DiAPREV-IT 2
Diabetes Prevention – Immune Tolerance 2

Clinical Trial Protocol

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

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1 INTRODUCTION AND RATIONALE

1.1 Background and diagnosis

Type 1 diabetes (former known as insulin dependent diabetes mellitus), is one of the most common serious chronic diseases in children. The disease is caused by immune-mediated destruction of the pancreatic islet beta cells resulting in loss of insulin production. Children developing type 1 diabetes become dependent on lifelong treatment with insulin, with enormous consequences for the child, its family and society. Late complications are common and life expectancy shortened. Years to months before the classical clinical symptoms of type 1 diabetes onset there has been an autoimmune sub-clinical destruction of beta cells. At the onset of clinical symptoms, only a small fraction of the beta cells are left. In young children the clinical onset of the disease is often dramatic, and sometimes life threatening.

The incidence rate of type 1 diabetes is increasing worldwide. It is reaching epidemic levels in some countries. In Sweden alone, two children per day are diagnosed with type 1 diabetes. Unfortunately the increase in type 1 diabetes is the greatest in children under five years of age.

The etiology of type 1 diabetes is still unknown. Genetic factors, such as Human Leucocyte Antigen (HLA) genotypes, confer susceptibility to the disease, but other factors, both genetic and environmental, are needed to initiate the autoimmune process that results in beta cell death. The environmental factors that either trigger the development of the autoimmune process or accelerate the beta cell destruction process are still to be defined.

About 50-60% of the genetic susceptibility is conferred by HLA-DQ [1,2]. Several other non-HLA genetic factors are known to affect the risk, but account for only 10-15 % of the genetic susceptibility [3]. HLA genotypes with increased risk of type 1 diabetes can be used to identify children at increased risk of the disease. HLA-DQA1*0301-B1*0302 (DQ8) and HLA-DQA1*0501-B1*0201 (DQ2) are the haplotypes associated with the highest risk for type 1 diabetes. About 90 % of children with type 1 diabetes have at least one of these HLA-DQ haplotypes, compared with about 20 % in the general population [2,4]. A child inheriting both of these two haplotypes, DQ2/8 has the highest risk for type 1 diabetes to represent about 30% of type 1 diabetes children compared to only 3% of the healthy population [2]. Still, the risk of developing the disease during a lifetime is only 6 % even with this genotype, emphasizing the importance of environmental triggering factors.

The destruction of the pancreatic beta cells in type 1 diabetes is associated with cellular immune responses against the islet beta cells and reflected by the appearance of autoantibodies against beta cell antigens. At the clinical onset of type 1 diabetes about 95% have one or several of autoantibodies against glutamic acid decarboxylase (GADA), insulinoma-associated protein 2 (IA-2A), insulin (IAA) or zinc T8 (ZnT8A) [5,6]. These autoantibodies precede the clinical onset of type 1 diabetes and are shown to be predictive markers of the disease.

Autoantibody positivity may appear early in life, and has been reported in children as young as 3 months [7,8]. While a single autoantibody may be harmless and often represents non-progressive beta cell autoimmunity, the appearance of multiple autoantibodies most often reflects a progressive process [9-13]. The number of detectable autoantibodies is unequivocally related to the risk of type 1 diabetes, both in first-degree relatives and in the general population. In studies of family members of type 1 diabetes patients, 60-100 % of individuals with three autoantibodies developed clinical diabetes over the next 5-6 years, and population-based studies indicate that the risk is similar in the general population [14-16]. The autoantibody pattern differs between age groups. IAA is the most frequent autoantibody in the very young children [17] and therefore tend to be the first autoantibody to appear. GAD65Ab is the most frequent antibody in older patients, adolescents and young adults, whereas positive IAA or IA-2Ab decreases with increasing age at onset [18].

1.2 Rationale for GAD65, Vitamin D and prevention of type 1 diabetes

1.2.1 GAD65 preclinical and clinical intervention studies

GAD65 is present in neurons and pancreatic islet beta cells and is an enzyme in which converts glutamic acid to γ-aminobutyric acid (GABA) – a major inhibitory transmitter substance in the nervous system. GAD65 is encoded on chromosome 10p11 and is a protein of 585 amino acids. The role of GAD65 within the islet cells is still unknown, as is the reason why GAD65 is a major autoantigen in type 1 diabetes. However, studies of the spontaneously diabetic non-obese diabetic (NOD) mouse have indicated that administration of GAD65 can prevent autoimmune destruction of the pancreatic beta cells [19-24]. These findings indicate that recombinant human GAD65 may be a preventive treatment of type 1 diabetes in individuals with positive GAD65 autoantibodies (GAD65Ab).

Two formulations of recombinant human GAD65 have been evaluated in preclinical and clinical studies. The bulk Drug Substance (formulated in buffer from the manufacturing process) was used for initial preclinical safety studies, a skin Prick Test in type 1 diabetes teenagers and a Phase I clinical trial (formulated in phosphate buffered saline). An adjuvanted formulation based on Alhydrogel® has also been developed to provide the Drug Product alum-formulated GAD65 (Diamyd®) used for evaluation in Phase II and Phase III clinical trials.

A preclinical safety evaluation program has been conducted by Diamyd Medical AB, Stockholm, Sweden to support the progression of Diamyd® into clinical development. This has included single- and repeat-dose toxicity, local tolerance, immuno-toxicity, neuro-toxicity, reproduction-toxicity and investigation of the potential for effects on behaviour and cardiovascular/respiratory function. Evaluation of all preclinical safety studies performed to date have not provided concerns for clinical safety even at multiples of the highest clinically-intended dose level, nor resulted in the observation of any target organs of toxicity. Likewise,

evaluation of the effects of Diamyd® in several different animal models of autoimmune disease did not indicate any potential for undesirable effects on the immune system.

A dose-finding Phase IIa study in 47 patients with Latent Autoimmune Diabetes in Adults (LADA) has extended experience with the Alhydrogel-formulated Diamyd® [24]. This randomized, double-blind and placebo-controlled Phase IIa study also demonstrated efficacy in preventing beta cell destruction in the group of patients receiving the 20 µg dose. There were no serious adverse events reported during the 6 month long main study period. The majority of reported adverse events were due to influenza-like symptoms. A minority of injections resulted in injection-site reactions, which were mild and occurred primarily on the day of the injections. These findings support the safety of immuno-modulation by treatment with alum-formulated GAD65. The 5-year final follow-up investigation did not reveal any study-related serious adverse events.

In a Phase IIb, randomized, double-blind, placebo-controlled multicenter study in 160 LADA-patients, the subjects received 20 µg of GAD65 or placebo on 2 occasions 4 weeks apart. The trial had a main study period of 18 months and was scheduled for un-blinding in June 2007. Unfortunately, as it was impossible to guarantee absolute identity of the contents of the injections administered to the patients the study was invalidated. However, no safety concerns were raised. None of the serious adverse events reported in the study were related to the treatment.

In another Phase IIb clinical trial, the efficacy and safety of Diamyd® were investigated in children 10-18 years of age, recently diagnosed with type 1 diabetes. A total of 70 GAD65 autoantibody-positive children with a recent diagnosis (<18 months duration) of type 1 diabetes and remaining beta-cell capacity measured by C-peptide levels, were included in the study. The study was randomized, double-blind and placebo-controlled and 20 µg Diamyd® was given twice four weeks apart. The study has provided strong support for the clinical safety of Diamyd® and statistically significant and clinically relevant positive effect on the preservation of beta cell function (production of C-peptide) was seen after 30 months[25]. The frequency and pattern of adverse events did not differ significantly between the placebo and active treatment group. There were no treatment-related serious adverse events reported.

A 3-armed Phase III, randomized double-blind, placebo-controlled multi-center intervention study has been completed. In this study a total of 334 children and adolescents in the EU, 10-20 years of age with recent onset of type 1 diabetes (within 3 months) was given placebo or 20 µg Diamyd® in a prime-and-boost regimen on days 1 and 30 to confirm previous Phase II results. Additionally, one arm of patients was given two additional single doses of 20 µg Diamyd® also on days 90 and 270, to evaluate optimization of the treatment. This study did not fulfil the efficacy goal at the 15 months follow-up, although a small positive effect was observed (16.4% treatment effect; p=0.10) [26]. A US sister study with identical study design, was closed early due to the EU study outcome. The safety analyses performed by the DSMB with data from both the EU Phase III study and the parallel US Phase III study further supports the clinical safety of Diamyd® administration in children and adolescents with T1D. In addition, an investigator initiated Phase II intervention clinical trial

performed by TrialNet, in recent-onset type 1 diabetes patients, reported results in June 2011 and this trial did not meet its efficacy endpoints [27].

All studies did confirm the previously determined favorable safety profile for Diamyd® and no neurological concerns have been identified.

A Phase II clinical intervention study (DIABGAD-1) with Linköping University in Sweden as sponsor is ongoing. This study will examine the ability of Diamyd®, combined with relatively high doses of vitamin D and the anti-inflammatory drug ibuprofen, to preserve beta cell function in children and adolescents newly diagnosed with T1D (10-18 years of age) The study will also evaluate the effect of a double dose of Diamyd®.

1.2.2 GAD65 and prevention of type 1 diabetes

Since positive effects in preservation of residual beta cell function was shown in newly diagnosed children and in LADA patients, we initiated a prevention study, where we intervene earlier in the autoimmune process when a majority of beta cells are still producing insulin. The hypothesis was that treating children with an ongoing subclinical autoimmune process against the beta cells may delay the clinical disease, or even stop the beta cell destructive process. Before any large-scale prevention studies with Diamyd® in children at risk for type 1 diabetes was started, we wanted to evaluate the safety of Diamyd® treatment in a smaller group of GADA positive subjects. The secondary objective would be to evaluate indications of reduced incidence or delay of the clinical onset of type 1 diabetes. The Diabetes Prevention – Immune Tolerance (DiAPREV-IT) study started in May 2009 and was fully recruited with 50 children 4-17.99 years of age in January 2012. All children were treated with 2 doses of 20 microgram Diamyd (n=25) or placebo (n=25). The children will be followed for 5 years. Up to date, no study related SAE have been reported. A total of 14/50 children have developed type 1 diabetes during follow-up. Full un-blinded analysis will be performed in Jan 2017 after completing the 5 year follow-up.

Since DiAPREV-IT was initiated as a small-scale study to primarily evaluate safety of Diamyd in non-diabetic children, a larger study will be important to be able to evaluate a possible preventive effect of the drug. In the current study, DiAPREV-IT 2, we will further investigate the preventive efficacy of Diamyd in children with multiple islet autoantibodies but not yet type 1 diabetes. The inclusion criteria and the outcome measurements will be identical between the two studies and the study protocol similar, with the following modifications. The first is to stratify randomization with glucose metabolism instead of number of islet autoantibody types and the second is to add supplementation with high dose Vitamin D to all participating children.

1.2.3 Vitamin D supplementation

Vitamin D have been shown to affect the maturation and activation of dendritic cells, leading to a suppressed T-cell activation[28]. Vitamin D-deficiency has been reported to be associated with increased incidence of type 1 diabetes. Previous studies have indicated that

Vitamin D supplementation from birth may protect the child from type 1 diabetes [29-31]. Additionally, low levels of vitamin D have been reported in children with newly diagnosed type 1 diabetes compared to healthy controls [32]. It is suggested that Vitamin D may have a direct effect on the beta cells, with improved insulin sensitivity[33] and a protection against cellular stress [34].

The rationale for the vitamin D treatment in this study is based on the above findings. The children will be treated for 30 days with vitamin D before the first injection with Diamyd® or placebo, to enhance the response to the first injection of the study substance and to make sure that all children are fully substituted with vitamin D at baseline. All children will be treated, unless Vitamin D levels exceed 125 nmol/L, to ensure that vitamin D levels in the treatment arm and the placebo arm are similar. Additionally, levels of Vitamin D will be measured and evaluated by a paediatrician after the first 30 days of treatment and then every 6th month during follow-up. Treatment with Vitamin D will continue during the full study period, unless levels are above 125 nmol/L. Calcium levels will for safety reasons be measured at baseline and every 3rd month during follow-up and treatment with Vitamin D will be omitted if hypercalcemia occurs.

An additional reason for treatment is that levels in children with known multiple islet autoantibodies and a high risk of diabetes may vary, since we know from previous studies (DiPiS, TEDDY and DiAPREV-IT) that some, but not all, parents may choose to give their children Vitamin D as a supplement in an attempt to prevent type 1 diabetes. Therefore substitution with Vitamin D will minimize the risk of differing Vitamin D levels as a confounder.

1.3 Objectives and Endpoints

1.3.1 Objectives

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

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

The subjects will be followed for 5 years.

1.3.2 Endpoints

1.3.2.1 Primary endpoints

The proportion of subjects diagnosed with clinical type 1 diabetes in the Diamyd® treated group, compared to the placebo treated group at five years after the first injection.

1.3.2.2 Secondary endpoints

To evaluate safety and the change in metabolic status from normal to impaired glucose metabolism in the group of children with normal glucose metabolism at baseline as well as the progression in metabolic status in the children with impaired glucose metabolism at baseline screening, in the non-diabetic children with multiple islet autoantibodies treated with Diamyd® compared to those treated with placebo.

Variables to evaluate safety:

- Injection site reactions
- Occurrence of adverse events (AEs)
- Laboratory measurements (biochemistry and haematology including complete blood count (CBC)), including Calcium and Vitamin D in serum
- Urine analysis
- Physical examinations, including neurological assessments
- Epitope-specific GADA titer, isotypes and subtypes as well as antiidiotypic autoantibodies to GADA

Metabolic status:

- Change from normal to impaired glucose metabolism, defined as any of

  a) F-glucose ≥ 6.1 mmol/L
  b) maximum p-glucose at 30, 60, 90 minutes ≥ 11.1 mmol/L in the OGTT
  c) 120 min p-glucose ≥ 7.8 mmol/L on OGTT
  d) HbA1c ≥ 39 mmol/mol.

  Impaired glucose metabolism has to be confirmed at a second visit. This endpoint will be used in the group of children with normal glucose metabolism at baseline screening

- Progression of impaired glucose metabolism from one or several of the above variables to additional signs of reduced glucose metabolism, confirmed at a second visit. This endpoint will be used in the group of children with impaired glucose metabolism at baseline.

1.3.2.3 Exploratory endpoints:

- Proportion of subjects diagnosed with clinical type 1 diabetes at 1, 2, 3 and 4 years of follow-up.
- Time from baseline visit to clinical type 1 diabetes diagnosis
- Change from baseline in the following key metabolic variables at various time points: HbA1c, First phase insulin response and K-value from IvGTT, AUC p-glucose and C-peptide from OGTT, 120 minutes glucose and C-peptide after OGTT, fasting C-peptide, insulin and glucose
- Change in other metabolic variables from baseline: AUC C-peptide, glucose and insulin from IvGTT, AUC insulin from OGTT, change in max p-glucose on OGTT.

1.4 Study population

Children repeatedly positive for more than one islet cell autoantibody, as a sign of an ongoing autoimmune process against the beta cells, will be invited to participate in DIAPREV-IT 2. The invited children have been screened for autoantibodies in a population based follow-up study such as Diabetes Prediction in Skåne (DiPiS) study, the Environmental Determinants of Diabetes in the Young (TEDDY) study or TEDDY relatives, screened as a relative to a type 1 diabetes patient in TrialNet or found positive for islet autoantibodies for example at screening at the clinic. All participating children have to be 4.00-17.99 years of age at screening and have autoantibodies to GAD65 and at least one more islet autoantibody (to IA-2A, IAA or ZnT8A).

DiPiS is a prospective population-based study of diabetes in children [35-37]. The aim of DiPiS is to determine the predictive value of genetic risk combined with islet cell autoantibody markers for type 1 diabetes and to identify factors before, during and after pregnancy that may trigger type 1 diabetes. In DiPiS, newborn children in the general population were screened during September 2000 to August 2004 for type 1 diabetes high-risk HLA genotypes. Children with increased risk of type 1 diabetes are thereafter followed from 2 to 15 years of age. Children with multiple islet autoantibodies are followed every 3rd month in the study. The study is approved by The Ethics Committee at Lund University, Sweden.

TEDDY is a prospective multicenter study sponsored by the National Institutes of Health. Skåne is participating as one of six centres in the world, along with Finland, Germany and the US states of Washington, Colorado and Georgia. Screening for the TEDDY began in September 2004 and was completed in February 2010. Children in the general population with diabetes high-risk HLA genotypes are followed from 3 months of age. The study was approved by the Regional Ethics Board at Lund University, Sweden. By now more than 2 000 children in Skåne are followed in the TEDDY study until 15 years of age.

Trial Net is a consortium providing screening for islet cell autoantibodies and HLA-typing of relatives of patients with type 1 diabetes. Relatives who are positive for autoantibodies will be HLA-typed to rule out the protective genotype DQ6. The participants are thereafter followed with annual OGTT´s to investigate the natural history of development of diabetes. Trial Net in Skåne, Sweden, was approved by the Central Ethics Committee 2009-08-27 (2009/470). The screening in Skåne started in March 2010. Participants in the Trial Net screening from 4 years of age who are positive for GAD65Ab and at least one more islet cell autoantibody and who have not the protective genotype DQ6 allele will be invited to participate in DiAPREV-IT 2. Between DiPiS, TEDDY and TrialNet we follow a large number of children positive for 2 or more islet cell autoantibodies who will be asked to participate in the study.

In TEDDY relatives, relatives to TEDDY children are screened for autoantibodies and followed if positive. Children found positive for GADA and at least one more islet autoantibody will also be asked to participate in DiAPREV-IT 2.

1.5 Sample size

Up to 80 children will be asked to participate in DIAPREV-IT 2. By including children followed in DiPiS, TEDDY, TEDDY relatives and TrialNet, who fulfil the inclusion criteria, we expect that we will be able to identify at least 80 children for inclusion in DiAPREV-IT 2. Although the number of subjects is somewhat limited, we expect that DiAREV-IT 2 will have power to evaluate the safety and the possible preventive effect of Diamyd®.

1.6 Dose and frequency of doses

In DIAPREV-IT 2 we will use the previously tested dose of 20 µg Diamyd® administered as a prime-and-boost at days 1 and 30. This dose regimen has been tested in several clinical trials in type 1 diabetes children and adolescents with no safety concerns**.**

A repeat administration of the same dose at day 30 is the 'prime-and-boost' regimen conventionally used to initiate and then promote immune response for most vaccines. A dosing interval of 4 weeks is considered sufficient to initiate and then recall specific cellular and humoral responses to antigens.

A relatively high dose of Vitamin D (2000 IU/day) will be given to all children in the study with start 30 +-7 days before the first subcutaneous injection of 20 µg Diamyd® or placebo. Samples for Vitamin D will be taken at screening and evaluated after analysis. If the levels at screening are above 125 nmol/L, the treatment with Vitamin D will be omitted.

The Vitamin D treatment will be given to all participating children below 125 nmol/L, regardless of treatment group. Vitamin D levels will be determined and evaluated at screening, randomization and every 6th month during follow up to secure compliance and for safety reasons. Treatment with Vitamin D will continue during the full study period, unless levels are above 125 nmol/L.

1.7 Choice of preparation and administration

Aluminum hydroxide is commercially called Alhydrogel®. The substance is commonly used in vaccines. Aluminium salts are well recognized and preferentially induce a humoral (Th2) rather than a cellular (Th1) immune response. Since the autoimmune process leading to type 1 diabetes is thought to be a mainly cellular (Th1) immune response, Aluminium salts could steer the process against a humoral (Th2) response and minimize the likelihood of exacerbating cell-mediated beta cell destruction.

The subcutaneous route of administration has been selected in previous studies of Diamyd®. Although intra-muscular route of administration is frequently used for vaccine administration, dermal tissues are more capable than muscle tissue to recognize and present antigens. In addition subcutaneous injections are less painful than intra-muscular injections.

2 RISK-BENEFIT ANALYSIS

Type 1 diabetes is a chronic lifelong disease that results from the immunological destruction of the pancreatic islet beta cells.. By the time a type 1 diabetes patient is diagnosed, up to 85-90% of pancreatic islet beta cells have been destroyed by autoimmune responses against specific beta cell autoantigens. The beta cells express autoantigen-peptides on HLA Class I proteins on the cell surface, which makes it possible for cytotoxic CD8 positive T cells to kill the beta cells. Over time this autoimmune response persists and the amount of endogenous insulin that is produced is reduced as the beta cells are killed. As this happens a type 1 diabetes patient is reliant on exogenous insulin and is at greater risk for both short and long term complications. In the more acute manifestation of type 1 diabetes, or the absence of treatment, the lack of insulin can result in diabetic ketoacidosis (DKA), a potentially life-threatening medical emergency. Insulin therapy dramatically reduces 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 a higher level of retained residual insulin production is associated with a reduction in DKA and hypoglycemic risk as well as the development of long-term complications [38].

Upon diagnosis of type 1 diabetes, the standard treatment is based on insulin replacement with injections or administered through insulin pump, and blood glucose monitoring through blood tests 5-10 times per day. Long acting insulin or basal dose in pump is complemented with doses to all meals. The doses are dependent on the glucose value, the carbohydrate content of the meal and on physical activity, among other. The treatment has immense implications on daily life. The continued beta cell destruction results in permanent loss of endogenous insulin secretion, as well as loss of any potential regeneration of islet beta cells. There is currently no approved method or medicament to halt or slow the immune destruction of remaining pancreatic islet beta cells.

It is estimated that only 10-15% of the beta cells are still functioning at time of clinical symptoms and diagnosis of type 1 diabetes. Saving these remaining insulin producing beta cells may result in clinically meaningful benefits including improved metabolic control and lower risk for hypoglycaemia and chronic complications. However, to save beta cells even earlier, before the clinical onset of type 1 diabetes, may result in delayed or prevented clinical onset of clinical disease. This would be of benefit for the child, family and society.

Diamyd® therapy aims at intervening in the autoimmune process 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 order to preserve residual endogenous insulin production a treatment to reduce or stop the autoimmune responses against remaining functional beta cells must be initiated in the period when a significant number of these cells still exist. Therefore, this study is proposed to include non-diabetic children with multiple islet antibodies, i.e. at high risk for the clinical onset of type 1 diabetes.

The dose of 20 μg of Diamyd®, administered as a prime dose with a booster dose 30 days 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. All clinical studies performed with Diamyd® to date indicates a favorable safety profile for Diamyd® and no neurological concerns have been identified (see section 1.2 above).

Risk-benefit Vitamin D: As reported previously in the rationale, Vitamin D may protect or delay the clinical onset of type 1 diabetes in children with multiple islet autoantibodies. Vitamin D in a dose of 2000 IU/day in children has been reported safe [39]. Additionally, a dose of up to 7000 IU/day to children from 5 years of age with HIV did not rise any safety concerns[40]. The dose can be compared to the supplementation dose of 400 IU/day (800 IU/day to children with darker skin) that are recommended to children up to 6 years of age to avoid vitamin D deficiency. For safety reasons, we will follow D-vitamin serum levels of both vitamin D and Calcium every 6th month. A paediatrician will dismiss the Vitamin D supplementation if Vitamin D levels are above 125 nmol/L.

3 STUDY DESIGN

3.1 Study procedure

Individual subjects will be exposed to the study drug Diamyd®/Placebo for a maximum of 1 month in the primary study. All participants will be monitored by study personnel at the study clinic for at least 30 minutes after administration of each study drug injection. In addition, the participants will be offered to stay for an additional 2 hours, or contact the physician/study nurse by phone.

We intend to treat 50% of the double antibody positive children with Diamyd® and the remaining 50% with placebo (Alhydrogel®). The randomisation will be stratified according to glucose metabolism at baseline screening. The tests and examinations scheduled for each visit during the first year are presented in Table 1. The participants will be followed for 5 years after the first injection with a first analysis carried out 3 years after the first injection. All patients will be supplemented with Vitamin D, regardless of randomization. If levels taken at screening or during follow-up are found to exceed 125 nmol/L, the treatment will be omitted as soon as the results come.

The rational to stratify this new study by glucose metabolism is based on findings from baseline data from the first cohort of 50 participants in DiAPREV-IT. The major finding in this analysis was that 43% of the first cohort of 50 children enrolled in the study, with GADA and at least one more islet autoantibody (inclusion criteria), had impaired glucose metabolism already at baseline, based on 2-OGTT plasma glucose and FPIR tests, despite normal k-values, HbA1c and fasting glucose and C-peptide levels. Hence, two distinct groups were identified in the study population at baseline; one group with impaired glucose metabolism (43 %, n=20) and one group with normal glucose metabolism (57%, n=27) [41]. In our expanded analyses of baseline data we additionally included p-glucose >11.1 mmol/L at 30, 60 and 90 min on OGTT in the diagnosis of impaired glucose metabolism (manuscript data). A total of 52 % had impaired glucose metabolism at baseline screening when including 30, 60, 90 and 120 min values from OGTT and 12/14 children who, up to date, have developed diabetes in the study were in this group (submitted manuscript).

3.2 Vitamin D Supplement:

In DiAPREV-IT 2 we will supplement all included children with Vitamin D. The children will get a prescription of vitamin D drops, or equivalent, by a paediatrician in a daily dose of 2000 IU. Samples for Vitamin D will be taken at screening and evaluated after analysis. If the levels at screening are above 125 nmol/L, the treatment with Vitamin D will be omitted as soon as the results come. Blood draws for Vitamin D will be taken at visit 0 (baseline), at visit 1 (after 30 days of treatment) at visit 4 (6 months from visit 1) and thereafter every 6 months. 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. Vitamin D levels will continue to be evaluated every 6th months and all results will be evaluated by the investigator. Vitamin D treatment will be started again if the levels fall <100 nmol/L.

3.4 Post diagnosis intervention protocol (PDIP)

Pending study drug shelf life, children diagnosed with type 1 diabetes within the study period may be offered to continue in the trial in a post-diagnosis intervention protocol (PDIP) to receive additional injections in the following manner:

- Within 4 months since diabetes diagnosis, participants will receive one injection of Diamyd® 20 µg on Day 1 in the Post Diagnosis Follow-up, followed by a second injection of Diamyd® on Day 30, in a prime and boost fashion (a total received dose of 40 µg Diamyd® for participants who pre-diagnosis was randomized to receive placebo and a total received dose of 80 µg Diamyd® for participants who pre-diagnosis was randomized to receive Diamyd®)

All children that are enrolled in the PDIP will be discontinued from the original prevention protocol to be followed thoroughly for safety and efficacy according to the post-diagnosis

intervention protocol for 15 months following the first injection of Diamyd® in the PDIP (Appendix 2).

Table 1. Examinations for each visit

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

Abbreviations: BP=Blood Pressure, OGTT=oral glucose tolerance test, IvGTT=Intravenous glucose tolerance test
Possibility for telephone contact with physician/study personnel in between the visits.

At all clinical visits the responsible paediatrician will fill out the Case Report Form (CRF) and also establish patient journals. After the first 6 months the clinical visits will be performed every 6th month with glucose tolerance tests, while blood sampling for autoantibodies, HbA1c, plasma glucose will be performed every 3rd month with the possibility of telephone contact with study personnel**.**

A glucose tolerance test will be performed at baseline and every 6th month; OGTT at baseline and every half year visit and IvGTT at the baseline visit and at every full year visit from the first vaccination. The estimated time for those visits is 2,5-3,5 hours.

3.3 Safety procedures

The investigator (in this section referred to as sponsor) reserves the right to discontinue further treatment within the study at any time for safety reasons or other reasons jeopardizing the justification of the study. If the study is prematurely terminated or suspended, the sponsor should promptly inform the subjects and assure appropriate therapy and follow-up. The sponsor will notify Regulatory Authorities of any plans to terminate the study, and notify the appropriate Regional Ethics Board. An independent Data Safety Monitoring Board (DSMB) is appointed. The DSMB will review the data and safety throughout the study period. The DSMB are responsible for safeguarding the interests of the trial participants and assessing the safety of the interventions during the trial. The DSMB will also be responsible for recommending the sponsor to discontinue further treatment within the study if the data would point towards 1) that children treated with Diamyd® develop type 1 diabetes at a higher frequency than the placebo group children (in reality the ability to terminate the study for this reason only applies during the active Diamyd®/placebo treatment phase of the trial) 2) that treatment with Vitamin D would cause serious unexpected adverse events (then the treatment with Vitamin D can discontinued in all children, while the follow-up in the study will continue as planned). Once all patients are recruited and have completed all injections the trial cannot be stopped because it has reached an observation only period.

The Sponsor 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 participant's wellbeing.

If the study is prematurely terminated or suspended, the investigator should promptly inform the participant's 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.

The objectives of the study is to to evaluate if there is an effect of the treatment in preventing beta-cell destruction and clinical onset of type 1 diabetes and to test that the

treatment is safe in children at risk for type 1 diabetes. The subjects will be followed for 5 years with a first analysis carried out 3 years after the first injection, but the study will be kept blinded for the entire study period to the parents and participating children, investigators and all study personell. The study will be unblinded groupwise to the biostatistician responsible for statistical analyses and if DSMB request unblinded data groupwise. Safety assessment will be conducted periodically during the study and will be reviewed by the DSMB.

3.4 Decision Criteria

The child is included in the study when the informed consent form has been signed at the information visit (Visit 0). No children will be enrolled until approvals from the appropriate Ethics Committees and Regulatory Authorities have been obtained. Prior to the study starting, it will be confirmed that all regulatory and ethical requirements for starting the study are met.

4 STUDY POPULATION

Children participating in DiPiS, TEDDY, TrialNet, TEDDY relatives or found to have autoantibodies in other settings fulfilling the inclusion criteria will be asked to participate. Up to 80 children are intended to be included in the study during the study years.

4.1 Major Inclusion criteria

1. Children from four (4) to 17,99 years of age.
2. Positive GADA and at least one additional type 1 diabetes-associated autoantibody (IA-2A, ZnT8R/W/QA or IAA).
3. Written informed consent from the child and the child's parents or legal acceptable representative(s) according to local regulations.

4.2 Major Exclusion criteria

1. Ongoing treatment with immunosuppressant therapy (topical or inhaled steroids are accepted).
2. Diabetes.
3. Treatment with any oral or injected anti-diabetic medications.
4. Significantly abnormal hematology results at screening.
5. Clinically significant history of acute reaction to vaccines or other drugs.
6. Treatment with any vaccine, other than influenza, within one month prior to the first dose of the study drug or planned treatment with vaccine up to two months after the last injection with the study drug.
7. A history of epilepsy, serious head trauma or cerebrovascular accident, or clinical features of continuous motor unit activity in proximal muscles.

8. Participation in other clinical trials with a new chemical entity within the previous 3 months.
9. History of hypercalcemia.
10. Unwilling to abstain from other medication with Vitamin D during the study period.
11. Significant illness other than diabetes within 2 weeks prior to first dosing.
12. Known human deficiency virus (HIV) or hepatitis.
13. Presence of associated serious disease or condition, including active skin infections that preclude subcutaneous injection, which in the opinion of the investigators makes the patient non-eligible for the study.
14. Diabetes-protective HLA-DQ6-allele.
15. Females who are lactating or pregnant (for females who have started menstruating the possibility of pregnancy must be excluded by urine βHCG onsite within 24 hours prior to the study drug administration)
16. Males or females not willing to use adequate contraception, if sexually active, until 1 year after the last Diamyd administration. 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)

4.3 Participant withdrawal criteria

The participant and the guardian will receive oral and written information about the study, which includes information about the right to withdraw from the trial at any time without prejudice to future treatment. In addition, the participant may be withdrawn at the investigator's discretion at any time if regarded in the child's best interest. Reasonable efforts should be made to contact any participant lost to follow-up during the course of the trial in order to complete assessments and retrieve any outstanding data. A subject that has withdrawn from the study may not be further evaluated in the study. However, the data obtained up to the date of withdrawal may be used in the study analyses.

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

- Symptoms of hypercalcemia such as tiredness, euphoria, drowsiness, nausea, weight loss, thirst, polyuria, nefrocalcinosis, renal failure
- ECG changes, arrhythmia

- Pancreatitis

Diamyd should not be given to the patient if the patient after inclusion in the study has got

- - brain damage, epilepsy, head trauma, neurological disease
- - other severe autoimmune disease that in the opinion of the investigator makes the subject non-eligible for the continuing study treatment
- - immune-suppressive treatment
- - cancer, cancer treatment
- - drug/alcohol abuse
- or if the patient has become pregnant or is no longer willing to use safe contraceptives during the study

5 STUDY VISIT ASSESSMENTS

5.1 Laboratory examinations

At each visit, blood will be drawn for efficacy and safety evaluations after application of EMLA cream to reduce pain. Blood samples during the first study year will be analyzed for hematological parameters, *i.e.* Hemoglobin, White Blood Cells, and Thrombocytes, and chemical variables, *i.e.* sodium, potassium, creatinin, calcium, vitamin D, liver functioning tests and specific diabetes-related parameters such as plasma-glucose, insulin, C-peptide, HbA1c, GADA, IA-2A, ZnT8A, IAA, CBC and analyses of lymphocytes by flow cytometry. To evaluate other autoimmunity than diabetes, autoantibodies to Thyroid peroxidase (TPO), Thyroglobulin (Thgl) and Tissue transglutaminase (tTg) will be analysed at visit 0 and yearly. The analyses will be performed at the Clinical Chemisty Department, University Hospital MAS, Malmö (hematology, chemical variables, insulin, C-peptide, HbA1c) and at Clinical Research Center, Skåne University Hospital SUS, Malmö (autoantibodies, lymphocyte analyses). Plasma-glucose will be analyzed locally with Hemocue. All analyses are standardized according to international recommendations. Blood volume taken will not exceed 100 ml per visit.

Urine will be analysed according to glucosuria, ketones, leucocytes and protein. During the 5 years follow up blood samples will be analyzed for plasma-glucose, HbA1c, GADA, IA-2A, IAA and ZnT8A.

5.2 Metabolic status

HbA1c and plasma-glucose will be measured every third month to be able to diagnose type 1 diabetes at an early stage. An increase in HbA1c values, even if within normal ranges, have been shown to precede clinical onset of type 1 diabetes in islet cell autoantibody-positive subjects [42]. Fasting and stimulated C-peptide levels will be measured biannually. An OGTT and IvGTT will be performed at baseline. During the 5 years follow-up, IvGTT will annually

be performed with analyses of insulin and C-peptide and first-phase insulin response (FPIR) and K-value. In addition an OGTT will be performed at visit 4 and every 6th month after baseline and thereafter annually in between the IvGTT. If a participant, who previously had a normal glucose metabolism develop impaired glucose metabolism, defined as any of a) F-glucose ≥ 6.1 mmol/L, b) maximum p-glucose 30, 60, 90 minutes ≥ 11.1 mmol/L in the OGTT, c) 120 min p-glucose ≥ 7.8 mmol/L on OGTT, d) HbA1c ≥ 39, a confirmatory OGTT will be performed within a month. Metabolic status will be evaluated from the baseline OGTT and IvGTT.

5.3 Safety assessments

The following parameters will be assessed during the study:

1. Laboratory analyses (chemistry, hematology, and urine analyses).
2. GADA titers, isotype, epitope and affinity, anti-idiotypic antibodies.
3. Clinical neurological examination*.
4. Vital signs and physical examination.
5. Occurrence of adverse events/serious adverse events with specific questions about treatment specific reactions, such as fever, muscle pain, discomfort, headache.
6. Injection site discomfort or local reaction.
7. Participant diary.

The results of these measures will be evaluated for clinically significant values.

*The patients will undergo a standardized clinical neurological examination. The neurological tests are performed in order to detect possible mild signs of neuromuscular disease such as disturbance of strength, balance, and coordination. The tests and the results may be modified and adapted to age in children younger than 6 years. The overall neurological status will also be assessed, since some children may refuse to perform all parts of the described neurological status and other kind of neurological assessment will be made during the examination of those children.

The neurological examination includes:

- Extremity reflexes
- Romberg (balance and coordination)
- Walk on a line, 2 meters (balance and coordination)
- Jumping on 1 leg 10 times, left and right (balance and coordination)
- Finger-nose (coordination)
- Mimic (cranial nerves)
- Babinski reflex (central function)
- Muscle strength (shake hands) biceps, triceps, distal extensors, and flexors
- Overall neurological status

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 paediatric neurologist for further evaluation.

6 SUBJECT MANAGEMENT

6.1 Screening visit and eligibility assessment

Children participating in DiPiS, TEDDY, TEDDY relatives or TrialNet and who have more than one positive islet cell autoantibody are followed at one of our sites in Malmö, Helsingborg or Kristianstad every 3rd month.

The children who are eligible to participate in DIAPREV-IT 2 and their parents will be informed about the study by the study personnel. Both oral and written information about the study will be given. If the children and parents agree to participate, a written consent will be signed.

Blood samples for islet cell autoantibodies, HbA1c and plasma-glucose are taken at the visit, all according to the study protocols. HLA-genotypes of all children in DiPiS and TEDDY were determined in their cord blood samples, while children followed in TrialNet are tested for HLA to rule out the protective HLA-DQ6. Children not tested for HLA in studies will be tested at screening for DiAPREV-IT 2.

6.2 Diamyd®

The Diamyd Drug Product is composed of the recombinant human GAD65 (rhGAD65) protein formulated in a sterile, non-pyrogenic phosphate buffered saline containing the aluminum hydroxide adjuvant, Alhydrogel®. The active ingredient, rhGAD65, is manufactured via a process involving expression in an insect cell line. The highly purified and unmodified form of rhGAD65 is currently indistinguishable from the native human protein. To maximize immunogenicity and bias towards a humoral rather than a cellular immune response, rhGAD65 is formulated with the adjuvant alum (Alhydrogel®). All study drugs will be kept between 2 and 8º C in a refrigerator, with limited access. The study nurses will administer the study medicine. After administration the used vial should be placed back into the vial box and kept between 2 and 8◦C in a refrigerator, with limited access until the site is instructed to return the study medication. Diamyd Medical AB, Stockholm, Sweden will ~~s~~upply Diamyd® for the present investigation (Supplier).

The Study Product is supplied in a 3-mL glass vial with either 20 µg Diamyd alum-formulated vaccine/mL or placebo. The dose volume is 0.5 mL each for subcutaneous administration. The vials have a slight overage (up to 0.8 mL) to allow withdrawal of 0.5 mL.

The study medication will be available in individually treatment numbered packages. The treatment numbered packages will be a box containing 1 or 2 glass vials with medication for one or two injections (as suggested by the packaging company), which corresponds to the study randomization list. Since each patient will receive 2 injections, each treatment number on the study randomization list will be used on 2 vials.

Each treatment numbered box will be identified by an injection number (either 1 or 2 for the injections in the original protocol and 3 or 4 for the injections in the post diagnosis protocol).

The Supplier will ship the study medication to a central distributor, who will ship the medication to study sites according to local laws and regulations. The procedures for shipment, ordering, and storage will be outlined in a Study Drug Accountability Document. Both the distributor and the investigator must keep record of all drugs received, used and returned. Both distributor and study sites are obliged to properly measure and record the storage temperature.

When the study is completed all unused investigational products must be returned to the central facility and subsequently to the study drug Supplier unless the Supplier has approved other arrangements. Any destruction of investigational products must be performed in accordance with the documented approved procedure from the Supplier.

7 STUDY MEDICATION

The following medication supplies will be used in the study:

A.	
Study medication:	GAD-Alum (Diamyd) subcutaneous injection or placebo Dosage and interval: One injection of 20 µg Diamyd or placebo will be administered 1 month apart
IMP supplier:	Diamyd Medical AB, Stockholm, Sweden.
B.	
Drug	Vitamin D (Calciferol) in oral solution or eqvivalent Dosage and interval: 2000 IE daily for 5 years
Drug supplier	Commercially available

8 ADVERSE EVENTS REPORTING AND SAFETY MONITORING

8.1 Definition of adverse event

In this clinical trial, an adverse event (AE) is 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 *i.e.,* 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.

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. As type 1 diabetes is the study outcome, this will not be reported as an AE. Neither will mild injection site reactions.

8.2 Definition of serious adverse event

A serious adverse event (SAE) is defined as an AE that:

1. results in death
2. is immediately life-threatening
3. requires in-patient hospitalization or requires prolongation of existing hospitalization
4. results in persistent or significant disability/incapacity
5. is a congenital anomaly/birth defect
6. is an important medical event

Important medical events are those, which may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the individual or may require intervention to prevent one of the other outcomes listed above.

8.3 Adverse events reporting and monitoring

All participants are carefully monitored for occurrence of any AE. At each clinical visit after the inclusion in the study AE will be collected by the investigators via non-leading questions as ‘Have you had any new or worsening health problems since the previous visit/last contact?’ as well as reporting events directly observed or spontaneously volunteered by the participant. The investigator will also ask the participants direct questions about treatment specific reactions, such as such as fever, muscle pain, discomfort and headache. In addition, any local reactions from the injection site will be asked for as well as recorded in the participant diary.

Early AEs after the vaccination will be documented in the participant diary. The family will also be encouraged to contact the responsible paediatrician if an AE, suspected to be related to the vaccine, occur between day 1 and 30. Any abnormal findings in physical examination, vital signs, and clinical laboratory tests must also be reported as AE. All reported AE are to be recorded in the CRF.

The participants will receive adequate therapy for concomitant diseases at the discretion of the investigator. Any concomitant medication must be recorded in the CRF. Note: if a new medication is introduced or an existing medication is changed due to a new medical condition or worsening of a pre-existing medical condition, the condition must be reported as an AE.

Symptoms and signs of development of type 1 diabetes (increased fasting glucose or postprandial glucose, urine-glucose, hypoglycemia in between meals, increased thirst and urination in combination with high plasma-glucose, weight loss in combination with high plasma-glucose) will be recorded separately.

Following parameters of the AE should be recorded:
Description, date of onset and date of resolution, intensity (mild, moderate, severe), action taken regarding the study drug, treatment of event, causality (unlikely, possibly, probably), outcome and sequel, seriousness (death, life-threatening, inpatient hospitalization or prolongation of hospitalization, result in persistent disability, congenital /birth defect, important medical event).
Intensity:
Mild: awareness of signs or symptoms, but easily tolerated (acceptable)
Moderate: discomfort to interfere with usual activity (disturbing)
Severe: incapacity to work or to do usual activity (unacceptable)
Causality:

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

Unlikely: Time relationship non-existent or doubtful and/or other factor(s) certain or probable to have been causative.
Possibly: Time relationship exists. Other possible causative factor(s) may exist (e.g., concurrent disease or concomitant medication). Improvement on dechallenges or dose reduction may or may not have been seen.
Probably: Time relationship exists. No other possible causative factor(s) may exist (not reasonably explained by the patient's known clinical state or concomitant medication). Improvement on dechallenges or dose reduction (if performed) has occurred. Recurrence of symptoms on rechallenge (if performed) has occurred. A specific laboratory investigation (if performed) has confirmed the relationship.

8.4 Timelines for reporting SAE

The investigator should report an SAE within 24 hours of the investigator becoming aware of the SAE. All SAE must be reported to the DSMB and to Diamyd Medical AB, whether or not considered attributable to the study drugs.

Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

All AEs/SAEs must be followed until final outcome is known or the condition is stable.

8.5 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.

8.6 Pregnancy:

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 as specified in exclusion criterion #14 above (section 4.2). At Visit 1 and 2 the need for birth control will be re-assessed. Patients and their partners will be strongly advised to avoid pregnancy for 1 year following the last dose of Diamyd and instructed to use adequate birth control.

A pregnancy occurring during the trial must be recorded on the Pregnancy Report Form and no further doses of Diamyd will be given. If the pregnancy is verified prior to any of the injections, no further injection shall be given. Any pregnancy must be followed until delivery or to the end of pregnancy.

9 DATA HANDLING

9.1 CRF

The online CRF will be completed at all visits by the local responsible pediatrician. The original CRF will be kept locally and under lock and key (printed, signed versions). Only study personnel will have access to the CRF. The online CRF directly transfer data to the database. Personal code number of the participants will be available in the study database (BC/GENE), where all parameters from the visits and CRF will be inserted. All laboratory parameters will also be kept locally and in the database. Login and Password will be limited to the principal investigator (Helena Elding Larsson), those personally working with the study and the DSMB.

9.2 Blood samples

All blood samples will be kept in -80 freezers at CRC, Malmö, Lund University, Sweden. The samples will be registered in the Region Skåne Biobank 136 in the Biobank Department BD31 under sample collection DIAPREV-IT 2. The samples will be analyzed regularly for islet cell autoantibodies and chemical analyses.

10 STATISTICAL ANALYSES

Children screened in DiPiS, TEDDY, TEDDY relatives or Trial Net will be invited from 4 years of age if repeatedly positive for GAD65Ab and at least one additional type 1 diabetes-associated autoantibody. About 10 % of the autoantibody positive children in DiPiS are expected to decline to participate. We expect that 50 % of children with more than one positive islet cell autoantibody will develop type 1 diabetes within 5 years.

10.1 Randomization of participants

Eligible participants will be randomized to active treatment or placebo, with 50 % of the participants in each of the two treatment arms. The distributor of Diamyd® (Penn Pharmaceuticals Ltd) will do the randomization for the study. Treatment will be stratified at randomization for the following groups:

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

An independent statistician will perform the randomization procedures. The randomization code for each child will be preserved in envelopes in a locked safe. The treating physician might break the code in cases of emergency. Further the DSMB will have access to a randomization list.

10.2 Statistical considerations and analytic plan

Analysis of study data will be conducted to address both safety and efficacy of the treatment. A Statistical Analytic Plan (SAP) will be developed before the statistical analyses.

The primary objective is to evaluate if Diamyd may delay or stop the autoimmune process leading to clinical type 1 diabetes in children with ongoing and persistent beta cell autoimmunity as indicated by multiple positive islet cell autoantibodies. The secondary objective is to demonstrate that Diamyd is safe in children at risk for type 1 diabetes. The subjects will be followed for 5 years. The study will be kept blinded for parents and participating children, investigators and study personnel during the entire study period.

Data from the 80 children in DiAPREV-IT 2 will be analysed when the full cohort have been followed for 5 years.

The analysis of efficacy will be carried out five (5) years after the first injection. The primary endpoint is the proportion of children developing clinical typ 1 diabetes during the five year follow-up in the two treatment groups.

Children will be staged for glucose metabolism at baseline screening. As secondary endpoints we will use

1) progression from normal to impaired glucose metabolism, defined as any of a) F-glucose ≥ 6.1 mmol/L, b) maximum p-glucose at 30, 60, 90 minutes ≥ 11.1 mmol/L in the OGTT, c) 120 min p-glucose ≥ 7.8 mmol/L on OGTT, d) HbA1c ≥ 39 mmol/mol. Impaired glucose metabolism has to be confirmed at a second visit. This endpoint will be used in the group of children with normal glucose metabolism at baseline screening
2) Progression of impaired glucose metabolism from one or several of the above variables to additional signs of reduced glucose metabolism, confirmed at a second visit. This endpoint will be used in the group of children with impaired glucose metabolism at baseline.

The exploratory endpoints are safety as well as the proportion of subjects in the two treatment groups who develop type 1 diabetes, according to the criteria below, after 1, 2, 3, 4 and 5 years as well as time to clinical type 1 diabetes onset from baseline. As exploratory endpoints we will use change from basline of 1) first-phase insulin response and K-value on IvGTT from baseline, 2) fasting, maximum and 2 hours C-peptide levels on OGTT as well as AUC, 3) fasting, maximum and 2 hours plasma-glucose and AUC glucose from OGTT and 4) HbA1c. Change in these variables will be compared across the two treatment groups after 1, 2, 3, 4 and 5 years.

The diagnosis of diabetes will follow the criteria developed by the World Health Organization (WHO) and the American Diabetes Association (ADA), also recommended by the International Society for Pediatric and Adolescent Diabetes (ISPAD):

1) A casual venous plasma glucose of ≥ 11.1 mmol/L (or capillary plasma glucose ≥ 12.2 mmol/L) and symptoms of diabetes (polyuria, polydipsia, unexplained weight loss)
   or
2) A venous fasting glucose of ≥ 7 mmol/L.
   or
3) A venous plasma glucose of ≥ 11.1 mmol/L (or capillary plasma glucose ≥ 12.2 mmol/L) 2 hours after glucose load in an oral glucose tolerance test (OGTT .

In the absence of unequivocal hyperglycaemia, the result must be confirmed on a subsequent day either by repeated sampling as above or by an OGTT. Since the children in DIAPREV-IT 2 will be closely followed, we expect to diagnose diabetes at an early stage, when the first

sign of diabetes usually is an increased postprandial plasma-glucose value or 2 hours after OGTT (values ≥ 11.1). Therefore, criteria 1 and/or 3, confirmed the subsequent day, will be used for diagnosis of diabetes in DIAPREV-IT 2.

Children with positive islet cell autoantibodies who develop type 1 diabetes have been reported to have gradually deteriorating glucose tolerance with declining C-peptide levels two hours after the glucose load in OGTT over a period of at least 2 years before onset of disease, despite the fact that fasting C-peptide levels remained stable [43]. Another early sign of progression towards type 1 diabetes is a reduced first-phase insulin response at IvGTT [9,43]. Therefore, analyses of change in C-peptide levels and plasma-glucose values two hours after OGTT as well as first-phase insulin response in the annual IvGTT will be compared between the treatment groups, as a measure of efficacy.

Analyses will be performed by using parametric statistics. If criteria of normality of variables are not met (e.g. Kolmogorov-Smirnov tests) non-parametric statistics of Wilcoxon type will be used. Four-fields tables will be analysed using the Fisher´s exact test. Time to diabetes will be analysed using life-table analyses, such as Kaplan-Meier and Cox regression. Analyses of efficacy will be performed as well on per-protocol (PP) as on intention-to-treat (ITT) basis. In ITT the last observation carried forward will be applied.

10.3 Power calculation

In this study we will include 80 children, 40 in each of the study arms.
We expect that 50 % of the untreated children with multiple autoantibodies will develop type 1 diabetes within 5 years. This frequency has previously been reported equal among relatives to diabetes patients and in the general population. If 20 % of the treated children will develop type 1 diabetes within the same period of time, we will have a power of 82 % with alfa=5 % with a group of 40+40=80 children. P value <0.05 will be used as significance level.

As exploratory values of effect, first phase insulin response, fasting and stimulated C-peptide levels, plasma-glucose and HbA1c, are used. These variables will be analysed to reveal differences of change from baseline between the untreated and treated group of children.

10.4 Drop out

Participating children may drop out from the study due to change in residence. The parents can also withdraw the informed consent whenever they want within the study period.

11 MONITORING AND QUALITY CONTROL

11.1 CRF

All data obtained at the visits will be reported in study specific online CRF's. The CRF's will be developed by the sponsor prior to the start of the study. All investigators who are authorized to fill out the CRF's will be listed with name and signature in the 'Investigator signature list' prior to the study start.

The online CRF enables tracking of changes and a full log of persons that have been editing data. The results from the CRF are forwarded directly into the database (BC/OS) The results from the CRF will be inserted in a database (BC/GENE). The printed and signed CRF will be kept locally at a safe place, to enable monitoring and quality control.

11.2 Patient journals

Patient journals will be established at each of the participant's visits.

11.3 Monitoring and Quality control

The monitoring of the study is made to ensure the scientific integrity, the data quality, the safety and integrity of the participating subjects and that the study is compliant with the current version of the Declaration of Helsinki (Appendix 1 in the protocol).

The Sponsor will delegate monitoring and quality control of the study to the Competence Centre for Clinical Research (RSKC) in Lund. RSKC is a center independent and detached from the study and will be responsible for on site monitoring of the study before, during and after the study. RSKC will control that the study is performed according to the rules of Good Clinical Practice (GCP).

To assure the accuracy and completeness of the data recorded in the trial, the monitor will compare CRFs with medical records and other relevant documentation during the on-site monitoring visits. The monitor must therefore be allowed direct access to all source data according to ICH GCP to confirm that required protocol procedures are being followed and check consistency between patient record and CRF data. Incorrect or missing entries into the CRFs will be queried and must be corrected. Study monitoring will not jeopardise patient confidentiality.

11.4 Quality Assurance

During or after the study are 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.

12 REGULATORY AN D ETHICAL ASPECTS

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 principles of informed consent as stated in the current revision of the Declaration of Helsinki (Edinburgh 2000) will be implemented in this study. The study will be conducted in

compliance with ICH GCP and local laws and regulations relevant to the use of new therapeutic agents.

All participating children and their parents/guardians are to provide written informed consent in accordance with the Declaration of Helsinki and the applicable national laws.

The participant and parent/guardian will sign and date the Informed Consent Form (ICF) before entering the trial, *i.e.*, before the first injection of substance is given. The investigator will explain the nature, purpose, and risks of the trial and provide the participant and the parents with a copy of the Patient Information Sheet. The participant and the parent/guardian will be given sufficient time to consider the trial's implications before deciding whether to participate. The participant and parent/guardian will be provided with a copy of the signed consent form. The investigator will document the process of consent. The investigator is responsible for obtaining the participant's and/or guardian's freely given written consent, including date, and thereafter sign and date the consent form by her/him before any study related procedure is performed.

It is the responsibility of the investigator to apply for and obtain written approval from the relevant Ethics Committee before the start of the study.

All participants are covered by the patient insurance in Region Skåne, Sweden.

12.1 Participant confidentiality

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

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 children 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.

12.2 Amendments

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 humans, 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 child's and parents/guardians 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.

Should amendment directly affect the participant'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 participant/parent must agree to sign this amended form verifying that they re-consent to continue their participation in the trial.

12.3 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.

12.4 Study report

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

12.5 GCP

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

13 PUBLICATION OF RESULTS

It is intended that the results from the study will be published in referee reviewed scientific medical journals.

14 DOCUMENTATION, RECORD KEEPING AND ARCHIVING

Every participant will be given a specific study-ID. The information from the visits will be recorded in Case Report Form (CRF). The information will thereafter be recorded in the database of DIAPREV-IT (BCOS). The study journal will be kept safely at each study site. The source documents will be made available to the DSMB and authority auditors upon request.

The investigator will arrange for the retention of the Investigator File for at least 10 years after the completion or discontinuation of the trial. Patient hospital files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 10 years.

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15 INVESTIGATOR SIGNATURE

Investigator's Statement:

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

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

Trial DIAPREV/2014 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 (Edinburgh 2000).

Coordinating Investigator's Name:
[redacted]

Coordinating Investigator's Title:
MD, PhD, Docent (Associate Professor)

Coordinating Investigator's Signature:

……..……

Date: ……..

APPENDIX 1, DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research, which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding,

sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the

impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should

state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists. (Note 1)

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study. (Note 2)

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new

information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Note 1 (Paragraph 29)

The WMA hereby reaffirms its position that extreme care must be taken in making use of a placebo-controlled trial and that in general this methodology should only be used in the absence of existing proven therapy. However, a placebo-controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:

- Where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety of a prophylactic, diagnostic or therapeutic method; or

- Where a prophylactic, diagnostic or therapeutic method is being investigated for a minor condition and the patients who receive placebo will not be subject to any additional risk of serious or irreversible harm.

All other provisions of the Declaration of Helsinki must be adhered to, especially the need for appropriate ethical and scientific review.

Note 2 (Paragraph 30)

The WMA hereby reaffirms its position that it is necessary during the study planning process to identify post-trial access by study participants to prophylactic, diagnostic and therapeutic procedures identified as beneficial in the study or access to other appropriate care. Post-trial access arrangements or other care must be described in the study protocol so the ethical review committee may consider such arrangements during its review.

APPENDIX 2: POST DIAGNOSIS INTERVENTION

POST DIAGNOSIS INTERVENTION AND FOLLOW-UP
Post-diagnosis intervention and follow-up of children participating in DiAPREV-IT 2, who develop type 1 diabetes during the study period.

Background:
Pending study drug shelf life, children developing type 1-diabetes within the study period may be offered to continue in the trial in a post-diagnosis intervention protocol (PDIP) where all participants diagnosed with type 1 diabetes will receive additional injections in the following manner:

Within 4 months since diabetes diagnosis, participants will receive one injection of Diamyd® 20 µg on Day 1 in the Post Diagnosis Follow-up, followed by a second injection of Diamyd® on Day 30, in a prime and boost fashion (a total received dose of 40 µg Diamyd® for participants who pre-diagnosis was randomized to receive placebo and a total received dose of 80 µg Diamyd® for participants who pre-diagnosis was randomized to receive Diamyd®).

All children that are included in the post diagnosis follow-up will be discontinued from the original prevention protocol to be followed thoroughly for safety and efficacy according to the post-diagnosis intervention protocol for 15 months following the first injection of Diamyd® in the PDIP.

Post-diagnosis intervention protocol:
At the time of clinical onset of type 1 diabetes, treatment with Diamyd® will be offered. The first injection will be administered within 4 months after diabetes diagnosis. The child will be followed according to the "Post-diagnosis intervention protocol" below.

Post-diagnosis intervention protocol:

Post-diagnosis visit P1 (within four months of diagnosis):
Fasting C-peptide and p-glucose
Insulin regimen and HbA1c
Mixed meal tolerance test
Hematology
Chemistry
Islet autoantibody analyses (GADA, IA-2A, IAA, ZnT8A)
T-cell analyses
Physical exam

Injection one of Diamyd®
Adverse event report
Diary

Post-diagnosis visit P2 (30 days ± 7 days from post-diagnosis visit 1)

Hematology
Insulin regimen and HbA1c
Chemistry
Physical exam including neurological assessment
Injection of Diamyd® or placebo pending assigned treatment group in DIAPREV-IT
Adverse event report
Diary

Post-diagnosis visit P3 (3 months ±14 days from post-diagnosis visit 1)

Fasting C-peptide and p-glucose
Mixed meal tolerance test
Hematology
Insulin regimen and HbA1c
Chemistry
T-cell analyses
Physical exam, including neurological assessment
Adverse event report
Diary

Post-diagnosis visit P4 (9 months ±14 days from post-diagnosis visit 1)

Fasting C-peptide, insulin and p-glucose
Mixed meal tolerance test
Hematology
Insulin regimen and HbA1c
Chemistry
T-cell analyses
Physical exam, including neurological assessment
Adverse event report
Diary

Post-diagnosis visit P5 (15 months' ±14 days from post-diagnosis visit 1)

Fasting C-peptide and p-glucose
Mixed meal tolerance test
Hematology
Insulin regimen and HbA1c
Chemistry
T-cell analyses
Physical exam, including neurological assessment
Adverse event report
Diary

	Visit P1 **Day 1** ***Within 4 month of diagnosis***	**Visit P2** **Month 1** **(Day 30)** ***30 +/-7 days from visit A1***	**Visit P3** **Month 3** ***3 months +/- 14 ~~7~~ days from visit P1***	**Visit P4** **Month 9** ***9 months +/- 14 days from visit P1***	**Visit P5** **Month 15** ***15 months +/- 14 days from visit P1***
Study Drug Administration	X	X			
Medical History	X	X	X	X	X
General Physical Exam	X	X	X	X	X
Concomitant Medication	X	X	X	X	X
Weight, Height	X	X	X	X	X
Vital signs (BP)	X	X	X	X	X
Blood Sampling:					
Hematology, Chemistry	X	X	X.	X	X
Cellular analyses	X	X	X	X	X
GADAb, IA-2Ab, ZnT8Ab, IAA	X	X	X	X	X
HbA1c, insulin regimen	X	X	X	X	X
Plasma Glucose	X		X	X	X
MMTT (Mixed Meal Tolerance Test) including C-peptide p-glucose	X		X	X	X
Neurological Assessment	X	X	X	X	X
Adverse Events	X	X	X	X	X
Diary	X	X	X	X	
Questionnaire to child about the study procedures					At the final visit

APPENDIX 3
PROTOCOL ADDENDUM 2017-08-25

As described in the original protocol, the aim of DiAPREV-IT 2 is to test if immune tolerance with two doses of Diamyd® prevent or delay type 1 diabetes in children with multiple islet autoantibodies.
The primary objective is to evaluate if Diamyd®, in children treated with relatively high dose vitamin D, may delay or stop the autoimmune process leading to clinical type 1 diabetes in children with ongoing persistent beta cell autoimmunity as indicated by multiple positive islet cell autoantibodies.
The secondary objective is to demonstrate that Diamyd® is safe in children at risk for type 1 diabetes.
Primary and secondary endpoints are described in detail in the protocol.

During 2017 we unblinded and analyzed data from the first study of Diamyd® in children with multiple islet autoantibodies Diaprev/2008, EuCT 2008-007484-16. In this group of 50 children we did not find any effect on delaying or preventing type 1 diabetes, while GADA increased. This has been reported, through a written report, to the MPA during June 2017.

Since the cohort of children, inclusion and exclusion criteria and dose of Diamyd® are all equal in the current study, with the exception that all children (unrelated to study arm) are treated with Vitamin D in the current protocol, the rational for the current study has changed on basis of the findings in the Diaprev/2008 study. We do not find it probable that the current study will delay or prevent type 1 diabetes, but it may have effects on the immune system that can be explored in mechanistic studies.

Therefore, from 2017-08-25, we propose the following addendum to the protocol:

- Inclusion is stopped – a total of 26 children have up to date been included and given study drug.
- Included children are followed up to two years (24 months) after the first dose of Diamyd®, according to revised protocol below.
- Children developing diabetes are not offered Diamyd® after diagnosis.
- Children already participating in the post diagnosis protocol are followed for 9 months according to revised post diagnosis protocol below.
- Main focus on analyses after the closure of the study will be secondary variables and mechanistic studies.
- All included study subjects will be informed and reconsent to the change in follow-up, if currently still followed in the study.

Schedule for the remaining two year follow-up:

Event	**Visit 0** **Information and consent**	**Visit 1** **Day 1** ***30 +/- 7 days from visit 0***	**Visit 2** **Month 1** ***30 +/-7 days from visit 1***	**Visit 3** **Month 3** ***3 months +/- 20 days from visit 1***	**Visit 4** **Month 6** ***6 months +/-20 days from visit 1***	**Visit 5** **Month 9** ***9 months +/- 20 days from visit 1***	**Visit 6** **Month 12** ***12 months +/- 20 days from visit 1***	**2 Years Follow up** **Every 3rd or 6th month** ***Every visit +/- 20 days from visit 1***
Informed Consent	X							
Randomization		X						
Diamyd/Placebo Administration[a]		X	X					
Start of Vitamin D	X							
Medical History	X							
General Physical Exam	X	X	X	X	X		X	Last visit
Concomitant Medication	X	X	X	X	X		X	
Weight, Height	X	X	X	X	X		X	Every 6th month
Vital signs (BP)	X	X	X	X	X		X	Every 6th month
Blood Sampling:								Every 6th month
Hematology, chemistry, including calcium	X	X	X	X	X	X	X	Every 3rd month
25 OH-vitamin D3	X	X			X		X	Every 6th month
Cellular analyses	X		X		X		X	Every 6th month
GADA, IA-2A, ZnT8A, IAA	X	X	X	X	X	X	X	Every 3rd month
C-peptide	X Fasting + stimulated	X Fasting + stimulated	X Random	X Random	X Fasting + stimulated	X Random	X Fasting + stimulated	Every 6th month Fasting + stimulated
HbA1c	X	X	X.	X	X	X	X	Every 3rd month
Plasma Glucose	X	X	X	X	X	X	X	Every 3rd month
OGTT/IvGTT	IvGTT	OGTT			OGTT		IvGTT	Every 6th month IvGTT/OGTT
TPOAb, ThglAb, tTGAb	X						X	Every 12th month
Urine Analysis	X	X	X	X	X		X	Every 6th month
Neurological Assessment	X		X	X	X		X	Last visit
Injection Site Inspection		X	X	X	X			

Adverse Events		X	X	X	X		X	Last visit
Diary		X	X	X	X		X	Last visit
Questionnaire to child about the study procedures		X						Last visit

Schedule for the remaining post diagnosis protocol:

	Visit P1 Day 1 ***Within 4 month of diagnosis***	**Visit P2 Month 1 (Day 30)** ***30 +/-7 days from visit A1***	**Visit P3 Month 3** ***3 months +/- 20 days from visit P1***	**Visit P4 Month 9** ***9 months +/- 20 days from visit P1***
Study Drug Administration	X	X		
Medical History	X	X	X	X
General Physical Exam	X	X	X	X
Concomitant Medication	X	X	X	X
Weight, Height	X	X	X	X
Vital signs (BP)	X	X	X	X
Blood Sampling:				
Hematology, Chemistry	X	X	X.	X
Cellular analyses	X	X	X	X
GADAb, IA-2Ab, ZnT8Ab, IAA	X	X	X	X
HbA1c, insulin regimen	X	X	X	X
Plasma Glucose	X		X	X
MMTT (Mixed Meal Tolerance Test) including C-peptide p-glucose	X		X	X
Neurological Assessment	X	X	X	X
Adverse Events	X	X	X	X
Diary	X	X	X	X
Questionnaire to child about the study procedures				At the last visit