A double-blind, randomized investigator-initiated study to determine the safety and the effect of Diamyd® in combination with Vitamin D on the progression to type 1 diabetes in children with multiple islet cell autoantibodies.

Study code: DiAPREV -IT 2

Phase IIa study

STATISTICAL ANALYSIS PLAN

Signatures:

Statistical Analysis Plan was prepared by:

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1 Abbreviations

AE	Adverse Event
AUC	Area Under the Curve
BDR	Blind Data Review
DSMB	Data Safety Monitoring Board
FPIR	First-phase insulin response
HbA1c	Hemoglobin A1c
ITT	Intent to Treat
IvGTT	Intravenous Glucose Tolerance Test
OGTT	Oral Glucose Tolerance Test
PP	Per-protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

2 Study objective(s)

The purpose of the study is to assess the safety and efficacy of Diamyd® 20 µg + vitamin D (2000 IU/day) in comparison with placebo + vitamin D (2000 IU/day) in non-diabetic children with multiple islet autoantibodies.

The primary objective of the study is to evaluate if Diamyd®, in children treated with relatively high doses of vitamin D, may delay or stop the autoimmune process leading to clinical type 1 diabetes in children with ongoing persistent beta cell autoimmunity as indicated by multiple positive islet cell autoantibodies.

The secondary objective is to demonstrate that Diamyd® is safe in children at risk for type 1 diabetes.

3 Design and type of the study

This is a phase IIa, double-blind, randomized, placebo-controlled, parallel group single centre study in children with multiple islet cell autoantibodies with a maximum of two-year follow-up.

3.1 Randomization and blinding

Eligible participants were randomized to active treatment or placebo, with 1:1 ratio. This randomization was stratified by glucose metabolism at baseline applying the following categorisation:

A) Impaired glucose metabolism defined as low first phase insulin response on IvGTT (≤30) and/or an increased 30, 60, 90 min glucose values (≥ 11.1 mmol/L) and/or 120 minutes glucose value (≥7.8 mmol/L) on OGTT, and/or HbA1c ≥ 39 and/or fasting glucose ≥6.1 mmol/L

B) Normal glucose metabolism

An independent statistician performed the randomization procedure. The randomization code for each child was preserved in envelopes in a locked safe. The treating physician had the possibility to break the code in cases of emergency. Further the Data Safety Monitoring Board (DSMB) had the possibility to request access to the randomization list.

4 Study variables

The following variables will be evaluated.

The demographic and baseline variables:

Age, Gender, Height, Weight, HLA genotypes, First degree relative or from general population, Autoantibodies, Other autoimmune diseases (celiac disease, thyroid disease).

The primary efficacy variable:

Type I diabetes status after 2-year follow-up

The secondary efficacy variables:

Proportion of children who progress from normal to impaired glucose metabolism and proportion of children who progress from impaired to more severely impaired glucose metabolism

The exploratory efficacy variables:

C-peptide, oGTT/IvGTT, plasma glucose, HbA1c

The safety variable:

Adverse events (AE), safety laboratory, physical and neurological examination, vital signs, GADA titers, isotype, epitope and affinity, anti-idiotypic antibodies, injection site reactions

5 Sample size considerations

The planned sample size of the study was 80 children, 40 in each of the study arms. This sample size was based on the expectation that 50% of the untreated children with multiple autoantibodies will develop type 1 diabetes within 5 years. This frequency had previously been reported equal among relatives to diabetes patients and in the general population. If 20% of the treated children would develop type 1 diabetes within the same period of time, there would have been a power of 82% with alpha=5% with a group of 40+40=80 children. P value <0.05 was to be used as the significance level.

However, the study was stopped prematurely ending up with a total sample size of 29 children. The study was stopped due to negative results received from another similar study (DiAPREV/2008). In that study no effect on delaying or preventing type 1 diabetes with Diamyd®-treatment was shown, while GADA increased in the study patients. Since the cohort of children, inclusion and exclusion criteria and dose of Diamyd® were all equal in DiAPREV/2008 and DiAPREV-IT 2, it is not probable that the current study would show that Diamyd® would delay or prevent type 1 diabetes, but it may have effects on the immune system that can be explored in mechanistic studies.

6 Statistical hypotheses

As the study was stopped prematurely with limited number of children within both treatment arms, no formal statistical hypotheses are defined.

7 Analysis sets

The database will be reviewed in a blinded manner prior to database lock. Patients are assigned to the respective analysis sets depending on the observed protocol violations.

7.1 Intention to Treat Population (ITT)

The ITT-population comprises all randomized (as planned) subjects.

7.2 Full Analysis Population

The Full Analysis Set (FAS) is a subset of ITT and comprises of all subjects who received any study drug and who participated in at least one post-baseline assessment. FAS is the primary efficacy population.

7.3 Per protocol (PP) Population

The PP population comprises of all subjects who did not substantially deviate from the protocol as to be determined on a per-subject basis. The PP population is used for sensitivity purposes.

7.4 Safety Population

The Safety Population comprises of all subjects who received at least one administration of the study drug at any dosage.

8 General statistical considerations

Descriptive statistics include at least the number of participants, mean, standard deviation (SD), minimum, median and maximum for continuous variables, and frequencies and percentages for categorical variables. Line listings of all participants are provided as well.

Missing values will not be imputed in the analyses.

The table and figure plan is presented in Appendix 18.2.

9 Demographic and other baseline characteristics

All background and other baseline characteristics will be tabulated with descriptive statistics. Medical history will be summarized with frequency distributions including both event and subject counts. Tabulations will be conducted by treatment group. No statistical tests will be carried out for any baseline measures between the treatments.

10 Concomitant medication/treatment

Concomitant medication and treatments will be summarized overall and by treatment group using ATC classification.

11 Extent of exposure and compliance

The extent of exposure and treatment compliance will be summarized descriptively overall and by treatment group.

12 Analysis of efficacy

Descriptive statistics of all the efficacy data will be presented by visit and treatment group. ITT-population will serve as the primary efficacy population.

12.1 Primary efficacy variables

The primary efficacy variable is the type 1 diabetes status (with vs without type 1 diabetes) at the end of the two-year follow-up. The proportion of children developing clinical type 1 diabetes during the two-year follow-up will be summarized with frequency tables. The proportions of children with type 1 diabetes will also be summarized for other study visits by treatment group. The analysis will be conducted for both ITT and PP populations.

In addition, time to the development of type 1 diabetes up to 2 years will be graphically illustrated by Kaplan-Meier curves by treatment.

12.2 Secondary efficacy variables

The secondary efficacy variables are the proportion of children who have progression from normal to impaired glucose metabolism (within children with normal glucose metabolism at screening) and the proportion of children who have progression from already impaired glucose metabolism from one or several criteria to additional signs of reduced glucose metabolism (within children with impaired glucose metabolism at screening).

Impaired glucose metabolism is defined as acceptance of at least one of the following criterion; a) F-glucose $\geq$ 6.1 mmol/L, b) maximum p-glucose at 30, 60, 90 minutes $\geq$ 11.1 mmol/L in the OGTT, c) 120 min p-glucose $\geq$ 7.8 mmol/L on OGTT, d) HbA1c $\geq$ 39 mmol/mol.

Both the secondary efficacy variables will be summarized descriptively by visit and treatment group.

12.3 Subgroup analysis

Subgroup analysis will be conducted for the primary and secondary efficacy variables, so that the above-mentioned analyses will also be assessed by HLA genotype (DR3-DQ2, present/absent). The DR3-DQ2 subgroup will be derived from the database so that patients that have HLA-DQ haplotype of DQ2 (DQ2/8 or DQ2/x) are classified as DR3-DQ2 (present) and the ones without DQ2 (DQ2.2/8; DQ8/8 or DQ8/x) are classified as DR3-DQ2 (absent).

12.4 Exploratory efficacy variables

The exploratory efficacy variables include

1) first-phase insulin response (FPIR) and K-value from IvGTT
2) fasting, maximum, 2 hours and $AUC_{0\text{-}120min}$ C-peptide levels from OGTT,
3) fasting, maximum, 2 hours and $AUC_{0\text{-}120min}$ plasma-glucose from OGTT,
4) HbA1c (mmol/mol),
5) Fasting and $AUC_{0\text{-}120min}$ insulin levels from OGTT,
6) AUC_{90min} of C-peptide, glucose and insulin from IvGTT

All the exploratory efficacy variables will be summarized descriptively including both the absolute and change from baseline values by visit and treatment group if feasible. In addition, the endpoints will be illustrated graphically if feasible.

The AUC values for C-peptide, plasma glucose and insulin will be calculated applying linear trapezoidal rule. In case of single missing time points for the C-peptide/plasma-glucose/insulin are detected, it will be assumed that missing values fall linearly between existing data points. In case the 0 min timepoint is missing, the corresponding AUC is set to missing.

13 Analysis of safety

All safety analyses will be conducted for the Safety population.

13.1 Adverse events

All adverse events (AEs) will be tabulated (according to the MedDRA dictionary) by treatment group, system organ class (SOC), preferred term (PT), severity and causality. Both participant and event counts will be calculated. For participants with more than one occurrence of the same preferred term, the strongest causality and severity will be used when participant counts are reported.

In addition, serious adverse events (SAE) and adverse events leading to discontinuation will be reported separately.

13.2 Laboratory safety variables

Descriptive statistics of laboratory safety variables (hematology, chemistry and urine analyses) will be presented by visit and treatment group, together with the changes from baseline (pre-dose) values.

13.3 Physical and neurological examination, vital signs

Vital signs (blood pressure, heart rate) will be summarized descriptively by visit and treatment group, together with changes from baseline (pre-dose). The abnormal findings in physical and neurological examinations will be presented as frequencies and percentages by visit and treatment group.

13.4 GADA titers, isotype, epitope and affinity, anti-idiotypic antibodies

GADA titers, isotype, epitope and affinity, anti-idiotypic antibodies (GADA (U), IA-2A (U), IAA ny (U/mL), ZnT8ArgA (U), ZnT8TrpA (U), ZnT8GluA (U)) will be summarized descriptively by visit and treatment group. In addition, GADA titers will be illustrated graphically.

13.5 Autoimmunity other than diabetes

Autoantibodies to Thyroid peroxidase (TPO), Thyroglobulin (Thgl) and Tissue transglutaminase (tTg), (Thyroid peroxidase Ab (U), Thyroglobulin Ab (U), tTG IgA (U) and tTG IgG (U) together with TSH (mU/L) and Free T4 (pmol/L), will be summarized descriptively at visit 0, 12 months and 24 months by treatment group.

13.6 Injection site reactions

The number of injection site reaction by severity will be summarized by visit and treatment group.

14 Completion and premature discontinuation

Completion and premature discontinuation will be listed. The reasons for premature discontinuation will be presented.

15 Post diagnosis intervention and follow-up

Study participants who developed type 1 diabetes during the study period were offered to participate in a post diagnosis intervention and follow up protocol. The collected post diagnosis data from the included participants will be summarized using data listings.

16 Deviations from the analyses planned in the study protocol

Due to the premature stop of the study, all the formal statistical analyses were removed from the statistical analysis plan compared to the protocol.

17 Execution of statistical analyses

Statistical analyses will be performed by 4Pharma Ltd.

18 Hardware and software

Statistical analysis, tables and patient data listings will be performed with SAS® version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). Pharmacokinetic parameters ($AUC_{0\text{-}120min}$ for C-peptide, glucose and insulin) will be calculated using Phoenix WinNonlin software (version 8.1; Certara USA, Inc; Princeton; NJ; USA).

19 References

Clinical Study Protocol, Version 3 (28.08.2017)

20 Appendices

20.1 Schedule of assessments

Event	**Visit 0 Information and consent**	**Visit 1 Day 1** *30 +/- 7 days from visit 0*	**Visit 2 Month 1** *30 +/-7 days from visit 1*	**Visit 3 Month 3** *3 months +/- 14 days from visit 1*	**Visit 4 Month 6** *6 months +/- 14 days from visit 1*	**Visit 5 Month 9** *9 months +/- 14 days from visit 1*	**Visit 6 Month 12** *12 months +/- 14 days from visit 1*	**5 Years Follow up Every 3rd or 6th month** *Every visit +/- 14 days from visit 1*
Informed Consent	X							
Randomization		X						
Diamyd/Placebo Administration		X	X					
Start of Vitamin D	X							
Medical History	X							
General Physical Exam	X	X	X	X	X		X	Last visit
Concomitant Medication	X	X	X	X	X		X	Every 6th month
Weight, Height	X	X	X	X	X		X	Every 6th month
Vital signs (BP)	X	X	X	X	X		X	Every 6th month
Blood Sampling:								
Hematology, chemistry, including calcium	X	X	X	X	X	X	X	Every 3rd month
25 OH-vitamin D3	X	X			X		X	Every 6th month
Cellular analyses	X		X		X		X	Every 6th month
GADA, IA-2A, ZnT8A, IAA	X	X	X	X	X	X	X	Every 3rd month
C-peptide	X Fasting + stimulated	X Fasting + stimulated	X Random	X Random	X Fasting + stimulated	X Random	X Fasting + stimulated	Every 6th month Fasting + stimulated
HbA1c	X	X	X.	X	X	X	X	Every 3rd month
Plasma Glucose	X	X	X	X	X	X	X	Every 3rd month
OGTT/IvGTT	IvGTT	OGTT			OGTT		IvGTT	Every 6th month IvGTT/OGTT
TPOAb, ThglAb, tTGAb	X						X	Every 12th month
Urine Analysis	X	X	X	X	X		X	Every 6th month
Neurological Assessment	X		X	X	X		X	Last visit
Injection Site Inspection		X	X	X	X			
Adverse Events		X	X	X	X		X	Last visit
Diary		X	X	X	X		X	Last visit
Questionnaire to child about the study procedures		X						Last visit

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20.2 Table and figure plan

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