CENTER FOR DRUG EVALUATION AND RESEARCH AND CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

APPLICATION NUMBER:

125141/0

PHARMACOLOGY REVIEW(S)

Comments on BLA 125141 Myozyme
From A. Jacobs 4/20/06
A. Jacobs

1. Pharm/tox Review: I agree with the comments in the Dr. Choudary supervisory memo of April 14, 2006 concerning what is clearly drug-related in animals (in contrast to some of the primary reviewer's conclusions) and with the Pharm/tox supervisor's recommendations for phase 4 commitments: a chronic tox study in neonatal/juvenile mice; a pre-postnatal study in rats; and a second species teratogenicity study in rabbits that have not received diphenhydramine. I concur with the supervisor that these studies can be conducted post approval.

2. Labeling:

a. I agree with the labeling comments of Dr. Choudary and with what should be included in the labeling (in contrast to some of the primary reviewer's recommendations) and concur with the latest proposed labeling, which Dr. Choudary shared with me.
b. I concur with the pregnancy category of B, (recommended by the Supervisor, but not the primary reviewer) pending results of the second species teratogenicity study in rabbits). This would be consistent with labeling for other similar products (with similar anaphylactoid effects) for this indication, although an anaphylactoid reaction of moderate severity might warrant a pregnancy category —

MEMORANDUM

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

DATE: April 14, 2006

FROM: Supervisory Pharmacologist
Division of Gastroenterology Products, HFD-180

SUBJECT: BLA STN 125141 (Myozyme)—Supervisory Addendum to Pharmacology Review

TO: BLA STN 125141

Under BLA STN 125141, the sponsor provided reports of a modest preclinical program in support of the indication of Myozyme for use in patients with Pompe disease. The submission included (1) pharmacology studies of Myozyme in GAA knockout mouse model for Pompe disease, (2) pharmacokinetics and tissue distribution studies in GAA knockout mice and rats and toxicokinetics in rats, mice and monkeys and (3) single dose i.v. toxicology studies in rats and dogs, repeated dose i.v. toxicology studies of 4-week duration in rats and mice, 26-week and 13-week repeated dose i.v. toxicology studies in monkeys, a Segment I. Fertility and reproductive performance study in male and female mice and a Segment II. Teratology study in pregnant mice. These studies have been reviewed by Dr. Barbara J. Wilcox of the Division of Neurology Products and her review is attached.

The following supervisory comments and recommendations should be noted.

1. Except for the anaphylactic and hypersensitivity responses in the 4-week rodent repeated dose i.v. toxicology studies, no specific target organs of toxicity were identified. Although Myozyme is intended for use in infantile onset Pompe disease, a chronic toxicology study in juvenile rodents is lacking. A 6-month chronic toxicology study in neonatal/juvenile mice is recommended. The dose selection for this study should be such that the high dose should elicit toxicity or it should be the maximum feasible dose.
2. As per sponsor's correspondence dated December 29, 2005, the disposition of the 4-week — rat toxicology study #6354-140 was in accord with the agreement between the Agency (CBER) and the sponsor. Under the circumstances, the matter should be brought to a closure. No further requests for histopathology examination of the retained tissues are warranted.
3. In the nonclinical overview of the submission, sponsor claims that in a safety pharmacology study in dogs, Myozyme did not exert any clinically relevant effects on electrocardiogram etc. No report of such a study is available in this submission. The only dog study in the submission is a single dose i.v. toxicity study — study #

6354-1342). There was no monitoring of ECG in this study. There was no indication of ECG monitoring in the 26-week and 13-week monkey toxicology studies (— studies 6354-152 & 6354-157). This is a major deficiency. Sponsor should be asked to conduct a cardiovascular safety pharmacology study in dogs or monkeys as a post marketing commitment.

4. There is no need to mention the low incidences of mild elevations of transaminases (ALT & AST) in the rat toxicology studies in the labeling under "PRECAUTION: Laboratory Tests" since the incidences are not exclusively treatment related or dose related. The incidences in the mouse toxicology study are also isolated occurrence. These 4-week toxicology studies are not sufficiently long to elicit any treatment related trends or identify the specific target organs of toxicity. Since the drug is intended for life long administration and the patient data base is limited, it is recommended that the sponsor should undertake a 6-month chronic toxicology study in neonatal/juvenile mice as a post marketing commitment.
5. Even though Myozyme is intended to be used in early onset patients, information on the prenatal and postnatal developmental toxicity is lacking. It is recommended that the sponsor should conduct a Segment III. Prenatal and postnatal study in rats as a post marketing commitment. Such a study would clearly identify potential postnatal developmental toxicities.
6. Segment I. Fertility and reproductive performance study in male and female mice — study # 6354-155). There were no significant differences in the reproductive performance of male mice and the fertility indices in female mice for the control and treated groups. Even though the number of pregnant females at autopsy on day 13 of gestation is generally low in all groups (41 to 60 %), it is known that pregnancy failures are highest (about 27%) during days 12 to 15 of gestation in White Swiss mice which coincides with transient hormonal imbalance before full placental function is established (Choudary, J.B. and Greenwald, G.S., Ovarian Activity in the Intact or Hypophysectomized Pregnant Mouse, Anatomical Record 63: 359-372, 1969). The sperm parameters from the epididymal samples are not optimal indices. They do not present any dramatic differences between control and treatment groups with respect to motility or % abnormal sperm cells. Overall, the study results do not demonstrate an adverse influence of Myozyme on reproduction in male and female mice. For further evaluation, the sponsor should be asked to (a) submit the final report of the additional — study 6354-163 titled "Intravenous Injection Study of Recombinant Human Acid-alfa-Glucosidase(rhGAA) on Female Fertility and Early Embryonic Development to Implantation in Mice", (b) conduct histopathology examination of the testes of male mice in study # 6354-155 and submit the full pathology report and (c) study the effects of Myozyme on spermatocytogenesis and spermiogenesis in male rabbits after treatment for a minimum of 90 days.
7. The characterization of the pregnancy category should be based on the outcome of the Segment II. Mouse teratology study (— ; study # 6354-153). In this study, there were no significant differences between control and treatment groups in regard to preimplantaion and postimplantation losses. Myozyme was clearly not teratogenic or contragestational in mice. Even in the Segment I. Fertility and reproductive performance study (— study # 6354-155), there were no treatment related adverse effects. The

category for pregnancy should be "Pregnancy: Teratogenic Effects. Pregnancy Category B." The sponsor in correspondence dated December 29, 2005 committed to initiate a Segment II. Teratology study of Myozyme in rabbits. Sponsor should first conduct a dose ranging study in rabbits for selecting appropriate doses. In the interest of clarity and precision for the data obtained, pretreatment with diphenhydramine should be avoided since it was implicated to be teratogenic in some teratology studies.

8. **RECOMMENDATION**: From a preclinical standpoint, approval of Myozyme for use in patients with Pompe disease is recommended, with the understanding that the sponsor undertakes the aforementioned post marketing studies.

Jasti B. Choudary, B.V. Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

Cc:
BLA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-120/Dr. Wilcox

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**BLA STN 125141**
SERIAL NUMBER:	**000/0008**
DATE RECEIVED BY CENTER:	**Original submission: 7/28/05**
	Serial #0008: 12/30/05
PRODUCT:	Alglucosidase alfa (recombinant human acid alpha-glucosidase, [rhGAA])
INTENDED CLINICAL POPULATION:	/
SPONSOR:	**Genzyme Corporation**
DOCUMENTS REVIEWED:	**Electronic submission. This review covers the Non-clinical sections**
REVIEW DIVISION:	**Division of Gastroenterology Drug Products (HFD-180)**
PHARM/TOX REVIEWER:	**Barbara J. Wilcox, Ph.D.**
PHARM/TOX SUPERVISOR:	**Jasti Choudary, B.V.Sc., Ph.D.**
DIVISION DIRECTOR:	**Brian Harvey, M.D., Ph.D.**
PROJECT MANAGER:	**Cristi Stark**

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

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The data reviewed here support approval of Alglucosidase alfa for the infantile onset patient population. This recommendation is made considering the absence of any available treatment for this disease, which is uniformly fatal within 18 months of birth. The product was generally well tolerated in the mouse and monkey and shows dose dependent efficacy in clearance of tissue glycogen in the Pompe GAA knockout mouse model.

B. Recommendation for nonclinical studies

Several issues remain regarding the toxicology package, however. Further reproductive toxicology studies are recommended using additional species to rule out questions about the sensitivity of the mouse model and confirm findings. Because manufacturing issues for the 2000 liter scale product having an impact on the pharmacokinetics and possibly toxicity remain unresolved, approval for that manufacturing process is not recommended at this time. Approval is recommended for only the 160 liter scale product for which clinical safety data are available.

Additional toxicology studies recommended:

Further reproductive toxicology studies are recommended prior to approval of Alglucosidase alfa for the late onset patient population for the following reasons:

- Only one species was studied for Segment II reproductive toxicology. The usual requirement is 2 species, one non-rodent. A rabbit study is recommended.
- No Segment III reproductive toxicology studies were performed. Studies of this type should be performed prior to approval of Alglucosidase alfa for the late onset patient population.

- Study # 6354-140 is a repeat dose study performed in Sprague-Dawley rats conducted for the purpose of comparing toxicity among three lots of rhGAA from different manufacturing processes. In that study, there were several unscheduled deaths and gross lesions noted at necropsy that were not sufficiently explained. The sponsor should be asked to perform the histopathology analysis that was not performed per the original protocol and submit the results for review.
- The integrated non-clinical summary states that an additional study (#6354-163) was underway and expected to be completed by Q3 2005. That study is not included in this BLA submission. The sponsor was asked to provide the study report but has not yet complied with that request.

C. Recommendations on labeling

Based on the non-clinical package as a whole, the following recommendations regarding labeling are given:

- In the Laboratory Tests section, the sponsor mentions only the two monkey toxicology studies where very little toxicity was noted. —

Although these findings are not clearly dose related, they do appear to be treatment related.

- In the section on Carcinogenesis, Mutagenesis, Impairment of Fertility, addition of a statement on effects of Alglucosidase alfa on male fertility parameters should be mentioned here. Results of study # 6354-155 (Fertility and Early Embryonic Development) showed a dose dependent decrease in sperm count and increase in abnormal morphology. This study was done in mice, which may not be the most sensitive species. Therefore, the toxicities may be underestimated. In addition, study #6354-140, treatment related finding of ovarian cysts were observed in 2 of 8 rats receiving 50 mg/kg Alglucosidase alfa for 4 weekly doses.
- Pregnancy Category: The sponsor suggests that this product be designated pregnancy category B. However, study #6354-153 showed a significant increase in post-implantation loss in the high dose group when pregnancy parameters are considered as a whole. The trends of reduced embryo viability in study #6354-155 and the increased post-implantation loss seen in study 6354-153, the lack of toxicokinetics to assure exposure to the test article in study #6354-155, the lack of a segment 2 study in a second (non-rodent) species, and the lack of justification of the high dose used in both studies render the Category B claim tenuous, at best. At this time, Pregnancy Category — would be more appropriate.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Primary Pharmacodynamics

The primary pharmacodynamic studies provided with this application consist primarily of repeated studies demonstrating dose dependent clearance of tissue glycogen load in the Pompe GAA knockout mouse model under varying conditions. These conditions included varying dose levels, treatment regimens, age of the animal and drug preparations. The doses tested ranged from 1 mg/kg administered weekly for four weeks to 100 mg/kg administered weekly for 4 weeks. The longest duration studies consisted of 10 and 20 mg/kg administered weekly for 16 weeks, and 10, 20, 40 mg/kg every other week (qow) for 16 weeks.

In general, the 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 the rhGAA were observed among the various muscles sampled. Glycogen was consistently cleared more readily from cardiac muscle than from skeletal muscle. 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. 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. The relative magnitude of depletion among muscle remained consistent from study to study. The differences in efficacy among the various muscles tested may reflect heterogeneity of mannose-6-phosphate receptor among those muscles.

When efficacy between 3 month old and 12 month old Pompe knockout mice was investigated, younger animals were significantly more responsive to glycogen clearance by rhGAA than the older animals. The relative magnitude of depletion among the muscle types was consistent with other studies. The 12 month old mice had higher tissue glycogen load at study initiation, which may 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 that are a common response of rodents to the rhGAA. However, some of the deaths could not be accounted for by hypersensitivity and no data on the potential cause are provided.

Due to the concern about hypersensitivity, rodents were routinely pre-treated with diphenhydramine (DPH), usually at 5 mg/kg, 20 minutes prior to infusion of the rhGAA. It is not clear whether the vehicle control animals also received DPH, but it appears that no study included a DPH only control group.

Secondary pharmacodynamics:

One study in Pompe 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. No detectable IgE titer was reported for any treatment group. Significantly elevated histamine levels were detectable in mice receiving rhGAA after the 8^{th} dose. Significant levels of IgG and IgG1 titers were reported in all mice receiving rhGAA, . These data are consistent with hypersensitivity response in the Pompe knockout mice.

Pharmacokinetics:

Both single and repeat dose pharmacokinetic studies were conducted in Pompe GAA knockout mice. When a semilogarithmic plot of concentration versus time was constructed from data produced by each of the 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-life was on the order of 2-3.5 hours for monkey, 1-2 hours for rat, 1.5 to 2 hours for Pompe knockout mouse 1-2 hours, approximately 75 minutes for CD-1 mouse, and approximately 75-100 minutes for beagle dog. 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 and AUC/dose. Both males and females in the high dose group showed significantly higher elimination half-life relative to the other dose groups, females for days 1-85 and males for days 1-169.

PK studies were conducted to investigate the potential differences between the 160 liter manufacturing process and the 2000 liter process. The results demonstrated that the ninety percent confidence intervals for either AUC_{last} or $AUC_{0-\infty}$ ratios between the 160L lot and the

2000 L lots evaluated in this study were not within a pre-determined acceptability range (80% to 125%). These data indicate that the pharmacokinetic equivalence was not established between the products from the two processes.

Biodistribution:

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 liter process was compared to that from the 2000 liter process, there appeared to be a higher magnitude of uptake in the liver for the 2000 liter product. When a lot from early harvest of the 2000 liter scale was compared to one from the late harvest, greater rhGAA activity levels were noted for the early harvest product. Changes in [redacted] among lots and during the course of manufacture might explain both the PK and biodistribution differences detected in these studies.

Toxicology:

The toxicology package for this BLA contains single dose studies in Sprague-Dawley rat and beagle dogs, repeat dose studies in Sprague-Dawley rat, cynomolgus monkey and C57Bl/6 mice as well as Segment I and Segment II reproductive toxicology studies in CD-1 mice. The rhGAA was well tolerated in monkey at doses up to 200 mg/kg, 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 male animals (of 3 in each group) from the two higher dose groups and ovarian cyst and unequal sized ovaries in one female of 3 from the high dose group as well as inflammation and degeneration of the quadriceps muscle in one female of three from the high dose group. A relationship to the test article cannot be ruled out but, due to the small number of animals per group, this relationship is difficult to establish.

The rhGAA was also generally well tolerated in C57Bl/6 mice at doses up to 100 mg/kg administered weekly for 4 weeks. The mouse study was intended to compare toxicities produced by product from three different manufacturing processes. The toxicity profile was similar among the three products, but an increase in severity seemed to be present for the BI2KrhGAA (2000 liter) and BI – rhGAA – liter) relative to the GENZrhGAA. The toxicities noted include 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 addition, a dose related mild increase in serum albumin was noted for all treatment groups and was more prevalent in females. Two of 6 females receiving 100 mg/kg each of GenzrhGAA had mildly increased AST and ALT levels. This finding may be related to the test article and suggests a potential adverse effect on the liver. In a similar study carried out in rats (6354-140, described above) 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 explained that study# 6354-140 was terminated at the end of the in-life portion due to the number of deaths that were thought to be due to hypersensitivity. The histopathology analysis was never performed but the tissues are archived with Genzyme.

An additional repeat dose toxicity study was performed in rat included only 5 per sex per group and investigated only one test article. The animals in this study showed a dose related statistically significant decrease in body weight. For males the weight loss was 23.3% and for females, 11.6%. 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 so apparently toxic in study 6354-140 relative to this additional rat study, except possibly the difference in lots used for study 6354-140.

Single Segment I and single Segment II reproductive toxicology studies were performed, both in CD-1 mice. For the Segment I study, mice received doses up to 40 mg/kg every other day beginning prior to mating and through early embryonic development. No clear rationale was given for the choice of dose and no pilot studies are included to support the choice of high dose. Results of this study showed a trend toward pre-implantation loss and late resorptions. These findings did not reach statistical significance and historical normal values for comparison were not found. Changes in male fertility parameters were also found. A statistically significant and dose related reduction in sperm count as well as an increase in abnormal sperm. The male mice also showed urine stained abdomens suggesting the presence of more generalized toxicity. In a toxicology study (#6354-140) female rats receiving 50 mg/kg weekly for 4 weeks showed an increased occurrence of ovarian cysts. No toxicokinetic analysis was performed so relative exposures to the test article cannot be established.

For the Segment II study, mated female mice received doses up to 40 mg/kg administered daily. No evidence of preimplantation loss was noted during this study. A small dose related increase in late resorptions was observed but did not reach statistical significance when considered alone. However, in considering pregnancy parameters as a whole, a significant increase in post-implantation loss was noted for the high dose group. There were no statistically significant adverse effects on fetal development reported.

A second segment 2 study in a non-rodent species is recommended to confirm and clarify the potential toxic effects. The sponsor has agreed to performance of such a study and this should be identified as a post-marketing commitment.

An additional non-clinical study is mentioned in the integrated summary of non-clinical studies in this BLA. That study, # 6354-163, was to be finished by Q3 2005, but is not included in this submission. Submission of this study report should be identified as a post-marketing commitment.

B. Pharmacologic activity

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 lysozomes of the cell. Enzyme deficiency results in accumulation of glycogen in the lysozomal compartment, a condition known as glycogenosis type II. The product, rhGAA, is administered intravenously (IV) as a form of enzyme replacement therapy (ERT). Once internalized by the cell, and passed to the lysosome by intracellular mechanisms, hGAA 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 uptake of exogenously administered enzyme by endocytosis (Raben, 2003, *Molecular Genetics and Metabolism*).

C. Nonclinical safety issues relevant to clinical use

Repeat dose studies demonstrated that this product is generally well tolerated in monkeys and mice. A small number of animals in various studies showed elevation of liver enzymes. As the highest uptake of Alglucosidase alfa is the liver, liver function parameters should be monitored during human use. As indicated above several issues remain to be addressed for reproductive toxicology. The reproductive toxicology issues should not be a relevant concern for the patients with infantile onset of Pompe's disease.

The variations in PK and biodistribution and potential variations in toxicity between the 160 liter and 2000 liter manufacturing processes should be resolved prior to approval of the 2000 liter process. However, approval of the 160 liter process, for which clinical safety data exists, should be acceptable as long as appropriate controls are in place.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 125141
Review number:
Sequence number/date/type of submission: BLA
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Genzyme Corporation
Manufacturer for drug substance: Genzyme Corporation

Reviewer name:	Barbara J. Wilcox, Ph.D.
Division name:	Division of Neurology Products
HFD #:	**120**
Review completion date:	3/20/06

Drug:

Trade name:
Generic name: alglucosidase alfa
Code name:
Chemical name: recombinant human acid alpha-glucosidase (rhGAA)
CAS registry number:
Molecular formula/molecular weight: This protein molecule is — in length. The — . with a calculated molecular weight of — kD.

Structure: The graphic below (provided by the sponsor) gives the — of the human recombinant acid alpha glucosidase (rhGAA).

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On Original

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2 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

___ § 552(b)(5) Deliberative Process

___ § 552(b)(4) Draft Labeling

Relevant INDs/NDAs/DMFs:
BB IND —, 10780

Drug class: biological therapeutic/recombinant protein/replacement enzyme

Intended clinical population: Alglucosidase alfa is indicated for —

Clinical formulation: The commercial formulation is composed of — , rhGAA, — mannitol, — , polysorbate 80 in — sodium phosphate. —

Route of administration: Intravenous

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

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of BLA 125141 are owned by Genzyme Corporation or are data for which Genzyme Corporation has obtained a written right of reference. Any information or data necessary for approval of BLA 125141 that Genzyme Corporation does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Genzyme Corporation does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of BLA 125141.

Studies reviewed within this submission: The studies reviewed within this submission include non-clinical pharmacology (pharmacodynamics, pharmacokinetics) and non-clinical toxicology (general toxicology and reproductive toxicology).

Study Title	Study Number	Lot number	Testing Lab	Page #
Clearance of glycogen in targeted tissues following 4 weekly doses of rhGAA in $6^{neo}/6^{neo}$Pompe knockout mice	**#01-0713pga**	GW10124094 E1585AM03 007774	Genzyme	18
Efficacy of rhGAA at 20 and 100 mg/kg in the $6^{neo}/6^{neo}$ knockout Pompe II mouse model.	**#01-0813pga**	GW10124095 E1585AM03	Genzyme	20
Analysis of glycogen depletion and re-accumulation after administration of a single dose of rhGAA in Pompe mice	**#02-0209pga**	E1585AM03 GA028	Genzyme	22
Efficacy of rhGAA in GAA knockout mice with every other week dosing and investigation of blood glucose levels in GAA knockout mice pre and post dosing	**#02-0314pga**	11284103	Genzyme	25
Investigation of glycogen depletion/re-accumulation in GAA knockout mice after 4 weekly doses of rhGAA	**#02-0359pga**	KSK11283091	Genzyme	28
A comparison of the efficacy of rhGAA in 3 month and 12 month GAA knockout mice	**#02-0380pga**	KsK11283092	Genzyme	29
Investigation of rhGAA KO mice treated with 10 or 20 mg/kg of enzyme for 16 weeks	**#02-0500pga**	KSK11283091	Genzyme	32
Efficacy of rhGAA in GAA knockout mice with every other week dosing at 10, 20 and 40 mg/kg	**#02-0715**	930018	Genzyme	34
Determining the optimal debulking dose of rhGAA in the Pompe knockout mouse	**#02-1136pga**	GA028	Not given	36
Long-term dose response of qow dosing in the Pompe knockout model	**#02-1165pga**	GA028	—	39
Investigation of a debulking and maintenance dosing regimen using rhGAA in the Pompe knockout mouse model	**#03-0255pga**	GA095	Genzyme	41
Efficacy of two recent rhGAA lots of formulated bulk	**#03-0317**	GAA1 03TP021 03TP022	Genzyme	44
A comparison of 3 and 12 month old Pompe mice treated with rhGAA	**#03-0462pga**	GA095	—	45
Efficacy of 2000L rhGAA in the Pompe knockout mouse model	**#04-0177pga**	93018 xGA179 xGA180	Genzyme	47

Investigation of the efficacy of processed rhGAA in Pompe knockout mice	**#04-0279pga**	GA139 GW12400008	Genzyme	48
Efficacy of 2000L and 160L rhGAA in the Pompe knockout mouse model	**#05-0217pga**	160L: 930018 2K:4573352 2K:5744693	Genzyme	50
The investigation of the immune response in Pompe mice treated with rhGAA	**#03-0665pga**	GA096	Genzyme	51

Pharmacokinetic studies:

Study title	**Study #**	**Lot #**	**Testing Lab**	**Page #**
Pharmacokinetics and biodistribution of three formulations of rhGAA in the GAA knockout mouse	**#02-0710pga**	930018 GA063 GA028	Genzyme	55
Pharmacokinetics of rhGAA in the GAA knockout mouse	**#02-0779pga**	E1585Am03 GA028 930018	Genzyme	57
Pharmacokinetics and biodistribution of two recent rhGAA lots of formulated bulk	**#03-0370pga**	GAA1 (ref.std) 03TP021 03TP022	Genzyme	59
Pharmacokinetics of 160L rhGAA in CD:1®(ICR) BR mice	**#04-0144pga**	751295 (160L)	Genzyme	61
Repeat pharmacokinetics of rhGAA 2000 liter in the Pompe knockout mouse	**#04-0424pga**	GA179 GA180 930018	Genzyme	62
Pharmacokinetics of Alglucosidase alfa produced at the 2000L scale	**#05-0414pga**	#930018 (160L 5744693 (2000L) 4573352 (2000L)	Genzyme	64
Pharmacokinetics and biodistribution of rhGAA 2000 liter in the Pompe knockout mouse	**#04-0152pga**	GA179 (2000L) GA180 (2000L) 930018 (160L)	Genzyme	68

Pharmacokinetics and biodistribution of Alglucosidase alfa produced at the 2000L scale	**#05-0252pga**	5744693 (2000L) 4573352 (2000L) 930018 (160L)	Genzyme	69
Pharmacokinetics and biodistribution of rhGAA on the Pompe knockout mouse with the presence of antibodies	**#03-0087pga**	GA095	Genzyme	71
Pharmacokinetics and biodistribution of rhGAA in the presence of antibody after intravenous dosing	**#03-0543pga**	GA096	Genzyme	71

Toxicology studies:

Study title	**Study #**	**Lot #**	**Testing Lab**	**Page #**
Effect of a single intravenous administration of rh-α-glucosidase to Sprague-Dawley rats	**#6354-134**	GW10124094	—	81
Effect of a single-dose intravenous administration of rh-α-glucosidase to beagle dogs	**#6354-132**	GW10124094	—	84
Effect of repeat intravenous infusion toxicity study with recombinant human acid-α-glucosidase in cynomolgus monkeys with a 14-day recovery period	**#6354-157**	996793	—	86
26-week toxicity study of recombinant human acid-α-glucosidase administered intravenously every other week to cynomolgus monkeys with a 2-week recovery phase	**#6354-152**	608341	—	91
Effect of repeated intravenous administration of three formulations of rh-α-glucosidase to C57Bl/6 mice	**#02009**	GA028	Genzyme	97
Effect of repeated intravenous administration of rh-α-glucosidase to Sprague-Dawley rats for 4 weeks	**#6354-133**	GW10124094	—	101
Effect of repeated intravenous administration of three formulations of rh-α-glucosidase to Sprague-Dawley rats	**#6354-140**	GA028 (160L) E1585AM03 — L) 105067 (2000L)	—	104
Intravenous injection study of recombinant human acid-α-glucosidase on fertility and early embryonic development to implantation	**#6354-155**	608341	—	110

in mice				
Intravenous injection study for effects on embryo-fetal development with recombinant human acid-α-glucosidase	#6354-153	751295	—	114

Studies not reviewed within this submission:

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The primary pharmacodynamic studies consisted primarily of repeated studies demonstrating dose dependent clearance of tissue glycogen load in the Pompe GAA knockout mouse model under varying conditions. These conditions included varying dose levels, treatment regimens, age of the animal and drug preparations. The doses tested ranged from 1 mg/kg administered weekly for four weeks to 100 mg/kg administered weekly for 4 weeks. The longest duration studies consisted of 10 and 20 mg/kg administered weekly for 16 weeks, and 10, 20, 40 mg/kg every other week (qow) for 16 weeks.

In general, the 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 the rhGAA were observed among the various muscles sampled. Glycogen was consistently cleared from cardiac muscle more readily than from skeletal muscle. 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. The relative magnitude of depletion among muscle remained consistent from study to study. The differences in efficacy among the various muscles tested may reflect heterogeneity of mannose-6-phosphate receptor among those muscles.

When efficacy between 3 month old and 12 month old Pompe knockout mice was investigated, younger animals were significantly more responsive to glycogen clearance by rhGAA than the older animals. The relative magnitude of depletion among the muscle types was consistent with other studies. 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 that are a common response of rodents to the rhGAA. However, some of the deaths could not be accounted for by hypersensitivity and no data on the potential cause are provided.

Due to the concern about hypersensitivity, 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.

One study in Pompe 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. No detectable IgE titer was reported for any treatment group. 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 levels of IgG and IgG1 titers were reported in all mice receiving rhGAA. These data are consistent with hypersensitivity response in the Pompe knockout mice.

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 lysozomes of the cell. Enzyme deficiency results in accumulation of glycogen in the lysozomal compartment, a condition known as glycogenosis type II. The product is administered intravenously (IV) 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 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 lysozomal 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 studies were included in the BLA submission as support for the primary pharmacodynamic activity of natalizumab.

Study #01-0713pga

Title: Clearance of glycogen in targeted tissues following 4 weekly doses of rhGAA in $6^{neo}/6^{neo}$ Pompe knockout mice.

Drug, lot#: The lot numbers for each of the enzymes are listed below as presented by the sponsor:

Test Article(s):	**Lot Number:**
1. Genzyme rhGAA	1. GW10124094
2. BI — rhGAA	2. E1585 AM03
3. BI 2K rhGAA	3. 007774

Methods:

The purpose of this study was to compare the clearance of glycogen from tissues of the Pompe GAA knockout mouse model following treatment with three different but similar rhGAA forms. Mice (4 per group) were treated with one of three enzymes (Genzyme, BI — or BI2K) at 1 mg/kg or 5 mg/kg weekly.

Animals: mouse, Pompe $6^{neo}/6^{neo}$, 4/group

Age: 20 gm at study initiation.

The table below, supplied by the sponsor, illustrates the basic study design. Animals were dosed weekly for 6 weeks followed by sacrifice.

Group #	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	[1]Dosing Regimen	Route of Administration
1	4	0	NA	No dosing	NA	No dosing	No Dosing
2	4	0	NA	Vehicle			
3	4	1	0.2	Genzyme rhGAA	— sodium phosphate, pH — mannitol.		IV
4	4	5	1.0	Genzyme rhGAA		Once weekly for 6 doses	
5	4	1	0.2	BI 2K rhGAA	25mM histidine pH 6.0 with 0.02% Tween-20	(see deviation)	
6	4	5	1.0	BI 2K rhGAA			
7	4	1	0.2	BI — rhGAA	— sodium phosphate — mannitol and —		
8	4	5	1.0	BI — rhGAA			

[1]Note: All animals received 5mg/kg diphenhydramine IP 15-20 minutes prior to treatment at the second dose and thereafter.

The table, below, supplied by the sponsor lists the scheme for sample collection:

Sample Collection:

Group	Timepoint	Tissue collection
1	Pre-dose	The following tissues were collected for glycogen analysis: Liver, spleen, kidneys, forelimb muscle, hindlimb muscle, diaphragm, heart and tongue.
2-8	24 hours after final dose	Terminal bloods were also taken for clinical chemistry and hematology analysis.

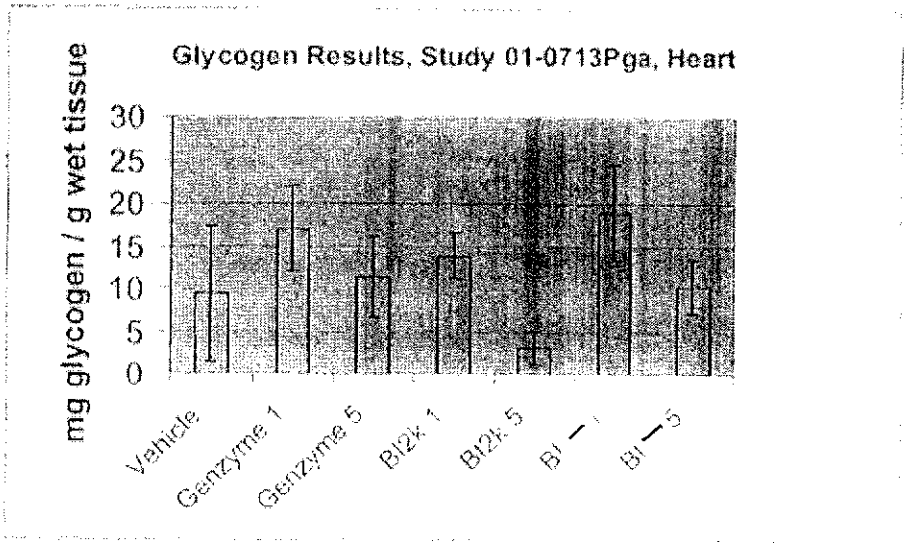
Results:

The results of in-life analyses showed no remarkable signs of toxicity. The glycogen load of heart and skeletal muscle was evaluated using computer assisted morphometry after appropriate processing and histological staining. No dose response pattern in tissue glycogen load was detected for the skeletal muscle samples. For heart muscle, a small degree of glycogen clearance was observed for all enzymes at the 5 mg/kg dose level. Due to large variability, significance is questionable. The largest effect was seen with the BI2K enzyme at 5 mg/kg in cardiac muscle. These results were confirmed using an enzymatic colorimetric assay to assess tissue glycogen in the muscle samples. One animal was found dead on the day of necropsy. However, no remarkable in-life observations were reported during the duration of the study.

Liver tissue showed approximately 33% and 67% reductions after treatment with 1 and 5 mg/kg, respectively

No dose response was noted in tissue glycogen clearance from skeletal muscle for any of the three preparations. A small percentage of clearance of glycogen from cardiac muscle was observed after 5 mg/kg dosing for all preparations. The changes did not appear to be statistically significant due to the large variation in results. The largest reduction was observed with the BI2K preparation.

The following graph, provided by the sponsor, summarizes the biochemical analyses of heart tissue glycogen levels after treatment with rhGAA:



Conclusion:

Depletion of glycogen from the various muscle tissues was small and statistical significance was not achieved.

Study # 01-0813pga

Title: Efficacy of rhGAA at 20 and 100 mg/kg in the $6^{neo}/6^{neo}$ knockout Pompe II mouse model.

Drug Lot#:

Project/Test Article(s):	**Lot Number:**
1. Genzyme rhGAA	1. GW10124095
2. BI — rhGAA	2. E1585 AM03

Methods:

The purpose of this study was to evaluate and compare efficacy of two doses of rhGAA after 2 and 4 weekly doses in a Pompe knockout mouse model. Efficacy was determined by analyzing tissue glycogen levels using biochemical methods as well as Metamorph analysis.

Animals: Mouse (Pompe $6^{neo}/6^{neo}$), female, 25 gm at study initiation

Dosing: 0, 20 or 100 mg/kg

Study design:

The basic study design is illustrated in the table below (supplied by the sponsor):

Group #	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Route of Administration
1	6	0	NA	Vehicle			
2	5	20	2	Genzyme rhGAA	sodium phosphate, pH mannitol,	Once weekly for 2 or 4 doses	IV
3	5	100	10	Genzyme rhGAA			
4	5	20	2	BI — rhGAA	20mM sodium phosphate pH 6.5, 2% mannitol and 0.5% sucrose		
5	5	100	10	BI — rhGAA			

[1]Note: All animals received 5mg/kg diphenhydramine IP 15-20 minutes prior to treatment at the second dose and thereafter.

The following samples were collected (table supplied by the sponsor):

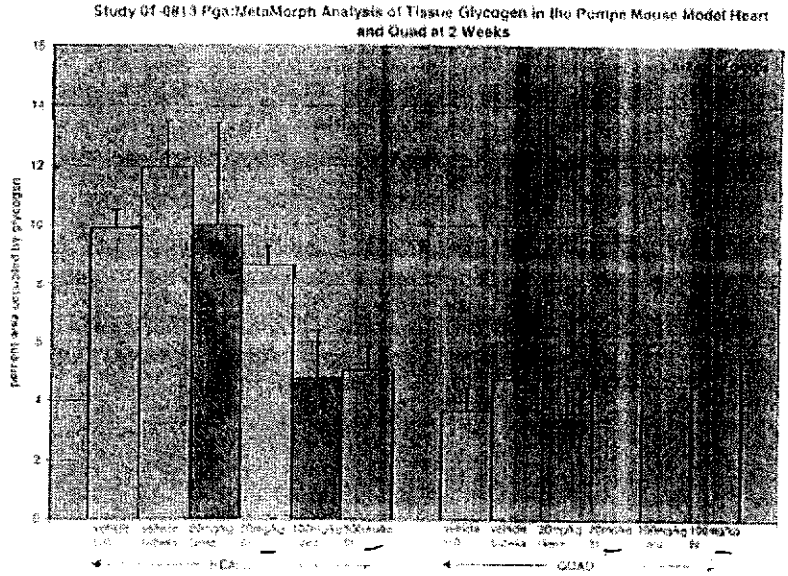
Group	Timepoint	Tissue collection
1	Pre-dose (n=2) 2 doses (n=2) 4 doses (n=2)	The following tissues were collected for glycogen analysis: Liver, forelimb muscle, hindlimb muscle, diaphragm, heart and tongue.
2-5	2 doses (n=2) 4 doses (n=3)	

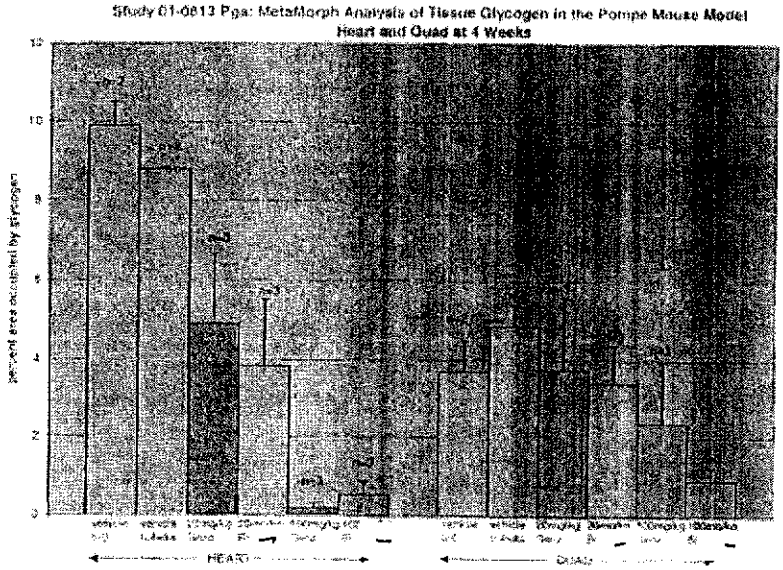
Results:

No significant test article-related adverse effects were noted during the in-life portion of this study. Histological analysis of the glycogen load in cardiac and skeletal muscle showed significant clearance of glycogen in a dose related pattern after 4 weeks of treatment (up to 95% clearance).

The glycogen load in each tissue sample (cardiac and skeletal muscle) was measured using computer assisted morphometry with MetaMorph software. A dose-related pattern of glycogen depletion was observed for both muscle types. Clearance was greater with the 100 mg/kg dose than the 20 mg/kg dose. The 100 mg/kg dose showed greater clearance in cardiac muscle than skeletal muscle (95% and 50-75%, respectively). The clearance after two weeks was much less robust. Clearance of glycogen from skeletal muscle was less robust (50 to 75% clearance after 4 weeks of therapy) relative to results for cardiac muscle. These results were

confirmed by analytical biochemical analysis of the tissue samples. The graphs below, provided by the sponsor demonstrate, as an example the depletion of glycogen in heart muscle after 2 and 4 weeks. The histological analysis gives results in % area occupied by glycogen.





Conclusion:

After treatment with rhGAA, tissue glycogen load is cleared in a dose and time-related manner. Cardiac muscle is the most sensitive to glycogen clearance by rhGAA. This finding may be due to higher density of mannose-6-phosphate receptors in this tissue type.

Study # 02-0209pga

Title: Analysis of glycogen depletion and re-accumulation after administration of a single dose of rhGAA in Pompe mice

Drug Lot#:

Project/Test Article(s):	Lot Number:
1. BI —	1. E1585 AM03
2. Genzyme rhGAA	2. GA028

Methods:

Animals: mouse, Pompe ($6^{neo}/6^{neo}$)
Age: 3-4 months
Dosing: 100 mg/kg for each enzyme. No control group was used.
The basic study design is illustrated in the table below, supplied by the sponsor: Each animal received a single dose and groups were sacrificed at 0, 1, 3, 6, 8, 14 and 21 post administration (n=3 per timepoint).

Group	Mouse strain	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	Pompe	21	100	10	BI — rhGAA	— Sodium Phosphate, — mannitol, —	Single dose	IV
2	Pompe	21	100	5	Genzyme rhGAA	— Sodium Phosphate, pH — mannitol, —		

The following table, supplied by the sponsor, lists the sample collection for this study.

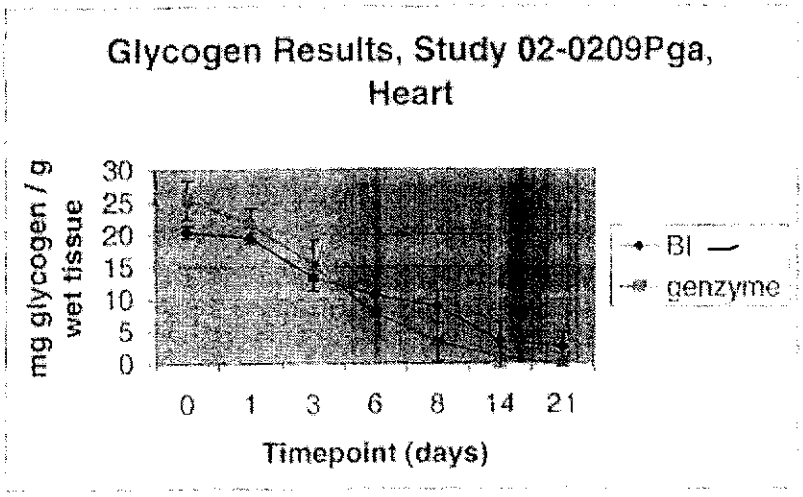
Group	Timepoint	Tissue collection
1 2	Tissues were collected at 0, 1, 3, 6, 8, 14 and 21 days after injection	The following tissues were collected for biochemical glycogen analysis and enzyme activity analysis[1] (snap frozen): Liver, heart, diaphragm, quadriceps, triceps and psoas muscle The following tissues were collected for histopathological glycogen analysis (3% gluteraldehyde): Liver, heart, diaphragm, quadriceps, triceps and psoas muscle

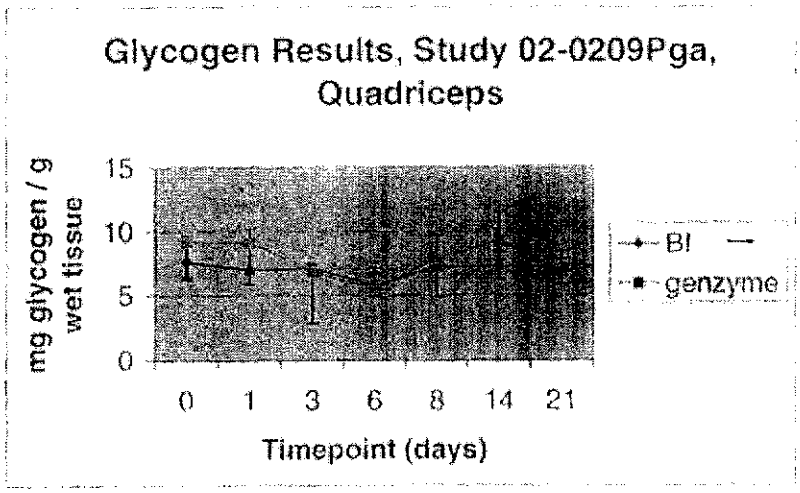
[1] Frozen tissue samples were sent to — or enzyme activity analysis.

Results:

The results of the histological analysis showed that, after a single dose of either BI — or rhGAA (100 mg/kg), glycogen is progressively cleared from cardiac muscle for up to 21 days with nearly complete depletion at 14 days. No re-accumulation was detected by 21 days post-injection. Genzyme rhGAA appeared to be equal to or slightly more effective than BI — n effectiveness. The treatment appeared to be less effective for other muscle tissue (triceps, psoas, diaphragm, quadriceps). The histological results were confirmed by biochemical

analysis of the tissues. The graphs below, provided by the sponsor illustrate the relative depletion observed in various tissues.





Tissue rhGAA activity: Enzyme activity analysis demonstrates that the two preparations of rhGAA have similar persistence time in the tissues. The graphs below, supplied by the sponsor illustrates the tissue rhGAA activity over time after a single dose.

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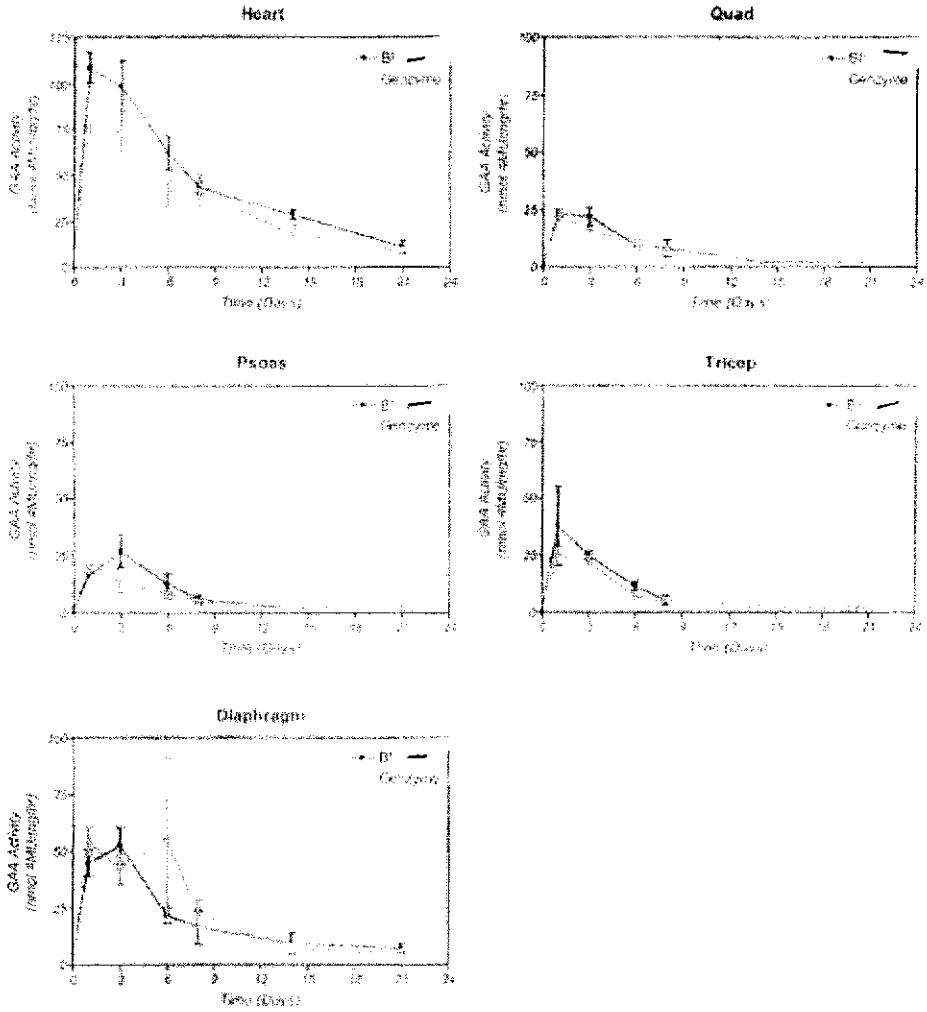


Conclusion:
The results of this study are consistent with other studies demonstrating that cardiac muscle is sensitive to the glycogen depleting activity of rhGAA than other muscle tissues examined. A single dose of rhGAA of 100 mg/kg can clear glycogen from cardiac tissue to below levels of detection for up to 21 days.

Study # 02-0314pga

Title: Efficacy of rhGAA in GAA knockout mice with every other week dosing and investigation of blood glucose levels in GAA knockout mice pre and post dosing.

Drug Lot#:

Project/Test Article(s):	Lot Number:
1. Genzyme rhGAA	1. 01284103
2. Vehicle (placebo buffer)	2. 11284102

Methods:

The objective of this study was to evaluate the efficacy of rhGAA in GAA knockout mice after every other week dosing for 2 or 4 doses. The intent of testing blood glucose was not successful because the [redacted] hat objective was not pursued after the first dose. Mice were treated with rhGAA or vehicle and sacrificed after 2 or 4 doses of 100 mg/kg. An additional control group that received no injections was also designated.

Animals: Mouse, C57B1/6 and mutant Pompe ($6^{neo}/6^{neo}$) mouse
Dosing: 0 or 100 mg/kg, every other week for two or 4 weeks.
Study design: Beginning with the second dose, mice were treated with 5 mg/kg diphenhydramine, IP, 20 minutes prior to dosing to combat expected hypersensitivity. Tissues were taken at sacrifice (liver, heart, diaphragm, quadriceps, triceps and psoas muscles) and analyzed for glycogen content histologically and biochemically. Then table below, provided by the sponsor, illustrates the basic study design.

Group	Mouse strain	# of Animals	Dose *mg/kg*	Conc *mg/ml*	Test Article	Vehicle	Dosing Regimen[1]	Dose Route
1	Pompe	8	100	8.33	Genzyme rhGAA	[redacted] Sodium Phosphate, [redacted] mannitol, [redacted]	Once every other week for two or four doses (4 mice each)	IV
2	Pompe	12	0	0	No dose or placebo *buffer*		Pre-dose, once every other week for two or four doses (4 *mice each*)	
3	C57Bl/6	8	No dose					
4	Pompe	3						

Group	Timepoint	Blood collection
1	Baseline, fasted (pre-dose), 1 hour post-dose, 2 hour post-dose (at first injection)	Tail vein blood spot was collected for glucose analysis using an Accu-Chek advantage diabetes monitor
2		
3	Baseline and fasted	
4	N/A	

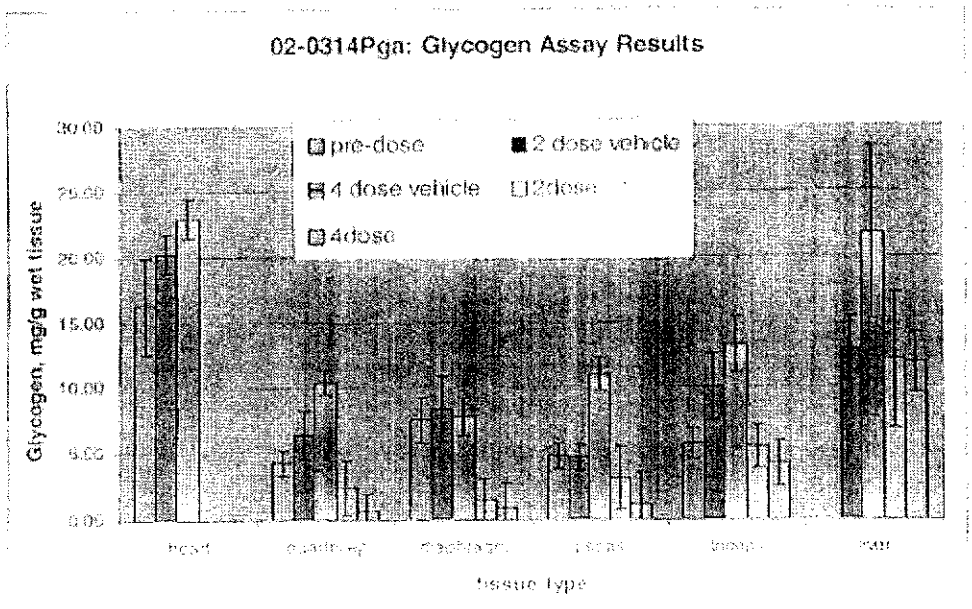
Group	Timepoint	Tissue collection
1	7 days after last injection (2 doses) or 24 hours after last injection (4 doses)	The following tissues were collected for biochemical glycogen analysis (snap frozen): Liver, heart, diaphragm, quadriceps, triceps and psoas muscle
2	Pre-dose, 7 days after last injection (2 doses) or 24 hours after last injection (4 doses)	The following tissues were collected for histopathological glycogen analysis (3% glutaraldehyde): Liver, heart, diaphragm, quadriceps, triceps and psoas muscle
3	No tissues collected – animals used only as controls for blood glucose analysis	
4	Pre-dose	The following tissues were collected for immunology department controls (snap frozen): Liver, heart, diaphragm, quadriceps, triceps and psoas muscle

Results:

No remarkable findings reported for clinical observations or gross necropsy examination. Biochemical results showed that glycogen was completely cleared from heart muscle after 2 or 4 doses of rhGAA. Blood glucose levels could not be evaluated in a meaningful way due to the presence of sucrose in the test article formulation. Skeletal muscle showed significant decrease in glycogen (63% in quadriceps, 33% in psoas, 83% in diaphragm, and 46% in triceps) relative to control. After 4 doses, the depletion was greater (range: 68% to 94%). Morphometric analysis confirmed these findings.

The following table and graphic, provided by the sponsor, illustrate the glycogen content of the various tissues examined. (Values represent the mean of each group in mg/gram of tissue.)

	heart	quadricep	diaphragm	psoas	triceps	liver	Hdev	Qdev	Ddev	Pdev	Tdev	Ldev
pre-dose	16.29	4.29	7.36	4.78	5.71		3.72	0.97	1.75	0.97	1.19	
2 dose vehicle	20.31	6.39	8.37	4.62	10.01	12.91	1.46	1.81	2.51	0.97	2.59	2.44
4 dose vehicle	22.95	10.40	7.71	10.97	13.24	21.83	1.49	0.89	1.48	1.22	2.18	6.66
2dose	0.00	2.39	1.44	3.11	5.45	12.07	0.00	2.09	1.66	2.35	1.57	5.21
4dose	0.00	0.56	0.91	1.16	4.23	11.85	0.00	1.32	1.52	2.32	1.69	2.3



Conclusion:
These findings agree with those of other studies showing greater efficacy of rhGAA treatment in cardiac muscle relative to skeletal muscle.

Study # 02-0359pga

Title: Investigation of glycogen depletion/re-accumulation in GAA knockout mice after 4 weekly doses of rhGAA

Drug Lot#:

Project/Test Article(s):	Lot Number:
1. Genzyme rhGAA	1. KsK1283091
2. Vehicle	2. KsK1283092

Methods:
Animals: mouse, Pompe KO ($6^{neo}/6^{neo}$), 25-30 grams at study initiation
Dosing: 100 mg/kg or vehicle
Study design:
Groups of mice were given weekly doses of rhGAA for 4 weeks, followed by sacrifice on SD1, 3, 7, 21, 28 and 42 after the last dose. Beginning with the second dose, mice were pre-treated with 5 mg/kg diphenhydramine, IP, to avoid hypersensitivity reactions. The table below, supplied by the sponsor, illustrates the basic study design.

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen[1]	Dose Route
1	21	100	9.48	Genzyme rhGAA	sodium phosphate, mannitol, polysorbate-80	Once weekly for 4 doses	IV
2	3	0	0	Vehicle			
3	3	N/A	N/A	Pre-dose control	N/A	N/A	N/A

[1]Note: All animals received 5mg/kg diphenhydramine IP 15-20 minutes prior to treatment at the second dose and thereafter.

Sample collection is described in the table below, supplied by the sponsor:

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Group	Timepoint	Tissue collection
1	Mice were sacrificed at day 1, 3, 7, 14, 21, 29 and 42 days after last injection	The following tissues were snap frozen for biochemical glycogen analysis: Heart, diaphragm, quadriceps, triceps and psoas muscle The following tissues were placed in 3% gluteraldehyde for histopathological glycogen analysis: Heart, diaphragm, quadriceps, triceps and psoas muscle
2	Mice were sacrificed at day 1 after last injection	
3	Pre-dose	

Tissue glycogen levels were determined using biochemical methods as well as histological methods (MetaMorph analysis).

Results:

No adverse effects attributable to test article administration were observed during the in-life portion of this study.

Biochemical results showed glycogen content was reduced to non-detectable levels in cardiac muscle and diaphragm by day 1 after the final injection of rhGAA. No significant re-accumulation was detected biochemically up to day 42. However, histological analysis detected slight re-accumulation beginning on day 21 for cardiac muscle. (Diaphragm was not analyzed histologically.) Quadriceps muscle samples showed partial depletion (52%) by day 1 and with continued decline on days 3, 7, 14 and 21. Complete depletion was noted on day 3. Slight re-accumulation was noted starting on days 28 and 42. Significant reduction was observed on day 3 with gradual re-accumulation up to day 42. However, glycogen levels remained significantly below control levels at day 42. Similar results were obtained for psoas muscle samples. Triceps muscle less depletion than quadriceps muscle but the reductions followed a similar pattern with the lowest levels observed on day 3 followed by slow re-accumulation. The data table below, provided by the sponsor, gives the biochemical analysis results for each tissue examined at each timepoint. Values are in units of mg/gram tissue.

Conclusion:

The results of this study agree with those of other pharmacology studies indicating that rhGAA administration results in near complete depletion of glycogen load in the cardiac muscle sample. The effect was less robust in other muscle types. The glycogen depletion persists for at least some time after termination of dosing. Gradual re-accumulation will occur at varying times after treatment is stopped.

Study #02-0380pga

Title: A comparison of the efficacy of rhGAA in 3 month and 12 month GAA knock out mice.

Drug Lot#:

Project/Test Article(s):
1. Genzyme rhGAA
2. *Vehicle*

Lot Number:
1. KsK11283091
2. KsK11283092

Methods:

The purpose of this study was to compare efficacy of rhGAA replacement in 3 and 12 month old Pompe mice. The test article was administered weekly for four weeks at a dose of 100 mg/kg, I.V. Glycogen levels in samples of a variety of muscle including cardiac, quadriceps, psoas and diaphragm were analyzed histologically using computer assisted morphometric analysis as well as biochemically for confirmation.

Animals: mouse, GAA knockout, n=5/group, gender not specified
Dosing: 0 or 100 mg/kg administered IV weekly for 4 weeks.
Study design: The basic study design is illustrated in the table below, provided by the sponsor. Groups 1-3 consisted of 3 month old mice; groups 4-6 contained 12 month old mice. Controls for both ages were either sacrificed pre-dose or received injections of vehicle only. All mice receiving injections of rhGAA or vehicle were sacrificed 7 days after the final dose.

Group	# of Animals	Age of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen[1]	Dose Route
1	5	3 months	100	9.48	Genzyme rhGAA	— sodium phosphate, — mannitol, — polysorbate -80	Once weekly for 4 doses	IV
2	5		0	0	Vehicle			
3	5		N/A	N/A	Pre-dose controls			
4	5	12 months	100	9.48	Genzyme rhGAA			
5	5		0	0	Vehicle			
6	5		N/A	N/A	Pre-dose controls			

[1]Note: All animals received 5mg/kg diphenhydramine IP 15-20 minutes prior to treatment at the second dose and thereafter.

Daily clinical observations were made and the following samples were collected (table provided by the sponsor):

Group	Timepoint	Tissue collection
1	Mice were sacrificed 7 days after fourth dose	The following tissues were snap frozen for biochemical glycogen and enzyme activity analysis. Heart, diaphragm, quadriceps, triceps and psoas muscle
2		
3	Pre-dose	The following tissues were placed in 3% glutaraldehyde for histopathological glycogen analysis: Heart, diaphragm, quadriceps, triceps and psoas muscle
4	Mice were sacrificed 7 days after fourth dose	
5		
6	Pre-dose	

Results:

No effects attributable to test article administration were noted during the in-life portion of this study. No abnormal findings were noted at gross necropsy.

Cardiac and quadriceps muscle were analyzed histologically for cellular morphology and glycogen content using computer assisted morphometry. After 4 weeks of dosing (100 mg/kg), 3 month old mice showed reductions in glycogen of 97.5% and 99.9% in quadriceps and cardiac muscle, respectively, relative to vehicle-treated controls.

After 4 weeks of dosing at 100 mg/kg, the 12 month old mice showed less dramatic reduction in tissue glycogen. Reductions reported for quadriceps and cardiac muscle were 65.0% and 78%, respectively, relative to vehicle-treated controls.

Biochemical analysis confirmed these findings. In addition, reductions of glycogen in diaphragm muscle displayed a similar pattern to cardiac muscle showing compete depletion in 3 month old mice after 4 weeks of treatment. Glycogen depletion in triceps, psoas and quadriceps in the 3 month old mice were 77%, 89% and 93%, respectively.

Biochemical analysis showed significantly more glycogen accumulation in cardiac and diaphragm muscles pre-dosing for the 12 month old mice relative to the levels at 3 months. This difference was not apparent for the other skeletal muscle samples (triceps, psoas or quadriceps). Glycogen depletion after 4 doses of rhGAA for the 12 month animals was 97% for cardiac muscle, 76% for diaphragm, 40% for the quadriceps, 45% for the psoas and 35% for the triceps. These data indicate that glycogen in the older animals is not as readily cleared as the in the younger animals. The table below, supplied by the sponsor, summarizes the final mean glycogen levels in the various tissue samples.

Study 02-0380Pga:
Glycogen Final Data, mg /g wet weight tissue

3 month	Heart	Quadriceps	Diaphragm	Psoas	Triceps	stdev Heart	stdev Quadriceps	stdev Diaphragm	stdev Psoas	stdev Triceps
100mg/kg	0	0.66	0	1.08	2.41	0	1.47	0	1.49	1.48
vehicle	15.96	9.22	6.41	9.89	10.84	1.84	1.33	1.01	1.31	2.00
pre-dose	13.94	10.33	6.42	8.71	11.68	2.55	2.54	1.61	0.25	1.17

12 month	Heart	Quadriceps	Diaphragm	Psoas	Triceps	stdev Heart	stdev Quadriceps	stdev Diaphragm	stdev Psoas	stdev Triceps
100mg/kg	1.02	6.94	2.30	5.76	6.70	2.28	1.60	2.50	1.85	2.71
vehicle	32.66	19.81	9.62	10.46	8.85	2.56	1.60	2.23	2.72	1.48
pre-dose	34.57	10.76	10.25	7.96	10.09	1.21	1.12	2.15	2.19	0.92

Analysis of rhGAA activity in muscle samples from treated mice was also performed. Enzyme activity in the 12 month old mice was increased relative to the 3 month old mice for cardiac and diaphragm muscles. The reverse was true for the other striated muscles. Results for the 12 month old mice were more highly variable relative to values for the 3 month old mice. The table below, provided by the sponsor, summarizes the mean rhGAA levels in the tissues examined.

Study 02-0380Tga, rhGAA activity Assay results, ug/g wet tissue

	Heart	Stdev	Quadriceps	Stdev	Triceps	Stdev	Diaphragm	Stdev	Psoas	Stdev
100 mg/kg 3 months	5.415	1.4134	18.503	16.0591	17.486	6.1722	2.906	0.6896	9.306	6.4039
Vehicle 3 months	0.144	0.1304	0.21	0.3303	0.040	0.069	0.058	0.1043	0.035	0.0552
Predose 3 months	0.059	0.0539	0.023	0.0143	0.000	0.0000	0.058	0.0360	0.043	0.0242
100 mg/kg 12 months	20.386	5.2102	5.678	5.8066	7.107	7.6805	10.358	3.1906	5.103	4.2559
Vehicle 12 months	0.168	0.0591	0.193	0.2537	0.086	0.0746	0.097	0.0239	0.060	0.0600
Predose 12 months	0.136	0.0351	0.031	0.0282	0.030	0.0278	0.137	0.0681	0.009	0.0090

Conclusion:

These results suggest that, skeletal muscle from older mice may be less responsive to the effects of rhGAA administration. However, the starting glycogen load was generally larger for the older animals and may have required larger doses or longer duration of dosing to see the full effects of the treatment.

Study # 02-0500pga

Title: Investigation of rhGAA in GAA KO mice treated with 10 or 20 mg/kg of enzyme for 16 weeks

Drug Lot#:

Project/Test Article(s): Genzyme rhGAA **Lot Number:** KsK11283091

Methods:

The purpose of this study was to explore efficacy of two doses of rhGAA in the Pompe GAA knockout mouse model. The doses compared were 10 and 20 mg/kg delivered IV weekly for 16 weeks.

Animals: mouse, Pompe KO ($6^{neo}/6^{neo}$), 5-6 month at study initiation, gender not specified

Dosing: 0, 10 or 20 mg/kg delivered IV weekly for 16 weeks.

Study design: Groups of animals were sacrificed after 4, 8, 12 or 16 doses. The basic study design is illustrated in the table below, supplied by the sponsor.

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen[1]	Dose Route
1	3	Pre-dose	N/A	N/A	N/A	N/A	N/A
2	4	vehicle	N/A	Vehicle	sodium phosphate, mannitol, polysorbate 80	Once weekly	IV
3	4	10	9.38	Genzyme rhGAA			
4	4	10	9.38				
5	4	10	9.38				
6	4	10	9.38				
7	4	20	9.38				
8	4	20	9.38				
9	4	20	9.38				
10	4	20	9.38				

[1]Note: All animals received 5mg/kg diphenhydramine IP 15-20 minutes prior to treatment at the third dose and thereafter.

The following table, supplied by the sponsor, illustrates the sacrifice schedule and samples taken:

Group #	Time Points	Tissue Collection
1	Pre-dose	The following tissues were snap frozen for biochemical glycogen analysis: Heart, diaphragm, quadriceps, triceps and psoas muscle The following tissues were placed in 3% glutaraldehyde for histopathological glycogen analysis. Heart, diaphragm, quadriceps, triceps and psoas muscle
2	Sacrifice after 16 doses	
3	Sacrifice after 4 doses	
4	Sacrifice after 8 doses	
5	Sacrifice after 12 doses	
6	Sacrifice after 16 doses	
7	Sacrifice after 4 doses	
8	Sacrifice after 8 doses	
9	Sacrifice after 12 doses	
10	Sacrifice after 16 doses	

Results:

Four mice died during the in vivo portion of this study. Two mice were found dead 24 hours after the third dose (one from group 3, one from group 6). One mouse from group 6 died 15 minutes after the 4th dose and one mouse from group 10 was found dead 24 hours after the 10th dose. The sponsor hypothesizes that the deaths were due to hypersensitivity responses to the rhGAA. Clinical observations consistent with hypersensitivity response were reported for other mice subsequent to the 3rd and 4th doses.

When mice were sacrificed after 4 weeks of treatment with 10 mg/kg rhGAA, cardiac glycogen was reduced by 27%. After 8, 12 and 16 doses, glycogen was reduced by 45%, 60% and 53%. Comparison is made relative to vehicle control mice. After treatment with 20 mg/kg for 4, 8, 12 and 16 weeks, glycogen levels were 57%, 75%, 69% and 66%, respectively.

For quadriceps muscle, after 4, 8, 12 or 16 weeks of dosing at 10 mg/kg glycogen depletions were 23%, 41%, 43% and 12%, respectively. After treatment for 4, 8, 12 or 16 weeks of 20 mg/kg, glycogen depletion for quadriceps was 29%, 28%, 61% and 35 %, respectively. The tables below, provided by the sponsor, summarizes the glycogen depletion in each of the tissues examined. (Expressed in mg/gram wet tissue.)

All Tissues:

02-0500; compiled data

10mg/kg

	heart	quadricep	diaphragm	psoas	triceps	stdev H	stdev Q	stdev D	stedv P	stdev T
pre-dose	22.12	10.80	8.75	8.50	10.63	1.52	1.55	0.85	1.01	0.24
vehicle	30.01	12.03	12.89	9.50	16.44	3.89	1.61	1.37	0.89	3.16
week 4	16.20	8.51	9.12	8.82	9.45	4.90	1.26	1.14	0.54	2.23
week 8	10.87	9.47	8.77	6.56	9.23	0.99	1.22	2.35	0.79	1.57
week 12	8.46	8.85	5.66	8.22	10.71	5.34	2.26	1.91	1.47	2.2*
week 16	8.16	9.59	6.95	8.96	8.72	3.04	0.89	4.25	0.41	1.91

20 mg/kg

	heart	quadricep	diaphragm	psoas	triceps	stdev H	stdev Q	stdev D	stedv P	stdev T
pre-dose	22.12	10.80	8.75	8.50	10.63	1.52	1.55	0.85	1.01	0.24
vehicle	30.01	12.03	12.89	9.50	16.44	3.89	1.61	1.37	0.89	3.16
week 4	10.15	7.77	6.56	7.02	8.60	3.91	0.80	0.90	1.98	0.52
week 8	4.70	6.60	6.02	7.82	8.39	1.05	0.99	1.27	1.07	1.59
week 12	3.84	7.35	5.75	8.21	7.96	3.40	3.29	1.42	1.76	1.32
week 16	2.36	7.57	7.91	8.56	8.88	2.06	4.07	1.18	0.38	3.34

Conclusion:

A time and dose dependent decrease in tissue glycogen levels was noted for cardiac and skeletal muscle. In general, glycogen depletion was greater for cardiac muscle than skeletal muscle and the 20 mg/kg dose was more effective than the 10 mg/kg dose. Depletion was greater after each subsequent dose up to week 12. For both dose levels, a small increase in tissue glycogen was noted after the 16th dose by morphometric analysis, indicating that re-accumulation had begun at this time point.

Study #02-0715pga

Title: Efficacy of rhGAA in GAA knockout mice with every other week dosing at 10, 20, and 40 mg/kg

Drug Lot#:

Project/Test Article(s): Genzyme rhGAA **Lot Number:** 930018

Methods:

The purpose of this study was to evaluate the efficacy of Genzyme rhGAA, 160 L product, in reducing tissue glycogen of the mutant GAA knockout mouse

Animals: GAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified, 25-30 g at study initiation

Dosing: 0, 10, 20, or 40 mg/kg

Study design: Mice received test article by IV administration every other week (qow) for 8 weeks. Prior to the 2nd and subsequent doses, animals were pre-treated with diphenhydramine

(5 mg/kg, IP) to prevent hypersensitivity reactions. The basic study design is illustrated in the table below, provided by the sponsor. The mice were sacrificed either pre-dosing or after 4 qow treatments.

Study Design Table

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen[1]	Dose Route
1	5	N/A	N/A	N/A	N/A	N/A	N/A
2	5	0	N/A	Vehicle	— sodium phosphate, — mannitol, — Tween-80	Every other week for four doses	IV
3	5	10	1	Genzyme rhGAA (1608)			
4	5	20	2				
5	5	40	4				

[1]Note: *All animals received* 5mg/kg diphenhydramine IP 15-20 minutes prior to treatment at the third dose and fourth dose.

Sample Collection

Group #	# of Animals	Time Points	Tissue Collection
1	5	Pre-dose	The following tissues were snap frozen for biochemical glycogen analysis: Heart, diaphragm, quadriceps, triceps and psoas muscle The following tissues were placed in 3% glutaraldehyde for histopathological glycogen analysis: Heart, diaphragm, quadriceps, triceps and psoas muscle
2	5	7 days after fourth dose	
3	5		
4	5		
5	5		

After sacrifice various muscle samples were retrieved for analysis of glycogen levels by biochemical and histological methods.

Results:

No adverse findings attributable to the test article were noted during the in-life portion of this study.

Histology results: Cardiac and quadriceps muscle samples were analyzed using computer assisted morphometry. A dose response effect of rhGAA was noted for glycogen depletion in both cardiac and quadriceps muscle samples. Cardiac muscle showed reductions in glycogen of 22%, 38% and 93% for the 10, 20 and 40 mg/kg dose groups, respectively, relative to vehicle control. A small reduction was noted for quadriceps muscle with reductions of 2%, 21% and 52%, relative to vehicle control.

Biochemical results: (Enzymatic colorimetric assay used for all studies.) For cardiac muscle, a clear dose response was observed for tissue glycogen levels. Complete depletion was reported for the 40 mg/kg group. A less dramatic effect was observed for the quadriceps muscle, although a trend of reduction demonstrating a dose related was present. Similar results were obtained for other skeletal muscle samples with greatest depletion observed at the 40 mg/kg dose. The table below, supplied by the sponsor, summarizes the glycogen levels for each tissue examined for each dose. (Data is expressed in mg glycogen/gram wet tissue.)

	Heart	Quadriceps	Psoas	Diaphragm	Triceps	stdev h	stdev Q	stdev P	stdev D	stdev T
pre-dose	14.23	7.24	5.28	5.50	9.55	2.32	0.66	0.63	0.86	2.32
vehicle	22.92	7.12	5.53	5.32	10.90	3.33	0.85	0.92	0.84	1.34
10 mg/kg	10.22	7.54	5.90	5.63	13.85	2.20	0.24	0.52	1.17	2.93
20 mg/kg	5.01	5.65	3.55	5.04	5.34	1.02	1.02	0.51	1.42	2.13
40 mg/kg	0.00	4.87	4.22	3.57	5.38	0.00	1.29	0.90	0.65	1.55

Conclusion:

A clear dose response effect in reduction of tissue glycogen content was apparent for both histological and biochemical analysis after 4 weeks of treatment with rhGAA. IN agreement with other studies, cardiac muscle is apparently more responsive to the glycogen depleting effects of rhGAA. AT 40 mg/kg, near complete depletion of glycogen was noted for cardiac muscle.

Study #02-1136pga

Title: Determining the optimal debulking dose of rhGAA in the Pompe knockout mouse

Drug Lot#:

Project/Test Article(s): Genzyme rhGAA **Lot Number:** GA028

Methods:

The purpose of this study was to explore dosing and regimen for clearing accumulated glycogen from cardiac and skeletal muscle of the GAA knockout mouse. Animals received 100 mg/kg in three different regimens: 2 weekly doses, 3 weekly doses or 2 doses qow.

Animals: Pompe GAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified.

Dosing: 0 or 100 mg/kg, IV administered weekly or every other week (qow)

Samples were taken at 3, 7 or 14 days post-dose. Three groups of nine mice each were designated.

Study design: The basic study design is illustrated in the tables below, provided by the sponsor.

Study Design Table

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	9	100	10	Genzyme rhGAA	– Sodium Phosphate, – mannitol, –	2 doses QW	IV
2						3 doses QW	
3						2 doses QOW	

Sample Collection

Group #	Time Points	Tissue Collection
1	Sacrifice mice 3, 7 and 14 days after last injection (n=3 per time point)	Serum ,heart, psoas, quadriceps, triceps, diaphragm
2		
3		

Results:

No findings attributable to test article administration were noted during the in-life portion if this study.

Glycogen was 100% depleted in cardiac muscle at all timepoints except the day 3 day point for the group receiving 2 weekly doses. In the remaining skeletal muscle samples, results for the groups receiving 3 weekly doses were similar to those of groups receiving 2 qow dosing. The tables below, supplied by the sponsor, summarize the glycogen levels in each tissue examined for each group. (Data given in units of mg/gram wet tissue.)

Final Data:

Heart	3 days post dose	Stdev 3 days	7 days post dose	Stdev 7 days	14 days post dose	Stdev 14 days
100 mg/kg 2 doses weekly injections	2.445	4.2340	0.000	0.0000	0.000	0.0000
100 mg/kg 3 doses weekly injections	0.000	0.0000	0.000	0.0000	0.000	0.0000
100 mg/kg 2 doses QOW injections	0.000	0.0000	0.000	0.0000	0.000	0.0000
Quadriceps	3 days post dose	Stdev 3 days	7 days post dose	Stdev 7 days	14 days post dose	Stdev 14 days
100 mg/kg 2 doses weekly injections	7.901	1.9663	6.159	0.3865	5.509	0.9755
100 mg/kg 3 doses weekly injections	4.012	0.5487	0.000	0.0000	0.000	0.0000
100 mg/kg 2 doses QOW injections	1.579	2.7353	0.000	0.0000	1.582	2.7403
Triceps	3 days post dose	Stdev 3 days	7 days post dose	Stdev 7 days	14 days post dose	Stdev 14 days
100 mg/kg 2 doses weekly injections	8.487	2.7822	6.946	2.3944	6.102	1.4230
100 mg/kg 3 doses weekly injections	4.758	1.5704	5.943	0.5876	3.729	0.3478
100 mg/kg 2 doses QOW injections	9.139	2.7960	3.952	0.7831	4.699	2.7353

Psoas	3 days post dose	Stdev 3 days	7 days post dose	Stdev 7 days	14 days post dose	Stdev 14 days
100 mg/kg 2 doses weekly injections	6.989	1.4115	4.390	3.9237	0.000	0.0000
100 mg/kg 3 doses weekly injections	4.030	0.4268	0.000	0.0000	0.000	0.0000
100 mg/kg 2 doses QOW injections	4.184	3.7124	1.827	3.1650	0.000	0.0000
Diaphragm	3 days post dose	Stdev 3 days	7 days post dose	Stdev 7 days	14 days post dose	Stdev 14 days
100 mg/kg 2 doses weekly injections	6.123	1.0208	3.243	1.5564	3.024	0.7273
100 mg/kg 3 doses weekly injections	2.277	0.4969	2.464	0.3837	0.501	0.4364
100 mg/kg 2 doses QOW injections	3.576	1.2098	2.967	1.0540	1.822	0.6314

Conclusion:
The least effective regimen for the 100 mg/kg dose level was the biweekly two dose regimen.

Study #02-1165pga

Title: Long-term dose response of qow dosing in the Pompe knockout model

Drug Lot#:

Project/Test Article(s): rhGAA **Lot Number:** GA028

Methods:
The purpose of this study was to evaluate efficacy of rhGAA administered every other week (qow) in clearing glycogen long-term in the Pompe GAA knockout mouse model. Five groups of mice received doses of 0, 10, 20 or 40 mg/kg, qow. One of the 2 groups receiving 20 mg/kg was dosed weekly. Tissue samples were analyzed for glycogen depletion using histological and biochemical methods as in previous studies.

Animals: rhGAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified.
Dosing: 0, 10, 20 or 40 mg/kg administered IV either weekly or qow
Study design: Five groups of mice were designated: group1 was sacrificed pre-dose or received vehicle, group 2 received 20 mg/kg weekly, group 3 received 10 mg/kg, qow, group 4 received 20 mg/kg, qow, group 5 received 40 mg/kg, qow. Mice were sacrificed 7 days after the final dose. Study duration was 16 weeks. Glycogen levels in selected muscle tissues were determined by biochemical and morphological (histological) methods. The following tables, provided by the sponsor, illustrate the basic study design.

Study Design Table

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	7	n/a	0	vehicle	— Phosphate, pH — mannitol, — / polysorbate-80	qow	IV
2	5	20	5.0	Genzyme rhGAA (reconstituted in — / polysorbate-80)		weekly	
3	5	10	1.0			qow	
4	5	20	5.0			qow	
5	5	40	5.0			qow	

Sample Collection

Group #	Time Points	Tissue Collection
1	Sacrifice 3 mice pre-dose and 4 mice 7 days after the last dose	Serum, Heart, Psoas, Quadriceps, Triceps, Diaphragm
2	Sacrifice 7 days after last dose	
3		
4	Sacrifice 14 days after last dose	
5		

Results:

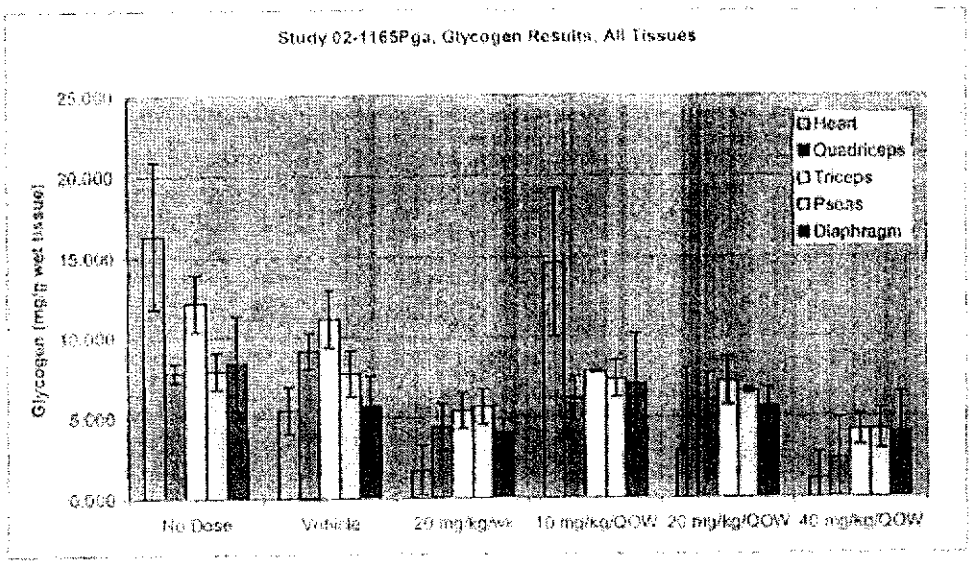
Seven deaths occurred during the study. A majority of these deaths were attributed to hypersensitivity reactions. Hypersensitivity is not unexpected with this product in mice. Treatment with diphenhydramine was able to prevent death in some cases where signs of hypersensitivity were recognized. One death from group 4 was determined to not be due to hypersensitivity but the cause was not determined.

Histology results: Cardiac and quadriceps muscle were analyzed by computer assisted morphometry (MetaMorph) for tissue glycogen levels. The results showed depletions in the quadriceps muscle of 48.7%, 15.3%, 12.1 % and 53% for weekly dosing at 20 mg/kg, qow dosing at 10, 20 or 40 mg/kg, respectively. Glycogen level changes for cardiac muscle for the same groups were 131.3%, +106%, -36.7% and -41.7%.

Biochemical results: A dose dependent reduction in glycogen levels was observed for qow dosing from 10 mg/kg to 40 mg/kg. Long-term qow dosing at 40 mg/kg was as effective at clearing glycogen accumulation as 20 mg/kg administered weekly. Depletion was apparently more efficient in cardiac muscle than skeletal muscle with glycogen levels consistently lower in cardiac muscle after similar dosing. The lowest glycogen levels were observed after 20 mg/kg administered weekly.

Serum anti-drug antibodies: ELISA assay was used to determine the level of anti-rhGAA antibodies in the mouse serum taken at weeks 4, 8, 12 and 15. Anti-drug antibodies were detected for all dosing regimens and dose levels. Highest levels were found in animals treated at 40 mg/kg, qow, at weeks 8, 12 and 15. The table and graph below, supplied by the sponsor, summarized the results of tissue glycogen loads and rhGAA levels for each tissue examined in each group.

Final Glycogen Graph, All Tissues:



rhGAA Activity results, ug/g wet tissue

	Heart	Stdev	Quadriceps	Stdev	Triceps	Stdev	Psoas	Stdev	Diaphragm	Stdev
Pre-Dose	0.068	0.0592	0	0	0.09	0.09	0.00	0.00	0.00	0.00
Vehicle Treated	0.073	0.0804	0	0	0.00	0.00	0.00	0.00	0.00	0.00
20 mg/kg/wk	0.529	0.0951	0.834	0.3129	1.842	0.2786	0.439	0.2509	2.569	2.4049
10 mg/kg/QOW	0.358	0.2168	1.265	0.4959	2.370	1.7263	0.684	0.5062	0.391	0.2206
20 mg/kg/QOW	0.691	0.3077	2.083	1.1527	2.607	2.1041	0.916	0.7056	0.465	0.3177
40 mg/kg/QOW	0.533	0.3679	4.785	2.1502	4.945	3.5530	1.710	0.5304	4.891	4.7616

Conclusion:
These data demonstrate that dosing at 40 mg/kg, qow, is as effective in cardiac muscle as 20 mg/kg weekly. Dosing of 40 mg/kg, qow, would be needed to equal the effect in the quadriceps muscle that is seen with 20 mg/kg weekly.

Study #03-0255pga

Title: Investigation of a debulking and maintenance dosing regimen using rhGAA in the Pompe knockout mouse model

Drug Lot#:
Project/Test Article(s): Genzyme rhGAA Lot Number: GA095

Methods:
Animals: rhGAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified.
Dosing: The dosing regimen included initial high dose (100 mg/kg X 2 qow) followed by lower doses (5, 10 or 20 mg/kg), qow, for 16 weeks. Test article was administered IV.
Study Design: The basic study design, dosing regimens and sample collection are illustrated in the tables below, provided by the sponsor. Tissue samples were analyzed for glycogen content using a biochemical colorimetric assay.

Study Design Table

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	15	100/0	10/0	rhGAA/ vehicle	20 mM Sodium Phosphate 150 mM mannitol, 0.005% Polysorbate 80	100mg/kg qow for 2 doses, then vehicle qow for 16 weeks	IV
2	5	100/5	10/1.25	rhGAA		100mg/kg qow for 2 doses, then 5mg/kg qow for 16 weeks	
3	5	100/10	10/2.5			100mg/kg qow for 2 doses, then 10mg/kg qow for 16 weeks	
4	5	100/20	10/5			100mg/kg qow for 2 doses, then 20mg/kg qow for 16 weeks	
5	5	100/20	10/5			100mg/kg qow for 2 doses, then 20mg/kg monthly for 16 weeks	

Sample Collection

Group #	Time Points	Tissue Collection
1	Sacrifice 5 mice at the start of the study, 5 mice at 14 days after dose #2 and 5 mice at study end	Serum was collected for antibody titers every 4 weeks The following tissues were snap frozen for biochemical glycogen analysis: heart, quadriceps, triceps, diaphragm and psoas muscle
2	Sacrifice 14 days after last maintenance dose	
3		
4		
5	Sacrifice 28 days after last maintenance dose	

Results:
Two mice died during the in-life portion of the study. The cause of death was not determined. However, the timing of the deaths was not consistent with an acute hypersensitivity response.
Glycogen assay results: A significant reduction in accumulated glycogen was observed for all samples after 2 doses qow at 100 mg/kg. Re-accumulation was noted in all tissues from animals receiving maintenance doses of 5 or 10 mg/kg. Re-accumulation was more robust in skeletal muscle samples that cardiac muscle or diaphragm. Re-accumulation for cardiac tissue

and diaphragm from animals receiving maintenance doses of 20 mg/kg, qow, or monthly was rated as moderate and re-accumulation for this group in skeletal muscle was rated as significant. The levels after re-accumulation were similar to those from the vehicle maintenance group.

The table below, provided by the sponsor, summarizes the mean levels of glycogen for each tissue for each dose regimen.

Study 03-0255Pga. Glycogen Results, mg/g wet tissue

Group	Dose	Heart	Quadriceps	Triceps	Psoas	Diaphragm
1a	pre dose	13.280	7.534	9.621	7.537	5.195
1b	100 mg/kg debulking	0.000	1.695	5.565	3.418	1.706
1c	100 mg/kg debulking and vehicle	11.672	10.461	12.318	7.942	6.390
2	100 mg/kg debulking and 5 mg/kg QOW	8.488	9.198	11.288	7.638	7.565
3	100 mg/kg debulking and 10 mg/kg QOW	5.890	8.521	10.739	8.241	7.064
4	100 mg/kg debulking and 20 mg/kg QOW	0.762	6.759	8.046	7.459	3.028
5	100 mg/kg debulking and 20 mg/kg monthly	2.855	8.162	8.637	8.957	3.830

Alpha glucosidase activity assay: A significant amount of rhGAA activity was detected in tissues after the 100 mg/kg, qow, initial clearance regimen. A dose response was noted in all tissues (except psoas muscle) during the maintenance phase of dosing. More rhGAA activity was observed after the 20 mg/kg monthly regimen than for the 20 mg/kg, qow, regimen. No explanation was given for this phenomenon. The table below, provided by the sponsor, summarizes the results of the rhGAA activity analysis.

Final Data Study 03-0255Pga. ug/g wet tissue

Group	Dose	Heart	Quadriceps	Triceps	Psoas	Diaphragm
1a	Pre Dose	0.000	0.000	0.000	0.000	0.000
1b	100 mg/kg debulking	1.885	1.058	0.462	0.395	22.116
1c	100 mg/kg debulking and vehicle	0.178	0.000	0.000	0.000	0.084
2	100 mg/kg debulking and 5 mg/kg QOW	0.058	0.033	0.133	0.000	1.121
3	100 mg/kg debulking and 10 mg/kg QOW	0.104	0.251	0.221	0.000	0.763
4	100 mg/kg debulking and 20 mg/kg QOW	0.177	0.349	0.330	0.024	1.677
5	100 mg/kg debulking and 20 mg/kg monthly	0.282	0.800	0.943	0.481	3.597

Serum anti-drug antibody levels: Significant anti-drug antibody titers were detected in all dose groups. A small increase in titer was observed between days 5 and 9 but no increases thereafter. No apparent correlation with rhGAA dose was noted.

Conclusion:

The results of this study are consistent with those of other similar studies indicating that rhGAA is more effective in clearing glycogen from cardiac muscle relative to other types of muscle and that reaccumulation does occur after termination of dosing. Enzyme activity studies confirm that rhGAA is retained in the tissues with highest levels of activity found in the liver.

Study #03-0317pga

Title: Efficacy of two recent rhGAA lots of formulated bulk.

Drug Lot#:

Project/Test Article(s): Genzyme rhGAA **Lot Numbers:** GAA1, 03TP021, 03TP022

Methods:

The purpose for this study was to compare efficacy of two lots of formulated bulk from the 160L scale. Biochemical analysis of lots 03TP021 and 03TP022 showed — — elative to the reference standard lot, GAA1. Animals received doses of one of three drug preparations: one group received reference material, two groups received drug from one each of the two lots that showed a deviation in — results.

Animals: rhGAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified.
Dosing: 0 or 100 mg/kg of each of the three lots to be compared for efficacy. Test article was administered IV, qow, for 2 doses.
Study design: The basic study design is illustrated in the tables below, provided by the sponsor. Animals were sacrificed 14 days after the second dose. Tissue samples were analyzed biochemically for glycogen clearance. Serum was stored for future reference as needed.

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	6	0	N/A	Vehicle	— Phosphate, pH — mannitol, — polysorbate 80	QOW for 2 doses	IV
2	6	100	9.447	GAA1			
3	6		9.375	03TP021			
4	6		10.409	03TP022			

Sample Collection

Group #	Time Points	Tissue Collection
1	Sacrifice 14 days after dose #2	Heart, Diaphragm, Triceps, Psoas, Quadriceps, Serum
2		
3		
4		

Results:

A thyroid neoplasm was noted at necropsy for one mouse from group 2. The mass was not present at the time of the second dose. This finding was thought to be spontaneous and not related to the test article. It is not clear why this judgment was made by the sponsor. White

spots were observed on the liver of one mouse from group 2. No information regarding this finding was given. The sponsor states that the finding is not related to administration of the test article.

No significant differences in glycogen clearance were detected among the three treated groups relative to control for any tissue analyzed. Complete depletion of tissue glycogen was observed for cardiac tissue, approximately 30 -40% reduction in quadriceps muscle, approximately 50% reduction in triceps, approximately 25% reduction in psoas muscle. The diaphragm showed a larger mean depletion effect relative to control than other skeletal muscle samples but the large variation resulted in no significant difference between the treated groups. The tables below, provided by the sponsor, summarize the results of tissue glycogen loads in each dose group for each tissue.

Final Results Heart:
mg glycogen / g wet tissue

Group	Dose	Group Average	Group Stdev.
1	Vehicle	19.789	3.9389
2	GAA1	0.000	N/A
3	03TP021	0.000	N/A
4	03TP022	0.000	N/A

Final Data Quadriceps:
mg glycogen / g wet tissue

Group	Dose	Group Average	Group Stdev.
1	Vehicle	9.121	1.6196
2	GAA1	0.488	2.1897
3	03TP021	5.759	1.2539
4	03TP022	7.170	0.7588

Final Results Triceps:
mg glycogen / g wet tissue

Group	Dose	Group Average	Group Stdev.
1	Vehicle	10.394	1.2949
2	GAA1	4.773	1.5791
3	03TP021	6.385	1.0643
4	03TP022	6.910	0.7534

Final Data Psoas:
mg glycogen / g wet tissue

Group	Dose	Group Average	Group Stdev.
1	Vehicle	9.653	0.309
2	GAA1	7.471	1.405
3	03TP021	7.350	1.055
4	03TP022	7.350	0.682

Final Data Diaphragm:
mg glycogen / g wet weight tissue

Group	Dose	Group Average	Group Stdev.
1	Vehicle	6.584	0.961
2	GAA1	2.218	2.533
3	03TP021	1.055	2.563
4	03TP022	0.722	1.118

Conclusion:
No significant difference in effectiveness in glycogen clearance was observed between the three lots. However, study # 03-0370pga demonstrated that the pharmacokinetics of lots 03TP021 and 03TP022 differed significantly from the reference standard lot GAA1. (AUC for lots 03TP021 and 03TP022 were 30-50% less than that for the standard lot GAA1). The apparent discrepancy between the two studies suggests that the glycogen clearance data is not a sufficiently sensitive measure for detecting potential variations among lots.

Study #03-0462pga

Title: A comparison of 3 and 12 month old Pompe mice treated with rhGAA

Drug Lot#:

Project/Test Article(s): *Genzyme* rhGAA **Lot Number:** GA095

Methods:
Animals: rhGAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified.
Dosing: 0 or 100 mg/kg, administered weekly for 4 weeks
Study design: Four groups of mice were designated: groups 1 and 2 contained 3 month old mice, groups 3 and 4 contained 12 month old mice. All mice were sacrificed 7 days after the final dose. The tables below, supplied by the sponsor, illustrate the basic study design:

Group	# of Animals	Dose mg/kg	Conc mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	12	100	10	rhGAA	Phosphate, pH mannitol polysorbate 80	Weekly	IV
2	6	0	0	Vehicle			
3	12	100	10	rhGAA			
4	6	0	0	Vehicle			

Sample Collection

Group #	Time Points	Tissue Collection
1	Sacrifice 7 days after the last dose	Serum, heart, quadriceps, triceps, diaphragm, spinal cord, liver, spleen, kidney, soleus
2		
3		
4		

Results:
A lump was observed on one mouse from group 3. The lump was removed at necropsy and examined histologically. The results identified the lump as a deep soft tissue abscess probably not related to test article administration. No other in-life findings were reported.
Histology report: The glycogen load in each tissue sample was quantified (as in the previous studies listed in this review) using computer assisted morphometry with MetaMorph software. The results showed that glycogen depletion is less robust in the older mice. Glycogen levels in cardiac and quadriceps muscles of 3 month old mice after 4 weeks of treatment at 100 mg/kg were reduced by 98.4% and 77.7%, respectively. The results for the 12 month old mice were 90.1% and 68.5% in cardiac muscle and quadriceps, respectively. These results are relative to vehicle treated controls.
Biochemical analytical report: The three month old mice showed glycogen levels in cardiac muscle to be below the level of detection (0.00%). A significant reduction of tissue glycogen was observed for skeletal muscle samples: 79%, 83%, 88% and 88% for triceps, psoas, diaphragm, and quadriceps, respectively. For the 12 month old mice, more glycogen accumulation was noted in cardiac muscle of vehicle treated mice than that of vehicle treated 3 month old mice. This increase of glycogen was not observed in triceps, quadriceps, psoas or diaphragm of the 12 month old vehicle treated mice. Cardiac muscle of the 12 month old rhGAA treated mice showed near complete clearance of tissue glycogen and moderate clearance for the skeletal muscle. This finding suggests that older mice are less efficient in

clearing accumulated glycogen. The table below, provided by the sponsor, summarizes these results.

Final Glycogen Results, mg / g wet tissue

Group	Dose	Heart	Quadriceps	Triceps	Psoas	Diaphragm
1	Vehicle 3 Months	26.366	9.254	10.626	8.859	5.065
3	Vehicle 12 Months	41.181	7.676	10.199	6.530	9.803
2	100 mg/kg 3 Months	0.000	1.120	2.105	1.516	0.597
4	100 mg/kg 12 Months	0.672	3.899	4.303	3.840	1.226

Anti-drug antibody report: Moderate levels of anti-drug antibodies were detected in both the 3 month old and 12 month old mice that received rhGAA at 100 mg/kg. The mean antibody titer appears to be generally lower for the 12 month old animals relative to the mean titer for the 3 month old animals. However, the large range of variability makes it difficult to determine if the difference is biologically significant.

rhGAA levels: Tissue levels of rhGAA were measured for each tissue for all animals in each group. Increased rhGAA activity was observed in cardiac muscle of 12 month old mice relative to the 3 month old mice. In contrast, rhGAA levels triceps psoas and quadriceps were lower in 12 month old mice relative to the 3 month hold mice. Enzyme levels are similar in both age groups for the diaphragm muscle. A high variability was observed in the results of this analysis, especially in the samples from the older animals. The table below, provided by the sponsor, summarizes those results.

Study 03-0462Pga
rhGAA Activity Assay Results, ug/g wet tissue

		Heart	Quadriceps	Triceps	Psoas	Diaphragm
Group	Dose (mg/kg)	ug/g	ug/g	ug/g	ug/g	ug/g
2	3 Months Vehicle	0.000	0.249	0.000	0.000	0.000
1	3 Months 100 mg/kg	18.795	24.336	15.653	9.919	13.290
4	12 Months Vehicle	0.084	0.043	0.000	0.000	0.093
3	12 Months 100 mg/kg	44.192	18.914	12.257	4.900	14.340

Conclusion:

These findings suggest that older mice are less efficient in clearing accumulated glycogen. However, the older mice had a higher glycogen load at the start of the study. Therefore, it is difficult to determine if the results reflect a true reduction in efficacy. Results of the biodistribution analysis indicate that a higher level of uptake in cardiac muscle is apparent for the 12 month old mice but is not apparent for the 3 month old mice.

Study #04-0177pga

Title: Efficacy of 2000L rhGAA in the Pompe knockout mouse model

Drug Lot#:

Project/Test Article(s): rhGAA (2000L & 160L) **Lot Number:** 930018, xGA179, xGA180

Methods:

The purpose of this study was to evaluate the efficacy of two lots (early and late harvest) of rhGAA produced by the scaled-up, 2000L manufacturing process, relative to the product manufactured with the 160L scale.

Animals: rhGAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified.

Dosing: 0 or 100 mg/kg, weekly for 4 weeks via IV administration

Study design: Groups 1 -3 received 100 mg/kg of rhGAA. Groups 1 and 2 each received from one of two lots of product produced by the 2000L process. Group 3 received 100 mg/kg of product produced with the 160L process. The tables below illustrate the basic study design and sample collection. Animals were dosed weekly for a total of 4 doses. Animals were sacrificed 6-7 days after the final dose. Samples were analyzed using a biochemical colorimetric assay to determine the relative levels of rhGAA. The tables below, provided by the sponsor, illustrate the basic design of this study:

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route	Tissue collection
1	4	100	9.34	xGA179	Sodium phosphate, mannitol, polysorbate 80	weekly	IV	Heart Quadriceps Triceps Diaphragm Psoas
2	4		9.64	xGA180				
3	4		10	160L				
4	4		N/A	vehicle				

Sample Collection

Group #	Time Points	Tissue Collection
1	7 days after 4th dose	Serum, heart, quadriceps, triceps, diaphragm were collected for biochemical and Metamorph glycogen analysis
2		
3		
4		

Results/conclusion:
Analytical results demonstrated that there was no detectable difference in efficacy between the products from the two different manufacturing processes. The table below, provided by the sponsor, summarizes these results for each group.

Study 04-0177Pga: Final data mg/g wet tissue

Test Article	Heart	Quadriceps	Triceps	Psoas	Diaphragm
rhGAA 2K lot xGA179	0.00	0.47	1.74	0.78	0.67
rhGAA 2K lot xGA180	0.00	0.58	0.91	2.03	0.55
rhGAA 160K lot 930018	0.00	0.00	3.08	1.85	0.00
Vehicle	19.81	7.84	10.23	7.70	7.97

Study #04-0297pga

Title: Investigation of the efficacy of processed rhGAA in Pompe knockout mice

Drug Lot#:

Project/Test Article(s):	Lot Number:
Myozyme	GA139 (Myozyme)
Processed rhGAA	GW12400008 (Processed rhGAA)

Methods:
Animals: rhGAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified, 5-6 months old.
Dosing: 0 or 60 mg/kg, administered IV weekly for 4 weeks

Study design: 3 groups were designated, each containing 4-5 animals. Group 1 received vehicle, group 2 received 60 mg/kg Alglucosidase alfa (rhGAA), group 3 received 60 mg/kg processed rhGAA species. Mice received 4 doses administered weekly for 4 weeks and were sacrificed 7 days after the final dose.

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	4	0	N/A	Vehicle	— sodium phosphate, pH —	4 weekly doses	IV
2	5	60	5.0 mg/ml	Myozyme	mannitol, — polysorbate 80		
3	5		4.83 mg/ml	Processed rhGAA preparation	— sodium phosphate, pH —		

[1] All mice received diphenhydramine (DPH, 5mg/kg) IP 15 minutes prior to dose #3 and 4

Sample Collection

Group #	Time Points	Tissue Collection
1	All mice sacrificed 7 days after the last dose	Serum, Heart, quadriceps, triceps, diaphragm, psoas, liver
2		
3		

The processing of the rhGAA modifies the terminal glycan and mannose-6-phosphate residues resulting in decreased binding to the mannose-6-phosphate receptor.

Results:

No remarkable findings attributable to the test article were reported for the in-life portion of the study.

Results of biochemical analyses: Significant glycogen clearance was observed for all samples from animals that received rhGAA relative to control. The reduction in cardiac muscle after treatment with Alglucosidase alfa was 95%, 54% in quadriceps, 65% in triceps, 47% in psoas muscles. The samples from animals treated with the processed species of rhGAA showed a much smaller response: 26% reduction in cardiac muscle, 27% reduction in quadriceps, 38% reduction in triceps and 23% reduction in psoas. Thus, it appears that the processed rhGAA species is less effective in glycogen clearance than Alglucosidase alfa.

Study 04-0297Pga: Final Data; Glycogen, mg/g wet tissue								
Group	Heart	Quadriceps	Triceps	Psoas	Heart stdev	Quadriceps stdev	Triceps stdev	Psoas stdev
60 mg/kg Vehicle	34.60	12.95	13.65	10.24	4.589	2.307	2.257	2.621
60 mg/kg Processed rhGAA	25.60	9.41	8.45	7.90	3.628	1.207	0.935	2.081
60 mg/kg rhGAA	1.69	5.96	4.75	5.46	2.585	0.814	0.649	1.340

Conclusion:

The processed rhGAA species is less effective in glycogen clearance than Alglucosidase alfa. This change in effectivness is most likely due to —

— during processing.

Study #05-0271pga

Title: Efficacy of 2000L and 160 L rhGAA in the Pompe knockout mouse model

Drug Lot#:

Project/Test Article(s): rhGAA (Myozyme)

Lot Number: 160L #930018
2000L #4573352
2000L #5744693

Methods:

This study was performed for the purpose of evaluating the efficacy of rhGAA from two different manufacturing processes (160L and 2000L scale manufacturing processes). Animals were dosed with 0 or 100 mg/kg of material taken from one of three lots of rhGAA weekly for 4 weeks. Tissue samples were taken at sacrifice and glycogen levels were determined biochemically. Histological analysis was not performed

Animals: rhGAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified, 4 months old.

Dosing: 0 or 100 mg/kg, administered weekly via IV infusion, for 4 weeks

Study design: Animals were divided into 6 groups of 6 mice per group. Group 1 received vehicle only, Group 2and 3 received 100 mg/kg of one of two lots of rhGAA manufactured by the 2000L manufacturing process, Group 4 received 100 mg/kg rhGAA manufactured by the 160L process. Samples were collected for biochemical and morphometric analysis. However, the morphometric analysis was not performed. The samples were stored for future use as needed. The tables below, provided by the sponsor illustrate the basic study design and sample collection.

Group	# of Animals	Dose (mg/kg)	Conc (mg/ml)	Test Article	Vehicle	Dosing Regimen[1]	Dose Route
1	6	100	N/A	Vehicle	Sodium phosphate, mannitol, polysorbate 80	weekly	IV
2	6		10	rhGAA (lot #4573352)			
3	6		10	rhGAA (lot # 5744693)			
4	6		10	rhGAA (lot #930018)			

[1]Note: All animals received 5mg/kg *diphenhydramine* IP approximately 15 minutes prior to treatment at the third dose and thereafter.

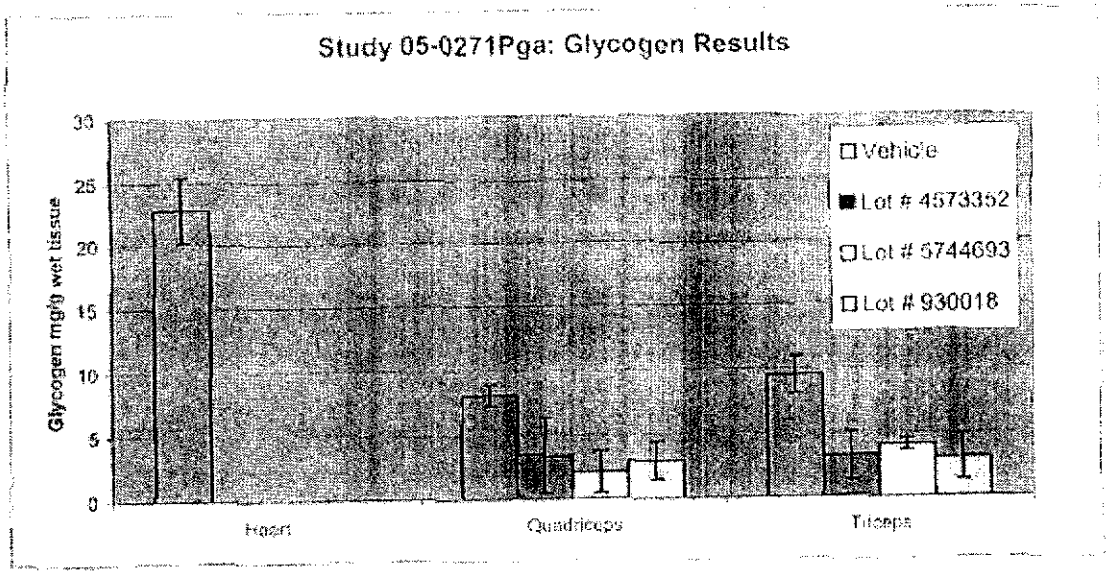
Sample Collection

Group #	Time Points	Tissue Collection
1	7 days after 4th dose	Serum, heart, quadriceps, triceps, diaphragm and psoas were collected for biochemical and Metamorph glycogen analysis.
2		
3		
4		

Results:

One animal from group 2 was found dead 5 days after the 3rd dose. The cause of death was not determined. No other in-life observations attributable to the test article were reported. Biochemical analysis detected no significant difference in efficacy of 100 mg/kg between products from the two processes. The table and graphic below, provided by the sponsor, illustrate the results of this analysis.

Study 05-0271Pga: Final Data; glycogen, mg/g wet tissue						
Group	Heart	Quadriceps	Triceps	Heart stdev	Quadriceps stdev	Triceps stdev
Vehicle	22.86	8.09	9.50	2.634	0.917	1.440
rhGAA 100 mg/kg 2000L Lot # 4573352	0.00	3.43	3.24	0.000	2.023	1.917
rhGAA 100 mg/kg 2000L Lot # 5744693	0.00	2.10	4.10	0.000	1.643	0.443
rhGAA 100 mg/kg 160L Lot # 930018	0.00	2.94	3.07	0.000	1.484	1.782



Conclusion:
Biochemical analysis showed no significant difference in efficacy among the three lots of rhGAA. However, these results are in contrast to results of study #05-0414pga that investigated potential differences in the pharmacokinetics among these lots. That study demonstrated that the lots from the 160L scale and the 2000L scale are not pharmacokinetically equivalent. The glycogen clearance is not a sufficiently sensitive measure for detecting inter-lot variability or comparability.

2.6.2.3 Secondary pharmacodynamics

Study #03-0665pga

Title: The investigation of the immune response in Pompe mice treated with rhGAA

Drug Lot#:

Project/Test Article(s): rhGAA Lot Number: GA096

Methods:

The purpose of this study was to evaluate the nature of the immune response mounted by the mice being treated with rhGAA. Rats and mice have been shown to develop a hypersensitivity reaction to rhGAA, thus requiring treatment of the animals with an anti-histaminic agent prior to dosing with rhGAA. To investigate the nature of the hypersensitivity reaction, 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.
Animals: rhGAA knockout mouse, Pompe KO ($6^{neo}/6^{neo}$), gender not specified, 3-4 months old
Dosing: 10 mg/kg weekly for 3 or 8 weeks.
Study design: Three groups of mice were designated: Group 1 received vehicle, Group 2 received 20 mg/kg rhGAA weekly for three doses, Group 3 received 20 mg/kg weekly for 8 weeks. Mice in group 3 were treated with diphenhydramine prior to receiving the test article for doses 3-8.

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen[1]	Dose Route
1	10	N/A	N/A	Vehicle	— sodium phosphate — mannitol, — polysorbate 80	Weekly x3 x8	IV
2	10	20	5	rhGAA		Weekly x 3	
3	10					Weekly x 8	

[1]Note: All animals in Group 1 and 3 received 5mg/kg diphenhydramine IP 15-20 minutes prior to treatment at the third through seventh dose.

Group #	Time Points	Tissue Collection
1	Pre-dose, and within 2-3 minutes of dose #3 and dose #8	Serum and plasma
2	Pre-dose and within 2-3 minutes of dose #3 Mice sacrificed after the 3rd dose	
3	Pre-dose and within 2-3 minutes of dose #8 Mice sacrificed after the 8th dose	

One deviation occurred: on 11/03/03, pre-dose samples were collected and accidentally discarded. The text does not state which samples were discarded or if all the pre-dose samples were discarded.

Results:

- 2 mice from group 3 were found dead after dose 4.
- 2 mice from group 3 appeared to be lethargic after dose 8.
- One mouse from group 2 appeared lethargic and cold to touch after dose 3.
- Animals receiving vehicle alone showed IgG titers below 100, no detectable specific anti-drug antibody, no detectable IgE titer.
- No detectable IgE titer was reported for all treatment groups.
- Moderate levels of histamine were detected in 2 of 4 mice in group 2 after the 3rd dose. Significantly elevated histamine levels were detected in group 3 after the 8th dose. Significant levels of IgG and IgG1 titers were reported in all mice receiving rhGAA. Indicating the formation of anti-drug antibodies

Histamine 3rd Dose			8th Dose		
Animal ID	*Group*	*Histamine (ng/mL)*	*Animal ID*	*Group*	*Histamine (ng/mL)*
1	1	0.76	6	1	101.23
2	1	67.50	7	1	66.55
3	1	0.86	26	1	682.50
11	2	QNS	22	2	935.00
12	2	509.50	29	2	1346.00
13	2	55.15	30	2	1678.00
14	2	101.05			
15	2	46.60			

Study 03-0665Pga rhGAA specific total IgG Antibody ELISA

	Group	Dose (mg/kg)	Timepoint	Titer
4	1	Vehicle	3	less than 100
5	1	Vehicle	3	less than 100
8	1	Vehicle	8	less than 100
9	1	Vehicle	8	less than 100
10	1	Vehicle	8	less than 100
16	2	20 mg/kg week	3	1:4783
17	2	20 mg/kg week	3	1:343
18	2	20 mg/kg week	3	1:4739
19	2	20 mg/kg week	3	1:2350
20	2	20 mg/kg week	3	1:5278
21	3	20 mg/kg week	8	1:9515
23	3	20 mg/kg week	8	1:361759
24	3	20 mg/kg week	8	Less than 100
25	3	20 mg/kg week	8	1:19131

Study 03-0665Pga rhGAA specific total IgG1 Antibody ELISA

	Group	Dose (mg/kg)	Timepoint	Titer
4	1	Vehicle	3	no detectable titer
5	1	Vehicle	3	no detectable titer
8	1	Vehicle	8	no detectable titer
9	1	Vehicle	8	no detectable titer
10	1	Vehicle	8	no detectable titer
16	2	20 mg/kg week	3	1:10,000
17	2	20 mg/kg week	3	1:5,000
18	2	20 mg/kg week	3	1:20,000
19	2	20 mg/kg week	3	1:20,000
20	2	20 mg/kg week	3	1:10,000
21	3	20 mg/kg week	8	1:320,000
23	3	20 mg/kg week	8	1:1,280,000
24	3	20 mg/kg week	8	no detectable titer
25	3	20 mg/kg week	8	1:320,000

Conclusion:

These data are consistent with hypersensitivity response in the Pompe knockout mice.

2.6.2.4 Safety pharmacology No studies performed.

2.6.2.5 Pharmacodynamic drug interactions No studies performed.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Tables omitted per Dr. Choudary

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 semilogarithmic plot of concentration versus time was constructed from data produced by each of the 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-life was on the order of 2-3.5 hours for monkey, rat 1-2 hours, Pompe knockout mouse 1-2 hours, CD-1 mouse approximately 75 minutes, and beagle dog approximately 1.5 to 2 hours. 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 and AUC/dose. Both males and females in the high dose group showed significantly higher elimination half-life relative to the other dose groups, females for days 1-85 and male for days 1-169. The table below, provided by Dr. Anil Rajpal summarizes the human pharmacokinetic parameters. ,

Table 1. Single-dose PK Parameters in Study 1602

Single-dose PK Parameters in Study 1602 (Mean ± SD)						
Dose [mg/kg]	N	Cmax [mcg/mL]	$AUC_{0-\infty}$ [mcg*hr/mL]	$t_{1/2,\lambda z}$ [hr]	Cl [mL/hr/kg]	Vss [mL/kg]
20	5	162 ± 31	811 ± 141	2.3 ± 0.4	25.2 ± 3.8	96.3 ± 15.7
40	8	276 ± 64	1781 ± 520	2.9 ± 0.5	24.1 ± 6.5	119.0 ± 28.1
Values above were calculated by this reviewer from concentration time data in dataset PKX_PL_1 PK 1602 using WinNonlin.						
Note: Plasma GAA levels were determined based on an activity assay.						

PK studies were conducted to investigate the potential differences between the 160 liter manufacturing process and the 2000 liter process. The results demonstrated that the ninety percent confidence intervals for either AUC_{last} or $AUC_{0-\infty}$ ratios between the 160L lot and the 2000 L lots evaluated in this study were not within a pre-determined acceptability range (80% to 125%). These data indicate that the pharmacokinetic equivalence was not established between the two lots.

Biodistribution:

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 liter process was compared to that from the 2000 liter process, there appeared to be a higher magnitude of uptake in the liver for the 2000 liter product. When a lot from — of the 2000 liter scale was compared to one from the — greater rhGAA activity levels were noted in the liver relative to those for the — — product. Changes in — among lots and during the course of manufacture might explain both the PK and biodistribution differences detected in these studies.

2.6.4.2 Methods of Analysis

Please refer to individual study reviews.

2.6.4.3 Absorption

The following studies were performed to evaluate non-clinical pharmacokinetics:

Study title: Pharmacokinetics and biodistribution of three formulations of rhGAA in the GAA knockout mouse

Study #: 02-0710Pga

The purpose of this study was to evaluate the pharmacokinetics (PK) and biodistribution (BD) of three sources of rhGAA: Genzyme lot# GA028, GA063 and lot#930018. Lot #930018 was produced by the 160L manufacturing process and was the same material used in the clinical study

Methods: For PK analysis: Mice (n=4/group) received a single dose of rhGAA via the tail vein at a dose of 20 mg/kg. Samples were collected via tail vein at 2, 5, 10, 15, 30, 60, 120 and 240 minutes post-dosing. For BD analysis: Mice (n=12/group) received test article via tail vein injection and 3 mice were sacrificed at each of the following 4 times: 1, 4, 8 and 24 hours post-dosing. Tissues were collected for determination of rhGAA activity. Whole tissue analysis of rhGAA activity was determined for three of 9 tissues collected. The basic study design is summarized in the tables below, supplied by the sponsor:

Study Design Table

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article (lot #)	Reconstitution buffer	Dosing Regimen	Dose Route
1	4	20	5	GA028	0.2% Tween-80 in WFI	Single dose	IV
2	4			GA063	WFI		
3	4			930018	WFI		
4	12			GA028	0.2% Tween-80 in WFI		
5	12			GA063	WFI		
6	12			930018	WFI		

Sample Collection

Group #	Time Points	Tissue Collection
1	2,5,10,15,30,60, 120,240 minutes	Serum
2		
3		
4	1,4,8,24 hours	Heart, lungs, diaphragm, liver, kidney, spleen, psoas, quadriceps, triceps
5		
6		

Results: The following table, provided by the sponsor, summarizes the results of the PK analysis.

	GA028	GA063
T ½ alpha (min)	13.4	14.4
T ½ beta (min)	143.8	141
Cl (ml/min/kg)	.37	.36
MRT (min)	182	192
AUC (min*ug/ml)	53141	55629
AUC/dose (min*ug/ml/mg/kg)	2657.1	2781.5
Vss (ml/kg)	68.6	69.1
Rsq	.98	.99

This table does not contain data for lot #930018 (160L manufacturing process). The sponsor states that there was a clear anomaly in the data from that lot so the results were not interpretable. A subsequent study (#02-0779) was planned to obtain appropriate data. However, the BD portion of the study was completed as well as PK analysis for the other 2 lots (GA028 and GA063).

- The PK data for the two remaining lots did not yield a straight line, indicating more that one disposition phase. The curves show behavior of a two compartment model and show no difference between the two formulations.

- For the BD comparison among the lots, all three appeared to distribute in a similar manner. The majority of activity is found in the liver at 1 hour for all test articles (14-23%). At 1 hour, 0.24 to 0.31% activity is found in the heart and 0.05 to 0.07% in the quadriceps muscle. The rate of decline in each tissue appears to be similar for all three test articles, with little decrease seen in 24 hours. The BD data is summarized in the table below, provided by the sponsor.

% of injected dose in three tissues

GA028				
	1 hour	4 hour	8 hour	24 hour
Liver	14.76	25.18	29.97	22.04
Heart	0.26	0.19	0.17	0.16
Quad	0.07	0.06	0.05	0.02
GA063				
	1 hour	4 hour	8 hour	24 hour
Liver	20.37	26.06	25.20	23.12
Heart	0.24	0.16	0.14	0.17
Quad	0.05	0.04	0.02	0.02
160L				
	1 hour	4 hour	8 hour	24 hour
Liver	23.21	31.45	35.35	31.04
Heart	0.31	0.21	0.15	0.21
Quad	0.06	0.03	0.02	0.04

Study conclusion:
The planned PK analysis was not performed for all three test articles due to data loss from lot#930018. The reasons for this loss were not described. PK analysis of the remaining two lots revealed parameters that were quite similar between the two preparations.
The BD analysis revealed that the three test articles had very similar distribution profiles.

Study title: Pharmacokinetics of rhGAA in the GAA knockout mouse

Study #: 02-0779Pga
Lot # : B[redacted]rhGAA lot #E1585AM03, Genzyme rhGAA lot# GA028 and Genzyme rhGAA lot #930018 (160L manufacturing process).

Methods: GAA knockout mice were separated into 3 groups of 4 mice each. The test article was administered by IV injection via the tail vein in a single dose of 20 mg/kg. Samples were collected at 2, 5, 10, 15, 30, 60, 120 and 240 minutes post-dosing.

Study Design Table

Group	# of Animals	Dose mg/kg	Conc mg/ml	Test Article[1]	Reconstitution buffer	Dosing Regimen	Dose Route
1	5	20	5	A	WFI	Single dose	IV
2	5	20		B	WFI + Tween-80 in WFI		
3	5	20		C	WFI		

[1]This study was undertaken in a blinded manner with vial A = BI – rhGAA, vial B = Genzyme rhGAA, lot #GA028 and vial C = Genzyme rhGAA, lot #930018.

Sample Collection

Group #	Time Points	Tissue Collection
1	2.5,10.15,30,60, 120,240 minutes	Serum
2		
3		

Results:

Pharmacokinetics: The PK analysis results are summarized in the table below, provided by the sponsor:

	Genzyme rhGAA (GA028)	Genzyme 160 L rhGA (930018)	BI – hGAA (E1585AM03)
T ½ alpha (min.)	12.5	12.9	28.3
T ½ beta (min.)	97.7	113.9	106.5
Cl (mL/min/kg)	.45	.45	.58
MRT (min.)	131	149	110
AUC (min*ug/mL)	44217	44607	34731
AUC/dose (min*ug/mL/mg/kg)	2210.8	2230.4	1736.6
Vss (mL/kg)	60	67	64
Rsq	.96	.99	.98

- The semilogarithmic plot of concentration versus time does not result in a straight line, suggesting more than one dispositional phase. Concentration in tissues takes longer to

reach equilibrium with the plasma concentration. The curves generated from the data exhibit characteristics of a two compartment model with first order elimination.

Study conclusion:

AUC and terminal half-life of the BI – material differs from the other two drug preparations. Therefore, it appears that lot#GA028 has a similar PK profile to the 160L material but he BI – product is not comparable to the other two preparations.

Study title: Pharmacokinetics and biodistribution of two recent rhGAA lots of formulated bulk

Study #: 03-0370Pga

Methods: The purpose of this study was to evaluate the pharmacokinetics (PK) and biodistribution (BD) of two lots of rhGAA from the 160L manufacturing scale. The two lots in question are lot # 03TP021 and 03TP022. These lots showed a variation in — when compared to the reference standard. Groups of Pompe rhGAA knockout mice, aged 3-4 months received one of three preparations of rhGAA as a single dose of 20 mg/kg by IV infusion. The tables below summarize the study design and sample collection scheme.

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Study Design Table

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	4	20	5	03TP021	– Sodium Phosphate, pH – mannitol, – polysorbate 80	Single dose	IV
2	4		5	03TP022			
3	4		5.1	GAA1	– Sodium Phosphate, pH –		
4	12		5	03TP021	– Sodium Phosphate, pH – mannitol, – polysorbate 80		
5	12		5	03TP022			
6	12		5.1	GAA1	– Sodium Phosphate, pH –		

Sample Collection

Group #	Time Points	Tissue Collection
1	0, 2, 5, 10, 15, 30, 60, 120, 240 and 480 minutes	Serum
2		
3		
4	1, 4, 8 and 24 hours	Serum, heart, lungs, diaphragm, liver, kidney, spleen, psoas, quadriceps, triceps
5		
6		

Results: No deviations from the protocol occurred. No abnormalities were noted during the in-life portion of this study.

Pharmacokinetics: The results of the PK analyses are summarized in the table below, provided by the sponsor:

	GAA1	03TP021	03TP022
T ½ alpha (min)	23.0	10.2	11.7
T ½ beta (min)	161.2	72.2	78.4
Cl (ml/min/kg)	0.46	0.77	0.55
MRT (min)	203	94	107
AUC (min*ug/ml)	43764	25964	36066
AUC/dose (min*ug/ml/mg/kg)	2188.2	1298.2	1803.3
Vss (ml/kg)	93.9	72.2	59.1
Rsq	0.99	0.99	0.99

The curves produced by semilogarithmic plots of concentration versus time exhibit behavior consistent with a two compartment model and first order elimination. These results are in agreement with results from other PK studies. PK parameters among the three lots appear to be somewhat different, including longer half-life and AUC for the reference standard relative to the two 160 L process lots.
Biodistribution results: No significant differences in biodistribution are reported.

Study conclusion:
The two lots produced by the 160L process that were tested in this study show shorter half-life and lower AUC relative to the reference standard. Although they show similar distribution patterns, they are not comparable to the reference standard and were not released for use in the clinic without further investigation.

Study title: Pharmacokinetics of 160L rhGAA in CD:1®(ICR)BR mice

Study #: 04-0144Pga

Methods: The purpose of this study was to evaluate the pharmacokinetics of rhGAA (160L process).Three groups of 4 mice each received 10, 20 or 40 mg/kg. Serum was collected at time 0, 10, 30, 60, 180 and 360 minutes post-dosing.

Project/Test Article(s): rhGAA 160L **Lot Number:** 751295

The tables below, provided by the sponsor, summarize the study design:

Study Design Table

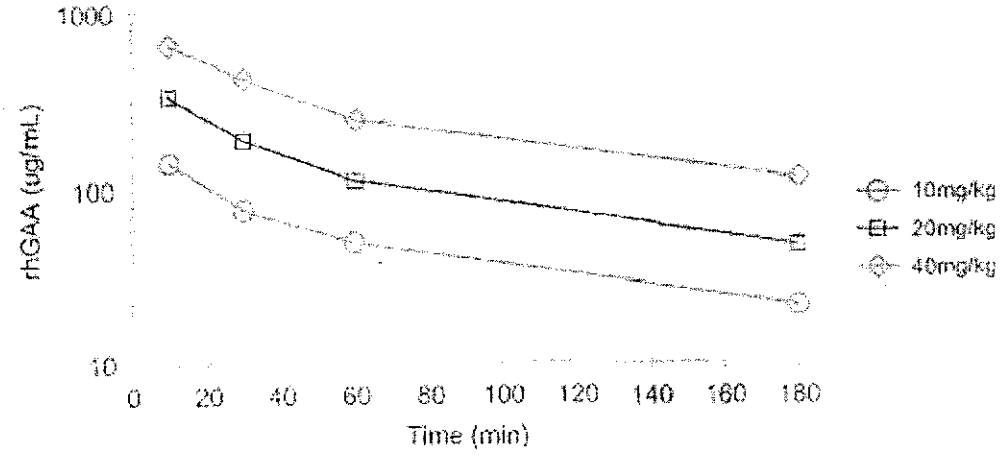
Group	# of Animals	Dose mg/kg	Conc mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	4	10	1.25	rhGAA	— sodium phosphate, — mannitol, — polysorbate 80	Single dose	IV
2	4	20	2.5				
3	4	40	5				

Sample Collection

Group #	Time Points	Tissue Collection
1	0, 10, 30, 60, 180 and 360 minutes post dose	Serum
2		
3		

Results:
The 360 minute serum timepoints were lost due to equipment malfunction. Therefore, the PK analyses were performed using data out to the 180 timepoint only. No abnormal clinical observations were reported for the in-life portion of this study.

PK parameters indicate a linear relationship among doses. No evidence of saturation kinetics was observed.



	10mg/kg	20mg/kg	40mg/kg
Elimination T ½ (min)	84.28 ± 8.4	81.13 ± 4.4	83.45 ± 15.0
Cl (ml/min/kg)	0.83 ± 0.16	0.70 ± 0.10	0.64 ± 0.09
MRT (min)	102.9 ± 10.8	99.2 ± 3.7	108.8 ± 22.3
AUC (min*ug/ml)	12474.8 ± 2624.3	28982.1 ± 4313.9	63694.7 ± 8675.7
AUC/dose (min*ug/ml/mg/kg)	1247.5 ± 262.1	1449.1 ± 215.7	1592.4 ± 216.9
Vss (ml/kg)	84.1 ± 11.7	69.4 ± 8.4	68.4 ± 10.8
Rsq	0.98 ± 0.01	0.95 ± 0.04	0.95 ± 0.04

Study conclusion:
At doses up to 40 mg/kg, there was no evidence of saturation kinetics as the clearance of the drug followed a first order process. There is no information on the gender of the animals used. Therefore, no conclusions can be drawn about potential gender differences in PK.

Study title: Repeat pharmacokinetics of rhGAA 2000 liter in the Pompe knockout mouse

Study #: 04-0424Pga

Methods:
The purpose of this study was to assess the pharmacokinetics of rhGAA produced by the 2000L manufacturing process compared to that produced by the 160 L process. Two lots of the 2000 L process were used: one from the — and one from the — Two groups were designated for each drug lot. Animals were dosed at 20 mg/kg and samples were collected for PK analysis at times from 2 minutes post-dose to 480 minutes post-dose. Early time points up to 30 minutes were taken from groups 1, 3 and 5. Late time points were taken

from groups 2, 4 and 6. Animals bled for early time points were randomly paired with animals bled for late time points to generate complete serum concentration/time data sets
The tables below, provided by the sponsor, illustrate the basic study design.

Study Design Table

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route	Tissue collection
1	5	20	5.074	rhGAA	sodium phosphate, mannitol, polysorbate 80	Single dose	IV	Serum
2	5							
3	5		5.172	rhGAA				
4	5							
5	5		5	rhGAA				
6	5							

Sample Collection

Group #	Time Points	Tissue Collection
1	2, 5, 10, 15 and 30 minutes post dose	Serum
2	60, 120, 240 and 480 minutes post dose	
3	2, 5, 10, 15 and 30 minutes post dose	
4	60, 120, 240 and 480 minutes post dose	
5	2, 5, 10, 15 and 30 minutes post dose	
6	60, 120, 240 and 480 minutes post dose	

Results:
The results of data analysis are summarized in the tables below, provided by the sponsor. Differences in AUC were noted among the three preparations of rhGAA. Statistical significance was achieved for the difference in AUC between both lots from the 2000L process relative to the lot produced by the 160L process. The curves resulting from the semilogarithmic plot of concentration versus time exhibit characteristics of a two-compartment model with first order elimination.

Study conclusion:
Ninety percent confidence intervals for either AUC_{last} or $Auc_{0-\infty}$ ratios between the 160L lot and the 2000 L lots evaluated in this study were not within a pre-determined acceptability range (80% to 125%). These data indicate that the pharmacokinetic equivalence was not established. Further analysis of the data was performed. The data were re-analyzed based upon individual animals necessitating the analysis of early and late time points separately. The following tables, provided by the sponsor, describe the results of these analyses.

Pharmacokinetic Parameters Using Early Time Points Only (2-30 minutes)

	2000 L rhGAA (xGA179)	2000 L rhGAA (xGA180)	160 L rhGAA (930018)
Elimination T ½ (min)	33.2 ± 5.35	33.5 ± 6.74	34.6 ± 9.41
Cl (ml/min/kg)	1.04 ± 0.13	0.86 ± 0.12	0.96 ± 0.22
MRT (min)	47.2 ± 4.34	48.2 ± 9.09	49.6 ± 13.5
$AUC_{0-\infty}$ (min*ug/ml)	19390 ± 2469	23568 ± 3552	21851 ± 5307
AUC/dose (min*ug/ml/mg/kg)	969 ± 123	1178 ± 178	1093 ± 265
Vss (ml/kg)	48.9 ± 1.39	40.8 ± 1.97	45.2 ± 3.14
Rsq	0.85 ± 0.15	0.98 ± 0.02	0.91 ± 0.14
90% CI of ratio to 160L. Parameter: AUClast			
Upper	100.47	120.06	-
Lower	85.37	102.01	-

Pharmacokinetic Parameters Using Late Time Points Only (60-480 minutes)

	2000 L rhGAA (xGA179)	2000 L rhGAA (xGA180)	160 L rhGAA (930018)
Elimination T ½ (min)	149 ± 24.8	152 ± 22.3	142 ± 10.8
Cl (ml/min/kg)	0.56 ± 0.04	0.38 ± 0.03	0.41 ± 0.05
MRT (min)	182 ± 43.6	163 ± 25.7	146 ± 22.9
$AUC_{0-\infty}$ (min*ug/ml)	35816 ± 2382	57069 ± 5814	49385 ± 5221
AUC/dose (min*ug/ml/mg/kg)	1791 ± 119	2853 ± 291	2469 ± 261
Vss (ml/kg)	101 ± 19.8	57.0 ± 4.17	59.8 ± 11.9
Rsq	0.91 ± 0.08	0.99 ± 0.01	0.99 ± 0.01
90% CI of ratio to 160L. Parameter: AUClast			
Upper	76.13	124.43	-
Lower	63.58	103.92	-

Study title: Pharmacokinetics of Alglucosidase alfa produced at the 2000L scale

Study #: 05-0414Pga

Lot #: 160L: 930018, 2000L: 5744693 and 4573352

Methods:

Three -4 month old Pompe knockout mice, 12 per group (gender not specified), were used for this study. Serum was collected for PK analysis from all groups at 5, 15, 30, 60, 120, 240, and 480 minutes post-dose.

Study Design Table

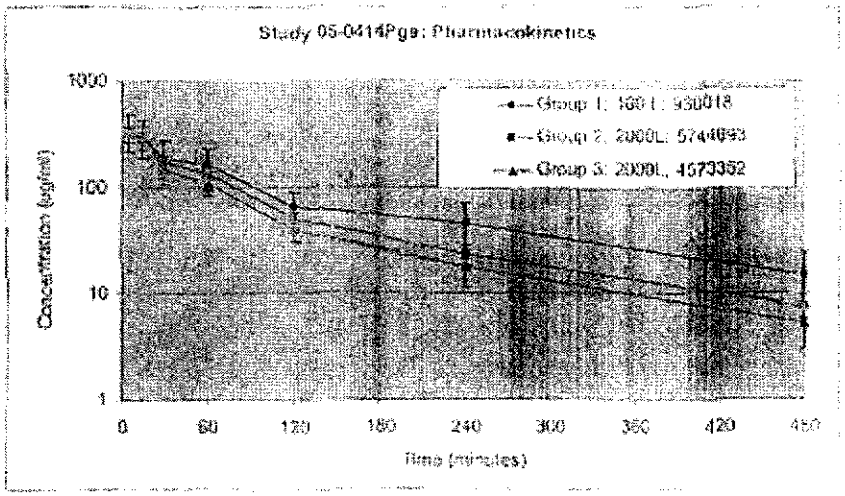
Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	12	20	5	lot 930018 (160L)	Sodium Phosphate, mannitol, polysorbate 80	Single dose	IV
2	12			lot 5744693 (2000L)			
3	12			lot 4573352 (2000L)			

Sample Collection

Group #	Time Points	Tissue Collection
1	5, 15, 30, 60, 120, 240 and 480 minutes post dose	Serum
2		
3		

Restults:

The summary tables for this study as provided by the sponsor are appended below.



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	Lot 030018	Lot 5744693	Lot 4573352
HL_lambda_z (min)	330.8 ± 39.3	115.5 ± 32.4	107.9 ± 17.5*
Cl (ml/min/kg)	0.57 ± 0.17	0.73 ± 0.09*	0.87 ± 0.2*
MRT (min)	163.8 ± 57.0	114.0 ± 26.1*	100.3 ± 13.2*
$AUC_{0-\infty}$ (min*ug/ml)	38530.5 ± 13144.2	27633.4 ± 3169.2*	24165.3 ± 5955.8*
AUC/dose (min*ug/ml/mg/kg)	1926.5 ± 657.2	1381.7 ± 158.3*	1208.3 ± 297.8*
Vss (ml/kg)	91.1 ± 39.8	82.6 ± 15.1	86.2 ± 17.8
Rsq	0.92 ± 0.14	0.98 ± 0.02	0.98 ± 0.01
90% CI of ratio to 160L, Parameter: $AUC_{0-\infty}$			
Upper	-	63.31	54.26
Lower	-	88.26	75.64
90% CI of ratio to 160L, Parameter: AUClast			
Upper	-	64.95	57.06
Lower	-	89.96	79.03

Students t-test: *p<0.05 between 2000L lots and 160L control

Conclusion:

The determination was made that, under the conditions of this study, the rhGAA produced by the 160L manufacturing process is not equivalent to that produced by the 2000L process.

2.6.4.4 Distribution

The tables below, provided by the sponsor, summarize the tissue distribution results for various lots of rhGAA as determined for the Pompe GAA knockout mouse. The product produced by the 2000L scale manufacturing process appears to undergo greater liver uptake than the product produced by the 160L process.

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2.6.5.5 **Organ Distribution**

Test Article: alglucosidase alfa (Myozyme)				
Study No.: 02-0710Pga (summarized in Section 2.6.4.4.1)		**Report Location: Section 4.2.2.2**		
Species: GAA knockout mice (6^{mo}-7^{mo}) Gender (M/F) / No. of animals: Male/Female/48 Feeding Condition: Ad Libitum Formulation: *Sodium Phosphate, mannitol* for GA028) *Sodium Phosphate, mannitol* polysorbate-80 (lots GA063 and 930018) Method of Administration: IV Dose (mg/kg): 20 Sampling time: 1, 4, 8 and 24 hours				
Tissues/organs:	% injected dose			
	1 hour	4 hours	8 hours	24 hours
Myozyme (30 L/60 L scale) lot GA028				
Liver	14.76 ± 2.98	25.18 ± 3.91	29.97 ± 3.54	22.04 ± 2.27
Heart	0.26 ± 0.01	0.19 ± 0.04	0.17 ± 0.05	0.16 ± 0.03
Quadriceps	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.02 ± 0.003
Myozyme (30 L/60 L scale) lot GA063				
Liver	20.37 ± 3.30	26.06 ± 3.99	25.20 ± 2.17	23.12 ± 3.79
Heart	0.24 ± 0.02	0.18 ± 0.04	0.14 ± 0.004	0.17 ± 0.02
Quadriceps	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.002	0.02 ± 0.01
Myozyme (160 L scale) lot 930018				
Liver	23.21 ± 2.33	31.45 ± 2.64	35.35 ± 4.77	31.04 ± 5.38
Heart	0.31 ± 0.14	0.21 ± 0.02	0.15 ± 0.02	0.21 ± 0.05
Quadriceps	0.06 ± 0.02	0.03 ± 0.005	0.02 ± 0.002	0.04 ± 0.01

Test Article: alglucosidase alfa (Myozyme)				
Study No.: 03-0370Pga (summarized in Section 2.6.4.4.2)		**Report Location: Section 4.2.2.2**		
Species: GAA knockout mice (6^{mo}-7^{mo}) Gender (M/F) / No. of animals: Male/Female/48 Feeding Condition: Ad Libitum Formulation: sodium phosphate, mannitol polysorbate-80 (03TP021 and 03TP022) sodium phosphate, (GAA1) Method of Administration: IV Dose (mg/kg): 20 Sampling time: 1, 4, 8 and 24 hours				
Tissues/organs:	% injected dose			
	1 hour	4 hours	8 hours	24 hours
Genzyme rhGAA drug substance lot 03TP021 (160 L scale)				
Liver	46.5 ± 3.3	45.6 ± 11.2	59.9 ± 5.6	50.1 ± 15.9
Heart	0.19 ± 0.03	0.13 ± 0.04	0.13 ± 0.02	0.13 ± 0.02
Quadriceps	0.050 ± 0.014	0.026 ± 0.002	0.018 ± 0.005	0.013 ± 0.003
Kidney	0.49 ± 0.07	0.22 ± 0.07	0.29 ± 0.14	0.19 ± 0.03
Spleen	0.83 ± 0.13	0.83 ± 0.03	0.72 ± 0.10	1.02 ± 0.13
Triceps	0.026 ± 0.006	0.007 ± 0.002	0.006 ± 0.002	0.006 ± 0.001
Genzyme rhGAA drug substance lot 03TP022 (160 L scale)				
Liver	33.8 ± 0.5	36.0 ± 6.5	38.8 ± 1.5	40.2 ± 5.7
Heart	0.27 ± 0.02	0.19 ± 0.03	0.16 ± 0.08	0.11 ± 0.01
Quadriceps	0.055 ± 0.014	0.033 ± 0.008	0.023 ± 0.007	0.016 ± 0.001

Test Article: alglucosidase alfa (Myozyme)				
Study No.: 03-0370Pga (summarized in Section 2.6.4.4.2)	**Report Location: Section 4.2.2.2**			
Kidney	1.19 ± 0.56	0.34 ± 0.03	0.30 ± 0.19	0.18 ± 0.04
Spleen	0.89 ± 0.31	0.96 ± 0.12	0.72 ± 0.18	0.78 ± 0.22
Triceps	0.022 ± 0.005	0.015 ± 0.001	0.011 ± 0.007	0.010 ± 0.004
GAA1 (30 L/60 L scale reference standard)				
Liver	29.5 ± 4.3	33.4 ± 4.4	33.8 ± 7.1	30.4 ± 7.3
Heart	0.28 ± 0.05	0.15 ± 0.03	0.12 ± 0.02	0.13 ± 0.01
Quadriceps	0.053 ± 0.028	0.031 ± 0.004	0.029 ± 0.022	0.022 ± 0.008
Kidney	0.99 ± 0.59	0.33 ± 0.13	0.20 ± 0.04	0.17 ± 0.01
Spleen	0.91 ± 0.13	1.09 ± 0.34	0.75 ± 0.22	0.81 ± 0.14
Triceps	0.020 ± 0.014	0.012 ± 0.002	0.010 ± 0.005	0.008 ± 0.002

Test Article: alglucosidase alfa (Myozyme)

Study No.: 04-0152Pga (summarized in Section 2.6.4.4.3) **Report Location: Section 4.2.2.3**

Species: GAA knockout mice (6[?]/6[?])

Gender (M/F) / No. of animals: Male/Female/36

Feeding Condition: Ad Libitum

Formulation: — sodium phosphate, — mannitol, — polysorbate-80

Method of Administration: IV

Dose (mg/kg): 20

Sampling time: 1, 4, and 8 hours

Tissues/organs:	% injected dose		
	1 hour	4 hours	8 hours
Genzyme rhGAA early harvest lot xGA179 (2000 L scale)			
Liver	40.71 ± 9.1	41.59 ± 11.8	57.60 ± 9.2
Heart	0.48 ± 0.15	0.26 ± 0.04	0.25 ± 0.07
Quadriceps	0.08 ± 0.030	0.05 ± 0.015	0.05 ± 0.034
Spleen	0.91 ± 0.15	0.83 ± 0.07	1.20 ± 0.12
Triceps muscle	0.02 ± 0.001	0.02 ± 0.003	0.02 ± 0.004
Genzyme rhGAA late harvest lot xGA180 (2000 L scale)			
Liver	34.68 ± 1.5	45.29 ± 1.5	39.64 ± 8.0
Heart	0.43 ± 0.12	0.37 ± 0.05	0.20 ± 0.05
Quadriceps	0.08 ± 0.009	0.05 ± 0.008	0.03 ± 0.004
Spleen	1.30 ± 0.07	1.69 ± 0.19	0.89 ± 0.37
Triceps muscle	0.02 ± 0.007	0.02 ± 0.007	0.01 ± 0.003

Test Article: alglucosidase alfa (Myozyme)			
Study No.: 04-0152Pga (summarized in Section 2.6.4.4.3)	Report Location: Section 4.2.2.3		
Tissues/organs:	% injected dose		
	1 hour	4 hours	8 hours
Myozyme (Genzyme rhGAA) lot 930018 (160 L scale)			
Liver	33.84 ± 2.1	26.39 ± 1.7	38.88 ± 9.5
Heart	0.38 ± 0.05	0.27 ± 0.05	0.27 ± 0.08
Quadriceps	0.06 ± 0.015	0.07 ± 0.050	0.06 ± 0.060
Spleen	1.20 ± 0.32	1.32 ± 0.07	1.02 ± 0.39
Triceps muscle	0.02 ± 0.012	0.02 ± 0.016	0.02 ± 0.024

Study title: Pharmacokinetics and biodistribution of rhGAA 2000L in the Pompe knockout mouse

Study #: 04-0152
Lot#: 160L: 930018, 2000L: GA179 and GA180
Methods:
The objective of this study was to evaluate the pharmacokinetics and biodistribution of rhGAA produced by the 2000L scale process in the Pompe GAA knockout mouse model. Three lots of rhGAA were compared: 2 lots at the 2000L scale (one from the — and one from the —, and one lot from the 160L scale.

Study Design Table

Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route	Tissue collection
1	4	20	5.074	xGA179	— sodium phosphate, — mannitol, — polysorbate 80	Single dose	IV	Serum
2	4		5.172	xGA180				
3	4		5	160L				
4	9		5.074	xGA179				Serum, heart, quadriceps, triceps, psoas, diaphragm, liver, spleen, lungs, kidney
5	9		5.172	xGA180				
6	9		5	160L				

Sample Collection

Group #	Time Points	Tissue Collection
1	2, 5, 10, 15, 30, 60 minutes post dose	Serum
2		
3		
4	1, 4 and 8 hours post dose (n=3 per time point). Serum also collected from 4 hour BD animals at 2 hours.	Serum, Heart, quadriceps, triceps, diaphragm, psoas, liver, kidneys, spleen, lungs
5		
6		

Results:
No PK analysis was conducted for this study due to extensive sample loss. Biodistribution analysis was conducted on liver, spleen, heart, quadriceps, and triceps. A high degree of variation was observed in rhGAA distribution for all three lots. No statistical significance between values was found. Biological significance of these results is questioned.

Study conclusion:
No PK parameters were determined due to sample loss. The high degree of variation in the biodistribution data does not allow any clear conclusions to be drawn regarding differences in behavior among the lots examined.

Study title: Pharmacokinetics and biodistribution of Alglucosidase alfa produced at the 2000L scale
Study #: 05-0252

Lot#: 160L: 930018, 2000L: 5744693 and 4573352

Methods

Study Design Table

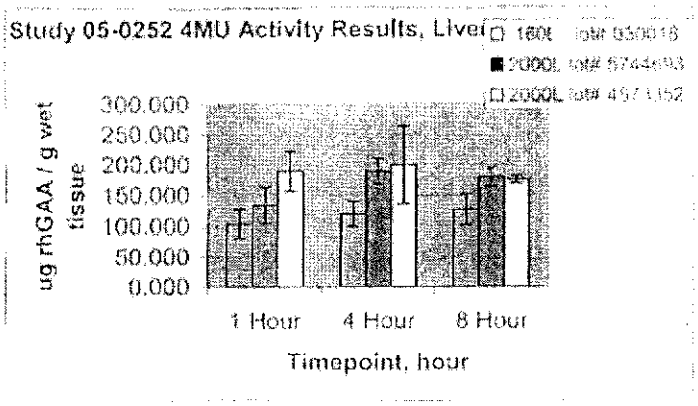
Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen	Dose Route
1	6	30	3	rhGAA, lot 930018 (160L)	Sodium Phosphate, mannitol, Polysorbate 80	Single Dose	IV
2	6			rhGAA, lot 5744693 (2000L)			
3	6			rhGAA, lot 4573352 (2000L)			
4	12			rhGAA, lot 930018 (160L)			
5	12			rhGAA, lot 5744693 (2000L)			
6	12			rhGAA, lot 4573352 (2000L)			

Sample Collection

Group #	Time Points	Tissue Collection
1	2, 5, 10, 15, 30 and 60 minutes post dose	Serum
2		
3		
4	1, 4 and 8 hours post dose	Heart, quadriceps, triceps, diaphragm, psoas, liver, spleen, kidney, brain and serum
5		
6		

Results:

- No remarkable in-life findings attributable to the test article are reported.
- Levels of rhGAA were lower tan expected. Therefore, PK parameters were not determined for this study.
- Biodistribution to muscle tissues was similar among the three lots examined.
- Significantly increased rhGAA levels are reported for the 2000L lots relative to the 160L lot. The table below, provided by the sponsor, summarizes the data.



Conclusion:

Although the biodistribution among the various muscle samples appears to be similar among the tree lots tested, there does appear to be a higher level of uptake in the liver for the 2000L product relative to the 160L product.

Title: Pharmacokinetics and biodistribution of rhGAA on the Pompe knockout mouse with the presence of antibodies.

Study #: #03-0087pga
Lot #:

Methods:
Two groups of Pompe knockout mice received either vehicle or 1 mg/kg rhGAA, i.p., weekly for 16 weeks. Serum was collected every 4 weeks to be analyzed for the presence of anti-drug antibodies. Mice with the highest titers of anti-drug antibodies were selected for the pharmacokinetic and biodistribution portion of the study. For this portion of the study two groups (group 1, n=4, PK and group 2, n=12, BD). The mice received 10 mg/kg radiolabeled rhGAA, i.v., followed by analysis for detection of radiolabel in the serum at 2, 5, 10, 15, 30, 60, 120, 240 and 1440 minutes post-dose. Tissues were examined at 1, 4 and 8 hours post dose for the presence of rhGAA activity.

Results:
- 8 of 16 mice in group 2 died within 1 hour of dosing from hypersensitivity reactions.
- Due to the loss of animals and the hypersensitivity reactions, no PK analysis was performed.

Conclusion:
Due to technical problems with hypersensitivity reactions in the mice the planed anlyses were not performed and no conclusions were drawn.

Title: Pharmacokinetics and biodistribution of rhGAA in the presence of antibody after intravenous dosing.

Study #: #03-0087pga
Lot #: GA096

Methods:
This study is a repeat of study #03-0089 (above). The tables below, supplied by the sponsor, illustrate the basic study design.

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Group	# of Animals	Dose mg/kg	Conc. mg/ml	Test Article	Vehicle	Dosing Regimen*	Dose Route
1	16	N/A	N/A	Vehicle	Sodium Phosphate, mannitol, polysorbate	Every other week for 4 doses (40 mg/kg) followed by a [illegible] (hGAA	IV
2	20	40/20	5/2.5	rhGAA			

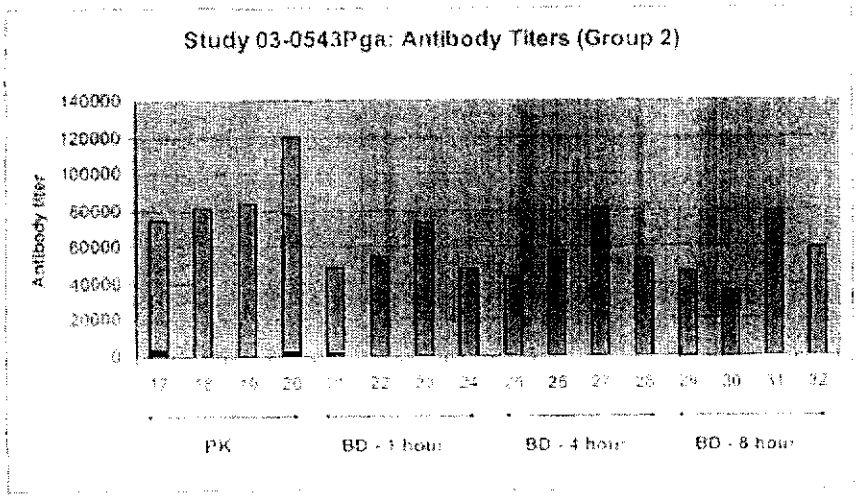
*All animals received Sweet-g duplex ethylenediamine (IV 10-15 minutes prior to rhGAA of dose 2 and thereafter)

Sample Collection

Group #	Time Points	Tissue Collection
1	Serum was taken for antibody titers every 4 weeks	Serum, heart, psoas, quadriceps, triceps, diaphragm, liver, spleen, kidneys, lungs
2	After the last dose: 0, 2, 5, 10, 25, 30, 60 and 1,90 minutes (PK, n=4)	
	After the last dose: 1, 4 and 8 hours (BD, n=4 per time point)	

Results:

- No in-life observations attributable to test article administration are reported.
- All animals developed high titers of anti-drug antibody



- Animals receiving vehicle had antibody titers of less than 1:100.

The table below, provided by the sponsor, summarizes the results of PK analysis:

	Naive	Injected
T ½ alpha (min.)	4.32	7.61
T ½ beta (min.)	69.88	72.44
Cl/mf (ml/min/kg)	0.59	0.83
MRT (min)	96.30	93.25
AUC (min*ug/ml)	126/69	120/65
AUC/dose (min*ug/ml/mg/kg)	126/1	125/1
Vss/mf (kg)	56.93	77.15
Rsq	0.93	

Conclusion:
The results indicate that the presence of anti-drug antibodies (in mice) does not significantly alter pharmacokinetic behavior of rhGAA.

2.6.4.5 Metabolism No studies performed.

2.6.4.6 Excretion No studies performed.

2.6.4.7 Pharmacokinetic drug interactions No studies performed.

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 for 2000L process when compared to the 160 liter process. Therefore, the product produced by these two processes cannot be considered comparable.
One hypothesis on why this difference occurred is the potential for lot to lot variation in

Under an agreement with the sponsor, the product from the 2000L process was withdrawn from consideration for approval at this time.

2.6.4.10 Tables and figures to include comparative TK summary

The tables below, provided by the sponsor, illustrate the toxicokinetic results for the various species used to investigate the toxicity of rhGAA. These species include cynomolgus monkey, CD:1®(ICR) BR mouse, Sprague-Dawley rat, beagle dog. Results from human pharmacokinetic analysis are also summarized for comparison.

Table 2.6.4.9-1:
Pharmacokinetic Parameters of Various Formulations and Scales of Myozyme in GAA Knockout Mice (Genzyme Studies 02-0710Pga, 02-0779Pga, 04-0424Pga and 05-0414Pga)

	Myozyme 30 L/60 L (Study 02-0779 Pga)*	Myozyme 160 L (Study 02-0779 Pga)*	Myozyme 2000 L (Study 04-0424Pga)	Myozyme 2000 L (Study 04-0424Pga)	Myozyme 160 L (Study 04-424Pga)	Myozyme 160 L (Study 05-0414Pga)	Myozyme 160 L (Study 05-0414Pga)	Myozyme 2000 L (Study 05-0414Pga)
Dose (Single admin.)	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg
Lot No.	GA628	930018	xGA179	xGA180	930018	930018	5744693	4523352
Elimination T ½ (min)	63.68 ± 19.9	69.63 ± 14.4	154.06 ± 24.3	151.8 ± 22.2	141.8 ± 30.8	176.8 ± 39.3	115.5 ± 32.4	107.9 ± 17.5
CL (mL/min/kg)	0.53 ± 0.1	0.55 ± 0.2	0.53 ± 0.05	0.58 ± 0.08	0.41 ± 0.04	0.57 ± 0.17	0.73 ± 0.09	0.87 ± 0.20
MRT (min)	88.09 ± 29.0	92.25 ± 24.6	176 ± 35.1	173 ± 27.2	158.06 ± 17.8	163.8 ± 57.9	114.6 ± 26.1	100.3 ± 13.2
AUC (min x µg/mL)	39333.6 ± 9546.1	39005.0 ± 11948.3	37973.3 ± 3588.9	53374.1 ± 5768.2	45723.6 ± 4333.9	38580.5 ± 13144.2	27633.4 ± 3169.2	24165.3 ± 5955.8
AUC/dose (min x µg/mL/mg/kg)	1966.7 ± 477.3	1950.3 ± 597.4	1898.7 ± 179	2668.7 ± 288	2286.2 ± 216.7	1926.5 ± 657.2	1381.7 ± 158.5	1208.3 ± 297.8
Vss (mL/kg)	46.26 ± 17.7	50.6 ± 11.0	92.3 ± 15.7	64 ± 4.8	69.2 ± 4.5	91.1 ± 39.8	82.6 ± 15.1	86.2 ± 17.8

* Note: Time points only collected out to 240 minutes

Beagle dogs (6354-132)

	1 mg/kg male	1 mg/kg female	10 mg/kg male	10 mg/kg female	100 mg/kg male	100 mg/kg female
Elimination T ½ (min)	171.8 ± 34.6	181.6 ± 2.2	92.7 ± 9.9	81.9 ± 11.5	110.9 ± 6.9	105.4 ± 8.9
Cl (ml/min/kg)	0.27 ± 0.06	0.29 ± 0.02	0.32 ± 0.02	0.31 ± 0.02	0.4 ± 0.01	0.39 ± 0.01
MRT (min)	243.2 ± 32.0	246.7 ± 9.5	163.8 ± 24.8	151.7 ± 17.0	153.5 ± 11.4	147.6 ± 11.6
AUC (min*ug/ml)	3760.4 ± 843.9	3414.6 ± 236.4	31606.2 ± 2009.1	31999.1 ± 1561.9	242221.3 ± 5230.7	255926.6 ± 6962.5
AUC/dose (min*ug/ml/mg/kg)	3760.4 ± 843.9	3414.6 ± 236.4	3161.6 ± 200.9	3199.9 ± 156.2	2422.2 ± 52.3	2559.3 ± 69.6
Vss (ml/kg)	65.9 ± 13.8	72.6 ± 7.6	51.2 ± 9.7	47.5 ± 5.7	63.5 ± 6.1	57.7 ± 4.5
Rsq	0.94 ± 0.02	0.98 ± 0.01	0.98 ± 0.01	0.92 ± 0.08	0.99 ± 0.01	0.99

Sprague-Dawley rats (6354-134)

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	1 mg/kg female	1 mg/kg male	10 mg/kg female	10 mg/kg male	100 mg/kg female	100 mg/kg male
Elimination T ½ (min)	129.7 ± 17.9	104.2 ± 53.9	85.6 ± 11.0	85.8 ± 14.0	80.1 ± 18.8	114.5 ± 49.8
Cl (ml/min/kg)	0.66 ± 0.16	0.94 ± 0.27	0.60 ± 0.08	0.55 ± 0.09	0.58 ± 0.10	0.51 ± 0.07
MRT (min)	170.4 ± 19.3	141.7 ± 78.6	111.5 ± 13.6	113.9 ± 17.8	104.7 ± 29.6	130.8 ± 62.4
AUC (min*ug/ml)	1592.3 ± 397.1	1137.3 ± 336.4	17100.9 ± 2587.4	18487.8 ± 3057.5	176441.3 ± 36588.8	201001.5 ± 28955.2
AUC/dose (min*ug/ml/mg/kg)	1592.3 ± 397.1	1137.3 ± 336.4	1710.1 ± 258.7	1848.8 ± 305.8	1764.4 ± 365.9	2010.0 ± 289.6
Vss (ml/kg)	110.2 ± 17.4	118.2 ± 34.7	66.8 ± 14.7	62.3 ± 10.6	62.9 ± 27.0	72.3 ± 39.6
Rsq	0.99	0.96 ± 0.03	0.97 ± 0.02	0.96 ± 0.04	0.98 ± 0.01	0.95 ± 0.04

Mice, CD:1®(ICR) BR (Reproductive toxicology 6354-153)

	10mg/kg	20mg/kg	40mg/kg
Elimination T ½ (min)	76.27 ± 11.5	75.77 ± 8.2	71.90 ± 5.5
Cl (ml/min/kg)	0.65 ± 0.03	0.60 ± 0.05	0.63 ± 0.07
MRT (min)	98.2 ± 14.4	100.3 ± 11.2	99.0 ± 6.2
AUC (min*ug/ml)	15364.1 ± 611.5	33663.8 ± 2461.0	64580.6 ± 7814.7
AUC/dose (min*ug/ml/mg/kg)	1536.4 ± 61.2	1683.2 ± 123.1	1614.5 ± 195.4
Vss (ml/kg)	63.7 ± 6.7	59.7 ± 6.8	59.8 ± 6.3
Rsq	0.99	0.98 ± 0.01	0.98

Sprague-Dawley rat (Repeat dose toxicokinetics, 6354-133)

Male

	Week 1			Week 4		
	1 mg/kg	10 mg/kg	100 mg/kg	1 mg/kg	10 mg/kg	100 mg/kg
Elimination T ½ (min)	123.8 ± 23.5	76.1 ± 4.1	68.3 ± 8.2	64.7 ± 35.0	71.9 ± 11.6	90.3 ± 25.1
Cl (ml/min/kg)	0.35 ± 0.02	0.52 ± 0.04	0.36 ± 0.05	0.91 ± 0.62	0.51 ± 0.15	0.38 ± 0.1
MRT (min)	172.4 ± 30.0	100.9 ± 5.5	93.2 ± 12.8	110.6 ± 27.3	98.1 ± 21.4	123.0 ± 34.4
AUC (min*ug/ml)	2857.3 ± 181.9	19202.4 ± 1463.1	272616.0 ± 39050.1	1545.1 ± 951.8	20597.0 ± 4633.6	282389.2 ± 78733.4
AUC/dose (min*ug/ml/mg/kg)	2857.3 ± 181.9	1920.2 ± 146.3	2726.2 ± 390.5	1545.1 ± 951.8	2059.7 ± 463.4	2823.9 ± 787.3
Vss (ml/kg)	60.1 ± 7.8	52.8 ± 3.5	33.8 ± 8.8	91.02 ± 50.2	48.6 ± 9.0	45.4 ± 14.3
Rsq	0.98 ± 0.01	0.97 ± 0.03	0.99 ± 0.005	0.93 ± 0.09	0.88 ± 0.19	0.94 ± 0.10

Female

	Week 1			Week 4		
	1 mg/kg	10 mg/kg	100 mg/kg	1 mg/kg[1]	10 mg/kg	100 mg/kg
Elimination T ½ (min)	114.7 ± 4.5	68.9 ± 6.5	64.4 ± 16.8	133.0	62.0 ± 3.5	74.4 ± 11.3
Cl (ml/min/kg)	0.41 ± 0.02	0.58 ± 0.06	0.53 ± 0.30	0.45	0.43 ± 0.04	0.40 ± 0.10
MRT (min)	160.7 ± 6.6	90.7 ± 9.2	87.8 ± 24.5	188.3	84.4 ± 4.7	101.3 ± 16.2
AUC (min*ug/ml)	2427.4 ± 136.9	17394.8 ± 1782.5	212595.8 ± 69111.9	2226.5	23265.3 ± 2244.5	266918.7 ± 77609.6
AUC/dose (min*ug/ml/mg/kg)	2427.4 ± 136.9	1739.5 ± 178.3	2126.0 ± 691.1	2226.5	2346.5 ± 224.4	2135.4 ± 1369.9
Vss (ml/kg)	66.4 ± 4.7	52.4 ± 4.9	51.0 ± 41.0	84.6	36.1 ± 1.8	39.4 ± 7.4
Rsq	0.98 ± 0.01	0.99	0.94 ± 0.05	0.99	0.99 ± 0.01	0.96 ± 0.04

[1] 4 out of 5 female rats administered 1 mg/kg were excluded from the analysis due to plasma rhGAA levels close to or below the level of detection in the assay.

Cynomolgus Monkey (Repeat dose TK, 6354-152)

Male

	Day 1			Day 85		
	4 mg/kg	20 mg/kg	100 mg/kg	4 mg/kg	20 mg/kg	100 mg/kg
Elimination T ½ (min)	88.6 ± 25.7	117.4 ± 41.1	124.0 ± 20.8*	84.4 ± 16.4	151.7 ± 24.1	108.4 ± 14.4*
Cl (ml/min/kg)	0.86 ± 0.4	0.52 ± 0.26	0.29 ± 0.15	1.34 ± 0.26	0.35 ± 0.02	0.24 ± 0.12
MRT (min)	124.2 ± 36.5	97.1 ± 2.3	113.0 ± 10.5	123.3 ± 24.8	106.2 ± 6.9	113.6 ± 5.6
AUC (min*ug/ml)	5761.5 ± 3568.4	45074.2 ± 22017.1	424509.3 ± 227093.1	3038.9 ± 590.8	57856.5 ± 3456.6	497863.4 ± 228685.2
AUC/dose (min*ug/ml/mg/kg)	1440.4 ± 892.1	2253.7 ± 1100.9	4245.1 ± 2270.9	759.7 ± 147.7	2892.8 ± 172.8	4978.4 ± 2286.9
Vss (ml/kg)	99.2 ± 40.9	50.8 ± 24.1	34.2 ± 19.4	162.1 ± 1.1	36.9 ± 4.6	26.7 ± 11.5*
Rsq	0.94 ± 0.02	0.99	0.99 ± 0.01	0.97 ± 0.04	0.99	0.99 ± 0.01

	Day 169		
	4 mg/kg	20 mg/kg	100 mg/kg
Elimination T ½ (min)	79.6 ± 30.4	138.0 ± 48.7	170.4 ± 36.1*
Cl (ml/min/kg)	1.81 ± 0.64	0.37 ± 0.01	0.35 ± 0.12
MRT (min)	108.7 ± 42.8	115.0 ± 16.5	132.6 ± 27.2
AUC (min*ug/ml)	2355.2 ± 827.2	53862.2± 1676.6	303881.9 ± 84572.0
AUC/dose (min*ug/ml/mg/kg)	588.8 ± 206.8	2693.1 ± 83.8	3038.8 ± 845.7
Vss (ml/kg)	183.0 ± 8.5	42.6 ± 4.8	45.5 ± 11.1*
Rsq	0.98 ± 0.02	0.99	0.99 ± 0.01

Students t-test: * p<0.05 between time points at 100 mg/kg

Female

	Day 1			Day 85		
	4 mg/kg	20 mg/kg	100 mg/kg	4 mg/kg	20 mg/kg	100 mg/kg
Elimination T ½ (min)	73.2 ± 2.5	115.0 ± 47.6	143.1 ± 9.3*	54.4 ± 2.5	163.4 ± 20.4	184.0 ± 22.6*
Cl (ml/min/kg)	1.33 ± 0.13	0.65 ± 0.43	0.37 ± 0.05*	2.46 ± 1.2	0.46 ± 0.04	0.53 ± 0.35
MRT (min)	107.4 ± 7.6	94.9 ± 9.7	106.9 ± 2.8	89.6 ± 5.5	107.5 ± 7.4	109.8 ± 5.0
AUC (min*ug/ml)	3030.9 ± 311.4	40219.8 ± 21271.2	276563.5 ± 40183.3*	2006.8 ± 1181.2	43834.1 ± 4152.3	229126.3 ± 89234.4
AUC/dose (min*ug/ml/mg/kg)	757.7 ± 77.8	2011.0 ± 1063.6	2765.6 ± 401.8*	501.7 ± 295.3	2191.7 ± 207.6	2291.2 ± 892.3
Vss (ml/kg)	143.3 ± 23.3	58.5 ± 32.8	39.2 ± 5.3*	224.0 ± 123.4	49.5 ± 6.5	57.5 ± 32.6
Rsq	0.76 ± 0.20	0.93 ± 0.10	0.99 ± 0.01	0.65 ± 0.11	0.99 ± 0.01	0.98 ± 0.02

	Day 169		
	4 mg/kg	20 mg/kg	100 mg/kg
Elimination T ½ (min)	59.8 ± 17.6	149.5 ± 30.9	158.6 ± 20.1
Cl (ml/min/kg)	2.78 ± 2.0	0.49 ± 0.08	0.49 ± 0.09*
MRT (min)	83.0 ± 26.2	114.2 ± 15.8	117.5 ± 12.3
AUC (min*ug/ml)	1929.5 ± 1048.9	41253.2 ± 7719.0	207845.9 ± 39263.5*
AUC/dose (min*ug/ml/mg/kg)	482.4 ± 262.2	2062.7 ± 385.9	2078.5 ± 392.6*
Vss (ml/kg)	196.2 ± 60.8	55.7 ± 2.6	57.3 ± 6.3*
Rsq	0.99 ± 0.01	0.99	0.99 ± 0.004

Students t-test: * $p<0.05$ between time points at 100 mg/kg

Human PK Summary (Study AGLU01602)

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Table 2.7.2.2-1:
Summary of Pharmacokinetic Parameters Measured in Study AGLU01602

Study	Subjects Treated (M/F) Subjects Included in the Analysis (M/F)	Median Age at First Infusion for Analyzed Subjects (months) Median Range for Analyzed Subjects (min, max)[2]	Summary of Pharmacokinetic Profile for GAA after IV Infusion of 20 mg/kg or 40 mg/kg qow to Patients With Infantile-Onset Pompe Disease		
			Parameter/Dose	Day 0	Week 12
AGLU01602: Open-Label Safety, Efficacy, Pharmacokinetics and Pharmacodynamics Study	18 (12M/6F) 15 (10M/5F)[1]	5.0 1.2, 7.3	C_{max} (ng/mL) 20 mg/kg 40 mg/kg	162,910 ± 27,598 271,253 ± 61,251	195,546 ± 75,190 256,096 ± 50,920
			AUC (hr*ng/mL) 20 mg/kg 40 mg/kg	937,896 ± 199,383 1,883,581 ± 407,002	1,017,138 ± 262,378 1,861,479 ± 497,002
			T_{max} (hr)	0.57 ± 0.081	0.39 ± 0.065
			$T_{1/2}$ (hr)	2.71 ± 0.58	2.80 ± 0.57
			CL (mL/hr) (mL/hr/kg)	133 ± 41 22.1 ± 4.2	154 ± 51 21.8 ± 5.1
			V_c (mL) (mL/kg)	264 ± 87 43.5 ± 8.4	398 ± 93 43.5 ± 8.6
			V_{ss} (mL) (mL/kg)	394 ± 139 66.9 ± 10.5	469 ± 100 85.5 ± 9.8

F = female; M = male
[1] For the purpose of the PK analysis, age was not adjusted for gestation.
[2] Patients 1602-312, 1602-314, and 1602-321 were excluded from the analysis because they did not have pharmacokinetic samples collected at Day 0 or Week 12.
Data are reported as mean ± standard deviation (SD) from individual estimates; test model-predicted average C_{max} was estimated based on direct examination of the observed data. AUC, $T_{1/2}$, and CL were secondary derived parameters based on model-predicted pharmacokinetic values. V_c and V_{ss} were estimated using the empirical Bayes estimates for each individual's pharmacokinetic parameters.
Reference: Table 4 and Section 9.1, Pharmacokinetic Analysis Report (Appendix 16.2.8) within the AGLU01602 26-Week Interim CSR and Section 11.4.1.7.4, AGLU01602 26-Week Interim CSR

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

Tables omitted per Dr. Choudary.

2.6.4.8

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The toxicology package for this BLA contains single dose studies in the Sprague-Dawley rat and the beagle dog, repeat dose studies in Sprague-Dawley rat, cynomolgus monkey and C57Bl/6 mice as well as Segment I and Segment II reproductive toxicology studies in the CD-1 mouse. The test article was delivered by IV infusion for all studies. The rhGAA was well tolerated in monkey 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 male animals (of 3 in each group) from the two higher dose groups and ovarian cyst and unequal sized ovaries in one female of 3 from the high dose group as well as inflammation and degeneration of the quadriceps muscle in one female of three from the high dose group. A relationship to the test article cannot be ruled out but, due to the small number of animals per group, this relationship is difficult to establish.

The rhGAA was also generally well tolerated in C57Bl/6 mice at doses up to 100 mg/kg administered weekly for 4 weeks. The mouse repeat dose toxicity study was intended

to compare toxicities produced by product from three different manufacturing processes. The toxicity profile was similar among the three products, but an increase in severity seemed to be present for the BI2KrhGAA (2000 liter) and BI – rhGAA – liter) relative to the GENZrhGAA (presumably the 160 liter product). The toxicities noted include 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 addition, a dose related mild increase in serum albumin was noted for all treatment groups and was more prevalent in females. Two females of 6 per group receiving 100 mg/kg of GenzrhGAA or B' – rhGAA had mildly increased AST and ALT levels (55-79%). This finding may be related to the test article and suggests a potential adverse effect on the liver.

In a similar 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.

In the major amendment to the BLA, (submitted 12/30/05), the sponsor reports that the microscopic analysis was removed from the protocol near the end of the in-life portion of the study. This change was documented with an amendment to the study protocol. They note that the study was terminated early and microscopic analysis was not performed due to clinical observations and unusual number of animal deaths. They report that the clinical signs were consistent with hypersensitivity reaction. Therefore the sponsor determined that the study results were not interpretable and followed this rat study with a similar study using mice. The strategy for follow-up was discussed with CBER in teleconference on 5/22/02. The sponsor reports that the tissues for the microscopic analysis that was not performed are retained and archived with Genzyme Research and Development Quality Assurance per GLP regulations.

An additional repeat dose toxicity study was performed in the rat (#6354-133) included only 5 per sex per group and investigated only one test article. Toxicities were less pronounced than those observed in 6354-140. The animals in this study showed a dose related statistically significant decrease in body weight. For males the weight loss was 23.3% and for females, 11.6%. 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 so apparently toxic in study 6354-140 relative to this additional rat study, except possibly the difference in lots used for study 6354-140.

The sponsor was asked to comment on the differences in toxicity seen between the two rat studies (6354-133 and 6354-140). They contend that the adverse effects were not significantly different between studies when consideration is limited to the groups receiving Alglucosidase alfa (160L product). The cause of the gross stomach findings was not explained but it was pointed out that the finding was observed in one of the control animals so may not be related to the test article administration. The sponsor points out that signs of hypersensitivity were noted in both studies although no deaths occurred in Study #6354-133. In addition, the sponsor suggests that the effect on liver function parameters (moderate to marked elevations increased aspartate aminotransferase and alanine aminotransferase) that were observed in study #6354-140 were also observed in study #6354-133 at the similar rate of incidence (2 of

62 rats and 3 of 40 rats, respectively). They also contend that these clinical chemistry findings are not dose related. However, the pathology report for study #6354-140 clearly states that the stomach lesions are interpreted as being test article-related (Page 27).

Rodents tend to launch a severe hypersensitivity reaction to the human recombinant 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 the DPH may have had or any interaction between DPH and the test article could not be assessed.

Genetic toxicology: As recombinant protein molecules, biological therapeutics do not normally have access to cellular DNA. Therefore, traditional genotoxicity studies are not thought to be relevant and are usually not required or conducted.

Carcinogenicity: No carcinogenicity studies were performed for this application. Biological therapeutics do not interact with cellular DNA. Therefore, traditional carcinogenicity studies are not thought to be relevant.

Reproductive toxicology:

Single Segment I and single Segment II reproductive toxicology studies were performed, both in CD-1 mice. For the Segment I study, mice received doses up to 40 mg/kg every other day beginning prior to mating and through early embryonic development. No clear rationale was given for the choice of dose and no pilot studies are included to support the choice of high dose. Results of this study showed a trend toward pre-implantation loss and late resorptions. These findings did not reach statistical significance and historical normal values for comparison were not found. Changes in male fertility parameters were also found. A statistically significant and dose related reduction in sperm count as well as an increase in abnormal sperm. The male mice also showed urine stained abdomens suggesting the presence of more generalized toxicity. No toxicokinetic analysis was performed so relative exposures to the test article cannot be established.

For the Segment II study, mated female mice received doses up to 40 mg/kg administered daily. No evidence of preimplantation loss was noted during this study. A small dose related increase in late resorptions was observed but did not reach statistical significance when considering this parameter alone. However, in considering pregnancy parameters as a whole, a significant increase in post-implantation loss was noted for the high dose group. There were no statistically significant adverse effects on fetal development reported. An additional finding of increased incidence of ovarian cysts was noted

A second segment 2 study in a non-rodent species is recommended to confirm and clarify the potential toxic effects.

An additional non-clinical study is mentioned in the integrated summary of non-clinical studies in this BLA. That study, # 6354-163, was to be finished by Q3 2005, but is not included in this submission.

Special toxicology: No studies performed for this category.

2.6.6.2 Single-dose toxicity

Study title: Effect of a single Intravenous Administration of rh-α-glucosidase to Sprague-Dawley rats.

Key study findings: The purpose of this study was to evaluate toxicity of rhGAA after a single intravenous dose in rats. No remarkable findings were observed for clinical observations, clinical pathology, body weight, food consumption, gross necropsy examination or microscopic examination. The NOAEL under the conditions of this study was determined to be 100 mg/kg.

Study no.: 6354-134 — 01025 (Genzyme)
Volume #, and page #: N/A
Conducting laboratory and location:

Date of study initiation: 8/16/2001
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity: rhGAA lot # GW10124094
Placebo lot # GW10124089 (— sodium phosphate, — mannitol —

Methods

Doses: 0, 1, 10 or 100 mg/kg
Species/strain: – CD (SD) IGS BR rats
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: IV administration in a volume of 10.87 ml
Satellite groups used for toxicokinetics or recovery:
Age: 42 days old at initiation of the study
Weight: males, 190-222g females, 126-171g
Sampling times:
Unique study design or methodology (if any):

Group	No. of Animals Male	Female	Dose Level (mg/kg)	Dose Concentration (mg/mL)[a]
1 (Control)[b]	5	5	0	0
2 (Low)	5	5	1	0.092
3 (Mid)	5	5	10	0.92
4 (High)	5	5	100	9.2

a Dose concentrations were based on actual values. The dose volume was 10.87 mL/kg.

b Animals in the control group received control article (placebo/diluent) only.

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: All animals survived to the scheduled sacrifice date.

Clinical signs: Recorded twice daily for mortality and moribundity. Cageside observations were performed once prior to treatment, 10, 30, 60 minutes and 2 hours post-dosing and daily thereafter.

Body weights: Recorded prior to study initiation, on the first day of treatment and weekly thereafter.

Hematology: Samples for clinical pathology were collected at termination.

Clinical chemistry: See above.

Gross pathology: Necropsy with gross examination was performed on day 15.

Organ weights (specify organs weighed if not in histopath table):

Histopathology: Adequate Battery: yes (X), no ( )---explain
Peer review: yes ( ), no ( ) not specified

adrenal (2)	eyes [(2) preserved in Davidson's solution]
brain	femur with bone marrow (articular surface of the distal end)
cecum	heart
colon	ileum
duodenum	injection site(s)
epididymis (2)	
esophagus	
jejunum	skeletal muscle (thigh)
kidney (2)	skin
lesions	spinal cord (cervical, thoracic, and lumbar)
liver	spleen
lung with mainstem bronchi	sternum with bone marrow
lymph node (mesenteric)	stomach
mammary gland (females only)	testis (2)
ovary (2)	thymus
pancreas	thyroid (2) with parathyroid
pituitary gland	tongue
prostate	trachea
rectum	urinary bladder
salivary gland [mandibular (2)]	uterus
sciatic nerve	vagina
seminal vesicle (2)	

Results

Mortality: All animals survived to the scheduled sacrifice.

Clinical signs: No test article-related effects are reported. No effects attributable to test article administration are reported.

Body weights: No effects on body weight or body weight change attributable to test article administration are reported.

Hematology: No effects on clinical pathology attributable to test article administration are reported at two weeks post-dosing.

- A significantly lower absolute neutrophil counts was noted for female animals that received 10 mg/kg. However, this effect was not noted in males from the same group nor was it noted in higher dose groups. Therefore, it is considered to not be related to test article administration.

Clinical chemistry: See above for clinical pathology results.

Gross pathology: No test article macroscopic findings are reported.

Organ weights (specify organs weighed if not in histopath table):

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )
Histopathology was not performed except for observed lesions. Tissues listed above were stored for future reference, as needed. A dilated renal pelvis was noted for two animals: one control female and one male receiving 100 mg/kg. One male receiving 10 mg/kg showed minimal hemorrhage in the thymus. These findings were not considered to be related to test article administration.

Toxicokinetics: Samples for toxicokinetics were collected prior to study initiation and 10 and 30 minutes post-dose, 1 and 3 hours post-dose on day 1. The data were analyzed using a non-compartmental model.

- Plasma concentrations of rhGAA decreased with time in a monophasic manner after all doses.
- TK parameters were linear with dose after normalization of the AUC to the dose administered (AUC/dose).
- The results indicate that the rats received expected exposure.
- There were no significant differences in exposure between genders.
- No evidence of saturation kinetics for doses up to 100 mg/kg was noted.

	1 mg/kg female	1 mg/kg male	10 mg/kg female	10 mg/kg male	100 mg/kg female	100 mg/kg male
Elimination T ½ (min)	129.7 ± 17.9	104.2 ± 53.9	85.6 ± 11.0	85.8 ± 14.0	80.1 ± 18.8	114.5 ± 49.8
Cl (ml/min/kg)	0.66 ± 0.16	0.94 ± 0.27	0.60 ± 0.08	0.55 ± 0.09	0.58 ± 0.10	0.51 ± 0.07
MRT (min)	170.4 ± 19.3	141.7 ± 78.6	111.5 ± 13.0	113.9 ± 17.8	104.7 ± 29.6	150.8 ± 62.4
AUC (min*ug/ml)	1592.3 ± 397.1	1137.3 ± 336.4	17100.9 ± 2587.4	18487.8 ± 3057.5	176441.3 ± 36588.8	201091.5 ± 28955.2
AUC/dose (min*ug/ml/ mg/kg)	1592.3 ± 397.1	1137.3 ± 336.4	1710.1 ± 258.7	1848.8 ± 305.8	1764.4 ± 365.9	2010.9 ± 289.6
Vss (ml/kg)	110.2 ± 17.2	118.2 ± 34.7	66.8 ± 14.7	62.3 ± 10.6	62.9 ± 27.0	77.3 ± 39.6
Rsq	0.99	0.96 ± 0.03	0.97 ± 0.02	0.96 ± 0.03	0.98 ± 0.01	0.95 ± 0.04

Study title: Effect of a single-dose intravenous administration of rh-α-glucosidase to beagle dogs

Key study findings: The purpose of this study was to evaluate the toxicity of rhGAA when administered as a single dose by intravenous injection to beagle dogs. The results indicate that rhGAA is generally well tolerated. Due to the tremors seen in dogs treated at 10 or 100 mg/kg, the NOAEL under the conditions of this study is determined to be 1 mg/kg.

Study no.: 6354-132/ Genzyme # 01026
Volume #, and page #: N/A
Conducting laboratory and location:

Date of study initiation: 8/23/2001
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity:
rhGAA lot # GW10124094 Placebo lot # GW10124089
vehicle: — sodium phosphate, – mannitol, ; —
Methods

Doses: 0, 1, 10 or 100 mg/kg
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics or recovery:
Age: 5 months old
Weight: 7.2 to 10.6 kg
Unique study design or methodology (if any):

Group	No. of Animals Male	Female	Dose Level (mg/kg)	Dose Concentration (mg/mL)[a]
1 (Control)[b]	3	3	0	0
2 (Low)	3	3	1	0.092
3 (Mid)	3	3	10	0.92
4 (High)	3	3	100	9.2

a Dose concentrations were based on actual values. The dose volume was 10.87 mL/kg.

b Animals in the control group received placebo/diluent only.

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: Observations were made twice daily for mortality/morbidity.

Clinical signs: Cageside observations were made once prior to treatment, 10, 15, 30 and 60 minutes post-injection, and daily thereafter. Observations included blood pressure, heart rate, respiration rate, and rectal body temperature.

Body weights: Body weights were recorded weekly prior to study initiation, on the day of treatment and twice weekly thereafter.

Hematology: Samples for clinical pathology were taken prior to study initiation, 24 hours post-dosing and prior to necropsy on SD16.

Clinical chemistry: Samples for clinical pathology were taken prior to study initiation, 24 hours post-dosing and prior to necropsy.

Gross pathology: Animals were sacrificed on SD16. Gross examination was performed, tissues were maintained for future use.

Organ weights (specify organs weighed if not in histopath table): Not done.

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )

The only tissue retained at necropsy was the injection site from each animal. These samples were preserved and stored for possible future examination.

Results

Mortality: All animals survived to scheduled sacrifice.

Clinical signs: Tremors were noted in one of three males and one of three females in the 10 mg/kg dose group and one female in the 100 mg/kg group at 60 minutes post-dosing. One male in the 10 mg/kg group had foamy vomitus 10 minutes post-dose. No other findings related to test article administration were noted.

Body weights: No effect on body weight attributable to the test article was noted.

Hematology: No effects in clinical pathology attributable to the test article administration were reported at 24 hours or 2 weeks after single dose administration.

Clinical chemistry: See above.

Gross pathology: No findings for macroscopic examination attributable to test article administration were reported.

Organ weights (specify organs weighed if not in histopath table): N/A

Histopathology: Adequate Battery: yes ( ), no ( )—explain Not performed.
Peer review: yes ( ), no ( )
No histopathology analysis was included in the study protocol. Only injection sites were retained for possible future examination.

Toxicokinetics: Blood samples were collected pre-dose, 5, 10, and 30 minutes post-dose; 1, 2, 4, and 6 hours post-dose on day 1. A fluorometric enzyme assay was used to determine rhGAA activity. The table below, provided by the sponsor summarizes the TK data analysis.

	1 mg/kg male	1 mg/kg female	10 mg/kg male	10 mg/kg female	100 mg/kg male	100 mg/kg female
T ½ beta (min)	116.7	96.6	69.7	74.9	105.1	102.5
Cl (ml/min/kg)	0.3	0.4	0.4	0.4	0.4	0.4
MRT (min)	168.5	139.1	100.6	108.0	151.7	147.9
AUC (min*ug/ml)	3119	2576	24622	27152	234099	248336
AUC/dose (min*ug/ml/mg/kg)	3119.0	2576.0	2462.2	2715.2	2340.9	2483.4
Vss (ml/kg)	54.0	54.1	40.8	39.8	64.8	59.6
Rsq	0.98	0.98	0.98	0.98	0.99	0.99

No significant difference in toxicokinetics was noted between genders. The parameters were linear with no evidence of saturation kinetics. Re-analysis did not alter these conclusions.

2.6.6.3 Repeat-dose toxicity

Study title: Effect of repeat intravenous infusion toxicity study with recombinant human acid-α-glucosidase (rhGAA) in cynomolgus monkeys with a 14-day recovery period

Key study findings: The purpose of this study was to evaluate the potential toxicity of rhGAA administered via a 12-hour intravenous infusion every other week to cynomolgus monkeys for at least 13 weeks. In addition, a 14-day recovery period allowed assessment of reversibility, persistence or delayed occurrence of such toxicities. The test article was well tolerated. All animals developed anti-drug antibodies but this immune response did not appear to affect the PK of the drug. No other effects attributable to rhGAA administration were reported. Therefore under the conditions of this study, the NOAEL was determined to be 200 mg/kg.

Study no.: #6354-157
Volume #, and page #: N/A
Conducting laboratory and location:

Date of study initiation: 6/24/04
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:

Test Article	Supplier	Lot No.	Storage[a]	Retest Date[b]	Reserve (Archive) Sample
Recombinant human acid-α-glucosidase (rhGAA)	Sponsor	996793	In a refrigerator set to maintain 2 to 8°C, protected from light	October 2004 and June 2005	1 vial

Control Article	Supplier	Lot No.	Storage	Expiration Date	Reserve (Archive) Sample
rhGAA Control	Sponsor	04-LS-6040	Refrigerated	4/27/2005	10 mL

Methods

Doses: 0 or 200 mg/kg

Species/strain: cynomolgus monkeys

Number/sex/group or time point (main study): Group 1: 2/sex/group, Group 2: 4/sex/group

Route, formulation, volume, and infusion rate: IV infusion at a rate of 3.33 ml/kg/hr

Satellite groups used for toxicokinetics or recovery: 1 monkey per sex per group were designated as recovery animals and sacrificed 14 days after the terminal sacrifice.

Age: 3-5.5 years old at study initiation

Weight: 3.0 to 4.4 for the males; 2.5 to 3.6 for the females

Unique study design or methodology (if any): Animals were dosed via 12 hour IV infusion once every other week for a total of 7 treatments.

Group Designation and Dose Levels

Group	Number of Animals[a] Male	Female	Dose Parameters[b] Level mg/kg/dose	Rate mL/kg/hr	Duration hr/dose	Concentration mg/mL
1 (vehicle control)	2	2	0	3.33	12	0
2 (rh-α-glucosidase)	4	4	200	3.33	12	5.0

a Animals designated for recovery sacrifice (last animal/sex/group) underwent at least 14 days of recovery following the final dose administration.

b Animals were dosed (12-hour infusion) once every other week for a total of 7 doses.

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: Animals were observed twice daily for mortality and moribundity.

Clinical signs: Detailed clinical exams were performed prior to study initiation and weekly during the study. On dosing days, observations were made prior to infusion, approximately hour after initiation of infusion, 10 minutes and 2 hours after infusion completion. Physical exams included heart rate, respiratory rate and rectal body temperature.

Body weights: Recorded twice prior to study initiation, one the first day of treatment (SD1) and weekly thereafter.

Food consumption: Qualitative assessment of food consumption was made once daily.

Ophthalmoscopy: Performed prior to study initiation, during week 13, and during week 15 for recovery animals.
EKG: Not performed.
Clinical pathology: Samples were taken for hematology, coagulation, and clinical chemistry upon animal arrival (clinical chemistry only), once prior to study initiation (at least 1 week after surgery to install instrumentation), once during Weeks 2 and 3 (hematology and clinical chemistry only), once during Week 12 (6 days prior to the last dose), and once during Week 16 (prior to recovery necropsy).
Urinalysis: Urine was collected from all surviving animals (over approximately 16 hours on wet ice) once prior to initiation of treatment (at least 1 week after surgery), once during Week 12 (6 days prior to the last dose), and once during Week 16 (prior to recovery necropsy).
Anti-drug antibody analysis: Samples were taken from Group 2 animals only within 24 hours prior to dosing on Days 1, 29, 57, and 85.
Toxicokinetics: Samples were taken from Group 2 animals only on Days 1 and 85 within 1 minute of the EOI, at 2, 5, 10, 30, 60, and 120 minutes (± 1 minute) after the EOI, and at 24 hours (±10 minutes) after the EOI.
Gross pathology: Sacrifice of the main study animals was carried out on day 93 (8 days after the final dose). Recovery animals were sacrificed on day 107 (22 days after the final dose). Gross examination included examination of catheterization sites, external features, external body orifices, all internal cavities, organs and tissues.
Organ weights (specify organs weighed if not in histopath table):

adrenal (2)	seminal vesicle (2)
brain	skeletal muscle – quadriceps
heart	skeletal muscle – triceps
kidney (2)	skeletal muscle – diaphragm
liver	spleen
lung	testis (2) with epididymis (2)
ovary (2)	thymus
pituitary gland	thyroid (2 lobes) with parathyroid
prostate	uterus
salivary gland [mandibular (2)]	

Histopathology: Adequate Battery: yes (X), no ( ) –explain
Peer review: yes ( ), no ( )

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ON ORIGINAL

adrenal (2)	ovary (2)
aorta	pancreas
brain	pituitary gland
cecum	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2) [2]	sciatic nerve
esophagus	seminal vesicle (2)
eye (2) [1]	skeletal muscle – quadriceps (2)
gallbladder	skeletal muscle – triceps (2)
heart	skeletal muscle – diaphragm
ileum	skin
infusion and catheterization sites	spinal cord (cervical, thoracic and lumbar)
jejunum	spleen
kidney (2)	sternum with bone marrow
lacrimal gland	stomach
lesions	testis (2) [2]
liver	thymus
lung with mainstem bronchi	thyroid (2) with parathyroid
lymph node (inguinal)	tongue
lymph node (submandibular)	trachea
lymph node (mesenteric)	urinary bladder
mammary gland	uterus
optic nerve (2) [1]	vagina

Results

Mortality: One female animal from group 2 was found dead on SD5. The previous day the clinical observations included hunched posture, hypoactivity and no food consumption. Post necropsy evaluation revealed that the probable cause of death was renal failure and cardiovascular collapse resulting from multiorgan embolic septicemia probably of pulmonary origin. Data from the pre-study examination as well as the post-necropsy examinations indicate that this death was probably due to a pre-existing bacterial infection. A replacement monkey was assigned to group 2 prior to the second dose (#I56847).

Clinical signs: No clinical observations attributable to the test article administration were reported.

Body weights: No effects in body weight attributable to test article administration were reported.

Food consumption: No apparent effects on food consumption attributable to the test article administration were noted.

Ophthalmoscopy: No ophthalmic observations attributable to test article administration were noted.

EKG: N/A

Clinical pathology: No effects on clinical pathology including hematology, clinical chemistry and urinalysis attributable to test article administration were noted.

Gross pathology: No effects on gross pathology attributable to the test article administration were noted.

Organ weights (specify organs weighed if not in histopath table):

Histopathology: Adequate Battery: yes (X), no ( )---explain
Peer review: yes ( ), no ( )
No dose related microscopic findings are reported.

Toxicokinetics: Samples were taken at SD1 and 85, at 1, 2, 5, 10, 30, 60, 120 and 1440 minutes post-infusion for detection of rhGAA activity. Serum samples for anti-drug antibody analysis were taken on SD1, 29, 57 and 85. Antibody detection was performed using an ELISA assay.

- The high dose of 200 mg/kg showed a significant increase in half-life in female monkeys from SD1 to SD85.
- AUC was higher in males than females at SD1 but the difference was not statistically significant. No difference was noted at SD85.
- Clearance parameters did not vary throughout the study. Calculated clearance parameters appeared to be slightly higher in females than males.
- All animals receiving rhGAA developed anti-drug antibodies. Titers appeared to increase up to day 85.
- rhGAA activity levels in tissues declined significantly at 22 days post-dose (200 mg/kg) relative to levels observed at SD8. rhGAA activity remained in the live at SD22 suggesting the possibility of accumulation in this organ. No detectable levels were observed in cardiac or skeletal muscle at 22 days post-dose. Therefore, it is concluded that repeat administration did not result in accumulation in muscle tissue.

The table below, provided by the sponsor summarizes the result of the toxicokinetic analysis.

	Male		Female	
	Day 1	Day 85	Day 1	Day 85
Elimination T ½ (min)	141.5 ± 13.5	208.0 ± 30.3*	146.5 ± 9.1	200.7 ± 71.2
Cl (ml/min/kg)	0.67 ± 0.18	0.68 ± 0.11	0.94 ± 0.47	0.93 ± 0.69
MRT (min)	105.8 ± 5.5	130.2 ± 39.7	110.1 ± 3.3	140.9 ± 57.8
AUC (min*ug/ml)	313242.2 ± 77478.2	302336.1 ± 50471.0	261885.8 ± 134483.0	301421.5 ± 170560.1
AUC/dose (min*ug/ml/ mg/kg)	1566.2 ± 387.4	1511.7 ± 252.4	1309.4 ± 672.4	1507.1 ± 852.8
Vss (ml/kg)	70.3 ± 14.7	85.0 ± 12.6	102.6 ± 48.7	123.3 ± 77.1
Rsq	0.99 ± 0.002	0.97 ± 0.01	0.99 ± 0.002	0.99 ± 0.02

Students t-test: * p<0.05 as compared to Day 1

The NOAEL for rhGAA under the conditions of this study was determined to be 200 mg/kg.

Study title: 26-week toxicity study of recombinant human acid-α-glucosidase (rhGAA) administered intravenously every other week to cynomolgous monkeys with a 2-week recovery phase

Key study findings:
The test article was administered QOW for 13 treatments, followed by a 2-week recovery period. A total of 13 doses of rhGAA were administered. Doses used were 0, 4, 20 or 100 mg/kg administered IV in a 6-hour infusion (a rate approximately twice the expected rate for human use). The rhGAA was generally well tolerated in monkeys. However, some toxicities with possible relationships to the test article administration were noted. On microscopic examination, two male animals, one in group 3 and one in group 4, showed thrombus formation in the atrium. The sponsor attributes this finding to the method of sacrifice (exsanguination). However, due to the small number of animals and the apparent dose related occurrence in the two higher dose groups, a test article contribution cannot be fully ruled out. One female of 3 from group 4 showed degeneration/atrophy and chronic inflammation of the quad muscle. In addition, one female of 3 in group 4 showed an ovarian cyst and unequal sized ovaries. A relationship to the test article is possible for these findings since they were observed only in the high dose group. Due to the small number of animals, this relationship is difficult to establish. However, one control animal also showed unequal sized ovaries at the recovery sacrifice, supporting the conclusion that this finding is not test article related.

TK analysis showed that female animals had consistently lower AUC and AUC/dose at all dose levels and at all time points relative to the males. This suggests possible reduced exposure for the females and was most pronounced for the high dose females on day 169 relative to day 1. A decrease in AUC and increase in clearance and V_{ss} were also noted in high dose males. These results are somewhat different than results of other studies. There may be a lot to lot difference in rate and amount of cellular uptake of the drug. No correlation is possible at this time. The sponsor has been asked to provide information on which manufacturing process was used to produce each lot used in the non-clinical studies.

All animals developed anti-drug antibodies. The titers peaked at day 85 and remained high until day 169 but were not dose related. The highest levels of rhGAA activity was observed in the liver with a dose response relationship. No indication of saturation was noted for repeat dosing at any dose. rhGAA activity was significantly lower at 14 days post-dose (recovery animals) but remained detectable above control.

Study no.: 6354-152
Volume #, and page #: N/A
Conducting laboratory and location:

Date of study initiation: 7/16/03
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:

Test Article	Supplier	Lot No.	Storage
Recombinant human acid-α-glucosidase (rhGAA)	Sponsor	608341	In a refrigerator set to maintain 2 to 8°C, protected from light

Methods

Doses: 0, 4, 20, or 100mg/kg

Species/strain: cynomolgus monkeys

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume, and infusion rate: Test article was administered by intravenous infusion at a rate of 3.33 ml/kg/hour (approximately a 6-hour infusion, 16.6 mg/kg/hr- a rate more than 2 times the maximum infusion rate anticipated for the clinical study).

Satellite groups used for toxicokinetics or recovery: 2/sex from groups 1 and 4 were designated recovery animals and survived an additional 15 days after the terminal sacrifice.

Age: 5 to 8 years old

Weight: males: 2.9 to 4.6 kg, females: 2.3 to 2.9 kg

Unique study design or methodology (if any): The test article was administered QOW for 13 treatments, followed by a 2-week recovery period. The table below, provided by the sponsor, illustrates the basic study design.

Group	No. of Animals[a]		Dose Parameters[b]			
	Male	Female	Level mg/kg/dose	Rate mL/kg/hr	Duration hr/dose	Concentration mg/mL
1 (Control)	5	5	0	3.33	6	0
2 (Low)	3	3	4	3.33	6	0.2
3 (Mid)	3	3	20	3.33	6	1.0
4 (High)	5	5	100	3.33	6	5.0

a Animals designated for recovery sacrifice (last two animals/sex in Groups 1 and 4) underwent at least 14 days of recovery following the final dose administration.

b Animals were dosed (6-hour infusion) once every other week for a total of 13 doses.

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: Observations made twice daily for mortality, moribundity and signs of distress.

Clinical signs: Detailed clinical observations recorded prior to study initiation and weekly thereafter. Daily cageside observations were done on dosing days prior to dosing, 1 hour after initiation of infusion (IOI), within 10 minutes and 2 hours after the end infusion. Physical exams were performed once prior to study initiation (post surgery), during week 24 and on week 26 (surviving recovery animals). The exams included body temperature, heart rate, and respiratory rate.

Body weights: Recorded twice prior to initiation of treatment, on day one of treatment and weekly thereafter.

Food consumption: Qualitative food consumption was recorded daily.

Ophthalmoscopy: Exams were performed prior to initiation of treatment and during week 24 as well as week 26.

EKG: Not performed.

Clinical pathology: Samples were taken for hematology, coagulation, and clinical chemistry once prior to initiation of treatment (at least 1 week after surgery), once during Week 12 (5 or

6 days prior to the 7th dose), once during Week 24 (5 or 6 days prior to the 13th dose), and once during the end of Week 27 (prior to recovery necropsy).
Urinalysis: Urine was collected from all surviving animals (over approximately 16 hours on wet ice) once prior to initiation of treatment, once during Week 24 (prior to the 13th dose), and once during the end of Week 27 (prior to recovery necropsy).
Anti-drug antibody analysis: Samples were collected 24 hours prior to dosing in SD 1, 29, 85, 127 and 169.

Gross pathology: On Day 170 (the day after the 13th dose), three animals/sex/group were sacrificed and necropsied. On Day 184 (15 days after the 13th dose), all remaining animals (two/sex in Groups 1 and 4) were sacrificed and necropsied.

Organ weights (specify organs weighed if not in histopath table):

adrenal (2)
brain
heart
kidney (2)
liver
lung
ovary (2)
pituitary gland
prostate
salivary gland [mandibular(2)]
seminal vesicle (2)
skeletal muscle – quadriceps (2)
skeletal muscle – triceps (2)
skeletal muscle – diaphragm
spleen
testis (2) with epididymis (2)
thymus
thyroid (2) with parathyroid
uterus

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( ) not noted

adrenal (2)
aorta
brain
cecum
colon
duodenum
epididymis (2) [2]
esophagus
eye (2) [1]
gallbladder
heart
ileum
infusion and catheterization sites
jejunum
kidney (2)
lacrimal gland
lesions
liver
lung with mainstem bronchi
lymph node (inguinal)
ovary (2)
pancreas
pituitary gland
prostate
rectum
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle (2)
skeletal muscle – quadriceps (2)
skeletal muscle – triceps (2)
skeletal muscle – diaphragm
skin
spinal cord (cervical, thoracic and lumbar)
spleen
sternum with bone marrow
stomach
testis (2) [2]
thymus
thyroid (2) with parathyroid
tongue

lymph node (submandibular)	trachea
lymph node (mesenteric)	urinary bladder
mammary gland	uterus
optic nerve (2) [1]	vagina

Results

Mortality: No unscheduled deaths were recorded.

Clinical signs: No observations attributable to test article administration were reported.

Body weights: No adverse effects on body weight or body weight change attributable to test article administration were noted.

Food consumption:

- All groups showed transient reduced food consumption regardless of group during this study. This finding is also reported for the control and high dose group during the recovery period.

Ophthalmoscopy: No findings attributable to test article administration are reported.

EKG: Not performed.

Clinical pathology:

- A small reduction relative to baseline in red cell parameters (RBC, HCT, HGB) was noted for all groups. No differences for these values between groups were reported. No relationship to test article administration is apparent.
- A small reduction relative to baseline for platelets was reported. Large standard error results in lack of statistical significance. However, since all groups were affected, a study drug effect is not likely.
- A small increase in WBC is noted for all groups, due mostly to increases in neutrophil counts. The significant differences reported among groups were evident prior to treatment. Therefore, no test article effect on WBC is likely.
- No other findings are noted for clinical pathology parameters.

Gross pathology: No macroscopic observations are reported for the male animals. One female of 3 in group 4 showed an ovarian cyst and unequal sized ovaries and one female of 3 in group 3 showed alopecia. Relevance of ovarian findings to test article administration cannot be determined due to the small number of animals. However, one control animal showed unequal sized ovaries at the recovery sacrifice.

Organ weights (specify organs weighed if not in histopath table): No significant effects attributable to test article administration were noted for organ weights.

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )

- One female of 3 from group 4 showed degeneration/atrophy, chronic inflammation of the quad muscle. Relationship to the test article is not determined due to the small number of animals.
- Signs of chronic inflammation were noted for multiple other organs in all groups, both male and females. This type of finding is common for this species and may be the result of a pre-existing condition. Therefore, it is not likely to be related to the study drug administration.
- Thrombus in the atrium of the heart was noted for 2 male animals: 1 of 3 from group 3 at the terminal sacrifice and 1 of 2 from group 4 at the recovery sacrifice. No female animals showed this finding. Animals were terminated by exsanguination. The thrombus formation in the atrium of the heart may be an artifact of the termination procedures. The animal from group 3 was one of two animals where drug levels were below the level of detection, indicating that exposure to the test article may have been reduced. This finding supports the hypothesis that the thrombus formation was not a test article effect.

Toxicokinetics: Samples were taken on SD 1, 85 and 169 within 1 minute of EOI, at 2, 5, 10, 30 60 and 120 minutes after EOI and at 24 hours after EOI.

- TK analysis showed that female animals had consistently lower AUC and AUC/dose at all dose levels and at all time points relative to the males. This suggests reduced exposure for the females.
- In the 100 mg/kg group, there was a significant increase in elimination half-life in males and females on days 1 to 85 and in males on days 1 to 169 relative to other dose groups. A similar trend was noted for the 20 mg/kg group but did not achieve statistical significance.
- However, the elimination half life for the 100 mg/kg group on days 85 to 169 was no different from other groups for either males or females.
- No effect on elimination half-life was observed for the 4 mg/kg group.
- These findings could be due to circulating ant-drug antibody or to receptor saturation at the higher doses. However, rhGAA activity analysis indicate that no saturation was apparent.
- Two animals (I55276 and I55279, 4 and 20 mg/kg, respectively) did not have detectable drug levels at all timepoints and were excluded from analysis. One of these animals was one of the male animals (group 3) that showed thrombus formation in the atrium.
- A statistically significant decrease in AUC and a slight increase in Cl and V_{ss} were noted for females in the high dose group on day 169 relative to day 1. No difference in AUC or Cl was noted for males in this same group although an increase in V_{ss} was observed in males in the high dose group for days 85 to 169.

The tables below, provided by the sponsor, summarize the calculated TK parameters for this study.

Male

	Day 1			Day 85		
	4 mg/kg	20 mg/kg	100 mg/kg	4 mg/kg	20 mg/kg	100 mg/kg
Elimination T ½ (min)	88.6 ± 35.7	117.4 ± 41.1	124.0 ± 26.8*	81.4 ± 16.4	283.7 ± 24.1	168.4 ± 19.4*
Cl (ml/min/kg)	0.86 ± 0.4	0.52 ± 0.26	0.29 ± 0.15	1.34 ± 0.26	0.35 ± 0.02	0.24 ± 0.12
MRT (min)	124.2 ± 36.5	97.1 ± 2.5	115.0 ± 10.5	123.3 ± 24.8	106.2 ± 6.9	113.6 ± 3.6
AUC (min*ug/ml)	5761.5 ± 3568.4	45074.2 ± 22917.1	421569.3 ± 227985.1	3038.9 ± 500.8	57856.5 ± 1356.6	403565.4 ± 235685.2
AUC/dose (min*ug/ml/ mg/kg)	1440.4 ± 892.1	2253.7 ± 1150.9	4215.7 ± 2279.9	759.7 ± 147.7	2892.8 ± 172.8	4035.4 ± 2356.9
Vss (ml/kg)	99.2 ± 40.9	59.8 ± 24.1	34.3 ± 19.4	162.1 ± 1.1	39.9 ± 4.6	26.7 ± 11.2*
Rsq	0.94 ± 0.02	0.99	0.99 ± 0.01	0.97 ± 0.04	0.99	0.99 ± 0.01

	Day 169		
	4 mg/kg	20 mg/kg	100 mg/kg
Elimination T ½ (min)	200.6 ± 30.4	158.0 ± 48.7	170.4 ± 26.1*
Cl (ml/min/kg)	1.81 ± 0.64	0.37 ± 0.01	0.25 ± 0.12
MRT (min)	108.7 ± 42.8	115.0 ± 16.5	132.6 ± 27.2
AUC (min*ug/ml)	2355.2 ± 827.2	53862.2 ± 1976.6	393881.9 ± 84572.9
AUC/dose (min*ug/ml/ mg/kg)	588.8 ± 206.8	2693.1 ± 83.8	3938.8 ± 845.7
Vss (ml/kg)	183.0 ± 8.5	42.6 ± 4.8	45.5 ± 11.1*
Rsq	0.98 ± 0.02	0.99	0.99 ± 0.01

Students t-test: * p<0.05 between time points at 100 mg/kg

Female

	Day 1			Day 85		
	4 mg/kg	20 mg/kg	100 mg/kg	4 mg/kg	20 mg/kg	100 mg/kg
Elimination T ½ (min)	75.7 ± 2.5	115.0 ± 47.6	147.5 ± 9.5*	54.4 ± 2.5	163.4 ± 20.4	184.0 ± 22.6*
Cl (ml/min/kg)	1.33 ± 0.23	0.65 ± 0.43	0.32 ± 0.05*	2.36 ± 1.2	0.46 ± 0.04	0.53 ± 0.35
MRT (min)	107.4 ± 7.6	94.9 ± 9.7	106.9 ± 2.6	89.6 ± 5.2	107.8 ± 7.4	104.8 ± 5.0
AUC (min*ug/ml)	3030.9 ± 311.4	4802.9 ± 2121.2	9705.5 ± 4018.3*	2006.8 ± 1281.2	43834.1 ± 4152.3	89234.4 ± 59234.4
AUC/dose (min*ug/ml/mg/kg)	757.7 ± 77.8	2014.6 ± 1063.6	2765.6 ± 401.8*	501.7 ± 295.3	2191.7 ± 207.6	3271.2 ± 893.3
Vss (ml/kg)	143.3 ± 23.3	58.3 ± 32.9	39.7 ± 8.3*	224.0 ± 123.4	39.5 ± 6.5	57.2 ± 32.6
Rsq	0.76 ± 0.20	0.93 ± 0.10	0.99 ± 0.01	0.65 ± 0.31	0.99 ± 0.01	0.98 ± 0.02

	Day 169		
	4 mg/kg	20 mg/kg	100 mg/Kg
Elimination T ½ (min)	59.8 ± 17.6	149.5 ± 30.9	158.6 ± 20.4
Cl (ml/min/kg)	1.78 ± 2.0	0.49 ± 0.08	0.40 ± 0.09*
MRT (min)	83.9 ± 26.2	114.2 ± 13.8	117.5 ± 12.3
AUC (min*ug/ml)	1926.5 ± 1048.9	41253.2 ± 7719.6	2078[illegible]9 ± 2978[illegible].5*
AUC/dose (min*ug/ml/mg/kg)	482.4 ± 262.2	2062.7 ± 385.9	2079.9 ± 392.6*
Vss (ml/kg)	196.2 ± 66.8	55.7 ± 2.6	57.3 ± 6.3*
Rsq	0.99 ± 0.01	0.99	0.99 ± 0.001

Students t-test: * p<0.05 between time points at 15.6 mg/kg

Other:

Anti-drug antibody assessment: Samples were taken within 24 hours prior to dosing on SD 1, 29, 85, 127 and 169.

- All animals developed anti-drug antibodies. The titers peaked at day 85 and remained high until day 169
- Antibody titers were not dose related.
- Antibody titers fro the two animals that had low drug levels did not differ from other animals, indicating that anti-drug antibodies most likely did not contribute to the low drug levels.

rhGAA activity levels in tissue:

- A dose response was noted for rhGAA activity in liver.
- No indication of saturation was noted for repeat dosing at any dose.
- rhGAA activity was significantly lower at 14 days post-dose but remained detectable above control.

Study title: Effect of repeated intravenous administration of three formulations of rh-α-glucosidase to C57Bl/6 mice.

Key study findings: The purpose of this study was to test the effects of each of three test articles when administered once weekly for 4 weeks to C57Bl/6 mice. Each lot was produced by a different manufacturing process (160 liter: lot # GA028, 2000 liter: Lot # 105067 and [illegible] liter: lot # E1585AM03). The sponsor wishes to market the 2000L scale product but PK

indicate that the product produced by the 2000L process is not comparable to the 160L scale product, which was used in the clinical studies. A similar study in rats was performed approximately 2 months earlier than this study (See review of — study number 6354-140. In contrast to the similar study done in rats, the rhGAA was well tolerated by the mice. Some lethargy was noted after dosing for a few mice in the high dose groups but the effect was inconsistent, occurred after only one of four doses for each animal and did not occur after the final dose. Therefore, if this finding is an effect of the test article it does not appear to be biologically significant. The instances of lethargy that occurred in three high dose mice after the third dose might be accounted for by the higher dose of DPH (10 mg/kg) that was administered prior to treatment. Other mouse studies used doses of 5 mg/kg to protect against the anticipated hypersensitivity to the test article that usually developed in rodents after the 3rd or 4th dose. The higher dose was most likely in response to the number of deaths in study 6354-140.

Clinical pathology results showed a dose related mild increase in serum albumin for GenzrhGAA at 10 and 100 mg/kg, for BI – rhGAA at all dose levels and for BI2KrhGAA at 10 and 100 mg/kg. This finding was more prevalent in females. Two females receiving 100 mg/kg each of GenzrhGAA or BI — rGAA had mildly increased AST and ALT levels (approximately 79% for AST and 69% for ALT). This finding may be related to the test article. These clinical pathology results suggest a test article effect on liver function. Effects on the liver are consistent with biodistribution results showing the highest rhGAA activity in the liver.

Very limited histopathology was performed. Aggregates of melanocytes were observed throughout the red pulp. This finding correlates with the macroscopic observation of dark spots seen in the spleen. This is not unusual for pigmented animals
No TK results are provided for this study.
Due to the liver findings, the NOAEL for GenzrhGAA is 1 mg/kg. For BI2K rhGAA the NOAEL is 1 mg/kg. No NOAEL is determined for BI — hGAA due to liver findings at all dose levels.

Study no.: 02009
Volume #, and page #: N/A
Conducting laboratory and location: Genzyme
Framingham, MA
Date of study initiation: 6/10/2002
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity:
Genzyme rhGAA: Lot # GA028, BI — ter rhGAA: lot # E1585AM03, BI2000 liter rhGAA: lot # 105067, vehicle 1: lot # KsK11283089D, vehicle 2: SWFI,
Control article: Lot #KsK11283140
Methods

Doses: 0, 1, 10 or 100 mg/kg for Genz rhGAA and BI — liter; 0, 1, 10 or 25 for BI 2000 liter product.

Species/strain: mouse, C57Bl/6; male and female Pompe knockout mice

Number/sex/group or time point (main study): 6/sex/group; knockout mice: 2/sex/group

Route, formulation, volume, and infusion rate: intravenous in a volume of 10 ml/kg.
Satellite groups used for toxicokinetics or recovery:
Age: 6-7 weeks old, knockout mice: 9-10 weeks old.
Weight: males: 20.06 to 24.83g; females: 15.9 to 20.36
Unique study design or methodology (if any):
The study design is illustrated in the table below, provided by the sponsor. Thirteen groups were designated with 6/sex/group in groups 1-4, 6-8 and 10-12. Groups 5, 9, and 13 consisted of 2/sex/group. Animals were euthanized on SD 23. Pompe knockout mice were used as reference only. In this study, the mice were treated with 10 mg/kg DPH prior to administration of the study drug. This is twice the dose administered in other mouse studies.

Group	No. of Animals		Substance	Dose Level	Dose Concentration
	Male	Female		(mg/kg/day)[a]	(mg/mL)[a]
1[b]	6	6	Control Article	0	0
2	6	6	Genz rhGAA	1	0.1
3	6	6		10	1
4	6	6		100	10
5[c]	2	2		100	10
6	6	6	BI — liter rhGAA	1	0.1
7	6	6		10	1
8	6	6		100	10
9[c]	2	2		100	10
10	6	6	BI 2000liter rhGAA	1	0.1
11	6	6		10	1
12	6	6		25	2.5
13[c]	2	2		25	2.5

a The dose volume will be 10 mL/kg.
b Animals in Group 1 will receive the control article only.
c GAA knockout (KO) mice will be used in groups 5, 9 and 13 as reference animals. High dose rhGAA has been successfully administered to these mice in the past and therefore they are included as reference animals as to the expected response.

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.
Mortality: Observations performed once daily.
Clinical signs: Performed once prior to initiation of treatment and 60 minutes post dosing on each day of treatment.
Body weights: Recorded once prior to initiation of treatment and prior to dosing on each day of dosing.
Clinical pathology: Samples were collected for hematology and clinical chemistry analysis at sacrifice.
Gross pathology:
Organ weights (specify organs weighed if not in histopath table): A limited number of organ weights were recorded: brain heart, kidney (2) liver, lung and spleen.
Histopathology: Adequate Battery: yes ( ), no (X)—explain Only a limited number of key tissues were collected. This study was intended to test variations in effect among three test articles and was not intended as a pivotal toxicity study.

Peer review: yes ( ), no ( )

- Brain, heart liver, lung, spleen, tongue, quadriceps.

Results

Mortality: All animals survived to the scheduled sacrifice.

Clinical signs: No clinical observations attributable to administration of the test article were reported. Mild lethargy was noted in 4 animals (25, 38, 91 and 132) after doses 1, 3, 3, and 3, respectively. This response was not considered by the sponsor to be related to the test article administration since it was inconsistent and occurred only after a single dose for each animal and did not occur after the last dose. However, each animal was in one of the groups receiving the highest dose for the specific formulation (groups 5, 5, 7 and 13). Therefore, relationship to test article administration is possible but probably not biologically significant.

Body weights: Two statistically significant body weight effects were noted. Neither of these results appears to be dose related. These same groups did not show similar weight changes at later timepoints.

Hematology:

- A decrease in WBC was observed for all three formulations: GenzrhGAA at 10 and 100 mg/kg, BI — hGAa at 1, 10 and 100 mg/kg, BI2KrhGAA at 1, 10 and 25 mg/kg. A clear dose-response is not apparent and all but one value (GenzrhGAA at 100 mg/kg in females) are within the historical normal. No pre-dosing values are available for comparison. Biological significance is not clear.

Clinical chemistry:

- A dose related mild increase in serum albumin was noted for GenzrhGAA at 10 and 100 mg/kg, for BI — hGAA at all dose levels and for BI2KrhGAA at 10 and 100 mg/kg. This finding was more prevalent in females.
- Two females receiving 100 mg/kg each of GenzrhGAA or BI — hGAA had mildly increased AST and ALT levels. This finding may be related to the test article.
-

Group #	AST	ALT
1 (control)	67	22
4 (100mg/kg	122	74
8 (100 mg/kg)	118	79

Gross pathology: Gross observations were limited to the finding of darkened areas of the spleen.

Organ weights (specify organs weighed if not in histopath table): Not reported.

Histopathology: Adequate Battery: yes ( ), no (X)--explain See above.
Peer review: yes ( ), no ( )

- Aggregates of melanocytes were observed throughout the red pulp. This finding correlates with the macroscopic observation of dark spots seen in the spleen. This is not unusual for pigmented animals
- No other test article-related findings are reported.

Toxicokinetics: Not found.

Study title: Effect of intravenous administration of rh-α-glucosidase to Sprague-Dawley rats for 4 weeks.

Key study findings: This study was an early toxicology study that was performed to investigate the safety of rhGAA administered weekly for 4 weeks. Sprague-Dawley rats received doses of a single lot of rhGAA weekly for 4 weeks in a range of doses up to 100 mg/kg. Prior to the third dose, the rats were pre-treated with 5 mg/kg DPH to prevent the expected hypersensitivity reaction. Much lower rates of toxicity are reported than was seen in study 6354-140.

The test article was generally well tolerated. No effects on clinical pathology related to rhGAA administration were reported. However, a dose related 23.3% decrease in body weight was observed for males in group 4 (100 mg/kg). The decrease was noted between weeks 1 and 2 as well as for the study duration, ruling out an effect of diphenhydramine, which was administered only after the 3rd and 4th doses. Females in group 4 also showed a decrease in body weight but lesser in magnitude (11.6%), and the results did not reach statistical significance.

Signs of chronic inflammation in the liver were observed for a total of 4 animals: one male group 4, one female in each of groups 1, 3 and 4. This finding could be related to the test article but the low incidence, lack of clinical chemistry correlate and occurrence in the control group suggests that the finding is not likely to be biologically significant.

No significant difference in toxicokinetics between genders was observed. For males, a small increase in AUC and elimination half-life was observed at the high dose at week 4 relative to week 1. This difference did not achieve statistical significance.

Testing for anti-drug antibodies was not performed.

Due to the effect on body weight observed in the high dose groups, the NOAEL under the conditions of this study is 10 mg/kg.

Study no.: — # 6354-133, Genzyme # 01027
Volume #, and page #: N/A
Conducting laboratory and location:

Date of study initiation: 8/29/01
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity:
Test article: Lot # GW10124094 Placebo: Lot #GW10124089 and KSK10125099

Methods

Doses: 0, 1, 10 or 100 mg/kg

Species/strain: Rat – CD®(SD)IGS BR

Number/sex/group or time point (main study): 4 groups, 5/sex/group

Route, formulation, volume, and infusion rate: Intravenous administration in a volume of 10.87 ml/kg

Satellite groups used for toxicokinetics or recovery:

Age: Approximately 57 days old at study initiation

Weight: males: 252 to 316 g; females: 165 to 206 g

Unique study design or methodology (if any): The study drug was administered IV once per week for at least 4 weeks via tail vein. Because allergic response is usually elicited after the second dose in rodents, diphenhydramine was administered (5 mg/kg, IV) pre-dose at the time of treatment for all animals after hypersensitivity was observed in at least one animal after the third dose.

	No. of Animals		Dose Level	Dose Concentration
Group	Male	Female	(mg/kg)	(mg/mL)[a]
1 (Control)[b]	5	5	0	0
2 (Low)	5	5	1	0.092
3 (Mid)	5	5	10	0.92
4 (High)	5	5	100	9.2

a Dose concentrations were based on actual values. The dose volume was 10.87 mL/kg.

b Animals in the control group received control article (placebo/diluent) only.

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: Observations made twice daily.

Clinical signs: Observations were made once prior to initiation of treatment, then 10, 30, 60 and 120 minutes post-dose on each treatment day and daily on non-treatment days. Detailed observations were made once weekly.

Body weights: Recorded prior to study initiation, on the day of first treatment and weekly thereafter.

Clinical pathology: Blood samples were collected at sacrifice. A standard panel of hematology and clinical chemistry analyses were performed.

Gross pathology: Necropsy was performed on SD23. Macroscopic exam of carcass, body cavities, organs and tissues was performed.

Organ weights (specify organs weighed if not in histopath table): Not reported

Histopathology: Adequate Battery: yes (X), no ( ) explain

Peer review: yes ( ), no ( )

Results

Mortality: All animals survived to scheduled sacrifice.

Clinical signs: Hypersensitivity response was observed in one and one male from group 4 after the third dose.

Body weights:

- A dose related 23.3% decrease in body weight was observed for males in group 4 (100 mg/kg). The decrease was noted between weeks 1 and 2 as well as for the study duration, ruling out an effect of diphenhydramine.
- Females in group4 also showed a decrease in body weight but lesser in magnitude (11.6%), but the results did not reach statistical significance.

Clinical pathology:

- No effects on clinical pathology parameters attributable to the test article are reported.

Gross pathology: No macroscopic effects attributable to test article administration are reported.

Organ weights (specify organs weighed if not in histopath table): Not reported.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

- Signs of chronic inflammation (red focus, hepatocellular necrosis, chronic inflammation, hemorrhage) in the liver were observed for a total of 4 animals: one male group 4, one female in each of groups 1, 3 and 4. This finding could be related to the test article but the low incidence, lack of clinical chemistry correlate and occurrence in the control group suggests that the finding is probably not biologically significant.

Toxicokinetics: Samples were collected at 10 and 30 minutes, 1 and 3 hours post-dosing during weeks 1 and 4.

Male

	Week 1			Week 4		
	1 mg/kg	10 mg/kg	100 mg/kg	1 mg/kg	10 mg/kg	100 mg/kg
Elimination T ½ (min)	123.8 ± 22.5	76.1 ± 4.1	68.3 ± 8.2	64.7 ± 35.0	71.9 ± 11.0	90.3 ± 25.1
CL (ml/min/kg)	0.35 ± 0.02	0.52 ± 0.04	0.36 ± 0.05	0.91 ± 0.42	0.51 ± 0.15	0.38 ± 0.1
MRT (min)	172.4 ± 30.0	105.9 ± 5.5	93.2 ± 12.8	110.6 ± 37.5	98.1 ± 21.4	125.0 ± 34.4
AUC (min*ug/ml)	2857.3 ± 181.9	19202.4 ± 1463.1	27261.6 ± 906.0.1	1545.1 ± 91.8	38597.0 ± 4633.6	38259.2 ± 78.73.2
AUC/dose (min*ug/ml/mg/kg)	2857.3 ± 181.9	1920.2 ± 146.3	272.6 ± 9.1	1545 ± 91.8	3859.7 ± 463.4	3823.9 ± 787
Vss (ml/kg)	60.1 ± 7.8	52.8 ± 3.5	53.5 ± 8.5	91.02 ± 50.2	48.6 ± 9.0	49.4 ± 14.1
Req	0.98 ± 0.01	0.97 ± 0.03	0.99 ± 0.005	0.91 ± 0.09	0.88 ± 0.19	0.94 ± 0.10

Female

	Week 1			Week 4		
	1 mg/kg	10 mg/kg	100 mg/kg	1 mg/kg	10 mg/kg	100 mg/kg
Elimination T ½ (min)	114.7 ± 4.5	68.0 ± 6.5	62.4 ± 16.3	133.6	62.0 ± 3.5	74.4 ± 1.3
Cl (ml/min/kg)	0.41 ± 0.02	0.58 ± 0.06	0.45 ± 0.10	0.45	0.43 ± 0.04	0.40 ± 0.10
MRT (min)	160.7 ± 6.6	96.7 ± 9.2	87.8 ± 23.5	188.5	80.4 ± 4.7	101.3 ± 16.2
AUC (min*ug/ml)	2427.4 ±	17394.8 ±	213595.8 ±	2226.5	23263.3 ±	169018 ±
	136.9	1352.5	69111.9		2214.0	9061.6
AUC/dose (min*ug/ml/mg/kg)	2427.4 ±	1739.5 ±	2135.9 ±	2226.5	2346.5 ±	1690.2 ±
	136.9	135.2	691.1		224.4	1349.0
Vss (ml/kg)	66.4 ± 4.3	52.4 ± 4.9	51.6 ± 21.0	84.6	36.1 ± 1.8	30.4 ± 7.4
Rsq	0.98 ± 0.01	0.99	0.93 ± 0.05	0.99	0.99 ± 0.01	0.96 ± 0.04

[illegible] out of 5 female rats administered 1 mg/kg were excluded from the analysis due to plasma rhGAA levels close to or below the level of detection in the assay.

- Under the conditions of this study, no difference in toxicokinetics between genders was observed.
- For males, a small increase in AUC and elimination half-life was observed at the high dose at week 4 relative to week 1. This difference did not achieve statistical significance.
- Findings for females administered 1 mg/kg could not be established since only one of 5 animals had detectable levels at week 4.
- The presence of anti-drug antibodies was not determined. Therefore, TK parameters may have been affected. There is no way to determine if this is the case.

Study title: Effect of repeated intravenous administration of three formulations of rh-α-glucosidase to Sprague-Dawley rats.

Key study findings: The purpose of this study was to compare the toxicity and toxicokinetics of three preparations of rhGAA (GenzrhGAA, BI2KrhGAA, BI −rhGAA) after weekly dosing for 4 weeks by intravenous administration in Sprague-Dawley rats.
The animals received doses of 1, 5, 10 or 50 mg/kg of GENZrhGAA or BI − hGAA, 0, 1, 10 or 25 of BI2KrhGAA.
A number of unexpected deaths occurred on this study. Nine males and 6 females died after the third or 4th dose. These deaths were determined to be related to hypersensitivity reactions. However, no details on how this was determined are provided.
Five animals were sacrificed moribund: one receiving GenzrhGAA at 50 mg/kg, three receiving BI − nGAA at 10 mg/kg and one receiving BI2KrhGAA at 25 mg/kg.
Observations recorded after administration of the third and fourth doses were attributed to hypersensitivity response to the test articles. These observations included hypoactivity, recumbancy, dilated pupils, clear discharge from eyes, abnormal respiration, blue skin, cold to touch. These findings were noted in some combination in all treated groups.

Clinical pathology results showed that each test article was associated with moderate to marked increases in mean liver enzyme levels for some animals relative to control. The effect was not clearly dose related. Because no baseline data are available, it is difficult to draw conclusions. The group receiving 10 mg/kg of BI −nGAA had the highest incidence (more females). However, the highest severity was observed in two females receiving BI2KrhGAA

(one at 10 mg/kg and one at 25 mg/kg). Other findings included lower glucose for males receiving BI2KrhGAA at 25 mg/kg, lower albumin to globulin ratio for males given BI2KrhGAA at 25 mg/kg.

Gross examination at necropsy revealed stomach lesions in animals from all treatment groups (except controls) and in those that were found dead or sacrificed early. The stomach lesions were characterized as dark or red areas or foci in the mucosa or diffusely red mucosa. The findings were not dose related but was most likely test article related since they occurred only in animals that received rhGAA.

Liver weights were increased relative to control in female animals receiving 50 mg/kg of GenzrhGAA, females receiving 10 or 50 mg/kg B[1] — nGAA and females given 25 mg/kg BI2KrhGAA. Lung weights were increased females receiving either 1 or 5 mg/kg of B[1] — nGAA or 1 or 25 mg/kg BI2KrhGAA. Unfortunately, no histopathology results were provided with this report, although the protocol indicates that tissues were harvested for that purpose. The sponsor has been asked to provide information on the fate of those tissues and an explanation on why the histopathology was not reported.

Due to adverse effects at all dose levels for all rhGAA preparations, no NOAEL was determined for any test article. However, there does appear to be a trend toward increased toxicities for the BI2KrhGAA and B[1] —.hGAA relative to GenzrhGAA.
It is recommended that further investigation be focused on this issue.

The sponsor was asked to comment on the differences in toxicity seen between the two rat studies (6354-133 and 6354-140) and the disposition of the tissues retained for histopathology. They responded in a major amendment submitted 12/30/05. In that document the sponsor contends that the adverse effects were not significantly different between studies when consideration is limited to the groups receiving Alglucosidase alfa (160L product). The cause of the gross stomach findings was not explained but it was pointed out that the finding was observed in one of the control animals and suggested that the finding may not be related to the test article administration. However, the pathology report for study #6354-140 clearly states that the stomach lesions are interpreted as being test article-related (Page 27, pathology report, Study #6354-140).

The sponsor points out that signs of hypersensitivity were noted in both studies although no deaths occurred in Study #6354-133. In addition, the sponsor suggests that the effect on liver function parameters (moderate to marked elevations increased aspartate aminotransferase and alanine aminotransferase) that were observed in study #6354-140 were also observed in study #6354-133 at the similar rate of incidence (2 of 62 rats and 3 of 40 rats, respectively). They also contend that these clinical chemistry findings are not dose related.

The information included in the major amendment to the BLA includes an explanation that the tissues retained for histopathology are currently archived with Genzyme Research and Development Quality Assurance. In addition, they explain that the original protocol indicates that only limited microscopic analysis would be performed and that this protocol was amended to eliminate the microscopic analysis completely when the decision was made to terminated the study. They have included a copy of the study protocol and amendments with the 12/30/05 submission to the BLA. Neither the protocol nor the amendments or indication of the protocol changes were included in the study report included in the original BLA.

Study no.: — # 6354-140
Volume #, and page #: N/A
Conducting laboratory and location:

Date of study initiation: 4/22/02
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity:

- Test article GENZrhGAA: lot #GA028, Test article BI – rhGAA: lot #E1585AM03, Test article BI2KrhGAA: lot # 105067
- Placebo/vehicle 1: Lot # KsK11283089D, Placebo/vehicle 2: lot #86-064-JT and 82-138-JT
- Diluent 1: Lot# KsK11283100, Diluent 2: lot 3 KsK11283099, Diluent 3: lot # KsK11283098
- Control article: Lot # KsK11283100

Methods

Doses: 0, 1, 5, 10 or 50 mg/kg of GENZrhGAA or BI – rhGAA, 0, 1, 10 or 25 of BI2KrhGAA.

Species/strain: Rat —CD®(SD)IGS BR

Number/sex/group or time point (main study): Groups 1-9 had 8/sex/group, Groups 10-12 5/sex/group.

Route, formulation, volume, and infusion rate: Intravenous in a volume of 10 ml/kg

Age: Approximately 7 weeks old

Weight: Males: 169-230 g, Females: 167-218 g

Unique study design or methodology (if any): Test articles were injected via tail vein once per week for 4 treatments. All animals were treated with 5 mg/kg diphenhydramine, i.p., 20 minutes prior to the 3rd dose and i.v. prior to the 4th dose.

Group	No. of Animals Male	Female	Substance	Dose Level (mg/kg/day)[a]	Dose Concentration (mg/mL)[a]
1[b]	8	8	Control Article	0	0
2	8	8	Genz rhGAA	1	0.1
3	8	8		5	0.5
4	8	8		10	1
5	8	8		50	5
6	8	8	BI — .iter rhGAA	1	0.1
7	8	8		5	0.5
8	8	8		10	1
9	8	8		50	5
10	5	5	BI 2000liter rhGAA	1	0.1
11	5	5		10	1
12	5	5		25	2.5

a The dose volume was 10 mL/kg.
b Animals in Group 1 received the control article only.

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: Observations were made twice daily.

Clinical signs: Cageside observations were made once prior to treatment, 10, 30, 60 and 120 minutes post-dosing, then daily on non-dosing days. Detailed observations were made weekly and prior to sacrifice.

Body weights: Recorded twice prior to study initiation, on SD1 and twice weekly thereafter.

Clinical pathology: On SD23, prior to sacrifice, blood was collected for hematology, clinical chemistry, and coagulation parameters. Animals were fasted overnight.

Gross pathology: Necropsies were performed on all animals that were sacrificed moribund or died prior to scheduled termination. Surviving animals were sacrificed on SD23. Gross exam was performed to include external carcass, all body orifices and cavities, organs and tissues.

Organ weights (specify organs weighed if not in histopath table): Organ weights were recorded for adrenals (2), brain, heart, kidney (2), liver, lung, ovary (2) pituitary, prostate, spleen, testis (2).

Histopathology: Adequate Battery: yes ( ), no (X) - explain A full panel of tissues was collected at necropsy (See below). However, no histopathology results are included. There is no explanation of what the fate of those tissues was or intended to be.

Peer review: yes ( ), no (X)

adrenal (2)	pancreas
brain	pituitary gland
cecum	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2)	sciatic nerve
esophagus	seminal vesicle (2)
eye (2)	skeletal muscle (thigh)
femur with bone marrow (articular surface of the distal end)	skin
	spinal cord (cervical, thoracic, and lumbar)
heart	spleen
ileum	sternum with bone marrow
injection site(s)	stomach
jejunum	testis (2)*
kidney (2)	thymus
lesions	thyroid (2) with parathyroid
liver	tongue
lung with mainstem bronchi	trachea
lymph node (mesenteric)	urinary bladder
mammary gland (females only)	uterus
ovary (2)	vagina

* Testis were preserved in Bouin's fixative (moribund and scheduled sacrifices only)

Results

Mortality: 9 males and 6 females died after the third or 4th dose. Deaths were determined to be related to hypersensitivity reactions.
Five animals were sacrificed moribund: one receiving GenzrhGAA at 50 mg/kg, three receiving B1 –rhGAA at 10 mg/kg and one receiving BI2KrhGAA at 25 mg/kg.

Clinical signs: Observations recorded after administration of the third and fourth doses were attributed to hypersensitivity response to the test articles. These observations included hypoactivity, recumbancy, dilated pupils, clear discharge from eyes, abnormal respiration, blue skin, cold to touch. These findings were noted in some combination in all treated groups.

Body weights: No effects on body weight or body weight change attributable to test article administration are reported.

Clinical pathology:

- Each test article was associated with moderate to marked increases in mean liver enzyme levels for some animals relative to control. The effect was not dose related. Because no baseline data are available, it is difficult to draw conclusions. The group receiving 10 mg/kg of B1 –hGAA had the highest incidence (more females). However, the highest severity was observed in two females receiving BI2KrhGAA (one at 10 mg/kg and one at 25 mg/kg). Although these findings do not appear to be dose related, they do appear to be test article related.
- Lower glucose was observed males receiving BI2KrhGAA at 25 mg/kg

- Lower albumin to globulin ratio for males given BI2KrhGAA at 25 mg/kg

Gross pathology:

- Stomach lesions were noted in animals from all treatment groups and those that were found dead or sacrificed early. The stomach lesions were characterized as dark or red areas or foci in the mucosa or diffusely red mucosa. The findings were not dose related but was most likely test article related.

Organ weights (specify organs weighed if not in histopath table):

- Liver weights were increased relative to control in female animals receiving 50 mg/kg of GenzrhGAA, females receiving 10 or 50 mg/kg BI —.GAA and females given 25 mg/kg BI2KrhGAA.
- Lung weights were increased females receiving either 1 or 5 mg/kg of BI — .GAA or 1 or 25 mg/kg BI2KrhGAA.

These organ weight changes are likely related to test article administration despite the fact that they are not clearly dose related. No such changes were noted for control animals. The following table taken from the pathology report summarizes the gross findings for this study:

Incidence of Selected Macroscopic Lesions in Rats given Genz rhGAA
Terminal Sacrifice

Dose level (mg/kg/day)	1	5	10	50
Number examined	16	16	15	15
Stomach, mucosa, red foci or areas	2	7	4	3
Ovarian cysts	0	0	0	2

Text Table 5
Incidence of Selected Macroscopic Lesions in Rats given BI — ner rhGAA
Terminal Sacrifice

Dose level (mg/kg/day)	1	5	10	50
Number examined	16	12	13	14
Stomach, mucosa, dark or red foci/areas or diffuse	0	6	8	5

Text Table 6
Incidence of Selected Macroscopic Lesions in Rats given BI 2000ther rhGAA
Terminal Sacrifice

Dose level (mg/kg/day)	1	10	25
Number examined	10	8	8
Stomach, mucosa, dark or red foci/areas or diffuse	0	1	4
Dark fluid in gastrointestinal tract	0	0	1
Perineal/perianal staining	0	0	1
Mesenteric lymph node, mottled or diffusely red	0	1	2

Histopathology: Adequate Battery: yes ( ), no (X)—explain
Peer review: yes ( ), no (X)
The protocol lists a complete panel of tissues to be collected for histopathology. However, the pathology report does not include any histopathology results.

Toxicokinetics: No data included.

2.6.6.4 Genetic toxicology

No genotoxicity studies are included with this BLA. Genotoxicity is not usually required for biological therapeutics.

2.6.6.5 Carcinogenicity

No carcinogenicity studies are included in this BLA. Traditional carcinogenicity studies are often not required for biological therapeutics. In this case, the product is a replacement enzyme that should not have access to nuclear DNA. It is taken up into the cell by endocytosis and remains within the lysosomal system.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Intravenous injection study of recombinant human acid a-glucosidase (rhGAA) on fertility and early embryonic development to implantation in mice

Key study findings: The purpose of this study was to evaluate potential adverse effects of rhGAA administration on fertility and embryonic development when given every other day in doses up to 40 mg/kg via IV injection to male and female mice. The dosing was initiated prior to mating and continued until termination for the males and through early gestation for the females. No toxicokinetic analyses were performed with this study.

Three unscheduled deaths occurred during this study. Two female deaths were attributed to hypersensitivity reactions, although no details on how this conclusion was reached are provided.

Findings included a slightly higher rate of pre-implantation loss in treated groups that appeared to be dose dependent. In addition, treated females showed a small increase in late resorptions. These findings did not reach statistical significance but in a more sensitive species, such as rat, the effect may be greater.

A statistically significant, dose-related reduction in sperm count per gram of epididymus is reported for the 20 and 40 mg/kg groups, representing a 6 and 22% reduction relative to controls, respectively. A treatment-related increase in the percentage of abnormal sperm was observed in the 40 mg/kg group. The increase in abnormal sperm morphology was characterized by 45% of the animals having at least 5% amorphous sperm heads, as compared to 18% of the control animals.

Clinical signs included swelling and irritation of the tail beginning around SD35. As dosing continued, scaly skin and sores appeared in all groups and eventually necrosis and loss of a portion of the tail near the end of the study. A low incidence of tail swelling was noted for all groups, but a slightly higher incidence of these findings was noted in dose groups receiving ≥20 mg/kg. In addition, hypoactivity was observed after approximately 2 weeks of dosing. The hypoactivity was resolved with administration of DPH.

The sponsor sets the NOAEL for embryo/fetal viability to be 40 mg/kg in this study. However, the small increase in pre-implantation loss and late resorptions relative to control, although not clearly dose related indicate that the test article may not be benign. The choice of mouse as the species for this study is unusual. Previous toxicology data (see above) indicates

that rat is more sensitive to the toxic effects of the test article. Therefore, the trends noted in embryo viability noted above should not be ignored. Additionally, there was no toxicokinetic analyses performed for this study. Therefore, the true exposure of the mothers and offspring to the test article is not substantiated as expected for such a study.

The doses chosen for the reproductive toxicology studies are in question. There is no evidence that any pilot dose ranging studies were performed in pregnant mice nor was any data provided comparing exposure of the high dose females in this study to those of the high doses in other toxicology studies or the expected human exposure. Additional justification for the choice of the high dose in this study was requested from the sponsor.

The sponsor responded to these inquiries in the major amendment submitted 12/30/05. The choice of species for the reproductive toxicology studies was chosen with concurrence from CBER. Nice were selected because rats had been shown in one study (#6354-140) to have a more severe problem with hypersensitivity reactions to Alglucosidase alfa. The doses were chosen with reference to the mouse toxicology study (#02009). No toxicokinetic analysis was performed with this study. Therefore, actual exposure of the animals is not verified.

Study no.: Genzyme 04006, — 6354-155
Volume #, and page #: N/A
Conducting laboratory and location: —
Date of study initiation: 5/8/04
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity:
rhGAA lot# 608341 placebo lot#04-LS-0040
Methods

Doses: 0, 10, 20 or 40 mg/kg
Species/strain: mice, — CD-1®(ICR)BR
Number/sex/group: 22/sex/group
Route, formulation, volume, and infusion rate: IV administration in a volume of 8 ml/kg
Satellite groups used for toxicokinetics:
Study design:

Group	No. of Animals		Dose Level (mg/kg/every other day)	Dose Concentration (mg/mL)
	Male	Female		
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

Due to the probability of allergic reaction to the rhGAA, 5/mg/kg of DPH was administered 10-20 minutes prior to test article administration. It is not clear whether or not the control animals also received the DPH. After dose #7, the DPH was administered prophylactically to both males and females prior to all remaining doses.

Parameters and endpoints evaluated: Observations recorded are included in the table below (provided by the sponsor):

Procedure	Frequency/Comment
Clinical Observations[a]	Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain and distress. Additional findings were recorded as they were observed. Detailed observations were done once prior to initiation of treatment and at each body weight interval thereafter. Once daily, during the first 2 weeks of dosing for each sex, cageside observations were done. On dosing days, postdose observations were conducted within 10 minutes after dosing (PDO-1) to check for anaphylaxis and/or postdose observations and approximately 1 hour after dosing (PDO-2) for postdose observations.
Body Weights[a]	For the males, body weights were recorded prior to initiation of treatment, on the first day of treatment, and twice weekly thereafter. For the females, body weights were recorded prior to initiation of treatment, on the first day of treatment, and twice weekly during the premating treatment phase, and during mating. Female body weights were also recorded on GD 0, 3, 7, 10, and 13.
Food Consumption	For the males, food consumption was determined weekly during the premating treatment period but was not recorded during or after mating. For the females, food consumption was determined weekly during the premating treatment period. Beginning on GD 0, food consumption was measured at gestation body weight intervals.
Estrous Cycle Determination[a]	During the 2-week premating period, vaginal smears were assessed for stage of estrus.
Mating Procedures	Animals from respective dose groups were mated by placing one female in the breeding cage of a male from the same dose 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.
Sacrifice at Cesarean Section[a]	Performed on GD 13. Mice were sacrificed by carbon dioxide inhalation and exsanguination. Uterine contents were examined.
Male Sacrifice	After at least 9 weeks of dose administration, all surviving males were rendered unconscious with carbon dioxide and exsanguinated.
Male Reproductive Assessment	At the terminal sacrifice, all surviving males in each group were evaluated for sperm motility, sperm counts, and sperm morphology. Evaluation was conducted by [redacted]
Organ Weights	Protocol-specified organ weights were recorded for the males at the terminal sacrifice.
Tissue Preservation	Abnormal viscera were saved in 10% neutral-buffered formalin. Protocol-specified male reproductive organs preserved in 10% neutral-buffered formalin.

Results

Mortality: Three unscheduled deaths occurred during this study.

- On SD12, a male animal receiving 20 mg/kg was found recumbent within 10 minutes of administration of dose #7. DPH, 5 mg/kg, was given i.p., but the animal subsequently died. Other males showing hypersensitivity: 1 male receiving 10 mg/kg showed hypoactivity and squinted eyes, several 40 mg/kg males showed similar signs. These mice received 5 mg/kg, i.p. and the symptoms resolved.
- One female in the 20 mg/kg group was found dead on the first day of gestation (GD1). No clinical observations or necropsy findings were reported. The cause of death is presumed to be due to anaphylactic-like reaction.
- A male mouse receiving 40 mg/kg was found dead on SD53. This was a non-dosing day. Clinical observations made prior to death include: hunched posture, hypoactivity, squinted eyes, rough coat, urine stains, reduced feces and a 10.6 gram weight loss.

This death was apparently not due to hypersensitivity. None of the other mice in this group had similar signs. The cause of death was presumed to be due to undetermined factors compromising the health of this animal.

- Due to the necrotic tails, all males were sacrificed early at 9 weeks of dosing.

Clinical signs:

- Clinical signs in the remaining male animals included urine stains in all groups and tail findings that related to the tail vein route of administration. The tail findings included swelling and irritation beginning around SD35. As dosing continued, scaly skin and sores appeared in all groups and eventually necrosis and loss of a portion of the tail near the end of the study. A slightly higher incidence of these findings was noted in dose groups receiving ≥20 mg/kg.
- In surviving females, hypoactivity was observed after approximately 2 weeks of dosing. The hypoactivity was resolved with administration of DPH.
- A low incidence of swollen tails in females was noted in all groups.

Body weight:

- No remarkable effects on maternal body weight attributable to the test article were observed.
- For males, no consistent effect on body weight change attributable to the test article was reported.

Food consumption: No effect on food consumption for either males or females attributable to the test article was reported.

Toxicokinetics: No toxicokinetics were performed.

Necropsy:

- No treatment related effects on organ weights is reported.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

No. of male/female pairs	22	22	22	22
No. of females mated	22	22	22	22
Male/Female Copulation Index (%)	100	100	100	100
No. of females successfully mated	9	11	10	13
Male/Female Fertility Index (%)	41	50	45	59

- A slightly higher rate of pre-implantation loss is noted in treated groups. The trend appears to be dose dependent. However, the finding did not achieve statistical significance.
- The table below summarizes the findings for pre-implantation loss:

Pre-Implantation loss	Group 1 0 mg/kg	Group 2 10 mg/kg	Group 3 20 mg/kg	Group 4 40 mg/kg
mean	4.2	4.8	4.7	8.5
SD	4.0	5.4	5.2	7.8

- Treated females showed a small increase in late resorptions. This finding did not reach statistical significance.
- No difference in live litter size was noted.
- A statistically significant, dose-related reduction in sperm count per gram of epididymus is reported for the 20 and 40 mg/kg groups relative to control and low dose groups.
- A dose-related trend toward increased percentage of abnormal sperm for the 40 mg/kg group. This finding did not reach statistical significance.

A treatment-related reduction in the epididymal sperm count was observed in the 20 and 40 mg/kg groups, representing a 6 and 22% reduction relative to controls, respectively. A treatment-related increase in the percentage of abnormal sperm was observed in the 40 mg/kg group. The increase in abnormal sperm morphology was characterized by 45% of the animals having at least 5% amorphous sperm heads, as compared to 18% of the control animals. A reduction in the sperm motility in all groups, compared to historical control, may be attributed to either the vehicle control or the IP administration with DPH1. Treatment with rhGAA did not exacerbate the reduction in motility; therefore, changes in sperm motility are not attributed to rhGAA. NOEL for embryo/fetal viability was determined by the sponsor to be 40 mg/kg. However, the small increase in pre-implantation loss and late resorptions relative to control, although not clearly dose related indicate that the test article may not be benign. The choice of mouse as the species for this study is unusual. Toxicology data indicates that rat is more sensitive to the toxic effects of the test article.

Embryofetal development

Study title: Intravenous injection study for effects on embryofetal development with recombinant human acid-α- glucosidase (rhGAA) in mice

Key study findings: The purpose of this study was to assess the potential embryo/fetal toxicity and teratogenic potential of rhGAA administered daily in doses up to 40 mg/kg to CD-1 mice. Mated mice were dosed on gestation days (GD) 6-15 and fetuses were delivered by Cesarean section on GD18. Analysis of pregnancy data demonstrated no treatment effects on preimplantation loss, number of implantation sites, or number of corpora lutea were noted for this study. A small, dose related increase in late resorptions is noted. This finding did not reach statistical significance. However, it is consistent with findings from the previous study (#6354-155). Due to the loss of one whole litter in the high dose group, a significant increase in post-implantation loss was noted for the 40 mg/kg/day group. The low frequency of the finding indicates that it may be sporadic or perhaps due to a problem with maternal health. However, because it occurred in the high dose group, a test article effect cannot be ruled out. The results would be more convincing, perhaps, if the study had been performed in a more sensitive species.

No statistically significant effects of the test article on external fetal variations are noted. In some cases a slight trend may be observed but the incidence is very low and does not reach statistical significance. A test article effect is questionable. The total for fetal external malformations is such a case: The incidence among groups is 2, 3, 3, and 6 for groups

1, 2, 3, and 4, respectively. The total number of fetuses examined was 264, 301, 315 and 297 for each group, respectively.

The low incidence of these findings suggests that it may not be above the normal incidence for this species. The sponsor has not supplied historical data that may resolve this question.

Toxicokinetic analysis showed that serum concentrations for all groups were linear with dose. No evidence of saturation kinetics was noted for any dose up to 40 mg/kg. No data are provided for fetal or amniotic fluid levels.

Analyses of rhGAA activity in maternal liver, fetal liver and placenta indicated that there was a dose dependent increase in enzyme activity in adult liver and placenta but very little found in fetal liver. This indicates that either little rhGAA crosses the placental barrier or that the doses were not large enough to provide significant exposure to the developing fetus.

Study no.: 6354-153
Volume #, and page #:
Conducting laboratory and location:

Date of study initiation: 1/6/04
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot #, and % purity:

Test Article	Lot No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
rhGAA	751295	refrigerated	—	May 2004	1 vial

Control Article	Lot No.	Storage	Expiration Date	Reserve (Archive) Sample
GAA Control	03-LS-91133	refrigerated	04 June 2004	5 ml

Vehicle	Lot No.	Storage	Expiration Date	Reserve (Archive) Sample
Sterile Water	C592165	4–30 C	30 September 2004	5 ml

Methods

Doses: 0, 10, 20 and 40 mg/kg

Species/strain: Mouse, CD-1, females were 10-12 weeks old

Number/sex/group: 25/females/group with additional 18 at each dose level (except control) for toxicokinetic analysis.

Route, formulation, volume, and infusion rate: Animals were dosed IV in a volume of 8 ml/kg/day.

Satellite groups used for toxicokinetics: 18 animals per dose group, except control.

Study design: Mated females were administered test article, IV, on SD6-15. The basic study design is summarized in the table below, provided by the sponsor. Cesarean delivery was performed on GD18.

Group	No. of Females Main/TK	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dosing Schedule Days of Gestation
1 (Vehicle Control)	25/-	0	0	6-15
2 (Low)	25/18	10	1.25	6-15
3 (Mid)	25/18	20	2.5	6-15
4 (High)	25/18	40	5	6-15

Parameters and endpoints evaluated: Endpoints evaluated included clinical observations (daily), body weights (GD0, 4, 6, 8, 10, 12, 14, 16, and 18), TK (samples taken GD6 at pre-dose, 10, 30, 60, 180, and 360 minutes post-dose), food consumption (measured at each body weight measurement), gross necropsy of mothers including placenta and amniotic sac, uterus examined for number and placements of implantation sites, number of live and dead fetuses, early and late resorptions, fetal abnormalities. Ovaries were examined for number of corpora lutea. Livers from the dams and placentas from 5 randomly selected litters in each group were retrieved and fixed for further evaluation.

Results

Mortality (dams): All dams survived to scheduled sacrifice.

Clinical signs (dams): No significant effects attributable to test article administration were reported.

Body weight (dams): No treatment effect noted.

Food consumption (dams): No treatment effect noted.

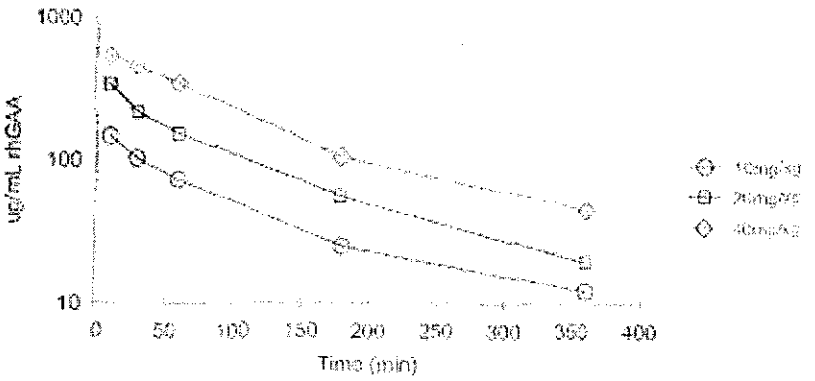
Toxicokinetics:

Table 1: Toxicokinetic parameters

	10mg/kg	20mg/kg	40mg/kg
Elimination T ½ (min)	76.27 ± 11.5	78.77 ± 4.3	71.99 ± 5.5
Cl (ml/min/kg)	0.69 ± 0.07	0.69 ± 0.05	0.65 ± 0.07
MRT (min)	98.2 ± 14.8	109.3 ± 11.1	96.6 ± 6.2
AUC (min*ug/ml)	15364.1 ± 631.5	31662.8 ± 2461.8	64380.6 ± 7814.7
AUC/dose (min*ug/ml/mg/kg)	1536.4 ± 64.1	1585.2 ± 123.1	1614.5 ± 195.4
Vss (ml/kg)	68.7 ± 6.7	59.7 ± 6.8	59.8 ± 6.3
Rsq	0.99	0.98 ± 0.02	0.99

Figure 1:

Log-linear plot of concentration-time data depicting the average serum rhGAA concentration as a function of time



Toxicokinetic analysis showed that serum concentrations for all groups were linear with dose. No evidence of saturation kinetics was noted for any dose up to 40 mg/kg. No data are provided for fetal or amniotic fluid levels.

Analyses of rhGAA activity in maternal liver, fetal liver and placenta indicated that there was a dose dependent increase in enzyme activity in adult liver and placenta but very little found in fetal liver. This indicates that either little rhGAA crosses the placental barrier or that the doses were not large enough to provide significant exposure to the developing fetus. The tables below, provided by the sponsor, summarize the levels of rhGAA activity in adult and fetal liver and placenta.

Adult Livers, Final Data

Study 6354-153 Adult Livers
4MU Activity Assay Analysis
Final
Data

Group	Dose	Mean, ug/g	Stdev	%CV
1	0	13.988	2.6113	18.7
2	10	194.289	31.5287	16.2
3	20	436.647	86.7523	19.8
4	40	1033.942	194.4826	18.8

Fetal Livers, Final Data

Study 6354-153 Fetal Livers
rhGAA 4MU Activity Assay
Analysis
Final
Data

Group	Dose	Mean, ug/g	Stdev	%CV
1	0	3.203	1.6326	51.0
2	10	4.199	1.8532	44.1
3	20	3.177	1.5968	50.3
4	40	3.967	1.0044	25.4

Placentas, Final Data

Study 6354-153 Fetal Placentas
rhGAA 4MU Activity Assay
Analysis
Final
Data

Group	Dose	Mean, ug/g	Stdev	%CV
1	0	8.139	1.0667	13.1
2	10	34.419	7.8323	23.0
3	20	70.178	27.1834	38.7
4	40	138.966	47.0724	33.9

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

- No treatment effects in preimplantation loss were noted for this study.
- No treatment effect on the number of implantation sites was reported.
- No treatment effects were noted for the number of corpora lutea.
- Small, dose related increase in late resorptions is noted. This finding did not reach statistical significance. However, it is consistent with findings from the previous study (#6354-155).
- A significant increase in post-implantation loss for the 40 mg/kg/day group.

The tables below, provided by the sponsor, summarize the cesarean section parameters examined and results:

Summary of Cesarean Section Data

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Females Mated	N	25	25	25	25
Pregnant	N	23	25	25	24
	%	92	100	100	96
Aborted	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Died	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Delivered Early	N	4	3	4	1
	%	16	12	16	4.0
Pregnant at C-section	N	22	23	24	24
Dams with Viable Fetuses	N	22	23	24	23
	%	100	100	100	96
Dams with no Viable Fetuses	N	0	0	0	1
	%	0.0	0.0	0.0	4.2
Corpora Lutea	MEAN	13.7	14.6	14.7	14.6
	S.D.	1.9	2.0	2.7	2.3
	N	22	23	24	24
	TOTAL	302	336	352	351
Implantation Sites	MEAN	12.9	13.9	13.4	13.5
	S.D.	1.3	1.7	2.1	2.5
	N	22	23	24	24
	TOTAL	283	319	322	324
Preimplantation Loss	MEAN%	6.2	4.8	7.7	8.2
	S.D.	5.4	5.6	7.4	9.8

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

TABLE 7
SUMMARY OF CESAREAN SECTION DATA

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Pregnant at C-section	N	22	23	24	24
Live Fetuses	MEAN	13.0	13.1	13.1	12.4
	S.D.	1.5	1.9	2.3	3.6
	N	22	23	24	24
	TOTAL	286	301	315	297
	MEAN*	93.4	94.5	97.6	86.3
	S.D.	6.3	8.5	4.6	22.5
Females	MEAN	6.1	7.4	7.2	6.7
	S.D.	1.7	2.4	2.3	3.6
	N	22	23	24	23
	TOTAL	134	170	174	154
	MEAN%	51.6	56.2	55.1	52.8
	S.D.	14.9	15.1	13.8	11.5
Males	MEAN	5.8	5.7	5.9	6.2
	S.D.	2.2	2.1	2.2	2.1
	N	22	23	24	23
	TOTAL	130	131	141	143
	MEAN*	48.5	43.8	44.9	47.2
	S.D.	14.9	15.1	13.8	11.5
Sex Ratio M:F		49:51	44:56	45:55	48:52

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

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Pregnant at C-section	N	22	23	24	24
Dams with Viable Fetuses	N	22	23	24	23
Resorptions: Total	MEAN	0.9	0.8	0.3	1.0
	S.D.	1.1	1.1	0.6	1.5
	N	22	23	24	23
	TOTAL	19	18	7	23
	MEAN%	6.6	5.5	2.4	7.9
	S.D	8.3	8.1	4.6	12.7
Early	MEAN	0.8	0.6	0.2	0.7
	S.D.	1.1	0.9	0.5	1.4
	N	22	23	24	23
	TOTAL	17	13	6	15
	MEAN%	5.6	4.1	2.1	5.2
	S.D.	8.0	6.9	4.5	9.7
Late	MEAN	0.1	0.2	0.0	0.3
	S.D	0.3	0.4	0.2	0.6
	N	22	23	24	23
	TOTAL	2	5	1	8
	MEAN%	0.8	1.5	0.3	2.7
	S.D	2.6	2.8	1.4	4.6
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	6.6	5.5	2.4	7.9
	S.D	8.3	8.1	4.6	12.7

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01
MEANS CALCULATED EXCLUDING DAMS WITH NO VIABLE FETUSES.

Offspring (malformations, variations, etc.):

- No statistically significant effects of the test article on external fetal variations are noted. In some cases a slight trend may be observed but the incidence is very low and does not reach statistical significance. A test article effect is questionable. The total for fetal external malformations is such a case: The incidence among groups is 2, 3, 3, and 6 for groups 1, 2, 3, and 4, respectively. The total number of fetuses examined was 264, 301, 315 and 297 for each group, respectively.

Reviewer comment: The low incidence of these findings suggests that it may not be above the normal incidence for this species. The sponsor has not supplied historical data that may resolve this question.

- One dam in the high dose group had 0 viable fetuses. No other groups showed this finding.

Reviewer comment: The low frequency of the finding indicates that it may be sporadic or perhaps due to a problem with maternal health. However, because it occurred in the high dose group, a test article effect cannot be ruled out. The results would be more convincing, perhaps, if the study had been performed in a more sensitive species.

- No treatment effect on offspring sex ratios is reported.

The tables below, provided by the sponsor, summarize the parameters examined in the fetuses and the results:

Summary of Mean Fetal Weights (g)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
of all Viable Fetuses	MEAN	1.33	1.33	1.33	1.32
	S.D.	0.10	0.08	0.08	0.06
	N	22	23	24	23
Covariate Adjusted	MEAN	1.32	1.33	1.33	1.32
of Male Fetuses	MEAN	1.35	1.37	1.35	1.34
	S.D.	0.13	0.10	0.09	0.08
	N	22	23	24	23
Covariate Adjusted	MEAN	1.34	1.37	1.35	1.34
of Female Fetuses	MEAN	1.30	1.30	1.31	1.30
	S.D.	0.13	0.09	0.08	0.09
	N	22	23	24	23
Covariate Adjusted	MEAN	1.30	1.30	1.32	1.30

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

Summary of Fetal External Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Litters Evaluated	N	22	23	24	23
Fetuses Evaluated	N	264	301	315	297
Live	N	264	301	315	297
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL VARIATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

N = NUMBER

Summary of Fetal External Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Litters Evaluated	N	22	23	24	23
Fetuses Evaluated	N	264	301	315	297
Live	N	264	301	315	297
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL VARIATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

N = NUMBER

Summary of Fetal External Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Litters Evaluated	N	22	23	24	23
Fetuses Evaluated	N	264	301	315	297
Live	N	264	301	315	297
Dead	N	0	0	0	0
MALFORMED HINDLIMBS					
Fetal Incidence	N	2	3	3	5
	%	0.8	1.0	1.0	1.7
Litter Incidence	N	2	2	2	2
	%	9.1	8.7	8.3	8.7
UMBILICAL HERNIA					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.3
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.3
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	2	3	3	6
	%	0.8	1.0	1.0	2.0
Litter Incidence	N	2	2	2	3
	%	9.1	8.7	8.3	13

N = NUMBER

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

Fetal Soft Tissue Variations

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Litters Evaluated	N	22	23	24	23
Fetuses Evaluated	N	135	149	155	149
Live	N	135	149	155	149
Dead	N	0	0	0	0
RENAL FOLD					
Fetal Incidence	N	1	0	0	0
	%	0.7	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.5	0.0	0.0	0.0
INCREASED RENAL PELVIS CAVITATION					
Fetal Incidence	N	1	0	1	2
	%	0.7	0.0	0.6	1.3
Litter Incidence	N	1	0	1	2
	%	4.5	0.0	4.2	8.7
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence	N	2	0	1	2
	%	1.5	0.0	0.6	1.3
Litter Incidence	N	2	0	1	2
	%	9.1	0.0	4.2	8.7

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

Fetal Soft Tissue Malformations

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Litters Evaluated	N	22	23	24	23
Fetuses Evaluated	N	135	149	155	149
Live	N	135	149	155	149
Dead	N	0	0	0	0
CLEFT PALATE					
Fetal Incidence	N	0	1	2	0
	%	0.0	0.7	1.3	0.0
Litter Incidence	N	0	1	2	0
	%	0.0	4.3	8.3	0.0
INTERNAL HYDROCEPHALY					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.7
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.3
KIDNEY-REDUCED IN SIZE (BILATERAL)					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.7
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.3
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	0	1	2	2
	%	0.0	0.7	1.3	1.3
Litter Incidence	N	0	1	2	1
	%	0.0	4.3	8.3	4.3

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

Fetal Skeletal Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Litters Evaluated	N	22	23	24	23
Fetuses Evaluated	N	129	152	160	148
Live	N	129	152	160	148
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF SKULL					
Fetal Incidence	N	1	0	4	2
	%	0.8	0.0	2.5	1.4
Litter Incidence	N	1	0	4	2
	%	4.5	0.0	17	8.7
UNOSSIFIED HYOID BODY					
Fetal Incidence	N	1	1	0	1
	%	0.8	0.7	0.0	0.7
Litter Incidence	N	1	1	0	1
	%	4.5	4.3	0.0	4.3
ACCESSORY BONE(S) IN SKULL					
Fetal Incidence	N	2	1	4	2
	%	1.6	0.7	2.5	1.4
Litter Incidence	N	1	1	3	2
	%	4.5	4.3	13	8.7
25 PRESACRAL VERTEBRAE					
Fetal Incidence	N	0	2	0	1
	%	0.0	1.3	0.0	0.7
Litter Incidence	N	0	2	0	1
	%	0.0	8.7	0.0	4.3
STERNEBRA(E) ASYMMETRICALLY OSSIFIED					
Fetal Incidence	N	15	10	15	10
	%	12	6.6	9.4	13
Litter Incidence	N	12	6	12	11
	%	55	26*	50	48

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

TABLE 13
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Litters Evaluated	N	22	23	24	23
Fetuses Evaluated	N	129	152	160	148
Live	N	129	152	160	148
Dead	N	0	0	0	0
STERNEBRAE EXTRA OSSIFICATION SITE(S)					
Fetal Incidence	N	6	11	4	9
	%	4.7	7.2	2.5	6.1
Litter Incidence	N	6	7	3	6
	%	27	30	13	26
SCH/6SH STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	0	1	1	0
	%	0.0	0.7	0.6	0.0
Litter Incidence	N	0	1	1	0
	%	0.0	4.3	4.2	0.0
SCH STERNEBRA UNOSSIFIED					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.7	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	4.3	0.0	0.0
SCH/6SH STERNEBRA(E) BIPARTITE					
Fetal Incidence	N	1	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.5	0.0	0.0	0.0
MINOR FUSION OF STERNEBRAE					
Fetal Incidence	N	0	1	1	0
	%	0.0	0.7	0.6	0.0
Litter Incidence	N	0	1	1	0
	%	0.0	4.3	4.2	0.0

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

Fetal Skeletal Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 20 MG/KG/DAY	GROUP 4 40 MG/KG/DAY
Litters Evaluated	N	22	23	24	23
Fetuses Evaluated	N	129	152	160	148
Live	N	129	152	160	148
Dead	N	0	0	0	0
MAJOR FUSION OF STERNEBRAE					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.6	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	4.2	0.0
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.6	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	4.2	0.0

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

Prenatal and postnatal development

No studies of prenatal and postnatal development were performed for this BLA. For the infantile indication, such studies should not be required prior to initial approval due to the dire nature of the disease. However, for the late onset indication such studies should be requested.

2.6.6.7 Local tolerance

No studies were performed.

2.6.6.8 Special toxicology studies

No studies were performed.

2.6.6.9 Discussion and Conclusions

The toxicology package for this BLA contains single dose studies in SD rat and beagle dogs, repeat dose studies in SD rat, cynomolgus monkey and C57bl/7 1 mice as well as segment 1 and segment 2 reproductive toxicology studies in CD-1 mice. A number of concerns regarding these studies have been identified during this review. These include the following:

- The potential for higher rate of toxicity in rats as evidenced by unexplained deaths and uninvestigated toxicities in study 6354-140.
- The potential lot-to-lot difference in rhGAA uptake in the liver, PK and toxicity. This is especially apparent in rhGAA from different manufacturing scales. These concerns have been discussed with the product review team.

- The choice of species for the reproductive toxicology may not be the most sensitive to the toxicities of rhGAA possible resulting in under-representation of adverse effects. However, the choice of the mouse was discussed with CBER and was agreed to based on the greater severity of the hypersensitivity reactions apparent in the rat toxicology studies.
- No second species for the Segment 2 reproductive toxicology studies. (*The sponsor has agreed to perform a second segment 2 toxicology study in a non-rodent species.)*
- The Segment 1 reproductive toxicology study did not include toxicokinetics so exposure to the test article cannot be verified.
- An additional toxicology study mentioned in the non-clinical summary that was to be finished by Q3 2005 6354-163 is not included in this submission.

In spite of these concerns, from the Pharmacology/Toxicology perspective, approval is recommended for alglucosidase alfa for use in the infantile onset indication and for the 160 liter manufacturing process only. It is recommended that the outstanding issues be resolved prior to approval for the late onset indication or the 2000 liter manufacturing process.

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

Tables omitted per Dr. Choudary.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Pompe's disease is an autosomal recessive disorder resulting in the failure of acid α-glucosidase to hydrolyze lysosomal glycogen. This failure leads to abnormal accumulation of glycogen within lysozomes of some tissues. Pompe's disease is a severe form in which rapidly progressive disease results in cardiac failure. The infantile onset form is uniformly fatal within 18 months of birth.

Alglucosidase alfa (rhGAA) is a replacement enzyme produced by recombinant technology using Chinese hamster ovary cells as the production cell line. The function of this product, human recombinant rhGAA, is degradation of glycogen to glucose, a process that normally takes place within the lysozomes of the cell. Enzyme deficiency results in accumulation of glycogen in the lysozomal compartment, a condition known as glycogenosis type II. The product, rhGAA, is administered intravenously (IV) as a form of enzyme replacement therapy (ERT). Once internalized by the cell, and passed to the lysosome by intracellular mechanisms, hGAA 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 uptake of exogenously administered enzyme by endocytosis (Raben, 2003, *Molecular Genetics and Metabolism.)*

A series of non-clinical efficacy studies was performed to identify an optimal dosing regimen. Those studies were performed in the Pompe knockout mouse model in which the GAA gene is disrupted by a *neo* insertion in exon 6 ($6^{neo}/6^{neo}$). These animals develop biochemical and pathological changes similar to those seen in the human disorder including reduced muscle strength and immobility. . The doses tested ranged from 1 mg/kg administered weekly for four weeks to 100 mg/kg administered weekly for 4 weeks. The longest duration studies consisted of 10 and 20 mg/kg administered weekly for 16 weeks, and 10, 20, 40 mg/kg every other week (qow) for 16 weeks.

In general, the 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 the rhGAA were observed among the various muscles sampled. Glycogen was consistently cleared from cardiac muscle more readily than from skeletal muscle. 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. The various skeletal muscles showed significant variation in response to rhGAA. The relative magnitude of depletion among muscle remained consistent from study to study. The differences in efficacy among the various muscles tested may reflect heterogeneity of mannose-6-phosphate receptor among those muscles.

When efficacy between 3 month old and 12 month old Pompe knockout mice was investigated, younger animals were significantly more responsive to glycogen clearance by rhGAA than the older animals. The relative magnitude of depletion among the muscle types was consistent with other studies. 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.

Both single and repeat dose pharmacokinetic studies were conducted in Pompe GAA knockout mice. When a semilogarithmic plot of concentration versus time was constructed from data produced by each of the 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-life was on the order of 2-3.5 hours for monkey, rat 1-2 hours, Pompe knockout mouse 1-2 hours, CD-1 mouse approximately 75 minutes, and beagle dog approximately 1.5 to 2 hours. 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 and AUC/dose. Both males and females in the high dose group showed significantly higher elimination half-life relative to the other dose groups, females for days 1-85 and male for days 1-169.

The toxicology package for this BLA contains single dose studies in the Sprague-Dawley rat and the beagle dog, repeat dose studies in Sprague-Dawley rat, cynomolgus monkey and C57Bl/6 mice. The test article was delivered by IV infusion for all studies. The rhGAA was well tolerated in monkey 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 male animals (of 3 in each group) from the two higher dose groups and ovarian cyst and unequal sized ovaries in one female of 3 from the high dose group as well as inflammation and degeneration of the quadriceps muscle in one female of three from the high dose group. A relationship to the test article cannot be ruled out but, due to the small number of animals per group, this relationship is difficult to establish.

The rhGAA was also generally well tolerated in C57Bl/6 mice at doses up to 100 mg/kg administered weekly for 4 weeks. The mouse repeat dose toxicity study (#2009) was intended to compare toxicities produced by product from three different manufacturing

processes. The toxicity profile was similar among the three products, but an increase in severity seemed to be present for the BI2KrhGAA (2000 liter) and BI — rhGAA — liter) relative to the GENZrhGAA (presumably the 160 liter product). The toxicities noted include 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 addition, a dose related mild increase in serum albumin was noted for all treatment groups and was more prevalent in females. Two females of 6 per group receiving 100 mg/kg of GenzrhGAA or BI — rhGAA had mildly increased AST and ALT levels (79% for AST, 69% for ALT). This finding may be related to the test article and suggests a potential adverse effect on the liver.

In a similar 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. The histopathology was not performed but the tissues are archived at Genzyme. (See toxicology summary for complete discussion.)

An additional repeat dose toxicity study was performed in the rat (#6354-133) included only 5 per sex per group and investigated only one test article. Toxicities were less pronounced than those observed in 6354-140. The animals in this study showed a dose related statistically significant decrease in body weight. For males the weight loss was 23.3% and for females, 11.6%. No clinical pathology or anatomical pathology results correlated with the weight loss finding. No other significant findings were reported.

Rodents tend to launch a severe hypersensitivity reaction to the human recombinant 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 the DPH may have had or any interaction between DPH and the test article could not be assessed.

A number of concerns regarding these studies have been identified during this review. These include the following:

- The potential for higher rate of toxicity in rats as evidenced by unexplained deaths and uninvestigated toxicities in study 6354-140.
- The potential lot-to-lot difference in rhGAA uptake in the liver, PK and toxicity. This is especially apparent in rhGAA from different manufacturing scales. These concerns have been discussed with the product review team.
- The choice of species for the reproductive toxicology may not be the most sensitive to the toxicities of rhGAA possible resulting in under-representation of adverse effects.
- No second species for the Segment II reproductive toxicology studies.

- The Segment I reproductive toxicology study did not include toxicokinetics so exposure to the test article cannot be verified.
- An additional toxicology study mentioned in the non-clinical summary that was to be finished by Q3 2005 6354-163 is not included in this submission.
- In considering all the non-clinical studies together, a pattern seems to emerge that may suggest the presence of a lot-to-lot variation in pharmacokinetics (PK) and toxicities. In study #6354-140, the incidence and severity of toxicities appear to be increased in rhGAA produced by 2000 liter and — liter manufacturing processes. In addition, biodistribution studies indicate that rhGAA from the 2000 liter process has increased uptake into the liver relative to the product from the 160 liter process. There was some suggestion that lot-to-lot variation (— in the 160 liter process resulted in observable variations in PK and toxicities among lots. Not enough data is available at this time to fully investigate this concern. However, these concerns reflect manufacturing issues that are being managed by the CMC review team. The non-clinical data has been provided to the product reviewers and discussed in detail. These issues will be followed up by the CMC review team.

Reproductive toxicology:

The reproductive toxicology package included in this submission is inadequate to support approval of alglucosidase alfa for the late onset indication. The studies performed include a single Segment I study and a single Segment II study, both performed in CD-1 mice. For the Segment I study, mice received doses up to 40 mg/kg every other day beginning prior to mating and through early embryonic development. No clear rationale was given for the choice of dose and no pilot studies are included to support the choice of high dose. Results of this study showed a trend toward pre-implantation loss and late resorptions. These findings did not reach statistical significance and historical normal values for comparison were not found. Changes in male fertility parameters were also found. A statistically significant and dose related reduction in sperm count as well as an increase in abnormal sperm. The male mice also showed urine stained abdomens suggesting the presence of more generalized toxicity. No toxicokinetic analysis was performed so relative exposures to the test article cannot be established.

For the Segment II study, mated female mice received doses up to 40 mg/kg administered daily. No evidence of preimplantation loss was noted during this study. A small dose related increase in late resorptions was observed but did not reach statistical significance. However, in considering pregnancy parameters as a whole, a significant increase in post-implantation loss was noted for the high dose group. There were no statistically significant adverse effects on fetal development reported.

A second Segment II study in a non-rodent species is recommended to confirm and clarify the potential toxic effects.

In addition, a Segment III study should be considered for the older patient population. These issues should be resolved prior to approval of alglucosidase alfa for the late onset indication.

Suggested labeling:

Based on the non-clinical package as a whole, the following recommendations regarding labeling are given:

- In the Laboratory Tests section, the sponsor mentions only the two toxicology studies monkey where very little toxicity was noted.
- In the section titled Carcinogenicity, Mutagenesis and Impairment of Fertility, addition of effects on male fertility parameters is recommended. Results of study # 6354-155 (Fertility and Early Embryonic Development) showed a dose dependent decrease in sperm count and increase in abnormal morphology. This study was done in mice, which may not be the most sensitive species. Therefore, the toxicities may be underestimated. In addition, increased occurrence of ovarian cysts was observed for mouse and monkey.
- Pregnancy Category: The sponsor suggests that this product be designated pregnancy category B. However, study #6354-153 showed a significant increase in post-implantation loss in the high dose group when pregnancy parameters are considered as a whole. The trends of reduced embryo viability in study #6354-155 and the increased post-implantation loss seen in study 6354-153, the lack of toxicokinetics to assure exposure to the test article in study #6354-155, the use of a potentially non-sensitive species for these studies, the lack of a segment 2 study in a second (non-rodent) species, and the lack of a clear justification of the high dose used in both studies render the Category B claim tenuous, at best. At this time, Pregnancy Category — would be more appropriate.
- A toxicology study in juvenile animals may be useful.

It is recommended that the outstanding issues be addressed prior to approval of alglucosidase alfa for the late onset indication.

Recommendation on approval

Based on the non-clinical data provided, from a non-clinical standpoint, approval is recommended for treatment of infantile onset Pompe's disease.

Signatures (optional):

Reviewer Signature Barbara J. Wilcox 3/22/06
Barbara J. Wilcox, Ph.D.

See the accompanying Supervisory Addendum.

Supervisor Signature Jasti Choudary Concurrence Yes ___ No ___
Jasti Choudary, B.V.Sc., Ph.D. 4/13/2006

CC:
BLA
HFD-180

HFD-181/CSO
HFD-180/Dr. Choudary
HFD-120/Dr. Wilcox
HFD-048/Dr. Viswanathan

R/D Init.:J. Choudary 3/10/06

APPENDIX/ATTACHMENTS