CLINICAL STUDY PROTOCOL

Study title

A pilot study on safety, feasibility and insulin-promotion by intra-inguinal lymph node injections of glutamic acid decarboxylase (GAD) in patients with LADA type of diabetes.

Protocol code: GADinLADA
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Sponsor
Dept of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway.

Sponsor's representative, project manager and investigator
Ingrid K Hals. PhD. Postdoctor at Dept of Clinical and Molecular Medicine, NTNU
Gastrosenter 3. et, St Olavs Hospital, Trondheim, Norway.
E-mail: [redacted]
Phone:

Sponsor's medical expert and Principle Investigator
Chandima Balasuriya, MD, PhD. Endocrinologist and specialist in internal medicine.
Dept of Endocrinology, St Olavs Hospital, Trondheim University Hospital, Norway.
E-mail: [redacted]
Phone:

Overall responsible for the trial site related medical decisions
Inger Karin Lægreid, MD, PhD. Head of Medisinsk Klinikk, St Olavs Hospital, Trondheim University Hospital, Norway.
E-mail: [redacted]
Phone:

Study site
Dept of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU, / St Olavs Hospital, Trondheim University Hospital. Norway.

STUDY PROTOCOL VERSION HISTORY
Version 1: 17th July 2019
Version 2: 10th September 2019
Version 3: 8th October 2019 (Substantial amendments to Version 2, Protocol amendment # 1)
Version 4: 12th November 2019
Version 5: 5th November 2019
Version 6: 19th December 2019
Version 7: 6th March 2020
Version 8: 10th July 2020 (Substantial amendments to Version 7, Protocol amendment #2)
Version 9: 29th January 2021 (Substantial amendments to Version 8, Protocol amendment #3)

List of abbreviations

AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area Under the Curve
BALAD	Behandling Av LADA (Treatment of LADA)
CCR	CC Chemokine Receptor
CD	Cluster of Differentiation Antigen
CRF	Case Report Form
CTLA-4	Cytotoxic T-Lymphocyte Associated Protein 4
DPP-4	Dipeptidylpeptidase-4
eCRF	Electronic Case Report Form
FOXp3	Forkhead box p3
GAD	Glutamic Acid Decarboxylase
GADA	Glutamic Acid Decarboxylase Antibody
GCP	Good Clinical Practice
GSCT	Glucagon-Stimulated C-peptide Test
HbA1c	Hemoglobin A1c
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
ICH	International Council for Harmonisation
Ig	Immunoglobulin
IL	Interleukin
IFN	Interferon
LADA	Latent Autoimmune Diabetes in Adults
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MHC	Major Histocompatibility Complex
MMTT	Mixed Meal Tolerance Test
NPH	Neutral Protamine Hagedorn
PBMC	Peripheral Blood Mononuclear Cells
RHA	Regional Health Authority
SAE	Serious Adverse Event
SDS	Source Data Verification
SPS	Stiff Person Syndrome
SUSAR	Suspected Unexpected Serious Adverse Reaction
TFS	Trial Form Support AB
Th2	T helper type 2
TNF	Tumor Necrosis Factor

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1. GENERAL INFORMATION

1.1 Persons authorized to sign the protocol and the protocol amendments

- Ingrid K Hals (Sponsor's representative, project manager and investigator)
  Dept of Clinical and Molecular Medicine, NTNU, Norway.
- Chandima Balasuriya (Sponsor's medical expert, Principle Investigator and responsible for all trial-site related medical decisions), St Olavs Hospital, Trondheim.
- Anneli Björklund (Principle Investigator), Karolinska Institutet, Stockholm.

1.2 Investigators responsible for conducting the trial

In Norway:

- Ingrid K Hals, PhD
  Dept of Clinical and Molecular Medicine, NTNU, Trondheim.
- Chandima Balasuriya, MD, PhD
  Dept of Endocrinology, St Olavs Hospital, Trondheim University Hospital.

In Sweden:

- Anneli Björklund, MD, PhD, Akademiskt Specialistcentrum, Centrum for Diabetes, Stockholm and Dept of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital Solna, 171 76 Stockholm.

1.3 Other Co-investigators

- Valdemar Grill. MD, Senior Professor, Department of Clinical and Molecular Medicine, NTNU, Trondheim, Norway. Specialist in internal medicine and chief physician at department of Endocrinology, St Olavs Hospital, until 2017.
- Johnny Ludvigsson. MD, Senior Professor, Div of Pediatrics, Dept of Clinical and Experimental Medicine, Linköping University Hospital, Linköping, Sweden.

1.4 Clinical laboratories and other medical and/or technical departments and/or institutions involved in the trial

Sampling for hematology and clinical chemistry:

- Avd for medisinsk biokjemi, Laboratoriemedisinsk klinikk, St Olavs Hospital, Trondheim, Norway.
- Karolinska Universitetslaboratoriet, Stockholm, Sweden.

For the administration of the investigational product GAD-alum:

- Dept of Radiology and Nuclear Medicine, St Olavs Hospital, Trondheim University Hospital, Norway.
- Dept of Function Picture and Function, Karolinska University Hospital, Solna, Sweden.

For the HLA classification:

- Oslo University Hospital Rikshospitalet, Dept of Immunology and Transfusion Medicine, Oslo, Norway.

For immunological analyses, including the classification and analyses of T-cells:

- Dept of Clinical Experimental Research, Linköping University Hospital, Linköping, Sweden.

1.5 Monitor

In Norway: Clinical Trial Unit, Faculty of Medicine and Health Sciences, St Olavs Hospital and NTNU.
In Sweden: Karolinska Trial Alliance.

1.6 Progress plan

Recruitment: Q1, 2020 – Q1, 2021
Intervention and follow-up: Q2, 2020 – Q1, 2022
End of trial: Q2, 2022

The trial will be conducted in compliance with the protocol, ICH GCP guidelines and the applicable regulatory requirements and in accordance with the Brazil (2013) amendment to the Declaration of Helsinki 1964.

2. SYNOPSIS

Latent autoimmune diabetes in adults (LADA) is a common type of diabetes which often masquerades as type 2 diabetes because patients clinically do not need insulin for a significant time period after diagnosis. Still these patients progress with time in large numbers to insulin dependency; this is due to the impact of autoimmunity, which is manifested by positivity for type 1 diabetes-like antibodies, in particular anti-GAD (GADA). Our recently completed 21 month clinical trial (BALAD) shows that patients displaying high levels of GADA successively lose their capacity for insulin secretion regardless of tested treatment alternatives (insulin before clinically needed or a DPP-4 inhibitor, both as add-ons to metformin). Treating autoimmunity directly is thus needed to halt diabetes progression in these high GADA titer LADA patients.

Therefore, together with co-investigators at Karolinska University Hospital (Anneli Björklund) and Linköping University (Johnny Ludvigsson) we propose a vaccination study testing for desensitization of the autoimmune process by intra-inguinal injections of GAD-alum. Encouraging results from a pilot study in type 1 diabetic children and adolescents support the rationale for a similar study in LADA.

We plan to enroll approximately 15 patients newly diagnosed with LADA and who display high levels of GADA to receive three intra-inguinal injections of GAD-alum one month apart. The company Diamyd Medical AB (Stockholm) will collaborate by providing the GAD-alum to be injected. We will follow patients during one year for safety (no safety concerns were raised in the afore-mentioned study) and immunological, mainly T-cell, responses. In addition, we will follow beta cell function as tested by C-peptide glucagon and mixed meal tolerance tests.

The study is a pilot one that does not include a placebo arm. A positive outcome (no safety concerns, a robust immunological response and a favorable time course of beta cell function compared to a historical control, i.e. BALAD, will pave the way for a placebo-controlled phase II/III study.

3. INTRODUCTION

3.1 Investigational medicinal product

The investigational product in this trial is GAD-alum (Diamyd®). The active ingredient is recombinant human glutamic acid dehydrogenase (rhGAD65), formulated in aluminium hydrogel. GAD-alum will be available from the company Diamyd Medical AB in Stockholm, Sweden.

3.2 Non-investigational medicinal product

The non-investigational medicinal product in this trial is vitamin D (1 tablet/day, total daily dose of 2000 IE) given per os from day -30 through day 90.

Trade name:" Divisun 2000 IE", Supplier: Study site Stockholm: Meda, Solna, Sweden. Study site Trondheim: Meda, Allerød, Denmark.

3.3 Background and rational

3.3.1 Urgent need for a treatment that counters autoimmunity in LADA

Latent Autoimmune Diabetes in Adults (LADA) encompasses approximately 10% of phenotypic type 2 diabetic patients [1]. LADA patients are by definition not insulin dependent at diagnosis. However, very many of these patients experience a successive decline of their own insulin secretion, something that renders these patients insulin dependent within a few years. The demise of LADA patients' insulin-producing beta cells is due to an autoimmune process that is similar to the autoimmune process in type 1 diabetes. The speed of beta cell demise is coupled with high levels of type 1 diabetes-like antibodies, in particular to antibodies against glutamic acid decarboxylase (GADA), which likely reflect the intensity of the autoimmune process [2, 3].

There is strong evidence that demise in beta cell capacity to release insulin couples to deterioration of metabolic control and that such deterioration in turn leads to an increased risk for complications of diabetes with serious consequences in terms of morbidity and mortality [4, 5]. In addition, reports indicate that LADA patients have worse metabolic control than other types of diabetes when comparing mortality [6, 7]. Consequently, there is an urgent need for therapies that counteract the ongoing destruction of beta cells in LADA patients. LADA is – as indicated above – a very common form of diabetes; this underscores the need for a therapy that works.

Very few studies, only 10 clinical trials listed on clinicaltrials.gov, have specifically investigated the extent to which different types of treatment could influence the decline of beta cell function in LADA patients (Clinicaltrials.gov as of February 15th 2019). The scarcity of trials and the resulting lack of evidence for best treatment in LADA has led to large regional variations in treatment.

In the recently published BALAD trial [8] we randomized LADA patients to add-on treatments with either insulin (primarily nighttime NPH insulin, i.e. intermediate acting insulin) or a DPP-4 inhibitor, sitagliptin (trade name Januvia), 100 mg daily. Here we show that early insulin treatment does not provide any added benefit when compared to sitagliptin. Further, following patient stratification at inclusion into low and high GADA titer groups, (cutoff 190 U/ml) results show a clear deterioration of stimulated C-peptide parameters in participants in those of the high GADA category. The decline in stimulated C-peptide amounted to a median of 38% compared with results obtained at the time of randomization.

Taken together these findings demonstrate the inability of standard therapeutic modalities to modify the impact of autoimmunity in LADA. We conclude that a new therapy is needed that directly counters autoimmunity in LADA patients displaying high titers of GADA.

3.3.2 GAD-vaccination – an alternative to other immunotherapies?

In the type 1 diabetes field, several immunomodulatory therapies have included both children at high risk to develop type 1 diabetes and children or adolescents with recent onset of the disease. Immunotherapies include testing the effects of antibodies against CD3 or CTLA-4 (abatacept). The results have been mixed, with trials showing no positive effects, and others showing improvement of insulin secretion (as assessed by stimulated C-peptide measurements). In general, beneficial effects have been short-lived and side effects frequently encountered [9].

Could a vaccination study with GAD provide an alternative to other trials that modulate diabetes-associated autoimmunity? Antigen therapy aims at presenting the antigen to the T-cells in the lymph nodes to get a new balance of the immune system and tolerance against the antigen. GAD can be regarded as an auto-antigen, as it is produced in the pancreatic islets from which this protein is released in response to beta cell stimulation. Hence, the effect of GAD-vaccination is achieved through antigen-specific reprogramming of immune cells to create tolerance to GAD rather than causing beta cell demise.

Studies have shown that administration of GAD can prevent diabetes in experimental animals [10]. The observed effect, which was observable even after the start of the autoimmune process, suggests that it might be possible to expect the same effect in humans.

3.3.3 Previous and ongoing GAD-vaccination trials

In a phase II study in LADA patients the subcutaneous administration of one low dose of GAD-alum, 20 µg, led to improved beta cell function for up to 2 years compared to the placebo treated group, with no side effects [11]. In addition, other doses were tried: 4 µg showed no effect, 100 µg showed a similar effect as 20 µg, while 500 µg showed no effect. None of the doses were associated with any adverse events. An association with changes in the ratio of CD4+CD25+/ CD4- CD25- cells was found, indicating a mechanism behind the beneficial effect [11].

These findings were followed up by a phase II study in children and adolescents with type 1 diabetes, which also showed beta cell preserving effects by subcutaneous GAD-alum injections. However, a phase III study did not reproduce these findings [12]. Further analysis of results from the phase III study indicated positive effects in subgroups of participants [13]. A meta-analysis of studies with subcutaneous GAD-alum injections showed altogether a positive effect on C-peptide parameters; these effects were however only modest and of questionable size to pursue [14].

However, the subcutaneous route of injection may be inferior to other routes in activating the desired immunological pathways. Desensitization studies in allergy patients indicate that intra lymphatic injections of antigen achieve desensitization better than other types of injections [15]. As this route of administration has the potential to maximize the immune response, lower doses of the allergen can be used. Inguinal lymph nodes are readily accessible in patients and are chosen as the site of administration of the GAD-alum.

Results with intranodal injections of GAD-alum are available from a 15-month proof-of-concept phase I/II clinical trial DIAGNODE-1 (NCT02352974) [16]. DIAGNODE-1, is an open-label trial of intralymphatic rhGAD therapy together with vitamin D supplementation for 12 patients aged 12 to 24 with residual beta cell activity diagnosed with type 1 diabetes within six months of inclusion. This study employs three low doses (4 µg each) of GAD-alum that are administered directly into an inguinal lymph node using ultrasound guided injections. The disease progression appears very positive in terms of partial remission, levels of stimulated C-peptide, HbA1c and insulin dose. Using the compound variable insulin dose-adjusted HbA1c (IDAAC) as a marker, 11 of 12 patients attained partial remission after 15 months (IDAAC ≤9) (Ludvigsson J, unpublished data). In comparison, less than 20% of children and adolescents with new-onset T1D in a cohort (N=3657) followed over six years were in partial remission 18 months after diagnosis [17].

After 15 months, stimulated C-peptide area under the curve (AUC) decreased by only 19% in treated patients. In contrast, a matched untreated control population of 27 patients (historical controls) selected from the placebo arm of a different Diamyd trial in the same patient age-range showed an average decrease of between 40% and 50% [18]. The positive effects on beta cell function reported in the DIAGNODE-1 trial are accompanied by a reduction in HbA1c by 18% (compared to a 15% increase in controls), and average insulin dose increase of only 6% (compared to more than a 50% increase in controls) (Ludvigsson J, unpublished data).

Immunological response to intralymphatic administration was characterized by a relative reduction of IgG1, with a shift towards IgG2, IgG3 and IgG4 subclasses after 3 intra-lymphatic injections of GAD-alum (Ludvigsson J, unpublished data). This finding is significant as both IgG2 and IgG4 have low respectively no ability to bind complement and an IgG4 response is associated with long-term immunotherapy response in the allergy field [15]. Further, while IgG1 is the dominant GAD antibody subclass in type 1 diabetes, IgG4 is more common in

LADA, suggesting that subclass distribution is an important aspect of the underlying disease mechanism [19].

Importantly, comparing a few patients in the DIAGNODE-1 trial to patients that had received a subcutaneous injection of GAD-alum, indicate that the clinical effects correlate with an immune response which is stronger and more pronounced in its Th2 responses than that from subcutaneous GAD-alum administration [20].

3.3.4 Rationale for the use of GAD-alum in the treatment of LADA

A large part of LADA patients will within a few years after diagnosis develop a need for insulin treatment. Exogenous insulin cannot cure diabetes, neither does it represent an ideal therapy, as even well-treated and monitored patients develop significant complications and have increased morbidity and mortality. A therapy that could counteract beta cell demise completely, or at least to an extent so that levels of blood glucose could be kept in the normal range, is therefore highly desirable.

The underlying pathology in type 1 diabetes and LADA is thought to be closely similar. Also, type 1 diabetes and LADA seem genetically similar, in that they share the same frequency of HLA genes conferring either susceptibility or protection to type 1 diabetes [21]. However, type 1 diabetes and LADA do represent two separate sub-groups of autoimmune diabetes. Differences between the two subgroups, like the age of onset (for type 1 diabetes usually in childhood/youth years vs. for LADA most often after 35 years of age) and the intensity of autoimmunity, might impact on the response to the GAD-vaccine. Hence, safety and feasibility of intranodal treatment with GAD-alum has to be confirmed also in LADA individuals in order to support a larger trial in the LADA population.

The proposed trial will be the first to test for the effect of intranodal injections of GAD-alum in LADA subjects. The study is a unique one and has the potential to provide a therapy that halts or delays autoimmunity-governed beta cell destruction in LADA patients.

3.3.5 Rationale for vitamin D supplementation

Oral vitamin D supplementation will be given only to those patients with vitamin D levels below 100 nmol/L at the screening visit.

Experimental evidence indicates that vitamin D may play a role in the defense against type 1 diabetes through its effects on the immune system. Vitamin D has been shown to affect antigen presenting cells (APCs) and T-cells in such a way that an anti-inflammatory and Th2 inclined immune response is favored, including an increase in the T-regulatory cell response and the secretion of IL-10 [22] [22-25]. Mechanistic studies have shown that vitamin D modulates dendritic cell maturation in vitro and in vivo [26-29], facilitates a shift from a Th1 to a Th2 immune response [30], and promotes a switch from M1 to M2 macrophages [31]. Because of these properties, vitamin D has been used e.g. in cell therapy protocols to produce tolerogenic

immune cells. Vitamin D has further been shown to direct the immune response to a GAD65 peptide from a Th1 towards a Th2 profile in NOD mice [32].
Pre-conditioning with vitamin D is intended to avoid the negative impact of vitamin D insufficiency on the tolerogenic effect of the antigen specific Diamyd immunotherapy.

3.4 Safety and feasibility of GAD-vaccination together with vitamin D supplementation

In contrast to traditional immune suppression, GAD-alum treatment (administered subcutaneously or intralymphatic) is regarded as safe, very tolerable and with little or no discomfort for the patients. No treatment-related adverse events of GAD-alum have been reported except from mild and transient reaction at the injection site. Also, intralymphatic injections are reported as less painful than a venous puncture [16, 33].

Vitamin D in a dose of 2000 IU/day in children has been reported safe [34]. Additionally, a dose of up to 7000 IU/day given to children from 5 years of age with human immunodeficiency virus (HIV) did not raise any safety concerns [35]. We do not foresee that the vitamin D supplementation to the GAD-alum treatment would increase the theoretical risks of GAD-alum, such as acceleration of the autoimmune process, undesirable effects on the immune system, or neurological disease.

3.5 Choice of study drug administration and dosage

As mentioned, studies in allergy immunotherapy show that, compared to other routes of administration, injections of antigens directly into the lymph nodes leads to better efficacy of the treatment [15]. Encouraging results from the DIAGNODE-1 trial (see Section 3.3.3) as well as unpublished blinded safety data from an ongoing phase II trial (DIAGNODE-2, NCT03345004) in type 1 diabetes (Johnny Ludvigsson, personal communication) support the rationale for a similar study in LADA. Based on the experiences from DIAGNODE-1 and -2, we will in the proposed trial use the same route of administration, dosage and treatment intervals as in the DIAGNODE trials (for details, see Section 7.1 and Table 1). The DIAGNODE-1 trial shows that potential treatment-related changes in the immunological parameters of interest are detectable within 12 months from baseline. The total study period from baseline in the proposed trial will therefore be 12 months.

3.6 Study population

Study participants are to be men and women between 30-70 years of age, that have been diagnosed with LADA within the last 18 months, i.e. being GADA positive and insulin independent at baseline. Based on the findings in our recent trial, BALAD (see 3.3.1), we will include only those with high GADA titers (>190 U/ml).

Altogether approximately 15 LADA patients will be recruited from one clinic in Norway (St Olavs Hospital, Trondheim) and one in Sweden (Karolinska University Hospital, Stockholm). Recruitment will be through advertisement in journals for diabetic patients and information through other media outlets as well as by referrals from physicians treating patients for

diabetes. Given that LADA patients constitute 10% of the total diabetes population we foresee that the study will be fully recruited within 1 year.

4. TRIAL OBJECTIVES AND PURPOSE

The purpose of the trial is to evaluate the effects of 3 intra-nodal injections of GAD-alum, together with oral vitamin D supplementation, in a population of LADA patients with high GADA titers. Effects will be summarized at 5 and 12 months after the first injection.

- The primary objective is to evaluate safety and feasibility of this treatment regimen.
- Secondary objectives are to test if the treatment induces a strong GAD-specific immune response similar to what has previously been observed in type 1 diabetes patients and to test for indications of preservation of endogenous insulin production.

The pilot study will be performed in order to support the launch of a larger placebo controlled clinical trial in the LADA population.

5. TRIAL DESIGN

5.1 Primary endpoint

Variables for the evaluation of safety and feasibility:

1) Injection site reactions, skin reactions 1 hour post injection vs. before injection.
2) Occurrence of AEs, continuously registered and status summarized at 5 and 12 months after the first injection.
3) Laboratory measurements (hematology and clinical chemistry), status summarized at 5 and 12 months after the first injection vs. baseline.
4) Physical examinations, including neurological assessments, status summarized at 5 and 12 months after the first injection vs. baseline.
5) GAD65A titer in serum, levels at 5 and 12 months after the first injection vs. baseline.
6) Vital signs (blood pressure), status summarized at 5 and 12 months after the first injection vs. baseline.

5.2 Secondary endpoints

Variables for the evaluation of beta cell insulin secretion capacity and metabolic control. Stimulation tests: glucagon-stimulated C-peptide test (GSCT) [36] and mixed meal tolerance test (MMTT) [37].

1) Insulin secretion measured by glucagon- and MMTT stimulated C-peptide at baseline, and at 5 and 12 months after the first injection
2) Change in HbA1c from baseline to 5 and 12 months after the first injection
3) Change in fasting glucose from baseline to 5 and 12 months after the first injection.
4) Change in Fasting C-peptide between baseline and 5 and 12 months after the first injection

5) Change in maximum C-peptide during MMTT between baseline and 5 and 12 months after the first injection.

Variables for the evaluation of immunological response at 5 and 12 months vs. baseline:

6) Measurement of serum concentration of GAD65-specific IgG1, IgG2, IgG3 and IgG4 antibodies for all included patients

7) Measurement of supernatant concentrations of IL-1, IL-2, IL-5, IL-13, IL-10, IL-17, IFN-γ and TNF secreted during cultivation of PBMCs isolated from all included patients

8) Characterization of PBMCs with FACS using CD3, CD4, CD8, CD45RA, CCR7, CD25, CD127, FOXp3 at baseline

9) Analyze the proliferation of PBMCs isolated from all included patients during cultivation with vehicle, GAD65 and control antibody.

10) Other relevant variables.

5.3 Trial description

The proposed study is an open label Phase IIa feasibility trial. It is a pilot study that does not include a placebo arm. The time course of beta cell function in the trial will be compared to that in our recent trial, BALAD (described in 3.3.1), as a historical control. A positive outcome (i.e. no safety concerns, a robust immunological response and a favorable time course of beta cell function compared to BALAD) will pave the way for a placebo-controlled phase III study.

5.4 Time line and main study events

The project is expected to take 2 years. Given that LADA patients constitute 10% of the total diabetes population, our expectation is that the study could be fully recruited within 1 year. Each patient will then be followed for 12 months. Study close out, data cleaning and analysis, compilation of final report, etc. will take place during the final 6 months of the project. The schedule of the main study events is given in Table 1. The end of the trial is defined as the last visit of the last patient included in the trial and the time point where all data have been collected.

Table 1: Schedule of main events for the trial												
Event	**V1 Screening**	**V2**		**V3 Baseline**		**V4[a] M1**	**V5 [a] M2**		**V6 M5**		**V7 M12**	
DAY	**-44 to -60**	**-31**	**-30**	**1**	**2 (+2)**	**30 (±5)**	**60 (±5)**	**90**	**150 (±14)**	**151 (+2)**	**360 (±14)**	**361 (+2)**
Informed consent	X	X										
Demographics	X											
GAD-alum (4 µg) [a, b, c,]					X	X	X					
Vitamin D [d] start /end			Start					Stop				
Neurological assessment	X			X		X	X		X		X	
Concomitant medication	X			X		X	X		X		X	
Vital signs (BP)	X			X		X	X		X		X	
Injection site inspection; investigator/study nurse[e]					X	X	X					
AEs				X		X	X		X		X	
Glucagon test[c,f]			X		X					X		X
MMTT[f]		X		X					X		X	
	Blood and urine sampling for safety, genetics, vitamin D levels and immunology:											
Hematology	X			X		X	X		X		X	
Clinical Chemistry	X			X		X	X		X		X	
GAD65A titer[g]	X			X		X	X		X		X	
HLA characterization				X								
Vitamin D level	X	X		X		X	X		X		X	
Other immunological parameters[h]		X		X		X	X		X		X	
Creatinine	X			X		X	X		X		X	
	Blood sampling for diabetes status:											
Fasting C-peptide	X	X	X	X	X	X	X		X	X	X	X
Fasting glucose	X	X	X	X	X	X	X		X	X	X	X
Glucagon stimulated C-peptide and glucose			X		X					X		X
MMTT stimulated C-peptide		X		X					X		X	
MMTT stimulated glucose		X		X					X		X	
HbA1c	X	X		X		X	X		X		X	

[Abbreviations for Table 1: V= visit; M=month; GAD= Glutamic Acid Decarboxylase; BP=Blood Pressure; AEs=Adverse Events; MMTT=Mixed Meal Tolerance Test; GAD65A=Glutamic Acid Decarboxylase Antibodies; HLA=Human Leukocyte Antigen; HbA1c=Hemoglobin A1c]

a. Study drug administration: For visit 4 and 5 the visit date must be set in accordance with visit 3 and 4, respectively, so that the first, second and third doses will be 30 days apart (± 5days).
b. The GAD-alum injection directly into the inguinal lymph node is to be done by an appropriately qualified radiologist at the X-ray department at the study site by help of ultrasound technique.
c. On day two at visit 2, the glucagon test needs to be done before the injection of GAD-alum.
d. Supplementation with vitamin D starts at day -30, after the glucagon test has been performed, if the vitamin D serum levels are below 100 nmol/L (40 ng/ml) at screening. If the patient has vitamin D serum levels above 100 nmol/L (40 ng/ml) at screening, no Vitamin D supplementation will be given for that patient.
e. The investigator/study nurse will inspect the injection site before and after the injection is given and record any injection site reactions in the Case Report Form (CRF).
f. The MMTT and the Glucagon test must be carried out on separate days, as each test has to be performed in the fasting state.
g. Antibodies against IA-2 (islet cell antigen 2), ZnT8 (Zink transporter 8) and insulin will be measured at Baseline and at Visit 7.
h. Antibodies against SARS-CoV-2 will be measured at Visit 6 and Visit 7.

6. SELECTION AND WITHDRAWAL OF SUBJECTS

6.1 Informed consent

Potential study patients will receive both written and oral information about the study procedures, potential risks, and benefits. Before any study specific procedures may be performed, the patients must sign an informed consent form.

6.2 Medications other than the study drug

Antidiabetic medication in the form of metformin is acceptable before and during the trial. Study participants must be insulin independent at baseline, but if the need for insulin treatment develops during the trial, such treatment will be given. The need for insulin treatment will be based on clinical judgement after considering the following parameters:

- Increased levels of HbA1c, i.e. two measurements, performed 1 month apart, showing an increase of 22 mmol/mol vs. baseline
- Fasting blood glucose >10 mmol/L in at least 3 occasions during a single week
- Weight loss >2 kg vs. baseline.

6.3 Inclusion and exclusion criteria

Inclusion criteria:

1. Signed informed consent by the patient.
2. Diagnosis of LADA and diabetes debut within the last 18 months before inclusion. LADA should be defined by the criteria of age ≥30 years at the onset of diabetes, anti-GAD positivity and no clinical need for permanent treatment of insulin during the first 3 months after the diagnosis of diabetes.
3. Male or female between 30-70 years of age

4. Fasting C-peptid levels ≥ 0.3 nmol/l
5. High GADA titers (>190 U/ml)
6. Patients must be insulin independent at baseline by clinical judgement and C-peptide criteria
7. Antidiabetic medication in the form of metformin is acceptable for inclusion as well as medications not mentioned under exclusion criteria
8. Females must agree to avoid pregnancy and have a negative urine pregnancy test.
   Patients of childbearing potential must agree to use adequate contraception, until one (1) year after the last administration of GAD-alum. Adequate contraception is as follows:

   For females of childbearing potential:

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

   For males of childbearing potential:

   a. condom (male)
   b. abstinence from heterosexual intercourse

Exclusion criteria:

1. Current or previous treatment with immunosuppressant therapy (topical or inhaled steroids are accepted)
2. Continuous treatment with anti-inflammatory drug (sporadic treatment e.g. because of headache or in connection with fever a few days will be accepted)
3. Systemic treatment with glucocorticoids
4. Treatment with any vaccine, including influenza vaccine, within 1 month prior to planned first study drug dose or planned treatment with any vaccine up to 1 month after the last injection with study drug
5. Antidiabetic medication (metformin excepted)
6. Significantly abnormal hematology results at screening (i.e. anemia with hemoglobin < 12 g/L).

7. A history of epilepsy, head trauma or cerebrovascular accident, or clinical features of continuous motor unit activity in proximal muscles
8. Clinically significant history of acute reaction to vaccines in the past.
9. Renal disease (as defined by serum creatinine >150 μmol/l)
10. Serious cardiovascular events (myocardial infarction, stroke) within the last year preceding recruitment.
11. Participation in other clinical trials with a new chemical entity within the previous 3 months
12. A history of alcohol or drug abuse
13. Known HIV or hepatitis
14. Presence of associated serious disease or condition, including active skin infections that preclude intralymphatic injection, which in the opinion of the investigator makes the patient non-eligible for the study
15. Other serious chronic disease as judged by investigator.
16. Females who are lactating, are pregnant or intend to become pregnant.
17. Inability or unwillingness to comply with the provisions of this protocol
18. Deemed by the investigator not being able to follow instructions and/or follow the study protocol
19. Treatment with any other supplementation of vitamin D, marketed or not, or unwilling to abstain from such medication during the 120 days daily intake of Divisun (non-investigational medicinal product)

6.4 Withdrawal criteria

a) When and how to withdraw subjects from the trial

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

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

Complete withdrawal (i.e. stopping investigational product(s) and also continued efficacy and safety evaluations). In order to achieve as complete as possible 12 month follow-up, whenever feasible, patients that are considered for complete withdrawal should be asked if they could consider to return for a 12-month visit. Attempts should be made to contact patients lost to follow-up with respect to this.

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

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

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

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

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

Criteria for terminating the investigational product treatment/trial treatment:
Rapid increase in HbA1c or decline in C-peptide levels vs. historical controls. Evidence for induction of stiff person syndrome (SPS) or any adverse neurological or behavioural effects.

GAD-alum should not be given to the patient if the patient after inclusion in the study develops/experiences:

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

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

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

b) The type and timing of the data to be collected for withdrawn subjects

Intention-to-treat population
Patients will be included in the primary intention to treat population for analysis of efficacy if they receive at least 1 dose of GAD-alum and are assessed at a later visit.

Per protocol population
The subjects must have followed the protocol without any major violations. Any examinations/tests missed will be substituted with the last observation carried forward, but examinations/tests from not more than 1 visit may be lost.

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

c) Follow up of subjects withdrawn from investigational product treatment/trial treatment.
Withdrawals due to non-attendance must be followed up by the investigator to obtain the reason for non-attendance. Withdrawals due to any illness or AEs must be fully documented in the CRF.

7. TREATMENT AND FOLLOW UP

7.1 Study drug: dosage and interval

Enrolled participants will receive an intra-nodal injection of 4 µg GAD-alum three times. The first, second and third injection will be 1 month apart (Table 1). GAD-alum will be injected directly into an inguinal lymph node by a qualified radiologist at the X-ray department of the study site by help of ultrasound technique. The responsible investigator or study nurse will inspect the injection site prior to administration of GAD-alum. The patient shall remain in the vicinity of the study site for the next hour after the injection, and the injection site will be investigated again by the responsible investigator or study nurse 1 hour post injection. Attempts will be made to inject GAD-alum into the same lymph node each time. The time of the day for injection will be decided by the cooperating radiologist.

Supplementation with vitamin D starts at day -30 if the vitamin D serum levels are below 100 nmol/L (40ng/ml) at screening. If the patient has vitamin D serum levels above 100 nmol/L (40 ng/ml) at screening, no vitamin D supplementation will be given to that patient. Patients with vitamin D levels below 100 nmol/L (40 ng/ml) at screening will receive vitamin D (2000 IE per day) administered orally for 4 months (120 days in total). The patient should be instructed to return all unused vitamin D blisters/boxes at Visit 6.

7.2 Intervention

Patients will be followed for a total of 12 months during which their endogenous insulin production and immune response will be evaluated at regular intervals throughout the study period. A physician and a study nurse will be responsible for carrying out the events of the study. Study procedures and assessments are described in Table 1. Urine and blood samples will be taken for safety, diabetes status assessments, vitamin D levels and immunological assessments. Concomitant medication and demographics will be collected.

7.3 Discontinuation criteria

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

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

7.4 Supply of study drug

GAD-alum will be supplied as prepacked medication from Diamyd Medical (Stockholm, Sweden) to a local pharmacy (in Trondheim: Sykehusapoteket at St Olavs Hospital, in Stockholm: Pharmacy at Karolinska University Hospital, Solna, Stockholm). All dosing will take place in the hospital and handled only by trained and authorized study personnel. GAD-alum will be stored in a refrigerator at 2-8°C in a secure area (e.g. a locked cabinet or drug store room), protected from unintended use. The GAD-alum solution must never be frozen (i.e. exposed to temperatures below 0ºC). If any temperature deviations occur, please contact Diamyd Medical immediately (clinicaltrials@diamyd.com) and do not administer any GAD-alum solution that have been exposed to temperatures outside the acceptable temperature range without prior approval from Diamyd Medical.

7.5 Supply of non-investigational medicinal product

Vitamin D will be purchased and labeled by the local hospital pharmacy (in Trondheim: Sykehusapoteket at St Olavs Hospital, in Stockholm: Pharmacy at Karolinska University Hospital, Solna, Stockholm). It will be ordered through the electronic prescription system by the PI and picked up by the study participant at the local hospital pharmacy. The vitamin D used in the study will be:

Trade name: ”Divisun 2000 IE”, Supplier: In Stockholm: Meda, Solna, Sweden, in Trondheim: Meda, Allerød, Denmark.

Vitamin D kits should be stored at room temperature (< 25 ºC) in a secure area (e.g. a locked cabinet or drug storage room), protected from unintended use at the pharmacy.
All study medication will be labelled with information according to national and local regulations.

7.6 Study drug and non-investigational medication product accountability

GAD-alum and vitamin D must be retained in a safe place at all times of the study. Only personnel authorized by the responsible investigator will dispense GAD-alum and vitamin D, and the accountability is the responsibility of the responsible investigator.

The patient should be instructed to return all unused vitamin D in the blisters/boxes at Visit 6 and compliance to the vitamin D supplementation should be checked.

GAD-alum and vitamin D inventory (dispersing records) must be maintained at all times and always kept current. Used and unused medication must be stored at the site or at the pharmacy throughout the study. The investigator/pharmacist must keep record of the GAD-alum and vitamin D received, used and returned. Both the pharmacy and the study site are obliged to properly measure and record the storage temperature.

When the study is completed all unused and used study medication must be returned to the drug supplier unless the drug supplier has approved other arrangements. The remaining vitamin D will be handled and disposed of by the local pharmacies. **NB!** Before any study drug and

non-investigational medication product is returned and destructed the used vials and blisters/boxes should be monitored and accountability performed.

8. ASSESSMENT OF EFFICACY

8.1 Effect parameters

See also Section 5.2, secondary endpoints. Endogenous insulin production will be measured by glucagon- and mixed meal stimulation of C-peptide at baseline and after 5 and 12 months of intervention. Metabolic control will be evaluated by measurements of HbA1c and fasting glucose at all study visits. Immunological response will be assessed by measurements of GADA and the immunological parameters listed in Section 5.2. Blood samples for the assessment of immunological response will be collected at all study visits.

8.2 Methods for the recording of effect parameters

The patient must attend all study visits in the morning following an overnight fast (>10 hours, water allowed). Each visit should be completed as scheduled (see Table 1). However, for patients with evidence of an infection, the visit should be postponed until the patient has recovered.

*8.2.1 **Endogenous insulin production: Visit 2, 3, 6 and 7***

The MMTT [37] and the GSCT [36] must be performed according to the instructions in the specific laboratory manuals. The tests must be carried out on separate days, as each test has to be performed in the fasting state. Also, if the patient develops need for insulin treatment during the study period, the patient must not take short acting/direct acting insulin within 6 hours before the test. The patient is allowed to take base-insulin the day/night before, but not in the test morning and during the MMTT and the GSCT. If the patient does not fulfill the above mentioned criteria, the MMTT and GSCT should be rescheduled and the patient return to the study site within 5 days if possible.

MMTT: The patient will be given a standardized liquid nutrition mixture. Blood samples will be secured for glucose and C-peptide measurements at before and 0, 30, 60, 90 and 120 (±5) minutes after the meal.

GSCT: The patient will receive an intravenous injection of 0.5 mg glucagon. Blood samples will be secured both before and 6 minutes after the injection for the measurements of glucagon-stimulated C-peptide and glucose.

*8.2.2 **Immunological tests: Blood samples collected at all visits: Visit 2-7***

Measurements of GADA titer and some of the relevant immunological parameters will be performed at the Department of Clinical and Molecular Medicine, NTNU. Measurements of Anti-SARS-CoV-2 will be performed at Laboratoriemedisinsk klinikk, St Olavs Hospital, Trondheim, and at Karolinska Universitetslaboratoriet, Stockholm. HLA classification will be

performed at Oslo University Hospital Rikshospitalet, Department of Immunology and Transfusion medicine. Classification and analyses of T-cells and other relevant immunological tests will be performed at Linköping University Hospital.

9. SAFETY AND ADVERSE EFFECTS

9.1 Safety parameters

See also Section 5.1, Primary endpoint, and Table 1.

The safety assessments include occurrence of adverse events (AEs), laboratory measurements, physical examinations including neurological assessments. Adverse events will be recorded by the physician at every visit throughout the study.

Blood tests for safety:

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

Urinalysis for safety:

- Urine pregnancy test as appropriate
- Microalbuminuria
- Creatinine

Other Variables which will be evaluated:

- Inflammatory markers, especially TNF-alfa, IL-1 beta, IL-2, IL-17
- Th2-deviation of cell-mediated immune response seen e.g. as increased ratio of IL-5,10, 13 in comparison with IFN-gamma, TNF-alfa, IL-1 beta and IL-17, and increase of T-regulatory cells
- C-peptide (90 minute value and AUC mean 0-120 min) during an MMTT
- Fasting C-peptide
- HbA1c
- D-vitamin
- Antibodies against SARS-CoV-2 (at Visit 6 and 7)

9.2 Definitions of adverse events and reactions

An adverse event (AE) is defined as any untoward medical occurrence in the clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

A serious adverse event (SEA) is defined as any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalization or prolongation of

existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.

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

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

A suspected unexpected serious adverse reaction (SUSAR) is defined as a SAE, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for the unauthorized investigational product).

The term 'severity' is used here to describe the intensity of a specific event. This has to be distinguished from the term 'serious'. The term severe is used to describe the intensity (mild, moderate or severe) of the event and the event does not necessarily need to be considered serious. The term serious is based on the patient/event outcome or action and serves as a guide for defining regulatory reporting obligations.

Mild intensity: The adverse event is transient and easily tolerated.
Moderate intensity: The adverse event causes the patient discomfort and interrupts the patient's usual activities.
Severe intensity: The adverse event causes considerable interference with the patient's usual activities and may be incapacitating or life-threatening.

9.3 Relationship to study medication

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

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

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

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

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

9.4 Reporting and follow up of adverse and serious adverse events (AE and SAE)

All AE must be recorded in the CRF, defining relationship to study medication, severity and seriousness. AE should also be recorded by the physician or study nurse in the patient file/notes.

Timelines and Reporting of SAE

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

TFS Trial Form Support AB (TFS) will be responsible for reporting all SAEs in accordance with ICH Good Clinical Practice (GCP) and local regulations. Diamyd and TFS will complete and sign a "Pharmacovigilance Working Agreement" agreement covering the safety reporting responsibilities in the study. This agreement will ensure the sponsor is directly informed of each SAE reported by the investigators.
In order to meet the specified reporting requirements investigators should adhere to the following process for recording and reporting SAEs.

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

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

It is TFS's responsibility to receive e-mail or fax copies of the SAE report form and other relevant eCRF pages from the investigators. The Drug Safety unit at TFS will review the information provided on the form and enter it into the safety data base. The SAE report will be

assigned a unique number that will be entered on the SAE Report Form and will be used to identify the report in all future communication. A notification of receipt of the report will be sent to the reporter, either by fax or e-mail within 48 hours. TFS will contact the investigator directly if there is any inconsistencies and missing information.

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

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

Pregnancy Report Form

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

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

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

9.5 Annually report of AE/SAE

Sponsor shall annually send the authorities in both Norway and Sweden
a) a list of all assumed serious adverse events that have occurred in the period in question, and
b) a report on the safety of the trial subjects.

10.STATISTICS

10.1 Statistical analysis plan

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

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

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

Data regarding immune response, beta cell function, AE and other data will be summarized descriptively.

Analyses of data will be performed after 5 and 12 months from baseline. Data from the BALAD study (see Section 3.3.1) will be used as a historical control for the evaluation of treatment-related effects on the evolution of beta cell function parameters between baseline and 5 and 12 months of intervention. We acknowledge that it is difficult to statistically compare data from BALAD as a historical control with data from this pilot study, as the participants in BALAD were randomized to active treatment. The comparison will still be valuable, primarily as a pointer for future research.

10.2 Estimation of sample size

The proposed study is an open-label pilot phase IIa trial and will include approximately 15 patients. No formal power analyses are performed for this pilot study.

10.3 Missing data

See Section 6.4b.

11. MONITORING AND ACCESS TO SOURCE DATA

Prior to the start of the study, the monitor will review the protocol and CRFs with the project manager and her staff. The project manager will be visited by the monitor, who will check study procedures, including safety assessments, study medication handling, data recording and source data verification (SDV). To assure the accuracy and completeness of the data recorded in the trial, the monitor will compare CRFs with medical records and other relevant documentation during the on-site monitoring visits. The monitor must therefore be allowed

direct access to all source data according to ICH GCP to confirm that required protocol procedures are being followed and check consistency between patient record and CRF data. Incorrect or missing entries into the CRFs will be queried and must be corrected. Study monitoring will not jeopardise patient confidentiality. After the last study subject has completed the last visit and all CRFs are completed a close-out monitoring visit will take place.

During or after the trial is completed, regulatory authorities or Diamyd Medical might wish to carry out an inspection and/or audit. These representatives must have the same access to study data and patient source data as the monitor.

12. QUALITY CONTROL AND SOURCE DATA

All patient information and data collected during a visit should, if possible, be entered directly into the electronic CRF (optimal) or paper CRF (see also Section 14.1) and should therefore be defined as source data. Data recorded in a paper CRF format should be transferred to the electronic version of the CRF as soon as possible after the visit. Other data pending results from analyses of biological samples (collected during the visit) to be performed after the visit should be entered to the patient's electronic CRF as soon as these data are available. Also, other relevant information that for some reason cannot be entered directly into the electronic CRF should be recorded in the electronic version as soon as possible. All original data records or certified copies of original records that contains information to be transferred into the CRFs are to be defined as source data and must be kept in a secured area (e.g. a locked cabinet).

Procedures for quality control and quality assurance will be performed in compliance with ICH GCP guidelines and the applicable regulatory requirements and within the principles of the latest revision of Declaration of Helsinki.

13. ETHICS

Regional ethical committees, drug administration agencies and data protection authorities in Norway and Sweden must approve the proposed trial. Informed consent must be obtained from all study participants.

13.1 Risk-benefit analyses

LADA cannot be cured and can hardly be prevented. A treatment that preserves the insulin secreting capacity of the LADA patient's beta cell, or at least decelerate the demise of beta cells, would be of great value for the patient (see also Section 3.3.1). A better preservation of beta cell function would result in better metabolic control, leading to lesser diabetic complications and thus a better self-management of their disease, i.e. an improved quality of life.

Any immune intervention in LADA patients must balance the possible benefits with any negative effects. One could argue that the increasing therapeutic possibilities for treatment of LADA (and other types of diabetes) that have emerged in recent years (such as new drugs and better possibilities for self-monitoring of blood glucose) will to some extent diminish the possible benefits of immune intervention in terms of better glucose control and better quality of life. In the face of better standard treatment for diabetes now than in previous years it becomes more important than ever that side effects (both short-term and long-term) do not overshadow benefits of treatment. Such would be the case if strong immune suppressants used in other autoimmune diseases (think rheumatoid arthritis or multiple sclerosis) would be administered. Long-term experience from vaccination with GAD-alum has so far shown negligible side effects [16, 33] (for details, see Investigator's Brochure), this being in contrast to most other agents that have so far been used to prevent beta cell deterioration in type 1 diabetes. Vaccination with GAD-alum would therefore seem to present an ideal way to intervene in LADA patients.

Some patients may balk at the prospect of injections given in lymph nodes rather than subcutaneous injections. However, experience in children and adolescents in previous and the ongoing trial (DIAGNODE-2) using this new mode of administration are favorable: no adverse events or added discomfort compared with subcutaneous injections (only mild transient reactions to the site of injection). The pain associated with the injections is reported to be less than taking intravenous blood samples. This will be thoroughly explained to potential study participants.

We acknowledge that approximately 15 study participants and no placebo arm provide limited statistical power to detect changes in C-peptide and other clinical parameters over time. However, with the clinical and immunological experience from the type 1 diabetes space including ongoing late-stage trials we feel comfortable in that this trial, focusing on patients with high GADA titers, will provide the required decision support for a placebo-controlled larger phase II/III trial in LADA.

Naturally, the basic motivation for the proposed study is an expected positive outcome. However, the possibility of a negative outcome with respect to beta cell preservation (i.e. no indications of a treatment-related protection of beta cell insulin-releasing capacity) cannot be excluded. In that scenario, one would still gain important knowledge (through measurements of multiple immunologic parameters) on the factors of autoimmunity that are involved in the demise of beta cells in LADA. Deepened knowledge of such factors would to our mind be very important for a better understanding of the disease and also for the design of any future clinical treatment trial in LADA.

The lack of agreement on treatment recommendations in LADA results in wide variations in treatment within and between countries. Results from the present project will therefore be of valuable and great interest for the treatment of LADA both nationally and internationally.

13.2 Recruitment and informed consent

Recruitment will be through advertisement in journals for diabetic patients and information through other media outlets as well as by referrals from physicians treating patients for diabetes.

Potential study patients will receive both written and oral information about the nature and purpose of the trial, study procedures, potential risks, and benefits. The patients will be notified that they are free to withdraw from the study at any time. The patients will be given reasonable time to read and understand the information before signing the informed consent. Before any study specific procedures may be performed, the patients must sign the informed consent form.

14. HANDLING AND FILING OF DATA AND BIOLOGICAL MATERIALS

14.1 Data management

Case report forms (CRFs) will be supplied for recording data from each patient. CRFs will be available in both electronic and paper format. Since it is important to have proper data collection in a timely manner, the investigator (or her designate) shall complete the CRFs promptly. To ensure legibility the paper CRFs (if relevant) should be completed in block capitals with a black or blue pen (not pencil). Any information noted in the paper version of the CRF shall be transferred into the electronic version as soon as possible. Any corrections to the CRFs (electronic or paper format) must be carried out by the investigator or his designate. The correction has to be dated and initialled. In the paper format (if relevant), incorrect entries must not be covered with correcting fluid, or obliterated, or made illegible in any way. Instead, a single stroke must be drawn through the original entry.

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

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

14.2 Record retention

The CRFs and all medical records upon which the CRFs are based (source data) must be kept for at least 15 years after completion of the study.

14.3 Collection and destruction of biological materials

A research biobank will be established for the storage of human biological materials (blood samples and urine samples) to be collected during the trial. Both the establishment and discontinuance of the biobank and procedures for destruction of the biological material must be approved by REC.

Any shipping of human biological materials from NTNU/St Olavs Hospital to Linköping/Sweden for analyses must be approved by REC.

15. ETHIC COMMITTEES AND COMPETENT AUTHORITIES

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

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

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

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

Minor amendments which do not affect the safety or conduct of the study from the patient viewpoint, and which do not significantly reduce the scientific value of the protocol, and which do not require a significant change to be made to the consent form and/or the information sheet, will not be submitted for formal ethics and regulatory review.

16. FUNDING AND INSURANCE

16.1 Funding

Sponsor (Ingrid K Hals) is funded by a 3-year post doc grant (2019-2021) from the Central Norway Regional Health Authority (RHA) that also includes funding for operating costs for parts of the proposed trial. In November 2019 the project received additional funding from the Central Norway RHA for major parts of the project for 2020 and 2021.

Remaining necessary funding (operating costs and salary expenditure) will be sought from the Central Norway HRA (Samarbeidsorganet and Felles Forskningsutvalg) – as well as from other relevant institutions like the Novo Nordisk Foundation and the Norwegian Diabetes Association.

Diamyd Medial AB (Stockholm, Sweden) will ensure the production and availability of the GAD-alum to be used for intranodal injections.

16.2 Insurance

Insurance will be covered through membership of the Drug Liability Association. Claims for compensation arising from drug injuries are submitted to and considered by, on behalf of Norsk Legemiddelforsikring AS, Norsk Pasientskadeerstatning and on behalf of Läkemedelsförsäkringen, Patientskadeförsäkringen (Sweden). Diamyd Medical is also part of Läkemedelsförsäkringen (Sweden).

16.3 Allowances and expenses

Study participants will not be payed to participate in the trial. They will however be encouraged to fill out a travel claim form after each visit in the study to receive full compensation for travel expenses and accommodation expenses. If relevant, lost wages will also be covered.

17.PUBLICATION AND DATA RIGHTS

17.1 Dissemination

We expect that the study results will, in due course and by mutual agreement, be published in a scientific journal and presented at scientific meetings. The authorship of the publication will be in accordance with the international guidelines for authorship (International Committee of Medical Journal Editors, 1997).

17.2 Final study report

The final study report will be sent to the Norwegian Medicines Agency, the Swedish Medical Products Agency, the Regional Committee for Medical and Health Research Ethics in Norway and the Ethical Review Authority in Sweden in accordance with the ICH Guidelines for GCP.

17.3 Registration

The trial is registered at ClinicalTrials.gov, NCT04262479.

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19. SIGNATURES

This Clinical Study Protocol is approved by:

Sponsor's Representative

Name: Ingrid K Hals

Title:

Affiliation:

Signature:

……..……

Date: ……..

Overall responsible for the trial site related medical decisions

Name: Inger Karin Lægreid

Title:

Affiliation:

Signature:

……

Date:…….

20. CLINICAL STUDY PROTOCOL AGREEMENT FORM

Investigator's Statement:

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

"A pilot study on safety, feasibility and insulin-promotion by intra-inguinal lymph node injections of glutamic acid decarboxylase (GAD) in patients with LADA type of diabetes."

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

Investigator's Name: Anneli Björklund

Investigator's Title:

Investigator's Affiliation:

Investigator's Signature:

……..……

Date: ……..

Investigator's Name: Chandima N. D. Balasuriya

Investigator's Title:

Investigator's Affiliation:

Investigator's Signature:

……..……

Date: ……..