Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:

A Phase III, Randomized, Double-blind, Placebo-controlled, Multicenter Trial to Evaluate the Efficacy and Safety of rhGAD65 to Preserve Endogenous Beta Cell Function in Adolescents and Adults with Recently Diagnosed Type 1 Diabetes, Carrying the Genetic HLA DR3-DQ2 Haplotype – The DIAGNODE-3 study protocol.

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DIAGNODE-3 Statistical Analyses Plan

Populations for analyses

The following analysis sets will be used for the statistical analysis and presentation of data:

- The screened set will consist of all patients who were screened for participation in this study. The screened set will be used for presentation of study disposition of patients.
- The randomized set will consist of all patients who were randomized.
- The safety set (SAF) will consist of all randomized patients who received at least one injection. Patients will be analyzed according to treatment received rather than randomized. If a patient received more than one randomized treatment, they will be analyzed and included in summaries according to the treatment they received the most. Patients receiving no study treatment will be excluded, as will patients who have no post-dose safety assessments. Safety analyses will be based on the SAF.
• The FAS will consist of all randomized patients who have received at least one dose of study medication, a baseline measurement and have at least one post-baseline assessment for any efficacy endpoint. The FAS is the primary analysis dataset, and will be used for all primary, secondary and exploratory efficacy endpoints. Patients in the FAS will contribute to the analysis "as randomized."

• The per protocol set (PPS) will consist of all patients in the FAS who meet the following criteria:
  - Have no important protocol deviations;
  - Completed the treatment phase (Month 24) for the primary end point (i.e., did not discontinue from the trial early);
  - Received all injections of study drug.

C-peptide

The null hypothesis ($H_0$) is that there is no difference versus the alternative hypothesis ($H_1$) that there is a difference in the geometric mean ratio (GMR) between the Diamyd-treated group and the placebo-treated group. The null and alternative hypotheses testing can be formalized as follows:

$$H_0: \text{GMR (Diamyd/placebo)} = 1 \quad \text{vs.} \quad H_1: \text{GMR (Diamyd/placebo)} \neq 1$$

where GMR (Diamyd/placebo) is the back-transformed least square mean (LSM) ratio in the relative change from baseline in $AUC_{\text{mean 0-120 min}}$.

Change from baseline will be analyzed using a Restricted Maximum Likelihood-based repeated measures approach (MMRM). The model for analysis will include fixed, categorical effects of treatment, stratification variables, visit, treatment-by-visit interaction, as well as the continuous, fixed covariate of log-transformed baseline C-peptide $AUC_{\text{mean 0-120 min}}$ during an MMTT and the interaction between baseline C-peptide-by-visit, and the fixed continuous covariate of baseline age. Patient identification number will be included as a categorical random effect. An unstructured covariance matrix will be assumed. If this analysis fails to converge, compound symmetry will be tested. The (co)variance structure converging to the best fit, as determined by Akaike’s information criterion will be used as the primary analysis. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom.

LSM estimates and 95% CIs will be back-transformed from the natural log scale to the original scale and presented together with nominal p-values. Back-transformed estimates of the treatment difference will provide an estimate of the (Diamyd/placebo)-ratio in the relative change from baseline in $AUC_{\text{mean 0-120 min}}$. A ratio of e.g., 0.8 will mean that the change from baseline to Month 24 in C-peptide level was 20% smaller for Diamyd than for placebo at Month 24.

The primary efficacy analyses will be repeated using the PPS.
HbA1c

If the null hypothesis for the first primary endpoint C-peptide is rejected, then the second primary endpoint HbA1c will be tested. The null hypothesis ($H_0$) is that there is no difference versus the alternative hypothesis ($H_1$) that there is a difference in the mean change from baseline to EoS in HbA1c between the Diamyd-treated group and the placebo-treated group. The null and alternative hypotheses testing can be formalized as follows:

$$H_0: \mu_{Diamyd} = \mu_{Placebo} \quad \text{vs.} \quad H_1: \mu_{Diamyd} \neq \mu_{Placebo}$$

where $\mu$ is mean change from baseline to EoS in HbA1c.

If the null hypothesis for the first primary endpoint is not rejected then the hierarchical testing in the overall DR3-DQ2-positive population stops; p-values for the second primary endpoint will be regarded as exploratory.

Change from baseline will be analyzed with the MMRM model and subject to the sensitivity analyses described in Section 0.

HLA DR4-DQ8-negative Subgroup

If either of the co-primary endpoints is not statistically significant in the overall population at a two-sided significance level of 0.04, the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.01 level of significance in an analogous manner to the primary analysis in the overall population. If both co-primary endpoints in the overall population are statistically significant at the two-sided 0.04 level, then the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.05 level of significance in an analogous manner to the primary analysis in the overall population.

Statistical Analyses of Other Endpoints

Analyses of Secondary Endpoints

The following secondary efficacy endpoint will be analyzed with a similar MMRM model as the primary efficacy endpoint (details on log-transformations will be provided in the SAP):

- Change in time in glycemic target range 3.9 to 10 mmol/L (70 to 180 mg/dL) [evaluated from CGM data] between baseline and 24 months.

A specific section of the SAP will lay out in detail the processing and statistical analysis of the raw CGM device data.

The following secondary efficacy endpoint will be analyzed using the Cochran/Mantel-Haenszel Test stratified by the stratification variables; 95% CIs will be calculated according to the Clopper-Pearson method:

- Proportion of patients with IDAA1c ≤9 (partial remission) at 24 months.

The following secondary efficacy endpoints will be assessed using Poisson regression, including stratification variables; rate ratios with 95% CI and p-value will be given:

- Number of episodes per patient of severe hypoglycemia between baseline and 24 months.
- Number of episodes per patient of DKA between baseline and 24 months.
Analyses of Exploratory Endpoints

The following exploratory endpoint variables will be analyzed with a similar MMRM model as the primary efficacy endpoint (details on log-transformations will be provided in the SAP):

- Change from baseline to Month 24 in IDAA1c.
- Change from baseline to Month 24 in exogenous insulin requirements based on total number of units of insulin per kilogram body weight per day.
- Change in time in severe hypoglycemic range <3.0 mmol/L (50 mg/dL) [evaluated from CGM data] between baseline and Month 24.
- Change in time in hypoglycemic range 3.0 to 3.8 mmol/L (50 to 69 mg/dL) [evaluated from CGM data] between baseline and Month 24.
- Change in glycemic variability as measured by %CV [evaluated from CGM data] between baseline and Month 24.
- Change in (fasting, maximal, and stimulated) C-peptide measured at 0, 30, 60, 90, and 120 minutes during MMTT at Month 24.
- Change in serum GAD65A titers between baseline and Month 24.
- Change in QoL evaluated by PRO measures (PedsQL), family impact, generic and diabetes module with parent proxy between baseline and Month 24.
- Change from baseline to Month 24 in BMI.

The following exploratory endpoint will be assessed using Poisson regression, including stratification variables; rate ratios with 95% CI and p-value will be given:

- Number of episodes per patient of mild/moderate hypoglycemia between baseline and Month 24.

The following exploratory endpoints will be analyzed using the Cochran/Mantel-Haenszel Test stratified by the stratification variables; 95% CIs will be calculated according to the Clopper-Pearson method:

- Proportion of patients with a stimulated 90 min C-peptide level above 0.2 nmol/L (0.6 ng/mL) at Month 24.
- Proportion of patients with new onset hyperthyroidism, hypothyroidism, and celiac disease.
- Proportion of patients with increase or decrease in medication usage for treatment of hyperthyroidism and hypothyroidism in those with such disorders at baseline.
- Proportion of patients who change insulin delivery method during the study (MDI/CSII/semi/closed loop system).

Analysis of Safety and Immunological Endpoints

The safety endpoints will be evaluated based on the SAF.

Immunological endpoints will be summarized descriptively, including p-values from non-parametric statistical tests (details to be provided in the SAP).