The committee will discuss the safety and efficacy of biologic license application (BLA) 125291, MYOZYME (alglucosidase alfa), Genzyme Corp., for the treatment of late onset Pompe disease.

Statistical Comments

BLA 125291

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1. **Overview**

This supplement to the FDA briefing package discusses statistical and design issues important to the understanding and interpretation of the results from Study AGLU02704 (LOTS).

These issues include:

- Change from a fixed design to the use of an adaptive strategy
- Change in the endpoint from 6MWT (FVC) at 52 weeks to average change in 6MWT (FVC) as measured by a linear slope
- Change in statistical analysis methods after data were shown to violate assumptions of prespecified model
- Use of a minimization algorithm to allocate subjects
- Results of re-randomization analyses

2. **Allocation of subjects to treatment groups**

A minimization algorithm was used to maintain a 2:1 (2000L:Placebo) treatment balance within study site (n=8), within 6MWT baseline strata (≤ 300 meters, > 300 meters) and within FVC baseline strata (≤55% predicted, >55% predicted).

The minimization algorithm is reproduced in Figure 1.
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Figure 1 Minimization Algorithm

Minimization Parameters

1. Number of patients: 90
2. Number of treatments: 2
3. Allocation ration: 2:1 (A:B)
4. Number of sites: 8
5. Number of strata: 4
6. Threshold percentage: 40%
7. Assignment probability: 90%
8. Site weight: 1
9. Strata weight: 3

Definitions

1. nsite(A) = number of subjects assigned to treatment A currently in Site i
2. nsite(B) = number of subjects assigned to treatment B currently in Site i
3. nstrata(A) = number of subjects assigned to treatment A currently in Strata i
4. nstrata(B) = number of subjects assigned to treatment B currently in Strata i

Algorithm Steps

1. Threshold check: if \( \frac{\max(nsite(A), 2^{nstrata(B)}) - \min(nsite(A), 2^{nstrata(B)})}{nsite(A) + 2^{nstrata(B)}} > 0.40 \) then patient is automatically assigned to the treatment out of balance. This essentially overrides the minimization allocation. Note that the multiplier of 2 is for the allocation ratio 2:1.

2. Compute site minimization score for treatment A: site(A) = 1^* \left( \frac{\max(nsite(A)+1, 2^{nstrata(B)}) - \min(nsite(A)+1, 2^{nstrata(B)})}{nsite(A) + 2^{nstrata(B)} + 1} \right).
3. **Statistical methodologies**

In the various statistical analysis plans, discussed below, the 6MWT (distance in meters) and FVC upright (% predicted) assessments formed the co-primary endpoints. A protocol amendment changed the definition of the endpoints to accommodate the adoption of an adaptive design strategy. In all plans, the measurement of the 6MWT was to be examined first. If the treatment effect were statistically significant at 0.05, then FVC upright would be evaluated.

3.1 **Original Statistical Analysis Plan, dated 9/27/05**

The original statistical analysis plan specified two co-primary variables:
- Number of meters walked in 6 minutes at Week 52, adjusted for baseline number of meters walked
- FVC upright (% predicted) at Week 52, adjusted for baseline FVC upright (% predicted)
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The treatment effect at 52 weeks for the 6MWT was to be assessed by a repeated measures linear model with covariates. The model was to have the following characteristics:

- Site is an explanatory variable
- Distance walked at baseline is a continuous covariate
- Time of assessment is an explanatory variable (categorical) in the model
- The model includes a term for the interaction between time and treatment, i.e., to assess evidence about whether the time effect is different for each treatment
- Response covariance is modeled by a compound symmetry structure
- The model contains a parameter for the treatment effect between Myozyme and placebo at Study Week 52, adjusted for baseline.

The parameter for the treatment effect at Study Week 52 would be tested to assess the significance of the treatment effect.

The model parameters would be estimated by a restricted maximum likelihood method (REML). Assumptions regarding the normality of errors and the form of the covariance matrix would be assessed. If the data indicated that model assumption of normality of errors were likely to be violated, then non-parametric methods may be used to carry out the significance testing.

Assessment of FVC upright would be carried out in a similar manner.

The sample size calculations for the original fixed design were based on variability in FVC over time in untreated subjects with late-onset Pompe disease in Study AGLU02303; there was no longitudinal data on 6MWT. With a 2:1 treatment allocation (2000L:placebo), a sample size of 63 (2000L, n=42; placebo n=21) would have 80% power to detect a treatment difference of 0.75 standard deviations using a two-sample t-test with a Type I error rate of 5%. The plan was to enroll at least 72 subjects (2000L, n=48; placebo n=24) to account for a 10 to 15% dropout rate.

3.2 Statistical Analysis Plan Amendment, dated 9/29/06

In May 2006, while the study was ongoing, the protocol was amended to an adaptive clinical trial design, and the primary endpoint was changed to a linear rate of change in distance walked estimated from a longitudinal model where response was to be modeled as a linear function of time of assessment. The rationale for these changes was to determine the optimal
duration of the study and to “compare the two treatments over the course of the study, rather than focusing on comparisons at 52 weeks.”

The statistical analysis plan contained methods and plans for the implementation of the adaptive design.

The adaptive design required changes to the primary endpoints. The co-primary efficacy variables were changed to slopes (average monthly increase):

- Average monthly increase in 6MWT.
- Average monthly increase in FVC upright (% predicted).

The interim analysis for the adaptive design was to be done when the last patient enrolled and continuing in the study completed Week 38 or when the statistical criterion for the analysis had been achieved. The study could continue to 52 weeks, as originally planned, or extended an additional 3 or 6 months. The maximum length of time any patient could participate in the study was 78 weeks.

The changes in the average monthly increases in 6MWT (and FVC upright) among subjects randomized to 2000 L product compared with changes in average monthly increases in 6MWT (and FVC upright) among subjects randomized to placebo were to be analyzed using a linear mixed effects (LME) model:

- Independent variables are site, treatment, time, and treatment-by-time interaction
- Outcome vector contains the observed measurements of 6MWT (or FVC upright) collected at baseline and at study visits
- Model to be fit using restricted maximum likelihood estimation
- Model to use unstructured variance-covariance matrix for the random effects (i.e., intercept and slope)

The model would be used to estimate the rate of change for each subject. Model assumptions would be assessed.

Differences between the average monthly increase between subjects randomized to 2000L and to placebo would be tested by a Wald statistic. The Haybittle-Peto alpha-spending function would adjust for the implementation of the adaptive design, resulting in a final nominal alpha of 0.04999 for declaring statistical significance.
Supportive analyses were to include a re-randomization analysis, which was to consist of running the minimization algorithm used for the treatment assignments 10,000 times. The LME would be applied and the p-values for the test of the treatment effect recorded. The resulting empirical distribution for the p-values would be compared with the observed p-value.

An analysis of covariance model (ANCOVA) was an additional supportive analysis. The ANCOVA would model the change from baseline and, separately, relative change from baseline to the last observed assessment. The model would include the baseline strata used in the minimization algorithm, the baseline observation, and treatment indicator.

3.3 Changes to the statistical methods after data were unblinded

After fitting the pre-specified LME to the data, diagnostics were done to determine the appropriateness of the LME model. These diagnostics were pre-specified in the SAP. Examination of the 6MWT data indicates significant departures from linearity and violations of the assumption of normality. Moreover, the applicant asserts the observed non-linearity also compromises the estimate of the model-dependent variance-covariance matrix.

Because of these findings, the applicant chose to use a sandwich estimator of the variance-covariance matrix. In addition, the applicant decided to test the difference in the monthly rate of change using GEE models and the repeated measures Wei-Lachin test, a nonparametric procedure. The applicant claims that GEE models relax the assumptions of linearity and a correctly specified variance-covariance matrix. Their GEE model uses a compound symmetric working correlation matrix. ANCOVA models were used in place of LME models to estimate treatment effects in subgroups.

The FVC efficacy analysis did not violate any of the assumptions of the LME model. However, GEE, Wei-Lachin and ANCOVA models were used to analyze FVC to maintain consistency with the analyses of the 6MWT.

I agree that the sandwich estimator of the variance-covariance matrix in the LME model is more appropriate for these data than is a model-based estimator. However, because of the numerous violations of the assumptions underlying the pre-specified LME model, I favor the use of the ANCOVA model to assess the efficacy of the 2000L product. The ANCOVA model makes fewer assumptions. Additionally, it answers the clinical question of interest: does the change from baseline to the last assessment in the 6MWT among subjects treated
with the 2000L product differ from the change from baseline to the last assessment in the 6MWT among subjects treated with placebo. The assessments between baseline and the last visit are not needed to answer this question.

4. Results

4.1 Interim analysis

An external independent statistical center (ISC) performed an interim analysis of the 6MWT data when the last patient enrolled and continuing in the study had completed Week 38 of the study. The analysis used the linear mixed effects model specified in the statistical analysis plan. Based on the amount of information accrued, the ISC recommend extending the study from 52 weeks to 78 weeks in order to increase the number of measurements per subject, allowing for sufficient information to attain 90% power to detect a difference between 2000L and placebo of 3.75 meters/month.

A representative of the ISC presented the results of the interim analysis to the Data and Safety Monitoring Board. After deliberating on this information the DSMB recommended to the applicant that the study continue for an additional 26 weeks.

4.2 6MWT

Figure 2 shows the means and standard errors of the total distance walked in the 6MWT at each study visit.

The results from the LME with a sandwich estimator (i.e. robust variance estimation) and from the ANCOVA indicate that the improvement among subjects treated with the 2000L product is significantly better than among subjects treated with placebo (Table 1).

The p-values from the re-randomization analyses are statistically non-significant at a nominal level of 0.05: LME with robust variance estimation (p=0.15) and ANCOVA (p=0.06); see Table 2.

The applicant asserts this re-randomization test is difficult to interpret because the discrete nature of the allocation algorithm restricts the space of possible treatment allocations. Moreover, they also assert the re-randomization test is an efficient test of the treatment effect because the
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The reference distribution of the test statistics from the 10,000 re-randomizations is skewed and not centered at zero; see Figure 3.

Because of the assumptions of the re-randomization test, the average t-score must be equal to zero. Further, the distribution shows that the t-score is not normally distributed. This feature calls into question the use of the p-values from the classical tests, which assume the t-scores are asymptotically normal. This lack of normality would argue in favor of the re-randomization test.

The re-randomization test results presented here assume subjects arrived in a fixed patient arrival sequence. The applicant argues that there is no reason to believe the arrival is not random. They did a re-randomization test assuming a random patient arrival sequence, resulting in a statistically significant p-value. However, I do not believe this approach is appropriate. The reasons for why patients arrive in the order that they do is unknowable.

Figure 2  Mean (+/- SEM) Change from baseline over time in six-minute walk test: total distance walked

Reference: Figure 14.2.1.1B

Source: Figure 11-1, Clinical Study Report
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Table 1 Change in distance walked in six-minute walk test.

<table>
<thead>
<tr>
<th></th>
<th>2000L N=60</th>
<th>Placebo N=30</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimates/Tests of Monthly Change in Distance Walked (linear mixed effects model)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LME with model-based variance estimation, meters/month (95% CI)</td>
<td>1.18 (0.34, 2.03)</td>
<td>-0.06 (-1.26, 1.14)</td>
<td>1.24 (-0.21, 2.70)</td>
<td>0.093</td>
</tr>
<tr>
<td>LME with robust variance estimation, meters/month (95% CI)</td>
<td>1.18 (0.26, 2.11)</td>
<td>-0.06 (-0.90, 0.78)</td>
<td>1.24 (0.02, 2.47)</td>
<td>0.046</td>
</tr>
<tr>
<td>Estimates/Tests of Monthly Change in Distance Walked from Baseline to Last Observation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANCOVA, meters (95% CI)</td>
<td>25.13 (10.07, 40.19)</td>
<td>-2.99 (-24.16, 18.18)</td>
<td>28.12 (2.07, 54.17)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Source: Table 11-3, Clinical Study Report

Table 2 Re-randomization p-values for the 6MWT

<table>
<thead>
<tr>
<th>Model</th>
<th>Re-randomization p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LME with robust variance estimation, meters/month</td>
<td>.15</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>.06</td>
</tr>
</tbody>
</table>

Source: Table 1, Errata 2, Clinical Study Report; e-mail communication
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Figure 3 Histogram of test statistics for LME with robust variance estimation

4.3 Results for FVC

According to the statistical analysis plan, FVC could be assessed if and only if the results for the 6MWT are statistically significant. I present the results here for the sake of completion.

The changes from baseline in FVC at each study visit are depicted in Figure 4. The results of the statistical analyses show that the differences between 2000L and placebo are statistically significant, regardless of the statistical test used; see Table 3 and Table 4.
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Figure 4 Mean (+/- SEM) Change from baseline over time in FVC upright (% predicted)

Reference: Figure 14.2.1.2B

Source: Figure 11-3, Clinical Study Report

Table 3 Change in FVC upright (% predicted)

<table>
<thead>
<tr>
<th></th>
<th>2000L N=60</th>
<th>Placebo N=30</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LME with model-based variance estimation, % predicted (95% CI)</td>
<td>0.03 (-0.05, 0.10)</td>
<td>-0.16 (-0.27, -0.05)</td>
<td>0.18 (0.05, 0.31)</td>
<td>0.0084</td>
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<tr>
<td>LME with robust variance estimation, meters/month (95% CI)</td>
<td>0.03 (-0.05, 0.10)</td>
<td>-0.16 (-0.25, -0.06)</td>
<td>0.18 (0.06, 0.30)</td>
<td>0.0041</td>
</tr>
</tbody>
</table>
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| Estimates/Tests of Monthly Change in % Predicted FVC from Baseline to Last Observation |
|------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| ANCOVA – mean change, % predicted        | 1.20            | -2.20           | 3.40            | 0.0055          |
| (95% CI)                                  | (-0.16, 2.57)   | (-4.12, -0.28)  | (1.03, 5.77)    |                 |

Source: Table 11-7, Clinical Study Report

Table 4 Re-randomization p-values for FVC

<table>
<thead>
<tr>
<th>Model</th>
<th>Re-randomization p-value</th>
</tr>
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<tbody>
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<td>LME with robust variance estimation, meters/month</td>
<td>.004</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>.004</td>
</tr>
</tbody>
</table>

Source: Table 1, Errata 2, Clinical Study Report; e-mail communication

5. Conclusions

From a statistical perspective, this application is unusual in several ways. While the study was ongoing, the study was changed from a fixed design to a design with an adaptive strategy. This necessitated a change to the endpoint, which became the linear rate of change over the duration of the study. For each subject, this rate of change was estimated by a slope. Thus, instead of comparing the 6MWT at 52 weeks, the primary analysis became a comparison of the slopes.

To evaluate the slopes, the primary analysis method was a linear mixed effects model, where the patient-level intercepts and slopes were random effects and other effects (e.g., treatment, baseline strata) were fixed effects. The statistical analysis plan specified a model-dependent estimator of the variance-covariance matrix.

The statistical analysis plan also specified ANCOVA models and re-randomization tests as supportive analyses. The re-randomization tests were included to address the minimization algorithm that was used to allocate subjects in a 2:1 ratio to either 2000L or placebo.

When the data were analyzed, diagnostic tests determined the 6MWT departed from the assumption of linearity and the assumption of normality as well. Moreover, the applicant asserts
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the observed non-linearity also compromises the estimate of the model-dependent variance-covariance matrix.

As a result, the applicant proposed the use of a “sandwich” estimator of the variance-covariance matrix. While I agree the “sandwich” estimator is more appropriate, its use leaves some unanswered questions. If the model assumptions are correct, the model-based estimator of the variance-covariance matrix is more efficient than a “sandwich” estimator.” Had the results been statistically significant, I wonder whether the applicant would have explored the use of the “sandwich” estimator and argued for its use if the results had been statistically non-significant.

The applicant also included results from analyses that were not pre-specified: generalized estimating equation (GEE) models and non-parametric assessment of the data. These tests gave statistically significant results for the 6MWT. However, I question the use of models that were not prespecified.

Because of the violations of the assumptions underlying the linear mixed effects model and the changes to the model after the data were unblinded, I believe the results of the ANCOVA should be emphasized. The ANCOVA is consistent with the clinical question of interest, which is whether the change from baseline to the last observation differs between 2000L-treated subjects and placebo-treated subjects.

Further complicating the interpretation of the study results is the scheme used to allocate subjects to 2000L and placebo. Instead of a blocked randomization, the study used a minimization allocation in order to maintain a 2:1 (2000L:placebo) ratio within study sites and within strata defined by baseline values for the 6MWT and FVC.

Re-randomization tests are the appropriate approach for assessing statistical significance when a minimization algorithm used. Usually, the result from a re-randomization test is consistent with the result from the classical test. However, that is not the case in this submission. For the ANCOVA of the 6MWT, the p-value changes from 0.035 to 0.06; and for the LME with the sandwich estimator, the p-value changes from 0.046 to 0.150. I discount the applicant’s argument that subjects can be assumed to have arrived in a random order, an assumption which leads to statistically significant results for the 6MWT. The results for FVC are statistically significant regardless of the test used.

The clinical team believes the 6MWT is the relevant parameter for deciding the efficacy of 2000L. The results for FVC were to be assessed only if the result for the 6MWT was statistically significant.
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Although the p-value of 0.06 for the re-randomization test, which I believe is the appropriate test, corresponding to the ANOVA is not statistically significant at the traditional alpha level of 0.05, I believe the orphan status of the indication needs to be entertained when deciding on the efficacy of this product for the treatment of adult subjects with Pompe disease.