CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125291

PHARMACOLOGY REVIEW(S)

ADDENDUM TO PHARMACOLOGIST'S REVIEW OF BLA 125, 291 DATED JANUARY 30, 2009

Reviewer: Niraj R. Mehta, Ph.D.

Date: February 13, 2009

In response to my labeling recommendations stated on pages 3 to 7 of the review dated January 30, 2009, the Sponsor suggested that in sections "8.1 Pregnancy" and "13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility", the calculation of animal (mouse and rabbit) to human dose ratio should be based on a bodyweight comparison (mg/kg) rather than a body surface area comparison (mg/m^2). In support of their recommendation, the Sponsor references the 2002 FDA Guidance "Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers", which recommends that biological products administered intravascularly (>100,000 daltons) be normalized to mg/kg. After conferring with Dr. Abigail Jacobs (ODE Associate Director for Pharmacology and Toxicology, Office of New Drugs), she suggested that we compare doses based on bodyweight (mg/kg) and body surface area (mg/m^2) to the plasma AUC for animals and humans. The table below shows a comparison of plasma AUC to mg/kg and mg/m^2 dose in animals and humans.

Species	Product	Dose (mg/kg)	Dose (mg/m^2)	AUC (mg·min/mL)	AUC/(mg/kg)	AUC/(mg/m^2)
Human	Lumizyme	20	740	162.6	8.1	0.2
Mouse*	Myozyme	40	120	64.6	1.6	0.5
Mouse**	Lumizyme	20	60	27.6	1.4	0.5
Mouse**	Lumizyme	20	60	24.2	1.2	0.4
Mouse**	Myozyme	20	60	38.5	1.9	0.6
Rabbit*	Myozyme	20	240	74.1	3.7	0.3
Rabbit*	Myozyme	40	480	85.0	2.1	0.2

*Data was taken from the teratogenicity studies described in the Pregnancy section of labeling.
**Data was taken from a pharmacokinetic study in mice using Lumizyme (2 lots) and Myozyme..

(b) (4)

(b) (4) After discussing these results with Dr. Jacobs, she suggested that since plasma AUC is available for both animals and humans, the animal to human dose ratios in sections 8.1 and 13.1 of the label should be expressed relative to human plasma steady state AUC.

Therefore, the "Recommended Version" for Sections 8.1 and 13.1 of the Lumizyme label should read as follows:

Recommended Version:

"8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Teratogenic Effects:

(b) (4)

Recommended Version:

"13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate carcinogenic potential or studies to evaluate mutagenic potential have not been performed with alglucosidase alfa.

Alglucosidase alfa at intravenous doses up to 40 mg/kg, administered every other day (plasma AUC of 64.6 mg•min/mL, 0.4 times the human exposure at the recommended bi-weekly dose) had no effect on fertility and reproductive performance in mice.

02/13/09

Niraj R. Mehta, Ph.D.
Pharmacologist
Division of Gastroenterology Products

Date

Comment:

David Joseph 2/13/09

David B. Joseph, Ph.D.
Acting Pharmacology Team Leader
Division of Gastroenterology Products

Date

cc:
BLA 125,291
DGP
DGP/CSO
DGP/Dr. Joseph
DGP/Dr. Mehta
ONDIO/Dr. Jacobs

R/D Init.: D. Joseph 02/12/09

NM/nrm: 02/13/09
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APPENDIX/ATTACHMENTS

None

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

FROM: David B Joseph, Acting Pharmacology Team Leader

DATE: February 12, 2009

SUBJECT: BLA 125,291 (serial # 000 dated May 30, 2008)

Sponsor: Genzyme Corporation

Drug Product: Lumizyme (alglucosidase alfa)

Comments:

1. As stated in Dr. Mehta's Pharmacology/Toxicology Review of BLA 125,291 dated January 30, 2009, all nonclinical studies that were needed to support approval of Lumizyme were incorporated in this application by reference to BLA 125,141 (Myozyme®, a licensed product indicated for use in patients with Pompe disease). This was done as per an agreement between the Sponsor and the Division of Gastroenterology Products. Both Lumizyme and Myozyme® contain recombinant human acid α-glucosidase (alglucosidase alfa) as the active ingredient. Both products are produced by recombinant DNA technology in a Chinese hamster ovary cell line. However, Lumizyme is manufactured using a 2000L bioreactor, whereas Myozyme® is produced using a 160L bioreactor. Lumizyme exhibits biochemical differences in comparison to Myozyme® that could have a negative impact on efficacy. Furthermore, there is a lack of clinical studies to allow for a meaningful comparison of these products. Therefore, the FDA deemed that Lumizyme should be marketed under its own BLA, rather than BLA 125,141 (Myozyme®). The proposed indication for Lumizyme is for use in patients 8 years and older with late-onset (non-infantile) Pompe disease (GAA deficiency), whereas Myozyme® is indicated for patients of all ages with Pompe disease.

2. Nonclinical studies of Lumizyme were not requested by the Agency, since the observed differences between Lumizyme and Myozyme® are not expected to result in a different toxicity profile. The available nonclinical studies with Lumizyme include pharmacology, pharmacokinetics, and a 4-week toxicity study in mice. Lumizyme was shown to produce glycogen clearance from skeletal and cardiac muscle in GAA knockout mice, indicative of pharmacologic activity (studies reviewed in the Pharmacology/Toxicology Review of BLA 125,291 dated January 30, 2009). The pharmacokinetic properties of Lumizyme and Myozyme® were compared in previous studies, using GAA knockout mice. The observed AUC values for various lots of Lumizyme were 27-37% lower than the Myozyme® AUC value, which indicates that these two products are not equivalent. The tissue distribution of Lumizyme and Myozyme® in GAA knockout mice was similar, with liver as the major

organ of distribution for both products. However, the amount of Lumizyme uptake in liver was slightly higher than that of Myozyme®. A comparative toxicity study of Myozyme® and Lumizyme was performed in C57B1/6 mice using weekly doses of up to 100 mg/kg for four weeks. The toxicity profile was similar between the two products (Pharmacology/ Toxicology Review of BLA 125,141 dated April 13, 2006).

Recommendations:

I concur with the recommendations of the pharmacology/toxicology reviewer, Dr. Niraj Mehta. There are no nonclinical issues which preclude the approval of Lumizyme for late-onset (non-infantile) Pompe disease.

David Joseph 2/12/09

David B. Joseph, Ph.D. Date
Acting Pharmacology Team Leader
Division of Gastroenterology Products

cc:
BLA 125,291
DGP
DGP/CSO
DGP/Dr. Joseph
DGP/Dr. Mehta
OND IO/Dr. Jacobs

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125,291**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**05/30/08**
PRODUCT:	**Lumizyme (alglucosidase alfa) intravenous infusion**
INTENDED CLINICAL POPULATION:	**Patients with late-onset Pompe disease**
SPONSOR:	**Genzyme Corporation**
DOCUMENTS REVIEWED:	**Amendment 0007**
REVIEW DIVISION:	**Division of Gastroenterology Products**
PHARM/TOX REVIEWER:	**Niraj R. Mehta, Ph.D.**
ACTING PHARM/TOX TEAM LEADER:	**David B. Joseph, Ph.D.**
DIVISION DIRECTOR:	**Donna Griebel, M.D.**
PROJECT MANAGER:	**Richard Ishihara, Lt., U.S.P.H.S**

Date of review submission to Division File System (DFS): N/A

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The application is recommended for approval.

B. Recommendation for nonclinical studies

None.

C. Recommendations on labeling

Recommendations are shown below for the following sections and subsections: "INDICATIONS AND USAGE" under "HIGHLIGHTS OF PRESCRIBING INFORMATION", "DRUG INTERACTIONS", "Pregnancy" under the section of "USE IN SPECIFIC POPULATIONS", "Mechanism of Action" under the section of "CLINICAL PHARMACOLOGY", and "Carcinogenesis, Mutagenesis, Impairment of Fertility" under the section of "NONCLINICAL TOXICOLOGY".

(b) (4)

[redacted]

Recommended Version:

2 pp withheld in full immed. after this page as (b) (4) Draft Labeling.

(b) (4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Lumizyme (alglucosidase alfa) is produced by recombinant DNA technology in a Chinese hamster ovary cell line at a 2000L bioreactor scale, whereas Myozyme® (alglucosidase alfa) is produced at a 160L bioreactor scale. As per an agreement between the sponsor and the Division of Gastroenterology Products, all nonclinical studies that are needed to support approval of Lumizyme were incorporated in the present BLA by reference to BLA 125,141 (Myozyme®). This review contains a summary of the nonclinical information presented in the review of BLA 125,141 (Myozyme®) by Barbara J. Wilcox, Ph.D. (review dated March 20, 2006). New studies presented in this review were submitted post-approval of Myozyme®. Two pharmacology studies were submitted, which showed that 2000L alglucosidase alfa produced similar pharmacological activity (glycogen clearance) as compared to the 160L alglucosidase alfa. In two other pharmacology studies, 2000L alglucosidase alfa (Lumizyme) was fractionated over a mannose-6-phosphate receptor (M6PR) column into various affinity fractions. Based on the observed glycogen level in tissues exposed to unfractionated Lumizyme or various M6PR affinity fractions, glycogen clearing activity appears to be directly correlated with M6PR binding affinity. New toxicology studies included a 6-month intravenous repeat-dose toxicity study in juvenile mice, which showed no treatment-related effects on developmental parameters. In addition, five reproductive toxicity studies in mice or rabbits were submitted. There were no treatment-related effects on fertility or early embryonic development in mice, or on fetal development in rabbits. Lastly, the sponsor submitted a special toxicology study evaluating various immune tolerance regimens to attenuate antibody responses to enzyme replacement therapies. The only treatment that induced a long-lived reduction in alglucosidase alfa-directed antibody responses was methotrexate.

B. Pharmacologic activity

Lumizyme is an enzyme replacement therapy indicated for patients who lack acid-α-glucosidase (GAA), an enzyme that degrades lysosomal glycogen.

C. Nonclinical safety issues relevant to clinical use

None.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125,291
Review number: 1
Sequence number/date/type of submission: 0001/May 30, 2008/Original

Information to sponsor: Yes (x) No ( )

Sponsor and/or agent: Genzyme Corporation
Cambridge, MA

Manufacturer for drug substance: Genzyme Corporation
Cambridge, MA

Reviewer name: Niraj R. Mehta, Ph.D.
Division name: Gastroenterology Products
HFD #: 180
Review completion date: January 30, 2009
Drug:

Trade name: Lumizyme
Generic name: Alglucosidase alfa
Code name: None
Chemical name: Recombinant human acid alpha-glucosidase (rhGAA)
CAS registry number: None
Molecular formula/molecular weight: 109,000 daltons
Structure: Lumizyme is a glycoprotein with a calculated mass of 99,377 daltons for the polypeptide chain, and a total mass of approximately 109,000 daltons, including carbohydrates. Lumizyme consists of the human enzyme acid α-glucosidase (GAA), (b) (4)

Relevant INDs/BLAs/DMFs: IND 010,780 (alpha-alglucosidase), BLA 125,141 (Myozyme® (alglucosidase alfa))

Drug class: Recombinant human protein/enzyme replacement therapy

Intended clinical population: Lumizyme is indicated for patients with Pompe disease.

Clinical formulation: Each 50 mg vial contains 52.5 mg alglucosidase alfa, 210 mg mannitol, 0.5 mg polysorbate 80, 9.9 mg sodium phosphate dibasic heptahydrate, 31.2 mg sodium phosphate monobasic monohydrate.

Route of administration: intravenous

Disclaimer: Tabular and graphical information were constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: As per an agreement between the sponsor and the Division of Gastroenterology Products (as per meeting with sponsor on April 28, 2008, STN BLA 125141/65), nonclinical studies for BLA 125,291 were cross referenced from BLA 125,141 (Myozyme®). Preclinical studies of Myozyme® (alglucosidase alfa manufactured at the 160L scale) were previously submitted and reviewed (Pharmacology/Toxicology review of BLA 125,141 dated March 20, 2006). The nonclinical section of this application contains a summary of the previously submitted studies. In addition, the sponsor submitted new preclinical studies in amendments to BLA 125,141. The reviews of the newly submitted studies are presented in the current review.

Studies not reviewed within this submission: None.

Preclinical Study	Testing Laboratory	Report # (Study #)	Lot #	Page
PHARMACOLOGY				
Effect on Glycogen Clearance of 160L and 2000L Myozyme at Different (b) (4) Levels in the GAA Knockout Mice	Genzyme, Framingham, MA	(Study No. 05-0823Pga)	2000 L: 4573352 3940564 2538399 160L: 608345	12
Effect on Glycogen Clearance of 160L and 2000L Myozyme in the GAA Knockout Mice	Genzyme, Framingham, MA	(Study No. 06-0276)	2000L: 2538399 160L: 608341	13
Glycogen Clearance Following Administration of 4-Weekly Doses of Mannose-6-Phosphate (M6P) Receptor Column Fractions of Myozyme (No Affinity Fraction, Pooled Bound Fraction, and High Affinity Fraction) as Compared to Unfractionated Myozyme	Genzyme, Framingham, MA	(Study No. 07-2015)	2000L: 9867120	14
An Evaluation of Glycogen Clearance Following Administration of 4-Weekly Doses of M6P-Receptor Column Fractions of Myozyme (Low Affinity and Medium Affinity) as Compared to the Unfractionated Myozyme	Genzyme, Framingham, MA	(Study No. 07-2053)	2000L: 9867120	17
GENERAL TOXICOLOGY				
Six-Month Intravenous Repeated-Dose Toxicity Study of Recombinant Human Acid-α-Glucosidase in Juvenile Mice	(b) (4)	FFA00067 (Reference No. 06032)	5930179, 7003108 and 7003108A	22
REPRODUCTIVE TOXICOLOGY				
Intravenous Injection Study of Recombinant Human Acid-α-Glucosidase on Fertility and Early Embryonic Development in Mice		FFA00051 (Reference No. 06010)	1369017	30
Intravenous Injection Study of		6354-155	608341	39

Recombinant Human Acid α-Glucosidase on Fertility and Early Embryonic Development to Implantation in Mice (Study Phase: Qualitative Spermatogenesis Evaluation and Testicular Pathology)	(b) (4)	(Reference No. 04006)		
Intravenous Dosage-Range Developmental Toxicity Study of Recombinant Human Acid-α-Glucosidase in Rabbits		FFA00052 (Reference No. 06022)	5930179	42
Intravenous Developmental Toxicity Study of Recombinant Human Acid-α-Glucosidase in Rabbits		FFA00053 (Reference No. 06030)	5930179 and 7003108A	47
Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Recombinant Human Acid-α-Glucosidase in Mice, Including Postnatal Behavioral/Functional Evaluation		FFA00058 (Reference No. 06023)	5930179	63
Intravenous Injection Study of Recombinant Human Acid-a''' Glucosidase (rhGAA) on Female Fertilty and Early Embryonic Development to Implantation in Mice		6354-163	1369017	74
SPECIAL TOXICOLOGY				
Evaluation of the Immune Tolerance Induction Regimens to Ameliorate Antibody Responses to Enzyme Replacement Therapies	Genzyme, Framingham, MA	ITR-333-1106	GA062, GA079, GA080, GA123	80

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The primary pharmacodynamic studies demonstrated clearance of tissue glycogen load in the Pompe GAA knockout mouse model under varying conditions. These conditions included varying dose levels, treatment regimens, ages of the animal, and product preparations. The doses tested ranged from 1 mg/kg administered weekly for four weeks to 100 mg/kg administered weekly for four weeks. The longest duration was 16 weeks, which consisted of either a 10 and 20 mg/kg dose administered weekly, or a 10, 20, 40 mg/kg dose administered every other week (qow).

In general, rhGAA showed consistent efficacy in depleting glycogen load from a range of muscles including cardiac, diaphragm, quadriceps, psoas and triceps, as demonstrated by both histological and biochemical detection methods. Differences in response to rhGAA were observed among the various muscles sampled. When rate of depletion and reaccumulation were investigated, cardiac muscle appeared to clear faster and more completely and the depletion appeared to last longer relative to skeletal muscle. When Pompe knockout mice were treated with 100 mg/kg weekly for 4 weeks, complete depletion was noted in cardiac muscle by Day 1,

in skeletal muscle by Day 3. The onset of reaccumulation was observed in skeletal muscle by Day 28, but no reaccumulation was detected biochemically for cardiac muscle at Day 42. The various skeletal muscles showed significant variation in response to rhGAA.

When efficacy in 3- and 12-month old GAA knockout mice was investigated, younger animals were significantly more responsive to glycogen clearance by rhGAA than the older animals. The 12 month old mice had higher tissue glycogen load at study initiation, which might have affected the resulting levels at study termination. When long term dosing regimens were compared, results indicated that 40 mg/kg, qow, was as effective as 20 mg/kg administered weekly.

Several unscheduled animal deaths occurred during the pharmacology studies with no details regarding cause of death provided. Some of these deaths can be accounted for by hypersensitivity reactions, which are a common response of rodents to rhGAA. However, some of the deaths could not be accounted for by hypersensitivity, and no data on the potential cause were provided. Due to the concern about hypersensitivity reactions, rodents were routinely pre-treated with diphenhydramine (DPH), usually at 5 mg/kg 20 minutes prior to infusion of the rhGAA. It is not known whether the vehicle control animals also received DPH, but it appears that no study included a DPH-only control group.

In newly submitted studies, the pharmacological activity of alglucosidase alfa manufactured at the 160L and 2000L scales was tested in GAA knockout mice (0, 20, 60 or 100 mg/kg once weekly for 4 weeks). The results showed that the 2000L product (Lumizyme) can produce similar pharmacological activity (glycogen clearance) as compared to the 160L product (Myozyme®) at doses of 60 and 100 mg/kg. Unfractionated 2000L product was compared to fractionated 2000L product with different affinities for the mannose-6-phosphate (M6P) receptor in GAA knockout mice (various doses once weekly for 4 weeks). At equivalent doses (40 mg/kg), pooled fractions, low affinity, medium affinity and the high affinity fractions cleared more glycogen than unfractionated product.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: The function of this product, human recombinant acid α-glucosidase (rhGAA), is degradation of glycogen to glucose, a process that normally takes place within the lysosomes of the cell. Enzyme deficiency results in accumulation of glycogen in the lysosomal compartment, a condition known as glycogenosis type II. The product is administered intravenously (i.v.) as a form of enzyme replacement therapy (ERT). Once internalized by the cell, and passed to the lysosome by intracellular mechanisms, rhGAA is proteolytically processed, forming an active, multi-subunit complex which degrades lysosomal glycogen at low pH (Van der Ploeg, 1988 *Pediatr Res*; Moreland, et. al., 2005, *J Biol Chem*). Intracellular trafficking of the 110 kD form of rhGAA to the lysosome is thought to occur through a mannose-6-phosphate receptor dependent mechanism. This receptor is also present on the surface of many cell types and is thought to play a role in the cellular uptake of exogenously administered enzyme by endocytosis (Raben, 2003, *Molecular Genetics and Metabolism*).

Drug activity related to proposed indication: Glycogen storage disease type II (GSDII), also

known as Pompe disease, is an inherited condition resulting in a disorder of glycogen metabolism due to defective function of the lysosomal hydrolase acid α-glucosidase in all tissues. The symptoms of Pompe disease are mainly due to functional impairment of skeletal and cardiac muscle, resulting in weakness, cardiomyopthy and cardiomegaly and respiratory impairment due to weakness of the diaphragm.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated March 31, 2008 by Dr. Ke Zhang.

Effect on Glycogen Clearance of 160L and 2000L Myozyme at Different (b) (4) Levels in the GAA Knockout Mice (Study No. 05-0823Pga)

Methods: To compare the pharmacological activity of Myozyme manufactured at the 160L and 2000L scales, GAA knockout mice (6/group) were treated with Myozyme manufactured at the 2000L scale and 160L scale by i.v. injection at 0, 20, 60 or 100 mg/kg once weekly for 4 weeks (4 doses). These animals were 3-5 months old when the treatment was initiated. To prevent the hypersensitivity reaction, these animals were also treated with diphenhydramine 5 mg/kg IP 15-20 minutes prior to the third dose. Myozyme manufactured at the 2000L scale (lots #4573352, 3940564, and 2538399) contains three levels of (b) (4) Myozyme manufactured at the 160L scale (lot #608345) contains (b) (4) and (b) (4). All animals were sacrificed 7 days after the last dose. Tissue glycogen levels in the heart, quadriceps and triceps were determined using the Glycogen Trinder assay.

Results: The results indicated that the glycogen levels were similar in the heart between the 160L and 2000L Myozyme in all treatment groups containing same level of (b) (4) The glycogen levels were in general higher in the triceps and quadriceps in the 160L Myozyme group as compared to the 2000L Myozyme group containing the same level of (b) (4) The results suggested that the 2000L Myozyme can produce same or better pharmacological activity (glycogen clearance) as compared to the 160L Myozyme with same (b) (4) levels. The (b) (4) level in the 160 L (b) (4) was much higher than those in the 2000 L (b) (4). The results were presented in the following table.

Best Possible Copy

Table 2. Mean glycogen levels in the heart, triceps and quadriceps following 4 weekly administrations of 160L or 2000L Myozyme.

Dose and Tissue	2000 L lot#4573352 SA = 4.7 Gr. 1-3	2000 L lot#3940564 SA = 5.5 Gr. 4-6	2000 L lot#2538399 SA = 6.4 Gr. 7-9	160 L lot#608345 SA = 5.5 Gr. 10-12
20 mg/kg				
Heart	8.58 ± 2.95	6.84 ± 2.39	4.09 ± 1.79[2]	5.86 ± 1.72
Quadriceps	5.90 ± 1.66	2.36 ± 1.38[1,2]	4.31 ± 1.76	7.93 ± 2.27
Triceps	8.37 ± 2.68	4.40 ± 1.30[2]	5.39 ± 1.77[2]	5.68 ± 1.57
60 mg/kg				
Heart	3.80 ± 2.58	0.00 ± 0.00[2]	0.00 ± 0.00[2]	0.23 ± 0.57
Quadriceps	4.15 ± 2.11	1.55 ± 1.14	4.21 ± 2.96	2.96 ± 2.10
Triceps	4.83 ± 1.87	2.76 ± 0.79[1]	3.89 ± 1.13	4.37 ± 1.14
100 mg/kg				
Heart	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Quadriceps	1.37 ± 1.20[3]	1.79 ± 1.45[3]	3.96 ± 1.57	1.00 ± 0.79
Triceps	3.66 ± 0.95	2.60 ± 0.50	3.57 ± 0.65	2.94 ± 0.47
Vehicle				
Heart	13.6 ± 2.32			
Quadriceps	7.52 ± 2.26			
Triceps	7.18 ± 2.28			

Values represent mean ± SD. Concentrations are in units of mg/g of wet tissue.
[1] - indicates $p<0.05$ compared to 160L lot at the same dose
[2] - indicates $p<0.05$ compared to the low SA 2000L group at the same dose
[3] - indicates $p<0.05$ compared to the high SA 2000L group at the same dose

Addendum: "Myozyme" was used inappropriately as a name for 2000L alglucosidase alfa. "Myozyme" is the tradename for the 160L product.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated March 31, 2008 by Dr. Ke Zhang.

Effect on Glycogen Clearance of 160L and 2000L Myozyme in the GAA Knockout Mice (Study No. 06-0276)

Methods: To compare the pharmacological activity of Myozyme manufactured at the 160L and 2000L scales, GAA knockout mice (8/group) were treated with Myozyme manufactured at the 2000L scale and 160L scale by i.v. injection at 0, 20, 60 or 100 mg/kg once weekly for 4 weeks. These animals were ~5 months old when the treatment was initiated. To prevent the hypersensitivity reaction, these animals were also treated with diphenhydramine 5 mg/kg IP 15-20 minutes prior to the second dose. Myozyme manufactured at the 2000L scale (lot #2538399) contains [redacted] (b) (4) and [redacted] (b) (4). Myozyme manufactured at the 160L scale (lot #608341) contains [redacted] (b) (4) and [redacted] (b) (4) All animals were sacrificed 7 days after the last dose. Tissue glycogen levels in the heart, quadriceps and triceps were determined using the Glycogen Trinder assay.

Results: The results indicated that the glycogen levels were not significantly different in the heart, triceps and quadriceps between the 160L and 2000L Myozyme groups at 60 and 100 mg/kg and in the heart between the 160L and 2000L Myozyme groups at 20 mg/kg. However, the glycogen levels were lower in the triceps and quadriceps with 160L Myozyme as compared to 2000L Myozyme at 20 mg/kg. Myozyme manufactured at the 2000L scale and at the 160L scale contains similar [redacted] (b) (4). The results were presented in the following table.

Table 2. Mean glycogen levels in the heart, triceps and quadriceps following 4 weekly administrations of 160L or 2000L Myozyme.

Best Possible Copy

Dose and Tissue	160 L lot#608341	2000 L lot#2538399
20 mg/kg		
Heart	9.34 ± 2.49	8.89 ± 2.91
Triceps	8.86 ± 1.82	11.7 ± 2.64*
Quadriceps	6.12 ± 1.07	7.86 ± 1.25*
60 mg/kg		
Heart	0.00 ± 0.00	1.08 ± 2.01
Triceps	5.16 ± 2.77	8.16 ± 2.94
Quadriceps	3.35 ± 0.65	4.46 ± 1.38
100 mg/kg		
Heart	0.00 ± 0.00	0.00 ± 0.00
Triceps	3.78 ± 3.28	5.12 ± 1.03
Quadriceps	2.60 ± 0.45	3.25 ± 1.44
Vehicle		
Heart	24.0 ± 6.30	
Triceps	10.9 ± 2.84	
Quadriceps	9.12 ± 1.22	

Values represent mean ± SD. Concentrations are in units of mg/g of wet tissue. * - indicates $p<0.05$ compared to 160L lot

The results suggested that the 2000L Myozyme can produce similar pharmacological activity (glycogen clearance) as compared to the 160L Myozyme at doses of 60 and 100 mg/kg.

Addendum: "Myozyme" was used inappropriately as a name for 2000L alglucosidase alfa. "Myozyme" is the tradename for the 160L product.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated August 28, 2008 by Dr. Niraj Mehta.

Glycogen Clearance Following Administration of 4-Weekly Doses of Mannose-6-Phosphate (M6P) Receptor Column Fractions of Myozyme (No Affinity Fraction, Pooled Bound Fraction, and High Affinity Fraction) as Compared to Unfractionated Myozyme (Study No. 07-2015)

Methods: The study design consisted of 66 acid alpha glucosidase (GAA) knockout mice (3-6 months old) divided into 11 groups of 3 males and 3 females per group except for Group 11 which contained 6 males. Myozyme 160L was fractionated over a M6P (mannose-6-phosphate) receptor column into three distinct fractions; unbound (no affinity), pooled bound, and a high affinity fraction. The pooled bound fraction contained rhGAA with low affinity, medium affinity, and high affinity for the M6P receptor column. The study design is shown in the table below (taken from the study report).

Group	Animal	Test Article	Dose (mg/kg)	Conc (mg/ml)	Dosing Regimen	Sample Collection
1	1-6	Vehicle	-	-	Once weekly for 4 weeks (4 total doses/animal)	heart, quadriceps, triceps, diaphragm, psoas, tibialis anterior, liver and serum
2	7-12	Myozyme	20	2.0		
3	13-18		40	4.0		
4	19-24		100	10		
5	25-30	Myo-M6P-NA	20	2.0		
6	31-36		40	4.0		
7	37-42		100	10.0		
8	43-48	Myo-M6P-Pooled	20	2.0		
9	49-54		40	4.0		
10	55-60	Myo-M6P-HA	20	2.0		
11	61-66		40	4.0		

Group 1 received vehicle treatment, Groups 2, 3, and 4 received 20, 40, or 100 mg/kg unfractionated Myozyme, Groups 5, 6, 7 respectively received 20, 40, or 100 mg/kg of Myo-M6P-NA (no affinity fraction), Groups 8 and 9 received 20 and 40 mg/kg of Myo-M6P-Pooled (pooled fraction), and Groups 10 and 11 received 20 and 40 mg/kg of Myo-M6P-HA (high affinity fraction). Test article, or vehicle, was intravenously administered weekly, via the tail vein, for 4 weeks. In order to prevent a test article related hypersensitivity reaction, 5 mg/kg diphenhydramine (DPH) was intraperitoneally administered 10-20 minutes prior to the second, third, and fourth doses in all groups. Animals were sacrificed 7-days after the last dose and the heart, quadriceps, triceps, diaphragm, psoas, tibialis anterior, liver and serum were collected. Biochemical glycogen content analysis was conducted on the heart, quadriceps, and triceps.

Results:

The results are shown in the table below (taken from the sponsor's submission).

Table 1. Tissue Glycogen Content Following Administration of Vehicle or Test Article
^P<0.05 compared to Vehicle; * P<0.05 compared to 20 mg/kg Myozyme; ** P<0.05 compared to 40 mg/kg Myozyme; # P<0.05 compared to 100mg/kg Myozyme

Group	Glycogen (mg/g of tissue)		
	Heart	Quadriceps	Triceps
Vehicle	35.88 ± 1.57	9.54 ± 0.40	11.25 ± 3.48
Myozyme - 20mg/kg	15.48 ± 2.97^	8.38 ± 0.73	11.32 ± 2.49
Myozyme - 40mg/kg	7.39 ± 2.38^	6.71 ± 1.68^	8.83 ± 2.38
Myozyme - 100mg/kg	0.40 ± 0.97^	5.06 ± 0.89^	7.50 ± 2.39^
Myo-M6P-NA - 20mg/kg	22.70 ± 2.93^*	10.02 ± 1.10	11.93 ± 2.98
Myo-M6P-NA - 40mg/kg	13.71± 2.23^**	9.32 ± 2.20**	13.01 ± 1.75**
Myo-M6P-NA - 100mg/kg	2.08 ± 2.38^	7.94 ± 1.00#	7.84 ± 1.55^
Myo-M6P-Pooled - 20mg/kg	11.70 ± 2.99^	8.20 ± 1.00	8.68 ± 1.43
Myo-M6P-Pooled - 40mg/kg	3.68 ± 2.53^**	6.31 ± 1.60^	7.30 ± 0.23^
Myo-M6P-HA - 20mg/kg	5.36 ± 0.73^*	6.00 ± 1.46^*	7.56 ± 1.32^*
Myo-M6P-HA - 40mg/kg	1.19 ± 1.85^**	4.35 ± 0.93^	5.82 ± 1.01^**

Heart: Glycogen levels were decreased in all unfractionated Myozyme dose groups and fractionated Myozyme dose groups ($p<0.05$). At 20 mg/kg, the reductions of glycogen in tissue (mg/g of tissue) were 57% in the Myozyme group, 37% in the no affinity fraction group, 67% in the pooled fractions group and 85% in the high affinity fraction group respectively, when compared to the vehicle control group. At 40 mg/kg, the reductions of glycogen in tissue were 79% in the Myozyme group, 62% in the no affinity fraction group, 90% in the pooled fractions group and 97% in the high affinity fraction group respectively, when compared to the vehicle control group. Over the dose range tested, the pooled fractions and the high affinity fraction reduced glycogen concentrations in the heart more robustly than Myozyme. The high affinity fraction of Myozyme produced the largest reduction in tissue glycogen levels.

Quadriceps: Groups that received 20 mg/kg Myozyme or 20 mg/kg pooled fractions did not significantly lower tissue glycogen compared to vehicle. However, treatment with 40 and 100 mg/kg Myozyme and 40 mg/kg pooled fractions, significantly ($p<0.05$) reduced glycogen levels by 30%, 47% and 34% respectively, compared to vehicle treatment. The high affinity fraction reduced glycogen levels by 37% and 54% in the 20 and 40 mg/kg groups, respectively. Groups that received the no affinity fraction at any dose level did not have significantly lower glycogen levels compared to vehicle. Thus, the high affinity fraction of Myozyme produced the largest clearance of glycogen.

Triceps: Glycogen levels were not significantly lower in the 20 mg/kg Myozyme, 20 mg/kg pooled fractions or 20 and 40 mg/kg no affinity fraction respectively, when compared to vehicle. However, tissue glycogen was significantly ($p<0.05$) reduced in dose groups that received 100 mg/kg Myozyme (↓ 33%), 100 mg/kg no affinity fraction (↓ 30%), 40mg/kg pooled fractions (↓

35%), and 20 and 40 mg/kg high affinity fraction (↓ 33% and ↓ 48%) respectively, compared to vehicle control. Therefore, high affinity Myozyme produced the largest clearance of glycogen.

Conclusions:

In the heart, quadricep and tricep tissues, the high affinity fraction of Myozyme produced the largest reduction in glycogen, followed by the pooled fractions, the unfractionated Myozyme and lastly, the no affinity fraction (based on 20 or 40 mg/kg doses). Therefore based on the observed glycogen levels in tissues treated with unfractionated Myozyme and various M6P affinity fractions, glycogen clearing activity appears to be directly correlated with M6P binding affinity. Interestingly, the data indicates that the no affinity fraction produced glycogen clearance in the heart (all doses) and triceps (100 mg/kg), based on comparison to the vehicle control glycogen tissue levels.

Addendum: The product used in the study was alglucosidase alfa manufactured at the 2000L scale. Myozyme (the 160L scale product) was not used.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated August 28, 2008 by Dr. Niraj Mehta.

An Evaluation of Glycogen Clearance Following Administration of 4-Weekly Doses of M6P-Receptor Column Fractions of Myozyme (Low Affinity and Medium Affinity) as Compared to the Unfractionated Myozyme (Study No. 07-2053)

Methods: The study design consisted of 7 groups (3 males and 3 females per group) of 3-6 month old acid alpha glucosidase (GAA) knockout mice. Each group received 4-weekly intravenous doses of vehicle, Myozyme, and fractionated Myozyme [Myo-M6P-LA (low affinity fraction) or Myo-M6P-MA (medium affinity fraction)]. In order to prevent a test article induced hypersensitivity response, 5 mg/kg diphenhydramine (DPH) was administered 10-20 minutes prior to test article administration at doses, two, three and four. One week following the 4th dose, animals were sacrificed and the heart, quadriceps, triceps, diaphragm, psoas, tibialis anterior, liver and serum were collected. The following analyses were conducted: biochemical glycogen content in heart, quadricep and tricep tissue. The study design is outlined in the sponsor's table below.

Group	Animal	Test Article	Dose (mg/kg)	Dosing Regimen	Sample Collection
1	1-6	Vehicle	-	Once weekly for 4 weeks (4 total doses/animal)	heart, quadriceps, triceps, diaphragm, psoas, tibialis anterior, liver and serum
2	7-12	Myozyme	40		
3	13-18	Myo-M6P-LA	20		
4	19-24	Myo-M6P-LA	40		
5	25-30	Myo-M6P-LA	100		
6	31-36	Myo-M6P-MA	20		
7	37-42	Myo-M6P-MA	40		

Results: Animal #42 was sacrificed following the 3rd dose due to observations consistent with hypersensitivity to the test article. All other animals appeared normal throughout the course of the study. The results are shown in the table below (taken from the study report).

Table 1 Tissue Glycogen Content Following 4 Weekly Doses Myozyme, Myozyme Column Fractions, or Vehicle to GAAKO Mice.
Values represent mean ± SD of Glycogen per gram of wet tissue

Test Article/Dose	Glycogen (mg/g of tissue)		
	Heart (Mean ± SD)	Quadriceps (Mean ± SD)	Triceps (Mean ± SD)
Vehicle (n=6)	26.9 ± 5.03	10.8 ± 1.23	14.5 ± 3.45
Myozyme 40mg/kg (n=6)	9.59 ±3.40^	8.30 ± 1.54^	10.6 ± 2.98^
Myo-M6P-LA 20mg/kg (n=6)	21.6 ± 2.60^	8.81 ± 1.96	10.6 ± 2.50^
Myo-M6P-LA 40mg/kg (n=6)	8.47 ± 1.80^	7.72 ± 1.19^	8.79 ± 0.56^
Myo-M6P-LA 100mg/kg (n=6)	0.00 ± 0.00^	4.78 ± 0.83^	5.60 ± 1.55^
Myo-M6P-MA 20mg/kg (n=6)	7.39 ± 3.15^	6.60 ± 1.00^	6.98 ± 1.84^
Myo-M6P-MA 40mg/kg (n=5)	2.70 ± 2.65^ **	6.74 ± 1.37^	7.44 ± 1.73^ **

^p<0.05 compared to Vehicle; ** p<0.05 compared to 40 mg/kg Myozyme

Heart: Tissue glycogen was significantly ($p<0.05$) reduced in dose groups that received 40 mg/kg Myozyme (↓ 64%), 20, 40, and 100 mg/kg low affinity fraction (↓ 20%, ↓ 69% and ↓ 100%, respectively), and 20 and 40 mg/kg medium affinity fraction (↓ 73% and ↓ 90%) respectively, compared to vehicle control. A comparison of Myozyme, low affinity Myozyme, and medium affinity Myozyme at 40 mg/kg shows that the medium affinity Myozyme produced the largest reduction in tissue glycogen.

Quadriceps: All treatment groups, with the exception of the 20 mg/kg low affinity fraction group, had significantly ($p<0.05$) reduced tissue glycogen, relative to the vehicle control value. There was a 23% decrease in the 40 mg/kg Myozyme group, a 29% and 56% decrease in the 40 and 100 mg/kg low affinity group, and a 39% and 38% decrease in the 20 and 40 mg/kg medium affinity group respectively, compared to vehicle control. A comparison of Myozyme, low affinity Myozyme, and medium affinity Myozyme at 40 mg/kg shows that the medium affinity Myozyme produced the largest reduction in tissue glycogen.

Triceps: Tissue glycogen was significantly ($p<0.05$) reduced in dose groups that received 40 mg/kg Myozyme (↓ 27%), 20, 40, and 100 mg/kg low affinity fraction (↓ 27%, ↓ 39% and ↓ 61%), and 20 and 40 mg/kg medium affinity fraction (↓ 52% and ↓ 49%) respectively, compared to vehicle control. A comparison of Myozyme, low affinity Myozyme, and medium affinity Myozyme at 40 mg/kg shows that the medium affinity Myozyme produced the largest reduction in tissue glycogen.

Conclusion:

In the heart, quadricep and tricep tissues, the medium affinity fraction of Myozyme produced the largest reduction in glycogen, followed by the low affinity fraction and lastly, the unfractionated Myozyme (based on a 40 mg/kg dose). Therefore, based on the observed glycogen level in tissues exposed to unfractionated Myozyme or various M6P affinity fractions, glycogen clearing activity appears to be directly correlated with M6P binding affinity.

Addendum: The product used in the study was alglucosidase alfa manufactured at the 2000L scale. Myozyme (the 160L scale product) was not used.

2.6.2.3 Secondary pharmacodynamics

No studies were submitted.

2.6.2.4 Safety pharmacology

No studies were submitted.

2.6.2.5 Pharmacodynamic drug interactions

No studies were submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Both single and repeat dose pharmacokinetic studies were conducted in Pompe GAA knockout mice. When a semilogaritlmic plot of concentration versus time was constructed from data produced by each or the pharmacokinetic (PK) studies, the curves exhibited characteristics of a two compartment model with first order elimination.

When PK parameters were analyzed after doses of 10, 20 or 40 mg/kg, results were linear with increasing doses. At doses up to 40 mg/kg, there was no evidence of saturation kinetics and the clearance of the drug followed a first order process. PK parameters were somewhat variable between species and between studies. Elimination half-lives were on the order of 2-3.5 hours for monkeys, 1-2 hours for rats, 1-2 hours for Pompe knockout mice, approximately 75 minutes for CD-1 mice, and approximately 1.5 to 2 hours for Beagle dogs. No consistent differences could be identified between male and female rodents due to the large variations. For the two monkey studies, the females consistently had lower AUC values. Both males and females in the high-dose group showed significantly higher elimination half-life relative to the other dose groups.

PK studies were conducted to investigate the potential differences between products of the 160 L

manufacturing process and the 2000 L process. The results demonstrated that the 90% confidence intervals for either AUC_{last} or $AUC_{0-\infty}$ ratios observed with the 160L and 2000 L lots were not within a pre-determined acceptability range (80% to 125%). These data indicate that pharmacokinetic equivalence was not established between the 160L and 2000L alglucosidase alfa.

Biodistribution studies in mice demonstrated that the highest levels of rhGAA activity are consistently found in the liver. Significantly lower levels (on the order of 30 X lower) were detected in spleen. Muscle results were lower still with cardiac muscle consistently demonstrating the highest levels of activity among the muscles studied. When the biodistribution of rhGAA from the 160 L process was compared to that from the 2000 L process, there appeared to be a higher magnitude of uptake in the liver for the 2000 L product.

2.6.4.2 Methods of Analysis

Not applicable.

2.6.4.3 Absorption

No studies were submitted.

2.6.4.4 Distribution

No studies were submitted.

2.6.4.5 Metabolism

No studies were submitted.

2.6.4.6 Excretion

No studies were submitted.

2.6.4.7 Pharmacokinetic drug interactions

No studies were submitted.

2.6.4.8 Other Pharmacokinetic Studies

No studies were submitted.

2.6.4.9 Discussion and Conclusions

The most significant finding derived from this collection of PK studies is the apparent difference in PK parameters among the various lots and manufacturing processes that were compared. A shorter half-life, lower AUC, and greater uptake by liver was observed with 2000 L

alglucosidase alfa, as compared to 160 L alglucosidase alfa. Therefore, these products do not appear to be comparable.

2.6.4.10 Tables and Figures

Not applicable.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

The sponsor submitted single dose studies in Sprague-Dawley rats and beagle dogs, and repeat dose studies in Sprague-Dawley rats, Cynomolgus monkeys and C57Bl/6 mice. In addition, Segment I and Segment II reproductive toxicology studies in the CD-1 mouse were also submitted. The test article was administered by i.v. infusion for all studies. rhGAA was well tolerated in monkeys at doses up to 200 mg/kg every other week (qow) for 13 weeks, and up to 100 mg/kg qow for 26 weeks. No adverse effects were reported for the 13 week study. For the 26 week study, a few findings were reported, including thrombus formation in the atrium of 2 of 3 male animals from the two higher dose groups. In female animals, ovarian cyst and unequal sized ovaries were noted in 1 of 3 females from the high dose group. Inflammation and degeneration of the quadriceps muscle occurred in 1 of 3 females from the high dose group. A relationship to the test article cannot be ruled out. Due to the small number of animals per group, this relationship is difficult to establish.

rhGAA was also generally well tolerated in C57Bl/6 mice at doses up to 100 mg/kg administered weekly for 4 weeks. The repeat dose toxicity study in mice was intended to compare toxicities produced by product from three different manufacturing processes (160L, 2000L and 4000L alglucosidase alfa). The toxicities noted included a small decrease in WBC for all three formulations. The biological significance for this finding is not clear since no baseline data is available and most values remained within normal limits for this species. In a similar 4-week study carried out in Sprague-Dawley rats (#6354-140), toxicity was much more apparent. Several unscheduled deaths occurred during the study that were not sufficiently explained. In addition, although a full panel of tissues was collected, no histopathology was reported even though gross examination at necropsy revealed several unexpected lesions. The sponsor was asked to explain the absence of the histopathology and the fate of those tissues. In addition, they were asked to provide information on the apparent increased toxicity in this rat study compared to study #6354-133, where the same dose levels and regimen was used. The sponsor reported that microscopic analysis was removed from the protocol near the end of the in-life portion of the study due to early termination of the study as a result of clinical observations and an unusual number of animal deaths. They reported that the clinical observations were consistent with hypersensitivity reactions. Therefore, the sponsor determined that the study results were not

interpretable and followed this rat study with a similar study using mice (meeting on May 22, 2002).

An additional repeat-dose toxicity study in rats (#6354-133) included only 5 rats/sex/group and investigated only one test article. Toxicities were less pronounced than those observed in study #6354-140. The animals in this study showed a dose related statistically significant decrease in body weight. The overall body weight change for males and females given 100 mg/kg was 23.3% and 11.6% lower than control animals, respectively. No clinical pathology or anatomical pathology results correlated with the weight loss finding. No other significant findings were reported. There is no indication of why the rhGAA was toxic in study #6354-140 relative to this additional rat study, except possibly the difference in lots used for study #6354-140.

Rodents tend to exhibit a severe hypersensitivity reaction to rhGAA, and numerous unscheduled deaths were attributed to this hypersensitivity. In most cases, the diagnosis was based upon clinical observations and the response to administered anti-histamine medications, and no in depth investigations were conducted. In response to the known hypersensitivity concern, for all rodent studies, the animals were pre-treated with 5 mg/kg diphenhydramine (DPH), i,p., approximately 20 minutes prior to administration of the test article. None of the study reports indicate that a separate control group receiving DPH only was used for any study, nor was there any indication of whether the control animals were also pre-treated with DPH. Therefore, any effects that DPH may have had or any interaction between DPH and the test article could not be assessed.

2.6.6.2 Single-dose toxicity

No studies were submitted.

2.6.6.3 Repeat-dose toxicity

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated September 15, 2008 by Dr. Niraj Mehta.

Study title: Six-Month Intravenous Repeated-Dose Toxicity Study of Recombinant Human Acid-α-Glucosidase (rhGAA) in Juvenile Mice

Key Study Findings: The no-observable-effects-level (NOEL) of rhGAA was 20 mg/kg/every other week, and 50 mg/kg/every other week was the tolerated dose.

Study #: FFA00067 (Reference No. 06032)

Seq. 74, Pg. 1-628 (electronic)

Conducting Laboratory and Location (b) (4)

Date of Study Initiation: January 8, 2007 (report dated September 26, 2007)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot #5930179, #7003108 and #7003108A; > 98% purity

Methods:

Doses: 0 (control/Saline), 0 (control/DPH), 10, 20, 50 mg/kg/every other week

Species/strain: Crl:CD1(ICR) mice

Number/sex/group or time point (main study): 12 /sex/group (Groups I to V)

Route, formulation, volume, and infusion rate: rhGAA or the rhGAA control article/vehicle was given by i.v. administration into the lateral tail vein. The treatment article, Diphenhydramine Hydrochloride (DPH) at 5 mg/mL and treatment article diluent, 0.9 % Sodium Chloride Injection, USP (saline), were administered to mice in Groups II through V and Group I, respectively. The prophylactic dose of DPH or saline was administered approximately 10 to 20 mins prior to the start of each infusion via i.p. injection at 5 mg/kg, beginning with the second dose, (1 mL/kg) based on the most recent body weight. The methodology (including dosage volume) is summarized in the sponsor's Table below:

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Dosage Group	Intraperitoneal Administration[a]	rhGAA Dosage[b] (mg/kg/every other week)	rhGAA Concentration (mg/mL)	rhGAA Dosage Volume[c] (mL/kg)	Number of Mice per Sex	Assigned F1 Generation Mouse Numbers	
						Male	Female
I	Saline	0 (Control Article)	0	10	12	1315[d], 102, 201, 202, 301, 302, 401, 402, 501, 502, 601, 602	701, 702, 1711[e], 802, 901, 902, 1001, 1002, 1101, 1102, 1201, 1202
II	DPH	0 (Control Article)	0	10	12	103, 104, 203, 204, 303, 304, 403, 404, 503, 504, 603, 604	703, 704, 803, 804, 903, 904, 1003, 1004, 1103, 1104, 1203, 1204
III	DPH	10	1	10	12	105, 106, 205, 206, 305, 306, 405, 406, 505, 506, 605, 606	705, 706, 805, 806, 905, 906, 1005, 1006, 1105, 1106, 1205, 1206
IV	DPH	20	2	10	12[f]	107, 108, 207, 208, 307, 308, 407, 1311[g], 507, 508, 607, 608, 1008[h]	707, 708, 807, 808, 907, 908, 1007, 1107, 1108, 1207, 1208
V	DPH	50	5	10	12[f]	109, 110, 209, 210, 309, 310, 409, 410, 509, 510, 609, 610, 1110[h]	709, 710, 809, 810, 909, 910, 1009, 1010, 1109, 1209, 1210

f. This group had 13 male and 11 female mice.

Satellite groups used for toxicokinetics: Blood samples (0.1 mL to 0.2 mL each) were collected for antibody analysis during the dosage period (one or two days prior to the seventh dosage administration) and on the day of the last dosage administration (prior to dosing) from each mouse via the sub-mandibular vein.

Study Design: The test article, recombinant human acid-α-glucosidase (rhGAA), and/or the control article, rhGAA Control Article, were administered once every other week beginning on day 21 postpartum (DP 21; Day 1 of study) to F1 generation mice/juvenile mice (F0 generation mice sacrificed day 21 of lactation). The mice were sacrificed after DP 203 (day of last dose) on DP 204-208.

Parameters and endpoints evaluated: mortality, clinical signs, body weight, food consumption, sexual maturation (female; vaginal patency, male: preputial separation), passive avoidance, open field observations, motor activity, antibody analysis, hematology, clinical chemistry, gross necropsy/pathology, organ weights and histopathology.

RESULTS:

Mortality: A total of 1, 0, 2, 5 and 1 mice were found dead (6/9) or sacrificed (3/9) early in the 0 (Control Article/Saline), 0 (Control Article/DPH), 10, 20 and 50 mg/kg/every other week dosage groups, respectively. None of these deaths were considered related the test article because the number of deaths were not dose-dependent and no pattern of deaths or adverse clinical signs could be found. The sponsor stated that there was no clear cause of death for 6/9 animals with the treatment procedure being the cause of death for 3 of the animals.

In Group III animals, male mouse 206 was found dead on DP 35. This mouse was found dead 2 mins after administration of its 2nd dose. The mouse had no adverse clinical observations and unremarkable bodyweight gain. All tissues appeared normal at necropsy. Female 1106 from Group III was found dead on DP 119, 61 mins after its 8th dosage administration. The mouse had decreased motor activity and necropsy revealed a pink colored pancreas, mesenteric lymph nodes and the renal pelvis was red in both kidneys. No clear cause of death could be determined for both of the animals.

In Group IV animals, male mouse 1311 was found dead on DP 203, before its 14 dosage administration (last dose DP 196). This mouse had no adverse clinical observations prior to death and unremarkable body weight gain. All tissues appeared normal at necropsy. No clear cause of death could be determined. Male mouse 608 was sacrificed on DP 133 due to adverse clinical observations, which started after the 8th dose. This mouse had paraphimosis, piloerection, ungroomed coat, cold to touch, hunched posture, severe dehydration, pale extremities, ptosis and decreased motor activity on DP 133. Body weight gain was unremarkable and all other tissues appeared normal at necropsy except for a small thymus. The moribund condition was presumed to be due to the treatment procedure, based on the on set of the clinical signs that started after dosing.

Female mouse 708 from Group IV was found dead on DP 189 (1 hr after 13th dose). This mouse had decreased motor activity starting on DP 161. All tissues appeared normal at necropsy. No clear cause of death could be determined. Female 1107 was sacrificed on DP 145 due to adverse clinical observations. This mouse had been dosed nine times (last dose DP 133). This mouse had tremors on DP 144, decreased motor activity, cold to touch, hunched posture, mild dehydration on DPs 144 to 145 and ungroomed coat on DP 145. Body weight gain was unremarkable and all tissues appeared normal at necropsy. No clear cause of death could be

determined. Female mouse 1207 was found dead on DP 21 (1 min after 1st dose). This mouse had no adverse clinical observations. All tissues appeared normal at necropsy.

In Group V animals, female mouse 710 was found dead on DP 34 after 1 dose. This mouse had no adverse clinical observations, normal tissues at necropsy with a moderate degree of autolysis. No cause of death could be determined.

Clinical Signs: Male mice in the Group III and V (10 and 50 mg/kg rhGAA dose) showed an increased incidence in microphthalmia when compared to control groups (Group I and II). The 50 mg/kg group also exhibited increased incidences of lacrimation compared to all other groups. Male animals treated with rhGAA had increased incidences of alopecia (not dose dependent). The male animals of the 10 and 20 mg/kg group had higher incidences of corneal opacity than the other groups. Males and females in the treatment groups had increased incidences of bent tails with the highest incidences for both sexes in the 10 mg/kg group. Females treated with rhGAA showed increased incidences of sparse hair coat compared to the control groups, and one female treated with 50 mg/kg of the test article had an increased incidence in head alopecia. Some other clinical observations observed in all groups throughout the study were red substance on the fur, dehydration, swollen face, cold to touch, ungroomed coat, excessive grooming, hunched posture, decreased motor activity, piloerection, purple pinna, hyperreactivity, vocalization, limited use of hindlimbs and tremors.

The adverse clinical observations in the male and female mice were unrelated to the administration of the test article because the incidences were not dosage dependent, the observation occurred in only one to three mice and some observations occurred in only the vehicle control groups.

Bodyweight: The mean body weights of Group I control males and females on DP 21 were 6.3 ± 0.28 and 8.4 ± 1.5 g, respectively. The mean body weights of Group I males and females at the end of the dosing period (DP 203) were 39.1 ± 3.2 and 31.2 ± 2.6 g, respectively. Body weights for the male and female mice were unaffected (up to and including terminal body weights) by doses of the test article as high as 50 mg/kg/every other week.

Food Consumption: The mean food consumption values of Group I males and females during the first week of dosing (DPs 28-35) were 5.9 ± 0.4 and 7.7 ± 1.1 g/animal/day respectively, and mean food consumption values during the last week of dosing (DP 196-203) were 5.7 ± 0.3 and 6.4 ± 1.0, respectively. There were minor increases in food consumption values in male and female mice administered doses of the test article as high as 50 mg/kg/every other week. Absolute food consumption values for male mice were increased ($p \leq 0.05$ or $p \leq 0.01$) in the 10 (↑ 27%) and 50 mg/kg (↑ 32%) groups on DPs 42 to 49 when compared to the saline control group. Absolute food consumption values were increased ($p \leq 0.01$) for male mice in the 20 mg/kg group on DPs 112 to 119 (↑ 17%) and 119-126 (↑ 13%).

Sexual Maturation: Sexual maturation was unaffected by maternal doses of the test article as high as 50 mg/kg/every other week. The average day on which preputial separation (~30.5 days) or vaginal patency (~ 27.5 days) occurred was comparable among the five dosage groups.

Passive Avoidance: There were no statistically significant or biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the male and female mice, at either of the testing periods (DPs 55 or 56 and DPs 167 or 168) as evaluated by performance in a passive avoidance paradigm. The data pertaining to passive avoidance performance are summarized in the sponsor's Table below:

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TABLE 12 (PAGE 1): PASSIVE AVOIDANCE PERFORMANCE - SUMMARY - F1 GENERATION MICE

DAY 55 OR 56 POSTPARTUM

DOSAGE GROUP		I	II	III	IV	V
INTRAPERITONEAL ADMINISTRATION		SALINE a	DPH b	DPH b	DPH b	DPH b
rhGAA DOSAGE (MG/KG/WEEK)c		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	50
MALE MICE						
SESSION 1d	N	6	6	5e	7f	7f
TRIALS TO CRITERION	MEAN±S.D.	5.3 ± 1.0	5.5 ± 1.6	5.8 ± 1.6	5.6 ± 1.7	5.1 ± 1.3
LATENCY TRIAL 1g	MEAN±S.D.	5.8 ± 4.5	10.7 ± 5.0	6.0 ± 2.2	9.3 ± 6.7	4.8 ± 6.5
LATENCY TRIAL 2g	MEAN±S.D.	21.3 ± 18.0	29.0 ± 22.0	15.2 ± 6.4	17.0 ± 8.2	18.6 ± 11.3
FAILED TO LEARN h	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2d	N	6	6	5e	7f	7f
TRIALS TO CRITERION	MEAN±S.D.	2.2 ± 0.4	2.0 ± 0.0	2.4 ± 0.5	2.6 ± 1.5	2.3 ± 0.5
LATENCY TRIAL 1g	MEAN±S.D.	53.7 ± 15.5	60.0 ± 0.0	45.0 ± 22.4	52.1 ± 20.8	49.7 ± 19.1
FEMALE MICE						
SESSION 1d	N	6	6	6	5	4e
TRIALS TO CRITERION	MEAN±S.D.	4.2 ± 0.8	4.2 ± 1.0	4.0 ± 0.6	4.2 ± 0.8	4.2 ± 1.2
LATENCY TRIAL 1g	MEAN±S.D.	20.0 ± 21.8	12.5 ± 9.8	15.7 ± 6.4	8.4 ± 7.3	16.0 ± 2.2
LATENCY TRIAL 2g	MEAN±S.D.	25.2 ± 20.1	33.2 ± 17.2	28.8 ± 18.0	43.6 ± 16.4	39.5 ± 23.0
FAILED TO LEARN h	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2d	N	6	6	6	5	4e
TRIALS TO CRITERION	MEAN±S.D.	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
LATENCY TRIAL 1g	MEAN±S.D.	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0

a. Administered saline intraperitoneally to match Diphenhydramine Hydrochloride (DPH) administration of Groups II through V.
b. Administered DPH intraperitoneally starting prior to the second dose of rhGAA.
c. Dosage occurred every other week day 21 through 203 postpartum.
d. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
e. Excludes values for mice that were found dead or sacrificed due to adverse clinical observations.
f. Includes mice that had their sex incorrectly identified.
g. The latency was recorded in seconds.
h. Number of mice that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these mice were excluded from summarization and statistical analyses.

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TABLE 12 (PAGE 2): PASSIVE AVOIDANCE PERFORMANCE - SUMMARY - F1 GENERATION MICE

DAY 167 OR 168 POSTPARTUM

DOSAGE GROUP		I	II	III	IV	V
INTRAPERITONEAL ADMINISTRATION		SALINE a	DPH b	DPH b	DPH b	DPH b
rhGAA DOSAGE (MG/KG/WEEK) c		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	50
MALE MICE						
SESSION 1d	N	6	6	6	6	6
TRIALS TO CRITERION	MEAN±S.D.	6.0 ± 4.4	5.3 ± 1.5	5.7 ± 1.9	5.7 ± 1.6	4.8 ± 1.3
LATENCY TRIAL 1e	MEAN±S.D.	12.0 ± 16.0	8.5 ± 4.6	8.7 ± 4.7	5.2 ± 4.1	9.2 ± 9.3
LATENCY TRIAL 2e	MEAN±S.D.	23.2 ± 18.4	19.0 ± 15.2	22.2 ± 9.3	21.5 ± 20.4	21.8 ± 19.6
FAILED TO LEARN f	N(%)	1(16.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2d	N	5	6	6	6	6
TRIALS TO CRITERION	MEAN±S.D.	3.4 ± 1.5	3.5 ± 1.0	2.8 ± 0.8	3.3 ± 2.0	3.0 ± 1.7
LATENCY TRIAL 1e	MEAN±S.D.	27.6 ± 29.7	41.7 ± 28.4	36.2 ± 23.4	50.3 ± 21.3	60.0 ± 0.0
FEMALE MICE						
SESSION 1d	N	6	6	5g	4g	6
TRIALS TO CRITERION	MEAN±S.D.	4.7 ± 0.8	4.2 ± 0.4	4.8 ± 1.1	4.5 ± 1.3	5.0 ± 1.8
LATENCY TRIAL 1e	MEAN±S.D.	9.0 ± 4.8	13.0 ± 6.7	14.0 ± 13.1	10.0 ± 7.4	12.0 ± 13.8
LATENCY TRIAL 2e	MEAN±S.D.	13.5 ± 8.4	35.0 ± 13.0	42.8 ± 11.0	31.2 ± 20.4	25.0 ± 23.5
FAILED TO LEARN f	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2d	N	6	6	5g	4g	6
TRIALS TO CRITERION	MEAN±S.D.	2.7 ± 0.5	2.2 ± 0.4	2.4 ± 0.5	2.2 ± 0.5	2.3 ± 0.5
LATENCY TRIAL 1e	MEAN±S.D.	26.2 ± 27.9	54.2 ± 14.3	45.8 ± 20.8	50.0 ± 20.0	44.0 ± 25.3

a. Administered saline intraperitoneally to match Diphenhydramine Hydrochloride (DPH) administration of Groups II through V.
b. Administered DPH intraperitoneally starting prior to the second dose of rhGAA.
c. Dosage occurred every other week day 21 through 203 postpartum.
d. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
e. The latency was recorded in seconds.
f. Number of mice that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these mice were excluded from summarization and statistical analyses.
g. Excludes values for mice that were found dead or sacrificed due to adverse clinical observations.

Open Field Observations: There was a statistically significant decrease ($p \leq 0.01$) in the number of awake, immobile male mice in Control Article/DPH group and 10 mg/kg group on DP 68. On DP 176, the number of female mice with no defecations was significantly increased ($p \leq 0.01$) and the number of female mice with normal defecations was significantly reduced ($p \leq 0.01$) in the Control Article/DPH, 10 and 20 mg/kg groups. Female mice from the 20 mg/kg group had increased startle reaction compared to all the other dosage groups. These differences were not considered related to the test article because they were not dose dependent or they occurred in the Control Article/DPH group that did not get the test article. The open field observations are summarized in the Table below.

Dosage Group	Saline Ctrl	DPH Ctrl	10 mg/kg	20 mg/kg	50 mg/kg
Male: Home cage behavior (awake, immobile, DP180)	4/11	0/12**	0/11**	2/12	5/13
Female: Home cage behavior (awake, immobile, DP68)	1/12	0/12	2/12	1/10	2/10
Female: No Defecation in open field, DP176	9/12	12/12**	11/11**	10/10**	7/11
Female: Auditory reaction, DP176 (startle)	1/12	1/12	2/11	4/10	0/11

** Significantly different from Group I value {$p \leq 0.01$}

Motor Activity: No statistically significant or biologically important differences occurred in either the number of movements or time spent in movement for the male and female mice. No statistically significant differences occurred among the groups.

Antibody Analysis: Results showed the presence of rhGAA antibodies in all three dose groups administered rhGAA prior to the seventh dose and prior to the last dose. Three animals

administered the control article, animals 201 and 1102 in Group I and animal 604 in Group II were positive for rhGAA. Animal 201 demonstrated a titer of 6580 prior to the seventh dose, with no sample available for analysis prior to the last dose. Animal 1102 presented with a titer of 219 prior to the last dose. Animal 604 presented with a titer of 5389 prior to the seventh dose, with no detectable titer prior to the last dose. The ELISA data for the presence of rhGAA antibodies in the test article treated groups is summarized in the Tables below provided by the sponsor:

Prior 7th Dose				
Dose	Number Positive	Titer Range	% Positive	Mean Titer
10 mg/kg	9 out of 23	1: 213427 -130	39	39365
20 mg/kg	8 out of 23	1: 99896 -170	35	28440
50 mg/kg	14 out of 23	1: 665050 - 109	61	82715

Prior Last Dose				
Dose	Number Positive	Titer Range	% Positive	Mean Titer
10 mg/kg	10 of 22	1: 173277 - 327	45	31539
20 mg/kg	7 of 20	1: 79692 - 558	35	19026
50 mg/kg	15 of 22	1: 228323 - 590	68	44933

There was a minor difference in the incidence or level of antibody response between 10 and 20 mg/kg dose groups at either time point. There was an increase in the incidence and level of antibody response at the 50 mg/kg dose group for both time points when compared to the 10 and 20 mg/kg group. The mean titer for the 50 mg/kg group was considerably higher than the 10 and 20 mg/kg group prior to the seventh dose and the 20 mg/kg group prior to the last dose. There was only a small increase in the mean titer in the 50 mg/kg group compared to 10 mg/kg group prior to the last dose. For unknown reasons, the majority of the animals across all groups demonstrated a low level of cross-reactivity following repeat administration of rhGAA.

Hematology: There were no statistically significant changes observed related to test article administration.

There was an increase (↑ 31%) of WBC in male mice from the 20 mg/kg group, while there was a 13% decrease of WBC in female mice from the same group when compared to the Saline/Control group. There was a 14% decrease in the number of neutrophils in male mice from the 50 mg/kg group when compared to the Saline/Control group. There was a 45% and 23% increase in the number of lymphocytes and a 121% and 33% increase in the number of large unstained cells in male mice from the 20 and 50 mg/kg groups respectively, when compared to the Saline/Control group. 20 mg/kg male mice also had a 37% increase in the number of monocytes and a 72% increase in eosinophils when compared to the Saline/Control group. There was a 39% and 61% increase in the number of basophils in male mice from the 10 and 50 mg/kg groups respectively, when compared to the Saline/Control group.

There was a 14% and 13% increase in platelet counts in female mice from the 10 and 50 mg/kg groups, respectively. There was a minor, 12% and 11% decrease in the number of lymphocytes in female mice from the 20 and 50 mg/kg groups when compared to the Saline/Control group. There was a 44% increase in the number of monocytes in female mice from the 50 mg/kg group when compared to the Saline/Control group. There was a decrease in the number of eosinophils by 35%, 42% and 45% and a decrease in the number of large unstained cells by 40%, 23% and 49% in female mice from the 10, 20 and 50 mg/kg groups respectively, when compared to the Saline/Control group. There was a 50% increase in the number of basophils in female mice from the 10 mg/kg group, while there was a 23% decrease in the number of basophils in female mice from the 50 mg/kg group when compared to the Saline/Control group.

Clinical Chemistry: There were no statistically significant changes observed related to test article administration. There was a decrease in glucose concentration by 30%, 28% and 24% and in the levels of triglycerides by 10%, 22% and 17% in male mice from the 10, 20 and 50 mg/kg groups respectively, when compared to the Saline/Control group. Cholesterol levels increased by 19% and blood urea nitrogen by 15% in male mice from the 10 mg/kg group when compared to the Saline/Control group. Creatinine levels decreased by 33-50% in all test article treated groups when compared to the saline group. There was a 16% decrease in the alanine aminotransferase (ALT) level in male mice from the 50 mg/kg group when compared to the Saline/Control group.

In female mice from the 10 mg/kg group, there was a 20% decrease in glucose concentration when compared to the Saline/Control group. There was a 21% and 32% increase of alkaline phosphatase (ALP) levels in female mice from the 20 and 50 mg/kg groups when compared to the Saline/Control group. Unlike the male mice there was an increase in triglyceride levels of 30%, 13% and 16% in the female mice from the 10, 20 and 50 mg/kg groups respectively, when compared to the Saline/Control group. Female mice from the 50 mg/kg group showed a 15% decrease in potassium concentrations when compared to the Saline/Control group.

Gross Necropsy/Pathology: There were no major treatment-related effects on gross necropsy/pathology because the incidences were not dose-dependent and the observed effects occurred in only 1 or 2 mice. The observations (excluding those described under 'mortality') included numerous yellow areas on the spleen in one Control Article/DPH male mouse and ovarian bursal cyst in one Control Article/Saline and one 10 mg/kg mouse and pitted areas of one kidney in one Control Article/DPH female mouse.

Organ Weights: There were some minor changes in absolute organ weights or the ratio of these weights to the terminal body weight or the brain weight at doses as high as 50 mg/kg/every other week. For example, the absolute weight of the right kidney and the ratio of this weight to the terminal body weight were significantly increased ($p \leq 0.05$) in males from the Control Article/DPH (↑ 12-14%), 10 (↑ 12-15%) and 20 mg/kg (↑ 11-13%) groups compared to the control group values. In the 20 mg/kg group, the absolute weight of the left testis and the ratio of the left testis weight to the terminal body weight were increased by 26% and 27%, respectively. The ratio of this weight to the brain weight was significantly increased ($p \leq 0.01$) in the 20 mg/kg group (↑ 23%).

The absolute weights of the thyroid/parathyroid, and the ratio of these weights to the terminal body weight and the brain weight for the male and female mice were significantly increased ($p \leq 0.01$) in the 10 and 20 mg/kg group compared to Control Article/Saline group values. The absolute thyroid/parathyroid weight in male mice from the 10 and 20 mg/kg groups increased by 116% and 133% respectively, while the ratio of these weights increased by 109% and 114%, respectively. In female mice, the absolute thyroid/parathyroid weight from the 10 and 20 mg/kg groups increased by 166% and 100%, while the ratio of these weights increased by 151% and 132%, respectively.

In female mice, there was a significant reduction ($p \leq 0.05$ or $p \leq 0.01$) in absolute weight of the left ovary and the ratio of this weight to terminal body weight and brain weight in the Control Article/DPH group. In addition, the absolute weight of the brain and the ratio of this weight to the terminal body weight in females from the Control Article/DPH group were significantly increased ($p \leq 0.05$). Finally, the ratio of the lungs and thymus weights to the brain weight were reduced ($p \leq 0.05$ or $p \leq 0.01$) in female mice compared to the Control Article/DPH group.

None of these differences were considered related to the test article because the change was not dose-dependent and the bulk of the changes occurred in the Control Article/DPH mice that were not administered the test article.

Histopathology: There was an increased number of male mice from the 50 mg/kg group with mild to minimal pigmented macrophages in the adrenal glands (8/13) compared to both the saline and DPH control groups (5/12), while the female mice had an increase of spindle-cell proliferation in the 50 mg/kg (8/10) compared to either of the control groups (7/12). Female mice in the 50 mg/kg group had an increased incidence of microgranuloma in the lungs (3/10) compared to either control group (0/12). Male mice from the 50 mg/kg group had an increased number of thymuses with atrophy/involution (5/12) compared to the Saline/Control (3/12) or the DPH/Control (1/12) and increased mononuclear-cell infiltration in the kidneys (7/13) compared to the Saline/Control (4/12) or the DPH/Control (3/12). In addition, in the parotid salivary gland and lacrimal gland, there were several changes related to the venipuncture procedure. These changes included areas of acute hemorrhage, atrophy, necrosis, inflammation, and granulomas or microgranulomas. These changes were unilateral and in the region of the submandibular vein. The histopathology data are summarized in the Table below.

Dose Group/Sex: **Number of animals/ group:**	**I-M** **12**	**II-M** **12**	**V-M** **13**	**I-F** **12**	**II-F** **12**	**V-F** **10**
Adrenal glands: pigmented macrophages (minimal)	5 (3/5)	5 (3/5)	8 (5/8)			
Subcapsular, spindle-cell proliferation (minimal)				7 (4/7)	7 (3/7)	8 (5/8)
Lung: Foreign body, microgranuloma				0	0	3
Thymus: Atrophy/involution (minimal)	3 (1/3)	1 (0/1)	5 (3/5)			
Kidneys: Mononuclear-cell infiltration (minimal/mild)	4	3	7			

Conclusions: Based on the results of this 6-month intravenous repeated-dose toxicity study in juvenile animals, the no-observable-effects-level (NOEL) of rhGAA was 20 mg/kg/every other week and 50 mg/kg/every other week was the tolerated dose. There were no treatment-related

effects on mortality, body weights, body weight gains, absolute food consumption values, sexual maturation, learning and memory, motor activity, open field performance, absolute organ weights and the ratio of organ weights to terminal body weights and brain weights.

There were no statistically significant changes in hematology or clinical chemistry. All clinical and necropsy observations in the male and female mice were unrelated to administration of the test article. There were histopathological effects in male's from the 50 mg/kg group compared to the control groups including increased incidence of mononuclear-cell infiltration into the kidneys (7/13 compared to 3/12 from the DPH control group) and an increased incidence of atrophy in the thymus (5/12) when compared to the saline and DPH control groups (1/12). The kidney and the thymus were the target organs of toxicity.

Addendum: The test article used in the study, "rhGAA", is Myozyme®.

2.6.6.4 Genetic toxicology

No studies were submitted.

2.6.6.5 Carcinogenicity

No studies were submitted.

2.6.6.6 Reproductive and developmental toxicology

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated September 15, 2008 by Dr. Niraj Mehta.

Study title: Intravenous Injection Study of Recombinant Human Acid-α-Glucosidase (rhGAA) on Fertility and Early Embryonic Development in Mice

Key Study Findings: The doses of rhGAA as high as 40 mg/kg administered every other day to male and female mice did not affect the mating and fertility. However, there were dose dependent decreases of sperm motility and sperm count in the 20 and 40 mg/kg groups when compared to the saline control group.

Study #: FFA00051 (Reference No. 06010)

Seq. 37, Pg. 1-295(electronic)

Conducting Laboratory and Location (b) (4)

Date of Study Initiation: May, 1 2006 (report dated September, 22 2006)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot #1369017; > 98% pure

Methods:

Doses: 0 (control article), 0 (control/DPH), 10, 20, 40 mg/kg/every other day

Species/strain: Crl:CD1 (ICR) mice

Number/sex/group or time point (main study): 22/sex/group (Groups I to V)

Route, formulation, volume, and infusion rate: rhGAA or the rhGAA control article/vehicle was given by intravenous administration into the lateral tail vein. The treatment article, Diphenhydramine Hydrochloride (DPH) at 5 mg/mL and treatment article diluent, 0.9 % Sodium Chloride Injection, USP (saline), were administered to mice in Groups II through V and Group I, respectively. The prophylactic dose of DPH or saline was administered approximately 10 to 20 mins prior to the start of each infusion via i.p. injection at 5 mg/kg, after the fourth (male mice) or sixth (female mice) dose, (1 mL/kg) based on the most recent body weight. The methodology (including dosage volume) is summarized in the sponsor's Table below:

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Dosage Group	Number of Mice per Sex	IP Administration	rhGAA Dosage (mg/kg/day)	rhGAA Concentration (mg/mL)	rhGAA Dosage Volume (mL/kg)	rhGAA Injection Rate (mL/min)	Assigned Numbers	
							Male Mice	Female Mice
I	22	Saline	0 (Control Article)	0	8	slow bolus	5201 - 5222	5311 - 5332
II	22	DPH	0 (Control Article)	0	8	slow bolus	5223 - 5244	5333 - 5354
III	22	DPH	10	1.25	8	slow bolus	5245 - 5266	5355 - 5376
IV	22	DPH	20	2.5	8	slow bolus	5267 - 5288	5377 - 5398
V	22	DPH	40	5.0	8	slow bolus	5289 - 5310	5399 - 5420

The test article was considered 100% active/pure for the purpose of dosage calculations.
IP = Intraperitoneal injection.

Satellite groups used for toxicokinetics: none

Study design: The test article, recombinant human acid-α-glucosidase (rhGAA), and/or the control article, rhGAA Control Article, were administered intravenously once every other day to male and female mice. Male mice were given the test article or the control article once every other day beginning nine weeks before cohabitation (maximum 14 days) and continuing through the cohabitation period. All surviving male mice were sacrificed within 1 to 2 days after the completion of the cohabitation period.

Female mice were given the test article or the control article once every other day beginning 15 or 16 days before cohabitation (maximum of 14 days) and continuing through day 8 of presumed gestation (DG 8). All surviving female mice were sacrificed on DG 13.

Parameters and endpoints evaluated: mortality, clinical signs, body weight, food consumption, sexual maturation (female; vaginal patency, male: preputial separation), and gross necropsy/pathology. Male specific data includes organ weights (right testis, left testis, left epididymis (whole and cauda), right epididymis, seminal vesicles and prostate), sperm evaluation (concentration, motility and morphology), and histopathology (examination of left epididymis, as well as the right epididymis, prostate and seminal vesicles). Female specific data includes mating and fertility (estrous cycle and pregnancy), and cesarean-sectioning observations (number and distribution of implantation sites, and viable and nonviable embryos).

RESULTS:

Mortality:

Male: The 5 (two male mice in the 10 mg/kg/every other day dosage group and three male mice in the 20 mg/kg/every other day dosage group) deaths that occurred were not considered related to rhGAA administration because the deaths were not dose-dependent and because 4 of the 5 mice died prior to rhGAA administration. In the 10 mg/kg/every other day (Group III) dosage group, mouse 5245 was found dead on Day 9 of the study (DS 9), two hours after the fourth dose was administered. There were no adverse clinical observations and although this was the smallest male of the group, the body weight gain and food consumption values for this mouse were comparable to other mice in the group. All tissues appeared normal at necropsy.

The rest of the animals (4 of 5 total) died right before rhGAA administration, but after administration of DPH. The other Group III animal, mouse 5262, was found dead on DS 61, prior to the 31st dosage administration with no adverse clinical observations, comparable body weight gain and food consumption, and normal tissues at necropsy.

In the 20 mg/kg/every other day group, mouse 5267, 5275 and 5283 died on DS 57 prior to the 29th dosage, DS 59 prior to the 30th dosage and DS 27 prior to the 14th dosage, respectively. All 3 mice had no adverse clinical observations, comparable body weight gain and food consumption, and normal tissues at necropsy.

Female: The 3 deaths that occurred in the 10 mg/kg/every other day dosage group were not considered related to rhGAA administration because the deaths were not dose dependent. Mouse 5359 was sacrificed due to adverse clinical signs on DS 2 (dosed once on DS 1). Adverse clinical signs noted for this mouse on DS 2 included decreased motor activity, splayed hindlimbs, dyspnea, labored breathing, pale extremities, cold to touch and scant feces. Body weight for this mouse was comparable to other mice in this dosage group. Necropsy revealed approximately 1 mL of dark red fluid in the thoracic cavity. All other tissues appeared normal. The sponsor presumed that the clinical observations and necropsy were secondary to the restraint used prior to the injection process of the test article. Another animal, mouse 5373, was accidentally killed on DG 7 when it fell to the floor and had its cage land on top of it. Lastly, mouse 5368 was found dead on DG 1, four hrs after its 9th test article administration. There were no adverse clinical observations and the body weight gain and feed consumption values for this mouse were comparable to other mice in the group. All tissues appeared normal at necropsy.

Clinical Signs:

Male: Clinical observations were generally related to the injection site or tail. The most common observation was a purple or black injection site that occurred in 9, 4, 6, 9 and 9 male mice in the 0 (Saline), 0 (DPH), 10, 20 and 40 mg/kg/every other day dosage groups, respectively (Groups I to V), and a bent tail that occurred in 1, 0, 1, 4 and 0 mice in the same respective dosage groups. Most adverse occurrences generally took place after DS 30.

Female: There were no test article-related adverse clinical observations.

Bodyweight:

Male: The initial (Day 1) mean body weight for Group I males was 29.2 ± 2.2 g, and the terminal body weight was 34.0 ± 2.2 g. Body weights and body weight changes were not affected by doses of rhGAA as high as 40 mg/kg/day.

Female: The initial (two weeks prior to cohabitation) mean body weight for Group I females was 26.0 ± 1.2 g, and the body weight on DG 13 was 40.1 ± 3.4 g. Body weights and body weight changes during both the precohabitation and gestation periods were generally not affected by doses of rhGAA as high as 40 mg/kg. There was a 14% increase in gestational body weight (DG 0-13) in the 10 mg/kg group when compared to the saline control group (Group I).

Food Consumption:

Male: The mean food consumption values of Group I males from Days 1-9 and Days 57-64 of the precohabitation period were 5.1 ± 1.0 and 5.4 ± 1.0 g/day, respectively. Food consumption values were not affected by doses of rhGAA as high as 40 mg/kg/every other day with the exception of a statistically significant ($p \leq 0.01$) increase of 24% when compared to the saline control group (Group I) in the absolute food consumption that occurred in the 40 mg/kg group from cohabitation Days 9 to 15.

Female: The mean food consumption values of the Group I females from Days 1-9 of the cohabitation period and DG 10-13 were 5.2 ± 0.9 and 7.2 ± 1.6 g/day, respectively. Food consumption values were not affected by doses of rhGAA as high as 40 mg/kg/day either during the precohabitation or gestation periods.

Mating and Fertility: Doses as high as 40 mg/kg/day did not affect any mating or fertility parameters including days in cohabitation, mice that mated, fertility index, or pregnancy rate. The mating and fertility data for the male and female mice are summarized in the sponsor's Tables below.

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TABLE A6 (PAGE 1): MATING AND FERTILITY - SUMMARY - MALE MICE

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (CONTROL ARTICLE)b	II 0 (CONTROL ARTICLE)c	III 10c	IV 20c	V 40c
MICE IN COHABITATION	N	22	22	20d	19d	22
DAYS IN COHABITATION e	MEAN±S.D.	2.4 ± 1.6	4.2 ± 4.3	3.2 ± 3.0	3.5 ± 3.5	4.6 ± 4.4
MICE THAT MATED f	N(%)	22(100.0)	22(100.0)	19(95.0)	19(100.0)	21(95.4)
FERTILITY INDEX g,h	N/N (%)	22/22 (100.0)	21/22 (95.4)	16/19 (84.2)	19/19 (100.0)	19/21 (90.5)
MICE WITH CONFIRMED MATING DATES	N	22	20	19	18	19
MATED WITH FEMALE i						
DAYS 1-7	N(%)	22(100.0)	17(85.0)	19(100.0)	17(94.4)	18(94.7)
DAYS 8-14	N(%)	0(0.0)	3(15.0)	0(0.0)	1(5.6)	1(5.3)
MICE PREGNANT/MICE IN COHABITATION h	N/N (%)	22/22 (100.0)	21/22 (95.4)	16/20 (80.0)	19/19 (100.0)	19/22 (86.4)

a. Dosage occurred every other day from day 1 of study through one or two days before sacrifice.
b. Administered saline intraperitoneally to match DPH administration of Groups II through V.
c. Administered DPH intraperitoneally from the fourth dose of rhGAA until one or two days before sacrifice.
d. Excludes mice that were found dead.
e. Restricted to mice with a confirmed mating date and mice that did not mate.
f. Includes only one mating for each male mouse.
g. Number of pregnancies/number of mice that mated.
h. Includes only one pregnancy for each mouse that impregnated more than one female mouse.
i. Restricted to mice with a confirmed mating date.

TABLE A7 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE MICE

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (CONTROL ARTICLE)b	II 0 (CONTROL ARTICLE)c	III 10c	IV 20c	V 40c
MICE EXAMINED d	N	22	22	22	22	22
FOUND DEAD	N	0	0	2e,f	3g,h,i	0
APPEARED NORMAL	N	21	21	20e,f	22g,h,i	20
SPLEEN: LARGE	N	1	0	0	0	0
KIDNEYS: RIGHT, SMALL	N	0	0	1	0	0
RIGHT, DEPRESSED AREA	N	0	0	1	0	0
SEMINAL VESICLES: LEFT LOBE, BROWN	N	0	1	0	0	0
TESTES: LEFT, SMALL	N	0	0	0	0	1
PROSTATE: SMALL	N	0	0	0	0	1

a. Dosage occurred every other day from day 1 of study through one or two days before sacrifice.
b. Administered saline intraperitoneally to match DPH administration of Groups II through V.
c. Administered DPH intraperitoneally from the fourth dose of rhGAA until one or two days before sacrifice.
d. Refer to the individual clinical observations table (Table A12) for external observations confirmed at necropsy.
e. Mouse 5245 was found dead on day 9 of study.
f. Mouse 5262 was found dead on day 61 of study.
g. Mouse 5267 was found dead on day 57 of study.
h. Mouse 5275 was found dead on day 59 of study.
i. Mouse 5283 was found dead on day 27 of study.

Gross Necropsy/Pathology: No necropsy observations related to rhGAA occurred in either male or female mice. No observation occurred in more than a single mouse in any group.

Organ Weights: Terminal body weights, organ weights and the ratio of these organ weights to the terminal body weight did not significantly differ among the groups. There was a minor decrease (12%) in the ratio of organ weight to the body weight in the left testis in the 40 mg/kg group compared to saline control animals.

Sperm Evaluation: There was a decrease in sperm motility and count for sperm evaluated from the vas deferens and cauda epididymis (sperm count only) across all the Groups II to V. There was an 11% and 14% decrease in the mean sperm count, and a 12% and 16% decrease in the mean sperm density in the 20 and 40 mg/kg groups respectively, compared to the control saline group. The sperm motility and sperm count data are summarized in the sponsor's Table below.

Best Possible Copy

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (CONTROL ARTICLE)b	0 (CONTROL ARTICLE)c	10c	20c	40c
MICE TESTED	N	22	22	20d	19d	22
CAUDAL EPIDIDYMAL SPERM MOTILITY						
NUMBER MOTILE	MEAN±S.D.	608.6 ± 350.1 [20]e	524.7 ± 383.1 [19]e	511.2 ± 226.1 [19]e	498.0 ± 351.8 [17]e	598.1 ± 347.4 [19]e
MOTILE PERCENT	MEAN±S.D.	79.1 ± 13.8 [20]e	67.9 ± 21.7 [19]e	76.5 ± 15.2 [19]e	69.6 ± 17.1 [17]e	74.9 ± 25.2 [19]e
STATIC COUNT (NONMOTILE)	MEAN±S.D.	135.7 ± 80.2 [20]e	177.9 ± 102.8 [19]e	144.6 ± 84.4 [19]e	170.7 ± 94.8 [17]e	148.6 ± 118.1 [19]e
TOTAL COUNT f	MEAN±S.D.	744.2 ± 367.9 [20]e	702.7 ± 354.5 [19]e	655.8 ± 243.0 [19]e	668.7 ± 368.0 [17]e	746.7 ± 315.1 [19]e
CAUDA EPIDIDYMAL SPERM COUNT						
SPERM COUNT g	MEAN±S.D.	58.3 ± 17.2	67.7 ± 17.1	57.9 ± 16.4	51.4 ± 19.0	50.1 ± 15.5
SPERM DENSITY h	MEAN±S.D.	1832.91 ± 529.93	2176.81 ± 443.59*	1729.55 ± 400.23	1615.67 ± 551.92	1534.66 ± 511.15

[ ] = NUMBER OF VALUES AVERAGED

a. Dosage occurred every other day from day 1 of study through one or two days before sacrifice.
b. Administered saline intraperitoneally to match DPH administration of Groups II through V.
c. Administered DPH intraperitoneally from the fourth dose of rhGAA until one or two days before sacrifice.
d. Excludes mice that were found dead.
e. Excludes values for mice that had motility data reflecting drifting debris.
f. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
g. Sperm count used in the calculation of sperm density. Ten fields were evaluated.
h. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.2×10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

Sperm morphology was comparable among the groups except there was a 30% decrease in flagella with no head in the 40 mg/kg dose group when compared to the saline control group. The numbers of normal sperm per 200 counted and the percent abnormal sperm per mouse did not differ significantly among the groups. In addition, there was a significant decrease ($p \leq 0.01$) in the number of coiled flagellum in the Control Article/DPH group (↓ 76%) and 20 mg/kg groups (↓ 74%) which were not considered related to the test article because the reduction occurred in a control group and was not dose-dependent. The sperm morphology data is summarized in the sponsor's Table below.

Best Possible Copy

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (CONTROL ARTICLE)b	II 0 (CONTROL ARTICLE)c	III 10c	IV 20c	V 40c
MICE EXAMINED	N	22	22	20d	19d	20e
NORMAL	MEAN±S.D.	178.7 ± 8.7	181.6 ± 11.9	180.5 ± 8.7	183.9 ± 10.4	181.3 ± 11.3
PERCENT ABNORMAL	MEAN±S.D.	10.6 ± 4.3	9.2 ± 6.0	9.8 ± 4.4	8.0 ± 5.2	9.4 ± 5.7
DETACHED HEAD	MEAN±S.D.	4.1 ± 4.4	8.0 ± 7.0	6.4 ± 6.2	6.8 ± 6.3	5.2 ± 3.7
NO HEAD	MEAN±S.D.	9.0 ± 4.8	8.3 ± 5.7	7.4 ± 4.4	7.1 ± 5.0	6.2 ± 4.3
BROKEN FLAGELLUM	MEAN±S.D.	0.6 ± 0.8	0.4 ± 0.7	0.4 ± 1.1	0.1 ± 0.3	1.0 ± 1.3
COILED FLAGELLUM	MEAN±S.D.	7.6 ± 7.0	1.8 ± 2.8**	5.3 ± 8.3	2.0 ± 2.5**	6.4 ± 6.2

a. Dosage occurred every other day from day 1 of study through one or two days before sacrifice.
b. Administered saline intraperitoneally to match DPH administration of Groups II through V.
c. Administered DPH intraperitoneally from the fourth dose of rhGAA until one or two days before sacrifice.
d. Excludes values for mice that were found dead.
e. Excludes values for mice 5289 and 5292; unable to find 200 sperm.
** Significantly different from the Group I value ($p \leq 0.01$).

Histopathology: There were no test article-related microscopic changes seen in the testes or epididymides from the male mice given the highest dosage level of the test article, rhGAA. One minor difference was increased incidences of exfoliated spermatogenic cells in the 40 mg/kg group (3/22) compared to either saline or DPH control group (1/22). The histomorphologic examinations showed that sporadic abnormal differences occurred equally amongst the control group animals (saline and DPH) and the 40 mg/kg group. The type, incidence, or severity of the changes were not influenced by administration of the test article.

Mating and Fertility/Caesarean-Sectioning Observations: Doses of rhGAA as high as 40 mg/kg/every other day did not affect estrous cycling, any mating parameters or fertility. The number of estrous cycles was comparable among the groups both prior to and after two weeks of dosing. No parameter evaluated at caesarean-section was affected by the test article. The number of viable and nonviable embryos was comparable among the groups.

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TABLE B10 (PAGE 1): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - FEMALE MICE

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (CONTROL ARTICLE)b	0 (CONTROL ARTICLE)c	10c	20c	40c
ESTROUS CYCLING OBSERVATIONS						
PREDOSAGE ESTROUS CYCLING						
MICE EVALUATED	N	22	22	22	22	22
ESTROUS STAGES/ 15 DAYS	MEAN±S.D.	2.6 ± 0.8	2.4 ± 0.8	2.5 ± 0.9	2.6 ± 0.9	2.5 ± 0.5
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	1	5	4	3	4
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	1	0	0
PRECOHABITATION ESTROUS CYCLING						
MICE EVALUATED	N	22	22	22	22	22
INCLUDED IN ANALYSES	N	22	22	21d	22	22
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	2.6 ± 0.6	2.7 ± 0.9	2.6 ± 0.9	2.1 ± 0.7	2.4 ± 0.7
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	3	2	3	2	3
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0	0

a. Dosage occurred every other day from day 1 of study through day 8 of gestation.
b. Administered saline intraperitoneally to match DPH administration of Groups II through V.
c. Administered DPH intraperitoneally from the sixth dose of rhGAA until one or two days before sacrifice.
d. Excludes values for mouse 5359, which was sacrificed on day 2 of study due to adverse clinical observations.

TABLE B10 (PAGE 2): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - FEMALE MICE

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (CONTROL ARTICLE)b	0 (CONTROL ARTICLE)c	10c	20c	40c
MATING OBSERVATIONS						
MICE IN COHABITATION	N	22	22	21d	22	22
DAYS IN COHABITATION e	MEAN±S.D.	2.4 ± 1.6	4.3 ± 4.3	3.2 ± 3.0	3.4 ± 3.3	4.6 ± 4.4
MICE THAT MATED	N(%)	22(100.0)	22(100.0)	20(95.2)	22(100.0)	21(95.4)
FERTILITY INDEX f	N/N (%)	22/22 (100.0)	21/22 (95.4)	17/20 (85.0)	22/22 (100.0)	19/21 (90.5)
MICE WITH CONFIRMED MATING DATES	N	22	20	20	21	19
MATED BY FIRST MALE g						
DAYS 1-7	N(%)	22(100.0)	17(85.0)	20(100.0)	20(95.2)	18(94.7)
DAYS 8-14	N(%)	0(0.0)	3(15.0)	0(0.0)	1(4.8)	1(5.3)
MICE PREGNANT/MICE IN COHABITATION	N/N (%)	22/22 (100.0)	21/22 (95.4)	17/21 (81.0)	22/22 (100.0)	19/22 (86.4)

a. Dosage occurred every other day from day 1 of study through day 8 of gestation.
b. Administered saline intraperitoneally to match DPH administration of Groups II through V.
c. Administered DPH intraperitoneally from the sixth dose of rhGAA until one or two days before sacrifice.
d. Excludes mouse 5359, which was sacrificed on day 2 of study due to adverse clinical observations.
e. Restricted to mice with a confirmed mating date and mice that did not mate.
f. Number of pregnancies/number of mice that mated.
g. Restricted to mice with a confirmed mating date.

Conclusions: The doses of rhGAA as high as 40 mg/kg administered every other day to male and female mice did not affect the mating and fertility. However, there were dose dependent decreases of sperm motility and sperm count in the 20 and 40 mg/kg groups when compared to the saline control group. There was also a decrease in flagella with no head in the 40 mg/kg dose group.

Addendum: The observed decrease in sperm motility and sperm count was not statistically significant. The reduction in sperm motility was not dose-dependent, and this effect, if real, may have been related to diphenhydramine administration rather than rhGAA. Furthermore, there

was no effect on fertility in males. Thus, the small changes in sperm parameters do not appear have biological significance. The test article used in the study, "rhGAA", is Myozyme®.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated September 15, 2008 by Dr. Niraj Mehta.

Study title: Intravenous Injection Study of Recombinant Human Acid α-Glucosidase (rhGAA) on Fertility and Early Embryonic Development to Implantation in Mice (Study Phase: Qualitative Spermatogenesis Evaluation and Testicular Pathology)

Key Study Findings: The test article given as outlined in this protocol had no effect on the macroscopic appearance of testicles at the terminal sacrifice on Week 9.

Study #: 6354-155 (Reference No. 04006)

Seq. 31, Pg. 1-36 (electronic)

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: August 5, 2004 (report dated October 23, 2006)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot #608341; > 98%

Methods:

Doses: 0, 10, 20, 40 mg/kg/every other day

Species/strain: Crl:CD 1®(ICR)BR mice

Number/sex/group or time point: 22/sex/group (Groups 1 to 4)

Route, formulation, volume, and infusion rate: Vehicle control or test article, rhGAA were administered via slow bolus i.v. injections into a tail vein at a volume of 8 mL/kg. All mice received a prophylactic dose of Diphenhydramine Hydrochloride (DPH) at 5 mg/mL, or saline approximately 10 to 20 mins prior to the start of each infusion. The prophylactic dose was administered via i.p. injection, beginning on the seventh dosing day. The group designation, dose levels and dose concentrations are summarized in the sponsor's Table below:

Group	Number of Animals		Dose Level	Dose Concentration
	Male	Female	(mg/kg/every other day)	(mg/mL)
1 (Vehicle Control)	22	22	0	0
2 (Low)	22	22	10	1.25
3 (Mid)	22	22	20	2.5
4 (High)	22	22	40	5

Satellite groups used for toxicokinetics: none

Study Design: Males were dosed over a 9-week period prior to sacrifice. Females were dosed for at least a 14 day-period prior to mating, throughout the mating (14-day) period, and through Gestation Day (DG) 7 or 8.

Parameters and endpoints evaluated: mortality, male gross necropsy/pathology (as listed in Table below), organ weights (as listed in table below), male histopathology (qualitative examination of spermatogenic stages)

Organs Weighed at Necropsy

Epididymis (left)
Epididymis (right)
Prostate
Seminal Vesicles (with coagulating gland)
Testis (left)
Testis (right)

Tissues Examined and Collected at Necropsy

Coagulating Gland
Epididymis (Left)
Epididymis (Right)
Injection site(s)
Lesions
Prostate
Seminal Vesicle (2)
Testis (left)
Testis (right)

RESULTS:

Mortality: One 20 mg/kg male (Group 3; A82545) and one 40 mg/kg male (Group 4; A82565) were found dead on Day 12 and Day 53, respectively. The left and right testicles from both animals were microscopically normal.

Gross Necropsy/Pathology: There were no gross lesions recorded at necropsy.

Histopathology: Microscopic changes seen in the testicles from all groups were few and considered incidental. The right testicle due to handling and fixation issues was deemed unacceptable for evaluation, however the left testicle was in very good condition with little artifact. The histopathology data is summarized in the sponsor's Table below.

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Group 1 (Vehicle Control - 0 mg/kg/every other day)

Terminal Euthanasia Week 9

TISSUE/DIAGNOSIS	A82497	A82498	A82499	A82500	A82501	A82502	A82503	A82504	A82505	A82506	A82507	A82508	A82509	A82510	A82511	A82512	A82513	A82514	A82515	A82516	A82517	A82518	INC
RIGHT TESTIS (H&E)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	22 / 22
Degeneration, germinal epithelium																							0 / 22
Testicular immaturity																							0 / 22
Intralumenal spermatid giant cells																							0 / 22
LEFT TESTIS (PASH)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N	N	21 / 22
Degeneration, germinal epithelium																			1				1 / 22
Testicular immaturity																							0 / 22
Intralumenal spermatid giant cells																							0 / 22

Group 2 (Low - 10 mg/kg/every other day)

Terminal Euthanasia Week 9

TISSUE/DIAGNOSIS	A82519	A82520	A82521	A82522	A82523	A82524	A82525	A82526	A82527	A82528	A82529	A82530	A82531	A82532	A82533	A82534	A82535	A82536	A82537	A82538	A82539	A82540	INC
RIGHT TESTIS (H&E)	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	21 / 22
Degeneration, germinal epithelium																							0 / 22
Hemorrhage, interstitial														1									1 / 22
Testicular immaturity																							0 / 22
Intralumenal spermatid giant cells																							0 / 22
LEFT TESTIS (PASH)	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N	N		N	N	N	20 / 22
Degeneration, germinal epithelium							1												1				2 / 22
Testicular immaturity																							0 / 22
Intralumenal spermatid giant cells																							0 / 22

Group 3 (Mid - 20 mg/kg/every other day)

Terminal Euthanasia Week 9

TISSUE/DIAGNOSIS	A82541	A82542	A82543	A82544	A82546	A82547	A82548	A82549	A82550	A82551	A82552	A82553	A82554	A82555	A82556	A82557	A82558	A82559	A82560	A82561	A82562	INC
RIGHT TESTIS (H&E)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	21 / 21
Degeneration, germinal epithelium																						0 / 21
Testicular immaturity																						0 / 21
Intralumenal spermatid giant cells																						0 / 21
LEFT TESTIS (PASH)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	21 / 21
Degeneration, germinal epithelium																						0 / 21
Testicular immaturity																						0 / 21
Intralumenal spermatid giant cells																						0 / 21

Group 4 High - (40 mg/kg/every other day)

Terminal Euthanasia Week 9

TISSUE/DIAGNOSIS	A82563	A82564	A82566	A82567	A82568	A82569	A82570	A82571	A82572	A82573	A82574	A82575	A82576	A82577	A82578	A82579	A82580	A82581	A82582	A82583	A82584	INC
RIGHT TESTIS (H&E)	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	20 / 21
Degeneration, germinal epithelium														1								1 / 21
Testicular immaturity																						0 / 21
Intralumenal spermatid giant cells														1								1 / 21
LEFT TESTIS (PASH)	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	20 / 21
Degeneration, germinal epithelium														1								1 / 21
Testicular immaturity																						0 / 21
Intralumenal spermatid giant cells																						0 / 21

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Group 3 (Mid - 20 mg/kg/every other day)					Group 4 High - (40 mg/kg/every other day)				
Found Dead, Day 12					Found Dead, Day 53				
TISSUE/DIAGNOSIS	A82545	INC			TISSUE/DIAGNOSIS	A82565	INC		
RIGHT TESTIS (H&E)	N	1	/	1	RIGHT TESTIS (H&E)	N	1	/	1
Degeneration, germinal epithelium		0	/	1	Degeneration, germinal epithelium		0	/	1
Testicular immaturity		0	/	1	Testicular immaturity		0	/	1
Intralumenal spermatid giant cells		0	/	1	Intralumenal spermatid giant cells		0	/	1
LEFT TESTIS (PASH)	N	1	/	1	LEFT TESTIS (PASH)	N	1	/	1
Degeneration, germinal epithelium		0	/	1	Degeneration, germinal epithelium		0	/	1
Testicular immaturity		0	/	1	Testicular immaturity		0	/	1
Intralumenal spermatid giant cells		0	/	1	Intralumenal spermatid giant cells		0	/	1

Conclusions: The test article given as outlined in this protocol had no effect on the macroscopic appearance of testicles at the terminal sacrifice on Week 9. All stages of the spermatogenic cycle were present in normal proportions. There were only a few minor microscopic changes seen in testicles from mice in Groups I to V. In addition, testicles from the two males found dead on Day 12 and Day 53 were microscopically normal.

Addendum: The test article used in the study, "rhGAA", is Myozyme®.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated September 15, 2008 by Dr. Niraj Mehta.

Study title: Intravenous Dosage-Range Developmental Toxicity Study of Recombinant Human Acid-α-Glucosidase (rhGAA) in Rabbits

Key Study Findings: The compound was not teratogenic in rabbits and 40 mg/kg/day dose appears to be a tolerable dose.

Study #: FFA00052 (Reference No. 06022)

Seq. 62, Pg. 1-146 (electronic)

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: September 15, 2006 (report dated January 29, 2007)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot #5930179; > 98% pure

Methods:

Doses: 0 (control/Saline), 10, 20, 40 mg/kg/day

Species/strain: New Zealand White [HRA:(NZW)SPF] rabbits

Number/sex/group or time point (main study): 6 time-mated females/group (Groups I to IV)

Route, formulation, volume, and infusion rate: rhGAA or the rhGAA control article/Saline was administered intravenously via the marginal ear vein once daily. The dosage volume, 8 mL/kg adjusted based on the individual body weights recorded immediately before dosage administration, was administered over approximately 10 mins. If rabbits developed anaphylactic response, the antihistamine Diphenhydramine Hydrochloride (DPH), was to be administered via i.p. injection at 5 mg/kg (1 mL/kg) based on the most recent body weight.

Satellite groups used for toxicokinetics: None

Study design: The control or test article were administered intravenously once daily to time-mated rabbits on days 7 through 19 of presumed gestation (DGs 7 through 19). Female rabbits were caesarean-sectioned on DG 29 and sacrificed.

Parameters and endpoints evaluated: mortality, clinical signs, bodyweight, food consumption, water consumption, gross necropsy/pathology, histopathology (only in does found dead or sacrificed early), caesarean-sectioning (number and distribution of corpora lutea, number and distribution of implantation sites, number of live and dead fetuses and early and late resorptions) and fetal observations (gross examinations of aborted fetuses, conceptuses *in utero* and/or delivered pups, and body and liver weights of each fetus).

RESULTS:

Mortality: A total of 0, 0, 2 and 1 rabbits were found dead or sacrificed early in the 0 (Control Article/Saline), 10, 20 and 40 mg/kg/day dosage groups, respectively.

Doe 8189 of the 20 mg/kg group delivered on DG 29 and was sacrificed. Adverse clinical observations included scant feces (DGs 26 and 28); soft or liquid feces (DG 29), excess shedding (DG 28), prevaginal substance and a substance in the cage pan (DG 29). The doe lost weight from DG 23 until DG 28 with a severe reduction in food consumption from DG 23. This doe had 7 implantation sites, which were, distributed as follows, one late resorptions, three pups in the cage pan, and three conceptuses that the sponsor presumed were cannibalized. The necropsy observations showed that the maternal tissues were normal, but of the 3 pups, one pup was partially cannibalized (no necropsy observations), one pup had flexed forepaws and one pup was normal. The maternal histological examination revealed lesions in the kidneys, liver and lungs. The kidneys had a moderate degree of cortical, tubular, multifocal nephropathy, and mild mononuclear-cell, interstitial, multifocal infiltration. The liver had mild diffuse hepatocellular cytoplasmic vacuolation and the lungs had mild alveolar edema, moderate multifocal acute congestion and mild scattered focal macrophages in the alveoli.

Doe 8190 of the 20 mg/kg group aborted and was found dead on DG 29. Adverse clinical observations included scant feces (DGs 26 and 28), soft or liquid feces (DGs 13-16, 21-22). The doe gained weight until DG 20 and then began losing weight until DG 28, which was accompanied by concomitant severe reduction of food consumption from DGs 21-28. The heart, liver and kidneys appeared pale at necropsy and a red prevaginal substance was observed at necropsy. The doe had 8 implantation sites and 8 delivered fetuses that were found dead, but appeared normal based on fetal weights (23-31 g). The maternal histological observations revealed lesions in kidneys, liver and heart. The liver had marked diffuse hepatocellular cytoplasmic vacuolation and mild mononuclear-cell periportal infiltration. The heart had mild chronic focal cardiomyopathy, which the sponsor stated was an incidental finding.

Doe 8197 of the 40 mg/kg group aborted on DG 27 and was sacrificed. Adverse clinical observations included scant feces (DGs 17-18, 26-27), soft or liquid feces (DGs 17, 20, 27); urine-stained abdominal fur (DGs 23-27) and ungroomed coat (DGs 24-27). This doe gained weight until DG 11, but lost weight each day from DGs 12-27 which was accompanied by a concomitant sever reduction of food consumption from DGs 15-27. The heart, liver and kidneys appeared pale at necropsy. The litter consisted of 8 grossly normal fetuses with the exception of 1 fetus that was partially cannibalized. The maternal histological evaluation revealed lesions in the kidneys and liver. The kidneys had a moderate degree of cortical, tubular, multifocal nephropathy, and minimal mononuclear-cell, interstitial, multifocal infiltration. The liver had marked diffuse hepatocellular cytoplasmic vacuolation.

Clinical Signs: No signs of anaphylaxis were observed in rabbits throughout the dosing period. Therefore, no DPH was administered to any doe. In the 20 and 40 mg/kg group, ungroomed coat occurred in a greater number of animals (2 and 2) when compared to the control article (0), and in higher frequency (15 and 27 incidences) when compared to the control article (0 incidences). Scant and soft or liquid feces occurred in 2-3 of the 6 does in the 10, 20, or 40 mg/kg dose groups compared to 1 doe in the control article group. All other adverse clinical observations occurred in single does within a group and were not considered related to the test article because the incidences were not dose dependent. These observations included urine-stained abdominal fur, sparse hair coat, excess shedding and localized alopecia.

Bodyweight: The initial mean body weight of Group I females was 3.67 ± 0.32 kg on Day 0, and 4.17 ± 0.43 kg on DG 29. Mean body weight gains during DGs 7-20 were reduced in the 20 and 40 mg/kg groups by 52% and 57% respectively, when compared to the control article group. The 40 mg/kg group showed a decrease in body weight gains of 67% during DGs 10-13 and a decrease of 87% during DGs 13-16 when compared to the control article group. During DGs 16-20, both the 20 and 40 mg/kg groups actually had a decrease in weight of -0.08 and -0.03 kg respectively, while the control article group increased in weight (+0.02 kg). This decrease in weight persisted during DGs 20-29 in the 20 mg/kg group. Body weight gains during DGs 20-29 were also robustly reduced (↓ 62% and 57%) in the 20 and 40 mg/kg groups respectively, when compared to the control article group. Total body weight gains for the entire study period (DGs 7-29) were decreased by 17%, 90% and 52% for the 10, 20 and 40 mg/kg/day rhGAA dosage groups respectively, when compared to the control group value.

Food Consumption: The initial mean food consumption for Group I females was 155.8 ± 16.2 g/day from DGs 7-10, and the mean food consumption at the end of the study (DGs 24-29) was 118.4 ± 14.7 g/day. During dose administration (DGs 7-20), the absolute food consumption in the 40 mg/kg dose group was decreased by 22% when compared to the control article group. During the postdosage period (DGs 20-29), the absolute food consumption was decreased by 11%, 40% and 21% in the 10, 20 and 40 mg/kg groups respectively, when compared to the control article group. Overall (DGs 7-29) the 20 and 40 mg/kg dosage groups had a decrease of 25% and 15% in mean absolute food consumption when compared to the control article group.

Gross Necropsy/Pathology: One 10 mg/kg and two 20 mg/kg dose group animals had pale kidneys at necropsy. All other gross lesions occurred in does that were found dead or sacrificed prior to scheduled sacrifice.

Histopathology: Summarized above in 'mortality' since histopathological examinations were only performed in does that were found dead or sacrificed prior to scheduled sacrifice.

Caesarean-Sectioning Observations: There was an 11%, 18% and 12% decrease in the mean number of identified corpora lutea in the 10, 20 and 40 mg/kg dose groups respectively, when compared to the control article group, and there was a 10%, 17.5% and 13% decrease in the mean number of implantations in the 10, 20 and 40 mg/kg dose groups respectively, when compared to the control article group. There was also a 10%, 16% and 20% decrease in the mean litter size/live fetuses of the 10, 20 and 40 mg/kg dose groups respectively, when compared to the control article group. There were 2 does with resorptions (early and late) in the 40 mg/kg group, while there was 1 doe with resorptions (early and late) in the control article group. One fetus in the 10 mg/kg group had extra digits on one limb. The average weights of the fetuses and fetal livers were comparable among the four groups. The caesarian observations are summarized in the Sponsor's Tables below.

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TABLE 8 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (CONTROL ARTICLE)	II 10	III 20	IV 40
RABBITS TESTED	N	6	6	6	6
PREGNANT	N(%)	6(100.0)	6(100.0)	5(83.3)	6(100.0)
FOUND DEAD	N(%)	0(0.0)	0(0.0)	1(20.0)	0(0.0)
ABORTED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	0(0.0)	1(16.7)
DELIVERED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	1(20.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	6	6	3	5
CORPORA LUTEA	MEAN±S.D.	9.8 ± 1.8	8.7 ± 1.6	8.3 ± 1.7	8.6 ± 1.3
IMPLANTATIONS	MEAN±S.D.	9.7 ± 2.0	8.7 ± 1.6	8.3 ± 1.7	8.4 ± 1.5
LITTER SIZES	MEAN±S.D.	9.5 ± 1.9	8.5 ± 1.5	8.0 ± 1.7	7.6 ± 1.8
LIVE FETUSES	N	57	51	24	38
	MEAN±S.D.	9.5 ± 1.9	8.5 ± 1.5	8.0 ± 1.7	7.6 ± 1.8
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0	0.8 ± 1.1
EARLY RESORPTIONS	N	1	1	0	2
	MEAN±S.D.	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0	0.4 ± 0.9
LATE RESORPTIONS	N	0	0	0	2
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.9
DOES WITH ANY RESORPTIONS	N(%)	1(16.7)	1(16.7)	0(0.0)	2(40.0)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	6(100.0)	6(100.0)	3(100.0)	5(100.0)
PLACENTAE APPEARED NORMAL	N(%)	6(100.0)	6(100.0)	3(100.0)	5(100.0)

a. Dosage occurred on days 7 through 19 of gestation.

TABLE 9 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (CONTROL ARTICLE)	II 10	III 20	IV 40
LITTERS WITH ONE OR MORE LIVE FETUSES	N	6	6	3	5
IMPLANTATIONS	MEAN±S.D.	9.7 ± 2.0	8.7 ± 1.6	8.0 ± 1.7	8.4 ± 1.5
LIVE FETUSES	N	57	51	24	38
	MEAN±S.D.	9.5 ± 1.9	8.5 ± 1.5	8.0 ± 1.7	7.6 ± 1.8
LIVE MALE FETUSES	N	18	25	14	18
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	31.5 ± 11.4	49.8 ± 15.3	55.5 ± 29.4	46.9 ± 8.0
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	44.71 ± 3.21	45.85 ± 5.40	37.55 ± 7.02	45.23 ± 4.94
MALE FETUSES	MEAN±S.D.	45.96 ± 3.18	46.72 ± 3.97	38.95 ± 8.31	47.09 ± 4.09
FEMALE FETUSES	MEAN±S.D.	43.99 ± 3.45	45.24 ± 7.00	37.28 ± 6.88	43.71 ± 5.56
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	1.5 ± 3.7	1.7 ± 4.1	0.0 ± 0.0	9.7 ± 13.6

a. Dosage occurred on days 7 through 19 of gestation.

TABLE 10 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (CONTROL ARTICLE)	II 10	III 20	IV 40
LITTERS EVALUATED	N	6	6	3	5
FETUSES EVALUATED	N	57	51	24	38
LIVE	N	57	51	24	38
LEFT HINDPAW: EXTRA DIGITS					
LITTER INCIDENCE	N(%)	0(0.0)	1(16.7)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(2.0)	0(0.0)	0(0.0)

a. Dosage occurred on days 7 through 19 of gestation

Conclusions: Based on the visceral and external malformation data from fetuses, rhGAA does not appear to be teratogenic in this dose-ranging study when administered to does from DG 7 to 29 at doses up to 40 mg/kg/day. One 20 mg/kg/day dosage group doe delivered on DG 29 and was sacrificed, 1 doe in this group aborted and was found dead on DG 29 and 1 doe in the 40 mg/kg/day dosage group aborted and was sacrificed on DG 27. Mean body weight gains and food consumption were generally decreased in these does prior to their deaths. Histological evaluation from these 3 does revealed significant microscopic changes in the kidneys and liver. There were small decreases in the mean number of identified corpora lutea and in the number of implantations in the 10, 20 and 40 mg/kg dose groups respectively, when compared to the control article group. There was also a dose-dependent decrease in the mean litter size/live fetuses of the 10, 20 and 40 mg/kg dose groups respectively, when compared to the control article group. However, the average weights of the fetuses and fetal livers were comparable among the groups.

Addendum: The test article used in the study, "rhGAA", is Myozyme®.

The following review was taken from the Pharmacology/Toxicology review of BLA 125141 dated September 15, 2008 by Dr. Niraj Mehta.

Study title: Intravenous Developmental Toxicity Study of Recombinant Human Acid-α-Glucosidase (rhGAA) in Rabbits

Key Study Findings: rhGAA was not teratogenic at doses up to 40 mg/kg/day. No caesarean-sectioning or litter parameters were affected by dosages of the test article as high as 40 mg/kg/day. The occurrence of fetal alterations were comparable in all groups except for the 20 mg/kg/day group, which showed an increased number of fetuses with alterations and the percentage of fetuses with alterations, however; this finding was not dosage-dependent.

Study #: FFA00053 (Reference No. 06030)

Seq. 62, Pg. 1-346 (electronic)

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: February 16, 2007 (report dated June 21, 2007)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot #5930179; > 98% pure, 7003108A; >98% pure

Methods:

Doses: 0 (control/Saline), 0 (control/DPH), 10, 20, 40 mg/kg/day

Species/strain: New Zealand White [HRA:(NZW)SPF] rabbits

Number/sex/group or time point (main study): 25 time-mated females/group (Groups I to V)

Route, formulation, volume, and infusion rate: rhGAA or the rhGAA control article/vehicle was administered intravenously via the marginal ear vein once daily. The treatment article, Diphenhydramine Hydrochloride (DPH) at 5 mg/mL and treatment article diluent, 0.9 % Sodium Chloride Injection, USP (saline), were administered to rabbits in Groups II through V and Group I, respectively. The prophylactic dose of DPH or saline was administered approximately 15 to 25 mins prior to the start of each infusion via intramuscular injection at 10 mg/kg, beginning with the fifth day of dosage, (0.2 or 1 mL/kg) based on the most recent body weight. There is a discrepancy in the sponsor's report which stated under 'Rationale for Dosage Selection' that DPH was not administered because there were no signs of anaphylaxis, but the Table below and under the 'Method and Frequency of Administration' presented specific times that DPH was administered. The methodology (including dosage volume and infusion time) is summarized in the sponsor's Table below:

Dosage Group	Intramuscular Administration[a]	rhGAA Dosage (mg/kg/day)	rhGAA Concentration (mg/mL)	rhGAA Dosage Volume (mL/kg)	rhGAA Infusion Time (min)	Number of Rabbits	Assigned Rabbit Numbers
I	Saline	0 (Control Article)	0	8	10	25	576 - 600
II	DPH	0 (Control Article)	0	8	10	25	601 - 624, 8794[b]
III	DPH	10	1.25	8	10	25	626 - 650
IV	DPH	20	2.5	8	10	25	651 - 675
V	DPH	40	5.0	8	10	25	676 - 700

The test article was considered 100% active/pure for the purpose of dosage calculations.

a. DPH [10 mg/kg (1 mL/kg)] or Saline [0 mg/kg (1 mL/kg)] was administered beginning on the 5th day of rhGAA dose administration. Beginning on the 10th day of rhGAA dose administration, DPH [10 mg/kg (0.2 mL/kg)] or Saline [0 mg/kg (0.2 mL/kg)] was administered. See Method and Frequency of Administration - Treatment Article section for more information.

b. Rabbit 625 was removed from study prior to dosage administration on DG 7 due to body weight loss and replaced with rabbit 8794.

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Satellite groups used for toxicokinetics: On DG 7, blood samples were collected prior to dosage and at 15 mins, 30 mins, 1, 2, 4, 8 and 24 hrs postdosage (end of infusion) or 5 mins, 15 mins, 30 mins, 1, 2, 4, 8 and 24 hrs postdosage (end of infusion). Also, on DGs 14 and 19, blood samples were collected for histamine analysis approximately 10 mins postdosage (end of infusion). On DG 20, 24 hrs after the end of infusion on the last day of dosage administration (DG 19), blood samples were collected for future antibody analysis.

Study design: The control or test article were administered intravenously once daily to time-mated rabbits on days 7 through 19 of presumed gestation (DGs 7 through 19). Female rabbits were caesarean-sectioned on DG 29 and sacrificed.

Parameters and endpoints evaluated: mortality, clinical signs, bodyweight, food consumption, water consumption, antibody analysis, urinalysis, gross necropsy/pathology, histopathology, caesarean-sectioning (number and distribution of corpora lutea, number and

distribution of implantation sites, number of live and dead fetuses, placentae examined, and early and late resorptions), toxicokinetics, and histamine analysis. Fetal observations included gross examinations of aborted fetuses, delivered pups, and fetuses removed from the uterus, body and liver weights of each fetus, and fetal alterations (malformations and variations).

RESULTS:

Mortality: A total of 0, 1, 1, 0 and 3 rabbits were sacrificed prior to scheduled sacrifice (DG 29) in the 0 (Control Article/Saline), 0 (Control Article/DPH), 10, 20 and 40 mg/kg/day dosage groups, respectively. No does were found dead. Doe 623, a Control Article/DPH doe, was sacrificed on DG 11 due to a broken forelimb caused by an accident during dosing and therefore not related to the test article. Necropsy revealed fractures anterior to one carpal, one metatarsal, and on the anterior surface of the radius and ulna. Other clinical observations included hyperactivity to touch and movement, mydriasis, 2 cm laceration on forelimb. In addition, doe 623 had a decrease in body weight of 81 g from DGs 7-11 with a concomitant decrease in food consumption on DGs 8-10. Histopathology of the tissues from this doe revealed a minimal degree of multifocal, tubular, cortical nephropathy.

The delivery in the 10 mg/kg/day group and the two abortions and one early delivery in the 40 mg/kg/day group were considered related to administration of the test article and/or the DPH administration.

Doe 637 in the 10 mg/kg group delivered its litter on DG 29. The litter consisted of 8 late resorptions. Clinical observation in this doe included: hyperpnea (DG 12); mydriasis (DGs 12 to 19); hyperactivity (DG 13); vocalization to touch (DGs 16 and 19); scant feces (DG 21); no feces in cage pan (DG 22); red substance in the cage pan (DGs 27 to 29); and a red substance on the fur (DG 29). This doe had a decrease in body weight of 180 g from DGs 7-28 while most other does in group gained weight within the same time period and its food consumption was reduced on DGs 12- 21 and 27. All tissues appeared normal at necropsy. Histopathology of the tissues from this doe revealed: a mild degree of multifocal, tubular, cortical nephropathy; a minimal degree of multifocal, interstitial, inflammation in the lung and a mild degree of lymphoid hyperplasia in the spleen.

Doe 693 in the 40 mg/kg group delivered six fetuses on DG 29. Four of the delivered fetuses were partially cannibalized, and soft tissue examination revealed that 2/4 partially cannibalized fetuses sustained visceral damage. Clinical observations in this doe included: hyperpnea (DGs 11 and 12); mydriasis (DGs 11-19); urine-stained abdominal fur (DG 16); scant feces (DGs 20 and 21 and DGs 24-29); and no feces in the cage pan (DG 22). Additionally, this doe had: limited use of the left hindlimb (DG 11); and purple injection sites (DGs 18-26) that were considered the result of the DPH injections. In addition, doe 693 had a decrease in body weight of 697 g from DGs 7-28 and its food consumption was reduced on DGs 11-28. Unlike the majority of does in the 40 mg/kg group, food consumption did not rebound in this doe in the postdosage period. All tissues appeared normal at necropsy. Histological evaluation of the tissues revealed: moderate hemorrhage and marked necrosis of the placenta at the implantation sites; marked diffuse, hepatocellular vacuolation of the liver cytoplasm and moderate multifocal necrosis with interstitial edema in the heart.

Doe 682 in the 40 mg/kg group was in the process of aborting and was sacrificed on DG 23. Clinical observations in this doe included: soft or liquid feces (DGs 9 and 10); mydriasis (DGs 12-19); increased motor activity, hyperpnea (DGs 14, 17 and 18) and a red substance in the cage pan (DG 23). Additionally, this doe had limited use of the left hindlimb on DG 14 that was possibly the result of the DPH injections. The doe lost 218 g of body weight from DGs 7-22 and its food consumption was reduced from DGs 12-22. All tissues appeared normal at necropsy. Histopathology of the tissue from this doe revealed: marked hemorrhage and necrosis of the placenta at the implantation sites; moderate diffuse, hepatocellular vacuolation of the liver cytoplasm with minimal multifocal anoxic centrilobular necrosis; minimal interstitial edema in the heart; and mild multifocal hemosiderosis in the spleen. These findings were considered to be related to the degradation of the pregnancy, rather than an overt test article effect.

The litter consisted of nine fetuses. Two of these fetuses were *in utero*, but each was detached from its implantation site. Gross external examination of the fetuses revealed one fetus with two absent digits on the left hind paw. Skeletal examination revealed non-ossified pubic bones in each fetus. All other fetuses appeared grossly normal with no soft tissue or any other skeletal alterations.

Doe 692 in the 40 mg/kg group aborted eight fetuses and one late resorption on DG 27. Clinical observations in this doe included: hyperpnea (DGs 11 to 12); mydriasis (DGs 11-19); scant feces (DGs 20, 21, 24 and 26); a 3 x 2 cm scab on the right hindpaw (DGs 21-27); no feces in the cage pan (DG 22 and DGs 25-27); and dehydration (DGs 26 to 27). Additionally, this doe had limited use of the left hindlimb (DG 11), purple injection sites (DGs 14-19); and limited use of the right hindlimbs (DGs 15 and 20) that were possibly the result of the DPH injections. This doe had a 853 g decrease in body weight from DGs 7-26 and its food consumption was severely reduced from DGs 11-26. Unlike the majority of does in the 40 mg/kg group, food consumption did not rebound in this doe in the postdosage period. All tissues appeared normal at necropsy. Histopathology of the tissues from this doe revealed: moderate hemorrhage and marked necrosis of the placenta at the implantation sites; moderate diffuse, hepatocellular vacuolation of the liver cytoplasm; mild, multifocal tubular, cortical nephropathy and moderate cortical tubular renal dilatation; minimal multifocal necrosis in the heart with moderate interstitial edema in the heart. These findings were considered to be related to decreased food consumption and/or degradation of the pregnancy, rather than an overt test article effect. The late resorptions could not be examined further due to autolysis. Skeletal examination revealed non-ossified pubic bones in each fetus; a normal condition for this fetal age. No gross, soft tissue or any other skeletal alterations occurred in these fetuses.

Clinical Signs: All Does (25/25) administered DPH, Groups II to V, had a statistically significant ($p \leq 0.01$) increase in incidences of mydriasis. Almost every incidence of mydriasis occurred on or after DG 12 (right after first dose of DPH delivered). There was a statistically significant, ($p \leq 0.05$ and $p \leq 0.01$) dose dependent increase in the number of animals (frequency) administered DPH with limited hindlimb use. Also animals from Groups II through V given DPH had a statistically significant increase ($p \leq 0.01$) in the incidences of hypernea when compared to the Control Article/Saline group. These adverse clinical observations appeared to be related to the administration of DPH.

Adverse clinical observations related to test article administration included increased motor activity, increased incidence of scant or no feces, vocalizations to touch/movement and urine-stained abdominal fur. There was a dose dependent increase in number of animals with increased motor activity (12, 13 and 15 does in Groups III to V respectively, compared to 0 animals in the Control Article/Saline group). In the 20 and 40 mg/kg group, there was an increase in the number of animals with scant feces (16 and 14) when compared to the Control Article/Saline group (8). Animals treated with rhGAA also showed an increased number of animals with no feces in the cage pan (8, 8 and 10 for Groups III through V, respectively) when compared to the saline control article groups (2). There was a statistically significant ($p \leq 0.01$) increase in vocalizations to touch/movement in the 40 mg/kg group (12 does with 39 incidences) when compared to the control saline group (2 does with 2 incidences). In addition, the 40 mg/kg group had 3 does with 3 incidences of urine-stained abdominal fur while no does from the other dose groups had such an observation (statistically significant; $p \leq 0.01$).

Other clinical observations were considered unrelated to the test article and/or DPH administration because the incidences were not dose-dependent and the observations occurred in only one or two does. These clinical observations included ungroomed coat, soft or liquid feces, scabs, a red substance in the cage pan, localized alopecia, sparse hair coat, ulcerations, intermittent head weaving, dehydration, red, green, tan and/or purple right hindpaw, missing toe nail on the first digit of the right hindpaw, swollen digit, red urine, head tilt, ataxia, red substance on fur, laceration and broken forelimb.

Bodyweight: The mean body weight of the control does on Day 0 was 3.60 ± 0.32 kg and 4.07 ± 0.34 kg at the end of the study period (Day 29). Around DG 16, does administered test article and/or DPH started showing decreased mean weight gains when compared to the does in the saline control group. Significant body weight losses ($p \leq 0.01$) in all groups administered the test article and/or DPH (Groups II through V) occurred for DGs 10 to 13, 13 to 16, 16 to 20 and for the dosage period calculated as DGs 7 to 20. During the dosing period, all groups administered test article and/or DPH had steadily decreased body weight through the entire period while the saline control group had increased body weight throughout the same time period.

During the postdosage period (DGs 20 to 29), body weight gains were increased for all groups administered the test article and/or DPH, with a significant increase ($p \leq 0.05$ to $p \leq 0.01$) in the Control Article/DPH (↑ 64%) and 20 mg/kg/day dosage groups (↑ 93%) compared to the Control Article/Saline dosage group. The 10 and 40 mg/kg groups had an increased body weight gain of 21% and 64%, respectively, when compared to the saline control article group. Overall, there was a statistically significant decrease ($p \leq 0.01$) in mean body weight gain throughout the study (DGs 0-29) of 62%, 72%, 60% and 76% in Groups II through V respectively, when compared to the Control Article/Saline group. The same statistically significant ($p \leq 0.01$) decreased body weight gains were seen on DGs 7-29.

Food Consumption: The initial mean food consumption value for control does on DGs 7-10 was 142.6 ± 21.4 g/day and food consumption value at the end of the study (DGs 24-29) was 85.6 ± 31.7 g/day. Significant reductions in food consumption values reflected the losses in body weight across all groups administered the test article and/or DPH (Groups II through V).

Significant ($p \leq 0.05$ to $p \leq 0.01$) decreases in food consumption for all groups administered the test article and/or DPH (Groups II through V) occurred for DGs 10-13 (↓ 31%, 31%, 36% and 35%), DGs 13-16 (↓ 78%, 78%, 79% and 83%) and DGs 16 to 20 (↓ 76%, 76%, 76% and 86%) when compared to the Control Article/Saline group. Overall for the dosage period calculated as DGs 7 to 20, there was a statistically significant ($p \leq 0.01$) decrease of 48%, 51%, 52% and 55% in Groups II through V respectively, when compared to the Control Article/Saline group.

During the postdosage period (DGs 20 to 29), food consumption rebounded after an initial decrease (DGs 20-24). During DGs 20-24 there was a statistically significant decrease ($p \leq 0.05$ to $p \leq 0.01$) of food consumption in Groups II through V (test article and/or DPH) of 29%, 29%, 33% and 42%, respectively, when compared to the Control Article/ Saline group. However in DG 24-29, there was a concomitant decrease in food consumption from the Control Article/ Saline group and an increase in all other groups. Groups II through V showed an increase of 25%, 23%, 37% and 22%, respectively, when compared to the saline control article group. Overall from DGs 7-29 there was still a statistically significant ($p \leq 0.01$) decrease in food consumption of 33%, 35%, 35% and 39% in Groups II through V respectively, when compared to the Control Article/ Saline group.

Water Consumption: Significant ($p \leq 0.05$ to $p \leq 0.01$) water consumption decreases in all groups administered the test article and/or DPH (Groups II through V) occurred for DGs 10-13 (↓ 20%, 27%, 34% and 28%), DGs 13-16 (↓ 54%, 55%, 67% and 57%) and DGs 16 to 20 (↓ 44%, 35%, 52% and 51%) when compared to the Control Article/Saline group. Overall for the dosage period (DGs 7 to 20), there was a statistically significant ($p \leq 0.01$) decrease of 32%, 34%, 44% and 38% in Groups II through V respectively, when compared to the control saline group.

During the postdosage period, DGs 20 to 29, absolute water consumption values were comparable among the groups. However, for the entire study (DGs 7-29), there was a statistically significant ($p \leq 0.05$ to $p \leq 0.01$) decrease of 20%, 19%, 31% and 24% in Groups II through V respectively, when compared to the Control Article/Saline group. Significant reductions in water consumption values reflected the losses in body weight across all groups administered the test article and/or DPH (Groups II through V). These reductions were not as great as for the food consumption values.

Gross Necropsy/Pathology: No gross lesions related to the test article or DPH administration occurred. The only gross necropsy observations were related to the doe that had a broken forelimb and were previously described.

Urinalysis: Urinalysis was performed in urine samples collected from does that had low food consumption for three or more consecutive days. There was no evidence of a test article-related increase in ketone bodies (acetoacetate) in the urine samples evaluated from does that had low food consumption. Selected samples (Groups II-V) were analyzed for the presence of beta-hydroxy butyrate. The analyzed samples demonstrated highly variable results; however, significantly elevated (above the normal range) hydroxy butyrate levels indicated that the rabbits with low food consumption were in a state of negative energy balance.

Histopathology: There were no microscopic changes observed in the stomach, small intestine, cecum or colon of any of the does that died, aborted or delivered prior to scheduled sacrifice.

Caesarean-Sectioning Observations: Pregnancy occurred in 24 to 25 of the does in each dosage group. The number of litters cesarean-sectioned on DG 29 were 24, 24, 23, 25 and 21 from Groups I through V, respectively. No caesarean-sectioning or litter parameters were affected by dosages of the test article as high as 40 mg/kg. The litter averages for corpora lutea, implantations, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable among the 5 dosage groups and did not significantly differ. There was a minor decrease in litter size of 15% and 12% from the 10 and 40 mg/kg dose groups when compared to the saline control group. One doe in the 20 mg/kg dosage group had all resorbed conceptuses and one doe in the 40 mg/kg/day dosage group had a dead fetus. All placenta appeared normal. Fetal liver weights and the ratio of these weights to the fetal body weight were comparable among the 5 dosage groups and did not significantly differ. The caesarean-sectioning observations are summarized in the sponsor's Tables below.

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TABLE 10 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
RABBITS TESTED	N	25	25	25	25	25
PREGNANT	N(%)	24(96.0)	25(100.0)	24(96.0)	25(100.0)	24(96.0)
UNSCHEDULED SACRIFICE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
ABORTED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(8.3)
DELIVERED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	1(4.2)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	24	24	23	25	21
CORPORA LUTEA	MEAN±S.D.	8.6 ± 1.7	8.9 ± 1.7	8.1 ± 2.3	8.6 ± 1.6	9.0 ± 2.3
IMPLANTATIONS	MEAN±S.D.	8.5 ± 1.7	8.8 ± 2.0	7.7 ± 2.9	8.2 ± 1.6	8.7 ± 2.7
LITTER SIZES	MEAN±S.D.	8.1 ± 1.7	7.7 ± 1.9	6.9 ± 2.6	7.4 ± 2.1	7.1 ± 3.2
LIVE FETUSES	N	194	185	158	186	148
	MEAN±S.D.	8.1 ± 1.7	7.7 ± 1.9	6.9 ± 2.6	7.4 ± 2.1	7.0 ± 3.2
DEAD FETUSES	N	0	0	0	0	1
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2
RESORPTIONS	MEAN±S.D.	0.4 ± 0.5	1.1 ± 1.4	0.8 ± 1.0	0.8 ± 1.3	1.6 ± 2.2
EARLY RESORPTIONS	N	7	8	14	12	16
	MEAN±S.D.	0.3 ± 0.5	0.3 ± 0.6	0.6 ± 1.0	0.5 ± 1.2	0.8 ± 1.5
LATE RESORPTIONS	N	4	18	5	7	17
	MEAN±S.D.	0.2 ± 0.4	0.8 ± 1.3	0.2 ± 0.5	0.3 ± 0.5	0.8 ± 1.7

a. DPH/Saline was administered by intramuscular injection at 10 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA. DPH/Saline was administered by intramuscular injection at 10 mg/kg (0.2 mL/kg) beginning with the 10th rhGAA dose administration.
b. Dosage occurred on days 7 through 19 of gestation.

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TABLE 10 (PAGE 2): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
RABBITS TESTED	N	25	25	25	25	25
PREGNANT	N(%)	24(96.0)	25(100.0)	24(96.0)	25(100.0)	24(96.0)
UNSCHEDULED SACRIFICE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
ABORTED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(8.3)
DELIVERED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	1(4.2)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	24	24	23	25	21
DOES WITH ANY RESORPTIONS	N(%)	11(45.8)	12(50.0)	11(47.8)	12(48.0)	11(52.4)
DOES WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	24(100.0)	24(100.0)	23(100.0)	24(96.0)	21(100.0)
PLACENTAE APPEARED NORMAL c	N(%)	24(100.0)	24(100.0)	23(100.0)	24(100.0)	21(100.0)

a. DPH/Saline was administered by intramuscular injection at 10 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA. DPH/Saline was administered by intramuscular injection at 10 mg/kg (0.2 mL/kg) beginning with the 10th rhGAA dose administration.
b. Dosage occurred on days 7 through 19 of gestation.
c. Excludes does with all early resorptions.

TABLE 11 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
LITTERS WITH ONE OR MORE LIVE FETUSES	N	24	24	23	24	21
IMPLANTATIONS	MEAN±S.D.	8.5 ± 1.7	8.8 ± 2.0	7.7 ± 2.9	8.3 ± 1.5	8.7 ± 2.7
LIVE FETUSES	N	194	185	158	186	148
	MEAN±S.D.	8.1 ± 1.7	7.7 ± 1.9	6.9 ± 2.6	7.8 ± 1.4	7.0 ± 3.2
LIVE MALE FETUSES	N	106	103	70	95	73
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	54.8 ± 17.6	56.3 ± 16.6	45.0 ± 20.8	51.8 ± 20.2	51.7 ± 25.5
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	44.50 ± 5.28	41.59 ± 6.06	43.12 ± 5.67	43.39 ± 5.41	40.10 ± 5.43
MALE FETUSES	MEAN±S.D.	45.32 ± 5.64	42.11 ± 6.65	43.06 ± 6.21 [22]c	43.57 ± 6.87	39.55 ± 6.08 [20]c
FEMALE FETUSES	MEAN±S.D.	43.80 ± 5.88 [23]d	40.55 ± 6.00 [23]d	42.63 ± 6.20 [22]d	42.86 ± 5.65	40.72 ± 5.38 [19]d
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	5.4 ± 6.2	11.3 ± 14.8	9.2 ± 11.7	6.2 ± 7.4	18.9 ± 25.9

[ ] = NUMBER OF VALUES AVERAGED
a. DPH/Saline was administered by intramuscular injection at 10 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA. DPH/Saline was administered by intramuscular injection at 10 mg/kg (0.2 mL/kg) beginning with the 10th rhGAA dose administration.
b. Dosage occurred on days 7 through 19 of gestation.
c. Litters 627 and 680 had no male fetuses.
d. Litters 593, 615, 636, 684 and 695 had no female fetuses.

Fetal Alterations: Fetal evaluations were based on 194, 185, 158, 186 and 148 live, DG 29 caesarean-delivered fetuses in 24, 24, 23, 24 and 21 litters in the 0 (Control Article/Saline), 0 (Control Article/DPH), 10, 20 and 40 mg/kg/day dosage groups, respectively. Each of these fetuses was examined for gross external, soft tissue and skeletal alterations and fetal ossification site averages. There were no treatment-related effects on fetal alterations.

No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by dosages of the test article as high as 40 mg/kg/day. There were no dose-dependent or

significant differences in the litter or fetal incidences of any gross external, soft tissues or skeletal alterations. The skeletal ossification averages were comparable among the groups.

In Groups I through V, litters with fetal alterations numbered 11 (45.8%), 11 (45.8%), 5 (21.7%), 14 (58.3%) and 9 (42.8%), respectively. The numbers of fetuses with any alteration observed were 11 (5.7%), 13 (7.0%), 6 (3.8%), 22 (11.8%)* and 13 (8.8%), respectively. There was one dead fetus (694-8) in the 40 mg/kg/day dosage group, which had downward-flexed forepaws and a domed head. The fetal alterations were not dose-dependent.

Gross Alterations:

Fetus 696-2 in the 40 mg/kg/day dosage group had multiple malformations of the head including micrognathia, a small oral opening and a right depressed eye bulge. Soft tissue evaluation revealed an absent right eye. Skeletal evaluation revealed irregular nasal suture, a short mandible, fused mandible and maxilla, not ossified zygomatic and squamosal bones on the right, small right eye socket, misaligned tympanic ring and an angulated hyoid ala. This fetus also had flat ribs bilaterally.

Fetus 586-8 in the 0 (Control Article/Saline) mg/kg/day group had flexed forepaws bilaterally. The sponsor stated that this alteration was considered a variation because the flexion had no effect on bone of the limb and no other alterations occurred in this fetus.

Fetus 661-6 in the 20 mg/kg/day dosage group had absent hindpaw digits (first and second). Skeletal alterations in this fetus included fused metatarsals, small metatarsals, a small phalanx, not ossified phalanx and absent digits.

Soft Tissue Malformations:

Eyes: Fetus 696-2 in the 40 mg/kg/day dosage group had an absent eye and other alterations as previously described.

Diaphragm: Fetus 674-1 in the 20 mg/kg/day dosage group had a diaphragmatic hernia, with a portion of the stomach and liver protruding into the thoracic cavity.

Kidneys: Fetus 614-13 in the 0 (Control Article/DPH) mg/kg/day dosage group had a low set right kidney. Skeletal evaluation revealed large sternal centra and flat ribs.

Soft Tissue Variations:

Fetus 582-2 in the 0 (Control Article/Saline) mg/kg/day dosage group had a circumcorneal hemorrhage in the left eye.

Fetal Skeletal Malformations:

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* Significantly different from the Group I value ($p \leq 0.01$).

Skull: Fetus 696-2 in the 40 mg/kg/day dosage group had multiple malformations of the right side of the skull including a short mandible, fused maxilla and mandible, not ossified zygomatic and squamosal arches, a small eye socket and a misaligned tympanic ring. Additionally, this fetus had an irregular nasal suture, an angulated right hyoid ala and flat ribs (bilateral 3rd to 5th). This fetus had other alterations as previously described.

Vertebrate/Ribs: Fetus 588-6 in the 0 (Control Article/Saline) mg/kg/day dosage group had a small arch, irregularly shaped arches and unilateral ossification of the centrum.

Fetus 670-2 in the 20 mg/kg/day dosage group had fused centra and a bifid centrum.

Fetus 696-9 in the 40 mg/kg/day dosage group had a hemivertebra (right 4th, arch with attached rib). Interrelated with this malformation were fused and split ribs.

Hindlimb: Fetus 661-6 in the 20 mg/kg/day dosage group had a left hindlimb that had fused and small metatarsals, small and not ossified phalanx and absent digits.

Fetal Skeletal Variations:

Skull: Small irregularities in ossification of the skull (the presence of small ossification sites within the sutures of the nasal or, frontal bones and/or irregular shaping or fusion of the sutures or bones) occurred in 3, 3, 3, 7 and 2 fetuses from 3, 3, 3, 4 and 2 litters in the Control Article/Saline, Control Article/DPH, 10, 20 and 40 mg/kg/day dosage groups, respectively.

Fetus 577-2 in the 0 (Control Article/Saline) mg/kg/day dosage group had a 2 mm hole in the parietal bone. This fetus also had an internasal bone, fused frontal bones, irregularly shaped scapulae ala and a misaligned caudal vertebrae.

Hyoid: One or both hyoid alae were angulated in 2, 5, 1, 5 and 4 fetuses from 2, 5, 1, 4 and 4 litters in the Control Article/Saline, Control Article/DPH, 10, 20 and 40 mg/kg dosage groups, respectively. One fetus in the 0 (Control Article/DPH) group (605-8) also had fused sternal centra. Additional alterations also occurred in one 40 mg/kg/day dosage group fetus (696-2) as described previously.

Vertebrae: A misaligned caudal vertebra occurred in 3, 4 and 3 fetuses each from a different litter in each of the Control Article/Saline, 20 and 40 mg/kg/day dosage groups, respectively. Only one of these fetuses, [577-2 in the Control Article/Saline group] had other alterations as previously described.

Fetus 607-11 in the Control Article/DPH group had a small caudal vertebrae.

Ribs: Fetuses 614-13 in the Control Article/DPH group and 696-2 in the 40 mg/kg group had flat ribs. Fetus 696-2 had alterations that have been previously described. Fetus 614-13 also had two large sternal centra (3rd and 4th).

Sternum: Fused sternal centrum occurred in 0, 4, 2, 3 and 4 fetuses from 0, 4, 2, 3 and 3 litters in the Control Article/Saline, Control Article/DPH, 10, 20 and 40 mg/kg dosage groups, respectively. Fetus 658-2 in the 20 mg/kg dose group also had an intranasal bone. Fetus 605-8 in the Control Article/DPH group had other alterations as previously described.

Fetus 661-1 in the 20 mg/kg dose group had asymmetric sternal centra.

Fetus 614-13 in the 0 Control Article/DPH mg/kg/ dose group had two large sternal centra. This fetus had other alterations as previously described.

Scapula: Fetus 577-2 in the Control Article/Saline dose group had irregularly shaped scapulae ala. This fetus had other alterations as previously described.

Fetal Ossification Site Averages: There were no statistically significant or biologically important differences among the 5 dosage groups in the average numbers of ossification sites per fetus for the hyoid, vertebrae (cervical, thoracic, lumbar, sacral and caudal), ribs, sternum (manubrium, sternal centers and xiphoid), forelimbs (carpals, metacarpals, and phalanges) or hindlimbs (tarsals, metatarsals, and phalanges).

The fetal external, skeletal and soft tissue malformations are summarized in the sponsor's Tables below.

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TABLE 12 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
ADMINISTRATION a		SALINE	DPH	DPH	DPH	DPH
rhGAA DOSAGE (MG/KG/DAY)b		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	40
LITTERS EVALUATED	N	24	24	23	24	21
FETUSES EVALUATED	N	194	185	158	186	149
LIVE	N	194	185	158	186	148
DEAD	N	0	0	0	0	1c
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	11(45.8)	11(45.8)	5(21.7)	14(58.3)	9(42.8)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	11(5.7)	13(7.0)	6(3.8)	22(11.8)**	13(8.8)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	5.7 ± 6.7	7.1 ± 9.7	3.4 ± 7.4	12.1 ± 12.8*	7.5 ± 10.3

a. DPH/Saline was administered by intramuscular injection at 10 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA. DPH/Saline was administered by intramuscular injection at 10 mg/kg (0.2 mL/kg) beginning with the 10th rhGAA dose administration.
b. Dosage occurred on days 7 through 19 of gestation.
c. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 26.
* Significantly different from the Group I value ($p \leq 0.05$).
** Significantly different from the Group I value ($p \leq 0.01$).

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TABLE 13 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
LITTERS EVALUATED	N	24	24	23	24	21
FETUSES EVALUATED	N	194	185	158	186	149
LIVE	N	194	185	158	186	148
DEAD	N	0	0	0	0	1c
HEAD: ORAL OPENING SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)d
EYE: BULGE DEPRESSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)d
JAW: MICROGNATHIA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)d
FORE AND/OR HINDLIMBS: FLEXED						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FORE AND/OR HINDLIMBS: DIGIT(S) ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)

a. DPH/Saline was administered by intramuscular injection at 10 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA. DPH/Saline was administered by intramuscular injection at 10 mg/kg (0.2 mL/kg) beginning with the 10th rhGAA dose administration.
b. Dosage occurred on days 7 through 19 of gestation.
c. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 26.
d. Fetus 696-2 had other gross external alterations.

TABLE 14 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
LITTERS EVALUATED	N	24	24	23	24	21
FETUSES EVALUATED	N	194	185	158	186	149
LIVE	N	194	185	158	186	148
DEAD	N	0	0	0	0	1c
EYES: CIRCUMCORNEAL HEMORRHAGE						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
EYES: ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)
DIAPHRAGM: HERNIA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
KIDNEYS: LOW SET						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)

a. DPH/Saline was administered by intramuscular injection at 10 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA. DPH/Saline was administered by intramuscular injection at 10 mg/kg (0.2 mL/kg) beginning with the 10th rhGAA dose administration.
b. Dosage occurred on days 7 through 19 of presumed gestation.
c. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 26.

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TABLE 16 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
LITTERS EVALUATED	N	24	24	23	24	21
FETUSES EVALUATED	N	194	185	159	186	149
LIVE	N	194	185	159	186	148
DEAD	N	0	0	0	0	1c
SKULL: IRREGULAR OSSIFICATION d (SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF THE SKULL e; INDIVIDUAL SUBCATEGORIES CITED BELOW)						
LITTER INCIDENCE	N(%)	3(12.5)	3(12.5)	3(13.0)	4(16.7)	2(9.5)
FETAL INCIDENCE	N(%)	3(1.5)	3(1.6)	3(1.9)	7(3.8)	2(1.4)
SKULL: NASAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF MIDLINE SUTURE DISPLACED, CONTAIN AN INTERNASAL, CONTAINS AN INTRANASAL AND SUTURE IRREGULAR)						
LITTER INCIDENCE	N(%)	3(12.5)	3(12.5)	3(13.0)	3(12.5)	2(9.5)
FETAL INCIDENCE	N(%)	3(1.5)	3(1.6)	3(1.9)	6(3.2)	2(1.4)
SKULL: NASALS, MIDLINE SUTURE DISPLACED						
LITTER INCIDENCE	N(%)	2(8.3)	1(4.2)	2(8.7)	1(4.2)	1(4.8)
FETAL INCIDENCE	N(%)	2(1.0)	1(0.5)	2(1.3)	1(0.5)	1(0.7)
SKULL: NASALS, CONTAIN AN INTERNASAL						
LITTER INCIDENCE	N(%)	1(4.2)	1(4.2)	0(0.0)	3(12.5)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)f	1(0.5)	0(0.0)	3(1.6)	0(0.0)
SKULL: NASAL, CONTAINS AN INTRANASAL						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	1(4.3)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	1(0.6)	2(1.1)j	0(0.0)
SKULL: NASALS, SUTURE IRREGULAR						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m

DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
LITTERS EVALUATED	N	24	24	23	24	21
FETUSES EVALUATED	N	194	185	159	186	149
LIVE	N	194	185	159	186	148
DEAD	N	0	0	0	0	1c
SKULL: FRONTAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF SUTURE IRREGULAR AND FUSED)						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
SKULL: FRONTALS, SUTURE IRREGULAR						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
SKULL: FRONTALS, FUSED						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)f	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SKULL - OTHER ALTERATIONS:						
SKULL: MANDIBLE, SHORT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m
SKULL: MANDIBLE AND MAXILLA, FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m
SKULL: ZYGOMATIC, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m
SKULL: SQUAMOSAL, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m
SKULL: EYE SOCKET, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m

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DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
LITTERS EVALUATED	N	24	24	23	24	21
FETUSES EVALUATED	N	194	185	158	186	149
LIVE	N	194	185	158	186	148
DEAD	N	0	0	0	0	1c
SKULL: TYMPANIC RING, MISALIGNED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m
SKULL: PARIETAL, CONTAINS A HOLE						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)f	0(0.0)	0(0.0)	0(0.0)	0(0.0)
HYOID: ALA, ANGULATED						
LITTER INCIDENCE	N(%)	2(8.3)	5(20.8)	1(4.3)	4(16.7)	4(19.0)
FETAL INCIDENCE	N(%)	2(1.0)	5(2.7)h	1(0.6)	5(2.7)	4(2.7)m
THORACIC VERTEBRAE: ARCH, SMALL						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)g	0(0.0)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: ARCH, IRREGULARLY SHAPED						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)g	0(0.0)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)g	0(0.0)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRA, FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)L	0(0.0)
THORACIC VERTEBRAE: CENTRUM, BIFID						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)L	0(0.0)

DOSAGE GROUP ADMINISTRATION a rhGAA DOSAGE (MG/KG/DAY)b		I SALINE 0 (CONTROL ARTICLE)	II DPH 0 (CONTROL ARTICLE)	III DPH 10	IV DPH 20	V DPH 40
LITTERS EVALUATED	N	24	24	23	24	21
FETUSES EVALUATED	N	194	185	158	186	149
LIVE	N	194	185	158	186	148
DEAD	N	0	0	0	0	1c
THORACIC VERTEBRAE: HEMIVERTEBRA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)n
CAUDAL VERTEBRAE: MISALIGNED						
LITTER INCIDENCE	N(%)	3(12.5)	0(0.0)	0(0.0)	4(16.7)	3(14.3)
FETAL INCIDENCE	N(%)	3(1.5)f	0(0.0)	0(0.0)	4(2.2)	3(2.0)
CAUDAL VERTEBRAE: SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)
RIBS: FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)n
RIBS: SPLIT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)n
RIBS: FLAT						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)i	0(0.0)	0(0.0)	1(0.7)m
STERNAL CENTRA: FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	4(16.7)	2(8.7)	3(12.5)	3(14.3)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.2)h	2(1.3)	3(1.6)j	4(2.7)
STERNAL CENTRA: ASYMMETRIC						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)

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DOSAGE GROUP		I	II	III	IV	V
ADMINISTRATION a		SALINE	DPH	DPH	DPH	DPH
rhGAA DOSAGE (MG/KG/DAY)b		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	40
LITTERS EVALUATED	N	24	24	23	24	21
FETUSES EVALUATED	N	194	185	158	186	149
LIVE	N	194	185	158	186	148
DEAD	N	0	0	0	0	1c
STERNAL CENTRA: INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
STERNAL CENTRA: LARGE						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)i	0(0.0)	0(0.0)	0(0.0)
SCAPULAE: ALA, IRREGULARLY SHAPED						
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.5)f	0(0.0)	0(0.0)	0(0.0)	0(0.0)
HINDLIMB: METATARSALS, FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)k	0(0.0)
HINDLIMB: METATARSAL, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)k	0(0.0)
HINDLIMB: PHALANX, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)k	0(0.0)
HINDLIMB: PHALANX, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)k	0(0.0)
HINDLIMB: DIGIT, ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)k	0(0.0)

FOOTNOTES:

a. DPH/Saline was administered by intramuscular injection at 10 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA. DPH/Saline was administered by intramuscular injection at 10 mg/kg (0.2 mL/kg) beginning with the 10th rhGAA dose administration.
b. Dosage occurred on days 7 through 19 of gestation.
c. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 26.
d. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summarization, except when alterations of other ossification sites were also present.
e. Includes all alterations noted for the skull except hyoid, ala, angulated, short mandible, mandible and maxilla, fused, zygomatic, not ossified, squamosal, not ossified, tympanic ring, misaligned, eye sockets, small and parietal, contains a hole. These categories are excluded because these alterations do not result from irregular ossification.
f. Fetus 577-2 had other skeletal alterations.
g. Fetus 588-6 had other skeletal alterations.
h. Fetus 605-8 had other skeletal alterations.
i. Fetus 614-13 had other skeletal alterations.
j. Fetus 658-2 had other skeletal alterations.
k. Fetus 661-6 had other skeletal alterations.
L. Fetus 670-2 had other skeletal alterations.
m. Fetus 696-2 had other skeletal alterations.
n. Fetus 696-9 had other skeletal alterations.

Toxicokinetics: The measured and calculated toxicokinetic parameters are presented in the sponsor's Table below:

Table 1. Toxicokinetic parameters for rhGAA following a single intravenous infusion to presumed-pregnant female rabbits on Day 7 of gestation.

Toxicokinetic Parameter	10 mg/kg Group III (n=10)	20 mg/kg Group IV (n=10)	40 mg/kg Group V (n=10)
$t_{1/2}$ (min)	78.8 ± 3.34	74.9 ± 5.31	68.6 ± 8.36
Cl (ml/min/kg)	0.34 ± 0.04	0.27 ± 0.03	0.50 ± 0.12
Vd (ml/kg)	38.8 ± 4.31	29.5 ± 3.88	48.4 ± 8.30
C_{max}(µg/ml)	334 ± 34.6	787 ± 89.2	1233 ± 162
C_{max} /Dose (kg*µg/ml/mg)	33.4 ± 3.46	39.4 ± 4.46	30.8 ± 4.05
t_{max} (min)	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
$AUC_{0\text{-}INF}$ (mg*min/ml)	29.6 ± 3.22	74.1 ± 8.14	84.9 ± 23.1
$AUC_{0\text{-}INF}$/Dose (mg*min*kg/ml/mg)	2.96 ± 0.32	3.71 ± 0.41	2.12 ± 0.58

Values represent mean ± SD. $t_{1/2}$, elimination half-life; Cl, clearance; Vd, apparent volume of distribution; C_{max}, maximal test article concentration; t_{max}, time after dosing C_{max} observed; $AUC_{0\text{-}INF}$, area under concentration versus time curve extrapolated to infinity.

rhGAA serum concentrations versus time profiles indicated that rhGAA toxicokinetics were predominantly biphasic with linear elimination from 1 hr through 8 hrs following test article administration. The time to reach t_{max} was 5 mins in all doses and 24 hrs following test article administration, rhGAA was quantifiable in 11 out of 30 animals. In these cases concentrations were not linear relative to those measured through the first 8 hrs of sampling. Thus, due to insufficient data between 8 and 24 hrs, elimination rate constants were not obtainable. Therefore, the 11/30 data points were not included in the estimations for the elimination rate constants.

The elimination half-life of rhGAA ranged between 68.6 and 78.8 mins and changes in maximal rhGAA concentration were linear over the doses administered. As the dose of rhGAA was increased from 10 to 20 mg/kg, there was a disproportionate decrease in clearance and increase in systemic exposure. However, such trends were not conserved as the rhGAA was further increased to 40 mg/kg. An explanation of the mechanisms underlying the trends in clearance and exposure across rhGAA doses is unknown, however the data demonstrated that sufficient rhGAA exposure for toxicological evaluation following the first intravenous administration to presumed-pregnant female rabbits.

Histamine Analysis: The results demonstrated that histamine levels were highly variable both within and among the groups; however, no significant difference between groups was noted. The highest average value occurred in the control group that was not receiving any DPH. The average group values are represented in the following sponsor's Table:

Group	Dosage (mg/kg/day)	DG 14 Mean Histamine Value (ng/mL)	DG 19 Mean Histamine Value (ng/mL)
I	0 (Control Article/Saline)	421.7	562.8
II	0 (Control Article/DPH)	309.4	350.9
III	10	152.9	212.8
IV	20	227.2	312.9
V	40	127.0	397.6

The results of this analysis suggest that a significant histamine release did not occur following test article administration at the time points evaluated in this study.

Conclusions: On the basis of these data, 10 mg/kg/day caused reductions in body weight gain or weight losses and reduced food and water consumption values, therefore the maternal no observable effect level (NOEL) of rhGAA is < 10 mg/kg/day. There were no adverse developmental effects noted up to 40 mg/kg/day in the fetuses delivered by test-article treated does. There were only minor adverse effects on embryo-fetal development evaluated in this study, and based on the data, rhGAA should not be identified as a developmental toxicant.

The early delivery in Group III (10 mg/kg/day) and the 2 abortions and one early delivery in Group V (40 mg/kg/day) were considered related to the test article and/or DPH because the adverse clinical signs such as mydriasis, limited hindlimb use, hypernea, increased motor activity, increase of scant or no feces, vocalizations to touch/movement and urine-stained abdominal fur, the reductions in body weight and food and water consumption occurred after the fifth dose of the test article when DPH administration was started.

No caesarean-sectioning or litter parameters were affected by dosages of the test article as high as 40 mg/kg/day. The litter averages for corpora lutea, implantations, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable among the five dosage groups and did not significantly differ. The occurrence of fetal alterations were comparable in all groups except for Group IV (20 mg/kg/day), which showed an increased number of fetuses with alterations and the percentage of fetuses with alterations, however; this finding was not dosage-dependent. There were no significant changes in the incidences of fetal soft tissue or skeletal malformations and alterations within the 5 dose groups.

rhGAA serum concentrations versus time profiles indicated that rhGAA toxicokinetics were predominantly biphasic with linear elimination from 1 hr through 8 hrs following test article administration. The effective elimination half-life of rhGAA ranged between 68.6 and 78.8 minutes and changes in maximal rhGAA concentration were linear over the doses administered. As the dose of rhGAA was increased from 10 to 20 mg/kg, there was a disproportionate decrease in clearance and increase in systemic exposure, however the trend was not conserved in the 40 mg/kg dose of rhGAA.

Addendum: The test article used in the study, "rhGAA", is Myozyme®.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated September 15, 2008 by Dr. Niraj Mehta.

Study title: Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Recombinant Human Acid-α-Glucosidase (rhGAA) in Mice, Including Postnatal Behavioral/Functional Evaluation

Key Study Findings: There was no effect on fertility at doses up to 40 mg/kg/every other day. There were minor effects of rhGAA on embryonic development in the 40 mg/kg group. F1 generation mice were cesarean-sectioned, and there fetuses were examined. There was an increased incidence of gross external alterations in the 40 mg/kg fetuses (3 fetuses with rotated hind limbs) when compared to Saline Control fetuses (0 fetuses with alterations). There were no statistically significant or biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F1 generation male and female mice, as evaluated by performance in a passive avoidance paradigm.

Study #: FFA00058 (Reference No. 06023)

Seq. 74, Pg. 1-375(electronic)

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: September, 27 2006 (report dated September, 20 2007)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot #5930179; > 98% pure

Methods:

Doses: 0 (control/Saline), 0 (control/DPH), 10, 20, 40 mg/kg/every other day

Species/strain: Presumed-pregnant female Crl:CD1 (ICR) mice

Number/sex/group or time point (main study): 25/group (Groups I to V)

Route, formulation, volume, and infusion rate: rhGAA or the rhGAA control article/vehicle was given by intravenous (i.v.) administration into the lateral tail vein. The treatment article, Diphenhydramine Hydrochloride (DPH) at 5 mg/mL and treatment article diluent, 0.9 % Sodium Chloride Injection, USP (saline), were administered to mice in Groups II through V and Group I, respectively. The prophylactic dose of DPH or saline was administered approximately 10 to 20 min prior to the start of each infusion via intraperitoneal (i.p.) injection at 5 mg/kg, beginning with the fifth dose, (1 mL/kg) based on the most recent body weight. The methodology (including dosage volume) is summarized in the sponsor's Table below:

4.5.1.1. F0 Generation Mice

Dosage Group	Number of Mice	IP Administration[a]	rhGAA Dosage (mg/kg/day)	rhGAA Concentration (mg/mL)	rhGAA Dosage Volume (mL/kg)	rhGAA Injection Rate (mL/min)	Assigned Mouse Numbers
I	25	Saline	0 (Control Article)	0	8	slow bolus	3701 - 3725
II	25	DPH	0 (Control Article)	0	8	slow bolus	3726 - 3750
III	25	DPH	10	1.25	8	slow bolus	3751 - 3775
IV	25	DPH	20	2.5	8	slow bolus	3776 - 3800
V	25	DPH	40	5.0	8	slow bolus	3801 - 3825

The test article was considered 100% active/pure for the purpose of dosage calculations.
IP = Intraperitoneal injection.
a. DPH/Saline administration at 5 mg/kg (1 mL/kg) began with the fifth dose administration as one mouse in Group 3 began to show signs of hypersensitivity reaction following its dose on DG 14.

4.5.1.2. F1 Generation Mice

Dosage Group	Number of Mice per Sex	Assigned Mouse Numbers	
		Male Mice	Female Mice
I	25	101 - 125[a]	301 - 325
II	25	126 - 150	326 - 350
III	25	151 - 164, 166 - 175, 779[b]	351 - 375
IV	25	176 - 200	376 - 383, 385 - 400, 777[c]
V	25	202 - 225, 778[d]	401 - 425

a. On postpartum day 27, 12 November 2006, mouse 105 was determined to be female and was excluded from study on postpartum day 28, 13 November 2006.
b. Mouse 165 was found dead on 9 November 2006 and excluded from study. Mouse 779 was added to study on postpartum day 25, 10 November 2006.
c. Mouse 384 was excluded from study on 7 November 2006 due to adverse clinical signs. Mouse 777 was added to study on postpartum day 24, 9 November 2006.
d. Mouse 201 died during passive avoidance testing and was excluded from study on 8 November 2006. Mouse 778 was added to study on postpartum day 23, 8 November 2006.

Satellite groups used for toxicokinetics: none

Study design: The test article, recombinant human acid-α-glucosidase (rhGAA), and/or the control article, rhGAA Control Article, were administered intravenously once every other day (even-numbered days of gestation) on days 6 through 22 of presumed gestation. If the mice delivered a litter, administration of dosing continued to day 20-21 postpartum (Day of lactation (DL) 20-21). F0 mice that did not give birth were sacrificed on DG 23 and mice that did give birth were sacrificed on postpartum day 29.

F1 generation pups were not directly given the test article, but may have been exposed to the test article during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period. At weaning on day 21 postpartum (DL 21), a table of random units was used to select 25 male and 25 female pups per group, resulting in a total of 250 F1 generation mice (125 per sex) chosen for continued evaluation. At least one male pup and one female pup per litter, when possible, were selected (all others sacrificed).

At approximately 90 days of age, the F1 generation mice within each dosage group were assigned to cohabitation, one male mouse per one female mouse (exclusion of sibling matings). The cohabitation period consisted of a maximum of 9 days. Female mice with a copulatory plug observed *in situ* were considered to be at DG 0 and assigned to individual housing. All male mice were sacrificed 1 or 2 days after the end of the cohabitation period and surviving females were sacrificed on DG 18.

Parameters and endpoints evaluated: mortality, clinical signs, body weight, sexual maturation (female; vaginal patency, male: preputial separation), passive avoidance, gross necropsy/pathology, organ weights (testes and epididymides), natural delivery observations of F0 mice, and mating and fertility of F1 mice. Female specific data includes caesarean-sectioning observations of F1 mice (corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent-resorbed conceptuses and percent live male fetuses).

RESULTS:

Mortality: There were no treatment-related deaths were observed in any group.

F0: A total of 1, 0, 2, 0 and 1 mice were found dead in the 0 (Control Article/saline), 0 (Control Article/DPH), 10, 20 and 40 mg/kg/every other day dosage groups, respectively. Each death occurred during the lactation period and none of these deaths was considered related to the test article because the number of deaths were not dose-dependent, and the time of deaths postdosage and/or the clinical observations in the mice indicated that the deaths might have been secondary to the injection procedure.

In the 10 mg/kg group, mouse 3763 and 3764 died on DL 13 and 15, respectively. Weight gain had been normal for both mice during gestation; however, mouse 3763 and mouse 3764 had lost 11 g and 5.3 g respectively, in the time between the last two doses administered. At necropsy, mouse 3763 had a purple area on the back and mouse 3764 had head swelling (mass revealed firm tan material when prosected). Mouse 3763 also had soft or liquid feces. At necropsy, mouse 3763 had 3 pups that died with no milk in their stomachs, but the rest of the pups from both dams appeared normal. A 40 mg/kg mouse was found dead on DL 2, 13 mins after i.p. injection of DPH. The mouse had no adverse clinical effects, normal tissue at necropsy, normal weight and weight gain compared to other mice in the study, and had delivered 12 healthy pups. Thus, death may have been due to the i.p. injection procedure.

F1: A total of 1, 0, 2, 1, and 1 male and female F1 generation were found dead or sacrificed early in groups administered Control Article/saline (Group 1), Control Article/DPH (Group 2), 10 mg/kg/every other day (Group III), 20 mg/kg/every other day (Group IV) and 40 mg/kg/every

other day (Group V), respectively. These deaths and unscheduled sacrifices were not related to the maternal administration of rhGAA because the incidences were not dose-dependent and no clear pattern to the deaths occurred.

Group III male mouse 157 and Group V female mouse 418 were sacrificed on Day 43 and 42 postweaning, respectively. The adverse clinical signs of the Group III mouse included a loss of weight (-7.4 g from day 36-43 post weaning) decreased motor activity, dehydration and labored breathing during week 7 postweaning (WP 7). The adverse clinical signs of the Group V mouse included minimal weight gain (0.2 g from days 29 to 36 postweaning) decreased motor activity, splayed limbs, dehydration, cold to touch and ungroomed coat prior to death during WP 6.

The other Group III mouse (female 351) died on DG18 with no adverse clinical observations, normal weight gain and appeared normal at necropsy. The uterus contained 13 implantations composed of one late and 12 early resorptions. Group IV male mouse 200 died on day 35 postweaning with no adverse clinical observations, normal weight gain and appeared normal at necropsy.

Clinical Signs:

F0: During gestation, 3 female mice in the 10 mg/kg group had a red perivaginal substance and 1 female mouse (not pregnant) in the 20 mg/kg group had decreased motor activity, splayed hind limbs, was cold to touch and dehydrated on DG 14.

During lactation, a purple area on the midline or back occurred in 3, 2 and 1 mice in the 10, 20 and 40 mg/kg/day rhGAA dosage groups, respectively. On DLs 27 to 29, mouse 3818 and 3824 in the 40 mg/kg group had masses, one in the perivaginal area and one in the axilla. Mouse 3777 (20 mg/kg) and 3802 (40 mg/kg) had scabs, one mouse in the 40 mg/kg group (3822) had a purple injection site and one mouse in the 10 mg/kg group (3774) had perivaginal swelling.

All clinical observations in the male and female mice were unrelated to the administration of the test article because the incidences were not dose dependent.

F1: The number of litters that had at least 1 pup with an adverse clinical observation in the Group I (Control Article/saline), Group II (Control Article/DPH), Group III (10), Group IV (20) and Group V (40) mg/kg groups were 6/24 (25%), 8/22 (36%), 10/21 (48%), 7/23 (30%), 10/24 (42%), respectively. Noteworthy changes in clinical observations are summarized in the Table below:

Clin. Observ. (Total Freq / Litters w/ Observations)	Saline (CTRL)	DPH (CTRL)	10 mg/kg rhGAA	20 mg/kg rhGAA	40 mg/kg rhGAA
Cold to touch	7/4	0/0	27/5	25/3	26/4
Dehydrated	15/2	4/3	4/2	1/1	28/4
Ungroomed coat	0/0	0/0	27/1	14/2	23/1
Decreased motor activity	0/0	0/0	0/0	0/0	11/1
No milk band present	0/0	0/0	14/3**	0/0	0/0

** Significantly different from the Saline (CTRL) value ($p \leq 0.01$).

There was a large increase in frequency (number of days clinical effect was observed multiplied by # of pups) of dehydration and decreased motor activity in mice from the 40 mg/kg group when compared to all other groups. There was only 1 litter, Group V litter # 3816 that showed decreased motor activity and ungroomed coat; however, the sponsor noted that the nesting box of this litter was flooded by a leaking water bottle. In addition, 3 litters dosed with 10 mg/kg (1/3 litters had a dead dam) had an increased frequency of pups without milk bands which was statistically different ($p \leq 0.01$) from the Saline/Control group. Other clinical observations that were minor in frequency and observed in all groups included scab, purple or black tip of tail, not nesting or nursing, whole body purple or gray, abdominal distension, red or purple area on the head or back, ulceration on the chest, no use of both hind limbs, yellow or black area on the lower midline, bent tail and abrasion.

Most of the clinical observations in the F1 generation pups were not attributed to dosages of the test article as high as 40 mg/kg/day because the incidences were not dose-dependent and the observation occurred in only one or two litters. However, the results show that there was an increased dehydration frequency in mice from the 40 mg/kg rhGAA group when compared to the Saline/Control group.

In post-weaned F1 animals, there was a statistically significant increase ($p \leq 0.05$) in bent tails of male mice from the 40 mg/kg group, and there was a statistically significant decrease ($p \leq 0.01$) in bent tails of male mice from the 10 mg/kg group compared to the Saline/Control group. Other clinical observations that were minor in number of incidences and observed in all groups included bent tail, ungroomed coat, torn ear, sparse hair coat, dehydration, cold to touch, black, purple or red tail or tip of tail, decreased motor activity, hunched posture, tip or portion of tail missing, urine-stained abdominal fur, scab, mass, scant feces, inguinal mass, red substance in the cage pan, labored breathing, lost righting reflex, swollen digit, alopecia, abrasion, swollen tail, ulceration, and splayed limbs.

Bodyweight:

Maternal administration of rhGAA at dosages as high as 40 mg/kg/every other day caused minor changes in the average body weight and body weight gains for the pre- and postweaned, F1 generation male and female mice. These changes may be attributed to the smaller litter sizes in Groups II through IV compared to group I. In addition, many of the changes appear transient and not dosage dependent.

F0: The mean body weight of the Group I (Control Article/saline) maternal females on Day 0 was 27.2 ± 1.4 g and at the end of the dosing period (Day 18) the mean body weight was 57.8 ± 4.9 g. Body weights and body weight gains during the gestation and lactation periods were unaffected by doses of the rhGAA as high as 40 mg/kg/day and the few statistically significant reductions that occurred were not dose-dependent. There was a statistically significant ($p \leq 0.01$) decrease in the average gestational body weight gain (↓ 15%) of mice in the 10 mg/kg dose group for DGs 0 to 18 when compared to the Saline/Control group. In addition, the 20 mg/kg group had a statistically significant decrease ($p \leq 0.05$) of 30% in lactational body weight gains

for DLs 2 to 4 compared to the Saline/Control group. Subsequently, on DLs 4 to 6 the 20 mg/kg group body weight gain was 118% greater than the Saline/Control group.

F1: The mean body weights of the Group I male and female pups on Day 1 were 7.4 ± 2.0 g and 6.9 ± 1.8 g, respectively. The mean body weights of the control male and female pups at the beginning of the cohabitation period (Day 89-94) were 33.9 ± 2.7 and 26.6 ± 1.9 g, respectively. At birth (DL 1) and for the majority of time during DLs 4, 7, 14 and 21, Group II (maternal DPH/Control group), Group III (10 mg/kg maternal rhGAA), Group IV (20 mg/kg maternal rhGAA) and Group V (40 mg/kg maternal rhGAA) mice had decreased litter size weights compared to Group I (Saline/Control group). However there were less pups/litter in Groups II through V. Thus when the pup weight/litter was calculated, there were no differences amongst the 5 groups on DL 1 and this value stayed at or above the Group I weights during DLs 4, 7, 14 and 21. There were statistically significant increases ($p \leq 0.05$) of pup weight/litter in Group III on DL 4 (↑ 15%) and in Group IV on DLs 14 and 21 (↑ 19% and ↑ 32%, respectively).

The average body weights on day 1 post weaning were significantly increased ($p \leq 0.05$ to $p \leq 0.01$) in the male and female mice from Group II (M: ↑ 24%; F: ↑ 25%), Group III (M: ↑ 20%; F: ↑ 23%) and Group IV (M: ↑ 32%; F: ↑ 36%). Similar statistically significant increases were seen on Day 8 postweaning (except in female Group I). The average body weight in male mice from Group IV was significantly increased ($p \leq 0.05$, ↑ 10%) on day 15 postweaning, but average body weight was significantly reduced during days 15 to 22 postweaning (↓ 41%).

Body weight gains for F1 generation female mice were significantly reduced ($p \leq 0.05$ to $p \leq 0.01$) in Group IV (↓ 14%) on postweaning days 8 to 15 and Groups III and IV (↓ 36% and ↓ 44%) on days 15 to 22. Although not statistically significant, there were also decreases in body weight gains of 36% observed in Group V on days 15 to 22. These sporadic differences resulted in an overall (Day 1 postweaning to cohabitation) significant reduction ($p \leq 0.05$ to $p \leq 0.01$) in body weight gain in Groups II, III and IV. Body weight gains during gestation were comparable among the groups.

Sexual Maturation: The average day of preputial separation and vaginal patency in male and female mice along with the body weight at sexual maturation are summarized in the table below:

Sexual Maturation (results Mean days or (g) ± S.D.)	Saline (CTRL)	DPH (CTRL)	10 mg/kg rhGAA	20 mg/kg rhGAA	40 mg/kg rhGAA
Male Mice					
Preputial Separation	33.6± 2.7	32.3±3.7	32.1±2.8	31.1±1.5**	33.3±2.5
Body Weight at Separation	20.9±2.1	21.4±2.2	21.0±2.0	20.7±1.8	21.2±1.9
Female Mice					
Vaginal Patency	32.5±3.6	30.8±2.5	31.6±2.8	30.2±2.5*	30.2±1.6*
Body Weight at Patency	16.7±2.6	16.6±2.8	17.3±2.8	17.1±1.7	15.2±2.9

* Significantly different from the Saline (CTRL) value ($p \leq 0.05$).
** Significantly different from the Saline (CTRL) value ($p \leq 0.01$).

The average day of sexual maturation for the treatment groups appears slightly accelerated when compared to the Saline/Control group. Statistically significant differences are seen in preputial

separation in the 20 mg/kg maternal rhGAA group and in vaginal patency in the 20 and 40 mg/kg maternal rhGAA groups. Although not statistically significant, sexual maturation was accelerated in the DPH control and 10 mg/kg maternal rhGAA group.

Passive Avoidance: There were no statistically significant or biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F1 generation male and female mice, as evaluated by performance in a passive avoidance paradigm.

Gross Necropsy/Pathology: There were single incidences of masses in the inguinal, pelvic and perivaginal area in the 40 mg/kg group in F0 mice. In F1 generation pups, there was a statistically significant increase ($p \leq 0.01$) in the number of pups with slight to extreme dilation of the renal pelvis (40 mg/kg maternal rhGAA group). However, the 5 abnormal pups came from 2 litters, which on a litter basis is not a significant increase and a number of weaned mice in all groups had similar findings when they were sacrificed. In terminally sacrificed F1 generation mice, there were no test article related necropsy observations. In the 20 mg/kg maternal rhGAA group, 2/25 female mice had a cyst on their ovary or in the uterus. Other singular necropsy observations included moderate dilation of the renal pelvis, black material in the stomach, a mass proximal to the vagina or a single conceptus in the vagina.

Organ Weights: There were no test-article related changes in absolute or relative epididymides and testes weights in F1 generation male mice.

Natural Delivery Observations of F0 mice: Pregnancy occurred in 24, 22, 21, 23 and 24 of the 25 mated female mice in Groups I, II, III, IV and V, respectively. All pregnant dams delivered litters and there was no change in duration of gestation between the 5 groups.

Groups II, III, IV and V had litter sizes that were 19%, 13%, 15% and 12% smaller (statistical significance; $p \leq 0.01$ to $p \leq 0.05$) than Group I litter sizes. However, litter sizes were similar between the DPH control group (Group II) and the test article treated groups. Group IV females had statistically more stillborn pups than compared to Group I (5/243 compared to 2/323). The number of pups dying on DLs 15 to 21 was significantly increased ($p \leq 0.05$) in Group V (12.2%) compared to the Group I (6.7%) value. Relative to Group I, the average number of implantation sites and the average number of live born per litter in Groups II, IV and V were significantly reduced ($p \leq 0.05$ to $p \leq 0.01$) by 14%, 12% and 11%, respectively. There was a significant increase ($p \leq 0.05$) in the percentage of pups found dead or presumed cannibalized in Group V (10.2%) from Days 8-14 and Days 15-21 when compared to Group I (6.64%) and Group II (3.3%). There were no other differences between Group II animals and the test-article treated groups. The natural delivery observations are summarized in the sponsor's Tables below.

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DOSAGE GROUP		I	II	III	IV	V
IP ADMINISTRATION a		SALINE	DPH	DPH	DPH	DPH
rhGAA DOSAGE (MG/KG/DAY)b		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	40
MICE ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25	25
PREGNANT	N	24	22	21	23	24
DELIVERED A LITTER	N(%)	24(100.0)	22(100.0)	21(100.0)	23(100.0)	24(100.0)
DURATION OF GESTATION c	MEAN±S.D.	19.8 ± 0.4	19.7 ± 0.6	19.8 ± 0.4	19.9 ± 0.4	19.8 ± 0.7
IMPLANTATION SITES	N	335	263	265	284	295
PER DELIVERED LITTER	MEAN±S.D.	14.0 ± 2.0	12.0 ± 2.9*	12.6 ± 2.4	12.3 ± 2.1*	12.4 ± 1.4*
MICE WITH STILLBORN PUPS	N(%)	2(8.3)	0(0.0)	1(4.8)	0(0.0)	1(4.2)
MICE WITH NO LIVEBORN PUPS	N	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX d	%	100.0	100.0	100.0	100.0	100.0
	N/N	24/ 24	22/ 22	21/ 21	23/ 23	24/ 24
MICE WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
MICE WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	3(12.5)	1(4.5)	1(4.8)	0(0.0)	4(16.7)

a. DPH/Saline was administered by intraperitoneal injection at 5 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA.
b. Dosage occurred every other day from day 6 of gestation to day 20 of lactation.
c. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the time the first pup was delivered.
d. Number of mice with live offspring/number of pregnant mice.
* Significantly different from the Group I value (p≤0.05).

DOSAGE GROUP		I	II	III	IV	V
IP ADMINISTRATION a		SALINE	DPH	DPH	DPH	DPH
rhGAA DOSAGE (MG/KG/DAY)b		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	40
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	22	21	23	24
PUPS DELIVERED (TOTAL)	N	323	237	243	260	285
	MEAN±S.D.	13.4 ± 2.2	10.8 ± 2.8**	11.6 ± 2.8*	11.3 ± 2.1**	11.9 ± 1.6*
LIVEBORN	MEAN±S.D.	13.4 ± 2.3	10.8 ± 2.8**	11.3 ± 3.0	11.3 ± 2.2**	11.8 ± 1.6*
	N(%)	321(99.4)	237(100.0)	238(97.9)	259(99.6)	284(99.7)
STILLBORN	MEAN±S.D.	0.1 ± 0.3	0.0 ± 0.0	0.2 ± 1.1	0.0 ± 0.0	0.0 ± 0.2
	N(%)	2(0.6)	0(0.0)	5(2.1)**	0(0.0)	1(0.3)
UNKNOWN VITAL STATUS	N	0	0	0	1	0
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED						
DAY 1	N/N(%)	1/321(0.3)	0/237(0.0)	0/238(0.0)	0/259(0.0)	1/284(0.4)
DAYS 2- 4	N/N(%)	11/312(3.5)c	7/237(3.0)	9/238(3.8)	15/259(5.8)	3/271(1.1)c
DAYS 5- 7	N/N(%)	13/301(4.3)	0/230(0.0)**	3/229(1.3)*	1/244(2.9)	17/267(6.4)d
DAYS 8-14	N/N(%)	19/288(6.6)	5/230(2.2)**	13/216(6.0)c	1/237(0.4)**	21/250(8.4)
DAYS 15-21	N/N(%)	18/269(6.7)	11/225(4.9)	4/191(2.1)c	0/236(0.0)**	28/229(12.2)*
VIABILITY INDEX e	%	96.2c	97.0	98.2	94.2	98.5c
	N/N	301/313c	230/237	229/233	244/259	268/272c
LACTATION INDEX f	%	83.4	93.0	91.5c	98.7	75.3d
	N/N	251/301	214/230*	187/204c	236/244**	201/267d

DAY(S) = DAY(S) POSTPARTUM
a. DPH/Saline was administered by intraperitoneal injection at 5 mg/kg (1 mL/kg) beginning on the day of the 5th dose of rhGAA.
b. Dosage occurred every other day from day 6 of gestation to day 20 of lactation.
c. Excludes pups that were sacrificed due to death of mouse.
d. Excludes a pup from litter 3003, which had an accidental death.
e. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
f. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 4 postpartum.
* Significantly different from the Group I value (p≤0.05).
** Significantly different from the Group I value (p≤0.01).

Values for the numbers of dams delivering litters, the duration of gestation, the gestation index (number of dams with one or more liveborn pups/number of pregnant mice), the numbers of dams with all pups dying, viability and lactation indices, surviving pups per litter, percent male pups per number of pups sexed per litter, live litter size at weighing and pup weight per litter were comparable among the five dosage groups.

Mating and Fertility: There were no biologically significant effects on the mating and fertility parameters evaluated in the F1 generation male and female mice. Values for the number of days in cohabitation, the number of mice that mated, the fertility index (number of pregnancies per number of mice that mated), the number of mice with confirmed mating dates during the first and second weeks of cohabitation, and the number of pregnancies per number of mice in cohabitation were comparable among the 5 dosage groups.

Caesarean-Section Observations of F1 mice: No caesarean-section or litter parameters were affected by maternal dosages of rhGAA as high as 40 mg/kg/day. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses and percent live male fetuses were comparable among the five dosage groups and did not significantly differ.

A total of 23 (92.0%), 23 (92.0%), 24 (96.0%), 23 (92.0%) and 22 (88.0%) mice were pregnant with one or more live fetuses in Group I (Saline/Control), Group II (DPH/Control), Group III (10 mg/kg), Group IV (20 mg/kg) and Group V (40 mg/kg) maternal rhGAA dosage groups, respectively. There were 1, 0, 0, 2 and 3 fetuses in Groups I to V that had a gross external alteration. One Group I fetus had hind limbs rotated, one Group IV fetus had body edema and one had hind limbs rotated; and three fetuses each from a different litter in Group V had hind limbs rotated. The caesarian-sectioning observations are summarized in the sponsor's Table below.

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TABLE B16 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE MICE

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL IP ADMINISTRATION		SALINE	DPH	DPH	DPH	DPH
MATERNAL rhGAA DOSAGE (MG/KG/DAY)		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	40
MICE TESTED	N	25	25	25	25	25
PREGNANT	N(%)	23(92.0)	23(92.0)	24(96.0)	23(92.0)	22(88.0)
DELIVERED AND SACRIFICED	N(%)	2(8.7)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 18 OF GESTATION	N	21	23	23	23	22
CORPORA LUTEA	MEAN±S.D.	14.5 ± 1.4	14.5 ± 2.3	13.5 ± 3.4	13.9 ± 2.6	14.1 ± 2.7
IMPLANTATIONS	MEAN±S.D.	14.1 ± 1.4	14.2 ± 2.4	13.1 ± 3.6	13.8 ± 2.7	13.9 ± 2.7
LITTER SIZES	MEAN±S.D.	13.1 ± 1.6	12.8 ± 3.3	12.0 ± 4.3	11.9 ± 2.8	12.9 ± 3.1
LIVE FETUSES	N	275	292	275	274	283
	MEAN±S.D.	13.1 ± 1.6	12.7 ± 3.4	12.0 ± 4.3	11.9 ± 2.8	12.9 ± 3.1
DEAD FETUSES	N	1	3	1	0	0
	MEAN±S.D.	0.0 ± 0.2	0.1 ± 0.3	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	1.0 ± 0.9	1.4 ± 1.9	1.1 ± 1.5	1.9 ± 2.8	1.0 ± 1.2
EARLY RESORPTIONS	N	18	30	26	40	21
	MEAN±S.D.	0.8 ± 0.8	1.3 ± 1.9	1.1 ± 1.5	1.7 ± 2.7	1.0 ± 1.2
LATE RESORPTIONS	N	2	2	0	3	2
	MEAN±S.D.	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.4	0.1 ± 0.3
MICE WITH ANY RESORPTIONS	N(%)	13(61.9)	16(69.6)	12(52.2)	14(60.9)	13(59.1)
MICE WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)	0(0.0)
MICE WITH VIABLE FETUSES	N(%)	21(100.0)	23(100.0)	22(95.6)	23(100.0)	22(100.0)
PLACENTAE APPEARED NORMAL a	N(%)	21(100.0)	23(100.0)	22(100.0)	23(100.0)	22(100.0)

a. Excludes mice with all early resorptions.

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TABLE B17 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL IP ADMINISTRATION		SALINE	DPH	DPH	DPH	DPH
MATERNAL rhGAA DOSAGE (MG/KG/DAY)		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	40
LITTERS WITH ONE OR MORE LIVE FETUSES	N	21	23	22	23	22
IMPLANTATIONS	MEAN±S.D.	14.1 ± 1.4	14.2 ± 2.4	13.5 ± 3.1	13.8 ± 2.7	13.9 ± 2.7
LIVE FETUSES	N	275	292	275	274	283
	MEAN±S.D.	13.1 ± 1.6	12.7 ± 3.4	12.5 ± 3.5	11.9 ± 2.8	12.9 ± 3.1
LIVE MALE FETUSES	N	123	129	127	128	126
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	43.7 ± 19.8	42.4 ± 13.4	45.1 ± 13.9	46.2 ± 13.7	43.1 ± 22.1
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	1.34 ± 0.10	1.32 ± 0.11	1.34 ± 0.12	1.35 ± 0.15	1.36 ± 0.10
MALE FETUSES	MEAN±S.D.	1.38 ± 0.09	1.36 ± 0.12 [22]a	1.38 ± 0.13	1.38 ± 0.14	1.38 ± 0.11
FEMALE FETUSES	MEAN±S.D.	1.30 ± 0.10	1.29 ± 0.11	1.32 ± 0.13	1.33 ± 0.16	1.33 ± 0.11
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	7.1 ± 6.8	11.6 ± 17.5	9.2 ± 15.5	12.3 ± 17.0	8.2 ± 9.2

[ ] = NUMBER OF VALUES AVERAGED
a. Litter 345 had no male fetuses.

TABLE B18 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY - F2 GENERATION LITTERS/FETUSES

MATERNAL DOSAGE GROUP		I	II	III	IV	V
MATERNAL IP ADMINISTRATION		SALINE	DPH	DPH	DPH	DPH
MATERNAL rhGAA DOSAGE (MG/KG/DAY)		0 (CONTROL ARTICLE)	0 (CONTROL ARTICLE)	10	20	40
LITTERS EVALUATED	N	21	23	22	23	22
FETUSES EVALUATED	N	276	295	276	274	283
LIVE	N	275	292	275	274	283
DEAD a	N	1	3	1	0	0
BODY: EDEMA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	0(0.0)
HINDLIMB(S): ROTATED						
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	1(4.3)	3(13.6)
FETAL INCIDENCE	N(%)	1(0.4)	0(0.0)	0(0.0)	1(0.4)	3(1.1)

a. Dead fetuses were excluded from summarization and statistical analyses. Observations for these conceptuses are cited on Table B23.

Conclusions: F0 generation female mice were given 10, 20, 40 mg/kg/day rhGAA intravenously every other day from DGs 6 through DL 21. There was no effect on fertility at doses up to 40 mg/kg/every other day. There were minor effects of rhGAA on embryonic development in the 40 mg/kg group. There was an increase in frequency (number of days clinical effect was observed multiplied by # of pups) of dehydration and decreased motor activity in F1 generation mice from the 40 mg/kg group when compared to all other groups. The average day of sexual maturation for the treatment groups appears to be slightly accelerated when compared to the Saline/Control group. There was no effect on learning or memory or the ability to produce another generation by the F1 generation mice at doses as high as 40 mg/kg/day of the test article.

Groups II, III, IV and V had litter sizes that were 19%, 13%, 15% and 12% smaller (statistical significance; $p \leq 0.01$ to $p \leq 0.05$) compared to Group I. However, litter sizes were similar between the DPH control group (Group II) and the test article treated groups. There was an increased number of pups dying on DLs 15 to 21 in Group V (12.2%) compared to the Group I (6.7%) value. After cesarean-sectioning of F1 generation mice, there was an increased incidence of

gross external alterations in Group V fetuses (3 fetuses with rotated hind limbs) when compared to Group I fetuses.

Addendum: The test article used in the study, "rhGAA", is Myozyme®.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated May, 16 2008 by Dr. Fang Cai.

Study title: Intravenous Injection Study of Recombinant Human Acid-α-Glucosidase (rhGAA) on Female Fertility and Early Embryonic Development Implantation in Mice

Key study findings: In this segment I study, female mice (22/group) were administered rhGAA intravenously (slow bolus) at doses of 10, 20 and 40 mg for 14 days premating, throughout the mating period and gestation day 7 or 8 Body weights, body weight gain and food consumption were not affected by rhGAA. There were no treatment-related effects on estrous cycle, fertility inc pregnancy rate, post-implantation, resorptions, and total live fetuses. It is inconclusive whether the higher frequency of pre-implantation loss in the rhG treated groups (10 and 40 mg/kg/every other day) is related to treatment because the change did not reach statistical significance and there was no d related pattern. Vehicle, DPH and repeated IV/IP injections had no effects o female fertility and early embryonic development in mice.

Study no.: [redacted] (b) (4) 6354-163

Volume #, and page # 1-209

Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation and completion: May 20, 2005 and December 12, 2005

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug, lot # 1369017, and % purity: >98%

Methods

Doses: 10, 20 or 40 mg/kg of rhGAA were dosed to females for at least a 14-day period prior to mating, throughout the mating period, and through Gestation Day (GD) 7 or 8. Diphenhydramine (DPH, 5 mg/kg) was administered approximately 26 minutes prior to each dose of test article (groups 4-7) for the duration of the study to prevent anaphylaxis starting on dosing day 7. rhGAA was administered by IV bolus every other day at a dose volume of 8 ml/kg. Vehicle control was provided by the sponsor (GAA control, the composition of the vehicle was not provided by sponsor. Lot #: GW12408163). The high dose used in this study was determined by the sponsor based on all available data including those obtained from pharmacology, other toxicology and kinetics studies.

Species/strain: Crl:CD-1®(ICR)BR mice

Age and body weight: females: 14 weeks of age; 26.6 to 35.3 g

Number/sex/group: 22 /sex/group

Route of administration: slow bolus i.v injection

Formulation: Sterile water was added to the lyophilized rhGAA to prepare a stock solution (1.25, 2.5 and 5 mg/ml). Diphenhydramine (DPH) was prepared by diluting with physiological saline to achieve a 5 mg/mL concentration.

Study design: Crl:CD-1®(ICR)BR mice were assigned to 7 groups (22/sex/group), however, only the females were treated in the study. Females in Group 1 were untreated and served as naïve controls, while the females in Groups 2-4 received saline or the rhGAA control article every other day by slow bolus IV injection into a tail vein at the volume of 8 mL/kg. The females in Groups 5, 6, and 7 received doses of 10, 20 or 40 mg/kg of rhGAA under the same dosing regimen, respectively. Beginning on the seventh dosing day, females in Groups 2-7 were administered saline or DPH (5 mg/kg) by IP injection just prior to the IV injections. The study design is depicted in the sponsor's table below.

Group	No. of Animals		IP Administration[b]	IV - Dose Level	Dose Concentration
	Male[a]	Female		(mg/kg/every other day)	(mg/mL)
1	22	22	N/A	No	0
2	22	22	Saline	Saline	0
3	22	22	Saline	Vehicle	0
4	22	22	DPH	Saline	0
5	22	22	DPH	10 mg/kg of rhGAA	1.25
6	22	22	DPH	20 mg/kg of rhGAA	2.5
7	22	22	DPH	40 mg/kg of rhGAA	5

a Males were not treated.
b DPH/saline administration began just prior to the seventh dosing day of rhGAA.

During the 2-week premating period, vaginal smears were assessed for stage of estrus. Animals from respective dose groups were mated by placing one female

in the breeding cage of an untreated male from the same group. During the mating period, a daily inspection was made for the presence of a retained copulatory plug or obvious copulatory plugs on the tray liner. The day of plug observation was designated as GD 0.

Parameters and endpoints evaluated: The animals were observed for mortality and signs of morbidity once daily during the first 2 weeks of dosing for the females, and twice daily during the rest of the study period. On dosing days, all animals were checked for anaphylaxis post dosing. If any anaphylactic symptoms appeared, the mice were treated with antihistamine DPH. All females were weighed twice weekly during the premating phase and during mating. Female body weights were recorded on gestation day 0, 3, 7, 10 and 13. Food consumption was recorded weekly during the premating treatment period, during mating and on gestation day 0, 3, 7, 10 and 13.

On day 13 of gestation, all females except for unscheduled death of one female (on sixth dosing day) were sacrificed and examined for gross abnormalities. The uterine contents were examined for the number of early- or late- resorptions, live/dead fetuses and implantation sites. The ovaries were examined for the number of corpora lutea. Abnormal viscera were saved in 10% neutral-buffered formalin. All cervical, thoracic, and abdominal tissues from the last 5 surviving control females were retained in 10% neutral-buffered formalin for possible evaluation.

Results

Mortality (dams): One unscheduled death occurred during the study. A 40 mg/kg female (Group 7; A86806) was found dead at the 1-hour postdose observation on Day 10 (sixth dosing day). Since no remarkable clinical signs were noted at 10 minutes postdose, DPH was not administered. There were no abnormal necropsy findings for this animal.

Clinical signs (dams): During the premating phase, a low incidence of sporadic clinical signs (hyperactivity, hunched appearance and sore tails) was reported with no apparent relationship to treatment. During gestation, remarkable clinical signs were limited to a low incidence of hyperactivity and hunched appearance, findings associated with DPH treatment.

Body weight (dams): During the premating phase, significantly lower mean body weight gain was observed in the sham control-treated groups (Groups 2-4: 0.4 ± 1.3g, 0.2 ± 1.3 g, 0.6 ± 1.1 g vs. 1.6 ± 1.3 g in naïve controls, $p \leq 0.05$ or 0.01), whereas there were no significant changes in mean body weights and body weight changes in mice of rhGAA treatment groups. No significant changes in mean maternal body weights and body weight changes were seen in gestation day 0-7 and gestation day 0-13 except for groups 3-4 that had

increased mean maternal body weight changes on gestation day 10-13 (group 3 7.69 ± 1.44; group 4: 7.77 ± 0.98 vs. 6.54 ± 1.55 in naïve controls, $p \leq 0.05$).

Food consumption (dams): Mean food consumption during premating and gestation were unaffected by vehicle, DPH and rhGAA treatments.

Macroscopic examination (dams): The principal thoracic, cervical and abdominal organs were subjected to a macroscopic post-mortem examination. No remarkable changes were seen in animals of all treatment groups.

Fertility parameters: Vehicle, DPH and rhGAA had no effects on estrous cycle fertility index and pregnancy rate (see sponsor's Tables below). The majority of females were confirmed mated within the first 4 days. When compared to the naïve or saline control, a slightly higher number of females in the rhGAA-treated groups, DPH and vehicle control group were confirmed mated at > 4 days (see sponsor's Figure 1 and Table 3 below. Table 3 is constructed based on the data provided by sponsor.). No dose-related pattern was seen.

Text Table 1
Estrous Cycle

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
No. of females with prolonged diestrus[a]	11	9	9	10	9	10	7
No. of nonpregnant females	3	3	3	1	3	2	2

a Four or more consecutive days in diestrus.

Text Table 2
Reproductive Indices

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
No. of male/female pairs	22	22	22	22	22	22	21
No. of females mated	22	22	21	22	21	22	21
Male/Female Copulation Index (%)	100	100	95	100	95	100	100
No. of females successfully mated	19	19	19	21	19	20	19
Male/Female Fertility Index (%)	86	86	86	95	86	91	90

Figure 1
Mean Preceital Histogram

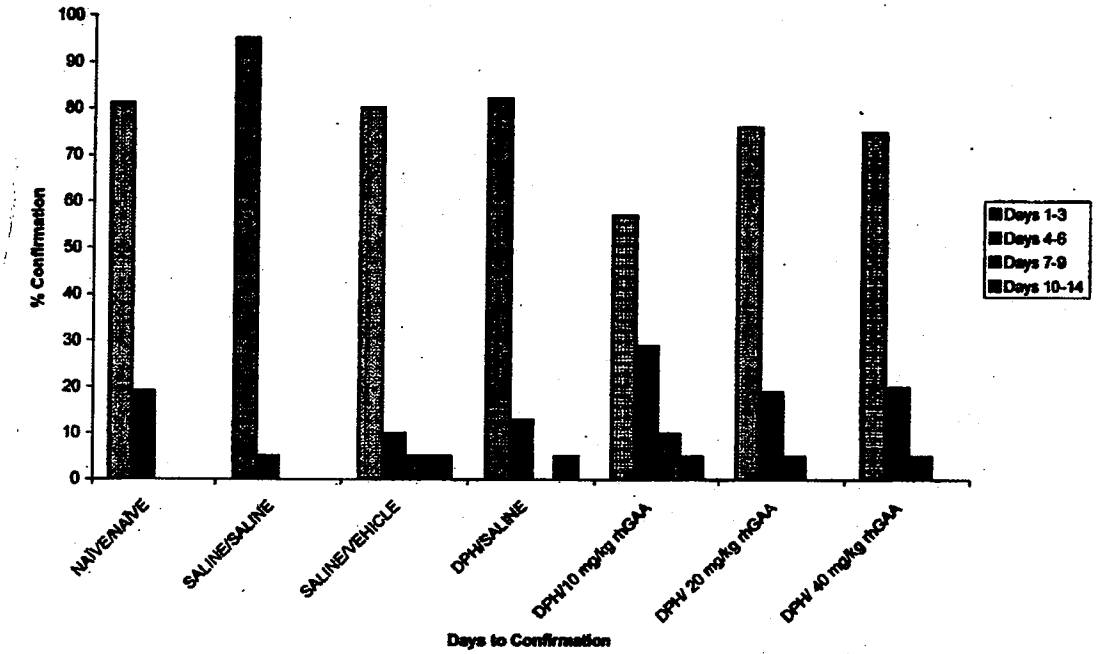

Table 3. Mating days to confirmation

Groups	Mating day = 4 days	Mating day > 4 days
	Numbers of females	
Naive	4	0
Saline	1	0
Vehicle	1	3
DPH	2	2
rhGAA 10 mg/kg	4	4
rhGAA 20 mg/kg	2	3
rhGAA 40 mg/kg	2	3

There were no abortions or early deliveries, and all dams had litters with viable fetuses. Slightly lower numbers in corpora lutea were seen in rhGAA treatment groups. A increase in the mean pre-implantation loss occurred in Groups of 10 and 40 mg/kg rhGAA, respectively, because one (A86746 of 10 mg/kg: 12 corpora lutea and 6 implant sites) or two litters (A86787of 40 mg/kg: 7 corpora lutea and 4 implant sites; A86797 of 40 mg/kg: 10 corpora lutea and 2 implant sites) had an unusually low number of implantations. There were no drug-related effects on post-implantation, resorptions, and total live fetuses. The cesarean section data is summarized in sponsor's Table 10 below.

The sponsor's no-observed-effect level (NOEL) for maternal and fertility indices and embryo/fetal viability was 40 mg/kg. It is inconclusive whether the higher frequency of pre-implantation loss in the rhGAA-treated groups (10 and 40

mg/kg/ every other day) is related to treatment because the change did not reac statistical significance and there was no dose-related pattern. Vehicle, DPH an repeated IV/IP injections had no effects on female fertility and early embryonic development in mice.

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Table 10
Summary of Cesarean Section Data

	DOSE LEVEL	GROUP 1 0 MG/KG (NAIVE)	GROUP 2 0 MG/KG (SAL/SAL)	GROUP 3 0 MG/KG (SAL/VEH)	GROUP 4 0 MG/KG (DPH/SAL)	GROUP 5 10 MG/KG (DPH/rhGAA)	GROUP 6 20 MG/KG (DPH/rhGAA)	GROUP 7 40 MG/KG (DPH/rhGAA)
Females Mated	N	22	22	21	22	21	22	21
Pregnant	N	19	19	19	21	19	20	19
	%	86	86	90	95	90	91	90
Aborted	N	0	0	0	0	0	0	0
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Died	N	0	0	0	0	0	0	1
	%	0.0	0.0	0.0	0.0	0.0	0.0	4.5
Delivered Early	N	0	0	0	0	0	0	0
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pregnant at C-section	N	18	17	18	21	19	19	18
Dams with Viable Fetuses	N	18	17	18	21	19	19	18
	%	100	100	100	100	100	100	100
Dams with no Viable Fetuses	N	0	0	0	0	0	0	0
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Corpora Lutea	MEAN	14.6	14.2	14.7	14.4	13.8	13.1	13.1
	S.D.	1.9	1.1	2.0	1.7	1.6	2.2	2.5
	N	18	17	18	21	19	19	18
	TOTAL	263	242	264	302	262	248	235
Implantation Sites	MEAN	13.7	13.5	13.9	13.4	12.3	12.2	11.6
	S.D.	1.8	1.2	2.1	1.4	2.2	2.5	3.5
	N	18	17	18	21	19	19	18
	TOTAL	247	229	250	282	233	231	209
Preimplantation Loss	MEAN%	5.9	5.2	5.3	6.3	11.2	7.3	13.1
	S.D.	6.5	6.5	6.2	6.3	11.7	8.3	19.2

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

TABLE 10
SUMMARY OF CESAREAN SECTION DATA

		DOSE LEVEL	GROUP 1 0 MG/KG (NAIVE)	GROUP 2 0 MG/KG (SAL/SAL)	GROUP 3 0 MG/KG (SAL/VEH)	GROUP 4 0 MG/KG (DPH/SAL)	GROUP 5 10 MG/KG (DPH/rhGAA)	GROUP 6 20 MG/KG (DPH/rhGAA)	GROUP 7 40 MG/KG (DPH/rhGAA)
Pregnant at C-section		N	18	17	18	21	19	19	18
Resorptions:	Total	MEAN	1.6	0.5	0.9	0.6	0.3	1.3	0.6
		S.D.	2.4	0.7	1.0	0.9	0.7	1.2	1.0
		N	18	17	18	21	19	19	18
		TOTAL	29	8	17	12	6	25	10
		MEAN%	11.3	3.5	7.1	4.4	2.5	10.4	4.4
		S.D.	16.0	5.6	7.4	6.7	5.1	9.3	7.5
	Early	MEAN	1.3	0.4	0.8	0.4	0.2	0.8	0.4
		S.D.	2.1	0.6	0.9	0.8	0.5	1.1	0.6
		N	18	17	18	21	19	18	18
		TOTAL	23	6	15	9	4	16	7
		MEAN%	9.0	2.6	6.1	3.3	1.6	6.5	3.2
		S.D.	14.6	4.5	6.5	6.2	4.0	8.0	4.8
	Late	MEAN	0.3	0.1	0.1	0.1	0.1	0.5	0.2
		S.D.	0.8	0.3	0.3	0.4	0.3	0.7	0.5
		N	18	17	18	21	19	19	18
		TOTAL	6	2	2	3	2	9	3
		MEAN%	2.2	0.9	0.9	1.1	0.9	3.8	1.3
		S.D.	5.0	2.6	2.7	2.7	2.7	5.7	3.9
Dead Fetuses		TOTAL	0	0	0	0	0	0	0
Postimplantation Loss		MEAN%	11.3	3.5	7.1	4.4	2.5	10.4	4.4
		S.D.	16.0	5.6	7.4	6.7	5.1	9.3	7.5
Live Fetuses		MEAN	12.1	13.0	12.9	12.9	11.9	10.8	11.0
		S.D.	2.5	1.4	2.4	1.8	2.2	2.3	3.5
		N	18	17	18	21	19	19	18
		TOTAL	218	221	233	270	227	206	198
		MEAN%	88.7	96.5	92.9	95.6	97.5	89.6	95.6
		S.D.	16.0	5.6	7.4	6.7	5.1	9.3	7.5

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

SUMMARY AND EVALUATION:

MYOZYME (alglucosidase alfa), the human enzyme acid α-glucosidase (GAA) is produced by recombinant DNA technology in a Chinese hamster ovary cell line. Myozyme has been approved to treatment of patients with infantile-onset Pompe disease on April 28, 2006. In this submission, sponsor submitted the final report of Segment I study in mice, in which the potential adverse effects of rhGAA and vehicle on female fertility and early embryonic development were evaluated.

In this Segment I study, female mice (22/group) were administered rhGAA intravenously at doses of 10, 20 and 40 mg/kg/ every other day for 14 days premating, throughout the mating period and throughout gestation day 7 or 8. There were no drug-related effects on estrous cycle, fertility index, pregnancy rate, post-implantation, resorptions, and total live fetuses. Vehicle, DPH and repeated IV/IP injections had no effects on female fertility and early embryonic development in mice.

Addendum: The test article used in the study, "rhGAA", is Myozyme®.

2.6.6.7 Local tolerance

No studies were submitted.

2.6.6.8 Special toxicology studies

One study in GAA knockout mice was conducted to investigate the nature of the hypersensitivity reaction seen routinely in mouse studies. Serum and plasma was collected from GAA knockout mice after administration of rhGAA and analyzed for the presence of IgG, IgG1, IgE, histamine, total complement and C3a/C5a. The results showed that animals receiving vehicle alone had low IgG titers, no detectable specific anti-drug antibody, and no detectable IgE titer. Moderate levels of histamine were detected in mice receiving rhGAA after the 3rd dose. Significantly elevated histamine levels were detectable in mice receiving rhGAA after the 8th dose. Significant elevation of IgG and IgG1 titers were reported in all mice receiving rhGAA. These findings are possibly related to the hypersensitivity response in the GAA knockout mice.

The following review was taken from the Pharmacology/Toxicology review of BLA 125,141 dated September 15, 2008 by Dr. Niraj Mehta.

Study title: Evaluation of the Immune Tolerance Induction Regimens to Ameliorate Antibody Responses to Enzyme Replacement Therapies (ERT)

Key Study Findings: 10 mg/kg MTX (methotrexate) administration, 0, 24 and 48 hrs following the first 8 of 16 weekly rhGAA treatments, reduced rhGAA-specific IgG titers.

Study #: ITR-333-1106

Seq. 37, Pg. 1-106 (electronic)

Conducting Laboratory and Location: Genzyme Corporation.
Framingham, MA 01701

Date of Study Initiation: Not given (report dated December, 26 2006)

GLP Compliance: A statement of compliance was not included.

QA Report: yes ( ) no (x)

Drug: Lot Nos. GA062, GA079, GA080, GA123

Methods:

Doses: See Table below.

Species/strain: $6^{neo}/6^{neo}$ GAA KO mice, C57Bl/6 mice, CBA/CaHN-Btk^{xid}/J (Xid) mice, CBA/CaJ mice

Number/sex/group or time point: 5-8 mice/treatment group

Route, formulation, volume, and infusion rate: The rhGAA dosage for immunogenicity studies and immune tolerance induction (ITI) regimens are summarized in Tables 1 and 2 provided by the sponsor:

Table 1. Immunogenicity models

Mouse strain	rhGAA Dose	Frequency of Dose	Treatment Duration
GAA KO	20mg/kg IV	weekly	16 weeks
GAA KO	20mg/kg IV	weekly	32 weeks
C57Bl/6	20mg/kg IV	weekly	12 weeks

Studies involving repeat dosing of rhGAA, 30mg/kg of diphenhydramine (DPH) IP was administered at most 15 minutes prior to each rhGAA treatment.

Table 2. ITI regimens investigated

Immunosuppressive Agent	Dose	Route	Regimen	Study Duration
MMF	100mg/kg	Oral	0, 24 and 48 hours after the first 8 weekly rhGAA tx.	16 weeks
CsA with	Dose I - 12.5mg/kg Dose II - 6.25mg/kg Dose III - 3.125mg/kg	Oral	twice daily: Dose I: day -18 to +14; Dose II: day +15 to +28; Dose III: day +29 to +42	
Aza	5 mg/kg	IP	every other day	12 weeks
ATG	25 mg/kg	IP	60 minutes prior to and 48 hrs after first rhGAA treatment	16 weeks
CTLA4-IgG fusion protein	100µg/mouse	IP	every other day for 12 days	12 weeks
MTX	10, 25 and 50 mg/kg	IP	24 and 48 hours after first 8 weekly rhGAA treatment	16 weeks

MMF = mycophenolate mofetil, CsA = cyclosporine A with azathioprine (Aza), ATG = murine anti-thymocyte globulin, and methotrexate (MTX)

rhGAA-specific IgG titers were evaluated over time and in different mouse strains to characterize the immunogenicity models used to evaluate different ITI regimens (5-8 mice/treatment group). The goal was to identify a short-term regimen that would induce immune tolerance to rhGAA and avoid long-term immunosuppression.

In all instances except for the CsA with Aza evaluation, 20 mg/kg of rhGAA was chronically administered i.v. once per week. In the CsA/Aza studies, rhGAA treatment began on day 18 at a sub-therapeutic dose of 2 mg/kg and was increased to 5, 10 and 20 mg/kg through the subsequent weekly treatments. The therapeutic dose of 20 mg/kg was reached by 4 weeks and this dose was maintained throughout the remainder of the studies. All agents were evaluated in GAA KO mice. In addition, CsA/Aza was evaluated in C57Bl/6 mice as well.

Age: 12-16 weeks

Sampling Times: In the immunogenicity studies, sera were collected and analyzed every other week for 16 weeks and then monthly from weeks 16 to 32 for the evaluation of rhGAA-specific IgG titers by ELISA. To ensure that ATG treatment depleted T cells and to characterize T cell repopulation, whole blood was analyzed by flow cytometry for circulating T cells via CD3 surface staining on days 4, 11, 18, 32 and 45 following ATG treatment.

Study Design (MTX treatment regimens): MTX was the only agent evaluated that induced a sustained decrease in rhGAA-specific IgG titers. In an effort to improve MTX efficacy while minimizing the dose of this potentially deleterious treatment, multiple doses and dosing regimens were evaluated. All studies involved chronic, weekly, i.v. treatments of 20 mg/kg rhGAA (0 hr) and conducted in GAA KO mice (5-8 animals/group). The MTX treatment regimens are summarized below in the sponsor's Table below:

Table 3. MTX treatment regimens

Dose of MTX	Weekly Regimen	Number of Weekly Treatments	Study Duration
25 vs. 50mg/kg	0 & 48 hours	8	12 weeks
10 vs. 20 mg/kg	0, 24 & 48 hours	8	32 weeks
20mg/kg	0 & 36 hours	8	16 weeks
10 mg/kg	0, 24 & 48 hours	4	16 weeks
5 vs.0.5mg/kg	0, 24 & 48 hours	3 vs. 8	32 weeks
5, 0.5, 0.05mg/kg	0, 24 & 48 hours	3	16 weeks
5 vs. 0.5mg/kg	0, 24 & 48 hours vs. -24, 0, & 24 hours vs. -48, -24 & 0 hours	3	12 weeks

B1 B cells: The involvement of B1 B cells in the rhGAA-induced immune response was examined. The study outline of B1 B cell assessments is provided in the sponsor's Table below:

Table 4. B1 B cell assessments

Study Assessment	Study Duration	rhGAA Administration	Mouse strain
Percentage of B1 B cells	24 hours	Single treatment - 20mg/kg IV	GAA KO
rhGAA-specific IgG titers	26 days	Single treatment - 20mg/kg IV	Xid & CBA/CaJ

Observations and Times:

Clinical Signs: Observed daily.

Bodyweight: Body weights were taken prior to the initiation of the study. Test article was administered based on an average body weight of 25 grams for the test animals.

Immunogenicity Studies: See Table 1 above.

ITI Regimens: See Table 2 above.

MTX Treatment Regimens: See Table 3 above.

T cell-independent immune responses: GAA KO mice were treated for 5 consecutive weeks with 20 mg/kg rhGAA i.v. and 10 mg/kg of MTX 0, 24 and 48 hrs following each of the first 3 rhGAA treatments. Animals were sacrificed either 1 day following the final MTX treatment (3 week time-point) or 2 weeks following the final MTX treatment (5 week time-point). Spleens were harvested at either 1 day following or 2 weeks following the last MTX treatment. Single cell suspensions of splenocytes were cultured *in vitro* and stimulated with 1 μg/mL of lipopolysaccharide (LPS). Cell proliferation was evaluated by measuring ^{3}(H)-thymidine incorporation 24 hrs following stimulation.

T cell-dependent immune responses: GAA KO mice were first treated with 1 mg/mL ovalbumin (OVA) in aluminum hydroxide gel i.p. Three weeks later, 20 mg/kg of rhGAA was given i.v. for

3 consecutive weeks alongside MTX treatment at 10 mg/kg 0, 24 and 48 hrs following the first 3 rhGAA administrations. Spleens were harvested 1 day following the last MTX treatment. Splenocytes were cultured *in vitro* and stimulated with OVA (2.5-20 μg/ml). Proliferation assays evaluating ^{3}(H)-thymidine incorporation were performed 5 days following stimulation.

Hematopoietic bone marrow cell populations: GAA KO mice were treated weekly with 20 mg/kg rhGAA i.v. for 5 consecutive weeks and with 10 mg/kg of MTX delivered i.p., 0, 24 and 48 hrs following each of the first 3 rhGAA treatments. Either 1 day or 2 weeks following the last MTX treatment (3 or 5 week time-point, respectively), mice were sacrificed and single cell suspensions of bone marrow cells were prepared for flow cytometry analysis.

B1 B cell Assessments: See Table 4 above. GAA KO and control mice as well as Xid mice lacking B1 B cells and their wild-type counterpart (CBA/CaJ mice) were immunized with 20 mg/kg of rhGAA intravenously. Percentages of peritoneal B1 B cells were evaluated 24 hrs following rhGAA treatment in GAA KO mice. rhGAA-specific IgG titers were assessed by ELISA (5-10 mice/group) for Xid and CBA/CaJ mice.

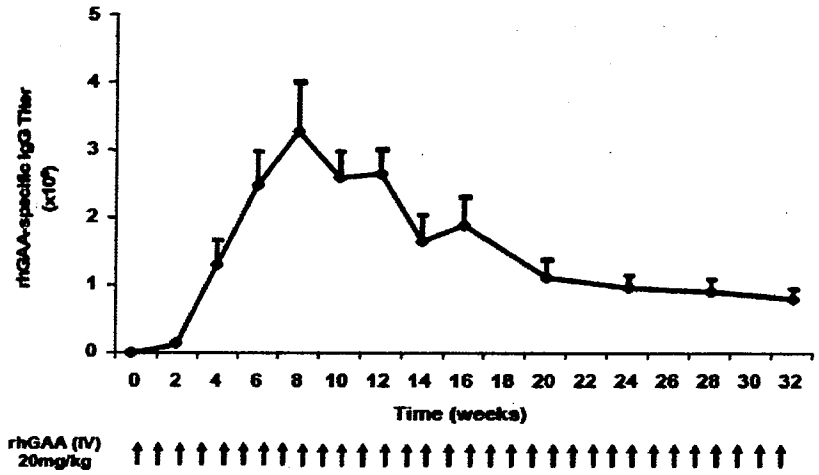
RESULTS:

Clinical Signs: No individual or group data were provided. The sponsor stated that the animals remained healthy throughout all studies, and on rare occasions when mice experienced lethargy, dyspnea, muscle spasms or lowering of body temperature following rhGAA administration, they were given a 15 to 30 mg/kg rescue dose of DPH.

Bodyweight: Body weights were only taken prior to the initiation of the study.

Immunogenicity Studies: In GAA KO mice treated weekly with 20 mg/kg of rhGAA i.v. once per week for 16 weeks, the rhGAA-specific IgG levels steadily increased over the initial 6 week period and then reached a plateau that continued through the remaining 10 weeks. All GAA KO mice developed rhGAA-specific IgG antibodies upon treatment.

In GAA KO mice treated weekly with 20 mg/kg of rhGAA i.v. once per week for 32 weeks, a decline in rhGAA-specific IgG levels over time by ELISA was observed, however complete tolerance as defined by the elimination of the specific antibodies, was not achieved (figure below).



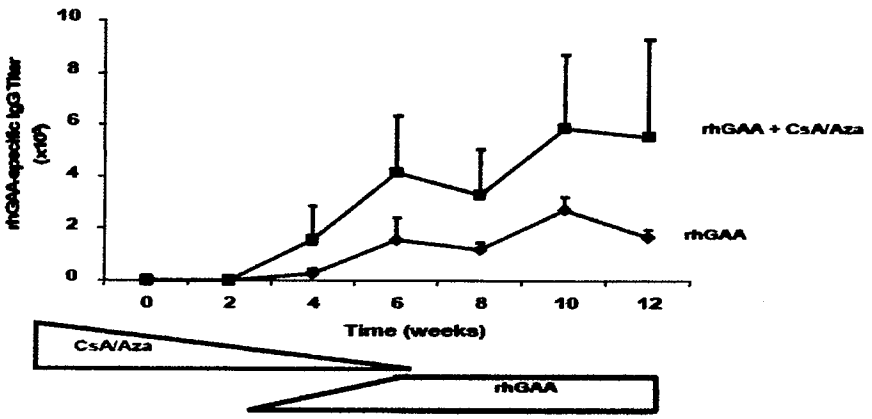
- These data represent the means and standard errors from two experiments.

In a wild-type mouse strain, C57Bl/6, treated with weekly i.v. injections of 20 mg/kg of rhGAA, rhGAA-specific IgG titers steadily increased over the 12 week study to a similar level as that observed upon rhGAA treatment of GAA KO mice

ITI Regimens:

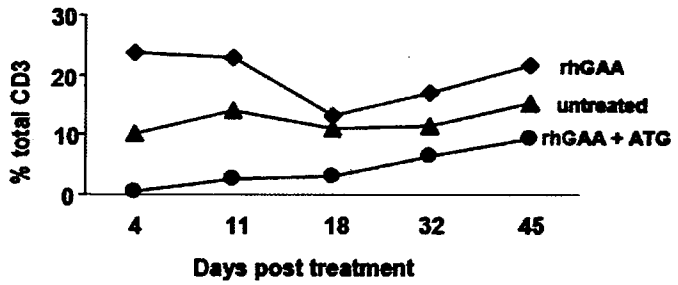
MMF (mycophenolate mofetil): To determine whether a short course of MMF treatment would induce rhGAA-specific tolerance, 100mg/kg of MMF given orally 0, 24 and 48 hrs following the first 8 of 16 weekly rhGAA treatments. This regimen of MMF treatment was ineffective at reducing rhGAA-specific immune responses in three separate experiments.

CsA with Aza: In three separate experiments conducted in both GAA KO and C57Bl/6 mice this regimen, there was no reduction of rhGAA-specific IgG titers when mice were treated with decreasing doses of CsA (p.o.) and Aza (i.p.) alongside increasing doses of rhGAA. There was actually an exacerbated rhGAA-specific antibody response as seen in figure below from one representative experiment.



ATG: In order to evaluate a potential effect of ATG treatment on rhGAA-specific IgG titers, 25 mg/kg of ATG was administered i.p. 60 minutes prior to and 48 hrs after the first rhGAA

treatment. T cell depletion following ATG treatment demonstrated that the percentage of circulating T cells (as measured by the percentage of CD3+ cells in whole blood) decreased in ATG treated mice by day 4 and remained significantly reduced when compared to untreated mice by day 45 (see sponsor figure below).

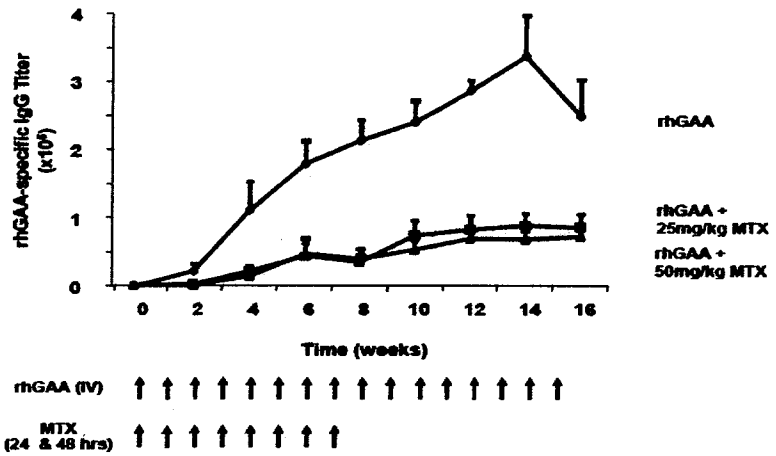


Despite ATG treatment and consequential T cell depletion, rhGAA-specific antibody titers increased over the 16 week study period and were not statistically different than titers achieved by weekly rhGAA treatment alone. The sponsor stated that since T cell ablation does not interfere with rhGAA-specific IgG responses, T cells are not the primary mediators of this response.

(b) (4)

Initially treatment with control antibody exhibited a robust reduction in rhGAA-specific antibody titers, however the sponsor hypothesized that this is attributed to the presence of sodium azide (can antagonize oxidative metabolism) in the isotype control antibody. This study is currently being repeated and preliminary results demonstrate a similar trend.

MTX: MTX effectively reduced rhGAA-specific titer at both 25 and 50 mg/kg given both 24 and 48 hrs following each injection of rhGAA for 8 consecutive weeks of a 16 week dosing regimen. Both doses significantly reduced rhGAA-specific IgG titers from week 6 onward (p= 0.0002 to 0.01). Moreover, this reduction was sustained throughout 16 weeks of chronic rhGAA dosing (see sponsor's figure below).

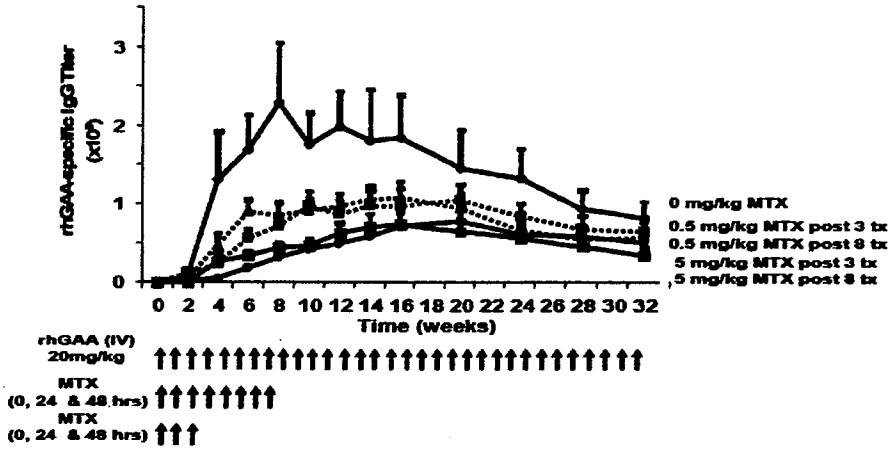


MTX treatment regimens: The different regimens evaluated are shown in the sponsor's Table below:

Table 9. MTX optimization studies and results.

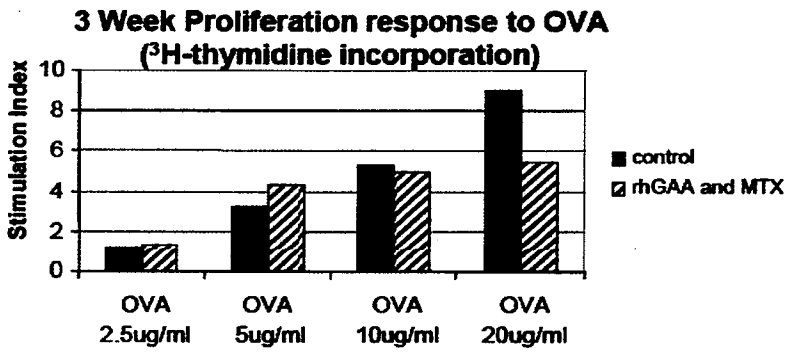
Dose of MTX	Weekly Treatments	Number of Weekly Treatments	Study Duration	Experimental Results – rhGAA-specific IgG titers
25 vs. 50mg/kg	0 & 48 hours	8	12 weeks	No effect.
10 vs. 20 mg/kg	0, 24 & 48 hours	8	32 weeks	Significantly reduced titers.
20mg/kg	0 & 36 hours	8	16 weeks	No effect.
10 mg/kg	0, 24 & 48 hours	4	16 weeks	Reduced titers.
5 vs.0.5mg/kg	0, 24 & 48 hours	3 vs. 8	32 weeks	Both 5 and 0.5mg/kg significantly reduced titers. Equivalent effects were observed with both 3 weeks and 8 weeks of MTX dosing.
5, 0.5, 0.05mg/kg	0, 24 & 48 hours	3	16 weeks	Only 5mg/kg significantly reduced titers.
5 vs. 0.5mg/kg	0, 24 & 48 hours vs. -24, 0, & 24 hours vs.-48, -24 & 0 hours	3	12 weeks	All regimens equally reduced titers. MTX pretreatment provides no added benefit.

Long-term suppression of rhGAA-specific IgG titers throughout the study period were reached with low dose (5 and/or 0.5 mg/kg), short-term treatment of MTX given 0, 24 and 48 hrs after each injection of rhGAA for either the first 3 or 8 consecutive weeks of a 32 week rhGAA dosing regimen (see sponsor's figure below).



T cell independent immune responses: MTX treatment did not exhibit an effect on LPS-induced immune responses from splenocytes that were harvested from mice at the 3- and 5-week time-points.

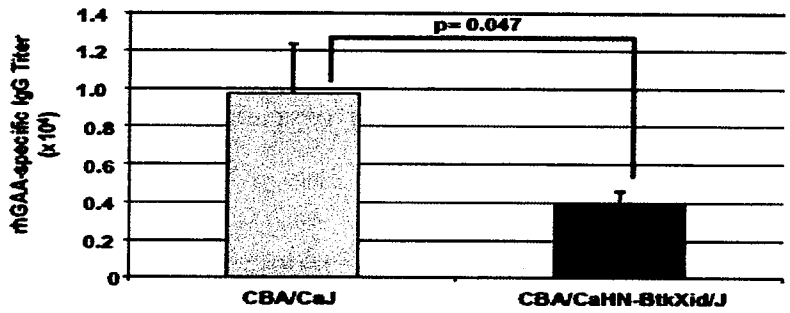
T cell-dependent immune responses: OVA-specific memory responses were not impaired by the treatment of 10 mg/kg of MTX following stimulation of 2.5 to 10 μg/mL of OVA. However, splenocytes that were exposed to rhGAA and MTX treatment were not as responsive to OVA stimulation (see sponsor's figure below). Cell death was not evaluated in this study.



Hematopoietic bone marrow cell populations: Hematopoietic cell populations did not vary significantly from control mice at either the 3 or 5 week time points following MTX treatment.

B1 B cell Assessments: In comparison to control-treated mice, GAA KO mice treated with rhGAA exhibited an expansion in B1 B cells 24 hrs following immunization. The sponsor stated that the level of B1 B cell expansion varied between experiments (individual data of 5 independent experiments not shown) possibly due to inherent genetic differences among backcrossed GAA KO mice. The sponsor's figure below demonstrated that rhGAA-specific IgG

titers are significantly lower in Xid mice (deficient in B1 B cells) than compared to CBA/CaJ mice.



Conclusions: Apart from MTX, all of the immunosuppressive agents, CsA/Aza, MMF, ATG, and (b) (4) fusion protein, failed to induce ITI. (b) (4) fusion did induce a short-lived reduction (first 4 weeks of a 12 week study) in the rhGAA-directed antibody response, thus a periodic dosing of (b) (4) fusion protein would be required throughout chronic weekly administration of this ERT in order to provide a sustained reduction in rhGAA-specific IgG titers.

The only treatment that induced a long-lived reduction in rhGAA-directed antibody responses was MTX, a dihydrofolate reductase antagonist, which interferes with the metabolism of proliferating cells (like T cells) resulting in cell death. The effective regimen which reduced rhGAA-specific IgG titers was 10mg/kg of MTX administered 0, 24 and 48 hrs following the first 8 of 16 weekly rhGAA treatments. Pretreatment with MTX either 24 or 48 hrs before rhGAA administration did not appear to improve the effectiveness of MTX. Various MTX treatment regimens (Table 9) demonstrated that the administration of MTX at 0 hrs post rhGAA dose was critical in reducing antibody levels at this dose. Also, since the 0 hr MTX treatment was critical in establishing the suppressive effect with low dose MTX, the sponsor hypothesized that a very rapid and likely T cell-independent B cell response may be implicated in the initial phase of the rhGAA-specific immune response. This may be due to the fact that T cell-independent B cell responses can occur as quickly as 24 hrs following immunization since such B cell differentiation is not dependent upon T cell activation. If not controlled immediately, higher doses of MTX were required to effectively reduce antibody levels. It is important to note that the MTX treatment did not completely abolish rhGAA-specific IgG levels. The sponsor stated that this may suggest that some B cell responses to rhGAA either occur too rapidly for MTX to exert an effect or are unresponsive to MTX treatment.

Due to MTX's potentially adverse effects on pre-existing immune responses and cellular differentiation, the effects of 10 mg/kg of MTX on bone marrow hematopoeisis along with T cell-independent and T cell-dependent immune responses were evaluated. These results suggested that at least in mice, MTX does not hamper bone marrow hematopoeisis, and T cell-independent or T cell-dependent immune responses.

The ATG study suggested that T cell ablation does not affect rhGAA-specific antibody titers, thus the sponsor investigated the potential involvement of B1 B cells, a unique subset of B cells that are capable of responding to antigen within 24 hrs of immunization, in the rhGAA-specific

immune response. In support of the hypothesis that MTX treatment within 30 mins of rhGAA administration interfered with rapid T cell-independent B cell responses, the sponsor observed a significant (p=0.03) expansion of B1 B cells within 24 hrs of rhGAA administration. In addition, rhGAA administration in mice that are deficient in B1 B cells resulted in significantly lower rhGAA-specific IgG titers.

Addendum: The test article used in the study, "rhGAA", is Myozyme®.

2.6.6.9 Discussion and Conclusions

The sponsor submitted seven new studies, including an i.v. repeat-dose toxicity study in juvenile mice, Segment I, II and III reproductive toxicology studies in mice, Segment II study in rabbits, and a study evaluating immune tolerance induction regimens to reduce anti-rhGAA IgG antibody production in various strains of mice to enzyme replacement therapies.

In a 6-month i.v. repeat-dose toxicity study in juvenile mice, mice were administered recombinant human acid-α-glucosidase (rhGAA) or control article once every week starting on postpartum Day 21 until postpartum Day 203. The no-observed-adverse-effect level (NOAEL) of rhGAA was 20 mg/kg/every other week. There were no treatment-related effects on mortality, body weights, body weight gains, food consumption, sexual maturation, learning and memory, motor activity, open field performance, and organ weights

In an i.v. study of fertility and early embryonic development, male and female mice were administered i.v. doses of rhGAA or control article every other day. Male mice were given the test article once every other day beginning nine weeks before cohabitation (cohabitation period was 14 days) and continuing through the cohabitation period. Female mice were given the test article once every other day, beginning 15 or 16 days before cohabitation and continuing through Day 8 of presumed gestation. The doses of rhGAA as high as 40 mg/kg administered every other day to male and female mice did not affect the mating and fertility.

In an i.v. study of the effect of rhGAA on spermatogenesis evaluation and testicular pathology, males were dosed with rhGAA every other day over a 9-week period, which included a pre-mating period and a 14-day mating period. Females were dosed for at least a 14 day-period prior to mating, throughout the mating period, and through gestation Day 7 or 8. The test article had no effect on the macroscopic appearance of testicles at the terminal sacrifice on Week 9.

In an i.v. developmental toxicity study in rabbits, time-mated female rabbits were administered daily intravenous doses of rhGAA or control article on Days 7 through 19 of presumed gestation (rabbits were caesarean-sectioned on gestation Day 29). rhGAA was not teratogenic at doses up to 40 mg/kg/day. No caesarean-sectioning or litter parameters were affected by dosages of the test article as high as 40 mg/kg/day. The occurrence of fetal alterations were comparable in all groups except for the 20 mg/kg/day group, which showed an increased number of fetuses with alterations and the percentage of fetuses with alterations, however this finding was not dose-dependent.

The sponsor submitted an i.v. developmental and perinatal/postnatal reproduction toxicity study in which presumed-pregnant female mice were administered rhGAA or control article once every other day on Days 6 through 22 of presumed gestation. If the mice delivered a litter, administration of dosing continued to Day 20-21 postpartum. F1 generation mice were mated and pregnant females were subsequently cesarean-sectioned. There was no effect on fertility of F1 females at doses up to 40 mg/kg/every other day. There were minor effects of rhGAA on embryonic development of mice in the 40 mg/kg group, which included an increased incidence of gross external alterations in the 40 mg/kg fetuses (3 fetuses with rotated hind limbs) when compared to control fetuses.

Lastly, the sponsor submitted a study to evaluate various immune tolerance induction (ITI) regimens to ameliorate antibody responses to enzyme replacement therapies. The only agent evaluated that induced a sustained decrease in anti-rhGAA IgG titers was methotrexate (MTX). The administration 10 mg/kg MTX at 0, 24 and 48 hrs following the first 8 of 16 weekly rhGAA treatments reduced rhGAA-specific IgG titers. However, MTX treatment did not completely abolish anti-rhGAA IgG levels.

2.6.6.10 Tables and Figures

Not applicable.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Lumizyme (alglucosidase alfa) is produced by recombinant DNA technology in a Chinese hamster ovary cell line at a 2000L bioreactor scale, whereas Myozyme® (alglucosidase alfa) is produced at a 160L bioreactor scale. The current submission for Lumizyme (alglucosidase alfa/recombinant human acid α-glucosidase/rhGAA) is for the treatment of late-onset Pompe disease. There were no nonclinical studies submitted in the present application. However, with prior approval from the Agency (as per meeting with the sponsor on April 25, 2008 for STN BL 125141/65), all nonclinical studies submitted for BLA 125,141 (Myozyme®) were cross-referenced to support the nonclinical study requirements for Lumizyme (BLA 125,291). Myozyme® is approved for the treatment of patients with Pompe disease. The sponsor submitted new pharmacology studies for BLA 125,141. In addition, the sponsor submitted a 6-month intravenous toxicity study in juvenile mice and reproductive toxicology studies as a part of the post-marketing commitments for BLA 125,141. These studies are summarized below.

The glycogen clearance activity of 160 L and 2000 L alglucosidase alfa were compared in the GAA knockout mice. The results suggested that the 2000L product can produce similar or superior pharmacological activity (glycogen clearance) as compared to the 160L product

(Myozyme) with the same [redacted] (b) (4) levels. An alternate study suggested that when [redacted] (b) (4) and [redacted] (b) (4) concentrations are similar, the glycogen clearance activity of 160 L and 2000 L products were not significantly different in the heart, triceps and quadriceps at 60 and 100 mg/kg and in the heart at 20 mg/kg. The clearance of glycogen from heart, quadricep and tricep tissue from GAA knockout mice was evaluated after treatment with unfractionated 2000L alglucosidase alfa, a no affinity fraction, pooled bound fractions, and high affinity fraction of 2000L alglucosidase alfa. The various 2000L alglucosidase alfa affinity fractions were prepared using a M6P receptor column. In all three tissues, the high affinity fraction of 2000L alglucosidase alfa produced the largest reduction in glycogen, followed by the pooled fractions, the unfractionated 2000L alglucosidase alfa, and the no affinity fraction (based on 20 or 40 mg/kg doses). In a related study, glycogen clearance was evaluated after treatment with unfractionated 2000L alglucosidase alfa, and low and medium affinity fractions of 2000L alglucosidase alfa, respectively. The medium affinity fraction of 2000L alglucosidase alfa produced the largest reduction in glycogen, followed by the low affinity fraction and the unfractionated 2000L alglucosidase alfa (based on 40 mg/kg dose). Therefore, based on the observed tissue glycogen level in mice treated with unfractionated 2000L alglucosidase alfa or various M6P receptor affinity fractions, glycogen clearing activity appears to be directly correlated with M6P receptor binding affinity.

In the 6-month i.v repeat-dose toxicity study of rhGAA in juvenile mice, rhGAA or control article was administered once every other week beginning on Day 21 postpartum (DP) (Day 1 of study) to juvenile mice at doses of 0 (saline), 0 (DPH), 10, 20 or 50 mg/kg. The mice were sacrificed after DP 203 (day of last dose). A total of 1, 0, 2, 5 and 1 mice were found dead or sacrificed early in the 0 (saline), 0 (DPH), 10, 20 and 50 mg/kg groups, respectively. None of these deaths were considered to be related to the test article because the number of deaths were not dose-dependent and no pattern of deaths or adverse clinical signs could be found. There were histopathological changes in male's from the 50 mg/kg group compared to the control groups, including increased incidence of mononuclear-cell infiltration in kidneys and an increased incidence of thymus atrophy when compared to the control groups. Based on the results, the NOAEL of rhGAA was 20 mg/kg/every other week. The kidney and the thymus were the target organs of toxicity.

In an i.v. study of rhGAA on fertility and early embryonic development in mice, rhGAA or control article was administered at doses of 0 (saline), 0 (DPH), 10, 20, 40 mg/kg/every other day to male and female mice. Male mice were given the test article beginning nine weeks before cohabitation and continuing through the cohabitation period. Female mice were given the test article or the control article beginning 15 or 16 days before cohabitation and continuing through Day 8 of presumed gestation (DG 8). The doses of rhGAA as high as 40 mg/kg administered every other day to male and female mice did not affect the mating and fertility.

In an i.v. study used to evaluate spermatogenesis and testicular pathology, rhGAA or control article was administered at doses of 0 (DPH), 10, 20, 40 mg/kg/every other day to male mice over a 9-week period prior to sacrifice, and to female mice for 14 days prior to mating, throughout the mating (14-day) period, and through DG 7 or 8. The test article had no effect on the macroscopic appearance of testicles at the terminal sacrifice on Week 9. Testicles from all

males, including the two males found dead on Day 12 and Day 53, were also microscopically normal. All stages of the spermatogenic cycle were present in normal proportions.

In an i.v. developmental toxicity study of rhGAA in rabbits, rhGAA or control article were administered once daily to time-mated rabbits on DGs 7 through 19 at doses of 0 (saline), 0 (DPH), 10, 20 and 40 mg/kg. Female rabbits were caesarean-sectioned on DG 29 and sacrificed. On the basis of these data, the maternal no observable effect level (NOEL) of rhGAA is < 10 mg/kg/day due to the observations that 10 mg/kg/day caused reductions in body weight gain or weight losses and reduced food and water consumption values. The early delivery in the 10 mg/kg does and the 2 abortions and one early delivery in the 40 mg/kg does were considered related to the test article and/or DPH because a number of adverse clinical signs occurred after the fifth dose of the test article when DPH administration was started. There were no fetal developmental effects noted in doses up to 40 mg/kg/day. No caesarean-sectioning or litter parameters were affected by dosages of the test article as high as 40 mg/kg/day. There were no significant changes in the incidence of fetal malformations or alterations within any treatment group.

In an i.v. prenatal/postnatal development study of rhGAA on female fertility and early embryonic development to implantation in mice, rhGAA or control article was administered to F0 generation female mice at 0 (saline), 0 (DPH), 10, 20, or 40 mg/kg every other day from gestation day (DG) 6 through lactation day (DL) 21. At DL 21, 25 male and female pups per group were chosen for continued evaluation, and at 90 days of age, the F1 generation mice within each dosage group were assigned to cohabitation, one male mouse per one female mouse. Following a 9-day cohabitation period, pregnant F1 mice were evaluated until DG 18. There was no effect on fertility of F1 mice at doses up to 40 mg/kg. Adverse clinical observations in the F1 generation included an increased frequency of dehydration and decreased motor activity in mice from the 40 mg/kg group when compared to all other groups. The average day of sexual maturation for the treatment groups appears to be slightly accelerated when compared to the Saline/Control group. There was no effect on learning or memory or the ability to reproduce in the F1 generation mice at doses as high as 40 mg/kg of the test article. There was a small but significant decrease in litter sizes of the control (DPH), and 10, 20 and 40 mg/kg groups when compared to the control (saline) group. However, litter sizes were similar between the DPH control group (Group II) and the test article treated groups. There was an increase in the number of pups dying on DLs 15 to 21 in the 40 mg/kg group compared to the control (saline) group. After cesarean-sectioning of F1 generation mice, there was an increased incidence of gross external alterations in fetuses delivered from the 40 mg/kg group (3 fetuses with rotated hind limbs) when compared to fetuses delivered from the control (saline) group.

A special toxicology study evaluating different immune tolerance induction (ITI) regimens to reduce anti-rhGAA IgG titers showed that apart from methotrexate (MTX), all of the immunosuppressive agents, CsA/Aza, MMF, ATG, and CTLA4-IgG fusion protein, failed to induce ITI. CTLA4-IgG fusion did induce a short-lived reduction (first 4 weeks of a 12 week study) in the rhGAA-directed antibody response, thus a periodic dosing of CTLA4-IgG fusion protein would be required throughout chronic weekly administration of rhGAA in order to provide a sustained reduction in anti-rhGAA IgG titers. The only treatment that induced a long-lived reduction in rhGAA-directed antibody responses was MTX, which interferes with the

metabolism of proliferating cells (including T cells) resulting in cell death. The effective regimen, which reduced anti-rhGAA IgG titers, was 10 mg/kg of MTX administered 0, 24 and 48 hrs following the first 8 of 16 weekly rhGAA treatments. The 0 hr MTX treatment was critical in establishing the suppressive effect with low dose MTX. The sponsor hypothesized that a very rapid and likely T cell-independent B cell response may be implicated in the initial phase of the anti-rhGAA immune response. It is important to note that the MTX treatment did not completely abolish anti-rhGAA IgG levels.

Unresolved toxicology issues:

None.

Recommendations:

1. From a preclinical viewpoint, the application should be approved, with the provision that the "INDICATIONS AND USAGE" subsection under "HIGHLIGHTS OF PRESCRIBING INFORMATION", the "Pregnancy" subsection under "USE IN SPECIFIC POPULATIONS", and the "Carcinogenesis, Mutagenesis, Impairment of Fertility" subsection under "NONCLINICAL TOXICOLOGY" of the proposed labeling will be changed as described in the "EXECUTIVE SUMMARY" section of this review.

2. Although the studies listed below were reviewed under the current submission for Lumizyme, they were originally submitted as part of the post-marketing commitments (PMC) for Myozyme® (approval letter dated April 28, 2006). These studies fulfill the PMCs stated after the study titles.

    a) Study No. FFA00067: Six-month intravenous repeated-dose toxicity study of Recombinant Human Acid-α-Glucosidase (rhGAA) in juvenile mice (PMC 8)
    b) Study No. FFA00051: Intravenous injection study of Recombinant Human Acid-α-Glucosidase (rhGAA) on fertility and early embryonic development in mice (PMC 12)
    c) Study No. 6354-155: Intravenous injection study of Recombinant Human Acid-α-Glucosidase (rhGAA) on fertility and early embryonic development to implantation in mice (Study Phase: Qualitative spermatogenesis evaluation and testicular pathology) (PMC 11)
    d) Study No. FFA00053: Intravenous developmental toxicity study of Recombinant Human Acid-α-Glucosidase (rhGAA) in rabbits (PMC 10)
    e) Study No. FFA00058: Intravenous developmental and perinatal/postnatal reproduction toxicity of Recombinant Human Acid-α-Glucosidase (rhGAA) in mice, including postnatal behavioral/functional evaluation (PMC 13)
    f) Study No. 6354-163: Intravenous Injection Study of Recombinant Human Acid-α-Glucosidase (rhGAA) on Female Fertility and Early Embryonic Development to Implantation in Mice (PMC 9)

Suggested labeling: The labeling should be changed as described in the "EXECUTIVE SUMMARY" section of this review.

Reviewer Signature 1/30/09
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Supervisor Signature 1/30/09 Concurrence Yes X No ___
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BLA 125,291
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R/D Init.: D. Joseph 11/30/08

NM/nrm: 01/30/2009
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APPENDIX/ATTACHMENTS

None