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**Memorandum**

Division of Clinical Trial Design and Analysis
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# BRIEFING DOCUMENT
## BLA STN 103977/0

# Transkaryotic Therapies, Inc.

# AGALSIDASE ALFA
# Alpha-Galactosidase A

# For Treatment of Fabry Disease

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## Acronyms

| | |
|---|---|
| AE | Adverse experience |
| ALDS | Active lipid damage score |
| ALT, AST | Amino transferase liver enzymes |
| AUC | Area-under-the-curve |
| BLA | Biological license application |
| BPI | Brief Pain Inventory Questionnaire |
| CDS | Chronic damage score |
| CNS/PNS | Central nervous system/peripheral nervous system |
| COPD | Chronic obstructive pulmonary disease |
| CR | Complete review |
| CRF | Case report form |
| CRIM | Cross-reactive alpha-galactosidase A-like material |
| CTH | Ceramidetrihexosides, same as $Gb_3$ (globotriasosylceramide) |
| EKG | Electrocardiogram |
| ELISA | Enzyme immunoassay |
| GI | Gastrointestinal |
| GFR/RPF | Glomerular filtration rate/renal plasma flow |
| HSA | Human serum albumin |
| H and E | Hematoxylin and Eosin stain |
| IND | Investigational new drug application |
| ID | Identification |
| ITT | Intent to treat |
| IV | Intravenous |
| LOCF | Last observation carried forward imputation method |
| LV | Left ventricle |
| MI | Myocardial infarction |
| MRI | Magnetic resonance image |
| M6P | Mannose-6-phosphate |
| NPS | Neuropathic pain scale |
| NIH | National Institutes of Health |
| PFT | Pulmonary function test |
| PT/APTT | Prothrombin time/activated partial thromboplastin time |
| QOL | Quality of Life |
| SAE | Serious adverse experience |
| SH | Standard histopathology |
| TKT | Transkaryotic Therapy, Inc. (sponsor) |
| URI | Upper respiratory tract infection |

## 1. Introduction:

This document is a summary of the clinical information contained within the Biological License Application (BLA) submitted to FDA by Transkaryotic Therapy, Inc (TKT) for alpha-galactosidase A (Agalsidase alfa), a recombinant enzyme product. Agalsidase is proposed for treatment of patients with Fabry disease.

This BLA contains a large amount of clinical data obtained from multiple studies. However, the most important data generally come from the application's two controlled clinical studies: the 26 subject Study TKT003 and, to a lesser extent, the 15 subject Study TKT005. All other studies were uncontrolled, open-label studies. Because of the extent of these clinical data, this document includes a summary overview section prior to the more detailed review of each individual study. The summary overview section is intended to describe solely the major observations from the series of clinical studies and to highlight the limitations of these observations. Substantially more detail is provided within the annotated review of each clinical study.

## 2. Materials Reviewed and Regulatory History:

This document includes a comprehensive review of all clinical information submitted with this application (including amendments and a response to an FDA Complete Review letter) and medical literature publications.

The original submission was on June 16, 2000, with multiple amendments submitted after that time. The clinical information contained within the original BLA filing included the results from a phase 1 study, two controlled clinical studies (Study TKT003 and Study TKT005) and interim data from Study TKT006, an uncontrolled, open-label study. Subsequent amendments supplied additional data clarification information, additional histopathological analyses and a complete report for Study TKT006. FDA reviewed this information and issued a Complete Review letter to the sponsor on December 22, 2000.

Following issuance of the FDA Complete Review letter the sponsor submitted an amendment to the BLA containing additional histopathology information. On May 23, 2002, the sponsor submitted a comprehensive response to the FDA Complete Review letter. This comprehensive response contained clarification information and data from two additional uncontrolled studies, Study TKT011 and Study TKT014. On November 22, 2002, FDA issued a second Complete Review letter. Table 1 contains a summary of the major regulatory time points for the BLA.

**Table 1. Major Regulatory History**

| Date | Action |
|---|---|
| June 16, 2000 | BLA filed |
| December 22, 2000 | FDA issues Complete Review Letter |
| May 23, 2002 | Response to Complete Review Letter |
| November 22 ,2002 | FDA Issues second Complete Review Letter |

## 3. Proposed Indication and dose:

At the time of the original BLA filing, the sponsor proposed that Agalsidase be indicated for long term enzyme replacement therapy of patients with Fabry disease with the claimed benefits of:

1. reduction of pain
2. stabilization or improvement in renal function
4. increase in weight
5. reduction of cardiac enlargement and improvement in cardiac function.

The original indication proposal for Agalsidase was subsequently modified. Within the May 23, 2002 BLA amendment the sponsor proposed that Agalsidase be indicated for "long term enzyme replacement therapy for patients with Fabry Disease," an indication that identified no specific clinical outcome.

The dose of Agalsidase proposed for marketing is 0.2 mg/kg (mixed within 100 mL normal saline), administered as an IV infusion over 40 minutes. This dose is to be repeated every two weeks. No specific duration of treatment is proposed so that the intent in this lifelong disease is for lifelong treatment.

## 4. Clinical Background:

Fabry disease is a congenital lipid storage disease attributed to a deficiency of the lysosomal enzyme, alpha-galactosidase A. This enzyme is thought to be involved in metabolic degradation of cellular membrane lipids. Alpha-galactosidase A is targeted to its lysosomal site of action by mannose-6-phosphate (M6P) residues on the alpha-galactosidase A molecule. The M6P moiety binds to a specific M6P receptor in the Golgi and is thus directed to prelysosomal compartments. Enzymes that escape this routing system are secreted by the cell via the constitutive secretory pathway and are often recaptured by cell surface M6P receptors that return the alpha-galactosidase A to the lysosome by the endocytic pathway. Specifically, alpha-galactosidase A deficiency leads to accumulation of ceramidetrihexosides (CTH, also known as globotriaosylceramide, GL-3 or $Gb_3$) within cellular lysosomes and the blood.

Fabry disease has been related to multiple mutations within the gene coding for alpha-galactosidase A. The gene is located on the X chromosome (Xq22). Consequently, the disease is inherited in an X-linked pattern and the vast majority of patients are male. The disease is rare and has been estimated to occur at an incidence of 1 in 40,000 persons. The disease impacts virtually all tissues within the body and results in a broad spectrum of clinical abnormalities.

The systemic manifestations of the disease have been largely attributed to the accumulation of precursor metabolic molecules in fibroblasts, muscle cells and endothelial cells. The endothelial cell engorgement with precursor molecules is an especially prominent pathological feature of the disease. The endothelial cell pathology has been implicated in the occlusive vascular abnormalities that result in glomerulopathy, neuropathy, and myocardial ischemia. Additionally, the development of lower extremity edema has been attributed to lymphatic endothelial cell pathology.

In general, the disease initially manifests with skin abnormalities and pain during adolescence.[1] Subsequently, patients may develop end organ failure, with renal failure being the most common major clinical sequela of the disease. Other late sequelae include cardiac failure related to myocardial infarction and/or valvular insufficiency and cerebrovascular infarction. Based upon published studies over the past few decades, death has been reported to generally occur during the fourth to sixth decade of life. The impact of newer treatment strategies, such as renal transplantation, upon mortality is unclear.

Pain is generally the most disabling early symptom of the disease. The most common form of pain is an acroparesthesia--pain in the extremities. The extremity pain has been recognized as taking two major forms--chronic dull pain and "Fabry crises." Fabry crises consist of intense acute episodes of pain that may be associated with low grade fever. Chronic pain has been treated with phenytoin and carbamazepine as well as analgesics.

Treatment of Fabry disease is symptomatic and supportive. Renal transplantation has been performed with successful results and long term survival of the renal grafts.

[1] Desnick, RJ, Ioannou, YA, Eng, CM. Alpha-galactosidase deficiency: Fabry Disease. In: The Metabolic and Molecular Basis of Inherited Disease, 7th edition. McGraw-Hill, New York. 2741, 1995.

## 5. Product Background:

TKT's alpha-galactosidase is produced by gene activation technology from an established human fibroblast cell line. Gene-activation uses homologous recombination to activate a portion of the endogenous alpha-galactosidase A coding sequence in the host human cell. Human alpha-galactosidase is then purified through multiple column chromatography and filtration steps. The enzyme is a homodimer comprised of approximately 50 kDa subunits, and contains three N-linked glycosylation sites. Formulated drug product is supplied as a sterile solution. Each vial of drug product (3.5 mL) contains 3.5 mg of alpha-galactosidase A, 12 mg sodium phosphate as a buffering agent, 0.8 mg polysorbate 20 as a stabilizing agent and 31 mg sodium chloride as an isotonic agent at a pH of 5.4 - 6.6. The vials contain no preservatives and are intended for single use. Drug product is delivered into sterile saline (0.9% sodium chloride) for intravenous administration.

Preclinical acute toxicity studies and the initial clinical study (TKT001), utilized alpha-galactosidase A enzyme that was manufactured using recombinant DNA technology (designated DRX005A). Complementary DNA for human alpha-galactosidase was inserted into an established human cell line and the enzyme was secreted into the cell culture medium. Alpha-galactosidase A was then purified from the conditioned cell culture media through a series of column chromatography and filtration steps.

All subsequent clinical studies have utilized alpha-galactosidase A produced from gene-activation of a human fibroblast cell line (designated DRX005B). The alpha-galactosidase enzyme is secreted into the cell culture medium and subsequently purified using column chromatography and filtration steps. Physicochemical and animal testing demonstrated comparability between DRX005A and DRX005B drug substance. During product development, DRX005B was manufactured by two different manufacturing facilities using two different manufacturing processes. Common to these processes was cell culture utilizing roller-bottle cell culture techniques. Comparability between drug substance produced by these two manufacturing processes was demonstrated by biochemical and animal studies. Commercial manufacturing, from one manufacturer and using one process, utilizes the gene activated human cell line and has been designated as DRX005B-BSCP.

## 6. Preclinical Studies:

Preclinical studies in several animal species supported the safety of the proposed clinical dose regimen. Notably, the sponsor performed two experiments using a knockout mouse model of Fabry disease. These animals develop excessive accumulation of CTH ($Gb_3$) in tissues throughout their body. A single injection was shown to decrease liver content of CTH ($Gb_3$) by 79% at 0.2 mg/kg and 90% at 1 mg/kg, while multiple doses were observed to reduce CTH ($Gb_3$) to levels similar to wild type mice. Heart CTH ($Gb_3$) accumulation was shown to decrease, by 56% for 0.1 mg/kg and 83% with 1.0 mg/kg given repeatedly. Renal CTH ($Gb_3$) was also observed to decrease with enzyme treatment, to 14% and 40% following multiple injections at 0.1 and 1.0 mg/kg, respectively.

## 7. Overview of Clinical Studies:

All major (non-phase 1) studies with Agalsidase tested a dose of 0.2 mg/kg delivered as an intravenous infusion every two weeks. The major clinical safety and efficacy data within the BLA come from two controlled clinical studies, Study TKT003 and Study TKT005. The BLA also includes data from three uncontrolled, open-label studies, Study TKT006, TKT011 and TKT014. Information from a single dose phase 1 study was also included within the BLA. Table 2 provides a summary of the overall clinical data submitted to the BLA.

**Table 2. Overview of clinical study data submitted to BLA**

| Study | Country | Design/notes | # subjects | Duration | Status |
|---|---|---|---|---|---|
| TKT001 | USA | Phase 1, uncontrolled | 10 | single dose | completed |
| TKT003 | USA | Phase 2, placebo-controlled | 26 | 6 months | completed |
| TKT005 | UK | Phase 2, placebo-controlled | 15 | 6 months | completed |
| TKT006 | USA | Uncontrolled maintenance, post TKT003 | 25 | 1 year | completed |
| TKT011 | USA | Uncontrolled maintenance, post TKT006 | 24 | 1 year + | ongoing |
| TKT014 | Germany | Uncontrolled study in females | 15 | $\leq$ 1 year | completed |

Overall, clinical outcome data from 47 subjects exposed to Agalsidase (manufactured using the methods to be used for the product proposed for marketing) have been submitted to the BLA (data from Studies TKT003, TKT005, TKT006, TKT011, TKT014). This includes data from 32 men and 15 women. All clinical studies were performed among adults.

*Comment: For convenience, this review occasionally cites the sponsor's study numbers without the "TKT" prefix, subject ID numbers are occasionally cited without the routine "00" prefix and Agalsidase alfa is generally referred to as "Agalsidase." Overall, most summary data are reported as mean $\pm$ standard error (se) unless otherwise noted.*

As described in Section 5 above, there have been 2 major versions of the product, with two subversions of the second form of the product, one subversion used in the larger controlled clinical study, Study 003, and a second subversion used in Studies 005, 006, 011 and 014 and proposed for marketing.

## 8. Summary of major observations from clinical studies:

This section provides an overview of the major clinical observations from the studies, and discusses limitations of these data. It is important to note that many important details are supplied within the subsequent review of each individual study and this summary is not intended to identify all the notable clinical outcomes nor all limitations or concerns associated with these outcomes.

The largest controlled clinical study was Study TKT003, a study of 26 adult male Fabry disease subjects in which the subjects were randomized to treatment with either Agalsidase or placebo for a six month treatment period. The study was double-blinded and focused upon the subject's assessment of pain using a pain questionnaire (Brief Pain Inventory, BPI). Major features of the study design included the use of a novel approach to score pain while subjects were temporarily "off pain medications" and the performance of baseline and end-of-study kidney biopsies. The approach to assessing pain was especially notable because the design anticipated that subjects would be "on pain medication" during some weeks of the study and "off pain medication" at other time points during the study. Specifically, subjects were to have their pain medications withdrawn following randomization (for a one week period) at the end of which the pain score was assessed, and subjects could then resume pain medications. This process was repeated at various follow-up time points. If subjects needed to resume their pain medications during the study off-medication weeks, they could do so, but only following early completion of the "off pain medication" pain questionnaire. The pain questionnaire was comprised of multiple questions, among them a question regarding the “worst pain” experienced by the subject in the preceding period. The primary endpoint was a comparison between the two study groups of the area-under-the-curve of change in "worst pain" between baseline and certain follow-up visits. There were a vast number of secondary and tertiary endpoints. Many of these related to other aspects of the pain questionnaire, but others examined renal function and changes in renal biopsy histopathology.

The other controlled clinical study was Study TKT005, a study of 15 adult male Fabry disease subjects that was also a randomized, double-blinded, placebo controlled study. This six month study included most of the features of Study TKT003 but focused upon cardiac outcomes. The most notable design feature included the performance of a baseline and follow-up endocardial biopsy. Kidney biopsies were not

performed. The primary endpoint was a comparison between the two study groups in the change of cardiac CTH ($Gb_3$) content as obtained from biochemical assay of the cardiac biopsy samples.

There were also three open-label, uncontrolled studies. Two of these were follow-up extension treatment studies to Study TKT003. Specifically, Study TKT006 allowed subjects who completed Study TKT003 to receive one year of Agalsidase and Study TKT011 allowed subjects who completed Study TKT006 to continue receiving Agalsidase indefinitely. One year interim data from Study TKT011 were submitted to the application such that, from the beginning of Study TKT003, the total Agalsidase exposure duration is either 24 months (for placebo group subjects in Study TKT003) or 30 months (for Agalsidase group subjects). The third open-label, uncontrolled study was Study TKT014, a study conducted in Germany. In this study, 15 adult Fabry disease female subjects received Agalsidase. The study was terminated once Agalsidase became commercially available in Germany so that subject treatment duration in this study varied considerably from subject to subject.

**A. Major efficacy observations:**

**(1). Pain outcomes:**

Study TKT003 was the main study assessing pain. The primary endpoint result for Study TKT003 (Table 3) was not statistically persuasive.

**Table 3. Primary endpoint result: area under the curve for change in pain at its worst while off pain medication**

| Assessment | Agalsidase n = 14 | Placebo n = 12 | p-value* |
|---|---|---|---|
| Change in Worst pain | - 22.4 ± 9.4 | - 1.0 ± 13.5 | 0.20 |

* t-test

As described in the detailed review of Study TKT003, there are substantial limitations to interpreting this primary endpoint outcome. These include the following:

- The primary endpoint was to be calculated from scores only obtained during periods while subjects were "off" pain medications. However, unexpected difficulties precluded verification of "off pain medication" status at the time scores were obtained. During course of the study, many subjects were "on" and "off" pain medications. There were many inconsistent and incomplete records between subject medication diaries, hospital records, case report forms, and subject questionnaires regarding use of pain medications. There was no prospectively defined process for determining which scores constituted the primary endpoint scores when uncertainties in pain medication use arose.

- A comprehensive study audit with correction of any data problems, and designation of which data values constitute the dataset for the primary endpoint was not done prior to the initial unblinded data analysis. Following an initial unblinded analysis of the primary endpoint result the sponsor recognized certain problems in determination of the “off medication” values, and revised which pain scores were designated the “off pain medication” scores for some assessments. The effect of this revision changed the primary endpoint's analysis p-value from 0.43 (initially) to 0.20 (final). It is not known to what extent this process introduced bias.

- There was no prospective, explicit definition of "pain medications" which would disqualify a pain score from being used as an “off pain medication” score. The sponsor used a post-hoc definition that focused only on non-traditional analgesics, such as anti-epileptic agents. Consequently, narcotics and certain other general analgesics were not to be classified as pain medications. It is not known to what degree use of such general analgesics confound interpretation of the “off medication” scores.

Consequently, no conclusions regarding a treatment effect on pain can be drawn based on the intended primary endpoint.

The vast majority of pain-related secondary endpoints in Study TKT003 showed no evidence of a difference between the two study groups.

**(2). Renal function outcomes:**

Study TKT003 and its follow-up open label studies provide the bulk of renal function data. The major creatinine clearance and GFR changes during Study TKT003 are shown in Table 4. There were no remarkable changes in other renal function outcomes.

**Table 4. Study TKT003 Creatinine Clearance (CC) and GFR Changes**

| **Outcome** | **Study TKT003** | | |
|---|---|---|---|
| | Agalsidase n = 14 | Placebo CC n = 11, GFR n = 10 | p-value |
| **CC change to Wk 24** | **0.07 ± 5.9** | **- 19.7 ± 9.1** | **0.05** |
| *Creatinine Clearance Components* | | | |
| *Wk 0* | *103.1 ± 7.6* | *107.3 ± 12.2* | *N/A* |
| *Wk 9* | *112.5 ± 9.4* | *105.6 ± 15.0* | |
| *Wk 17* | *111.7 ± 9.9* | *104.1 ± 9.5* | |
| *Wk 23* | *106.8 ± 9.9* | *100.7 ± 16.1* | |
| *Wk 24* | *103.1 ± 7.5* | *85.7 ± 11.2* | |
| **GFR change** | **- 8.8 ± 3.8** | **- 19.8 ± 7.9** | **0.65** |
| *GFR Components* | | | |
| *Wk 0* | *81.0 ± 6.4* | *98.0 ± 10.8* | *N/A* |
| *Wk 24* | *72.2 ± 4.3* | *78.2 ± 7.7* | |

CC change = change in creatinine clearance from baseline to week 24, in mL/min,
Note that for CC: Agalsidase n=12 Wk 17; Placebo n=12 Wk 0, n=10 Wk 23, but 1 missing value scattered among subjects also on Wk 9, 17, 24. Analysis of patient subset of no missing data shows similar pattern between groups and between weeks.

The major limitations in assessing a possible Agalsidase-treatment effect related to the Study TKT003 renal function outcomes include the following:

- The nominal statistical difference between the two study groups in creatinine clearance at week 24 is not a robust finding.

- The creatinine clearance data are especially notable for the markedly lower mean value in the placebo group at week 24 compared to week 23. This large a difference in the space of one week is physiologically unlikely The placebo group's mean creatinine values during the open label treatment extension study are similar to the week 23 value, suggesting that the week 24 values is likely to represent an aberrant value.

- The GFR data appear relatively inconsistent with the creatinine clearance data with regards to the comparability at baseline between groups, comparability of group means at week 24, and change within group expressed as a fraction of baseline.

- Study TKT005 GFR data suggest no difference between the placebo and treatment group in renal function.

While the renal function data obtained during the open-label follow-up studies (follow-up to Study TKT003 in which subjects received Agalsidase for a period of 24 or 30 months) generally show no remarkable changes from the baseline values, this outcome is impossible to interpret due to the lack of an untreated control group.

**(3). Renal biopsy outcomes:**

Study TKT003 subjects underwent a baseline and end-of-study renal biopsy. The study's prospectively defined analytical methods for assessing the histopathology used two composite scores. One of these scores summarized the values for several components related to the detection of CTH ($Gb_3$) deposits within the kidney (the Acute Lipid Damage Score, ALDS) while the other score summarized a number of components related to chronic pathology (the Chronic Damage Score, CDS). The study results for these scores showed no difference between the two study groups. However, two components of the ALDS (those measuring vascular endothelial CTH ($Gb_3$) deposition) appeared to show a difference between the two study groups (bolded in table). Table 5 shows the ALDS composite and component scores.

**Table 5. ALDS score in subset of subjects with available data**

| Outcome | Agalsidase, n = 11<br>m ± sem | Placebo, n = 9<br>m ± sem | p-value for change<br>ANCOVA |
|---|---|---|---|
| ALDS at baseline | 9.4 ± 0.6 | 8.3 ± 1.2 | 0.11 |
| ALDS change to week 24 | - 1.5 ± 0.8 | 0.9 ± 0.9 | |
| ***Components of ALDS*** | | | |
| *Glomerular epithelial cells* | | | |
| Baseline | 2.3 ± 0.2 | 2.4 ± 0.3 | 0.83 |
| Change to week 24 | 0.0 ± 0.3 | 0.0 ± 0.3 | |
| ***Endocapillary cells*** | | | |
| Baseline | 1.2 ± 0.1 | 1.1 ± 0.2 | 0.04 |
| Change to week 24 | - 0.7 ± 0.2 | 0.0 ± 0.3 | |
| *Proximal tubules* | | | |
| Baseline | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.31 |
| Change to week 24 | - 0.1 ± 0.1 | 0.1 ± 0.1 | |
| *Distal tubules* | | | |
| Baseline | 1.8 ± 0.2 | 1.9 ± 0.4 | 0.72 |
| Change to week 24 | 0.2 ± 0.4 | 0.0 ± 0.2 | |
| ***Vascular endothelium*** | | | |
| Baseline | 2.0 ± 0.2 | 1.6 ± 0.3 | 0.003 |
| Change to week 24 | - 1.2 ± 0.3 | 0.2 ± 0.3 | |
| *Vascular media* | | | |
| Baseline | 2.3 ± 0.2 | 1.8 ± 0.3 | 0.80 |
| Change to week 24 | 0.0 ± 0.2 | 0.2 ± 0.4 | |

The degree to which these particular histologic changes correlate with renal function changes, especially well-defined quantitative correlations, is unknown. In addition, these outcomes were "one" among a large number of secondary and tertiary outcomes so that comparison multiplicity concerns impair interpretation of the reported p-values.

The histopathology data analyses are also hampered by a lack of rigor in the slide grading. There were ad hoc changes of the slide grading plan. In addition, there were no prospective descriptions of the following:

- Procedures to be employed by the pathologists in reviewing the slides (ie., review of all slides at a single "sitting," independent or interactive review of slide findings by pathologists, plans for reconciling disagreements among pathologists);

- Definitions of pathological findings or explicit criteria to be systematically followed with respect to assigning pathological outcomes;

- The minimum or maximum number of slides (sections) to be used in assessing a pathological outcomes;

- The types of stained slides to be reviewed by the pathologists as they ascertained the study data;

- Training provided to pathologists in order to ensure systematic consistency among the reviewing pathologists or consistency of their interpretations over time.

Additionally, the source datasheet containing the original pathological grade assessment is missing.

**(4). Cardiac outcomes:**

Study TKT005 provides most of the controlled clinical data relating to cardiac outcomes. The study's primary endpoint, a comparison of the change in heart content of CTH ($Gb_3$), showed no significant difference between the two study groups.

Secondary endpoints were numerous. Included among them were left ventricular mass as assessed using MRI and echocardiography. The MRI outcomes are summarized in Table 6, which shows the outcome among the subject subset that had both heart outcomes and the set of all subjects (using imputation for placebo subject number 5, a subject who had a baseline and week 13 result but no end-of-study result).

**Table 6. Left ventricular mass**

| Group | Left ventricular mass | | | |
|---|---|---|---|---|
| | Baseline (gm) | Week 24 (gm) | Change from baseline | p-value |
| ***Among subjects with baseline and week 24 day (excludes subject 5)*** | | | | |
| Agalsidase, n = 7 mean ± SE | 276.2 ± 19.4 | 264.8 ± 19.3 | - 11.5 ± 11.2 | 0.04 |
| Placebo, n = 7 mean ± SE | 248.2 ± 26.0 | 270.0 ± 23.1 | + 21.8 ± 5.9 | |
| ***Among all subjects (LOCF imputation for placebo subject 5)*** | | | | |
| Agalsidase, n = 7 mean ± SE | 276.2 ± 19.4 | 264.8 ± 19.3 | - 11.5 ± 11.2 | 0.10 |
| Placebo, n = 8 mean ± SE | 274.4 ± 34.5 | 285.6 ± 25.4 | + 11.3 ± 11.7 | |

The major limitations to assessing an Agalsidase-treatment effect upon Study TKT005 left ventricular mass determinations include the following:

- The primary endpoint of the study failed to demonstrate a treatment associated effect. Secondary endpoints were numerous, and Type I error related to comparison multiplicity is a concern.

- The nominal statistical significance of a difference between the two study groups is not a robust finding, as indicated by the sensitivity analysis (all subject set).

- Numerous other MRI outcomes, including left ventricular posterior wall thickness, indicated no difference between the two study groups.

- Echocardiographic assessments of left ventricular mass showed no statistically significant difference between the two study groups.

**B. Major safety concerns:**

The major safety concerns generally relate to the incidence of infusion reactions and immunogenicity outcomes.

**(1). Infusion reactions:**

Study TKT003 and its follow-up series of open-label studies generally provide the bulk of clinical data relating to infusion reactions. During the six months of Study TKT003, Agalsidase administrations were temporarily halted for a comprehensive review of infusion reactions. In general, these reactions consisted of various combinations of rigors, facial flushing, throat tightness and eyelid edema. A revised, systematic approach to the management of infusion reactions in Study TKT003 was instituted following this interim safety review. Steroids and antihistamines were administered and the infusion duration was lengthened to 40 minutes from the prior 20 minutes.

During Study TKT003 approximately 57% of the subjects receiving Agalsidase experienced an infusion reaction, of which two required overnight hospitalization of the subjects. During the series of open-label studies that allowed subjects completing Study TKT003 to receive two years of Agalsidase, the incidence of infusion reactions appeared to decrease, such that the infusion reaction rate was approximately 25% during the final year of observation. All but one of the Study TKT003 subjects who experienced infusion reactions also had antibody formation to Agalsidase.

Notably, no female subjects (Study TKT014) experienced infusion reactions and no female subjects had antibody formation to Agalsidase.

**(2). Antibody formation:**

Study TKT003 and its follow-up series of open-label studies also provide the most informative antibody formation data. Overall, 13 of the 25 (52%) subjects who received Agalsidase in the series of studies developed antibodies after one year of Agalsidase exposure. During the subsequent year of Agalsidase, the antibody formation appeared to lessen in four subjects. However, other subjects appeared to have progressive increases in antibody formation. The following are especially notable observations from this series of studies (TKT003/006/011):

- Approximately one-third of the subjects who received Agalsidase experienced progressive increases in the magnitude of antibody formation.

- An increasing magnitude of antibody formation appeared to be associated with the loss of activity in two biomarkers: urine sediment CTH ($Gb_3$) content and plasma CTH ($Gb_3$) concentration.

- Female (heterozygote) subjects in Study TKT014 exhibited no antibody formation. While symptomatic from enzyme deficiency, these subjects are thought to have some low levels of endogenous enzyme. This may influence the propensity to form antibodies. However, since study participation was progressively limited beyond 6 months of treatment, robust conclusions cannot be formed.

## 9. Study TKT001 (single dose):

### A. Overview:

Data from this phase 1, single center, open label, single dose, dose-ranging study was used in combination with certain preclinical study results to support the choice of dose for all subsequent clinical studies.

This phase 1 study was conducted between January 11, 1997 and September 18, 1997 at the National Institutes of Health (NIH) in Bethesda, MD. The study was an open label, single dose, dose-ranging study in which 10 Fabry disease male subjects received an IV dose of one of the dosages shown below:
-cohort 1, 0.007 mg/kg
-cohort 2, 0.014 mg/kg
-cohort 3, 0.028 mg/kg
-cohort 4, 0.056 mg/kg
-cohort 5, 0.110 mg/kg.

Each dose cohort was to consist of two subjects. Subjects were to undergo a liver biopsy prior to the Agalsidase dose and a second liver biopsy approximately 44 hours later. The liver biopsy samples were to be assayed for CTH ($Gb_3$) content and alpha-galactosidase enzyme activity. Subjects were followed for 28 days after the enzyme infusion in order to obtain pharmacodynamic, pharmacokinetic and safety data. The study did not have a formal, prospective analytic plan. All study results are a consequence of exploratory, post-hoc analyses. The study was designed solely to obtain pilot clinical data.

*Comment: It is notable that this study did not examine the effect of a 0.2 mg/kg dose--the dose used within all subsequent clinical studies.*

### B. Results:

#### 1. Subject disposition and baseline characteristics:

Ten subjects participated in the study, with two subjects in each of the five dose cohorts. All subjects were male and nine of the 10 subjects were Caucasian. One subject was Hispanic. The subjects aged in range from 21 to 46 years with a mean of 34.5 years.

#### 2. Blood pharmacokinetics:

All subjects received Agalsidase as an IV infusion over 20 minutes. Plasma concentrations of alpha-galactosidase activity peaked at approximately 22 minutes (range 20 to 24 minutes). Plasma alpha-galactosidase concentrations began to decline in a biphasic manner after peaking. A plateau in plasma enzyme activity was not achieved.

The observed $C_{max}$ increased proportionally with dose. The ratio of $C_{max}$ to dose (U/kg) was relatively constant across the range of doses. The $AUC_{\infty}$ also increased proportionally with dose and the ratio of $AUC_{\infty}$ to dose (U/kg) was also relatively constant across the range of doses. The apparent volume of distribution at steady state ranged from 7.3 L to 14.6 L.

The clearance of administered protein ranged from 122 mL/min to 208 mL/min with a mean of 158 mL/min. When normalized for body weight, clearance ranged from 1.3 to 3.1 mL/min/kg with an average of 2.2 mL/min/kg. The average clearance was approximately 65% higher than the average creatinine clearance for the 10 subjects--a difference possibly related to at least a second mode of clearance (non-renal) for the protein, possibly via cellular mannose-6-phosphate receptors.

The terminal half-life of elimination, obtained from the non-compartmental model, ranged from 42 to 117 minutes with an average of 83 minutes.

The sponsor notes that only infusions of 0.056 mg/kg and higher resulted in a peak plasma concentration significantly greater than the (*in vitro*) $K_d$ for the mannose-6-phosphate receptor.

**3. Liver pharmacokinetics:**

Baseline and follow-up liver samples were assayed for content of alpha-galactosidase and were also examined immunochemically for the presence of the enzyme in tissue sections.

The sponsor utilized an estimate of each subject's liver size to estimate the percent of each total enzyme dose that was taken up by the liver. Table 7 shows the liver pharmacokinetics, including the sponsor's estimated "% total dose in liver."

**Table 7. Liver pharmacokinetics of alpha-galactosidase A**
(n = 2 subjects at each dose level)

| ***Parameter*** | ***Dose level (mg/kg)*** | | | | |
|---|---|---|---|---|---|
| | 0.007 | 0.014 | 0.028 | 0.056 | 0.11 |
| Liver $\alpha$-galactosidase, pre-dose (nmol/h/mg protein, mean) | 0.6 | 2.6 | 2.2 | 3.2 | 2.2 |
| Liver $\alpha$-galactosidase, post-dose (nmol/h/mg protein, mean) | 5.0 | 18.6 | 18.9 | 48.2 | 52.2 |
| Estimated % Total dose in liver 44 hours post-dose | 25.0 | 28.7 | 16.8 | 19.4 | 13.7 |

The sponsor notes that the estimated percent of total dose that was deposited in the liver was decreased at the highest dose--a finding which the sponsor interprets to mean that the liver may be "saturated" with respect to uptake of the enzyme. Consequently, the sponsor concludes that doses in excess of 0.056 mg/kg may saturate the liver uptake of the enzyme and allow higher concentrations of the enzyme to be achieved in the plasma and coincidentally made available for uptake by other tissues.

Immunohistochemical staining also detected the presence of alpha-galactosidase A in the liver. Liver biopsy specimens were stained with a polyclonal anti-human alpha-galactosidase A antibody. Baseline immunostaining was minimal. Follow-up liver biopsy samples showed significant staining in Kupffer and sinusoidal endothelial cells. Within the hepatocytes, staining was present in the intracellular compartment near the plasma membrane.

**4. Plasma CTH ($Gb_3$):** Subjects had baseline and follow-up plasma CTH ($Gb_3$) levels determined. In general, these results showed no change following administration of any dose of the enzyme.

**5. Liver CTH ($Gb_3$):** There was no dose-related decrease in liver CTH ($Gb_3$) content following Agalsidase administration. However, there was a statistically significant decrease in the liver CTH ($Gb_3$) content when data from all cohorts were pooled and compared to baseline. Using the Wilcoxon signed rank test to compare the post-dose result to the pre-dose result for all subjects pooled, the decrease in liver CTH ($Gb_3$) content resulted in a p-value of 0.05.

**6. 24 hour urine sediment CTH ($Gb_3$):** 24 hour urine samples were obtained at baseline, day 1, day 7 and day 28. The volume of the samples was recorded and the concentration of creatinine determined. Cellular contents were concentrated by centrifugation and the pellets assayed for CTH ($Gb_3$). Table 8 shows the 24 hour urine CTH ($Gb_3$) results.

**Table 8. 24 hour urine CTH (nmoles/g creatinine)**

| ***Result*** | ***Pre-dose*** | ***Day 1*** | ***Day 7*** | ***Day 28*** |
|---|---|---|---|---|
| Mean ± SD | 1554 ± 590 | 1416 ± 943 | 1977 ± 831 | 964 ± 475 |
| Min, max | 868, 2579 | 256, 3022 | 1031, 3766 | 513, 2019 |

Using the Wilcoxon signed rank test, the change from baseline to 28 days was statistically significant ($p < 0.01$). All other values were not statistically different from baseline. There was no dose response effect upon the change in 24 hour urine CTH ($Gb_3$).

**7. Safety results:** All subjects completed the enzyme infusions without any immediate adverse events or need for interruption of the dose. The most common adverse event was discomfort/injection site reaction related to performance of the liver biopsy (three subjects). One adverse event was assigned as possibly related to the study agent--myalgia developed on day seven in a subject. The myalgia resolved without any specific therapy.

There were no deaths in the study and one serious adverse event (SAE). The SAE was an overnight hospitalization on day 22 because of a fever that developed following a dental procedure.

There were no significant alterations in clinical hematology or clinical chemistry and no subject developed antibodies to the enzyme.

**8. Conclusions from the study:**

The sponsor made the following conclusions from this study:

-the doses were safe and tolerated well
-liver CTH ($Gb_3$) content decreased following enzyme administration
-24 hour urine sediment CTH ($Gb_3$) content was decreased on day 28 following enzyme administration
-alpha-galactosidase A pharmacokinetic parameters were dose proportional across the dose range studied and systemic clearance was similar for all doses
-an estimate of enzyme delivery to the liver suggested a greater than 24 hour *in vivo* tissue half-life
-estimates of enzyme delivery to the liver demonstrated a dose-dependent decrease in the fraction of enzyme recovered in the liver

*Comment:*

*This was a pilot, exploratory study that examined the effect of an alpha-galactosidase product produced from cDNA-transduced diploid fibroblasts. This study was remarkable for the lack of evidence of dose-response pharmacodynamic effects in several outcomes. The only evidence of a potential pharmacodynamic effect (alteration of liver CTH ($Gb_3$) content, 24 hour urine CTH ($Gb_3$) content) comes from the pooling of results across all the study dose cohorts.*

*The sponsor utilizes liver biopsy measurements of enzyme activity (with estimates for liver size) to estimate that the putative liver-uptake fraction of total dose administered appeared to decrease at the highest dose level. Based on this, the sponsor proposes that the liver may be "saturated" with enzyme. Consequently, the sponsor hypothesizes that any higher dose levels would also "saturate" the liver and possibly expose other parts of the body to higher concentrations of the enzyme. This hypothesis is based upon animal radiolabeled biodistribution studies which show maximal uptake in the liver.*

*The sponsor utilized these data to partially support the dose selection for all subsequent clinical development. It is notable that the sponsor chose a dose of 0.2 mg/kg for subsequent clinical development--a dose not evaluated in the phase 1 study.*

**10. Basis for dose selection:**

All clinical studies of the Agalsidase product proposed for marketing examined the effect of one dose regimen: 0.2 mg/kg IV every two weeks. The sponsor based this dose selection upon the following:

1. The animal studies of the product proposed for marketing showed no toxicity related to the product through a dose of 1.0 mg/kg administered weekly for six months.

2. A dose of the fibroblast-derived alpha-galactosidase A enzyme product of 0.056 mg/kg or greater appears to be necessary to achieve a plasma $C_{max}$ that is greater than the $K_d$ for the mannose-6-phosphate receptor.

3. The Phase 1 study results suggest that the liver may be "saturated" with respect to fractional uptake of the administered enzyme. Consequently, the sponsor hypothesizes that doses higher than those utilized in the phase 1 study may allow more Agalsidase to become available to the body.

4. Estimates from the phase 1 study suggest that the tissue half-life of the enzyme may be in excess of 24 hours.

*Comment: Several aspects of the sponsor's basis for selection of the Agalsidase dose intended for clinical testing are appropriate for comment:*

*1. The dose selection for clinical development is based upon an assumption that the Agalsidase produced using gene-activated technology will exhibit human pharmacokinetics and pharmacodynamics that are similar to those of the cDNA transfection-derived enzyme which was tested in Study TKT001. This was not tested.*

*2. Selecting the clinical dose based upon "saturating" the liver with enzyme activity assumes liver "saturation" may be a logical approach to selecting the minimum dosage to make the enzyme available to other tissues. However, it is conceivable that impacting the clinical disease may require dosages considerably in excess of those required for liver "saturation."*

*3. The Fabry disease knockout mouse studies are notable for suggesting that that the 1 mg/kg dose Agalsidase exhibited more complete resolution of lipid storage than the lower dose.*

*4. The overall clinical development plan for Agalsidase has focused upon the single dose regimen of 0.2 mg/kg, IV, every two weeks. No other doses have been evaluated on a sustained basis, precluding any assessment of dose-response relationships of clinical safety, bioactivity or efficacy.*

## 11. Study TKT003:

### A. Overview:

This clinical study was the initial introduction into humans of the Agalsidase produced using gene-activated technology. This study, along with Study TKT005, are the controlled studies performed with the product and provide the majority of data assessing the safety and efficacy of Agalsidase.

This study was conducted between December 12, 1998 and September 2, 1999 at the National Institutes of Health (NIH) in Bethesda, MD with Drs. Raphael Schiffmann and Roscoe Brady as the principal investigators.

Following this six month, placebo-controlled study, completing subjects were eligible for enrollment in Study 006, an open label study in which all subjects received Agalsidase for one year. Following completion of Study 006, the subjects were eligible to enroll in Study 011, another open label study in which all subjects could receive Agalsidase indefinitely. Study 006 and one year of data from Study 011 have been submitted to the BLA such that the application contains an additional two years of uncontrolled clinical data from the group of subjects who completed Study 003. Consequently, the BLA contains 30 months of Agalsidase exposure data for some of the Study 003 subjects (the group randomized to Agalsidase) and 24 months of Agalsidase exposure data for others (the group randomized to placebo). However, the most important efficacy and safety information come from the six month controlled study, Study 003.

### B. Protocol:

The following information highlights the main aspects of the clinical protocol.

**1. Title and amendments:** "Alpha-galactosidase A replacement therapy in Fabry Disease (Clinical protocol number TKT003). A phase 2 randomized double-blind placebo-controlled safety and efficacy clinical trial of alpha-galactosidase A replacement therapy in patients with Fabry Disease." The final initial protocol was dated October 27, 1998.

The protocol was amended twice, as follows:

**Amendment 1** was prepared following the suspension of the study drug administration by the investigators and sponsor because of safety concerns related to infusion reactions. Amendment 1 was dated June 4, 1999 and noted that several subjects had experienced apparent allergic reactions. This amendment described a premedication regimen that was to be utilized during subsequent conduct of the study. These regimens included administration of the product over 40 minutes (instead of 20 minutes) in all subjects unless the subject had previously experienced facial edema, in which case the infusion was to be administered over one hour and possibly even longer periods of time. If a subject had not had a reaction to the study drug, no premedication was to be given. All subjects who had any reaction to the study drug were to receive premedication prior to subsequent doses. The premedication regimen was to be:

-night before the infusion--oral prednisone 50 mg "may be taken"
-morning of the infusion--oral prednisone 50 mg "may be taken," oral ranitidine 150 mg was to be taken
-one hour prior to infusion--Solumedrol 50 mg IV push, diphenhydramine 50 mg IV push, ranitidine 50 mg IV

*Comment: Administration of the steroids was to be solely to patients who had infusion reactions. This is likely only the active treatment arm and thereby assigned an important additional therapy to the active treatment arm that would be little or not utilized in the control arm. This change in the protocol may impact the ability to distinguish Agalsidase effects from the steroid effects and complicates interpretation of data from the study. There is also potential that this exacerbated any unblinding effects of infusion reactions by*

*heightening attention to them and instituting sustained differential management of subjects who had infusion reactions.*

**Amendment 2** was dated July 23, 1999 and contains a description of the statistical analytical plan for the study.

**Statistical Analytical Plan**
This separate document was dated October 3, 1999. The final subject evaluation visit had occurred on September 2, 1999. The database for unblinded analysis was locked on October 10, 1999.

**2. Design:** Randomized, double-blinded, placebo-controlled phase 2 study to be conducted among 24 Fabry disease subjects. Subjects were to receive the study drug every two weeks for 24 weeks. The primary endpoint was a comparison of pain assessment changes between the two groups.

**3. Objectives:** To evaluate the safety and efficacy of the product. Efficacy was to be determined "primarily by measuring the effect of enzyme replacement therapy on serious debilitating pain, as measured by a quantitative, validated pain assessment scale. Efficacy will also be assessed by measuring the effect of enzyme replacement therapy on several critical secondary endpoints, including kidney pathology (including analysis of both glomerular and tubular pathologic changes, kidney CTH content), and cardiac structure and function (as determined by cardiac echocardiogram and cardiac magnetic resonance imagine)."

*Comment: Although the primary endpoint is described as "serious debilitating pain" in several places within the protocol, the eligibility criteria did not ensure subjects were experiencing "debilitating pain" and the primary endpoint was an assessment only of "pain at its worst", irrespective of whether or not it was associated with any debilitation. The pain assessment scale did include components which might be interpreted as impacting the functional significance of the pain (the "debilitation"--"interference" scores), but these components were not part of the primary endpoint for this study.*

**4. Subjects:** 24 subjects with the following criteria:

-inclusion criteria:
- -male hemizygote with Fabry disease documented by clinical evidence and by evidence of alpha-galactosidase deficiency as assayed on white blood cells or cultured skin fibroblasts
- -neuropathic pain consistent with Fabry disease
- -age ≥ 18 years
- -adequate general health to undergo phlebotomy and two kidney biopsies
- -evidence of CRIM as evidenced by any one of the following:
  - -positive western blot on patient cells or serum using anti-alpha-galactosidase A antibodies
  - -positive enzyme immunoassay (ELISA) using anti-alpha galactosidase A antibodies
- -low levels of alpha-galactosidase A (> 0 but < 15% of normal) by sensitive enzymatic assay
- -PT/APTT normal and platelet count > 100,000/mcL
- -consent

-exclusion criteria:
- -diabetes mellitus or any disorder associated with peripheral neuropathy
- -permanent cardiac pacemaker
- -receipt of treatment with another investigational therapy within past 30 days
- -medical condition making protocol adherence or interpretation of study results difficult

*Comment: Note that there was no criterion for a specific, minimal degree of pain or pain impact on the subject (e.g., debilitating pain).*

**5. Treatment assignment/randomization:** A blocked (in 4's) 1:1 randomization list generated by a contract research organization (----------) was provided to the unblinded pharmacist at the study site. Subjects were to be assigned randomization numbers by the NIH research pharmacy in the order in which they were enrolled after completion of the first baseline evaluation. Randomization was not stratified by any factors.

*Comment: Subjects were to be enrolled into the study and then undergo the baseline evaluations. These were to be conducted during a 10 day hospitalization at the NIH. Following the completion of the first baseline evaluation, subjects were to be randomized. Only one screened subject was found to be ineligible for the study.*

**6. Dose:** Agalsidase (0.2 mg/kg) was to be diluted in 100 mL saline and initially planned to be administered over 20 minutes. Protocol amendment number 1, following the development of infusion reactions, changed the infusion time to 40 minutes. The dilution was to be prepared in the research pharmacy by the unblinded pharmacist. The placebo was to consist of the product formulation without the active drug and was to be identical to the active product. These doses were to be administered every two weeks for a total of 26 weeks (13 administrations).

**7. Evaluations:** The study was divided into two major sections: the baseline evaluations and the subsequent evaluations.

The baseline evaluations were all to be performed within one week prior to administration of the study drug. The subjects were to be hospitalized for a 10 day period--the baseline evaluation period plus the time associated with the first infusion of the study drug. The first baseline evaluation was to begin immediately after enrolling the subject into the study. All pain medications which were administered on a continuous basis at the time of the first baseline evaluation were discontinued. Aspirin and non-steroidal anti-inflammatory agents were also discontinued at this time until two weeks following the renal biopsy. Eligibility to participate in the study was to be confirmed by the results of the first baseline evaluation and subjects were to be randomized immediately following eligibility confirmation.

Pain medications were to remain discontinued after the first baseline evaluation. However, if needed for pain relief, the pain medicine could be resumed if the subject notified the investigator. In this event, the investigator was to administer the short form Brief Pain Index (BPI short form) at that time (just prior to pain medication resumption). The BPI was utilized in two forms: a long form and a short form. The major difference between the two forms is additional information collected on the long form, however the actual pain scale measurements are the same on the two forms. The BPI requested subjects to rate their pain in the past week on a numeric scale of 0 (no pain) to 10 (worst imaginable pain).

If the subject was unable to contact the investigator, the subject was to complete the BPI short form prior to resuming the pain medication. Pain medications were not explicitly defined in the protocol. The protocol stated, "Patients will be instructed to discontinue all pain medications which are administered on a continuous basis [e.g., phenytoin (Dilantin), carbamazepine (Tegretol), or gabapentin (Neurontin)]...]."

This process of cessation of pain medication and recording of BPI short form data was to be repeated at weeks 9, 17 and 24, providing the intended assessments of pain "off medication".

There were many evaluations to be performed during the clinical study and the most important include the following:

-BPI assessment of "off pain medication" pain scores (with subjects required to be off pain medication) was

to be recorded at baseline and weeks 9, 17 and 24; BPI assessments of pain were also to be performed at each visit, at least every other week for study drug infusion (these would be either off or on pain medication, depending on the subject's need for pain medication). The BPI would also be obtained immediately preceding the resumption of pain medications (following cessation of pain medications in anticipation of the requisite week 9, 17 and 24 "off pain medication" visit).

Consequently, each subject was to have a set of 13 or more BPI pain assessment scores (some the requisite "off pain medication" scores and others obtained while subjects were potentially on pain medication)

-creatinine clearance was evaluated at the first and second baseline evaluations (prior to renal biopsy) and at weeks 9, 17, 23 and 24 (prior to renal biopsy)
-renal biopsy was to be performed at baseline and week 24
-GFR (inulin clearance) was to be measured at first baseline evaluation and week 23
-cardiac MRI and echocardiography were to be performed at baseline and week 23
-antibodies to Agalsidase were to be measured at baseline and weeks 9, 17 and 24.

The BPI assessment of pain at its worst (item 12 on the BPI Long Form, item 3 on the short form) asked for a rating of severity pain at its worst over the past week, ranging from no pain (0) to “pain as bad as you can imagine” (10).

**8. Endpoints:** The protocol and statistical analytical plan described a large number of exploratory analyses of the primary endpoint and secondary/tertiary endpoints. Neither the protocol nor the subsequent Statistical Analytic Plan were fully specific on the nature of all the endpoints. An explanation of what can be recognized follows:

Primary Endpoint
The primary endpoint was described as "Efficacy will be determined primarily by measuring the effect of enzyme replacement therapy on serious debilitating pain...The primary analysis of pain will utilize the pain at its worst scale of the BPI...Changes in pain will be measured by patient's ratings of pain severity on the BPI at baseline and throughout the study. … The other symptoms scales of the BPI and NPS will be utilized in the secondary analysis." The primary endpoint used the “off pain medication “ scores only.

*Comment: Issues with the BPI instrument and study plan for use may limit the interpretation of the results. These include:*

*1. The study used the BPI scale in a novel method for assessing pain: The study required the abrupt cessation of certain "pain medications," followed by measurement of the pain experienced while "off pain medication." The impact of abrupt pain medication cessation upon the BPI assessment of pain has not been evaluated in prior studies. No data to support the validity of the BPI with this specific type of study procedure were submitted.*

*2. The BPI instrument appears to have an inherent inability to assess the putative "off pain medication" BPI score: The format of the BPI short form is such that it is impossible to determine whether the time point of "worst pain" actually occurred while the patient was off pain medication since the form does not ask this question. The form asks the subject to assign a score to the worst pain within the past seven days--regardless of the use of pain medication (or not) during the preceding seven days. The study design allowed subjects to resume pain medications at any time, if their pain reappeared. A BPI form was to be completed immediately prior to the resumption of the pain medication. Consequently, it is inherently impossible to verify that "worst pain" scores occurred while patients were off pain medications. The study design appears to assume that the time point of "worst pain" was following the cessation of pain medication, a clinically logical assumption but unverifiable process.*

*3. There was no prospective definition of "pain medications:" Review of aggregate medication listings led to a post-hoc designation of "pain medications" limited to certain non-traditional neuropathic-type pain medications--this definition excluded traditional analgesics from being regarded as a "pain medication." Consequently, using this definition of "pain medication," a patient could be receiving Fentanyl or Percocet for pain relief and still be regarded as "off pain medication."*

*The cumulative effect of these design limitations is to profoundly limit the interpretability of the BPI pain score data intended for the primary endpoint.*

Secondary Endpoints

Secondary endpoints were described as the following: "Efficacy will also be assessed by measuring the effect of enzyme replacement therapy on several critical secondary endpoints, including kidney pathology (including analysis of both glomerular and tubular pathologic changes), CTH content, and cardiac structure and function (as determined by cardiac echocardiogram and cardiac magnetic resonance imaging)." The protocol states that the following "secondary variables will be analyzed:

1. kidney pathology
2. kidney CTH
3. cardiac echocardiogram/UTC
4. cardiac MRI
5. urine sediment CTH
6. plasma CTH
7. pain (BPI measurements not utilized in primary analysis)
8. pain medication usage
9. renal function tests (24 hour urine chemistry, serum creatinine and urea nitrogen, calculated creatinine clearance, GFR/RPF)
10. PFT
11. Quality of Life
12. weight
13. cerebral MRI
14. cerebral PET examination
15. TCD
16. EKG
17. urinalysis
18. ocular examination, photography and cytology
19. skin innervation density
20. quantitative sensory testing

The evaluation of renal structural changes is especially important and the following details are notable. The changes consisted of numerous endpoints:

-from the protocol:

-the protocol noted that "kidney pathology" was the first secondary endpoint listed. This endpoint was to involve "both glomerular and tubular pathologic changes"

-an appendix to the protocol ("Procedures") provided more detail:

> "The extent of renal injury and damage will be assessed throughout the entire renal cortex. Parameters to be evaluated include: glomeruli (segmental sclerosis, global sclerosis, and segmental hyalinosis), tubular atrophy, interstitial fibrosis, and vascular structures (hyalinosis, thickening, fibrosis, and fibrin change). All parameters will be rated for pathological changes using a scoring system from 0 - 3 (for none, mild, moderate, and severe pathological changes). Separate pathology scores will be calculated for glomerular, tubular, interstitial, and vascular structures for each sample. In addition, the examiner will provide a global qualitative assessment of the pathology changes in each sample. The pathological inclusions characteristic of Fabry Disease will also be assessed in each of three cellular compartments. The glomeruli (epithelial cells, endothelial cells, and mesangial

cells), cortical tubules (proximal and distal) and vasculature (endothelium and media) will be assessed separately, and each cell compartment will be scored for both the area density and tinctorial density of inclusions. Scoring will be on a 0 - 3 scale (normal, mild, moderate and severe) for each cellular compartment. Paired samples, blinded to order of biopsy, will be evaluated for each patient."

-the Statistical Analytical Plan provided additional detail:

"By analogy with lupus nephritis, we propose to measure both a Fabry Disease active damage score and a Fabry Disease chronic damage score similar to that described by Austin, et al. for lupus, but based on renal pathology scores as described by Gubler et al. for Fabry Disease. The kidney biopsy light microscopic analysis will be conducted in a blinded manner with the biopsy samples coded to maintain blinding. For both indices, a score on a four-point scale will be assigned to changes in the glomeruli, tubules, and vasculature; the final damage score (acute or chronic) will be the sum of the individual component scores. The individual component scores will be as follows: 0 = normal, 1 = mild, 2 = moderate, 3 = severe (diffuse).

Active Lipid Damage Score (six elements): In the glomeruli, the extent of vacuolization of individual glomerular epithelial cells and endocapillary cells will be assessed on the 0 to 3 scale. The extent of foamy cell appearance in both the proximal and distal tubules will be assessed on the 0 to 3 scale as described by Gubler, et al. For the arterioles, an assessment of both endothelial vacuolization and vascular media involvement will be made on the 0 to 3 scale. The final active damage score will be the combined sum (18 point maximum) of all six individual component measurements (i.e., glomerular epithelium, glomerular endothelium, proximal tubules, distal tubules, vascular endothelium and vascular media.)

Chronic Damage Score (seven elements): In the glomeruli, the extent of glomerulosclerosis or hyalinosis based on capillary collapse and mesangial matrix expansion will be assessed on the 0 to 3 scale essentially as described. In addition, the extent of global glomerulosclerosis will be assessed. A score on the 0 to 3 scale will be given to the extent of tubular atrophy. Both interstitial fibrosis and interstitial inflammation will be assessed essentially as described. For the chronic vascular changes, both vascular hyalinosis and vascular medial thickening will be assigned a score from 0 to 3. The chronic damage score will be the sum (21 point maximum) of the seven individual component scores (i.e, glomerulosclerosis or hyalinosis, global glomerulosclerosis, tubular atrophy, interstitial fibrosis, interstitial inflammation, vascular hyalinosis, and vascular medial thickening). For both scores, the change from baseline will be calculated."

*Comment: Because portions of the protocol and Statistical Analytical Plan textual description are nonspecific and open to differing interpretations identification of all intended endpoints with certainty is infeasible. In addition to the Active Lipid Damage Score and the Chronic Damage Score, the histopathological outcomes could also include at least 17 additional outcomes as listed below:*

1. *A 0 to 3 overall pathological grade for extent of each of the following:*
   - *-glomerular segmental sclerosis*
   - *-glomerular global sclerosis*
   - *-glomerular segmental hyalinosis*
   - *-tubular atrophy*
   - *-interstitial fibrosis*
   - *-vascular hyalinosis*
   - *-vascular thickening*
   - *-vascular fibrosis*
   - *-vascular "fibrin change"*
   - *-global assessment in glomeruli*
   - *-global assessment of tubules*
   - *-global assessment of vascular structures*
2. *A 0 to 3 assessment of CTH ($Gb_3$) deposition within each of the following:*
   - *-glomerular epithelium*
   - *-glomerular endothelium*
   - *-glomerular mesangial cells*
   - *-proximal tubule cells*
   - *-distal tubule cells*
   - *-vascular endothelium*
   - *-vascular media*

*Comment: There were substantial limitations in the extent of prospective plans for histopathological data collection and analysis. These limitations are described in detail within the study Results section.*

**9. Statistical analyses:**

The analytic plan was described in two documents, Amendment 2 of the protocol dated July 23, 1999 and the subsequent separate "Statistical Analytical Plan", dated October 3, 1999 and containing the more detailed plan. A large number of endpoints were identified for analysis in the statistical analytic plan, raising concern regarding multiplicity of comparisons.

**The "Statistical Analytical Plan" formulated on October 3, 1999 contained the following major points:**

-All randomized subjects were to be included in the primary and secondary endpoint analyses (intention-to-treat analyses)

-The Last Observation Carried Forward method (LOCF, using the last same type of observation) was to be the method of imputing missing data.

-Whereas the protocol identified only primary and secondary endpoints, the Statistical Analytical Plan identified primary, secondary and tertiary variables. The plan contained a variably detailed description of these endpoints and the various statistical analyses.

-The analytical plan did not provide details on the specific methods for assigning or standardizing the scores given in the histological analysis. The individual component scores were only stated to be as follows: 0 = normal, 1 = mild, 2 = moderate, 3 = severe (diffuse).

-The "active lipid damage score" (ALDS) consisted of six elements in which the extent of vacuolization was to be assessed as 0 to 3, such that the maximal score was 18 (the most severe). The six elements include: glomerular epithelium, glomerular endothelium, proximal tubules, distal tubules, vascular endothelium, vascular media.

-The "chronic damage score" (CDS) consisted of seven elements which were also to be graded on a 0 to 3 scale, such that the maximum score was 21. The seven elements included the following: glomerulosclerosis or hyalinosis, global glomerulosclerosis, tubular atrophy, interstitial fibrosis, interstitial inflammation, vascular hyalinosis, vascular medial thickenining.

**C. Study conduct:**

The study began on December 12, 1998 and the final subject evaluation occurred on September 2, 1999.

Clinical monitoring and clinical data management were performed by the sponsor and ----------. Statistical analyses were performed by the sponsor. All standard laboratory analyses were performed by the NIH clinical laboratories. CTH ($Gb_3$) assays on plasma, urine sediment and kidney tissue were performed by the sponsor. Pathological specimens were analyzed at the Armed Forces Institute of Pathology. During the study, site monitoring visits were performed by ----------. and by the sponsor. The case report forms were received by ----------.

The study was temporarily halted in May of 1999 (approximately mid-study) because of safety concerns related to infusion reactions. Consequently, no clinical evaluations were performed from the period of May 25, 1999 to June 7, 1999. There were no interim analyses of the data.

**(1) Protocol violations:**

Individual protocol deviations of study conduct were comprised of the following: three subjects were granted an inclusion criterion waiver of coagulation laboratories for minor abnormalities; one placebo subject missed three doses of study medication due to the onset of renal failure requiring peritoneal dialysis; and one placebo subject failed to return for follow-up (withdrew at week 21).

Systematic deviations include 17 subjects missing one dose of study drug (10 Agalsidase, 7 placebo) during the two week halt in the study due to infusion reaction safety concerns. Additionally, electron microscopy analyses of the renal biopsy specimens were not performed for technical reasons.

Other minor protocol deviations included missed or miss-timed assessments. No subject was excluded from the analyses due to a protocol deviation.

**(2) FDA site inspectional findings:**

The clinical site was inspected by FDA during an assignment that ended on November 3, 2000. The following were the most significant findings from this inspection:

-of 15 subject records reviewed, 11 did not have EKGs performed at time points required by the protocol

-there were multiple inconsistencies in case report form (CRF) pain medication usage, patient diaries, hospital records, physician and nurses notes. In general, these inconsistencies related to inability to determine the start and stop date of certain pain medications, the timing of BPI questionnaire completion and contradictions among the various records. Pain medication use was prominent among the contradictory records. This issue is central to interpretation of the study primary endpoint. These problems underscore the complexity of the primary endpoint assessment (see following issues).

It is important to note that this inspection focused upon the pain outcomes and safety issues, with lesser emphasis upon the secondary and exploratory endpoint data verification. However, random renal laboratory findings were assessed for accuracy and no deviations detected. Renal histopathological documents were not inspected.

**(3) Primary endpoint appropriate dataset determination:**

Each subject had a set of at least 13 BPI scores, of which baseline and weeks 9, 17, 24 were intended to be off “pain medication” and used in the primary endpoint analysis. Because of the potential increased pain to cause a necessary early resumption of medications, ad-hoc BPI assessments might also be obtained just before the early resumption of medications. The scheduled assessment would still occur, but would then actually be an on-medication pain score. Thus, from amongst the many BPI values obtained for each subject, 4 values needed to be designated as the “off medication” values for the primary endpoint analysis.

The blank BPI forms were laid out to ensure ascertainment of whether the assessment was actually on or off medications. Consequently, compilation of the set of true "off pain medication" BPI scores required a review of many study records (BPI scheduled scores, BPI ad-hoc scores, CRF medication records of type and date of medication, patient diaries, etc.). Several problems limit the adequacy with which this could be accomplished. There was no prospective plan for this review process. Analgesics used on an as-needed basis were particularly poorly ascertained. Patient diaries were inconsistent and often unreliable with regard to medication use.

An important perspective on this problem is that this was the first-known randomized, controlled, intervention study in Fabry disease which attempted to have an impact on pain as the primary focus. The sponsor drew upon existing knowledge of methods for pain assessment, but desired to ascertain the ‘total magnitude’ of subject pain, not only that which was residual beyond the existing analgesic use. Therefore,

the sponsor devised new procedures to assess pain in Fabry disease. There was no body of prior experience with this novel pain assessment approach that could have been used to provide well defined, effective procedures for this study to employ. The study procedures that were employed were, however, not adequately adaptable to the unforeseeable complexities that occurred.

The complexities of the study and the inconsistent and inadequately recorded information connote that it is not possible to accurately determine a set of BPI true "off pain medication" scores for all patients.

**(4) Primary endpoint -- database construction:**

There were also multiple modifications of the clinical datasets in the period between the initial review of the unblinded study results and the date of the "final" database lock, including modifications to the dataset designation for the primary endpoint. These database alterations are described briefly here, and in more detail in the appendices (Appendix A).

After study completion, the contract research organization (CRO) created the study database files. Each file would have the component data audited, data queries resolved, and then that database file could be finalized ("locked"). The study database files were not completed and locked as a single entirety. In early October 1999 a portion of the clinical study database was locked by ----------. following audit of all BPI "worst pain" score values. Audits were still incomplete on other clinical data at that date, including data relating to pain medication use, and these other database files in the full database remained unlocked. This limited database, including the full set of all BPI data and the other study data was transmitted to the sponsor. The CRO did not have the responsibility for designating the specific values comprising the primary endpoint dataset. The BPI data file did not identify which of the approximately 450 BPI worst pain scores were the "off pain medication" scores. The responsibility for designating which values to include was assumed by the sponsor, TKT.

The sponsor evaluated the BPI data according to the primary endpoint analytic plan at that time and designated a set of scores as a "preliminary" primary endpoint dataset of BPI values. The sponsor had the analysis performed for the primary endpoint results which revealed a p-value of 0.43.

The sponsor indicated that at the end of October 1999, "review of the clinical data by the TKT medical monitor revealed numerous errors and inconsistencies in the clinical database." This finding prompted the sponsor to authorize a TKT clinical research associate to conduct an audit of the clinical site with two goals--to review concomitant pain medication usage and urine chemistry results.

Based on this audit report, the sponsor modified the "preliminary" primary endpoint database file to designate certain other pain values as the off-medication values, and thereby to construct the final primary endpoint database. The primary endpoint database was again finalized in February 2000 (the second database "lock"), along with all other clinical dataset information. This was the database used by the applicant to perform the final analyses, and provided a p-value of 0.19 for the primary endpoint.

It is not known to what extent the special additional audit, planned with knowledge of the preliminary unblinded study results, and the re-designation of the revised primary endpoint dataset involving TKT personnel who had knowledge of the unblinded data, may have introduced bias into the process.

**(5) Histopathology Assessment Conduct Issues :**

There were also problems with the conduct and analysis of the histopathology data. These problems are complex and described within the Histopathology Results section of this document.

**D. Results:**

**(1) Subject disposition:**

Twenty-seven subjects were enrolled. One subject (subject 24) was withdrawn from the study prior to randomization because he was discovered to be in atrial fibrillation at the first baseline evaluation. Table 9 shows the subject disposition.

**Table 9. Subject disposition**

| ***Subject Status*** | ***Agalsidase*** | ***Placebo*** | ***Total*** |
|---|---|---|---|
| Subjects enrolled | - | - | 27 |
| Subjects randomized | 14 | 12 | 26 |
| Subjects who completed study | 14 | 11 | 25 |
| Subjects who withdrew | 0 | 1 | 1 |

Subject number 20 (a placebo subject) withdrew consent and failed to return for follow-up visits beyond the week 21 infusion. The intent-to-treat patient population includes all 26 randomized subjects. Twenty-five subjects received the study drug infusions through week 24. However, subject number 5 (placebo) was excluded from the "completed study" dataset as a result of missing three consecutive infusions (as described in the statistical analytical plan) on weeks 17, 19 and 21. No subject was discontinued from the study because of an adverse event.

**(2) Baseline Characteristics:**

The statistical analytical plan described 16 parameters to be utilized in summarizing the baseline characteristics. These results are shown in Table 10.

**Table 10. Baseline characteristics identified in statistical analytical plan**
***(Unless otherwise noted, n = 14 for Agalsidase, n = 12 for Placebo)***

| **Characteristic** | **Agalsidase** | **Placebo** |
|---|---|---|
| Age (years), mean ± SE | 34.0 ± 2.3 | 34.4 ± 2.2 |
| Race | | |
| Caucasian, n | 13 | 11 |
| Hispanic, n | 1 | 1 |
| BPI Worst Pain scores | | |
| On pain medications, mean ± SE (long form) | 4.2 ± 0.5 | 6.3 ± 0.7 |
| Off pain medications, mean ± SE (short form) | 6.2 ± 0.5 | 7.3 ± 0.6 |
| Number of patients on pain medications | 10 | 12 |
| Mean BPI severity score, off medication, mean ± SE | 3.8 ± 0.4 | 5.4 ± 0.5 |
| Mean BPI interference items score, off pain medication, mean ± SE | 3.2 ± 0.6 | 4.8 ± 0.6 |
| Serum creatinine (mg/dL), mean ± SE | 1.0 ± 0.1 | 1.2 ± 0.2 |
| 24 hour urine protein (g), mean ± SE | 1.1 ± 0.5 | 1.5 ± 0.7 |

| | | |
|---|---|---|
| Creatinine clearance (mL/min), mean ± SE | 103.1 ±7.6 | 107.3 ± 12.2 |
| GFR (mL/min), mean ± SE | 81.0 ± 6.4 | 93.3 ± 11.3**, n = 10 |
| Plasma CTH (nmole/mL), mean ± SE | 12.1 ± 0.9 | 11.0 ± 1.1, n = 11 |
| Urine sediment CTH (nmoles/g creatinine), mean ± SE | 2369 ± 308 | 2162 ± 383, n = 11 |
| Kidney CTH (nmoles/mg creatinine), mean ± SE | 19.5 ± 1.7, n = 11 | 19.0 ± 3.6, n = 9 |
| Kidney, active lipid damage score, mean ± SE | 9.4 ± 0.6, n = 11 | 8.3 ± 1.2, n = 9 |
| Kidney, chronic damage score, mean ± SE | 7.0 ± 0.9, n = 12 | 7.0 ± 1.6, n = 9 |

**This GFR is for all 12 placebo subjects, including two subjects who did not have end of study GFR values (subjects 5 and 20); the mean and SE for the 10 placebo subjects (excluding subjects 5 and 20) is 98.0 ± 10.8 mL/min

The GFR was estimated using inulin clearance. The normal range for creatinine clearance in this study was 90 to 125 mL/min. The upper limit of normal protein excretion was 0.1 g/24 hours.

*Comment: In general, it appears the subjects randomized to the active product may have had less pain at baseline than the placebo subjects as evidenced by lower worst pain scores, severity pain scores and interference pain scores.*

*It is notable that the renal functional data show somewhat contradictory observations between the two groups. For example, the serum creatinine is slightly higher in the placebo group (1.2 vs 1.0) and the 24 hour urine protein excretion also slightly higher in this group (1.5 vs 1.1)--suggesting the Agalsidase group might have somewhat better renal function at baseline. However, the creatinine clearance data are similar between the two groups. Together, these three findings suggest the baseline renal function was similar between the two groups. Notably, the GFR data suggest the Agalsidase group has more impairment of renal function (and the magnitude of this GFR difference is especially striking in light of the other two measures suggesting renal function is either similar between the two groups or better within the Agalsidase groups). This observation raises questions about the accuracy of the notably lower GFR for the Agalsidase group.*

**(3) Extent of exposure:**

The study was planned to involve the administration of 12 study drug infusions during 13 patient visits. However, in mid-May, 1999 a series of infusion reactions prompted cessation of subject dosing for approximately a week. Consequently, most patients missed at least one dose. The extent of exposure is shown in Table 11.

**Table 11. Extent of exposure**

| **Number of subjects who:** | **Agalsidase n = 14** | **Placebo n = 12** |
|---|---|---|
| received 12 infusions | 3 | 4 |
| received 11 infusions | 11 | 6 |
| received 10 infusions | 0 | 1 |
| received 9 infusions | 0 | 1 |
| Had infusion interrupted | 5 | 0 |

**(4). Primary endpoint:**

**(A) Prospectively defined primary endpoint result:**

The primary endpoint for the study was the AUC1 method (trapezoidal area under the curve) analysis of the change in pain score by visit for the "off pain medication" visits compared between groups using the two-

sample t-test. All randomized subjects were to be included in the analysis. There were four placebo subjects who each had one value imputed due to missing values. For two of these four subjects the needed scores were designated as "missing" because the available scores were categorized as invalid due to use of pain medication. Last observation carried forward (LOCF) was used for imputation. Table 12 shows the result of the primary endpoint.

**Table 12. Primary endpoint result: area under the curve for change in pain at its worst while off pain medication (AUC1)**

| Assessment | Agalsidase n = 14 | Placebo n = 12 | p-value* |
|---|---|---|---|
| Worst pain | - 22.4 ± 9.4 | - 1.0 ± 13.5 | 0.195 |

* t-test

The sponsor also submitted a post-hoc analysis of the primary endpoint using an analysis of covariance (ANCOVA) with baseline pain score as covariate, which resulted in a p-value of 0.08.

*Comment: It is important to note several points regarding this analysis.*

1) *The study data do not permit an identification with certainty of the BPI Worst Pain values that should be used for the "off pain medication" analysis intended by the analytic plan. It is impossible to ascertain if any true "off medication" set of data does exist (see Study Conduct section, above).*

2) *The analysis in Table 12 (p = 0.195) is the sponsor's revised analysis following the modifications to the dataset as described above (Study Conduct). The comparison between the two groups prior to the database revision provided a p-value of 0.43.*

3) *The "pain medications" selected by the applicant include only the non-traditional analgesics often used for neuropathic pain, and excludes attention to standard, effective analgesics. Thus, analgesic use, in the broad sense, was uncontrolled and not standardized when the BPI assessments are done.*

4) *Consequently, interpretation of the primary endpoint with regard to demonstration of efficacy is not possible.*

*Systemic steroids were utilized in some subjects during the study. There was more pervasive use of steroids in the Agalsidase subjects, a usage almost entirely related to either infusion premedication or treatment of infusion reactions: 8 of 14 Agalsidase subjects received steroids (median total dose 800 mg) while only 2 of 12 placebo subjects received steroids (200 mg in one and an unknown amount between 360-720 mg in the other). There is potential that this might have resulted in some bias favorable toward the active treatment group if the steroids lessened pain via an anti-inflammatory mechanism. It is difficult to assess the impact of steroids upon "off pain medication" pain scores. Subjects who had a 1.0 or greater decrease in Worst Pain score from baseline at weeks 9, 17 or 24 were examined for steroid use. No excess predominance of steroid use in these subjects is apparent, but the sample sizes within the study are too small to adequately explore this possibility.*

**(B) Exploratory analyses prospectively defined to be supportive of the primary analysis:**

The statistical analytical plan identified several analyses to be performed in order to explore the primary endpoint result, as summarized in Appendix B. These exploratory/supportive analyses may be classified into three groups: analyses of the component scores of the primary endpoint, sensitivity analyses for missing value imputation, or analyses of the pain scores from all study visits (i.e., both "on" and "off" medication scores).

These analyses showed that some alternative statistical methods provide p-values that are smaller than the prospective analytic method. Some of these p-values are numerically less than 0.05, but the meaning of such analyses is uncertain, given both the post-hoc nature and the multiplicity of analyses.

Of particular interest is the analysis of the all-visits pain score. This analysis does not differentiate between scores intended to be “on” or “off” pain medication and pools scores from all visits. This all-visits analysis showed an AUC of 2.1 for the Agalsidase group, -26.4 for placebo (p=0.97), where negative values imply improvement. Although this analysis should not be over-interpreted to regard this as an indication that Agalsidase subjects had increased pain (or lessened analgesic effectiveness) this analysis does indicate that no pain improvement compared to placebo was apparent in the Agalsidase group. This analysis has the advantage of being unaffected by the uncertainties of whether or not any particular subject completed the BPI evaluation while on or off medication when intended to be off medication. This analysis may also be more reflective of what patients might experience in actual use of the product, since the “off medication” state is "artificial," and patients are likely to use pain medications to the degree needed in daily life.

*Comment: The analyses show that there was no clear evidence of a treatment related difference between the two study groups using the prospectively defined primary endpoint analysis or the prospectively defined exploratory analyses. The totality of the data provide no evidence that Agalsidase treatment is associated with improvement in the "worst pain" BPI scores.*

**(5) Secondary endpoints:**

As described in the section on Statistical Analyses above, the protocol and statistical analytic plan directed there be a large number of data comparisons, many imprecisely stated, and without prioritization of importance. This review will focus upon those secondary and tertiary endpoints which are directly applicable to the sponsor’s proposed labeling indications and which have the potential to be evidence of efficacy.

The Statistical Analytical Plan stated that these analyses would be performed utilizing the LOCF method of imputation for missing data points and the following results utilize this method, unless otherwise noted. Details of the secondary endpoint results are shown in the Appendices (Appendix B).

**(A) Change in pain scores that assess "severity" of pain:**

There were five secondary endpoint analyses subtended by assessments of pain "severity." All these endpoints used the mean of the four severity items from the BPI questionnaire.

The "severity" items of the BPI refer to questions 3 through 6 of the BPI short form. These questions asked for ratings of 0-10 for the following:

Pain at its worst in the last week (This was also the primary endpoint assessment)
Pain at its least in the last week
Pain on average
Pain you are having right now

These were analyzed as “off medication” scores and all visits-pooled analyses (similar to the methods for the primary endpoint).

All of the prospectively defined secondary endpoints that assess the "severity" scores from the BPI questionnaire show no difference between the two treatment groups. While the “off medication” scores and responder analysis suggest the possibility of treatment associated benefit, these data provide no robust evidence of this. It is notable that the "All Visits" severity scores in this average pain analysis, as in the primary endpoint analysis, suggested no benefit from Agalsidase.

**(B) Change in pain scores that assess "interference" of pain with activities:**

The multiple secondary endpoints within this heading assessed responses to the "interference" components of the BPI questionnaire. The "interference items" of the BPI refers to questions 9A through 9G of the BPI short form. Subjects were to respond to these questions also by a 0 to 10 response . These questions asked how, during the past week, pain has interfered with:

General Activity
Mood
Walking ability
Normal Work
Relations with other people
Sleep
Enjoyment of life

The average interference score was analyzed as had been done for the average severity score. The "interference" pain scores, like the "severity" and "worst" pain scores, reveal no statistically significant difference between the two groups.

*Comment: Overall, the BPI pain scores show no evidence of a favorable impact of Agalsidase upon pain. This failure to impact the pain scores may be related not only the potential lack of any efficacy of Agalsidase in the prevention or treatment of pain, but also to possible deficiencies within the scoring system or the manner in which it was used in this study. Conceivably, the practice of abruptly stopping and restarting neuropathic pain medications throughout the study may have resulted in physiological changes which obscured any evidence of an Agalsidase treatment effect. There are multiple other considerations in the failure to demonstrate a treatment effect upon the pain scores--such as inappropriate dose or dose regimen, alterations due to concomitant medications (especially steroids), the extent of underlying irreversible neuropathic disease within the subjects, etc.*

**C) Pain medication usage:**

The Statistical Analytical Plan stated that the time to pain medication permanent discontinuation was to be analyzed with a Kaplan-Meier analysis and a comparison using a Log-rank statistical test. However, the number of subjects who discontinued pain medications was small and this determination was also confounded by the difficulties with data recording and narrow data definitions.

*Comment: Using post-hoc compilations of medications used during the study, the sponsor designated neuropathic medications to be the following: Tegretol, carbamazepine, Neurontin, Dilantin, phenytoin, Lamictal, Lamotrigine, Nortriptyline and amitriptyline. Within the sponsor's analyses, subjects were considered as being on pain medications if these medications were described in the response to question 18 of the BPI long form (BPILF) or if the medication was started or stopped in the baseline period. However, this analysis is subject to the same uncertainties and inconsistencies in the study data that render the "off medication" dataset unreliable.*

*It is important to note that the sponsor's post-hoc definition of "pain medications" does not include narcotics and other general analgesics. Consequently, a subject who was weaned from designated pain medications but began the use of a narcotic or other general analgesic was apparently assessed as a success.*

The sponsor reports that four Agalsidase subjects successfully discontinued pain medications during the study, versus no placebo subjects. Although FDA inspection of the data and existing site records supports an assessment that two Agalsidase subjects and one placebo subject discontinued pain medications, the data recording difficulties described above lead to the conclusion that no truly accurate analysis of this endpoint can be achieved with this study dataset.

(**D) Renal histopathology and renal function outcomes:**

Few secondary endpoints appeared to be related to renal function. Indeed, the analytical plan was specific in stating that "secondary analyses" were related to effects of Agalsidase upon pain and renal *structure.* Some assessments of renal functional change were listed as tertiary and exploratory analyses in the Statistical Analytical Plan. Nevertheless, because the renal structural changes may correlate with renal functional changes, both outcomes are reviewed in this section. In addition, pertinent findings from Study TKT006 (the one year open label study that allowed all TKT003 patients to receive Agalsidase) and the interim report from Study TKT011 (the report of an additional year of Agalsidase administration to subjects who completed Study TKT006 and chose to continue the enzyme). Certain data from Study TKT005 (the cardiac study) are also included here for comparison purposes. The renal structural changes (histopathology) will be described first, followed by renal functional changes. The renal structural changes were based upon the results from percutaneous kidney biopsies.

**((1)) Renal histopathology:**

Histopathological data analyses from this study may be broadly grouped into three categories: data related to the Active Lipid Damage Score (ALDS), the Chronic Damage Score (CDS), and "Standard Histopathology" (SH). All three categories of data analyses were obtained from light microscopic evaluation of kidney tissue slides, and two of these data categories (ALDS and CDS) were secondary endpoints within the study.

*Comment: The renal histopathological outcomes include both prospectively defined endpoints (the lipid damage scores) and outcomes that were entirely post-hoc.*

*There were few prospectively defined details relating to the performance of the histopathological evaluations and data analyses, as described below. Perhaps in response to the few prospective details, there were ad-hoc modifications of the plans and generation of new slide grading procedures during the conduct of the study. These ad-hoc modifications involved an extensive revision of the CDS and the creation of a new category of assessments, the SH. These ad-hoc changes were made while the applicable investigators and reviewing pathologists were still blinded to the treatment assignment code. During a portion of the deliberations regarding the ad-hoc changes, the investigators were performing "preliminary" (blinded) review of microscopic slides from the biopsies. The specific prospective analyses to be performed are unclear due to lack of detail within the protocol. The Statistical Analytical Plan appears to indicate that the two composite scores (the ALDS and CDS) were the only prospectively defined endpoints to be analyzed. This is important to note because these scores are composites of several component scores. The ALDS component scores grade the extent of CTH ($Gb_3$) deposition within the various cellular compartments while the CDS components score various markers of chronic disease.*

**a. Kidney biopsy sample processing and slide review procedures:**

Kidney biopsies were performed at baseline and the end of the study. The kidney tissue samples were to be initially divided into three portions, each portion prepared for one of the following: electron microscopy, light microscopy and CTH ($Gb_3$) content assay. The Armed Forces Institute of Pathology (AFIP) prepared the slides, and provided sets to both the NIH investigators and TKT. These blinded slides were reviewed by the NIH investigators during the process of generating the ad-hoc slide grading plans. The final slide review procedures were performed in a blinded manner (reviewing pathologists blinded to subject and biopsy sequence).

The renal histopathology slides were reviewed by two AFIP pathologists in a process that involved both pathologists reviewing the same slide through a two-headed microscope simultaneously. The slides were reviewed over a several day period in which the pathologists completed a spread sheet that contained columns headed by the specific outcome and the rows identifying the biopsy sample number. This spread sheet was composed during the study by the NIH investigators (and includes their ad-hoc changes).

The pathologists recorded a count of the number of affected glomeruli, the total number of glomeruli, the number normal, and numbers with several different types of abnormality. There were scores of 0 to 3+ for each of the components of Chronic Damage Score, as follows (using the score sheet terminology):

Tubules
Int Inflammation
Int fibrosis
Arterial Hyalinosis
Arterial Medial thickening
Arteriolar Hyalinosis
Arteriolar Medial thickening

Similarly , the components of the Active Lipid Damage Score were each grades 0 to 3+ (using score sheet terminology):

Deposits GEC
Endocapillaries
Proximal tubule
Distal tubule
Vascular Endothelium
Vascular Media

Notably, there were no prospective description of the following procedures:

-detailed, systematic plans for light microscopic data ascertainment by pathologists; specifically, there was no identification of the number of reviewing pathologists or the procedures to be employed by the pathologists in reviewing the slides (ie., review of all slides at a single "sitting", independent or interactive review of slide findings by pathologists, plans for reconciling disagreements among pathologists)

-definitions of pathological findings or provision of criteria to be systematically followed with respect to assigning pathological outcomes; specifically, there were no criteria for assessing the following outcomes: extent of "vacuolization," extent of "foamy cell appearance," "endothelial vacuolization," "vascular media involvement," glomerulosclerosis, hyalinosis, "capillary collapse," "mesangial matrix expansion," tubular atrophy, interstitial fibrosis, interstitial inflammation.

-there was no description of the minimum or maximum number of slides (sections) to be used in assessing a pathological outcomes (for example, renal biopsy sample number 21 might have four toluidine blue slides, while sample number 97 might have three toluidine blue slides and some or all of the slides could be reviewed at the discretion of the pathologist)

-there was no description of the types of stained slides to be reviewed by the pathologists as they ascertained the study data (for example, was the extent of "foamy cell appearance" to be based only upon the H and E-stained sections, or based on findings from other sections also?)

-there was no training provided to pathologists in order to ensure systematic consistency among the reviewing pathologists or consistency of their interpretations over time

-plans for "interim" review of microscopic slides in order to finalize the slide grading procedures

*Comment: There are deficiencies within the prospective plan for histopathological assessments. The lack of specific guidelines on the procedures for data ascertainment severely limits the quality of the data. For example, there was no external reference standard to define "mild, moderate or severe" grades. Conceivably, a pathologist's assessment of "severe" foamy cell appearance may, in the absence of criteria, vary from day to day (reading to reading). Similarly, certain findings from an H and E-stained slide (e.g.,*

*extensive "foamy cell appearance") might influence one pathologist (but not the other) to change a "mild" toluidine blue-stained section score of CTH ($Gb_3$) deposition from "mild" to "moderate."*

*There was only a single spread sheet supplied to the two reviewing pathologists and few directions provided on the time line or method for completion of this sheet. The sum effect of this lack of detail is an inability to verify multiple aspects of the data ascertainment procedures--e.g., the ability to identify any disagreements among the reviewing pathologists or the ability to ascertain whether interpretations/judgments used in assigning pathological outcomes changed over the several day period involved in pathological scoring, or the impact of pathologist discretion in reviewing the totality of available slide data. Additionally, the source data spread sheet (the original) is missing.*

**b. Histopathology results:**

Of the 26 subjects in the study, one subject (subject number 18, Agalsidase) had neither a baseline nor a week 24 kidney biopsy performed. Four subjects did not have week 24 biopsies performed (placebo subjects 5, 11, 20 and Agalsidase subject 16). Subject 16 had a hemorrhagic event following the initial renal biopsy.

Consequently, there were 46 renal tissue biopsy samples submitted for pathological review (including those with no follow-up samples). These data included complete sets of renal biopsy samples for 21 subjects: 12 (86%) Agalsidase subjects and 9 (75%) of the placebo subjects.

There were two prospectively defined analyses of histopathology results: the ALDS composite score and the CDS composite score. The post-hoc outcomes relating to Standard Histopathology (SH) are also described in this section.

**1) ALDS:**

The ALDS score was a sum of six different components, in which the severity of disease was graded within each component on a scale from 0 (normal) to 3 (severe involvement). The six components were the following: -glomerular epithelium; -glomerular endothelium; -proximal tubules; -distal tubules; -vascular endothelium; -vascular media.

Consequently, the maximum (worst disease) corresponded with a score of 18. Table 13 summarizes the ALDS analyses (including the post-hoc analyses of ALDS component scores). Three subjects (out of the 21 subjects with paired biopsy sample data) had missing scores for the various components of the ALDS. Subject 26 (Agalsidase) had all baseline ALDS component scores missing (insufficient tissue) and this subject is excluded from Table 13 ALDS composite analyses (hence the "n" for Agalsidase is 11 instead of 12). Two other subjects had missing components for the ALDS and these missing values were imputed as "0" within the ALDS composite score. However these two subjects were excluded from analyses of the applicable component scores, as follows: subject 2 (Agalsidase) and subject 25 (placebo) both had two baseline ALDS component scores missing (glomerular epithelium and glomerular endothelium). The source data sheet lists "tissue insufficient" for subject 26 (the subject missing all baseline ALDS component scores) but no basis for the other two subject's missing data points.

**Table 13. ALDS score in subset of subjects with available data***

| Outcome | Agalsidase, n = 11<br>m ± sem | Placebo, n = 9<br>m ± sem | p-value for change<br>ANCOVA** |
|---|---|---|---|
| ALDS at baseline | 9.4 ± 0.6 | 8.3 ± 1.2 | 0.11 |
| ALDS change to week 24 | - 1.5 ± 0.8 | 0.9 ± 0.9 | |
| ***Components of ALDS*** | | | |
| *Glomerular epithelial cells* | | | |
| Baseline | 2.3 ± 0.2 | 2.4 ± 0.3 | 0.83 |
| Change to week 24 | 0.0 ± 0.3 | 0.0 ± 0.3 | |

| | | | |
|---|---|---|---|
| *Endocapillary cells* | | | |
| Baseline | 1.2 ± 0.1 | 1.1 ± 0.2 | 0.04 |
| Change to week 24 | - 0.7 ± 0.2 | 0.0 ± 0.3 | |
| *Proximal tubules* | | | |
| Baseline | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.31 |
| Change to week 24 | - 0.1 ± 0.1 | 0.1 ± 0.1 | |
| *Distal tubules* | | | |
| Baseline | 1.8 ± 0.2 | 1.9 ± 0.4 | 0.72 |
| Change to week 24 | 0.2 ± 0.4 | 0.0 ± 0.2 | |
| *Vascular endothelium* | | | |
| Baseline | 2.0 ± 0.2 | 1.6 ± 0.3 | 0.003 |
| Change to week 24 | - 1.2 ± 0.3 | 0.2 ± 0.3 | |
| *Vascular media* | | | |
| Baseline | 2.3 ± 0.2 | 1.8 ± 0.3 | 0.80 |
| Change to week 24 | 0.0 ± 0.2 | 0.2 ± 0.4 | |

* n = 10 Agalsidase and n = 8 Placebo, as described above
**with baseline as covariate

*Comment: Of the six components of the ALDS score, only two components showed nominal (post-hoc) statistically significant differences between the two groups: the endocapillary and vascular endothelium scores. Both scores suggested improvement within the active treatment group. The ALDS, per the analytical plan and the spreadsheet, was a measure of "deposits"--ie., CTH ($Gb_3$) deposits. There was no prospective plan describing how the "deposits" were to be assessed, although the sponsor reports that the pathologists had the discretion to review all slides--including those slides in which CTH ($Gb_3$) deposits were potentially not stained (PAS, H and E, Masson) and those slides in which CTH ($Gb_3$) deposits may have been stained (toluidine blue).*

**2) CDS:**

The CDS score could not be calculated as prospectively defined because the biopsy assessment spread sheet had been changed during the study conduct to delete two CDS components and to modify two other components. The result of these alterations was that the seven CDS components the pathologists scored were not the seven components prospectively planned for scoring. In post-hoc analyses, the sponsor used these modified seven components to form the modified CDS. These changes are summarized in Table 14.

**Table 14. CDS component changes**

| Prospectively defined | Actually performed |
|---|---|
| Glomerulosclerosis or hyalinosis | Not scored |
| Global glomerulosclerosis | Not scored |
| Tubular atrophy | Tubular atrophy |
| Interstitial fibrosis | Interstitial fibrosis |
| Vascular hyalinosis | Arterial hyalinosis |
| | Arteriolar hyalinosis |
| Vascular medial thickening | Arterial medial thickening |
| | Arteriolar medial thickening |

Table 15 shows the results of the post-hoc modified composite CDS score and each of the seven components. Neither the CDS overall, nor any of the components, reached a p-value of less then 0.05 in the statistical comparisons.

**Table 15. Modified CDS score in subset of subjects with available data***

| Outcome | Agalsidase, n = 12 m ± sem | Placebo, n = 9 m ± sem | p-value for change ANCOVA** |
|---|---|---|---|
| CDS at Baseline | 7.0 ± 0.9 | 7.0 ± 1.6 | 0.81 |
| CDS change to week 24 | 0.1 ± 0.7 | 0.4 ± 1.6 | |

| Components of CDS | | | |
|---|---|---|---|
| *Tubular atrophy* | | | |
| Baseline | 0.9 ± 0.3 | 0.8 ± 0.3 | 0.37 |
| Change to week 24 | - 0.2 ± 0.2 | 0.1 ± 0.3 | |
| *Interstitial inflammation* | | | |
| Baseline | 0.6 ± 0.2 | 1.0 ± 0.4 | 0.78 |
| Change to week 24 | 0.1 ± 0.2 | - 0.1 ± 0.2 | |
| *Interstitial fibrosis* | | | |
| Baseline | 1.0 ± 0.3 | 1.0 ± 0.3 | 0.44 |
| Change to week 24 | 0.0 ± 0.2 | 0.2 ± 0.3 | |
| *Arterial hyalinosis* | | | |
| Baseline | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.30 |
| Change to week 24 | - 0.4 ± 0.2 | 0.0 ± 0.3 | |
| *Arterial medial thickening* | | | |
| Baseline | 1.3 ± 0.3 | 1.1 ± 0.4 | 0.30 |
| Change to week 24 | 0.3 ± 0.2 | - 0.1 ± 0.5 | |
| *Arteriolar hyalinosis* | | | |
| Baseline | 0.9 ± 0.2 | 1.1 ± 0.3 | 0.46 |
| Change to week 24 | - 0.1 ± 0.2 | 0.1 ± 0.5 | |
| *Arteriolar medial thickening* | | | |
| Baseline | 1.6 ± 0.2 | 1.2 ± 0.3 | 0.74 |
| Change to week 24 | - 0.1 ± 0.2 | 0.2 ± 0.2 | |

*subject 26 (Agalsidase) was missing baseline scores for the components of tubular atrophy, interstitial fibrosis, interstitial inflammation; the CDS score within this table imputes these three missing values as 0; within the component analyses, this subject is eliminated from the analysis in which the component is missing so the Agalsidase n = 11 for these three components;
**baseline is covariate

**3) Standard histopathology (SH):**

The sponsor established a series of post-hoc SH analyses. The SH refers to the use of the seven columns of the source data spread sheet that required the pathologists to record (within each biopsy sample) the number of glomeruli that met the following criteria:

-the number on the "LM" sections
-the number on the "EM" sections
-the number on both the "LM" and "EM" sections (i.e., the total number of glomeruli)
-the number on both "LM" and "EM" sections that were normal glomeruli
-the number on both "LM" and "EM" sections with segmental sclerosis
-the number on both "LM" and "EM" sections with mesangial widening
-the number on both "LM" and "EM" sections with global sclerosis

The post-hoc designed analyses consisted of four glomerular endpoints in which the two groups were compared for the following changes from baseline:

-in the fraction of normal glomeruli (fraction being normal/total)
-in the fraction of glomeruli with mesangial widening (fraction being affected/total)
-in the fraction of glomeruli with segmental sclerosis (fraction being affected/total)
-in the fraction of glomeruli with obsolescence (fraction being affected/total)

SH results are shown in Table 16.

**Table 16. SH results from the subset of subjects with available data**

| Outcome | Agalsidase, n = 12<br>m ± sem | Placebo, n = 9<br>m ± sem | p-value for change* |
|---|---|---|---|
| *Fraction of normal glomeruli* | | | |
| Baseline | 0.40 ± 0.07 | 0.60 ± 0.07 | 0.01 |
| Change to week 24 | 0.08 ± 0.04 | - 0.16 ± 0.08 | |
| *Fraction of glomeruli with mesangial widening* | | | |
| Baseline | 0.38 ± 0.04 | 0.24 ± 0.04 | 0.01 |
| Change to week 24 | - 0.13 ± 0.05 | 0.17 ± 0.08 | |
| *Fraction of glomeruli with segmental sclerosis* | | | |
| Baseline | 0.03 ± 0.01 | 0.06 ± 0.02 | 0.05 |
| Change to week 24 | 0.04 ±0.02 | - 0.03 ± 0.02 | |
| *Fraction of glomeruli with obsolescence* | | | |
| Baseline | 0.20 ± 0.07 | 0.11 ± 0.05 | 0.87 |
| Change to week 24 | 0.00 ± 0.05 | 0.02 ± 0.03 | |

*ANCOVA with baseline as covariate

*Comment: Of the four SH components, two appeared to favor improvement within the active treatment group (the fraction of normal glomeruli and the fraction of glomeruli with mesangial widening), one suggested no difference between the two groups (fraction of obsolescent glomeruli) and one suggested worsening within the active treatment group (fraction of glomeruli with segmental sclerosis).*

*Overall, there was a lack of rigor in the collection of histopathological data. This deficiency, combined with striking imbalances in the extent of evaluable tissue and missing data, largely precludes the ability to reach any verifiable conclusions based upon these histopathological data.*

*There were no prospective definitions of important histopathological outcome terms, no training of pathologists in slide reviews, no prospective detailed procedures to be employed during the pathological reviews and there were deficiencies within the analytical plans. The investigator-initiated revision of the histopathological assessment procedures during the study so that certain prospectively planned assessment procedures were not followed exemplifies the difficulties that ensued from the lack of adequate prospectively detailed procedures.*

*The available findings are open to many speculative and exploratory interpretations. However, one of the exploratory interpretations could be that Agalsidase administration lessened the anatomical extent of CTH ($Gb_3$) deposition within the vascular endothelium (as assessed by the components of the ALDS). It is interesting to note that multiple other cellular components of the kidney did not show appreciable changes in the extent of CTH ($Gb_3$) deposition, including the glomerular epithelium. The failure of all cellular components to "change" in a pattern consistent with the vascular endothelium is notable, especially given the finding that biochemical measures of CTH ($Gb_3$) deposition within the kidney biopsy samples showed no difference between the two groups. One interpretation of this discordant observation might be that Agalsidase shifted the CTH ($Gb_3$) burden from vascular endothelium to other parts of the kidney. Alternatively, the vascular endothelium reservoir of CTH ($Gb_3$) might have been so small that biochemical assays would not have been impacted by changes in endothelial content.*

**((2)) Changes in creatinine clearance and GFR:**

**a. Creatinine clearance and GFR in Study TKT003**:

The most important renal functional assessments in the study were measures of creatinine clearance and GFR. Renal plasma flow (PAH clearance) was also measured in the study but there was no difference between the two groups. Hence, the renal plasma flow results are not presented.

Creatinine clearance and GFR were supportive endpoints within Study TKT003 and are presented here because of the large impact renal function decline ultimately has on Fabry disease patients. In addition, data from two follow-up studies are also presented in this section: Study 006 (the post-TKT003 open label study that allowed all subjects to receive one year of Agalsidase exposure) and Study 011 (the post-TKT006 study that allowed subjects to continue receiving Agalsidase). Table 17 shows the most important renal functional outcomes from Study TKT003 and, for comparison purposes, the similar data from Study TKT005 (a randomized, controlled study of similar design). It is important to note that the creatinine clearance data from Study TKT005 are reported by TKT to be inaccurate due to errors in sample procurement. However, these errors do not apply to the Study TKT005 GFR data.

**Table 17. Study TKT003 and Study TKT005 Creatinine Clearance (CC) and GFR Changes**

| **Outcome** | **Study TKT003** | | | **Study TKT005** *(see legend for missing data notes)* | | |
|---|---|---|---|---|---|---|
| | Agalsidase n = 14 | Placebo CC n = 11**, GFR n = 10*** | p-value* | Agalsidase CC n = 6#, GFR n = 7 | Placebo CC n = 8, GFR n = 8 | p-value* |
| **Creatinine Clearance Evaluations** | | | | | | |
| **CC change** | **0.07 ± 5.9** | **- 19.7 ± 9.1** | **0.05** | **- 7.3 ± 26.7** | **- 2.3 ± 18.1** | **0.84** |
| *Creatinine Clearance Components* | | | | | | |
| *Wk 0* | *103.1 ± 7.6* | *107.3 ± 12.2* | *N/A* | *107.7 ± 17.8* | *91.0 ± 17.7* | *N/A* |
| *Wk 9* | *112.5 ± 9.4* | *105.6 ± 15.0* | | *121.0 ± 17.1* | *91.8 ± 18.6* | |
| *Wk 17* | *111.7 ± 9.9* | *104.1 ± 9.5* | | *125.7 ± 14.4* | *92.8 ± 21.5* | |
| *Wk 23* | *106.8 ± 9.9* | *100.7 ± 16.1* | | *109.0 ± 22.3* | *87.7 ± 22.2* | |
| *Wk 24* | *103.1 ± 7.5* | *85.7 ± 11.2* | | *100.3 ± 15.0* | *88.7 ± 21.0* | |
| **GFR Evaluations** | | | | | | |
| **GFR change** | **- 8.8 ± 3.8** | **- 19.8 ± 7.9** | **0.65** | **25.4 ± 6.4** | **14.3 ± 8.4** | **0.34** |
| *GFR Components* | | | | | | |
| *Wk 0* | *81.0 ± 6.4* | *98.0 ± 10.8* | *N/A* | *106.3 ± 13.9* | *100.6 ± 16.0* | *N/A* |
| *Wk 24* | *72.2 ± 4.3* | *78.2 ± 7.7* | | *131.7 ± 15.5* | *114.9 ± 22.4* | |

CC change = change in creatinine clearance from baseline to week 24, in mL/min,
GFR change = change in GFR from baseline to week 24, in mL/min
*ANCOVA with baseline as covariate
** the missing value is for subject number 20, a subject who had a creatinine clearance of 127 mL/min at baseline and 107 mL/min at week 17 and no week 24 data (imputing the week 24 value using LOCF does not change the p-value for treatment group comparison)
***the missing values are subjects 5 and 20, placebo subjects who had baseline GFR but not end of study GFR
Study TKT005 contained approximately 20% missing or physiologically improbable (CC > 200 mL/min) CC data points and the table presents the (LOCF imputed) data for all patients with a first baseline and any subsequent data point (it excludes 1 Agalsidase subject who had no baseline CC value)
#the missing value is for subject 1, a subject who did not have a baseline CC
N/A = not applicable; Wk = week
Note that for CC within Study TKT003 Agalsidase n=12 Wk17; Placebo n=12 Wk0, n=10 Wk23, but 1 missing value scattered among subjects also on Wk9, 17, 24.
Note that for CC within Study TKT005: Placebo n=7 Wk 17; Agalsidase n=5 Wk 23.

*Comment: The average creatinine clearance was normal for both study groups at baseline. However, the average GFR was reportedly low at baseline for the Agalsidase group (normal GFR is 90 to 130 mL/min). It is also notable that, while there were many creatinine clearance measurements in Studies 003 and 006, there were only three time points that assessed GFR. Hence, the impact of a small number of errors within the ascertainment or analyses of the GFR data may have more profound apparent impact than a similar number of errors within the creatinine clearance data. Also very notable is the inconsistency within the placebo group's end of study TKT003 creatinine clearance results--in that the mean week 23 creatinine clearance result was 100.7 mL/min and the mean week 24 result was 85.7 mL/min. However, such a large decline within a one week period seems physiologically improbable. Consistent with this expectation of no dramatic changes within a one week period were the average serum creatinine values of 1.7 at week 23 and 1.8 at week 24 within the placebo group.*

Figure 1 shows the creatinine clearance and GFR changes over both Study TKT003 and TKT006 (for subjects with available data). Within this figure, for creatinine clearance: Agalsidase n = 14 (except for week 17 where n = 12, and weeks 37 and 63 where n = 13, missing data) and Placebo n = 12 (except for weeks 9, 17, 24 and 51 where n = 11 and weeks 23, 37, 63 and 76 where n = 10, missing data). For GFR, Agalsidase n = 14 and Placebo n = 12 (except for weeks 23 and 76, where n = 10, missing data).

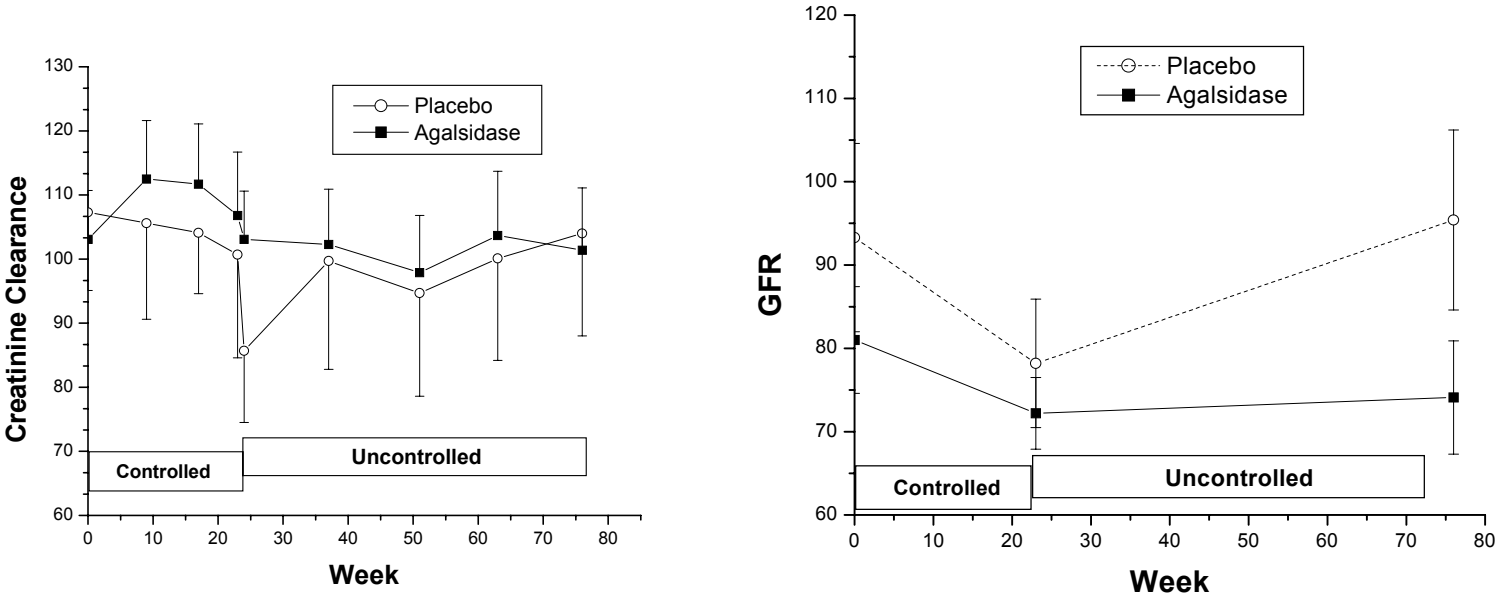



**Figure 1. Creatinine Clearance (left) and GFR (right) in Studies TKT003 (controlled period) and TKT006 (open label extension period)**

*Comment: The Study TKT003 Week 24 data statistically suggest that Agalsidase administration may have prevented a decline in creatinine clearance (p = 0.05). However, it is important to note that the placebo group's deterioration in creatinine clearance occurred during the single time period between week 23 and week 24. Notably, for the placebo subjects shown in Figure 1, the mean (± SE) week 23 serum creatinine value was 1.9 ± 0.8 (n = 10) and the group's corresponding week 24 serum creatinine value was also 1.9 ± 0.8 (n = 11).*

*The relatively small sample sizes result in the possibility that almost any error within the week 24 data could profoundly influence the statistical analysis of week 24 change from baseline. Consequently, the Study TKT003 data do not provide robust evidence of any beneficial impact upon renal function within 6 months of treatment for patients who begin with nearly normal (averaged) renal function.*

Because of the inaccuracy within the Study TKT005 creatinine clearance dataset, Study TKT003 provides the bulk of meaningful creatinine clearance data and all subsequent analyses relate to this study. The sponsor proposes that the creatinine clearance data may be skewed by a hemorrhagic event following the first kidney biopsy in subject number 16, an Agalsidase subject. This subject required two interventional radiology procedures (emboli) to control the bleeding and it is conceivable that the embolization may have culminated in progressive renal dysfunction. Creatinine clearance results, excluding subject number 16 are not substantially different then the results which include Subject 16. Notably, the physiologically improbable time course of changes in the placebo group remains.

The appropriateness of excluding subject 16 from creatinine clearance analyses is questionable. The totality of the renal function data suggest that the renal emboli done prior to Week 3 had little impact upon

the subject's renal function. The decline in renal function of this subject occurred at Week 17 and later, having been largely unchanged at week 9 (see Appendix C).

*Comment: Overall, the creatinine clearance results from Study TKT003 appear problematic for the following reasons:*

*a. Creatinine clearance was measured at six time points during the study. Only the baseline and week 24 values form the basis for a claim of a treatment effect. However, graphical presentation of all creatinine clearance time point outcome data shows that the week 24 outcomes may be an aberrancy of certain outlier effects. Without this time point's impact, the creatinine clearance values for the two groups generally parallel each other.*

*b. The putative decline in creatinine clearance values for the placebo group at week 24 are not robust and may be due to chance alone. This conclusion is exemplified by the examination of placebo subject 17. Subject 17 had a week 24 creatinine clearance value of 75 mL/min but a week 23 value of 149 ml/min, a value similar to the preceding values (all but that one of this subject's creatinine clearance values were above 133 mL/min). Recalculation of the comparison of the change from baseline to week 24 using a week 23 imputation for subject 17 yields a non-statistically significant outcome (p = 0.20). Consequently, since it is physiologically unlikely that this subject's true renal function declined by this amount in a one week period, there is no robustness to the proposed evidence of treatment effect.*

*c. The sponsor's basis for eliminating subject 16 (the Agalsidase subject who experienced a renal embolism) from analyses is not substantiated by the data.*

*d. The comparison of the change in creatinine clearance between the two groups should also be viewed within the context of the assessment reliability. There were two baseline measurements, and two study-end measurements that occurred within 1 week of each other. Since renal function should not substantially change within this short time period, the one week difference within subjects provides a general estimate of the amount of variability of the assessment. The difference between these paired measurements generally spans a range of approximately 19 mL/min. This potential within-subject variability should be borne in mind in interpreting any observed differences.*

*e. The GFR data show no significant difference between the two treatment groups. In general, one would have anticipated that important changes in creatinine clearance would have been accompanied by changes in GFR.*

**b. Creatinine clearance and GFR in Study TKT006:**

Study TKT006 was an open label study that allowed all subjects who completed Study TKT003 to receive Agalsidase for one year. Because of the relevance of the Study TKT006 renal function tests to Study TKT003, these data are presented below. All but one of the 26 subjects enrolled in Study TKT003 were also enrolled in Study TKT006. As shown in Table 18, there was no statistically significant change in creatinine clearance (from end of Study TKT003 to end of Study TKT006) for either the prior Agalsidase or placebo group.

*Comment: Table 18 is presented in the manner of analysis as prospectively described for the primary endpoint in Study TKT003 (Study TKT003 Week -1 compared to Study TKT003 week 24 and Study TKT006 week 52 values, with LOCF imputation for missing values). The sponsor presents the Study TKT003/006 data in a slightly different format in which comparisons are made between the average of two baseline values, the average of week 23 and 24 values and the final week 52 value. The conclusions from this type of analysis do not differ substantially from those evident within Table 18.*

*Overall, the change from baseline analyses of renal function in Study TKT006 support the Study TKT003 findings of no difference in the change of creatinine clearance related to Agalsidase administration. As can*

*be seen in Figure 1, and consistent with Study TKT006 findings, the low Study TKT003 week 24 data point for the placebo group appears to represent a spurious decline.*

**Table 18. Study TKT003/006 change in creatinine clearance**

| Assessment | Mean ± SE (mL/min) | p-value |
|---|---|---|
| ***Subjects treated with placebo in 003 and Agalsidase in 006**** | | |
| Baseline of 003 (n = 12) | 107.3 ± 12.2 | - |
| End of 003 (n = 12) | 87.5 ± 10.4 | |
| End of 006 (n = 11) | 101.6 ± 14.7 | |
| Change: 003 Baseline to end of 003 (n = 12) | - 19.7 ± 8.3 | 0.04 |
| Change: End of 003 to end of 006 (n = 11) | 15.9 ± 10.4 | 0.16 |
| ***Subjects treated with Agalsidase in 003 and Agalsidase in 006*** | | |
| Baseline of 003 (n = 14) | 103.1 ± 7.6 | - |
| End of 003 (n = 14) | 103.1 ± 7.5 | |
| End of 006 (n = 14) | 101.4 ± 9.7 | |
| Change: 003 Baseline to end of 003 (n = 14) | 0.07 ± 5.9 | 0.99 |
| Change: End of 003 to end of 006 (n = 14) | - 1.7 ± 5.7 | 0.77 |
| ***All subjects in 006**** | | |
| Baseline of 003 (n = 26) | 105.0 ± 6.8 | - |
| End of 003 (n = 26) | 95.9 ± 6.3 | |
| End of 006 (n = 25) | 101.5 ± 8.3 | |
| Change: End of 003 to end of 006 (n = 25) | 6.0 ± 5.7 | 0.30 |

*Subject 20 was not enrolled into Study TKT006, this subject is excluded from Study TKT006 analyses
p-values are from paired t-tests

The GFR data of Study TKT006 present an anomalous result. Unlike their changes in creatinine clearance, the prior-placebo patients who received Agalsidase in Study TKT006 appeared to show an improvement in GFR (compared to their end of Study TKT003 value), as shown in Table 19. Normal GFR (inulin clearance) is 90 to 130 mL/min. It is important to note that the nominal p-values are calculated for changes from baseline.

**Table 19. Study TKT003/006 change in GFR**

| Assessment | Mean ± SE (mL/min) | p-value |
|---|---|---|
| ***Subjects treated with placebo in 003 and Agalsidase in 006 (n = 10*)*** | | |
| Baseline of 003 | 98.0 ± 10.8 | - |
| End of 003 | 78.2 ± 7.7 | |
| End of 006 | 95.4 ± 10.8 | |
| Change: 003 Baseline to end of 003 | - 19.8 ± 7.9 | 0.03 |
| Change: End of 003 to end of 006 | 17.2 ± 6.4 | 0.03 |
| ***Subjects treated with Agalsidase in 003 and Agalsidase in 006 (n = 14)*** | | |
| Baseline of 003 | 81.0 ± 6.4 | - |
| End of 003 | 72.2 ± 4.3 | |
| End of 006 | 74.1 ± 6.8 | |
| Change: 003 Baseline to end of 003 | - 8.8 ± 3.8 | 0.04 |
| Change: End of 003 to end of 006 | 1.9 ± 3.4 | 0.58 |
| ***All subjects in 006 (n = 24)*** | | |
| Baseline of 003 | 88.1 ± 6.0 | - |
| End of 003 | 74.7 ± 4.0 | |
| End of 006 | 83.0 ± 6.2 | |
| Change: End of 003 to end of 006 | 8.3 ± 3.6 | 0.03 |

*excludes subject 5 who began peritoneal dialysis in Study 003 and was unable to complete GFRs

*Comment: The GFR results of Study TKT006 were illustrated in Figure 1 along with the results of Study TKT003. The GFR data remain difficult to reliably interpret. The apparent sizable difference in TKT003-*

*baseline GFR between the two groups was not reflected in serum creatinine or creatinine clearance measurements at that time. Furthermore, while in TKT003 the subjects receiving initial exposure to Agalsidase showed essentially little change in GFR over 6 months or 18 months of exposure, the subjects receiving initial exposure to Agalsidase in TKT006 showed an apparent sizable improvement in GFR over one year of treatment. This however, merely restored their mean GFR to the level of baseline of Study TKT003.*

*The small sample sizes within the study profoundly limit the robustness of the GFR results. One or two spurious values may alter the analytical outcome. Examination of the individual GFR results reveals, for example, a large increase in reported GFR for subject 13 (prior placebo group, see appendix for details). A reanalysis of the GFR data substituting an imputed value of no change for this single subject from the start of Study TKT006 substantially decreases the mean "improvement" in GFR in the prior placebo subjects during TKT006, and results in the absence of a nominally significant p-value. The absence of robustness to the uncontrolled GFR findings largely confirms that the controlled data (Study TKT003) are likely to be correct in concluding that there was no Agalsidase treatment effect upon GFR.*

**c. Creatinine clearance and GFR in Study TKT011:**

Study TKT011 was an open label, uncontrolled extension study that allowed all subjects who completed Study TKT006 to continue receiving Agalsidase indefinitely (until licensure of the product or termination of the study by the sponsor). The BLA contains an interim report comprising one year of follow-up in Study TKT011. This report includes a summary of data cumulative for 2 years of Agalsidase administration for some subjects (Study TKT003 placebo subjects) and 2.5 years of Agalsidase administration for other subjects (Study TKT003 Agalsidase subjects).

A total of 24 subjects were enrolled into Study TKT011. At the end of the Study TKT011 interim reporting period, a total of 22 subjects continue to receive Agalsidase. Four of the original 26 subjects randomized into Study TKT003 did not complete receiving Agalsidase through the Study TKT011 one year interim and the basis for these "drop outs" is described in another section of this review. However, no "drop out" was related to adverse events or progressive renal insufficiency. Indeed, during the series of three studies, only one subject (subject 5, a placebo subject from Study TKT003) developed ESRD. This subject began dialysis during Study TKT003 and underwent a renal transplant (successfully) during Study TKT011. Subject 5 had received approximately one year Agalsidase administration prior to the renal transplant and in the analyses below the subject's renal function data are truncated at the end of this one year treatment period (immediately prior to transplant).

Overall, the subjects may be divided into two major groups, those receiving at least 2 years of Agalsidase treatment through one year of Study TKT011 (n = 21) and those receiving less than two years of treatment through prior studies (n = 4).

**Table 20. Creatinine clearance among subjects receiving at least two years of Agalsidase**

| Outcome | mL/min; Mean ± SE | p-value (t-test) |
|---|---|---|
| Baseline for all subjects, n = 21 | 98.5 ± 6.4 | not applicable |
| 2 year value for all subjects, n = 21 | 107.7 ± 10.3 | |
| 2 year value for subjects receiving only 2 years, n = 9 | 109.7 ± 19.6 | |
| 2.5 year value for subjects receiving 2.5 years, n = 12 | 112.2 ± 13.6 | |
| Change over 2 years for all subjects, n = 21 | 9.2 ± 7.5 | 0.23 |
| Change over 2 years for "2 year subjects", n = 9 | 18.6 ± 15.1 | 0.25 |
| Change over 2.5 years for "2.5 year subjects", n = 12 | 8.2 ± 9.8 | 0.42 |

Among the 21 subjects who received at least two years of Agalsidase treatment:

- 10 subjects had improvement in creatinine clearance at the last available data point (mean ± SE change of 45.5 ± 8.3 mL/min or a change of 45 ± 9% from baseline).

- 11 subjects had either no change or a deterioration in creatinine clearance at the last available data point (mean ± SE change of -17.3 ± 5.2 or a change of - 19 ± 4% from baseline).

- The mean change at the last available data point was 12.7 ± 8.4 mL/min (± SE, n = 21. p = 0.15) .

At baseline (immediately prior to Agalsidase), nine subjects had creatinine clearances less than 90 mL/min. Of these nine subjects, three had improvement in the creatinine clearance following two years of Agalsidase while the other six continued to have deteriorations.

The uncontrolled creatinine clearance data from the series of studies following Study TKT003 are difficult to interpret because of the lack of a control group. It is notable that, of all the subjects who received at least two years of treatment, half had improvement in renal function and half had deterioration. It is also especially notable that, among the nine subjects with renal insufficiency within the ≥ 2 year treatment group, two years of Agalsidase treatment resulted in improvement in three subjects, while the other six continued to decline. The GFR data are also interesting to compare to these creatinine clearance results, because, of the three subjects with renal insufficiency (subjects 13, 17 and 18) who appeared to improve after at least two years of Agalsidase treatment, GFR improved in one of two subjects with GFR evaluations (subject 13). Additionally, the marked increase (80 mL/min) in subject 17's creatinine clearance is inconsistent with the GFR result of no change for this subject.

Within Study TKT011, GFR (inulin) was to be measured yearly. Consequently, the interim analysis from Study TKT011 adds one data point to the series of results from the preceding two studies (a total of four data points are available, baseline and end of 003, end of 006 and end of 011). However, eight of the 24 subjects enrolled in Study 011 did not have follow-up GFR values.

Among the 24 subjects with some follow-up GFR data, the available data may be broadly divided into two groups based on duration of Agalsidase exposure: subjects receiving at least two years of Agalsidase and subjects receiving less than two years of Agalsidase. Within Table 21, the baseline value is the value obtained immediately before beginning Agalsidase treatment (nine subjects received 2.5 years Agalsidase and 7 subjects received 2.0 years Agalsidase).

**Table 21. GFR among subjects receiving at least two years Agalsidase (n = 16)**

| Outcome | mL/min; Mean ± SE | p-value |
|---|---|---|
| Baseline, n = 16 | 82.1 ± 6.4 | not applicable |
| 2 years only, n = 7 | 95.1 ± 17.6 | |
| 2.5 years only, n = 9 | 93.1 ± 13.0 | |
| Change over 2 years, n = 7 | 21.1 ± 14.9 | 0.21 |
| Change over 2.5 years, n = 9 | 4.8 ± 9.2 | 0.62 |

Among the 16 subjects who received Agalsidase for at least two years:

- the mean change from baseline to the last available data point was 11.9 ± 8.3 mL/min.

- Nine of the 16 subjects had an improvement in the GFR at the last available data point. The average increase was 33.6 ± 9.2 mL/min ( ± SE, n = 9), a change of 45 ± 14% from the baseline value.

- Seven of the 16 had either no change or a deterioration in the GFR at the last available data point. The average change was - 15.9 ± 4.1 (± SE, n = 7) or a change of - 26 ± 8% from the baseline value.

- Of the nine subjects who had improvement in GFR, creatinine clearance increased during the same time period for five subjects, while deterioration was noted for the other four subjects.

- 10 subjects had baseline GFR < 90 mL/min and of these 10 subjects, six improved (3 after 2 years and 3 after 2.5 years). However, creatinine clearances improved in only two of the six subjects with GFR improvement. The subject with the greatest increase in GFR (subject 7 had an increase of 84 mL/min after 2 years Agalsidase, from 78 to 162) had creatinine clearance values that showed a slight decline over this time period (- 8 mL/min, from 131 to 123).

*Comment: The GFR data are notable for showing similar results as the creatinine clearance data: after Agalsidase treatment approximately half the subjects increase their GFR values and half have decreases in these values. Among the subjects who appear to have increases in GFR after two years of Agalsidase, approximately half the subjects have inconsistent changes in creatinine clearance results over the same time period. Consequently, Study TKT011 creatinine clearance and GFR data fail to provide any robust evidence for a clinical benefit of Agalsidase treatment.*

**((3)) Serum creatinine concentrations and other renal function outcomes in Studies TKT003/006/011:**

There was no statistically significant difference in the changes in serum creatinine between the two treatment groups within Study TKT003. Table 22 shows the serum creatinine values for all patients who completed studies TKT003/006 (excludes placebo subject 20 who failed to complete Study TKT003).

**Table 22. Study TKT003/006 serum creatinine values**

| Group | 003 Baseline mean ± SE | 003 Week 23 mean ± SE | 006 Week 52 mean ± SE |
|---|---|---|---|
| Agalsidase, n = 14 | 1.04 ± 0.06 | 1.07 ± 0.09 | 1.18 ± 0.13 |
| Placebo, n = 11 | 1.26 ± 0.27 | 1.77 ± 0.70 | 1.86 ± 0.77 |

During Study TKT003 no subject had a decrease in serum creatinine of more than 0.1, while 4/14 Agalsidase subjects and 3/12 placebo subjects had increases in serum creatinine of more than 0.1.

Study TKT006 also showed no statistically significant changes in serum creatinine values from baseline for either the prior placebo or Agalsidase group.

The one year interim result from Study TKT011 provides substantial additional follow-up data for the Study TKT003/006 subjects. Notably, there were no remarkable changes (from baseline) in the following outcomes over the series of studies TKT-003-006-011: serum creatinine, BUN, 24 hour urine protein excretion and microalbumin. Change in serum creatinine from pre-Agalsidase to 1 year of TKT011 averaged 0.0 in 12 subjects with 2.5 years exposure and + 0.1 in 9 subjects with 2 years exposure.

Of the 21 subjects who received two or more years of Agalsidase, at the last available data point:

- Twelve subjects had decreases in serum creatinine with an average decline of - 0.18 ± 0.06 mg/dL (Mean ± SE, n = 12) or a change of - 15 ± 6% from baseline.

- Nine subjects had an increase in serum creatinine with an average increase of 1.6 ± 0.3 mg/dL (Mean ± SE, n = 9) or a change of + 26 ± 6% from baseline.

*Comment: Comparing follow-up serum creatinine values to baseline values yields a pattern that is largely consistent with GFR and creatinine clearance data: there generally is little change in the renal function data over the sequential periods of Agalsidase administration. Since most of these time periods involve*

*uncontrolled Agalsidase administration, no inferences can be made to interpret this information. While Fabry disease has a well recognized component of loss of renal function over time, no quantitative predictions of what might have happened to these subjects had they not been receiving Agalsidase can be formed.*

*Therefore, these data do not provide evidence of efficacy for a benefit in renal function.*

**((4)) Natural history of renal function decline:**

*Comment: As part of the May 23, 2002 submitted response to the first Complete Review letter, the sponsor performed a search of the published literature regarding renal function deterioration in Fabry disease. This literature search was performed in order to contrast the findings from Studies TKT003/006/011 to a potential "natural history" of renal functional deterioration. The literature search identified 11 subjects with sufficiently described sequential GFR and/or creatinine clearance values such that an estimate of renal function deterioration could be formulated. An additional publication was also cited (Branton et.al.), whose authors included the NIH clinical site Principal Investigator where TKT003 was conducted. This publication provided renal function data for 14 additional subjects[2]. For completeness, all these findings are reviewed here.*

From the sponsor's published literature search two major renal outcomes were identified:

- the average age of onset of end stage renal disease (ESRD), and
- the average rate of decline in creatinine clearance and/or GFR.

The literature search resulted in identification of 116 Fabry disease patients with age and creatinine clearance or GFR identified. Only few of these patients had sequential measures of renal function described (n = 11) and some had the age of onset of ESRD described (n = 62). The sponsor also notes that other "case series" reports describe a total of approximately 300 Fabry disease patients who have the average age of onset of ESRD identified. The sponsor also cites the Branton publication as providing sequential renal function data for an additional 14 patients.

The summary of published reports suggest that the average yearly creatinine clearance and/or GFR rate of decline is approximately - 21 mL/min among Fabry disease patients (n = 11 patients) and the average age of onset of ESRD is 37 years (n = 62 patients). The separate "case series" reports from approximately 300 Fabry disease patients describe a mean age of ESRD onset from 35 to 43 years. The Branton publication describes an average rate of renal function decline of 12.2 mL/min/year for 14 patients. The sponsor also states that the average change in creatinine clearance in the Study TKT003 placebo group (- 20 mL/min over six months) was somewhat similar to the -21 mL/min/yr derived from the natural history reports. Taken together, the sponsor proposes that these observations suggest a "natural history" average rate of renal function decline of approximately 19 mL/min/year in Fabry disease patients and an average age of 38 years for onset of ESRD. The sponsor contrasts the published literature analysis ("natural history") of an average yearly decline in renal function of 19 mL/min to their clinical study findings that "patients treated with Replagal in Studies TKT003, TKT006 and TKT011 for 2 to 2.5 years have experienced an improvement in renal function."

*Comment: As noted previously, the totality of renal function data generally shows no remarkable change for the Study TKT003/006/011 subjects who completed 2 to 2.5 years of Agalsidase administration (see Table 20). The basis for the sponsor's claim of "improvement in renal function" is apparently based upon comparisons showing modestly numerically higher values of creatinine clearance that do not reach even nominal statistical significance.*

The sponsor notes that by their comparison of the published reports to the average age of subjects enrolled in Study TKT003 (34 years at baseline) and receiving Agalsidase during the series of studies following

[2] Branton, M., Schiffman, R. et al. Natural history of Fabry renal disease: influence of alpha-galactosidase activity and genetic mutations on clinical course. Medicine 2002;81:122-138.

Study TKT003 (003/006/011) they would have expected a number of Agalsidase subjects to have developed ESRD during the total observation period of 2 to 2.5 years. However, only one subject in the sponsor's series of three studies actually developed ESRD--subject 5, a placebo subject from Study TKT003. The sponsor proposes the ESRD age-of-onset comparison suggest a treatment benefit to Agalsidase in the prevention of ESRD.

*Comment: Several aspects of the sponsor's published database review are notable:*

*-Detailed, individual patient data were not provided for the "natural history" Fabry patients. The appropriateness of using assembled natural history data to compare to subjects in a prospective clinical trial cannot be assessed without complete details. These include, but are not limited to, the manner of patient ascertainment, demographic and clinical status information during time of renal data observation and complete individual renal function measurements. None of this information is provided.*

*-The sample size for determination of rate of renal function decline is small. There were only 11 subjects from pooled reports and 14 in the Branton publication. Confidence intervals on the proposed rate of deterioration were not provided, but are likely to be large. There is also a marked difference in the point estimate of deterioration rate between the 11 published patient reports and Branton et.al.*

*-The vast majority of the published data describing the rate of renal function decline were from patients who had advanced renal failure at baseline. Approximately half the subjects in Study TKT003 had normal renal function at baseline and the impairment was generally not profound in those with abnormal function. The baseline renal function status of these subjects was substantially different from those of the published reports. All but three of the 11 subjects within the review of published literature had profound renal impairment at baseline. Notably, all 14 subjects within the Branton report were in chronic renal insufficiency at baseline (defined as a serum creatinine $\geq$ 1.5 mg/dL). Overall, the sponsor's published reports provide little or no information regarding the rate of renal function decline prior to the diagnosis of profound renal impairment.*

*-Although TKT cites an average age of onset of ESRD, it is notable that Branton et.al. explictly state that after examining the question, they were unable to identify a meaningful correlation of renal function decline with age.*

*-The placebo observation period in Study TKT003 was a relatively short 6 months, and this data is pooled with the 11 subjects from the published reports and 14 subjects from Branton, et. al. to form a "historical database." It is important to note that the approximately - 20 mL/min decline in GFR/creatinine clearance among placebo subjects in Study 003 is from only six months of observation and is extrapolated to be sustained for 2 to 3 years. The 11 patient published reports suggested a rate of decline of - 20 mL/min, a rate that appears inconsistent with the cited Study 003 finding of a decline of - 20 mL/min over six months. Perhaps more importantly, as described previously, the change in renal function results for the placebo group are vastly different depending upon whether the Week 23 or Week 24 observation is used. Thus, no reliable estimates can be formed if these data are included.*

*Overall, the inherent publication bias, the lack of detail regarding individual patients, the observation that the baseline renal function in the literature report patients is strikingly different from that of most subjects in Study 003, and the doubtful accuracy of the Study 003 placebo period results, makes any comparisons of the sponsor's uncontrolled agalsidase-treatment renal function data to the "historical" results uninterpretable. .*

**(E) Kidney CTH ($Gb_3$) content:**

Two Study TKT003 secondary endpoints examined the change in kidney content of CTH ($Gb_3$), analyzing the biochemical assay of tissue homogenates. One analysis was a comparison of the change in kidney

CTH ($Gb_3$) content and the other endpoint was a "responder analysis" in which the number of patients with a decrease in the kidney CTH ($Gb_3$) content were compared. Of the 21 subjects who had baseline and week 24 kidney biopsies, one subject (subject number 26, Agalsidase), had uncertain baseline CTH ($Gb_3$) results (trace amounts). Consequently, 20 subjects are included among the analyses of kidney CTH ($Gb_3$) content. Kidney CTH ($Gb_3$) content changes are shown in Table 23.

**Table 23. Change from baseline in kidney CTH ($Gb_3$) content**

| **Outcome** / **Kidney CTH ($Gb_3$) values (nmoles/mg)** | **Agalsidase, n = 11** | **Placebo, n = 9** | **p-value** |
|---|---|---|---|
| Baseline, mean ± SE | 19.53 ± 1.682 | 18.99 ± 3.588 | - |
| Baseline - week 24, mean ± SE | - 3.98 ± 2.169 | - 0.87 ± 1.763 | 0.27* |
| **Responders with Lower CTH n (%)** | 9 (82%) | 4 (44%) | 0.16** |

*ANCOVA with baseline as covariate;**Fisher's exact test

*Comment: These data do not show a statistically significant treatment effect upon the change in kidney CTH ($Gb_3$) content. However, the responder analysis "trends" toward lower CTH($Gb_3$) content within the Agalsidase group.*

**(F) Urine CTH ($Gb_3$) content:**

Two Study TKT003 secondary endpoint analyses examined the impact of the study agent upon 24 hour urine CTH ($Gb_3$) content. These analyses included a comparison of urine CTH ($Gb_3$) content at various time points and a "responder" analysis in which the proportion of patients with a decrease in urine CTH ($Gb_3$) (from baseline) were compared between the two groups. These results are shown in Tables 24.

All analyses of urine, plasma and kidney CTH ($Gb_3$) content were performed with blinding to subject treatment assignment and the designated subject number. The baseline value is the mean of values from the two baseline evaluations and the values at week 24 are the mean of week 23 and 24 values.

**Table 24. Urine sediment CTH ($Gb_3$) (nmole/gm creatinine)**

| **Outcome** | **Baseline** | **Week 9** | **Week 17** | **Week 24** | **p-value between groups** |
|---|---|---|---|---|---|
| ***CTH ($Gb_3$) content*** | | | | | |
| Agalsidase, n = 14 mean ± SE | 2369 ± 308 | 737 ± 176 | 1534 ± 337 | 1683 ± 443 | Repeated measures (ANOVA) = 0.09; Week 24 - baseline ANCOVA = 0.05 |
| Placebo, n = 12 mean ± SE | 2162 ± 383 | 2618 ± 438 | 2118 ± 317 | 2494 ± 553 | |
| ***Responder analysis*** | | | | | |
| n (%) responders Agalsidase, n = 14, | - | 14 (100%) | 12 (86%) | 12 (86%) | p-values shown in last row - |
| n (%) responders Placebo, n = 12 | - | 6 (50%) | 5 (42%) | 4 (33%) | |
| p-value* | - | < 0.01 | 0.09 | 0.02 | |

*Fisher's exact test

*Comment: There was a large variation in the urinary CTH ($Gb_3$) content. There appears to have been a decrease in urine CTH ($Gb_3$) excretion at week 9 for the Agalsidase group. It is notable that subsequent Agalsidase group urine CTH ($Gb_3$) values increased from the week 9 nadir. Nevertheless, there is evidence of a bioactivity effect upon urinary CTH ($Gb_3$) excretion.*

**(6) Tertiary and exploratory endpoints:**

These numerous analyses were given low emphasis in the prospective plans, and some use several analytic methods for a single type of data. Therefore, nominal p-values require careful interpretation.

**(A) Other renal laboratory results:**

There were no statistically significant differences (or notable trends) between the two study groups for changes in BUN, 24 hour protein excretion, microalbumin, and urine specific gravity. There was also no difference in these results using "responder" analyses. These data are not repeated here.

**(B) Tertiary and exploratory endpoints related to "reduce pain:"**

**1. Neuropathic Pain Scale (NPS) results:**

The NPS was administered at the two baseline evaluations, then at weeks 1, 5, 9, 13, 17, 21 and 24 (a total of 9 evaluations). The NPS is a series of 10 questions in which patients rate the "intensity" of their pain as well as certain characteristics (the quality) of the pain. Rather than a rating encompassing any specific prior period of time, the NPS requests a generic assessment of "your pain" at the time of the rating. The scales range from 0--no pain (or not sharp, not hot, not dull, not cold, not sensitive, not itchy, not unpleasant, no deep pain, no surface pain) to 10--with 10 being the most intense pain response. The tertiary endpoint in the Statistical Analytical Plan stated that the "scales of the NPS will be compared for each patient." The outcomes at weeks 9, 17 and 24 were to be compared to baseline and "responder" analyses performed.

The NPS was not administered concurrently with the BPI at the time of "off pain medication" assessments. Consequently, there are no "off pain medication" NPS measurements which correspond to the "off pain medication" BPI measurements used in the primary endpoint analysis.

Of these 10 assessments, 5 show larger decline in mean score from baseline to Week 24 for the Agalsidase group than the placebo group, 3 show larger declines in the placebo group, and 2 (nearly) no difference. Only 3 of these have nominal p-values less than 0.06 on a repeated measures analysis. A table of the results is shown in the appendix (Appendix D).

*Comment: The NPS data consist of multiple questions and no single overall score. The finding of positive statistical results ($p < 0.05$) from only two of the 10 questions highlights the no apparent treatment effect conclusion from these data. For the most part, the NPS scores confirm the primary endpoint's finding of no significant difference between the two study groups.*

**2. Quantitative sensory testing:**

Quantitative sensory testing included evaluations of the threshold for vibration, cold, and warmth sensation. The change between baseline and week 24 was compared for each patient. There were no significant differences between treatment groups and these data are not repeated here.

**C) Tertiary and exploratory endpoints related to "reduce cardiac enlargement and improve cardiac function:"**

In Study TKT003, cardiac changes were to be evaluated by changes in three outcomes--magnetic resonance (MRI) measurements, echocardiographic measurements and electrocardiographic changes.

In general, Fabry disease is thought to be associated with increases in cardiac mass over time, such that a decrease in cardiac mass might represent a favorable response. There are two (relatively) common methods for assessing cardiac mass: MRI and echocardiography.

The presence of cardiac disease was not a requirement for study eligibility and no subject had signs or symptoms of cardiac disease. Nevertheless, baseline abnormalities of increased echocardiographic estimates of left ventricular mass/$m^2$ were detected in seven Agalsidase subjects and six placebo subjects.

Follow-up was incomplete for one of these, so data from subjects with echocardiographic evidence of left ventricular muscle enlargement are limited to 12 subjects. Other missing data include the following:

-One Agalsidase subject failed to have a baseline cardiac MRI determination of end systolic mass.

-Although the Statistical Analytical Plan stated that left ventricular posterior wall thickness as assessed by MRI would be analyzed, these data were not obtained in the study.

**1. MRI results of left ventricular wall thickness, cardiac mass, left ventricular mass, T1 and T2 signal assessments:**

Overall MRI assessments of left ventricular mass revealed similar results for the two groups, with no apparent treatment associated effect. A subset of subjects based on those who showed increased LV mass at baseline (by echocardiography, not MRI) was also examined (5 - 7 subjects per group). This subset also failed to show significant treatment associated effects (see Appendix D).

**2. Echocardiographic results.**

Echocardiography was used to examine left ventricular mass/$m^2$, aortic root diameter, interventricular septum in diastole, and posterior wall thickness in diastole.

As with the MRI data, the echocardiographic average values of the left ventricular mass/$m^2$, aortic root diameter, interventricular septal diastolic thickness and posterior wall diastolic thickness were similar for the two groups and were all within the normal value range for these measures. However, the mean results all tend to favor the placebo group. While raising the possibility of Agalsidase administration association with cardiac hypertrophy, the totality of the cardiac muscle mass data (MRI and echocardiography) suggest there is no evidence of a treatment associated effect (see Appendix D).

The subset of subjects with increased LV mass at baseline again failed to show any different effects based on echocardiographic outcomes.

**3. Electrocardiographic results:**

The Statistical Analytical Plan stated that electrocardiographic results would be used to "correlate measurements of changes in cardiac hypertrophy as assessed by the imaging methods." Conduction system measurements (QRS) duration were also to be recorded. The change from baseline to week 24 was specified as the time points to be analyzed in these analyses.

Since the imaging methods showed similar findings between the two groups, it was not meaningful to correlate these results to the electrocardiographic findings of left ventricular hypertrophy.

Only 7 of the 26 randomized subjects had a week 24 electrocardiograph performed. Consequently, in a post-hoc alteration of this exploratory endpoint, the outcome was assessed with substantial amounts of missing data imputation (using the latest electrocardiogram obtained among week 21, 23 or week 24 as the imputed value). Mean QRS duration change from baseline was - 2.4 in the Agalsidase group and + 3.6 in the placebo group, $p = 0.05$. However, this result is not robust and contingent upon one single value--subject number 26 (Agalsidase) had an intermittent right bundle branch block, and very variable QRS duration is recorded for this subject (see Appendix D).

Overall, the data do not suggest a treatment effect of Agalsidase upon cardiac outcomes.

**(D) Tertiary endpoints and exploratory endpoints related to "increase weight:"**

Of the 26 study subjects, three subjects (one Agalsidase and two placebo subjects) did not have week 24 weights measured. Consequently, the weight change analysis was performed among the subset of subjects with available baseline and week 24 weight data (n = 23). While the change from baseline to week 24 results suggested a possible treatment associated effect (+ 1.6 Agalsidase, - 1.4 placebo, p = 0.03), the repeated measures analysis incorporating data from Week 9 and 17 along with Week 24 showed no trend for a treatment effect.

Irrespective of the numeric results, however, the underlying data are difficult to interpret. Review of the detailed study data suggests that in some of these subjects changes in body fluid mass may have contributed substantially to the observed changes. Body mass index or other nutritional status data were not recorded during the study. Thus, interpretation of these data is difficult at best.

**(E) Additional tertiary endpoint data:**

**Plasma CTH ($Gb_3$) content:**

Consistent with what was observed in Study TKT001, plasma CTH(Gb3) was decreased by the study agent throughout several study time points.

**Analyses of Cerebrovascular blood flow:**

Controlled clinical study data evaluating cerebrovascular blood flow were obtained within Study 003. The sponsor's analyses of these data show no treatment effect associated with Agalsidase administration, as shown below.

**Table ZZ. Change in Cerebral PET measures of resting global cerebral blood flow**

| Outcome | Agalsidase, n = 14 | Placebo, n = 11 | p-value* |
|---|---|---|---|
| Baseline value | 43.2 ± 0.7 | 41.7 ± 1.6 | not applicable |
| Change to end of study | 0.50 ± 1.3 | 2.1 ± 2.1 | 0.88 |

*ANCOVA with baseline as covariate
Blood flow presented as mL/min per 100 g

Cerebrovascular blood flow was also measured in Study 003 via transcranial doppler of numerous blood vessels. Specifically, the following vessels were assessed: anterior cerebral arteries, middle cerebral arteries, internal carotid arteries, ophthalmic arteries, posterior cerebral arteries. The flow velocity and pulsatility indices were measured. Overall, the sponsor's analyses of the transcranial dopper findings showed no treatment effect upon cerebrovascular blood flow.

*Comment: Two publications submitted to the BLA report that Agalsidase treatment results in improved cerebral blood flow. These findings cannot be verified based upon the data submitted to the BLA. The BLA data provide no evidence of a treatment effect upon cerebral blood flow.*

**(7). Summary of secondary, tertiary and exploratory endpoint results:**

As the prior text noted, there was a very large number of secondary and tertiary endpoints. Tables 25 and 26 attempt to summarize these endpoints. The shading is by similar types of endpoints and solely to facilitate reading the table.

**Table 25. Summary of secondary endpoint results**

| Parameter | Method | Time points | Agal'dase | Placebo | Stat test | P value |
|---|---|---|---|---|---|---|
| **"Pain related" (n = 14 for Agalsidase and n = 12 for Placebo)** | | | | | | |
| 1. Severity items, off pain meds | AUC, Chg fr Bsln | Wks 9, 17, 24 | - 14.3 ± 5.3 | - 6.2 ± 10.2 | t-test<br>ANCOVA | 0.47<br>0.39 |
| 2. Severity items, off pain meds | % not worsened | Wk 9 | 71% | 42% | Fisher's x | 0.23 |
| | | Wk 17 | 71% | 50% | Fisher's x | 0.42 |
| | | Wk 24 | 64% | 50% | Fisher's x | 0.69 |
| 3. Severity items, off pain meds | Re Meas Chg fr Bsln | Wks 9, 17, 24 | See Table 59 | | ANCOVA<br>ANOVA | 0.40<br>0.02* |
| 4. Severity items, all visits | AUC, Chg fr Bsln | All visits | - 9.6 ± 7.8 | - 21.6 ± 12.3 | t-test<br>ANCOVA | 0.41<br>0.95 |
| 5. Severity items, all visits | Re Meas Chg fr Bsln | All visits | See Table 59 | | ANCOVA<br>ANOVA | 0.70<br>0.28 |
| 6. Interference items, off pain m | AUC, Chg fr Bsln | Wks 9, 17, 24 | - 6.9 ± 9.4 | - 9.1 ± 9.7 | t-test<br>ANCOVA | 0.88<br>0.58 |
| 7. Interference items, off pain m | % not worsened | Wk 9 | 50% | 50% | Fisher's x | 1.00 |
| | | Wk 17 | 64% | 50% | Fisher's x | 0.69 |
| | | Wk 24 | 64% | 50% | Fisher's x | 0.69 |
| 8. Interference items, off pain m | Re Meas Chg fr Bsln | Wks 9, 17, 24 | See Table 60 | | ANCOVA<br>ANOVA | 0.22<br>0.05* |
| 9. Interference items, all visits | AUC, Chg fr Bsln | All visits | - 20.1 ± 7.1 | - 34.3 ± 13.1 | t-test<br>ANCOVA | 0.33<br>0.81 |
| 10. Interference items, all visits | Re Meas Chg fr Bsln | All visits | See Table 60 | | ANCOVA<br>ANOVA | 0.46<br>0.35* |
| 11. Worst pain, off pain meds | Scores at week 24 | Wk 24 | 4.3 ± 0.7 | 6.8 ± 0.6 | ANCOVA | 0.05 |
| 12. Worst pain, off pain meds | Ch fr Bsln to wk 24 | Wk 24 | - 1.9 ± 0.5 | - 0.4 ± 0.9 | t-test<br>ANCOVA | 0.14<br>0.05* |
| 13. Pain med D/C | Time to event | | 30.5 ± 10.2 | 0 | log-rank | 0.03 |
| 14. Pain med | Days off pain med | | 93.5 ± 20.2 | 25.4 ± 13.7 | t-test | 0.01 |
| **"Renal Structure" (n = 11 for Agalsidase, n = 9 for placebo)** | | | | | | |
| 15. Active lipid damage score | Ch fr Bsln | Wk 24 | -1.5 ± 0.8 | 0.9 ± 0.9 | ANCOVA | 0.11 |
| 16. Improved active damage sc | improved, % all 26 pts | Wk 24 | 57% | 25% | Fisher's x | 0.12 |
| 17. Chronic lipid damage score | Ch fr Bsln | Wk 24 | 0.1 ± 0.7 | 0.4 ± 1.6 | ANCOVA | 0.81 |
| 18. Improved Chronic dama sc | improved % all 26 pts | Wk 24 | 36% | 33% | Fisher's x | 1.00 |
| **"Kidney CTH content" (n = 11 for Agalsidase, n = 9 for placebo)** | | | | | | |
| 19. Kidney content | Ch fr Bsln | Wk 24 | - 4.0 ± 2.2 | - 0.9 ± 1.8 | ANCOVA | 0.27 |
| 20. Lower content "Responders" | % improved | Wk | 82% | 44% | Fisher's x | 0.16 |
| **"Urine CTH content" (n = 14 for Agalsidase, n = 12 for placebo)** | | | | | | |
| 21. Urine CTH | Re Meas Chg fr Bsln | Wks 9, 17, 24 | See Table 24 | | ANOVA<br>ANCOVA | 0.09<br>0.05 |
| 22. Lower urine CTH content "Responders" | % improved | Wk 9 | 100% | 50% | Fisher's x | < 0.01 |
| | | Wk 17 | 86% | 42% | Fisher's x | 0.09 |
| | | Wk 24 | 86% | 33% | Fisher's x | 0.02 |

*post-hoc analyses;
Re Meas Chg fr Bsln = repeated measures analysis for change from baseline
Fisher's x = Fisher's exact test; ANCOVA utilized baseline value as covariate
Ch fr Bsln = change from baseline

**Table 26. Summary of tertiary endpoint results**

| Parameter | Method | Time points | Agalsidase | Placebo | Stat test | P Value |
|---|---|---|---|---|---|---|
| **Renal function (n = 14 for Agalsidase, n = 12 for placebo, unless otherwise noted)** | | | | | | |
| 1. Cr Cl | Ch fr Bsln | W 24 | - 0.1 ± 5.9 | - 19.7 ± 9.1 | ANCOVA | 0.05 |
| 2.Improv Cr Cl "Responders" | % improved | Wk 9 | 57% | 17% | Fisher's x | 0.10 |
| | | Wk 17 | 50% | 17% | Fisher's x | 0.09 |
| | | Wk 24 | 57% | 25% | Fisher's x | 0.23 |
| 3.GFR *A n=14, pl n= 10* | Ch fr Bsln | Wk 24 | - 8.8 ± 3.8 | - 19.8 ± 7.9 | ANCOVA | 0.65 |
| 4. RPF *A n=11, pl n=7* | Ch fr Bsln | Wk 24 | - 49.4 ± 28.3 | - 83.8 ± 32.2 | ANCOVA | 0.39 |
| **Neuropathic pain scale results (n = 14 for Agalsidase, n = 11 for placebo)** | | | | | | |
| 5. Intensity | Ch fr Bsln | Wk 24 | - 2.4 ± 1.0 | - 1.4 ± 0.7 | ANOVA | 0.04 |
| 6. Sharpness | Ch fr Bsln | Wk 24 | - 1.4 ± 1.1 | - 0.8 ± 0.4 | ANOVA | 0.03 |
| 7. Hotness | Ch fr Bsln | Wk 24 | - 3.7 ± 1.0 | - 1.9 ± 0.7 | ANOVA | 0.18 |
| 8. Dullness | Ch fr Bsln | Wk 24 | - 2.2 ± 0.8 | - 3.0 ± 1.5 | ANOVA | 0.57 |
| 9. Coldness | Ch fr Bsln | Wk 24 | 0.0 ± 0.5 | - 1.6 ± 0.8 | ANOVA | 0.90 |
| 10. Sensitivity | Ch fr Bsln | Wk 24 | - 1.6 ± 0.6 | 0.5 ± 1.0 | ANOVA | 0.09 |
| 11. Itchiness | Ch fr Bsln | Wk 24 | - 0.1 ± 0.3 | 0.0 ± 0.3 | ANOVA | 0.85 |
| 12. Unpleasant | Ch fr Bsln | Wk 24 | - 1.8 ± 1.0 | - 1.7 ± 0.8 | ANOVA | 0.11 |
| 13. Deep pain | Ch fr Bsln | Wk 24 | - 1.6 ± 1.0 | - 2.2 ± 0.9 | ANOVA | 0.30 |
| 14. Surface pain | Ch fr Bsln | Wk 24 | - 2.5 ± 0.5 | - 1.5 ± 0.9 | ANOVA | 0.06 |
| **Cardiac data (n as noted)** | | | | | | |
| ***MRI*** | | | | | | |
| 15. LVED mass | Ch fr Bsln | Wk 24 | 3.5 ± 2.7 n = 14 | 4.1 ± 5.7 n = 11 | ANCOVA | 0.93 |
| 16. LVES mass | Ch fr Bsln | Wk 24 | 6.2 ± 4.0 n = 13 | - 2.2 ± 6.4 n = 11 | ANCOVA | 0.25 |
| 17. T1 | Ch fr Bsln | Wk 24 | 13.1 ± 59.1 n = 14 | - 39.5 ± 38.3 n = 11 | ANCOVA | 0.57 |
| 18. T2 | Ch fr Bsln | Wk 24 | 0.8 ± 1.9 n = 13 | 1.5 ± 1.8 n = 11 | ANCOVA | 0.89 |
| ***Echocardiography*** | | | | | | |
| 19. LV mass/$m^2$ | Ch fr Bsln | Wk 24 | 13.9 ± 4.2 n = 14 | - 7.9 ± 13.2 n = 11 | ANCOVA | 0.06 |
| 20. D LV septum | Ch fr Bsln | Wk 24 | 0.4 ± 0.3 n = 13 | - 0.9 ± 0.7 n = 11 | ANCOVA | 0.03 |
| 21. D LV p wall | Ch fr Bsln | Wk 24 | 0.4 ± 0.3 n = 14 | - 1.1 ± 0.7 | ANCOVA | 0.07 |
| 22. Ao root diam | Ch fr Bsln | Wk 24 | 0.9 ± 1.1 n = 14 | - 2.5 ± 1.5 n = 11 | ANCOVA | 0.49 |
| ***Electrocardiography, n = 14 for Agalsidase, n = 12 for placebo*** | | | | | | |
| 23. QRS duration | Ch fr Bsln | Wk 24 | - 2.4 ± 3.9 | 3.6 ± 1.2 | ANCOVA | 0.05 |
| ***Weight (n = 13 for Agalsidase, n = 10 for placebo)*** | | | | | | |
| 24. Wgt change | Ch fr Bsln | Wk 24 | 1.6 ± 0.6 | - 1.4 ± 1.3 | ANCOVA | 0.03 |
| ***Plasma CTH content (n = 14 for Agalsidase, n = 11 for placebo)*** | | | | | | |
| 25. CTH content | Ch fr Bsln, Repeat Mes | Wk 9 | - 5.8 ± 0.8 | 0.2 ± 0.6 | ANOVA | 0.01 |
| | | Wk 17 | - 6.4 ± 0.8 | - 1.9 ± 0.8 | | |
| | | Wk 24 | - 7.0 ± 0.8 | - 0.6 ± 0.7 | | |

A = Agalsidase; pl = placebo; D = diastolic; Ao root diam = aortic root diameter; Wgt = weight; LVED = left ventricular end-diastolic; LVES = left ventricular end systolic; Improv Cr Cl= improvement in creatinine clearance; other abbreviations as in Table 25.

Table 25 excludes the secondary endpoints which the sponsor concurs as clearly showing no difference between the two groups (quantitative sensory testing, serum creatinine, other urine chemistry).

*Comment: The secondary and tertiary endpoint summary tables illustrate the very large number of endpoint analyses--indeed, the tables include only the most "important" outcomes. Inherent in such a large number of analyses is the potential meaninglessness of the nominally significant p-values--due to multiplicity concerns.*

**(8). Safety:**

Adverse events were summarized by treatment group and frequency, the severity and relationship to study drug tabulated and the outcomes compared by the treatment group.

**A. Adverse events:**

There were no withdrawals due to an adverse event. There were no deaths during the study.

Overall, 657 WHOART preferred term adverse events were reported among the 26 subjects in the study, with all subjects experiencing at least one adverse event. The adverse events which occurred in a higher proportion of the Agalsidase group than the placebo group are shown in Table 27.

**Table 27. Adverse events occurring in a higher proportion of Agalsidase subjects**

| Adverse event | Agalsidase, n = 14 | Placebo, n = 12 |
|---|---|---|
| Headache | 13 (92.5%) | 9 (75.0%) |
| Rigors | 9 (64.3%) | 2 (16.7%) |
| Allergic reaction | 7 (50.0%) | 3 (25.0%) |
| Fever | 7 (50.0%) | 4 (33.3%) |
| Diarrhea | 7 (50.0%) | 4 (33.3%) |
| Peripheral edema | 6 (42.9%) | 5 (41.7%) |
| Influenza-like syndrome | 6 (42.9%) | 5 (41.7%) |
| Arthralgia | 5 (35.7%) | 3 (25.0%) |
| Skeletal pain | 5 (35.7%) | 1 (8.3%) |
| Chest pain | 4 (28.6%) | 2 (16.7%) |
| Increased sweating | 4 (28.6%) | 3 (25.0%) |
| Erythematous rash | 4 (28.6%) | 0 |
| Infection | 4 (28.6%) | 3 (25.0%) |
| Rhinitis | 3 (21.4%) | 2 (16.7%) |
| Hearing decreased | 3 (21.4%) | 2 (16.7%) |
| Sinusitis | 3 (21.4%) | 2 (16.7%) |
| Inflicted injury | 3 (21.4%) | 1 (8.3%) |
| Upper respiratory tract infection | 3 (21.4%) | 2 (16.7%) |
| Flushing | 3 (21.4%) | 0 |
| Eye abnormality | 2 (14.3%) | 0 |
| Skin discoloration | 2 (14.3%) | 0 |
| Periorbital edema | 2 (14.3%) | 0 |
| Anxiety | 2 (14.3%) | 1 (8.3%) |
| Emotional lability | 2 (14.3%) | 0 |
| Insomnia | 2 (14.3%) | 1 (8.3%) |
| Nervousness | 2 (14.3%) | 1 (8.3%) |
| Somnolence | 2 (14.3%) | 0 |
| Tinnitus | 2 (14.3%) | 1 (8.3%) |
| Dyspepsia | 2 (14.3%) | 1 (8.3%) |
| Melena | 2 (14.3%) | 1 (8.3%) |
| Hyperkinesia | 2 (14.3%) | 0 |
| Involuntary muscle contractions | 2 (14.3%) | 1 (8.3%) |
| Bronchospasm | 2 (14.3%) | 0 |
| Neuralgia | 2 (14.3%) | 1 (8.3%) |
| Constipation | 2 (14.3%) | 1 (8.3%) |
| Allergy | 2 (14.3%) | 1 (8.3%) |

*Comment: The profile of adverse events is especially notable for what appears to be higher numbers of "allergic-type" reactions among the Agalsidase group--rash, bronchospasm, skeletal muscle pain, arthralgia, flushing and fever--events which may have an immunologic basis.*

None of the preferred term adverse events were graded as life-threatening by the site investigator. However, 33 of the adverse events were graded as severe: five adverse events in the Agalsidase group (2 subjects) and 28 adverse events in the placebo group (five subjects). The large number of severe adverse events in the placebo group were skewed by the development of renal failure in subject number 5 (a subject who experienced many severe adverse events).

The severe adverse events among the two subjects in the Agalsidase group consisted of allergic reactions in subject 16, headache in subject 4 and among the five subjects in the placebo group consisted of the following: subject 5 experienced renal failure, tremor, pain and myalgia, subject 1 experienced headache, pain and abnormal vision, subject 3 experienced pain and dysaesthesia, subject 14 experienced syncope and subject 22 experienced paresthesia and pain.

Of the 657 preferred term adverse events, 18 were graded by the site investigator as "probably related to the study drug" while none of the placebo adverse events were graded as "probably related." Three of the adverse events within the Agalsidase group were graded as "unknown" relationship to study drug, while 11 adverse events in the placebo group were recorded as "unknown" relationship.

There were 10 serious adverse events (SAE) reported during the study (serious adverse events occurring to the same patient on the same day being counted as a single serious adverse event). The 10 SAEs occurred among six subjects (four Agalsidase subjects with seven SAE and two placebo subjects with three SAE). These SAEs are listed in Table 28.

**Table 28. Serious Adverse Events (SAE)**

| Group | Subject number | SAE |
|---|---|---|
| **Agalsidase:** | | |
| | 16 | Post renal biopsy bleeding (February 14, 1999) |
| | 16 | Infusion reaction (May 25, 1999) |
| | 16 | Chest pain (June 13, 1999) |
| | 18 | Infusion reaction (April 1, 1999) |
| | 21 | Bilateral hearing loss (August 4, 1999) |
| | 27 | Anemia (March 19, 1999) |
| | 27 | Fever (March 23, 1999) |
| **Placebo:** | | |
| | 5 | Renal failure (March 5, 1999) |
| | 5 | Constipation (May 7, 1999) |
| | 22 | Fever, nausea, abdominal pain, diarrhea (April 1, 1999) |

The infusion reaction in Agalsidase subject number 16 was graded as "probably related" by the site investigator and the infusion reaction in Agalsidase subject number 18 was graded as "possibly related" by the site investigator. The fever in Agalsidase subject number 27 was also graded as "possibly related" by the site investigator. Other SAEs were graded as "not related" by the site investigator.

Infusion reactions were common during the study. A total of eight subjects treated with Agalsidase reported at least one infusion-related adverse event. These reactions all occurred after a minimum of three Agalsidase infusions (generally between weeks 7 to 17). These reactions consisted of various combinations of rigors, facial flushing, throat tightness, and eyelid edema. These infusion reactions prompted a cessation of dosing and a safety review. Although the sponsor proposes that these were not immune mediated infusion reactions, the sponsor notes that all eight subjects who experienced infusion reactions were able to continue the study drug infusions with diphenhydramine and steroid premedication.

The sponsor also notes that lengthening the infusion period from 20 to 40 minutes appears to have lessened the number of infusion reactions.

There were no remarkable alterations in clinical chemistry or hematology among comparisons of the two treatment groups.

**B. Antibody development:**

The sponsor performed four different assays in order to assess antibody formation: antibody detection by ELISA in which Agalsidase was the capture antigen, an immunoprecipitation assay, an assay in which neutralization of Agalsidase enzymatic activity was monitored and an assay that assessed the "internalization" of Agalsidase into human foreskin fibroblasts.

Blood samples for antibody formation were obtained at baseline and weeks 9, 17 and 24. The sponsor reports, that, of the 14 Agalsidase subjects, nine (64%) were positive by the immunoprecipitation assay, eight (57%) were positive by the neutralization assay and three (21%) were positive by the enzyme immunoassay (ELISA). Antibody positivity was defined (post-hoc) by a 300% increase in absorbance over baseline and neutralization was defined (post-hoc) as a 50% decrease in enzyme activity over baseline. Internalization data were incomplete for 12 subjects, largely precluding a meaningful interpretation of these data.

**Table 29. Antibody results**

| Assay | Agalsidase, n = 14 | Placebo, n = 12 |
|---|---|---|
| **ELISA** | | |
| *Subjects with any absorbance increase over baseline* | 13 (93%) | 6 (50%) |
| *Subjects with increase in absorbance more than 2 SD above placebo mean OD** | 8 (57%) | 1 (8.3%) |
| **Neutralization** | | |
| *Subjects with 50% inhibition of enzyme activity* | 7 (50%) | 0 |
| **Immunoprecipitation** | | |
| Number of patients with any immunoprecipitation** | 9 (64%) | 0 |

*> 85% increase in absorbance (study 003 + 005 result)

** all titers were either 1:2 or 1:20

Table 30 summarizes the results of the immunoreactivity and infusion reaction data for the Agalsidase subjects. This table lists all the subjects who had at least one of: an infusion reaction, an increase in ELISA absorbance of > 85%, a 50% neutralization result or a positive immunoprecipitation result (the table highlights the subjects with infusion reactions).

**Table 30. Agalsidase subjects with at least one of the following: infusion reaction, positive ELISA, positive immunoprecipitation or positive neutralization results.**

| *Subject* | *Agalsidase Lot number* | *50% neutralization* | *IP* | *% increase absorbance (ELISA)* | *Infusion reaction* |
|---|---|---|---|---|---|
| *2* | *GA8J001* | *yes* | *1:20* | *95%* | *yes* |
| *4* | *GA8J001, GA8K001* | *no* | *1:2* | *115%* | *yes* |
| *8* | *GA8J001, GA8K001* | *no* | *none* | *0* | *yes* |
| *10* | *GA8J001, GA8K001* | *yes* | *1:20* | *114%* | *no* |
| *12* | *GA8J001, GA8K001* | *yes* | *none* | *0* | *no* |
| *16* | *GA8J001, GA8K001* | *no* | *1:2* | *41%* | *yes* |
| *18* | *GA8J001* | *yes* | *1:20* | *290%* | *yes* |
| *19* | *GA8J001, GA8K001* | *yes* | *1:20* | *292%* | *no* |
| *21* | *GA8J001* | *yes* | *1:10* | *158%* | *yes* |
| *23* | *GA8J001, GA8K001, GA9E001* | *yes* | *1:20* | *427%* | *yes* |
| *27* | *GA8J001, GA8K001, GA9E001* | *yes* | *1:20* | *750%* | *yes* |

*Comment: In general, there appears to be some correlation between infusion reactions and immunoreactivity assays in that, of the eight subjects with infusion reactions, only one (subject number 8) had negative immunoprecipitation, ELISA and neutralization results.*

*Overall, there appears to be a relatively high incidence of antibody formation (approximately 50%). However, this clinical study was too brief to assess much of the clinical impact of this antibody formation. There were allergic-type reactions to the product which largely resolved with a premedication regimen and lenghthening the duration of the infusion from 20 to 40 minutes. The contribution of antibodies versus the impact of the infusion duration upon the occurrence of the allergic/infusion reactions is unclear. These allergic/infusion reactions appear to be the most significant safety concern with the product. Additionally, the potential for antibodies to interfere with enzyme activity over longer periods of time remains a concern.*

**E. Summary of Study TKT003:**

Study TKT003 provides most of the controlled clinical data assessing the safety and efficacy of Agalsidase. The study used a randomized, double-blind, placebo-controlled design. The Agalsidase dose was 0.2 mg/kg administered IV every two weeks. The study's primary endpoint was a comparison of the change from baseline in the area-under-the-curve of "worst pain" while "off pain medication" as assessed by the use of a pain scale (the BPI). During the study, subjects were to have certain pain medications discontinued and the BPI completed at certain sequential time points. The pain medications could be resumed following completion of the "off pain medication" pain scores. There were a large number of secondary and tertiary endpoints, the most notable focusing upon changes from baseline in various other components of the pain scale results, renal function outcomes (creatinine clearance and GFR) and renal histopathology (as assessed from a baseline and end-of-study renal biopsy).

Within the study, 26 adult male Fabry disease subjects were randomized (14 to Agalsidase, 12 to placebo) and 25 subjects completed the six month study. One subject (a placebo subject) withdrew consent and withdrew from the study after week 20.

This study was reviewed within the Original Submission of the BLA, and FDA comments included in the Complete Response letter to TKT. FDA noted that the study's primary endpoint results showed no statistically significant difference between the Agalsidase and placebo groups (the sponsor's p-value assessment reported as 0.20). The study results disclosed multiple problems with the primary endpoint data procedures such that it appears impossible to verify the primary endpoint result. In general, these problems related to inconsistencies within the study source documents (especially those related to determination of when a subject was "off pain medication"), lack of explicit prospective plans for determining the primary endpoint data points and a notable alteration of the primary endpoint database following a preliminary analysis of the primary endpoint outcome. Exploratory analyses of the primary endpoint generally supported the finding of no difference between the two study groups.

The secondary endpoints related to other pain scale outcomes generally showed no statistically significant difference between the two study groups (differences in pain "severity" and "interference" of pain with activities). Thus, this study does not provide substantial evidence supporting a conclusion of reduction in pain with Agalsidase treatment.

The secondary endpoints related to renal histopathology showed no statistically significant difference between the two study groups for the two prospectively defined composite scores (ALDS and CDS) of pathological change. Exploratory analyses of certain components of these scores suggested that Agalsidase administration was associated with decreased CTH ($Gb_3$) deposition within the vascular endothelium (but not certain other cellular components of the biopsies). The histopathology data were noted to be of limited utility because of a lack of rigor in ascertainment of the data. There was substantial lack of explicit prospective plans for biopsy slide interpretation such that the investigators generated ad-hoc procedures during the study. Additionally, the source data records for histopathology results are missing.

FDA concludes that these data are insufficient to reach a conclusion that Agalsidase alfa has demonstrated an effect on renal structure.

The secondary/tertiary endpoints related to renal function mainly consisted of analyses in creatinine clearance and GFR changes. The creatinine clearance data are especially notable for a substantial decline in the reported average values for a single time point within the placebo group, the week 24 data point (as compared to the group's week 23 data point). This is notable because the statistical comparison of change from baseline to end-of-study in creatinine clearance showed a p-value of 0.05, with an average decline in creatinine clearance of approximately 20 mL/min for the placebo group and generally no change for the Agalsidase group. The apparent aberrancy of the placebo group's week 24 data point, combined with the lack of robustness to the statistical analysis (the nominal p-value of 0.05 may be substantially altered by an assessment of "no change" for one subject) limits the ability to assign substantial meaningfulness to an apparent decline in creatinine clearance by the placebo group and minimal change in the Agalsidase group.

The GFR data showed no statistically significant difference between the two study groups.

Other renal function data (serum creatinine, BUN, 24 hour urine protein) also showed no statistically significant difference between the two study groups.

The secondary endpoints also included assessment of cardiac structure by MRI and echocardiography. In general, there was no difference between the two study group in these various outcomes (LV mass, septal size, etc.). Electrocardiographic results suggested that the Agalsidase group had a decline in the QRS duration although this was not a robust finding in that the result appeared related to a subject with intermittent bundle branch block.

Comparisons of weight changes (a tertiary endpoint) suggested the Agalsidase group (on average) gained weight while the placebo group (on average) lost weight. However, the use of steroids within the Agalsidase group, combined with other concomitant medications made interpretation of this finding unclear. Additionally, the meaningfulness of the apparent weight change was noted to be unclear since no nutritional data were assessed.

Two of the study's main bioactivity markers of CTH ($Gb_3$) content (urine sediment content and plasma concentration) generally showed decreased values from baseline to end-of-study for the Agalsidase group compared to the placebo group. However, there was no difference between the two study groups in the measures of kidney biopsy CTH ($Gb_3$) content.

The main safety findings from the study were within the Agalsidase group with the most notable findings being a 57% incidence of infusion reaction and an approximately 50% incidence of antibody formation. During the study, the infusion time was lengthened from 20 to 40 minutes and a prophylactic regimen of steroids/antihistamines was used in an attempt to decrease the infusion reaction incidence. In general, these measures appeared to lessen the frequency and severity of the infusion reactions.

The high rate of antibody formation has the potential to impact pharmacokinetics and pharmacodynamics, and consequently may impact efficacy. These are further evaluated in extension study TKT006.

## 12. Study TKT005:

### A. Overview:

The focus of this second controlled study was an assessment of the impact of Agalsidase upon cardiac structure and function. The randomized, double-blind study enrolled 15 adult Fabry disease subjects with echocardiographic evidence of left ventricular hypertrophy. The dose regimen for this study (0.2 mg/kg every other week for 24 weeks) was identical to Study TKT003.

This study was conducted between July, 1999 and March, 2000 at the Royal Free Hospital in London, United Kingdom. The first subject was enrolled on July 15, 1999 and the last evaluation was completed on March 3, 2000. The study report was finalized on August 12, 2000. The principal investigator for this study was Dr. Kay MacDermot.

The study enrollment was targeted to 24 subjects. However, enrollment was terminated at 15 subjects.

### B. Protocol:

There were no protocol amendments. However, there was a statistical analytical supplement to the protocol.

#### 1. Title:

"A phase II randomized double-blind placebo-controlled clinical trial of the effect of alpha-galactosidase A replacement therapy on cardiac structure and function in Fabry disease" (TKT clinical protocol number TKT005, June 9, 1999)

#### 2. Design:

This was to be a randomized, double-blind, placebo-controlled study in which 24 adult subjects with Fabry disease were to receive 0 or 0.2 mg/kg of alpha-galactosidase every other week for a total of 24 weeks (12 doses). Subjects were to have baseline, week 13 and week 24 cardiac biopsies. The primary endpoint was a comparison of the cardiac content of CTH ($Gb_3$) in the biopsy samples.

#### 3. Objectives:

The protocol stated objective was to evaluate the safety and efficacy of Agalsidase. The protocol stated “Efficacy will be determined primarily by measuring the effect … on heart CTH content. Efficacy will also be assessed by measuring the effect of enzyme replacement therapy on several critical secondary endpoints, including heart function and pathology, kidney function, pain and urine CTH concentration."

#### 4. Patients/eligibility criteria:

24 subjects who satisfy the following criteria:

-Inclusion criteria:
- -male with Fabry disease as documented by clinical evidence and by evidence of alpha-galactosidase A deficiency, as assayed on white blood cells or cultured skin fibroblasts.
- -left ventricular enlargement on echocardiographic measurement of left ventricular mass
- -≥ 18 years of age
- -adequate general health to undergo all study procedures

-PT/APTT less than 1.5 X normal and platelet count > 100,000/mcL

-Exclusion criteria:
-permanent cardiac pacemaker
-treatment with another investigational therapy or any approved therapy for investigational use within the past 30 days
-medical condition contraindicating a study procedure or which makes implementation of a study procedure difficult

**5. Evaluations:**

Subjects were to be randomized to a treatment assignment when the first baseline evaluation was completed and final eligibility criteria confirmed. Randomization was to be blocked. Subjects underwent two baseline evaluations--each separated by approximately one week. The first evaluation could be performed as an outpatient, while the second evaluation had to be performed during a hospitalization.

The study evaluation plan was to largely parallel that of Study TKT003, including “off medication” periods and use of BPI forms for assessing pain scores).

Cardiac biopsy (endocardial), cardiac MRI and echocardiography were to be performed at baseline and weeks 13 and 24. Creatinine clearance and GFR were to be measured at the same time points as in Study TKT003. All other evaluations were also generally the same as in Study TKT003.

**6. Dose:**

Agalsidase was to be diluted in 100 mL of saline and administered intravenously over 40 minutes. The placebo was to consist of the identical study drug formulation, without the enzyme. The study drugs were to be prepared by the unblinded pharmacist at the site. No subjects were to receive routine premedications. However, subjects who had any reaction to study drug were to receive the following premedication: prednisone 50 mg PO and ranitidine 150 mg PO on the morning of the infusion, then solumedrol 50 mg IV, benadryl 50 mg IV and ranitidine 50 mg IV one hour before the infusion.

Note that the Agalsidase product used in Study TKT005 was not manufactured by the same source as that of Study TKT003. Clinical study TKT003 used Agalsidase that was produced by the contractor, Abbott, Inc., and is referred to as DRX005B-Abbott. Clinical studies TKT005 and TKT006 used a product manufactured at Bio Science Contract Production Company and is referred to as DRX005B-BSCP. The sponsor performed physicochemical and animal studies to demonstrate comparability between these two products. The DRX005B-BSCP is the product intended for marketing.

**7. Endpoint/assessments:**

The primary endpoint was to be a comparison of the change in the heart biopsy content of CTH ($Gb_3$) from baseline to study weeks 13 and 24.

Secondary endpoints were numerous and included the following:

1. MRI assessment of Cardiac mass (approximately 5 different comparisons)
2. Heart pathology (multiple comparisons)
3. Pain (multiple comparisons)
4. Urine sediment CTH ($Gb_3$)
5. Pain medication usage
6. Echocardiography (approximately 7 different comparisons)
7. Renal function tests (5 different comparisons)
8. Quality of life

9. EKG (multiple features compared)
10. Cerebral MRI
11. Plasma CTH
12. Audiograms
13. Weight
14. Urinalysis

Cardiac CTH ($Gb_3$) content was to be determined by the sponsor in a central facility.

Cardiac Pathology
The cardiac pathology assessment was to be conducted in a blinded manner, with the biopsy samples coded to maintain blinding. The extent of lipid deposition in myocardial, endocardial and endothelial cells was to be measured in light microscopy sections and graded using a 0 - 4 grading system, as follows:

0 -normal cellular structure
1 -fewer than 10% cells affected
2 -clusters of affected cells, or 10 - 25% cells affected
3 -many cells affected, 25-60% of cells affected, or moderate diffuse deposition
4 -severe and diffuse deposition with more than 60% of cells affected.

Total lipid deposition score for each sample were to be calculated and an attempt made to calculate separate lipid deposition scores for myocardial, endocardial and endothelial cells.

Cardiac electron microscopic analysis was also to be performed in a blinded manner.

Renal Function Tests
Included inulin-based GFR, 24-hr urine protein, serum creatinine and BUN, calculated creatinine clearance.

MRI-based Cardiac Mass
Included comparisons of: posterior left ventricular wall thickness, cardiac mass, left ventricular mass, T1 signals, and T2 signals.

Echocardiographic endpoint
Consisted of seven different outcomes including left ventricular mass, aortic root diameter and others.

**8. Statistical analytical plan:**

The protocol stated the change from baseline scores for the two treatment groups were to be compared at the end of the study using ANOVA or t-test for continuous variables, if applicable, or a non-parametric test when necessary assumptions could not be met.

Treatment group change from baseline pain comparisons were to be assessed by using area under the curve methods whenever possible, or descriptive statistics.

The subsequent Statistical Analytical Plan (a separate document) included the following statements:

The primary and secondary efficacy variables were to be based on the intent-to treat patient population. However, this was defined as including all randomized patients with the exception of subjects who never received any study drug.

The efficacy variables were to compare the two groups using ANCOVA, adjusting for baseline values.

The primary endpoint (cardiac CTH ($Gb_3$) content) was to be analyzed using ANCOVA for data at week 13 and 24. There was no prospective adjustment for multiple comparisons to limit Type I error to 0.05.

The baseline measurements was stated to be the first measurement during the baseline week (-1) for the following evaluations: weight, exam, NPS, quality of life questionnaires, serum chemistry, blood hematology, urinalysis, 24 hour urine chemistry and urine chemistry.

For the plasma CTH($Gb_3$), urine sediment CTH ($Gb_3$) and creatinine clearance, the mean of the two baseline period evaluations was to serve as the baseline.

The plan contained multiple definitions relating to pain scores which were the same as those utilized in Study TKT003

For missing data the last observation carried forward (LOCF) principle was to be used for imputation where possible, or in case of missing data within a sequence, linear interpolation would be used if there were available values before and after the missing time point.

All efficacy comparisons, other than the primary endpoint, were to be considered as supportive. Thus, no compensation for multiplicity of secondary endpoints was to be performed.

*Comment: Because there were so many secondary endpoints in this study, it is important to note that the Statistical Analytical Plan described all the secondary endpoints as supportive only, i.e, if the primary endpoint failed, the meaningfulness of certain individual secondary endpoint p-values was undefined.*

**C. Study conduct:**

**1. Overview:**

The first subject was enrolled into the study on July 15, 1999 and the last subject completed the final evaluation on March 3, 2000.

*Comment: No alteration of the study database occurred following the database lock.*

The sponsor notes that "because of Fabry disease rarity," the study did not complete enrollment. Instead of enrolling 24 subjects, 15 subjects were enrolled. There were several protocol violations. All related to missed or miss-timed evaluations and do not significantly impact the interpretation of the study results.

The sponsor notes that multiple secondary endpoints were not analyzed or were not analyzed by the method proposed in the Statistical Analytical Plan because of technical reasons. These altered analyses included the following:

- -cardiac MRI T1 and T2 data were not collected
- -echocardiographic determinations of wave E/A ratio, LV relaxation time, mitral valve thickness were not determined
- -unplanned echocardiographic measures were obtained and analyzed--including left atrial size, left ventricular diameter in diastole and systole
- -the cardiac pathology scoring system was found to be "meaningless" because of the severe pathological findings and was not analyzed
- -certain EKG findings (QRS duration) were analyzed in post hoc analyses in addition to the proposed analyses
- -the time to discontinuation of pain medication was not performed because only four patients were taking pain medications at baseline (2 in the Agalsidase group and 2 in the placebo group)
- -certain creatinine clearance outcomes were not analyzed as described in the statistical analytical plan (i.e, the repeated measures analysis was not performed because of missing data). Instead, the change from baseline to week 24 was analyzed.
- -one subject (subject number 6, a subject in the Agalsidase group) did not have

cardiac biopsies performed and this subject is eliminated from the analyses of cardiac CTH ($Gb_3$) content.

The blind was broken for one subject during the clinical study. Subject number 5 (placebo) developed end stage renal failure early in the course of the subsequent maintenance study (TKT007) and, at the investigator's request, the blind for this patient was broken. This unblinding occurred after the last subject visit, but prior to the database lock (hence, outside Study TKT005). The medical monitor remained blinded until the database lock.

Both echocardiographic data and cardiac MRI data were recorded directly on the CRF at the time of each visit. Consequently, the cardiac MRI and echocardiographic evaluations were performed and interpreted with knowledge of the sequence of the examinations (i.e., the baseline evaluation was known to be baseline, the week 24 known to be week 24, etc).

**2. FDA inspection:**

The clinical site was inspected and the major findings from this inspection were the following:

A failure to accurately collect urine for creatinine clearance determinations, from the baseline period through November 10, 1999 (after which time the samples were obtained appropriately)

A failure to formally "reconsent" subjects when a revision in the consent document occurred.

*Comment: The inspectional findings explain the basis for so many physiologically impossible " early and mid-study" creatinine clearance outcome data points.*

**D. Results:**

**1. Subject disposition:**

The study enrolled 15 subjects, 7 in the Agalsidase group and 8 in the placebo group. All 15 subjects completed the study and all subjects are included in the efficacy and safety analyses.

**2. Baseline characteristics:**

Table 31 describes certain baseline characteristics.

**Table 31. Baseline characteristics**

| Characteristic | Agalsidase (n = 7) | Placebo (n = 8) |
|---|---|---|
| Age, years, mean ± SE | 36.4 ± 3.8 | 36.9 ± 2.8 |
| Race, n<br>Caucasian<br>Asian | <br>7<br>0 | <br>7<br>1 |
| Weight, kg, mean ± SE | 72.5 ± 4.0 | 69.6 ± 6.0 |
| MRI cardiac mass, gm, mean ± SE | 276.2 ± 19.4 | 248.2 ± 26.0 |
| Creatinine clearance, mL/min, mean ± SE | 107.7 ± 17.8 (n = 6) | 91.0 ± 17.7 |
| No pain medications*, n | 5 | 6 |

*As in TKT003, there was no prospective definition of "pain medications." The sponsor notes that the "operational definition" of "pain medications" includes: tegretol, carbamazepine, neurontin, dilantin, phenytoin, lamictal, lamotrigine, nortriptyline or amitriptyline and were considered as used at the start of the study if the medication was started or stopped in the baseline period or was indicated as used on the BPI long form, question 18.

**3. Study drug exposure:**

All subjects received all 12 study drug infusions with no interruption of the infusion. There were no infusion reactions and no subjects received premedication prior to the infusion reaction.

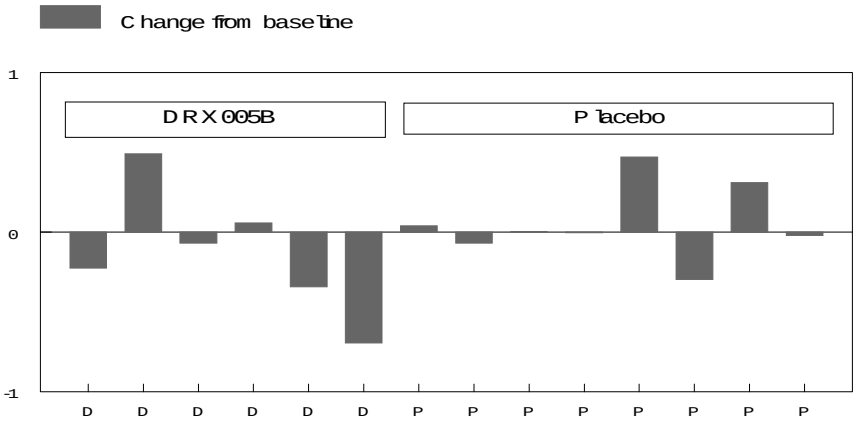
**4. Primary endpoint results:**

The primary endpoint was cardiac CTH ($Gb_3$) content. The treatment groups were to be compared with respect to the change from baseline to weeks 13 and 24 using ANCOVA with the baseline measurement as an independent variable. There were no prospectively defined exploratory analyses of the primary endpoint. Table 32 shows the primary endpoint result.

**Table 32. Primary endpoint: change from baseline to week 13 and week 24 cardiac CTH ($Gb_3$) content (nmol/mcg protein)**

| Group | Baseline | Week 13 | Week 24 | p-value |
|---|---|---|---|---|
| **Agalsidase**, n = 6<br>mean ± SE | 0.71 ± 0.18 | 0.70 ± 0.19 | 0.58 ± 0.18 | 0.42 for baseline to week 24 |
| **Placebo**, n = 8<br>mean ± SE | 0.58 ± 0.08 | 0.63 ± 0.10 | 0.63 ± 0.11 | 0.73 for baseline to week 13 |

The change from baseline to week 24 for each subject is shown in Figure 2.




*Change from Baseline*

**Figure 2. Cardiac CTH Change (nmole/mcg protein) from baseline to week 24 (each subject is shown, group indicated as D = Agalsidase, P = placebo)**

*Comment: The study fails to show a difference in the cardiac content of CTH($Gb_3$) at either week 13 or 24 (change from baseline). This outcome somewhat parallels certain preclinical studies which showed that a higher dose of enzyme may be necessary in order to significantly lessen cardiac CTH ($Gb_3$) content.*

**5. Secondary endpoints:**

The protocol and statistical analytical plan identified 14 major headings for secondary endpoints, generally with multiple endpoints beneath each heading. Because of the high multiplicity of these endpoints, all were to be regarded as supportive only in the event of a significant finding on the primary endpoint.

**A. Cardiac mass (as determined by cardiac MRI):**

Cardiac MRIs were performed at baseline, week 13 and week 24. Multiple MRI parameters were to be assessed, but the statistical analytical plan noted that "the major consideration will be left ventricular mass."

**a. Left ventricular mass:**

The sponsor performed an analysis of the left ventricular mass in which only subjects who had a baseline and a week 24 left ventricular mass determination were included in the patient population. This post-hoc revised analysis eliminated subject number 5, a placebo subject who had a baseline and week 13 result but did not have a week 24 result (the CRF notes the examination was abandoned due to claustrophobia). This manipulation is especially notable since subject number 5 had the largest baseline left ventricular mass of all the patients (baseline value of 457 gm) and by week 13 the mass had decreased to 395 gm. Table 33 shows the left ventricular mass results for both the "available data" subset and the results for all subjects using the prospective plan of LOCF imputation for subject 5.

*Comment: The nominal statistical success of Agalsidase upon lessening the left ventricular mass is not a robust finding, as illustrated by the change when all subjects are included within the analysis.*

**Table 33. Left ventricular mass at baseline and Week 24**

| Group | Left ventricular mass | | | |
|---|---|---|---|---|
| | Baseline | Week 24 | Change from baseline | p-value* |
| ***Among subjects with baseline and week 24 data (excludes subject 5)*** | | | | |
| Agalsidase, n = 7 mean ± SE | 276.2 ± 19.4 | 264.8 ± 19.3 | - 11.5 ± 11.2 | 0.04 |
| Placebo, n = 7 mean ± SE | 248.2 ± 26.0 | 270.0 ± 23.1 | + 21.8 ± 5.9 | |
| ***Among all subjects (LOCF from Wk 13 imputation for placebo subject 5)*** | | | | |
| Agalsidase, n = 7 mean ± SE | 276.2 ± 19.4 | 264.8 ± 19.3 | - 11.5 ± 11.2 | 0.10 |
| Placebo, n = 8 mean ± SE | 274.4 ± 34.5 | 285.6 ± 25.4 | + 11.3 ± 11.7 | |

LV Mass measurements in grams

*p-values from ANCOVA with baseline and treatment as independent variables

**b. Other cardiac MRI outcomes:**

The sponsor performed multiple other analyses of cardiac MRI outcomes. All were calculated using the subset of subjects with available data. None of these analyses showed evidence of effect of Agalsidase. The outcomes among subjects with available data are shown in Appendix E.

An analysis which Included all randomized subjects for a true ITT analyses by using imputation does not change the outcomes. On the whole, there appears to have been no convincing evidence of a treatment effect upon cardiac MRI outcomes.

**B. Echocardiographic outcomes:**

The Statistical Analytical Plan listed seven echocardiographic outcomes to be assessed. Two of these outcomes (mitral valve thickness and wave E/A ratio) were not assessed. The five remaining prospective endpoints and other post-hoc exploratory outcomes submitted by the sponsor all fail to show evidence of a treatment effect at a nominal 0.05 level of significance (see Appendix E).

Even though none of the echocardiographic findings demonstrate a convincing impact of Agalsidase, the sponsor has highlighted the observation that "Patients treated with placebo had an approximately 20 gm increase in their cardiac mass, but patients treated with DRX005B had an approximately 20 gm decrease in their cardiac mass (p = 0.26)."

*Comment: Overall, the echocardiographic data provide no evidence of a treatment effect. The sponsor's perception of a possible treatment effect is not substantiated by the data, and appears inconsistent with a reasonable interpretation of this multitude of statistical analyses.*

**C. Electrocardiography outcomes:**

Electrocardiograms were obtained at multiple visits throughout the study. QRS duration showed no statistically significant evidence of a treatment effect (see Appendix E)

*Comment: EKG analyses reveal no evidence of a treatment effect. This outcome in patients with heart disease at baseline supports an interpretation of the Study TKT003 result of marginal evidence of a treatment effect upon QRS duration (in subjects without cardiac disease were eligible for enrollment) as not being significant.*

**D. Cardiac pathology:**

Cardiac pathology was to be evaluated using a severity grading scale. The sponsor notes these results showed no evidence of a treatment effect.

**E. Renal function tests:**

Creatinine clearance and GFR results have already been included and discussed within Study TKT003 review sections. Note that errors in urine sample collection early in the study invalidate the creatinine clearance results from Study TKT005. GFR results showed a small increase in GFR in both groups, not significantly different between groups.

**F. Plasma and urine sediment CTH ($Gb_3$) content:**

Changes in plasma and urine sediment CTH ($Gb_3$) content were to be analyzed using a repeated measure analysis (baseline, weeks 9, 17, 24). The sponsor performed this analysis as well as an analysis that compared the change from baseline to week 24 (ANCOVA). Both analyses revealed similar results of a larger decrease in plasma CTH ($Gb_3$) and urine sediment CTH ($Gb_3$) associated with Agalsidase treatment. The details of the analyses are shown in Appendix E.

*Comment: As in study TKT003, there was a decrease in plasma CTH ($Gb_3$) content in the active treatment group. Unlike the marginal results from Study TKT003, there was also a decrease in the urine sediment CTH($Gb_3$) content in this group. These results support the conclusion that Agalsidase was a biologically active treatment. The efficacy of this treatment cannot be assessed from these evaluations.*

**G. Pain outcomes:**

Multiple analyses of pain scores were to be performed, most of which were the same analyses as were performed in Study TKT003. The most notable of these analyses was the assessment of "worst pain" score while off pain medication, the same evaluation as that chosen for the primary endpoint in Study TKT003. The "worst pain" scores while off pain medication are shown in the appendix. Subjects were not required to have pain as a requirement for enrollment in this study. Consequently, mean worst pain scores were 1 to 2 points lower in this study than in Study TKT003. Additionally, only two subjects in each group were receiving pain medications at baseline. Subject number 2 (a placebo subject) is eliminated from these analyses because of the lack of a baseline off pain medication score.

Mean worst pain scores were slightly greater in the placebo group than Agalsidase group at baseline, and during the study decreased in the placebo group while increasing in the Agalsidase group, so that by Week 24 the Agalsidase group had a slightly greater mean pain score than the placebo group. These between group differences in this small study were not statistically significant. Details of the scores and between group comparisons are shown Appendix E.

*Comment: It is notable that the placebo group tends to have more improvement in their pain scores than the Agalsidase group. This finding, although not statistically significant, is similar to the Study TKT003*

*analysis for "all visits" (i.e., most visits with normal use of medications as needed). Since the majority of Study TKT005 subjects were not receiving pain medications, their "off medication" evaluations are also their "as needed" state. These observations support the interpretation of the findings from Study TKT003 suggesting no impact of Agalsidase upon the BPI pain scores within 24 weeks of treatment.*

Neither interference scores nor mean pain scores from the combined pain severity questions showed a significant treatment associated effect.

Time to discontinuation of pain medications was not compared between the two groups since only two subjects in each group were receiving pain medications at baseline.

**H. Weight:**

The statistical analytical plan did not describe the specific analyses to be performed for changes in weight. The sponsor chose to present a table in which the weight change was analyzed in a repeated measure ANOVA. This analysis resulted in a p-value of 0.77 for the comparison of the two groups (data not shown here). Change from baseline to week 24 was a mean of 0.7 kg in the Agalsidase group, 1.3 kg in the placebo group (p = 0.3, ANCOVA with baseline included as an independent variable).

*Comment: The finding of no statistical difference in the weight change between the two groups of subjects in this study is informative because no subjects received systemic steroids. Consequently, this study appears more likely to assess the effect of the study agent upon weight changes than Study TKT003 where systemic steroids were widely utilized. The finding of no significant impact of the active study agent upon weight change in this study, combined with the equivocal results from Study TKT003, suggests that Agalsidase may not increase weight.*

**I. Other secondary endpoints and summary of secondary endpoints:**

The protocol and Statistical Analytical Plan listed multiple additional study endpoints which included results of cerebral MRI scan, quality of life measures, audiogram measures, urinalysis changes and scores on the Fabry Symptom Scale. None of these endpoints achieved nominal statistical success. These results are not repeated here in detail, but following the sponsor's example, the summary of the statistical results for their selection of the most notable secondary endpoints are shown. Note that the sponsor's analysis of cardiac MRI mass (the only analysis with a nominally significant p-value) is a post-hoc analysis performed in the subset of patients with available data.

<u>Analyses of hearing loss</u>

Controlled clinical study data evaluating hearing loss were obtained in Study 005. Multiple aspects of audiologic function were assessed and the sponsor's analyses are all consistent in showing no treatment effect associated with Agalsidase administration.

*Comment: A publication (an abstract) has been submitted to the BLA assessing hearing loss. This publication reports an Agalsidase treatment benefit based upon audiometric data. It is important to note that the controlled clinical study data show no treatment effect and the abstract's claim appears to be based upon selected, uncontrolled clinical data.*

**Table 34. Summary of most notable secondary endpoint statistical analyses**

| Measure | | p-value | |
|---|---|---|---|
| | | ANCOVA week 24 | Repeated measures |
| **Plasma CTH ($Gb_3$)** | | <0.01 | <0.01 |
| **Urine sediment CTH ($Gb_3$)** | | 0.05 | 0.04 |
| **Cardiac MRI** | | | |
| Cardiac mass (*subset analysis*) | | 0.04 | 0.74 |
| Left ventricular posterior wall | | 0.95 | 0.54 |
| Left ventricular systolic volume | | 0.89 | 0.30 |
| Left ventricular ejection fraction | | 0.78 | 0.42 |
| Left ventricular end diastolic volume | | 0.22 | 0.23 |
| Left ventricular end systolic volume | | 0.87 | 0.20 |
| **Echocardiographic outcomes** | | | |
| Left Ventricular mass | | 0.26 | 0.74 |
| Left ventricular mass index | | 0.66 | 0.68 |
| Aortic root diameter | | 0.54 | 0.09 |
| Left atrial size | | 0.45 | 0.90 |
| Left ventricular diameter in diastole | | 0.28 | 0.66 |
| Left ventricular diameter in systole | | 0.27 | 0.68 |
| Ventricular septum in diastole | | 0.15 | 0.76 |
| Ejection fraction | | 0.46 | 0.88 |
| Left ventricular posterior wall thickness | | 0.94 | 0.81 |
| **EKG (QRS duration)** | | 0.81 | 0.88 |
| **BPI Scores** | | | |
| Pain at its worst | ANCOVA, week 24 | | 0.13 |
| | Repeated measures | | 0.95 |
| | AUC | | 0.12 |
| Interference scores | ANCOVA, week 24 | | 0.85 |
| | Repeated measures | | 0.70 |
| | AUC | | 0.59 |
| Severity scores | ANCOVA, week 24 | | 0.40 |
| | Repeated measures | | 0.64 |
| | AUC | | 0.12 |
| **Pain medication use** | | | **t-test** |
| | | | 0.33 |
| **Renal** | **ANCOVA** | **Repeated measures** | |
| Serum creatinine | 0.15 | 0.40 | |
| BUN | 0.18 | 0.21 | |
| creatinine clearance | 0.35 | not done (missing data) | |
| GFR | 0.34 | not done | |
| specific gravity | 0.34 | 0.08 | |
| **Weight** | | 0.773 | |

AUC = area under the curve

*Comment: Among the secondary endpoints, the only ones with strong evidence of a treatment effect are the plasma CTH ($Gb_3$) and urine sediment CTH ($Gb_3$) findings. Virtually all other findings reveal no treatment effect.*

**6. Safety:**

**Termination of study agent due to adverse events:**

All patients completed all study agent administrations. There were no infusion reactions. No patients required premedication.

*Comment: The finding of no infusion reactions with the Agalsidase used in Study TKT005 but an approximately 50% rate of infusion reaction in Study TKT003 (a study which used Agalsidase manufactured by another company) suggests that the products utilized between these two studies may have properties that differ clinically. Note that it is the Study TKT005 product that is to be marketed. However, the infusion times were also different (40 minutes in Study TKT005 and initially 20 minutes in Study TKT003). Because comparability data suggest the products manufactured by the different companies are very similar, it may be reasonable to, at least in part, attribute the difference in the rate of infusion reactions to the lengthening of Agalsidase infusion time.*

**A. Adverse events and serious adverse events:**

All subjects experienced at least one adverse event (AE). Those event types more frequent in Agalsidase subjects are shown in Table 35. Overall, 213 AEs were reported among the 15 subjects. Four AEs were graded as severe (3 placebo group, 1 Agalsidase group). The one severe event in the Agalsidase group was also a serious AE. Subject number 9 developed a severe pharyngitis approximately one month after beginning the study agent administration, requiring an overnight hospital stay for intravenous antibiotic therapy. The AE was attributed by the site investigator as unrelated to the study agent.

**Table 35. Adverse events occurring in more Agalsidase subjects than placebo subjects**

| Event | Agalsidase, n = 7 | Placebo, n = 8 |
|---|---|---|
| Influenza-like symptoms | 6 (85.7%) | 4 (50.0%) |
| Pharyngitis | 5 (71.4%) | 3 (37.5%) |
| Infection | 4 (57.1%) | 3 (37.5%) |
| Paresthesia | 3 (42.9%) | 1 (12.5%) |
| Vomiting | 3 (42.9%) | 1 (12.5%) |
| Chest pain | 3 (42.9%) | 3 (37.5%) |
| Coughing | 3 (42.9%) | 2 (25.0%) |
| Nausea | 3 (42.9%) | 1 (12.5%) |
| Creatinine clearance decrease | 3 (42.9%) | 1 (12.5%) |
| Skeletal pain | 2 (28.6%) | 1 (12.5%) |

**1. Adverse events by causality:**

The investigators assessed no adverse events as "probably" related to the study agents. Two adverse events in the Agalsidase group were assessed as "possibly" related--one case of nausea and vomiting and a case of dry skin. Five adverse events in the Agalsidase group were assessed as "unknown" relationship to study agent--one case of paresthesia, one case of influenza-like symptoms, and one case of pharyngitis, one case of renal dysfunction and one case of flushing.

**2. Laboratory data:**

Overall, there were no remarkable differences in laboratory findings between the two groups.

**B. Antibody results:**

The sponsor reports that "in the screening ELISA assay for IgG, no patient developed an IgG antibody response to Agalsidase. Two patients were mildly positive by immunoprecipitation (at 1:2 titers) and these same patients were also mildly positive by the in vitro neutralization assay (1:2 titers)."

*Comment: It is notable that there appears to be somewhat lower immunoreactivity and infusion reaction rate detected in this study than in Study TKT003. This raises the possibility that the Agalsidase utilized within Study TKT005 differs from that utilized within Study TKT003 (as noted above) as well as the possibility that the length of infusion time may influence the rate. Nevertheless, the sample sizes in Study TKT005 are very small and it is difficult to reach solid conclusions related to the apparently lower rate of immunogenicity in this study than Study TKT003.*

## E. Summary of Study TKT005

Study TKT005 was a study conducted at a single site in Great Britain that focused upon cardiac disease. The study design was similar to Study TKT003 in that it was a randomized, double-blind, placebo-controlled study conducted among adult subjects with Fabry disease. The Agalsidase dose was also the same as that tested in Study TKT003 (all Agalsidase was to be infused over 40 minutes in Study TKT005). Unlike Study TKT003, all Study TKT005 subjects had to have left ventricular enlargement on echocardiography. Within Study TKT005 subjects underwent a series of endocardial biopsies: baseline, mid-study and at the end of the six month study. The study's primary endpoint was a comparison of the change from baseline in cardiac biopsy content of CTH ($Gb_3$) as measured by a biochemical assay of the tissue sample. Subjects also underwent pain score evaluations as was performed in Study TKT003. Notably, the Agalsidase product used in this study was manufactured by a company that differed from the company manufacturing the Agalsidase product used in Study TKT003. The Study TKT005-manufactured product is to be marketed. Biochemical comparability has been demonstrated between the Agalsidase products manufactured by the two companies.

Overall, 15 subjects were randomized, seven to Agalsidase and 8 to placebo. One Agalsidase subject did not undergo a cardiac biopsy and this subject was eliminated from the primary endpoint analyses.

The study's primary endpoint (a comparison of the change from baseline in cardiac CTH ($Gb_3$) content) showed no statistically significant difference between the Agalsidase and placebo groups.

The most notable secondary endpoints included cardiac structure outcomes as assessed by MRI and echocardiography, EKG changes, renal function changes and weight changes. Using the subset of subjects with available data, the MRI assessment of change in left ventricular mass suggested a difference between the two study groups, with (on average) a decrease in the Agalsidase group and (on average) an increase in the placebo group. However, using the entire study population (one placebo subject had a week 13 result but no end-of-study result), there was loss of this nominal statistical significance. Echocardiographic assessments of left ventricular mass showed no statistically significant difference between the two study groups. Similarly, there was no statistically significant difference between the two study groups in changes from baseline in the QRS duration.

Renal function results revealed no statistically significant difference in GFR. The creatinine clearance data points were determined to be inaccurate.

Pain was also assessed in Study TKT005 and no significant differences in pain were observed between the groups.

Similar to Study TKT003, Study TKT005 showed decreases (on average) in the (urine sediment/plasma concentration) content of CTH ($Gb_3$) among subjects receiving Agalsidase as compared to those receiving placebo.

There was no statistically significant difference in weight changes between the two study groups.

In general, the major safety findings related to infusion reactions and antibody formation. Unlike Study TKT003, no subjects in Study TKT005 experienced infusion reactions. Some evidence of antibody formation was detected among approximately one third of subjects receiving Agalsidase.

**13. Study TKT006:**

**A. Overview:**

This study was a single arm, open label, continuation of Study TKT003. The 25 subjects who successfully completed study TKT003 were eligible for enrollment. Subjects were to receive, for one year, the same dose of Agalsidase as was studied in Study TKT003. Because 14 of the Study TKT006-eligible subjects had completed 6 months of therapy with the enzyme in Study TKT003, the TKT006 results provide data extending through 18 months of Agalsidase administration for these subjects as well as the entire 12 month Agalsidase exposure data from subjects who had received placebo within Study TKT003. Following completion of this study, subjects were eligible to continue receiving open label enzyme under Study TKT011 (a review of interim data follows this summary of Study TKT006). Both TKT006 and TKT011 were performed at the NIH with the same investigators as Study TKT003.

**B. Protocol:**

There were three protocol amendments. Amendment number 1 allowed the cessation of premedication therapies (steroids, histamine antagonists), required a Week 27/28 GFR/renal plasma flow, and clarified the infusion regimen and safety monitoring procedures. Amendment 2 provided additional details on evaluations after week 41 and required conducting an interim analysis to include all data through week 27 (14 infusion visits). Amendment three allowed continued administrations of Agalsidase for up to six doses (as Study TKT011 plans were being finalized).

**1. Title:** "A phase 2 open-label maintenance safety and efficacy clinical trial of alpha-galactosidase A replacement therapy in patients with Fabry disease"

**2. Design:** Open-label, single arm, single center continuation study. The baseline evaluations were to be the week 23/24 evaluations from Study TKT003. Subjects were to receive Agalsidase every other week for a total of 52 weeks.

**3. Objectives:** To evaluate the safety and activity of maintenance doses of Agalsidase in subjects who had completed Study TKT003.

**4. Patients:** All subjects who had completed Study TKT003 were to be eligible. Hence, enrollment was limited to a maximum of 26 subjects.

**5. Dose:** Agalsidase was to be administered at 0.2 mg/kg IV over 40 minutes every other week for 52 weeks.

**6. Evaluations:**

The study included continued "off pain medication" evaluations. These evaluations were to be performed in a manner very similar to that utilized in Studies TKT003 and TKT005. Subjects were to abruptly discontinue all designated pain medications which were administered on a continuous basis (e.g. Dilantin, Tegretol or Neurontin) four times during the study: one week prior to the visits at weeks 13, 27, 39 and 52. If a patient felt he needed to resume his pain medications, he was to do so only following the completion of a BPI short form assessment.

The most notable planned evaluations were the following:

-GFR at baseline and week 52
-Creatinine clearance at baseline and weeks 13, 27, 29 and 52
-Cardiac MRI and echocardiography at baseline and weeks 27 and 52

Agalsidase antibodies and blood levels were collected throughout the study.

**7. Endpoints and statistical assessments:**

The primary endpoints were: cardiac mass (by MRI), GFR and pain (BPI). A single primary endpoint was not identified.

The secondary endpoints included the following:

-urine sediment CTH ($Gb_3$)
-plasma CTH ($Gb_3$)
-renal function tests (24 hour urine chemistry, serum creatinine, BUN, creatinine clearance, RPF)
-pain (other measurements)
-pain medication usage
-cardiac echocardiogram (other measurements)
-cardiac MRI
-cerebral MRI examination
-PFT
-quality of life questionnaires
-EKG
-urinalysis (specific gravity)
-quantitative sensory testing
-skin biopsy for innervation density
-endothelial activation markers

Efficacy analyses: The three primary endpoints were to be each analyzed using a one sample paired t-test to test the hypothesis of no change from baseline.

The secondary endpoints were to be analyzed using similar methods as the primary endpoints. In addition, the change in pain from baseline over the entire study period was to be descriptively analyzed.

*Comment: All evaluations conducted during the study appear to have been generally unblinded as to study week. Cardiac mass via MRI and echocardiography were to be recorded directly on CRF. Similarly, creatinine clearance and GFR were all to be calculated and recorded on the CRF at the time of performance.*

*There was little detail of the analytic plan within the study protocol and protocol amendments. A single primary endpoint was not identified. The sponsor finalized two Statistical Analytical Plans for the study. The plan for an interim statistical analysis was dated June 9, 2000, a time point after which subjects had completed 6 months of the study. A final Statistical Analytical Plan was dated November 12, 2000, two months after the last subject's final evaluation. The report of the interim study analysis following six months of therapy to all subjects was dated June 11, 2000. Consequently, the analytical plans for both the study's interim and final analyses may have been formulated with knowledge of study results. The lack of explicit prospective descriptions of the types of analyses to be performed upon the data from this study, combined with the lack of a controlled design, underscores the relatively limited usefulness of the data for drawing conclusions regarding efficacy. However, the study does provide additional safety data assessing the more long-term effects of the product.*

**C. Study conduct:**

The study was initiated on June 15, 1999 and the last subject evaluation was performed on September 9, 2000. The findings from Study TKT006 were not evaluated by FDA site inspections.

**D. Results:**

**1. Extent of exposure and protocol deviations:**

All but nine of the 25 enrolled subjects received all 26 infusions, and all but 3 subjects received at least 24 infusions. Some protocol deviations occurred consisting of missed or mistimed assessments.

*Comment: One prior placebo subject (subject 25) missed 12 infusions. The basis for this is unclear. However, the subject completed all infusions for which he attended the visits (with no infusion reactions). The subject generally attended clinic for infusions in a pattern of missing every other scheduled visit (he received week 47 and 51 infusions with no problems). The subject experienced no SAE. The findings suggest the compliance problems may not have been related to safety concerns.*

**2. Subject disposition:**

Twenty five subjects were enrolled (from Study TKT003, 14 Agalsidase subjects and 11 placebo subjects). All subjects completed the study. The intent-to-treat and safety population are identical.

**3. Baseline characteristics:**

The major baseline characteristics of the patients were described within the TKT003 study report. At the time of enrollment into Study 006, 17/25 (68%) subjects were receiving designated neuropathic pain medications. At the beginning of Study TKT003, 22/26 (85%) of subjects were receiving designated neuropathic pain medications.

*Comment: The definition of "pain medication" used within the sponsor's Study TKT006 report is identical to that utilized in the Study TKT003 report--"Neuropathic pain medications may have included Tegretol, Carbamazepine, Neurontin, Dilantin, Phenytoin, Lamictal, Lamotrigine, Nortriptyline and Amitriptyline and were considered as used at the start of study if the medication was started or stopped in the baseline period or was indicated as used on the BPI short form, question 7 at week 1." It is important to note that the problems with this definition of "neuropathic pain medications" that were identified in Study TKT003 are also true for Study TKT006--hence the meaningfulness, as well as the accuracy, of the "neuropathic pain medication use" data are questionable.*

**4. Pharmacokinetic analysis:**

Following the first dose of Agalsidase within Study TKT006, plasma enzyme activity levels were measured over 24 hours. Mean (± SD) clearance of Agalsidase was 193 ± 47 mL/min in the prior-Placebo group and 1772 ± 3084 mL/min in the prior-Agalsidase group. AUC of the first dose was 0.4 ± 0.1 and 0.2 ± 0.2 in the prior-placebo and Agalsidase groups, respectively. These data are shown in Appendix F.

Eight of the 13 subjects who had previously received Agalsidase in Study TKT003 (14 subjects had received Agalsidase but one subject had missing data) had more rapid clearance and lower AUC/dose than any of the TKT003-prior-placebo subjects. Six of these prior Agalsidase subjects were also the six subjects with the maximum immunoprecipitation titers (1:20) (subjects 2, 10, 18, 19, 23 and 27).

Enhanced clearance associated with progressive exposure to Agalsidase is also suggested by analyses that examined the change in pharmacokinetics from the beginning to end of Study TKT006 (Table 36).

**Table 36. Study TKT006 pharmacokinetics**

| Group | Clearance (mL/min, mean ± SD) | | AUC/dose (mean ± SD) | |
|---|---|---|---|---|
| | 006 start | 006 end | 006 start | 006 end |
| Prior placebo, n = 10 | 193 ± 47 | 703 ± 1064 | 0.40 ± 0.1 | 0.58 ± 0.7 |
| Prior Agalsidase, n = 12 | 1772 ± 3084 | 1320 ± 1815 | 0.20 ± 0.2 | 0.34 ± 0.4 |

In general, the accelerated clearance correlated with antibody formation. Four subjects had increasing antibody formation during the study. Three of these subjects had PK evaluations at the end of TKT006 and all three showed increased clearance and decreased AUC. These data are listed Appendix F.

*Comment: This alteration of pharmacokinetics raises concerns about potential alterations of pharmacodynamics as well as any potential clinical efficacy. However, in the absence of any clear demonstration of efficacy, it is impossible to assess any impact upon efficacy. Certain bioactivity outcomes, such as urine or plasma concentrations of precursor molecules may estimate the potential impact of immunoreactivity and altered pharmacokinetics (as noted in Study TKT011).*

**5. Primary endpoint results:**

**a. Cardiac mass:**

The Statistical Analytical Plan noted that MRI mass was the main cardiac mass outcomes. Cardiac mass by echocardiography was also assessed (as a secondary endpoint).

The mean normal cardiac mass is approximately 148 gm with 2 standard deviations yielding a reference range of 98 - 198 gm. Table 37 shows the MRI results and Table 38 shows the echocardiographic results.

**Table 37. Study TKT006 Change from baseline in left ventricular end diastolic mass by MRI**

| Assessment | Mean ± SE (g) | p-value |
|---|---|---|
| ***Subjects treated with placebo in 003 and Agalsidase in 006, n = 11*** | | |
| Baseline of 003 | 211.8 ± 12.6 | - |
| End of 003 | 215.9 ± 11.2 | |
| End of 006 (n = 10)* | 181.3 ± 9.7 | |
| Change: 003 Baseline to end of 003 | 4.1 ± 5.7 | 0.49 |
| Change: End of 003 to end of 006 (n = 10)* | - 27.7 ± 10.1 | 0.02 |
| ***Subjects treated with Agalsidase in 003 and Agalsidase in 006, n = 14*** | | |
| Baseline of 003 | 226.1 ± 17.4 | - |
| End of 003 | 229.6 ± 17.0 | |
| End of 006 | 207.9 ± 18.3 | |
| Change: 003 Baseline to end of 003 | 3.5 ± 2.7 | 0.21 |
| Change: End of 003 to end of 006 | - 21.7 ± 4.7 | < 0.01 |

*subject 14 did not have a week 52 MRI

*Comment: Only one echocardiographic outcome (aortic root diameter ) showed a nominally statistically significant change from baseline. The echocardiographic data suggest no impact of Agalsidase upon potentially important cardiac outcomes. The apparent decrease in cardiac mass by MRI during Study TKT006 is not supported by echocardiographic findings.*

*Note that both MRI and echocardiography assessments appear to have been performed in an unblinded manner. A potential suggestion of unblinding-related bias may appear within the results pattern of MRI changes. Note that prior Agalsidase subjects did not show a decrease in mass following 6 months of blinded study treatment in Study TKT003, but did show a decrease in mass during the additional 12 months of unblinded study treatment, and this effect was similar in size to the mass decrease shown by the prior-*

*placebo subjects following 12 months of unblinded treatment. The available data do not persuasively demonstrate an impact upon cardiac mass, a finding consistent with the bulk of clinical data.*

**Table 38. Study TKT006 summary of echocardiographic outcomes**

| Assessment | Change; 003 end to 006 end | | p-value on within group chg | |
|---|---|---|---|---|
| | Prior placebo | Prior Agalsidase | Prior placebo | Prior Agalsidase |
| LV mass index ($g/m^2$) | 27.6 ± 42.7 n = 7 | 6.5 ± 11.3 n = 12 | 0.54 | 0.58 |
| Septal thickness (mm) | 0.1 ± 1.1 n = 7 | 0.3 ± 0.5 n = 12 | 0.90 | 0.50 |
| LV post wall thickness (mm) | 0.3 ± 1.0 n = 8 | 0.3 ± 0.5 n = 12 | 0.80 | 0.49 |
| Aortic root diameter (mm) | - 0.6 ± 2.4 n = 8 | - 2.4 ± 0.8 n = 12 | 0.80 | 0.01 |

All p-values are from paired t-tests; chg = change

**b. GFR:**

GFR data were reviewed with Study TKT003 Renal Function data (see above).

**c. Pain outcomes:**

The BPI Worst Pain assessment while “off pain medication” outcome was analyzed. During Study TKT006 the prior-placebo subjects had a decrease in mean score, while the prior-Agalsidase subjects had a slight increase in mean score (detailed values shown in appendix).

*Comment: It is largely impossible to interpret the pain scores in this open label study. The uncertainties of data collection and meaningfulness of the definitions of “pain medication” and abrupt discontinuation paradigm further prevent drawing any conclusions based on these data.*

**6. Secondary endpoints:**

There were many secondary endpoints, the most important being the renal functional outcomes that were reviewed with the Study TKT003 data, and are not further discussed in this section.

**a. EKG outcomes:**

There was no change from baseline in the QRS duration for either the prior placebo group or prior Agalsidase group (data not shown).

**b. Severity and interference pain scores:**

In general, these scores paralleled the "worst pain" outcomes for the study--with apparent improvement for the prior placebo group and no change during the time period for the prior Agalsidase group (data not shown). As noted in the section on "worst pain" scores, no conclusions can be drawn from these data.

**c. Urine sediment CTH ($Gb_3$) content and plasma CTH ($Gb_3$) concentration:**

Urine sediment concentration of CTH ($Gb_3$) declined in both the prior-placebo and the prior-Agalsidase groups during Study TKT006. Plasma CTH ($Gb_3$) concentrations also declined in the prior-placebo subjects first introduced to Agalsidase in TKT006, but further reduction in plasma CTH ($Gb_3$) was not observed in the prior-Agalsidase subjects. Details of these results are shown in Appendix F.

*Comment: The urine sediment changes from one year of follow-up in Study TKT006 do not reveal evidence of loss of bioactivity among the prior Agalsidase subjects at 18 months of treatment compared to 6 months*

*of treatment. However, the plasma CTH($Gb_3$) concentration changes for this group raise the possibility that enzyme's effects may be waning (perhaps related to immunogenicity) but not in a magnitude that results in effects upon the urine CTH($Gb_3$) outcome. These effects are more concerning in the further extension of treatment, Study TKT011.*

**d. Weight changes:**

In general, prior placebo subjects gained weight during Study TKT006 while prior Agalsidase subjects were unchanged (data not shown). No conclusions can be drawn from these data in an open label study that did not have well planned procedures to control for any external influences.

**7. Safety outcomes:**

**A. Antibody formation:**

Of the 25 subjects exposed to Agalsidase in Study TKT006, 17 (68%) tested positive for antibodies (by any assay method) to the product. The sponsor proposes that there is some evidence of immunotolerance to the product developing because of the following observations:

-among subjects with antibodies by ELISA, 3 of 12 subjects were negative by week 52
-among subjects with antibodies by immunoprecipitation, 9 of 16 were negative by week 52
-among subjects with antibodies by in vitro neutralization assays, 10 of 13 were negative by week 52

*Comment: The intra-subject variability over time of these evaluations has not been well determined, nor the intra-subject concordance of changes between these evaluations. While some of the subjects exposed to Agalsidase may develop immunotolerance to the protein, a notable number do not, and some develop increases in antibody titer. This is especially notable for the subjects shown in Table 39.*

***Table 39. Study TKT006 subjects with increasing antibody formation to Agalsidase***

| Subject | ELISA, Week 52 OD/baseline* | Immunoprecipitation titer | | Serum neutralizing assay (% retained activity) | |
|---|---|---|---|---|---|
| | | 003 end | 006 end | 003 end | 006 end |
| 22 | 3.8 | 0 | 1:10 | 124 | 81 |
| 16 | 13.6 | 1:2 | 1:10 | 83 | 46 |
| 19 | 15.1 | 1:2 | 1:20 | 39 | 11 |
| 23 | 13.8 | 1:20 | 1:100 | 30 | 79 |

*baseline is end of 003 for prior placebo subject or baseline of 003 for prior Agalsidase subjects
Of the four subjects, only subject 22 was a prior placebo subject

*Subject number 19's immunoreactivity to the product is especially striking. Overall, at least 4/25 (16%) of the subjects appear to become progressively more immunoreactive to the product. This number may actually be higher because several subjects did not have all three immunoassays performed.*

*Of the four subjects who had progressive increases in antibody titers, only one had an increase in urine CTH($Gb_3$) content during the study (possibly indicating loss of a pharmacodynamic effect). Notably, subject 19 (the subject with generally the greatest increase in antibody titer) had a decrease in urine CTH($Gb_3$) content during Study TKT006, as shown below (values in nmol/gm creatinine). Overall, the immunogenicity results may impact pharmacokinetic results (as assayed by enzyme activity), but the findings generally do not appear to be impacting the bioactivity outcomes over the one year within this study.*

*Urine CTH($Gb_3$) content during Study TKT006*

| *Subject* | *Study TKT006 baseline* | *Study TKT006 end* |
|---|---|---|
| *22* | *622* | *605* |
| *16* | *314* | *787* |
| *19* | *4372* | *1489* |
| *23* | *1967* | *561* |

**B. Adverse events:**

All 25 subjects experienced at least one adverse event. Eleven subjects (44%) experienced adverse events that were assessed as "probably related" to the study drug by the site investigator. Table 40 shows the important adverse events, with those events possibly indicative of infusion reactions highlighted.

Although the vast majority of adverse events were of mild to moderate grade severity, ten subjects experienced adverse events (AE) that were graded as severe. These severe grade AE included (number of subjects with AE): back pain (2), chest pain (1), flu-like symptoms (1), peripheral edema (1), pain not otherwise specified (2), dizziness (1), abdominal pain (2), diarrhea (1), tenesmus (1), decreased hearing (2), dyspnea (1), renal calculus (1).

AE which were graded as probably related to study drug included (number of subjects with AE): back pain (1), chest pain (3), **fever (4)**, **rigors (6)**, hypertension (1), dizziness (1), dysaesthesia (1), dysphonia (1), headache (1), neuralgia (1), abdominal pain (2), nausea (2), myalgia (1), cough (1), laryngitis (1), **respiratory insufficiency (1)**, rhinitis (1), heat intolerance (1), pruritis (1), erythematous rash (2), skin disorder (2), increased sweating (3), **flushing (6)**, eye abnormality (1), abnormal lacrimation (1).

**Table 40. Study TKT006 adverse events that occurred in more than eight (32%) subjects**

| Event | Number with event (n, %), n = 25 |
|---|---|
| Headache | 23 (92%) |
| Nausea | 22 (88%) |
| Diarrhea | 22 (88%) |
| Peripheral edema | 21 (84%) |
| Abdominal pain | 20 (80%) |
| Increased sweating | 18 (72%) |
| Sinusitis | 17 (68%) |
| Vomiting | 17 (68%) |
| Pain | 16 (64%) |
| Fatigue | 16 (64%) |
| Rhinitis | 15 (60%) |
| Dyspnea | 15 (60%) |
| Skin disorder | 15 (60%) |
| Flu-like symptoms | 15 (60%) |
| Insomnia | 15 (60%) |
| Asthenia | 14 (56%) |
| Fever | 14 (56%) |
| URI | 14 (56%) |
| Chest pain | 13 (52%) |
| Rigors | 13 (52%) |
| Dizziness | 13 (52%) |
| Flushing | 12 (48%) |
| Hyperkinesia | 12 (48%) |
| Back pain | 12 (48%) |
| Gastro-intestinal disorder, NOS | 11 (44%) |
| Pharyngitis | 10 (40%) |
| Tinnitus | 10 (40%) |
| Malaise | 10 (40%) |
| Erythematous rash | 9 (36%) |
| Skeletal pain | 9 (36%) |

*Comment: The AE possibly associated with infusion reactions are highlighted above. These are notable in that there is evidence for infusion reactions persisting among some subjects over the year long therapy--although the pattern is generally one of decreasing severity with time. Contrary to this assessment, the sponsor states that "no subjects were experiencing infusion reactions at the end of Study TKT006."*

Of the 11 subjects who had received placebo during Study TKT003, two experienced infusion reactions during Study TKT006. Subjects 3 and 14 developed rigors 20 - 56 minutes after the end of the enzyme infusion. These episodes were treated with IV diphenhydramine and hydrocortisone. One subject also had back pain and the other subject had flushing. Neither subject experienced hypotension. Subsequently, the two subjects were premedicated with hydrocortisone and diphenhydramine.

Of the 8 subjects in the prior TKT003 Agalsidase group who had experienced infusion reactions, all had their premedication regimens tapered in Study TKT006.

Overall, at the end of Study TKT006, 10 of 25 subjects were receiving premedications for infusion reactions (40%).

*Comment: The incidence of infusion reaction was approximately 57% in Study TKT003, an outcome which the sponsor relates to the infusion of the study drug over 20 minutes. In Study TKT006, the study drug was infused over 40 minutes and this appears to have decreased the infusion reaction rate modestly (40%, 10/25)--albeit most of these infusion reactions were early in the clinical study. Thus there does appear to have been at least a lessening of frequency and severity of infusion reactions, although a complete elimination of infusion reactions with the lengthening of infusion duration does not seem to have occurred.*

**C. Serious adverse events:**

There were no deaths during the study and no study drug discontinuations due to adverse events.

Eight subjects experienced a total of 11 serious adverse events. In addition, there were two serious adverse events that were continuing from Study TKT003. None of the serious adverse events were assessed as study drug-related by the site investigator.

**Table 41. Study TKT006 serious adverse events**

| Subject | SAE | 006 treatment duration at onset of SAE (days) |
|---|---|---|
| ***Continuing from Study TKT003*** | | |
| 05 (placebo) | Renal failure | N/A |
| 21 (Agalsidase) | Hearing decreased | N/A |
| ***SAEs with onset during Study TKT006*** | | |
| 01 | Ataxia,<br>chest pain | 163<br>222 |
| 05 | Hearing decreased | 49 |
| 07 | Hearing decreased | 34 |
| 10 | Viral hepatitis (C) | 23 |
| 12 | Abdominal pain | 215 |
| 14 | Coronary artery disorder<br>Burn | 98<br>197 |
| 16 | B12 deficiency<br>Muscle weakness | 14<br>37 |
| 21 | Cerebrovascular disorder | 282 |

**D. Clinical laboratory:**

In general, there are no safety signals from review of laboratory data

## E. Summary of Study TKT006

Study TKT006 was the first "extension" study following Study TKT003. Study TKT006 allowed all subjects who completed Study TKT003 to receive Agalsidase for approximately one year. The dose was the same as that tested in Study TKT003 (Agalsidase infused over 40 minutes). Study TKT006 used an open-label, uncontrolled design in which various outcomes (changes from baseline of the study) were assessed sequentially during the study. The study used three primary endpoint headings: cardiac mass, GFR and pain scores (subjects had "pain medications" stopped in a manner similar to that used in Study TKT003). A large number of secondary endpoints were to be analyzed. Additionally, pharmacokinetic outcomes were to be analyzed.

Overall, 25 subjects completed Study TKT003, all 25 enrolled and completed Study TKT006.

The study's major outcomes included comparisons from baseline of the study (end of Study TKT003) to the one year follow-up time point. In general, subjects were divided into two groups: "prior placebo" subjects (n = 11) and "prior Agalsidase" subjects (n = 14).

The study's primary endpoints were comparisons of changes from baseline in the following: cardiac mass by MRI, GFR and pain. Overall, the primary endpoint results were generally similar for all three primary endpoints in showing that open-label administration of Agalsidase to prior placebo subjects resulted in nominally statistically significant changes from baseline. There were no remarkable changes in these primary endpoint results for the prior Agalsidase subjects (subjects who had already received six months of Agalsidase).

A large number of secondary endpoints were analyzed in a manner similar to that for the primary endpoints. The most remarkable of these outcomes was the finding that echocardiographic assessments of left ventricular mass showed no nominally statistically significant difference for either the prior Agalsidase group or the prior placebo group. The assessments of changes in CTH ($Gb_3$) biomarkers showed that prior placebo subjects had decreases in both the urine sediment CTH ($Gb_3$) content and plasma CTH ($Gb_3$) concentration. The prior Agalsidase subjects had a decrease in the urine sediment CTH ($Gb_3$) content and no nominally statistically significant change in plasma CTH ($Gb_3$).

Especially notable was the finding that antibody formation to Agalsidase appeared to impact Agalsidase pharmacokinetics. In general, subjects with Agalsidase antibodies appeared to have accelerated clearance and decreased AUC of the enzyme as compared to subjects without antibodies.

Safety findings were notable for showing that the incidence of infusion reactions was lower in this study than in Study TKT003 and that the severity of these reactions also lessened. Antibody formation data were notable for showing that slightly more than half the subjects developed some evidence of antibody formation. In a small proportion of these subjects the magnitude of the antibody formation appeared to decrease during the study while a similar proportion appeared to have an increase in the magnitude of the antibody formation.

## 14. Study TKT011 (interim report):

### A. Overview:

Study TKT011 was an open label, uncontrolled study that allowed all subjects who completed Study TKT006 to continue receiving Agalsidase indefinitely (until licensure of the product or termination of the study by the sponsor). Consequently, the sponsor submitted an interim report (one year of follow-up in Study TKT011). This report included a summary of data cumulative for 2 years of Agalsidase administration for some subjects (Study TKT003 placebo subjects) and 2.5 years of Agalsidase administration for other subjects (Study TKT003 Agalsidase subjects). Notably, this study differed from prior studies by the allowance for administration of Agalsidase in subject homes (using trained home health care nurses) or local private physician medical offices. Studies TKT003 and TKT006 had permitted study drug infusion only at the NIH investigative site. However, subjects, at their request, could also continue receiving the infusions at NIH during TKT011.

### B. Protocol:

The study protocol was entitled, "An open label clinical study of alpha-galactosidase A replacement therapy for patients who completed study TKT006" (Study TKTCSR011-1). The major aspects of the study protocol are summarized below.

**1. Design:** Open label, uncontrolled study in which all subjects (potentially 25) who completed Study TKT006 were to receive Agalsidase at the same dose as previously tested, indefinitely. The study was to be divided into 25 week cycles with adverse event data collected at every infusion and comprehensive evaluations performed every 25 weeks.

**2. Objectives:** The primary objective was the collection of safety information and the secondary objectives were the detection of certain sentinel clinical events and efficacy information.

**3. Dose:** Agalsidase 0.2 mg/kg/IV administered over 40 minutes every other week. Vials of product were to be diluted in 100 mL saline and administered with in-line 0.2 micron filters. No premedication was to be routinely administered.

**4. Evaluations:** During weeks 1 - 23 of each cycle, subjects were to have adverse events, concomitant medications and vital signs recorded. During the last week of each cycle (week 25), subjects were to undergo evaluations at NIH, as follows: height, weight, vital signs, physical examinations, adverse event and concomitant medication recording, EKG, serum chemistry, hematology, urinalysis, creatinine clearance, plasma CTH ($Gb_3$), serum anti-Agalsidase concentration, pharmacokinetic data. At week 51 a cardiac MRI, quantitative sensory (with nerve conduction velocities) testing, cerebral MRI and GFR was to be performed.

**5. Endpoints and statistical assessments:** The primary endpoint was to be a summary of the major safety outcomes: vital signs, adverse events, EKG, laboratory data, creatinine clearance and anti-Agalsidase blood concentrations.

The secondary endpoints were divided into two groups: sentinel clinical events and efficacy data. The sentinel clinical events included renal dialysis, stroke or myocardial infarction. The efficacy endpoints included plasma CTH ($Gb_3$) outcomes and GFR.

The endpoint results were to be summarized and change from baseline analyzed with statistical methods using paired t-tests or Wilcoxon signed-rank tests.

### C. Study conduct:

This report is an interim study report to an on-going study. The most remarkable protocol violations were relatively minor and included two subjects who missed a single infusion.

**D. Results:**

**1. Subject disposition:**

A total of 24 subjects were enrolled into Study TKT011. At the end of the Study TKT011 interim reporting period, a total of 22 subjects continue to receive Agalsidase. A summary of subjects disposition through all three studies is shown in Table 42.

**Table 42. Study 003, 006, 011 subject disposition**

| Group | Study 003 Agalsidase group | Study 003 placebo group |
|---|---|---|
| Study TKT003 | | |
| Enrolled | 14 | 12 |
| Completed | 14 | 11 |
| Study TKT006 | | |
| Enrolled | 14 | 11 |
| Completed | 14 | 11 |
| Study TKT011 | | |
| Enrolled | 13 | 11 |
| On-going | 12 | 10 |

The subjects who did not complete the series of three studies (Study TKT003, TKT006 and TKT011), included the single subject who withdrew from Study TKT003 at week 21 and three subjects (not enrolling into or withdrawing from Study TKT011) who moved or returned to non-US home countries. All three of the latter subjects reportedly continue to receive Agalsidase in their home country.

**2. Baseline characteristics:**

The baseline characteristics for the group were described in detail within the review of Study TKT003. All subjects were male and 22 of the 24 were Caucasian (two subjects were Hispanic). At the beginning of Study TKT011, the mean age was 36.4 years (range of 21 to 49) and the mean duration of illness was 13.6 years (range 2 to 35).

**3. Primary endpoints: major safety outcomes:**

**a. Concomitant medication usage:**

Table 43 shows the most notable concomitant medication usage during the study.

**Table 43. Study TKT011 major concomitant medication usage (by drug class)**

| Drug | Number, (%), n = 24 |
|---|---|
| Any antiepileptic/neuropathic medications | 22 (92%) |
| Ace-inhibitors | 5 (21%) |
| Any analgesics (includes aspirin, acetaminophen) | 19 (79%) |
| Any systemic antibiotics | 13 (54%) |
| Any systemic antihistamines | 9 (38%) |
| H2 receptor antagonists | 8 (33%) |

*Comment: It is notable that neuropathic medication usage was substantial during the one year interim study reporting period (92% of subjects)--an observation suggesting that prolonged Agalsidase administration had little impact upon "neuropathic pain medication" usage. During Study TKT003, the sponsor had reported that four of the 26 enrolled subjects discontinued neuropathic pain medications (subjects 6, 8, 12 and 15). Subject 12 did not enroll in Study TKT011. Subjects 6, 8 and 15 all had to use*

*neuropathic pain medications in Study TKT011. This suggests the cessation of the pain medication for these subjects during Study TKT003 was transient, either a transient improvement or a trial-participation event unrepresentative of their true status.*

**b. Adverse events:**

All 24 subjects experienced at least one adverse event. The most common (occurring in more than 1/3 of the subjects) adverse events are summarized within Table 44 (number of subjects experiencing the event).

**Table 44. Study TKT011 Adverse events occurring in more than 1/3 of the subjects**

| Body system/preferred term | number (%), n = 24 |
|---|---|
| Body as a whole | |
| Pain | 18 (75%) |
| Fatigue | 17 (71%) |
| Fever | 16 (67%) |
| Headache | 14 (58%) |
| Rigors | 11 (46%) |
| CNS/PNS disorders | |
| Dizziness | 15 (63%) |
| Neuropathy | 11 (46%) |
| GI system disorder | |
| Nausea | 17 (71%) |
| Diarrhea | 14 (58%) |
| Vomiting | 13 (54%) |
| Abdominal pain | 12 (50%) |
| Anorexia | 9 (38%) |
| Musculoskeletal disorders | |
| Myalgia | 14 (58%) |
| Arthralgia | 11 (46%) |
| Psychiatric disorders | |
| Insomnia | 13 (54%) |
| Resistance mechanism disorders | |
| Infection | 12 (50%) |
| Respiratory system disorder | |
| Coughing | 13 (54%) |
| Congestion | 13 (54%) |
| Sinusitis | 11 (46%) |
| URI | 11 (46%) |

Most adverse events were graded as mild to moderate severity. Fourteen (58%) of the subjects experienced adverse events of severe grade. The most notable severe grade adverse events were also serious adverse events and are discussed below.

Most adverse events were assessed as "not related to" or "possibly related to" the study drug by the site investigator. However, four subjects experienced adverse events (all infusion reactions) that were assessed as "related." These events are also summarized below.

*Comment: In general, the most common adverse events were consistent with the natural history of Fabry disease.*

**c. Infusion reactions:**

Six subjects (25%) experienced at least some form of infusion reaction. All infusion reactions were graded as mild to moderate severity and no reaction required cessation of the infusion. In general, the most common reactions were rigors and flushing (in 3 subjects), fever and dizziness (in 2 subjects). The scope

of infusion reactions are shown in Table 45 (where the number of subjects experiencing each event is summarized).

**Table 45. Study TKT011 Infusion reaction adverse events**

| Event | n (%), n = 24 |
|---|---|
| Rigors | 5 (21%) |
| Fever | 3 (13%) |
| Flushing | 3 (13%) |
| Dizziness | 2 (8%) |
| Gastritis | 1 (4%) |
| Malaise | 1 (4%) |
| Hypertension | 1 (4%) |
| Dysphonia | 1 (4%) |
| Nausea | 1 (4%) |
| Esophagitis | 1 (4%) |
| Rhinitis | 1 (4%) |
| Coughing | 1 (4%) |
| Dyspnea | 1 (4%) |
| Throat tightness | 1 (4%) |
| Increased sweating | 1 (4%) |

**d. Serious adverse events:**

Six subjects experienced serious adverse events during one year of Study TKT011. Two of these subjects also experienced serious adverse events following week 52 (the cut off of the interim report) and these events were also described by the sponsor. The serious adverse events are summarized within Table 46. None of these events were attributed to the study drug by the site investigator.

**Table 46. Study TKT011 Serious adverse events**

| Subject | Event |
|---|---|
| 0005 | Urinary tract infection; renal transplant |
| 0006 | Pacemaker implantation; pneumonia |
| 0010 | Abdominal pain |
| 0011 | Transient ischemic event; cellulitis; another cellulitis episode |
| 0014 | Chest pain; atrial fibrillation; prostatitis; pneumonia; myocardial infarction; acute mental illness |
| 0026 | Cerebellar stroke; Transient ischemic attack |

**e. Antibody formation:**

The sponsor performed ELISA assays for all but one of the month 6 and 12 samples. A positive response was defined as "an increase in absorbance at 490 nm of 2 fold over the baseline and a absorbance $\geq$ 0.04."

Overall, of the 22 subjects who completed one year of Study TKT011, eight subjects (33%) had positive ELISA antibody results. Of these eight subjects, seven subjects had progressive increases in the antibody magnitude (based upon absorbance units) during the series of three clinical studies (Studies TKT003, TKT006, TKT011). No subject who had negative ELISA results prior to enrollment in Study TKT011 developed positive antibody results.

Overall, 13 of the 25 (52%) subjects from Studies TKT003 and TKT006 had positive ELISA results at some point prior to the beginning of Study TKT011. All 13 of these subjects enrolled in Study TKT011 and at the one year interim time of Study TKT011, nine were still positive (this includes subject 27 who provided blood samples but was receiving Agalsidase outside Study TKT011). One subject (subject 22) did not have a Study TKT011 week 49 sample but the week 25 sample was positive (hence, possibly 10 of the original 13 positive subjects may have remained positive).

The sponsor also performed *in vitro* enzyme neutralization assays for the blood samples. In general, these results were similar to the ELISA results.

**f. Clinical laboratory results:**

In general, there were no remarkable alterations in clinical laboratory results during one year of Study TKT011.

**4. Secondary endpoints:**

**a. Sentinel clinical events:**

The protocol identified the incidence of three sentinel clinical events for analyses: end stage renal disease, myocardial infarction and stroke.

-Only one subject progressed to end stage renal disease during the totality of the three sequential studies (Studies TKT003, 006 and 011). This subject began dialysis during Study TKT003 and underwent a renal transplant during Study TKT011.

*Comment: Within the BLA submission, the sponsor notes that published literature suggests 36.7 years is the mean age of onset of end stage renal disease in Fabry disease and that the mean age of Study TKT011 subjects at the end of the one year follow-up was 37.4 years. Consequently, the sponsor suggests that more subjects in Study TKT011 would be expected to be in end stage renal disease unless Agalsidase had a treatment effect. However, the appropriateness of this interpretation is unclear since the published reports are selected clinical data and the subjects in the TKT studies were selected with potentially different criteria. It is conceivable that published reports represent patients more similar to subject 5--the one subject who developed end stage renal disease during the series of TKT clinical studies. Historical comparisons in this case are unable to provide any clear conclusions.*

-Two subjects experienced strokes during one year of Study TKT011 (subject 11 and 26, see serious adverse event summary, above). Overall, during the 30 months of this sequential series of TKT clinical studies, three subjects experienced strokes (subject 21 had a stroke during Study TKT006).

-Only one subject experienced a myocardial infarction during Study TKT011 (subject 14, see serious adverse event report, above). This is the only report of a myocardial infarction during this sequential series of TKT clinical studies.

**b. Other efficacy endpoints:**

*Comment: The protocol listed plasma CTH ($Gb_3$) and GFR as the "efficacy endpoints." In order to be comprehensive, a number of additional endpoints (post hoc) are summarized here.*

**1. Creatinine clearance, GFR and other renal function tests:**

These results were summarized with Study TKT003 data.

**2. Plasma CTH ($Gb_3$) content:**

Agalsidase treatment during Study TKT011 did nor result in any further decreases in mean plasma CTH ($Gb_3$) concentrations. Both the prior-placebo and prior-Agalsidase subjects (from Study TKT003) had small increases in mean plasma levels during Study TKT011 (results shown in Appendix G).

There were eight subjects (two placebo subjects from 003 and six Agalsidase subjects from 003) who completed the series of studies with persistently positive immunological reactions throughout Study TKT011

(blood ELISA results). The plasma CTH ($Gb_3$) results for these eight subjects during their course of receiving Agalsidase are shown in Table 47. Tables 47 and 48 use the baseline value as the mean of the two values immediately prior to initiation of Agalsidase administration (consistent with the Study TKT003 analytical plan).

**Table 47. Plasma CTH ($Gb_3$) in subjects persistently ELISA positive throughout Study TKT011**

| Outcome | nmol/ mL; Mean ± SE |
|---|---|
| Baseline, n = 8 | 12.6 ± 1.4 |
| Change after: | |
| 6 months, n = 8 | - 7.1 ± 1.5 |
| 1 year, n = 8 | - 4.3 ± 1.5 |
| 2 years, n = 8 | - 3.6 ± 1.5 |
| 2.5 years, n = 6 | - 3.2 ± 1.8 |

There were 14 subjects who completed the series of studies without persistently positive immunological reactions in Study TKT011 (blood ELISA results). The plasma CTH ($Gb_3$) concentration results for these subjects are shown in Table 48. Table 48 excludes subjects 20, 12, 22, 27 (subjects who did not complete the series) and the eight subjects listed in Table 47, but includes subjects who were intermittently positive.

**Table 48. Plasma CTH in subjects not persistently ELISA positive in Study TKT011**

| Outcome | nmol/mL; Mean ± SE |
|---|---|
| Baseline, n = 14 | 10.8 ± 1.0 |
| Change after: | |
| 6 months, n = 14 | - 5.3 ± 0.8 |
| 1 year, n = 14 | - 5.6 ± 0.9 |
| 2 years, n = 14 | - 5.4 ± 0.8 |
| 2.5 years, n = 6 | - 5.4 ± 0.6 |

*Comment: Tables 47 and 48 suggest there is an impact of immunoreactivity upon blood CTH ($Gb_3$) concentrations: i.e., antibody formation appears to be associated with a trend of return toward the baseline values of this bioactivity marker. These changes are illustrated more clearly in Figure 2, below.*

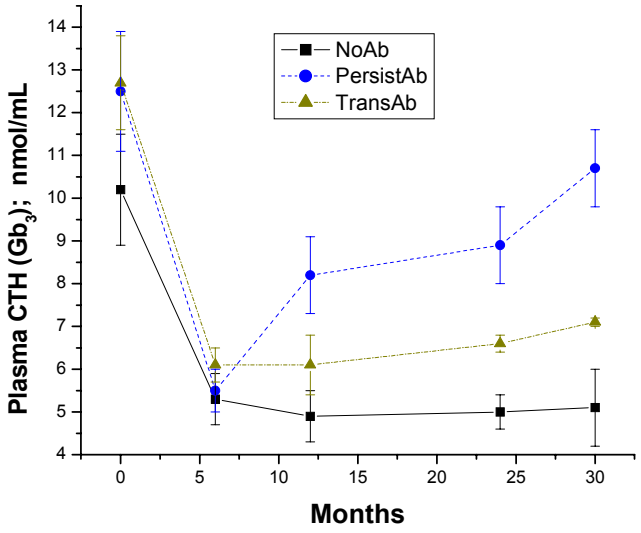



**Figure 2. Plasma CTH ($Gb_3$) results among subjects who completed Study TKT011**

*Within Figure 2 the subjects who completed Study TKT011 are divided into three groups:*

*-"No Ab"--those with no antibody formation during Agalsidase administration*
*(n = 11, except for the month 30 time point where n = 4)*

*-"Persist Ab"--those with persistent antibody formation during Agalsidase administration*
*(n = 8, except for the month 30 time point where n = 6)*

*-"Trans Ab"--those with transient antibody formation during Agalsidase administration*
*(n = 3, except for month 30 time point where n = 2)*

**3. Urine sediment CTH ($Gb_3$) content:**

Agalsidase treatment resulted in initial decreases in urine CTH ($Gb_3$) content in all subjects compared to the pre-Agalsidase values. However, during Study TKT011 the urine CTH ($Gb_3$) content did not decrease further from the start of Study TKT011 values in either group, and may have increased somewhat in the group with the longest Agalsidase exposure (the prior-Agalsidase group), as shown in Table 49 (a copy of the sponsor's table that shows the results for the subjects treated in the series of 3 studies).

**Table 49. Change from baseline in urine sediment $Gb_3$ content**

| Month of treatment/Study | nmol/g creatinine; Mean ± SE | p-value |
|---|---|---|
| ***Subjects treated with placebo in 003 and Agalsidase in 006 and 011 (n = 11)*** | | |
| Month 0/003 baseline, n = 11 | 2162 ± 383 | not applicable |
| Month 6/003 end, n = 11 | 2495 ± 553 | |
| Month 12/006, n = 11 | 624 ± 124 | |
| Month 18/006, n = 11 | 456 ± 135 | |
| Month 24/011, n = 10 | 597 ± 280 | |
| Month 30/011, n = 10 | 477 ± 190 | |
| Change during 003, n = 11 | 333 ± 400 | 0.42 |
| Change from 003 end to: | | |
| Month 12 (6 months Agalsidase) n = 11 | - 1871 ± 506 | < 0.01 |
| Month 18 (1 year Agalsidase) n = 11 | - 2039 ± 496 | < 0.01 |
| Month 24 (1.5 years Agalsidase) n = 10 | - 1992 ± 396 | < 0.01 |
| Month 30 (2 years Agalsidase) n = 10 | - 2112 ± 472 | < 0.01 |
| ***Subjects treated with Agalsidase in 003, 006 and 011*** | | |
| Month 0/003 baseline, n = 13 | 2496 ± 303 | not applicable |
| Month 6/003 end, n = 13 | 1809 ± 459 | |
| Month 12/006, n = 13 | 1159 ± 219 | |
| Month 18/006, n = 13 | 868 ± 191 | |
| Month 24/011, n = 12 | 1494 ± 401 | |
| Month 30/011, n = 12 | 1323 ± 352 | |
| Change from 003 baseline to: | | |
| Month 6 (6 months Agalsidase) n = 13 | - 687 ± 321 | < 0.01 |
| Month 12 (1 year Agalsidase) n = 13 | - 1337 ± 238 | < 0.01 |
| Month 18 (1.5 years Agalsidase) n = 13 | - 1628 ± 231 | < 0.01 |
| Month 24 (2 years Agalsidase) n = 12 | - 1065 ± 312 | < 0.01 |
| Month 30 (2.5 years Agalsidase) n = 12 | - 1236 ± 274 | < 0.01 |

The urine CTH ($Gb_3$) content results for the eight subjects who completed the series of studies with persistently positive antibody results are shown in Table 50. In tables 50 and 51, the baseline value is the first of the two baseline results immediately prior to beginning Agalsidase administration.

*Comment: In the urine sediment CTH ($Gb_3$) content analyses, the baseline measure is the first of the two consecutive urine sediment CTH ($Gb_3$) measurements. The statistical plan for Study TKT003 indicated that*

*the mean of the two baseline urine sediment value was to be used unless the kidney biopsy "confounded" the result ("for example, gross hematuria"). But, because the plan had no specific identification of the criteria other than gross hematuria, the first baseline value is used.*

**Table 50. Urine sediment CTH ($Gb_3$) content in subjects persistently ELISA positive in Study TKT011**

| Outcome | nmol/g creatinine; Mean ± SE |
|---|---|
| Baseline, n = 8 | 3751 ± 561 |
| Change after: | |
| 6 months, n = 8 | - 1250 ± 813 |
| 1 year, n = 8 | - 2207 ± 623 |
| 2 years, n = 8 | - 1552 ± 738 |
| 2.5 years, n = 6 | - 1021 ± 474 |

There were 14 subjects who completed the series of studies without persistently positive immunological reactions in Study TKT011 (blood ELISA results). The urine sediment CTH ($Gb_3$) content results for these subjects are shown in Table 51. Table 51 excludes subjects 20, 12, 22, 27 (subjects who did not complete the series) and the eight subjects listed in Table 50.

**Table 51. Urine sediment CTH ($Gb_3$) in subjects not persistently ELISA positive in Study TKT011**

| Outcome | nmol/g creatinine; Mean ± SE |
|---|---|
| Baseline, n = 14 | 1996 ± 347 |
| Change after: | |
| 6 months, n = 14 | - 1465 ± 258 |
| 1 year, n = 14 | - 1600 ± 317 |
| 2 years, n = 14 | - 1631 ± 328 |
| 2.5 years, n = 6 | - 1711 ± 646 |

*Comment: Similar to the plasma CTH ($Gb_3$) results, Tables 50 and 51 suggest there may be an impact of immunoreactivity upon urine sediment CTH($Gb_3$) concentrations: positive antibody formation appears to be associated with loss of Agalsidase impact upon this bioactivity marker.*

**4. Weight:**

The sponsor notes that, during Study TKT003, placebo group subjects lost weight while Agalsidase group subjects gained weight (p = 0.03). The sponsor notes that during the subsequent series of studies to the interim point of TKT011, the group of subjects receiving 2.5 years Agalsidase experienced a net increase of 1.5 kg from the baseline of Study TKT003 and the prior-placebo subjects who had received 2 years of Agalsidase had 2.7 kg weight increase from the pre-Agalsidase time. These results are shown in Appendix G.

*Comment: The sponsor interprets the weight gain associated with Agalsidase administration in Study TKT003 as indicative of improved nutrition. However, the details of the individual subject course raise uncertainties regarding the impact of fluid balance upon these values (see Weight endpoint discussion in Study TKT003 review section). There were also no other measures of nutrition examined in the study. Consequently, no conclusions regarding a beneficial effect upon subject weight in any of these studies can be formed.*

**E. Summary:**

One year of Study TKT011 provided follow-up clinical data from 22 subjects who received a total of either 2 or 2.5 years of Agalsidase administration during a series of three studies beginning with Study TKT003. Study TKT003 was the only controlled study among this group of studies and randomized subjects to either Agalsidase or placebo with follow-up over a six month period. Subsequently, all subjects who completed Study TKT003 were eligible to receive Agalsidase for one year in Study TKT006, and then indefinitely in

TKT011. Twenty-four subjects were enrolled into Study TKT011 and 22 completed the study up to the one year interim time point. All 25 subjects who received Agalsidase at any time point are reported by the sponsor as continuing to receive the product (three subjects receive the product outside Study TKT011).

Study TKT011 results provide solely uncontrolled clinical data. These data are most remarkable for providing safety information, especially with respect to antibody formation and its impact upon certain bioactivity outcomes. The major safety findings are the following:

- The pattern of adverse events and serious adverse events is most remarkable for an apparent decrease in the incidence of infusion reactions over time. Whereas the infusion reaction rate was approximately 57% during Study TKT003, the rate had decreased to approximately 25% during Study TKT011 interim analysis. In general, the infusion reactions were mild to moderate grade severity.

- Approximately one-third of the subjects who received Agalsidase in the series of studies experienced progressive increases in the magnitude of antibody formation. Most remarkably, this increasing extent of antibody formation appeared to be associated with the loss of activity in two biomarkers: urine sediment CTH ($Gb_3$) content and plasma CTH ($Gb_3$) concentration.
- The only subject who progressed to renal failure during the series of subjects was subject 5, a subject who began dialysis during Study TKT003. This subject underwent transplantation during Study TKT011 and continued to receive Agalsidase.

- Renal function data suggest that, during the sequence of studies, approximately half the subjects had increases in renal function values and half had decreases in the values following Agalsidase administration. The overall pattern was generally one of no remarkable change from baseline.

## 15. Study TKT014:

### A. Overview:

Study TKT014 was an open label, uncontrolled study that examined the safety of Agalsidase administration to a group of 15 female adult Fabry disease subjects at the Children's Hospital, University of Mainz in Mainz, Germany. The study was initially designed to examine safety over a long term (more than one year). However, Agalsidase became available in Germany as a marketed product and the study was terminated early. Consequently, the duration of study participation varied from approximately 15 to 55 weeks. The first subject enrolled on December 27, 2000 and the last subject completed the study on January 30, 2002. The dose of Agalsidase was identical to that studied in the sponsor's other major studies: 0.2 mg/kg every other week.

This study is especially notable because it provides some data from females, subjects who had clinical disease consistent with Fabry disease and genotypic documentation of heterozygosity for a mutation in the Fabry disease gene. The sponsor did not perform blood or tissue measures of alpha-galactosidase enzyme content but it is conceivable that all subjects may have had some degree of intrinsic enzyme activity.

As an X-linked disorder, Fabry disease is more common among males. However, published reports describe certain females (heterozygotes) with clinical manifestations of the disease that largely mirror those of men with Fabry disease.[3, 4] The incidence of serious or debilitating complications of the disease have been estimated to occur in approximately 30% of heterozygotes.[5] As in men, these manifestations include renal failure, neuropathy, stroke and cardiac disease.

### B. Protocol:

The study protocol was entitled, "An open label safety and efficacy clinical trial of Replagal enzyme replacement therapy in female patients with Fabry disease" (Study TKT014). The major aspects of the study protocol are summarized below.

**1. Design:** Open label, uncontrolled study in which all subjects (potentially 15) were to receive Agalsidase at the same dose as previously tested. The dose was to be administered indefinitely (until marketing approval or sponsor's termination of the study). The study was to be divided into 13 week cycles with adverse event data collected at every infusion and comprehensive evaluations performed every 13 weeks.

**2. Objectives:** "to evaluate the safety and efficacy of Replagal enzyme replacement therapy in heterozygous female patients with Fabry Disease."

**3. Dose:** Agalsidase 0.2 mg/kg/IV administered over 40 minutes every other week. Vials of product were to be diluted in 100 mL saline and administered with in-line 0.2 micron filters. Infusions were to be administered at the clinical site on an out-patient basis.

**4. Evaluations:** During weeks 1 - 13 of each cycle, subjects were to have adverse events, concomitant medications and vital signs recorded at the time of each infusion. At baseline and during the last week of each cycle (week 13), subjects were to undergo evaluations at the site, as follows: height, weight, vital signs, physical examinations, adverse event and concomitant medication recording, EKG, serum chemistry, hematology, urinalysis, creatinine clearance, plasma CTH ($Gb_3$), serum anti-Agalsidase concentration,

[3] Whybra, C, Kampmann, C, Willers, I, et.al. Anderson-Fabry disease: clinical manifestations of disease in female heterozygotes. J Inherit Metab Dis; 24:715-24; 2001.
[4] Whybra, C, Wendrich, K, Ries, M, et.al. Clinical manifestation in female Fabry Disease patients; in Rare Kidney Diseases; Schieppati, et. al. ed. Basel, Karger, 2001.
[5] MacDermot, K, Holmes, A, Miners, A. Anderson-Fabry disease: clinical manifestations and impact of disease in a cohort of 60 obligate carrier females. J Med Genet; 38:769-807; 2001.

echocardiogram, BPI pain scale assessment, SF36 assessment and EuroQoL (EQ-5D) assessment. Pharmacokinetic data were to be collected at the time of the first dose.

**5. Endpoints and statistical assessments:** The safety outcomes were to be summarized. The primary "efficacy" endpoint was to be a summary of the urine sediment CTH ($Gb_3$) content. Secondary efficacy endpoints included summaries of the following: plasma CTH ($Gb_3$) concentration, BPI pain scale, SF-36 and EQ-5D outcomes and cardiac mass as measured by echocardiography.

**C. Study conduct:**

The study's principal investigator was Dr. Michael Beck at the Children's Hospital, University of Mainz, Germany. The sponsor notes that "all echocardiograms were performed by one experienced cardiologist, digitally saved and later analyzed, and blinded to the name and clinical status of the patient." Dr. Christoph Kampmann at the University of Mainz read the echocardiograms. All clinical laboratory assays were performed at the site. CTH ($Gb_3$) content and concentration assays, pharmacokinetic and antibody assays were performed by the sponsor. The study conduct was monitored by PPD Germany, Karlstrasse, Germany and PPD Development, Nuremberg, Germany and the sponsor. The most remarkable protocol violations were relatively minor and included subjects who missed certain enzyme infusions, as follows: four subjects missed one infusion, one subject missed two infusions and another subject missed three infusions.

**D. Results:**

**1. Subject disposition:**

A total of 15 subjects were enrolled into the study. One subject (subject 2) died during the study and all other subjects were still participating at the time of study termination. As previously noted, the duration of participation in the study varied substantially (Table 52).

**Table 52. Duration of participation in Study TKT014**

| ***Outcome*** | ***Study weeks*** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 | 17 | 27 | 33 | 35 | 40 | 43 | 44 | 45 | 47 | 51 | 55 |
| n completing | 15 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 |

*Comment: Most subjects (approximately two-thirds) received Agalsidase for at least six months. Study participation after the six month time point was widely staggered over the following six months.*

**2. Baseline characteristics:**

All subjects were Caucasian women with clinical manifestations of Fabry disease and genotypically confirmed heterozygosity. The average age was 45 years (range from 20 years to 66) and the average duration of Fabry illness was 14 years (range from initial diagnosis).

**3. Safety endpoint results:**

**a. Concomitant medication usage:**

Table 53 shows the most notable concomitant medication usage during the study.

**Table 53. Study TKT014 major concomitant medication usage (by drug class)**

| Drug | Number, (%), n = 15 |
|---|---|
| Any antiepileptic/neuropathic medications | 2 (13%) |
| Ace-inhibitors and antagonists | 4 (27%) |
| Any analgesics (includes aspirin, acetaminophen) | 4 (27%) |
| Any systemic antibiotics | 9 (60%) |
| Any systemic antihistamines | 3 (20%) |

*Comment: It is notable that few subjects used neuropathic medication, an observation suggesting that most subjects may not have had very much chronic pain or that the pain was not being treated with medication because the baseline BPI pain scores were similar to those of subjects within Study TKT003 (where most subjects received pain medications).*

**b. Adverse events:**

All 15 subjects experienced at least one adverse event. The vast majority of these events were of mild or moderate severity and assessed as either "not related" to the study drug or only "possibly" related. There were only two subjects who experienced severe grade adverse events and these events also qualified as serious adverse events (described below). The most common (occurring in more than three subjects) adverse events are summarized within Table 54 (number of subjects experiencing the event).

**Table 54. Study TKT011 Adverse events occurring in more than three subjects**

| Body system/preferred term | number (%), n = 15 |
|---|---|
| Body as a whole | |
| Flu-like symptoms | 10 (67%) |
| Leg pain | 9 (60%) |
| Other pains | 6 (40%) |
| Back pain | 6 (40%) |
| Fatigue | 3 (20%) |
| Fever | 3 (20%) |
| Cardiovascular disorder | |
| Heart murmur | 3 (20%) |
| CNS/PNS disorders | |
| Headache | 8 (53%) |
| Vertigo | 3 (20%) |
| GI system disorder | |
| Diarrhea | 5 (33%) |
| Dyspepsia | 4 (27%) |
| Nausea | 3 (20) |
| Tooth disorder | 3 (20%) |
| Musculoskeletal disorders | |
| Arthralgia | 6 (40%) |
| Skeletal pain | 3 (20%) |
| Psychiatric disorders | |
| Depression | 5 (33%) |
| Resistance mechanism disorders | |
| Herpes simplex | 3 (20%) |
| Respiratory system disorder | |
| Coughing | 4 (27%) |
| Urinary system disorder | |
| Urinary tract infection | 4 (27%) |

*Comment: In general, the most common adverse events are consistent with the natural history of Fabry disease.*

**c. Infusion reactions:**

No subjects experienced infusion reactions.

*Comment: The lack of infusion reactions may relate to the possibility that the subjects had some intrinsic enzyme activity. Hence, additional enzyme administration (Agalsidase) may not have been viewed as a "foreign" antigen.*

**d. Serious adverse events:**

Three subjects experienced serious adverse events: one subject died due to a stroke following a myocardial infarction, another subject suffered a nonfatal stroke and a third subject had an overnight hospitalization because of hypertension-associated dizziness. All events were regarded by the site investigator as unrelated to the study drug. Notably, Agalsidase was administered during the course of these events.

*Comment: The details of the serious adverse events are not repeated here. In general, the pattern of serious adverse events appears consistent with the subjects' underlying clinical disease, especially for the two stroke subjects (one had severe chronic lung disease and the other had a history of prior strokes).*

**e. Antibody formation:**

No subjects developed antibodies to Agalsidase.

*Comment: As noted in the comment on lack of infusion reactions, the Fabry disease heterozygosity may also have influenced the lack of antibody formation.*

**f. Clinical laboratory results:**

In general, there were no remarkable alterations in clinical laboratory results during Study TKT014.

**4. Efficacy endpoints:**

**a. Primary endpoint of change in urinary sediment CTH ($Gb_3$) content:**

As shown in Table 55, there was no statistically significant change in urinary CTH ($Gb_3$) content. P-values, as in all subsequent study report tables, represent changes from baseline (paired t-test).

**Table 55. Change from baseline in urine sediment CTH ($Gb_3$) content**

| Week of treatment | nmol/24 hours; Mean ± SE | p-value |
|---|---|---|
| Baseline, n = 15 | 400 ± 112 | not applicable |
| Week 13, n = 15 | 246 ± 36 | |
| Week 27, n = 11 | 301 ± 51 | |
| Week 41, n = 7 | 368 ± 81 | |
| **Change from baseline to:** | | |
| Week 13, n = 15 | - 154 ± 80 | 0.08 |
| Week 27, n = 11 | - 140 ± 139 | 0.34 |
| Week 41, n = 7 | - 201 ± 236 | 0.43 |

*Comment: The baseline urine sediment CTH ($Gb_3$) in these subjects is notably lower than in the males in Study TKT003. The urine sediment results for Studies TKT003/006/011 are presented in term of nmol/gm creatinine. If analyzed as nmol/24 hours (as in Study TKT014), the baseline for all subjects in Study TKT003 was 3,336 nmol/24 hours, a value considerably higher than that of the female subjects in Study TKT014. Table 56 compares the urine sediment precursor molecule content among the subjects receiving six month of Agalsidase in Study TKT003 to the baseline value of the subjects in Study TKT014.*

***Table 56. Urine Sediment CTH ($Gb_3$) content in Study TKT003 Agalsidase group & Study TKT014, as nmol/24 hrs***

| *TKT003, n = 14 (Mean ± SE)* | | *TKT014, n = 15 (Mean ± SE)* |
|---|---|---|
| *Baseline* | *Study end* | *Baseline* |
| *3523 ± 458* | *2609 ± 818* | *400 ± 112* |

*These observations raise questions about the meaningfulness of the urine sediment CTH ($Gb_3$) values as a predictor of clinical course or possible gender differences in the pathophysiological correlates of precursor molecule measurements. Although the women of TKT014 had definite clinical symptoms, their baseline urine CTH ($Gb_3$) was lower than achieved in the treatment of men.*

**b. Secondary efficacy endpoints:**

*Comment: The protocol listed plasma $Gb_3$ and GFR as the "efficacy endpoints." In order to be comprehensive, a number of additional endpoints (post hoc) are summarized here.*

**1. Echocardiographic assessment of left ventricular (LV) mass:**

The sponsor states that all four echocardiographic measures of left ventricular mass demonstrated decreases (results shown in Appendix H).

The sponsor notes that the normal LV mass index is < 125 g/$m^2$ and that all subjects with an index above this normal range experienced a decline in the value. There was no statistically significant change in the left ventricular ejection fraction at the various follow-up time points. The average baseline ejection fraction was 71% and the average ejection fraction was 74% at week 27.

*Comment: Echocardiographic assessments may be operator-dependent with respect to assessing the quantitative aspects of certain measures. The lack of a control group, the unblinded treatment and an unverifiable echocardiographic blinding procedure substantially limits the interpretability of these data.*

**2. SF-36, Brief Pain Inventory pain scale (BPI) and EQ-5D changes:**

The protocol indicated that scores from three different scoring systems would be used to measure various components of "quality of life (QOL)." These three scoring systems were the SF-36, BPI and EQ-5D. These systems consist of multiple component subscores with no single composite score for any system. Consequently, a large number of scores resulted from these assessments.

Overall, the QOL measures revealed very variable results with inconsistent changes from baseline at the various follow-up time points. In general, no meaningful patterns were evident. For example, there was no statistically significant change from baseline in the BPI measures of worst pain item, the average pain severity score or the average pain interference score. Similarly, the EQ-5D analyses showed a pattern of no notable changes from baseline in the various components: mobility, self-care, usual activities, pain or anxiety.

*Comment: The various measures of physical, social, mental functioning and pain show no consistent pattern of changes from baseline over time.*

**3. Plasma CTH ($Gb_3$) content:**

The baseline mean plasma CTH ($Gb_3$) content in this study ( 5.7 nmol/mL) is approximately half of the value observed for men in Study TKT003. The mean changes from baseline were - 0.9, - 0.7, - 0.7 nmol/mL at weeks 13, 27, 41 respectively, with successively decreasing numbers of subjects (n = 15, 11, 8). Both the

absolute and fractional decrease in TKT014 plasma CTH (Gb3) content were less than that seen in TKT003.

A notable observation is that, although these women had symptoms at enrollment, the women's mean baseline plasma CTH ($Gb_3$) is similar to that in the Agalsidase group in TKT003 after treatment.

**4. Exploratory efficacy endpoints:**

**(A) EKG changes:**

A statistically significant change in QRS duration from baseline was noted at week 27 while the changes at the other time points were very variable, see table of results in Appendix H .

**(B) Creatinine clearance, serum creatinine, urine total protein:**

There were no remarkable changes from baseline in creatinine clearance, serum creatinine or 24 hour urine total protein. The creatinine clearance data are summarized in Appendix H.

**E. Summary:**

Study TKT014 was an open label, uncontrolled study conducted in Germany at a single clinical center among 15 women. The study was terminated by the sponsor once Agalsidase became commercially available. Consequently, the extent of participation of the subjects ranged from 13 to 55 weeks. The major safety findings were notable for two subjects experiencing strokes, with one fatality related to the stroke. The strokes were assessed as consistent with the underlying Fabry disease natural history and each subject's past medical history. Notably, no subjects had antibody formation to Agalsidase and no subjects experienced infusion reactions. The major exploratory efficacy endpoints were notable for showing that the baseline urine sediment and plasma CTH ($Gb_3$) values were substantially less than those for the men participating in the Study TKT003. There were very variable changes from baseline in these values with no pattern of consistent statistically significant reduction of the average values from baseline. The clinical endpoints also showed no consistent pattern of improvement from baseline except in the echocardiographic assessment of left ventricular mass where four different measures of the mass generally suggested a pattern of loss of mass.

**16. Additional clinical studies (including the design of Study TKT010):**

The sponsor has multiple on-going open-label clinical studies as well as one recently completed study. In general, the on-going studies examine pharmacokinetics and pharmacodynamics in special populations or allow subjects who completed the controlled studies to continue receiving Agalsidase. The one recently completed study is especially notable because it was a double-blind, controlled clinical study (Study TKT010) conducted among a relatively large target subject population. This study design is summarized below.

*Comment: Study TKT010 was completed in 2002 and the sponsor reports that the results are being analyzed. The sponsor has publicly reported that "preliminary review of the six-month data did not show a statistically significant difference between treated and placebo patients for the primary endpoint of renal function, as measured by glomerular filtration rate." As of the date of this briefing document, no additional preliminary reports from Study TKT010 have been provided and no detailed data or analyses have been submitted for FDA review.*

**Study TKT010 Title:**

"A phase 3 randomized, double-blind, placebo-controlled, multi-center clinical trial of alpha-galactosidase A replacement in patients with Fabry disease (Study TKT010)."

The study protocol was submitted to FDA in 2001 and multiple discussions between TKT and FDA regarding the design of this study ensued. The study protocol was amended several times, including after initiation of the study. The notable amendments included an increase in sample size, and a change in the primary endpoint to a comparison of GFR change from baseline. Data analysis from this study is underway. As of the time of this document, results have not been submitted to FDA. If results are known prior to the Advisory Committee Meeting, FDA will not have had adequate opportunity to review any results, and will be largely unable to comment on the reported results.

**Design:**

The protocol called for a multicenter, international, double-blind study in which approximately 74 male Fabry disease subjects were to be randomized (1:1) to a six month treatment period with either placebo or Agalsidase. The Agalsidase dose was 0.2 mg/kg (the same as tested in earlier controlled clinical studies) infused over 40 minutes.

**Subjects:**

Eligible subjects were to have been male subjects ≥ 18 years of age who had Fabry disease evidenced by both clinical and laboratory findings. Clinical findings required the presence of at least one of the following:

-neuropathic pain (subjects with non-Fabry causes of neuropathy were ineligible)
-renal insufficiency or proteinuria (subjects on dialysis or those with a transplant were ineligible)
-cardiomyopathy
-history of stroke
-angiokeratoma
-malabsorption and weight loss.

Laboratory evidence was defined as < 15% of control level of alpha-galactosidase A activity in plasma, serum, leukocytes or cultured fibroblasts.

**Evaluations:**

The major study evaluations related to renal function assessments and pain assessments. GFR was measured at baseline and end-of-study. Creatinine clearance was measured at baseline and weeks 9, 17 and 24. GFR was to be measured using $^{51}$Cr-EDTA outside the USA and $^{99}$Tc-DTPA within the USA. Pain assessments are to be performed in a manner identical to that used in Study TKT003, including the abrupt cessation of pain medications at certain designated follow-up time points and the recording of "off medication" scores. The protocol defined pain medications as tricyclic antidepressants and anticonvulsants.

**Endpoints and analyses:**

The primary endpoint was a comparison of the change in GFR between the two study groups.

The secondary endpoints included the following:

-"Worst pain scores off pain medication"
-pain medication usage
-creatinine clearance
-urine sediment CTH ($Gb_3$) content.

*Comment: Shortly prior to initiating the study analyses, the sponsor revised the study's statistical analytical plan. As of the date of this briefing document, the study's final statistical analytical plan was not available for FDA review, and some details of endpoint selection or analysis may have been changed..*

The primary endpoint of change from baseline in GFR was to be analyzed using an analysis of covariance with the baseline GFR value as a covariate. The analytical plans for the secondary endpoints were to be largely similar to those for the primary endpoint analysis.

There were several limitations identified within the study design that may impair the study's ability to demonstrate efficacy, including the following:

- Due to issues of availability differences in Europe vs North America, the GFR assessment for the primary endpoint was obtained using $^{51}$Cr-EDTA in some subjects and other subjects will have the measurement obtained using $^{99}$Tc-DTPA. The appropriateness of using two different assessment methods is unclear.

- The change in renal function is determined over only a six month period of time. Based upon the findings from Studies TKT003 and TKT005, 6 months may be too brief a period to successfully assess renal function alterations.

- The 6 month duration is especially notable given the potential heterogeneity of the population with regard to baseline renal function and the potential heterogeneity of the rate of progression of renal dysfunction. The study population was not focused upon subjects in a disease stage likely to show renal impairment that was amenable to slowing of progression.

- Most of the systematic problems associated with assessment of "off pain medication" pain scores that were evident from Study TKT003 were present within this study's design. A notable exception is that "pain medication" was prospectively defined as limited to tricyclic antidepressants and anticonvulsants. However, difficulties with this exclusion of traditional analgesics remained.

## 17. Appendices

### Appendix A. Study TKT003 Primary Endpoint Database Construction

The study began on December 12, 1998 and the final subject evaluation occurred on September 2, 1999. During the study, site monitoring visits were performed by a Contract Research Organization (CRO) and by the sponsor. The case report forms were received by the CRO Clinical Data Management group. There were multiple changes of the clinical datasets in the period between the initial review of the unblinded study results and the date of the "final" database lock--the most notable being the dataset for the primary endpoint. These database alterations are described below:

The primary endpoint of the study involved computation of the numeric results of "off medication" worst pain scores at four occasions: baseline, week 9, week 17 and week 24. However, the study was designed such that worst pain scores were required to be obtained at all out-patient visits (alternate weeks) and at any (ad-hoc) time points immediately preceding the resumption of pain medications (at weeks 9, 17 and 24) because of recurrent pain symptoms. Consequently, any single subject had a set of 13 or more BPI scores (depending on the number of ad-hoc scores), but only four of these scores were to be utilized for calculation of the primary endpoint (scores from baseline and weeks 9, 17 and 24, "off medications"). Because subjects could complete ad-hoc BPI forms (at baseline and weeks 9, 17 and 24, based on the need for resumption of pain medication), any single subject could have a set of up to eight possible BPI forms from which the four definitive primary endpoint forms had to be selected (for example, if a subject discontinued pain medication at week eight, as directed, but the subject had to resume pain medication one day later, the subject would complete an ad-hoc week nine BPI form--yet when the subject returned to the clinic for visit number nine, the subject completed the usual week nine BPI form--resulting in two week "nine" BPI forms).

BPI forms were not designed to ascertain whether a patient was "off pain medication" at the time of the recording of the score. Consequently, accurate compilation of the set of true "off pain medication" BPI scores required a review of many study records--and there was no prospective plan for this review methodology. In order to construct the primary endpoint database, it appears that it was necessary for the sponsor to review several items following study completion:

- -review all worst pain scores from the four applicable times (baseline, week 9, week 17 and week 24)
- -review all worst pain scores from the four possible ad-hoc time points
- -review all responses to questions on the BPI short form (especially "What treatments or medications are you receiving for pain?"), information contained on case report forms
- -review the case report form record of concomitant medication usage in order to assess pain medication usage and the specific dates of usage
- -review all medical records and patient medication diaries for concomitant medication usage, including the dates of usage.

*Comment: Conceivably, subjects could have been receiving "as needed" analgesics (such as codeine, narcotics, aspirin) at the time of completion of the "off pain medication" BPI pain scores. It is inherently impossible to assess whether patients were taking "as-needed" analgesics because the study case report forms were not designed to accurately obtain this information. Patient diaries were utilized, but FDA site inspection findings revealed that the dates/times on these diaries were incompletely and inconsistently detailed such that it is impossible to verify whether or not patients were actually "on" or "off" either "as-needed" analgesics or more chronic pain medications at the time of completion of the "off pain medication" BPI pain scores.*

Hence, the primary endpoint dataset was a dataset derived from selection among the many BPI forms.

There was no prospective, standard operating procedure in place describing:

-the organization or individual responsible for constructing the primary endpoint dataset

-the definition of "pain medications"

-the process or timeline for construction of the primary endpoint dataset

The contract research organization responsible for managing the study data was not assigned the responsibility for establishing the primary endpoint dataset. This responsibility appears to have been assumed by the sponsor.

On October 10, 1999 a portion of the clinical study database was locked by the CRO following audit of all BPI "worst pain" score clinical data, but no or incomplete audits of all other clinical data, including pain medication usage data (case report forms and medication diaries). On October 14, 1999 this limited but unblinded database was transmitted to the sponsor. This database consisted of multiple files, including a file of the BPI pain scores containing approximately 450 BPI worst pain scores. However, the BPI data file did not identify which scores were the true "off pain medication" scores. The BPI data file did identify which scores were baseline, weeks 9, 17, and 24 and ad-hoc scores--hence, it was possible to estimate which scores might constitute the primary endpoint dataset and this estimate was utilized in initial data analyses.

The sponsor reviewed these study results and established a "preliminary" primary endpoint dataset by designating scores for inclusion. On October 14, 1999, the sponsor submitted his "preliminary" primary endpoint dataset to Dr. Robert Makuch at Yale University. Dr. Makuch performed analyses of the primary endpoint results (revealing a p-value of 0.43 for the primary endpoint result) and conveyed these results to the sponsor. On October 23, 1999, the sponsor submitted a preBLA submission to FDA which contained the analyses performed by Dr. Makuch as the "preliminary primary endpoint dataset" from the unblinded dataset.

Subsequent to the submission of these data, the sponsor states that, "an initial review of the clinical data by the TKT medical monitor revealed numerous errors and inconsistencies in the clinical database." This finding prompted the sponsor to authorize a TKT clinical research associate to conduct an audit of the clinical site with two goals--to review concomitant pain medication usage and urine chemistry results. This audit was begun on October 29, 1999 and completed on November 4, 1999.

Following the report from the audit, the sponsor utilized certain data from this report and the "preliminary" primary endpoint dataset to construct the final primary endpoint database. This final primary endpoint database was again "locked" on February 18, 2000 (the second database "lock"), along with all other clinical dataset information.

Consequently, the primary endpoint data listings submitted with the BLA consist of a derived primary endpoint dataset (the February 18, 1999 database constructed by the sponsor following a review of the preliminary data) containing all 104 primary endpoint data points and three datasets which were used to generate the derived primary endpoint dataset. These three primary endpoint source datasets consist of the dataset of all BPISF worst pain scores and datasets listing all concomitant medications. In addition to the case report tabulations, the case report forms were also used in deriving the primary endpoint dataset and all case report forms were submitted electronically.

Following completion of the second database lock on February 18, 2000, the sponsor identified three less notable errors within the database and the locked database was manually changed on June 1, 2000 to correct these errors. The three errors consisted of the following: subject 11, week 24 total urine creatinine was changed from 70.0 to 1.41 gms, subject 11, week 17 beta 2 microglobulin was changed from 449 to 0.449 mg/L and subject 14, week 21 lymphocyte count was changed from 256% to 25.6%.

*Comment: The construct of the primary endpoint dataset appears to have substantially involved the study's medical monitor. The medical monitor authorized an audit of certain data following review of the preliminary findings. Consequently, this audit was inherently susceptible to uncontrolled bias. The sponsor generally acknowledges that selective changes of the preliminary primary endpoint dataset was performed.*

*The consequence of the alteration of the "preliminary" primary endpoint dataset was to decrease the primary endpoint p-value from 0.43 to 0.19. This was accomplished largely by changing certain placebo data points to "missing data" such that imputation resulted in a higher pain score than that originally contained in the "preliminary" primary endpoint database.*

*Utilizing the "BPISF" database, alone, to calculate the primary endpoint is impossible because the dataset does not identify the specific data point to be used in the primary endpoint calculation. For example, subject number 1 has a baseline score, then scores for weeks 9, 17 and 24. But subject number 1 also has three scores coded as visit numbers "29," and the "29" encryption defines these scores as an "off pain medication scores." The dataset does not identify which of the six follow-up time points constitute the "true" scores to be utilized in the primary endpoint calculation for this subject--the scores identified as corresponding to visits at week 9, 17 and 24 or the scores identified as ad-hoc "off pain medication" results (the "29" coded visits). The problems with subject number 1 are exemplary of those associated with many other subjects.*

*The difficulty in verifying the accuracy of the primary endpoint database is illustrated by the following two cases (found through random checks):*

*1. Subject number 3 (a placebo subject) had the baseline off pain medication worst score recorded as 10. This BPI completion took place on December 14, 1998. The case report form for the BPI from December 14, 1998 confirms (by the subject's response to question number 7 and by the clinical study monitor's record of concomitant medication usage on the case report form and by the concomitant medication file in the datasets) that this score was obtained while the subject was receiving Tegretol. Consequently, this subject appears to have had no baseline off pain medication score--however, the sponsor elected to include the December 14, 1998 BPISF score as the "off pain medication worst pain baseline score." FDA site inspection of the hospital record for this subject found that the subject had been hospitalized on December 12, 1998 and Tegretol was discontinued at that time. An "off pain medication" worst pain score was recorded on December 14, 1998, prior to resumption of Tegretol. Consequently, the December 14, 1998 "worst pain score off pain medication" appears accurate but the case report form and the case report tabulations appear inaccurate. This observation illustrates the potential errors and inconsistencies within the study data. It is also very important to note that this subject was "off pain medication" for only two days, not the intended seven days.*

*2. Subject number 5 (a placebo subject) had the week 9 off medication worst pain score recorded as 7 in the primary endpoint dataset. This BPI completion took place on March 7, 1999. The case report form for the BPI from March 7, 1999 confirms (by the subject's response to question 7 and by the concomitant medication record) that this score was obtained while the subject was receiving Tegretol. This suggests the subject may have no true off pain medication score for week 9. This illustrates the complexity of identifying the primary endpoint dataset because the case report tabulations and case report forms provide no way to verify that the March 7, 1999 worst pain score was recorded while the subject was off pain medication. Indeed, the study data suggest the subject was receiving Tegretol. Inspection of the on-site hospital records indicate that the Tegretol had been discontinued on March 5, 1999 and the subject completed the BPI shortly prior to resuming Tegretol on March 7, 1999. This is verifiable only because the subject happened to have been hospitalized at this time point. As with the subject described above (subject 1), subject number 5 had been off pain medication for only two days prior to recording the off pain medication BPI score, not the intended seven days. The effect of the shorter off-medication duration is unknown.*

*The sponsor's post-hoc changes of the primary endpoint dataset illustrates the profound limitations of these data and the inability to accurately interpret any of the "off pain medication" results. Conceivably, examination of all pain scores, regardless of whether patients were "off" or "on" pain medications may*

*reveal some useful information. But even this approach has limitations because of the unknown physiological/clinical consequences of the abrupt cessation of neuropathic pain medications.*

## Appendix B. Study TKT003 Pain Evaluations

The following tables provide additional detail regarding the pain outcomes within Study TKT003.

**Table 57. Study TKT003 Pain at its worst while "off pain medications" (Components and Exploratory analyses)**

| Outcome/Components | Agalsidase n = 14 | Placebo n = 12 | Statistical test | p-value |
|---|---|---|---|---|
| Prospective Primary Endpoint Analysis, AUC1 | - 22.4 | - 1.0 | t-test | 0.20 |
| Post-hoc Primary Endpoint Analysis, AUC1 | - 22.4 | - 1.0 | ANCOVA | 0.08 |
| ***Components of Primary Endpoint*** | | | | |
| Score (average ± SE) at: | | | | |
| Baseline | 6.2 ± 0.5 | 7.3 ± 0.6 | N/A | N/A |
| Week 9 | 5.6 ± 0.7 | 7.1 ± 0.8 | | |
| Week 17 | 4.9 ± 0.8 | 7.5 ± 0.6 | | |
| Week 24 | 4.3 ± 0.7 | 6.8 ± 0.6 | | |
| Average change from baseline score at: | | | | |
| Week 9 | - 0.6 | - 0.2 | | |
| Week 17 | - 1.4 | 0.3 | | |
| Week 24 | - 1.9 | - 0.4 | | |
| ***Exploratory Analyses of "off medication" scores*** | | | | |
| Repeated Measures* | Average scores listed above | | ANCOVA | 0.05 |
| Post-hoc Reptd. Meas. | Average scores listed above | | ANOVA | 0.02 |
| Week 24 change | - 1.9 | - 0.4 | t-test | 0.14 |
| ***Missing Value Imputation Sensitivity Analyses*** | | | | |
| AUC1, near value | - 22.4 | - 3.2 | t-test | 0.03 |
| AUC1, worst case | - 22.4 | - 0.4 | t-test | 0.18 |
| AUC1, near value* | - 22.4 | - 3.2 | ANCOVA | 0.11 |
| AUC1, worst case* | - 22.4 | - 0.4 | ANCOVA | 0.08 |
| ***All Visit Assessments ("on" and "off" medication pooled)*** | | | | |
| Area Under the Curve* | 2.1 | - 26.4 | ANCOVA | 0.97 |
| Repeated Measures* | Average scores listed in Table 58 | | ANCOVA | 0.70 |

N/A = not applicable

* Baseline pain score as covariate

The last lines of Table 57 show an exploratory analysis of pain scores that does not differentiate between scores intended to be "on" or "off" pain medication and employs scores from all visits. This analysis has the advantage of being unaffected by the uncertainties of whether or not any particular subject completed the BPI evaluation while on or off medication when intended to be off medication. This analysis may also be more reflective of what patients might experience in actual use of the product, since the "off medication" state is artificial, and patients are likely to use pain medications to the degree needed in daily life. This analysis is unsupportive of any impact of Agalsidase upon pain in this disease. The biweekly mean scores, shown in Table 58, show no suggestion of a beneficial impact on pain.

**Table 58. Study TKT003 Change in all worst pain scores (not just "off pain medication") from baseline to the set of results from all visits**

| Change from baseline to: | Agalsidase, n = 14 (mean) | Placebo, n = 12 (mean) |
|---|---|---|
| Week 1 | 1.9 | - 0.5 |
| Week 3 | - 0.9 | - 1.6 |
| Week 5 | 0.4 | - 0.8 |
| Week 7 | 0.9 | - 1.6 |
| Week 9 | 0.4 | 0.0 |
| Week 11 | 0.6 | - 1.8 |
| Week 13 | - 0.2 | - 0.8 |
| Week 15 | - 0.1 | - 1.2 |
| Week 17 | 0.1 | 0.3 |
| Week 19 | - 0.9 | - 2.1 |
| Week 21 | - 0.9 | - 2.1 |
| Week 23 | - 0.9 | - 1.6 |
| Week 24 | - 0.5 | - 1.2 |

*Comments: Table 58 shows that, at 11 of the 13 assessment time points, the change from baseline was more favorable in the placebo group than the Agalsidase group. The totality of the data provide no evidence that Agalsidase treatment is associated with improvement in the "worst pain" BPI scores.*

Tables 59 and 60 provide additional details upon the secondary endpoints of "Severity" and "interference" aspects of pain in Study TKT003.

**Table 59. Study TKT003 "Severity" of pain secondary endpoint results**

| Outcome/Components | Agalsidase n = 14 | Placebo n = 12 | Statistical test | p-value |
|---|---|---|---|---|
| **"Off Medication" Scores Analysis** | | | | |
| **1. Area Under Curve (mean ± SE)** | -14.3 ± 5.3 | - 6.2 ± 10.2 | t-test<br>ANCOVA | 0.47<br>0.39 |
| **2. "Responders"** | | | | |
| Week 9 | 10 (71%) | 5 (42%) | Fisher's exact test | 0.23 |
| Week 17 | 10 (71%) | 6 (50%) | Fisher's exact test | 0.42 |
| Week 24 | 9 (64%) | 6 (50%) | Fisher's exact test | 0.69 |
| **3. Repeated Measures** | shown below | shown below | ANCOVA<br>ANOVA | 0.40<br>0.02 |
| *AUC and RM Components (means)* | | | | |
| Baseline | 3.8 | 5.4 | N/A | N/A |
| Week 9 | 3.1 | 5.2 | | |
| Week 17 | 3.3 | 5.2 | | |
| Week 24 | 2.7 | 4.7 | | |
| Week 9 change | - 0.8 | - 0.2 | | |
| Week 17 change | - 0.5 | - 0.2 | | |
| Week 24 change | - 1.1 | - 0.7 | | |
| **All Visits Pooled Analysis** | | | | |
| **4. Area Under Curve (mean ± SE)** | -9.6 ± 7.8 | -21.6 ± 12.6 | t-test<br>ANCOVA | 0.41<br>0.95 |
| **5. Repeated Measures** | shown below | shown below | ANCOVA<br>ANOVA | 0.70<br>0.28 |
| *RM2 Components (means)* | | | | |
| Change from baseline to: | | | | |
| Week 1 | 0.4 | - 0.8 | N/A | N/A |
| Week 3 | - 0.8 | - 1.3 | | |
| Week 5 | - 0.1 | - 0.5 | | |
| Week 7 | - 0.2 | - 1.2 | | |
| Week 9 | - 0.5 | - 0.3 | | |
| Week 11 | - 0.3 | - 1.2 | | |
| Week 13 | - 0.6 | - 0.8 | | |
| Week 15 | - 0.6 | - 0.9 | | |
| Week 17 | - 0.3 | - 0.5 | | |
| Week 19 | - 0.8 | - 1.3 | | |
| Week 21 | - 0.7 | - 1.1 | | |
| Week 23 | - 0.5 | - 1.1 | | |
| Week 24 | - 0.8 | - 0.9 | | |

N/A = not applicable

**Table 60. "Interference" of pain secondary endpoint results**

| Outcome/Components | Agalsidase n = 14 | Placebo n = 12 | Statistical test | p-value |
|---|---|---|---|---|
| **"Off Medication" Scores** | | | | |
| **1. Area Under Curve (mean ± SE)** | - 6.9 ± 9.4 | - 9.1 ± 9.7 | t-test<br>ANCOVA | 0.88<br>0.58 |
| **2. "Responders"** | | | | |
| Week 9 | 7 (50%) | 6 (50%) | Fisher's exact test | 1.00 |
| Week 17 | 9 (64%) | 6 (50%) | Fisher's exact test | 0.69 |
| Week 24 | 9 (64%) | 6 (50%) | Fisher's exact test | 0.69 |
| **3. Repeated Measures** | shown below | shown below | ANCOVA<br>ANOVA | 0.22<br>0.05 |
| *AUC1 and RM1 Components (means)* | | | | |
| Baseline | 3.2 | 4.8 | N/A | N/A |
| Week 9 | 3.2 | 4.1 | | |
| Week 17 | 2.8 | 4.6 | | |
| Week 24 | 2.1 | 4.2 | | |
| Week 9 change | - 0.0 | - 0.7 | | |
| Week 17 change | - 0.4 | - 0.2 | | |
| Week 24 change | - 1.1 | - 0.6 | | |
| **All Visits Pooled Analysis** | | | | |
| **4. Area Under Curve (mean ± SE)** | - 20.1 ± 7.1 | - 34.3 ± 13.1 | t-test<br>ANCOVA | 0.33<br>0.81 |
| **5. Repeated Measures** | shown below | shown below | ANCOVA<br>ANOVA | 0.46<br>0.35 |
| *RM2 Components (means)* | | | | |
| Change from baseline to: | | | | |
| Week 1 | - 0.1 | - 0.9 | N/A | N/A |
| Week 3 | - 1.2 | - 1.3 | | |
| Week 5 | - 0.4 | - 1.1 | | |
| Week 7 | - 0.3 | - 1.8 | | |
| Week 9 | - 0.5 | - 1.4 | | |
| Week 11 | - 0.6 | - 1.5 | | |
| Week 13 | - 1.1 | - 1.6 | | |
| Week 15 | - 1.0 | - 1.5 | | |
| Week 17 | - 0.8 | - 0.9 | | |
| Week 19 | - 1.4 | - 1.9 | | |
| Week 21 | - 1.4 | - 1.8 | | |
| Week 23 | - 1.3 | - 1.6 | | |
| Week 24 | - 1.4 | - 1.4 | | |

N/A = not applicable

## Appendix C. Study TKT003/006 Renal Function Data

In Study TKT003, subject 16 experienced a renal hemorrhage following the baseline kidney biopsy. Table 61 shows the change in creatinine clearance results when this subject is excluded from the analyses.

**Table 61. Study TKT003 Change from baseline in creatinine clearance (excluding subject 16)**

| Creatinine clearance values | Agalsidase, n = 13 (mL/min) | Placebo, n = 11 (mL/min) |
|---|---|---|
| Baseline, mean ± SE | 99.3 ± 7.2 | 107.3 ± 12.2 |
| Change from baseline to week 24, mean ± SE | 4.4 ± 4.3 | - 19.7 ± 9.1 |
| ANCOVA p-value | 0.02 | |

*Comment: The appropriateness of excluding subject 16 is unclear. The totality of creatinine, creatinine clearance and GFR data for this subject suggest the renal emboli may have had little impact upon the subject's renal function. This 19 year old subject had been hospitalized on February 8, 1999 to begin the baseline evaluations for the study. On February 10, 1999, the second series of baseline lab tests was begun and on this date a renal biopsy was performed which resulted in intraparenchymal hemorrhage. The subject experienced further bleeding which prompted renal arterial embolization on February 18, 1999 and again on February 20, 1999. The major renal events for this subject are summarized in Table 62.*

***Table 62. Notable Renal Events for subject number 16 in Study TKT003***

| *Date* | *Week/event* | *Creatinine (mg/dL)* | *Cr CL (mL/min)* | *GFR (mL/min)* |
|---|---|---|---|---|
| *2/8/99* | *Week 1* | *0.7* | *152* | *130.0* |
| *2/10/99* | *Renal biopsy* | | *-* | *-* |
| *2/12/99* | *Renal bleed* | *0.8* | *-* | *-* |
| *2/16/99* | *Renal bleed* | *0.9* | *162* | *-* |
| *2/18/99* | *Renal embolization* | | | |
| *2/20/99* | *Renal embolization* | | | |
| *3/5/99* | *Week 3* | *1.0* | *-* | *-* |
| *3/19/99* | *Week 5* | *0.9* | *-* | *-* |
| *4/16/99* | *Week 9* | *0.9* | *148* | *-* |
| *5/14/99* | *Week 13* | *0.9* | *-* | *-* |
| *6/11/99* | *Week 17* | *0.8* | *129* | *-* |
| *7/9/99* | *Week 21* | *0.8* | *-* | *-* |
| *7/19/99* | *Week 23* | *1.0* | *120* | *88* |
| *7/25/99* | *Week 24* | *0.9* | *96* | *-* |

*One might have expected the impact of the renal emboli to be evident within two months following the procedure. However, the data show little, if any evidence of an acute impact of the emboli. The lack of impact of subject 16 is also illustrated by reanalyzing GFR result by excluding subject 16 (Table 63)--even excluding subject 16, the analyses show no statistical difference in GFR between the two treatment groups.*

**Table 63. GFR results, excluding subject number 16**

| GFR value | Agalsidase, n = 13 (mL/min) | Placebo, n = 11 (mL/min) |
|---|---|---|
| Baseline, mean ± SE | 77.2 ± 5.6 | 90.9 ± 12.1 |
| Change from baseline to week 23, mean ± SE | - 6.2 ± 3.1 | - 19.8 ± 7.1 |
| p-value* | 0.17 | |

*ANCOVA with baseline as independent variable

The following two tables provide Study TKT006 GFR data as compared to the data from the end of Study TKT003.

*Table 64 shows the distribution of changes in GFR and highlights the large increase in GFR by (prior placebo group) subject number 13. Reanalyzing the change in GFR data using an imputation of no change for subject number 13 shows that this single subject may, in large part, be responsible for the nominal improvement in GFR detected among the group of prior placebo subjects who received Agalsidase in Study TKT006--emphasizing the lack of robustness of this group's putative improvement in GFR. The absence of robustness to the uncontrolled GFR findings largely confirms that the controlled data (Study TKT003) are likely to be correct in concluding that there was no Agalsidase treatment effect upon GFR.*

**Table 64. Change in GFR from end of TKT003 to end of TKT006, distribution of changes**

| **Change in GFR by: (mL/min)** | **Number of subjects** | |
|---|---|---|
| | **Prior placebo, n = 10** | **Prior Agalsidase, n = 14** |
| - 10 to - 20 | 0 | 3 |
| 0 to - 10 | 2 | 3 |
| 0 to 10 | 1 | 4 |
| 10 to 20 | 2 | 3 |
| 20 to 30 | 2 | 1 |
| 30 to 40 | 2 | 0 |
| 40 to 50 | 0 | 0 |
| 50 to 60 | 1* | 0 |

*subject 13 had an increase in GFR by 56 mL/min, this is similar to the subject's change in creatinine clearance from an 003 end value of 72 mL/min to an 006 end value of 129 mL/min

**Table 65. Change in GFR values in Studies TKT003/006**

| Group | 006 Week 52 - 003 final Mean ± SE |
|---|---|
| Prior Agalsidase, n = 14 | 1.9 ± 3.4<br>p = 0.58 |
| Prior Placebo, n = 10 | 17.2 ± 6.4<br>p = 0.03 |
| Prior Placebo, n = 10<br>*Imputing no change in wk 003 final value for subject 13* | 10.2 ± 5.5<br>p = 0.09 |

## Appendix D. Study TKT003 Additional Efficacy Endpoint Analyses

Table 66 shows the repeated measures assessment of the change in the various NPS question score results. Note that the table shows the change from baseline to week 24, but the nominal p-value is calculated using a repeated measures comparative analysis of all time points.

**Table 66. Study TKT003 NPS Changes**

| NPS question | Agalsidase mean ± SE | Placebo mean ± SE | p-value* |
|---|---|---|---|
| Intensity of pain (Q1) | N = 14 | n = 11 | |
| Baseline | 5.5 ± 0.7 | 6.5 ± 0.9 | |
| Change to week 24 | - 2.4 ± 1.0 | - 1.4 ± 0.7 | 0.04 |
| Sharpness of pain (Q2) | N = 14 | n = 11 | |
| Baseline | 3.9 ± 1.0 | 5.5 ± 1.3 | |
| Change to week 24 | - 1.4 ± 1.1 | - 0.8 ± 0.4 | 0.03 |
| Hotness of pain (Q3) | N = 14 | n = 11 | |
| Baseline | 6.6 ± 0.8 | 6.4 ± 0.9 | |
| Change to week 24 | - 3.7 ± 1.0 | - 1.9 ± 0.7 | 0.18 |
| Dullness of pain (Q4) | N = 14 | n = 11 | |
| Baseline | 3.8 ± 0.9 | 5.6 ± 1.0 | |
| Change to week 24 | - 2.2 ± 0.8 | - 3.0 ±1.5 | 0.57 |
| Coldness of pain (Q5) | N = 14 | n = 12, n = 11 | |
| Baseline | 1.4 ± 0.6 | 2.3 ± 0.9 | |
| Change to week 24 | 0.0 ± 0.5 | - 1.6 ± 0.8 | 0.90 |
| Sensitivity (Q6) | N = 14 | n = 12, n = 11 | |
| Baseline | 3.4 ± 0.7 | 4.67 ± 1.1 | |
| Change to week 24 | - 1.6 ± 0.6 | 0.5 ± 1.0 | 0.09 |
| Itchiness of pain (Q7) | n = 14 | n = 12, n = 11 | |
| Baseline | 1.0 ± 0.4 | 1.6 ± 0.9 | |
| Change to week 24 | - 0.1 ± 0.3 | 0.0 ± 0.3 | 0.85 |
| Unpleasantness of pain (Q9) | n = 14, n = 13 | n = 12, n = 11 | |
| Baseline | 5.8 ± 0.7 | 6.8 ± 0.7 | |
| Change to week 24 | - 1.8 ± 1.0 | - 1.7 ± 0.8 | 0.11 |
| Intensity of deep pain (Q10a) | n = 14 | n = 12, n = 11 | |
| Baseline | 5.6 ± 0.7 | 6.8 ± 0.9 | |
| Change to week 24 | - 1.6 ± 1.0 | - 2.2 ± 0.9 | 0.30 |
| Intensity of surface pain (Q10b) | n = 14 | n = 12, n = 11 | |
| Baseline | 4.8 ± 0.7 | 6.0 ± 0.9 | |
| Change to week 24 | - 2.5 ± 0.5 | - 1.5 ± 0.9 | 0.06 |

*repeated measures ANOVA

Table 67 provides analyses of the Study TKT003 cardiac MRI data.

**Table 67. Study TKT003 MRI Left ventricular mass and myocardial T1 and T2 results**

| Outcome/group | Baseline | Week 23 | Change | p-value* |
|---|---|---|---|---|
| **LVED mass, g** | | | | |
| Agalsidase, n = 14; mean ± SE | 226.1 ± 17.4 | 229.6 ± 17.0 | + 3.5 ± 2.7 | 0.93 |
| Placebo, n = 11; mean ± SE | 211.8 ± 12.6 | 215.9 ± 11.2 | + 4.1 ± 5.7 | |
| **LVES mass, g** | | | | |
| Agalsidase, n = 13; mean ± SE | 217.8 ± 16.7 | 224.0 ± 15.9 | + 6.2 ± 4.0 | 0.25 |
| Placebo, n = 11; mean ± SE | 218.8 ± 13.3 | 216.6 ± 11.7 | - 2.2 ± 6.4 | |
| **Myocardial T1, msec** | | | | |
| Agalsidase, n = 14; mean ± SE | 753.1 ± 43.3 | 766.1 ± 39.8 | + 13.1 ± 59.1 | 0.57 |
| Placebo, n = 11; mean ± SE | 846.6 ± 30.2 | 807.1 ± 34.6 | - 39.5 ± 38.3 | |
| **Myocardial T2, msec** | | | | |
| Agalsidase, n = 13; mean ± SE | 39.8 ± 1.4 | 40.6 ± 1.1 | + 0.8 ± 1.9 | 0.89 |
| Placebo, n = 11; mean ± SE | 39.0 ± 1.4 | 40.5 ± 1.0 | + 1.5 ± 1.8 | |

*ANCOVA with baseline as independent variable
LVED = left ventricular end diastolic
LVES = left ventricular end systolic

Table 68 presents an exploratory analysis of the results of the LV mass among the subset of subjects with abnormal LV mass as determined by echocardiography. The upper limit of normal LV mass is 115 g/m$^2$.

**Table 68. LV mass among the subset of subjects with echocardiographic assessment of increased LV mass at baseline**

| Outcome/group | Baseline | Week 23 | Change from baseline | p-value* |
|---|---|---|---|---|
| **LVES mass, g** | | | | |
| Placebo, n = 5; mean ± SE | 244.4 ± 23.4 | 241.2 ± 17.2 | - 3.2 ± 12.4 | 0.53 |
| Agalsidase, n = 6; mean ± SE | 254.8 ± 23.8 | 260.5 ± 24.5 | + 5.7 ± 7.1 | |
| **LVED mass, g** | | | | |
| Placebo, n = 5; mean ± SE | 236.8 ± 20.6 | 239.8 ± 16.1 | + 3.2 ± 11.1 | 0.71 |
| Agalsidase, n = 7; mean ± SE | 268.8 ± 21.5 | 269.4 ± 23.4 | - 0.571 ± 2.9 | |

* ANOVA

Tables 69 and 70 presents analyses of the echocardiographic results from Study TKT003.

**Table 69. Study TKT003 Echocardiographic results**

| Outcome/group | Baseline | Week 23 | Change from baseline | p-value* |
|---|---|---|---|---|
| **LV mass/m$^2$, g/m$^2$** | | | | |
| Agalsidase, n = 14; mean ± SE | 111.7 ± 13.7 | 125.6 ± 10.9 | + 13.9 ± 4.2 | 0.06 |
| Placebo, n = 11; mean ± SE | 114.4 ± 10.9 | 106.5 ± 9.1 | - 7.9 ± 13.2 | |
| **Diastolic LV Septum, mm** | | | | |
| Agalsidase, n = 13; mean ± SE | 10.3 ± 0.6 | 10.7 ± 0.5 | + 0.4 ± 0.3 | 0.03 |
| Placebo, n = 11; mean ± SE | 10.5 ± 0.6 | 9.5 ± 0.6 | - 0.9 ± 0.7 | |
| **Diastolic LV post wall, mm** | | | | |
| Agalsidase, n = 14; mean ± SE | 10.2 ± 0.6 | 10.6 ± 0.7 | + 0.4 ± 0.3 | 0.07 |
| Placebo, n = 11; mean ± SE | 10.9 ± 0.7 | 9.8 ± 0.6 | - 1.1 ± 0.7 | |
| **Aortic root diameter, m** | | | | |
| Agalsidase, n = 14; mean ± SE | 34.0 ± 1.2 | 34.9 ± 0.9 | + 0.9 ± 1.1 | 0.49 |
| Placebo, n = 11; mean ± SE | 37.7 ± 1.7 | 35.3 ± 1.3 | - 2.5 ± 1.5 | |

*ANCOVA with baseline and treatment as independent variables

**Table 70. Study TKT003 Echocardiographic results among subset of patients with increased LV mass index**

| Outcome/group | Baseline | Week 23 | Change from baseline | p-value* |
|---|---|---|---|---|
| **LV mass/m$^2$, g/m$^2$** | | | | |
| Agalsidase, n = 7; mean ± SE | 148.2 ± 18.0 | 150.0 ± 15.7 | - 1.8 ± 3.3 | 0.25 |
| Placebo, n = 5; mean ± SE | 145.1 ± 12.3 | 120.0 ± 16.6 | -25.1 ± 26.0 | |
| **Diastolic LV Septum, mm** | | | | |
| Agalsidase, n = 7; mean ± SE | 12.0 ± 0.6 | 11.9 ± 0.7 | + 1.4 ± 0.3 | 0.12 |
| Placebo, n = 5; mean ± SE | 11.8 ± 0.6 | 10.6 ± 0.9 | + 1.6 ± 1.2 | |
| **Diastolic LV post wall, mm** | | | | |
| Agalsidase, n = 7; mean ± SE | 50.1 ± 1.6 | 51.4 ± 1.4 | - 1.3 ± 1.2 | 0.70 |
| Placebo, n = 5; mean ± SE | 48.6 ± 1.5 | 48.8 ± 2.5 | - 0.2 ± 2.7 | |

**ANCOVA with baseline as covariate*

Table 71 shows analyses of Study TKT003 EKG data.

**Table 71. Electrocardiographic results of QRS duration (msec)**

| Group | Baseline | Week 24 | Change from baseline | p-value* |
|---|---|---|---|---|
| Agalsidase, n = 14; mean ± SE | 94.1 ± 4.9 | 91.7 ± 2.1 | - 2.4 ± 3.9 | 0.05 |
| Placebo, n = 12; mean ± SE | 94.0 ±3.4 | 97.6 ± 3.4 | + 3.6 ± 1.2 | |

*ANCOVA with baseline and treatment as independent variables

*Comments: This statistical test outcome (p = 0.05) appears largely contingent upon one single value--subject number 26 (Agalsidase) had an intermittent right bundle branch block. Table 72 shows the QRS duration results for subject number 26.*

**Table 72. QRS duration for subject number 26 by visit**

| QRS, msec | Visit | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | -1 | 1 | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 17 | 19 | 21 | 23 | 23 |
| | 150 | 103 | 154 | 107 | 101 | 103 | 103 | 110 | 104 | 100 | 106 | 107 | 106 | 99 |

*By utilizing the 103 msec value (pre-first dose) as the baseline value for patient number 26, the ANCOVA p-value (with baseline as covariate) changes from 0.05 to 0.08.*

## Appendix E. Study TKT005 Analyses

Tables 73 and 74 provide analyses of cardiac MRI and echocardiographic results, respectively, within Study TKT005.

**Table 73. Study TKT005 Additional cardiac MRI outcomes in the subset of patients with baseline and week 24 data**

| Group | Baseline | Week 24 | Change from baseline | p-value* |
|---|---|---|---|---|
| **LV posterior wall thickness (mm)** | | | | |
| Agalsidase, n = 7<br>mean ± SE | 17.0 ± 0.5 | 17.7 ± 0.9 | + 0.7 ± 0.4 | 0.95 |
| Placebo, n = 7<br>mean ± SE | 16.3 ± 1.0 | 16.9 ± 1.2 | + 0.6 ± 0.6 | |
| **"LVSV"--Left ventricular stroke volume (mL)*** | | | | |
| Agalsidase, n = 6<br>mean ± SE | 127.4 ± 10.0 | 118.6 ± 5.5 | - 8.8 ± 6.8 | 0.89 |
| Placebo, n = 6<br>mean ± SE | 116.2 ± 7.2 | 114.3 ± 5.0 | - 1.9 ± 5.0 | |
| **"LVEF" Left ventricular ejection fraction (%)** | | | | |
| Agalsidase, n = 6<br>mean ± SE | 73.8 ± 3.9 | 75.4 ± 1.9 | + 1.7 ± 2.3 | 0.78 |
| Placebo, n = 6<br>mean ± SE | 76.2 ± 2.4 | 77.3 ± 2.6 | + 1.2 ± 2.4 | |
| **"LVEDV"--Left ventricular end diastolic volume (mL)** | | | | |
| Agalsidase, n = 7<br>mean ± SE | 168.5 ± 11.7 | 149.3 ± 10.7 | - 19.2 ± 6.7 | 0.22 |
| Placebo, n = 7<br>mean ± SE | 147.4 ± 8.8 | 148.4 ± 4.1 | + 1.0 ± 7.2 | |
| **"LVESV"--Left ventricular end systolic volume** | | | | |
| Agalsidase, n = 6<br>mean ± SE | 46.2 ± 9.1 | 39.0 ± 4.5 | - 7.2 ± 5.1 | 0.87 |
| Placebo, n = 6<br>mean ± SE | 36.2 ± 3.9 | 33.6 ± 4.1 | - 2.6 ± 4.2 | |

*ANCOVA with baseline and treatment as independent variables.

**Table 74. Study TKT005 Echocardiographic changes from baseline to week 24**

| Group | Baseline | Week 24 | Change from baseline | p-value* |
|---|---|---|---|---|
| **Left ventricular mass/body surface ($g/m^2$)** | | | | |
| Agalsidase, n = 7 mean ± SE | 182.2 ± 10.3 | 185.8 ± 27.6 | 3.5 ± 26.2 | 0.66 |
| Placebo, n = 8 mean ± SE | 152.8 ± 24.7 | 192.3 ± 23.1 | 39.5 ± 28.1 | |
| **Aortic root diameter (m)** | | | | |
| Agalsidase, n = 7 mean ± SE | 35.6 ± 1.5 | 35.6 ± 1.8 | 0.0 ± 1.7 | 0.54 |
| Placebo, n = 8 mean ± SE | 31.6 ± 1.2 | 32.1 ± 0.9 | 0.42 ± 0.8 | |
| **Left atrium size (mm)** | | | | |
| Agalsidase, n = 7 mean ± SE | 36.8 ± 1.8 | 33.7 ± 2.2 | - 3.1 ± 1.7 | 0.45 |
| Placebo, n = 8 mean ± SE | 38.6 ± 2.4 | 36.6 ± 1.9 | - 2.0 ± 1.4 | |
| **Left ventricle (diastole, mm)** | | | | |
| Agalsidase, n = 7 mean ± SE | 50.8 ± 1.7 | 48.5 ± 2.6 | - 2.3 ± 1.0 | 0.28 |
| Placebo, n = 8 mean ± SE | 52.4 ± 3.2 | 52.1 ± 3.2 | - 0.3 ± 1.3 | |
| **Left ventricle (systole, mm)** | | | | |
| Agalsidase, n = 7 mean ± SE | 30.4 ± 1.7 | 27.8 ± 2.4 | - 2.6 ± 1.1 | 0.27 |
| Placebo, n = 8 mean ± SE | 30.6 ± 2.2 | 30.5 ± 2.8 | - 0.1 ± 1.7 | |
| **Ventricular septum (diastolic, mm)** | | | | |
| Agalsidase, n = 7 mean ± SE | 14.2 ± 0.4 | 13.5 ± 1.1 | - 0.7 ± 1.0 | 0.15 |
| Placebo, n = 8 mean ± SE | 12.9 ± 1.0 | 14.0 ± 1.1 | 1.0 ± 0.5 | |
| **Left ventricular posterior wall (diastolic, mm)** | | | | |
| Agalsidase, n = 7 mean ± SE | 12.1 ± 0.7 | 12.6 ± 0.9 | 0.6 ± 0.6 | 0.94 |
| Placebo, n = 8 mean ± SE | 13.0 ± 0.6 | 13.5 ± 1.2 | 0.5 ± 1.0 | |
| **Ejection fraction (%)** | | | | |
| Agalsidase, n = 7 mean ± SE | 78.7 ± 1.7 | 81.0 ± 2.7 | 2.3 ± 2.2 | 0.46 |
| Placebo, n = 8 mean ± SE | 80.0 ± 1.4 | 79.1 ± 1.9 | - 0.9 ± 2.5 | |
| **Left ventricular mass (not adjusted for body size, g)** | | | | |
| Agalsidase, n = 7 mean ± SE | 327.5 ± 21.9 | 307.0 ± 43.9 | - 20.4 ± 27.2 | 0.26 |
| Placebo, n = 8 mean ± SE | 342.3 ± 39.7 | 363.8 ± 51.8 | 21.5 ± 20.4 | |

*ANCOVA with baseline measure and treatment as independent variables

Study TKT005 Statistical Analytical Plan stated that the QRS duration would be analyzed using a "repeated measures" analysis throughout the study. The sponsor presented the summary data in a table which highlights the change from baseline to week 24. These data are shown in Table 75.

**Table 75. Study TKT005 QRS duration (msec)**

| Group | Baseline | Week 24 | Change from baseline |
|---|---|---|---|
| **Agalsidase, n = 7** mean ± SE | 111.0 ± 10.5 | 98.1 ± 1.9 | - 12.9 ± 11.7 |
| **Placebo, n = 8** mean ± SE | 94.9 ± 2.6 | 99.5 ± 3.6 | 4.6 ± 1.9 |
| p-value for repeated measures analysis (ANOVA) = 0.88 | | | |
| p-value for change from baseline to week 24* = 0.81 | | | |
| Difference of means | - 17.5 | | |
| 95% CI | -27.0, - 7.9 | | |

*ANCOVA with baseline as covariate

Table 76 shows the results of Study TKT005 urine and plasma CTH ($Gb_3$) content/concentration assessments.

**Table 76. Study TKT005 Plasma and urine sediment CTH ($Gb_3$) content**

| Measures | Agalsidase, n = 7 | Placebo, n = 8 |
|---|---|---|
| **Plasma CTH (nmole/mL)** | | |
| Baseline, mean ± SE | 12.6 ± 1.6 | 13.3 ± 1.6 |
| Change from baseline to week 24, mean ± SE | - 6.2 ± 1.1 | - 0.6 ± 0.4 |
| p-value* | < 0.01 | |
| Difference of means | - 5.7 | |
| 95% CI | - 7.6, - 3.7 | |
| **Urine sediment CTH (nmole/24 hours)** | | |
| Baseline, mean ± SE | 2037.3 ± 409.3 | 1995.8 ± 459.4 |
| Change from baseline to week 24, mean ± SE | - 1052 ± 475 | - 25 ± 167 |
| p-value* | 0.05 | |
| Difference of means | - 1027 | |
| 95% CI | - 2024.9, -29.1 | |

*ANCOVA with baseline as independent value

Table 77 shows "worst pain" scores within Study TKT005.

**Table 77. Study TKT005 "Worst pain" scores while off pain medications**

| Assessment | Agalsidase, n = 7 | Placebo, n = 7 |
|---|---|---|
| Baseline, mean ± SE | 3.6 ± 1.2 | 5.4 ± 1.5 |
| Week 9, mean ± SE | 4.6 ± 1.3 | 4.4 ± 1.4 |
| Week 17, mean ± SE | 4.3 ± 1.2 | 3.1 ± 1.3 |
| Week 24, mean ± SE | 4.1 ± 1.2 | 3.1 ± 1.3 |

Unlike the prespecified analysis for the worst pain scores in Study TKT003, the change in the worst pain score AUC was to be compared using ANCOVA with the baseline value as covariate. An additional analysis was to be a repeated measure ANOVA analysis. The result of these analyses are shown in Table 78.

**Table 78. Study TKT005 AUC change from baseline to week 24 and repeated measure analysis of worst pain scores while off pain medications**

| Group | Change from baseline | | | p-value* for change from baseline to week 24 | p-value (ANOVA) for repeated measure comparisons |
|---|---|---|---|---|---|
| | week 9 | week 17 | week 24 | | |
| Agalsidase, n = 7 mean ± SE | 1.0 ± 1.1 | 0.7 ± 0.7 | 0.6 ± 1.0 | 0.13 | 0.95 |
| Placebo, n = 7 mean ± SE | - 1.0 ± 1.0 | - 2.3 ± 1.1 | - 2.3 ± 1.0 | | |

*ANCOVA using baseline measure as covariate

## Appendix F. Study TKT006 Analyses

Table 79 shows the pharmacokinetic data from Study TKT006.

**Table 79. Study TKT006 Pharmacokinetic (PK) analysis of Agalsidase**

| Patients from 003 placebo arm | | | Patients from 003 Agalsidase arm | | |
|---|---|---|---|---|---|
| ID number | Clearance (mL/min) | AUC/dose | ID number | Clearance (mL/min) | AUC/dose |
| 1 | 304 | 0.23 | 2 | 438 | 0.14 |
| 3 | 206 | 0.28 | 4 | 289 | 0.22 |
| 5 | ND | ND | 6 | 235 | 0.30 |
| 7 | 185 | 0.51 | 8 | 91 | 0.85 |
| 9 | 231 | 0.44 | 10 | 2493 | 0.03 |
| 11 | 168 | 0.42 | 12 | 261 | 0.30 |
| 13 | 156 | 0.56 | 15 | 174 | 0.38 |
| 14 | 176 | 0.38 | 16 | 467 | 0.16 |
| 17 | 190 | 0.36 | 18 | 2710 | 0.02 |
| 22 | 170 | 0.35 | 19 | 1720 | 0.06 |
| 25 | 140 | 0.48 | 21 | 568 | 0.12 |
| | | | 23 | 2039 | 0.05 |
| | | | 26 | not done | not done |
| | | | 27 | 11545 | 0.01 |
| **Mean** | 193 | 0.40 | **Mean** | 1772 | 0.20 |
| **SD** | 47 | 0.10 | **SD** | 3084 | 0.23 |

The alteration of pharmacokinetics is especially notable for the four subjects who appeared to have increasing antibody formation during the study, as shown in Table 80.

**Table 80. Study TKT006 pharmacokinetic changes in subjects with most the profound immunoreactivity to Agalsidase**

| Subject | Clearance (mL/min) | | AUC/dose (mean ± SD) | |
|---|---|---|---|---|
| | 003 end | 006 end | 003 end | 006 end |
| 22 | 170 | 2725 | 0.35 | 0.02 |
| 16 | 467 | 1291 | 0.16 | 0.06 |
| 19 | 1720 | 5296 | 0.06 | 0.02 |
| 23 | 2039 | not done | 0.05 | not done |

Table 81 shows the Study TKT005 urine sediment CTH ($Gb_3$) content analyses.

**Table 81. Study TKT006 Change from baseline in urine sediment CTH ($Gb_3$)**

| Assessment | Mean ± SE (nmole/g creatinine) | p-value |
|---|---|---|
| ***Subjects treated with placebo in 003 and Agalsidase in 006, n = 11*** | | |
| Baseline of 003 | 2162 ± 383 | - |
| End of 003 | 2495 ± 553 | |
| End of 006 | 456 ± 135 | |
| Change: Baseline to end of 003 | 333 ± 400 | 0.425 |
| Change: End of 003 to end of 006 | - 2039 ± 496 | 0.002 |
| ***Subjects treated with Agalsidase in 003 and Agalsidase in 006, n = 14*** | | |
| Baseline of 003 | 2369 ± 308 | - |
| End of 003 | 1683 ± 443 | |
| End of 006 | 812 ± 185 | |
| Change: Baseline to end of 003 | - 686 ± 298 | 0.04 |
| Change: End of 003 to end of 006 | -871 ± 306 | 0.01 |

Table 82 shows the Study TKT005 plasma CTH ($Gb_3$) concentration changes.

**Table 82. Study TKT006 Change from baseline in plasma CTH ($Gb_3$)**

| Assessment | Mean ± SE (nmole/mL) | p-value |
|---|---|---|
| ***Subjects treated with placebo in 003 and Agalsidase in 006, n = 11*** | | |
| Baseline of 003 | 11.0 ± 1.1 | - |
| End of 003 | 10.2 ± 1.3 | |
| End of 006 | 5.1 ± 0.7 | |
| Change: Baseline to end of 003 | - 0.8 ± 0.5 | 0.14 |
| Change: End of 003 to end of 006 | - 5.1 ± 1.2 | 0.002 |
| ***Subjects treated with Agalsidase in 003 and Agalsidase in 006, n = 14*** | | |
| Baseline of 003 | 12.1 ± 0.9 | - |
| End of 003 | 5.6 ± 0.5 | |
| End of 006 | 6.3 ± 0.5 | |
| Change: Baseline to end of 003 | - 6.6 ± 0.8 | < 0.001 |
| Change: End of 003 to end of 006 | 0.7 ± 0.43 | 0.13 |

## Appendix G. Study TKT011 Analyses

Study TKT011 plasma CTH ($Gb_3$) concentration analyses are shown in Table 83.

**Table 83. Study TKT011 Change from baseline in plasma CTH ($Gb_3$) concentration**

| Month of treatment/Study | nmol/mL; Mean ± SE | p-value |
|---|---|---|
| ***Subjects treated with placebo in 003 and Agalsidase in 006 and 011 (n = 11)*** | | |
| Month 0/003 baseline, n = 11 | 11.0 ± 1.1 | not applicable |
| Month 6/003 end, n = 11 | 10.2 ± 1.3 | |
| Month 12/006, n = 11 | 6.0 ± 0.4 | |
| Month 18/006, n = 11 | 5.1 ± 0.7 | |
| Month 24/011, n = 10 | 6.0 ± 0.7 | |
| Month 30/011, n = 10 | 5.6 ± 0.5 | |
| Change during 003, n = 11 | - 0.8 ± 0.9 | 0.14 |
| Change from 003 end to: | | |
| Month 12 (6 months Agalsidase) n = 11 | - 4.2 ± 1.0 | < 0.01 |
| Month 18 (1 year Agalsidase) n = 11 | - 5.1 ± 1.2 | < 0.01 |
| Month 24 (1.5 years Agalsidase) n = 10 | - 4.1 ± 1.2 | < 0.01 |
| Month 30 (2 years Agalsidase) n = 10 | - 4.6 ± 1.1 | < 0.01 |
| ***Subjects treated with Agalsidase in 003, 006 and 011*** | | |
| Month 0/003 baseline, n = 13 | 12.6 ± 0.9 | not applicable |
| Month 6/003 end, n = 13 | 5.7 ± 0.6 | |
| Month 12/006, n = 13 | 7.6 ± 0.6 | |
| Month 18/006, n = 13 | 6.4 ± 0.6 | |
| Month 24/011, n = 12 | 7.6 ± 0.8 | |
| Month 30/011, n = 12 | 8.2 ± 0.9 | |
| Change from 003 baseline to: | | |
| Month 6 (6 months Agalsidase) n = 13 | - 6.8 ± 0.8 | < 0.01 |
| Month 12 (1 year Agalsidase) n = 13 | - 5.0 ± 0.9 | < 0.01 |
| Month 18 (1.5 years Agalsidase) n = 13 | - 6.2 ± 0.8 | < 0.01 |
| Month 24 (2 years Agalsidase) n = 12 | - 4.9 ± 1.0 | < 0.01 |
| Month 30 (2.5 years Agalsidase) n = 12 | - 4.2 ± 0.9 | < 0.01 |

Study TKT011 weight changes are shown in Table 84.

**Table 84. Study TKT011 Change from baseline in body weight**

| Month of treatment/Study | kg; Mean ± SE | p-value |
|---|---|---|
| ***Subjects treated with placebo in 003 and Agalsidase in 006 and 011 (n = 11)*** | | |
| Month 0/003 baseline, n = 11 | 77.0 ± 5.3 | not applicable |
| Month 6/003 end, n = 10 | 72.5 ± 3.8 | |
| Month 12/006, n = 11 | 78.3 ± 4.4 | |
| Month 18/006, n = 11 | 79.3 ± 4.5 | |
| Month 24/011, n = 10 | 80.8 ± 4.8 | |
| Month 30/011, n = 10 | 78.6 ± 4.7 | |
| Change during 003, n = 10 | - 1.4 ± 1.3 | 0.31 |
| Change from 003 end to: | | |
| Month 12 (6 months Agalsidase) n = 10 | 2.7 ± 0.9 | 0.01 |
| Month 18 (1 year Agalsidase) n = 10 | 4.1 ± 1.2 | 0.01 |
| Month 24 (1.5 years Agalsidase) n = 9 | 4.4 ± 1.5 | 0.02 |
| Month 30 (2 years Agalsidase) n = 9 | 2.7 ± 1.6 | 0.13 |
| ***Subjects treated with Agalsidase in 003, 006 and 011*** | | |
| Month 0/003 baseline, n = 13 | 73.8 ± 3.4 | not applicable |
| Month 6/003 end, n = 12 | 74.7 ± 3.8 | |
| Month 12/006, n = 12 | 77.7 ± 3.6 | |
| Month 18/006, n = 13 | 76.3 ± 3.8 | |
| Month 24/011, n = 12 | 76.7 ± 3.9 | |
| Month 30/011, n = 12 | 76.1 ± 3.8 | |
| Change from 003 baseline to: | | |
| Month 6 (6 months Agalsidase) n = 12 | 1.4 ± 0.6 | 0.04 |
| Month 12 (1 year Agalsidase) n = 12 | 3.5 ± 0.7 | < 0.01 |
| Month 18 (1.5 years Agalsidase) n = 13 | 2.5 ± 0.8 | 0.01 |
| Month 24 (2 years Agalsidase) n = 12 | 2.1 ± 1.1 | 0.08 |
| Month 30 (2.5 years Agalsidase) n = 12 | 1.5 ± 1.1 | 0.21 |

## Appendix H. Study TKT 014 Analyses

Study TKT014 echocardiographic analyses are shown in Table 84 (p-values are from t-tests).

**Table 84. Study TKT014 Echocardiographic changes in left ventricular mass (Mean ± SE)**

| Outcome | Week: | | | | Change to Week: | | |
|---|---|---|---|---|---|---|---|
| | 0<br>n = 15 | 13<br>n = 15 | 27<br>n = 11 | 41<br>n = 7 | 13<br>n = 15 | 27<br>n = 11 | 41<br>n = 7 |
| LV mass, gm | 254 ± 18 | 243 ± 18 | 213 ± 20 | 225 ± 22 | - 11 ± 10 | - 39 ± 10 | - 43 ± 16 |
| p-value | | | | | 0.29 | < 0.01 | 0.04 |
| | | | | | | | |
| LV mass index, g/m$^2$ | 148 ± 10 | 143 ± 12 | 124 ± 11 | 123 ± 9 | - 6 ± 6 | - 23 ± 6 | - 25 ± 8 |
| p-value | | | | | 0.37 | < 0.01 | 0.01 |
| | | | | | | | |
| Septum, mm | 12 ± 0.6 | 11 ± 0.6 | 10 ± 0.6 | 10 ± 0.6 | - 1 ± 0.2 | - 2 ± 0.4 | - 2 ± 0.6 |
| p-value | | | | | < 0.01 | < 0.01 | 0.01 |
| | | | | | | | |
| LV wall, mm | 13 ± 0.7 | 12 ± 0.8 | 11 ± 0.7 | 11 ± 0.4 | - 1 ± 0.6 | - 2 ± 0.6 | - 2 ± 1.1 |
| p-value | | | | | 0.31 | 0.03 | 0.09 |

LV wall = left ventricular posterior wall

The following text explains the blinding procedure for the echocardiographic assessments: One physician was responsible for blinding himself and then unblinding himself for the echocardiographic review process. This process included the following (as described by the sponsor):

The sponsor reports that one individual performed all the echocardiograms. The echocardiograms were reviewed and interpreted on an on-going basis during the study by a site cardiologist (Dr. Kampmann). Notable findings were reported to the site investigator. However, these preliminary findings were not recorded on the CRF. Following completion of the study, Dr. Kampmann initiated a process of rereading the echocardiograms in a blinded fashion. In order to do this, he blanked out the ID number and date from the echocardiographic digital recordings, composed a blinded ID/sequence code and designated each echocardiographic recording by a unique (blinded) identifier. He then reread the blinded echocardiograms, recorded the findings and then unblinded himself. Following his unblinding, he relayed the echocardiographic information to the site investigators who placed the information (according to the date of performance of the echocardiogram) into the CRF. Consequently, the information in the CRF is the source of the echocardiographic data.

*Comment: It is impossible to verify the integrity of the echocardiographic blinding procedures because of multiple limitations in documentation but it is especially notable that one individual was apparently responsible for all aspects of the process.*

Table 85 shows the Study TKT014 EKG outcomes (p-values are from t-tests).

**Table 85. Study TKT014 Change from baseline in QRS duration**

| Week of treatment | Mean ± SE, msec | p-value |
|---|---|---|
| Baseline, n = 15 | 101 ± 4 | not applicable |
| ***Change from baseline to:*** | | |
| Week 13, n = 15 | - 5.5 ± 3.0 | 0.09 |
| Week 27, n = 11 | - 8.7 ± 2.6 | < 0.01 |
| Week 41, n = 5 | - 3.6 ± 1.8 | 0.12 |

Table 86 shows the Study TKT014 creatinine clearance outcomes.

**Table 86. Study TKT014 Creatinine clearance changes**

| Week of treatment | Mean ± SE, mL/min | p-value |
|---|---|---|
| Baseline, n = 15 | 70 ± 5.2 | not applicable |
| ***Change from baseline to:*** | | |
| Week 13, n = 15 | 2.9 ± 2.7 | 0.29 |
| Week 27, n = 11 | - 0.5 ± 4.0 | 0.90 |
| Week 41, n = 5 | - 2.7 ± 2.7 | 0.38 |

## Appendix I. Financial disclosure:

The sponsor submitted 3454 forms (financial disclosure) for all investigators who participated in the controlled clinical studies described in this submission. This information discloses that, of the two controlled studies, one investigator received financial compensation from the sponsor. Specifically:

-for Study TKT005, Dr. Kay MacDermot (PI) received compensation for services during 1999 and 2000. No indication of bias in conduct of the study was apparent in exploration of the submitted data.

For Study TKT003, Dr. Raphael Schiffmann (PI) received no compensation. However, Dr. Roscoe Brady, a sub-investigator for Study 003, received compensation for services during 1999 and 2000. Dr. Brady is employed by the National Institutes of Health.

Study TKT003 was the principal randomized controlled study and was the subject of an extensive. FDA site inspection revealed no evidence of bias in conduct of the study by any site personnel.

*Comment: Overall, there is no evidence of impact of any financial influence upon the study outcomes in the two controlled studies (Studies TKT003 and TKT005).*