CLINICAL STUDY PROTOCOL

Open Label trial to evaluate the tolerability of a combination therapy consisting of GAD-alum (Diamyd®), etanercept and vitamin D in children and adolescents newly diagnosed with type 1 diabetes

Acronym: EDCR (Etanercept Diamyd Combination Regimen)

Study No. EDCR IIa
EudraCT Number: 2014-001323-76
Study Development Phase: II

Version 4.2, 2015-03-17

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Contract Research Organization: Linköping Academic Research Centre (LARC)
Planned start date: Q1, 2015

VERSION TABLE*

Version 4.2:	Final including Non-Substantial Information Amendment #0.2	Mar 17, 2015
Version 4.1:	Final including Non-Substantial Information Amendment #0.1	Mar 03, 2015
Version 4:	Final	Nov 11, 2014

**Version 1-3, prior to MPA approval, not applicable.*

STUDY ADMINISTRATION		
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CONFIDENTIALITY STATEMENT

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STUDY INFRASTRUCTURE

The study will be conducted at approximately 9 clinical sites in Sweden.

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- Bang Peter, Professor of Pediatrics, Linköping University Hospital
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- Ingela Johansson, MSC, Clin Exp Centre, Div of Pediatrics, Linköping university
- Gosia Smolinska, Biomedical Assistant, Clin Exp Centre, Div of Pediatrics, Linköping university

Facilities and Equipment

- *Laboratory:* Clinical Experimental Research, Linköping university hospital, has localities with all necessary modern equipment. Div of Pediatrics has laboratory facilities, with an experienced and skilful staff
- *Clinical infrastructure*: All participating clinics have experience from previous clinical studies, with experienced pediatric 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*: A Contract Research Organization (CRO) will be assigned

Data Safety Monitoring Board

- Anders Fasth, MD, PhD, Dept of Pediatrics, University of Gothenburg
- Ulf Smith, MD, PhD, Department of Internal Medicine, SU/Sahlgrenska
- Jesper Peterson, MD, Department of Infectious Diseases, Malmo University Hospital, MAS

- SYNOPSIS OF THE PROPOSED PILOT STUDY

<table>
<tr><td>Name of Sponsor
Johnny Ludvigsson</td><td rowspan="2">Individual Study Table Referring to Part of Dossier in Which the Individual Study or Study Table is Presented:

Volume:
Reference:</td><td rowspan="2">(For National Authority Use Only)</td></tr>
<tr><td>Name of Finished Product:
DIAMYD®
ETANERCEPT
VITAMIN D

Name of Active Ingredient:
Recombinant Human Glutamic Acid Decarboxylase (rhGAD65)
Calciferol (Vitamin D)
Etanercept</td></tr>
<tr><td colspan="3">Title of Study: Open Label trial to evaluate the tolerability of a combination therapy consisting of GAD-alum (Diamyd®), etanercept and vitamin D in children and adolescents newly diagnosed with type 1 diabetes</td></tr>
<tr><td colspan="3">Protocol Number:
EDCR IIa</td></tr>
<tr><td colspan="3">Investigators and Study Centre:
Approximately 9 sites in Sweden, including approximately 20 patients.</td></tr>
<tr><td colspan="3">Phase of Development: Phase IIa</td></tr>
<tr><td colspan="3">Objectives:
• Evaluate the tolerability of a combination therapy with Diamyd, vitamin D and etanercept
• Evaluate how the above mentioned treatments influence the immune system and endogenous insulin secretion</td></tr>
<tr><td colspan="3">Study Design:
The study is a multicenter, open-label, pilot clinical trial. All patients will from Day 1 receive 2 000 IU vitamin D per os per day during 15 months, and from Days 1-90 receive etanercept (Enbrel) injected subcutaneously 0.8 mg/kg body weight (max 50 mg) once a week, and receive 2 subcutaneous injections of 20 µg Diamyd in a prime-and-boost regimen on Days 30 and 60. The patients will be evaluated for tolerability for 6 months (main study period, 6 visits) and followed for additionally 24 months (extension study period, 3 visits). The total study period is 30 months.</td></tr>
<tr><td colspan="3">Selection of Subjects: Patients must be age 8.00 to 17.99 years old, and diagnosed with type 1 diabetes (T1D) within the previous 100 days 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.</td></tr>
<tr><td colspan="3">Number of Subjects Planned: Approximately 20 patients will be enrolled.</td></tr>
<tr><td colspan="3">Description of Treatment Groups:
There is one single treatment group. The patients will be assessed for eligibility at the screening visit (Visit 1) 2 to 4 weeks prior to the start of the treatment. On Visit 2 (Day 1), patients eligible for the study will start the treatment as mentioned above.</td></tr>
</table>

Name of Sponsor Johnny Ludvigsson	**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® ETANERCEPT VITAMIN D		
Name of Active Ingredient: Recombinant Human Glutamic Acid Decarboxylase (rhGAD65) Calciferol (Vitamin D) Etanercept	**Volume:** **Reference:**	

Endpoints

Primary endpoints:
To Evaluate the tolerability of a combination therapy with Diamyd, Vitamin D and etanercept at Month 6 (main study Period), 9, 15 and 30 (extension study period)

Variables to evaluate tolerability:

- Reactions at the injection site
- Infections
- Occurrence of Adverse Events (AEs)
- Occurrence of Serious Adverse Events
- Physiological and Neurology assessments
- Laboratory measurements (biochemistry and haematology), including Calcium and Vitamin D in serum
- GAD65AB titer (GADA)

Secondary endpoints:
To evaluate how the above mentioned treatments influence the immune system and endogenous insulin secretion at Month 6 (main study Period), 9, 15 and 30 (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 $AUC_{mean\ 0\text{-}120\ min}$) during an MMTT
- Proportion of patients with a stimulated maximum C-peptide level above 0.2 nmol/L
- 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 just to see if the treatment is tolerable and does not cause negative effects on beta cell function and/or immune system.

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
EC	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
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
SAP	Statistical Analysis Plan
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 in our country, and 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 soon becomes 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 a 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 child usually has near normal blood glucose values, not even mild hypoglycemia and no episodes of keto-acidosis. The quality of life is very good as the child feels well, grows normally, needs few restrictions if any with regard to food, 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 70-90% 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 T1D, 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 increase of certain cytokines such as IFNg and decrease of IL-10, IL-13.

Interventions that may preserve 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 doubtlessly 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-CD3 antibodies in an attempt to block the destructive immune process have been performed. Result 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 (Macrogenics 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 exogenous insulin requirement to reach good HbA1c (27). 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 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 (reported at IDF, Dubai dec 2011 and at ADA June 2012) suggests that treatment with Diapep277 may preserve beta cell function in adults with mild T1D. However, the results are a bit unclear, as there was only a weak C-peptide preservation 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 some effect (12).

1.1.4 Previous clinical studies with GAD-Alum (Diamyd)

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 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.

1.1.4.1 GAD-Alum (Diamyd) vaccination

GAD-alum (Diamyd) therapy aims at intervening in the autoimmune process in T1D in order to preserve beta cell function and endogenous insulin secretion by modulating the immune system in a discrete, antigen-specific fashion to stop the destruction of beta cells. Thus, the goal of Diamyd therapy would be to dramatically slow or halt the ongoing autoimmune destruction of pancreatic islet beta cells in order to preserve the largest possible amount of endogenous insulin production.

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 T1D 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 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 (16.4% treatment effect; p=0.10); Fasting C-peptide p=0.07) (46). This prompted the company (Diamyd Medical + Johnson&Johnson) to close the Phase III program 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 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! (47) 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).

1.1.5. Vitamin D and type 1 diabetes

Experimental evidence indicates that vitamin D may play a role in the defence against T1D as well as type 2 diabetes (T2D). Epidemiological data suggest that there is a link between vitamin D deficiency and an increased incidence of T1D. Thus a multinational case-control study and a birth cohort follow-up study from Finland with (48,49) have both concluded that vitamin D3 supplementation at birth protects from T1D later in life, and a meta-analysis supports similar conclusions (50). Others report lower serum levels of 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3, calcitriol] in patients with recently diagnosed T1D than in healthy control subjects (51) 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 (52-55) and facilitates a shift from a Th1 to a Th2 immune response (56). An increasing amount of evidence suggests that vitamin D also affects beta cells directly thereby rendering them more resistant to cellular stress (57), and there are results indicating that vitamin D may also improve insulin sensitivity (58), which in turn decrease beta cell stress.

With this background vitamin D has been used in patients with recent onset T1D in an effort to preserve residual insulin secretion. However, so far Vitamin D alone has not been efficacious enough (59,60). Therefore there is reason to try Vitamin D, both in somewhat higher dose, and in combination with other therapy.

1.1.6 Etanercept and Type 1 diabetes

Although T1D is regarded to be an autoimmune disease, there are several studies both in experimental animals and in humans suggesting that an existing inflammation when autoantigen are presented to T-cells play an important role for which response the immune system will take.

We know that Tumor Necrosis Factor – α (TNFα) plays an important role for the destructive process in T1D. TNFα is involved in the autoimmune process leading to beta cell destruction (61,62). In the non-obese diabetic mouse, TNFα mRNA is produced by CD4+ T-cells within inflamed islets during the development of diabetes (63). In vitro models show that TNFα potentiates the direct functional inactivation and destruction of beta cells by other cytokines such as interleukin-1β and interferon-γ (64-68). Transgenic mice with increased beta cell expression of TNFα have significant lymphocytic insulitis (69).

Etanercept is a recombinant soluble TNFα receptor fusion protein that binds to TNFα. It acts by clearing TNFα from the circulation, thereby blocking the biological activity of this inflammatory cytokine. Although etanercept is used in the treatment of many autoimmune diseases including ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, and rheumatoid arthritis, (70,71) it has only been tested in young people with T1D (72). This was a 24-week double-blind, randomized, placebo-controlled study conducted at the Diabetes Center, Women and Children's Hospital of Buffalo. Eighteen subjects (11 male and 7 female, aged 7.8-18.2 years) were randomly assigned to receive either placebo or etanercept. The effect was quite impressive: The beta cell function was not only preserved but the percent change in C-peptide area under the curve from baseline to week 24 showed a 39% increase (!) in the etanercept group compared to a 20% decrease in the placebo group ($P < 0.05$). From baseline to week 24 insulin dose decreased 18% in the etanercept group compared with a 23% increase in the placebo group ($P < 0.05$). At the same time, with lower insulin dose, HbA1c at week 24 was lower in the etanercept group (5.91 +/- 0.5%) compared with that in the placebo group (6.98 +/- 1.2%; $P < 0.05$) with a higher percent decrease from baseline than in the placebo group (etanercept 0.41 +/- 0.1 vs. placebo 0.18 +/- 0.21; $P < 0.01$). Seventeen patients completed the study, and none withdrew because of adverse events. In fact the adverse events were minimal in that study.

1.1.7 Rationale for etanercept and GAD-alum (Diamyd) in type 1 diabetes

There is consensus among diabetes researchers today that the key to curing T1D will be to simultaneously tackle the disease on several fronts by combining various therapeutics and that an autoantigen-specific treatment such as Diamyd will constitute an essential component in future combination therapies.

In this EDCR IIa trial, we propose that etanercept and GAD-alum will synergize in a novel manner to produce extended beta cell function in those newly diagnosed with T1D.

More specifically, although Phase III studies indicate GAD-alum alone does not preserve beta cell function enough, it does have a potent effect on the immune system. The data indicate that GAD-alum activates *both* TH2/immunoregulatory *and* TH1/proinflammatory components (73). Immunologically, TNFα plays an integral role in the initiation and progression of the innate and beta cell-specific autoimmunity (74). Blocking TNFα with etanercept in autoimmune patients has resulted in downregulation of Th1 and Th17 activation and an upregulation of Th2 and regulatory T-cell (Treg) subsets of immunocytes (75). If we can induce quiescence in the inflammatory immunologic milieu present in and around islets via combination treatment with etanercept, we could both *enhance* the TH2/immunoregulatory GAD-alum response *and* lessen the TH1/proinflammatory response.

Furthermore, metabolically, TNFα appears to inhibit insulin secretion, and acts in synergy with other cytokines such as IL1-β and IFNγ to enhance beta cell stress and destruction (74, 76, 77, 64). Experience with etanercept and other TNFα blockers in T1D animal models and clinical patients with T1D suggests that beta cell destruction can be arrested leading to better metabolic control (78, 79, 80, 81). Because it is a potent proinflammatory cytokine (74) blocking soluble TNFα (sTNFα) reduces the activation state of immunocytes (75), but additionally, because Etanercept preferentially binds sTNFα over membrane bound TNFα (mTNFα) expressed on Dendritic Cells (DC) (unlike other anti-TNFα agents), a DC-mediated antigen specific Treg generation is preserved (82, 83). By uniquely blocking B-cell borne Lymphotoxin-α, disrupting Follicular Dendritic cells and Germinal Center architecture in lymphoid tissue, anti-TNFα reduces B cell numbers and impairs activation (84), and autoantibody production should be reduced.

Preclinical data has shown that beta cell antigen delivered via quiescent DCs induce T-cell unresponsiveness and arrest beta cell destruction (85, 86).

We believe, therefore, that when etanercept therapy is added to GAD-alum treatment, GAD-specific autoantibody levels and effector T cell activity and number will be reduced while GAD-specific Treg number and function will be maintained. Due to its acute beta-cell-protective and metabolic effects, etanercept will also preserve beta cells directly. This will produce a deviation of the diabetes autoimmune response towards a regulatory phenotype of sufficient critical mass to lead to a significant and prolonged effect on beta cell preservation. This is not unlike the original rationale for the GAD-alum monotherapy approach evaluated in a number of clinical studies, but with critical improvements based on the knowledge gained from those trials.

1.1.8 Rationale for Vitamin D supplementation

As explained in the rational above, experimental evidence indicates that vitamin D may play a role in the defence against T1D and epidemiological data suggest that there is a link between vitamin D deficiency and an increased incidence of T1D. Mechanistic studies show that 1,25(OH)2D3 modulates dendritic cell maturation in vitro and in vivo (52-55) and facilitates a shift from a Th1 to a Th2 immune response (56). An increasing amount of evidence suggests that vitamin D also affects beta cells directly thereby rendering them more resistant to cellular stress (57), and there are results indicating that Vitamin D may also improve insulin sensitivity (58), which in turn decreases beta cell stress. Thus, there is reason to include Vitamin D in the combination with etanercept and Diamyd®.

1.2 Summary

- The encouraging results of the Phase II GAD-alum trial and the partly positive results of the Phase III European Trial, support the concept that administration of GAD-alum (Diamyd) twice, at Day 1 and 30, may decrease the autoimmune process and contribute to preservation of residual insulin secretion
- Injection of GAD-alum (Diamyd) subcutaneously in the stomach area closer to pancreas and its neighbouring lymph nodes will likely increase the effect
- 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

- Treatment with etanercept, before and/or during treatment with GAD-alum will decrease the ongoing inflammation and improve/maintain the effect of GAD-alum treatment enforced by vitamin D.

With this background we believe that etanercept should be tried in combination with GAD-alum therapy and Vitamin D in rather large doses. Thus, we want to make an open pilot-trial where our first aim is to study safety, but then also elucidate efficacy and immunological mechanisms.

2 Risk-Benefit Analysis

2.1 Type 1 diabetes

The onset of human T1D is the clinical manifestation of beta-cell failure caused by T-cell mediated autoimmune destruction of the insulin producing pancreatic beta cells, a pathologic feature involving an inappropriate immunological recognition of the body's own tissues. This results in a life-long dependence on daily insulin injections and exposure to both the acute and late complications of T1D. T1D is a particular burden to children and their families, representing by far the most common chronic, serious, life-threatening disease in our country, and tends to become an extremely serious global problem.

By the time a T1D patient is diagnosed, up to 70-90% of pancreatic islet beta cell function has been lost due to autoimmune responses against specific beta cell antigens. Over time this autoimmune response persists and the amount of endogenous insulin that is produced is reduced. As this happens a T1D patient is reliant on exogenous insulin and is at greater risk for both short and long term complications of T1D. In the more acute manifestation of T1D, or in the absence of treatment, the lack of insulin can result in diabetic ketoacidosis (DKA), a potentially life-threatening medical emergency. Insulin therapy can dramatically reduce the possibility of death from DKA but even patients adequately treated with insulin are still at increased risk to a number of acute and long-term (chronic) complications. Acute complications include DKA and severe hypoglycaemia. Long-term complications of T1D include microvascular, macrovascular and neurologic complications that increase morbidity as well as mortality. Studies have shown that retained residual insulin production is associated with a reduction in DKA and hypoglycemic risk as well as in the development of long-term complications (6).

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

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

In this new pilot-trial (EDCR IIa) it is proposed to use a treatment period with the TNF-α inhibitor etanercept of three months at a dose of 0.8 mg/kg body weight (max 50 mg) per week (as for children with JIA), in combination with Diamyd® at a dose of 20µg administered as a prime dose with a booster dose of 20µg one month later (2 doses of 20µg in total), and Vitamin D at a dose of 2000 IU per day during 15 months.

We argue that etanercept, Diamyd® and vitamin D in the proposed doses, and with the proposed exclusion and inclusion criteria, and careful follow-up are justified to try to stop the devastating progress of autoimmune beta cell destruction in new onset T1D, aiming to preserve blood glucose control and endogenous insulin secretion.

2.2 Risks of Diamyd®

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

Theoretical risks of Diamyd® such as acceleration of the autoimmune process, undesirable effects on the immune system and neurological disease have been discussed and also thoroughly evaluated during several clinical trials in both children and adults, and all clinical studies performed with Diamyd® to date indicate a favorable safety profile for Diamyd® and no neurological concerns have been identified (see section 1.1.4 above and the Investigator's Brochure of Diamyd®). It is however possible that not yet detected side effects may be revealed in this or future clinical trials.

The dose of 20 µg of Diamyd®, administered as a prime dose with a booster dose one month later or as a prime and boost on days 1 and 30 followed by one or two additional single doses (days 90 and 270) have been shown to be safe in man (children and adolescents) with few and mild adverse reactions. Recently approximately 60 patients have been randomized in the ongoing DIABGAD-1 trial and beside mild transient (a few hours) irritation at the injection site we have seen no adverse events related to the Diamyd treatment.

2.3 Risks of Etanercept (Enbrel®)

Etanercept (Enbrel®) is one of the clinically most well-used biologics in the world and is regarded as justified in the treatment of Juvenile Idiopathic Arthritis (JIA) also in children down to 2 years of age.

Etanercept has documented side effects and the most commonly reported side effects are injection site reactions, infections (URI, bronchitis, cystitis and skin infections, reactivation of tuberculosis and other serious infections such as tuberculosis, and opportunistic infections, including invasive fungal infections, listeriosis and legionellosis), allergic reactions, development of autoantibodies, itching, pruritus and fever.

TNF blockers such as etanercept affect the immune system and its use can affect the body's immune response towards infections. Serious infections affect less than 1 of 100 patients that are treated with etanercept. Deadly and life-threatening infections as well as sepsis have been reported. Different kinds of malignancies have also been reported while treated with etanercept,

even if this AE has been questioned by Swedish researchers as the incidence of malignancy in children and adults with rheumatic disorders is increased even without treatment. Additionally hematological, neurological and autoimmune reactions have been reported, including rare cases of pancytopenia (less than 1 of 1000) and very rare cases of aplastic anemia (less than one of 10 000). Central and peripheral demyelination has been reported in both rare and very rare cases. Also, rare cases of lupus and lupus-like syndrome and vasculitis have been reported

There are also reports that the risk for hypoglycemia may increase in patients with diabetes being treated with etanercept.

2.4 Risks of Vitamin D supplementation

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

In DIABGAD-1 we give vitamin D 2000 IU per day for 15 months. This dose given to children and adolescents aged 10-18 years can be compared with the dose of 400 IU per day given to babies as a health recommendation to avoid vitamin D insufficiency. Approximately 60 patients in DIABGAD-1 have been randomized of whom ¾ should have received Vitamin D, and we have noticed no adverse event which can be regarded as related to Vitamin D.

Vitamin D in a dose of 2000 IU/day in children has been reported safe (87). Additionally, a dose of up to 7000 IU/day to children from 5 years of age with HIV did not raise any safety concerns (88).

However, as an extra safety measure, vitamin D serum levels and Calcium will be analyzed at most visits. Patients will only be included in the trial if their baseline Vitamin D is below 100 nmol/L and the Vitamin D treatment will be dismissed if Vitamin D levels are above 125 nmol/L or if the patient develops hypercalcemia. Vitamin D treatment will be started again if the levels fall <100 nmol/L.

2.5 Risks of combining Diamyd® and etanercept and vitamin D supplemenetation

We would not foresee that the addition of an anti-inflammatory treatment to the Diamyd® treatment would increase the theoretical risks of Diamyd®, such as acceleration of the autoimmune process, undesirable effects on the immune system, or neurological disease. We also do not believe that the risks of anti-TNFα therapy, which are mostly the side-effects of compromised immunity, should be increased by the presence of a native antigen. There are no anticipated risks supplementing with vitamin D in the proposed doses.

2.6 Justification

We argue that etanercept (Enbrel), Diamyd and vitamin D in the proposed doses, and with the proposed exclusion and inclusion criteria, and careful safety plan and follow-up are justified to try to stop the devastating progress of autoimmune beta cell destruction in those recently diagnosed with T1D, aiming to preserve blood glucose control and endogenous insulin secretion and thereby significantly reduce both acute and long-term complication of the disease.

We propose that etanercept and Diamyd will synergize in a novel manner to produce extended beta cell function in those newly diagnosed with T1D.

As explained in the rational, we believe, that when etanercept therapy is added to GAD-alum treatment, GAD-specific autoantibody levels and effector T cell activity and number will be reduced while GAD-specific Treg number and function will be maintained. Due to its acute beta-cell-protective and metabolic effects, etanercept will also preserve beta cells directly. This will produce a deviation of the diabetes autoimmune response towards a regulatory phenotype of sufficient critical mass to lead to a significant and prolonged effect on beta cell preservation.

It is true that there is no documented pre-clinical data on the combination of etanercept and GAD immunotherapy in T1D. There are however positive results from the use of TNFα inhibitors in T1D models as well as positive clinical data on etanercept in both children and adults with T1D, suggesting that beta cell destruction can be arrested leading to better metabolic control (78-81). We do not expect the individual risks of GAD-alum and etanercept therapy to be increased by combining them.

There are no anticipated risks with the proposed vitamin D supplementation.

When summarizing the pros and cons, we argue that there is a clear possibility of therapeutic benefit of great importance whereas the adverse events and risks are reasonable as well as ethically and medically justified in the efforts to save residual insulin secretion at onset of T1D, as we know that T1D without residual insulin secretion is a dangerous disease in spite of very heavy and advanced treatment, during the whole life.

3 Aim of Present Study

Our primary aim of the present pilot study is to ensure the combination of etanercept, Vitamin D and GAD-alum is tolerable in children newly diagnosed with T1D. Secondly we want to study how this regimen influences the immune system, block TFN-alfa, and other dangerous cytokines but rather cause the desired Th-2 deviation, increase of T-regulatory cells, and hopefully preservation of residual beta cell function although we do not expect to show statistically convincing proof with this small pilot group of patients in an open trial.

A third aim is to receive more information on how GAD-alum could be combined with other types of treatment to improve efficacy without unacceptable non-justified risk of adverse events.

Thus we want to evaluate the tolerability of a combination therapy of GAD-alum, vitamin D in rather large dose and etanercept given in the same dose as used in the treatment of arthritis also in children.

Based on the results of this open pilot study we then want to use this knowledge for design of a larger Phase II trial to get more robust information. The main long-term goal is then to find a treatment at onset of T1D in young patients which is easy to administer at any clinical setting, tolerable for the patients, reasonably 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 tolerability of a combination therapy with Diamyd**,** Vitamin D and etanercept
- Evaluate how the above mentioned treatments influence the immune system and endogenous insulin secretion

4.2 Endpoints

4.2.1 Primary endpoints

Primary endpoints:
To Evaluate the tolerability of a combination therapy with Diamyd, Vitamin D and etanercept at Month 6 (main study Period), 9, 15 and 30 (extension study period)

Variables to evaluate tolerability:

- Reactions of the injection site
- Infections
- Occurrence of Adverse Events (AEs)
- Occurrence of Serious Adverse Events
- Physiological and Neurology assessments
- Laboratory measurments (biochemistry and haematology), including Calcium and Vitamin D in serum
- GAD65AB titer (GADA)

4.2.2 Secondary endpoints

Secondary endpoints:
To evaluate how the above mentioned treatments influence the immune system and endogenous insulin secretion at at Month 6 (main study Period), 9, 15 and 30 (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 $AUC_{mean\ 0\text{-}120\ min}$) during an MMTT
- Proportion of patients with a stimulated maximum C-peptide level above 0.2 nmol/L
- Fasting C-peptide
- Hemoglobin A1c (HbA1c)

- Exogenous insulin dose per kg body weight and 24 hours

5 Population

Consecutive patients with recent-onset T1D patients at Swedish pediatric clinics are given information about the study and they and their parents are asked to participate in the trial.

5.1 Inclusion Criteria

1. Informed consent given by patients and parent(s)/legal guardian(s)
2. Type 1 diabetes according to the ADA classification, diagnosed within the previous 100 days at the time of screening
3. Age 8.00 -17.99 years at time of screening
4. Fasting C-peptide at time of screening ≥0.12 nmol/L
5. Positive for GADA but < 50 000 Units
6. Menarchal females must agree to avoid pregnancy and have a negative urine pregnancy test
7. Immunity against Varicella, either through previous infection or vaccination
8. Patients must follow the Swedish vaccination programme
9. Patients of childbearing potential must agree to using adequate contraception, if sexually active, until 1 year after the last administration of GAD-alum and etanercept. Adequate contraception is as follows:

For females of childbearing potential:

a. oral (except low-dose gestagen (lynestrenol and norestisteron), injectable, or implanted hormonal contraceptives (females)
b. intrauterine device (females)
c. intrauterine system (for example, progestin-releasing coil) (females)
d. vasectomized male (with appropriate postvasectomy documentation of the absence of sperm in the ejaculate)

For males of childbearing potential:

a. 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 anti-inflammatory drug (sporadic treatment e.g. because of headache or in connection with fever a few days will be accepted)
3. Treatment with any oral or injected anti-diabetic medications (especially hypoglycemic agents) 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 hypercalcemia
6. A history of anaemia or significantly abnormal haematology results at screening
7. A history of epilepsy, head trauma or cerebro-vascular accident, or clinical features of continuous motor unit activity in proximal muscles
8. Clinically significant history of acute reaction to vaccines or other drugs in the past
9. Treatment with any vaccine within 4 months prior to planned first administration of GAD-Alum or planned treatment with vaccine up to 4 months after the last injection with GAD-Alum, including influenza vaccine
10. Participation in other clinical trials with a new chemical entity within the previous 3 months
11. Inability or unwillingness to comply with the provisions of this protocol
12. A history of alcohol or drug abuse
13. A significant illness other than diabetes within 2 weeks prior to first dosing
14. Known human immunodeficiency virus (HIV)
15. Prior or active viral hepatitis B or C infection
16. Females who are lactating or pregnant (for females who have started menstruating the possibility of pregnancy must be excluded by urine βHCG on-site within 24 hours prior to the GAD-Alum and etanercept administration, respectively)
17. Males or females not willing to use adequate contraception, if sexually active, until 1 year after the last GAD-Alum and etanercept administration, respectively
18. 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.
19. Deemed by the investigator not being able to follow instructions and/or follow the study protocol
20. Active infection, including chronic and local infection or a history of previous tendency to serious infections, recent or ongoing uncontrolled bacterial, viral, fungal or other opportunistic infections, or known infection with active EBV or CMV
21. Hypersensivity to the active substance in Enbrel (etanercept) or other ingredients in Enbrel
22. Active or inactive (latent) tuberculosis (TBC) at screening
23. History of malignancy or significant cardiovascular disease
24. Current or history of leukopenia, anemia and/or thrombocytopeni
25. Liver disease (clinical or hepatic enzymes >3 times the upper limit of normal (ULN))
26. Renal insufficiency (clinical or creatinine >3 times the upper limit of normal (ULN))
27. MS, undefined neurologic condition or known SLE, or anti-nuclear or known double-stranded DNA antibody positivity
28. Arrhythmia
29. Pancreatitis
30. Vitamin D serum levels >100 nmol/L at screening

5.3 Recruitment and Screening

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

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

5.3.1 Screening for tuberculosis (exclusion criterion)

All patients will, at the screening visit, be screened for tuberculosis according to established Swedish and international guidelines. Patients with active or inactive (latent) tuberculosis cannot be included in the study.

The evaluation will include

- detailed medical history
- previous potential exposure for tuberculosis
- recent travel to countries with possible high exposure to tuberculosis
- previous and/or current immunosuppressive treatments
- pulmonary X-ray
- QuantiFeron® test

The investigator must be aware of the risk for a false negative tuberculin result in patients with serious disease or with impaired immune response.

Any indication of active or latent tuberculosis will exclude the patient from the study. These patients should be further investigated according to respective pediatric clinic's standard routines for tuberculosis diagnosis and excluded from enrollment in this study.

These patients cannot be enrolled in the study and cannot receive etanercept.

5.3.2 Screening for viral hepatitis, liver disease and renal insufficiency

All patients will be tested for Hepatitis B and C. Patients with prior or active hepatitis B and C will be excluded from the study.

All patients will, at the screening visit, be tested for liver disease (clinical and/or hepatic enzymes ≥3 times the upper limit of normal (ULN) and renal insufficiency (clinical and/or creatinine ≥3 times the upper limit of normal (ULN))

Patients with liver disease, renal insufficiency or tested positive for hepatitis B and/or C cannot be included in the study.

5.4 Patient Withdrawal

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

There will be two main 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 decision (withdrawal of consent to participate)
- Patient lost to follow-up

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

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

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

Thus subcutaneous GAD-Alum should not be given to the patient if the patient after inclusion in the study develops/experiences

- Brain damage, epilepsia, head trauma, neurological disease
- Any active, serious hormonal disease other than T1D
- Other severe autoimmune disease (except celiac disease which is accepted for inclusion.)
- Immune-suppressive treatment
- Cancer, cancer treatment
- Any other diabetes drugs other than insulin
- Any vaccination
- Drug/alcohol abuse
- Become pregnant or is no longer willing to use safe contraceptives during the study

Etanercept should not be continued if the patient after inclusion in the study develops/experiences

- Allergic reactions to etanercept or any of the excipients
- Other severe reactions to etanercept injections
- Sustained elevations of creatinine of liver function tests,
- Evidence of cardiac disorder
- Severe infections, such as sepsis, meningitis or osteomyelitis

- Suspected, latent or manifest tuberculosis
- Suspected or manifest viral hepatitis
- Severe lymphopenia
- Anemia
- Thrombocytopenia
- Malignancy
- Urticaria
- Varicella Zoster virus exposure or infection
- Live vaccines such as morbilli, parotitis, rubella, live polio, BCG and others
- Alcohol abuse or alcohol induced hepatitis
- Other autoimmune disease than T1D, such as SLE
- Pancytopeni or aplastic anemia (cautiousness should be taken with patients that have previously experienced blood dyscrasias).
- Become pregnant or is no longer willing to use safe contraceptives during the study
- Heart disease
- Impaired renal or liver function
- Neurological disturbances

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

- Symptoms of hypercalcemia such as tiredness, euphoria, drowsiness, nausea, weight loss, thirst, polyuria, nefrocalcinosis, renal failure
- Arrhythmia
- Pancreatitis
- Vitamin D serum levels above 125 nmol/L.

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 report form, with the addition of supplementary information if available and/or appropriate.

6 Treatment Procedures

6.1 Study Design and Treatment

The trial is multicenter, open-label, pilot study in GADA positive T1D patients of either gender, 8.00 to 17.99 years old, diagnosed with T1D within 100 days at time of screening (Visit 1) and fasting C-peptide levels equal to or above 0.12 nmol/L. In total, approximately 20 patients will be recruited at approximately 10 sites in Sweden.

The patients will be assessed for eligibility at the screening visit (Visit 1) 10 to 21 days prior to the start of treatment. At each site, the screened patients will be assigned a sequential screening number (starting with 01) and this screening number together with the site number will be used as patient identification throughout the study (i.e. XX-XX).

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 9 visits to the site.

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

Table 1. Study Visits

EDCR IIa	**Screening**	**Intervention – Main Study Period**					**Follow-up – Extension Study Period**		
	Day -10 to -21 Screening	**Day 1 Base-line**	**Day 30 Month 1**	**Day 60 Month 2**	**Day 90 Month 3**	**Day 180 Month 6**	**Day 270 Month 9**	**Day 450 Month 15**	**Day 900 Month 30**
	Visit 1	**Visit 2**	**Visit 3 ± 3**	**Visit 4 ± 3**	**Visit 5 ± 7**	**Visit 6 ± 7**	**Visit 7 ± 14**	**Visit 8 ± 14**	**Visit 9 ± 14**
Appr 20 subjects		Vitamin D 2 000 IU daily, from Day 1 to Day 450							
		Etanercept injections once weekly from Day 1 to 90							
			Prime injection GAD-Alum 20 µg	*Boost injection* GAD-Alum 20 µg					

6.2 Assessments and Procedures

1. Standard insulin treatment, education and psychosocial support for newly diagnosed T1D patients.
2. Normalization of fluid, electrolyte and acid-base balance.
3. Thereafter information about the study.
4. When the patients and parent(s) / legal guardian(s) have given their informed consent, at the latest 100 days 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). Patients are also tested for tuberculosis, renal or liver disease at this visit (see section 5.3.1 and 5.3.2 above).
5. At Visit 2 (Baseline), Visit 6 (6 months), Visit 8 (15 months) and Visit 9 (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 the first GAD-alum treatment) and at Visit 8 (15 months)
7. HbA1c, safety (haematology and chemistry), autoantibody titres (GAD65, IA-2), immunology are followed by blood samples at every visit.
8. Exogenous insulin dose/24 hours, AEs and concomitant medication are registered at every 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 problems should be treated at the discretion of the clinical doctor. Thus if a patient experiences symptoms and/or signs of infection that should be treated lege artis.

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 9

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 Injections with GAD-Alum (Diamyd), Visits 3 and 4

Injections of GAD-alum (Diamyd) will be administered at Visits 3 and 4, subcutaneously in the stomach area.

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

6.2.3 Mixed Meal Tolerance Test (MMTT), Visits 2, 6, 8 and 9

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 are done and genes related to diabetes development
    b. Array studies to elucidate the importance of diabetes-related genes
3. Growth factors:
    a. IGF-1 and related binding proteins and growth factors possibly related to beta cell growth and regeneration

4. Diabetes status:
    a. HbA1c
    b. Fasting glucose and fasting C-peptide
    c. Meal stimulated glucose and C-peptide

5. Blood sampling for safety:
    a. Hematology
    b. Chemistry, including calcium

6. Urinalysis
    a. Microalbuminuria
    b. Creatinine

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 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 T1D diagnosis date and family history of T1D will also be documented.

6.5 Physical Examination and 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 physical examinations by the physician, undergo a standardized clinical neurological examination at 0, 6, 9, 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 Patient Diary

The patients will be supplied with a diary at visit 2, 3, 4 and 5 respectively (during the Diamyd and etanercept treatment period).

All patients and their parent(s)/legal guardian(s) will be informed, during etanercept treatment, to carefully follow the patient's general condition, and if affected, to measure the patient's morning

body temperature daily, and in case it is elevated also measure it at least once more that day. The body temperature should be documented in the patient diary.

Since fever is one of the first signs of infection, all patients experiencing fever during the etanercept treatment period should, in addition to contacting medical health care and the study team, continue to measure the body temperature daily and document the temperature in the patient diary.

Note: For safety reasons, during the etanercept treatment, this diary must be collected and reviewed by the investigator at every visit to detect potential side effects of etanercept.

The patient will also be asked to use diary for documentation of injection site reactions from the Diamyd subcutaneous injections during the week following injection.

The patient will carefully be instructed by study personnel on how to complete the diary and the investigator will review these together with the patient at the corresponding visit to sort out any inconsistencies and record any AE/SAE information onto the relevant CRF.

6.7 Safety Plan

To ensure subject safety a Safety Plan is in place describing the study specific safety measures that are to be taken during the study. This Safety Plan must be used in conjunction with this protocol.

The purpose of the Safety Plan is to ensure:

- the safety of patients receiving etanercept, Diamyd and vitamin D treatment in the study.
- that appropriately qualified medical personnel are readily available to advise on trial related medical questions or problems regarding the study drug treatment in the above mentioned study.
- that study personnel are educated about know side effects of the study drug treatment and how to handle them.
- that patients and their parents/legal guardian(s) are given information about possible side effects.

6.7.1 General precautions during etanercept treatment (see Safety Plan for details)

To detect infections and other side effects as early as possible it is very important the investigator/study team cautiously collects symptoms and signs indicating any of the withdrawal criteria, as well as adverse events, and also be responsive to any spontaneous symptom/adverse event reporting in between study visits and at study visits. Patients who develop a new infection under etanercept treatment should be closely monitored. Fever is one of the first signs of infections.

The Medical Adviser will be contacted and the etanercept treatment will be interrupted, until a decision is taken by the responsible physician to continue or withdraw, if a patient during etanercept treatment develops or experiences

- Fever, long-lasting and/or low-grade
- Infection with fever

- Sore throat, especially with fever
- Fever with any kind of symptoms e.g. symptoms from a joint, red skin, difficulties to breath, abnormal tiredness
- Unexpected productive cough lasting two-three weeks or more
- Any abnormal signs such as pronounced tiredness, paleness, difficulties to breath, hematomas in the skin, bleedings, bruises, weight loss or any worrying symptom or sign
- Suspicion of exposure to tuberculosis or symptoms or signs of tuberculosis
- Suspicion of exposure to Varicella Zoster virus or symptoms or signs of chickenpox/shingles

All patients and their parent(s)/legal guardian(s) must be informed by the investigator and study team that they should seek medical help immediately in case the patient treated with etanercept develops signs and/or symptoms of blood dyscrasia or infections (long-lasting low-grade fever, sore throat, bruises, bleedings, weight loss, unexpected productive cough lasting two-three weeks or more and/or paleness).

Since fever is one of the first signs of infection, all patients and their parent(s)/legal guardian(s) will be informed to, during etanercept treatment, carefully follow the patient's general condition, and if affected, to measure the patient's morning body temperature daily, and in case it is elevated also measure it at least once more that day. The body temperature should be documented in the patient diary and the study team should be contacted.

As there are reports that the risk for hypoglycemia may increase in patients with diabetes being treated with etanercept, there should be extra awareness among the diabetes team members and among patient and parent(s)/legal guardian(s) regarding the possible need to decrease insulin doses.

It is desirable that patients that are to start with etanercept treatment have followed the national vaccination program. Live vaccines must not be given during etanercept treatment.

6.7.2 General precautions during Vitamin D treatment

Blood draws for Vitamin D will be taken at screening (Visit 1), month 1 (Visit 3), month 6 (Visit 6), month 9 (Visit 7) and month 15 (Visit 8) and be analyzed continuously. Blood draws for Calcium will be taken at every visit. Should the blood concentration during Vitamin D treatment exceed 125 nmol/L or if the child would develop hypercalcemia, the Vitamin D treatment will be stopped. The subject will however continue in the study and complete all the other study related evaluations, including sampling for Vitamin D and Calcium levels.

Vitamin D treatment will be started again if the levels fall <100 nmol/L.

Vitamin D treatment will be stopped and not started again if the subject develops hypercalcemia.

6.8 Data Safety Monitoring Board (DSMB)

An independent Data Safety Monitoring Board (DSMB) will be appointed. A DSMB charter will be written to outline the working procedures and the duties of the DSMB. The DSMB will review the safety twice per year as long as patients are being treated with etanercept and thereafter annually.

6.9 Adverse Events

See section 9.2.2 below.

6.10 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 EDCR IIa Study, Schedule of Study Events, DIABETES patients

Study Period	**Scree-ning**	**Intervention Main Study Period**					**Follow-up Extension Study Preiod**		
Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Day from Baseline (Visit 2) (Day/Month)	**D** -10 to -21	**D1**	**M1** (D30 ± 3)	**M2** (D60 ± 3)	**M3** (D90 ± 7)	**M 6** (D180 ± 7)	**M9** (D270 ± 14)	**M 15** (D450 ± 14)	**M30** (D900 ± 14)
Informed Consent	X								
Inclusion/Exclusion Criteria	X								
Etanercept Administration starts		Start Day 1			Stop Day 90				
Vitamin D administration starts		Start Day 1						Stop Day 450	
GAD-Alum (Diamyd) injections			X	X					
Medical History	X								
General Physical Examination	X					X	X	X	X
Brief Physical Examination		X	X	X	X				
Neurological Examination	X					X	X	X	X
Concomitant Medication	X	X	X	X	X	X	X	X	X
Weight, height, BMI	X	X	X	X	X	X	X	X	X
Urine pregnancy test (females)	X	X	X	X	X	X	X	X	X
Injection site inspection [a]			X	X					
Insulin dose		X	X	X	X	X	X	X	X
Self-Reported Severe Hypoglycemia [b]		X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X
Patient Diary review and collection			X	X	X	X			
Screening for tuberculosis, including blood tests, medical history and pulmonary X-ray	X								
Screening for Hepatitis B and C (blood tests)	X								
Blood sampling for safety									
Hematology	X	X	X	X	X	X	X	X	X
Chemistry, including Calcium	X	X	X	X	X	X	X	X	X
Urinalysis									
Microalbuminuria	X	X	X	X	X	X	X	X	X
Creatinine	X	X	X	X	X	X	X	X	X
Blood sampling for Auto-antibodies									
GADA	X	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	X
Fasting glucose/C-peptide	X	X	X	X	X	X	X	X	X
MMTT glucose/ C-peptide		X				X		X	X
Blood sampling for genetics									
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
Blood sampling for viruses									
Enteroviruses[c]		X	X	X	X	X	X	X	X
Blood sampling additional									
IGF-1		X	X	X	X	X	X	X	X
Vitamin D in serum	X		X			X	X	X	
Blood sampling for biobank		X	X	X	X	X	X	X	X

[a] Inspection of injection site before and 60 minutes after GAD-Alum injection by investigator or nurse
[b] Severe Hypoglycemia is defined as needing help from others and/or seizures and/or unconscious
[c] Especially certain types of Coxsackie virus may play a role for the beta cell destruction. Details will depend on results in the ongoing DIABGAD trial.

7.1 Visits

The first visit, the screening visit (Visit 1) should be performed 10 to 21 days before planned Visit 2 (Baseline). Visit 3 and 4 should then be scheduled with a visit window of ±3days (first and second injection with GAD-Alum) and of ±7 days for Visit 5 and 6. For Visit 7, 8 and 9 the visit window is extended to ± 14 days.

Please note that all visits, except Visit 4 must be calculated from the baseline visit (Visit 2) according to the visit schedule. 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). Please also note that the visits must be performed within the visit windows to comply with the study protocol.

8 Study Medication

8.1 Study Medications

The following medication supplies will be used in the study:

A.	
Study medication:	GAD-Alum (Diamyd) subcutaneous injection Dosage and interval: 20 µg will be given subcutaneously at two occasions with one month interval (at Days 30 and 60).
IMP supplier:	Diamyd Medical AB, Stockholm, Sweden.
B.	
Study Medication	Etanercept (Enbrel) Dosage and interval: 0.8 mg/kg body weight, max 50 mg, given subcutaneously once per week from Day 1-90
IMP supplier	Commercially available
C	
Study Medication	Vitamin D (Calciferol) in oral solution Dosage and interval: 2 000 IU daily for 450 days
IMP supplier	Commercially available

8.2 Supply, Packaging, Handling and Storage

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

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

Etanercept will be handled and distributed by Apoteket AB who will then distribute to the clinical site or local pharmacy.

Vitamin D (Calciferol) will be handled and distributed by Apoteket AB, who will then distribute to the clinical site or local pharmacy.

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

8.3 Dosage and Administration

Etanercept	0.8 mg/kg body weight (max 50 mg) given subcutaneously once weekly from Day 1-90
Vitamin D	2 000 IU per day, given per os from Day 1 to Day 450
GAD-Alum:	20 µg given subcutaneously in the stomach area two times with one month interval.

8.4 Duration of Treatment

See 8.3

8.5 Concomitant Medication

No systemic immune modulating medication, no other diabetes medication 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 study 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 tolerability and safety assessments includes occurrence of adverse events (AEs), laboratory measurements, physical examinations and 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
- Proportion of patients with a stimulated maximum C-peptide level above 0.2 nmol/L
- Fasting C-peptide
- Hemoglobin A1c (HbA1c)
- Exogenous insulin dose per kg body weight and 24 hours
- Vitamin D levels in serum

Outcomes above are in agreement with the general Guidelines for Intervention Trials in subjects with newly diagnosed T1D defined by the Immunology of Diabetes Society (89).

9.2 Adverse events, Serious Adverse Events and Pregnancy

9.2.1 Definitions of Adverse Events (AE)

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 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 exisiting 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.

9.2.3 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.

9.2.4 Relationship to study medication

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

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

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.

9.2.5 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.

9.2.6 Timelines and Reporting of SAE

All SAEs must be reported, whether or not considered attributable to the study drug on a separate SAE Report Form.

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 a copy of the SAE reporting form to the assigned CRO at the numbers/e-mail address provided in the investigator Site File and on the SAE Report Form. The faxed/e-mailed SAE Reporting Form should be received by the assigned 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 respoinsibility to report SUSARs to investigators according to ICH Good Clinical Practice and to local regulations and to notify the Competent Authorities will be notified of all SUSARs through the Eudravigilance database.

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

9.2.7 Unresolved Events

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

9.2.8 Pregnancy Report Form

Pregnant and lactating women will not be included in the study. Menarchal females must have a negative urine pregnancy test prior to randomization and a negative urine pregnancy test at each study visit with 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 EDCR IIa study is an open controlled pilot intervention trial of the safety, and evaluation of the immune system response and residual insulin secretion, by a combined treatment with etanercept, Vitamin D and GAD-Alum (Diamyd).

Study Participants:

Newly diagnosed classic type 1 diabetes patients: N=20. Age 8.00-17.99 years. Recruited from approximately 10 pediatric clinics in Sweden

10.2 Estimation of Sample Size

Power analysis:

No formal power analyses is done for this pilot study

10.3 Statistical Analysis Plan

In brief the following analysis is 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 treatment group and visit. Where appropriate, baseline (screening) descriptive statistics will also be included.

Demographic and other baseline characteristics

Demographics such as age and gender will be described for Intention-to-treat and the Total population. Measures for other baseline characteristics are medical history, current medical conditions (at baseline), and current diabetes medication (at baseline). The baseline characteristics will be presented for the safety population.
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 safety data will be summarized descriptively.

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

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

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

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 in compliance with the protocol and according to the latest international (ICH) guidelines for Good Clinical Practice (GCP) as well as the applicable regulatory requirement(s).

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 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 Source Data

Source data is defined as any information in original records and certified copies of original records of clinical findings, observations or other activities necessary for reconstruction and evaluation for the study (e.g. CRFs, medical records (including EMRs) lab reports, patient information sheets, patient diaries, etc.).

For this study, a separate Source Data Identification Agreement will be written for each site, defining the source data for this study.

11.9 Quality Assurance and Insurance

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.

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.

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 trial to evaluate the tolerability of a combination therapy consisting of GAD-alum (Diamyd®), etanercept and vitamin D in children and adolescents newly diagnosed with type 1 diabetes"

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

Investigator's Printed Name:

……..……

Investigator's Signature:

……..……

Date: ……..

Sponsor and Coordinating Investigator's signature:

Name and function	Signature	Date
Johnny Ludvigsson Coordinating Investigator and Sponsor		