CLINICAL STUDY PROTOCOL

Open Label Pilot Trial in patients with recent-onset T1D to evaluate the safety, diabetes status and immune response of GAD-antigen (Diamyd®) therapy administered into lymph nodes in combination with an oral vitamin D regimen

Acronym: DIAGNODE-1. (Trial to evaluate the safety and diabetes status in patients with recent-onset Type 1 **dia**betes by giving **G**AD-antigen (Diamyd®) therapy into lymph **NODE**s in combination with an oral Vitamin D regimen)

Study No. DIAGNODE-1
EudraCT Number: 2014-001417-79
Study Development Phase: I/II

Version 5.1: 2018-06-11

Sponsor: [redacted]
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Contract Research Organization: Linköping Academic Research Centre (LARC)

Planned start date: Q4, 2014

VERSION TABLE

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Version 2:	Version 1.1, including Substantial Amendment #2	Jan 15, 2016
Version 1.1:	Version 1, including Information Amendment #1.1	Nov 24, 2014
Version 1:	Version 1	Aug 14, 2014

STUDY ADMINISTRATION		
	Name and address	**Telephone and fax numbers, e-mail address**
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CONFIDENTIALITY STATEMENT

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Study infrastructure

Principal Investigator and sponsor:

- [redacted]

Co-investigators:

- [redacted]

Study nurses:

- [redacted]

Laboratory staff:

[redacted]

Facilities and Equipment:

- *Laboratory:* Clinical Experimental Research, Linköping university hospital, has localities with all necessary modern equipments. Div of Pediatrics has laboratory facilities, with an experienced and skilful staff
- *Clinical infrastructure*: Participating clinics have experience from previous clinical studies, with experienced diabetologists, nurses with knowledge and experience of MMTT and collection of samples, how to fill in CRFs etc
- *Study Monitors*: Clinical Study Monitors, are available at the Linköping Academic Research Centre (LARC)
- *Computers, information technology*: All necessary facilities are available at the Faculty of Health Sciences, Linköping university and at the involved hospitals
- Safety Reporting: Contract Research Organization (CRO) to be decided

SYNOPSIS OF THE PROPOSED PILOT STUDY

Name of Sponsor [redacted] **Name of Finished Product:** DIAMYD® **Name of Active Ingredient:** Recombinant Human Glutamic Acid Decarboxylase (rhGAD65)	**Individual Study Table Referring to Part of Dossier in Which the Individual Study or Study Table is Presented:** **Volume:** **Reference:**	**(For National Authority Use Only)**

Title of Study:
Open Label Pilot Trial in patients with recent-onset T1D to evaluate the safety, diabetes status and immune response of GAD-antigen (Diamyd®) therapy administered into lymph nodes in combination with an oral and vitamin D regimen

Protocol Number:
DIAGNODE-1

Investigators and Study Centre:
2 sites including approximately 15 patients

Phase of Development: Phase I/II

Objectives:

- Evaluate the safety of giving GAD-Alum (Diamyd) directly into lymph glands in combination with an oral and vitamin D regimen
- Evaluate how the above mentioned treatments influence the immune system and endogenous insulin secretion.

Study Design: The study is a double center open-labelled pilot clinical trial. Eligible patients will be treated with 4 µg GAD-Alum into an inguinal lymph gland at three occasions, with one month intervals in combination with vitamin D (14 000 IE/week) for 4 months, starting 1 month prior to first GAD-Alum injection.

Selection of Subjects: Patients must be 12.00-29.99 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 GAD65 antibodies are present.

Number of Subjects Planned: Approximately 15 patients will be enrolled.

Description of Treatment Groups:
There is one single treatment group. The patients will be assessed for eligibility at the screening visit (Visit 1) 10 to 21 days prior to the start of the treatment. All patients will from Day 1 (Visit 2) receive 14 000 I/E vitamin D per os per week during 4 months. In addition, all patients will at Month 1 (Visit 3) receive their first GAD-Alum treatment (4 µg) into a lymph node, where after a 2nd and 3rd dose of GAD-Alum will be administered at Months 2 and 3 (Visits 4 and 5). The patients will be evaluated at 6 months (main study period, 6 visits) and will then be followed for additionally 24 months (extension study period, 2 visits). The total study period is 30 months.

Name of Sponsor [redacted]	**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®		
Name of Active Ingredient: Recombinant Human Glutamic Acid Decarboxylase (rhGAD65)	**Volume:** **Reference:**	

Endpoints:

Primary endpoints:
To evaluate the safety of giving Diamyd directly into lymph glands in combination with an oral vitamin D regimen at Month 6 (main study period) and Month 15 and 30 (extension study period).

Variables to evaluate safety:

- Reactions of the injection site
- Occurrence of adverse events (AEs)
- Laboratory measurements (biochemistry and haematology), including Calcium and Vitamin D in serum
- Urinalysis (microalbuminuria, creatinine)
- Physical examinations, including neurological assessments
- GAD65AB titer (GADA)

Secondary endpoints:
To evaluate how the above mentioned treatment influences the immune system and endogenous insulin secretion from baseline to Month 6 (main study period) and subsequent visits during the extension study period.

Variables to evaluate the influence on the immune system:

- Inflammatory markers, especially TNF-alfa, IL-1 beta, IL-2, IL-17
- Th2-deviation of cell-mediated immune response seen e.g. as increased ratio of IL-5,10, 13 in comparison with IFN-gamma, TNF-alfa, IL-1 beta and IL-17
- Increase of T-regulatory cells

Variables to evaluate the effect of endogenous insulin secretion:

- C-peptide (90 minute value and AUCmean 0-120 min) during an MMTT)
- Fasting C-peptide
- Hemoglobin A1c (HbA1c)
- Exogenous insulin dose per kg body weight and 24 hours

Sample size:
No real sample size calculation is done as this is an open-label pilot study to study safety and summarize changes in the immune system and beta cell function descriptively. All variables will be summarized descriptively.

LIST OF ABBREVIATIONS AND DEFINITION OF STUDY SPECIFIC TERMINOLOGY

ADA	American Diabetes Association
AE	Adverse Event
Alum	Aluminum hydroxide
AUC	Area Under the Curve
$AUC_{mean\ 0\text{-}120\ min}$	AUC mean 0-120 minutes
BMI	Body Mass Index
CRF	Case Report Form
CRA	Clinical Research Associate
CRO	Contract Research Organization
DCCT	Diabetes Control and Complications Trial
DCF	Data Clarification Form
DSMB	Data Safety Monitoring Board
IEC	Independent Ethics Committee
GAD	Glutamic acid decarboxylase
GADA	Antibodies to GAD with molecular mass 65,000
GAD65	GAD with molecular mass 65,000
GCP	Good Clinical Practice
HbA1c	Hemoglobin A1c
HLA	Human Leukocyte Antigen
IAA	Insulin Autoantibody
IA2	Islet cell Antigen 512
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ITT	Intent-to-Treat
LADA	Latent Autoimmune Diabetes in Adults
MMTT	Mixed Meal Tolerance Test
NOD	Non-obese Diabetic
PP	Per-Protocol
rhGAD65	Recombinant Human GAD with molecular mass 65,000
SAE	Serious Adverse Event
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes

1 Introduction

1.1 Background and rational

The incidence of Type 1 diabetes (T1D) in children is next to Finland highest in Sweden in the world, and is increasing rapidly. T1D is by far the most common chronic, serious, life-threatening disease among children and adolescents in our country, and the incidence of Type 1 diabetes is high also in young adults. The disease tends to become an extremely serious global problem. The disease is characterized by lack of insulin. Even though several patients at diagnosis have rather impressive residual beta cell function (1) the deficiency becomes soon very pronounced and finally complete (2,3). Residual insulin secretion is of crucial importance. In rare cases the beta cell function improves so much shortly after diagnosis that glucose metabolism normalizes and no insulin is required for some time, that is the patient goes into so called complete remission (4). As long as the patient is in a complete remission there is no need of active treatment, more than perhaps some recommendation of sound life style regarding physical exercise and diet. There are no symptoms, no acute complications and if somebody stayed in complete remission it is unlikely that such an individual would ever develop late complications. Slight abnormality of glucose or lipid metabolism might increase the risk of macrovascular complications in the same way as for individuals with chemical diabetes or impaired glucose tolerance.

Complete remission is rare, but partial remission it is not (4). During this period the patient usually has near normal blood glucose values, not even mild hypoglycemia and no episodes of ketoacidosis. The quality of life is very good as the patient feels well, children grow normally, few restrictions are needed, if any with regard to food, the patients can exercise with great variation without getting hypoglycemia, and experiences very good home blood glucose tests. Only some residual insulin secretion is enough to diminish the risk of ketoacidosis (5). Furthermore, it has been shown in the DCCT trial that even quite modest residual insulin secretion, a response to a beta cell stimulation with serum C-peptide >0.20pmol/ml, plays an important role for prevention of complications (6). This effect may be due to the fact that residual insulin secretion should reasonably make it easier to reach good blood glucose balance, but it is also possible that C-peptide per se has a physiological function. It has in fact been reported that C-peptide influences vascular permeability, decreases leakage in retinal vessel, and not least has a positive effect on nerve function (7) although the effect of C-peptide per se still is under debate.

1.1.1 Factors influencing the natural course

At diagnosis of T1D it has been claimed that 80-90% of the beta cells in pancreas have been destroyed. However, the proof for this is scarce, and it may well be that the main problem is deterioration of function. Furthermore there is great difference between patients, as some have quite good residual insulin secretion and others have not. Shortly after diagnosis, especially when an active insulin treatment is given, there is an increase of C-peptide production, and at the same time an improvement of insulin sensitivity. Good metabolic control seems to improve the milieu and metabolism for the beta cells and the beta cell function is preserved, which in turn contributes to better metabolic control, and vice versa. The intensity of the autoimmune process plays a role, and it seems evident that children have a more aggressive immune process than adults with Type 1 diabetes, but it is still difficult to predict the course. Some studies have suggested that high concentrations of auto-antibodies are followed by a more rapid loss of insulin secretion, while others have not found such a relationship, or even the opposite. No special signs of cell-mediated

immunity have so far been proven to predict beta cell loss but our own studies have shown that disease process is related to a T-helper-1 (Th-1) deviation of the immune system with increases of certain cytokines such as IFNg and decrease of IL-10, IL-13.

The effect of insulin treatment on beta cell function.
Active insulin treatment during the first period of the disease prolonged the partial remission long time ago, and this finding could be confirmed and validated by improved residual insulin secretion (2). Intensified treatment seems to improve residual beta cell function at least for some time (8), but it may also have long-term positive effects (9). Active treatment has been shown not only to prevent or postpone diabetes in experimental animals, but studies have indicated that such treatment could prevent diabetes in high risk individuals (10). However, when tried at a larger scale in the Diabetes Prevention Trial, parenteral insulin treatment did not prevent diabetes (11). Oral treatment with insulin might have an effect (12) and therefore further studies are needed.

1.1.2 Interventions

In the 1970ies it became clear that T1D is an autoimmune disease and therefore immune interventions were tried. We performed the first immune intervention studies in the world on diabetic children when we already 30 years ago used plasmapheresis in newly-diagnosed children and adolescents with some positive effects (13). As a side effect of that treatment a new protein with the weight 64kD was found in plasma (14), which later showed to be Glutamic Acid Decarboxylase (GAD). The breakthrough, taken as a proof for the concept of immune intervention, was cyclosporin, which doubtless slowed down the autoimmune destructive process and gave improved residual insulin secretion, while other trials with immune suppression had minimal effect, especially so in children (15, 16,17), or showed too serious adverse events or risks (18, 19). In an effort to modulate the immune system we used photopheresis. Although clear effects on the immune system were demonstrated in a double blind placebo-controlled trial (20), the clinical effect was minimal and almost no improvement of residual beta cell function could be seen (21). Thus, with no successful immune intervention, our interest was directed to protective agents such as Nicotinamide and Diazoxide, with no or transient effect (22, 23, 24). With increasing knowledge of the immune process leading to beta cell destruction, it has become possible to direct more precisely the immune intervention to target the important T-cells. Promising studies using anti-CD3antibodies in an attempt to block the destructive immune process have been performed. Results from both North-American and French trials with anti-CD3 have shown that it is possible to block the destructive autoimmune process and thereby at least postpone the decline of the beta cell function (25,26). The decline of residual insulin secretion was significantly slowed down, but unfortunately it looks as if the decline was just delayed a year, and thereafter the declining C-peptide curve went parallel to the declining curve in the placebo group. Furthermore, a majority of the patients experience some Cytokine Release Syndrome (CRS), which may be quite serious, and in addition a number of side effects were seen in most of the patients. We have participated in one of two recent Phase III trials (Protegé trial), which failed to reach the primary endpoint, although the arm with the most intense treatment indeed showed some preservation of residual insulin secretion and lower insulin requirement to reach good HbA1c (27; Sherry, Hagopian, Ludvigsson et al Lancet 2011). New studies are needed but it is difficult to believe that this type of treatment alone will be the accepted solution for general clinical use. Even less likely is such a treatment accepted as a preventive treatment in otherwise healthy individuals of whom many never would develop diabetes.

1.1.3 Immune therapy with auto-antigens

In the treatment of allergic diseases, immunotherapy with small amount of disease specific antigen has been efficiently used during many years.The mechanism for this treatment remains unclear, although immune modulation of the immune responses and induction of regulatory cells have been suggested. In autoimmune diseases no such treatment has been successful, but should be tried (28). Experiments in diabetes prone animals have shown that treatment with a heat shock protein could delay or postpone development of diabetes. The use of Diapep277 peptide in a study in adults showed significant preservation of insulin secretion without almost any adverse events (29). Later trials in children and adolescents with T1D (30), however, have shown no effect. Studies with Diapep277 treatment in so called LADA (Latent Autoimmune Diabetes in the Adult) are ongoing, and preliminary results (report at IDF, Dubai Dec 2011 and at ADA June 2012) suggests that treatment with Diapep277 may preserve beta cell function in adults with mild Type 1 diabetes. However, the results are a bit unclear, as there was a weak C-peptide preservation only seen after Glucagon stimulation, but no effect at all after Mixed Meal Tolerance Test, and there was no differences whatsoever between the actively treated group and placebo in immune markers.

Active treatment with insulin has been shown not only to prevent or postpone diabetes in experimental animals but preliminary open studies indicated that such treatment could prevent diabetes in high risk individuals (10). Insulin, clearly a beta cell specific auto-antigen, has been parentally administrated (DPT) to prevent diabetes in high risk individuals with no effect, while oral insulin administration with the same purpose may have a slight effect (12).

1.1.4 Previous clinical studies with GAD-Alum

1.1.4.1 GAD-vaccination

GAD (Glutamic Acid Decarboxylase), can be regarded as an auto-antigen, as it is produced in the islets with increased release as response to beta cell stimulation. This protein has been shown to deeply influence the autoimmune immune process (31,32,33,34). Several studies have shown that indeed GAD can prevent diabetes in experimental animals (35-42). The similarity of GAD with viral proteins may be important for the therapeutic action. The observed effect, even after the start of the immune process, suggests that it might be possible to expect the same effect in humans after the start of the immune process. In a phase II study in LADA patients the administration of one low dose, Diamyd 20 µg, led to improved beta cell function for up to 2 years compared to the placebo treated group, with no side effects. Also other doses were tried: 4 µg showed no effect, 100 µg showed a similar effect as 20 µg, while 500 µg showed no effect. None of the doses showed any adverse events, still so after several years follow-up (43). Association with change in the ratio of CD4+CD25+/ CD4- CD25- cells was found, indicating a mechanism for the effect. With this promising background we started a Phase II study in Type 1 diabetic patients 10-18 years with recent onset. Based on the idea that the treatment earlier had effect in slowly progressive LADA patients we included patients with up to 18 months duration of T1D diabetes at intervention. The patients were randomized to either 20 µg GAD-Alum (Diamyd) sc at Day 1 and 30, or placebo**.** The effect still after 30 months was remarkable, and clearly both statistically and clinically significant (44), with about half of the C-peptide decline in the GAD treated group compared with the placebo group. Patients with a diabetes duration < 3 months had a remarkably good effect with no or minimal decline of beta cell function during the follow-up of the first 15 months. Almost all effect was seen in patients with < 6 months duration at vaccination**.** Even more, in contrast to other intervention treatments**,** this effect was

gained with no adverse events at all, making the treatment very encouraging! Still after 48 months the patients treated with < 6 months duration hade significantly preserved C-peptide and still no adverse events (45). So far GAD-treatment looked very promising. Two Phase III trials were performed, one European with Johnny Ludvigsson (JL) as PI, and one in USA with Jerry Palmer as PI and JL as co-investigator. In the European trial 334 patients were recruited into three arms, one arm with GAD-Alum (Diamyd) 20 µg at Day 1,30, 90 and 270, another arm with GAD-Alum 20 µg at Day 1 and 30, and placebo at Day 90 and 270 and a third arm with Placebo at Day 1,30,90 and 270. Primary endpoint, serum C-peptide AUC after a Mixed Meal Tolerance Test (MMTT) at 15 months was not met! (C-peptide AUC p=0.1; Fasting C-peptide p=0.07) (46). This prompted the company (Diamyd Medical + Johnson&Johnson) to close the phase III trials early. However, the Phase III trial did show several positive effects. Thus statistically significant efficacy was seen in several pre-specified subgroups. Furthermore, 45 Swedish patients had passed the 30 month's visit when the study was stopped, and those 15 patients who had received two doses of GAD-Alum (Diamyd) 20 µg showed a significant preservation of C-peptide after 30 months compared with placebo! This is especially remarkable as the Swedish patients were the ones without efficacy after 15 months, while efficacy was found after 15 months in the non-Nordic countries.

1.1.4.2 Possible Reasons to the different results Phase II and Phase III

In Phase III randomization, patients receiving active drug were more often in the 10-11 year age group than in the 16-18 year age group whereas placebo was more frequent than active drug in the higher age group. This may have influenced the result. The Phase II patients were treated in March–April and those patients in Phase III who were treated in March-April had also significant effect of GAD-treatment. In the Phase II trial no vaccinations were accepted, but in Phase III Influenza-vaccination was allowed. Unfortunately, an epidemic of H1N1-flu lead to that almost all patients were vaccinated, many of them in connection with the GAD-vaccinations. In Sweden and Finland the vaccine contained squalen, suspected to influence the immune system towards auto-immunity, and in these two countries there was no efficacy of GAD-treatment, while the efficacy was significant in other European countries. Patients in Sweden who did not get the influenza vaccination close to the GAD-treatment, had better efficacy of GAD-treatment (46).

1.1.4.3 Ongoing DIABGAD-1 trial

Since Jan 2013 the Phase II DIABGAD-1 trial is ongoing in Sweden. This is a trial in 64 patients, 10-18 years old, with recent-onset Type 1 diabetes, who are treated in a double-blind placebo-controlled randomized study with GAD-Alum 20 µg resp 40 µg given twice with 30 days interval, in combination with Vitamin D, 2000 units daily for 450 days, and Ibuprofen 400 mg daily for 90 days. Recruitment is fulfilled and now closed. There are so far no Serious Adverse Events judged as related to study medication, and very slight adverse events, not related to the treatment except for mild transient reactions on the site of injection of GAD-Alum. An interim analysis is planned already after 6 months follow-up of all patients while more extended analyses will be performed after 15 and 30 months.

1.1.5 Intra lymph-node immunotherapy

Antigen therapy 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 so far antigen has been given either orally, intra-nasally or subcutaneously, in order to present the antigen to antigen presenting/dendritic cells which in turn are expected to present the antigen to the T-cells of the immune system. However, animal studies have shown that intralymphatic

injections induce a strong and relevant T-cell response (47,48) and in the allergy field clinical studies have shown that presentation of the antigen/allergen directly into the lymph nodes seems to be more effective than traditional administration (49). Lower doses of the allergen can be used, the number of treatments can be radically reduced, and there have been no treatment-related adverse events. Inguinal lymphnodes are readily accessible in patients and the pain associated with the injection is rated as below that of venous puncture (50). With this background it is our aim to study whether the same approach can be used in patients with the autoimmune form of Type 1 diabetes.

1.1.6 Vitamin D and type 1 diabetes

Experimental evidence indicates that vitamin D may play a role in the defence against Type 1 diabetes (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 Type 1 diabetes. Thus a multinational case-control study and a birth cohort follow-up study from Finland with (51,52) have both concluded that vitamin D3 supplementation at birth protects from type 1 diabetes later in life, and a meta-analysis supports gives similar conclusions (53). Others report lower serum levels of 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3, calcitriol] in patients with recently diagnosed type 1 diabetes than in healthy control subjects (54) 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 (55-58) and facilitates a shift from a Th1 to a Th2 immune response (59). An increasing amount of evidence suggests that vitamin D also affects beta cells directly thereby rendering them more resistant to cellular stress (60), and there are results indicating that Vitamin D may also improve insulin sensitivity (61), which in turn decrease beta cell stress.

With this background vitamin D has been used in patients with recent onset Type 1 diabetes in an effort to preserve residual insulin secretion. However, so far Vitamin D alone has not been efficacious enough (62,63). Therefore there is reason to try Vitamin D, both in somewhat higher dose, and in combination with other therapy.

1.2 Hypothesis

- The encouraging results of the Phase II GAD trial and the partly positive results of the Phase III European Trial, support the concept that administration of GAD-Alum (Diamyd) may decrease the autoimmune process and contribute to preservation of residual insulin secretion
- As previous studies have indicated that the dose should be somewhere between 20 and 100 µg Diamyd**,** a lower dose of 4 µg given three times should be adequate when administrated directly into lymph nodes.
- Injection of GAD-Alum (Diamyd) directly into lymph nodes will give no serious adverse events, have desired immunological effects and will (shown in future studies) improve efficacy
- Addition of rather large doses of Vitamin D may improve the efficacy both via effects on the immune system and mechanism directly on the beta cells

2. Risk-Benefit Analyses

The incidence of Type 1 diabetes is next to Finland higher in Sweden than in any other country of the world. The incidence is continuously increasing for decades. The disease cannot be cured and cannot be prevented. In spite of a very heavy, intensive, expensive treatment many patients get life-threatening serious both acute and late complications, and the mortality is much increased. At diagnosis many patients have some slight residual insulin secretion. As long as this is the case it is much easier to keep blood glucose stable, the incidence of hypoglycaemia decreases as well as the risk of ketoacidosis. Quality of life for the patient as well as for parents of children with diabetes is better as long as there is some residual insulin secretion.

It is evident that there is a great benefit of preservation of residual insulin secretion, and therefore therapies aiming at preservation of this function justify treatments that are quite heavy, even dangerous and expensive. Thus it has been regarded as justified to treat Type 1 diabetes at onset with drugs like monoclonal antibodies against the CD3-receptor, which causes adverse events in principally all patients, some even quite serious adverse events and risks. Even pure cytostatics have been used.

In our proposed study we use GAD-Alum (Diamyd) 4µg x 3, a treatment which has been used in much larger doses given to children and adults with almost no adverse events seen during follow-up of thousands of patient years. In our study we plan to use a very low dose, which means that the general risk can be expected to be even lower, but the administration will be directly into a lymph node which might give local reactions. The effect on the immune system may become more pronounced but should not lead to any adverse effects. Previous studies in allergy (where Dr. Helene Zachrisson has given the intra lymph node injections of alum-formulated allergens) have not shown any adverse effect neither generally nor locally).

In DIABGAD-1 we give vitamin D 14000 IU per week 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. 64 patients in DIABGAD-1 have been randomized of whom ¾ should have received Vitamin D, and we have noticed no adverse event which can be regarded as related to Vitamin D.

When summarizing the pros and cons, there is a clear possibility of therapeutic benefit of great importance, both for the participating patients, for patients in future studies, whereas the risk is very small. If these studies contribute to the development of a good treatment, this will be of enormous value for a great number of patients.

3. Aim of present study

Our aim of the present pilot study is to get information on whether GAD-Alum can be given into lymph nodes without treatment related serious adverse events, in combination with an oral Vitamin D regimen, to allow future Phase II-studies with the same technique to improve efficacy in preserving residual insulin secretion in Type 1 diabetes. Thus we want to see whether this treatment give any adverse events, and how treatment regimens influence the immune system, cause the desired Th-2 deviation, increase of T-regulatory cells, and hopefully indications of preservation of residual beta cell function. Based on the short-term results of this pilot study (6 months main study period) we then may want to design a larger Phase II trial, to get more robust information. The main long-term goal is then to find a treatment at onset

of Type 1 diabetes in young patients which is tolerable for the patients, safe, and which can preserve residual insulin secretion and give the patients a better quality of life, with less acute complications and in the long run less risk of late complications.

4. Objectives and Endpoints

4.1 Objectives

- Evaluate the safety of giving Diamyd directly into lymph glands in combination with an oral vitamin D regimen
- Evaluate how the above mentioned treatments influence the immune system and endogenous insulin secretion.

4.2 Endpoints

4.2.1 Primary endpoints

To evaluate the safety of giving Diamyd directly into lymph glands in combination with an oral vitamin D regimen at Month 6 (main study period) and Month 15 and 30 (extension study period).

Variables to evaluate safety:

- Reactions of the injection site
- Occurrence of adverse events (AEs)
- Laboratory measurements (biochemistry and haematology), including Calcium and Vitamin D in serum
- Urinalysis (microalbuminuria, creatinine)
- Physical examinations, including neurological assessments
- GAD65AB titer (GADA)

4.2.2 Secondary endpoints

To evaluate how the above mentioned treatment influences the immune system and endogenous insulin secretion from baseline to Month 6 (main study period) and subsequent visits during the extension study period.

Variables to evaluate the influence on the immune system

- Inflammatory markers, especially TNF-alfa, IL-1 beta, IL-2, IL-17
- Th2-deviation of cell-mediated immune response seen e.g. as increased ratio of IL-5,10, 13 in comparison with IFN-gamma, TNF-alfa, IL-1 beta and IL-17
- Increase of T-regulatory cells

Variables to evaluate the effect of endogenous insulin secretion:

- C-peptide (90 minute value and AUCmean 0-120 min) during an MMTT)
- Fasting C-peptide
- Hemoglobin A1c (HbA1c)
- Exogenous insulin dose per kg body weight and 24 hours

5. Population

Patients with recent-onset Type 1 diabetes and guardian(s) (where applicable) at Linköping university hospital are given information about the study and they are asked to participate in the trial.

5.1 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. Type 1 diabetes according to the ADA classification with < 6 months diabetes duration
3. Age 12.00-29.99 years at diagnosis of Type 1 diabetes
4. Fasting C-peptide ≥0.12 nmol/L
5. Pos GADA but < 50 000 random units
6. Females must agree to avoid pregnancy and have a negative urine pregnancy test
7. Patients of childbearing potential must agree to using adequate contraception, until 1 year after the last administration of GAD-Alum. 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. intrauterine device
   c. intrauterine system (for example, progestin-releasing coil)
   d. vasectomized male (with appropriate postvasectomy documentation of the absence of sperm in the ejaculate)

   For males of childbearing potential:

   e. condom (male)

5.2 Exclusion criteria

1. Previous or current treatment with immunosuppressant therapy (although topical or inhaled steroids are accepted)
2. Continuous treatment with any 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 anaemia or significantly abnormal haematology results at screening
6. A history of epilepsy, head trauma or cerebro-vascular 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 human immunodeficiency virus (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 GAD-Alum treatment)
15. Males or females not willing to use adequate contraception until 1 year after the last GAD-Alum treatment
16. Presence of associated serious disease or condition, including active skin infections that preclude subcutaneous injection, which in the opinion of the investigator makes the patient non-eligible for the study
17. Deemed by the investigator not being able to follow instructions and/or follow the study protocol

5.3 Recruitment and screening

Potential study patients and guardian(s) (where applicable) 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, the patient/guardian(s) will have the opportunity to ask questions to the investigational team. If, after this, the subjects agree to participate, patient and/or guardian(s) will personally sign and date the written informed consent form.

The patients and/or guardian(s) will then receive a copy of the signed and dated patient information/informed consent form.

5.4 Patient withdrawal

In accordance with the Declaration of Helsinki, the investigator must explain to the patient and guardian(s) (where applicable) 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 CRF.

There will be different categories for withdrawals from the study:

Complete withdrawal (i.e. stopping investigational product and also continued efficacy and safety evaluations)

Standard reasons for withdrawing from further participation in the study and from the follow-up visits (and <e.g. blood tests>) may be:
- Patient's and/or guardian(s) decision (withdrawal of consent to participate)
- Patient lost to follow-up

Standard reasons from withdrawing from taking further investigational product, but continuing follow-up visits and safety evaluations may be:
- Unacceptable adverse events
- Patient and/or guardian(s) request
- Investigator's discretion
- Patient lost to follow-up/non-attendance
- Intercurrent illness
- The patient becomes pregnant

Thus intra lymph node GAD-Alum should not be given to the patient if the patient after inclusion in the study has got
- brain damage, epilepsia, head trauma, neurological disease

- any active, serious hormonal disease other than Type 1 diabetes
- 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
- any vaccination
- drug/alcohol abuse

or if the patient has

- become pregnant or is no longer willing to use safe contraceptives during the study

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 (visit <>) - 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 adverse events must be fully documented in the case record form, with the addition of supplementary information if available and/or appropriate.

6. Treatment procedures

6.1 Study Design and Treatment

The trial is double center, open-label, pilot study in GADA positive T1D patients of either gender, 12.00 to 29.99 years old, diagnosed with T1D within 6 months at time of screening (Visit 1) and fasting C-peptide levels equal to or above 0.12 nmol/L. In total, approximately 15 patients will be recruited at one site in Linköping, Sweden.

The patients will be assessed for eligibility at the screening visit (Visit 1) 10 to 21 days prior to the start of treatment. The screened patients will be assigned a sequential screening number and this screening number will be used as patient identification throughout the study.

Patients who qualify for inclusion in the study will then be enrolled in the study to receive investigational study drug at the subsequent visits according to table 1 below.

The patients will be followed for a total study period of 30 months which includes 8 visits to the site.

See table 1 below for an overview of the study visits.

Table 1 Schedule of Patient Visits, Visit Windows and Study Drug Administration

Study	**Screening**	**Intervention – Main Study Period**					**Follow-up – Extension Study Period**	
	Day -10 to - 21 Screening	**Day 1 Baseline**	**Day 30 Month 1**	**Day 60 Month 2**	**Day 90 Month 3**	**Day 180 Month 6**	**Day 450 Month 15**	**Day 900 Month 30**
	Visit 1	**Visit 2**	**Visit 3** ± 3	**Visit 4** ± 3	**Visit 5** ± 3	**Visit 6** ± 14	**Visit 7** ± 14	**Visit 8** ± 30
Appr 15 subjects		Oral Vitamin D 14 000 IE per week from Day 1 to Day 120						
			1st GAD-Alum treat-ment	2nd GAD-Alum treat-ment	3rd GAD-Alum treat-ment			

6.2 Assessments and procedures

1. Standard insulin treatment, education and psychosocial support for newly diagnosed Type 1 diabetes patients.
2. Normalization of fluid, electrolyte and acid-base balance.
3. Thereafter information about the study.
4. When the patients and/or guardian(s) have given their informed consent, at the latest 6 months after diagnosis, screening is performed with a fasting venous sample from patients who are eligible according to other criteria than C-peptide and GADA concentrations (Visit 1).
5. At Baseline (Visit 2), 6, 15 and 30 months, assessment of residual endogenous insulin secretion by MMTT.
6. Blood sampling for Vitamin D in serum at Visit 1 and then after one month of treatment at Visit 3 (before first GAD-Alum treatment) and at Visit 7 (15 months)
7. HbA1c, safety (haematology and chemistry), autoantibody titres (GAD65, IA-2), immunology, are followed by blood samples at every study visit.
8. Exogenous insulin dose/24 hours four days prior to each visit, AEs and concomitant medication is registered at every study visit.
9. Self-reported hypoglycaemia (defined as needing help from others and/or seizures and/or unconscious) registered at every study visit.
10. Any symptoms or signs of other medical problem should be treated at the discretion of the clinical doctor.

Examinations will be performed according to Table 2 in Section 7 below, and in the order outlined in the case report form (CRF).

6.2.1 All Visits, Visit 1 through 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.

6.2.2 Administrations of GAD-Alum, Visits 3, 4 and 5

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

6.2.3 Mixed Meal Tolerance Test (MMTT), Visits 2, 6, 7 and 8

The MMTT must be performed according to the instructions in the CRF.

The patient should:

- Come to the study site following an overnight fast (>10hr), 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 for safety reasons, subjects need to eat or take insulin, the visit should also be rescheduled.

6.3 Laboratory tests and examinations:

1. Immunological tests:
    a. Autoantibodies (Anti-GAD65, Anti-Insulin, Anti-IA-2, ZnT8)
    b. Relevant cytokines and chemokines are determined (see Table 2 and section 9.1.1 below)
    c. T-cells are classified and studied (see Table 2 and section 9.1.1 below)
2. Genetics:
    a. HLA determinations is done and genes related to diabetes development
    b. Array studies to elucidate the importance of diabetes-related genes
3. Diabetes status:
    a. HbA1c
    b. Fasting glucose and fasting C-peptide
    c. Meal stimulated glucose and C-peptide
4. Blood sampling for safety:
    a. Hematology
    b. Chemistry
5. Urine analysis
6. Urine pregnancy test as appropriate
7. Other:
    a. Vitamin D in serum

6.4 Medical history

A complete review of the subject's past medical history will be undertaken by the investigator and documented on the Medical History CRF.

All pre-existing conditions/diseases will be reported on the Medical History CRF page at the screening visit (Visit 1).

The subject's Type 1 diabetes diagnosis date and family history of Type 1 diabetes will also be documented.

6.5 Physical Examination Including Neurological Examination

At the screening visit (Visit 1) the patient will undergo a general physical examination and a neurological examination and any findings will be reported as pre-existing conditions on the Medical History CRF 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 CRF and any medication given on the concomitant medication pages.

The patients will, in addition to the limited physical examination by the physician, undergo a standardized clinical neurological examination at screening, 6, 15 and 30 months. The neurological tests are performed in order to detect possible mild signs of neuromuscular disease such as disturbance of strength, balance, and coordination.

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.

6.6 Adverse events

See section 9.2.2 below.

6.7 Concomitant Medication

Any concomitant medication used during the study, whether considered relevant for the study or not by the investigator must be reported on the concomitant medication log of the CRF. Please also see section 8.5, below.

7. Scheduling of procedures

Table 2 Pilot DIAGNODE-1 Study, Schedule of Study Events, T1D patients

Study Period	**Scree-ning**	**Intervention**					**Follow-up**	
Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Time (Day/Week/Month)	**D** -10 to -21	**D1** Base-line	**M1** (D30 ± 3)	**M2** (D60 ± 3)	**M3** (D90 ± 3)	**M 6** (D180 ± 14)	**M 15** (D450 ± 14)	**M 30** (D900 ± 30)
Informed Consent	X							
GAD-alum (Diamyd) treatment			X	X	X			
Vitamin D administration start and stop		Start Day 1			Stop Day 120			
Medical History	X							
Physical Examination	X	X	X	X	X	X	X	X
Neurological Assessment	X					X	X	X
Concomitant Medication	X	X	X	X	X	X	X	X
Height [a]	X	X	X	X	X	X	X	X
Weight, BMI	X	X	X	X	X	X	X	X
Pubertal Stage, if applicable [b]	X				X	X	X	X
Urine pregnancy test (females)	X	X	X	X	X	X	X	X
Injection site inspection [c]			X	X	X			
Insulin dose [d]	X	X	X	X	X	X	X	X
Self Reported Hypoglycemia [e]		X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X
Blood sample safety:								
* *Hematology*	X	X	X	X	X	X	X	X
* *Chemistry*	X	X	X	X	X	X	X	X
Urinalysis:								
* *Microalbuminuria*	X	X	X	X	X	X	X	X
* *Creatinine*	X	X	X	X	X	X	X	X
Blood sampling for Auto-antibodies:								
* *GADA*	X	X	X	X	X	X	X	X
* *other diabetes-related autoantibodies*	X	X	X	X	X	X	X	X
Blood sampling for Diabetes status:								
* *HbA1c*	X	X	X	X	X	X	X	X
* *Fasting glucose/C-peptide*	X	X	X	X	X	X	X	X
* *MMTT glucose/ C-peptide*		X				X	X	X
Blood sampling for genetics	X	X	X	X	X	X	X	X
* *HLA*		X						
Blood sampling for immunology								

** TNF-alfa, IL-1 beta, IL-2, IL-17, IL-5,10, 13 IFN-gamma and T-regulatory cells*	X	X	X	X	X	X	X	X
Vitamin D in serum		X	X				X	
Blood sampling for biobank		X	X	X	X	X	X	X

[a] Hight will only be documented for adult patients at visit 1. For patients <18 years of age the height will be documented at all visits
[b] Pubertal Stage should be measured when applicable. Not applicable >1.5 years after the patient have reached final height
[c] Inspection of injection site before and 60 minutes after GAD-Alum injection by investigator or nurse
[d] Insulin dose should be collected 4 days prior to each visit
[e] Severe hypoglycemia defined as needing help from others and/or seizures and/or unconscious

7.1 Visits

The first visit, the screening visit (Visit 1) should be performed 10 to 21 days before planned Visit 2 (Baseline), when Vitamin D treatment starts. One month (30 days) later, at Visit 3, the first injection of GAD-Alum will be administered. For Visit 4 (second GAD-Alum administration), the visit date must be set in accordance with Visit 3 (i.e. the first GAD-Alum administration) so that the first and second GAD-Alum doses will be 30 days apart (±3 days). For Visit 5 (third GAD-Alum administration) the visit date must be set in accordance with Visit 4 (i.e. the second GAD-Alum administration) so that the second and third GAD-Alum doses will be 30 days apart (± 3 days). For Visit 6 and 7, the visit date must be calculated from baseline (Visit 2) ±14 days and ±30 days from baseline for Visit 8.

Please see table 1 and 2 above, for schedule of patient visits, visit windows and study drug administration.

8. Study medication

8.1 Study medication

The following medication supplies will be used in the study:

A.	
Study medication:	GAD-Alum (Diamyd) subcutaneous injection Dosage and interval: One injection of 4 µg Diamyd will be administered once per month during three months (4 µg given with one month interval three times)
IMP supplier:	Diamyd Medical AB, Stockholm, Sweden.
B.	
Study medication	Vitamin D (Calciferol) in oral solution, 14 000 IE/week until day 120 Dosage and interval: 2000 IE daily for 120 days
IMP supplier	Commercially available

8.2 Supply

GAD-Alum (Diamyd) will be supplied by Diamyd Medical. It will be supplied as pre-packed medication from Diamyd Medical to a local pharmacy. All dosing will take place in the hospital, and handled only by trained and authorised study personnel.

GAD-Alum will 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 (Calciferol) will be handled and distributed by a local pharmacy.

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

8.3 Dosage and administration

GAD-Alum: 4 µg given into lymph node in the inguinal area (by help of ultra sound technique) three times with one month interval

Vitamin D: 14 000 IE per week, given per os until Day 120

8.4 Duration of treatment

See 8.3

8.5 Concomitant medication

No systemic immune modulating medication, and no other diabetes medication other than insulin, and no Vitamin D, whether marketed or not, are allowed.

8.6 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 authorised by the investigator should dispense the study medication, and the accountability is the responsibility of investigator.

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.

9. Response variables and outcomes

9.1 Assessments

9.1.1. Variables

The safety assessments includes occurrence of adverse events (AEs), laboratory measurements, physical examinations including neurological assessments (See section 6.3-6.7).

Adverse events will be recorded by the physician at every visit throughout the study.

Blood tests for safety:

- Chemistry: Creatinine, Calcium, Liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase, bilirubin)
- Haematology (MHC, MCV, MCHC, Hemoglobin, Platelets, Leukocytes)

Urinalysis for safety:

- Urine pregnancy test as appropriate
- Microalbuminuria
- Creatinine

Other Variables which will be evaluated:

- Inflammatory markers, especially TNF-alfa, IL-1 beta, IL-2, IL-17
- Th2-deviation of cell-mediated immune response seen e.g. as increased ratio of IL-5,10, 13 in comparison with IFN-gamma, TNF-alfa, IL-1 beta and IL-17, and increase of T-regulatory cells
- C-peptide (90 minute value and $AUC_{mean\ 0\text{-}120\ min}$) during an MMTT
- Fasting C-peptide
- Hemoglobin A1c (HbA1c)
- Exogenous insulin dose per kg body weight and 24 hours

9.2 Adverse events and Serious Adverse Events

9.2.1 Definitions of Adverse Events

An Adverse Event (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.

9.2.2.1 Seriousness

A Serious Adverse Events (SAE) is defined as: an Adverse Event that is fatal, life-threatening, significant or persistent disabling or requires hospitalisation or prolongation of hospitalisation, 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 jeopardise 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.

9.2.2.2 Intensity

Mild*:*	The adverse event is transient and easily tolerated.
Moderate*:*	The adverse event causes the patient discomfort and interrupts the patient's usual activities.
Severe*:*	The adverse event 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.

9.2.2.3 Relationship to study medication

Relationship to study medication will be assessed for the two treatments (GAD-Alum and Vitamin D) separately.

Unrelated*:*
This category is applicable to those adverse events which, after careful medical consideration at the time of evaluation, 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.

Remote*:*
In general, this category is applicable to those adverse events which, after careful medical consideration at the time they are evaluated, are judged to be remotely related to the test study medication. An adverse event may be considered remote if, or when, for example: (i) it does not follow a reasonable temporal sequence from administration; (ii) it could readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it does not follow a known response pattern to the suspected study medication; (iv) it does not reappear or worsen when the study medication is re-administered.

Plausible:
This category is applicable to those adverse events which, after careful medical consideration at the time they are evaluated, the connection with the test study medication administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered plausible if, or when: (i) it follows a reasonable temporal sequence from administration; (ii) it could not readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it follows a known response pattern to the

suspected study medication; (iv) it does not reappear or worsen when the study medication is re-administered.

Probable*:*
This category is applicable to those adverse events, which, after careful medical consideration at the time they are evaluated, the connection with the test study medication administration appears to, with a high degree of certainty, be related to the test study medication. An adverse event may be considered probable if: (i) it follows a reasonable temporal sequence from administration; (ii) it could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the study medication, yet study medication-relatedness exists; e.g. bone marrow depression, fixed study medication eruptions, and tardive dyskinesias; (iv) it follows a known response pattern to the suspected study medication; (v) it reappears upon re-challenge.

9.2.2.4 Reporting of Adverse Events

All Adverse Events must be recorded in the CRF, defining relationship to study medication, severity and seriousness. Adverse Events should also be recorded by the investigator in the patient file/notes.

9.2.2.4.1 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.

An assigned CRO, contracted by Diamyd Medical AB, will be responsible for reporting all Serious Adverse Events (SAEs) in accordance with ICH Good Clinical Practice and local regulations. The sponsor, Diamyd Medical AB and the assigned CRO will complete and sign a “Pharmacovigilance Working Instructions” agreement covering the safety reporting responsibilities in the study. This agreement will also ensure the sponsor and Diamyd Medical AB 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 Serious Adverse Event (SAE), he/she should contact the assigned CRO 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 copy of the SAE reporting form to the CRO at the numbers/e-mail addresses provided in the Investigator Site File and on the SAE report form The faxed/e-mailed SAE report form should be received by The CRO within 24 hours of the initial notification of the event.

It is the investigator’s responsibility to report to the CRO follow-up information on an existing SAE that is fatal or life-threatening within 5 days after the initial report. Where appropriate, hospitalisation or autopsy reports should be made available. All Serious Adverse Events will be followed up until resolution (i.e., asymptomatic,

stabilisation or death).

It is the CRO's responsibility to receive e-mail or fax copies of the SAE report form and other relevant CRF pages from the Investigators. The Drug Safety unit at the CRO 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. The CRO will contact the Investigator directly if there is any inconsistencies and missing information.

The CRO is responsible for the timely submission of Suspected Unexpected Serious Adverse Reactions (SUSARs) to the Competent Authority and Ethics Committee according to appropriate Competent Authority and Ethical Committee requirements. It is the CRO's responsibility to report SUSARs to investigators according to ICH Good Clinical Practice and to local regulations ant to notify the Competent Authorities of all SUSARs through the Eudravigilance database.

Fatal and life-threatening SUSARs should be reported by the CRO as soon as possible to the Competent Authorities and Ethical Committee, and in any case no later than seven (7) calendar days, after first knowledge by the Sponsor/CRO 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 the Sponsor/CRO.

9.2.2.4.2 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.

9.2.2.5 Pregnancy Report Form:

Pregnant and lactating women will not be included in the study. Females must have a negative urine pregnancy test prior to randomization and a negative urine pregnancy test at each study visit with study drug 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 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 the assigned CRO. 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.

10. Statistical methodology and data management

10.1 Study design

The DIAGNODE-1 study is an open-label pilot Phase I intervention study

Study Participants:

Newly diagnosed classic type 1 diabetes patients: N=15. Age 12.00-29.99 years. Recruited from one hospital in Sweden.

10.2 Estimation of sample size

Power analysis:

No formal power analyses are done for this pilot study.

10.3 Statistical analysis plan

In brief the following analyses are planned:

All continuous variables will have the following descriptive statistics displayed: number of observations (n), mean value, standard deviation, minimum, median, and maximum. All variables of a categorical nature will be displayed with frequencies and percentages. The tabulation of the descriptive statistics will be split by visit. Where appropriate, baseline (screening) descriptive statistics will also be included.

Demographic and other baseline characteristics

Demographics and baseline characteristics will be presented using descriptive statistics (summary tables).

Variables

The AE/SAE data will be presented using a standardized tabulation of the frequency and incidence rate of all observed AEs/SAEs. The frequencies and incidence rates are calculated on a per patient basis. Adverse events will be summarized by body system, causality, and severity. Other safety data will be presented by descriptive statistics.

Data regarding C-peptide and immune system as well as Adverse events and other data will be summarized descriptively.

After 6 months analysis of the data. (The results will be used for design of a Phase II DIAGNODE trial).

10.4 Study populations

Intention-to-treat population
Patients will be included in the primary intention-to-treat population for analysis of efficacy if they receive at least 1 dose of all study drugs in that arm, and are assessed at a later visit.

Per protocol population
In order to qualify for the stringent per protocol population, the subjects must have followed the study protocol without any major violations. Any examinations missed will be substituted with the last observation carried forward, but examinations from not more than 1 visit may be lost.

Total population
Any patient who withdraws from the study will be included in the safety analysis (adverse events and safety parameters). Data for all patients will be listed, and a list of withdrawn patients, with all reasons for withdrawal, will be given.

10.5 Data collection / case report forms

Case report forms (CRFs) will be supplied for recording data from each patient. Since it is important to have proper data collection in a timely manner, the investigator or assigned designee shall complete the CRFs promptly. When the monitor requests additional data or clarification of data for the CRF, the request must be answered satisfactorily in due time.

It is the responsibility of the investigator to ensure that these case report forms are properly completed. The investigator will sign the designated signature pages to confirm that the case report form is accurate and complete.

To ensure legibility the CRFs should be completed in block capitals with a black or blue ballpoint pen (not pencil, felt-tip or fountain pen).

Any corrections to the CRFs must be carried out by the investigator or his designate. A single stroke must be drawn through the original entry. The correction has to be dated and initialled. Incorrect entries must not be covered with correcting fluid, or obliterated, or made illegible in any way.

Even if there are no changes from a previous examination, in the interests of completeness of data acquisition the questions, which are repeated in each section of the CRFs should be answered in full. A reasonable explanation must be given by the investigator for all missing data.

10.6 Data management

Data will be entered into a computer database. The handling of data, including data quality control, will comply with regulatory guidelines (e.g., International Conference on Harmonization [ICH] and Good Clinical Practice [GCP]).

11. Regulatory and administrative procedures

11.1 Ethics committee 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 Informed Consent Form 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.

11.2 Patient Information / Informed Consent

The investigator is responsible for giving the patients and guardian(s) (where applicable) full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the study. Patients and/or guardian(s) must also be notified that they are free to withdraw from the study at any time. The patients and/or guardian(s) should have reasonable time to read and understand the information before signing. The investigator is responsible for obtaining signed informed consent from all patients and/or guardian(s) 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 and/or guardian(s) 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 Informed Consent Form will be given to the patients and/or guardian(s).

11.3 Patient confidentiality

The investigator must ensure that patient's confidentiality will be maintained. CRFs 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 Case Report Forms. 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.

11.4 Patient treatment plan

All patients will continue to receive standard care for Type 1 diabetes during the study.

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

11.5 GCP

The study will be managed and conducted according to the latest international (ICH) guidelines for Good Clinical Practice

11.6 Record retention

The CRFs and all medical records upon which the CRFs are based (source data) must be kept for at least 10 years after completion of the study.

11.7 Monitoring / Quality Control

Prior to the start of the study, the monitor will review the protocol and CRFs 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 CRFs with medical records and other relevant documentation during the on-site monitoring visits. The monitor must therefore be allowed 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 CRF data. Incorrect or missing entries into the CRFs will be queried and must be corrected. Study monitoring will not jeopardise patient confidentiality.

11.8 Quality Assurance

During or after the study is completed, regulatory authorities, Diamyd Medical, assigned CRO 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.

11.9 Insurance

Patients insurance is covered by the ordinary Patientskadeförsäkringen.

12. 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.

12.1 Study report

A clinical study report will be prepared covering clinical and statistical aspects and summarising all findings of the clinical study. The content has to be treated as strictly confidential.

12.2 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 Ethics Committee of any plans to terminate the study.

12.3 Publication and Data Rights

It is envisaged that the findings of the study will, in due course and by mutual agreement, be published in a scientific journal and/or presented at a scientific meeting. The authorship of this publication/ will remain as specified below.

The published international guidelines for authorship (International Committee of Medical Journal Editors, 1997) will be adhered to; i.e. 'All persons designed as authors should qualify for authorship. Each author should have participated sufficiently in the work to take public responsibility for the content.' Authorship credit will therefore be based only on substantial contributions to 1) conception and design, or analysis and interpretation of data; and to 2) drafting the article or revising it critically for important intellectual content; and on 3) final approval of the version to be published. Conditions 1), 2) and 3) must all be met. Participation solely in acquisition of funding or the collation of data does not justify authorship. General supervision of the research group is not sufficient for authorship. It is intended that information on what each author has contributed will be published. It is emphasised however, that only those who entirely meet the above mentioned criteria will be listed as authors. The principal investigator, who will be first or last author of publications based on this trial, has the final decision of the list of authors.

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14. Investigator/Sponsor signatures

Investigator's Statement:

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

"Open Label Pilot Trial in patients with recent-onset T1D to evaluate the safety, diabetes status and immune response of GAD-antigen (Diamyd®) therapy administered into lymph nodes in combination with an oral and vitamin D regimen"

Trial number DIAGNODE-1 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).

Principal Investigator's Printed Name:

……..……

Principal Investigator's Signature:

……..……

Date: ……..

Sponsor and Principal Investigator's signature:

Name and function	Signature	Date
[redacted] Coordinating & Principal Investigator and Sponsor		

APPENDIX TO CLINICAL TRIAL PROTOCOL (AMENDMENT NO 5):

"Prolonged Extension Study Period for study DIAGNODE-1"

Open Label Pilot Trial in patients with recent-onset T1D to evaluate the safety, diabetes status and immune response of GAD-antigen (Diamyd®) therapy administered into lymph nodes in combination with an oral vitamin D regimen

Acronym: DIAGNODE-1. (Trial to evaluate the safety and diabetes status in patients with recent-onset Type 1 **dia**betes by giving **G**AD-antigen (Diamyd®) therapy into lymph **NODE**s in combination with an oral Vitamin D regimen)

Study No. DIAGNODE-1
EudraCT Number: 2014-001417-79
Study Development Phase: I/II

Version 5.1: 2018-06-11

Sponsor:
[redacted]
Div of Pediatrics, Linköping university,
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Coordinating & Principal Investigator:
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Contract Research Organization: Linköping Academic Research Centre (LARC)

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1 Introduction

This is an amendment to Study Protocol DIAGNODE-1 and describes the prolongation of the Extension Study Period from approx. 31 months to 43 months. During the Prolonged Extension Study Period three adult patients will be offered treatment with a fourth injection with 4 µg GAD-Alum (Diamyd) into an inguinal lymph gland. The Prolonged Extension Study Period includes five visits. Although the treatment with three GAD-Alum (Diamyd) injections have shown encouraging results with preservation of the residual beta cell function, decreased insulin requirement parallel to a low HbA1c, we see tendencies to a gradual decline of the desired effect[1, 2]. One can fear that the immunological effect fades away. To prolong an immune response it is usual to give a booster dose in vaccinations, and it is reasonable to try this approach also when auto-antigen is used to modify the autoimmune process destroying the insulin producing pancreatic beta cells.

This Prolonged Extension Study Period will include three adult patients in the study DIAGNODE-1 and evaluate safety after a fourth injection with 4 µg GAD-Alum into an inguinal lymph gland. The Prolonged Extension Study Period is 12 months and will start 14 days (±5 days) after Visit 8 in the Main study (approx. 31 months after baseline). At the end of the Prolonged Extension Study Period the patients will have been followed for approx. 43 months.

2 Objectives

The objective of this Prolonged Extension Study Period is to evaluate safety after a fourth injection with 4 µg GAD-Alum direct into an inguinal lymph gland and to evaluate how the above mentioned treatment influences the immune system and endogenous insulin secretion.

3. Population

Adult patients who participated in DIAGNODE-1 (Visit 1-8) are given information about the Prolonged Extension Study Period and will be asked to participate in the Prolonged Extension Study Period.

3.1 Inclusion criteria

1. Informed consent given by patients according to national regulations
2. Participated in DIAGNODE-1 (Visit 1-8)
3. Females must agree to avoid pregnancy and have a negative urine pregnancy test
4. Patients of childbearing potential must agree to using adequate contraception, until 1 year after the fourth administration of GAD-Alum (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

3.2 Exclusion criteria

1. Previous or current treatment with immunosuppressant therapy (although topical or inhaled steroids are accepted)
2. Continuous treatment with any 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 anaemia or significantly abnormal haematology 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 fourth injection of study drug dose or planned treatment with any vaccine up to 4 months after the fourth 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. Known human immunodeficiency virus (HIV) or hepatitis
13. Females who are lactating or pregnant (the possibility of pregnancy must be excluded by urine βHCG on-site within 24 hours prior to the GAD-Alum treatment)
14. Males or females not willing to use adequate contraception until 1 year after the last GAD-Alum treatment
15. Presence of associated serious disease or condition, including active skin infections that preclude subcutaneous 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

4. Treatment procedures

4.1 Study visits and schedule of events

The Prolonged Extension Study Period comprises of 5 study visits:

- Visit 9: Day 1 (Screening for Prolonged Extension Study Period and Informed Consent, corresponds to approximately Month 31 from Baseline Visit (Visit 2))
- Visit 10: Day 30, Month 1 (Fourth injection with GAD-Alum, corresponds to approximately Month 32 from Baseline Visit (Visit 2))
- Visit 11: Day 90, Month 3 (Safety visit, corresponds to approximately Month 34 from Baseline Visit (Visit 2))
- Visit 12: Day 180, Month 6 (Follow-up and MMTT, corresponds to approximately Month 37 from Baseline Visit (Visit 2))
- Visit 13: Day 360, Month 12 (Follow-up and MMTT, corresponds to approximately Month 43 from Baseline Visit (Visit 2))

See table 3 below for an overview of the study visits.

Table 3 Schedule of Patient Visits, Visit Windows and Study Drug Administration

Study	**DIAGNODE-1 Prolonged Extension Study Period**				
Visit number	**Visit 9***	**Visit 10**	**Visit 11**	**Visit 12**	**Visit 13**
Time (Month/Day)	**Day 1**	**M 1** (D30 ± 3)	**M 3** (D90 ± 14)	**M 6** (D180 ± 14)	**M 12** (D360± 14)
3 adult patients		4th GAD-Alum treatment			

*Visit 9 should be scheduled 14 days (+/- 5 days) after Visit 8 in the main study period.

The procedures and the timelines for data collection are given in Table 4.

Table 4. Schedule of Procedures and Assessments

Study Period	**Prolonged Extension Study Period**				
Visit number	**9**[a]	**10**	**11**	**12**	**13**
Time (Day/**W**eek/**M**onth)	**Day 1**	**M 1** (D30 ± 3)	**M 3** (D90 ± 14)	**M 6** (D180 ± 14)	**M 12** (D360± 14)
Informed Consent	X				
GAD-Alum (Diamyd) treatment		X			
Vitamin D administration start and stop	Start at Visit 9	Stop 60 days after Visit 9			
Physical Examination	X	X	X	X	X
Neurological Assessment	X	X	X	X	X
Concomitant Medication	X	X	X	X	X
Weight, BMI	X	X	X	X	X
Urine pregnancy test (females)	X	X	X	X	X
Injection site inspection [b]		X			
Insulin dose [c]	X	X	X	X	X
Self-Reported Hypoglycemia [d]	X	X	X	X	X
Adverse Events	X	X	X	X	X
Blood sample safety:					
* *Hematology*	X	X	X	X	X
* *Chemistry*	X	X	X	X	X
Urinalysis:					
* *Microalbuminuria*	X	X	X	X	X
* *Creatinine*	X	X	X	X	X
Blood sampling for Auto-antibodies:					
* *GADA*	X	X	X	X	X
* *other diabetes-related autoantibodies*	X	X	X	X	X
Blood sampling for Diabetes status:					
* *HbA1c*	X	X	X	X	X
* *Fasting glucose/C-peptide*	X	X	X	X	X
* *MMTT glucose/ C-peptide*				X	X
Blood sampling for immunology:					
* *TNF-alfa, IL-1 beta, IL-2, IL-17, IL-5,10, 13 IFN-gamma and T-regulatory cells*	X	X	X	X	X
Vitamin D in serum	X		X		
Blood sampling for biobank	X	X	X	X	X

a) Visit 9 should be scheduled 14 days (+/- 5 days) after Visit 8 in the main study period.
b) Inspection of injection site before and 120 minutes after GAD-Alum injection by investigator or nurse
c) Insulin dose should be collected 4 days prior to each visit
d) Severe hypoglycemia defined as needing help from others and/or seizures and/or unconscious

4.2 Assessments and procedures

1. Information about the Prolonged Extension Study Period.
2. The patient gives their informed consent.
3. When the patients have given their informed consent, all inclusion and exclusion criteria will be checked for eligibility according to the listed inclusion and exclusion criteria in section 3.1 and 3.2
4. At Visit 12 and 13 assessment of residual endogenous insulin secretion by MMTT.
5. HbA1c, safety (haematology and chemistry), autoantibody titres (GAD65, IA-2), immunology, are followed by blood samples at every study visit.
6. Exogenous insulin dose/24 hours, AEs and concomitant medication is registered at every study visit.
7. Self-reported hypoglycaemia (defined as needing help from others and/or seizures and/or unconscious) registered at every study visit.
8. Any symptoms or signs of other medical problem should be treated at the discretion of the clinical doctor.

Examinations will be performed according to Table 4 above, and in the order outlined in the case report form (CRF).

4.2.1 All Visits, Visit 9, 10, 11, 12 and 13

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.

4.2.2 Administrations of GAD-Alum, Visits 10

After administration, the patient shall remain in the vicinity of the study site for the next two hours, and the administration site will be examined by investigator/study nurse 2 hours post injection.

4.2.3 Mixed Meal Tolerance Test (MMTT), Visits 12 and 13

The MMTT must be performed according to the instructions in the CRF.

The patient should:

- Come to the study site following an overnight fast (>10hr), 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 for safety reasons, subjects need to eat or take insulin, the visit should also be rescheduled.

4.3 Laboratory tests and examinations

1. Immunological tests:
    a. Autoantibodies (Anti-GAD65, Anti-Insulin, Anti-IA-2, ZnT8)
    b. Relevant cytokines and chemokines are determined (see Table 4)
    c. T-cells are classified and studied (see Table 4)
2. Genetics:
    a. HLA determinations is done and genes related to diabetes development
    b. Array studies to elucidate the importance of diabetes-related genes
3. Diabetes status:
    a. HbA1c
    b. Fasting glucose and fasting C-peptide
    c. Meal stimulated glucose and C-peptide
4. Blood sampling for safety:
    a. Hematology
    b. Chemistry
5. Urine analysis
6. Urine pregnancy test as appropriate
7. Other:
    a. Vitamin D in serum

4.4 Physical Examination Including Neurological Examination

At Visit 9, 10, 11, 12 and 13 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 CRF and any medication given on the concomitant medication pages.

The patients will, in addition to the limited physical examination by the physician, undergo a standardized clinical neurological examination at Visit 9, 10, 11, 12 and 13. The neurological tests are performed in order to detect possible mild signs of neuromuscular disease such as disturbance of strength, balance, and coordination.

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.

4.5 Adverse events

See section 6.2 below.

4.6 Concomitant Medication

Any concomitant medication used during the study, whether considered relevant for the study or not by the investigator must be reported on the concomitant medication log of the CRF. Please also see section 5.5, below.

5. Study medication

5.1 Study medication

The following medication supplies will be used in the Prolonged Extension Study Period:

A.	
Study medication:	GAD-Alum (Diamyd) subcutaneous injection Dosage and interval: the fourth injection of 4 µg GAD-Alum (Diamyd) will be administered at Visit 10 (approx. 32 months from baseline, i.e. 1 month after Visit 9)
IMP supplier:	Diamyd Medical AB, Stockholm, Sweden.
B.	
Study medication	Vitamin D (Calciferol) in oral solution, 14 000 IE/week for 60 days in this Prolonged Extension Study Period. Dosage and interval: 2000 IE daily (25 drops per day) for 60 days starting at Visit 9 (approx. 31 months from baseline, i.e. 14 days (± 5 days) after Visit 8)
IMP supplier	Commercially available

5.2 Supply

GAD-Alum (Diamyd) will be supplied by Diamyd Medical. It will be supplied as pre-packed medication from Diamyd Medical to a local pharmacy. All dosing will take place in the hospital, and handled only by trained and authorised study personnel.

GAD-Alum will 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 (Calciferol) will be handled and distributed by a local pharmacy.

Vitamin D (Calciferol) 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.

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

5.3 Dosage and administration

GAD-Alum:	4 µg given into lymph node in the inguinal area (by help of ultra sound technique) fourth injection will be administrated at Visit 10 (approx. 32 months from baseline, i.e. 1 month after Visit 9)
Vitamin D:	14 000 IE per week, given per os for 60 days (start at Visit 9)

5.4 Duration of treatment

See section 5.3 above.

5.5 Concomitant medication

No systemic immune modulating medication, and no other diabetes medication other than insulin, whether marketed or not, are allowed.

5.6 Study medication accountability

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

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.

6. Response variables and outcomes

6.1 Assessments

6.1.1. Variables

The safety assessments include occurrence of adverse events (AEs), laboratory measurements, physical examinations including neurological assessments (See section 4.2-4.6).

Adverse events will be recorded by the physician at every visit throughout the Prolonged Extension Study Period.

Blood tests for safety:
- Chemistry: Creatinine, Calcium, Liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase, bilirubin)
- Haematology (MHC, MCV, MCHC, Hemoglobin, Platelets, Leukocytes)

Urinalysis for safety:
- Urine pregnancy test as appropriate
- Microalbuminuria
- Creatinine

Other Variables which will be evaluated:

- Inflammatory markers, especially TNF-alfa, IL-1 beta, IL-2, IL-17
- Th2-deviation of cell-mediated immune response seen e.g. as increased ratio of IL-5,10, 13 in comparison with IFN-gamma, TNF-alfa, IL-1 beta and IL-17, and increase of T-regulatory cells
- C-peptide (90-minute value and $AUC_{mean\ 0\text{-}120\ min}$) during an MMTT
- Fasting C-peptide
- Hemoglobin A1c (HbA1c)
- Exogenous insulin dose per kg body weight and 24 hours

6.2 Adverse events and Serious Adverse Events

6.2.1 Definitions of Adverse Events

An Adverse Event (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.

6.2.1.1 Seriousness

A Serious Adverse Events (SAE) is defined as: an Adverse Event that is fatal, life-threatening, significant or persistent disabling or requires hospitalisation or prolongation of hospitalisation, 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 jeopardise 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.

6.2.1.2 Intensity

Mild*:*	The adverse event is transient and easily tolerated.
Moderate*:*	The adverse event causes the patient discomfort and interrupts the patient's usual activities.
Severe*:*	The adverse event 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.

6.2.1.3 Relationship to study medication

Relationship to study medication will be assessed for the two treatments (GAD-Alum and Vitamin D) separately.

Unrelated*:*
This category is applicable to those adverse events which, after careful medical consideration at the time of evaluation, 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.

Remote*:*
In general, this category is applicable to those adverse events which, after careful medical consideration at the time they are evaluated, are judged to be remotely related to the test study medication. An adverse event may be considered remote if, or when, for example: (i) it does not follow a reasonable temporal sequence from administration; (ii) it could readily have been

produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it does not follow a known response pattern to the suspected study medication; (iv) it does not reappear or worsen when the study medication is re-administered.

Plausible:
This category is applicable to those adverse events which, after careful medical consideration at the time they are evaluated, the connection with the test study medication administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered plausible if, or when: (i) it follows a reasonable temporal sequence from administration; (ii) it could not readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it follows a known response pattern to the suspected study medication; (iv) it does not reappear or worsen when the study medication is re-administered.

Probable:
This category is applicable to those adverse events, which, after careful medical consideration at the time they are evaluated, the connection with the test study medication administration appears to, with a high degree of certainty, be related to the test study medication. An adverse event may be considered probable if: (i) it follows a reasonable temporal sequence from administration; (ii) it could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the study medication, yet study medication-relatedness exists; e.g. bone marrow depression, fixed study medication eruptions, and tardive dyskinesias; (iv) it follows a known response pattern to the suspected study medication; (v) it reappears upon re-challenge.

6.2.1.4 Reporting of Adverse Events

All Adverse Events must be recorded in the CRF, defining relationship to study medication, severity and seriousness. Adverse Events should also be recorded by the investigator in the patient file/notes.

6.2.1.4.1 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.

An assigned CRO, contracted by Diamyd Medical AB, will be responsible for reporting all Serious Adverse Events (SAEs) in accordance with ICH Good Clinical Practice and local regulations. The sponsor, Diamyd Medical AB and the assigned CRO will complete and sign a “Pharmacovigilance Working Instructions” agreement covering the safety reporting responsibilities in the study. This agreement will also ensure the sponsor and Diamyd Medical AB 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 Serious Adverse Event (SAE), he/she should contact the assigned CRO 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 copy of the SAE reporting form to the CRO at the numbers/e-mail addresses provided in the Investigator Site File and on the SAE report form. The faxed/e-mailed SAE report form should be received by The CRO within 24 hours of the initial notification of the event.

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

It is the CRO's responsibility to receive e-mail or fax copies of the SAE report form and other relevant CRF pages from the Investigators. The Drug Safety unit at the CRO 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. The CRO will contact the Investigator directly if there is any inconsistencies and missing information.

The CRO is responsible for the timely submission of Suspected Unexpected Serious Adverse Reactions (SUSARs) to the Competent Authority and Ethics Committee according to appropriate Competent Authority and Ethical Committee requirements. It is the CRO's responsibility to report SUSARs to investigators according to ICH Good Clinical Practice and to local regulations ant to notify the Competent Authorities of all SUSARs through the Eudravigilance database.

Fatal and life-threatening SUSARs should be reported by the CRO as soon as possible to the Competent Authorities and Ethical Committee, and in any case no later than seven (7) calendar days, after first knowledge by the Sponsor/CRO 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 the Sponsor/CRO.

6.2.1.4.2 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.

6.2.1.5 Pregnancy Report Form

Pregnant and lactating women will not be included in the study. Females must have a negative urine pregnancy test at each study visit with study drug administration, prior to injection of study drug. Patients will be required to use an adequate form of birth control during the study. At Visit 9 the need for birth control will be re-assessed. Patients will be strongly advised to avoid pregnancy for 1 year following the last dose 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 the assigned CRO. 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.

7. Statistical methodology and data management

7.1 Statistical analysis plan

In brief the following analyses are planned:

All continuous variables will have the following descriptive statistics displayed: number of observations (n), mean value, standard deviation, minimum, median, and maximum. All variables of a categorical nature will be displayed with frequencies and percentages. The tabulation of the descriptive statistics will be split by visit. Where appropriate, baseline (screening) descriptive statistics will also be included.

Variables

The AE/SAE data will be presented using a standardized tabulation of the frequency and incidence rate of all observed AEs/SAEs. The frequencies and incidence rates are calculated on a per patient basis. Adverse events will be summarized by body system, causality, and severity. Other safety data will be presented by descriptive statistics.

Data regarding C-peptide and immune system as well as Adverse events and other data will be summarized descriptively.

7.2 Study populations

Intention-to-treat population
Patients will be included in the primary intention-to-treat population for analysis of efficacy if they receive at least 1 dose of all study drugs in that arm, and are assessed at a later visit.

Per protocol population
In order to qualify for the stringent per protocol population, the subjects must have followed the study protocol without any major violations. Any examinations missed will be substituted with the last observation carried forward, but examinations from not more than 1 visit may be lost.

Total population
Any patient who withdraws from the study will be included in the safety analysis (adverse events and safety parameters). Data for all patients will be listed, and a list of withdrawn patients, with all reasons for withdrawal, will be given.

7.3 Data collection / case report forms

Case report forms (CRFs) will be supplied for recording data from each patient. Since it is important to have proper data collection in a timely manner, the investigator or assigned designee shall complete the CRFs promptly. When the monitor requests additional data or clarification of data for the CRF, the request must be answered satisfactorily in due time.

It is the responsibility of the investigator to ensure that these case report forms are properly completed. The investigator will sign the designated signature pages to confirm that the case report form is accurate and complete.

To ensure legibility the CRFs should be completed in block capitals with a black or blue ballpoint pen (not pencil, felt-tip or fountain pen).

Any corrections to the CRFs must be carried out by the investigator or his designate. A single stroke must be drawn through the original entry. The correction has to be dated and initialled. Incorrect entries must not be covered with correcting fluid, or obliterated, or made illegible in any way.

Even if there are no changes from a previous examination, in the interests of completeness of data acquisition the questions, which are repeated in each section of the CRFs should be answered in full. A reasonable explanation must be given by the investigator for all missing data.

7.4 Data management

Data will be entered into a computer database. The handling of data, including data quality control, will comply with regulatory guidelines (e.g., International Conference on Harmonization [ICH] and Good Clinical Practice [GCP]).

8. Regulatory and administrative procedures

8.1 Ethics committee 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 Informed Consent Form 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.

8.2 Patient Information / Informed Consent

The investigator is responsible for giving the patients full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the Prolonged Extension Study Period. Patients must also be notified that they are free to withdraw from the study at any time. The patients 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 Informed Consent Form will be given to the patients.

8.3 Patient confidentiality

The investigator must ensure that patient's confidentiality will be maintained. CRFs 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 Case Report Forms. 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.

8.4 Patient treatment plan

All patients will continue to receive standard care for Type 1 diabetes during the study.

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

8.5 GCP

The study will be managed and conducted according to the latest international (ICH) guidelines for Good Clinical Practice

8.6 Record retention

The CRFs and all medical records upon which the CRFs are based (source data) must be kept for at least 10 years after completion of the study.

8.7 Monitoring / Quality Control

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 CRFs with medical records and other relevant documentation during the on-site monitoring visits. The monitor must therefore be allowed 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 CRF data. Incorrect or missing entries into the

CRFs will be queried and must be corrected. Study monitoring will not jeopardise patient confidentiality.

8.8 Quality Assurance

During or after the study is completed, regulatory authorities, Diamyd Medical, assigned CRO 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.

8.9 Insurance

Patients insurance is covered by the ordinary Patientskadeförsäkringen.

9. End of Trial

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

9.1 Study report

A clinical study report will be prepared covering clinical and statistical aspects and summarising all findings of the Prolonged Extension Study Period. The content must be treated as strictly confidential.

9.2 Study Stopping Criteria

The Sponsor and the investigators reserve the right to discontinue the Prolonged Extension Study Period 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 Prolonged Extension Study Period 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 Ethics Committee of any plans to terminate the study.

9.3 Publication and Data Rights

It is envisaged that the findings of the Prolonged Extension Study Period will, in due course and by mutual agreement, be published in a scientific journal and/or presented at a scientific meeting. The authorship of this publication/ will remain as specified below.

The published international guidelines for authorship (International Committee of Medical Journal Editors, 1997) will be adhered to; i.e. 'All persons designed as authors should qualify for authorship. Each author should have participated sufficiently in the work to take public responsibility for the content.' Authorship credit will therefore be based only on substantial contributions to 1) conception and design, or analysis and interpretation of data; and to 2) drafting the article or revising it critically for important intellectual content; and on 3) final

approval of the version to be published. Conditions 1), 2) and 3) must all be met. Participation solely in acquisition of funding or the collation of data does not justify authorship. General supervision of the research group is not sufficient for authorship. It is intended that information on what each author has contributed will be published. It is emphasised however, that only those who entirely meet the above mentioned criteria will be listed as authors. The principal investigator, who will be first or last author of publications based on this trial, has the final decision of the list of authors.

10. References

1. Ludvigsson J, Wahlberg J, et al, N Engl J Med 2017 Feb 16;376(7):697-699
2. Ludvigsson J, Tavira B, et al, N Engl J Med 2017 Jul 27;377(4):403-5

11. Investigator/Sponsor signatures

Investigator's Statement:

I have read and understand this amendment to the foregoing protocol with the title:

"Open Label Pilot Trial in patients with recent-onset T1D to evaluate the safety, diabetes status and immune response of GAD-antigen (Diamyd®) therapy administered into lymph nodes in combination with an oral and vitamin D regimen"

Trial number DIAGNODE-1 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).

Principal Investigator's Printed Name:

……..……

Principal Investigator's Signature:

……..……

Date: ……..

Sponsor and Principal Investigator's signature:

Name and function	Signature	Date
[redacted] Coordinating & Principal Investigator and Sponsor		