NCT: 03345004

DIAMYD MEDICAL

CLINICAL TRIAL PROTOCOL

A Phase IIb, 2-Arm, Randomized, Double-blind, Placebo-Controlled, Multicentre Study to Optimize Diamyd® Therapy Administered into Lymph Nodes Combined with Oral Vitamin D to Investigate the Impact on the Progression of Type 1 Diabetes

Study No. DIAGNODE-2[redacted]
EudraCT Number: 2017-001861-25
Study Development Phase: IIb
Version 8: May 15, 2020

Sponsor: Diamyd Medical AB
[redacted]

Coordinating investigator:
[redacted]

Contract Research Organization:
[redacted]

Planned start date: Q4, 2017

STUDY PROTOCOL VERSION TABLE

Version 1:	May 03, 2017
Version 2:	June 16, 2017
Version 3:	August 21, 2017
Version 4:	November 03, 2017
Version 5:	February 26, 2018
Version 6:	June 28, 2018
Version 7:	June 18, 2019
Version 8:	May 15, 2020

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2 SYNOPSIS OF PROPOSED DIAGNODE-2 STUDY

Name of Sponsor/Company Diamyd Medical AB	**Individual Study Table Referring to Part of Dossier in which the Individual Study or Study Table is Presented:**	**(For National Authority Use Only)**
Name of Finished Product: DIAMYD® D-vitamin [redacted]		
Name of Active Ingredient: [redacted]	**Volume:** **Reference:**	

Title of Study: A Phase IIb, 2-Arm, Randomized, Double-blind, Placebo-Controlled, Multicentre Study to Optimize Diamyd® Therapy Administered into Lymph Nodes Combined with Oral Vitamin D to Investigate the Impact on the Progression of Type 1 diabetes

Protocol Number: DIAGNODE-2[redacted]

Investigators and Study Centre: Approximately 22 sites in Sweden, Spain, Czech Republic and the Netherlands including approximately 106 patients

Phase of Development: Phase IIb

Objectives:

Primary objective

The primary objective is to evaluate the efficacy of Diamyd, administered into lymph nodes in combination with an oral vitamin D regimen, compared to placebo in terms of preserving endogenous insulin secretion as measured by C-peptide.

Secondary objectives

The secondary objectives are to compare Diamyd, administered into lymph nodes in combination with an oral vitamin D regimen and placebo treatment with respect to the effects on the diabetes status, treatment safety, immune system and quality of life (QoL) of the patients.

Study Design:

The study is a 2-arm, randomized, double-blind, placebo-controlled, multicenter, clinical trial. Eligible patients will receive injections of Diamyd/placebo into an inguinal lymph gland at three occasions, with one month intervals in combination with an oral vitamin D/placebo regimen (starting 1 month ahead of injections) during 4 months. All patients will continue to receive intensive insulin treatment from their personal physicians during the whole study period. The patients will be followed in a blinded manner for a total of 15 months. All patients that are ongoing, i.e. have not performed Visit 7 (15 months visit) when protocol version 7 is approved and implemented, will be asked to participate in the Extension Study Period which include Visit 8 at month 24.

Selection of Patients: Patients must be ≥12 and <25 years old, and diagnosed with Type 1 diabetes (T1D) within the previous 6 months at the time of screening. Patients will be eligible for enrolment if fasting C-peptide is ≥0.12 nmol/L (0.36 ng/mL) and elevated levels of GAD65A, i.e. antibodies to glutamic acid decarboxylase (GAD) with molecular weight of 65 kDa (GAD65) are present.

Number of Patients Planned: Approximately 106 patients will be enrolled. To ensure that 106 patients are screened and enrolled approximately 127 patients will be screened.

Name of Sponsor/Company Diamyd Medical AB	**Individual Study Table Referring to Part of Dossier in which the Individual Study or Study Table is Presented:**	**(For National Authority Use Only)**
Name of Finished Product: DIAMYD® D-vitamin [redacted]		
Name of Active Ingredient: [redacted]	**Volume:** **Reference:**	

Description of Treatment Groups:
The patients will be assessed for eligibility at the screening visit (Visit 1) 2 to 4 weeks prior to start of oral treatment with vitamin D. On Visit 2 (Day 1), patients eligible for the study will be randomized to 1 of 2 treatment groups:

- Approximately 53 patients will be assigned to receive i) three (3) intralymphatic injections with 4 µg Diamyd on Days 30, 60, and 90 and; ii) oral vitamin D 2000 IE daily for 4 months (from Day 1 through Day 120)
- Approximately 53 patients will be assigned to receive i) three (3) intralymphatic injections of Placebo for Diamyd on Days 30, 60, and 90 and; ii) oral Placebo for vitamin D once a day for 4 months (from Day 1 through Day 120)

Name of Sponsor/Company Diamyd Medical AB	**Individual Study Table Referring to Part of Dossier in which the Individual Study or Study Table is Presented:**	**(For National Authority Use Only)**
Name of Finished Product: DIAMYD® D-vitamin [redacted]		
Name of Active Ingredient: [redacted]	**Volume:** **Reference:**	

Endpoints:

Primary Endpoint:

- Change in C-peptide (Area Under the Curve $[AUC]_{mean\ 0\text{-}120\ min}$) during a Mixed Meal Tolerance Test (MMTT) between baseline to 15 months.

Key Secondary Endpoints:

- Change in insulin-dose-adjusted HbA1c (IDAA1c) between baseline and 15 months
- Change in Hemoglobin A1c (HbA1c) between baseline and 15 months
- Change in daily exogenous insulin consumption between baseline and 15 months.

Secondary endpoints:

- Other variables that indicate Diabetes Status such as plasma C-peptide, variability of blood sugar, and number of self-reported hypoglycemia.
- Variables that indicate treatment safety such as occurrence of adverse events (AEs), physical examinations, hematology, urine analysis, injection site reactions, GAD65A titer, vital signs and clinical chemistry.
- Variables that indicate effects on the immune system such as serum autoantibodies (and isotypes) to GAD65, serum cytokine levels, secretion of cytokines by immune cells in response to GAD65-stimulation, and proportions of immune cells in blood.
- Measurements of patient QoL by questionnaire.

Exploratory Endpoints:

- All data collected at the 24-month follow-up visit will be regarded as exploratory endpoints.

Sample size:

A total sample size of 106 patients is planned for a 1:1 randomization in order to provide 90% power to detect a 50% difference in geometric mean C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) at 15 months between the active arm and placebo arm using a two-sided test at the 0.05 level, with 10% loss to follow-up. This is based upon a t-test employing ln(X+1) normalizing transformation of C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at 15 months and assumed mean and standard deviation estimates, on the transformed scale, of 0.134 and 0.20 respectively.

Analysis of Primary endpoint variable:

The mean change in log-transformed values will be analyzed using Mixed Model Repeated Measures (MMRM). The model for analysis will include fixed, categorical effects of treatment, randomization strata (GAD65A level), visit and treatment by visit interaction, as well as the continuous, fixed covariates of baseline value.

Analysis of Key Secondary endpoint variables:

Key secondary efficacy endpoint variables will be analysed with the same MMRM model as the primary efficacy endpoint.

Analysis of Secondary endpoint variables:

Continuous secondary diabetes status variables will be compared using the same MMRM model as for the primary efficacy endpoint.

Name of Sponsor/Company Diamyd Medical AB	**Individual Study Table Referring to Part of Dossier in which the Individual Study or Study Table is Presented:**	**(For National Authority Use Only)**
Name of Finished Product: DIAMYD® D-vitamin [redacted]		
Name of Active Ingredient: Recombinant Human Glutamic Acid Decarboxylase (rhGAD65) Calciferol	**Volume:** **Reference:**	

Categorical secondary endpoints will be presented using summary statistics and p-value from stratified Cochran/Mantel-Haenszel Test.

For immunological secondary endpoints summary statistics will be accompanied with p-values from non-parametric statistical tests

Analysis of the QoL data obtained within the study will follow the EQ-5D-5L User guide.

3 LIST OF ABBREVIATIONS AND DEFINITION OF STUDY SPECIFIC TERMINOLOGY

1,25(OH)2D3	1α,25-dihydroxyvitamin D3 also called calcitriol
AE	Adverse Event
Alum	Aluminum hydroxide
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
$AUC_{mean\ 0\text{-}120\ min}$	AUC mean 0-120 minutes
BMI	Body Mass Index
BP	Blood Pressure
DKA	Diabetic Ketoacidosis
CI	Confidence Interval
CRO	Contract Research Organization
DCCT	Diabetes Control and Complications Trial
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
eDiary	Electronic Diary
ELISPOT	Enzyme Linked Immunosorbent Spot Forming Cell Assay
FAS	Full analysis set
FGM	Flash Glucose Monitoring
GABA	Gamma-amino Butyric Acid
GAD	Glutamic acid decarboxylase
GAD65	GAD with molecular mass 65 kDa
GAD65A	Antibodies to GAD65
GCP	Good Clinical Practice
HbA1c	Hemoglobin A1c
HCG	Human Chorionic Gonadotropin
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IAA	Insulin Autoantibody
IA-2	Insulinoma-associated Protein 2

IA-2A	Insulinoma-associated Protein 2 antibodies
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IDAA1c	Insulin-Dose-Adjusted HbA1c
IEC	Independent Ethics Committee
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IMP	Investigational Medicinal Product
IWRS	Interactive Web Response System
LADA	Latent Autoimmune Diabetes in Adults
LS	Least Square
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model Repeated Measures
MMTT	Mixed Meal Tolerance Test
NCS/CS	Not Clinically Significant/Clinically Significant
NOD	Non-obese Diabetic
PBMC	Peripheral Blood Mononuclear Cells
PPS	Per-Protocol Set
QALY	Quality Adjusted Life Years
QoL	Quality of Life
rhGAD65	Recombinant Human GAD65
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SOC	System Organ Class
SOP	Standard Operating Procedure
[redacted]	[redacted]
SUSAR	Suspected Unexpected Serious Adverse Reaction
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
TEAE	Treatment-Emergent Adverse Event
TESAE	Treatment-Emergent Serious Adverse Event
[redacted]	[redacted]
Th1	T Helper Cell Type 1
Th2	T Helper Cell Type 2
TNF	Tumour Necrosis Factor

4 INTRODUCTION AND RATIONALE FOR THE STUDY

4.1 Introduction

Type 1 diabetes (T1D) belongs to the group of medical disorders classified as "autoimmune" due to a pathologic feature involving an inappropriate immunological recognition of the body's own tissues. This abnormal immune response, in the case of T1D, results in the destruction of insulin-secreting pancreatic beta cells which in turn leads to a lifelong dependence on exogenous insulin treatment and exposure to both the acute and late complications of T1D. T1D is a particular burden to children and their families, representing by far the most common chronic, serious, life-threatening disease among children and adolescents in Sweden, but common also among adults, and tends to become an extremely serious global problem.

By the time a T1D patient is diagnosed, 70-90% of pancreatic islet beta cell function has been lost due to autoimmune responses against specific beta cell antigens. Over time the ability to produce endogenous insulin is reduced even further. As this happens a T1D patient is more and more reliant on exogenous insulin and is at greater risk for both acute and long term complications of T1D. In the more acute manifestation of T1D, or in the absence of treatment, the lack of insulin can result in diabetic ketoacidosis (DKA), a potentially life-threatening medical emergency. Insulin therapy can dramatically reduce the possibility of death from DKA but even patients adequately treated with insulin are still at increased risk to a number of acute and long-term (chronic) complications. Acute complications include DKA and severe hypoglycemia. Long-term complications of T1D include microvascular, macrovascular and neurologic complications that increase morbidity as well as mortality. Studies have shown that retained residual insulin production is associated with a reduction in DKA and hypoglycemic risk as well as in the development of long-term complications.

Although the use of insulin has dramatically improved the survival of patients with T1D, insulin does not represent a cure as individuals with the disorder, even if well managed, display marked increases in the frequency of debilitating and even life-threatening complications. The severity of these complications makes the development of a therapy allowing for beta cell preservation of great urgency for patients and caregivers. The world-wide incidence of T1D is increasing and despite the significant progress that has been made in its treatment, T1D still represents a severe burden on the individual and on society as well. Any intervention, which can stop or delay the complete loss of functional residual beta-cell mass would be significant as it would provide protection against hypoglycemia and keto-acidosis, improve metabolic control, and a delay and reduction in the micro and macro-vascular complications of diabetes.[1,2,3,4].

In summary, T1D without residual insulin secretion is a dangerous disease with increased mortality despite a lifetime of intense treatment. There is currently no approved method or medicament to halt or slow the immune destruction of remaining pancreatic islet beta cells. Such a treatment would be of great benefit for all newly diagnosed T1D patients in the world, as well for the society.

The etiology of T1D is still unknown. Genetic factors, such as Human Leukocyte Antigen (HLA) genotypes, confer susceptibility to the disease, but other factors, both

genetic and environmental, are needed to initiate the autoimmune process that results in beta cell death. The environmental factors that either trigger the development of the autoimmune process or accelerate the beta cell destruction process are still to be defined.

The destruction of the pancreatic beta cells in T1D is associated with cellular immune responses to the pancreatic islet cells, genetic susceptibility involving genes thought to modulate the immune response, and the presence of autoantibodies against several islet beta cell components (i.e., autoantigens)[5,6] In addition, as these T1D-associated autoantibodies often precede the clinical onset of disease, GAD65A, i.e. autoantibodies directed against glutamic acid decarboxylase (GAD) with a molecular weight of 65 kDa (GAD65A), insulinoma-associated protein 2 (IA-2A), insulin autoantibody (IAA) or zinc T8 (ZnT8A) are widely recognized not only as diagnostic markers for autoimmune beta cell destruction, but as predictive markers for the disease[7].

Animal studies and early clinical trials have shown promise in targeting the autoimmune process and reversing the progress of the disease through the use of anti-inflammatory, immunomodulatory or immunosuppressive regimens. However, no monotherapies have reached their primary end-points when tested in late-phase clinical trials.

The current consensus around developing a cure for T1D is to target the etiology of the disease using a combination of different therapies. These therapies should optimally down-regulate the acute inflammatory response, tolerize the immune system against islet cell autoantigens and stimulate the proliferation, neogenesis and insulin secretion of the remaining beta cells[8].

The combination therapy to be evaluated in this study involves the investigational Antigen Specific Immunotherapy Diamyd administered directly into lymph node and Vitamin D administered orally.

Diamyd is an investigational drug composed of the recombinant human GAD65 (rhGAD65) protein formulated in a sterile, non-pyrogenic phosphate buffered saline containing the aluminum hydroxide (alum) adjuvant, Alhydrogel. The active ingredient, rhGAD65, is manufactured via expression in an insect cell line, followed by stringent purification.

The underlying basis of T1D involves a “pro-inflammatory” T-cell response against insulin-secreting beta cells that is sufficient to cause their destruction and result in insufficient production of endogenous insulin. Diamyd therapy aims at intervening in this destructive process by modulating the immune system in a discrete, antigen-specific fashion to prevent the destruction of beta cells. Thus, the goal of Diamyd therapy would be to slow down or halt the ongoing autoimmune destruction of pancreatic islet beta cells in order to preserve the largest possible amount of endogenous insulin production.

In the proposed treatment regimen, exogenous oral Vitamin D treatment may improve the efficacy both via effects on the immune system and mechanism directly on the beta cells to increase beta cell function and limit the autoimmune reaction. This may

improve blood sugar control and at the same create a fertile field for Diamyd to induce long term tolerability.

4.1.1 GAD and Diamyd, Completed Studies

Although the involvement of GAD in neural transmission is understood by its function as an enzyme converting glutamate to gamma-amino butyric acid (GABA), its specific and unequivocal function in pancreatic beta cells, as well as its role in the pathogenesis of either form of diabetes remains unclear. Indeed, the reason why GAD is a major autoantigen in autoimmune diabetes is not known. However, a broad body of the scientific community has produced convincing data from the non-obese diabetic (NOD) mouse model of T1D that administration of an isoform of GAD with a molecular mass of approximately 65kDa (GAD65) can prevent autoimmune destruction of pancreatic beta cells and subsequent need for exogenous insulin replacement[9,10,11,12,13,14,15,16,17,18,19]. These findings indicate the potential of Diamyd administration as a treatment for T1D.

GAD65 is a major autoantigen in autoimmune diabetes and clinical administration of Diamyd aims to intervene in the autoimmune process in T1D by modulating the immune system via discrete, antigen-specific tolerization in order to arrest or delay the autoimmune destruction of pancreatic beta cells. In recent-onset T1D patients, such induced immune tolerization is proposed to prevent the destruction of remaining beta cells thereby maintaining residual insulin secretion, improving metabolic control and reducing the risk of acute and long-term diabetes complications. Available data demonstrate that even relatively modest retained insulin secretion capability is associated with clinically meaningful benefits including improved glycemic control, reduced hypoglycemia, retinopathy and nephropathy[1,2,3,4]. Additionally, intervening in the destruction of beta cells could allow for beta cell replenishment, either by stimulating regeneration or transplantation[20].

Thus, the goal of Diamyd therapy would be to dramatically slow or halt the ongoing autoimmune destruction of pancreatic islet beta cells in order to preserve the largest possible amount of endogenous insulin production.

Two formulations of Diamyd have been evaluated in preclinical and clinical studies. The bulk Drug Substance (formulated in buffer from the manufacturing process) was used for initial preclinical safety studies, a skin Prick Test in T1D patients and a Phase I clinical trial (formulated in phosphate buffered saline). An adjuvant formulation based on Alhydrogel® was then developed (Diamyd®) and used in Phase II clinical trials.

A preclinical safety evaluation program has been conducted by Diamyd Medical AB, Stockholm, Sweden to support the progression of Diamyd into clinical development. This has comprised single- and repeat-dose toxicity, local tolerance, immunotoxicity, and investigation of the potential for effects on behavior and cardiovascular/respiratory function. Evaluation of all preclinical safety studies performed to date have not provided concerns for clinical safety of either the bulk Drug Substance used for Diamyd or Diamyd itself, even at multiples of the highest clinically-intended dose level, nor resulted in the observation of any target organs of toxicity. Likewise, evaluation of the effects of Diamyd in several different animal

models of autoimmune disease did not indicate any potential for undesirable effects on the immune system.

A dose-finding Phase IIa study in 47 patients with Latent Autoimmune Diabetes in Adults (LADA) demonstrated the safety and efficacy of Diamyd. This randomized, double-blind and placebo-controlled study demonstrated efficacy in preventing beta cell destruction in the group of patients receiving the 20 µg dose. There were no serious adverse events (SAEs) reported during the 6 month long main study period. The majority of reported Adverse Events (AEs) were due to influenza-like symptoms. A minority of injections resulted in injection-site reactions, which were mild and occurred primarily on the day of the injections. These findings support the safety of immuno-modulation by treatment with alum-formulated rhGAD65. The 5-year final follow-up investigation did not reveal any study-related SAEs[21,22].

In a Phase IIb, randomized, double-blind, placebo-controlled multicenter study in 160 LADA-patients, the patients received 20 µg of rhGAD65 or placebo on 2 occasions 4 weeks apart. The efficacy analysis was discontinued due to quality concerns pertaining to randomization. However, no safety concerns were raised. None of the SAEs reported in the study were related to the treatment.

In another Phase IIb clinical trial, the efficacy and safety of Diamyd were investigated in children 10-18 years of age, recently diagnosed with T1D. A total of 70 GAD65A-positive children with a recent diagnosis (<18 months duration) of T1D and remaining beta-cell capacity measured by C-peptide levels, were included in the study. The study was randomized, double-blind and placebo-controlled and 20 µg Diamyd was given twice 4 weeks apart. The study has provided support for the clinical safety of Diamyd and statistically significant and clinically relevant positive effect on the preservation of beta cell function (production of C-peptide) was seen after 30 months[23]. The frequency and pattern of AEs did not differ significantly between the placebo and active treatment group. There were no treatment-related SAEs reported.

A 3-armed Phase III, randomized double-blind, placebo-controlled multi-center intervention study has been completed. In this study a total of 334 children and adolescents in the EU, 10-20 years of age with recent onset of T1D (within 3 months) was given placebo or 20 µg Diamyd in a prime-and-boost regimen on days 1 and 30 to confirm previous Phase II results. Additionally, one arm of patients was given two additional single doses of 20 µg Diamyd also on days 90 and 270, to evaluate optimization of the treatment. This study did not fulfil the efficacy goal at the 15 months follow-up, but significant treatment effects were observed in several subgroups[24]. A US study run in parallel with identical study design, was closed early due to the EU study outcome. The safety analyses performed by the Data Safety Monitoring Board (DSMB) with data from both the EU Phase III study and the parallel US Phase III study further supports the clinical safety of Diamyd administration in children and adolescents with T1D. In addition, an investigator initiated Phase II clinical intervention trial in the US and Canada, performed by T1D TrialNet (with National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK] as sponsor), reported results in June 2011 and this trial did not meet its efficacy endpoints[25].

A meta-analysis of several clinical studies performed with Diamyd antigen specific immunotherapy was published in 2017 showing that there is a 98% probability that 20 µg rhGAD65 with alum administered twice yields a positive biological effect[26]. All studies also confirm the previously determined favorable safety profile for Diamyd and no neurological concerns have been identified, supporting further development using new alternative approaches aiming to enhance the effect.

Currently five investigator initiated clinical studies are ongoing in T1D patients, investigating how to improve the efficacy of Diamyd though different combinations with other substances such as vitamin D, etanercept, and GABA, or as a prevention therapy, four in Sweden and one in the USA. One of these are a Swedish open label pilot study investigating the safety and impact of administering Diamyd as intralymphatic injections (DIAGNODE-1) in combination with oral vitamin D. The current status of this trial is summarized below.

The intention of the proposed trial, DIAGNODE-2, is to verify the effects of oral vitamin D in combination with Diamyd that has been observed in the open label study DIAGNODE-1 where Diamyd is administered directly into the lymph node. It is proposed that a combination of Diamyd (administered directly into lymph node) with vitamin D may prove efficacious in saving residual insulin secretion in T1D.

4.1.2 Ongoing Study with Diamyd Administered as Intralymphatic Injections – DIAGNODE-1

An open label, single-center, Phase I/II combination clinical study (DIAGNODE-1), with [redacted] in Sweden as sponsor started enrolling subjects in February 2015. The study objectives are to evaluate the safety of giving Diamyd directly into lymph glands in combination with an oral vitamin D regimen, and to evaluate how the combination treatment influences the immune system and endogenous insulin secretion. A correspondence letter in the New England Journal of Medicine was published discussing preliminary interim findings in 2017[27].

[redacted]

[redacted]

4.2 Rationale for Treatment of Type 1 Diabetes Patients with Diamyd and Vitamin D

Experimental evidence indicates that vitamin D may play a role in the defense against T1D as well as type 2 diabetes (T2D). Epidemiological data suggest that there is a link between vitamin D deficiency and an increased incidence of T1D. A multinational case-control study and a birth cohort follow-up study from Finland[28,29] have both concluded that vitamin D3 supplementation at birth protects from T1D later in life, and a meta-analysis supports gives similar conclusions[30]. Others report lower serum levels of 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3, calcitriol] in patients with recently diagnosed T1D than in healthy control subjects[31]. The protective effects of vitamin D are mediated through the regulation of several components such as the immune system and calcium homeostasis. Thus, mechanistic studies show that 1,25(OH)2D3 modulates dendritic cell maturation in vitro and in vivo[32,33,34,35] and facilitates a shift from a T helper cell type 1 (Th1) to a (T helper cell type 2) Th2 immune response[36]. An increasing amount of evidence suggests that vitamin D also affects beta cells directly thereby rendering them more resistant to cellular stress[37], and there are results indicating that vitamin D may also improve insulin sensitivity[38], which in turn decreases beta cell stress.

Vitamin D has been used in patients with recent onset T1D in an effort to preserve residual insulin secretion. So far vitamin D alone has not been efficacious enough[39,40]. Therefore, there is reason to evaluate vitamin D, both in somewhat higher dose, and in combination with other therapies, as has been done in the DIAGNODE-1 study. In our proposed treatment regimen exogenous oral Vitamin D treatment may improve the efficacy both via effects on the immune system and directly on the beta cells to increase beta cell function and limit the autoimmune reaction. This may improve blood sugar control and at the same create a fertile field for Diamyd to induce long term tolerability.

4.3 Rationale for Use of Intralymphatic Route of Administration of Diamyd

Antigen specific immunotherapy aims at presenting the antigen to the T-cells in the lymph nodes to get a new balance of the immune system and tolerance against the antigen. In the treatment of autoimmune diseases antigen has most often been given either orally, intranasally or subcutaneously after which antigen-presenting cells having moved to the lymphatic tissues will ingest and present the antigen to the cells of the immune system.

Animal studies have shown that direct intralymphatic injections of antigen, induce a strong and relevant T-cell response[41,42,43]. In the allergy field, clinical studies have shown that presentation of the antigen directly into the lymph nodes seems to be more effective than traditional administration, allowing for lower doses and radically fewer numbers of treatments, and there have been no treatment-related AEs[44,45,46,47]. Inguinal lymph nodes are readily accessible in patients and the pain associated with the injection is rated as below that of venous puncture[45]. With this background one of the currently ongoing studies with Diamyd is investigating whether the same approach can be used to increase efficacy of antigen specific immunotherapy in patients in T1D in an open label pilot study (DIAGNODE-1). As previous studies have indicated that a dose of 20 µg Diamyd administered sc twice in a prime-and-boost regimen has a positive effect on preservation of endogenous insulin producing capacity[26], and other studies have shown that intralymphatic administration can achieve adequate efficacy with 3 injections of lower antigen doses, a lower dose of 4 µg Diamyd injected directly into the inguinal lymph node (by help of ultrasound technique) 3 times at 1 month intervals is expected to be adequate. This was the treatment regimen chosen for DIAGNODE-1. The larger proposed double-blind clinical study DIAGNODE-2 will use the same treatment regimen to verify the results of the previous trial.

5 RISK-BENEFIT ANALYSIS

5.1 Risks of Intralymphatic Diamyd

Diamyd is an investigational experimental study drug (not marketed or approved) that has been studied in preclinical and clinical trials (Phase I through III). There are no risks of particular severity or seriousness anticipated based on the toxicological data in animals or prior studies in humans, except that patients have reported injection site reactions, such as e.g. itching, edema, tenderness, bruises, and pain. All patients recovered, and no patient was withdrawn from further treatment due to injection site reactions.

Theoretical risks of Diamyd such as acceleration of the autoimmune process, undesirable effects on the immune system and neurological disease have been discussed and also thoroughly evaluated during several clinical trials in both children and adults, all clinical studies performed with Diamyd to date indicate a favorable safety profile for Diamyd and no neurological concerns have been raised. It is still possible that not yet detected side effects may be revealed in this or future clinical trials.

The dose of 20 µg of Diamyd, administered as a prime dose with a booster dose 1 month later or as a prime and boost on days 1 and 30 followed by 1 or 2 additional single doses (days 90 and 270) have been shown to be safe in man (children and adolescents) with few and mild adverse reactions. All dosing will take place in the hospital-based clinic, and handled only by trained, authorized study personnel, by help of ultrasound technique.

Drug Products consisting of alum-formulated proteins have been injected intralymphatically in clinical trials in the allergy fields without concerns[45,46,47]. The immunological data gathered in the DIAGNODE-1 trial suggest that the immune response of intralymphatic injections are stronger than previous subcutaneous administrations, and the levels of antibodies to GAD65 in patient serum appear to be increased further than previously. This withstanding there have been no negative effects reported in any of the patients of DIAGNODE-1[27].

For further detailed information about previous studies and safety please refer to the Diamyd Investigator's Brochure vs 21, 2019.

5.2 Risks of Vitamin D

There are no anticipated risks of vitamin D supplementation at the dose to be administered in this study, although toxic levels may induce hypercalcemia with symptoms such as tiredness, euphoria, nausea, drowsiness, weight loss, thirst, polyuria, nefrocalcinosis and renal failure. Additional symptoms of vitamin D toxicity include electrocardigraph changes, arrhythmia and pancreatitis.

In the study DIABGAD-1, vitamin D 2000 IU per day is administered orally for 15 months. This dose given to children and adolescents aged 10-18 years can be compared with the dose of 400 IU per day given to babies as a health recommendation to avoid vitamin D insufficiency. Approximately 60 patients in DIABGAD-1 have been randomized of whom ¾ should have received vitamin D, and only 6 AEs were assessed by the investigator as related to vitamin D (in 4 patients). One (1) of these

patients received placebo of vitamin D (2 reported AEs). All 4 AEs (patients received active vitamin D) were also assessed as related to ibuprofen and/or Diamyd and all is reported as recovered (1 dizziness, 2 injection site reaction, and 1 common cold).

Vitamin D in a dose of 2000 IU/day in children has been reported safe[48]. Additionally, a dose of up to 7000 IU/day given to children from 5 years of age with human immunodeficiency virus (HIV) did not raise any safety concerns[49].

Patients will only receive vitamin D/placebo if their screening vitamin D is below 100 nmol/L (40 ng/ml) and the vitamin D/placebo treatment will be dismissed if the patient develops hypercalcemia. So, if the patient has Vitamin D serum levels above 100 nmol/L (40 ng/ml) at screening, no Vitamin D/placebo treatment will be given to that patient and only Diamyd/placebo injections will be given.

5.3 Risks of Combining Diamyd and Vitamin D

We would not foresee that the addition of vitamin D treatment to the Diamyd treatment would increase the theoretical risks of Diamyd, such as acceleration of the autoimmune process, undesirable effects on the immune system, or neurological disease.

5.4 Justification – Risk-Benefit

The hypothesis is that the proposed treatment regime will rebalance islet cell interactions and the pancreatic immune environment to increase beta cell function and limit the autoimmune reaction. This will improve blood sugar control and at the same create a more fertile field for the GAD65 antigen specific immunotherapy Diamyd to induce long term tolerability. Administering Diamyd directly into lymph nodes may enhance the tolerizing effect even more (see rationale in section 4.3).

The individual risks of Diamyd and oral vitamin D are not expected to be increased by combining them in the proposed manner.

The justification for the proposed study with the combination treatment regimen, combined with the proposed exclusion and inclusion criteria and 15-months follow-up investigate if the combination of Diamyd and vitamin D slow down or halt the progress of autoimmune beta cell destruction in T1D, aiming to preserve blood glucose control and endogenous insulin secretion and thereby possibly reduce both acute and long-term complication of the disease. As Diamyd has proven to be well tolerated in a large number of patients in previous studies, the possibility of therapeutic benefit outweighs the risks. In order to safeguard the patients, a DSMB will review data from the study twice a year.

6 TRIAL OBJECTIVES AND PURPOSE

6.1 Aim of Present Study

The main goal is to find a reasonably safe and tolerable treatment for young and adult patients with T1D which can preserve residual insulin secretion, improve the patients' quality of life (QoL) and reduce the risk of both short- and long term complications.

6.2 Primary Objective

The primary objective is to evaluate the efficacy of Diamyd, administered into lymph nodes in combination with an oral vitamin D regimen, compared to placebo in terms of preserving endogenous insulin secretion as measured by C-peptide.

6.3 Secondary Objectives

The secondary objectives are to compare Diamyd, administered into lymph nodes in combination with an oral vitamin D regimen and placebo treatment with respect to the effects on the diabetes status, treatment safety, immune system and quality of life (QoL) of the patients.

6.4 Endpoints

6.4.1 Primary Endpoint

The primary endpoint in this study is:

- Change in C-peptide ($AUC_{mean\ 0\text{-}120\ min\text{-}}$) during a Mixed Meal Tolerance Test (MMTT) between baseline and 15 months.

6.4.2 Secondary Endpoints

The key secondary endpoints to evaluate diabetic status are:

- Change in insulin-dose-adjusted HbA1c (IDAA1c) between baseline and 15 months.
- Change in HbA1c between baseline and 15 months.
- Change in daily exogenous insulin consumption between baseline and 15 months.

The other secondary endpoints to evaluate diabetic status are:

- Change in glycemic variability/fluctuations (evaluated from data from continuous glucose monitoring FreeStyle LibrePro, Flash Glucose Monitoring [FGM]) over 14 day period between Screening and 15 months.
- Proportion of patients with IDAA1c ≤ 9 at 15 months.
- Proportion of patients with a stimulated maximum C-peptide level above 0.2 nmol/L (0.6 ng/ml) at 15 months.
- Proportion of patients with a stimulated 90min C-peptide level above 0.2 nmol/L (0.6 ng/ml) at 15 months.
- Number of self-reported episodes of severe hypoglycemia (Severe hypoglycemia defined as needing help from others and/or seizures and/or unconscious) between baseline and 15 months.
- Change in Rate of hypoglycemic events between baseline and 15 months.

- Number of patients having at least 1 severe hypoglycemic event between baseline and 15 months.
- Change in maximum C-peptide during MMTT between baseline and 15 months.
- Change in Fasting C-peptide between baseline and 15 months.
- C-peptide measured at 30, 60, 90, and 120 minutes during MMTT at 15 months.
- Change in body weight and body mass index (BMI) between baseline and 15 months

The secondary endpoints to evaluate safety are:

- Injection site reactions
- Occurrence of AEs
- Laboratory measurements (hematology and clinical chemistry)
- Urine analysis (microalbuminuria, creatinine)
- Physical examinations, including neurological assessments
- GAD65A titer
- Vital signs (blood pressure [BP])

The secondary endpoints to evaluate the influence on the immune system are:

- Concentrations of serum autoantibodies towards GAD65 and IA-2.
- Concentrations of serum autoantibody isotypes towards GAD65.
- Secretion of cytokines interleukin (IL)-1, IL-2, IL-5, IL-13, IL-10, IL-17, interferon (IFN)γ, and tumour necrosis factor (TNF)α by peripheral blood mononuclear cells (PBMCs) upon stimulation with GAD65.
- Serum concentrations of cytokines IL-1, IL-2, IL-5, IL-13, IL-10, IL-17, IFNγ, and TNFα.
- Secretion of cytokines IL-1, IL-2, IL-5, IL-13, IL-10, IL-17, IFNγ, and TNFα by PBMCs upon stimulation with anti-CD3 and anti-CD28.
- Proliferation of PBMCs upon stimulation with GAD65.
- Further exploratory immunological characterization.

The secondary endpoints of Quality of Life are:

- Change in QoL as measured by questionnaire EQ-5D-5L between baseline and Month 15.
- Quality adjusted life years (QALYs) based on the EQ-5D-5L questionnaire.

6.4.3 Exploratory Endpoints

All data collected at the 24-month follow-up visit (Visit 8) will be regarded as exploratory endpoints and will be presented using summary statistics including data from the main study period where only the patients that participated in the Extended Study Period will be included. The following statistical analyses will be repeated for the whole study period:

- The change in log-transformed C-peptide $AUC_{mean\ 0\text{-}120}$ min during an MMTT from baseline to Month 24

- Change in HbA1c between baseline and 24 months
- Change in daily exogenous insulin consumption between baseline and 24 months
- Change in Fasting C-peptide between baseline and 24 months.
- Change in glycemic variability/fluctuations (evaluated from data from continuous glucose monitoring FreeStyle LibrePro, FGM) over 14 day period between Screening and 24 months.
- Rate of hypoglycemic events using Poisson regression including randomization strata (GAD65A)
- Number of patients having at least 1 severe hypoglycemic event using Cochran/Mantel-Haenszel Test stratified for randomization strata (GAD65A)
- Change in body weight and body mass index (BMI) between baseline and 24 months

7 TRIAL DESIGN

7.1 Description of the Study Design and Procedures

The study is a 2-arm, randomized, double-blind, placebo-controlled study in GAD65A positive T1D patients aged ≥12 and <25 years old, diagnosed within 6 months prior to screening with fasting C-peptide levels ≥0.12 nmol/L (0.36 ng/ml). In total, the aim is to recruit 106 patients at approximately 22 sites. The patients will be followed for 15 months with 7 visits to the clinic (Table 2) in the Main Study Period. All patients that are ongoing, i.e. have not performed Visit 7 (Month 15) when protocol version 7 is approved and implemented, will be asked to participate in the Extension Study Period which include Visit 8 at month 24 (Table 3).

Patients and parent(s)/guardian(s), as applicable, will provide written informed consent before any study-related procedures are performed. On Day 1 (Visit 2), 2-4 weeks after the Screening Visit, patients eligible for the study will be randomized in a 1:1 ratio stratified by GAD65A level and country to receive either:

- i) three (3) intralymphatic injections with 4 µg Diamyd on Days 30, 60, and 90 and; ii) oral vitamin D 2000 IE daily for 4 months (from Day 1 through Day 120)
- i) three (3) intralymphatic injections of Placebo for Diamyd on Days 30, 60, and 90 and; ii) oral Placebo for vitamin D daily for 4 months (from Day 1 through Day 120)

The treatment schedule is given in Table 1.

At the screening visit patients will be assigned a 3-digit country/site number and a 3-digit sequential screening number and these numbers together will be used as a 6-digit patient identification (Patient Number), i.e. XXX-YYY.

Individual patients will be exposed to Diamyd for a maximum of 3 months and vitamin D for a maximum of 4 months. All patients will continue to receive intensive insulin treatment from their personal physicians during the whole study period. All patients will be monitored for at least 1 hour after administration of each Diamyd/placebo injection.

When the last patient has completed the double-blind study period, the study code will be broken and results analyzed.

Table 1 Schedule of Patient Visits for DIAGNODE-2

	Main Study Period							Extension Study Period
	Screening	Intervention and Follow-up						Follow-up
	Day -14 to -28 Screening	Day 1 Baseline	Day 30 Month 1 ±5 days	Day 60 Month 2 ±5 days	Day 90 Month 3 ±5 days	Day 180 Month 6 ±14 days	Day 450 Month 15 ±14 days	Day 720** Month 24 ±14 days
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Active			Diamyd 4 µg intra-lymphatic injection	Diamyd 4 µg intra-lymphatic injection	Diamyd 4 µg intra-lymphatic injection			
		Vitamin D 2000 IE daily, administered orally during 4 months (Day 1 to day 120)*						
Placebo			Placebo for Diamyd, intra-lymphatic injection	Placebo for Diamyd, intra-lymphatic injection	Placebo for Diamyd, intra-lymphatic injection			
		Placebo for vitamin D daily, administered orally during 4 months (Day 1 to day 120)*						

*Treatment with vitamin D/placebo starts at Visit 2 if the Vitamin D serum levels are below 100 nmol/L (40 ng/ml) at screening.
If the patient has Vitamin D serum levels above 100 nmol/L (40 ng/ml) at screening, no Vitamin D/placebo treatment will be given for that patient and only Diamyd/placebo injections will be given.
**Patients that are ongoing, i.e. have not performed Visit 7 when protocol version 7 (Month 15) is approved and implemented, will be asked to participate in the Extension Study Period which include Visit 8 at Month 24.

Table 2 Schedule of Study Events, Main Study Period

Event	V1 Screening	V2 Baseline Visit	V3 Month 1	V4 [a] Month 2	V5 [a] Month 3	V6 Month 6	V7[n] Month 15
DAY	**-14 to -28**	**1**	**30 (±5)**	**60 (±5)**	**90 (±5)**	**180 (±14)**	**450 (±14)**
Informed Consent	X						
Eligibility check[b]	X	X					
Demographics	X						
Randomization		X					
Diamyd/placebo [a]			X[c]	X[c]	X[c]		
Vitamin D/placebo [d] start /end		X				X (day 120)	
Medical History	X						
Family history of T1D	X						
General Physical Exam [e]	X	X	X	X	X	X	X
Neurological Assessment	X	X	X	X	X	X	X
Concomitant Medication	X	X	X	X	X	X	X
Vital signs (BP)	X	X	X	X	X	X	X
Pubertal Stage, if applicable [f]	X					X	X
Distribution of patient eDiary		X					
eDiary use compliance			X	X	X	X	X
Injection Site Inspection; investigator/study nurse [g]			X	X	X		
Injection Site Inspection; in eDiary [h]			X	X	X		
Insulin Dose Collected [i]		X	X	X	X	X	X
FreeStyle LibrePro, FGM[j]	X					X	X
AEs		X	X	X	X	X	X
Urine Pregnancy Test (menarchal females only)	X	X	X	X	X	X	X
Self-reported severe hypoglycemia [k]		X	X	X	X	X	X
QoL questionnaire		X				X	X
MMTT[l]		X				X	X
Blood Sampling for Safety, Genetics, Vitamin D levels and Immunology:							
Hematology	X	X	X	X	X	X	X
Clinical Chemistry	X	X	X	X	X	X	X
GAD65A titer	X	X	X	X	X	X	X
HLA characterization			X				
Vitamin D level	X	X	X	X	X	X	X
Covid-19 serological test[m]							X
Other immunological parameters as specified in Section 6.4.2		X	X	X	X	X	X
Urine Analysis:							
Microalbuminuria	X	X	X	X	X	X	X
Creatinine	X	X	X	X	X	X	X
Blood Sampling for Diabetes Status:							
Fasting C-peptide	X	X	X	X	X	X	X
MMTT-induced C-peptide[l]		X				X	X
HbA1c	X	X	X	X	X	X	X
Fasting Glucose	X	X	X	X	X	X	X
MMTT-induced Glucose[l]		X				X	X

Abbreviations: AEs=Adverse Events; BP= Blood Pressure; eDiary=electronic Diary; GAD65A=Antibodies to glutamic acid decarboxylase with molecular mass 65kDa; HBA1c=Hemoglobin A1c; HLA=Histocompatibility antigen; MMTT=mixed meal tolerance test; FGM=Flash Glucose Monitoring; GAD65=Glutamine acid decarboxylase with a molecular mass of 65kDa; GAD65A= antibodies to GAD65; MMTT=Mixed Meal Tolerance Test; T1D=Type 1 Diabetes; QoL=Quality of Life

a. Study drug administration: For Visit 4 and 5 the visit date must be set in accordance with Visit 3 and 4, respectively, so that the first, second and third doses will be 30 days apart (± 5days).
b. If the fasting c-peptide concentration is not ≥0,12 nmol/L (0.36 ng/ml) at the screening visit this test can be repeated on another day within a period of 2 weeks (maximum 2 tests on different days within a 2-week period).
c. The Diamyd/placebo injection directly into the inguinal lymph node is to be done by an appropriately qualified person at the X-ray department by help of ultrasound technique. For more information regarding the injection please refer to the operations manual for the injection procedure.
d. Treatment with vitamin D/placebo starts at Visit 2 if the Vitamin D serum levels are below 100 nmol/L (40 ng/ml) at screening. If the patient has Vitamin D serum levels above 100 nmol/L (40 ng/ml) at screening, no Vitamin D/placebo treatment will be given for that patient.
e. General Physical Exam also to include height and weight
f. Pubertal Stage should be measured when applicable. Not applicable >1 5 years after the patient have reached final height
g. The investigator/study nurse will inspect the injection site before and after the injection is given and record any injection site reactions in the electronic Case Report Form (eCRF)
h. The patient will record injection site reactions in the eDiary starting the day after injection and for 7 days following the injection. Injection site reactions reported by patients in the eDiary during day 1-7 following an injection should not be reported as an AE in the eCRF. Any injections site reactions reported after the 1 week recording period will be recorded as AEs in the eCRF.
i. Patients will record insulin dose in the eDiary for 4 days prior each visit starting from Visit 2. Insulin dose collected at Visit 2 (for the 4 days prior to Visit 2) will be done by the Investigator and recorded in the eCRF.
j. FGM during 2 weeks post visit for Visits 1, 6 and 7. Patients will be given instructions on when and where to return the device after the testing period. For instruction on how to return the Freestyle Libre Pro sensor, please refer to the Freestyle Libre Pro Worksheet.
k. The number of self-reported severe hypoglycemia (defined as needing help from others and/or seizures and/or unconsciousness) should be recorded in the eCRF and each event will be recorded separately.
l. See Procedure for MMTT in the Operations Manual
m. Only applicable to patients completing their Visit 7 after 01 January 2020.
n. Patients that have completely withdrawn from the study will be asked to return for a final visit at month 15, in order to achieve as complete as possible follow-up. See section 8.5 for further details.

Table 3 Schedule of Study Events, Extension Study Period

Event	**V8[g] Month 24**
DAY	**720 (±14)**
General Physical Exam [a]	X
Neurological Assessment	X
Concomitant Medication	X
Vital signs (BP)	X
Pubertal Stage, if applicable [b]	X
eDiary use compliance	X
Insulin Dose Collected [c]	X
FreeStyle LibrePro, FGM [d]	X
AEs	X
Urine Pregnancy Test (menarchal females only)	X
Self-reported severe hypoglycemia [e]	X
QoL questionnaire	X
MMTT[f]	X
Blood Sampling for Safety, Genetics, Vitamin D levels and Immunology:	
Hematology	X
Clinical Chemistry	X
GAD65A titer	X
Vitamin D level	X
Covid-19 serological test	X
Other immunological parameters as specified in Section 6.4.2	X
Urine Analysis:	
Microalbuminuria	X
Creatinine	X
Blood Sampling for Diabetes Status:	
Fasting C-peptide	X
MMTT-induced C-peptide[f]	X
HbA1c	X
Fasting Glucose	X
MMTT-induced Glucose[f]	X

Abbreviations: AEs=Adverse Events; BP= Blood Pressure; eDiary=electronic Diary; GAD65A=Antibodies to glutamic acid decarboxylase with molecular mass 65kDa; HBA1c=Hemoglobin A1c; HLA=Histocompatibility antigen; MMTT=mixed meal tolerance test; FGM=Flash Glucose Monitoring; GAD65=Glutamine acid decarboxylase with a molecular mass of 65kDa; GAD65A= antibodies to GAD65; MMTT=Mixed Meal Tolerance Test; T1D=Type 1 Diabetes; QoL=Quality of Life

a. General Physical Exam also to include height and weight
b. Pubertal Stage should be measured when applicable. Not applicable >1.5 years after the patient have reached final height
c. Patients will record insulin dose in the eDiary for 4 days prior each visit starting from Visit 2.
d. FGM for 2 weeks post visit for Visits 8 (for patients participating in this extra follow-up visit). Patients will be given instructions on when and where to return the device after the testing period. For instruction on how to return the Freestyle Libre Pro sensor, please refer to the Freestyle Libre Pro Worksheet.
e. The number of self-reported severe hypoglycemia (defined as needing help from others and/or seizures and/or unconsciousness) should be recorded in the eCRF and each event will be recorded separately.See Procedure for MMTT in the Operations Manual

f. See Procedure for MMTT in the Operations Manual
g. Patients that are ongoing, i e. have not performed Visit 7 (Month 15) when protocol version 7 is approved and implemented, will be asked to consent for the Extension Study Period an additional visit at Month 24.

7.1.1 Study Procedures

The study procedures and assessments are described in the section below and presented in detail in Section 9.1 (diabetes status assessments), Section 9.2 (safety), Section 9.3 (immunological assessments), Section 9.4 (genetic status assessments), Section 9.5 (vitamin D status), Section 9.7 (Quality of Life) Section 9.7 (pubertal stage, concomitant medication assessments), and Section 9.9 (demographics). Recording and reporting of AEs are described in detail in Section 11.
The timing of all study events is shown in Table 2 and Table 3 in Section 7.1.

*7.1.1.1 **All Visits, Visit 1 through Visit 8***

Note that the patient should attend all study visits in the morning following an overnight fast (>10 hours, water allowed). For patients with evidence of an infection (including fever), the complete visit should be postponed for 5 days or until the patient has recovered.

*7.1.1.2 **Timing of all visits, Visit 1 through Visit 8***

The first visit, the screening visit (Visit 1) should be performed 14 to 28 days before planned Visit 2 (Baseline), when Vitamin D treatment starts. One month (30 days) later, at Visit 3, the first injection of Diamyd will be administered. For Visit 4 (second Diamyd administration), the visit date must be set in accordance with Visit 3 (i.e. the first Diamyd administration) so that the first and second Diamyd doses will be 30 days apart (±5 days). For Visit 5 (third Diamyd administration) the visit date must be set in accordance with Visit 4 (i.e. the second Diamyd administration) so that the second and third Diamyd doses will be 30 days apart (± 5 days). For Visit 6, 7 and 8, the visit date must be calculated from baseline (Visit 2) ±14 days.

*7.1.1.3 **Visit 1, Screening (Day -14 to -28)***

The screening examination will take place within 6 months of diagnosis of T1D. Potential study patients and guardian(s), where applicable, will receive both written and oral information about the study procedures, potential risks, and benefits. Before any study specific procedures may be performed, the patients and guardian(s), where applicable, must sign the informed consent form (ICF). After written informed consent has been obtained, the following activities and assessments will be performed:

- Eligibility will be checked versus the inclusion/exclusion criteria
- Demographics (including age and gender)
- Medical history
- Family history of T1D
- General Physical Examination, including weight and height
- Neurological Assessment
- Concomitant medication
- Vital signs (BP)
- Pubertal stage, if applicable

- Glycemic variability/fluctuations device (FreeStyle LibrePro, FGM) will be given to the patient
- Urine pregnancy test (menarchal females only)
- Blood sampling for hematology, clinical chemistry and GAD65A
- Blood sampling for vitamin D status
- Urine analysis for microalbuminuria and creatinine
- Blood samples for diabetes status (fasting C-peptide, HbA1c and fasting glucose)
- The patient will be instructed to collect the insulin dose 4 days prior to the next visit and the site will send text message/call to remind the patient

If the fasting c-peptide concentration is not ≥0,12 nmol/L (0.36 ng/ml) at the screening visit this test can be repeated on another day within a period of 2 weeks (maximum 2 tests on different days within a 2-week period).

Examinations will be performed according to Table 2, and in the order outlined in the electronic case report form (eCRF).

7.1.1.4 ***Visit 2, Baseline (Day 1)***

At Visit 2 (baseline) the following activities and assessments will be performed:

- Eligibility will be checked versus the inclusion/exclusion criteria
- Randomization in Interactive Web Response System (IWRS)
- Start of treatment with the oral treatment of vitamin D/Placebo (if the Vitamin D serum levels are below 100 nmol/L [40 ng/ml] at screening)
- General Physical Examination, including weight and height
- Neurological Assessment
- Concomitant medication
- Vital signs (BP)
- Insulin dose collected (Exogenous insulin dose/kg/24 hours) for 4 days prior to visit
- AEs
- Urine pregnancy test (menarchal females only)
- Self-reported severe hypoglycemia (defined as needing help from others and/or seizures and/or unconsciousness)
- QoL questionnaire
- MMTT
- Blood sampling for hematology, clinical chemistry and GAD65A
- Blood sampling for other immunological parameters
- Blood sampling for vitamin D status
- Urine analysis for microalbuminuria and creatinine
- Blood samples for diabetes status (fasting C-peptide, MMTT-induced C-peptide, HbA1c, fasting glucose and MMTT-induced glucose)
- Electronic Diary (eDiary) activated and patients trained in how to use it

Examinations will be performed according to Table 2, and in the order outlined in the eCRF.

7.1.1.5 ***Visit 3, Month 1 (Day 30 ±5)***

At Visit 3 (Month 1) the following activities and assessments will be performed:

- Treatment with Diamyd/Placebo injection
- Injection site inspection by the Investigator/Study Nurse
- Patient instructed on how to record injection site injections in the eDiary
- General Physical Examination, including weight and height
- Neurological Assessment
- Concomitant medication
- Vital signs
- AEs
- Urine pregnancy test (menarchal females only)
- Self-reported severe hypoglycemia (defined as needing help from others and/or seizures and/or unconsciousness)
- Blood sampling for hematology, clinical chemistry, GAD65A and HLA characterization
- Blood sampling for other immunological parameters
- Blood sampling for vitamin D status
- Urine analysis for microalbuminuria and creatinine
- Blood samples for diabetes status (fasting C-peptide, HbA1c and fasting glucose)
- Check of eDiary use compliance

Examinations will be performed according to Table 2, and in the order outlined in the eCRF.

7.1.1.6 ***Visit 4, Month 2 (Day 60 ±5) and Visit 5, Month 3 (Day 90 ±5)***

For Visit 4 and 5 the visit date must be set in accordance with Visit 3 and 4, respectively, so that the first, second and third doses will be 30 days apart (± 5days).

At Visit 4 (Month 2) and Visit 5 (Month 3) the following activities and assessments will be performed:

- Treatment with Diamyd/Placebo injection
- Injection site inspection by the Investigator/Study Nurse and check for patient reported injection site recordings for previous visit in eDiary
- General Physical Examination, including weight and height
- Neurological Assessment
- Concomitant medication
- Vital signs (BP)
- AEs
- Urine pregnancy test (menarchal females only)
- Self-reported severe hypoglycemia (defined as needing help from others and/or seizures and/or unconsciousness)
- Blood sampling for hematology, clinical chemistry and GAD65A
- Blood sampling for other immunological parameters
- Blood sampling for vitamin D status
- Urine analysis for microalbuminuria and creatinine
- Blood samples for diabetes status (fasting C-peptide, HbA1c and fasting glucose)
- Check of eDiary use compliance

- Confirm with the patient that last dose of vitamin D/placebo will occur at month 4 (before next visit month 6)

Examinations will be performed according to Table 2, and in the order outlined in the eCRF.

7.1.1.7 ***Visit 6, Month 6 (Day 180 ±14)***

At Visit 6 (Month 6) the following activities and assessments will be performed:

- General Physical Examination, including weight and height
- Neurological Assessment
- Concomitant medication
- Vital signs
- Pubertal stage, if applicable
- Glycemic variability/fluctuations device (FreeStyle LibrePro, FGM) will be given to the patient
- AEs
- Urine pregnancy test (menarchal females only)
- Self-reported severe hypoglycemia (defined as needing help from others and/or seizures and/or unconsciousness)
- QoL questionnaire
- MMTT
- Blood sampling for hematology, clinical chemistry and GAD65A
- Blood sampling for other immunological parameters
- Blood sampling for vitamin D status
- Urine analysis for microalbuminuria and creatinine
- Blood samples for diabetes status (fasting C-peptide, MMTT-induced C-peptide, HbA1c, fasting glucose, and MMTT-induced glucose)
- Check of eDiary use compliance
- Collection of used and unused vitamin D/placebo for accountability

Examinations will be performed according to Table 2, and in the order outlined in the eCRF.

7.1.1.8 ***Visit 7, Month 15 (Day 450 ±14)***

At Visit 7 (Month 15) the following activities and assessments will be performed:

- General Physical Examination, including weight and height
- Neurological Assessment
- Concomitant medication
- Vital signs
- Pubertal stage, if applicable
- Glycemic variability/fluctuations device (FreeStyle LibrePro, FGM) will be given to the patient
- AEs
- Urine pregnancy test (menarchal females only)
- Self-reported severe hypoglycemia (defined as needing help from others and/or seizures and/or unconsciousness)
- QoL questionnaire
- MMTT
- Blood sampling for hematology, clinical chemistry and GAD65A

- Blood sampling for other immunological parameters
- Blood sampling for vitamin D status
- Urine analysis for microalbuminuria and creatinine
- Blood samples for diabetes status (fasting C-peptide, MMTT-induced C-peptide, HbA1c, fasting glucose and MMTT-induced glucose)
- Serological test for Covid-19 (for patients completing their Visit 7 after 01 January 2020)
- Check of eDiary use compliance and inactivate the account (only applicable for patients that are not continuing in the Extension Study Period)

Examinations will be performed according to Table 2, and in the order outlined in the eCRF.

*7.1.1.9 **Visit 8, Month 24 (Day 720 ±14)***

Visit 8 will only be performed by patients that are ongoing i.e. have not performed Visit 7 (Month 15) when protocol version 7 is approved, implemented and gives their consent to enter the Extension Study Period. Study patients and guardian(s), where applicable, will be asked to sign the updated ICF describing Visit 8 (Extension Study Period).
At Visit 8 (Month 24) the following activities and assessments will be performed:

- General Physical Examination, including weight and height
- Neurological Assessment
- Concomitant medication
- Vital signs
- Pubertal stage, if applicable
- Glycemic variability/fluctuations device (FreeStyle LibrePro, FGM) will be given to the patient
- AEs
- Urine pregnancy test (menarchal females only)
- Self-reported severe hypoglycemia (defined as needing help from others and/or seizures and/or unconsciousness)
- QoL questionnaire
- MMTT
- Blood sampling for hematology, clinical chemistry and GAD65A
- Blood sampling for other immunological parameters
- Blood sampling for vitamin D status
- Urine analysis for microalbuminuria and creatinine
- Blood samples for diabetes status (fasting C-peptide, MMTT-induced C-peptide, HbA1c, fasting glucose and MMTT-induced glucose)
- Serological test for Covid-19
- Check of eDiary use compliance and inactivate the account

Examinations will be performed according to Table 3, and in the order outlined in the eCRF.

7.2 Patient Diary

The patients will be supplied with an eDiary at Visit 2. The diary will be internet based and the patients will be able to access the diary from either a computer or a smart phone. From Visit 3 until Visit 8 the patient will record insulin doses 4 days before each visit (at Visit 2 the insulin dose will be collected at the visit and will be done by the Investigator and recorded in the eCRF). The patient will also use the eDiary for documentation of injection site reactions during the week following injection (Visits 3, 4 and 5), starting the day after the injection.

The patient will be carefully instructed by study personnel on how to log in and complete the eDiary. The investigator/study nurse will review the eDiaries at each visit to check that the patient is completing the eDiary as instructed. Injection site reactions reported by patients in the eDiary during day 1-7 following an injection should not be reported as AEs in the eCRF.

7.3 Study Stopping Criteria

The Sponsor and the investigators reserve the right to discontinue the study at any time for safety reasons or other reasons jeopardizing the justification of the study. Such a termination will be implemented in a time frame that is compatible with the patient's wellbeing.

If the study is prematurely terminated or suspended, the investigator should promptly inform the patients and assure appropriate therapy and follow-up. The Sponsor will notify the Regulatory Authorities and the Independent Ethics Committees (IECs) of any plans to terminate the study.

7.4 Decision Criteria

A patient is included in the study when the ICF has been signed at the screening visit (Visit 1). No patients will be enrolled until approvals from the appropriate IECs and Regulatory Authorities have been obtained.

Prior to the study starting, it will be confirmed that all regulatory requirements for starting the study are met. All key documents must be on file in the Sponsor and Investigator's File, respectively.

7.5 Management of the Disease

All patients will continue to receive intensive insulin treatment through their personal physicians via multiple daily injections of insulin or via insulin pump and should not use any oral or injected non-insulin pharmaceuticals for glycemic control. If, by mistake, such medication is introduced during the study, this is not a reason for discontinuation of the patient. The primary responsibility for diabetes management will be the treating or referring diabetes care provider, but the research study team will provide close additional support through interaction by phone as needed. Diabetes management will be monitored by the study staff with phone calls between study visits as needed. Patients will fill in their insulin doses 4 days before each visit in the patient diary.

The following diabetes treatment goals will be given to patients and treating physicians in accordance with the current recommendations of the American Diabetes Association (ADA) [27]

- Pre-meal glucose 4.4 to 7.2 mmol/L
- Bedtime/overnight glucose 5 to 8.3 mmol/L
- HbA1c <53 mmol/mol (Diabetes Control and Complications Trial [DCCT]-aligned)

These goals are only for guidance and are not considered as violating the protocol if not achieved, but it is regarded as important that every patient and treating physician make their best effort to achieve these recommendations.

The investigator/diabetes team/study nurse will provide support for T1D management by making scheduled phone calls to patients if HbA1c results are ≥53 mmol/mol at any study visit.

7.6 Data Safety Monitoring Board

An independent DSMB will be appointed. The DSMB will review the safety data throughout the study period twice a year. A DSMB charter will be written to outline the working procedures and the duties of the DSMB.

8 STUDY POPULATION

Approximately 22 sites in Sweden, Spain, Czech Republic and the Netherlands will include up to 106 patients. T1D patients, diagnosed within 6 months are given information about the study and they (and their guardian(s) if applicable) are asked to participate in the trial.

8.1 Inclusion Criteria

1. Informed consent given by patients and/or patient's parent(s) or legal acceptable representative(s) (guardian(s)) according to national regulations
2. T1D according to the ADA classification diagnosed ≤6 months at the time of screening
3. Age: ≥12 and <25 years old
4. Fasting C-peptide ≥0.12 nmol/L (0.36 ng/ml) on at least one occasion (maximum 2 tests on different days within a period of 2 weeks)
5. Positive for GAD65A but < 50 000 IU/ml
6. Females must agree to avoid pregnancy and have a negative urine pregnancy test.
   Patients of childbearing potential must agree to use adequate contraception, until one (1) year after the last administration of Diamyd. Adequate contraception is as follows:

   For females of childbearing potential:
   a. oral (except low-dose gestagen (lynestrenol and norestisteron)), injectable, or implanted hormonal contraceptives
   b. combined (estrogen and progestogen containing)
   c. oral, intravaginal or transdermal progesterone hormonal contraception associated with inhibition of ovulation
   d. intrauterine device
   e. intrauterine hormone-releasing system (for example, progestin-releasing coil)
   f. bilateral tubal occlusion
   g. vasectomized male (with appropriate post vasectomy documentation of the absence of sperm in the ejaculate)
   h. male partner using condom
   i. abstinence from heterosexual intercourse

   For males of childbearing potential:
   a. condom (male)
   b. abstinence from heterosexual intercourse

8.2 Exclusion Criteria

1. Previous or current treatment with immunosuppressant therapy (although topical or inhaled steroids are accepted)
2. Continuous treatment with anti-inflammatory drug (sporadic treatment e.g. because of headache or in connection with fever a few days will be accepted)
3. Treatment with any oral or injected anti-diabetic medications other than insulin
4. Treatment with Vitamin D, marketed or not, or unwilling to abstain from such medication during the trial

5. A history of anemia or significantly abnormal hematology results at screening
6. A history of epilepsy, head trauma or cerebrovascular accident, or clinical features of continuous motor unit activity in proximal muscles
7. Clinically significant history of acute reaction to vaccines or other drugs in the past
8. Treatment with any vaccine, including influenza vaccine, within 4 months prior to planned first study drug dose or planned treatment with any vaccine up to 4 months after the last injection with study drug.
9. Participation in other clinical trials with a new chemical entity within the previous 3 months
10. Inability or unwillingness to comply with the provisions of this protocol
11. A history of alcohol or drug abuse
12. A significant illness other than diabetes within 2 weeks prior to first dosing
13. Known HIV or hepatitis
14. Females who are lactating or pregnant (the possibility of pregnancy must be excluded by urine βHCG on-site within 24 hours prior to the Diamyd/placebo treatment)
15. Presence of associated serious disease or condition, including active skin infections that preclude intralymphatic injection, which in the opinion of the investigator makes the patient non-eligible for the study
16. Deemed by the investigator not being able to follow instructions and/or follow the study protocol

8.3 Recruitment and Screening

Eligible patients and/or their parent(s)/legal guardian(s) will have the study explained to them, and will receive the written patient information. After having had the time to review the nature of the study, they will have the opportunity to ask questions to the investigational team. If, after this, the patients agree to participate, they will personally sign and date the written ICF. Patients and/or their parent(s)/legal guardian(s) will provide written informed consent before any study-related procedures are performed.

The patients and/or their parent(s)/legal guardian(s) will then receive a copy of the signed and dated patient information/ICF.

8.4 Patient Withdrawal Criteria

The patient and the parent(s) / legal guardian(s) (where applicable) will receive oral and written information about the study, which includes information about the right to withdraw from the trial at any time without prejudice to future treatment. In addition, the patient may be withdrawn at the investigator's or Sponsor's discretion at any time if regarded in the patient's best interest. In the event that the patient drops out of the trial or is withdrawn from the trial, the appropriate withdrawal page (Study Termination Report) in the eCRF must be completed. Please note that a Study Termination Report must be completed for all patients who have given informed consent and who have been assigned a patient number.

Reasonable efforts should be made to contact any patient lost to follow-up during the course of the trial in order to complete assessments and retrieve any outstanding data.

Patients withdrawn after randomization will not be replaced.

Note that any changes in insulin requirement are not a reason for withdrawal of the patient from the study.

8.5 Patient Withdrawal

In accordance with the Declaration of Helsinki, the investigator must explain to the patient that they have the right to withdraw from the study at any time, and that this will in no way prejudice their future treatment. The reason for any kind of withdrawal must be recorded on the appropriate section of the eCRF.

There will be two main categories for withdrawals from the study:

Complete withdrawal (i.e. stopping investigational product(s) and also continued efficacy and safety evaluations). In order to achieve as complete as possible 15 month follow-up, whenever feasible, patients that are considered for complete withdrawal should be asked if they could consider to return for a 15-month visit, preferable complete visit but as a minimum do the MMTT, C-peptide and HbA1c sampling, and collection of AEs. Attempts should be made to contact patients lost to follow-up with respect to this.

Standard reasons for withdrawing from further participation in the study and from the follow-up visits may be:

- Patient's decision (withdrawal of consent to participate)
- Patient lost to follow-up

Withdrawals from investigational product(s) (i.e. stopping one or several investigational products, but continuing follow-up visits, including efficacy and safety evaluations)

Standard reasons from withdrawing from taking further investigational product, but continuing follow-up visits and safety evaluations may be:

- Unacceptable AEs
- Patient request
- Investigator's discretion
- Patient lost to follow-up/non-attendance
- Intercurrent illness
- The patient becomes pregnant

Diamyd/placebo should not be given to the patient if the patient after inclusion in the study develops/experiences:

- Brain damage, epilepsy, head trauma, neurological disease
- Any active, serious hormonal disease other than T1D
- Other severe autoimmune disease (except celiac disease which is accepted for inclusion)
- Immune-suppressive treatment
- Cancer, cancer treatment
- Any other diabetes drugs other than insulin (and the combination regimen administered in this study)
- Any vaccination

- Drug/alcohol abuse
- Becomes pregnant or is no longer willing to use safe contraceptives during the study

Vitamin D/placebo treatment should not be continued if the patient after inclusion in the study develops/experiences:

- Symptoms of hypercalcemia such as tiredness, euphoria, drowsiness, nausea, weight loss, thirst, polyuria, nefrocalcinosis, renal failure
- Arrhythmia
- Pancreatitis

However, whenever a patient is withdrawn from a study, or for whatever reason is not coming to any further visits, a final study evaluation must be completed for that patient stating the reason(s) why the patient was withdrawn from the study. All documentation concerning the patient must be as complete as possible.

Withdrawals due to non-attendance must be followed up by the investigator to obtain the reason for non-attendance. Withdrawals due to intercurrent illnesses or AEs must be fully documented in the eCRF, with the addition of supplementary information if available and/or appropriate.

9 STUDY ASSESSMENTS

9.1 Assessment of Variables of Diabetes Status

9.1.1 Mixed Meal Tolerance Test

Meal stimulated glucose and C-peptide will be assessed using the MMTT.
The MMTT must be performed according to the instructions in the Operations Manual.
The timing of the assessments is described in Section 7.1.1 and Table 2 and Table 3.
The patient should:

- Come to the study site following an overnight fast (>10 hours), i.e. the patient may not eat but is permitted to drink water
- Not take short acting/direct acting insulin within 6 hours before the MMTT. The patient is allowed to take base-insulin day/night before, but not in the morning before the MMTT.
- Patients with CSII (insulin pump) must continue with their basal dose insulin, but not add bolus dose during the last 6 hours before the MMTT
- Have a fasting plasma glucose level in the range defined by 4-12 mmol/L on the patient's home blood glucose meter in the morning of the test

If the patient does not fulfill all of the above criteria, the MMTT should be rescheduled and the patient should return to the study site within 5 days if possible.

If a patient has a blood sugar level <4 mmol/L in the morning of the scheduled visit, the patient is allowed to take dextrose tablets and then come to the study site. The patient is allowed to perform the MMTT if the blood sugar level is >4 mmol/L when the patients arrive at the study site. If the blood sugar level is still <4 mmol/L the visit should be rescheduled according to the instructions below.

For patients with clinical signs of ketoacidosis or unequivocal elevation of fasting plasma glucose (>12 mmol/L [216 mg/dl]) on the patient's home blood glucose meter, the complete visit should be postponed and the patient should return to the study site within 5 days of the scheduled visit. Please note that all study procedures/examinations must be postponed.

If for safety reasons, patients need to eat or take insulin, the visit should also be rescheduled.

9.1.2 Hemoglobin A1c

Blood samples will be taken to measure HbA1c levels and will be analyzed at a central laboratory, [redacted] The timing of the assessments is described in Section 7.1.1 and Table 2 and Table 3.

9.1.3 Fasting Glucose and Fasting C-Peptide

Blood samples will be taken to measure fasting glucose and fasting C-peptide levels and will be analyzed at [redacted] The timing of the assessments is described in Section 7.1.1 and Table 2 and Table 3.

9.1.4 Glycemic Fluctuations/Variability

The FreeStyle Libre Pro FGM System is a professional continuous glucose monitoring (CGM) device indicated for detecting trends and tracking patterns and glucose level excursions above or below the desired range, facilitating therapy adjustments in persons with diabetes. Readings from the FreeStyle Libre Pro FGM System are not made available directly to patients in real time. The FreeStyle Libre Pro FGM System aids in the detection of episodes of hyperglycemia and hypoglycemia, facilitating therapy adjustments.

Patients will be instructed to return the FreeStyle to the site after each of the testing periods following Visit 1,6, 7 and 8. For instruction on how to return the Freestyle Libre Pro sensor, please refer to the Freestyle Libre Pro Worksheet. The timing of the distribution of the glucose monitoring system and assessments is described in Section 7.1.1 and Table 2 and Table 3.

9.2 Assessment of Safety Variables

The safety assessments include:

- Reactions at the injection site
- Occurrence of AEs
- Laboratory measurements (hematology and clinical chemistry)
- Urine analysis
- Physical examinations, including neurological assessments
- GAD65A titer
- Vital signs

9.2.1 Adverse Events

AEs will be recorded throughout the study period from Visit 2 (baseline) to completion of Visit 8. Events occurring until the day before first administration of investigational medicinal product (IMP) (Visit 2) will be recorded as medical history and as AEs from start of first administration of IMP (day of Visit 2 and onwards).

SAE will be recorded from signing ICF.

AEs will be coded using the current version of Medical Dictionary for Regulatory Activities (MedDRA).
For further information of definitions and reporting of AEs and SAEs and Incidents, see Section 11.

*9.2.1.1 **Injection Site Reactions***

After each Diamyd/Placebo injection, the injection sites will be inspected by the Investigator/Study Nurse and reactions recorded in the eCRF. Patients will also inspect the injection site and record their observations in the patient eDiary, see Section 7.2.

9.2.2 Physical Examination

All patients will undergo a general physical examination (general appearance including skin, mouth, throat, cardiovascular, abdomen, lymphatic glands, and neurological/musculoskeletal [including reflexes]).
The timing of the assessments is described in Section 7.1 and Table 2 and Table 3.

The outcome of the assessments will be recorded as “normal” or “abnormal”. If the outcome is assessed as abnormal, the finding will be classified as “clinically significant” or “non-clinically significant”.
Any findings at the screening visit (Visit 1) will be reported as pre-existing conditions on the Medical History eCRF page.

During the subsequent study visits the patient will be examined for any new medical conditions or worsening of the pre-existing ones. Any change in pre-existing conditions or new conditions must be entered on the AE page in the eCRF and any medication given on the concomitant medication pages.

9.2.3 Neurological Examination

All patients will undergo a standardized clinical neurological examination at all visits. The neurological tests are performed in order to detect possible mild signs of neuromuscular disease such as disturbance of strength, balance, and coordination. The outcome of the assessments will be recorded as “normal” or “abnormal”.

The neurological examination includes:

- Extremity reflexes
- Romberg (balance and coordination)
- Walk on a line, 2 meters (balance and coordination)
- Standing on 1 leg, left and right, 15 seconds per leg (balance and coordination)
- Finger-nose (coordination)
- Mimic (cranial nerves)
- Babinski reflex (central function)
- Muscle strength (shake hands) biceps, triceps, distal extensors, and flexors

These examinations may also be repeated between scheduled visits at the discretion of the investigator. Screening for neurological disease with electroencephalogram (EEG) is not included due to low sensitivity and specificity. However, if any signs of neurological dysfunction are detected, the patient should be referred to a neurologist for further evaluation.

During the subsequent study visits the patient will be examined for any new medical conditions or worsening of the pre-existing ones. Any change in pre-existing conditions or new conditions must be entered on the AE page in the eCRF.

9.2.4 Vital Signs

The following vital signs will be monitored as safety variables:

- Supine systolic and diastolic BP (mmHg), after 5 minutes lying down

Weight, height and BMI will also be recorded.

The outcome of the assessments will be recorded as “normal” or “abnormal”. Abnormal findings will be assessed as “clinically significant” or “not clinically significant”. The timing of the assessments is described in Section 7.1 and Table 2 and Table 3.

9.2.5 Laboratory Safety Assessments

- Hematology: (mean corpuscular hemoglobin [MCH], mean corpuscular volume [MCV], mean corpuscular hemoglobin concentration [MCHC], Hemoglobin, Platelets, Total Leukocyte Count and Differential)
- Clinical Chemistry: Creatinine, Calcium, Liver function tests (alanine aminotransferase [ALT], Aspartate Aminotransferase [AST], Alkaline Phosphatase [ALP], Bilirubin)
- Urine analysis: Microalbuminuria Creatinine
- Urine pregnancy tests (menarchal females only)

The outcome of the assessments will be recorded as "normal" or "abnormal". Abnormal findings (values outside the reference ranges) will be assessed as "clinically significant" or "not clinically significant".
The timing of the assessments is described in Section 7.1 and Table 2 and Table 3.

The laboratory tests listed above (hematology, clinical chemistry and urine analysis) will be analyzed at the central laboratory [redacted] Full details on blood sample collection, processing, storage, and shipping requirements (as applicable) are provided in the study specific Laboratory Manual.

9.3 Immune System Assessments

9.3.1 GAD65 Antibody Titer

The timing of the assessments is described in Section 7.1 and Table 2 and Table 3.
The variables to evaluate the influence on the immune system will be analyzed at [redacted]

9.3.2 Bioanalytical Method

Assessment of serum autoantibodies
The quantification of serum autoantibodies towards GAD65 and IA-2, as well as antibody isotypes, will be performed with standard techniques. The levels of GAD65A indicate the general effect of the vaccine. The relative concentrations of GAD65A immunoglobulin G (IgG) subtypes indicate diverging effects on the immune reaction, and e.g. a rise in IgG4 is associated with positive effects of allergy immunotherapy.

Assessment of antigen specifically stimulated cytokine levels
Changes induced by immune modulation are studied with a broad range of cytokines involved in the regulation of the immune system. At minimum a set including IL-1, IL-2, IL-5, IL-13, IL-10, IL-17, IFN-γ, and TNF-α will be monitored to assess general and antigen specific immune responses over time. Luminex 200 will be used.

Assessment of immune response to antigen by cell proliferation
Proliferation of PBMCs is used to indicate changes in total immune response to autoantigens. Incorporation of 3H thymidine will be used, and provides an estimate of cell proliferation by measuring deoxyribose nucleic acid (DNA) synthesis. It is a robust technique for assessment of responses to various stimuli.

Assessment of immune cell frequency
Flow cytometric analysis allows the quantification of specific immune based on the presence of biomarkers before and after proliferation and activation by specific

antigens, and indicates changes to the immune response. It can be done by fluorescent activation or CyTOF based detection cells and the monitoring of changes over time. Enzyme Linked Immunosorbent Spot Forming Cell Assay (ELISPOT) analysis allows the measurement of the number of cells secreting specific cytokines upon stimulation with antigen (GAD65).

Assessment of cell mediated immune response
To monitor potentially favorable changes in the immune response to GAD65 and changes in the quality of the immune response post treatment versus baseline certain effects will be analyzed. A potential important biomarker is the increase of the antigen specific secretion of regulatory cytokine IL-10, or the Th2 cytokines IL-5 and IL-13. Another potential biomarker is the decrease in antigen specific secretion of the proinflammatory cytokines IL-17, IFN-γ, and TNF-α, or the proportion of Th2 cytokines compared to Th1 cytokines. A potential marker is also the frequency of IL-10 secreting cells, or a change in a certain cellular subtype as characterized by flow cytometry. Further compound analysis will be done in order to detect and evaluate other potentially important biomarkers.

9.4 Assessment of Genetic Status

All patients will undergo testing for HLA determination to assess the presence of genes related to diabetes development. Blood samples will be taken for HLA determination and will be analyzed a [redacted]
The timing of the assessments is described in Section 7.1 and Table 2 and Table 3.

9.5 Measurement of Vitamin D Status

Blood samples will be taken to measure serum Vitamin D levels and will be analyzed at [redacted] The timing of the assessments is described in Section 7.1 and Table 2 and Table 3. Only analysis results from screening visit will be reported to investigational sites to confirm eligibility and need to assign to Vitamin D /Placebo treatment. This information will be collected and maintained at the laboratory until after database lock of the Main Study Period.

9.5.1 Blood Sampling Procedures

Blood samples for assessments of diabetes status, laboratory safety assessments, vitamin D status, GAD65 antibody titer, genetic status and immunological testing should be collected in accordance with Table 2 and Table 3.
The total volume of blood that will be collected from each patient for diabetes status, laboratory safety assessments, vitamin D status, GAD65 antibody titer assessments and immunological testing will be approximately 438 mL for Main Study Period and additional approximately 80 mL for Extension Study Period.

9.6 Covid-19 serological testing

Covid-19 is a condition that greatly affects the immune system and therefore its influence on the safety and efficacy of the GAD65 treatment as well as a potential direct effect on diabetes disease status in affected patients will be assessed. Serological testing for Covid-19 will allow the analysis of the infection's effect on GAD65 treatment efficacy and additionally inform actions to be taken in future trials.

Blood samples will be used to analyze the presence of antibodies (IgG and/or Immunoglobulin M [IgM]) against SARS-CoV2 viral proteins using validated antibody detection tests.
Samples from patients completing their Visits 7 (after the 1st January 2020) and Visit 8 will be analyzed.

9.7 Assessment of Quality of Life

QoL will be assessed by including Questionnaire EQ-5D-5L at visits between baseline and Month 24 as described in Section 7.1 and Table 2 and Table 3.

9.8 Assessment of Pubertal Stage and Concomitant Medication

Variables which will be evaluated include:

- Pubertal Stage:
  For boys: tanner genital development (G1-G5), pubertal hair (PH1-PH5) and testicular volume (ml).
  For girls: Tanner breast (B1-B5) and pubertal hair (PH1-PH5)
- Concomitant medication

The timing of the assessments is described in Section 7.1 and Table 2 and Table 3.

9.9 Demographics and Other Baseline Characteristics

9.9.1 Demographics and Baseline Data

The following demographic and baseline data will be collected at the Screening visit (Visit 1):

- Age
- Sex

9.9.2 Medical History

Medical history will be recorded at the Screening visit (Visit 1).
A complete review of the patient's past medical history will be undertaken by and documented on the Medical History eCRF at the screening visit (Visit 1).
All pre-existing conditions/diseases will be reported on the Medical History eCRF page at the screening visit (Visit 1).
Medical histories will be coded using the current version of MedDRA.

9.9.3 Family History of T1D

The patient's T1D diagnosis date and family history of T1D will also be documented.

10 TREATMENT OF PATIENTS

10.1 Study Treatment

Study medication: Diamyd® intralymphatic injection

Diamyd/placebo will be injected directly into the inguinal lymph node at Visits 3, 4 and 5 (Days 30, 60 and 90). The Diamyd/placebo injection directly into the inguinal lymph node need to be done by an appropriately qualified person at the X-ray department by help of ultrasound technique. All possible efforts should be made to use the same lymph node for all 3 injections. For more information regarding the injection please refer to the operations manual for the injection procedure. Upon trouble finding a suitable inguinal lymph node for injection on one side, the other side should be inspected. Only under exceptional circumstances can the injection be given into an axillary lymph node.

After the injection, the patient shall remain in the vicinity of the study site for the next hour, and the injection site will be examined by investigator/study nurse 1 hour post injection.

Study Medication: Vitamin D

Treatment with vitamin D/placebo starts at Visit 2 if the Vitamin D serum levels are below 100 nmol/L (40ng/ml) at screening. If the patient has Vitamin D serum levels above 100 nmol/L (40 ng/ml) at screening, no Vitamin D/placebo treatment will be given to that patient (the patient will only be given the Diamyd/placebo injection). Patients with vitamin D levels below 100 nmol/L (40 ng/ml) at screening will receive vitamin D/placebo administered orally (2000IE per day) for 4 months (120 days in total). The patient should be instructed to return all used and unused vitamin D/placebo bottles at Visit 6.

10.1.1 Method of Assigning Patients to Treatment Groups

A computer-generated randomization list will be produced by the IWRS system. After confirmation of eligibility at Visit 2 patients will be randomized in a 1:1 ratio (Arm 1:Arm 2) across all study sites. Randomization will be stratified by country and GAD65A level to ensure that an equal number of patients in respective age group are included in each arm.

- Arm 1: Diamyd + vitamin D
- Arm 2: Placebo for Diamyd + Placebo for vitamin D

10.1.2 Blinding and Code Breaking

Patients, investigator site staff, persons performing the assessments, and data analysis will remain blind to the identity of the treatment from the time of randomization until database lock, using the following methods:

(1) Randomization data are kept strictly confidential until the time of unblinding, and will not be accessible by anyone else involved in the study, except for the independent, unblinded statistician who will have access to the randomization scheme and for the purpose of providing unblinded safety data to the DSMB.

(2) The identity of the treatments will be concealed by the use of matching placebo to the study drug that are identical in packaging, labeling, appearance and schedule of administration.

All respective study drugs will have the same treatment number printed on respective label. All three vials of Diamyd/placebo will be packed into one box per treatment number. The vial box and vials will all be labeled with the same treatment number. Allocation of treatment to Diamyd/placebo and vitamin D/placebo will follow a pre-produced randomization list.

Assignment of treatment number will be performed via a IWRS at Visit 2 for each individual patient. The randomization in IWRS will allocate the patient to one of the treatment arms in random order.

Study medication will be shipped from the central depot to the study sites according to country specific laws and regulations, and the procedures will be described in the Study Drug Accountability Document. Eligible patients will be given study medication on Visit 2 (Day 1), 3, 4 and 5 according to the treatment number provided by the IWRS.

The treatment code may be broken on an individual basis, in case of an SAE for which the investigator must know the study agent identity to initiate appropriate treatment. The investigator will be supplied with necessary information to break the study blind on an individual patient basis. If the study blind is broken, the investigator must inform the medical monitor or designee and report the reason for unblinding. The date, time, and reason for unblinding, together with the investigator's signature, must be recorded. Patients will continue in the study unless this is deemed inappropriate.

SUSARs will be unblinded for reporting to regulatory agencies and IECs. [redacted] pharmacovigilance center will be supplied with necessary information to break the study blind for individual patients as required for regulatory reporting purposes. However, the investigator, Sponsor, and study team will be kept blinded to treatment allocation. The information with regards to unblinding will be stored in a secure environment within the pharmacovigilance department and accessible only to pharmacovigilance staff.

The code will be broken when all patients have completed the Main Study Period of 15 months, and all data for this period has been entered into the database and the database has been locked. After database lock, the results will be analyzed and unblinded to the Contract Research Organization (CRO) statistician, CRO programmer, CRO personnel involved in the preparation of the clinical study report, Coordinating Investigator, and Sponsor. The study will be kept blinded to patients, investigators, study nurses and study personnel (i.e. monitors, study coordinators etc.) during the 9-month Extension Study Period, i.e. the whole study period of 24 months. After the whole study period is completed a 24-month analysis will be performed.

10.1.3 Study Medication

The following medication supplies will be used in the study:

A.

Study medication:	Diamyd® intralymphatic injection
	Dosage and interval: 4 µg (0.1 ml) will be administered directly into the inguinal lymph node (by an appropriately qualified person by help of ultrasound technique) at 3 occasions with one month intervals (at Days 30, 60 and 90).
Comparator:	Placebo intralymphatic injection
IMP supplier:	Diamyd Medical AB, [redacted].

B.

Study Medication:	Vitamin D, 25 drops
	2 000 IU per day, given per os from Day 1 through Day 120 Trade name: [redacted]
Comparator:	Placebo oral drops, solution
IMP supplier:	[redacted]

The doses are chosen according to Section 4 (Introduction and rational for the study)

10.1.4 Supply, Packaging, Handling and Storage

[redacted]

Diamyd/Placebo will be supplied as pre-packed medication to a central depot who will distribute to the clinical site or local pharmacy. All dosing will take place in the hospital, and handled only by trained and authorized study personnel.

Diamyd/Placebo must be stored in a refrigerator at 2-8 ºC in a secure area (e.g. a locked cabinet or drug storage room), protected from unintended use.

Vitamin D/Placebo should be stored at room temperature (< 25 ºC) in a secure area (e.g. a locked cabinet or drug storage room), protected from unintended use.

Vitamin D/Placebo will be distributed by a central depot, who will then distribute to the clinical site or local pharmacy.

All study medication will be labelled with information according to national and local regulations.

10.2 Treatment Compliance

The Diamyd/Placebo administered directly into the inguinal lymph node (by an appropriately qualified person) need to be done at the X-ray department by help of ultrasound technique. For more information regarding the injection please refer to the operations manual for the injection procedure.

10.3 Study Medication Accountability

All study medications supplied for this study must be retained in a safe place at all times of the study. Only personnel authorized by the investigator should dispense the study medication, and the accountability is the responsibility of investigator.

The patient should be instructed to return all used and unused vitamin D/placebo bottles at Visit 6.

A study medication inventory (dispensing records) for all medication dispensed must be maintained at all times and always kept current. Used and unused medication must be stored at the site or pharmacy throughout the study. The investigator/pharmacist must keep record of all drugs received, used and returned. Both pharmacies and study sites are obliged to properly measure and record the storage temperature.

When the study is completed all unused and used study medication containers must be returned to the drug supplier unless the drug supplier has approved other arrangements.

10.4 Concomitant Therapy

The patients will receive adequate therapy for concomitant diseases at the discretion of the investigator. Patients receiving a drug listed under exclusion criteria at screening should not be included in the study. Patients who are prescribed a drug listed in the exclusion criteria during the period between screening and first injection should be withdrawn from the study.

Any concomitant medication must be recorded in the eCRF. Note: if a new medication is introduced or an existing medication is changed due to a new medical condition or worsening of a pre-existing medical condition, the condition must be reported as an AE.

10.5 Post Study Treatment

No study medication will be offered to the patients after completion of the last injection. Patients will thereafter receive therapy at the discretion of the investigator and according to prescriptions.

11 ADVERSE EVENTS

11.1 Definitions of Adverse Events (AE)

An AE is defined as any untoward medical occurrence in a subject during a clinical study administered a medicinal product and which does not necessarily have a causal relationship with this treatment(s).

An AE can therefore be any unfavorable and unintended clinical sign or symptom, any illness or disease, which develops or worsens in intensity during the course of the trial. It also includes an abnormal laboratory finding, if e.g., the abnormality results in trial withdrawal, is serious, is associated with clinical signs or symptoms, or is considered being of clinical relevance.

It could also include accidents and reasons for changes in medication (drug and/or dose), any medical/nursing/pharmacy consultation and admission to hospital/surgical operations.

Any new findings, clinically significant laboratory values or worsening of pre-existing condition must be reported as an AE by the investigator, whether or not considered related to the medicinal product(s).

Note that hospital admission and/or surgical operations for illness, which existed before the study drug was given or the subject was enrolled in the clinical trial and did not worsen during the study, are not AEs.

T1D-related events

The number of self-reported episodes of severe hypoglycemia (Severe hypoglycemia defined as needing help from others and/or seizures and/or unconscious) between baseline and subsequent visits will be collected in the eCRF. Hypoglycemia, changes in C-peptide, changes to insulin dosage, and increases in HbA1c/blood glucose will not be reported as AEs unless the definition of SAEs is met.

11.2 Seriousness

A SAE is defined as: an AE that is fatal, life-threatening, significantly or persistently disabling, requiring hospitalization or prolongation of existing hospitalization or that is a congenital anomaly or birth defect.

These characteristics/consequences have to be considered at the time of the event. For example, regarding a life-threatening event, this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Some medical events may jeopardize the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events (referred to as important medical events) should also be considered as serious in accordance with the definition.

11.3 Intensity

Mild: The AE is transient and easily tolerated.

Moderate: The AE causes the patient discomfort and interrupts the patient's usual activities.
Severe: The AE causes considerable interference with the patient's usual activities, and may be incapacitating or life-threatening.

Note: a distinction should be drawn between serious and severe AEs. The term severe is used to describe the intensity of the event and the event does not necessarily need to be considered serious. The term serious is based on the patient/event outcome or action and serves as a guide for defining regulatory reporting obligations.

11.4 Relationship to Study Medication

Relationship to study medication will be assessed for the two treatments (Diamyd and Vitamin D) separately. AEs with a causal relationship assessment of Unlikely related, Possibly related and Probably related will be considered to be Adverse Reactions.

Not related: This category is applicable to those AEs which, after careful medical consideration at the time they are evaluated, are judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc.) and do not meet the criteria for study medication relationship listed under remote, plausible or probable.

Unlikely related: Time relationship non-existent or doubtful and/or other factor(s) certain or probable to have been causative.

Possibly related: Time relationship exists. Other possible causative factor(s) may exist (e.g., concurrent disease or concomitant medication). Improvement on dechallenges or dose reduction may or may not have been seen.

Probably related: Time relationship exists. No other possible causative factor(s) may exist (not reasonably explained by the patient's known clinical state or concomitant medication). Improvement on dechallenges or dose reduction (if performed) has occurred. Recurrence of symptoms on rechallenge (if performed) has occurred. A specific laboratory investigation (if performed) has confirmed the relationship.

11.5 Reporting of Adverse Events

All AEs must be recorded in the eCRF, defining relationship to study medication, intensity, seriousness, action taken with study drug, and outcome. AEs should also be recorded by the investigator in the patient file/notes.

11.6 Timelines and Reporting of SAE

All SAEs must be reported, whether or not considered attributable to the study drug on a separate SAE Report Form. SAEs will be reported from signing of informed consent.

[redacted] will be responsible for reporting all SAEs in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and local regulations. The sponsor and [redacted] will complete and sign a "Pharmacovigilance Working Agreement" agreement covering the safety reporting responsibilities in the

study. This agreement will ensure the sponsor is directly informed of each SAE reported by the investigators.
In order to meet the specified reporting requirements investigators should adhere to the following process for recording and reporting SAEs.

It is the investigator's responsibility to, as soon as he/she is aware of a potential SAE, he/she should contact [redacted] by fax or e-mail, and in any case no later than 24 hours after the knowledge of such a case.
At the time of initial reporting, the investigator must provide as a minimum requirement, the patient number, birth date, nature of the SAE, and a preliminary assessment of causality. The investigator should follow-up the initial notification of the potential SAE by faxing or e-mailing a copy of the SAE reporting form to [redacted] at the numbers/e-mail address provided in the investigator Site File and on the SAE Report Form. The faxed/e-mailed SAE Reporting Form should be received by [redacted] within 24 hours of the initial notification of the event.

It is the investigator's responsibility to report to [redacted] follow-up information on an existing SAE that is fatal or life-threatening within 5 days after the initial report. Where appropriate, hospitalization or autopsy reports should be made available. All SAEs will be followed up until resolution (i.e., asymptomatic, stabilization or death).

It is [redacted] responsibility to receive e-mail or fax copies of the SAE report form and other relevant eCRF pages from the investigators. The Drug Safety unit at [redacted] will review the information provided on the form and enter it into the safety data base. The SAE report will be assigned a unique number that will be entered on the SAE Report Form, and will be used to identify the report in all future communication. A notification of receipt of the report will be sent to the reporter, either by fax or e-mail within 48 hours. [redacted] will contact the investigator directly if there is any inconsistencies and missing information.

[redacted] is responsible for the timely submission of SUSARs to the Competent Authorities and IECs according to appropriate Competent Authority and IEC requirements. It is [redacted]s responsibility to report SUSARs to investigators according to ICH GCP and to local regulations. Competent Authorities will be notified of all SUSARs through the EudraVigilance database.

Fatal and life-threatening SUSARs should be reported by [redacted] as soon as possible to the Competent Authorities and Ethical Committees, and in any case no later than seven (7) calendar days, after knowledge by the Sponsor/[redacted] of such a case. Relevant follow-up information on the case will be subsequently communicated within an additional eight (8) days. All other SUSARs shall be reported to the Competent Authorities concerned and to the Ethics Committee concerned as soon as possible but within a maximum of fifteen (15) days of first knowledge by [redacted]

11.7 Unresolved Events

If an AE/SAE is present when the patient has completed the study the course of the event must be followed until final outcome is known or the condition is stable.

11.8 Pregnancy Report Form

Pregnant and lactating women will not be included in the study. Menarchal females must have a negative urine pregnancy test prior to randomization and a negative urine pregnancy test at each study visit with Diamyd/Placebo administration, prior to injection of study drug. Patients will be required to use an adequate form of birth control during the study. At Visit 2 the need for birth control will be re-assessed. Patients and their partners will be strongly advised to avoid pregnancy for 1 year following the last dose of Diamyd/Placebo and instructed to use adequate birth control.

A pregnancy occurring during the trial must be recorded on the Pregnancy Report Form and no further drug doses will be given. If the pregnancy is verified prior to any of the injections, no further injection shall be given.

The Pregnancy Report Form should be faxed or e-mailed within 24 hours of awareness to [redacted]. A copy of the report should be filed at the study site for follow-up until delivery. Any pregnancy must be followed until delivery or to the end of pregnancy.

11.9 Direct Access to Source Data/Documents

The sponsor should ensure that it is specified in the protocol or other written agreement that the investigator(s)/institution(s) will permit trial-related monitoring, audits, IEC review, and regulatory inspection(s), providing direct access to source data/documents.

12 DATA HANDLING

12.1 Data Management

Data management and handling of data will be conducted according to the study specific Data Management Plan with ICH guidelines and [redacted] standard operating procedures (SOPs).

An eCRF system will be used to capture data from the study. Data entry will be performed by the study site personnel. Validation and data queries will be handled by the [redacted] Data Management Team. The data will be subjected to validation according to [redacted] SOPs in order to ensure accuracy in the collected eCRF data.

Changes to the data in the eCRF will be made at the site by the study site personnel. The eCRF will have an audit trail with appropriate functionality for data capture, tracking and documentation of any queries or changes. Electronic signatures will be used to lock the data and identify the person entering or changing the data.
Before database closure a reconciliation will be performed between the SAEs entered in the safety database and the study database. After database closure, the database will be exported as SAS® data sets.

Any deviations, i.e. discrepancies and additions from the process defined in the Data Management Plan, will be described in a study specific Data Management Report.

13 STATISTICAL METHODS

13.1 Estimation of Sample Size

A total sample size of 106 patients is planned for a 1:1 randomization in order to provide 90% power to detect a 50% difference in geometric mean C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at 15 months between the active arm and placebo arm using a two-sided test at the 0.05 significance level. The sample size has been adjusted to allow for 10% loss to follow-up. This is based upon a t-test employing ln(X+1) normalizing transformation of C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at 15 months and assumed mean and standard deviation estimates, on the transformed scale, of 0.134 and 0.20, respectively[1]
It is estimated that there will be a screening failure rate of 16%. To ensure that 106 patients are screened and enrolled approximately 127 patients will be screened.

13.2 Statistical Analyses

A detailed statistical analysis plan (SAP) will be written and finalized well in advance of database lock. The plan will follow the outline of the statistical analyses presented below, but details necessary to complete the statistical analyses will be given.

13.2.1 Data Sets to be Analyzed

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

- The **screened set** will consist of all patients that were screened for participation in this study
- The **randomized set** will consist of all patients that were randomized.
- **The Safety Set** will consist of all randomized patients who received at least one injection.
- **The Full Analysis Set (FAS)** will consist of all randomized patients who have received at least one dose of study medication and have at least one post-baseline assessment and corresponding baseline measurement of any efficacy variable.
- The **Extension set** will consist of the subset of the FAS that participated in the Extension Study Period.
- **The Completers Set** will consist of all patients in the FAS who have completed the main study period and who have primary efficacy data available at Visit 7.
- **The Per Protocol Set (PPS)** will consist of all patients in the FAS who received all three study drug injections, and do not have any other major

[1] Lachin et.al Sample Size Requirements for Studies of Treatment Effects on Beta-Cell Function in Newly Diagnosed Type 1 Diabetes, 2011, 6(11):e26471

protocol violations which will affect the assessment of efficacy. Major protocol violations include:

- Missed at least one scheduled injection of IMP
- C-peptide samples received by lab later than 48 hours after sampling

The final criteria for PPS, regarding which protocol deviations that warrant exclusions, will be determined when all data on protocol violations/deviations are available and before breaking the blind. The final criteria will be documented in the SAP or in the Pre-Analysis Review form issued by the statistician prior to unblinding of the data.

The Screened set will be used for presentation of study disposition of patients.

The FAS is considered as the primary analysis dataset, and will be used for all primary, secondary and exploratory efficacy endpoints. The primary efficacy analyses will be repeated using the PPS and the completers set and these analyses will be regarded as sensitivity analyses. Any discrepancy between the results from the FAS and the PPS or the completers set will be analyzed and discussed in the Clinical Study Report.
The secondary variables will be analyzed based on FAS only.
The analysis datasets will be applied at the main study analyses as well as the whole study period.

Baseline presentations will be based on the safety set and on the FAS. Safety presentations will be based on the safety set. FAS will be analysed according to randomised (planned) treatment. Safety and PPS will be analysed on actual treatment received.

13.2.2 Statistical and Analytical Plan

In general, data will be presented using summary statistics. For continuous variables number of observations, mean, standard deviation, minimum, median, and maximum will be given. Categorical variables will be presented as frequencies and percentages. For all statistical analyses, p-values and 95% confidence intervals will be given. The tabulation of the descriptive statistics will be split by treatment group and visit, as applicable.

13.2.2.1 Demographic and Other Baseline Characteristics

Demographics and baseline characteristics will be presented using summary statistics for each treatment group and in total.

13.2.3 Hypotheses and Statistical Methods

13.2.3.1 Analysis of Primary Endpoint Variable

The primary efficacy variable will be change in log-transformed C-peptide $AUC_{mean\ 0\text{-}120}$ min during an MMTT from baseline to Month 15. Mean changes from baseline will be analyzed using Mixed Model Repeated Measures (MMRM). The model for analysis will include fixed, categorical effects of treatment, randomization

strata (GAD65A level), visit and treatment by visit interaction, as well as the continuous, fixed covariates of log-transformed baseline C-peptide $AUC_{mean\ 0\text{-}120}$ during an MMTT. An unstructured (co)variance structure will be used to model the within-patient errors. 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. The primary treatment comparison will be the contrast between treatments at Month 15 for active treatments versus placebo.

Least Square (LS) Mean estimates and 95% confidence intervals (CIs) will be back-transformed from the log scale to the original scale and given 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. Here a ratio of e.g. 0.8 will mean that the change from baseline to month 15 in C-peptide level was 20% smaller for Diamyd than for placebo at Month 15.

A sensitivity analysis where the hypotheses will also be assessed using Analysis of Covariance (ANCOVA) adjusted for baseline value of C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT between baseline to 15 months and randomization strata (GAD65A level) using the completers set.

Further sensitivity analyses will be given in the SAP.

13.2.3.2 Analysis of Secondary Endpoint Variables

13.2.3.2.1 Key Secondary Variables of Diabetes Status

The following key secondary efficacy endpoint variables will be analysed with the same model (MMRM) as the primary efficacy endpoint:

- Change in IDAA1c between baseline and 15 months.
- Change in HbA1c between baseline and 15 months.
- Change in daily exogenous insulin consumption between baseline and 15 months.

The analysis of key secondary endpoints will be subject to the same sensitivity analyses as the primary efficacy analysis. However, exploration of key secondary endpoints by covariate and subgroups analyses will be based on the observed results for respective endpoint.

13.2.3.2.2 Other Secondary Variables of Diabetes Status

Summary statistics (including Student's t based 95% CIs and p-value for the difference between Diamyd and Placebo) for change in glycemic variability/fluctuations (evaluated from data from continuous glucose monitoring FreeStyle LibrePro, FGM) over 14-day period between Screening and 15 months. Further analysis of the data from the 14-day FreeStyle LibrePro, periods will be presented in the SAP.

The following secondary efficacy endpoint variables will be analyzed with the same model as the primary efficacy endpoint (details on log-transformations will be given in the SAP):

- Proportion of patients with by IDAA1c ≤9 at 15 months.
- Change in Fasting C-peptide between baseline and 15 months.
- Change in C-peptide measured at 30, 60, 90, and 120 minutes during MMTT at 15 months.
- Change in maximum C-peptide during MMTT between baseline and 15 months.

The following secondary efficacy endpoints will be presented using summary statistics and p-value from Cochran/Mantel-Haenszel Test stratified for randomization strata (GAD65A level) will be given along with 95% CIs calculated according to the Clopper-Pearson method:

- Proportion of patients with a stimulated maximum C-peptide level above 0.2 nmol/L (0.6 ng/ml) at 15 months.
- Proportion of patients with a stimulated 90min C-peptide level above 0.2 nmol/L (0.6 ng/ml) at 15 months.
- Number of patients having at least 1 severe hypoglycemic event using Cochran/Mantel-Haenszel Test stratified for randomization strata (GAD65A level).
- Change in body weight and BMI between baseline and 15 months

In addition, the number of self-reported episodes of severe Hypoglycaemia (severe Hypoglycaemia defined as needing help from others and/or seizures and/or unconscious) between baseline and 15 months will be assessed using Poisson regression including randomization strata (GAD65A level) where rate ratios with 95% CI and p-value will be given.

13.2.3.3 Analysis of Exploratory Endpoint Variables

All data collected at the 24-month follow-up visit (Visit 8) will be regarded as exploratory endpoints and will be presented using summary statistics including data from the Main Study Period where only the patients that participated in the Extension Study Period will be included. The following statistical analyses will be repeated for the whole study period:

- The change in log-transformed C-peptide $AUC_{mean\ 0\text{-}120}$ min during an MMTT from baseline to Month 24
- Change in HbA1c between baseline and 24 months
- Change in daily exogenous insulin consumption between baseline and 24 months
- Change in Fasting C-peptide between baseline and 24 months.

- Change in glycemic variability/fluctuations (evaluated from data from continuous glucose monitoring FreeStyle LibrePro, FGM) over 14 day period between Screening and 24 months.
- Rate of hypoglycemic events using Poisson regression including randomization strata (GAD65A level)
- Number of patients having at least 1 severe hypoglycemic event using Cochran/Mantel-Haenszel Test stratified for randomization strata (GAD65A level)
- Change in body weight and BMI between baseline and 24 months

13.2.3.3.1 Secondary Safety Variables

Adverse events:

AEs will be coded according to the MedDRA system and tabulated by System Organ Class (SOC) and by preferred term.

A summary table will be presented with total number and No. of patients with:

- AEs
- serious AEs
- related AEs
- AE leading to discontinuation

AE will only be counted once within each patient on a preferred term level.

The total number of patients with at least one AE and the number of AEs will be derived and summarized by SOC and preferred term.

AEs will also be tabulated versus intensity and relationship to treatment (classified as related for outcomes “Remote”, “Plausible” and “Probable” and not related for outcome “Unrelated”. If a patient has more than one event classified with the same preferred term, then the worst intensity and the worst relationship will be used. In this table, patients having AEs will be identified by their patient number.

A treatment-emergent AE (TEAE) is an event emerging after treatment start or worsening during treatment. Hence, events with an onset time on or after the time of treatment start are treatment-emergent. Only TEAEs will be included in summary tables. Pre-treatment AEs will be listed.

Treatment-emergent serious adverse events (TESAEs) will be listed separately.

In listings of AEs the relative day of occurrence will be given. Relative day will be calculated as (Date of first administration of IMP – AE start date) + 1 for AEs occurring on date of first administration of IMP or later and as (Date of first administration of IMP – AE start date), otherwise.

AEs will be presented for the main study period and for the whole study period.

Other safety endpoints:

All other safety endpoints will be presented using summary statistics. These presentations will be done for the Main Study Period and the whole study period where only the patients that participated in the Extension Study Period will be included. For laboratory measurements and vital signs change from baseline will also be summarized. Shift tables will present shifts for all endpoints for which normal/abnormal (Not Clinically Significant/Clinically Significant [NCS/CS]) judgements exist.

13.2.3.3.2 Secondary Immunological Variables

Immunological secondary endpoints will be presented in summary statistics and will be accompanied with p-values from non-parametric statistical tests (details to be given in SAP).

13.2.3.3.3 Secondary Variables of Quality of Life

Analysis of the QoL data obtained within the study will follow the EQ-5D-5L User guide.

13.2.4 Level of Significance, Multiple Comparisons and Multiplicity

The primary endpoint and the key secondary endpoints will be tested hierarchically at the 5% significance level. This implies the following testing sequence:

1. Change in C-peptide $AUC_{mean\ 0\text{-}120\ min}$ during a MMTT between baseline and 15 months.
2. Change in IDAA1c between baseline and 15 months.
3. Change in HbA1c between baseline and 15 months.
4. Change in daily exogenous insulin consumption between baseline and 15 months.

13.2.5 Adjustment for Covariates

Change from baseline analyses will be adjusted for baseline and all other analyses will be adjusted for randomization strata (GAD65A level).

13.2.6 Handling of Dropouts and Missing Data

This MMRM suggested for the primary efficacy analysis does not require imputation of missing data. Instead missing data is modeled based on the patient's available data and on other patients' developments over time.

13.2.7 Multicenter Studies

As the number of centers are large in relation to the number of patients to be included, no by-center displays are planned.

13.2.8 Examination of Subgroups

Efficacy variables will be explored within the subgroup of patients with HLA haplotype DR3-DQ2 (versus not DR3-DQ2). The details of the analyses will be specified in the SAP.

13.2.9 Interim Analysis and Data Monitoring

No formal interim analysis will be performed.
An independent DSMB will be appointed. The DSMB will review the safety data throughout the study period twice a year. For further details, please see the DSMB charter and the DSMB SAP.

13.2.10 Deviation(s) from the Statistical Analysis Plan

Any deviation from the final SAP should be stated in the final clinical report.

14 REGULATORY AND ADMINISTRATIVE PROCEDURES

14.1 Ethics Committees and Competent Authorities

Any regulatory requirements must have been met before starting the study. The Sponsor will apply for the regulatory approval to the appropriate authorities.

Study sites, facilities, laboratories and all data (including source data) and documentation must be made available for inspection by the authorities.

The study will be conducted in accordance with the Brazil (2013) amendment to the Declaration of Helsinki 1964.

The Protocol and Patient Information and ICF will be approved by the Ethics Committee before commencement. If a substantial protocol amendment is necessary, this will be signed and submitted by the Sponsor for ethical and regulatory approval. The approval from the Ethics Committee and Competent Authority should be obtained before any implementation of the amendment is done. When the change or deviation is to eliminate or reduce risk to human patients, the amendment may be implemented before review of approval by the Ethics Committee and Competent Authority. The sponsor should notify the Ethics Committee and Competent Authority of the change or deviation in writing within 10 working days after implementation.

Minor amendments which do not affect the safety or conduct of the study from the patient viewpoint, and which do not significantly reduce the scientific value of the protocol, and which do not require a significant change to be made to the consent form and/or the information sheet, will not be submitted for formal ethics and regulatory review. These will be sent to the Ethics committee and Competent Authority on an ‘information only’ basis.

14.2 Patient Information / Informed Consent

The investigator is responsible for giving the patients and his/her parents/caregivers (if applicable) full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the study. Patients and, if applicable, his/her parents/caregivers must also be notified that they are free to withdraw from the study at any time. The patients and parents/caregivers should have reasonable time to read and understand the information before signing. The investigator is responsible for obtaining signed informed consent from all patients before including the patient in any study related procedures.

Should there be any amendments to the final protocol, such that would directly affect the patient's participation in the trial e.g., a change in any procedure, or if new data is obtained during the trial that may influence the standpoint to participate in the study, the patient information will be amended to incorporate this modification and the patient must agree to sign this amended form verifying that they re-consent to continue their participation in the trial.

A copy of the Patient Information and of the ICF will be given to the patients and parents/caregivers.

14.3 Patient Confidentiality

The investigator must ensure that patient's confidentiality will be maintained. eCRFs or other documents submitted to the Sponsor should only identify patients by their initials and study number. The investigator should keep a separate log of patient codes and names.

Documents not for submission to the Sponsor, e.g. patient's completed Consent Forms, should be retained by the investigator in strict confidence.

The investigator is required to record safety data, concomitant medication and patient progress in the patient's file/notes/medical record.

The patient's medical records will be reviewed by the study monitor to verify adequate source documentation, accuracy and completeness of eCRFs. The review will be conducted with strict adherence to professional standards of confidentiality.

The investigator must keep a screening log, recording all patients who were screened, whether they were enrolled or not, and a separate Patient Identification List showing code numbers, names, and dates of birth to allow unambiguous identification of each patient included in the study.

14.4 Patient Treatment Plan

All patients will continue to receive standard care for T1D during the study.

After the individual completion of the study, the patient will return to the standard treatment received prior to study participation.

14.5 GCP

The study will be managed and conducted in compliance with the protocol and according to the latest international (ICH) guidelines for GCP as well as the applicable regulatory requirement(s).

14.6 Record Retention

The eCRFs and all medical records upon which the eCRFs are based (source data) must be kept for at least 10 years, or the length of time according to local legislations, whichever is longer, after completion of the study.
The Investigator/institution should maintain essential documents (as defined in ICH E6 GCP, Section 8) as required by the applicable regulatory requirement(s). The Investigator/institution should take measures to prevent accidental or premature destruction of the documents.
Essential documents should be retained according to applicable regulatory requirements of the country(ies) where the product is approved, and/or where the Sponsor intends to apply for approval.
It is the responsibility of the Sponsor to inform the Investigator/institution in writing as to when the documents no longer need to be retained.

14.7 Monitoring / Quality Control

Prior to the start of the study, the monitor will review the protocol and eCRFs with the investigator and his/her staff. The investigator will be visited by the monitor, who will check study procedures, including safety assessments, study medication handling, data recording and source data verification (SDV). To assure the accuracy and completeness of the data recorded in the trial, the monitor will compare eCRFs with medical records and other relevant documentation during the on-site monitoring visits. The monitor must therefore direct access to all source data according to ICH GCP to confirm that required protocol procedures are being followed and check consistency between patient record and eCRF data. Incorrect or missing entries into the eCRFs will be queried and must be corrected. Study monitoring will not jeopardize patient confidentiality.

14.8 Source Data

Source data is defined as any information in original records and certified copies of original records of clinical findings, observations or other activities necessary for reconstruction and evaluation for the study (e.g. eCRFs, medical records (including EMRs) lab reports, patient information sheets, patient diaries, etc.).
The origin of source data in the study will be further specified for each study site in a separate document (“Origin of Source Data”).
It is the responsibility of the Investigator to record essential information in the medical records in accordance with national regulations and requirements. The following information should be included as a minimum:

- A statement that the patient is in a clinical study
- The identity of the study e.g. Study code
- Patient screening number and/or patient number
- That informed consent was obtained and the date
- Diagnosis
- Dates of all visits during the study period

- Any information relating to AEs
- All treatments and medications prescribed/administered (including dosage)
- Date of study termination
- Patient health service identification number

The Investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the data recorded in the eCRFs. Data reported in the eCRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained. Signed sections of eCRFs will be monitored and collected on a regular basis.

14.9 Quality Assurance and Insurance

During or after the study is completed, regulatory authorities, the Sponsor, [redacted] or other involved party may wish to carry out an audit. These representatives must have the same access to study data and patient source data as the monitor.

The Sponsor is covered by a liability insurance that also covers liability towards patients in clinical trials

15 END OF TRIAL

The end of the trial is defined as the last visit of the last patient included in the trial and all data have been collected.

15.1 Study Report

A clinical study report will be prepared covering clinical and statistical aspects and summarizing all findings of the Main Study Period. **The** data from the Extension Study Period will be summarized in an addendum to the final clinical **s**tudy report from the Main Study Period.

15.2 Study Stopping Criteria

The Sponsor and the investigators reserve the right to discontinue the study at any time for safety reasons or other reasons jeopardizing the justification of the study. Such a termination will be implemented in a time frame that is compatible with the patient's wellbeing.

If the study is prematurely terminated or suspended, the investigator should promptly inform the patients and assure appropriate therapy and follow-up. The Sponsor will notify the Regulatory Authorities and the IECs of any plans to terminate the study.

15.3 Publication

It is intended that the results of the trial will be published in the scientific literature. Results may also be used for submissions to Regulatory Authorities. The following conditions are to protect commercial confidential materials (patents, etc.) and not to restrict publication.

All information concerning trial drug (such as patent applications, formulae, manufacturing processes, or formulation information supplied to the investigator by the Sponsor and not previously published) is considered confidential by the Sponsor and shall remain the sole property of the Sponsor. The investigator agrees not to use this for other purposes without the Sponsor's written consent.

It is understood by the investigator that the Sponsor will use the information obtained in this clinical trial in connection with the development of the trial drug and therefore may be disclosed as required to other of the Sponsor's investigators or any appropriate international Regulatory Authorities. In order to allow for the use of the information derived from this clinical trial, the investigator understands that he/she has an obligation to provide the Sponsor with complete test results and all data obtained during this trial.

Prior to submitting the results of this trial for publication or presentation, the investigator will allow the Sponsor 30 days in which to review and comment upon the publication manuscript. The Sponsor agrees that before it publishes any results of this trial it shall provide the investigator at least 30 days for full review of the manuscript. Coordinating investigator will decide who should be authors/coauthors, and the order of authors. In accordance with generally recognized principles of scientific publication rules and collaboration, co-authorship with investigators and any of the Sponsor's personnel will be discussed and mutually agreed upon before submission of a manuscript to a publisher.

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17 SIGNATURES

This Clinical Study Protocol is approved by:

Sponsors Representative:
(Name, Title, Affiliation)

(Signature)

……..……

Date: ……..

Principal/Coordinating Investigator:
(Name, Title, Affiliation)

(Signature)

……..……

Date: ……..

Other(s), as applicable:
(Name, Title, Affiliation)

(Signature)

……..……

Date: ……..

18 CLINICAL STUDY PROTOCOL AGREEMENT FORM

Investigator's Statement:

I have read and understand the foregoing protocol with the title:

"A Phase IIb, 2-Arm, Randomized, Double-blind, Placebo-Controlled, Multicentre Study to Optimize Diamyd® Therapy Administered into Lymph Nodes Combined with Oral Vitamin D to Investigate the Impact on the Progression of Type 1 diabetes".

with study number DIAGNODE-2 (D/P2/17/6) and agree to conduct the trial, in compliance with ICH notes on Good Clinical Practice (CPMP/ICH/135/95), designated Standard Operating Procedures, National Laws and regulations and within the principles of the current revision of Declaration of Helsinki (Brazil 2013).

Investigator's Name:

Investigator's Title:

Investigator's Signature:

……..……

Date: ……..