CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761047Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

ADDENDUM TO CLINICAL PHARMACOLOGY REVIEW

BLA	**761047 (eCTD sequence 0001)**
Drug Name	**Vestronidase alfa (Mepsevii)**
Formulation	(b) (4) **mgmL solution in vials**
Dosing Regimen	**4 mg/kg every other week as an IV infusion**
Description of Submission	**Original BLA**
Submission Date	**3/16/17**
PDUFA Date	**11/16/17**
Primary Reviewer	**Christine Yuen-Yi Hon, Pharm.D.**
Team Leader	**Yow-Ming Wang, Ph.D.**
OCP Division	**DCP3**
OND Division	**DGIEP**
Applicant	**Ultragenyx**
Proposed Indication	**Treatment of Patients with Mucopolysaccharidosis VII (MPS 7, Sly syndrome)**

This addendum provides an updated summary table for immunogenicity data up to the 120-Day Safety Update. The revised table contains immunogenicity data that are used to inform Section 6.2 of the product labeling. It replaces Table 16 in the Clinical Pharmacology Review dated September 13, 2017.

Table 16. Summary of immunogenicity data up to the 120-Day Safety Update

Study/IND	ID	Age	Pre-Existing ADA Titer at Baseline/ Randomization	ADA+	NAb+	Highest ADA Titer Post Treatment	Anaphylaxis
UX003-CL201	1	5.5	-	Y	Y	10240	Suspected
	2	9.4	-	Y	N	5120	
	3	25.1	-	N	N	-	
UX003-CL301 and its extension study UX003-CL202	4	14.7	-	Y	Y	12800	
	5	8.5	-	Y	Y	65610	Y
	6	13.4	-	Y	Y	65610	
	7	12.8	-	Y	Y	2560	Y
	8	10.1	10	Y	N	640	
	9	17.4	160	Y	Y	10240	
	10	10.5	20	Y	Y	7290	
	11	25.3	-	N	N	-	
	12	22.6	10	N	N	-	
	13	22.5	80	N	N	80	
	14*	16.5	-	Y	N	2430	
	15*	11.5	80	Y	Y	40	
UX003-CL203	16	2.9	-	N	N	-	
	17	3.0	-	Y	N	2560	
	18	1.7	1280 (previously treated through an emergency IND)	Y	N	1280	
	19	3.4	-	Y	N	160	
	20[$]	3.9	-	Y	Y	10240	
	21[$]	2.0	-	Y	Y	10240	
	22[$]	5.0	-	Y	N	40	
	23[$,#]	4.7	-	-	-	-	
Emergency IND 119935	24	15	80	Y	N	5120	

*Subjects not enrolled in extension study UX003-CL202; [$]subjects from 120-Day Safety update; [#]Subject had a baseline immunogenicity sample only and was not included in the calculation of ADA incidence

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/s/

CHRISTINE Y HON
11/13/2017

YOW-MING C WANG
11/14/2017

Office of Clinical Pharmacology Review

NDA or BLA Number	BLA 761047
Link to EDR	\\CDSESUB1\evsprod\BLA761047\761047.enx
Submission Date	3/16/17
Submission Type	Original BLA (New Molecular Entity) – Priority Review
Brand Name	Mepsevii
Generic Name	Vestronidase alfa
Dosage Form and Strength	10 mg/5 mL solution in vials
Dosage and Administration	4 mg/kg every two weeks via intravenous infusion over approximately 4 hours
Proposed Indication	Treatment of patients with Mucopoly-saccharidosis VII (MPS VII, Sly syndrome)
Applicant	Ultragenyx
Associated IND	IND 123788
OCP Review Team	Christine Hon, Pharm.D. Yow-Ming Wang, Ph.D.
OCP Final Signatory	Hae Young Ahn, Ph.D. Deputy Director Division of Clinical Pharmacology 3

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1. EXECUTIVE SUMMARY

The Applicant is seeking marketing approval of vestronidase alfa (UX003), a recombinant human beta-glucuronidase (rhGUS), as an enzyme replacement therapy (ERT) for the treatment of mucopolysaccharidosis VII (MPS VII, Sly syndrome). MPS VII is a rare, multisystem lysosomal storage disease (LSD) with an estimated prevalence of < 1:1,000,000 caused by mutations in the *GUSB* gene. The clinical presentation may occur at birth with hydrops foetalis, or not until adolescence or adulthood with skeletal disease and other manifestations. Patients have variable degree of severity and progression of symptoms which include abnormal coarsened facies, pulmonary disease, cardiovascular complications, hepatosplenomegaly, joint stiffness, short stature, cognitive impairment, and the MPS skeletal disease known as dysostosis multiplex. Most patients die before the second or third decade of life due to complicating medical problems; death may also occur in the first year of life due to hydrops. Currently, there is no approved therapy for the treatment of MPS VII.

The vestronidase alfa program includes six clinical studies: two non-interventional and four interventional studies, and additional information collected from patients treated in early access programs. A total of 23 subjects were enrolled in the four interventional studies as of the 120 days safety update, which include the Phase 1/2 dose-finding study UX003-CL201 (n = 3), Phase 3 study UX003-CL301 (n = 12), a study in pediatric subjects < 5 years of age (n = 8), and the extension study for subjects who completed participation in any UX003 study (n =10, all from UX003-CL301).

Vestronidase alfa was granted Orphan Drug Designation for the treatment of MPS VII on February 16, 2012. Fast Track Designation was granted for the investigation of vestronidase alfa as an ERT for the treatment of MPS VII on July 15, 2015.

The Applicant's proposed dosing regimen is 4 mg/kg body weight administered every other week (QOW) as an intravenous (IV) infusion over approximately 4 hours. The to-be-marketed drug product (DP), vestronidase alfa for intravenous infusion, is a concentrate for solution containing (2 mg/mL) of vestronidase alfa in single-use glass vials filled with 5 mL solution.

1.1 Recommendations

From a clinical pharmacology perspective, information submitted to this BLA provides supportive evidence for the effectiveness of vestronidase alfa by demonstrating the pharmacological activity of vestronidase alfa, i.e., the reduction of urinary glycosaminoglycan (uGAG) levels increased with increasing dose and exposure of vestronidase alfa. However, the observed pharmacological activity may or may not be predictive of clinical outcomes because uGAG is not a qualified biomarker, i.e., the relationship between uGAG response and clinical outcome has not been established. In addition, deficiencies in the GAG assays are yet to be

addressed to ascertain the reliability of the observed GAG data. The demonstrated pharmacological activity of vestronidase alfa, being supportive instead of pivotal evidence of the effectiveness of vestronidase alfa, should be considered in the context of the-totality-of-the-evidence for regulatory decision with respect to the effectiveness of vestronidase alfa for the treatment of MPS VII.

The key review findings with specific recommendations/comments are summarized below:

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Exposure-response (E-R) analyses provide supportive evidence of effectiveness in terms of pharmacodynamic (PD) response in the reduction of biomarkers (urinary GAGs). Because uGAG is not a qualified biomarker and the quality of the PD data were suboptimal, the uGAG data are not sufficient as primary data and should be considered in the context of the-totality-of-the-evidence for regulatory approval.
General dosing instructions	Based on the E-R relationships of the biomarkers uGAG, the proposed dosing regimen of 4 mg/kg QOW appeared appropriate for both adult and pediatric subjects with MPS VII.
Dosing in patient subgroups (intrinsic and extrinsic factors)	Therapeutic individualization is not recommended for subjects of different ages because age did not appear to have an impact on PK and PD. No alternative dosing is recommended for subjects of different race because the sample size was too small to make a definitive conclusion of the effect of race on PK. Dose adjustment is not necessary with regard to sex. While the mean clearance (CL) value was lower in female subjects than in male subjects, the PD response in uGAG appeared similar between the two groups.
Immunogenicity	Seventeen of 22 subjects (77.3%) were tested positive for antidrug antibodies (ADA). Nine of the 17 (52.9%) ADA+ subjects developed neutralizing antibodies (NAb). The assessment of the impact of immunogenicity on PK was inconclusive because of the limited number of subjects. While

	between-subject comparison showed lower CL in ADA+ (NAb+) than in ADA- (NAb-) subjects, comparison of CL values at different study weeks within ADA+ or NAb+ subjects did not reveal consistent increase or decrease in CL values. Immunogenicity did not appear to have an impact on PD and safety in clinical trials. It remains to be evaluated the impact of immunogenicity on efficacy and safety after long-term treatment of vestronidase alfa.
Bridge between the to-be-marketed and clinical trial formulations	Not applicable. The to-be-marketed formulation was used in the clinical trials.
Labeling	The review team recommends revision of Sections 6.2 Immunogenicity, 12.2 Pharmacodynamics, and 12.3 Pharmacokinetics in the labeling (See Section 2.4 below)

1.2 Post-Marketing Requirements and Commitments

The Clinical Pharmacology review team recommends the following Post-Marketing Commitment (PMC) studies.

PMC or PMR	Key Issue(s) to be Addressed	Rationale	Key Considerations for Design Features
PMC	Impact of immunogenicity on efficacy and safety	Although immunogenicity did not appear to have an impact on PD or safety in clinical trials, the impact after long-term treatment of vestronidase alfa is not known.	Evaluation of immunogenicity and clinical assessments should be performed regularly to allow evaluation of the impact of immunogenicity on efficacy and safety after long-term therapy.
PMC	Method validation of the two PD assays, i.e., NRE and LC-MS/MS methods	Incurred sample reanalysis (ISR) was not performed for the method validation of the two PD assays. The Applicant has agreed to conduct ISR in response to FDA Information Request dated July 31, 2017 (see Section 4.1) The assessment of long-term sample storage stability of the LC-MS/MS method is ongoing. The Applicant should provide additional long-term sample storage stability data to	For the LC-MS/MS method, freshly prepared calibrator standards and quality controls should be used in conducting the ISR and long-term sample storage stability analysis.

		support storage durations for all samples from the clinical trials.	

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action

MPS VII is caused by mutations in the *GUSB* gene leading to deficiency of the lysosomal enzyme GUS which catalyzes the glucuronic acid-containing GAGs including chondroitin 6-sulfate (CS), dermatan sulfate (DS), and heparan sulfate (HS). Deficiency of GUS results in GAG accumulation in numerous cells throughout the body leading to multisystem tissue and organ damage.

Vestronidase alfa is intended to provide exogenous GUS enzyme to the body. It undergoes cellular uptake via cation-independent mannose-6-phosphate receptor (CI-M6PR) into the lysosomes of affected tissues, where accumulated GAGs in tissue are catabolized.

Pharmacokinetics (PK)

Vestronidase alfa concentrations declined multi-exponentially and remained quantifiable up to the last sampling time-point at 3 or 6 hours post infusion following IV infusion. Vestronidase alfa exposure appeared to increase more than dose proportionally from 1 mg/kg to 4 mg/kg dose in three subjects received 1, 2, and 4 mg/kg doses. However, this result might have been confounded by the small number of subjects as well as PK variability.

After repeated dosing of 4 mg/kg QOW, there was no accumulation of vestronidase alfa in the serum. The mean ± standard deviation (SD) maximal concentration (C_{max}) was 19.8 ± 8.00 µg/mL, and the mean area under the concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) was 57.4 ± 23.9 µg*h/mL. The PK characteristics at the 4 mg/kg QOW are summarized as below.

Distribution: The mean volume of distribution was 12.6 L.

Metabolism: The putative catabolism of vestronidase alfa is via peptide hydrolysis.

Elimination: Vestronidase alfa has a short mean half-life ($t_{1/2}$) of 2.59 h across treatment weeks. The mean CL value was 3.74 L/h. The inter-subject variability of CL was 33.7%, and the intra-subject variability ranged from 0.98% to 27.6%.

There appeared no significant difference in CL values among subjects of different race, but the number of subjects was too limited to make a definitive conclusion. Mean CL value was lower

in female subjects than in male subjects (3.66 vs. 5.09 L/h, p = 0.050). Vestronidase alfa PK did not appear to be different among different age groups.

Pharmacodynamics (PD)

The PD changes of GAG were determined by two methods (i.e., LC-MS/MS method and the NRE method) after the initiation of vestronidase alfa treatment. Because both methods were not fully validated, the extent of GAG reductions from baseline could not be ascertained quantitatively and the % GAG reductions are to be considered qualitative or semi-quantitative measurements only. Nonetheless, all subjects displayed consistent reductions from baseline in the excretion of total uGAG and urinary CS, DS, and CS/DS combined; all these GAG species demonstrated consistent E-R relationship as well (see Section 3.3.1). Taken together, the PD data demonstrate pharmacological activity and provide supportive evidence for the effectiveness of vestronidase alfa.

Following the administration of vestronidase alfa at 4 mg/kg QOW, excretions of urinary CS, DS, combined CS/DS, and total uGAG declined to reach nadirs at around four weeks and then plateaued. Thereafter, reductions in these urinary GAG excretions maintained at approximately 60% to 80% from baseline. Serum concentration of combined CS/DS decreased slowly to approximately 23% from baseline over a longer period of time. However, the temporal profile of serum HS concentration and urinary HS excretion did not show consistent PD response to vestronidase alfa treatment.

The observed PD responses were dose-dependent from 1 mg/kg to 4 mg/kg. The excretion of urinary CD, DS, combined CS/DS, and total uGAG declined with increasing dose of vestronidase alfa. The % uGAG reduction from baseline increased with vestronidase alfa exposure until uGAG response plateaued at a vestronidase alfa C_{max} value of approximately 15 ng/mL and an AUC_{0-t} value of approximately 40 ng*h/mL. No such dose-response or exposure-response was observed for serum CS/DS or HS concentration, or urinary HS excretion.

Vestronidase alfa PD responses did not appear to be different between males and females or among subjects of different age groups.

Immunogenicity

Seventeen of 22 subjects (77.3%) enrolled in the clinical trials developed antibodies to vestronidase alfa. The antidrug antibodies (ADA) titers varied among ADA+ subjects, with the highest ADA titers ranging from 10 to 65610 in individual subjects. Nine of the 17 (52.9%) ADA positive subjects developed neutralizing antibodies (NAb).

Between-subject comparison showed that the mean CL value was lower in ADA+ subjects than in ADA- subjects (4.21 vs. 5.79 L/h, p = 0.0103). Likewise, the mean CL value was lower in NAb+ subjects compared with NAb- subjects (3.77 vs. 5.03, p = 0.0443). However, comparison

of CL values at different study weeks within ADA+ subjects or within NAb+ subjects did not reveal consistent increase or decrease in CL values. There was no apparent correlation between CL values and ADA titers. Due to the limited sample size, the assessment of the impact of immunogenicity on PK is inconclusive.

Immunogenicity did not appear to have an impact on uGAG reduction or safety in the clinical trials. The impact of immunogenicity on efficacy and safety after long-term treatment remains to be evaluated.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The Applicant proposed QOW dosing of vestronidase alfa at 4 mg/kg via IV infusion over approximately 4 hours for the treatment of patients with MPS VII.

Vestronidase alfa has a half-life of 2.5 h in serum. However, intracellular half-life of vestronidase alfa is approximately 40 days in MPS VII fibroblasts *in vitro*, supporting the QOW dosing frequency.

The proposed dose of 4 mg/kg is supported by the dose-response and exposure-response relationships for the reductions in urinary GAG excretion, including those of the individual urinary GAGs such as CS and DS as well as the total uGAGs. Among the three doses evaluated in clinical trials, the 4 mg/kg dose achieved the greatest reductions of uGAG excretion compared to the 1 mg/kg and 2 mg/kg doses. Because the PD response (i.e., uGAG reductions) appeared to have plateaued at 4 mg/kg over the observed exposure range in three clinical studies, it is not necessary to explore doses higher than 4 mg/kg with an aim to achieve greater exposure.

2.2.2 Therapeutic individualization

Therapeutic individualization is not recommended for subjects of different ages because age did not appear to have an impact on PK and PD.

No alternative dosing is recommended for subjects of different race because the sample size was too small to make a definitive conclusion of the effect of race on PK.

Dose adjustment is not necessary with regard to sex. While the mean CL value was lower in female subjects than in male subjects, the PD response in uGAG appeared similar between the two groups.

2.3 Outstanding Issues

The duration of long-term storage stability was inadequate for samples analyzed at the (b) (4) which conducted the uGAG analysis using the LC-MS/MS method. Evaluation of the long-term sample storage stability is ongoing at (b) (4) the validation results for the 16-month and 20-month storage stability are not available at this time and will not

be available prior to the PDFUA date of November 16, 2017. While the Applicant has not committed to submitting the 16-month and 20-month storage stability data, these additional stability results could be addressed after approval in a post-marketing commitment (PMC) study.

In addition, ISR was not performed for the NRE method by the (b) (4) and for the LC-MS/MS method by (b) (4). Upon requested by the Agency, the Applicant agreed to perform ISR for both the NRE method and LC-MS/MS method (see Section 4.1 for more information about the ISR analysis plan). The ISR results will be the subject of a PMC study.

2.4 Summary of Labeling Recommendations

The underlined text is the proposed addition, while the ~~strikethrough text~~ is the proposed deletion. The changes recommended below are for the team's consideration. Additional modifications may be incorporated by the team in the final label.

6.2 Immunogenicity

(b) (4)

Four treatment naïve patients in Study UX003-CL301 had pre-existing ADA at baseline, and three of them had post-treatment ADA titer values less than fourfold increase from the baseline titer values. One of the three subjects was positive for NAb.

The presence of ADA (b) (4) does not appear to affect reduction in the pharmacodynamic marker, urinary glycosaminoglycans (uGAGs) during clinical trials.

12.2 Pharmacodynamics

(b) (4)

12.3 Pharmacokinetics

The pharmacokinetics of vestronidase alfa-vjbk were evaluated in a total of 129 MPS VII patients including 15 pediatric patients and 4 adults (b) (4) (b) (4)

[redacted] —Serum exposures of vestronidase alfa-vjbk appeared to increase [redacted] more than dose proportionally from 1 mg/kg (0.25 times the approved recommended dosage) to [redacted] in three subjects who received 1 mg/kg (0.25 times the approved recommended dosage), 2 mg/kg (0.5 times the approved recommended dosage), and 4 mg/kg. The mean ± standard deviation (SD) maximal concentration (C_{max}) was 19.8 ± 8.00 µg/mL, with a range of 6.56 to 34.9 µg/mL, and the mean area under the concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) was 57.4 ± 23.9 µg*h/mL, with a range of 18.8 to 97.0 µg*h/mL after repeated dosing of 4 mg/kg every other week. Vestronidase alfa concentrations in pediatric patients less than 5 years of age were similar to the concentrations in older children and adults.

Distribution:

The mean volume of distribution after repeated dosing was 12.6 L, with a range of 6.52 to 22.9 L across treatment weeks.

Metabolism:

The putative catabolism of vestronidase alfa is via peptide hydrolysis.

Elimination:

Following multiple dose administration of MEPSEVII 4 mg/kg every other week in 12 MPS VII patients (9 pediatric patients and 3 adults), the mean clearance value was 3.74 L/h with a range of 1.91 to 6.54 L/h. The inter-subject variability of clearance was 32.8% and the intra-subject variability ranged from 0.98% to 27.6%. Vestronidase alfa-vjbk has a short half-life of 2.59 h, with a range of 0.857 to 3.55 h across treatment weeks.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Drug Product

Vestronidase alfa is a recombinant form of human rhGUS produced in a Chinese Hamster Ovary cell culture line. The protein is expressed as a 651 amino acid precursor with an N-terminal signal sequence of 22 amino acids. The enzyme contains mannose 6-phosphate (M6P) and undergoes cellular uptake and internalization into the lysosome by the CI-M6PR. The mature rhGUS enzyme forms a homotetramer intracellularly after glycosylation. Each vestronidase alfa

monomer consists of 629 amino acids, and the calculated molecular mass of each non-glycosylated peptide chain is approximately 72.5 KDa. The determined molecular weight for each glycosylated monomer is approximately 79.2 kDa.

The to-be-marketed DP, vestronidase alfa for intravenous infusion, is a concentrate for solution containing 2 mg/mL of vestronidase alfa in single-use glass vials filled with 5 mL solution. Vestronidase alfa is the only active ingredient in the DP; other formulation excipients include 20 mM sodium phosphate, 135 mM sodium chloride, 20 mM L-histidine, and 0.01% polysorbate 20 at a pH of 6.0. The visual appearance of the DP solution is clear and colorless to slightly yellow, and essentially free of visible particles. The vestronidase alfa DP does not contain preservatives and should be used immediately after dilution.

The clinical develop program used two formulations of DP, one in pH 6.0 (to-be-marketed) and one in pH (b) (4). The initial pH (b) (4) formulation product was changed to the pH 6.0 formulation product to optimize long-term stability. Both formulations were used in the clinical studies, including UX003-CL201, UX003-CL301, and UX003-CL203. The pH 6.0 formulation is the to-be-marketed product.

Reviewer's Comments

According to the CMC Reviewer Dr. Rukman De Silva, the pH 6.0 and pH (b) (4) products are comparable from a product quality perspective. Hence, additional evaluation between the two products is not necessary from a clinical pharmacology perspective. Please refer to Dr. De Silva's review for more information.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology

Mucopolysaccharidosis VII is a lysosomal disorder characterized by the deficiency of GUS that results in GAG accumulation in cells throughout the body leading to multisystem tissue and organ damage. Vestronidase alfa is intended to provide exogenous GUS enzyme for uptake into cellular lysosomes. M6P residues on vestronidase alfa allow binding of the enzyme to the CI-MPR on the cell surface, leading to cellular uptake of the enzyme. Subsequently, the enzyme is internalized into the lysosomes causing catabolism and reduction of accumulated GAGs in affected tissues.

In vitro, cellular uptake of rhGUS by human MPS VII fibroblasts is saturable with a K_{uptake} of approximately 0.8 – 1.8 nM. Vestronidase alfa intracellular half-life in human MPS VII fibroblasts was determined to be approximately 40 days.

PK

Following a single IV administration of vestronidase alfa, serum concentrations increased and reached maximum concentrations around the end of the infusion at 4 hours. After the infusion,

vestronidase alfa concentrations declined multi-exponentially and remained quantifiable up to the last sampling time-point at 3 or 6 hours post infusion. Following repeated dosing of 4 mg/kg QOW, there was no accumulation of vestronidase alfa in the serum; all but one trough concentrations were below the limit of quantitation (BLQ).

Vestronidase alfa exposure appeared to increase more than dose proportionally from 1 mg/kg to 4 mg/kg dose in a small study (n = 3) with intra-subject dose-escalation/de-escalation design. However, this result might have been confounded by the small number of subjects as well as PK variability.

After repeated dosing of 4 mg/kg QOW in Study UX003-CL301, the mean ± standard deviation (SD) maximal concentration (C_{max}) was 19.8 ± 8.00 µg/mL, with a range of 6.56 to 34.9 µg/mL. The mean area under the concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) was 57.4 ± 23.9 µg*h/mL, with a range of 18.8 to 97.0 µg*h/mL. The PK characteristics at the 4 mg/kg QOW are summarized as below.

Distribution: The mean volume of distribution after repeated dosing was 12.6 L, with a range of 6.52 to 22.9 L across treatment weeks.

Metabolism: The putative catabolism of vestronidase alfa is via peptide hydrolysis.

Elimination: Vestronidase alfa has a short mean (range) half-life ($t_{1/2}$) of 2.59 (0.857 – 3.55) h across treatment weeks. The mean CL value was 3.74 L/h, with a range of 1.91 to 6.54 L/h. Following repeated dosing, the inter-subject variability of CL was 33.7% and the intra-subject variability ranged from 0.98% to 27.6%.

There appeared no significant difference in CL values among subjects of different race, but the number of subjects was too limited to make a definitive conclusion. Mean CL value was lower in female subjects than in male subjects (3.66 vs. 5.09 L/h, p = 0.050). Vestronidase alfa PK did not appear to be different among different age groups.

PD

The PD changes of urinary GAG excretion as determined by the LC-MS/MS method, and those of serum GAG concentration and urinary GAG excretion as determined by the NRE method after the initiation of vestronidase alfa treatment are described below. However, because the NRE method and the LC-MS/MS method were not fully validated, the extent of GAG reductions from baseline could not be ascertained quantitatively and the % GAG reductions are to be considered qualitative or semi-quantitative measurements only.

Following the administration of vestronidase alfa at 4 mg/kg QOW, excretions of urinary CS, DS, combined CS/DS, and total uGAG declined to reach nadirs at around four weeks and then plateaued. Thereafter, reductions in these urinary GAG excretions maintained at approximately 60% to 80% from baseline. The decline is qualitatively substantive although the assay limitations

preclude a quantitative determination of uGAG reduction. Serum concentration of combined CS/DS decreased slowly to approximately 23% from baseline over a longer period of time. However, the temporal profile of serum HS concentration and urinary HS excretion did not show consistent PD response to vestronidase alfa treatment.

The observed PD responses were dose-dependent from 1 mg/kg to 4 mg/kg. The excretion of urinary CD, DS, combined CS/DS, and total uGAG declined with increasing dose of vestronidase alfa. The % uGAG reduction from baseline increased with vestronidase alfa exposure until uGAG response plateaued at a vestronidase alfa C_{max} value of approximately 15 ng/mL and an AUC_{0-t} value of approximately 40 ng*h/mL. No such dose-response or exposure-response was observed for serum CS/DS or HS concentration, or urinary HS excretion.

Vestronidase alfa PD responses did not appear to be different between males and females or among subjects of different age groups.

Although uGAG data cannot be considered quantitative measurements, all subjects displayed consistent reductions from baseline in the excretions of total uGAG and urinary CS, DS, and CS/DS combined. Similarly, consistent E-R relationship was demonstrated for these aforementioned GAG species (see Section 3.3.1). Taken together, the PD data demonstrate pharmacological activity and provide supportive evidence for the effectiveness of vestronidase alfa.

Immunogenicity

Seventeen of 22 subjects (77.3%) enrolled in the clinical trials developed antibodies for vestronidase alfa. The antidrug antibodies (ADA) titers varied among ADA+ subjects, with the highest ADA titers ranging from 10 to 65610. Nine of the 17 (52.9%) ADA positive subjects developed neutralizing antibodies (NAb). Among the nine subjects who are NAb+, one subject had pre-existing ADA titer of 80 at baseline prior to vestronidase alfa treatment and no increase in ADA titer post-treatment.

Between-subject comparison showed that mean CL value was lower in ADA+ subjects than in ADA- subjects (4.21 vs. 5.79 L/h, $p = 0.0103$). Likewise, mean CL value was also lower in NAb+ subjects compared with NAb- subjects (3.77 vs. 5.03, $p = 0.0443$). However, comparison of CL values at different study weeks within ADA+ subjects or within NAb+ subjects did not reveal consistent increase or decrease in CL values. There was no apparent correlation between CL values and ADA titers. Due to the limited sample size, the assessment of the impact of immunogenicity on PK is inconclusive.

Immunogenicity did not appear to have an impact on uGAG reduction or safety in the clinical trials. The impact of immunogenicity on efficacy and safety after long-term treatment remains to be evaluated.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The Clinical Pharmacology data demonstrated pharmacological activity of vestronidase alfa in patients with MPS VII based on the response of the PD markers, i.e., the reduction of urinary GAGs, including total uGAG, and urinary CS, DS, and CS/DS combined. Furthermore, the PD data showed similar dose-response and E-R relationships for the reductions in the aforementioned GAG species (see below). Overall, the demonstration of pharmacological activity constitutes supportive evidence, rather than pivotal evidence, of effectiveness for the treatment of MPS VII patients because the PD markers are not qualified biomarkers, i.e., the reductions in urinary GAGs may or may not be associated with efficacy based on clinical outcomes. In addition, deficiencies were observed in the two assays (NRE and LC-MS/MS methods) used to measure the GAG concentrations in study samples from the clinical trials. As a result, the reliability of the data could not be ascertained.

Specifically, the clinical pharmacology review team identified that ISR was not performed as part of the method validation for both assays, and the long-term sample storage stability data were inadequate to cover the entire duration of sample storage for the LC-MS/MS method. Furthermore, FDA's inspection teams identified objectionable conditions at the two laboratories conducting the GAG analysis that impacted the reliability of the data for UX003-CL301. According to the review from Dr. Srinivas Rao Chennamaneni dated August 23, 2017, the NRE method was not fully validated as quality controls (QCs) were not used during sample analysis; therefore, the NRE method is considered to be semi-quantitative.

On the other hand, there was a lack of sample storage and tracking records, as well as documentation on the purity of the reference standards during the LC-MS/MS method validation and sample analysis. Freshly prepared calibration standards and QCs were not used during method validation, and run acceptable criteria were higher than those recommended in the FDA guidance. Reanalysis was not performed for samples with concentration values at and above the upper limit of quantification. Please see the review from Dr. Himanshu Gupta dated August 18, 2017 for detailed information of the inspection.

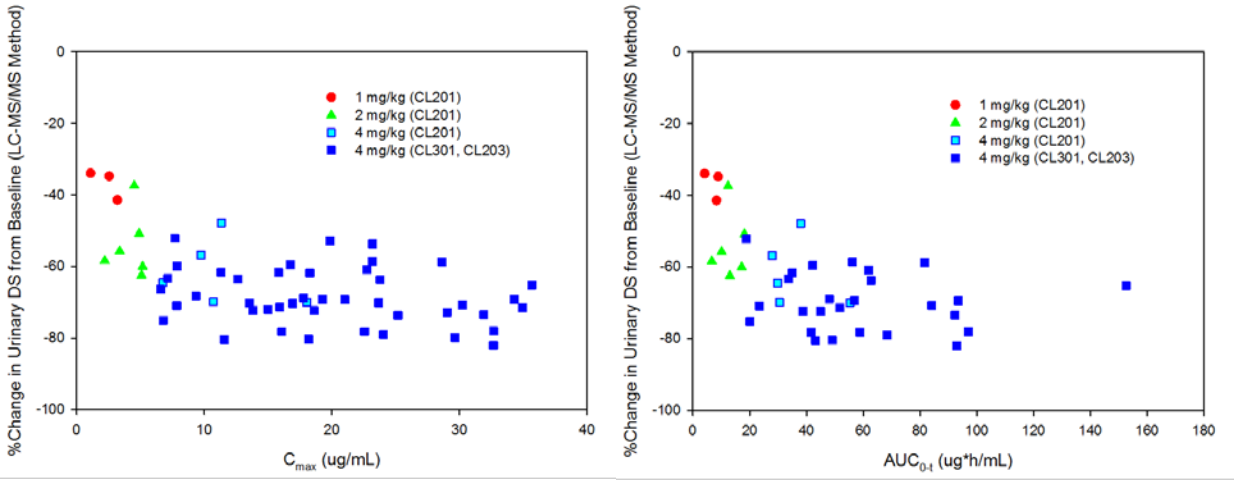
Figure 1 showed the E-R relationship using time-matched PK exposure (measured as C_{max} and AUC_{0-t} of serum concentration of vestronidase alfa) and uGAG response (measured as percent [%] change from baseline) for DS and for combined CS/DS. There was dose-dependent reduction in % uGAG from baseline, and the % uGAG reduction increased with vestronidase alfa exposure until uGAG response plateaued at vestronidase alfa C_{max} and AUC_{0-t} values of approximately 15 ng/mL and 40 ng*h/mL, respectively. The maximal % uGAG reductions from baseline were about 60% - 80% at the 4 mg/kg in 19 subjects, compared to the 25% - 40% reduction at the 1 mg/kg dose and 40% - 60% reduction at the 2 mg/kg dose from 3 subjects who had intra-subjects dose-escalation and/or de-escalation. Importantly, there was a consistent E-R

relationship across individual uGAG species (i.e, urinary CS, DS, and CS/DS combined) and total uGAG (i.e., total of CS, DS, and HS in urine) as measured by two different GAG analysis methods (NRE method by [redacted] (b) (4) and LC-MS/MS method by [redacted] (b) (4) see Section 4.1 for details). We note that an E-R relationship was not observed for urinary HS and serum GAG data measured with either method (Table 1).

Note that Figure 1 presents representative E-R correlation plots using urinary DS as measured by the LC-MS/MS method and urinary CS/DS combined as measured by the NRE method. The E-R analysis results for other GAG species and total uGAG are described in Section 4.4.

Figure 1. E-R analysis using (a) urinary CS/DS combined by the NRE method and (b) urinary DS by the LC-MS/MS method

(a) Urinary DS vs C_{max} and AUC_{0-t}



(b) Urinary CS/DS vs. C_{max} and AUC_{0-t}

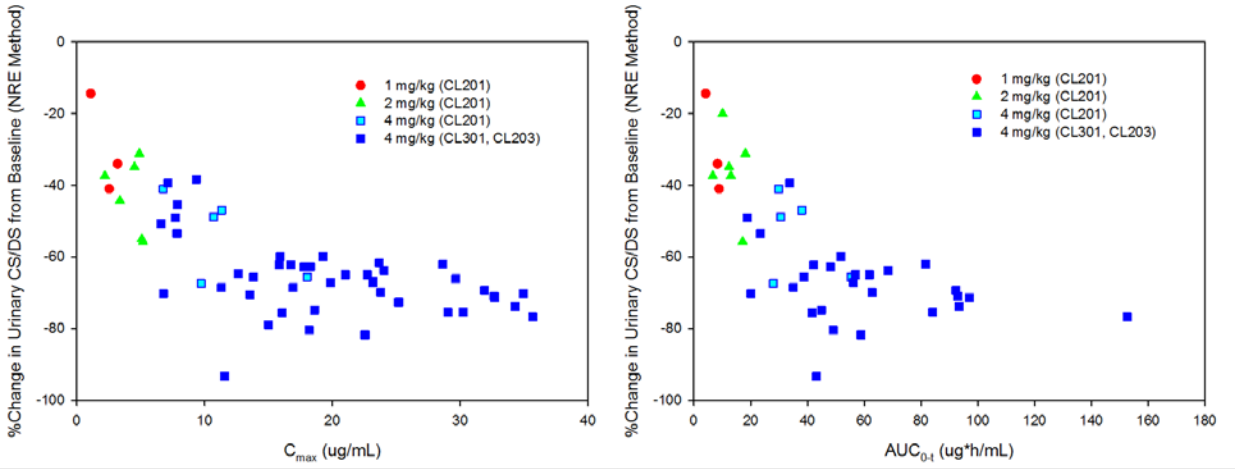


Table 1. Correlations of % reduction in GAG measurements, including individual specific GAG species and total GAG with vestronidase alfa dose or AUC

Biomarker	Positive Correlation with Dose or AUC	
	UPLC-MS/MS Method	NRE Method
Urinary CS	Yes	-
Urinary DS	Yes	-
Urinary HS	No	No
Urinary CS+DS	-	Yes
Urinary CS+DS+HS (i.e., total uGAG)	Yes	-
Serum CS	-	No
Serum CS+DS	-	No

ND: not performed

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimen of 4 mg/kg QOW administered as IV infusion over approximately 4 hours appears appropriate for the treatment of patients with MPS VII based on the E-R relationships of the uGAG biomarkers.

As seen in Figure 1, the greatest % reduction in uGAG from baseline was observed at the 4 mg/kg dose among the three dose levels (1 mg/kg, 2 mg/kg, and 4 mg/kg) evaluated. The PD response increased with increasing exposure and plateaued at vestronidase alfa C_{max} and AUC_{0-t} values of approximately 15 ng/mL and 40 ng*h/mL, respectively, with the observed range being 10 – 30 ng/mL for C_{max} and 40 – 100 ng*h/mL for AUC_{0-t}. These results suggest that the use of higher vestronidase alfa doses resulting in C_{max} higher than 15 ng/mL and AUC_{0-t} higher than 40 ng*h/mL is unlikely to result in further increase in uGAG response.

The non-clinical study UGNX-013 in MPS VII fibroblasts demonstrated an intracellular half-life of approximately 40 days for vestronidase alfa, supporting the QOW dosing frequency. In addition, the study showed that cellular uptake of vestronidase alfa by human MPS VII fibroblasts was saturable with a K_{uptake} (extracellular vestronidase alfa concentration corresponding to 50% of the maximum rate of cellular uptake by target tissues) of approximately 0.8 – 1.8 nM. The mean C_{max} at the 4 mg/kg dose ranged from 15 to 20 µg/mL (equivalent to approximately 47.3 – 63.1 nM) and was approximately 26- to 35-fold higher than the K_{upake}. Hence, uptake of vestronidase alfa is likely to have been saturated at the C_{max} levels achieved at the 4 mg/kg dose as evidenced by the plateau of the E-R curves (Figure 1).

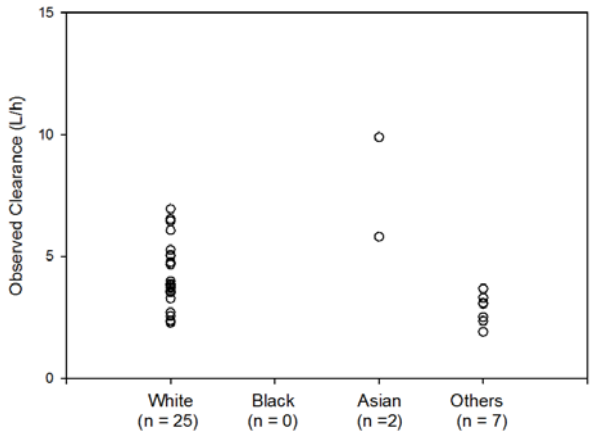
3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

No, an alternative dosing regimen is not necessary for patient subpopulations based on intrinsic factors.

Patients' race did not appear to affect vestronidase alfa PK, but there were very few non-White subjects to draw a definitive conclusion. Specifically, there were 12 White, 1 Asian, 1 Black,

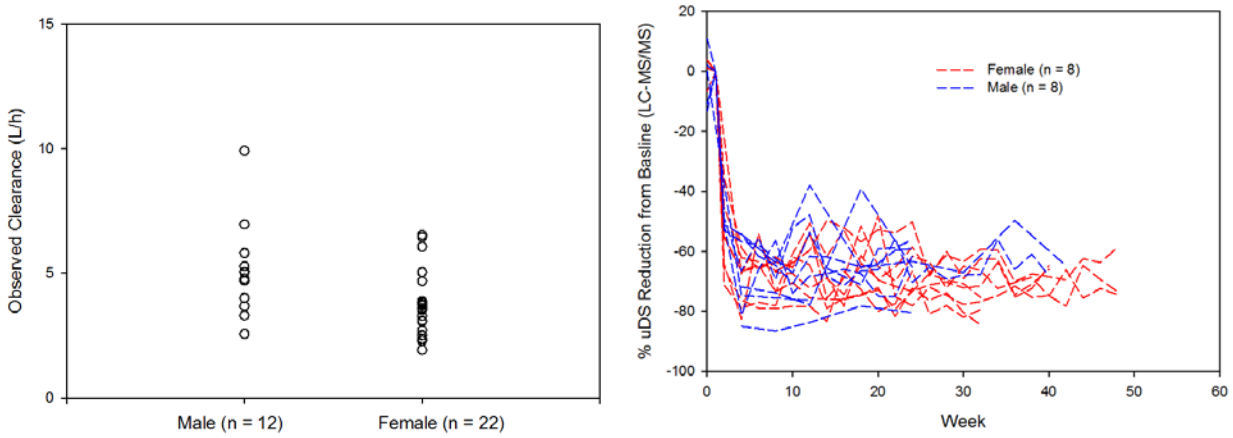
and 5 subjects whose race was described as “others” in the vestronidase alfa program. Among the 19 subjects whose efficacy was evaluated, 15 subjects had reportable CL at the 4 mg/kg dose. The mean ± SD (range) CL values in 11 White subjects across study treatment weeks (a total number of 25 PK profiles) was 4.24 ± 1.35 (2.28 – 6.96) L/h, whereas the mean CL in the 3 “others” with a total of seven PK profiles was 2.85 ± 0.611 (1.91 – 3.67) L/h (Figure 2). The Asian subject had CL values of 5.81 and 9.90 L/h from two study weeks.

Figure 2. Observed CL values among subjects with different race



The CL values at the 4 mg/kg dose were compared between eight male and eight female subjects ≥ 5 years of age across all treatment weeks. Of note, CL value was not available for 4 male subjects from Study UX003-CL203. The mean ± SD (range) CL was lower in female subjects [3.66 ± 1.35 (1.91 – 6.540), n = 22 observations] compared with male subjects [5.09 ± 1.91 (2.56 – 9.90), n = 12 observations] (p = 0.016, t-test) (Figure 3, left panel). However, the reduction in urinary DS did not appear different between eight male and eight female subjects receiving only the 4 mg/kg dose from UX003-CL301 and UX003-203 (Figure 3, right panel). Of note, one male subject had a high CL value of approximately 10 L/h. Excluding this CL value did not affect the statistical significance of the gender difference.

Figure 3. Observed CL value (left panel) and urinary DS response (right panel) in male and female subjects



Note: One of the male subjects < 5 years of age was previously enrolled and treated with vestronidase alfa in an emergency IND. The % reduction in urinary DS level was calculated based on baseline urinary DS level that was measured from a local laboratory rather than the (b) (4)

Figure 4 shows graphical comparisons of vestronidase alfa exposure at the 4 mg/kg dose among subjects of different age groups. In subjects < 5 years of age, vestronidase alfa concentrations were within the concentrate range in subjects 5 – 17 years of age, except for a few early data points (Figure 4, left panel). Concentrations in these youngest subjects were mostly within the concentration range in adults as well, except that a few data points were outside the upper and lower concentration bounds in adults (Figure 4, right panel). Of note, there were only 4 subjects each for adults and for subjects younger than 5 years of age in these comparisons.

Further comparison of data between adults and subjects 5 – 17 years of age showed that concentration-time profiles in the four adult subjects were within the concentration range in subjects 5 – 17 years of age (blue curves in left panel and those in right panel, respectively). Taken together, PK exposure did not seem to differ between adult and pediatric patients.

Likewise, graphical representations of urinary DS reductions after administration of 4 mg/kg dose in different age groups did not reveal significant differences in PD response between the adult and pediatric populations (Figure 5).

Figure 4. Graphical comparisons of vestronidase alfa exposure at the 4 mg/kg dose between subjects < 5 years old and subjects 5 – 17 years old (left panel) and between subjects < 5 years old and adults

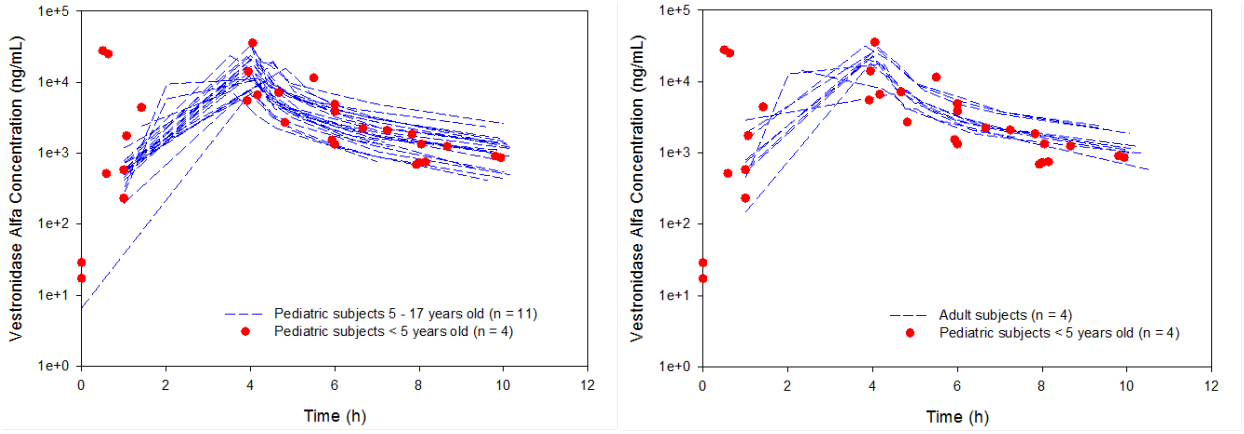
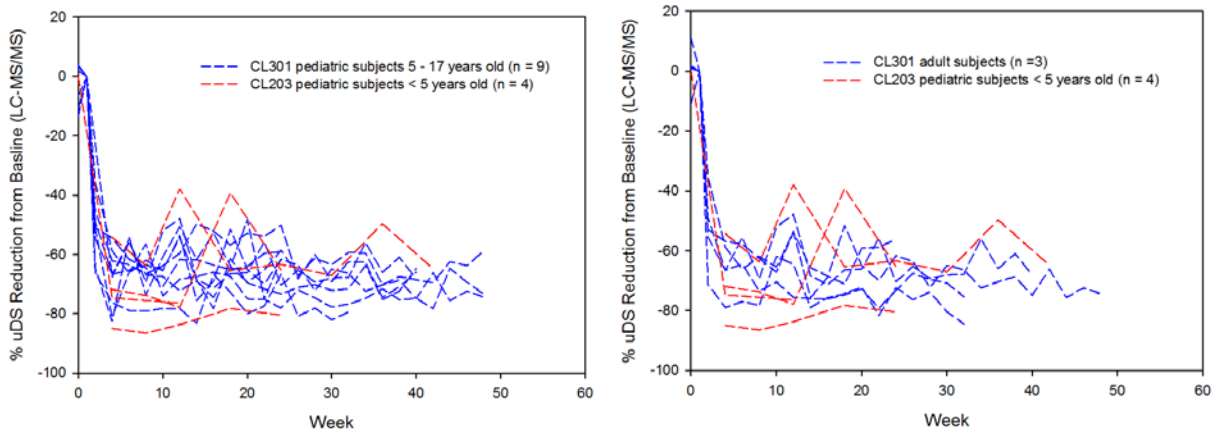


Figure 5 Graphical comparisons of PD response (% urinary DS reductions) at the 4 mg/kg dose between subjects < 5 years old and subjects 5 – 17 years old (left panel) and between subjects < 5 years old and adults



Note: One of the pediatric subjects < 5 years of age was previously enrolled and treated with vestronidase alfa in an emergency IND. The % reduction in urinary DS level was calculated based on baseline urinary DS level that was measured from a local laboratory rather than the (b) (4)

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

No clinically relevant food-drug or drug-drug interactions are anticipated because vestronidase alfa is a therapeutic protein administered intravenously and it is expected to be catabolized via peptide hydrolysis.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

4.1.1 PD Assays

There are various approaches for assessing GAG accumulation in MPS patients and its use as a biomarker. In this BLA, the Applicant used three different methods to analyze clinical samples (serum and urine) for GAGs at three bioanalytical laboratories (Table 2). The three methods are:

1. DMB (1, 9-dimethyl methylene blue) method from (b) (4) ,
2. Sensi-Pro non-reducing ends (NRE) method measuring GAGs by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) from (b) (4), and
3. Liquid chromatography coupled with MS/MS detection (LC-MS/MS) from (b) (4)

As MPS patients excrete significant amounts of GAG fragments in the urine, the most common assay involves measurement of total GAGs in urine samples using the DMB assay. However, because of the low specificity and sensitivity of the DMB assay, the Applicant used the DMB method for measuring screening urinary samples only and the results were not used for data analysis to assess the treatment response. Instead, the Applicant assessed vestronidase alfa PD effects using GAG data obtained from two different GAG analysis methods (LC-MS/MS method and NRE method) (Table 2).

Table 2. Bioanalytical assays for GAG measurements in clinical trials (Source: Table 2.7.1.1.1.1.2 of Module 2.7.1 Summary of Biopharmaceutical and Associated Analytical Methods)

		Samples		
Laboratory	**Assay**	**Serum**	**Urine**	**Analyzed**
(b) (4)	DMB	-	Yes	Screening samples in UX003-CL201
	Urine creatinine	-	Yes	Screening samples in UX003-CL201
(b) (4)	DMB	-	Yes	Screening samples in UX003-CL301
	NRE	Yes	Yes	All samples from UX003-CL201, -CL301, and -CL203
	Urine creatinine	-	Yes	All samples from UX003-CL201, -CL301, and -CL203
(b) (4)	LC-MS/MS	Yes	Yes	All samples from UX003-CL201, -CL301, and -CL203
	Urine creatinine	-	Yes	All Samples from UX003-CL201, -CL301, and -CL203

(b) (4)

Both the LC-MS/MS method and the NRE method have the capability of measuring the individual disaccharide subunit of the GAG species, i.e., CS, DS, and HS. However, the methodology differs between the two assays, and the two methods measure different fragments of GAGs (Table 3). As such, results from the LC-MS/MS method and the NRE method cannot be compared directly.

Table 3. Summary of the two methodologies used for GAG measurements.

	NRE Method	LC-MS/MS Method
Laboratory	(b) (4)	(b) (4)
Digestion steps	Pronase (a mixture of proteases) digestion followed by enzymatic (heparinases or chondroitinase) digestion	Methanol digestion
Derivation of the disaccharide subunits	Tagged with ^{12}C-aniline	No derivation
Internal standards	^{13}C-aniline disaccharide subunits	^{2}H disaccharide subunits
Separation and detection	UPLC-MS/MS	UPLC- MS/MS
GAG species quantified	Serum and urinary GAGs: CS/DS combined and HS	Urinary GAGs: CS, DS, HS, and total GAG (i.e., CS + DS + HS)

The concentration of GAGs in serum samples from clinical studies were analyzed by the NRE method only. On the other hand, urine samples were measured by both the LC-MS/MS and the NRE methods. The results of the urine samples were reported as uGAG excretion which was calculated using the reported urinary concentrations of GAGs divided by the creatinine concentrations in the urine samples. That means concentration values from two separate assays (i.e., GAG analysis method and urine creatinine assay) were used to generate one single value of urinary GAG measurement. In order to provide a reliable value for each uGAG measurement, adequate validation for the GAG analysis method as well as the urine creatinine assay is needed.

During the review cycle, the clinical pharmacology review team sent multiple Information Requests (IRs) on April 28, 2017, June 16, 2017, June 23, 2017, June 27, 2017, and July 31, 2017 regarding the different GAG analysis methods. The issues conveyed in these IRs as well as the Applicant's responses to the IRs are described below for each method. At the time of this review completion, there are two outstanding issues (long term stability and ISR). The Applicant has ongoing experiments to generate data to address these outstanding issues.

GAG Measurement Using DMB Method ((b) (4) ***)***

The DMB assay is a spectrophotometric method that is based on the binding of the DMB dye to uGAGs. The assay has low specificity due to non-specific binding of the dye to other polyanions, including nucleic acids, and inability to distinguish the type of uGAG analyte excreted without further enzymatic or separation methods. The DMB method was used for analyzing screening urinary samples only and the results were not used for data analysis. Therefore, validation of the DMB method (from (b) (4)) and the urine creatinine assay ((b) (4)) is not discussed and included in this review.

GAG Measurement Using NRE Method ([redacted])

The NRE method uses the Sensi-Pro diagnostic system to analyze GAGs in urine and serum. In this method, GAGs are enzymatically depolymerized releasing unique mono-, di-, or trisaccharides (mostly disaccharides) from the non-reducing ends of the chains. The assay quantifies GAGs, CS and DS combined together (CS/DS) as well as HS, after derivatization of the disaccharides with a ^{12}C-aniline, followed by analysis using UPLC-MS/MS.

The NRE assay was validated in urine and serum matrices. Table 4 presents the summary of validation results for the NRE assay.

Table 4. Summary of validation results for the NRE assay performed at [redacted] (Source: Table 2.7.1.1.1.3.1 of Module 2.7.1 Summary of Biopharmaceutical and Associated Analytical Methods)

Validation Parameter	Results
Calibration Curve Range (prepared in water and used for both urine and serum)	HS: 0.04 to 0.60 mg/L CS/DS: 0.02 to 1.0 mg/L
Urine	
LLOQ HS LLOQ CS/DS	0.04 mg/L 0.02 mg/mL
Intra Assay Precision	Low QC: HS: ND CS/DS: 7.1 %CV High QC: HS: 6.1 %CV CS/DS: 4.8 %CV
Inter Assay Precision	Low QC: HS: ND CS/DS: 6.1 %CV High QC: HS: 5.5 %CV CS/DS: 4.0 %CV
Serum	
LLOQ HS and CS/DS	0.02 mg/L
Intra Assay Precision Low QC High QC	Low QC: HS: 2.1 %CV CS/DS: 4.5 %CV High QC: HS: 5.2 %CV CS/DS: 2.9 %CV
Inter Assay Precision Low QC High QC	Low QC: HS: 15.0 %CV CS/DS: 9.7 %CV High QC: HS: 8.4 %CV CS/DS: 2.8 %CV
Urine and Serum	
Carryover, selectivity and Matrix Effect	Acceptable
Reinjection Stability	30 hours at 10°C
Maximum Valid Dilution	5 fold
Freeze Thaw Stability	2 Cycle Freeze-Thaw (Both urine and serum)
Bench Top Stability	2 hours ambient, 24 hours at 2 to 8°C
Long Term Stability	2 years at -80°C
Validation report number	MPSVII-BAMV-REPORT01

HS: HS G0A0 NRE (non reducing end)
CS/DS: CS/DS G0a0 NRE
QC: Quality Control
ND: Not detected
LLOQ : Lower limit of quantification

During the review cycle, the clinical pharmacology review team identified several issues related to the NRE method, which were conveyed to the Applicant through IRs. The issues included freeze-thaw stability, long-term storage stability, reinjection reproducibility, and ISR.

In response to FDA IR dated June 16, 2017, the Applicant submitted (SDN 24 on June 23, 2017) the Addendum dated October 14, 2016 to the Validation Report for the MPS VII SensiPro Assay to provide the updated long-term stability data. The addendum shows that long-term storage stability for serum control samples stored at -60 to -90^{0}C was 708 days for HS and 728 days for CS/DS. The long-term storage stability of urine control samples stored at -60 to -90^{0}C was 709 days for HS and 728 days for CS/DS.

In the IR dated June 23, 2017, the Agency requested that the Applicant perform additional freeze-thaw stability assessment to cover three freeze-thaw cycles experienced by the clinical study samples. The Applicant provided response on July 14, 2017 (SDN 29) to demonstrate that serum and urine samples for GAG measurement were stable up to five freeze-thaw cycles.

In response to FDA IR dated June 23, 2017, the Applicant also submitted (SDN 27 on June 30, 2017) the Addendum dated June 27, 2017 to the Validation Report for the MPS VII SensiPro Assay: Reinjection Reproducibility to update the reinjection stability data. Results demonstrated that reinjection reproducibility in extracts from serum control samples stored at 5 to 15°C was 114 hours for HS internal disaccharides and was 96 hours for CS/HS internal disaccharides. These data ascertain the stability of clinical samples that were stored in the autosampler before injection.

In their response on June 30, 2017 (SDN 27), the Applicant clarified that ISR was not performed for the analyses via the NRE method for serum and urine GAGs (see FDA IR dated June 23, 2017). The Agency further requested that the Applicant perform ISR to address the insufficiency (IR dated July 31, 2017). The Applicant responded on August 25, 2017 (SDN 42) and agreed to conduct ISR for the NRE method. The plan is to perform ISR on 10% of total samples in UX003-CL301, as well as 10% of total existing samples in UX003-CL203 because the NRE was discontinued in December 2016 and is no longer used. The Applicant proposed to submit the ISR report for UX003-CL301in March 2018. The date for the final ISR report of UX003-CL203 is not available because the study is still ongoing.

Urine Creatinine Assay by (b) (4)

Table 5 depicts the summary of validation results for the urine creatinine assay performed at (b) (4).

On June 30, 2017 (SDN 27), the Applicant submitted the report (b) (4)-0401003e to show that urine creatinine control samples were stable for 2 years and 6 months when stored at -10^{0}C or below. The data support the maximum urine sample storage duration ranged from 211 days to

501 days across clinical studies UX003-CL201, -CL301, and -CL203 (all UX003 samples were stored at -80^{0}C) as described in the response to FDA IR dated June 27, 2017.

Table 5. Summary of validation results for the urine creatinine assay performed at (b) (4) (Source: Table 2.7.1.1.1.5.2 of Module 2.7.1 Summary of Biopharmaceutical and Associated Analytical Methods)

Validation Parameter	Results
Analytical Measurement Range	1.1 to 610 mg/dL
Intra-run Precision (% CV)	Low : 0.9 High: 1.07
Inter-run Precision (% Dev)	Low: 4.0 High: 3.4
Accuracy (%Dev)	63 samples covering a range of 3-245 mg/dL were tested in parallel on the Cobas c 501 and on the current method [TOX Olympus (sn3102643)]. The current method was plotted as x values and the new method was plotted as y values resulting in a regression analysis of **y=1.062x -2.506, R^2=0.998**
Sensitivity	LLOQ: 1.5 mg/dL Slope 1.035; intercept -0.656 and observed error of 0.11 mg/dL. The reportable range has been determined to be 1.5-604 mg/dL
At the upper limit of AMR	Upper limit: 604mg/dL Slope, 1.013; intercept, -7.182; and observed error of 3.82 mg/dL.
Sample storage stability	6 months at -20°C
Validation report	(b) (4) VAL-AS-3507

AMR: Analytical measurement range
CV: Coefficient of variation
Dev: Deviation
LLOQ: Lower limit of quantification

Reviewer's Comment

The validation of the urine creatinine assay performed by (b) (4) is acceptable.

GAG Measurement using the LC-MS/MS Method ((b) (4)

The levels of three GAG species (CS, DS, and HS) in human urine were determined by LC-MS/MS at (b) (4) The intact GAGs are treated with methanolic acid to produce uronic or iduronic acid-N-acetylhexosamine or iduronic acid-N-sulfoglucosamine dimers, which are then mixed with internal standards derived from deuteriomethanolysis of GAG standards. Specific dimers derived from CS, DS and HS are separated by UPLC and analyzed by electrospray ionization MS/MS using selected reaction monitoring for each targeted GAG product and its corresponding internal standard. This assay reports the concentration of CS, DS and HS separately relative to the creatinine concentration in the subject's urine.

The LC-MS/MS assay was validated in urine matrix. Table 6 presents the summary of validation results for the LC-MS/MS assay performed at (b) (4)

Table 6. Summary of validation results for the LC-MS/MS assay performed at (b) (4) (Source: Table 2.7.1.1.1.4.1 of Module 2.7.1 Summary of Biopharmaceutical and Associated Analytical Methods)

Validation Parameter	Results
Calibration Standards: Accuracy	At least 4/6 (66.7%) calibrators were within 20% deviation (25% for LLOQ) from expected values in all runs
Calibration Standards: Linearity	$R^2 > 0.98$
Lower Limit of Quantification (LLOQ =5 µg/mL for all analytes)	Inter-run accuracy = -1.5%dev (CS), 7.4%dev (DS) & 1%dev (HS) Inter-run precision = 12.7%CV (CS), 1.3%CV (DS) & 14.3%CV (HS) Intra-run accuracy = 13.6%dev (CS), 5.6%dev (DS) & 15.5%dev (HS) Intra-run precision = 11%CV (CS), 7.4%CV (DS) & 14.8%CV (HS)
Upper Limit of Quantification (ULOQ = 500 µg/mL for all analytes)	Inter-run accuracy = 7.6%dev (CS), 8.2%dev (DS) & 5.6%dev(HS) Inter-run precision = 5.7%CV (CS), 6.5%CV (DS) & 8.5%CV (HS) Intra-run precision not done for ULOQ
Accuracy & Precision: Core Validation	For 25 µg/mL QC: Inter-run accuracy = 6.9%dev (CS), 5%dev (DS) & 6.1%dev (HS) Inter-run precision = 6.4%CV (CS), 1.5%CV (DS) & 10.6%CV (HS) For 250 µg/mL QC: Inter-run accuracy = 7.4%dev (CS), 8.6%dev (DS) & 8.7%dev(HS) Inter-run precision = 7.5% CV (CS), 1.8%CV (DS) & 9.1%CV (HS) Intra-run precision not done for 25 µg/mL & 250 µg/mL QC, but both inter-run and intra-run precision analyzed for four patient samples (%CV < 20% for all analytes except HS in MPS VI samples (below LLOQ) & 24% for DS in MPS III patient (near LLOQ)
Freeze/Thaw Stability	Three concentration levels: 25, 250 & 500 µg/mL: All DS & CS values within 20% of initial value after 3 freeze-thaw cycles. HS value was >20% (22.5%) lower than initial value after 3rd freeze/thaw in the 25 µg/mL sample, but acceptable at other concentrations.
Benchtop Stability	Percent deviation of value from benchtop sample stability run compared to value from original run ("expected value") was <20% for 30/32 samples for CS, 29/30 for DS (2 other values below LLOQ) and 10/12 for HS (20 other values below LLOQ or above ULOQ). Samples are stable for up to 24 hours at room temperature
Long Term Sample Stability	9 months at -20^{0}C, long term stability ongoing
Validation report	ULGX-GAG-01

CV: Coefficient of variation
Dev: Deviation
LLOQ: Lower limit of quantification
ULOQ: Upper limit of quantification
QC: Quality Control

The clinical pharmacology review team identified two issues with the LC-MS/MS method, including inadequacy of long-term sample storage stability evaluation and the lack of ISR in the method validation report.

As shown in Table 6, the long-term storage stability of samples analyzed with the LC-MS/MS method was established for 9 months at -20^{0}C. However, urine samples were stored up to 800 days and 526 days in Studies UX003-CL201 and UX003-CL301, respectively, prior to analysis (based on Appendix 16.1.10 of the Clinical Study Report from both studies). Hence, the Agency

requested that the Applicant provide additional stability data to ascertain the reliability of the urinary GAG data (see FDA IR dated June 27, 2017).

The Applicant provided response to the IR on June 30, 2017 (SDN 29). The Applicant clarified that the long-term storage stability assessment for urine GAGs samples is currently ongoing and further submitted the stability data from the 13-month timepoint. The % difference of the average result obtained after 13 months of storage at -20^{0}C for three human urine samples was within 20% of the initial result for CS and DS in each sample. The HS result for one of the three samples deviated by slightly over 20% from the initial result. While 279 out of 307 (90.9%) samples from Study UX003-CL301 tested were within the 13-month stability time period, only 28 out of 94 (29.8%) samples from Study UX003-CL201 were tested within the 13-month stability time period.

The Applicant further clarified that data from the 16-month stability time-point are expected in early August, at which time all urine samples from the UX003-CL301 study included in PK/PD analysis will be covered by the validated sample stability. Approximately 41% of samples from study UX003-CL201 will be covered by the 16-month stability time-point. From there, the next stability timepoint would be 20 months and the corresponding data will not be available prior to the PDFUA date of November 16, 2017. Sample stability testing will be continued in order to cover the UX003-CL201 sample storage period of 800 days. The Applicant did not provide the timeline for the submission of the 16-month and 20-month stability data.

On July 14, 2017 (SDN), the Applicant provided a list of urine samples analyzed outside the storage stability limits of 13 months and 16 months in Studies UX003-CL201 and UX003-CL301 per FDA request (IR dated June 27, 2017).

In response to FDA IR dated June 23, 2017, the Applicant clarified that ISR was not performed for the analyses via the LC-MS/MS method for urine GAGs (see SDN 27 on June 30, 2017). The Agency further requested that the Applicant perform ISR to address the insufficiency (IR dated July 31, 2017). The Applicant responded on August 25, 2017 (SDN 42) and agreed to conduct ISR for the LC-MS/MS method. The plan is to perform ISR on 10% of total samples in UX003-CL301, -CL202, and –CL203. The Applicant will not conduct ISR for UX003-CL201 because study samples were stored longer than validated sample stability. The Applicant proposed to submit the ISR report for UX003-CL301in January 2018.

Reviewer's Comments

Based on the list of urine samples analyzed outside of the storage stability limit of 13-month provided by the Applicant, all PD data points from Study UX003-CL201 and three PD data points from Study UX003-CL301 are deemed not reliable for E-R analysis. Excluding these data points does not significantly affect the E-R analysis results however, as data from Study UX003-CL301 constitute majority of the data points used in the E-R analysis. In addition, similar E-R relationship was observed using data from the NRE method despite the semi-quantitative nature

of the assay. Consistency across uGAG data from the LC-MS/MS method and the NRE method provides assurance that the uGAG data support demonstration of pharmacological activity of vestronidase alfa.

Nonetheless, the Applicant should provide additional long-term sample storage stability data to support storage durations for all samples from the clinical trials.

Urine Creatinine Assay by (b) (4)

Table 7 depicts the summary of validation results for the urine creatinine assay performed at (b) (4)

Table 7. Summary of validation results for the urine creatinine assay performed at (b) (4) (Source: Table 2.7.1.1.1.5.2 of Module 2.7.1 Summary of Biopharmaceutical and Associated Analytical Methods)

Validation Parameter	Results
Analytical Measurement Range	0.05- 5 mg/L
Intra-run Precision (% CV)	Control: 1.49 Low : 5.47 Medium : 5.42 High: 2.56
Inter run Precision (% Dev)	Control: 12.66
Accuracy (%Dev)	Low : -6.6 Medium : -8.88 High: -14.19
Sensitivity	LLOQ: 0.05 mg/L %Dev: -6.6 % CV: 5.47
At the upper limit of AMR	Upper limit: 5mg/mL %Dev: -14.19 %CV: 2.56
Sample stability	3 years at -20°C
Validation summary	B-7730

AMR: Analytical measurement range
CV: Coefficient of variation
Dev: Deviation
LLOQ: Lower limit of quantification

Reviewer's Comment

The validation of the urine creatinine assay performed by (b) (4) *is acceptable.*

4.1.2 PK Assay

A beta-glucuronidase activity assay was used to measure GUS concentration in human serum. Serum samples are diluted in assay buffer and incubated with the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (4-MUG), which is hydrolyzed by endogenous and/or vestronidase alfa to produce the fluorescent product 4-methylumbelliferone (4-MU). The 4-MU-related fluorescence is quantified (excitation at 365nm; emission at 445nm), and the amounts of

enzyme are determined using a 4-MU standard curve. Standard curves were fitted by 4-parameter curve fitting (Softmax® PRO software). The activity unit (i.e., IU) is defined as the amount of enzyme that converts 1 nmole of 4-MUG into 4-MU per hour at 37ºC.

Table 8 presents the summary of validation results for the PK assay of GUS activity in human serum.

Table 8. Summary of validation results for PK assay of GUS activity in human serum (Source: Table 2.7.1.1.2.1.1 of Module 2.7.1 Summary of Biopharmaceutical and Associated Analytical Methods)

Validation Parameter	Results
Accuracy	%RE ≤ 2.9%
Intra Assay Precision	%CV ≤ 5.3%
Inter Assay Precision	%CV ≤ 14.0%
Linearity	r2 range 0.996 to 1.000
Back-calculated Standards	%RE -1.9 to +3.3%
Minimal serum dilution	10 fold
Maximal serum dilution	16,000 fold
Standard Curve Range	1 to 100 µM
Sample stability	12 months at -80°C
Validation report number	(b) (4) -000675

CV: Coefficient of variation
RE: Relative error

The ISR results are included in the respective CSR Section 16.1.10, titled "Documentation of Inter-Laboratory Standardisation Methods and Quality Assurance Procedures if Used", for Studies UX003-CL301 and UX003-CL203; the ISR results for samples analyzed in Studies UX-CL301 and UX003-CL203 are acceptable. ISR was not performed during the bioanalytical sample analysis study for UX003-CL201.

4.1.3 Immunogenicity Assays

ADA Assay

ADA was determined using a bridging electrochemiluminescent assay (ECL) assay in which the bridging format utilizes the bivalency of the antibody molecule. A Meso Scale Discovery (MSD) Streptavidin Gold 96-well plate is prepared by blocking with 3% BSA. A mixture of biotinylated and ruthenylated vestronidase alfa is prepared in assay buffer. Test samples and controls are diluted 10-fold in assay buffer, pre-incubated with a mixture of biotinylated and ruthenylated vestronidase alfa, and then added to duplicate wells of the streptavidin-coated MSD plate. The samples and controls are incubated for approximately 1 hour at room temperature. Then the wells are aspirated, washed and read buffer added to each well. The plates are read on the MSD plate reader after brief shaking. ADA form a bridge between the biotinylated

vestronidase alfa bound to the plate and the ruthenium-labeled vestronidase alfa that is quantified.

NAb Assay

The NAb assay is a modified version of a bioassay by cellular uptake in fibroblasts. It evaluates the potential for anti-rhGUS antibodies present in patient serum to impact the uptake and activity of rhGUS into GUS-deficient fibroblasts based on the measurement of enzyme activity in fibroblasts. In brief, patient serum samples and antibody controls are pre-incubated with 5 nM vestronidase alfa for a minimum of 30 minutes. Cells are then treated with the samples and controls in assay medium for approximately 2 hours, rinsed and treated with assay medium, and frozen at <10°C for 2 hours to 1 week. The thawed lysate is then incubated with 25 µL of 4 mM 4MUG solution for approximately 30 minutes. The fluorescence signal developed is then detected after treatment with the stop buffer.

The cell based assay is qualitative and not quantitative; the results are either positive or negative. A positive result leads to very low enzyme activity and a very low signal. A negative result, with no neutralizing activity, will have high enzyme activity and a high signal.

Table 9 summarizes the sensitivity and drug tolerance of the ADA and NAb assays.

Table 9. Summary of ADA and NAb sensitivity and drug tolerance (Adapted from Tables 2.7.1.1.2.2.4 and 2.7.1.1.2.3.1 of Module 2.7.1 Summary of Biopharmaceutical and Associated Analytical Methods)

	ADA	NAb
Sensitivity	3.66 ng/mL	17.8 µg/mL
Drug tolerance	5 µg/mL rhGUS	1.13 µg/mL rhGUS

Reviewer's Comments

The ADA assay is sensitive, but the NAb assay is not given that it has a sensitivity of 17.8 µg/mL. Nonetheless, nine of the 16 subjects in UX003-CL301 were tested positive for NAb. Therefore, the CMC Review Team considers the NAb assay acceptable and will not recommend PMC/PMR study for a more sensitive NAb assay. Please refer to CMC review by Dr. Jacek Cieslak for more information regarding the immunogenicity assays.

4.2 Evaluation of Clinical Relevance of GAG Measurements

Background - GAGs and MPS VII

MPS consists of a subset of lysosomal storage diseases in which deficiencies occur in one or more enzymes involved in the degradation of GAGs. Five types of GAGs exist: HS, CS, DS, hyaluronan (HA), and keratan sulfate (KS). A family of at least 11 enzymes catalyzes the lysosomal degradation of GAGs. Degradation of the GAG chains occurs in a directional manner by removal or processing of the terminal sugar on the NRE of the GAG chain. Due to the

sequential nature of the degradative process, mutations in any enzyme in the pathway result in accumulation of lysosomal storage of undegraded GAGs; the composition of which depends on the specific enzyme deficiency. In addition to the lysosomal enzymes, an extracellular endoglycosidase (heparanase) can cleave HS chains at specific sites, giving rise to new NREs that are acted on by the catabolic exo-enzymes.[1]

In MPS VII, the deficiency of GUS enzyme leads to the accumulation of the glucuronic acid-containing GAGs, including CS, DS, and HS. CS is a major component of the extracellular matrix (ECM) of cartilage; it is involved in the regulation of assembly and maintenance of the ECM and participates in cell proliferation through interaction with growth factors, including fibroblast growth factors, hepatocyte growth factors, brain-derived growth factor, pleiotrophin, and midkine. Several CS-containing proteoglycans are known to regulate collagen fibrillogenesis and to play an important role in the physiology and biomechanical function of tendons and ligaments.[2]

DS is the predominant GAG of skin, but it is expressed in many mammalian tissues including blood vessels, heart valves, tendons, and lungs. This tissue distribution coincides with the involvement of DS in cardiovascular disease, tumorigenesis, infection, wound healing, and fibrosis.[2]

HS is found at the cell surface and in the ECM and binds to a plethora of ligands, regulating a wide variety of biological activities, including developmental processes, angiogenesis, blood coagulation, cell-ECM attachment, cell-cell interactions, and cell motility. HS-containing proteoglycans can also act as receptors for proteases and protease inhibitors, regulating their spatial distribution and activity.[2]

While the enzymatic, genetic, and molecular bases have been determined for many of the lysosomal diseases, the downstream or secondary biochemical and cellular pathways that are affected in these diseases, and that result in cell and tissue dysfunction and therefore pathology are not known.

Evaluation of CS, DS, and HS Data from Study Samples at Baseline

Using study samples at baseline prior to vestronidase alfa treatment, the reviewer summarizes the endogenous serum concentrations and baseline urinary excretion of CS, DS, and HS in 19 subjects enrolled in the clinical studies. As depicted in Table 10, the median urinary CS/DS excretion as determined by the NRE method was approximately 100-fold lower than the combined median excretion of urinary CS and urinary DS as determined by the LC-MS/MS method. In addition, the median (range) urinary CS/DS to urinary HS ratio was 23.3 (5.67 - 117) as determined by the NRE method and was 245 (84.5 - 562) as determined by the LC-MS/MS method, demonstrating the relatively limited contribution of urinary HS excretion in the overall total uGAG excretion. We note that direct comparisons of results from both methods are not

appropriate. Rather, the NRE method and the LC-MS/MS method offer orthogonal approaches to evaluating the GAGs in patients with MPS VII.

Table 10. Summary of GAG measurements at baseline prior to treatment

GAG Analyte	Mean	SD	Median	Min	Max
NRE					
Serum CS/DS (mg/L)	0.714	0.136	0.675	0.530	0.950
Urinary CS/DS (mg/mg creatinine)	0.0227	0.0627	0.0058	0.0016	0.279
Serum HS (mg/L)	0.0278	0.0055	0.0300	0.0200	0.0400
Urinary HS (mg/mg creatinine)	0.0005	0.0005	0.0003	0.0002	0.0024
Serum CS/DS to HS Ratio	27.4	10.4	23.0	14.5	47.5
Urinary CS/DS to HS Ratio	27.7	25.0	23.3	5.67	117
LC-MS/MS					
Urinary CS (g/g creatinine)	1.18	1.78	0.679	0.236	8.175
Urinary DS (g/g creatinine)	1.80	0.926	1.51	0.834	4.90
Urinary HS (g/g creatinine)	0.0119	0.0066	0.0098	0.00446	0.0272
Total uGAG (g/g creatinine)	2.99	2.65	2.15	1.45	13.1
Urinary (CS+DS) to HS Ratio	264	132	245	84.5	562

Note: g/g creatinine = mg/mg creatinine

Reference Range for GAGs in Serum and Urine

The normal reference ranges for serum and urine GAGs from the NRE method and the LC-MS/MS method are included in Table 11 and Table 12 for comparison. Refer to the Applicant's response to FDA IR received May 9, 2017 (SDN 15 on May 15, 2017) for more information about the reference ranges.

Table 11. Normal reference ranges for serum and uGAGs from the NRE assay at (b) (4) (Source: response to FDA IR received May 9, 2017)

	Serum GAG Normal Reference Range (mg/L)	
	CS/DS	HS
	0.00221 – 0.00831	< 0.00354
	Urine GAG Normal Reference Range (mg/mg creatinine)	
Age	CS/DS	HS
< 1 years	0.000530 – 0.00486	< 0.00124
1 – 10 years	0.000084 – 0.00115	< 0.00265
>10 years	< 0.00530	< 0.00265

Table 12. Normal reference ranges for uGAGs from the LC-MS/MS assay at (b) (4) (Source: response to FDA IR received May 9, 2017)

	Urine GAG Normal Reference Range (g/g creatinine)		
Age	CS	DS	HS
0 – 4 months	< 0.479	< 0.192	< 0.0450
5 – 18 months	< 0.277	< 0.105	< 0.0450
18 months – 2 years	< 0.120	< 0.0796	< 0.0450
3 – 5 years	< 0.0990	< 0.0698	< 0.0450
6 – 10 years	< 0.0751	< 0.0707	< 0.0450
11-14 years	< 0.0636	< 0.0539	< 0.0450
>14 years	< 0.0230	< 0.0513	< 0.0450

Reviewer's Comments

At baseline, median urinary HS excretion was 23.3 fold lower than the concentrations of urinary CS/DS excretion as determined by the NRE method and was 245 fold lower than the combined urinary CS and urinary DS excretion as determined by the LC-MS/MS method. In addition, urinary HS levels were in the normal range for all subjects in UX003-CL201 and were mildly elevated in UX003-CL301 subjects. Hence, the % changes from baseline in urinary HS excretion were minimal when compared to the % changes from baseline in combined urinary CS and urinary DS excretion. Because the pattern and magnitude of reductions in urinary CS and urinary DS were similar (data not shown), the reductions in total uGAG excretion are driven mostly by the combined excretion of urinary CS and urinary DS excretion.

Evaluation of GAGs by Age Group

The reviewer has performed comparison of serum and urine GAG data among subjects in three different age groups. Whereas median serum CS/DS concentrations appeared similar among the three age groups, median urinary GAG excretion was highest in subjects < 5 years of age, followed by subjects aged 5 – 17 years, and then adult subjects (Table 13). This trend seemed to be consistent among different urinary GAG measurements regardless of the assay method used. Similar trends of decreasing GAG values with increasing age are also observed with the normal reference ranges of uGAGs (Table 12).

Table 13. Median (range) of GAG measurements among different age groups

	Median (Range)			
GAG Analyte	< 5 years old (n = 4)	5 – 17 years old (n = 11)	≥ 18 years old (n = 4)	P value*
NRE				
Serum CS/DS	0.770 (0.580 – 0.950)	0.6500 (0.530 – 0.910)	0.7200 (0.670 – 0.835)	0.394
Urinary CS/DS (g/g creatinine)	0.0306 (0.00587 – 0.279)	0.0074 (0.0016 – 0.0106)	0.0025 (0.0018 – 0.0053)	0.015
Serum HS	0.0200 (0.0200 – 0.0300)	0.0300 (0.0200 – 0.0400)	0.0300 (0.0200 – 0.0300)	0.261
Urinary HS (g/g creatinine)	0.0009 (0.0003 – 0.0024)	0.0003 (0.0002 – 0.0004)	0.0002 (0.0002 – 0.0005)	0.031
Serum CS/DS to HS Ratio	29.0 (25.7 – 47.5)	21.7 (14.5 – 45.5)	24.0 (22.3 – 41.8)	0.126
Urinary CS/DS to HS Ratio	42.7 (13.5 – 117)	23.5000 (9.00 – 30.9)	10.5833 (5.67 – 30.0)	0.161
LC-MS/MS				
Urinary CS (g/g creatinine)	2.06 (0.810 – 8.17)	0.679 (0.369 – 1.01)	0.412 (0.236 – 0.623)	0.010
Urinary DS (g/g creatinine)	2.72 (0.834 – 4.90)	1.51 (1.15 – 2.40)	1.23 (1.14 – 1.75)	0.190
Urinary HS (g/g creatinine)	0.0151 (0.0074 – 0.0247)	0.0118 (0.0045 – 0.272)	0.0070 (0.0054 – 0.206)	0.417
Total uGAG (g/g creatinine)	4.80 (1.65 – 13.1)	2.15 (1.62 – 3.32)	1.63 (1.45 – 2.38)	0.067
Urinary (CS+DS) to HS Ratio	399 (168 – 562)	208 (115 – 434)	224 (84.5 – 419)	0.375

*Kruskal-Wallis one-way analysis of variance

4.3 Clinical PK and PD Assessments

PK and PD assessments were performed in all three clinical studies UX003-CL201, UX003-CL301, and UX003-CL203. The Applicant used the AUC_{0-t} values as the exposure metric in the E-R analyses; these AUC_{0-t} values were 85.4% to 95.7% of the AUC_{0-inf} values. In other words, the % AUC extrapolated ranged from 4.28% to 14.6% for PK exposure. On the other hand, the reviewer used dose-normalized AUC_{0-inf} values for evaluating dose proportionality due to different sampling durations among PK profiles in UX003-CL201.

Table 14 summarized the study design as well as PK, PD, and immunogenicity assessment schedules in the three clinical studies. Of note, in Study UX003-CL201, PK sampling were initially performed up to 180 minutes post-infusion, and most of the PK profiles had a duration of approximately 7 hours from the start of the infusion (i.e., 3 hours after the end of infusion).

Table 14. Study design of clinical studies UX003-CL201, -CL301, and CL-203 (Source: Tables 3-1 and 3-2 of Module 5.3.4.2 PK/PD Report)

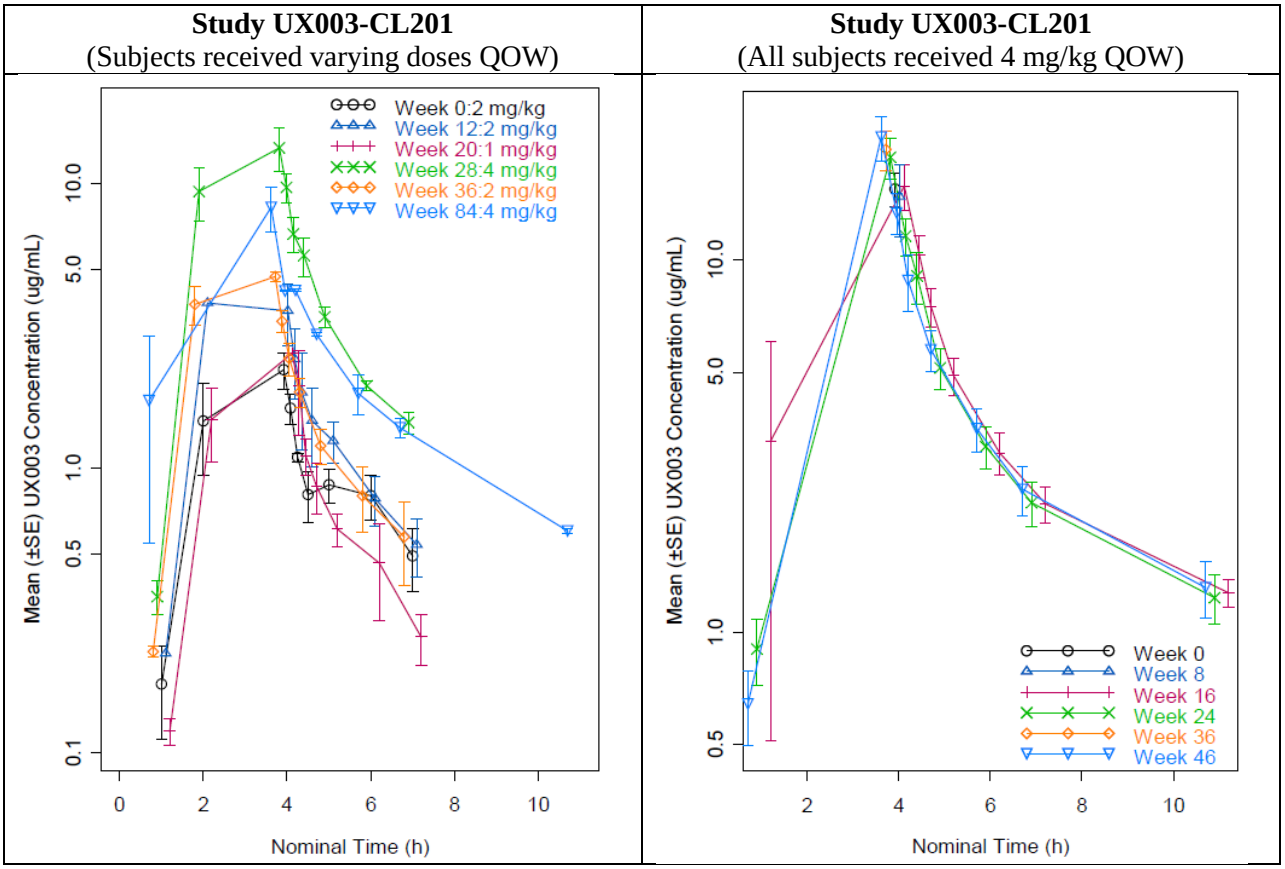
Study	Design	Population	Dose	PK/Immunogenicity Assessment	PD Assessment
UX003-CL201	Open-label, intra-subject dose titration study to assess the safety, efficacy, and the PK and PD	MPS 7 patients aged 5-30 years (n = 3)	Initial Period W0 - W12: 2 mg/kg QOW Forced-Dose Titration Period W12 - W20: 1 mg/kg QOW W20 - W28: 4 mg/kg QOW W28 - W36: 2 mg/kg QOW Continuation Period W36 – W84: 2 mg/kg QOW Long-Term Extension Period W84 onwards: 4 mg/kg QOW	PK W0 (baseline), 12, 20, 28, 36 and 84: pre-dose, 60 and 120 minutes after start of infusion, at the end of infusion, 5, 15, 30, 60, 120, 180 minutes post-infusion, and (6-8 hours post-infusion after Amendment 2) Immunogenicity W0 (baseline), 4, 8, 12, 22, 30, 36, 48, 60, 72, 84, 96, 108 and 120	Urinary GAG excretion W0, 2, 4, 6, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 48, 60 and 72 Liver and spleen volumes W0, 12 and 36
UX003-CL301	Randomized, placebo-controlled, blind-start, single-crossover Phase 3 study to assess the efficacy and safety	MPS 7 patients aged 5-35 years (n = 12)	Group A W0 - W48: 4 mg/kg QOW Group B W0 - W8: placebo QOW W8 - W48: 4 mg/kg QOW Group C W0 - W16: placebo QOW W16 - W48: 4 mg/kg QOW Group D W0 - W24: placebo QOW W24 - W48: 4 mg/kg QOW	PK W0, 8 and 36: pre-dose and end of infusion W16, 24 and 46: pre-dose, 60 minutes after start of infusion, at the end of infusion, 15, 30, 60, 120, 180 minutes post-infusion, and 6-8 hours post-infusion Immunogenicity W0 (baseline), 8, 12, 16, 20, 24, 28, 32, 36, 40, 46	Urinary GAG excretion W0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48 Liver and spleen volumes W0 and 48
UX003-CL203	Open-label, single-arm, multicenter, Phase 2 study to assess the safety and efficacy	MPS 7 patients < 5 years (n = 4)	W0 - W48: 4 mg/kg QOW	PK: W (baseline), 24 and 48: pre-dose, 60 minutes after start of infusion, at the end of infusion, 30-120 minutes post-infusion and 4-6 hours post-infusion Immunogenicity W0 (baseline), 8, 16, 24, 32, 40	Urinary GAG excretion W0, 4, 8, 12, 18, 24, 30, 36, 42 and 48 Liver and spleen volumes W0, 12, 24 and 48

With Protocol Amendment 2, an additional PK sampling timepoint was added at 6 – 8 hours post infusion, and the PK profile at Week 84 from two subjects was extended for approximately 10 hours from the start of infusion. In comparison, the duration of the PK profiles was approximately 10 hours from the start of the infusion (i.e., 6 hours after the end of infusion) in UX003-CL301 and approximately 8 hours from the start of the infusion (i.e., 4 hours after the end of infusion) in UX003-CL203.

PK Assessments

Following IV infusion of vestronidase alfa, serum concentrations increased and reached maximum concentrations around the end of the infusion at 4 hours (Figure 6). After the infusion, vestronidase alfa concentrations declined multi-exponentially and remained quantifiable up to the last sampling time-point at 10 hours post infusion in Study UX003-CL201. All but one of the pre-dose concentrations in Studies UX003-CL201 and UX003-CL301 were BLQ, indicating little to no accumulation of vestronidase alfa in the serum upon repeated QOW dosing.

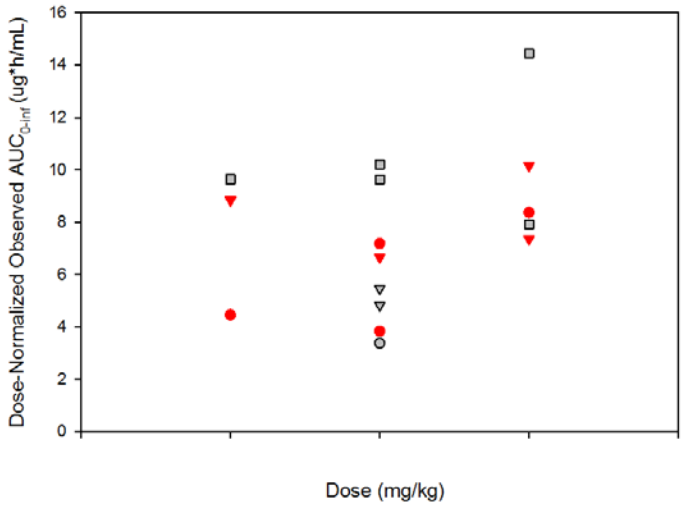
Figure 6. Mean vestronidase alfa concentration versus time profiles by study week in UX003-CL201 (left panel) and UX003-CL301 (right panel) (Source: Figures 5-1 and 5-5 of Module 5.3.4.2 PK/PD Report)



The reviewer evaluated dose proportionality graphically using PK data from three subjects in Study UX003-CL201, who received doses of 1 mg/kg, 2 mg/kg and 4 mg/kg via intra-subject dose-escalation and de-escalation. Overall, dose-normalized $AUC_{0\text{-inf}}$ ranged from 3.37 – 10.2 µg*h/mL across doses of 1 mg/kg to 4 mg/kg. Dose-normalized $AUC_{0\text{-inf}}$ values appeared to be higher in the 4 mg/kg dose compared to the 1 mg/kg and 2 mg/kg doses, but this may be due to one high AUC value in the 4 mg/kg dose group. Without this data point, vestronidase alfa exposure appeared to increase dose proportionally across 1 mg/kg to 4 mg/kg because the range of data were similar across three dose levels (Figure 7).

Study samples from two of the three subjects (square and triangle symbols in Figure 7) in UX003-CL201 were tested positive for ADA at least once during the study. Comparison of dose-normalized $AUC_{0\text{-inf}}$ values within these two subjects showed higher AUC values when ADA was positive at the PK assessment weeks (red squares and triangles) compared to the AUC values when ADA was negative at the PK assessment week (grey squares and triangles). However, similar AUC difference was also observed in AUC values between two ADA negative study weeks at the 4 mg/kg dose (grey squares). Overall, the data suggest minimal impact of immunogenicity on exposure. Due to the small number of subjects in this analysis, the assessment of the impact of immunogenicity on PK is inconclusive. Please refer to Section 4.5 for more information regarding the assessment of the impact of immunogenicity on PK.

Figure 7. Dose-normalized $AUC_{0\text{-inf}}$ over time in Study UX003-CL201. Each symbol represents AUC data for each individual subject. The grey color symbols represent AUC values at study weeks when ADA was negative and red color symbols represent AUC values at study weeks when ADA was positive.



In Study UX003-CL301, all 12 subjects received the 4 mg/kg QOW dosing but each of the four cohorts (n=3 each) was treated for a different duration of vestronidase alfa. Specifically, treatment started on Week 0 for Group A, Week 8 for Group B, Week 16 for Group C, and Week 24 for Group D. Table 15 summarizes the PK parameter values after the first dose and following multiple dosing of vestronidase alfa.

Table 15. Mean ± S.D. PK parameter values at the 4 mg/kg dose by treatment weeks in UX003-CL301

Treatment Week (Group)	Mean ± S.D Median (Min, Max)								
	C_{max} (µg/mL)	$^{a}T_{max}$ (h)	N	$t_{1/2}$ (h)	AUC_{0-t} (µg*h/mL)	AUC_{0-inf} (µg*h/mL)	CL (L/h)	V_{ss} (L)	N
1st dose (A*, B*, C, D)	14.5 ± 5.05 14.4 (7.24, 23.2)	3.80 ± 0.495 3.92 (2.33, 4.32)	12	3.01 ± 0.784 2.85 (2.27, 4.36)	45.8 ± 14.9 51.7 (21.8, 60.4)	50.2 ± 17.0 58.2 (23.1, 66.6)	4.50 ± 1.48 4.31 (3.07, 6.96)	14.3 ± 4.95 13.4 (8.60, 21.9)	6
8 (A, B, C)	19.6 ± 7.60 21.0 (6.56, 31.9)	3.87 ± 0.147 3.92 (3.52, 4.00)	9	3.00 ± 0.590 3.11 (2.23, 3.55)	69.1 ± 16.3 63.6 (56.9, 92.3)	75.4 ± 16.7 72.1 (63.3, 98.4)	3.49 ± 0.100 3.42 (2.35, 4.77)	12.3 ± 3.55 10.8 (10.0, 17.6)	4
12 (D*)	16.1 ± 6.93 15.8 (9.34, 23.2)	4.14 ± 0.313 3.97 (3.95, 4.50)	3	--	--	--	--	--	--
16 (A, B)	15.8 ± 4.23 16.9 (7.69, 19.8)	4.10 ± 0.365 3.97 (3.87, 4.83)	6	2.36 ± 0.693 2.60 (1.38, 2.86)	40.1 ± 14.8 44.9 (18.8, 51.7)	44.0 ± 16.3 49.6 (20.3, 56.4)	3.83 ± 1.38 3.88 (2.28, 5.27)	12.8 ± 5.59 12.3 (7.98, 18.6)	4
20 (C*)	24.6 ± 8.29 29.0 (15.0, 29.7)	3.93 ± 0.058 3.90 (3.90, 4.00)	3	--	--	--	--	--	--
22 (D)	17.4 ± 11.0 16.7 (6.77, 28.6)	4.01 ± 0.100 4.00 (3.92, 4.12)	3	2.72 ± 0.280 2.65 (2.48, 3.03)	48.0 ± 31.2 42.2 (20.1, 81.6)	52.6 ± 35.3 45.0 (21.7, 91.0)	4.12 ± 0.516 4.00 (3.68, 4.69)	13.6 ± 2.36 12.5 (12.0, 16.3)	3
24 (A)	22.4 ± 10.7 23.2 (11.3, 32.7)	3.95 ± 0.058 3.92 (3.92, 4.02)	3	2.38 ± 0.644 2.75 (1.64, 2.76)	62.7 ± 31.5 56.2 (35.0, 97.3)	68.3 ± 32.2 62.7 (39.2, 103)	4.06 ± 1.87 3.75 (2.37, 6.07)	13.1 ± 7.64 10.3 (7.31, 21.8)	3
28 (B*)	13.6 ± 5.70 13.5 (7.86, 19.3)	3.96 ± 0.051 3.97 (3.90, 4.00)	3	--	--	--	--	--	--
30 (C)	27.0 ± 7.76 30.2 (18.2, 32.7)	4.02 ± 0.098 3.98 (3.95, 4.13)	3	2.98 ± 0.034 2.99 (2.94, 3.00)	75.3 ± 23.2 84.1 (49.0, 92.9)	84.2 ± 26.9 94.5 (53.7, 104)	3.10 ± 1.05 3.52 (1.91, 3.88)	11.3 ± 3.39 12.8 (7.41, 13.6)	3
36 (A*)	25.2 ± 9.10 23.7 (16.9, 34.9)	3.94 ± 0.025 3.93 (3.92, 3.97)	3	--	--	--	--	--	--
38 (B)	16.4 ± 7.69 18.6 (7.84, 22.7)	4.10 ± 0.126 4.08 (3.98, 4.23)	3	2.59 ± 0.360 2.60 (2.22, 2.94)	43.4 ± 19.2 44.9 (23.4, 61.8)	46.8 ± 20.8 49.0 (25.0, 66.4)	3.31 ± 1.52 2.51 (2.36, 5.06)	11.0 ± 4.89 8.55 (7.79, 16.6)	3
46 (A)	24.0 ± 10.2 23.8 (13.8, 34.3)	4.03 ± 0.044 4.02 (4.00, 4.08)	3	2.03 ± 1.02 2.50 (0.857, 2.72)	65.0 ± 27.4 62.7 (38.8, 93.5)	69.8 ± 28.6 67.8 (12.3, 99.3)	4.31 ± 1.99 3.70 (2.70, 6.54)	13.8 ± 8.33 12.1 (6.52, 22.9)	3

*Estimation of C_{max} and t_{max} only

PK parameter values after the first dose were not significantly different from those after repeated dosing, indicating no systemic accumulation following QOW dosing of vestronidase alfa. Because of this result, the estimated PK parameters from Week 8 to Week 46 were pooled to derive descriptive statistical summary of vestronidase alfa PK parameters after repeated dosing. Following multiple dose administration, the mean ± SD (range) CL value was 3.74 ± 1.26 (1.91 – 6.54) L/h and mean $t_{1/2}$ was 2.59 ± 0.614 (0.857 – 3.55) h. The mean C_{max} and AUC_{0-t} values were 19.8 ± 8.00 (6.56 – 34.9) µg/mL and 57.4 ± 23.9 (18.8 – 97.0) µg*h/mL, respectively. Mean V_{ss} value was 12.6 ± 4.72 (6.52 – 22.9) L.

The inter-subject variability of CL was 33.7%. The intra-subject variability of CL across two to three treatment weeks within subjects ranged from 0.98% to 27.6%.

See Section 3.3.3 regarding vestronidase alfa PK with regard to age, gender, and race.

PD Assessments

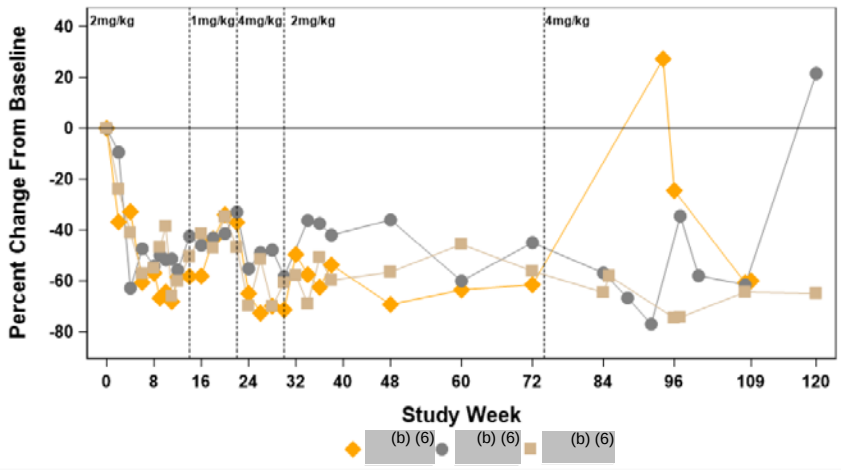
This review uses urinary DS as the representative biomarker for the evaluation of PD response because the E-R analysis results were consistent among urinary DS and other individual GAG species as well as total uGAG, despite the limitation of the LC-MS/MS method as described in Section 4.1. This is consistent with the Statistical Analysis Plan in Study UX003-CL301 which specified urinary DS as the primary analysis of uGAG. The PD response of serum GAG from the NRE method is also assessed.

Urinary GAGs (urinary DS)

Upon initial vestronidase alfa treatment at 2 mg/kg QOW in three subjects in UX003-CL201, mean urinary DS excretion as measured by the LC-MS/MS method reduced rapidly to reach a nadir of approximately 60% at around four to eight weeks and then plateaued (Figure 8). During the Forced-Dose Titration Period (i.e., Week 14 to Week 38), dose-dependent changes in DS were observed with a mean uGAG reduction of 42.2% at the 1 mg/kg QOW, 52.4% reduction at 2 mg/kg QOW, and 61.8% reduction at 4 mg/kg QOW. A mean reduction of 54.8% in urinary DS was maintained during the Continuation Period at 2 mg/kg QOW (i.e., Week 38 to Week 72). During the Long-term Extension Period at 4 mg/kg QOW, there was a sustained reduction in urinary DS with periodic brief increases in urinary DS when doses were missed for Subjects (b) (6) (Weeks 88, 90, and 92), (b) (6) (Weeks 74, 96, 118, and 122) and (b) (6) (Weeks 112 and 116).

A similar pattern and magnitude in urinary reductions in CS (by the LC-MS/MS method) and combined CS/DS (by the NRE method) were also observed (data not shown). While urinary HS excretion was also reduced after treatment initiation, the reduction was less consistent than those of urinary CS and DS excretion. This is partly due to the fact that urinary HS was within the normal range at baseline in all three subjects.

Figure 8. Percent Change in urinary DS excretion from Week 0 (Baseline) up to Week 120 by subject (Source: Figure 10.1.1.1 of UX003-CL201 CSR)



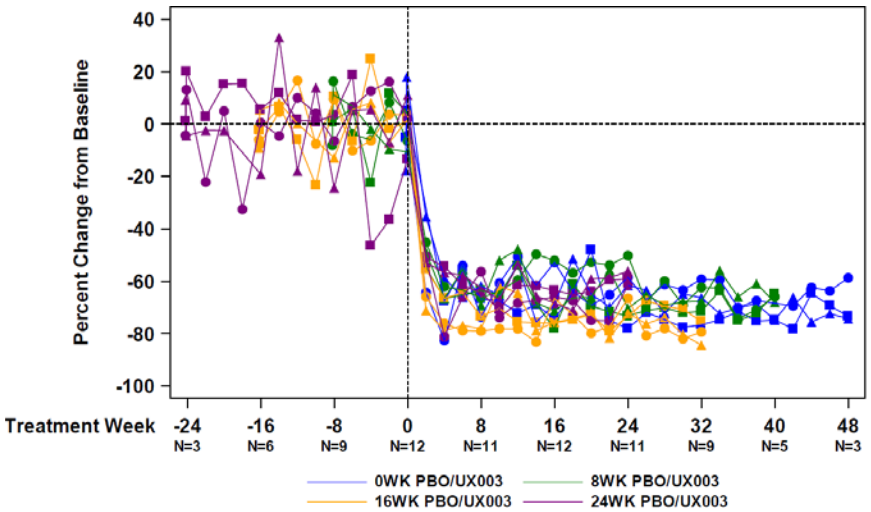
Abbreviations: DS = dermatan sulfate; LC-MS/MS = liquid chromatography-mass spectrometry/mass spectrometry

Note: Subject (b) (6) completed dosing through Week 118 with an incomplete dose at Week 86 (2.5% completion) and with missed doses at Weeks 88, 90, and 92. Subject (b) (6) completed dosing through Week 132 with missed doses at Weeks 74, 96, 118, and 122. Subject (b) (6) completed dosing through Week 124 with missed doses at Weeks 112 and 116. (Listing 16.2.5.1 and Listing 16.2.5.3)

In Study UX003-301, urinary DS excretion fluctuated within ±20% change from baseline during the placebo period. Following the initiation of vestronidase alfa treatment at 4 mg/kg QOW, urinary DS excretions declined to reach nadirs at around Week 4 and then plateaued. Thereafter, reductions in urinary DS excretion maintained at 60% to 80% from baseline, with fluctuations of 20% around the plateau (Figure 9).

See Section 3.3.3 for urinary DS excretion in subjects < 5 years of age in Study UX003-203.

Figure 9. Percent change from baseline in uGAG DS (LC-MS/MS) excretion by treatment week and subject (Source: Figure 10.1.2 of CL301 CSR)

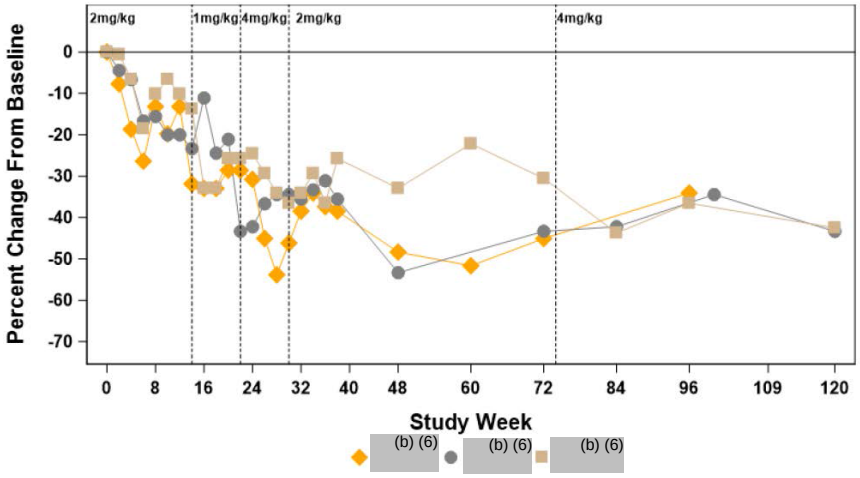


Serum GAGs

Upon the initiation of verstronidase alfa 2 mg/kg QOW in three subjects in UX003-201, mean combined serum CS/DS levels as determined by the NRE method dropped slowly to 14.5% over 12 weeks. During the Forced-Dose Titration Period, the concentrations further declined in a similar rate regardless the dose administered, with a mean decline from baseline of 40.8% at Week 28. Thereafter, serum CS/DS stabilized between 30% to 50% reduction from baseline through the end of study (Figure 10). The degree of serum GAG reduction from baseline was smaller than the reduction of uGAG from baseline.

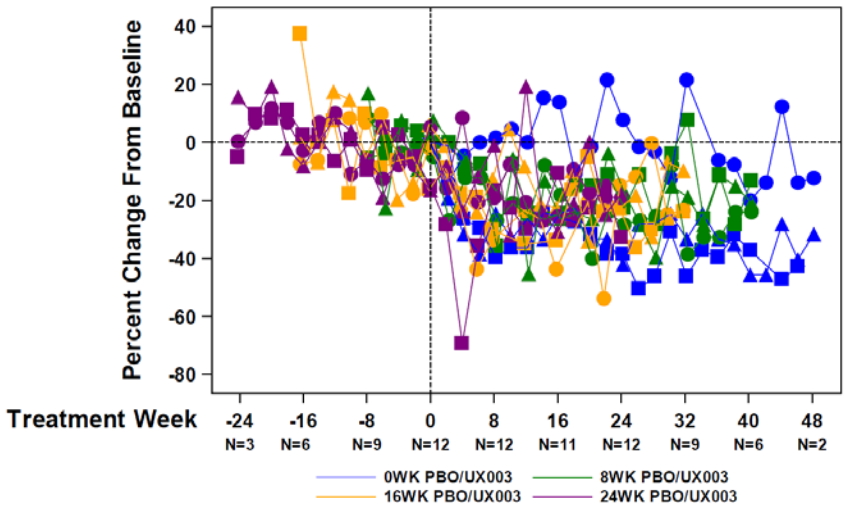
Serum HS measurements were at the lower limit of quantitation of the assay at baseline for all three subjects, and no change was demonstrated following treatment.

Figure 10. Percent change in serum GAG (NRE-CS/DS) from Week 0 (Baseline) to Week 120 by subject (Source: Figure 10.3.1.1 of UX003-CL201 CSR)



In UX003-301, the magnitude of change in serum GAG was less than that observed with uGAG (Figure 11). The combined serum CS/DS levels showed a least squares (LS) mean (±SE) percentage change from baseline of -22.50% (3.52%) after 24 weeks of vestronidase alfa treatment.

Figure 11. Percentage change in serum GAG (NRE-CS/DS) from baseline by subject (Source; Figure 14.2.13.2 of UX003-CL301 CSR)



4.4 Exposure-Response Analyses

The Applicant performed E-R analyses using time matched PK (C_{max} and AUC_{0-t}) and PD data from three dose levels (i.e., 1, 2, and 4 mg/kg QOW) from Study UX003-CL201 and 4 mg/kg QOW from Studies UX003-CL301 and UX003-CL203. The PD endpoints used in the analyses included % changes from baseline in urinary CS, DS, and HS by the LC-MS/MS method, as well as % reduction from baseline in combined CS/DS and in HS in both urine and serum by the NRE method.

As noted in Section 4.3, PK sampling duration in Study UX003-CL201 (i.e., 3 hours after the end of infusion for all but two PK profiles) was shorter than the duration in UX003-CL301 (i.e., 6 hours after the end of infusion. The PK sampling duration was also shorter in Study UX003-CL203 (i.e., 4 hours after the end of infusion). Hence, the AUC_{0-t} values in Studies UX003-CL201 and UX003-CL203 underestimated the actual exposure by 15% or less. Nonetheless, this underestimation was unlikely to adversely affect the analysis results because of the consistent E-R analysis results across different urinary GAG species.

E-R Analyses Using GAG Measurements from LC-MS/MS Method

Figure 12 shows 8 plots from the E-R analyses performed for urine samples measured by the LC-MS/MS method. A positive correlation was observed (Figure 12a, Figure 12b, and Figure 12d) between reductions of urinary CS, DS, and total uGAG excretion (i.e., PD response) and exposure (i.e., C_{max} and AUC_{0-t}.) The 4 mg/kg QOW was associated with greater PK exposures and PD responses, as compared with the two lower doses (1 and 2 mg/kg QOW). The E-R relationship plateaued at vestronidase alfa C_{max} and AUC_{0-t} values of approximately 15 µg/mL and 40 µg*hr/mL, respectively, suggesting that the use of higher vestronidase alfa doses resulting

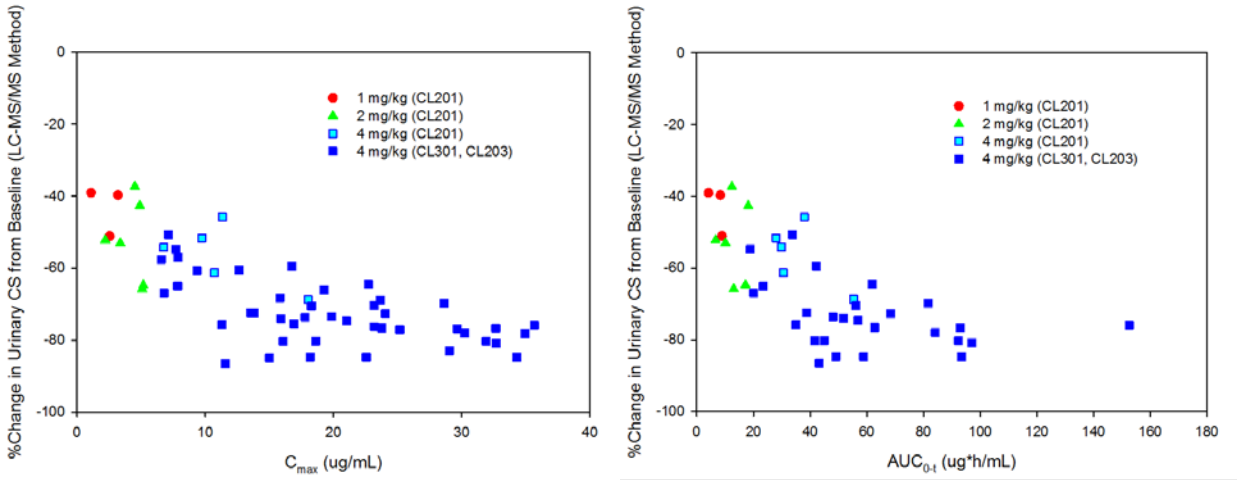
in C_{max} higher than 15 ng/mL and AUC_{0-t} higher than 40 ng*h/mL is unlikely to result in further increase in uGAG response.

Of note, the E-R relationship from total uGAG (i.e., urinary CS+DS+HS) was similar to that of the urinary CS or urinary DS. This finding is not unexpected, as urinary HS excretion was relatively normal and much lower than the excretion of urinary CS and urinary DS. As such, the total uGAG response was mostly driven by the responses in urinary CS and urinary DS excretions.

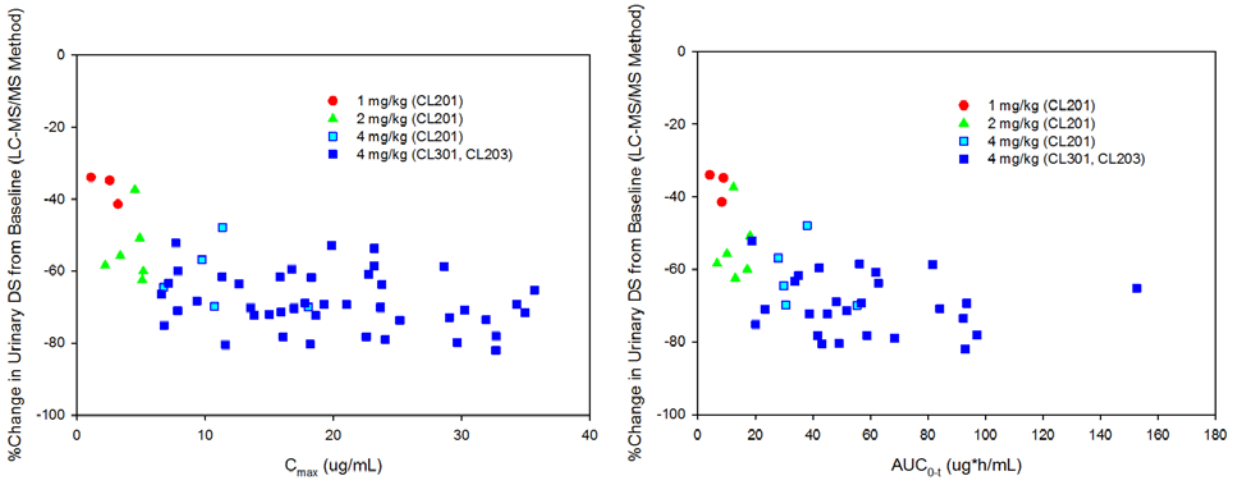
There was no E-R relationship between % reduction in urinary HS excretion and PK exposure (Figure 12c).

Figure 12. E-R analysis for (a) urinary CS, (b) urinary DS, (c) urinary HS, and (d) total uGAG by the LC-MS/MS method

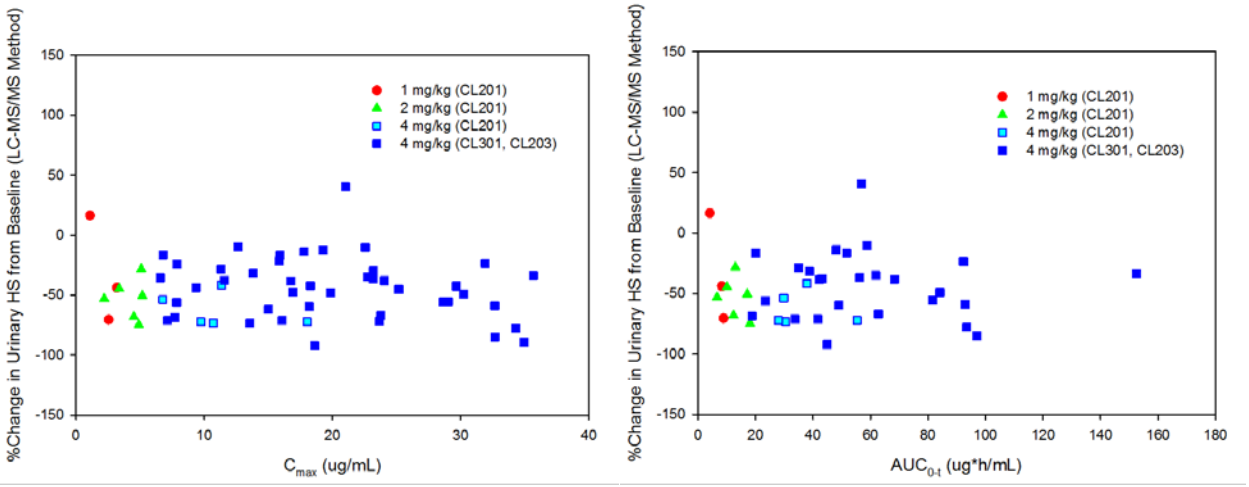
(a) Urinary CS vs. C_{max} and AUC_{0-t}



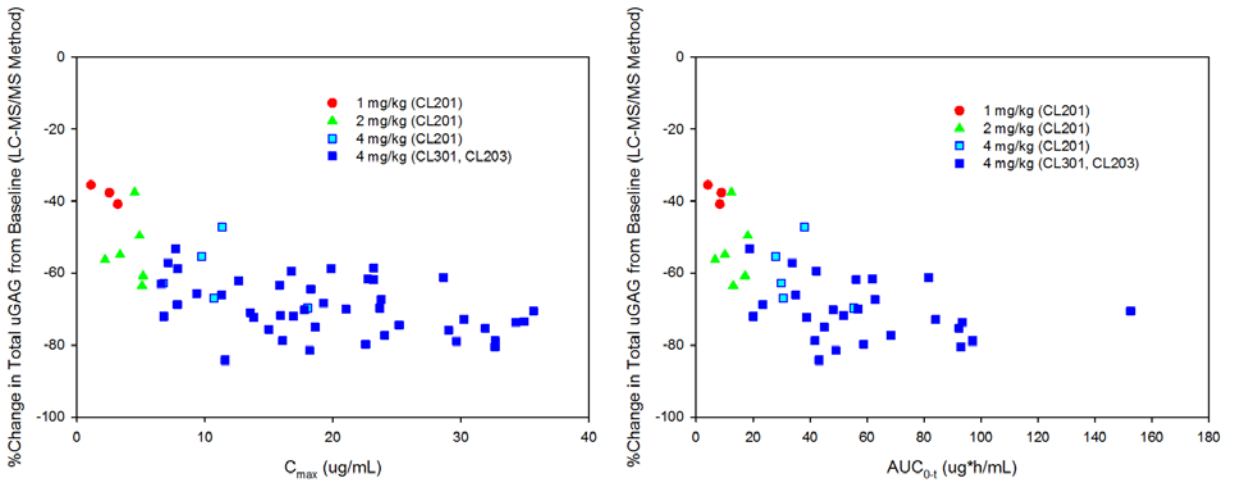
(b) Urinary DS vs. C_{max} and AUC_{0-t}



(c) Urinary HS vs. C_{max} and AUC_{0-t}



(d) Total uGAG vs. C_{max} and AUC_{0-t}

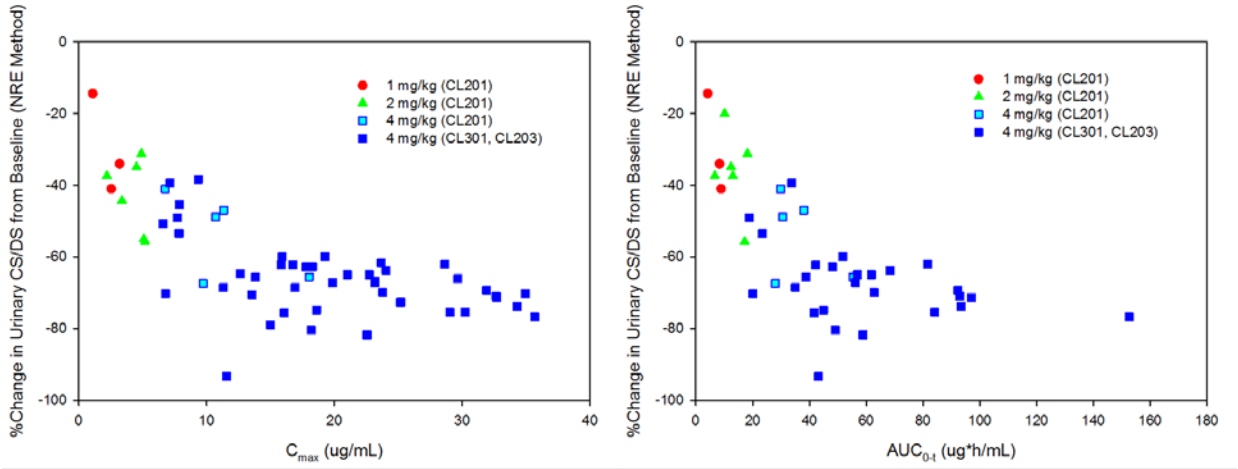


E-R Analyses Using GAG Measurements from NRE Method

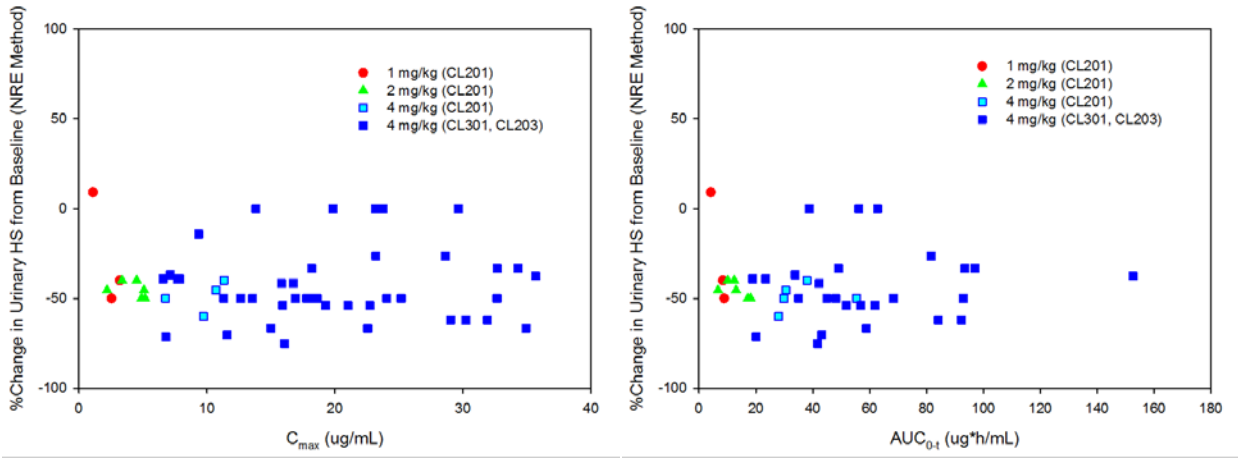
Similar E-R relationship was observed for urinary CS/DS combined as measured by the NRE method (Figure 13a). There was E-R relationship for the reduction from baseline in urinary HS excretion (Figure 12c) or serum CS/DS and HS concentrations (Figure 12c and Figure 12d). The lack of E-R relationship for urinary HS excretion is partly related to the relatively normal levels of urinary HS at baseline. The reason for the lack of E-R relationship for serum CS/DS is not known.

Figure 13. E-R analysis for (a) urinary CS/DS, (b) urinary HS, (c) serum CS/DS, and (d) serum HS by the NRE method

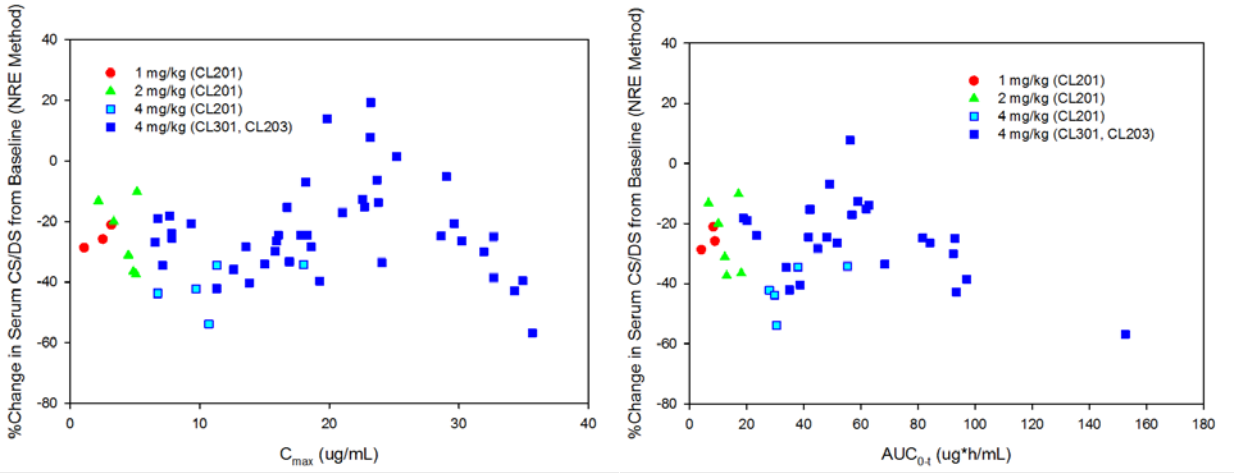
(a) Urinary CS/DS vs. C_{max} and AUC_{0-t}



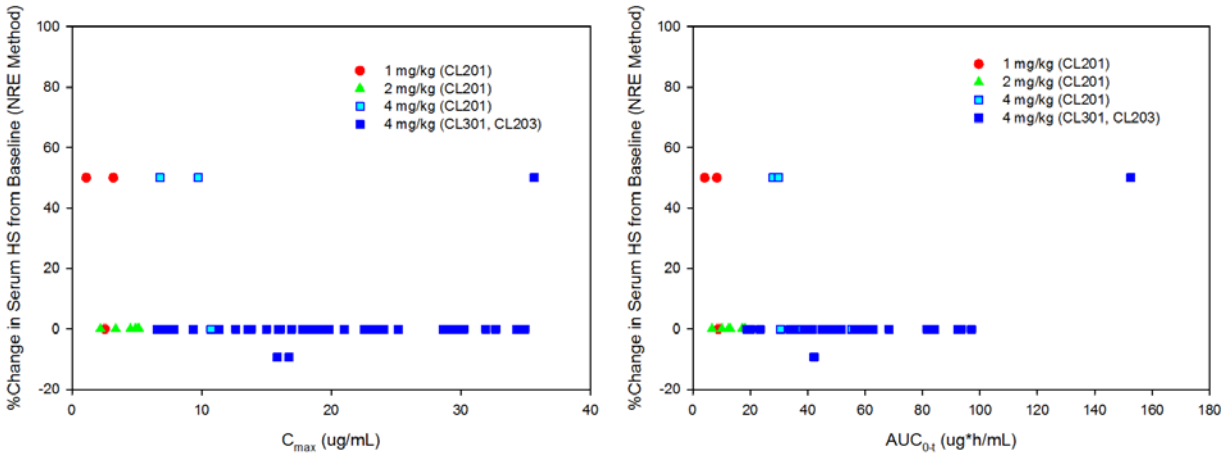
(b) Urinary HS vs. C_{max} and AUC_{0-t}



(c) Serum CS/DS vs. C_{max} and AUC_{0-t}



(d) Serum HS vs. C_{max} and AUC_{0-t}



4.5 Immunogenicity Assessment

The incidence of immunogenicity is 77.3% in subjects treated with vestronidase alfa based on data from the original submission (n = 19) and the 120 safety update (4 additional subjects). Specifically, 17 of 22 subjects (77.3%) in the three clinical studies developed ADA to vestronidase alfa (Table 16). This incidence takes into consideration four subjects with pre-existing ADA and one excluded subject as described below.

A total of 23 subjects were enrolled in the three clinical studies. Subject 23 from UX003-CL203 was not included in calculating the incidence of ADA because this subject had a baseline immunogenicity sample only. Four treatment naïve subjects in Study UX003-CL301 had pre-existing ADA at baseline, and three of these subjects had their post-treatment ADA titer values less than fourfold increase from the baseline titer values. The Applicant considered these three subjects negative for ADA because they defined a subject as ADA+ only if the subject showed at least a fourfold increase from baseline in titer values in the ADA titer assay.[3] On the other hand,

the clinical pharmacology reviewer considered one of these three subjects (Subject 15) positive for ADA because the subject was positive for NAb at one of the study visits.

The ADA titers varied among ADA+ subjects, with the highest ADA titers ranging from 10 to 65610 in the individual study subjects. Nine of the 17 (52.9%) ADA positive subjects developed NAb.

Table 16. Summary of immunogenicity data up to 120 Safety Update

Study	ID	Age	Pre-Existing ADA	ADA+	NAb+	Highest ADA Titers	Anaphylaxis
UX003-CL201	1	5.5	-	Y	N	10240	Suspected
	2	9.4	-	Y	N	5120	
	3	25.1	-	N	N	-	
UX003-CL301 (UX003-CL202)	4	14.7	-	Y	Y	12800	
	5	8.5	-	Y	Y	65610	Y
	6	13.4	-	Y	Y	65610	
	7	12.8	-	Y	Y	2560	Y
	8	10.1	1:10	Y	N	640	
	9	17.4	-	Y	Y	10240	
	10	10.5	-	Y	Y	7290	
	11	25.3	-	N	N	-	
	12	22.6	1:10	N	N	10	
	13	22.5	1:80	N	N	160	
	14*	16.5	-	Y	N	2430	
	15*	11.5	1:80	Y	Y	80	
UX003-CL203	16	2.9	-	N	N	-	
	17	3.0	-	Y	N	2560	
	18	1.7	2180 (previously treated through an emergency IND)	Y	N	1280	
	19	3.4	-	Y	N	160	
	20[$]	3.9	-	Y	Y	10240	
	21[$]	2.0	-	Y	Y	10240	
	22[$]	5.0	-	Y	N	40	
	23[$,#]	4.7	-	-	-	-	

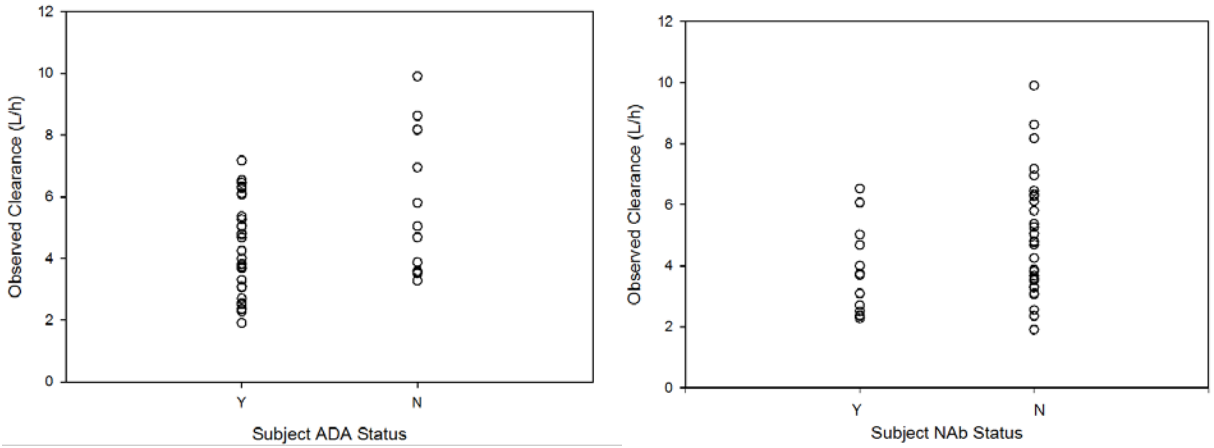
*Subjects not enrolled in extension study UX003-CL202; [$]subjects from 120 days update; [#]Subject had a baseline immunogenicity sample only and was not included in the calculation of ADA incidence

Impact of immunogenicity on PK

The clinical pharmacology reviewer performed the assessment of the impact of immunogenicity on PK using PK data from all PK assessment study weeks, together with immunogenicity data within a 2-week time period of the PK assessment study weeks. See Table 14 for sampling schedules of PK and immunogenicity assessments. In this evaluation, the reviewer considered subjects with at least one study sample tested positive for ADA and NAb as ADA+ and NAb+, respectively, with the exception that the three subjects with pre-existing ADA were considered ADA- (see above).

Based on between-subject comparison, the mean ± SD CL value was lower in ADA+ (4.21 ± 1.53 L/h, n = 32) subjects than ADA- subjects (5.79 ± 2.32, n = 13) (t-test, p = 0.0103) (Figure 14, left panel). The mean CL value was also lower in the NAb+ subjects (3.77 ± 1.44 L/h, n = 13) compared with the NAb- subjects (5.03 ± 1.97 L/h, n = 32) (t-test, p = 0.0443) (Figure 14, right panel).

Figure 14. Comaprison of CL values between ADA+ and ADA- subjects (left panel) and between NAb+ and NAb- subjects (right panel)



Using an alternative approach of within-subject comparison, the effects of immunogenicity were also evaluated between study weeks with respect to ADA status. Mean CL value was lower in ADA+ study weeks compared to ADA- study weeks (3.87 ± 1.35 L/h, n = 28 vs. 5.97 ± 2.00 L/h, n = 17) (t-test, p < 0.001) (Figure 15, left panel). However, comparison of CL values between ADA+ and ADA- study weeks within each ADA+ subject did not reveal consistent increase or decrease in CL values (Figure 15, right panel). A similar evaluation based on NAb status was not performed because NAb was tested positive only at one study week each for two subjects (i.e., a total of two NAb+ study weeks) when PK parameter values are available. However, graphical comparison of limited CL values showed no apparent different between NAb+ and NAb- study weeks within these two subjects (Figure 16, left panel).

No apparent correlation was observed between CL values and ADA titers (Figure 16, right panel).

Figure 15. Compariosns of CL values between ADA+ and ADA- study weeks (left panel) and between ADA+ and ADA- study weeks within each ADA+ subject with ADA status change (right panel)

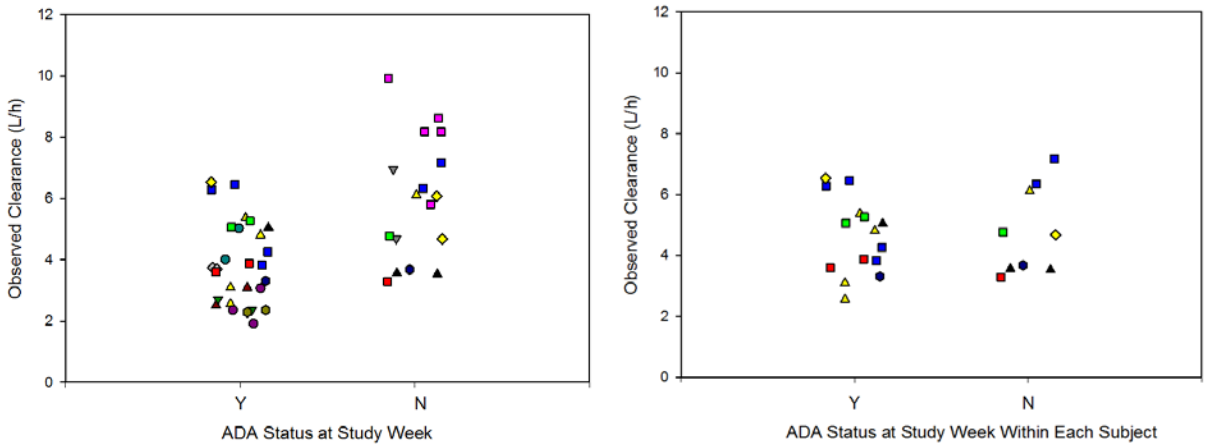
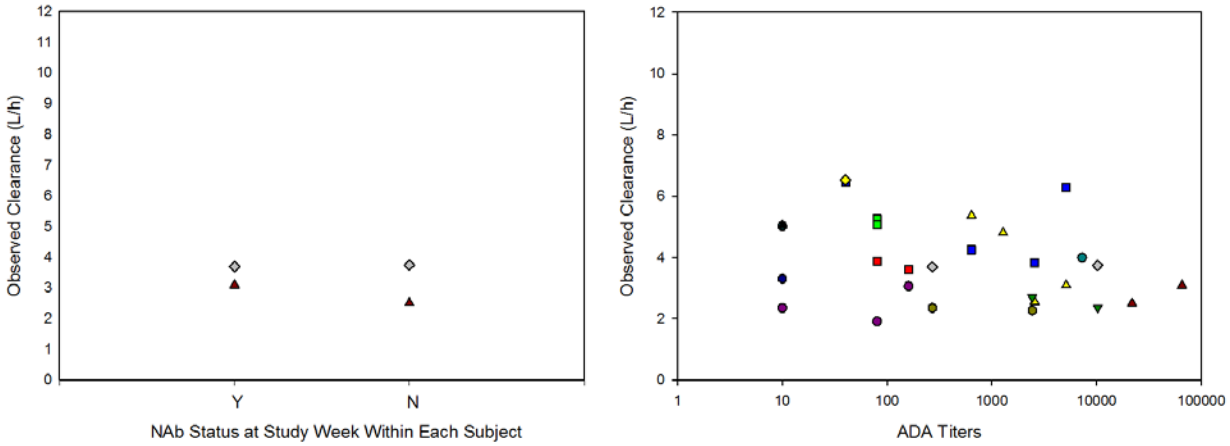


Figure 16. Comparison of CL values between NAb+ and NAb- study weeks within two ADA+ subjects who had NAb status change (left panel); correlation between CL values and ADA titers (right panel)



Impact of Immunogenicity on PD

Graphical comparison of the change in urinary DS levels as determined by the LC-MS/MS method in Study UX003-CL301 did not reveal significant difference in the PD response between ADA+ and ADA- subjects (Figure 17, left panel). Similarly, urinary DS response did not appear to be different between NAb+ and NAb- subjects (Figure 17, right panel).

Visual inspection of the individual subject's temporal profile of changes in urinary DS and the ADA titers did not show any consistent changes or trends in urinary DS reduction with ADA titers. Figure 18 shows example profiles from two subjects in Study UX003-CL301.

Figure 17. Changes in urinary DS levels in ADA+ and ADA- subjects (left panel) and in NAb+ and NAb- subjects (right panel) in Study UX003-CL301

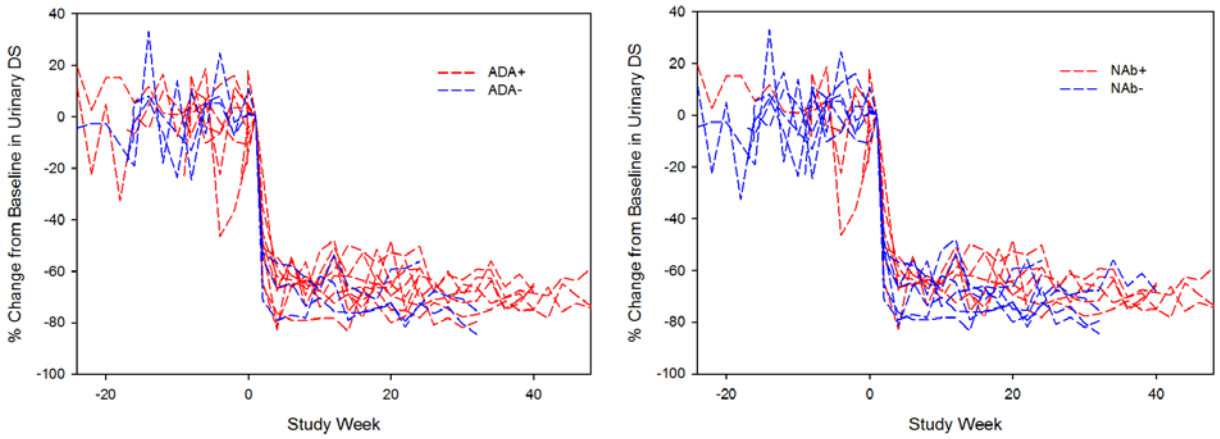
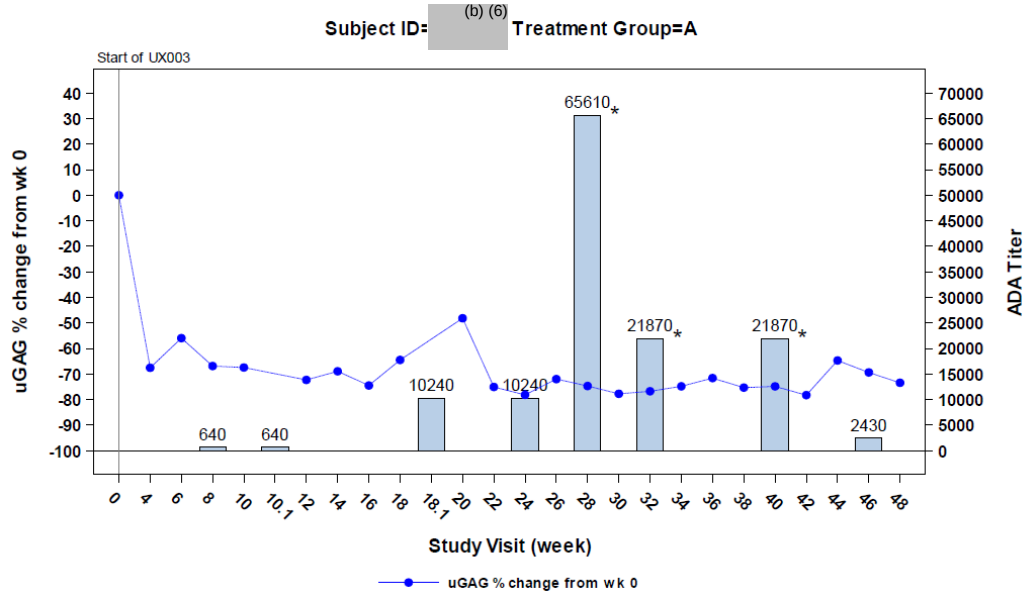
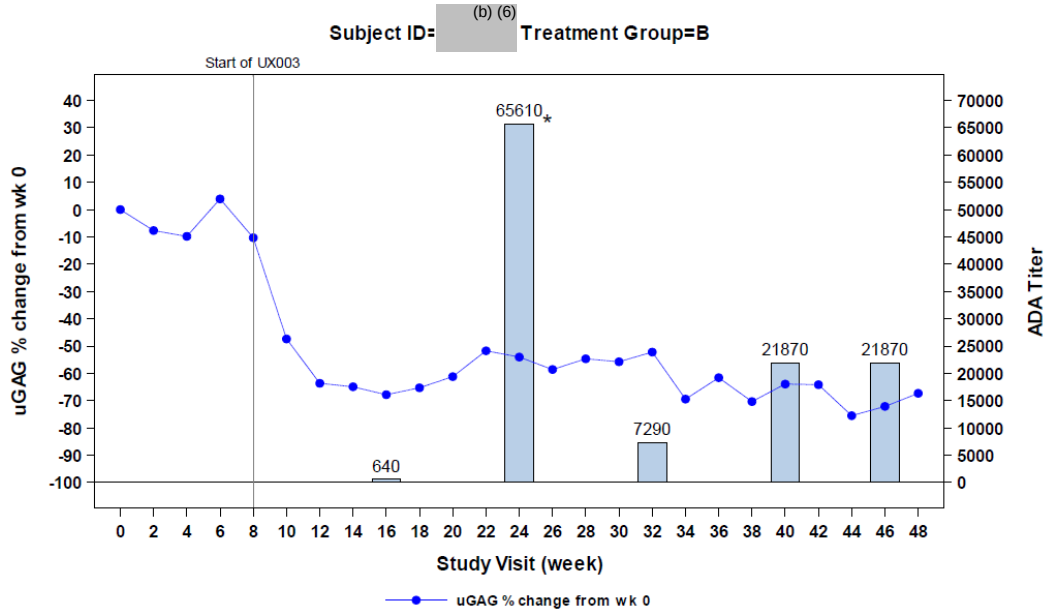


Figure 18. Examples of individual subject profile of urinary DS changes with ADA titers (Source: Figure 14.99.3.6.2.1 of UX003-CL301 CSR)





*NAb positive

Impact of Immunogenicity on Safety

Anaphylaxis was observed in three subjects (one in UX003-CL201 and two in UX003-CL301) in vestronidase alfa clinical studies (Table 16). The occurrence of anaphylaxis did not appear to the associated with ADA and NAb status, as well as ADA titers.

5. REFERENCES

1. Lawrence R, Brown JR, Lorey F, et al., Glycan-based biomarkers for mucopolysaccharidoses. Mol Genet Metab 2014;111:73-83.
2. Sasarman F, Maftei C, Campeau PM et al., Biosynthesis of glycosaminoglycans: associated disorders and biochemical tests. J inherit Metab Dis 2016;39:173-88.
3. Shankar G, Cocea AL, Devanarayan V et al., Assessment and reporting of the elinical immunogenicity of therapeutic proteins and petpides – harmonized terminology and tactical recommendations. The AAPS J 2014;16:658-73.

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CHRISTINE Y HON
09/13/2017

YOW-MING C WANG
09/13/2017

HAE YOUNG AHN
09/13/2017