 4-step purified clinical lot (ZYA DS 99309001) at doses of 25 and 500 U/kg. Doses were administered in two mice per genotype per group an interval of 3.5 days between doses. PBS was administered in WT and KO mice as controls.

Two days post-last dose, mice were euthanized (except one 500U/kg animal that received the material from the clinical process, Lot ZYA DS99309001, which died after the last injection) and organs were harvested for an assessment of oligosaccharide content as assessed by thin layer chromatography (TLC). As shown in Figure 7, the levels of residual oligosaccharides were low in the spleen at all dose levels with both products, but higher in the brain. Levels in the brain were relatively unchanged in animals that received the low dose but were lower in animals that received the high dose of both products. These TLC results were not non-quantitative, lacked loading controls, and an internal calibration curve, so there is little that can be concluded from this study; however, at the level evaluated, there appear to be no marked differences between the two sources of materials. This information, however, is not sufficient to conclude that the material used in animals was comparable to that used in humans. Additional information about the batches used and their release characteristics (e.g.,

potency, purity, stability, etc.) would be needed to make that determination. It should also be noted that the material used in published studies is not necessarily supported by these studies, as key information such as lot numbers and material quality attributes, were not provided in the publications. Therefore, how the material used in the papers by Roces, 2004; Damme et al. (2015); and Stroobants, et al. (2017) compares to the material evaluated in this study, is unclear.

Figure 7: Comparability of Oliogsaccharide Content in the Spleen and Brain of WT and KO Mice After Administration Preclinical or Clinical rhLAMAN



(Excerpted from the Applicant's BLA)

1.1.2 Development of an immunotolerant α-mannosidosis mouse model and its treatment with enzyme replacement therapy (Report z-2012-09-17)

Numerous gross errors were identified in this report, which compromised interpretation of the data; thus, this report was excluded from the review. Most of the data relating to the use of the Tg+KO mouse model was published in the papers by Stroobants, et al., 2017 (reviewed below) and Damme, et al., 2015.

1.1.3 Biodistribution of LAMAZYM (Study ERT-36)

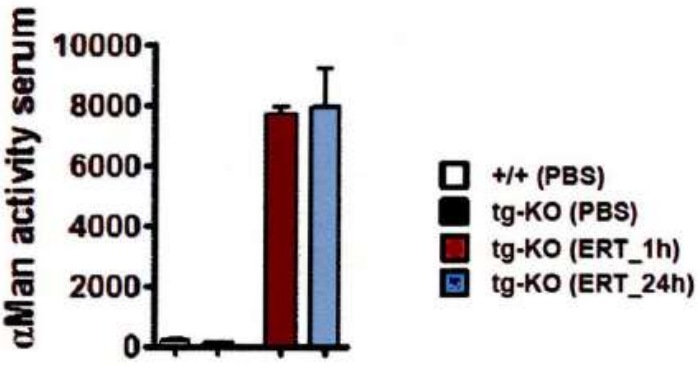
The purpose of this study was to evaluate biodistribution of rhLAMAN in an immune-tolerant strain of *MAN2B1*-deficient mice (KO mice). This *MAN2B1* KO mouse strain was constructed to permit evaluation of the efficacy of rhLAMAN by inserting an inactive human LAMAN gene into the KO mouse (Tg+KO mice), which was expected to partially tolerize mice to the human protein and reduce mortality secondary to anti-product antibody formation.

Female Tg+KO and wildtype (+/+) mice received one injection of either PBS or 1000 U/kg rhLAMAN, and biodistribution was measured in tissue lysates using enzyme activity as a marker of uptake at 1- and 24-hours post-injection (n = 3F/group/timepoint). The lot of material that was used for this study was 2M230 (64U/mL). A blood sample was collected at 5 minutes post-dose to confirm successful administration.

Following injection, perfused tissues were harvested and rhLAMAN activity was measured in protein lysates; however, the method by which they measured the enzyme levels was not described.

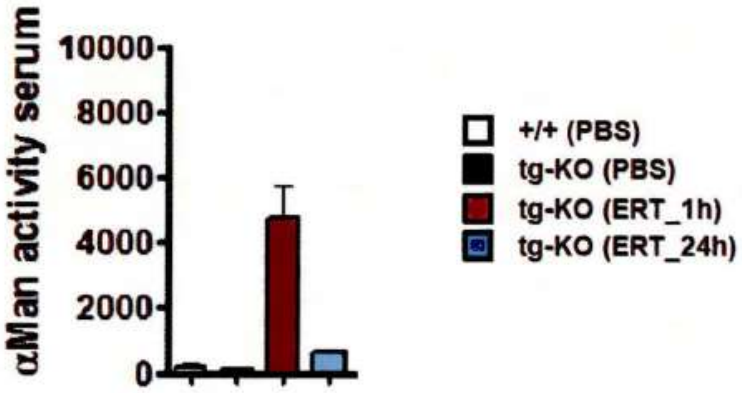
As shown in Figure 8, exposure was confirmed in Tg+KO animals. All animals had measurable concentrations in the blood at 5 minutes post-dose. At 1 hour, exposures in Tg+KO mice were reduced by about half of those measured at 5 minutes (Figure 9). Elimination was largely complete by 24 hours (Figure 9). Little activity was observed in +/+ mice in this assay, suggesting that the assay exhibited greater specificity for rhLAMAN (LAMAZYM), and no detectable activity was observed in PBS-treated Tg+KO mice.

Figure 8: LAMAZYM Activity Measured in Blood Samples at 5 Minutes Post-Dose Following Administration of PBS or 1000 U/kg LAMAZYM in Tg+KO or +/+ Mice



(Excerpted from the Applicant's BLA)

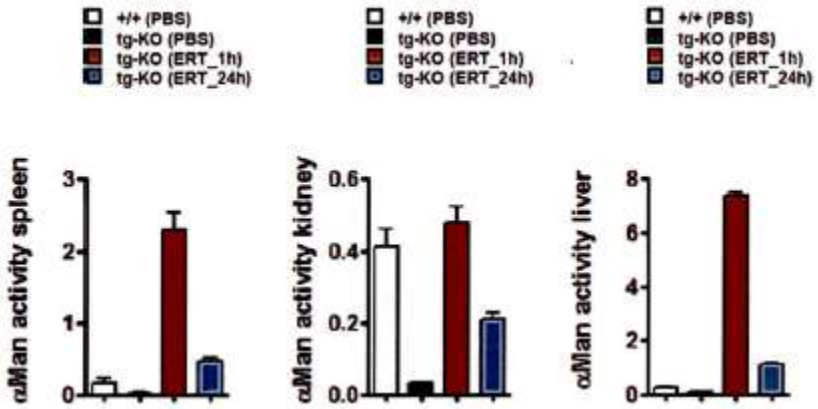
Figure 9: LAMAZYM Activity Measured in Blood Samples at 1- and 24-Hours Post-Dose Following Administration of PBS or 1000 U/kg LAMAZYM in Tg+KO or +/+ Mice



(Excerpted from the Applicant's BLA)

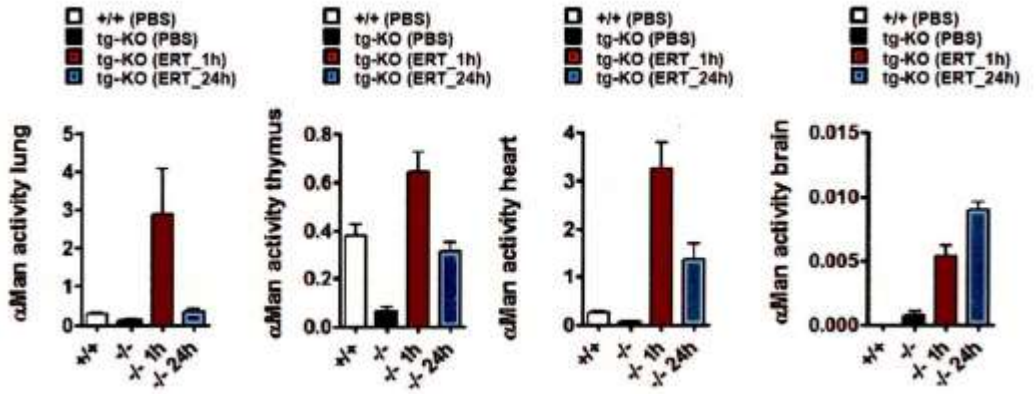
Exposure at 1 hour was greatest in the liver, heart, lung, and spleen. Exposures were lower in the kidney, thymus, and brain (Figure 10 and Figure 11). By 24 hours, exposures were highest in the heart, liver, and spleen. Levels in the kidney, lung, and thymus were the lowest. Levels in the brain at 24 hours post-dose, although low, had increased relative to the levels observed at 1-hour post-dose.

Figure 10: LAMAZYM Activity Measured in Spleen, Kidney, and Liver at 1- and 24-Hours Post-Dose Following Administration of PBS or 1000 U/kg LAMAZYM in Tg+KO or +/+ mice



(Excerpted from the Applicant's BLA)

Figure 11: LAMAZYM Activity Measured in Lung, Thymus, Heart, and Brain at 1- and 24-Hours Post-Dose Following Administration of PBS or 1000 U/kg LAMAZYM in Tg+KO or +/+ mice



(Excerpted from the Applicant's BLA)

Liao Y-F, et al., 1996.

Liao Y-F, Lal, A, Moreman KW. 1996 Cloning, Expression, Purification, and Characterization of the Human Broad Specificity Lysosomal Acid α-Mannosidase. J. Biol. Chem. 271(45):28348-58

This paper describes the cloning and expression of two cDNAs encoding the hLAM gene. MAN2B1 is required for the degradation of N-linked carbohydrates during glycoprotein catabolism in eukaryotes. The authors cloned the gene from human spleen fibroblasts by RT-PCR using primers derived by comparison of conserved sequences from other putative α-mannosidase genes. The resulting product was compared to amplimers from other tissues.

The resulting enzyme was characterized and had highest activity at pH 4.25-6.0 with a Km of 2.4 mM for pNP-Man and a swainsonine (an inhibitor of alpha mannosidase) IC50 of 0.11 μM. They performed a series of activity assays and showed that the enzyme was capable of cleaving α1,2Man, α1,3Man, and α1,6Man linkages on Man5GlcNAc and Man9GlcNAc substrates but that it did not recognize one of the α-linked mannose residues remaining on the β-linked core mannose.

Oligosaccharides accumulating in α-mannosidosis fibroblasts have a low content of the α1,6Man linkage because of the action of the α1,6 core-specific mannosidase in these cells. All of the other α-linked mannose residues are susceptible to cleavage by broad-specificity α-mannosidase.

Tissue distribution revealed that the transcript was present in all tissues but that the abundance varied. The expression is highest in WBCs and low in the brain. Also expressing high levels were the spleen and thymus, and the testis expressed multiple isoforms.

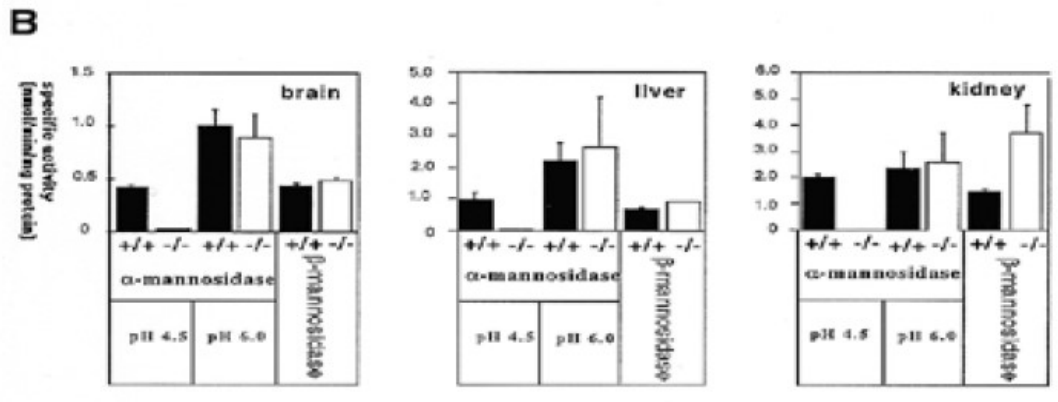
Chromosome mapping placed the gene on chromosome 19.

Stinchi S, et al., 1999

Stinchi S, Lullmann-Rauch R, Hartmann D, Coenen R, Beccari T, Orlaccio A, von Figura K, Saftig P. 1999. Targeted disruption of the lysosomal α-mannosidase gene results in mice resembling a mild form of human α-mannosidosis. Human Molecular Genetics 8(8):1365-1372

The purpose of this study was to create a model of human lysosomal α-mannosidase. The authors created a deficiency in murine lysosomal α-mannosidase using targeted gene disruption by homologous recombination in embryonic stem cells. The targeting vector contained a neomycin phosphotransferase gene that introduced a transational stop codon into the *Man2b* gene. Confirmation of the absence of lysosomal α-mannosidase enzyme activity was confirmed at pH 4.5 in brain, liver, and kidney. α-mannosidase activity at pH 6.0 was observed, which confirmed the presence of nonlysosomal mannosidases in the endoplasmic reticulum, Golgi, and cytosol (Figure 12).

Figure 12: Activity of α-Mannosidase at pH 4.5 and 6 in the Brain, Liver, and Kidney from WT and KO mice



(Excerpted from the Sponsor's BLA)

Homozygous lysosomal α-mannosidase-deficient animals exhibited an (~2x) increase in the content of mannose-containing oligosaccharides in the kidney, spleen, testes, and brain. In the liver, the observed increase was small (about 10%; Table 3).

Table 3: Increased SugarContent in KO Mouse Tissues

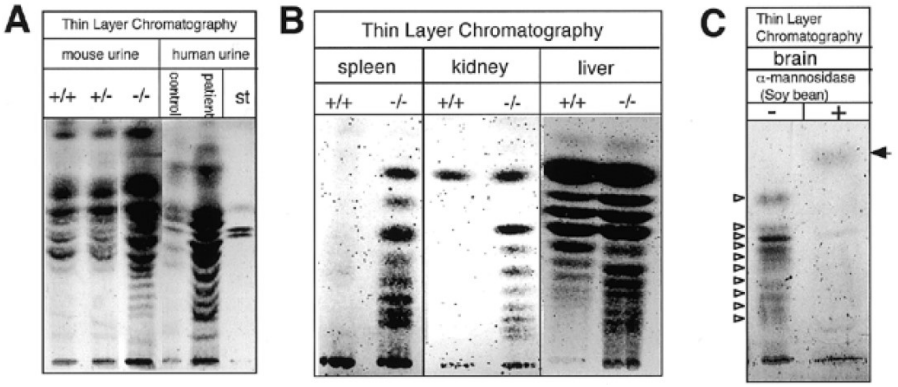
	Neutral carbohydrate (mg neutral carbohydrate/g wet weight) (± SD)	
Tissue	α-mannosidase$^{+/+}$	α-mannosidase$^{-/-}$
Kidney	0.34 (±0.1)	0.95 (±0.13)
Spleen	0.36 (±0.08)	0.93 (±0.06)
Brain	0.32 (±0.13)	0.71 (±0.08)
Liver	12.22 (±2.91)	13.61 (±2.31)
Testis	0.42 (±0.16)	0.67 (±0.33)

(Excerpted from the Sponsor's BLA)

The authors state that homozygous animals were fertile and had no malformations, unexpected behaviors, or elevated mortality up to 10 months of age, but exibited a mild increase in urinary oligosaccharide excretion.

Homogenates of spleen, kidney, and liver (as detected by treating plates in 0.2% orcinol in sulfuric acid) were also shown by TLC. Since the orsinol/sulfuric acid reaction is not specific for mannose (Brückner, 1955), it is difficult to determine how to quantify the analyte of interest in this assay (Figure 13). Nevertheless, the Applicant states that an increase in orcinol staining in the brain was also observed as shown in panel C. Digesting the extracts with jack bean α mannosidase overnight degraded the material to mannosidase monomers (Panel C, arrow) suggesting that the oligosaccharide contained mannose..

Figure 13: TLC Detection of Saccharide Content in Mouse and Human Urine and in WT and KO Mouse Tissues

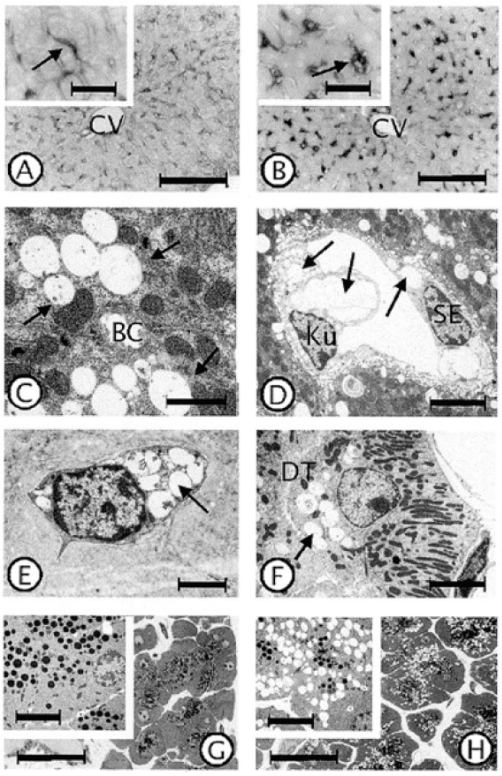


(Excerpted from the Sponsor's BLA)

The authors provide histological evidence of lysosomal storage (as demonstrated by increased vacuolation) in the liver, pancreas, kidney, eye, thyroid, smooth muscle, bone, the CNS and in peripheral nerves. As shown in Figure 14, Histological evidence of increased vacuolation, albeit modest, was observed in the livers of a α–mannosidase-deficient mouse (Panel B) versus control mice (Panel A). Panels C and D show the ultrastructural appearance of these vacuoles in a 6-month-old KO mouse. Panel E

shows vacuolation of osteocytes in a 2-month-old KO mouse and Panel F shows vacuoles in the kidney of a KO mouse at 6 months. Panel G shows the exocrine pancreas of a wildtype mouse vs. a 2-month-old KO mouse (Panel H).

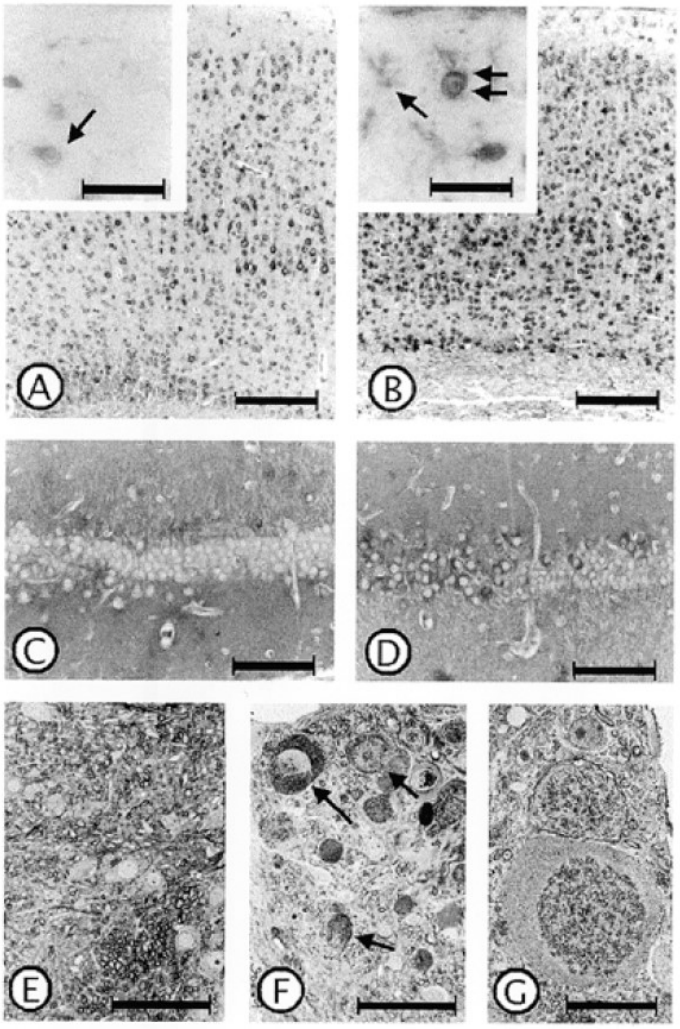
Figure 14: Micrographs of Wildtype and Tg+KO Mice



(Excerpted from the Sponsor's BLA)

Vacuolated neurons were also observed in the brains of KO-mice compared with concurrent controls (Figure 15 Panel B vs. A, below). Tissues were stained with *L. culinaris* lectin to reveal mannose-containing material associated with the clear vacuoles in the hippocampus (Panel D vs. control tissue in Panel C). The authors also reported axonal swellings in KO mice that were not observed in wildtype mice (Panels F & G vs. E from a control animal), which were filled with an accumulation of polymorphous material.

Figure 15: CNS Histopathology in WT and KO Mice



(Excerpted from the Sponsor's BLA)

Given the relatively benign clinical presentation exhibited in homozygous KO mice, the extent to which this model replicates the human disease is unclear. The authors state that in humans, the disease is associated with early (in the 1st year of life) psychomotor delays and facial changes which were not present in the 12-month observation period in mice, suggesting that the phenotype in mice is less pronounced than occurs in cases of spontaneous human disease. They suggest that the reduced severity may relate to metabolic compensation and that the phenotype may increase in severity with age as the accumulation increases; however, data were not provided from aging mice.

Roces DP, et al., 2004.

Roces DP, Lullmann-Rauch R, Peng J, Balducci C, Andersson C, Tollersrud O, Fogh J, Orlacchio A, Beccari T, Saftig P, von Figura K. 2004. Efficacy of enzyme replacement therapy in a-mannosidosis mice: a preclinical animal study. Human Molecular Genetics. 2004. 13(18): 1979-88

The purpose of this series of studies was to show the efficacy of enzymatic replacement of α-mannosidase in a murine α-mannosidosis model. The authors administered lysosomal α-mannosidase (LAMAN) from bovine kidney, or recombinant human, or mouse α-mannosidase in a mouse α-mannosidase knockout model. Mannosidase concentration was measured by incubation with *p*-nitrophenyl-α-mannopyranoside as a substrate. Absorbance was read at 405 nM.

As shown in Table 4, knockout mice had very little α-mannosidase activity at baseline compared to normal (+/+) controls. Following injection of mice with a dose of 100 mU/g body weight enzyme levels were measured in lysates of perfused tissues. Levels were highest in the liver and spleen where they exceeded physiological levels in WT mice. In the heart, levels also exceeded physiological levels in WT animals, but in the brain, they were about 10% of those present in WT tissues, suggesting poor brain penetrance. Levels declined rapidly in all tissues by the end of the 10-hour sampling phase.

Table 4: Biodistribution of LAMAN in Normal and KO Mice

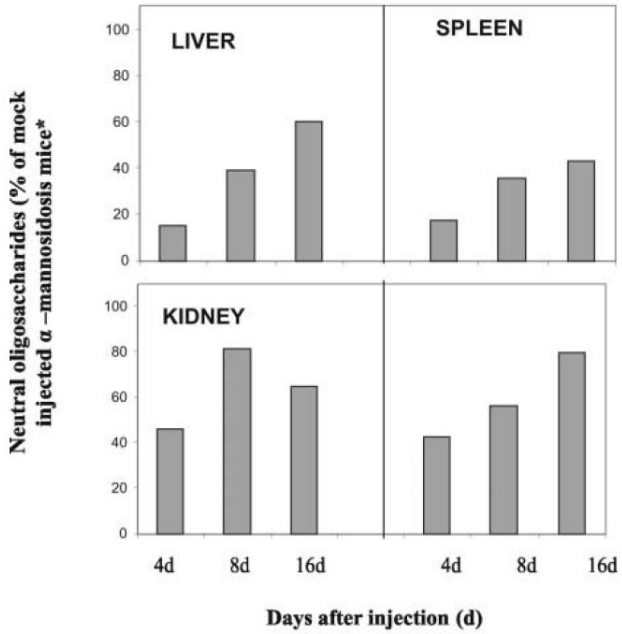
Genotype	LAMAN injected (100 mU/g body weight)	LAMAN (mU/g wet weight)				
		Liver	Spleen	Kidney	Heart	Brain
(+/+) (*n*)	–	136.7 ± 29.1 (19)	116.3 ± 44.5 (3)	168.4 ± 34.5 (9)	12.6 ± 3.1 (3)	25 ± 1 (2)
(−/−) (*n*)	–	2.9 ± 2.5 (21)	2.8 ± 2.1 (4)	2.5 ± 1.1 (10)	0.6 ± 0.5 (3)	0.4 ± 0.3 (3)
(−/−)[a]	2 h[a]	1057	185	26	22	2.9
	4 h[a]	883	183	40	5	1.4
	10 h[a]	197	36	10	2	1.0

+/+ refers to control mice, −/− refers to α-mannosidosis mice and *n* refers to the number of animals investigated.
[a]All values were corrected for the mean α-mannosidase activity in serum at t_0 (2350 mU/ml serum). The correction factors were 0.98, 1.07 and 0.96 for 2.4 and 10 h, respectively.

(Excerpted from the Applicant's BLA)

The authors also evaluated the timecourse of oligosaccharide clearance in tissues and found that the maximum PD effect was observed after 4 days in all tissues, but that effects persisted through 16 days in all tissues evaluated. In Figure 16, the unlabeled panel depicts clearance observed in the heart.

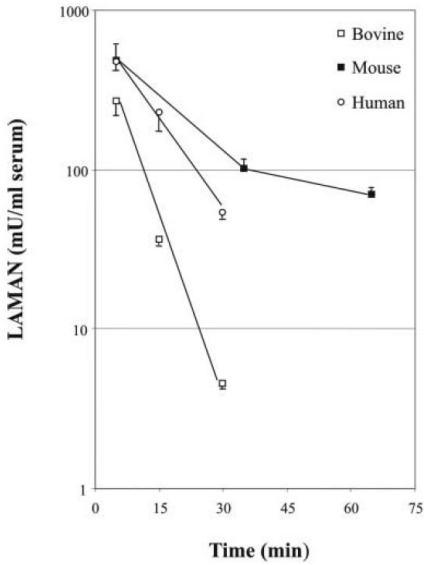
Figure 16: Timecourse of Oligosaccharide Clearance in MAN2B KO Mice Following Administration of LAMAN



(Excerpted from the Sponsor's BLA)

Clearance of the enzyme was dependent on the species from which the enzyme was derived (Figure 17). Injection of mice with 50 mU bovine and human LAMAN/g body weight was cleared quickly, with half-lives of 4 and 8 min, respectively. The clearance of the murine isoform, which is more heavily phosphorylated, was biphasic. Most of the material was cleared rapidly, with a half-life (HL) of 12 minutes, and the remainder had a HL of 47 minutes.

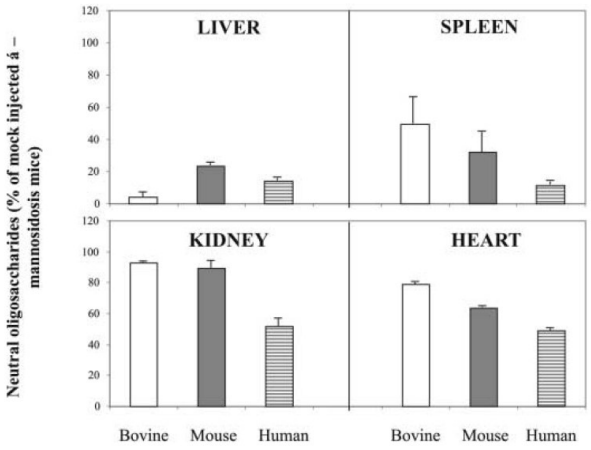
Figure 17: Kinetics of LAMAN Clearance in KO Mice



(Excerpted from the Sponsor's BLA)

Activity was best for human LAMAN and lowest for bovine. Murine LAMAN had intermediate activity (Figure 18).

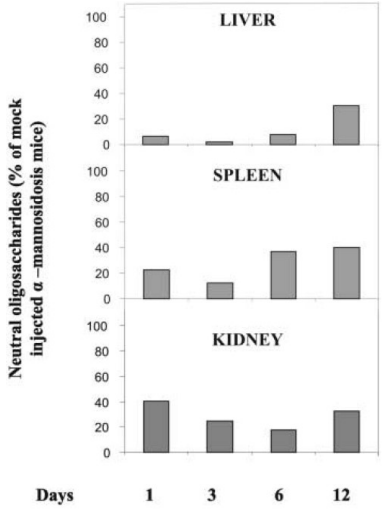
Figure 18: Effect of LAMAN from Different Sources on Oligosaccharide accumulation in KO Mice



(Excerpted from the Sponsor's BLA)

To evaluate effects after a higher dose, the Applicant administered 250 mU human LAMAN per gram of body weight. Oligosaccharide reduction was greatest in liver, but accumulation resumed between Days 6-12 (Figure 19). In the kidney and spleen, oligosaccharide levels were stated to fall to 12 and 18% of baseline, respectively. Peak reductions in the spleen and kidney occurred after 3 and 6d of treatment but began to re-accumulate after 6-12 days, respectively. Oligosaccharide levels in the brain were not affected (Data not shown). The lack of methodological details, and lack of information about variability makes interpretation of these data difficult.

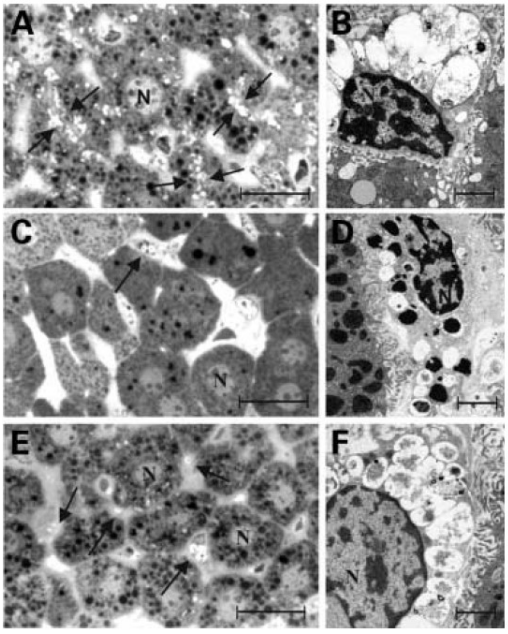
Figure 19: Oligosaccharide Reduction in Mice Treated with 250 mU/g rhLAMAN



(Excerpted from the Sponsor's BLA)

To determine whether the biochemical oligosaccharide reduction correlated reduced vacuolation, authors examined a timecourse of liver and kidney tissues from mock- and LAMAN-injected KO mice by light and electron microscopy. As shown in Figure 20, the hepatocytes and Kupffer cells of mock-treated animals were extensively vacuolated (A, arrows). At 1-day post-treatment with 250 mU/g body weight, vacuoles were largely absent from the hepatocytes and Kupffer cells (C arrows), but they reappear by Day 12 post-dose (E, arrows). Panels B, D, and F illustrate the abundance and morphology of the vacuoles at an ultrastructural level. The kinetics of clearance differed between organs, however. In the spleen and kidney, the maximal effect was observed between Days 3-6 post-dose, after which the materials began to reaccumulate.

Figure 20: Effect of rhLAMAN Treatment on Tissue Vacuolation in KO Mice



(Excerpted from the Sponsor's BLA)

After repeated administration, histological evidence of reduced vacuolation was improved in multiple tissues when administered 3.5 and 7 days prior to tissue collection(data not shown).

Stroobants S, et al., 2017

Stroobants S, Damme M, Van der Jeugd A, Vermaercke B, Andersson C, Fogh J, Saftig P, Blanz J, D'Hooge R. 2017. Long-term enzyme replacement therapy improves neurocognitive functioning and hippocampal synaptic plasticity in immune-tolerant alpha-mannosidosis mice. Neurobiology of Disease 106:255-268

This publication describes disease progression in an immune-tolerant murine model of α–mannosidase deficiency. Administration of rhLAMAN in the original KO strain was associated with a high rate of mortality that was attributed to anaphylaxis. To tolerize

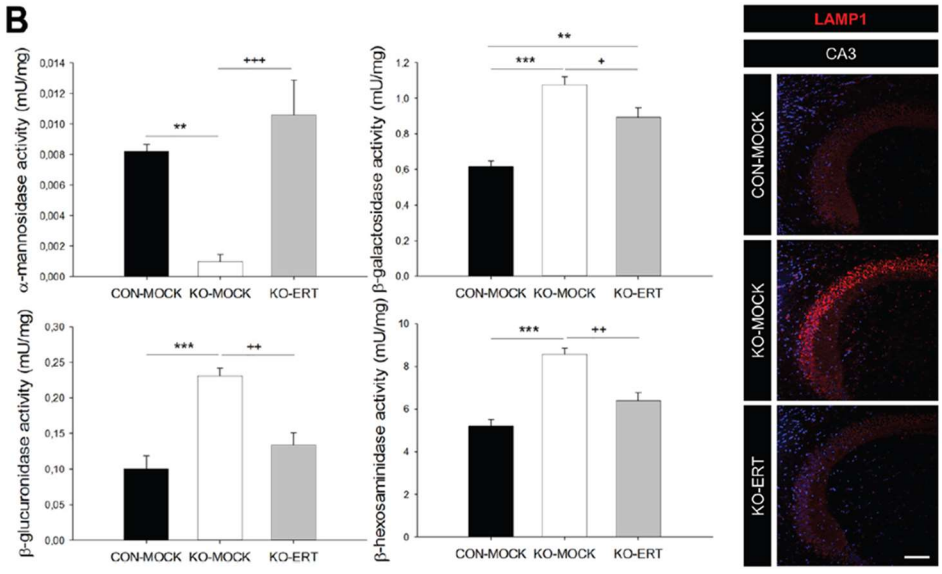
the animals to rhLAMAN, the Applicant inserted a defective human *MAN2B1* allele, which was found to permit long-term administration of rhLAMAN.

The authors treated Tg+KO mice with rhLAMAN for mid- (10 week) and long-durations (30 weeks) and evaluated on markers of glial cell activation, hippocampal histology, performance on a treadmill test, and performance in the Morris water maze test (MWMT), and they evaluated other in vitro and ex-vivo endpoints in isolated hippocampal preparations to assess endpoints potentially reflective of effects on learning and memory.

rhLAMAN (designated as ERT), administered at a weekly dose of 500 mU/g body weight (15.6 mg/kg), was derived from a CHO cell line. Three groups were evaluated: control animals that received vehicle (designated CON-MOCK); a knockout that received vehicle (designated KO-MOCK); and a knockout that received the active LAMAN drug (designated KO-ERT). Both KO-MOCK and KO-ERT were the Tg+KO strain. Treatment began at 2 months of age and continued for up to 30 weeks (presumably by the IV route), after which animals were euthanized for histological, biochemical, and electrophysiological analysis. During the treatment period, animals underwent behavioral training and neurocognitive evaluations using a treadmill and a water maze.

As in Figure 21, administration of ERT for 9 months led to increased levels of α-mannosidase and decreased levels of other lysosomal enzymes in the brains of KO-ERT mice compared with KO-MOCK-treated mice, which the authors claim suggests normalization of lysosomal function. This correlated with a decrease in the lysosomal marker, LAMP-1 hippocampal CA3 cells of KO-ERT mice compared with KO-MOCK-treated animals.

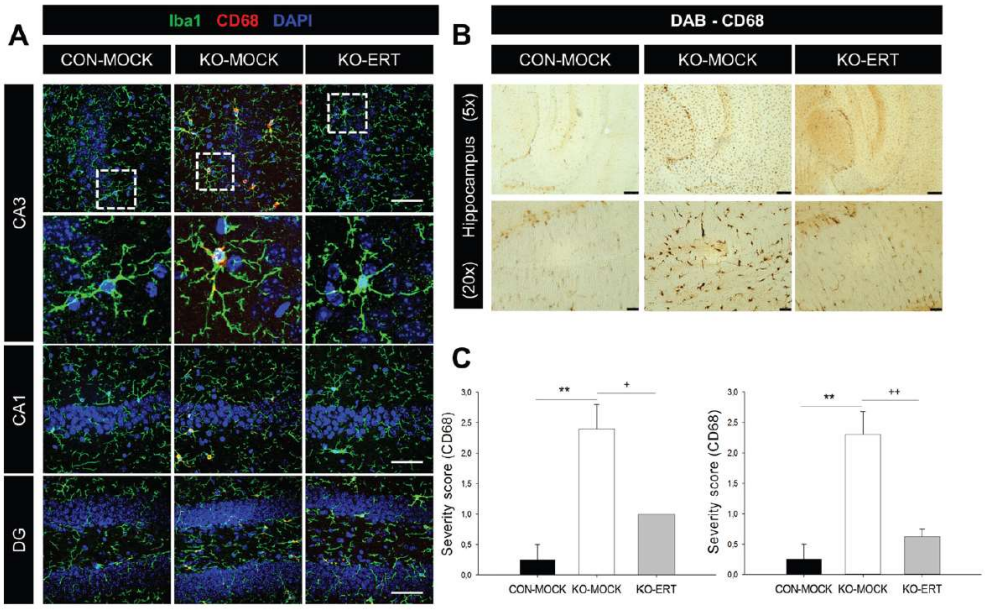
Figure 21: Effect of rhLAMAN Administration on α-Mannosidase levels in the Brain and Markers of Glial Activation



(Compiled from the Sponsor's BLA)

The authors then evaluated brain histopathology and immunochemical markers of glial cell activation in treated and mock-treated animals. As shown in Figure 22 Panel A, histological evidence of decreased CD68 immunoreactivity was observed in KO-ERT animals vs. KO-MOCK animals (red and yellow spots correlate with CD68 and CD-68/IBA1-positivity, respectively). An apparent decrease in CD68 immunoreactivity is also shown histologically in Panel B. There was an overall decrease in DAB-staining in the hippocampal tissues of KO-ER animals vs. KO-MOCK mice. As shown in Panel C, levels of CD68-positivity in treated Tg-KO animals were higher than those of wildtype mice, but lower than those of mock-treated Tg-KO mice, suggesting some improvement in gliosis.

Figure 22: Effect of rhLAMAN Administration on Markers of Glial Activation



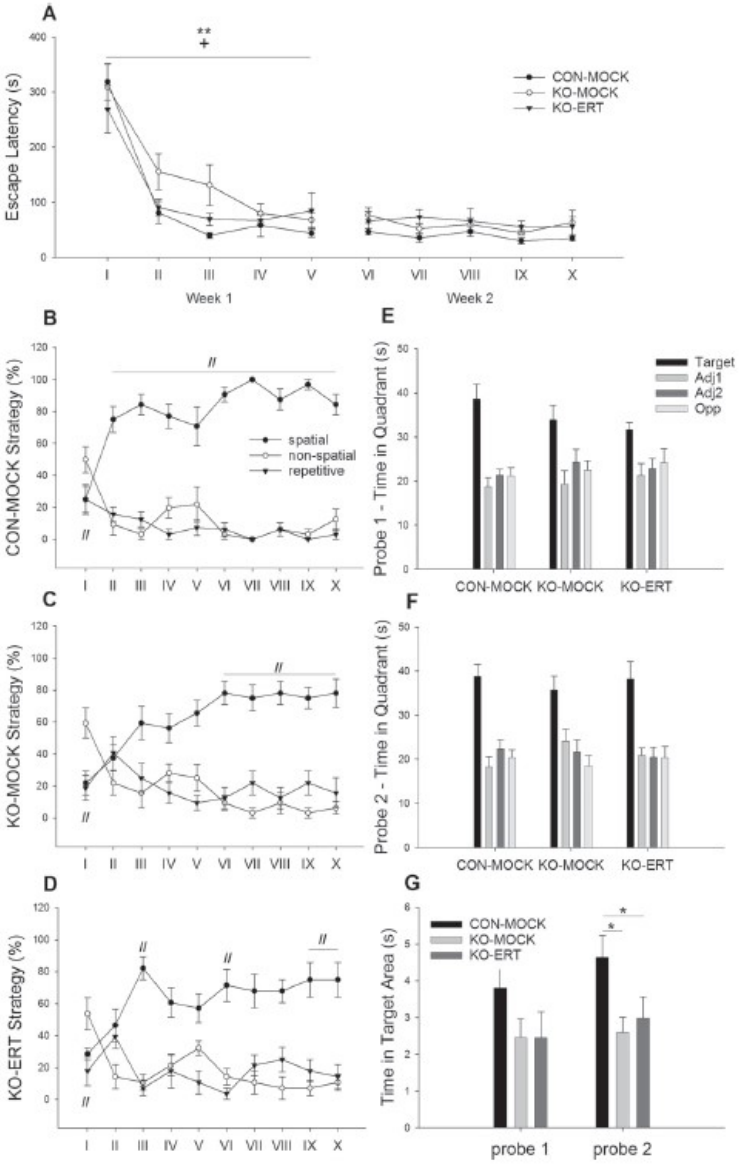
(Excerpted from the Sponsor's BLA)

To determine if there was an effect of treatment on learning and memory, animals that had received 10 weeks of treatment with rhLAMAN or vehicle were subjected to the Morris Water Maze Test (MWMT). In this test, animals were initially trained to locate a visible rescue platform in a tank of water, then they were tested in a tank in which the rescue platform has been removed. Animals received two sessions of repetitive training each consisting of five trial blocks, separated by two days of rest, after which the first probe trial was conducted. During the probe trial, the paths taken by the animals to the location of the former platform were recorded for 100 seconds. The endpoints measured are the time to reach the escape platform and the navigation strategies used (direct, non-spatial, or repetitive).

During the acquisition phase, KO-ERT-treated animals performed better that KO-MOCK-treated animals and performed similarly to the wildtype controls, exhibiting an escape latency (considered indicative of learning) to controls; however, all animals exhibited an apparent ability to learn, and the latency time became indistinguishable after 3 sessions as the efficiency of the search improved with repetition (Figure 23).

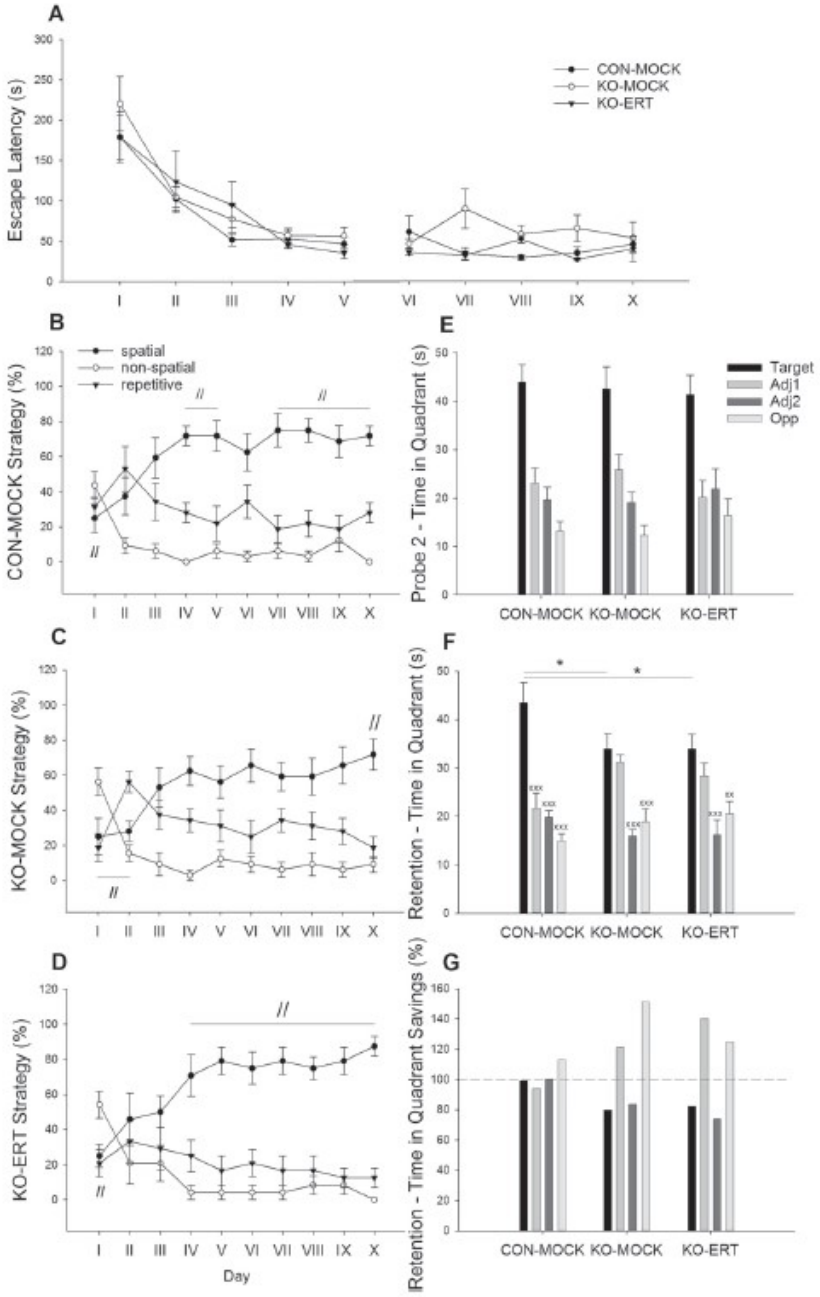
During the first probe trial, animals spent more time in the target quadrant (described as an indicator of spatial accuracy) compared with the other quadrants and there was no difference between any of the groups in either the accuracy or the time to reach the target (escape latency). Subtle differences between the groups in the amount of time spent in the target region were observed in subsequent trials, and the evidently increased accuracy appeared to favor the control mice vs either of the knockout groups (treated or untreated). There were also no apparent differences between the two KO groups (treated or mock-treated) in either the time that they spent in the vicinity of the target or the position that they assumed in relation to the target (opposite or adjacent the target). The authors also evaluated long-term spatial memory by giving a 2-week break between the probe tests. The performance in this component of the assay did not appear to favor the ERT-treated group (Figure 24, Panel G).

Figure 23: Effect of 10 weeks of α-mannosidase treatment on performance in the MWMT



// = preference for the denoted strategy
(Excerpted from the Sponsor's BLA)

Figure 24: Effect of 30 weeks of α-mannosidase treatment on performance in the MWMT



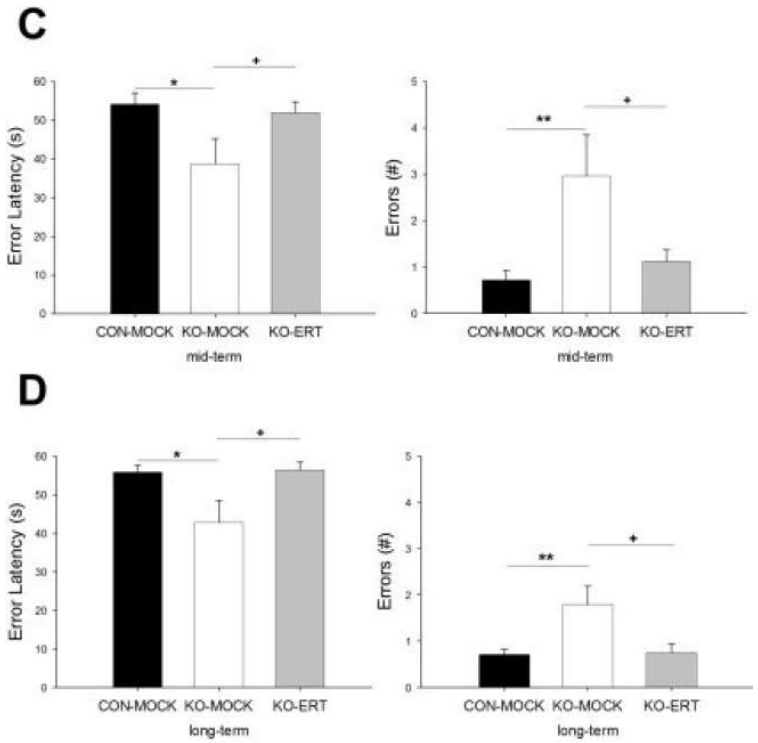
// = preference for the denoted strategy
(Excerpted from the Sponsor's BLA)

For the treadmill test, animals that were treated for 10 or 30 weeks with rhLAMAN were taught to walk on a moving belt for up to 60 seconds or until they made an error. The time between initiation of walking and the first error was denoted the latency period. Animals underwent several habituation exercises during which they were familiarized with the procedures, prior to being tested in the challenge phase. The latency (in

seconds) to an error was the endpoint measured. After both 10 and 30 weeks of treatment, there was a significant effect of treatment on the duration of latency. KO-MOCK animals exhibited shorter latencies compared to CON-MOCK and KO-ERT animals; thus, treatment appeared to improve motor performance in this assay (data not shown).

As shown in Figure 25 Panel C, there was an effect on the error latency; mock-treated animals exhibited more errors, and the latency to first error was shorter in mock-treated animals compared with rhLAMAN-treated animals. Another challenge was performed after long-term treatment. As shown in Figure 25 Panel D, long-term treatment was also associated with an improvement in the number of errors and an improvement in the latency to first error. The two regimens used were different, however. The animals treated for 10 weeks trials were exposed to different combinations of speed, slope, and duration than animals that were treated for 30 weeks.

Figure 25: Effect of rhLAMAN Administration on Performance in the Treadmill Test in Tg+KO Mice



(Excerpted from the Sponsor's BLA)

4.2 Secondary Pharmacology

None

4.3 Safety Pharmacology

No stand-alone safety pharmacology studies were conducted. CNS and respiratory safety pharmacology endpoints were collected in the juvenile toxicity study and cardiovascular safety pharmacology endpoints were collected in the 13-week repeat-dose toxicity study in the cynomolgus monkeys.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

PK/ADME Studies were not conducted

5.2 Toxicokinetics

1.1.4 5.2.1 Pharmacokinetics from rhLAMAN single dose toxicokinetic study in rats ((b) (4) Study #171-011)

The Applicant conducted this single dose toxicokinetic study in rats to assess exposure following IV administration of 5, 15.8, or 50 mg/kg (141, 446, or 1410 U/kg) in four male and four female animals per group. A sparse sampling method was employed, and K_2EDTA-anticoagulated blood was collected pre-dose and at 5, 20, 60, 360, and 1440 minutes post-dose.

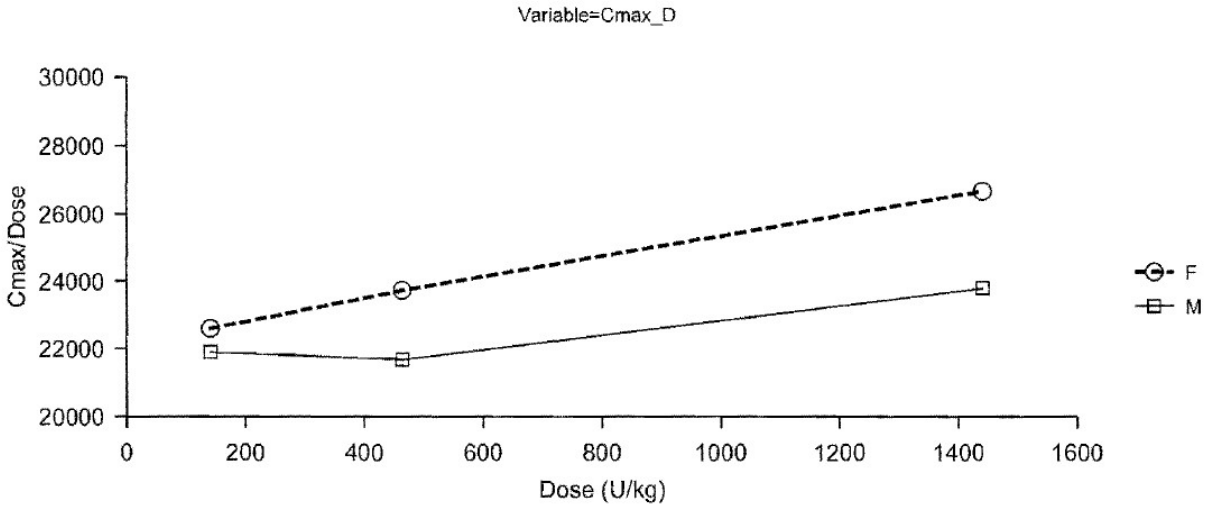
Concentrations of rhLAMAN were quantified in plasma samples using an ELISA-based method. The lower limit of quantitation (LLOQ) of the method was 152 ng/mL. Group mean concentration profiles were evaluated with Win-NonLin using a non-compartmental analysis to estimate the toxicokinetic parameters.

Table 5: Summary of Mean Toxicokinetic Parameters of rhLAMAN in the Rat Following Administration of a Single IV Bolus Dose

Group	Dose (U/kg)_	Sex	C_{max} (ng/mL)	T_{max} (min)	AUC (ng*min/mL)	$T_{1/2}$ (min)	V_z (mL/kg)	CL mL/min/kg)
1	141	F	113000	5	97075742	762	56.6	0.052
		M	109500	20	103112398	953	66.7	0.048
2	464	F	375000	5	298002238	719	56.7	0.055
		M	342500	20	309804071	665	49.0	0.051
3	1441	F	1335000	20	1106705562	719	46.8	0.045
		M	1190000	20	1086515487	684	45.4	0.046

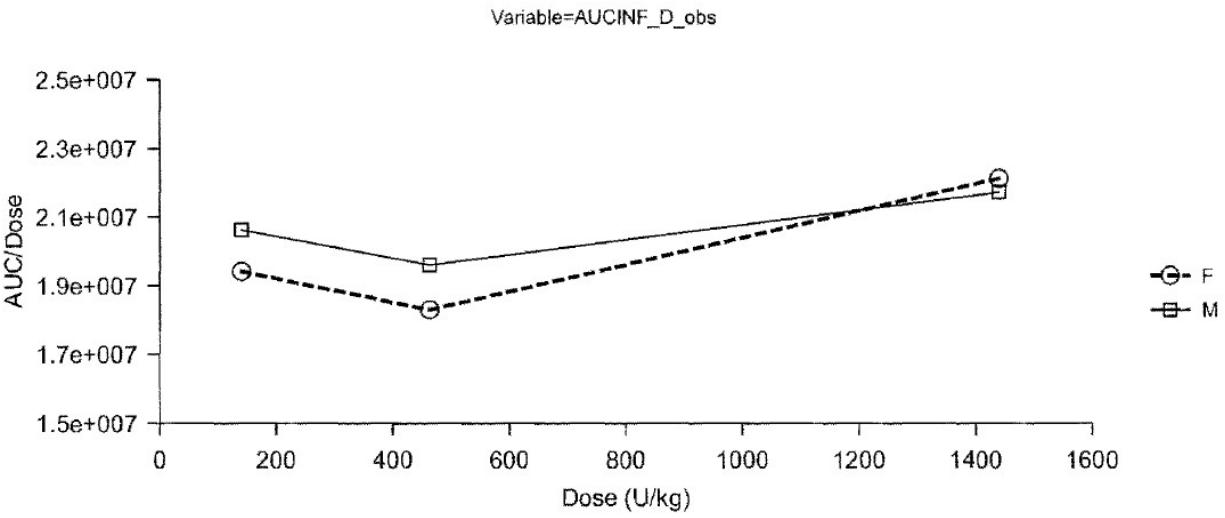
Predose samples from two, Group 2 males were at but not below the limit of quantitation. As shown in Table 5 and Figure 24 through Figure 28, Exposures were generally proportional with dose and there was no significant effect of sex on exposure, as estimated mean exposures were generally within 20% between sexes. Half-life appears to be independent of dose (Figure 28)

Figure 26: Plot of Cmax versus Dose Following IV Administration of rhLAMAN in Male and Female Rats



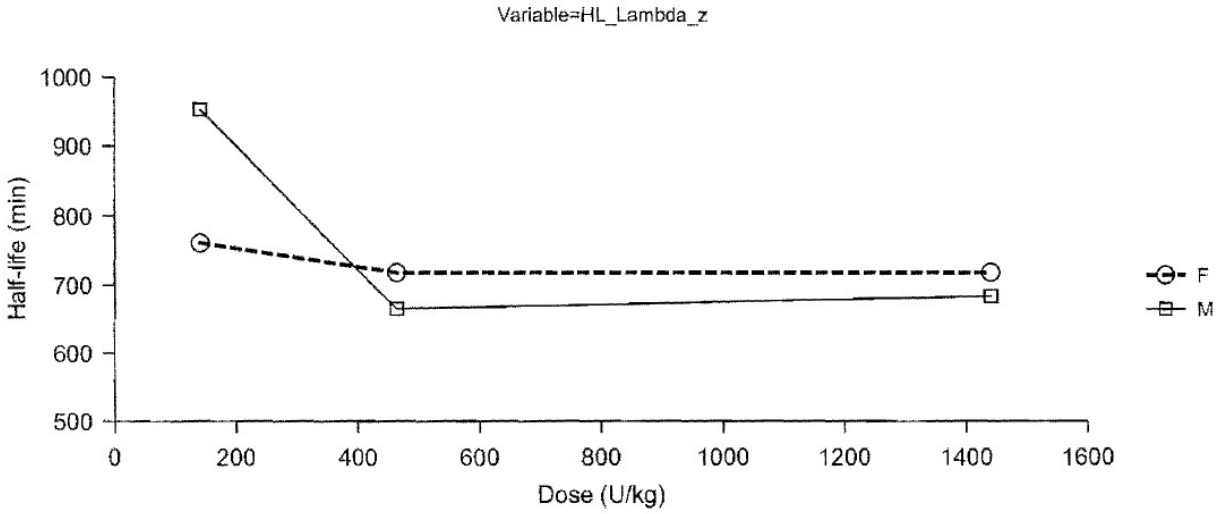
(Excerpted from the Sponsor's BLA)

Figure 27: Plot of AUC versus Dose Following IV Administration of rhLAMAN in Male and Female Rats



(Excerpted from the Sponsor's BLA)

Figure 28: Plot of $T_{1/2}$ versus Dose Following IV Administration of rhLAMAN in Male and Female Rats



(Excerpted from the Sponsor's BLA)

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: rhLAMAN: Single-Dose Intravenous (Slow Bolus) Tolerability Study in the Rabbit

Study no.:	IZUOOO1
Study report location:	4.2.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not stated in the report
GLP compliance:	No
QA statement:	None
Drug, lot #, and % purity:	rhLAMAN, 2M210, Purity not provided

1.1.5 Key Study Findings

This study was a single-dose, single dose-level embryofetal tolerability study in 2 animals. There were no findings noted.

Methods

Doses:	20 mg/kg
Frequency of dosing:	Single Dose
Route of administration:	IV Slow Bolus (2 minutes)
Dose volume:	10 mL/kg
Formulation/Vehicle:	Not formulated – used as supplied
Species/Strain:	*Oryctolagus cuniculus*; New Zealand White (Source: (b) (4))
Number/Sex/Group:	2F
Age:	6m
Weight:	4.25-4.66 kg on day of dosing
Satellite groups:	None
Unique study design:	No clinical pathology, TK, or histopathology was evaluated. Gross necropsy at 24 hours post-dose
Deviation from study protocol:	Protocol not included, no deviations reported

6.2 Repeat-Dose Toxicity+

6.2.1 Study Title: 26-WEEK CHRONIC TOXICITY STUDY OF hrLAMAN BY REPEATED INTRAVENOUS ADMINISTRATION (TWICE WEEKLY) TO TG+/KO MICE

Study no.:	24963
Study report location:	4.2.3.2
Study initiation date:	June 16, 2010
Conducting laboratory and location:	(b) (4)
Duration:	26
Duration Units:	weeks
GLP compliance:	Y
Drug, lot #, and % purity:	2L300, 99.6 (HPLC % Area)
Target Organs	None

Methods

Doses:	0, 144, 433, 1300 U/kg
Frequency of dosing:	Twice Weekly
Number/Sex/Group:	10/Sex; 9/sex for TK (3/sex for control)
Dose volume:	10, 1.1, 3.3, and 10 mL/kg for Groups 1, 2, 3, and 4, respectively
Formulation/Vehicle:	Not described, material was administered as supplied
Route of administration:	INTRAVENOUS
Species:	MOUSE
Strain:	129SVJ/C57B6J
Age / Sexual Maturity:	5-6 Weeks at dosing
Comment on Study Design and Conduct:	• α-mannosidase-deficient with an inactive human α-mannosidase allele (KI/KO) • TK Days 1 and 179 • Hematology and coagulation Weeks 5 and 25 • Urinalysis weeks 4 and 25 • Clinical Chemistry Week 7 and Test Day 184 • Auditory Examinations
Dosing Solution Analysis:	Not performed; materials were dosed as administered and were not formulated

Biomarker[+]

Biomarker Evaluated:	None

Observations and Results

Mortality

There were 3 preterm deaths: Control Animal #19 (female) died on Day 128. Animal #74, a high-dose female died on Day 117, and Animal #80, a high-dose female, died on Day 105. Preterminal clinical signs were only observed for Animal #19. These included reduced mobility, abdominal respiration, a thickened abdomen, and lateral recumbency on Days 127 and 128.

- Gross findings in preterm Animal #19 (a control) included pale liver with rough surface, thickened kidney, enlarged/discolored ovary, stomach with hemorrhagic focus; enlarged femoral lymph nodes; and abdominal cavity filled with red fluid. No cause of death was ascribed to this animal.

- Gross findings in Animal #74 (high dose) included thickened spleen; reddened lungs; and empty stomach and intestinal tract. The cause of death was considered spontaneous renal failure (chronic progressive glomerulopathy)

- Gross findings of Animal #80 (high dose) included discolored lungs; reddened thymus; and empty stomach and intestinal tract. The cause of death was considered spontaneous renal failure (chronic progressive glomerulopathy)

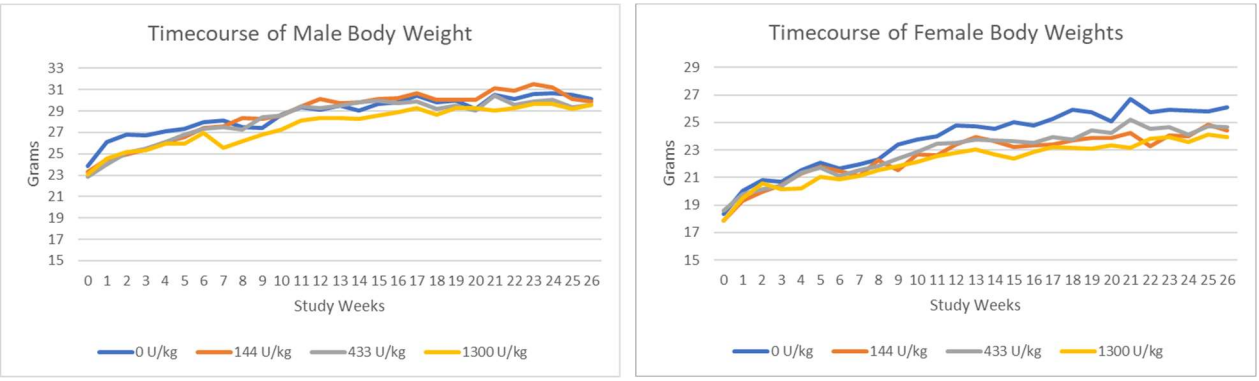
Clinical Signs

- One high-dose male (Animal #61) exhibited hair loss beginning on Day 106
- Eschar formation was observed in two high-dose males (Animals #62 and 68) from Day 172

Body Weights

Unremarkable. There were no statistically-significant differences between treated and untreated animals.

Figure 29: Timecourse of Body Weights in the 26-Week Mouse Toxicology Study



Feed Consumption

Generally unremarkable. A small increase in feed consumption was reported for Group 4 males during Week 7; however, the effect was transient.

Ophthalmoscopy

Unremarkable

ECG

Not performed

Hematology

Generally unremarkable. Statistically significant observations were noted but were considered unrelated to treatment either because they lacked a relationship to dose, were small in magnitude, or because they were the result of an aberrant result in a single animal.

Clinical Chemistry

Generally unremarkable. Statistically significant observations were noted but were considered unrelated to treatment either because they lacked a relationship to dose, or because they were small in magnitude.

Urinalysis

Generally unremarkable. There was an increase in urinary volume in high-dose females during Week 4 and Week 25 but the values were considered to be within the range of normal variability and there was no increase in males.

Gross Pathology

	Male (U/kg)				Female (U/kg)			
Observation	0	144	433	1300	0	144	433	1300
Abdomen, red liquid					1			
Eye, reduced size	1							
Intestines, empty								2
Kidney, thickened					1			
Liver, pale, rough surface					1			
Lungs, indurated, thickened						1		
Lungs, reddened								2
LN, femoral, enlarged					1			
LN, mesenterial thickened			1					
LN, Mandibular, thickened			1					1
Ovary, enlarged					1			
Spleen, partly hemorrhagic	1		1					
Spleen, hemorrhagic focus			1					
Spleen, enlarged			1					
Spleen, thickened						1		1
Stomach, empty								2
Stomach, hemorrhage					1	1		
Tail, eschar				1				
Thymus, reddened								1
Thymus, thickened			1					
Uterus, brown focus								1

Organ Weights

Unremarkable

Histopathology

A full tissue list was examined. In addition, frozen sections of the heart, liver, and one kidney were prepared and stained with scarlet red for demonstration of fat.

Adequate Battery: Yes
Peer Review: No

		Male		Female	
Tissue, observation	**Severity**	**0 U/kg**	**1300 U/kg**	**0 U/kg**	**1300 U/kg**
Adrenal, cortex; hemorrhage	Moderate				1
	Marked			1	
--cortex; hemorrhage	Moderate				
--Pigment-laden macrophages	A few		1		
Bone; metaphysis; fibrosis; focal	Marginal			1	
	Slight			2	
Bone; os femoris; myeloid hyperplasia	Present		1		
Bone; metaphysis; adipose replacement	Present		1	3	3
Brain; cerebrum/midbrain; gliosis	**Marginal**	**3**		**2**	
	Slight	**4**		**5**	
	Moderate	**2**		**1**	
Brain Stem; gliosis	**Marginal**	**4**		**3**	
Cerebellum; demyelination	**Marginal**	**1**		**2**	**3**
	Slight	**6**		**5**	
	Moderate	**3**		**2**	
Brain Stem; Demyelination	**Marginal**	**5**		**6**	
	Slight	**3**		**3**	
Cerebellum; neuronal vacuolation	**Marginal**	**3**		**2**	
	Slight	**4**		**4**	
	Moderate	**1**		**1**	
Cerebrum/midbrain; neuronal vacuolation	**Marginal**	**1**		**2**	
	Slight	**6**		**3**	
	Moderate	**3**		**3**	
Brain Stem; neuronal vacuolation	**Marginal**	**1**		**2**	
	Slight			**1**	
Eye; Cornea; erosion; unilateral; focal	Slight			2	1
Eye; optic nerve inflammation; unilateral	Marked		1		
Harderian glands; granulomatous inflammation; unilateral	Moderate			2	
	Marked	3			
	Severe			1	1

Harderian Glands; chronic inflammation; bilateral	Marked	1		2	
Harderian Gland; aggregate of mononuclear cells; unilateral	One	2		1	
	A few	3	3		1
	Several			1	
Harderian Glands; aggregate of mononuclear cell; bilateral	A few	1	1	1	2
	Several	1	1		
Gallbladder; cholecystitis; acute	Marginal	3	5	1	
	Slight		1	3	
Heart; Scarlet Red; Fatty Infiltration	**Marginal**	**4**	**2**	**5**	**2**
	Slight	**5**	**4**	**2**	**3**
	Moderate				
Injection Site; stroma; acute inflammation; focal	Marginal	1			
	Slight		1		
--Epidermis; acute inflammation	Severe		1		
--Muscle; fibrosis; focal	Slight	1			
--stroma; fibrosis; focal	Slight	1			1
--stroma; fibrosis; diffuse	Moderate		1		
--stroma; hemorrhage	Marginal				1
	Slight				2
	Moderate			1	
	Marked		1	1	
--Stroma; pigment deposition	Marginal				2
	Slight			1	
--ulceration	Severe		2		
--stroma; chronic inflammation; multifocal	Marginal				2
	Slight			1	1
--stroma; chronic inflammation; diffuse	Marginal			1	
	Slight			2	3
	Moderate		1	2	1
Kidney; foci of mononuclear cells	**One**				**1**
--corticomedullary junction; hyaline casts	**Two**			**1**	
	Few	**2**	**1**	**2**	**2**
	Several	**1**	**1**	**3**	**3**
--papilla; mineralization	**Marginal**			**4**	**3**
	Slight				**1**
--chronic progressive glomerulopathy; bilateral	**Moderate**			**1**	
	Marked		**1**		
	Severe				**1**

--cortical tubules; tubule necrosis	**Moderate**			**1**	
--cortex; basophilic tubules	**Few**	**1**	**2**		
--scarlet red; fatty infiltration	**Marginal**	**1**		**6**	**2**
	Slight	**1**	**1**		**3**
	Moderate	**4**	**4**	**4**	**4**
	Marked	**4**	**5**		**1**
Liver; fatty change/microvesicular vacuolation	**Marginal**	**3**	**5**		
	Slight	**3**	**2**	**2**	**2**
	Moderate	**2**		**3**	**5**
--foci of mononuclear cells	**Few**	**1**		**2**	
	Several	**1**		**1**	
--foci; necrosis	**slight**		**1**		
--pigment deposition	**Slight**				**1**
--vacuolated cells	**Few**			**2**	**1**
	Several	**5**		**3**	**1**
	Many			**4**	
--scarlet red; fatty infiltration	**Marginal**				**1**
	Slight			**1**	
	Moderate	**3**	**2**	**4**	
	Marked	**7**	**8**	**5**	**9**
--sinuses; infiltration, lymphocytes; increased	Moderate			1	
--hepatocellular atrophy	Present				1
Lungs; Acute Inflammation	**Marginal**		**2**	**1**	
	Slight	**2**	**2**		**1**
	Moderate		**1**		
--congestion	**Slight**		**3**	**2**	**3**
	Moderate	**3**	**2**	**2**	**2**
	Marked	**1**			**1**
	Severe				**1**
--chronic inflammation	**Slight**			**1**	
	Moderate	**1**			
--pulmonary edema; multifocal	**Slight**	**1**			**1**
LN, deep cervical; pigment deposition	Marginal	3	1	1	1
	Slight	1	1		1
--sinus histiocytosis	Marginal	1	2	2	
	Slight	4	4	4	3
	Moderate		1	1	1
--vacuolated cells	Several			1	
	Moderate		1		

LN, mesenteric; acute inflammation	Marked				
--sinus histiocytosis	Marginal	2	1	1	
	Slight	4	1	1	4
	Moderate		1	1	
--vacuolated cells	Several			1	
Mammary Gland; mammary development	Not Seen			2	1
	Marginal			8	9
Muscle; focus of mononuclear cells	One		1		
Ovary; hemorrhagic cysts	Present			1	
Pancreas; exocrine; focus of mononuclear cells	One	1		1	
Salivary Gland; parotid; aggregates; mononuclear cell	Two			1	
--mandibular; aggregates, mononuclear cell	One	2		2	
	Two	2	1	1	1
	A Few	3	3	1	2
Spinal Cord; demyelination	**Marginal**	**4**		**4**	**3**
	Slight	**4**		**3**	
	Moderate	**1**		**1**	
Spleen; atrophy	Present				1
--congestion	Present				1
--extramedullary hematopoiesis	Moderate			1	
--pigment deposition	Slight				1
	Moderate	1			1
	Marked	1	1		
--red pulp; vacuolation	Marginal			1	
	Slight	3		2	
	Moderate	6		4	
	Marked	1		1	
Stomach; glandular mucosa; dilated crypts	One		1		
Testicle; seminiferous tubules; germinal epithelium; degeneration/necrosis	**Marginal**		**1**		
	Slight		**4**		
	Moderate	**1**	**4**		
	Marked	**7**	**1**		
	Severe	**2**			
Thymus; atrophy	Slight			1	
	Moderate	6	6	6	4
	Marked	4	4	1	4
	Severe				1

--cortex; lymphocytosis	Marginal			3	3
	Slight	1		2	
	Moderate		1		
Parathyroids; cyst; unilateral	Few		1		
--Pigment Deposition	Slight		1		
Uterus; endometrial stromal polyp; benign	Present			1	
Vagina; diestrus	Present			4	4
--metestrus	Present			2	
--mucosa; mucification	Present			1	
--estrus	Present			4	6

Special Evaluation
Auditory examinations performed during Weeks 0 and 26 were unremarkable.

Sample Collection
- TK was collected on Days 1 and 179. Day 1 timepoints were at 5 and 30 minutes, 1, 6, and 24 hours. Day 179 timepoints were predose, 5 and 30 minutes, 1, 6, and 24 hours. 50- μL of blood were collected via the retrobulbar venous plexus under light anesthesia and anticoagulated with K_2EDTA.
- Antibody analysis was collected from the retrobulbar venous plexus at necropsy on Day 184. Samples were processed to serum.

Bioanalysis:
- Samples were analyzed ELISA for determination of rhLAMAN concentration using a plate-capture method. Briefly, plates were coated with a polyclonal rabbit anti-rhLAMAN antibody and incubated with diluted sample. After washing, the analyte was detected with a polyclonal biotin-conjugated antibody and reacted with a streptavidin-HRP colorimetric substrate. Absorbance was detected by spectrophotometric absorption at 450 nm.
- Incurred sample reanalysis was performed and 100% of the samples met the ISR acceptance criteria.

Toxicokinetics
- Control samples were all BLQ (224 ng/mL)
- All pre-dose samples during Week 26 from treated groups had measurable concentrations except for one female each in Groups 2 and 3.
- There were no clear effects of sex on exposure
- Exposure (AUC) appeared to be generally proportional with dose on Day 1 but was dose-proportionality was less evident during Week 26.

Table 6: Summary of Mean rhLAMAN Toxicokinetic Parameters in Male and Female Tg+KO Mice on Day 1 and During Week 26

Dose Group	Dose (U/kg)	Visit	Gender	Rsq	C_{max} (ng/mL)	t_{max} (min)	AUC (min·ng/ml)	$AUC_{\%Extrap}$ (%)	$t_{½}$ (min)	V_z (ml/kg)	CL (ml/min/kg)
2	144	Day 1	F	0.92	90900	5	42934266	16.1	510	92.4	0.126
			M	0.83	94500	30	*52712100*	*20.2*	*550*	*81.2*	*0.102*
		Week 26	F	0.13	57117	30	*57935062*	*59.9*	*1709*	*229.6*	*0.093*
			M	0.33	42090	60	*35018771*	*44.9*	*1023*	*227.3*	*0.154*
3	433	Day 1	F	0.93	354000	5	149367558	14.2	495	77.5	0.109
			M	0.98	423333	5	162605054	13.0	490	70.5	0.100
		Week 26	F	0.77	152433	60	*95698038*	*22.6*	*615*	*150.3*	*0.169*
			M	0.01	267000	30	*419326993*	*79.6*	*4404*	*245.8*	*0.039*
4	1300	Day 1	F	0.90	906667	30	524636702	16.8	516	69.1	0.093
			M	0.93	990000	30	599280411	15.8	507	59.5	0.081
		Week 26	F	1.00	626333	30	520835999	38.1	1088	146.7	0.093
			M	0.83	865333	30	*1013377267*	*65.3*	*2348*	*162.7*	*0.048*

Shadowed figures in italics are less reliable

(Excerpted from the Sponsor's BLA)

Anti-Drug Antibodies

Anti-rhLAMAN Antibodies were detected using an ELISA-based plate-capture method in which plates were coated with rhLAMAN and incubated with serum samples from main study mice. After washing, protein G-conjugated HRP was reacted with the colorimetric substrate, tetramethylbenzidine. Absorbance was detected by spectrophotometric absorption at 450 nm.

As shown in Table 7, ADAs were not detected in control animals but were present in all treated groups. The incidence of ADA-positivity in high-dose animals may be under-estimated by the relatively high amount of residual circulating drug at the end of the study, which may have interfered with the detection of ADA in the assay. It is not known whether the presence of ADA affected estimates of toxicokinetic exposure, or if the ADA inhibited activity of the drug in these animals.

Table 7: Summary of Anti-rhLAMAN Antibodies Detected in Main Study Animals

Group	Sex	No. of animals	No. of samples	No. samples/animals tested positive for anti-rhLAMAN	Range of antibody titres (if applicable)
1	males	10	10	0	-
	females	10	9#	0	-
2	males	10	10	5	179 - 230
	females	10	10	4	241 - 309
3	males	10	10	2	205 - 276
	females	10	10	8	142 - 2671
4	males	10	10	1	903
	females	10	8#	4	233 - 418

#: The female animals nos. 19 (group 1), 74 and 80 (group 4) died prematurely. Therefore, no samples for antibody determination were taken.

(Excerpted from the Sponsor's BLA)

6.2.1 Study Title: rhLAMAN: Intravenous Maximum Tolerated Dose in Cynomolgus Monkeys

Study no.:	(b) (4) 515896 (Report 30553)
Study report location:	4.2.3.2
Study initiation date:	April 20, 2009
Conducting laboratory and location:	(b) (4)
Duration:	8
Duration Units:	weeks
GLP compliance:	N
Drug, lot #, and % purity:	2L12Z, 89.6% (HPLC % Area of 3 main peaks)
Target Organs	None

Methods

Doses:	155, 466, and 1400 U/kg/dose
Frequency of dosing:	Twice Weekly
Number/Sex/Group:	1M, 1F
Dose volume:	1.31, 3.95, and 11.86 mL/kg for the 155, 466, and 1400 U/kg dose levels, respectively
Formulation/Vehicle:	(b) (4)
Route of administration:	INTRAVENOUS
Species:	MONKEY
Strain:	CYNOMOLGUS
Age / Sexual Maturity:	2.5 years upon initiation of dosing
Comment on Study Design and Conduct:	This was a rising dose study. 2 animals (1M and 1F) received 3 weeks of dosing at 155 U/kg, 4 weeks of dosing at 466 U/kg and one week of dosing at 1400 U/kg. Doses were administered twice weekly.
Dosing Solution Analysis:	Not conducted
Biomarker+	None

Observations and Results

Mortality

None

Clinical Signs

Salivation at doses of ≥466 U/kg

Body Weights
Despite the described reduction in feed consumption, there were no effects on body weight over the course of the study.

Feed Consumption
In both animals, a reduction in feed consumption was observed during the study. The magnitude of the effect appeared to be dose-related, as at a dose of 155 U/kg, the effect was transient; however, at 466 U/kg, the effect was about 25-30%. At 1400 U/kg, the first dose showed no reduction in feed consumption but by the second day, animals were inappetent. Animals were euthanized on Day 54 after receiving 3 dose levels (twice-weekly doses for 3 weeks at 155 U/kg, 4 weeks at 466 U/kg and a single dose of 1440 U/kg) due to poor tolerability.

Ophthalmoscopy
Not evaluated

ECG
Unremarkable

Hematology
Increased neutrophils, WBCs, and lymphocytes were increased compared with baseline measurements in the male Days 39 and 50 (at 466 and 1400 U/kg respectively); however, given the limited number of animals, the relationship to treatment is unclear.

Clinical Chemistry
Unremarkable

Coagulation
Unremarkable

Urinalysis
It is unclear if urine was collected or analyzed.

Gross Pathology
Unremarkable

Organ Weights
Data were not reported

Histopathology

Adequate Battery: Yes, for this limited study

There were no histopathological findings of note. Both animals had Balantidiasis in the cecum and/or colon.

Peer Review: No

Special Evaluation
None
Toxicokinetics
TK and ADA samples were collected but data were not reported.

6.2.1 Study Title: Feeding Study in Cynomolgus Monkeys

Study no.:	PWM0001
Study report location:	4.2.3.2
Study initiation date:	November 10, 2009
Conducting laboratory and location:	(b) (4)
Duration:	4
Duration Units:	weeks
GLP compliance:	N
Drug, lot #, and % purity:	2L200, Purity by HPLC (% Area of 3 peaks): 91.5%

Methods

Doses:	1310 U/kg
Frequency of dosing:	Twice Weekly
Number/Sex/Group:	3F
Dose volume:	10 mL/kg
Formulation/Vehicle:	Not stated; the report states that the item was preformulated
Route of administration:	INTRAVENOUS
Species:	MONKEY
Strain:	CYNOMOLGUS
Age / Sexual Maturity:	Approximately 2-4 Years
Comment on Study Design and Conduct:	Nonterminal; the purpose was to assesses effects on feeding and tolerability endpoints
Dosing Solution Analysis:	Not conducted; samples were sent to the Sponsor for possible analysis. No analytical results were reported.

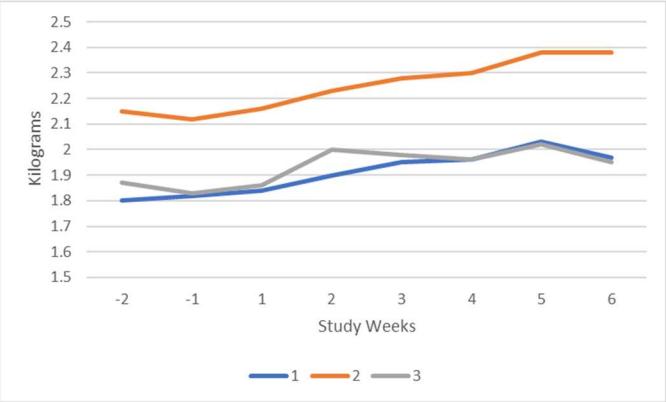
Biomarker+	None

Observations and Results
Mortality
None

Clinical Signs
None

Body Weights
Unremarkable

Figure 30: Timecourse of Body Weights in Three Female Monkeys



Feed Consumption
There was no apparent effect of treatment on feed consumption. Compared with baseline values, animals reportedly consumed as much or more feed offered during the study that they consumed for the 3-week pre-dose period over which feed consumption was monitored.

Ophthalmoscopy
Not evaluated

ECG
Not evaluated

Hematology
Generally unremarkable at Week 6 when compared to baseline values

Clinical Chemistry
Generally unremarkable at Week 6 when compared to baseline values

Urinalysis
Generally unremarkable. At the end of the study, animals showed urinary leukocytosis that was not present pre-dose; however, urinary erythrocytes were not elevated compared with pre-dose values.

Gross Pathology
Not Applicable – the study was nonterminal.

Organ Weights
Not Applicable – the study was nonterminal.

Histopathology
Not Applicable – the study was nonterminal.

Special Evaluation
N/A
Toxicokinetics
Not conducted

6.2.1 Study Title: 13 Week Intravenous Toxicity Study with rhLAMAN in Cynomolgus Monkeys

Study no.:	PWM0002
Study report location:	4.2.3.2
Study initiation date:	February 12, 2010
Conducting laboratory and location:	(b) (4)
Duration:	13
Duration Units:	weeks
GLP compliance:	Y
Drug, lot #, and % purity:	2L200, 99.7% by HPLC (%Area for 3 peaks)
Target Organs	None

Methods

Doses:	0, 131, 414, and 1310 U/kg
Frequency of dosing:	Twice weekly
Number/Sex/Group:	4/Sex/Group
Dose volume:	1.02, 3.21, and 10.16 mL/kg
Formulation/Vehicle:	Placebo: (b) (4) and Water for injection
Route of administration:	INTRAVENOUS
Species:	MONKEY
Strain:	CYNOMOLGUS
Age / Sexual Maturity:	2-4 years
Comment on Study Design and Conduct:	None Not conducted; the material was used as supplied
Dosing Solution Analysis:	Met pre-specified acceptance criteria

Biomarker

Biomarker Evaluated:	None

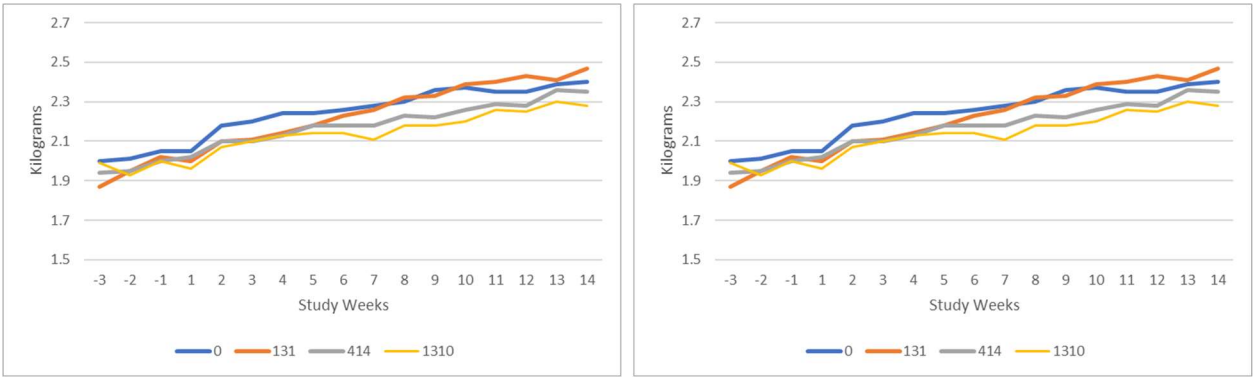
Observations and Results

Mortality
None

Clinical Signs
Generally unremarkable. There was one low-dose male and one high-dose female with what was described as reddish urine or reddish-brown urine. The effect was transient but in the male, was recurrent. It is not possible to determine if these observations coincided with dose-administration because they did not provide information about the days on which the doses were administered.

Body Weights
Generally unremarkable. In males, there was a small decrease in mean body weight compared with concurrent controls, but the effect was not statistically significant.

Figure 31: Timecourse of Mean Body Weights in Males (L) and Females (R)



Feed Consumption
Generally unremarkable. Intermittently reduced feed consumption was observed in mid- and high-dose males. Intermittently increased feed consumption was observed in high-dose females.

Ophthalmoscopy
Unremarkable

ECG
Unremarkable

Hematology
Unremarkable

Coagulation
Unremarkable

Clinical Chemistry
Unremarkable

Urinalysis
Unremarkable

Gross Pathology
Black discoloration was observed in several lymph nodes across females of all treatment groups. Based on incidence, the findings do not appear to be treatment-related. All other gross findings also appear to be incidental.

Organ Weights
There was an increase in the absolute mean paired kidney weight in high dose females, and an increase in the absolute spleen weights of low-dose females. No similar changes were noted in males and there was no effect on the relative kidney and spleen weights. The report does not state whether relative organ weights were normalized to body or brain weights.

Histopathology
Adequate Battery: Yes
Peer Review: No
A complete list of histopathological findings observed in this study is provided below. Nematodes and parasites/protozoal infection were present in a number of tissues from multiple animals. The pathologist concluded that the primary findings were those observed at the injection sites and that a number of other findings, including pigment deposition, were secondary to the phlebotomy and/or catheterization that the animals underwent during dosing and other study-related procedures. All other findings were considered incidental.

		Male				Female			
	Grade	0	131	414	1310	0	131	414	1310
	1				1	2			
Adrenal; mineralization	2								1
Bone Marrow, Femur; lymphoid infiltration	1								1
Brain; mononuclear infiltration	1		1			1		1	
Cecum; protozoa	3	1		2	1	2	1	2	
--Granuloma, submucosa	3	1	2	4	2	2	2	1	2
	2								1
Colon; granuloma, submucosa	3	1		2	1	1			1
Eye with Optic Nerve; nerve fiber degeneration	1			1					
Heart; mononuclear infiltration	1			1			1		
Infusion site; medial degeneration	2	1				1			
	1			1	1	1		1	2
--inflammation; chronic	2	1							
	1			1	1		2		
--inflammation; acute	2	1			1				
--fibrosis; adventitial	1	1		1	1				1

	2	2	2	1	2		2		2
	3					1			1
	1	1					1	1	1
--hemorrhage; perivascular	2			1	1		1		
	1								1
--thrombus	2					1			
--pigment; perivascular	1		1		2	1	1		1
Kidney; mononuclear infiltration	1	1			1			1	1
Larynx; inflammation, acute	1	1						1	
Liver; mononuclear infiltration	1		2		2	1	2		1
--increased pigment	1		1		2			1	
	1						1		
--fibrosis; capsule	2					1			
--trematode; bile duct	P		1	1					
--vacuolation; hepatocellular	1					1			
	1				1				
Lung; fibrosis; pleura	2				1				
--increased pigment	1							1	
--inflammation; chronic	1		1		1		1	1	
--Giant cell	1		1						
	1							1	1
LN, Axillary; pigment, macrophages	2					1	1		
--hyperplasia, lymphoid	2							1	1
	1							1	1
LN, Inguinal; pigment, macrophages	2					1	1		
--hyperplasia, lymphoid	2							1	1
	1	1		2	1	1	1	1	
LN, Mandibular; lymphoid hyperplasia	2	3	4	2	3	3	3	3	4
--plasmacytosis	2		1						
--erythrophagocytosis	1		1	1			1		
	1					1			
--extramedullary hematopoiesis	2							1	
	1	2	3	2	3	4	2	1	3
LN, Mesenteric; lymphoid hyperplasia	2		1	2	1		2	3	1
	1	3	3	2	1	1	4	3	2
LN, Tracheobronchial; pigment	2	1	1	2	3	3		1	2
	1		2	4	2	3	2	4	3
--lymphoid hyperplasia	2		1				1		
Salivary Gland, Mandibular; concretions, duct	1			2		1	1		
salivary gland; parotid; concretions, duct	1		1	1	1		1	1	

--atrophy; acinar cell	1			1			1		
Salivary Gland; Submandibular; mononuclear infiltration	2	1		1		2	1		1
Sciatic nerve; mononuclear infiltration	1		1	1		1			
--nerve fiber degeneration	1		1						
Skeletal Muscle; protozoa	P	1	2		1		2	1	
--mononuclear infiltration	1		2				2	1	
--myofiber degermation	1		1				2		
	1	3	3	3		2	2	2	4
Spleen; lymphoid hyperplasia	2		1	1	2	1	1	2	
--increased pigment	1	1	1	2	3	2	1	1	2
--fibrosis; capsule	1		1						
	1	2	1	1	1	4	1	3	1
Stomach; lymphoid follicles	2	1	2	3	2		2	1	2
--nematode	P			1					
Thyroid Gland; mononuclear infiltration	1	2		1		1	1		1
--Ectopic thymus	P	2	1	1	3	1	1	3	1
--cyst	P		1	1	1				
Tongue; protozoa	P	1					1		1
--mononuclear infiltration	2							1	
Urinary Bladder; mononuclear infiltration	1	2	3	1	2		2	1	1

Special Evaluation
None

Bioanalytical Sample Collection:

- Samples were collected on Days 1, 25, and 88 predose, at 5 and 30 minutes, and at 2, 6, 24, and 48 hours after the end of infusion.
- Samples were transferred to tubes containing K_2EDTA and processed for separation of plasma.

Bioanalysis:

- rhLAMAN was measured using a plate-capture ELISA method in which samples were incubated with a plate pre-treated with a polyclonal anti-product antibody.
- Detection of bound analyte was accomplished a polyclonal biotin-conjugated antibody followed by addition of HRP-streptavidin produced an enzymatic color reaction that was read spectrophotometrically at 450 nm.
- The method was found to be reproducible, as 94.1% (64/68) of the incurred samples met the acceptance criteria
- Concentrations in control samples were all below the limit of quantitation (BLQ; <486 μg/L
- Concentrations in pre-dose animals were generally BLQ; however, in 3 animals (14, 15, and 31), detectable levels were observed pre-dose during Week 13.

Toxicokinetics:

- There was no effect of sex on exposure; thus TK parameters were calculated for both sexes combined.
- Exposure was generally proportional with dose on Day 1.
- Evidence of an impact of ADA on exposure was observed in all treated groups by Week 4.

Table 8: Summary of Mean Toxicokinetic Parameters in Cynomolgus Monkeys Treated with rhLAMAN Twice Weekly for 13-Weeks

Dose Group	Dose (U/kg)	Visit		C_{max} (ng/mL)	t_{max} (min)	AUC(0-last) (min·ng/ml)	AUC (min·ng/ml)	$t_{½}$ (min)	V_z (ml/kg)	CL (ml/min/kg)
2	131	Day 1	N	8	8	8	8	8	8	8
			Mean	98288	65	66892325	73170950	767	78.6	0.074
			SD	13659	0	14853707	19011204	162	12.7	0.022
		Week 4	N	8	8	8	7	7	7	7
			Mean	56950	65	7373181	8765165	77	57.0	1.007
			SD	21757	0	7347139	7861178	83	14.2	0.615
		Week 13	N	8	8	8	8	8	8	8
			Mean	79538	65	14662081	15598028	123	52.0	0.523
			SD	23786	0	10248923	10474652	99	10.4	0.434
3	414	Day 1	N	8	8	8	8	8	8	8
			Mean	335875	65	254923750	279364568	832	70.1	0.060
			SD	47136	0	47090210	54013814	152	11.5	0.014
		Week 4	N	8	8	8	7	7	7	7
			Mean	168415	109	29013679	33114725	67	65.6	0.894
			SD	102523	126	36371029	37490644	48	40.0	0.612
		Week 13	N	8	8	8	8	8	8	8
			Mean	325500	65	88872375	99725814	184	42.3	0.197
			SD	44388	0	53243143	54279965	105	9.4	0.100
4	1310	Day 1	N	8	8	8	8	8	8	8
			Mean	1095375	65	840574313	983687436	891	64.5	0.060
			SD	128249	0	300744246	293737517	306	14.0	0.033
		Week 4	N	8	8	8	7	7	7	7
			Mean	168415	109	29013679	33114725	67	65.6	0.894
			SD	102523	126	36371029	37490644	48	40.0	0.612
		Week 13	N	8	8	8	8	8	8	8
			Mean	1196000	68	597182657	685901257	410	39.1	0.090
			SD	197298	9	357719906	464844258	415	14.0	0.035

(Excerpted from the Sponsor's BLA)

Anti-Drug Antibodies

- Anti-drug antibody samples were collected from all animals pre-study and on Days 25 and 8. Samples were processed for separation of serum.
- Anti-Drug antibody results were not included in this report. These Data were reported separately under Report 2010-P-106 in Module 4.2.3.7.
- Serum samples were incubated with rhLAMAN-coated plates and detection of bound antibody was accomplished using protein-G- conjugated to HRP. TMB was added for colorimetric detection of the HRP, which was quantified spectrophotometrically at 450 nm.
- Of the 96 samples screened, 79 samples gave absorbance values above the assay cut-point; thus, by these criteria, all treated animals were positive for anti-drug antibody formation during Weeks 4 and 13.
- The report states that the reproducibility of the titer values was 2.4%

7 Genetic Toxicology

Velmanase alpha-tycv is a biologic that is not expected to interact with DNA or other chromosomal material; therefore, the standard genotoxicity studies are not considered appropriate and were not performed.

8 Carcinogenicity

Standard bioassays were not conducted with velmanase alpha-tycv; however, according to the Catalogue of Somatic Mutations in Cancer, dysregulation (mutations and/or overexpression) of MAN2B1 has been observed in numerous tumors, including tumors of the adrenal, breast, CNS, cervix, endometrium, hematopoietic/lymphoid systems, kidney, intestinal tract, liver, lung, esophagus, pancreas, parathyroid, prostate, skin, stomach, thyroid, and urinary tract. Overexpression of MAN2B1 has also been determined to be a prognostic biomarker in some tumors (Lin, et al., 2022).

One high-dose (30 mg/kg) female in the rat pre- and postnatal development study developed a malignant histiocytoma of the ovary. According to (b) (4), the supplier of rats for that study, the incidence of this tumor in In 4-6 month old rats (the presumed age of rats in this study) was zero. The incidence in all control female Wistar rats was 1 in 4,962. The incidence in all Wistar rats (control + treated) was 2 in 13,550; therefore, this is a rare tumor and given that it occurred in a high-dose dam, a relationship to treatment cannot be excluded. A post-marketing commitment for the Applicant to evaluate the tumorigenic potential of exogenous rhLAMAN administration will be requested.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development+

9.1.1 Study Title: rhLAMAN: Intravenous (Slow Bolus) Fertility and Early Embryonic Development Study in the Rat

Study no.:	IZU0006
Study report location:	4.2.3.5.1
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	2O200, 99.5% (HPLC % area for 3 peaks)

Methods

Doses:	0, 3.3, 10, or 30 mg/kg
Frequency of dosing:	Twice weekly
Number/Sex/Group:	20/Sex/Group
Dose volume:	10, 1.1, 3.3, or 10 mL/kg
Formulation/Vehicle:	(b) (4) [redacted]
Route of administration:	INTRAVENOUS
Species:	Rattus norvegicus
Strain:	Crl:WI(Han) rats
Comment on Study Design and Conduct:	• Males were dosed twice weekly throughout the study; females were dosed twice weekly during pre-pairing (2 weeks) and during the pairing period, and on GDs 1 and 6 • Daily vaginal lavage was performed 10 days before pairing • Pairing was performed for 10 days or until evidence of mating • If mating did not occur, the sire was replaced for up to 3 days with a male that had successfully mated with another dam. • Mated dams were individually housed • Mated dams were killed on GD 13; Males were killed 2-weeks post completion of mating • Gross necropsy and limited tissue collection was performed on main study animals; histopathology was performed on control and high-dose animals • Bioanalytical sample collection and toxicokinetic analysis were not conducted
Dosing Solution Analysis:	Not conducted; material was administered as supplied.

Observations and Results

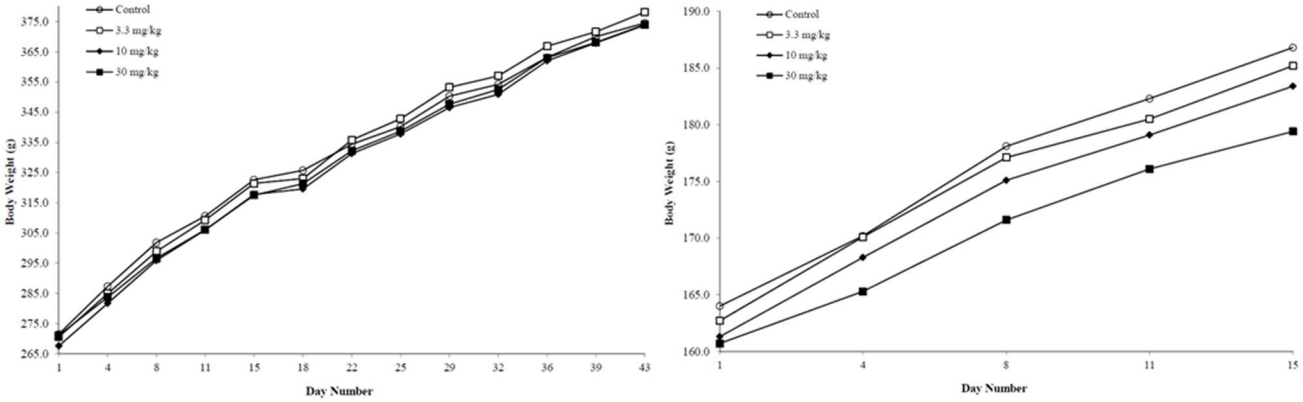
Mortality

Two females at the 30 mg/kg dose level were euthanized prematurely due to adverse clinical signs including convulsions (Female 160 before the 2nd dose), decreased activity, recumbency, labored breathing, abnormal gait, and body cool-to-touch (Female 151, after the 5th dose).

Clinical Signs
Dose-related clinical signs included: swollen muzzles (all high-dose males and 17/20 high-dose females); piloerection (3F at 10 mg/kg and 1F at 30 mg/kg)

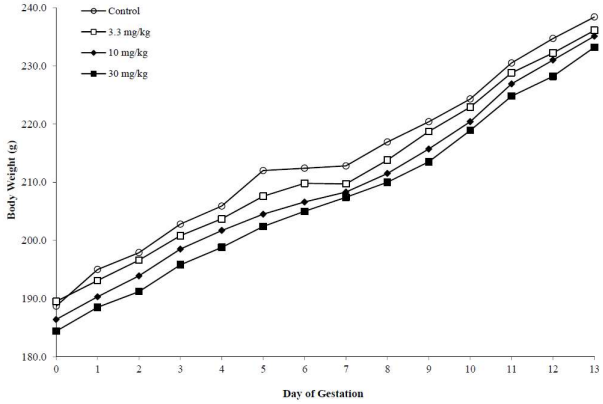
Body Weight
There was no effect of treatment on mean body weight in males, but there was a decrease of rhLAMAN treatment on mean body weights in females at the high dose level.

Figure 32: Timecourse of Male (L) and Female (R) Body Weights



(Compiled from the Applicant's BLA)

Figure 33:Timecourse of Female Gestational Body Weights



(Excerpted from the Applicant's BLA)

Feed Consumption

- Feed consumption in males was lower during Week 1 but was lower but generally comparable to controls thereafter.
- Feed consumption in females was lower in mid-and high-dose groups during pre-mating and slightly and intermittently reduced during gestation.

Fertility and Pregnancy Parameters

- There was no effect on cycle length or numbers of cycles, and no effect on the length of the pre-mating period between treated and control groups.

- There was no effect of treatment on the number of fertile females; the number of live embryos; the number of dams with resorptions; the number of fertile males or females; or the copulation index for males.

Table 9: Summary of Mean Pregnancy Data

Females	0 mg/kg	3.3 mg/kg	10 mg/kg	30 mg/kg
Group Size	20	20	20	20
Preterm Decedents	0	0	0	2
Not Pregnant	0	1	0	2
Not Pregnant (%)	0	5	0	10
Not Pregnant at Termination	0	1	0	1
Pregnant	20	19	20	18
Pregnant (%)	100	95	100	90
Pregnant Died/Killed/Aborted	0	0	0	1
Pregnant with Total Resorption	0	0	0	1
Number with Live Embryos	20	19	20	17

Table 10: Summary of Mean Uterine and Implantation Data

		0 mg/kg	3.3 mg/kg	10 mg/kg	30 mg/kg
		20	19	20	18
Preterm Decedent Dams	Sum	0	0	0	2
Number of Corpora Lutea	Sum	256	242	261	224
	Mean	12.8	12.7	13.1	12.4
	SD	1.6	1.7	1.2	1.6
Number of Implantations	Sum	232	227	241	214
	Mean	11.6	11.9	12.1	11.9
	SD	2.2	1.6	1.8	2.0
% Pre-Implantation Loss	Mean	8.9	5.9	7.3	4.7
Number of Early Deaths	Sum	16	5	5	12
	Mean	0.8	0.3	0.3	0.7
	SD	1.2	0.5	0.6	1.5
Number of Dead Embryos	Sum	0	0	0	0
	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
Number of Live Embryos	Sum	213	222	236	202
	Mean	10.7	11.7	11.8	11.2
	SD	2.0	1.6	1.7	3.2
% Post-Implantation Loss	Mean	7.8	2.2	1.9	8.4
Mean % of Implantations	Mean	92.2	97.8	98.1	91.6

Necropsy

There were no effects of treatment on mean body weight or the weights of the ovaries. There were no treatment-related gross necropsy findings.

Histopathological findings with an apparent relationship to treatment or for which a relationship to treatment cannot be excluded, were noted in the kidneys, stomach, and pancreas.

Table 11: Summary of Histopathological Findings in Females

	Severity	0 mg/kg	30 mg/kg
Kidneys; basophilic cortical tubules	Minimal	0	3
--pelvic distension	Slight	0	1
Pancreas; acinar degeneration/inflammation	Minimal	0	1
Stomach; distended mucosal glands	Minimal	0	1

Toxicokinetics

Toxicokinetic sampling was not performed; however, samples were collected for characterization of anti-drug antibody formation. Of the 159 samples collected, 103 samples were positive in Groups 2-4. Insufficient volume was available to titer the majority of positive samples.

9.2 Embryonic and Fetal Development+

9.2.1 Study Title: rhLAMAN: Intravenous Preliminary Embryo-Foetal Development Study in the Rat

Study no.:	TZU0003
Study report location:	4.2.3.5
Conducting laboratory and location:	(b) (4)
GLP compliance:	No
Drug, lot #, and % purity:	0OB40, Purity not stated, and a CoA was not provided

Methods

Doses:	0, 20 mg/kg/dose
Frequency of dosing:	Daily
Number/Sex/Group:	7 Females/Group
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4)
Route of administration:	INTRAVENOUS
Species:	*Rattus norvegicus*
Strain:	Crl: WI (Han)
Comment on Study Design and Conduct:	• Doses were administered daily from GDs 6-17 • Animals were euthanized on GD 20 for gross maternal examination, weights of the placentae and gravid uteri, and evaluation of fetal parameters (external evaluation, sex and weight of fetuses)
Dosing Solution Analysis:	Not Conducted

Observations and Results

F_0 Dams

Mortality

None

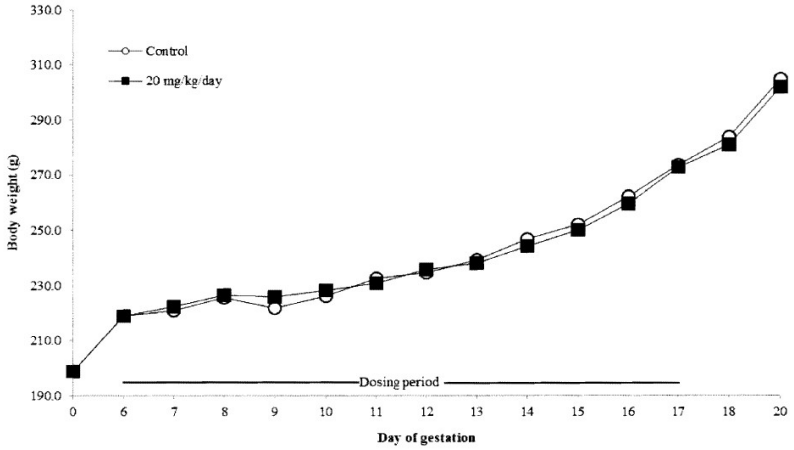
Clinical Signs

- Swollen muzzles in 6/7 females dosed with the 20 mg/kg dose of rhLAMAN on Day 1. There were no recurrences or other clinical signs for the remainder of the dosing period.
- One control female convulsed on Days 10 and 12. The female was euthanized on Day 12. Because the animal was in the control group, the effect is considered unrelated to rhLAMAN. There were also convulsions in high-dose animals in the FEED study in treated animals. Given the apparent anaphylactoid response (swollen muzzles), it was assumed to be related to hypersensitivity; however, its assignment to the control group suggests that there may be a component of the vehicle (e.g., glycine) involved.

Body Weight

Unremarkable

Figure 34: Timecourse of Mean Bodyweights in Pregnant Rats



(Excerpted from the Applicant's BLA)

Feed Consumption
Unremarkable

Cesarean Section Data
One control dam was euthanized due to convulsions; one high-dose dam was found to not be pregnant. All other dams were found to have live fetuses at the terminal timepoint.

Measurement	Control	20 mg/kg
Number of surviving pregnant animals	6	6
Number of females not pregnant	0	1
Number of females with implantations	6	6
Number of corpora lutea	70	75
Mean number of corpora lutea per dam	11.4	12.5
Standard deviation	2.0	1.0
Number of implantations	63	70
Mean number of implantations per dam	10.5	11.7
Standard deviation	1.2	1.4
Mean % Pre-Implantation Loss	9.2	6.8
Number of early embryo/fetal deaths	1	4
Number of late embryo/fetal deaths	0	0
Number of dead fetuses	0	0
Mean % post-implantation loss	1.9	5.8
Number of live fetuses	62	66
Mean number per female	10.3	11.0
Standard deviation	1.5	1.4
Mean % of implantations	98.1	94.2

Necropsy/ Histopathology
Unremarkable

Toxicokinetics
Not conducted

F_1 Offspring
Terminal Observations

Measurement	Control	20 mg/kg
Number of dams with live fetuses	6	6
Number of live fetuses	62	66
Mean number of live fetuses per dam	10.3	11.0
Standard deviation	1.5	1.4
Number of male fetuses	34	30
Number of female fetuses	28	36
Mean % male fetuses	54	45.2
Mean litter weight	37.6	39.8
Standard deviation	7.1	5.0
Mean fetal weight	3.62	3.63
Standard deviation	0.19	0.38
Mean fetal weight – males only	3.74	3.77
Standard deviation	0.19	0.46
Mean fetal weight – females only	3.51	3.53
Standard deviation	0.20	0.28
Mean placental weight	0.50	0.48
Standard deviation	0.04	0.05
Mean gravid uterus weight	56.5	58.7
Standard deviation	9.8	7.1

Fetal Malformations/ Variations (external, visceral, skeletal)
There were no malformations noted

9.2.1 Study Title: rhLAMAN: Intravenous (Slow Bolus) Preliminary Embryo-Foetal Development Study in the Rabbit with Toxicokinetic Sampling

Study no.: IZU0002
Study report location: 4.2.3.5
Conducting laboratory and location: (b) (4)
GLP compliance: No
Drug, lot #, and % purity: 0OB40, Purity not stated, and a CoA was not provided

Methods

Doses:	0, 20 mg/kg
Frequency of dosing:	Daily
Number/Sex/Group:	5 females/group
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4)
Route of administration:	INTRAVENOUS
Species:	*Oryctolagus cuniculus*
Strain:	NZ White Rabbit
Comment on Study Design and Conduct:	• Doses were administered daily from GDs 6-18 • Animals were euthanized on GD 28 for gross maternal examination, weights of the placentae and gravid uteri, and evaluation of fetal parameters (external evaluation, sex and weight of fetuses)
Dosing Solution Analysis:	Not conducted

Observations and Results

F_0 Dams

Mortality

One dam in the 20 mg/kg dose group experienced seizures on Day 13 and was euthanized. From Day 9, this dam had observations of discolored (red) ears, suggesting hypersensitivity. There was no cause of death ascribed to this animal; however, the report states that the effect was likely not due to toxicity. Given that this is a human protein and that rabbits are known to mount strong antibody responses when challenged with foreign proteins, it is likely that this death was the result of an immune response.

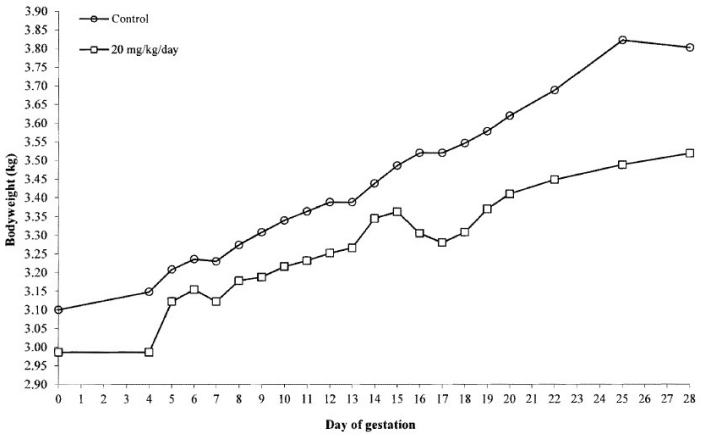
Clinical Signs

- Occasional observations of small/reduced feces were observed in treated and control animals.
- Discolored ears (see Mortality)

Body Weight

There was a reduction in mean body weights in treated dams compared with controls. The reduction in body weight did not reverse after cessation of dosing on PND18

Figure 35: Timecourse of Mean Body Weights in Pregnant Rabbits



(Excerpted from the Applicant's BLA)

Feed Consumption

Treated dams generally consumed less food than control dams; however, feed consumption was highly variable in both groups, so no statistically significant effects of treatment were observed.

Cesarean Section Data

All dams that survived to scheduled termination were pregnant at the time of termination.

Measurement	Control	20 mg/kg
Number of surviving pregnant animals	5	4
Number of females not pregnant	0	0
Number of females with implantations	5	4
Number of corpora lutea	48	41
Mean number of corpora lutea per dam	9.6	10.3
Standard deviation	0.9	2.5
Number of implantations	48	35
Mean number of implantations per dam	9.6	8.8
Standard deviation	0.9	0.5
Mean % Pre-Implantation Loss	0	11.7
Number of early embryo/fetal deaths	1	1
Number of late embryo/fetal deaths	0	0
Number of dead fetuses	0	0
Mean % post-implantation loss	2.0	3.1
Number of live fetuses	47	34
Mean number per female	7.4	8.5
Standard deviation	0.9	1.0
Mean % of implantations	98.0	96.9

Necropsy/ Histopathology
Unremarkable

Toxicokinetics
Blood for toxicokinetic analysis was collected on Days 6 and 16 at the following timepoints: pre-dose, 5 and 30 minutes, and at 1, 3, and 24 hours post-dose.

Antibody samples were also collected on Day 28 at necropsy.

Neither toxicokinetic data nor antibody data were included in the report.

F_1 Offspring

Terminal Observations

Measurement	Control	20 mg/kg
Number of dams with live fetuses	5	4
Number of live fetuses	47	34
Mean number of live fetuses per dam	9.4	8.5
Standard deviation	0.9	1.0
Number of male fetuses	23	13
Number of female fetuses	24	21
Mean % male fetuses	49.9	39.3
Mean litter weight	325.7	283.8
Standard deviation	25.8	36.0
Mean fetal weight	34.7	33.6
Standard deviation	1.6	3.9
Mean fetal weight – males only	35.7	34.3
Standard deviation	1.8	5.4
Mean fetal weight – females only	33.8	32.9
Standard deviation	1.6	2.8
Mean placental weight	3.61	3.52
Standard deviation	0.36	0.45
Mean gravid uterus weight	486.8	420.0
Standard deviation	37.8	45.4

Fetal Malformations/ Variations (external examination)
There were no malformations observed.

9.2.1 Study Title: rhLAMAN: Intravenous (Slow Bolus) Embryo-Foetal Development Study in the Rat with Toxicokinetic Sampling

Study no.:	IZU0004
Study report location:	4.2.3.5
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	2O200; 99.5% (HPLC % area, sum of 3 peaks)

Methods

Doses:	0, 3.3, 10, or 20 mg/kg/day
Frequency of dosing:	Daily
Number/Sex/Group:	20 presumed pregnant females/group
Dose volume:	3 mL/kg
Formulation/Vehicle:	(b) (4)
Route of administration:	INTRAVENOUS
Species:	Rattus norvegicus
Strain:	Crl:WI(Han) rat
Comment on Study Design and Conduct:	• Animals were dosed between GD 6-17 and euthanized on GD 20 • TK samples were collected on Days 6 and 17 in main study animals • Terminal antibody samples were taken • Terminal examinations included confirmation of pregnancy, number of corpora lutea, distribution of implantations from each uterine horn, assessment of resorption (early/late), assessment of fetal viability (alive/dead) and fetal external, visceral, and skeletal fetal examinations. Weights of placentae and gravid uteri were also taken.
Dosing Solution Analysis:	Not evaluated; the material was pre-formulated and administered as received and was therefore characterized by its labeling.

Conclusions:

- Clinical signs included swollen mouths beginning on GD8

- Minor reduction in feed consumption and mean maternal weights, particularly at the high dose level
- No effects on pregnancy endpoints (number or precent pregnant, number of corpora lutea, etc.), and no effects on fetal survival (fetal deaths, pre- or post-implantation losses)
- Several major malformations that occurred in the study. All occurred in fetuses of high-dose dams; therefore, the role of rhLAMAN in induction of these malformations cannot be excluded.
- An increase in the incidence of numerous minor malformations and variations. The role of rhLAMAN in induction of these malformations and variations also cannot be excluded due to the lack of the facility's historical control data for these endpoints. A lack of a dose-response for these observations is not adequate to preclude a relationship to treatment, as pharmacology may be saturated at all dose levels.
- Malformations at exposures that were approximately 7-fold those recommended in patients at the 1 mg/kg dose level.

Observations and Results

F_0 Dams

Mortality

None

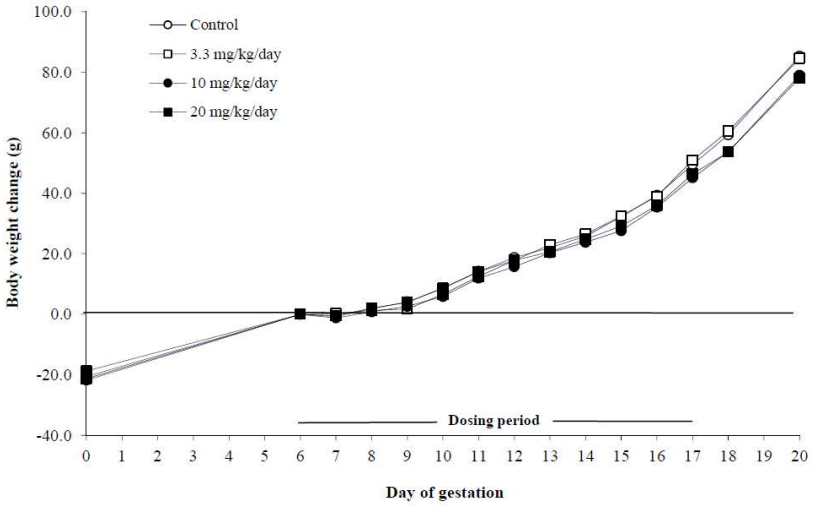
Clinical Signs

All high-dose dams had swollen mouths on GDs 6 and 7. On GD8, three dams had swollen mouths. On GD13, one dam had labored breathing with piloerection, and on GD 14, one dam had piloerection. All findings were in high-dose dams. Other clinical observations included hair loss (limbs and ventral surface in control dams), and perinasal hair staining (in Group 3 dams). One group 3 dam also had hair loss on the forelimbs. Group 4 dams also had perinasal fur staining beginning on GD18/19.

Body Weight

Generally unremarkable. On Day 20, mean body weights were significantly lower than control in high-dose dams, but prior to that day, the differences were not statistically significant. There was no difference between the groups in mean body weight when adjust for the weights of the gravid uteri.

Figure 36: Timecourse of Mean Body Weights in Pregnant Rats



(Excerpted from the Applicant's BLA)

Feed Consumption

Mean feed consumption was intermittently lower in high-dose dams (i.e., statistically significant between Days 12-15 and overall, between GDs 6-18)

Cesarean Section Data

- There were no treatment-related effects on pregnancy and caesarean section parameters. All parameters fell within the range of historical controls.

Table 12: Summary of Pregnancy Data in Rats

Measurement	0	3.3	10	20
Group Size	20	20	20	20
Not Pregnant	0	0	0	0
Not Pregnant (%)	0.0	0.0	0.0	0.0
Not Pregnant Died/Killed	0	0	0	0
Not Pregnant Scheduled Termination	0	0	0	0
Pregnant	20	20	20	20
Pregnant (%)	100.0	100.0	100.0	100.0
Pregnant Did/Killed/Aborted	0	0	0	0
Pregnant with Total Resorptions	0	0	0	0
Number with Live Fetuses	20	20	20	20

Table 13: Summary of Uterine and Implantation Data in Rats

Measurement		0	3.3	10	20
Number of Dams with Fetuses		20	20	20	20
Number of corpora lutea	Sum	261	243	243	250

	Mean	13.1	12.2	12.2	12.5
	SD	1.8	1.6	1.5	1.8
Number of implantations	Sum	233	232	227	223
	Mean	11.7	11.6	11.4	11.2
	SD	1.4	1.5	2.1	1.5
Mean % Pre-Implantation Loss	Mean	9.4	4.2	6.3	9.2
Number of early embryo/fetal deaths	Sum	6	10	15	16
	Mean	0.3	0.5	0.8	0.8
	SD	0.5	0.8	0.8	1.3
Number of later Intrauterine Deaths	Sum	0	0	0	1
	Mean	0.0	0.0	0.0	0.1
	SD	0.0	0.0	0.0	0.2
Number of Dead Fetuses	Sum	0	0	0	0
	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
Number of Live Fetuses	Sum	227	222	212	206
	Mean	11.4	11.1	10.6	10.3
	SD	1.7	1.9	2.5	23
% Post-Implantation Loss	Mean	2.8	4.6	8.1	8.3
Mean % of Implantations	Mean	97.2	95.4	91.9	91.7

Necropsy/ Histopathology
Unremarkable

Toxicokinetics

- Peak (Cmax) and overall (AUC) exposures increased in a generally dose-related fashion on GD 6.
- All control samples were BLQ (<152 ng/mL) at all timepoints evaluated.
- There was a decrease in measures of exposure (C_{max} and/or AUC) and a decrease in half-life with increasing duration of dosing.
- In one or more low- and mid-dose animals, concentrations were BLQ at by 6 hours post-dose on GD 17, suggesting an effect of immunogenicity on clearance.
- Exposure was reduced relative to GD6 but measurable in animals treated on GD17 in the mid- and high-dose levels but was BLQ in pre-dose samples on GD17 from all animals.

Table 14: Summary of Mean Toxicokinetic Parameters in Pregnant Rats

Dose (mg/kg/Day	Gestation Day	C_{max} (ng/mL)	$T_{1/2}$ (Hr)	AUC_{0-24} (ng*hr/mL)
3.3	6	79100	8.6	446000
	17	42500	--	67800
10	6	720000	9.0	1350000
	17	144000	1.7	230000
20	6	467000	8.9	2630000
	17	431000	1.8	996000

Immunogenicity

- All samples from control animals were negative for anti-drug antibody.
- Most animals in treated groups were ADA positive on Day 17; however, they were not able to titer the samples due to limited sample volume.

Table 15: Summary of Immunogenicity Data in Pregnant Rats

Dose (mg/kg/Day)	Serum Positive	Serum Negative	Total Samples
3.3	18	2	20
10	18	2	20
20	18	2	20

F_1 Offspring

Terminal Observations

There were no effects on the litter size, viability, or body weights of the fetuses in dams treated with rhLAMAN at any dose level.

Table 16: Summary of Fetal Litter Weight Data in Rats

		Dose (mg/kg/Day)			
Endpoint	**Measure**	**0**	**3.3**	**10**	**20**
Number with Live Fetuses		20	20	20	20
Number of Live Fetuses	Sum	227	222	212	206
Number of Male Fetuses	Sum	111	113	97	102
Number of Female Fetuses	Sum	116	109	115	104
% Male Fetuses	Mean	47.8	51.3	47.6	49.0
Litter Weight (grams)	Mean	42.33	41.61	39.88	37.92
Fetal Weight (M+F; grams)	Mean	3.73	3.77	3.83	3.65
Fetal Weight (M; grams)	Mean	3.85	3.84	3.95	3.77
Fetal Weight (F; grams)	Mean	3.63	3.67	3.66	3.58
Placental Weight (grams)	Mean	0.53	0.49	0.51	0.54

Fetal Malformations/ Variations (external, visceral, skeletal)

As shown in Table 17, several major malformations were observed in fetuses of high-dose dams. There was also a statistically significant increase in the number of litters with major malformations in high dose dams vs control dams.

Table 17:Fetal Examination Summary (Rat)

	Dose (mg/kg/Day)			
Observation*	**0**	**3.3**	**10**	**20**
Total number of litters examined	20	20	20	20
Total number of fetuses examined	227	222	212	206
Number with major abnormalities	0	0	0	4
Mean % of fetuses examined	0	0	0	3.1
Number of litters affected	0	0	0	3*

Number with minor abnormalities	43	43	41	44
Mean % of fetuses examined	18.6	20.3	20.8	23.0
Number of litters affected	18	18	17	17
Number with variations	133	135	121	126
Mean % of fetuses examined	58.3	60.9	56.8	61.9
Number of litters affected	20	20	20	20

*Significant at $p < 0.05$

A summary of all variations and malformations, including variations that were significantly elevated in treated groups, is provided in Table 18. This table lists all variations and malformations for which the incidence exceeded that of historical controls. The data are presented as incidence and group mean percentage (in parentheses) for each finding. The items listed in italics are major malformations that exceed facility historical controls (1664 fetuses from 318 litters covering the period between January 2009 and December 2014).

Table 18: Summary of Potentially Treatment-Related Fetal Malformations and Variations in Rats

		Dose (mg/kg/Day)			
Observation*	**Type**	**0**	**3.3**	**10**	**20**
***Oral Cavity**; cleft palate*	*Major*	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*	*1 (1.3)*
Forelimb; Forepaw- uni- or bilateral abnormal flexure	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)
Abdominal Cavity; ureter uni- or bilateral; dilated	Variant	1 (0.8)	0 (0.0)	0 (0.0)	2 (1.7)
Oral Cavity; palate; irregular ridging	Minor	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.8)
Abdominal Cavity; kidney- uni- or bilateral: increased pelvic cavitation	Variant	4 (3.8)	7 (6.0)	2 (1.7)	5 (4.8)
Abdominal Cavity; ureter- uni- or bilateral: dilated	Variant	2 (2.0)	3 (2.7)	1 (0.8)	5 (5.2)
Abdominal Cavity; umbilical artery: left sided	Variant	17 (14.3)	16 (15.4)	13 (11.7)	17 (18.2)
Abdominal Cavity; abdomen: hemorrhage	Minor	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)
Abdominal Cavity; liver- one or more lobe: accessory lobe present	Minor	1 (0.8)	4 (3.3)	0 (0.0)	0 (0.0)
Skull; nasal- uni- or bilateral: incomplete ossification	Minor	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.1)
Skull; frontal- uni- or bilateral: incomplete ossification	Minor	4 (3.3)	4 (3.8)	8 (7.4)	8 (8.0)
Skull; parietal- uni- or bilateral: incomplete ossification	Variant	40 (35.0)	33 (31.7)	29 (29.9)	37 (36.8)
Skull; interparietal: incomplete ossification	Variant	42 (36.6)	41 (38.1)	38 (38.5)	40 (38.8)

		Dose (mg/kg/Day)			
Observation*	**Type**	**0**	**3.3**	**10**	**20**
Skull; occipital: incomplete ossification	Variant	21 (17.8)	27 (25.4)	18 (16.1)	27 (29.3)
Skull; zygomatic arch- uni- or bilateral: incomplete ossification Number of litters affected	Minor	3 (3.5)	2 (2.5)	4 (3.2)	9 (8.8)
Skull; squamosal- uni- or bilateral: incomplete ossification	Minor	15 (12.9)	16 (16.0)	9 (7.7)	19 (19.8)
Skull; zygomatic arch and maxilla uni- or bilateral: partial fusion	Minor	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)
Skull; hyoid: not ossified	Minor	3 (2.5)	3 (2.8)	3 (2.5)	2 (3.5)
Skull; hyoid: incomplete ossification	Variant	4 (4.2)	4 (3.8)	2 (1.7)	5 (4.5)
***Skull**; palatine- uni- or bilateral: cleft*	*Major*	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*	*1 (2.5)*
Cervical Vertebra; one or more centra (1-3): ossified	Variant	58 (50.4)	70 (61.5)	60 (52.5)	57 (53.6)
Cervical Vertebra; one or more centra (4-7): ossified	Variant	109 (95.8)	110 (97.2)	104 (96.7)	102 (99.0)
Cervical Vertebra; one or more neural arch: incomplete ossification	Minor	0 (0.0)	0 (0.0)	2 (1.7)	1 (1.0)
Thoracic vertebra; one or more centra: bipartite ossification	Minor	2 (1.5)	2 (1.7)	1 (0.7)	0 (0.0)
Thoracic vertebra; one or more centra: asymmetrically ossified	Minor	1 (1.0)	0 (0.0)	2 (1.8)	1 (1.0)
Lumbar vertebra; one or more neural arch: incomplete ossification	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
Sacral vertebra; one or more neural arch: incomplete ossification	Minor	0 (0.0)	3 (2.8)	5 (4.4)	2 (1.8)
Caudal Vertebra; number of centra: <=2 ossified	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
Caudal Vertebra; number of neural arches: 0 ossified	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
Rib; rib- uni- or bilateral: cervical	Minor	8 (6.8)	13 (11.8)	5 (8.7)	4 (3.3)
Rib; one or more: wavy	Minor	9 (8.5)	14 (12.8)	18 (20.8)	20 (22.1)
Rib; one or more: incomplete ossification	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
Rib; 14th- uni- or bilateral: vestigial	Variant	44 (38.7)	41 (37.5)	45 (39.6)	43 (43.2)
***Pectoral girdle**; scapula- uni- or bilateral: bent severe*	*Major*	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*	*2 (2.5)*
***Sternum**; one or more sternebra: duplicated*	*Major*	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*	*1 (1.0)*

		Dose (mg/kg/Day)			
Observation*	**Type**	**0**	**3.3**	**10**	**20**
Sternum; 2nd sternebra: incomplete ossification	Minor	0 (0.0)	0 (0.0)	0 (0.0)	3 (4.5)
Sternum; 3rd sternebra: incomplete ossification	Minor	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)
Sternum; 4th sternebra: incomplete ossification	Minor	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)
Sternum; 5th sternebra: not ossified	Variant	2 (1.5)	1 (0.8)	2 (1.7)	5 (5.4)
Sternum; 5th sternebra: incomplete Ossification	Variant	9 (8.1)	12 (9.9)	10 (8.6)	9 (10.2)
Sternum; 6th sternebra: not ossified	Variant	0 (0.0)	1 (0.8)	1 (1.0)	0 (0.0)
Sternum; 6th sternebra: incomplete ossification	Variant	10 (9.0)	7 (5.8)	9 (8.2)	8 (9.3)
Pelvic Girdle; entire: asymmetric insertion; slight	Minor	1 (0.7)	0 (0.0)	1 (0.8)	8 (9.3)
Forelimb; one or more digit: phalanges ossified	Variant	26 (22.6)	32 (28.0)	37 (33.2)	32 (30.4)
Forelimb; one or more metacarpal 1st-4th: incomplete ossification	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
Forelimb; 5th metacarpal- uni- or bilateral: not ossified	Variant	9 (8.0)	14 (12.0)	8 (7.0)	14 (16.4)

*For each observation, the table lists the number of affected fetuses and the group mean percent in parentheses by group.

9.2.1 Study Title: rhLAMAN: Intravenous (Slow Bolus) Embryo-Foetal Development Study in the Rabbit with Toxicokinetic Sampling

Study no.: IZU0005
Study report location: 4.2.3.5
Conducting laboratory and location: (b) (4)
GLP compliance: Yes
Drug, lot #, and % purity: Drug: rhLAMAN; Lot: 2O200; Purity: 99.5% (HPLC % Area of 3 peaks)

Methods

Doses:	0, 3.3, 10, or 30 mg/kg/Day
Frequency of dosing:	Daily
Number/Sex/Group:	22 presumed pregnant females/group
Dose volume:	1.1, 3.3, 10 mL/kg
Formulation/Vehicle:	(b) (4) [redacted]
Route of administration:	INTRAVENOUS
Species:	Oryctolagus cuniculus
Strain:	NZ White Rabbit
Comment on Study Design and Conduct:	• Animals were dosed between GDs 6-18 and euthanized on G 28. • TK was taken from 3 main study dams/Group on GDs 6 and 16 • Terminal examinations included confirmation of pregnancy, number of corpora lutea, distribution of implantations from each uterine horn, assessment of resorption (early/late), assessment of fetal viability (alive/dead) and fetal external, visceral, and skeletal fetal examinations. Weights of placentae and gravid uteri were also taken.
Dosing Solution Analysis:	Not evaluated; the material was pre-formulated and administered as received and was therefore characterized by its labeling.

Observations and Results

F_0 Dams

Mortality

There were two preterm deaths.

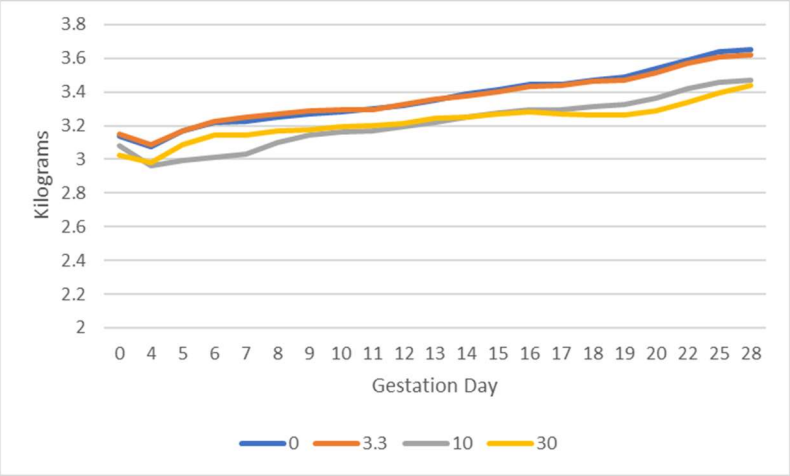
- Control female was killed on GD 21 due to declining condition and evidence of abortion (red material on the vulva and vaginal prolapse). Total litter loss was confirmed. This death was not considered treatment-related
- Female 90 in the high-dose group began to abort (stained bedding) and was euthanized on GD 23. The report stated that this death was considered this unrelated to treatment.

Clinical Signs

Clinical signs in treated animals included: Reduced/absent/loose/liquid feces; hair loss; anormal color, pinna; abnormal color, urine (red).

Body Weight
Compared with controls, body weights were significantly lower for mid- and high-dose dams at the end of the study. There was no significant difference in gravid uterine weights across the dose range.

Figure 37: Timecourse of Maternal Bodyweights in Rabbits



Feed Consumption
There was a decrease in mean feed consumption of the mid- and high-dose groups from GDs 12-24. The decrease was intermittently significant in mid-dose animals but generally consistent in high-dose animals. There was also an overall decrease (from GDs 6-18) in daily mean and total mean feed consumption in mid- and high-dose dams.

Cesarean Section Data

Table 19: Summary of Pregnancy Data in Rabbits

Measurement	0	3.3	10	30
Group Size	22	22	22	22
Not Pregnant	1	1	0	0
Not Pregnant (%)	4.5	4.5	0	0
Not Pregnant Died/Killed	0	0	0	0
Not Pregnant Scheduled Termination	1	1	0	0
Pregnant	21	21	22	22
Pregnant (%)	99.5	99.5	100	100
Pregnant Did/Killed/Aborted	1			
Pregnant with Total Resorptions	20	0	0	0
Number with Live Fetuses	20	21	22	21

Table 20: Summary of Uterine and Implantation Data in Rabbits

Measurement		0	3.3	10	30
Number of Dams with Fetuses		20	21	22	21
Number of corpora lutea	Sum	201	204	213	202
	Mean	10.1	9.7	9.7	9.6
	SD	2.3	1.6	1.5	1.8
Number of implantations	Sum	179	287	194	187
	Mean	9.0	8.9	8.8	8.9
	SD	2.1	1.9	1.6	2.0
Mean % Pre-Implantation Loss	Mean	9.9	8.2	8.6	7.3
Number of early embryo/fetal deaths	Sum	13	6	4	9
	Mean	0.7	0.3	0.2	0.4
	SD	1.2	0.6	0.4	0.9
Number of later Intrauterine Deaths	Sum	1	0	1	4
	Mean	0.1	0	0	02
	SD	0.2	0	0.2	0.4
Number of Dead Fetuses	Sum	0	0	0	0
	Mean	0	0	0	0
	SD	0	0	0	0
Number of Live Fetuses	Sum	165	181	189	174
	Mean	8.3	8.6	8.6	8.3
	SD	2.1	2.2	1.7	1.7
% Post-Implantation Loss	Mean	7.2	4.0	2.6	6.2
Mean % of Implantations	Mean	92.8	93.0	97.4	93.8

Necropsy/ Histopathology
Maternal necropsy observations were unremarkable.

Toxicokinetics
All samples from control animals were BLQ (<242 ng/mL) at all timepoints, as were all pre-dose samples on Day 6. All low-dose samples on Day 16 were BLQ at all timepoints. Most samples from animals in the mid-dose (10 mg/kg) level were BLQ after 1-hour post-dose on Day 16. Exposure in high-dose (30 mg/kg) animals was maintained in 2/3 animals at 6 hours post-dose on Day 16 but were BLQ by 24 hours post-dose. Rapid loss of exposure was suggestive of a strong immunogenicity response in all dose groups.

Summary of Mean Toxicokinetic Parameters in Pregnant Rabbits

Dose (mg/kg/Day	Gestation Day	C_{max} (ng/mL)	$T_{1/2}$ (Hr)	AUC_{0-24} (ng*hr/mL)
3.3	6	122000	--	635000
	16	--	--	--
10	6	273000	--	1950000

	16	6090	--	3500
30	6	815000	8.8	6430000
	16	320000	1.2	666000

-- = not evaluable

Immunogeincity

All animals were evaluated for an immunogenic response to the test article. All treated animals were ADA positive and had generated a strong antibody response to the drug by the end of the study. 15/21 control samples were also ADA positive; however, all control samples exhibited low titers.

Table 21: Summary of Immunogenicity Data in Pregnant Rabbits

Dose (mg/kg)	Serum Positive	Serum Negative	Total Samples
0	15	6	21
3.3	22	0	22
10	22	0	22
30	21	0	21

F_1 Offspring

Terminal Observations

There were no effects on the litter size, viability. There was a statistically significant decrease in fetal litter weight (combined M+F) and in placental weight.

Table 22: Summary of Fetal Litter Weight in Rabbits

	Dose (mg/kg/Day)			
Observation	**0**	**3.3**	**10**	**30**
Group Size	20	21	22	21
Number with Life Fetuses	20	21	22	21
Number of Live Fetuses	165	181	189	174
Number of Male Fetuses	80	98	104	78
Number of Female Fetuses	85	83	85	96
% Male Fetuses	47.5	54.9	55.6	45.7
Litter Weight (g)	285.66	295.2	294.96	265.20
Fetal Weight (M+F) (g)	35.14	34.66	34.48	32.59*
Fetal Weight (M) (g)	35.37	34.83	35.09	33.55
Fetal Weight (F) (g)	34.84	34.43	33.42	31.86
Placental Weight (g)	3.60	3.57	3.32	3.21*

** = $p < 0.05$*

Fetal Malformations/ Variations (external, visceral, skeletal)

Table 23:Fetal Examination Summary (Rabbit)

	Dose (mg/kg/Day)			
Observation	**0**	**3.3**	**10**	**30**
Total number of litters examined	20	21	22	21
Total number of fetuses examined	165	181	189	174
Number with major abnormalities	5	6	1	4
Mean % of fetuses examined	3.2	3.1	0.5	2.4
Number of litters affected	4	3	1	3
Number with minor abnormalities	74	97	79	93
Mean % of fetuses examined	43.6	56.0	41.5	52.6
Number of litters affected	20	201	21	20
Number with variations	165	181	189	172
Mean % of fetuses examined	100.0	100.0	100.0	98.3
Number of litters affected	20	21	22	21

There were several variations that were significantly ($p < 0.05$) increased in high-dose fetuses compared with controls. A summary of all variations and malformations, including variations that were significantly elevated in treated groups, is provided in Table 24. This table lists all variations and malformations for which the incidence exceeded that of historical controls. The data are presented as incidence and group mean percentage for each finding.

Table 24: Summary of Potentially Treatment-Related Fetal Malformations and Variations in Rabbits

		Dose (mg/kg/Day)			
Observation	**Type**	**0**	**3.3**	**10**	**30**
Tail; entire: stump only	**Major**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Head; eye uni- or bilateral: periorbital hemorrhage	**Minor**	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Thoracic Cavity; common carotid artery- left: arising from innominate artery	**Variant**	15 (9.9)	17 (12.6)	18 (9.8)	21 (12.7)
Thoracic Cavity: aortic arch: enlarged slight	**Minor**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Thoracic Cavity; pulmonary arch: enlarged slight	**Minor**	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Thoracic Cavity; intraventricular septum: incomplete	**Major**	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
Thoracic Cavity; lung- one or more lobe: reduced in size severe	**Major**	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Abdominal Cavity; kidney- uni- or bilateral: increased pelvic cavitation	**Minor**	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Abdominal Cavity; kidney- uni- or bilateral: absent	**Major**	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Abdominal Cavity; ureter- uni- or bilateral: absent	**Major**	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)

		Dose (mg/kg/Day)			
Observation	**Type**	**0**	**3.3**	**10**	**30**
Abdominal Cavity; liver- one or more lobe: diaphragmatic hernia	**Major**	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Abdominal Cavity; gall bladder: reduced in size	**Minor**	1 (0.7)	2 (0.9)	3 (1.8)	1 (0.7)
Abdominal Cavity; gall bladder: bilobed	**Minor**	0 (0.0)	2 (1.3)	0 (0.0)	0 (0.0)
Brain; one or more lobe: hydrocephaly	**Major**	0 (0.0)	1 (1.6)	0 (0.0)	0 (0.0)
Brain; olfactory lobe: single lobe	**Major**	0 (0.0)	1 (1.6)	0 (0.0)	0 (0.0)
Brain; cerebellum: cystic dilatation	**Major**	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
Brain; sub-arachnoid space: enlarged slight	**Minor**	1 (1.7)	3 (2.9)	1 (1.1)	1 (1.2)
Oral Cavity; Palate: Irregular ridging	**Minor**	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
Skull; premaxilla- uni- or bilateral: incomplete ossification	**Minor**	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Skull; fontanelle- anterior: increased in size	**Minor**	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Skull; one or more: fissure/plaque of bone integral to normal structure of bone	**Variant**	1 (1.3)	2 (2.0)	3 (3.8)	0 (0.0)
Skull; parietal- uni- or bilateral: incomplete ossification	**Minor**	0 (0.0)	2 (2.1)	0 (0.0)	1 (1.0)
Skull; interparietal: incomplete ossification	**Minor**	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)
Skull; maxilla- uni- or bilateral: incomplete ossification	**Minor**	4 (5.2)	5 (9.2)	11 (10.1)	4 (4.9)
Skull; hyoid: incomplete ossification	**Variant**	20 (24.2)	34 (37.3)	32 (32.7)	38 (43.9)
Skull; hyoid: cornua bent	**Minor**	0 (0.0)	3 (4.8)	2 (2.7)	3 (3.5)
Vertebra; number of presacral vertebra: 27	**Minor**	5 (3.6)	10 (5.0)	9 (4.9)	13 (7.2)
Vertebra; number of presacral vertebra: 25	**Minor**	0 (0.0)	1 (0.6)	2 (0.9)	1 (0.8)
Cervical Vertebra; one or more centra: not ossified	**Minor**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Cervical Vertebra; one or more centra: incomplete ossification	**Minor**	1 (0.8)	3 (1.5)	0 (0.0)	1 (0.5)
Cervical Vertebra; one or more centra: asymmetrically ossified	**Minor**	1 (0.7)	0 (0.0)	0 (0.0)	2 (1.3)
Cervical Vertebra; one or more centra: hemicentric	**Minor**	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.8)
Cervical Vertebra; one or more neural arch: fused severe	**Minor**	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.8)
Cervical Vertebra; one or more neural arch: malformed	**Major**	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.8)

		Dose (mg/kg/Day)			
Observation	**Type**	**0**	**3.3**	**10**	**30**
Cervical Vertebra; one or more neural arch: absent	**Major**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
Cervical Vertebra; additional ossification centre: present	**Minor**	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Thoracic Vertebra; number of vertebra: 13	**Variant**	62 (37.9)	47 (25.3)	86 (45.3)	66 (37.1)*
Thoracic Vertebra; one or more centra: bilobed ossification	**Minor**	1 (0.6)	2 (0.9)	0 (0.0)	2 (1.0)
Thoracic Vertebra; one or more centra: absent	**Major**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Thoracic Vertebra; one or more centra: asymmetrically ossified	**Minor**	0 (0.0)	3 (1.3)	0 (0.0)	1 (0.5)
Thoracic Vertebra; one or more centra: fused severe	**Major**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Thoracic Vertebra; one or more neural arch: malformed	**Major**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Thoracic Vertebra; one or more neural arch: misaligned	**Minor**	1 (0.7)	3 (1.5)	0 (0.0)	2 (1.3)
Thoracic Vertebra; additional neural arch- right: present	**Major**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Thoracic Vertebra; additional ossification centre: present	**Minor**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Lumbar Vertebra; number of vertebra: 6	**Variant**	57 (34.3)	39 (21.3)	79 (41.2)	55 (31.4)
Lumbar Vertebra; number of vertebra: 8	**Minor**	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.7)
Sacral Vertebra; one or more centra: fused severe	**Major**	0 (0.0)	2 (0.9)	0 (0.0)	0 (0.0)
Sacral Vertebra; one or more neural arch: misshapen	**Minor**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Sacral Vertebra; one or more neural arch: misaligned	**Minor**	1 (1.3)	1 (0.4)	3 (1.7)	1 (0.5)
Sacral Vertebra; additional ossification centre: present	**Minor**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Caudal Vertebra; one or more: fused	**Major**	0 (0.0)	2 (0.9)	0 (0.0)	0 (0.0)
Caudal Vertebra; number of centra: <=14	**Variant**	5 (3.7)	13 (7.1)	15 (7.8)	15 (8.5)
Caudal Vertebra; one or more centra: misshapen	**Minor**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Caudal Vertebra; one or more centra: fused severe	**Major**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Caudal Vertebra; one or more centra: offset	**Minor**	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
Caudal Vertebra; one or more neural arch- uni- or bilateral: bifid	**Major**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Rib; rib- uni- or bilateral: cervical	**Minor**	2 (2.0)	5 (2.7)	0 (0.0)	2 (1.6)
Rib; one or more: fused severe	**Major**	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)

		Dose (mg/kg/Day)			
Observation	**Type**	**0**	**3.3**	**10**	**30**
Rib; one or more: absent	**Major**	1 (0.7)	0 (0.0)	0 (0.0)	2 (1.3)
Rib; one or more: vestigial	**Major**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Rib; one or more: arising from same neural arch	**Major**	1 (0.7)	2 (1.0)	0 (0.0)	0 (0.0)
Rib; one or more: fused slight	**Minor**	1 (0.7)	2 (1.0)	0 (0.0)	0 (0.0)
Rib; 13th- uni- or bilateral: vestigial	**Variant**	18 (11.1)	17 (10.1)	21 (12.5)	20 (11.8)
Rib; 13th- uni- or bilateral: extra	**Variant**	62 (37.9)	47 (25.3)	86 (45.3)	66 (37.1)*
Rib; 13th- uni- or bilateral: floating	**Variant**	3 (1.7)	11 (6.2)	14 (8.0)	5 (3.2)
Rib; one or more: discontinuous	**Minor**	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)
Rib; additional rib- one or more: ossified	**Minor**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Sternum; 1st sternebra: incomplete ossification	**Minor**	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)
Sternum; one or more sternebra: mis-shapen or misaligned	**Minor**	2 (1.2)	5 (2.4)	1 (0.5)	4 (2.4)
Sternum; 2nd sternebra: incomplete ossification	**Minor**	0 (0.0)	2 (1.2)	1 (0.6)	0 (0.0)
Sternum; one or more: bilobed or bipartite ossification (sternebra 1-4)	**Minor**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Sternum; one or more sternebra: fused slight	**Minor**	1 (0.6)	4 (2.4)	0 (0.0)	0 (0.0)
Sternum; 5th sternebra: incomplete ossification	**Variant**	48 (30.1)	39 (24.3)	55 (29.4)	39 (22.2)
Sternum; 6th sternebra: not ossified	**Variant**	19 (12.7)	28 (14.7)	13 (6.9)	20 (10.3)
Sternum; 6th sternebra: incomplete ossification	**Variant**	31 (19.8)	22 (11.8)	39 (19.3)	29 (15.5)
Pelvic Girdle; entire: asymmetric insertion slight	**Minor**	3 (1.3)	4 (1.7)	2 (2.7)	4 (2.0)
Pelvic Girdle; pubis- uni- or bilateral: not ossified	**Minor**	3 (1.5)	1 (0.5)	3 (1.7)	5 (2.3)
Pelvic Girdle; pubis- uni- or bilateral: incomplete ossification	**Minor**	25 (14.0)	48 (26.7)	29 (15.5)	47 (24.1)
Forelimb; epiphyses- uni- or bilateral: not ossified	**Variant**	37 (22.1)	37 (21.1)	28 (15.2)	36 (19.1)
Forelimb; proximal or distal epiphyses of humerus only- uni- or bilateral: not ossified	**Variant**	65 (38.8)	80 (43.9)	103 (53.3)	83 (47.1)
Forelimb; one or more digit: phalanges not ossified	**Minor**	1 (0.6)	1 (0.5)	3 (1.5)	7 (4.5)
Forelimb; one or more digit: phalanges incomplete ossification	**Variant**	62 (38.1)	103 (57.7)	91 (50.1)	97 (54.7)
Forelimb; one or more metacarpal: not ossified	**Minor**	15 (8.1)	28 (15.3)	25 (13.1)	29 (15.7)

		Dose (mg/kg/Day)			
Observation	**Type**	**0**	**3.3**	**10**	**30**
Forelimb; one or more metacarpal: incomplete ossification	**Minor**	1 (0.6)	1 (0.5)	1 (0.5)	4 (2.7)
Hindlimb; epiphyses- uni- or bilateral: not ossified	**Variant**	123 (73.4)	137 (73.6)	128 (67.3)	115 (64.2)
Hindlimb; astragalus- uni- or bilateral: not ossified	**Minor**	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Hindlimb; one or more digit: phalanges incomplete ossification	**Variant**	2 (1.3)	13 (6.5)	9 (4.8)	20 (11.4)*

* = $p < 0.05$

9.3 Prenatal and Postnatal Development (PPND)+

9.3.1 Study Title: rhLAMAN: Intravenous (Slow Bolus) Pre-and Post-Natal Development Study in the Rat

Study no.: IZU0007
Study report location: 4.2.3.5
Conducting laboratory and location: (b) (4)
GLP compliance: Yes
Drug, lot #, and % purity: 2O200; Purity: 99.5% (HPLC % Area of 3 peaks)
Enhanced PPND Study: N

Methods

Doses:	0, 3.3, 10, 30 mg/kg/Day
Frequency of dosing:	Every 3 Days
Number/Sex/Group:	22 Presumed pregnant rats
Dose volume:	1.1, 3.3, or 10 mL/kg
Formulation/Vehicle:	(b) (4) [redacted]
Route of administration:	INTRAVENOUS
Species:	*Rattus Norvegicus*
Strain:	Crl:WI(Han) rat
Comment on Study Design and Conduct:	• Animals were dosed from GD6 to PND 20 • Litters were observed (#/sex) on PND 4 and pups were randomly culled to 4/sex/litter • Pups were evaluated daily for mortality, toxicity, and malformations • Maturation parameters (eye opening, righting, startle, and pupillary light reflexes) were evaluated • Surviving F1 pups were culled on Day 21; 20 F1 pups/Sex/Dose were selected for further evaluation • Non-sibling F1 mating pairs were established at 10 weeks of age until evidence of mating was obtained • F1 pups were euthanized on GD 13 and pregnancy status, # corpora lutea, number and distribution of implantations, and vital status of embryos was recorded
Dosing Solution Analysis:	Not evaluated; the material was pre-formulated and administered as received and was therefore characterized by its labeling.

Observations and Results

F0 Dams

Several adverse clinical signs were observed in pregnant F0 dams, and three dams died or were euthanized prematurely for adverse clinical signs. The report states that the causes of morbidity/death were not treatment-related; however, they do not state

why they were considered unrelated to treatment. Given that the underlying conditions necessitating preterm euthanasia were not determined, it is not possible to exclude the possible effect of rhLAMAN in these deaths.

- One mid-dose female (#57) was found dead on GD 16. This animal exhibited piloerection on Day 15.
- Another two high-dose dams (#78 and #87) were euthanized on Days 15 and 16, respectively, for declining condition. The causes of morbidity were not determined for these dams. Animal #78 exhibited pale coloration and piloerection, was cool-to-touch, had red discharge from the anus and vagina on Day 15. Animal #87 exhibited piloerection, palpebral closure, prostration, and red-stained bedding on Days 15 and/or 16.
- Another low-dose dam (#41) was euthanized on Lactation Day 1 due to adverse clinical signs (clonic seizure). The cause of morbidity was not determined.
- Another mid-dose dam (#60) was euthanized on LD 2 after the entire litter was found dead. This animal exhibited piloerection on GD 15.

As a result of the preterm deaths, the gestation index in the 10 and 30 mg/kg dose levels were lower than controls but because they were related to maternal tolerability, they were not considered indicative of effects on development.

Table 25: Summary of Clinical Signs in F0 Dams

	Dose (mg/kg)			
	0	**3.3**	**10**	**30**
Decreased Activity	0	0	3	3
Cold-to-touch	0	0	0	4
Swollen muzzle	0	0	0	1
Piloerection	0	0	5	6
Prostration	0	0	2	3
Slow breathing	0	0	2	3
Discharge, anus	0	0	0	1
Discharge, vulva	0	0	0	2
Stained bedding	0	0	0	1
Gait, abnormal	0	0	0	3
Gait, unsteady	0	0	0	2
Abnormal color	0	0	0	2
Abnormal color, tail	0	0	0	1
Palprebal closure	0	0	0	1

Lactation

There were no effects on the ability of F0 dams to rear offspring, however, on Days 1 (Animal #35) and 8 (Animal #41) of lactation, two low-dose dams experienced clonic convulsions.

	Dose (mg/kg)

	0	3.3	10	30
Convulsion, clonic	0	2	0	0
Eye, protruding	0	0	0	1

Table 26: Mean Pregnancy and Litter Data

	Dose (mg/kg)			
Parameter	**0**	**3.3**	**10**	**30**
Gestation Length (Days)	22.3	22.23	22.55	22.2
Gestation Index (%)	100.0	100.0	864	90.9
No. Implantation Scars	11.0	11.0	11.6	11.3
Pups Born	10.6	10.5	10.5	10.2
Live Pups on Day 0	10.5	10.4	10.2	9.9
Live Pups on Day 1	10.5	9.7	9.8	9.8
Live Pups on Day 4 (pre-cull)	10.5	9.7	9.8	9.7
Live Pups on Day 7	8.0	7.5	7.2	7.5
Live Pups on Day 14	8.0	7.5	7.2	7.5
Live Pups on Day 21	8.0	7.5	7.2	7.5
Live Birth Index (%)	99.55	98.99	97.5	95.45
Viability Index 1 (%)	100.00	95.04	90.58	97.42
Viability Index 2 (%)	100.00	100.00	100.00	100.00
Viability Index 3 (%)	100.00	100.00	100.00	100.00
Viability Index 4 (%)	100.00	100.00	100.00	100.00
Lactation index (%)	100.00	100.00	100.00	100.00
Cumulative survival index (%)	99.55	98.51	93.85	93.90
% Males	52.23	49.03	45.53	48.07

Table 27: Necropsy Data P Generation Pups

	Dose (mg/kg)			
Necropsy Data	**0**	**3.3**	**10**	**30**
Total Number of Litters	22	22	19	20
Number of Pups Born	233	230	199	204
Found Dead/Killed Prematurely	1	17	8	9
Missing (presumed cannibalized)	0	0	6	1
Scheduled Termination	192	173	147	154
Culled Day 4	56	47	48	45
Killed Post-Weaning	136	126	99	109
Retained for Rearing	40	40	38	40
Findings: dead/moribund/sacrificed pups				
No abnormalities	0	14	0	0
No Milk in Stomach	1	1	4	7
Cannibalized			2	
Left Ureter Dilated, Left Kidney Pelvic Dilation			1	

Visceral Autolysis				3
Pups Post-Weaning				
No Abnormalities	135	126	96	108
Hermaphrodite		1		
Visceral Autolysis		1		
Right Kidney Pelvic Dilatation Severe				1

Table 28: Summary of Necropsy Findings in F1 Dams

		Dose (mg/kg)			
Histopathology Data	**Severity**	**0**	**3.3**	**10**	**30**
Cecum; inflammatory infiltrate, mixed	Moderate				1
--Ulceration	Moderate				1
Eyes, retinal degeneration	Slight				1
Jejunum; GALT: distended Lacteals	Minimal	4			5
	Slight	2			3
--Mineralization, GALT	Minimal	2			1
	Slight	1			0
Kidneys; pyelitis: chronic	Moderate				1
--Hyperplasia: transitional cell	Slight				1
--Pelvic distension	Slight	2			
Liver; focal hepatocyte degeneration/inflammation	Minimal	2			1
--inflammatory infiltrate: peribiliary	Minimal				1
Ovaries; histiocytic sarcoma, malignant tumor	**Present**				**1**
Vagina; metestrus	Present	4			2
--Estrus	Present	4			3
--diestrus	Present	5			7
--proestrus	Present	5			4
--Epithelial mucification	Present	4			4

F1 Generation

There were no premature deaths, and no adverse clinical signs in F1 pups. There were also no effects on pup body weight or body weight gain. Initial survival after birth was also lower than controls but was reportedly within the facility's historical control range.

In culled F1 pups, there were no macroscopic abnormalities related to maternal administration of rhLAMAN.

As indicated in Table 29, aside from a small decrease in static righting reflex, there were no effects on attainment of developmental milestones in F1 animals that were selected to be reared. The effect on righting reflex was not statistically different from controls.

Table 29: Summary of Developmental Milestones

	Dose (mg/kg)			
Parameter	**0**	**3.3**	**10**	**30**
Number	22	21	18	20

Pinna detachment day	44.0	3.8	3.1	3.8
Eyelid separation day	15.0	14.8	14.8	15.1
Static righting reflex (% positive)	98.8	99.3	97.9	89.9
Pupillary light reflex (% positive)	100.0	100.0	100.0	100.0
Startle reflex (% positive)	100.0	100.0	94.4	100.0

There were no effects on body weight or body weight gain in F1 pups selected for rearing (Figure 38); no differences in learning and memory (Table 30 and Table 31), auditory function (Table 29), or age of attaining indices of sexual development (Table 33). Aside from an initial increase in activity during the first observation period in treated males (all dose levels) and females (mid- and high-dose groups), there was no difference in motor activity compared with controls (Table 32).

Figure 38: Mean Body Weight in F1 Males (L) and Females (Pre-Pairing, R)

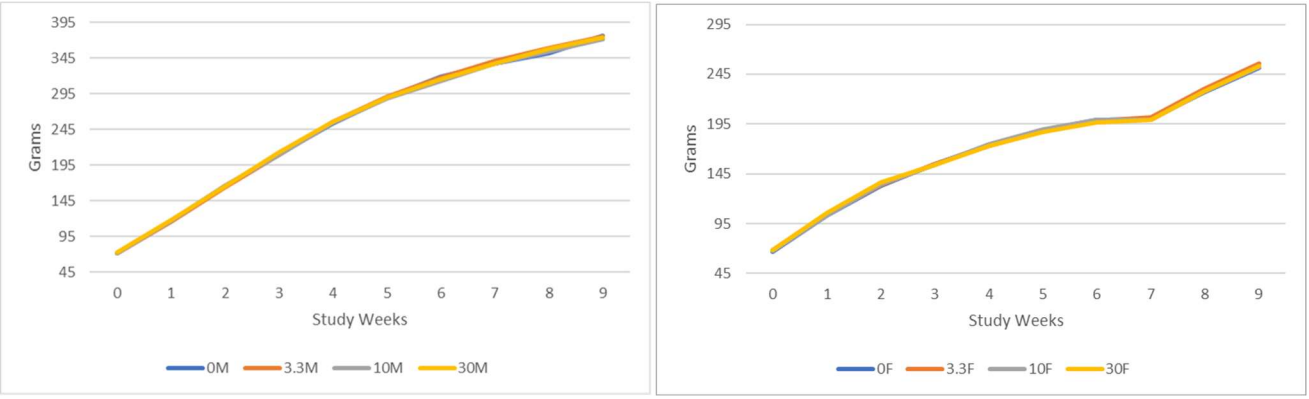


Table 30: E-Maze Learning test: Run Times – Session 1 (Seconds)

		Session 1 Exit Time Run Number				Session 1 Difference
Dose	Sex	1	2	3	4	1-4
0	Male	17.5	22.1	11.5	7.8	9.8
	Female	21.2	21.2	14.4	8.9	12.3
30	Male	23.5	17.2	10.5	9.1	14.4
	Female	19.1	23.6	17.0	10.0	9.1

Table 31: E-Maze Learning test: Run Times – Session 2 (Seconds)

		Session 2	Session 2 Exit Time Run Number				Session 2 Difference	Difference Session 1 & 2 Memory
Dose	Sex		1	2	3	4	1-4	--
0	Male	9.5	28.4	13.2	11.2	7.7	20.7	-1.8

	Female	5.9	25.0	14.7	11.2	8.3	16.7	3.1
30	Male	9.5	28.8	15.5	10.5	8.0	20.8	0.1
	Female	8.9	22.7	12.0	10.7	9.7	13.0	1.2

Table 32: Mean F1 Motor Activity Counts Over 10 Minute Observation Periods

Dose (mg/kg)	Sex	1	2	3	4	5	6	Total
0	Male	769.0	408.3	348.9	163.8	275.9	133.6	2099.3
	Female	857.6	414.4	382.2	218.6	247.9	184.6	2251.2
3.3	Male	914.2*	560.9	360.6	395.1	233.0	279.3	2743.0
	Female	834.0	467.2	410.3	191.6	175.9	178.2	2257.0
10	Male	1117.4*	565.8	350.6	268.5	83.3	313.6	2699.1
	Female	987.9*	513.5	418.8	197.4	192.5	169.9	2479.8
30	Male	1025.1*	538.5	544.3	282.5	313.8	238.2	2942.2
	Female	1038.6*	490.0	373.6	272.5	223.9	268.7	2267.2

* = $p < 0.05$

Table 33: Sexual Development Indices in F1 Males and Females

	Preputial Separation in Males		Vaginal Opening in Females	
Dose (mg/kg)	**Age**	**Weight**	**Age**	**Weight**
0	42.0	185.8	30.0	97.7g
3.3	41.2	180.7	31.4	100.8
10	41.9	186.9	31.5	100.4
30	42.0	187.1	31.8	104.2

There were no differences in the timing of mating in F1 animals (Table 34), and no differences in fertility parameters (Table 35).

Table 34: F1 Mating Timecourse

	Dose (mg/kg)			
Females Mated	**0**	**3.3**	**10**	**30**
Mated on Day 1	5	4	4	5
Mated on Day 2	6	2	3	4
Mated on Day 3	4	9	6	8
Mated on Day 4	5	5	7	3
Mated on Day 5	0	0	0	0
# Females Not Mated	0	0	0	0
Pre-coital interval	2.5	2.8	2.8	2.5

F2 Generation

There were also no differences in uterine evaluations in F1 females (Table 37).

Table 35: Summary of Mean F1 Fertility and Mating Data

	Dose (mg/kg)			
Fertility and Mating	**0**	**3.3**	**10**	**30**
Number of Females Paired	20	20	20	20
Number of Females Mated	20	20	20	20
Number of Fertile Females	19	19	19	19
Copulation Index Female %	100.0	100.0	100.0	100.0
Female Fertility Index %	95.0	95.0	95.0	95.0
Number of Males Paired	19	20	20	20
Number of Males Mated	19	20	20	20
Number of Fertile Males	18	19	19	19
Copulation Index Male %	100.0	100.0	100.0	100.0
Male Fertility Index %	94.7	95.0	95.0	95.0

Table 36: Summary of F1 Pregnancy Outcomes

	Dose (mg/kg)			
Pregnancy Outcomes	**0**	**3.3**	**10**	**30**
Not Pregnant	20	20	20	20
Not Pregnant (%)	1	1	1	1
Not Pregnant Died/Killed	0	0	0	0
Not Pregnant Scheduled Termination	1	1	1	1
Pregnant	19	19	19	19
Pregnant (%)	95.0	95.0	95.0	95.0
Pregnant Died/Killed/Aborted	0	0	0	0
Pregnant with Total Resorptions	0	0	0	0
Number with Live Embryos	19	19	19	19

Table 37: Summary of F1 Uterine and Implantation Data

	Dose (mg/kg)			
Uterine & Implantation Endpoints	**0**	**3.3**	**10**	**30**
Number with Implantations	19	19	19	19
Number of Corpora Lutea	241	256	250	243
Number of Implantations	211	244	235	234
% Pre-Implantation Loss	13.5	4.9	6.0	3.8
Number of Early Deaths	9	9	7	18
Number of Dead Embryos	0	0	0	0
Number of Live Embryos	202	235	228	216
% Post-Implantation Loss	5.9	3.5	3.0	7.7
Mean % of Implantations	94.1	96.5	97.0	92.3

Toxicokinetics and Lactation (exposures)
Not evaluated

9.4 Juvenile Animal Studies+

9.4.1 Study Title: rhLAMAN: Preliminary Juvenile Toxicity Study in Rats

Study no.:	(b) (4) 495110
Study report location:	4.2.3.5
Conducting laboratory and location:	(b) (4)
Duration:	10
Duration Units:	Weeks
GLP compliance:	Yes
Drug, lot #, and % purity:	ZYA TR UFDF2 20090325 001; 89.5% pure (HPLC % area of 3 peaks)

Scientific Justification for Study:
Potential pediatric Indication

JAS Specific Toxicity:
None

Methods

Doses:	0, 466, and 1400 U/kg
Frequency of dosing:	Twice Weekly
Number/Sex/Group:	4/Sex/Group **(see note below)
Dose volume:	3.95 or 11.86 mL/kg
Formulation/Vehicle:	The placebo formulation was not provided in the report; however, it was likely the same as other studies conducted with this agent. The test article used was not re-formulated for administration; different dose levels were administered by adjusting the volume of the stock.
Route of administration:	INTRAVENOUS
Species:	*Rattus norvegicus*
Strain:	Crl:CD®(SD)
Age at start of experiment:	Day 33 post-coitum (~11 days of age); PCD 50 (~28 days of age)
Period of development studied:	Neonatal to adult
Comment on Study Design and Conduct:	**(see Table 38 below) Briefly: Groups 1-3: Animals were dosed Tuesday and Friday from PCD 33 for 10 weeks at doses of 466 or 1400 U/kg twice-weekly; Groups 4-5 began treatment on PCD 50 at a dose of

	466, escalating to 1400 U/kg twice-weekly for 3 weeks, then animals received twice daily doses for 3 consecutive days at the same dose level.
Parameters and Key Endpoints Evaluated:	Body weight; feed consumption; FOBs; clinical pathology; ADA; organ weights; and gross and histopathological evaluations (Groups 1-3)
Dosing Solution Analysis:	

Table 38: Design of the Preliminary Rat Juvenile Animal Toxicity Study

Group	Treatment (U/kg/twice weekly)		Dose Volume (mL/kg)	Dosing Period (post coitum)	Dosing Routine
1	Control	0	11.86	Day 33 for 10 weeks	Twice weekly
2	Low dose	466	3.95	Day 33 for 10 weeks	Twice weekly
3	High dose	1400	11.86	Day 33 for 10 weeks	Twice weekly
4	Phase 2, dose 1	466/1400*	3.95/ 11.86	Day 50 for 3 weeks	Twice weekly
5	Phase 2, dose 2	1400	11.86	Day 50 for 3 weeks	Twice weekly and then twice daily over Days 75-77 post coitum

* Animals were dosed at 466 U/kg/twice weekly for one week and were then escalated to 1400 U/kg/twice weekly for the remaining dose period.

(Excerpted from the Applicant's BLA)

Observations and Results

Mortality

None

Clinical Signs

Swollen feet and/or muzzles, excessive grooming, and piloerection

Body Weight

Generally unremarkable. Small differences between treated and control groups were not considered biologically meaningful.

Figure 39: Summary of Mean Male (L) and Female (R) Body Weights for Phase 1 in the Preliminary Juvenile Animal Toxicity Study

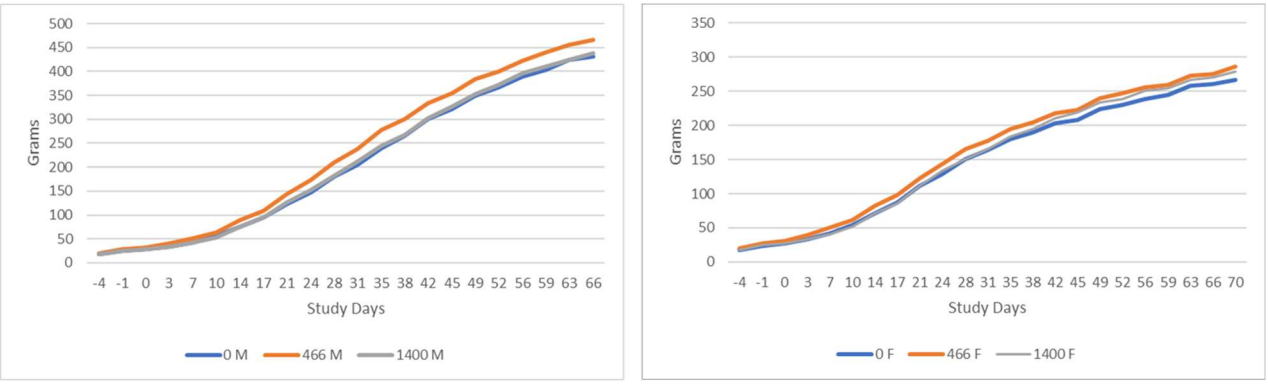
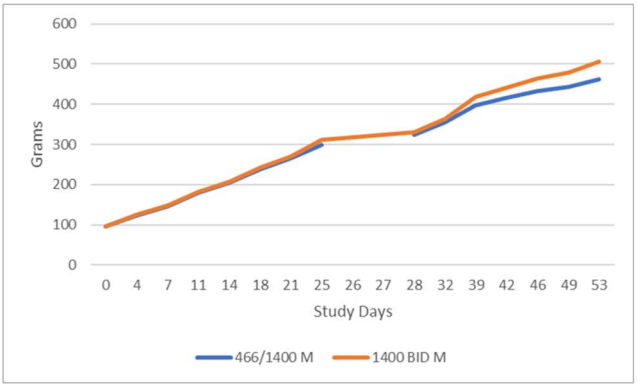
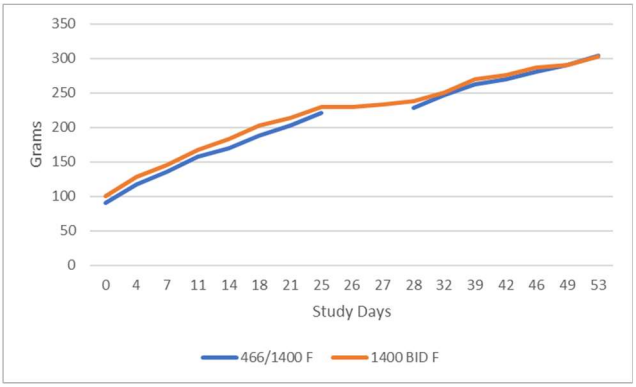


Figure 40: Summary of Mean Male (L) and Female (R) Body Weights for Phase 2 in the Preliminary Juvenile Animal Toxicity Study





Feed Consumption
Unremarkable

Ophthalmoscopy

Hematology
Hematology was collected for Groups 1-3 only. There were no effects on mean hematology or coagulations parameters.

Clinical Chemistry
Unremarkable. An individual glucose analysis was also performed from Group 5 animals, which was unremarkable.

Urinalysis
Not evaluated

Sexual Maturation
Not evaluated

Reproductive Capacity
Not evaluated

CNS/ Neurobehavioral Assessment
A modified FOB was performed on Group 3 animals on two occasions: Once, when the animals were about 3 weeks of age (on the day of the 5th dose) and on the day of the 20th dose. Animals were assessed for alertness, locomotor activity, exploratory behavior, grooming behavior, diarrhea, tremors, muscle spasms, gait, posture, presence of piloerection, respiration, skin color, startle response, body temperature, salivation, appearance of the eyes (palpebral closure, pupillary diameter, exophthalmos, lacrimation, crusting), vocalization, and reflexes (corneal, pinna, pupillary), and pain

response (tail flick). General handling observations (e.g., biting and aggression) were also noted. Table 39 and Table 40 provides a summary of the observations from Group 1-3 animals.

Table 39:Clinical Observations From the Modified Functional Observational Battery in Juvenile Animals (Groups 1-3)

	Males (U/kg/dose)			Females (U/kg/dose)		
Observations	**0**	**466**	**1400**	**0**	**466**	**1400**
Week 3						
Piloerection	1	2	2	1	1	2
Week 10						
Posture (hunched)	2	0	3	0	1	0
Gait (walking on tip toes)	1	0	1	3	3	2
Vocalization (upon handling)	0	0	0	1	0	0
Palpebral closure (partial)	0	0	1	0	0	0
Number Examined	4	4	4	4	4	4

Table 40: Modified Functional Observational Battery in Juvenile Animals (Groups 1-3)

		Males (U/kg/dose)			Females (Ukg/dose)		
Observations	**Week**	**0**	**466**	**1400**	**0**	**466**	**1400**
Locomotor Activity	3	14	12	11	17	16	12
	10	13	10	17	23	22	25
Number of Grooming Sessions	3	1	2	1	1	1	1
	10	1	1	1	0	0	0
Body Temperature	3	38.1	37.7	38.0	37.7	38.1	38.3
	10	38.2	38.5	37.2	38.6	3.6	38.6
Pain Response	3	1.4	1.9	1.9	1.4	1.8	2.2
	10	2.5	2.4	2.9	2.2	3.2	3.7

Bone Evaluation
Not conducted

Gross Pathology
Unremarkable

Organ Weights
Unremarkable

Histopathology
Generally unremarkable. Animals in Groups 4-5 did not undergo histopathological evaluation. A summary of the histological findings noted in Group 1-3 animals, is summarized below. Severity scores were not given for these findings.

	Male			Female		
	0	466	1400	0	466	1400
Heart; myocarditis, focal		1				
Kidney; hyaline droplsets		1				
--basophilic tubules		4	4	4	4	2
--mineralization				2		2
Liver; centrilobular hepatocyte vacuolation		1				
--inflammatory cell infiltration	1		1	1	2	

Toxicokinetics
Toxicokinetic analysis was not performed; however, sample collection for an evaluation of anti-drug antibody was undertaken on pre-dose samples obtained during Weeks 4 and 9, and from animals in Group 5 at about 2.5 weeks after the final dose. Samples were collected but not analyzed.

9.4.1 Study Title: rhLAMAN: Juvenile Toxicity Study in Rats

Study no.: 495126
Study report location: 4.2.3.5
Conducting laboratory and location: (b) (4)
Duration: 10
Duration Units: Weeks
GLP compliance: GLP
Drug, lot #, and % purity: rhLAMAN, ZYA DS 99309001; purity: 99.7% (HPLC % Area for 3 peaks)

Scientific Justification for Study:
Potential pediatric indication

JAS Specific Toxicity:
None

Methods

Doses: 0, 142, 426, and 1290 U/kg
Frequency of dosing: Twice Weekly
Number/Sex/Group: 12/Sex/Group
Dose volume: 1.1, 3.3, or 10 mL/kg
Formulation/Vehicle: Not stated

Route of administration:	INTRAVENOUS
Species:	*Rattus norvegicus*
Strain:	Crl:CD®(SD)
Age at start of experiment:	33d post-coitum (approximately 11 days old)
Period of development studied:	Neonatal to adult
Comment on Study Design and Conduct:	Dose formulation analysis was not conducted. Material was used as supplied and was characterized by its labeling.
Parameters and Key Endpoints Evaluated:	• Rotarod was conducted at 4-5 weeks of age • Open field assessments were performed at 5-6 weeks of age • Multiple Y Water Maze test was performed at 6-8 weeks of age • Modified FOB was performed at 3-4 weeks of age and toward the end of the study • Blood was taken from main study animals during Weeks 4 and 9 for TK and ADA, and clinpath was taken at necropsy
Dosing Solution Analysis:	

Observations and Results

Mortality

None

Clinical Signs

Swollen feet and base of tail for many animals in the 426 and 1290 U/kg dose groups, which began after the 5th dose and continued throughout the dosing period. The report states that these signs occurred immediately after dosing and generally subsided within 3-4 hours post-dose.

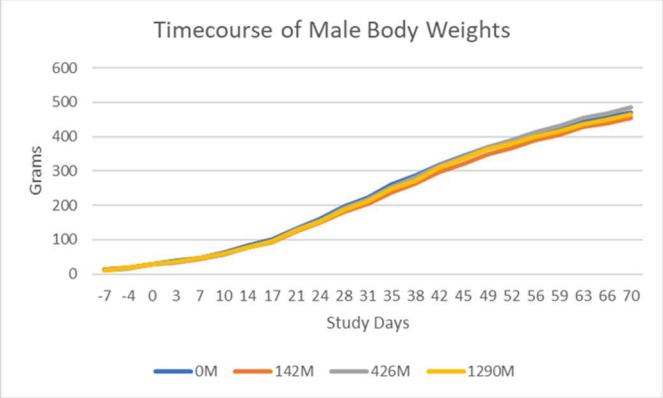
	Male (U/kg/dose)				Female (U/kg/dose)			
Observation	**0**	**142**	**426**	**1290**	**0**	**142**	**426**	**1290**
Swollen feet/hindlimbs	0	0	2	12	0	0	2	12
Swollen muzzle	0	0	0	4	0	0	0	3
Swollen tail base	0	0	0	0	0	1	0	4
Sparse hair	0	0	0	2	0	0	0	1
Pale skin/redness, extremities	0	0	0	0	0	0	0	2

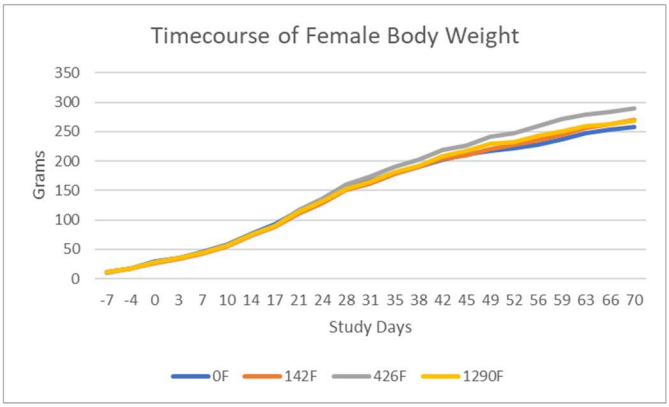
Body Weight

Generally unremarkable. In males, small decreases in mean male body weights were observed in treated groups; however, the effect was intermittent, and the magnitude was not dose-related. In females, mean body weights were higher at the 462 U/kg dose

level than in controls for most of the study; however, body weights in high-dose (1290 U/kg) animals and in low-dose groups (142 U/kg) groups were similar to controls; thus, the effect at the mid-dose level does not appear to be a treatment-related effect.

Figure 41: Time course of Mean Male (L) and Female (R) Body Weight





Feed Consumption
There were no apparent differences in feed consumption among treated males compared with controls; there was a small increase in feed consumption in mid-dose females that parallels and likely explains the increase in female body weight in that dose group; however, the effect was small and non-dose-related and is therefore unlikely to be a treatment-related effect.

Ophthalmoscopy
Not evaluated
Hematology and Coagulation
Unremarkable.

Clinical Chemistry
A few statistically significant differences in mean clinical chemistry parameters were noted compared with the concurrent control group, however, they did not suggest a pattern of target organ effects, and/or were of a magnitude that was not toxicologically significant.

Urinalysis
Not evaluated

Sexual Maturation
There was no apparent effect of rhLAMAN administration on the timing of sexual maturation, as measured by age or body size. A minor increase in the age of preputial separation at the low dose in males lacked a relationship to dose and was ascribed to normal variation.

Table 41: Assessment of of rhLAMAN Administration on Attainment of Sexual Developmental Milestones

	Male (U/kg/dose)				Female (U/kg/dose)			
Measurement	**0**	**142**	**426**	**1290**	**0**	**142**	**426**	**1290**
Age at vaginal opening (Days)					34.9	35.2	35.5	35.9
Weight at vaginal opening (g)					127	125	139	133
Age at preputial separation (Days)	41.0	44.2	42.3	41.7				
Weight at preputial separation (g)	205	218	219	204				

Reproductive Capacity
Not assessed

CNS/ Neurobehavioral Assessment
There were no effects of rhLAMAN between the groups in the performance on the rotarod.

Table 42: Summary of Results from the Rota Rod Test

	Male (U/kg/dose)				Female (U/kg/dose)			
Measurement	**0**	**142**	**426**	**1290**	**0**	**142**	**426**	**1290**
Maximum*	95.7	117.6	95.5	104.1	115.8	107.1	93.4	106.2
Mean**	74.8	88.8	66.1	68.4	80.1	78.7	65.7	73.5

*Mean of the longest time achieved over 3 trials by each animal
**Mean of the length of time achieved over 3 trials by each animal

There were generally no differences between treated and control animals in the functional observational battery parameters evaluated. A statistically significant increase in the mean body temperature of males (range: 37.2-39.6º C) in the high-dose group vs concurrent controls (range: 36.3-38.6º C) during week 9 was observed. The value was similar to that of high-dose females (range: 37.5-39.0º C). The high mean body temperatures in males during Week 9 were driven by two males that had values outside of the control range (Male 42: 39.6C and Male 46: 39.1º C). Other slight differences between treated and control animals in various FOB parameters were attributed to normal variation and not treatment-related.

Bone Evaluation
Not evaluated

Gross Pathology
The following gross necropsy observations were noted:

	Male (U/kg/dose)				Female (U/kg/dose)			
Measurement	**0**	**142**	**426**	**1290**	**0**	**142**	**426**	**1290**

Epididymis, right, small	0	1	0	0	0	0	0	0
Foot, abnormal shape	0	1	0	0	0	0	0	0
Foot, swelling	0	0	0	1	0	0	0	0
Kidney, depressed focus, right	0	0	1	0	0	0	0	0
Kidney, discolored, both	0	1	2	0	0	0	0	0
Kidney, pelvic dilation, one/both	1	2	0	0	0	0	0	0
Lungs, dark focus	1	4	3	6	0	0	1	2
Lungs, dark	0	0	0	1	0	0	0	1
Lungs, enlarged	0	0	2	0	0	0	0	0
LN (cervical), enlarged	0	0	0	0	0	0	0	1
LN (mandibular) speckled	0	0	0	0	0	0	0	1
LN (mandibular) enlarged	1	1	4	1	0	0	0	1
LN (Renal), reddened, left	0	0	1	0	0	0	0	0
Skin, pale	0	0	0	0	0	0	0	1
Stomach, raised focus	0	0	1	0	0	0	0	0
Tail, abnormal shape	0	0	0	0	0	0	1	0
Uterus, dilated with fluid	0	0	0	0	2	2	4	0

Organ Weights
Generally unremarkable. Except for a small increase in the absolute and body weight-normalized weights of thyroid glands, which was observed in both males and females, other statistically significant differences in mean absolute and/or relative organ weights were present in only one sex and/or the magnitude of the effect was not dose-related and are therefore considered incidental.

Histopathology
Generally unremarkable. Minor histological findings for which the incidence in treated groups exceeded those of concurrent controls were noted. Histopathological severity scores were not provided. Other than the findings in the tail and foot, for which correlating clinical signs were noted, none of the histopathological findings was considered toxicologically significant.

	Male (U/kg/dose)				**Female (U/kg/dose)**			
Histopathology Data	**0**	**3.3**	**10**	**30**	**0**	**3.3**	**10**	**30**
Foot/leg; dermatitis, focal	0	0	0	2	0	0	0	0
Kidney, chronic progressive nephropathy	1	0	0	2	1	0	0	1
--Mineralization, multifocal, medulla	1	0	0	2	1	0	0	1
Liver, inflammatory cell foci	3	0	0	4	3	0	0	4
--mineralization, focal	0	0	0	1	0	0	0	0
Lung, inflammation, multifocal	1	0	0	3	2	0	0	4
--agonal congestion/hemorrhage	4	0	0	9	3	0	0	4
LN, cervical, plasmacytosis	0	0	0	0	0	0	0	1
Prostate, prostatitis, focal	0	0	0	1	0	0	0	0
Skin, folliculitis, focal	0	0	0	0	0	0	0	1
Tail, serocellular crust, focal	0	0	0	1	0	0	0	1

	Male (U/kg/dose)				Female (U/kg/dose)			
Histopathology Data	**0**	**3.3**	**10**	**30**	**0**	**3.3**	**10**	**30**
--hemorrhage with inflammation, perivascular	2	0	0	3	4	0	0	5

Toxicokinetics
Exposure was generally proportional to dose. While initial (C_0) and peak (C_{max}) exposures appeared to be slightly higher in males than in females, overall (AUC) exposures were generally similar between the sexes. The half-life was similar across the dose range.

Dose (U/kg)	**Sex**	**C_0 (ng/mL)**	**C_{max} (ng/mL)**	**T_{max} (hr)**	**AUC (ng*hr/mL)**	**$T_{1/2}$ (hr)**
142	F	6027	96533	0.08	2194253	20.4
	M	9330	164667	1	3001783	14.4
426	F	22067	490333	0.08	7660354	12.6
	M	30200	597667	0.08	8508547	13
1290	F	52950	1336667	0.3	21229261	12.7
	M	83467	1430000	0.08	25285783	13.6

11 References

Brantova O, Asfaw B, Sladkova J, Poupetova H, Zivny J, Magner M, Krusek J, Vesela K, Hansikova H, Ledvinova J, Tesarova M, Zeman j. 2009. Ultrastructural and functional abnormalities of mitochondriain cultivated fibroblasts fromα-mannosidosis patients. Biologia. 64(2): 394-401. DOI: 10.2478/s11756-009-0054-2

Brückner, J. Estimation of Monosaccharides by the Orcinol-Sulphuric Acid Reaction. 1955. Biochemical Journal. 60(2): 200–205.

Damme M, Stroobants S, Iudemann M, Rothaug, M, Luellmann-Rauch R, Beck, H-C, Ericsson A, Andersson C, Fogh J, D'Hooge R, Saftig P, and Blanz J. 2015.Chronic Enzyme Replacement Therapy Amerliorates Neuropathology in Alpha-Mannosidosis Mice. Annals of Clinical and Translational Neurology. 2(11): 987-1001. doi: 10.1002/acn3.245

Diering GH, Numata M. 2014 Endosomal pH in neuronal signaling and synaptic transmission: role of Na+/H+ exchanger NHE5. Frontiers in Physiology;4:412.

Hansen G. Berg T, Stensland HMFR, Heikinheimo P, Klenow H, Evjen G, Nilssen O, Tollersrud OK. 2004. Intracellular transport of human lysosomal α-mannosidase and α-mannosidosis-related mutants. Biochem J. 2004 Jul 15; 381(Pt 2): 537–546. https://doi.org/10.1042%2FBJ20031499

Roces DP, Lullmann-Rauch R, Peng J, Balducci C, Andersson C, Tollersrud O, Fogh J, Orlacchio A, Beccari T, Saftig P, von Figura K. 2004. Efficacy of enzyme replacement therapy in a-mannosidosis mice: a preclinical animal study. Human Molecular Genetics, 2004, 13(18):1979–1988 doi:10.1093/hmg/ddh220

Stinchi S, Lullmann-Rauch R, Hartmann D, Coenen R, Beccari T, Orlaccio A, von Figura K, Saftig P. 1999. Targeted disruption of the lysosomal α-mannosidase gene results in mice resembling a mild form of human α-mannosidosis. Human Molecular Genetics 8(8):1365-1372

Stroobants S, Damme M, van der Jeugd, A, Vermaercke ZB, Andersson C, Fogh J, Saftig P, Blanz J, d'Hooge R. 2017. Long-term enzyme replacement therapy improves neurocognitive functioning and hippocampal synaptic plasticity in immune-tolerant alpha-mannosidosis mice. Neurobiology of Disease. 106: 255–268

Thomas GH. 2019. The Online Metabolic and Molecular Bases of Inherited Disease. *in* The Online Metabolic and Molecular Bases of Inherited Disease. New York, NY: McGraw Hill.

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