Effects of Recombinant Human Glutamic Acid Decarboxylase (rhGAD65) Formulated in Alum (GAD-alum) on the Progression of Type 1 Diabetes in New Onset Subjects

(Protocol TN08)

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Sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute of Allergy and Infectious Diseases (NIAID), the National Institute of Child Health and Human Development (NICHD), the National Center for Research Resources (NCRR), the Juvenile Diabetes Research Foundation International (JDRF), and the American Diabetes Association (ADA)

PREFACE

The Type I Diabetes TrialNet Protocol TN08, *Effects of Recombinant Human Glutamic Acid Decarboxylase (rhGAD65) Formulated in Alum (GAD-alum) on the Progression of Type 1 Diabetes in New Onset Subjects*, describes the background, design, and organization of the study. The protocol will be maintained by the TrialNet Coordinating Center over the course of the study though new releases of the protocol, or issuance of updates either in the form of revisions of complete chapters or pages thereof, or in the form of supplemental protocol memoranda.

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1. INTRODUCTION

1.1. Study Overview

Title	Effects of Recombinant Human Glutamic Acid Decarboxylase (rhGAD65) Formulated in Alum (GAD-alum) on the Progression of Type 1 Diabetes in New Onset Subjects
IND Sponsor	Type 1 Diabetes Trial Network (TrialNet)
Conducted By	Type 1 Diabetes Trial Network (TrialNet)
Protocol Chair	Diane Wherrett, MD
Accrual Objective	126 subjects over 2 years
Study Design	The study is a three-arm, multicenter, randomized, double-masked, placebo-controlled clinical trial. All groups will receive standard intensive diabetes treatment with insulin and dietary management.
Treatment Description	Recombinant human glutamic acid decarboxylase (rhGAD65), formulated in aluminum hydroxide is an antigen-specific immune modulator that has been shown to slow or prevent autoimmune destruction of pancreatic beta cells by inducing immune "tolerance". Participants will receive 3 injections consisting of 20µg of GAD-Alum x 3, 20µg of GAD-Alum x 2 plus one injection of placebo-Alum, or placebo-Alum x 3.
Study Duration	Total duration is approximately 4 years (2 years accrual and 2 years follow-up). Follow-up for up to 4 years may continue for those who have persistence of beta cell function after 2 years and/or detectable immunologic effects of treatment by descriptive analysis.
Objective	It is hypothesized that multiple injections with 20µg GAD-alum preserves endogenous insulin production in type 1-diabetes patients 3-45 years of age, when diagnosed within 3 months prior to the first injection.
Primary Outcome	The primary statistical hypothesis to be assessed in this study is whether the mean C-peptide value at one year for study subjects receiving three injections with GAD-Alum vaccine differs significantly from the mean value for placebo subjects, or if the mean C-peptide value for study subjects receiving two injections with GAD-Alum vaccine differs significantly from the mean value for placebo subjects.
Secondary Goals	The study will examine the effect of the proposed treatment on surrogate markers for immunologic effects, namely disease-specific outcomes and immunological outcomes.
Major Inclusion Criteria	Type 1 diabetes within past 3 months Age 3-45 years** Presence of GAD65 antibody

2. BACKGROUND AND SIGNIFICANCE

2.1. Rationale for intervention trials to preserve beta cell function in subjects with Type 1 diabetes

The onset of human Type 1 diabetes mellitus is the clinical manifestation of the β-cell failure caused by T cell mediated autoimmune destruction. This results in a life long dependence on daily insulin injections and exposure to both the acute and late complications of Type 1 diabetes mellitus (T1DM). T1DM is a particular burden to children and their families, representing one of the most common chronic childhood diseases. While T1DM can occur into adulthood, there is a bimodal peak age of onset, between ages 4-7 and ages 14-16 years. The world-wide incidence of T1DM is increasing, with the greatest increase in children under the age of 5 years. Despite the significant progress that has been made in its treatment, diabetes mellitus represents a severe burden on the individual and on society as well. Any intervention, which can stop or delay the complete loss of functional residual β-cell mass is significant as it may provide protection against hypoglycemia and provide improved metabolic control resulting in a delay in the micro and macro-vascular complications of diabetes.

Type 1 diabetes mellitus is an immune-mediated disease in which insulin-producing beta cells are completely destroyed resulting in life-long dependence on exogenous insulin. While this beta cell destruction process begins before clinical onset and continues after development of hyperglycemia and diagnosis, at the time of diagnosis subjects retain a significant amount of beta cell function as measured by C-peptide responses to a mixed meal tolerance test. However, this beta cell function deteriorates after diagnosis with the presumed eventuality of absent function over time. As shown in the Diabetes Control and Complications Trial, the persistence of residual beta cell function has been associated with important clinical outcomes, specifically reduction in severe hypoglycemia and complications. In this study, the rate of retinopathy and severe hypoglycemia were reduced by greater than 50% in those with sustained C-peptide when compared with those with undetectable C-peptide (1-3). Additionally, there is growing evidence that residual beta cell function shortly after the diagnosis of diabetes may be much greater than previously thought and may be as much as 50% of subjects without diabetes (4). Thus, an intervention that can enable continued endogenous insulin production would significantly improve the day-to-day management for subjects with diabetes and therefore reduce long-term complications.

2.2. Rationale for the use of GAD-alum

The destruction of the pancreatic beta cells in Type 1 Diabetes (T1D) is associated with cellular immune responses to the pancreatic islet cells, genetic susceptibility involving genes thought to modulate the immune response, and the presence of autoantibodies against several islet beta cell components (i.e., autoantigens). In addition, as these T1D associated autoantibodies often precede the clinical onset of disease, those antibodies directed against glutamic acid decarboxylase (GAD65 ab), IA2 ab, and insulin (IAA) are widely recognized not only as diagnostic markers for autoimmune beta cell destruction but in addition, as predictive markers for the disease (5-6).

Although the involvement of glutamic acid decarboxylase (GAD) in neural transmission is understood by its function as an enzyme converting glutamate to gamma-amino butyric acid (GABA), its specific and unequivocal function in pancreatic beta cells, as well as its role in the pathogenesis of either form of diabetes remains somewhat unclear. Indeed, the reason why GAD is a major autoantigen in autoimmune diabetes is not known. However, despite this lack of understanding, convincing (i.e., reproduced by a broad body of the scientific community) data from the NOD mouse model of type 1 diabetes has shown that an isoform of GAD with molecular mass 65,000 (GAD65) can prevent autoimmune destruction of pancreatic beta cells and subsequent need for exogenous insulin replacement (5-18). These findings indicate the potential of GAD65 administration to provide a preventive treatment for the forms of diabetes involving autoimmunity, most notably those occurring in patients with T1D.

The product is a highly purified and unmodified form of recombinant human glutamic acid decarboxylase (rhGAD65), formulated in aluminum hydroxide. It is an antigen-specific immune modulator that has been shown to slow or prevent autoimmune destruction of pancreatic beta cells by inducing immune "tolerance". In "recent-onset" T1D patients, tolerization is proposed to prevent the destruction of remaining beta cells and thereby maintain residual insulin secretion to minimize the likelihood of acute and long-term diabetic complications as well as improving metabolic control. Available data demonstrate that even relatively modest treatment effects on residual insulin secretion will result in clinically meaningful benefits (1-3).

As described below, pre-clinical and clinical data studies to date suggest that GAD65 in alum immunization is safe, and preliminary data indicates that it may be efficacious in diminishing beta cell autoimmune destruction among subjects with autoimmune diabetes.

3. CLINICAL AND PRE-CLINICAL DATA

3.1. Preclinical Data

3.1.1. Preclinical Safety Data

Two formulations have been evaluated in preclinical and clinical studies.
Diamyd® bulk biologic substance (formulated in buffer from the manufacturing process) was used for initial preclinical safety studies, a skin Prick Test and a Phase I clinical trial (formulated in PBS). An adjuvant formulation of Diamyd® based on Alhydrogel® has also been developed for evaluation in Phase II clinical trials (Diamyd® Product).

A preclinical safety evaluation program has comprised single and repeat dose toxicity, local tolerance, immunotoxicity and investigation of the potential for effects on behavior, cardiovascular/respiratory and central nervous systems. Evaluation of all preclinical safety studies performed to date have not provided concerns for clinical safety of Diamyd® biologic substance (even at multiples of the highest clinically-intended dose level) or Diamyd® Product, or identified any target organs of toxicity. Evaluation of the effects of Diamyd® in several different animal models of autoimmune diseases did not indicate any potential for undesirable effects on the immune system.

3.1.2. Preclinical Efficacy Data

The pharmacological effects proposed have also been confirmed using specialized and highly informative co-adoptive transfer experiments in NOD mice. This model involves transfusion of a large number of splenocytes and autoreactive T cells from newly diabetic NOD mice into NOD-Severe Combined Immunodeficiency (SCID) mice that have not yet developed the disease spontaneously, in conjunction with transfer of T cells from treated mice. The results clearly showed a protective effect induced by GAD65 administration (Diamyd, data on file).

3.2. Clinical Data

3.2.1. Clinical Safety Data

The first clinical study used "laboratory grade" Diamyd® bulk substance in a skin "prick test" study in selected volunteers. This was followed by a Phase I clinical trial in volunteers using GLP-grade Diamyd® bulk substance and for Phase II clinical trials an Alhydrogel® adjuvanted formulation of cGMP bulk Diamyd® substance (i.e. the Diamyd® Product). Evaluation of all clinical studies performed to date has not provided concerns for clinical safety of Diamyd®.

Skin-Prick Test Study

Skin-prick tests using at least 1 µg laboratory grade Diamyd® were performed in teenage children, both healthy controls (n=8) and those with a short duration of T1D (n=7). No cutaneous reactions or other adverse events were recorded in any of the subjects as a result of the prick test.

Phase I Trial in Healthy Volunteers

A Phase I randomized, double-blind, placebo controlled, rising dose, study was performed in 24 healthy male subjects ages 24-45 years to assess safety and tolerability after administration of a single subcutaneous injection of Diamyd® biologic substance (20, 100, 250 or 500 µg). In each dose group, four subjects received a single dose of active Diamyd® bulk substance and two received placebo. There were no treatment-related Adverse Events (AEs) or Serious Adverse Events (SAEs) at any dose level. The outcome supports safety and tolerability after subcutaneous administration of a single dose of Diamyd® over the dose range of 20-500 µg.

Phase IIa Dose-Finding Trial in LADA Patients

Approximately 10% of Type 2 Diabetes patients (T2D) have GAD65 ab or other diabetes associated autoantibodies, with patients characterized by their subsequent progression to insulin dependence (19-26). This subset of T2D patients has been characterized as having a unique blend of T1D and T2D known as Latent Autoimmune Diabetes in Adult

(or LADA) (23). The pathological mechanisms leading to insulin dependence in LADA are considered to be closely related to the autoimmune destruction of pancreatic islets in T1D. Thus, the LADA subgroup of autoimmune diabetes patients was selected as the first patient group for clinical trials to evaluate the potential of the GAD immunization. In the LADA subgroup of T2D, tolerization is proposed to prevent the destruction of pancreatic beta cells that results in insulin insufficiency and the need for treatment with exogenous insulin. The therapeutic mechanism of action is proposed to be the same in T1D and LADA because the underlying destructive mechanism in both diseases is considered to be the same in each.

A Phase IIa randomized, double-blind, placebo-controlled, group-comparison dose - finding study was performed in a total of 47 Latent Autoimmune Diabetes in Adults (LADA) patients. The main (6 month) part of the study to evaluate the safety of Diamyd® was completed in April 2003 and the study is currently in follow-up. In each of the 4 dose groups (each intended with 12 patients) patients received either placebo or 4, 20, 100 or 500 µg Diamyd® (using a 1:3 ratio of placebo:treated) via subcutaneous administration on 2 occasions 4 weeks apart. In addition, a maximum of two additional booster injections was allowed in the 500 µg dose group if the GAD65ab titer was unchanged at week 8. Among this group, two subjects received one additional boost for total dose of 1.5 mg, and one subject had two boosts for a total of 2.0 mg.

Outcome Safety:
There were no SAEs during the main study period. The majority of AEs were due to influenza-like symptoms, with nasopharyngitis the most common AE. During the follow-up period to date (August 1, 2007), there have been 11 SAEs in 7 patients (4 patients in the placebo group, 1 patient in the 20 µg group, and 2 patients in the 100 µg group). None of these are considered to be treatment related.

A minority of injections resulted in injection site reactions which were mild and most (in particular, "tenderness") occurred primarily on the day of the injections. These findings support the safety of immunomodulation by GAD65 immunization (19).

Phase II Trial in Patients Newly Diagnosed with T1D

To investigate safety and efficacy in type 1 Diabetes, a Phase II, multicenter, randomized, placebo-controlled clinical trial in 70 GAD65ab positive children and adolescents ages 10-18, diagnosed with T1D within the previous 18 months, was conducted in Sweden starting in November 2004. Administration was by subcutaneous injection of 20 µg Diamyd® Product or placebo (Alhydrogel® alone) on 2 occasions 4 weeks apart. The primary efficacy endpoint was change in fasting C-peptide level from baseline to Month 15. Long-term follow up data was obtained until 30 months (27).

During the 30 month period there were a total of 12 SAEs, 5 of which were in four subjects in the placebo group and 7 of which were in five subjects in the treated group. None of the SAEs were considered to be treatment-related. The frequency and pattern of reported Adverse Events during the 15-month main study period does not differ significantly between placebo and active treatment groups. In 2 patients the adverse event was judged as possibly related to study drug; both in the active treatment group, one mild and one moderate hypoglycemia. In both study groups, mild discomfort was reported at the site of injection. A neurological examination was performed at study day 1 as well as at month 15. There were no differences between treatment groups (27).

Phase IIb Clinical Trial in LADA Patients

A Phase IIb multi-center, randomized, double-blind, placebo controlled study in 160 LADA-patients initiated in December 2004, is currently on-going in Sweden. Subjects received 20 µg of GAD65 or placebo on two occasions four weeks apart. The trial had a main study period of 18 months and was scheduled for unblinding in June 2007. Unfortunately the study had to be invalidated. An independent audit of the central pharmacy for the trial concluded that it was impossible to guarantee absolute identity of the contents of each vial of the Investigational Product administered to the patients. The follow-up period is 3.5 years, and patients are currently followed to look at safety data only.

Safety:
As of August, 2007, there have been 21 SAEs, none of which are considered likely to be treatment-related. Two of the reported unrelated SAEs were two cases of pancreatic carcinoma. The time interval between injection of the therapy and manifestation of disease was short. A 70 year old male received two injections and reported abdominal pain the same day as the second injection was given, 25 days after the first injection. Sixty-four days after the first injection an ultrasound confirmed pancreatic cancer with metastasis to the liver. A second 69 year old male, received two injections of study drug. 55 days after first injection pancreatic cancer with metastasis to the liver was confirmed.

Although pancreatic carcinoma is a relatively rare disease the incidence has been reported to be higher in patients with type 2 diabetes compared to the general population (28-29). The short time interval between drug exposure and manifestation of disease makes it unlikely that the Diamyd® treatment was a primary cause for the pancreatic carcinomas. Furthermore, both patients had elevated levels of CA19-9, the best documented tumor marker of pancreatic carcinoma, prior to receiving study drug. In each case, neither the investigator nor the Safety Committee judged these events as likely to be treatment-related. All of the data available on these two patients was evaluated by an independent expert in pancreatic cancer, Dr. Nils Wilking, Associate Professor of Oncology at the Karolinska Institute, who also concluded that the study drug was not related to the development of pancreatic cancer in either patient.

3.2.2. Clinical Efficacy Data

Phase II clinical trials in LADA- and T1D-patients have been conducted, and have demonstrated efficacy in preserving pancreatic beta cell function.

Phase IIa, Clinical Trial in LADA Patients

In this clinical trial, LADA patients in the 20µg dose group showed statistically significant increases by 6 months in both fasting C-peptide and meal-stimulated C-peptide levels in conjunction with an improvement in HbA1c. The increases in C-peptide levels and decrease in HbA1c were sustained over 2 year's follow-up (Figures 1-2). The error bars in the graphs from the 47 patient LADA study represents SEM. These data were used to select the 20 µg dose group alone for further clinical investigation.

Fig. 1 **HbA1c**

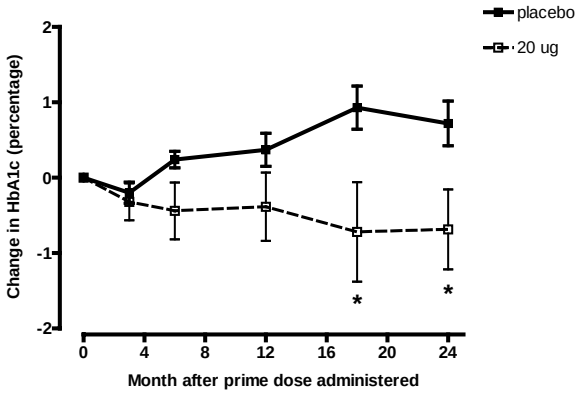
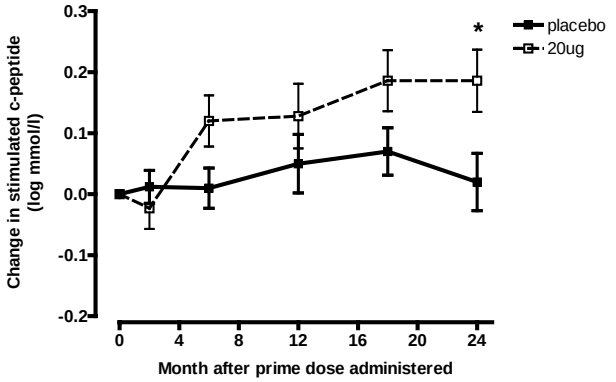


Fig. 2 **Stimulated C-peptide**



Phase II Trial in Patients Newly Diagnosed with T1D

In this Phase II trial, in patients with T1D, both placebo and active treatment groups showed a progressive decrease from baseline for both fasting and stimulated C-peptide secretion, indicating a continuous loss of endogenous insulin production. However, over the 15 months stimulated C-peptide secretion, as measured by Area Under the Curve (AUC), decreased only half as much in the experimental treated group as in the placebo group (p=0.01) (table 1). Also, maximum stimulated C-peptide deteriorated significantly less in the experimental treated group (p=0.04) (Table 1).

Table 1. Efficacy endpoints, mean change from baseline to month 15

(Treatment effect estimated using least square means methodology with baseline value as covariate.)

Mean change in characteristic ± SD	**Diamyd® (n = 35)**	**Placebo (n = 34)**	**Treatment Effect (95% C.I.)**	**p-value (ANCOVA)**
Δ Fasting C-peptide, pmol/ml	-0.12 ± 0.18	-0.17 ± 0.20	0.04 (-0.04, 0.12)	0.28
Δ Stimulated C-peptide Maximum, pmol/ml	-0.24 ± 0.26	-0.42 ± 0.40	0.16 (0.01, 0.31)	0.04
Δ Stimulated C-peptide AUC, pmol/ml*2hour	-0.38 ± 0.46	-0.75 ± 0.61	0.30 (0.07, 0.54)	0.01

The raw data for this trial was evaluated independently by the TrialNet Coordinating Center who confirmed, after adjusting for baseline AUC, the above overall treatment effect and moreover, that the treatment effect was more pronounced in those with shorter duration of diabetes. They also evaluated if additional baseline factors modified the effect of treatment on the C-peptide AUC. There was no such effect of age, age at

diagnosis, HbA1c, weight, BMI, insulin dose per kg, GAD titer, gender, and Tanner stage on AUC differences between treatment groups. They also evaluated if additional time-dependent factors have an effect on the efficacy as measured by the C-peptide AUC. The treatment group effects remained significant after allowing for changes over time in HbA1c, weight, BMI, insulin dose/kg, and GAD titer.

Full report of this trial recently published demonstrating continued benefit up to 30 months from treatment (27).

3.2.3. Other Information

GAD is prevalent in neural cells and antibodies to GAD are found in some neurologic diseases. These include Stiff Persons Syndrome (SPS) (30), previously known as Stiff Man Syndrome (SMS), and its variants, Batten disease, epilepsy, and cerebral ataxia. Though approximately 55-80% of SPS patients have anti-GAD65 and or GAD67 antibodies (30-32) it is unlikely that GAD65 auto-antibodies have a causative role. Neurological symptoms are not different between patients with and without detectable anti GAD antibodies, the level of anti-GAD antibodies in serum or CSF has no correlation with disease severity, duration, or fluctuations (33), and,T1D patients with elevated anti-GAD65 antibodies do not have elevated SPS susceptibility (32-34). Patients affected with Batten disease (35) frequently have GAD autoimmunity (35-37) but like SPS, the GAD antibodies are not thought to be causative. Batten disease is the result of a defect in the CLN3 gene (38). Neuronal death in patients with Batten disease probably allows the presentation of intracellular epitopes, including GAD65, to the immune system, with resultant GAD antibodies in susceptible patients. Indeed, multiple auto-antigens are recognized in Batten patients (39). While no association was found between GAD65ab and juvenile myoclonic epilepsy (41), 3/139 (2.1%) of patients with drug resistant epilepsy were positive for anti-GAD antibodies (40). Similarly, some reports have found anti-GAD65 antibodies in occassional patients with cerebellar ataxia, including some who also had T1D (42,43).

In summary, not unexpectedly, since GAD is present in neurons, GAD65 autoantibodies can be seen in neurologic diseases, but these antibodies are felt to be a result of neurologic injury and not to have a causative role. Nonetheless, due to this association, subjects with pre-existing neurologic disease will be excluded from this trial, and a structured neurologic exam will be included in study visits.

3.3. Rationale for Dose Regimen

3.3.1. Dose and dosing frequency

A Phase IIa Dose-Finding study was conducted in 47 patients using 4, 20, 100 and 500 µg dose groups. Analysis of outcome data from this study has provided evidence for safety (at all doses) and best efficacy in the patient group receiving 20 µg. Evidence for improved beta cell function (via increased C-peptide levels both before and after mixed meal tolerance test) and improved patient diabetic status (via lowered HbA1c levels) was

found only in the patient group receiving 2x20 µg. Further, the increase in a specific T cell subset associated with immunoregulatory functions (increase in CD4+CD25+/CD4+CD25- cell ratio) was found only in this dosing group.

A repeat administration of the same dose at +/- day 30 (with day 0 being the first dose) 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, and was used between "prime" and "boost" doses to enable immunomodulation. The third dose at 8 weeks after the 2nd is patterned after dosing frequency studies with anthrax vaccine and is designed to test whether thus additional boost will enhance the response.

3.3.2. Use of Alum as Adjuvant for Immunomodulation

Aluminum hydroxide (alum) is a conventional adjuvant that is commercially sold as Alhydrogel®. US licensed vaccines for children that contain aluminium adjuvants include DTP, DTaP, Pneumococcal conjugate, Hepatitis B, Hepatitis A, Anthrax, and Rabies. Alum was the only adjuvant approved for vaccines by the FDA prior to the start of Diamyd® clinical development. Aluminum salts are well-recognized as preferentially inducing a humoral (Th2) rather than cellular immune response (44). As patients with ongoing autoimmunity resulting in T1D are likely to be biased towards a Th1 (or cellular) immune response to autoantigens, alum is used to overcome this bias and "steer" the response induced by Diamyd® away from a cellular towards a humoral response in order to minimize the likelihood of exacerbating (cell-mediated) beta cell destruction. Inclusion of adjuvant was also rationalized to minimize the quantity of antigen required for treatment by maximizing its immunogenicity.

3.3.3. Use of Subcutaneous Route of Administration

The subcutaneous route was selected for drug administration. Although the intra-muscular route is frequently used for vaccine administration, muscle tissue is among those least capable of antigen presentation while dermal tissues are recognized as proficient at this. Furthermore, a significant number of commercially available vaccines are administered by the subcutaneous route (e.g. the Anthrax vaccine "Biothrax®", "Zostavax®" with live, attenuated varicella-zoster, the "ProQuad®" MMRV vaccine, and the Meningococcal Polysaccharide Vaccine "MPV"). Also subcutaneous administration is less painful than intramuscular.

4. STUDY DESIGN

4.1. Overview

It is hypothesized that multiple injections with 20µg GAD-alum, preserves endogenous insulin production in Type 1-diabetes patients diagnosed within 3 months prior to the first injection.

4.2. Summary of Inclusion and Exclusion Criteria

4.2.1 Inclusion Criteria

Potential participants must **meet all** of the following inclusion criteria:

1. Be between the ages of 3 and 45 years*
2. Type 1-diabetes mellitus diagnosed within the previous 3 months
3. Must have stimulated C-peptide levels ≥ 0.2 pmol/ml measured during a mixed meal tolerance test (MMTT) conducted at least 21 days from diagnosis of diabetes and within one month (37 days) of randomization
4. Presence of GAD65 antibodies
5. At least one month from last immunization
6. Must be willing to comply with intensive diabetes management
7. If participant is female with reproductive potential, she must be willing to avoid pregnancy for 2 years and have a negative pregnancy test
8. Willing to forgo routine clinical immunizations during the first 100 days after initial study drug administration

* *Enrollment will proceed in age cohorts as described below*

4.2.2 Exclusion Criteria

Potential participants must **not** meet any of the following exclusion criteria:

1. Be currently pregnant or lactating or anticipate getting pregnant for 24 months after first injection
2. Ongoing use of medications known to influence glucose tolerance
3. Require use of systemic immunosuppressant(s)
4. Have a history of malignancies
5. Be currently using non-insulin pharmaceuticals to affect glycemic control
6. Have any acute or chronic complicating medical issues or abnormal clinical laboratory results that interfere with study conduct or cause increased risk including neurological abnormalities.
7. Have a history of epilepsy, significant head trauma or cerebrovascular accident or clinical features of continuous motor unit activity in proximal muscles
8. Inability or unwillingness to comply with the provisions of this protocol
9. Have an active infection or positive PPD test result.
10. Have serologic evidence of current or past HIV, Hep B, or Hep C infection.

4.3. Staggered Enrollment

There will be tiered enrollment by age group. Initially enrollment will be limited to subjects between 16 and 45 years of age. When 20 subjects in this age group randomized to one of the experimental treatment arms have completed three months follow-up, review of the safety data will be done by the FDA and TN DSMB.

If the first formal safety review is acceptable, enrollment will then expand to include subjects age 10 to 45. When 20 subjects between the ages of 10 and 15 randomized to one of the experimental treatment arms have completed three months follow-up, a second review of the safety data will be done by the FDA and TN DSMB.

If this second formal safety review is acceptable, enrollment will then expand to include subjects age 3-45.

In order to maintain similar proportions in this study to other TrialNet studies, enrolment of those age 16 or above may be closed when about 70 such subjects have been enrolled, or 55% of the planned sample size for this trial. Then the remaining subjects would be limited to younger age groups.

4.4. Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written communication. Written material will include a Patient Handbook and written consent forms. The consent form will be reviewed with participants (and their guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB requirements differ in procedure). As part of the informed consent process, the participant and/or parent or guardian (if the participant is less than 18 years of age) will also be required to complete a short, written Volunteer Understanding Assessment that is designed to ensure that the subject understands the study, as well as what is being asked of him/her. The participant will be given a copy of their signed consent/assent forms.

4.5. Description of Treatment Groups

This protocol will enroll a total of 126 participants who will be randomly assigned to the following three groups:

- 42 participants will be assigned to receive 3 injections with 20µg GAD-alum
- 42 participants will be assigned to receive 2 injections of 20µg GAD-Alum followed by one injection with Alum alone

- 42 participants will be assigned to receive 3 injections of Alum only

All patients will be monitored at least for one hour after administration of each injection.

4.6. Treatment Assignment and Double Masking

After participants sign the consent form they will be randomized to one of the treatment arms. The randomization method will be stratified by TrialNet study site. The participants will not be informed regarding the intervention assignment until the end of the study. The investigator and clinic personnel will also be masked as to study assignment. Laboratories performing assays for this protocol will be masked as to the identity of biological material to be studied.

4.7. Study Assessments

During the course of the study, participants will frequently undergo assessments of their insulin production, immunologic status, overall health and well being and diabetes care (see schedule of assessments in Appendix A). The participant's insulin production will be measured by a series of mixed meal glucose tolerance tests (MMTT) conducted regularly during the study. The participant's diabetes control will be evaluated by measuring glycosylated hemoglobin (HbA1c) every three months and clinical records including insulin types, doses, and timing and SBGM records.

During the course of the study, samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Sites for future analysis. These samples will be collected only with the subject's permission. Subjects who decline consent for these sample collections will still be eligible to participate in this study.

4.8. Quality Assurance

During the study, duplicate collections of blood samples for assays will be obtained in a small sample of subjects for the purpose of external quality surveillance of the performance of the central laboratories.

4.9. Post-treatment Follow-up

All subjects treated with injections of 20µg GAD-Alum or Alum alone will be evaluated over a two year period. Subjects may subsequently be asked to undergo additional follow-up for an additional two years with a visit every 6 months until study end. Subjects with undetectable levels of C-peptide on the 30 month visit will not undergo any further MMTTs for assessment of C-peptide levels at subsequent visits.

5. PATIENT MANAGEMENT

5.1. Screening

After informed consent, subjects will undergo assessments to determine if they meet eligibility criteria. Documentation of the subjects understanding of the risks and benefits of the study will be collected through the Volunteer Understanding Assessment.

5.2. Randomization

Eligible study participants will be randomized by the TrialNet Coordinating Center at the baseline visit, and will be assigned a study randomization number corresponding to the treatment group assignment. The subject will receive the initial immunization of GAD-Alum or Alum alone at the baseline visit.

5.3. Intensive Diabetes Management

During the study period, all participants will receive "intensive" management of their diabetes. The goal of the treatment will be to keep the HbA1c levels within the currently recommended American Diabetes Association age-specific target range in the absence of significant or severe hypoglycemia or diabetic ketoacidosis. The primary responsibility for diabetes management will be the treating or referring diabetes care provider, but the research study team will provide close additional support through regular interaction. Subjects will not be permitted to use non-insulin pharmaceuticals for glycemic control.

Glucose levels should be checked frequently and records of the glucose levels communicated regularly to the study team. Records of communication with the participant will provide source documentation of this interaction.

The Clinic Monitoring Group (or designated TrialNet Committee) will be evaluating the HbA1c data and provide additional guidance to the clinical site as needed to bring diabetes control within goals. Any episodes of severe hypoglycemia will be promptly reviewed by the Safety Monitoring Committee with recommendations for changes in diabetes management, if any, conveyed to the clinical site in conjunction with the Clinic Monitoring Group.

5.4. Administration of GAD-Alum or Alum alone

5.4.1. Dosing

All patients will receive 3 subcutaneous injections of 20ug of GAD65-alum or placebo. The first injection is given at baseline (day 0), the second injection is given 4 weeks later, and the third injection is given at 8 weeks after the second. Dosing will not be done in subjects with a febrile illness within the previous 24 hours. These subjects will be rescheduled for another day within a five day window.

6. STUDY ASSESSMENTS

6.1. General Assessments

Study visits for all patients will occur for screening, at baseline, and subsequently for the injections and for the metabolic and immunologic monitoring (see Appendix A). General assessments include:

• Medical history and routine or directed Physical examination
• Concomitant medications
• Adverse events

6.2. Laboratory Assessments

The following laboratory assessments will be performed during the study:

- Chemistry (sodium, potassium, chloride, CO2, glucose, BUN, creatinine)
- Liver function tests (ALT, AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin)
- Hematology (complete blood count with differential and platelets)
- Urine pregnancy test as appropriate
- Islet Autoantibodies
- Viral serology: antibodies to HIV, hepatitis B (antiHBcAb, HbsAg,), hepatitis C (HCV),Cytomegalovirus (CMV IgG)) Epstein-Barr Virus (EBV IgG and IgM). Viral load will be determined if indicated.
- PPD
- Samples for titers from routine childhood immunizations.

6.3. Mechanistic Outcome Assessments

TrialNet will perform immune and genetic assays to further understand mechanisms that may be underlying the Type 1 disease process and response to therapy. For this purpose, samples for PBMC, DNA, RNA, plasma, and serum will be obtained. HLA testing will be done.

The primary mechanistic studies are those which measure GAD65-specific immune responses. The principle objective is to identify measures of immunity to GAD which distinguish between subjects receiving two or three injections of GAD in alum, as compared to those receiving alum injections alone.

Additional objectives are:
(i) To determine whether these measures of GAD immunity reflect immune deviation, changes in GAD epitope recognition or amplification of GAD regulatory function;
(ii) To evaluate other T1D-associated immune responses, such as anti-proinsulin or anti-IA2, as markers for antigen-spreading and bystander effects; and
(iii) To correlate changes in GAD65-specific immune responses with levels of c-peptide over the 24-month duration of the trial.

6.4. Metabolic Outcome Assessments

Metabolic assessments will consist of:

- Glucose records and reports of hypoglycemia including those that are symptomatic and confirmed with capillary glucose measurement, symptomatic and unconfirmed, and major hypoglycemia
- Insulin dose
- HbA1c
- Mixed meal tolerance test (MMTT)
-

6.5. Neurologic Assessments

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 neurological examination includes:

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

6.6. Visit Windows

The screening MMTT must occur at least 21 days after the day of diagnosis. Randomization must occur within 100 days from the date of diagnosis and the first injection should be administered within 37 days of the MMTT. The subsequent treatment visits should be no longer than +7 days from the target date. Immunization earlier than the target date is limited to -2 days. The target window for each subsequent injection will be based on the actual date the previous injection was given. There is a +/- 3 day window around the target date for the visit after the first injection and a +3 day window around the target dates for the visits immediately after the 2nd and 3rd injections. The window for all subsequent visits is +/- 2 weeks.

Patients who receive at least one injection will be considered active participants. If patients are febrile when scheduled for injection, treatment will be postponed until the patient is afebrile.

7. PARTICIPANT SAFETY

7.1. Expected Side Effects and Adverse Events

7.1.1. Influenza-like Symptoms

In previous studies, the majority of Adverse Events were due to mild influenza-like symptoms, with nasopharyngitis the most common Adverse Event. However, no significant differences were seen between the treatment groups.

7.1.2. Injection Site Reactions

In previous studies, injection site reactions occurred in a limited number of patient visits. All reactions were mild and most (in particular, "tenderness") occurred primarily on the day of the "prime" injection and "boost" injection four weeks later. No significant differences were seen between the treatment groups.

7.2. Other safety issues

7.2.1. Pregnancy

Pregnant and lactating women will not be included in the study. Females must have a negative pregnancy test prior to enrolling in the study and will be required to use birth control during the study. At every study visit the sexual activity of female participants of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, she will be counseled about the need to use a reliable form of birth control. Female subjects will also be required to undergo urine pregnancy tests at regular intervals and prior to each dosing. Subjects will be requested to avoid pregnancy for 2 years.

8. ADVERSE EVENT REPORTING AND SAFETY MONITORING

8.1. Adverse Event Definition

8.1.1. Adverse Event

In this clinical trial, an adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom or disease whether or not associated with the treatment and study procedures.

Throughout the study, the investigator must record all adverse events on source documentation, and those that are Grade 2 or greater must be recorded on the appropriate adverse event form as described below. The investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

- observation of the participant;
- questioning the participant;
- unsolicited complaint by the participant.

In questioning the participant the questioning should be conducted in an objective manner.

8.1.2. Serious Adverse Event

For this trial, an adverse event associated with the treatment or study procedures that suggests a significant hazard, contraindication, side effect or precaution (as described below) is to be reported as a serious adverse event (SAE). A serious adverse event (experience) or reaction is any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient and/or may require medical or surgical intervention to prevent one of the outcomes listed above.

8.1.3. Unexpected Adverse Event

An adverse event is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the protocol or informed consent document for a particular protocol required intervention.

8.1.4. Grading Event Severity

TrialNet has adopted usage of the National Cancer Institute (NCI) Common Technology Criteria for Adverse Events (CTCAE) with the exception of hypoglycemia and hyperglycemia for classification to describe the severity of adverse events. For this study, a reportable hypoglycemic event is defined as those resulting in loss of consciousness, seizure, or requiring assistance of others due to altered state of consciousness. A hyperglycemic event is one resulting in DKA.

8.2. Adverse Event Reporting and Monitoring

Study personnel will assess adverse events and the use of concomitant medications throughout the study. All adverse events will be recorded on source documents and those ≥ grade 2 will be reported to the TrialNet Coordinating Center as described below. They will be graded as to severity according to common toxicity criteria or study-specific criteria and the investigator will make a determination as to the relation to therapy. Events will be assessed and reported in accordance with the ICH Guideline for Good Clinical Practice and per the guidance of the DHHS Office for Human Research Protections (OHRP). An adverse event report must be completed for all adverse events (AE) of Grade 2 or greater severity regardless of relationship to therapy. For reporting serious adverse events (SAE), the TrialNet MedWatch Form should also be completed and faxed to the TNCC *within 24 hours of when the site was notified of the event.* This will be reviewed by the TrialNet Medical Monitor, the TrialNet Safety Committee, and the DSMB as appropriate. Deaths must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and reported when available, if not known at the time the event is reported. The follow-up information should contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

Adverse events will be assessed by the TrialNet Medical Monitor. The DSMB will conduct regular safety reviews approximately every three to six months (and, as needed) of adverse events by treatment group assignment. Serious adverse events as well as adverse events leading to study discontinuation will be reviewed by the DSMB.

8.3. Protecting Against or Minimizing Potential Treatment Risks

Subjects will not be enrolled who have other active serious medical problems. Frequent monitoring of patients with history, physical examination, and laboratory studies will allow for early identification of adverse events. Subjects with pre-existing neurologic disease will be excluded from the study, and a structured neurologic exam will be performed at study visits. Every attempt will be made to minimize the number of venipunctures.

All dosing will take place in a facility that has resuscitation capabilities, and subjects will be closely monitored during and after the injection.

Subjects will be counseled about the need to report any change in health status between

or at the time of visits.

Subjects will be counseled by study personnel and requested to avoid pregnancy for 2 years

9. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address all objectives of the trial and other interrelationships among data elements of interest to the investigators and of relevance to the objectives of the study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

Primary analysis of treatment effect will be conducted under the intention-to-treat principle of eligible patients whereby outcome data from all eligible patients will be included regardless of treatment compliance.

9.1. Primary Outcome and Analyses

The primary outcome of each participant is the area under the stimulated C-peptide curve (AUC) over the first 2 hours of a mixed meal glucose tolerance test conducted at the one-year visit. The AUC is computed using the trapezoidal rule that is a weighted sum of the C-peptide values over the 120 minutes. The weighted mean C-peptide is simply AUC/120 (pmol/mL). Let $Y_{Cp}^{GAD\times3}, Y_{Cp}^{GAD\times2}$, and $Y_{Cp}^{Control}$ represent the mean C-peptide value for study patients receiving three injections with 20µg GAD-Alum, those receiving two injections with 20µg GAD-Alum and those receiving placebo, respectively. Likewise, let $\mu_{Cp}^{GAD\times3}, \mu_{Cp}^{GAD\times2}$, and $\mu_{Cp}^{Control}$ represent the mean of Y_{Cp} for these groups, respectively.

The primary statistical hypotheses to be assessed in the study are:

$$\mathrm{H}_0\colon \mu_{Cp}^{GAD\times3} = \mu_{Cp}^{Control} \text{ versus } \mathrm{H}_a\colon \mu_{Cp}^{GAD\times3} > \mu_{Cp}^{Control}$$

$$\mathrm{H}_0\colon \mu_{Cp}^{GAD\times2} = \mu_{Cp}^{Control} \text{ versus } \mathrm{H}_a\colon \mu_{Cp}^{GAD\times2} > \mu_{Cp}^{Control}$$

The primary analysis will be conducted on the transformed Y_{Cp} using the log function: $\log(Y_{Cp}+1)$. This provides better normal distributional behavior by the test statistic. The comparison between any two treatment arms will be based on a t-test of treatment effect in an ANCOVA model adjusting for gender, baseline age, and baseline $\log(Y_{Cp}+1)$ (49).

The Holm closed-sequential procedure for multiple tests (46) will be used to control the type I error probability to not exceed 0.05, one-sided, for the set of two pairwise group comparisons.

9.2. Secondary Outcome and Analyses

Additional analyses of the primary outcome will include:

- a log rank test of the difference in the hazard function between groups in the first occurrence of a loss of the 2 hour peak C-peptide < 0.2 pmol/ml on a semi-annual MMTT (48), and
- longitudinal analyses (45) using mixed effects models with a random intercept and slope of the C-peptide values over the post-treatment period, adjusted for the baseline level of C-peptide. The average intercept and slope will be compared between groups adjusting for age, gender and the baseline $\log(Y_{Cp}+1)$.
- Analyses will also be conducted to adjust for baseline $\log(Y_{Cp}+1)$ and HbA1c levels, and by age, gender and race/ethnicity, as appropriate.
- Analyses will also be conducted to examine the effect of HLA or other genotype and immune phenotypes

Additional secondary objectives are to examine how the GAD-Alum vaccine affects the following:

- Mean C-peptide (as previously described) from the stimulated C-peptide curve over both 2 and 4 hours at 24 months for those age 12 or older
- Mean C-peptide (as previously described) from the stimulated C-peptide curve over 2 hours at 18 months
- HbA1c
- Insulin dose (units/kg)
- Blood glucose
- Number and severity of adverse events
- Hypoglycemia
  - Number of major hypoglycemic events (defined as loss of consciousness, seizure, or requiring assistance from another person because of altered state of consciousness)
  - Reported hypoglycemic events confirmed with capillary blood glucose measurement less than 70 mg/dl.
- For individuals for whom continuous glucose monitoring data is available
  - Area under the curve and number of events less than 70 mg/dl on the continuous glucose monitoring record
  - Hyperglycemia as measured as the area under the curve and number of events greater than 180 mg/dl on the CGMS record prior to each study visit
- Glycemia and glycemic variability prior to each MMTT visit
  - The daily mean level of glucose, as well as the levels before and after meals will be computed.
  - Measures of diurnal variability in glucose will be measured by the J-value, standard deviation of glucose values, and the mean amplitude of glycemic excursion (MAGE)

The mean HbA1c, insulin dose (units/kg), and blood glucose over all follow-up values will be compared between groups using a normal errors longitudinal analysis (45). The rates of severe hypoglycemic and adverse events will be computed (total number of events divided by total patient years of follow-up) and the rates compared using a Poisson regression model, allowing for over-dispersion using a quasi-likelihood model as appropriate. Both sets of analyses will be adjusted for age, gender, baseline *log(C-peptide+1)* and baseline HbA1c.

9.3. Additional Outcomes and Analyses

Additional outcomes of interest include the effects of the GAD-Alum vaccine on antigen specific immune activity which will be assessed from blood draws as outlined in the Schedule of Assessments (Appendix 1). The specimens will allow an assessment of whether reactivity can be found for autoantigen-specific peptides. Proliferation, cytokine production, and other methodologies will measure immune responses. T cell lines and clones may be made from antigen-reactive individuals.

Additional analyses will compare the results in this trial to other trials using GAD-alum vaccine and other TrialNet studies. Data in this trial will be used in conjunction with other TrialNet data for exploratory analysis.

Additional analysis will be conducted evaluating primary, secondary, and other outcomes using the subjects that have received their intended treatment ("per-protocol analysis).

9.4. Sample Size and Power Calculations

The primary analysis will compare the difference between each treated group separately versus the placebo group (two pair-wise comparisons) in the levels of $\log(Y_{Cp}+1)$ using an ANCOVA model adjusting for gender, baseline age, and baseline $\log(Y_{Cp}+1)$. Estimates of the mean and standard deviation of $\log(Y_{Cp}+1)$ (expressed algebraically as: $\hat{\mu}_{\log(Y_{Cp}+1)}$ and $\hat{\sigma}_{\log(Y_{Cp}+1)}$) in the placebo group were obtained from prior studies. Among patients with baseline C-peptide > 0.2 pmol/ml and ≥ age 12 years, $\hat{\mu}_{\log(Y_{Cp}+1)} = 0.248$ and $\hat{\sigma}_{\log(Y_{Cp}+1)} = 0.179$ (3). An approximation of the mean of Y_{Cp} for the placebo group is calculated by applying the inverse transformation; this yields 0.282 pmo/mL.

Using standard equations for the comparison of two means, a sample size of 38 patients with complete data needed for the primary analysis per group, would provide power of 85% to detect a 60% increase (on the original scale) in the geometric mean C-peptide with either treatment relative to the placebo group using a test at the 0.025 level (one-sided).

Assuming that 10% of the patients will be "lost" (unavailable for the primary analysis), this study will require enrolling 42 eligible patients per group (126 total). The plan is to follow these patients two years from baseline and for up to 2 additional years post-treatment if residual beta-cell function or immunological effects are detected.

The exact final sample size can not be fixed to be exactly because of staggered patient entry. The study will be closed to new patients entering the screening phase when the total number then randomized plus a fraction of those in screening is expected to provide the proper number of eligible patients. Patients who had already conducted the initial screening visit at that time will be allowed to complete screening and be randomized if both consenting and eligible.

9.5. Interim Monitoring Plan

Interim analyses will be conducted periodically during the study and will be reviewed by the TrialNet DSMB for assessment of effectiveness and safety. The Lan-DeMets spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability (47) and to assess the significance of the interim results that emerge during the trial. The spending function that approximates the O'Brien-Fleming boundaries is:

$$\alpha_1(t^*) = 2 - 2\Phi\left[\frac{Z_{\alpha/2}}{\sqrt{t^*}}\right]$$

where t^* is the information fraction ($0 < t^* \leq 1$), α_1 is the α-*level* of the interim (one-sided) test and α is the over-all type I error.

The monitoring plan will allow for early termination based on the treatment effects on C-peptide values at 1 year of follow-up. The DSMB will also consider early termination due to absence of a treatment effect (i.e. futility) based on the method suggested by Ellenberg et al (49). The stopping rule is: if the t-test (as described in the primary analysis and positive values reflect a higher Y_{Cp} values among the experimental group) is less than or equal to 0 at $t^* \geq 0.5$, the study should be stopped based on the futility of rejecting the null hypothesis at the full completion of the trial. Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events.

10. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

10.1. Statement of Compliance

This study will be conducted in compliance with the protocol and generally consistent with current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Independent Ethics Committee (IEC) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented.

10.2. Participating Centers

Participating TrialNet clinical sites must have an appropriate assurance, such as a Federal-wide Assurance (FWA), with the Office for Human Research Protections (OHRP), since they are actively engaged in research and provide informed consent. The protocol and consent forms will be approved by Institutional Review Boards at each of the participating clinical sites. HIPAA regulations will be followed by each participating institution in accordance with each institution's requirements. The participating international sites will obtain approval from their corresponding review boards in accordance with their local procedures and institutional requirements.

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants participating in this study. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identify individuals. The investigational site will normally be notified in advance of auditing visits.

10.3. Informed Consent

The consent process will be conducted by qualified study personnel (the Trial or Study Coordinator and/or Investigator or other designee). All participants (or their legally acceptable representative) must read, sign and date a consent form prior to participation in the study, and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever important new safety information is available, when indicated for a protocol amendment, and/or whenever any new information becomes available that may affect a patient's participation in the study.

10.4. Study Subject Confidentiality

Study records with the study subject's information for internal use at the clinical sites will be secured at the study site during the study. At the end of the study, all records will continue to be kept in a secure location. There are no plans to destroy the records.

Study subject data, which is for reporting purposes, will be stored at the TrialNet Coordinating Center. Electronic Case report forms submitted to the Coordinating Center will identify participants by the unique TrialNet Identification Number. The data entry system at the Coordinating Center is a secured, password protected computer system. At the end of the study, all study databases will be archived at the Coordinating Center.

HLA genotyping is for research purposes only. The HLA genotyping result will not be made available to the participant and his or her physician. DNA will be stored for future use with the permission of the study subject.

Stored samples could be utilized to learn more about causes of Type 1 diabetes, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment. The results of these future analyses will not be made known to the participant.

10.5. Risks, Benefits, and Inclusion of Children

The risks of this study are presented in this protocol and in the informed consent form. This study will examine whether the GAD vaccine will preserve beta cell function, but there is no guarantee that this will occur.

There is the prospect of direct benefit to the individual subjects for their participation in the study. These potential benefits include the recognized benefits of being in a clinical study, including close monitoring and additional resources available to maintain tight glycemic control offered to all subjects, regardless of group assignment. Further, the intervention has the prospect of direct benefit to a given subject and is likely to yield general knowledge about T1DM which is of importance for the understanding and amelioration of T1DM in children.

The study procedures, while possibly slightly greater than minimal risk, offer the possibility of benefit in the close monitoring for all children. Assent of children along with consent of the parents will be obtained prior to any study procedures. This research proposal in children is therefore consistent with United States Department of Health and Human Services, Protection of Human Subjects, subpart D, section 46.405 (research involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects) and with Subpart D. 50.52 (Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects).

11. STUDY ADMINISTRATION

This study is part of Type 1 diabetes TrialNet, which is funded by the National Institutes of Health. Funding will cover the costs of administration and laboratory tests associated with this study during the participant's period of follow-up. Diamyd, Inc., will provide GAD-Alum free of charge for the participant's entire length of treatment.

11.1. Groups and Committees

11.1.1. GAD Vaccine Study Committee

The GAD Vaccine Study Committee, the TrialNet Clinic Monitoring Group, Laboratory Monitoring Group, Steering Committee and Data and Safety Monitoring Board will receive periodic reports from the TrialNet Coordinating Center on the progress of the study. These will include accrual rates and baseline demographic characteristics.

As appropriate, abstracts and manuscripts dealing with the progress of the GAD Vaccine Study shall be prepared by the GAD Vaccine Study Committee under the guidance of the TrialNet Publications and Presentations Committee under the policies established by TrialNet.

11.1.2. TrialNet Chairman's Office and TrialNet Coordinating Center

The TrialNet Chairman's Office and TrialNet Coordinating Center (TNCC) will collaboratively provide leadership to the TrialNet study group to include protocol and manual preparation, training for clinical sites, development of statistical design for each study, analysis of study results and the preparation of publications and presentations. The TNCC will also coordinate interactions among the participating TrialNet Clinical sites, laboratories including TrialNet core laboratories and other subcontract laboratories, NIDDK, and other sponsoring agencies.

11.1.3. Clinical Sites

Each Principal Investigator at the participating TrialNet clinical site will oversee all operations. The clinical sites will forward all laboratory and data collection form information to The TrialNet Coordinating Center for analysis. Conference calls and site visits, as needed, will facilitate evaluation of the trial management.

11.1.4. Clinical Site Monitoring

In order to conduct this study consistent with established research principles and ICH-GCP guidelines, there may be site visits conducted during the study to evaluate study conduct. All sites will be monitored by the Coordinating Center and appropriate TrialNet committees for patient enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the case report forms (CRFs), and the occurrence and reporting of adverse events (AEs) and serious adverse events (SAEs).

11.1.5. Data and Safety Monitoring Board (DSMB)

The DSMB will meet approximately every 6 months to review efficacy issues and adverse events prepared by the Coordinating Center. All adverse events will be recorded on the adverse event forms, which will be sent to the local IRBs, per their reporting requirements, and to the Coordinating Center. The DSMB will independently evaluate whether the adverse events constitute grounds to discontinue the study.

11.2. Sample and Data Storage

Samples to be stored for research purposes will be located at the NIDDK Repository and at TrialNet Sites. While TrialNet is active, the use of the samples will be restricted to TrialNet researchers unless researchers from outside of TrialNet obtain approval from the TrialNet Steering Committee and the NIDDK to utilize the samples. The samples will be coded with unique study numbers, but TrialNet researchers will be able to identify samples if it is necessary to contact participants for reasons of health or for notification to them about future studies. Approval from the TrialNet Steering Committee and the NIDDK would be required before such linkage could occur. Researchers from outside of TrialNet will not be permitted to identify samples.

Data collected for this study will be sent to the TrialNet Coordinating Center. After the study is completed, de-identified data will be stored at the NIDDK Repository, under the supervision of the NIDDK/NIH, for use by researchers including those outside of TrialNet.

When TrialNet is completed, samples will continue to be stored at the NIDDK Repository Sites. Since the stored data will be fully de-identified upon the completion of TrialNet, it will no longer be possible to identify samples. Thus, whereas a sample can be destroyed upon a participant's request during the existence of the TrialNet, it can no longer be destroyed once TrialNet is completed. However, there will still be the potential to link data derived from the samples with data that had been derived from TrialNet studies. Once TrialNet is completed, researchers will only obtain access to samples through proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

11.3. Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual center will not report the data collected from its center alone. All presentations and publications using TrialNet trial data must protect the main objectives of the trial. Data that could be perceived as threatening the masking will not be presented prior to release of the primary study outcomes. Approval as to the timing of presentations of data and the meetings at which they might be presented will be given by the TrialNet Steering Committee. Study results should be discussed with the news media only upon authorization of the Steering Committee, and never before the results are presented. Any written statements about this study that are shared with national media should be approved by the Steering Committee before release. All publications and presentations must be approved by the TrialNet Publications Committee.

11.4. Participant Reimbursement and Compensation

Participants will be compensated for each visit attended in the study.

12. STUDY TIMELINE

It is anticipated that patient enrollment will occur during the first two years of the trial. Subjects will be followed until two years after initial treatment. Subjects will be asked to undergo additional follow-up after two years for up to an additional two years with a visit every 6 months. Subjects with undetectable levels of C-peptide on the 30 month or any subsequent visits, will not undergo any further MMTT for assessment of C-peptide.

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APPENDIX 1 - Schedule of Assessments

Visit :	-1	0	1	2	3	4	5	6	7	8	9	10
~Month of Trial		0		1		3		6	9	12	18	24
~Day of Trial:	-X	0	14	28[1]	35[1]	84[1]	91[1]					
History	X	X	X	X		X		X		X	X	X
Physical Exam	X	X		X		X		X		X	X	X
Adverse Events Assessments		X	X	X	X	X	X	X	X	X	X	X
CBC with Differential	X						X					X
Chemistries	X					X						X
Viral serology	X											
Urine Pregnancy Test	X	X		X		X		X		X	X	X
Serum for Autoantibodies	X					X		X		X	X	X
PPD	X											
Study drug Administration		X		X		X						
Hemoglobin A1c		X				X		X	X	X	X	X
MMTT (4-hour)[2]	X											X
MMTT (2-hour)						X		X	X	X	X	
HLA Determination	X											
Immunologic Assessments	X	X	X	X	X	X	X	X		X		X
Samples for NIDDK repository [3]	X	X	X	X	X	X	X	X		X		X

NOTE: The schedule for these assessments may vary as appropriate according to the subject's age and body weight. At no time will the blood draw volume exceed 3ml/kg for a single draw and 7ml/kg over a 6 week period for subjects <18 years old.

[1] The target date for the 2nd and 3rd study drug administration will be set in accordance with the previous dose so that the first and second doses will be 4 weeks apart and the third dose will be 8 weeks later. Similarly, the visit target dates after the 2nd and 3rd study drug administration will be scheduled in relation to the actual date the injection was done.

[2] A 2 hour MMTT will be done for subjects less than age 12.

[3] With permission of subject