Effect of GABA or Combination GABA/GAD on the Progression of Type1 Diabetes Mellitus in Children

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IRB Protocol Number: F130807009

IND Number: 122749

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Name of Sponsor ***Kenneth McCormick, MD***
Name of Finished Product: GABA (Gamma-Aminobutyric Acid) DIAMYD® 20µg, subcutaneous injection (GAD-alum)
Name of Active Ingredient: Gamma-Aminobutyric Acid (GABA) Recombinant Human Glutamic Acid Decarboxylase (rhGAD65)
Title of Study: Effect of GABA or Combination GABA/GAD on the Progression of Type1 Diabetes Mellitus in Children
IRB Protocol #: F130807009
Investigators and Study Centre: One site including approximately 110 patients.
Phase of Development: Pilot study in humans
Objectives: • Evaluate the safety and influence of treatment with GABA on preservation of residual insulin secretion in recent-onset type 1 diabetes. • Evaluate the safety and influence of treatment with two doses of GAD-alum (Diamyd®) plus GABA on preservation of residual insulin secretion in recent-onset Type 1 Diabetes.
Study Design: The study is a 3-arm, randomized, double-blind, placebo-controlled, clinical trial. Patients will receive either i) oral GABA, dosed per kg, twice daily for 12 months plus 2 subcutaneous injections of 20 µg Diamyd in a prime-and-boost regimen over a period of 30 days ii) oral GABA, dosed per kg, twice daily for 12 months, Placebo GAD-alum 2 subcutaneous injections over a period of 30 days. iii) placebo. The patients will be followed for a total of 12 months.
Selection of Subjects: Patients must be age 4 to 18years old, and diagnosed with Type 1 Diabetes (T1D) within the previous 5 weeks of randomization. Patients must be positive for GAD-65 autoantibody to be enrolled. They may, or may not be positive for the other autoantibodies at the time of enrollment, but all will be followed during the study.
Number of Subjects Planned: Approximately **110** patients will be enrolled.
Description of Treatment Groups: The patients will be assessed for eligibility prior to randomization. On Visit 1 (Day 1, baseline), patients eligible for the study will be randomized to 1 of 3 treatment groups: • 30 patients will be assigned to receive oral GABA twice daily, dosed per kg, from Day 1 through Month 12. In addition 2 subcutaneous injections with 20 µg Diamyd (GAD-alum) will be given at Day 1 and Month 1, i.e., 1 prime and 1 booster dose (providing a total dose of 40 µg Diamyd). • 43 patients will be assigned to receive oral GABA twice daily, dosed per kg, from Day 1 through Month 12. In addition 2 subcutaneous injections with Placebo GAD-alum will be given at Day 1 and Month 1 • 37 patients will be assigned to receive oral Placebo GABA twice daily, dosed per kg, from Day 1 through Month 12. In addition 2 subcutaneous injection with Placebo GAD-alum will be given at Day 1 and Month 1

Name of Sponsor *Kenneth McCormick, MD*
Name of Finished Product: GABA (Gamma-Aminobutyric Acid) DIAMYD® 20µg, subcutaneous injection (GAD-alum)
Name of Active Ingredient: Gamma-Aminobutyric Acid (GABA) Recombinant Human Glutamic Acid Decarboxylase (rhGAD65)

Primary Endpoint:

- Evaluate the effect of GABA and GABA + GAD-alum combination on pancreatic beta cell function, as measured by meal stimulated C-peptide secretion levels compared to age-matched placebo controls, before and after one year of treatment.

Secondary Endpoints:

- Evaluate the effect of GABA and GABA + GAD-alum combination on autoimmune diabetes autoantibodies: GAD-65, ICA512, and Zinc Transporter 8(ZnT8A) and the effect on HbA1c, fasting and stimulated glucose & glucagon levels, fasting C-peptide and the amount of daily insulin usage by participants, from baseline through subsequent visits
- Evaluate the safety of GABA and GABA/GAD-alum combination

Safety

The safety assessment includes observation of reactions at the injection site, occurrence of adverse events (AEs), laboratory measurements, neurological assessments, and limited physical examinations.

Sample size:

The sample size for the proposed study is 110 children; 30 in the treatment group of active GABA and active GAD-alum, 43 in the treatment group receiving active GABA and placebo GAD-alum, and 37 in the placebo group. For the primary comparison of the 12-month post-baseline C-peptide measurements between these groups, assuming an α of 0.05 and a mean (SD) C-peptide AUC of 1.0 (0.4) this sample size yields a ~97% power to detect a 50% difference. Adverse events and other data will be summarized descriptively.

3. Introduction and Rationale for Study

The primary defect in autoimmune Type 1 Diabetes Mellitus (T1DM) involves infiltration of the pancreatic islet cells by T-lymphocytes, macrophages, and other immune cells, and consequent loss of β-cells [1-3]. At the onset of T1DM, more than 70% of β-cells are destroyed [4], whereas the residual β-cells most likely represent the only reservoir for the potential regeneration of islet β-cell mass [5]. A series of immunological abnormalities have been reported in those with T1DM including, but not limited to, the production of autoantibodies (i.e., glutamic acid decarboxylase (GAD-65), tyrosine phosphatase-related islet antigen 2 (IA2), Zinc Transporter 8 (ZnT8A), or insulin (IAA)) as well as alterations in the capacity of regulatory T cells (Treg) to suppress the action of effector T cells (Teff); the latter population thought as playing a key role in the immune destructive processes. Therefore, a vast majority of studies attempting to prevent or reverse this disease have focused on immune suppression [6]. While these efforts have shown limited promise, most (including those investigated in children) have imparted multiple side effects which, in turn, have resulted in questioning the value for the short term benefits associated with utilizing these drugs [6-9]. Hence, the identification of agents with improved safety profiles, alongside the ability to preserve the metabolic capacity of β-cells, represents an important strategy. To that end, we hypothesize that gamma-aminobutyric acid (GABA), a naturally occurring substance in physiology, has the potential to locally reduce inflammation and protect pancreatic β-cells from auto-immune destruction.

GABA, synthesized from glutamate by GAD, is a well-known major neurotransmitter in the CNS [10] and acts mainly through the GABAA receptor (GABAAR) [11]. GABA is also locally produced by pancreatic β-cells [12]. GABAARs are also expressed in various immune cells, including T-cells, peripheral blood mononuclear cells, and are known to exert immune-inhibitory effects [13-15]. Interestingly, GABA appears to play multiple roles in the pancreas. Firstly, GABA promotes β-cell growth and survival [12]. Secondly, GABA can act on GABA(A) receptors in the pancreatic α-cells, in so doing suppressing glucagon secretion [16]. Lastly, GABA suppresses inflammation and increases regulatory T-cell numbers [12]. In vitro assays determined that GABA suppressed the production of IL-12 by macrophages, and of IFN-γ by CD8 T-cells concluding that GABA produces an anti-inflammatory effect by reducing the levels of these cytokines [12]. There are reports that in type 1 diabetic mouse models, GABA prevents and reverses the disease [12]. Soltani et al reported that mice with severe T1DM treated with GABA reduced lymphocytic islet infiltration, restored the β-cell mass and completely reversed hyperglycemia in these mice [12]. This was associated with increased serum insulin, decreased glucagon levels in the circulation, and improved glucose homeostasis.

1.1 Significance (composed for layperson)

As noted previously, T1DM is an autoimmune disease in which the body's immune system attacks and destroys the insulin producing β-cells of the pancreas. Therefore, the children affected by the condition present with high blood sugars. This condition is prevalent, affecting up to 1:400/500 persons worldwide. T1DM, previously known as juvenile diabetes, usually strikes in childhood, adolescence, or young adulthood, but lasts for a lifetime. To date, there have been no innocuous treatments that can arrest or reverse the ongoing β-cell destruction. Children with diabetes require multiple daily injections and, despite this, normal glucose regulation is exceedingly challenging. Moreover, they are at risk for heart disease, kidney failure, eye problems, and other complications from this life-long condition.

Based on the aforementioned information, we envisage that administering GABA to those with new onset T1DM may preserve or increase residual insulin production, suppress glucagon release, and decrease the inflammation surrounding the pancreas. With this, GABA may prolong the β-cell life after diagnosis. Combining with GAD-alum injections, which aim to halt the autoimmune attack by inducing tolerance thereby saving residual insulin production, may improve glycemic control even more and significantly decrease the risk of hypoglycemia and long-term complications in the future. There have been many other intervention trials for T1DM (including those involving children), but we believe this study has many clear advantages. First, GABA is widely considered safe and has very few side effects [17] and GAD-alum has been studied in approximately 700 patients in clinical trials with no safety concerns. In contrast, previously conducted studies of other interventions have typically involved some form of immunosuppression, which causes significant side effects that often outweigh the benefits [6-9]. Second, GABA is sold over -the -counter at many local food/health stores at a very reasonable cost (i.e., approximately $5-7 per bottle) and GAD-alum is easy to use (subcutaneous injection twice over a 1 month period). If the hypothesis is substantiated, the economic and social impact would be profound as T1DM, besides its noxious effects, is an extremely costly disease to manage. Lastly, GABA is an oral treatment and GAD-alum only involves two subcutaneous injections in contrast to many other intervention trials in this subject population, which required multiple intravenous injections/ infusions of medications, even to the point of those requiring extensive in-patient hospital days [18]. In sum, this pilot study may increase our current understanding of T1DM and may direct future therapies for new onset disease.

4 Specific Aims

Conduct a randomized, double-blind, placebo controlled one year clinical trial utilizing GABA or GABA/GAD combination in patients with newly diagnosed T1DM.

4.1 Objectives

- *Evaluate the safety and influence of treatment with GABA on the preservation of residual insulin secretion in recent-onset type-1 diabetes.*
- *Evaluate the safety and influence of treatment with two doses of GAD-alum (Diamyd®) plus GABA on preservation of residual insulin secretion in recent-onset type-1 diabetes.*

4.2 Primary Endpoint: Evaluate the effect of GABA or GABA/GAD combination administration on pancreatic beta cell function, as measured by meal stimulated C-peptide secretion levels compared to age-matched placebo controls, before and after one year of treatment.

4.3 Secondary Endpoint:

- Determine the effect of oral GABA or GABA/GAD combination administration on autoimmune diabetes autoantibodies: GAD-65, ICA512, and Zinc Transporter 8(ZnT8A) and the effect it has on HbA1c, fasting and stimulated glucose and glucagon levels, fasting C-peptide the amount of daily insulin usage by participants from baseline through subsequent visits.
- Evaluate the safety of GABA and GABA/GAD –alum combination.

5.0 Study Design

5.1 Subject Recruitment:

The patients will be approached regarding the study at the time of admission for new onset T1DM at The Children's Hospital of Alabama, a tertiary care university referral care center with greater than 150 new -onset diabetics referred per year. Participants willing to enroll will be consented prior to discharge from the hospital, or at/before the one month hospital follow up visit. Approximately 110 patients, 4-18yrs of age, will be enrolled, 30 of which will be on both the oral(GABA) and injected(GAD-alum), 43 on oral GABA and 37 on placebo-see outline below. The endocrine research nurses will aide in identifying study candidates and contacting families to remind them of appointments. Both the PI and other faculty have been successful in recruiting subjects for clinical trials at this institution.

There will be 3 arms of the study:

- Placebo = all placebo, placebo oral GABA and placebo GAD-alum
- GABA = oral GABA plus placebo GAD-alum
- GABA and GAD-alum= oral GABA plus GAD-alum

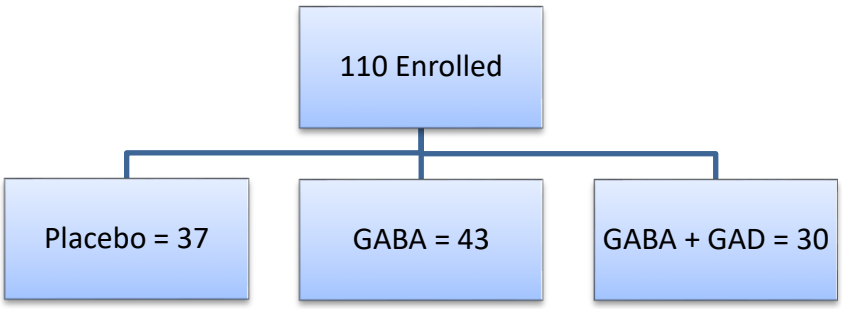
5.2 *Study Outline*

1) Males and females ages 4-18yrs will be recruited

2) Must meet ADA definition of Type I Diabetes

3) Must be positive for autoantibody GAD-65

COHORTS



5.3 Inclusion Criteria

- Males and Females 4-18 years of age.
- Be positive for autoantibody GAD-65. If not collected. Collect and send at visit 1.
- They must meet the ADA criteria for diabetes : classic symptoms, plus blood sugar >200mg/dL or fasting blood sugar >126mg/dL
- Enrolled within 4 weeks of diagnosis (+ 1 week window)
- If the participant is female and post-menarchal two forms of contraception must be used during the study if not abstinent. The types of contraception deemed acceptable would be oral contraceptive pills, intrauterine devices and barrier methods.
- Signed informed consent form

5.4 Exclusion Criteria

- Chronic systemic steroid use, including inhaled compounds, or any medication which can alter glucose metabolism
- Treated with any other oral or injectable hyperglycemic medication other than insulin.
- Obesity, defined as BMI >95 percentile, or BMI>27 in adolescents with acanthosis score between 1-1.5
- Pregnant and/or breastfeeding
- History of seizure disorder (epilepsy, head trauma, cerebral vascular accident), or any clinical features of continuous motor unit activity in proximal muscles
- Patients on medications which may disturb GABA action, such as Baclofen, Valium, Acamprosate, Neurontin, or Lyrica
- History of alcoholism or any substance abuse
- Chronic disease (such as liver, cancer, cystic fibrosis, or renal failure)
- Chromosome abnormality (such as trisomy 21, Turner Syndrome,..etc)
- History of anemia, or significantly abnormal hemoglobin results at screening
- Clinically significant history of acute reaction to vaccines and other drugs in the past.
- Known history of HIV or hepatitis
- Unwillingness to comply with conditions of the protocol
- History of illness, besides diabetes within 2 weeks of first GAD-alum/placebo injection
- Any condition that the PI feels would not be beneficial for the subject to be on study.

5.5 Informed Consent:

Potential participants will be approached by research personnel during initial hospitalization for new onset Type I Diabetes at The Children's Hospital of Alabama to provide information regarding the study. If interested, the informed consent document will be provided to the potential participant and contact information will be provided for study personnel. Subjects choosing to participate will be asked to consent for study participation prior to, or at the first clinic visit post diagnosis. Before the first follow up clinic appointment at one month, written consent from the parents and patient 14 years or older, or assent from children aged 7-13, will be obtained in a quiet setting prior to the initiation of any study procedures. Patients will not be consented in writing until they are able to demonstrate adequate understanding of all aspects of the study and consent process. A copy of the consent form will be given to the patient. The signed consent form remains in the patients study files at the clinical center. Consented participants will then be randomized

5.6 Randomization:

A total of 110 patients, with 30 in the GABA + GAD-alum group 43 in the GABA + placebo GAD-alum group, and 37 in the placebo GABA and placebo GAD-alum group will be enrolled. The study will be double-blinded with randomization under the stewardship of the Children's Hospital research pharmacist. Subjects will be randomized to one of the three treatment groups in a 1:1:1 ratio using a pre-set randomization list (generated by using a computerized procedure) known only to the un-blinded pharmacist.

The patients will be followed for a total study period of 12 months which includes 5 visits to the clinic.

See table 1 below for an overview of randomization groups and study visits.

Table 1. Randomization and study visits

Study	**Day 1 Baseline Randomization**	**Mo 1**	**Mo 5**	**Mo 8**	**Mo 12**
	Visit 1 <5 weeks from diagnosis	**Visit 2 ± 7**	**Visit 3 ± 7**	**Visit 4 ± 7**	**Visit 5 ± 7**
Group 1 GABA-GAD group (GABA+GAD-alum)	GABA twice daily, every day from Baseline to Month 12				
30 subjects	*Prime injection* GAD-alum 20 µg	*Boost injection* GAD-alum 20 µg			
Group 2	GABA twice daily, every day from Baseline to Month 12				
GABA group (GABA+ Placebo GAD-alum)	*Prime injection* Placebo GAD-alum	*Boost injection* Placebo GAD-alum			
43 subjects					
Group 3	Placebo GABA twice daily, every day from Baseline to Month 12				
Placebo group (Placebo GABA + Placebo GAD-alum)	*Prime injection* Placebo GAD-alum	*Boost injection* Placebo GAD-alum			
37 subjects					

5.7 Treatment Doses and Schedule

1) Oral Gamma-Aminobutyric Acid (GABA)

We shall not exceed the recommended adult dosages (up to 1.5 grams/day) and we will adjust the dose to the body surface area of the children enrolled in our study. For example, the average adult has a body surface area of 1.5 - 2 m2. Therefore, the current recommended adult dosage is equivalent to 1 grams/m2/day, and this is the dose we will be giving to our trial participants. This total daily amount will be divided in two daily doses to be given with breakfast and dinner. The GABA will be supplied by the manufacturer NOW Foods in 200 mg capsules. Each dose will be rounded to the nearest 100 mg. The placebo will also be provided by NOW Foods and will be indistinguishable in appearance, taste, and smell from the GABA capsules. The capsules will be opened and dissolved in water, or sprinkled on food immediately before consumption. Documentation of these products by site personnel will be documented in the case report form (CRF).

2) Subcutaneous GAD-alum

The Diamyd Drug Product (GAD-alum) is composed of the recombinant human GAD65 (rhGAD65) protein formulated in a sterile, non-pyrogenic phosphate buffered saline containing the aluminum hydroxide adjuvant, Alhydrogel, and will be provided by Diamyd Medical, Sweden (GAD-alum). The active ingredient, rhGAD65, is manufactured via a process involving expression in an insect cell line. The Drug product is supplied in a 3 mL glass vial with either 20µg Diamyd alum-formulated vaccine/mL or placebo. The dose volume is 0.5 mL for each subcutaneous administration. The vials have a slight overage to allow a withdrawal of 0.5mL. All dosing will take place in the hospital-based clinic, and handled only by trained, authorized study personnel.

All subjects will receive an injection of either GAD-alum or placebo at baseline (within 1 month after diagnosis), and a booster vaccine approximately 1 month later. The 20µg of GAD-alum (Diamyd Medical) is administered subcutaneously and, prior to exiting the clinic, the patient is observed for 1 hour post injection. The placebo injection has the equivalent components save, of course, the recombinant GAD, and is prepackaged in identical-appearing vials. The vials will be packaged into identical vial boxes. Both the vial box and the vial will be labeled with the same treatment number. Allocation of treatment to GAD-alum or placebo will be accomplished through Children's of Alabama Research Pharmacist.

The Diamyd Drug Product and Placebo Diamyd drug Product will be administered by the site personnel, and the administration will be documented in the site source documentation.

All vials of Diamyd and placebo will be kept between 2 and 8°C in a refrigerator that is behind a locked door in the Research Pharmacy to protect from unintended use. The temperature of the refrigerator is monitored constantly for accuracy. Any temperature deviations will be reported to Diamyd as soon as the site becomes aware of it. Vials should never be frozen, and any frozen vials should be rejected and is not allowed to be used clinically.

Diamyd product and placebo will be shipped from the distributor to the study site according to country specific laws and regulations. Diamyd product will not be offered to patients after the completion of the study. Participants will thereafter receive therapy at the discretion of the investigator.

Study Medication Accountability:

All study medication supplied for this study will be retained in the locked Research Pharmacy at the Children's Hospital of Alabama. The only persons with access to this room are the Research Pharmacists. Dispensing of the drug will occur only by the Research Pharmacist to authorized study personnel. A study medication inventory (dispensing records) for all medications dispensed will be kept in the Research Pharmacy and will be kept up to date. Used and unused study medication will be stored at the site or pharmacy throughout the study. The investigator/pharmacist will keep record of all drugs received, used, and returned.

At study end, all used and unused vials of Diamyd product and placebo must be returned to the central facility and subsequently to the supplier (Diamyd Medical), unless the supplier has approved other arrangements. Any destruction of the vials must be approved by Diamyd Medical and performed in accordance with the documented approved procedure from the supplier.

All research medications will be labeled with the following:

- Subjects name
- Date of dispensing
- Name of investigational drug
- Directions for use
- Prescribers name
- Lot # (if applicable)
- Expiration date (if applicable)
- Caution label for Investigational Use Only

5.8 Blinding and Code Breaking

Investigator site staff, persons performing assessments, and data analysts will remain blind to the identity of the treatment from the time of randomization until database lock, using the following methods. (1) randomization data are kept strictly confidential until the time of un-blinding and will not be accessible by anyone else involved in the study, with the exception of the independent, un-blinded pharmacist approving the randomization scheme and providing unblinded safety data to the DSMB per request. (2) the identity of treatment will be concealed by the use of matching placebo to the study drugs that are identical in packaging, labeling, and appearance and scheduled administration. Participants will be blinded in the same manner from randomization until their 12 month research visit. At that time, they will be un-blinded to their GABA treatment by the means of a sealed letter from the research pharmacist. This is done so that those who have received active GABA can continue their treatment, if they so choose, by purchasing GABA over the counter and not potentially losing beta cells as a result of discontinued therapy until results are available (2018/2019). It also allows those who have received placebo to not feel obligated to purchase GABA over the counter since we do not feel there would be benefit after 12 months of placebo therapy. The GAD status will remain blinded throughout the study for all participants. The participants and their families are asked to keep the letter in strictest confidence by not discussing with any study or clinic staff, and present or potential participants to the study.

The treatment code may be broken on an individual basis, in case of an SAE for which the investigator must know the study agent identity to initiate appropriate treatment. The investigator will be supplied the necessary information to break the study blind on an individual patient basis. If the code is broken for one individual subject, the date, time, and reason for un-blinding, together with the investigators signature, must be recorded and the sponsor and Diamyd, or its designee must be notified within 24 hours. Patients will continue in the study unless it is deemed inappropriate.

Suspected unexpected serious adverse reactions (SUSARS) will, if required, be un-blinded for reporting to regulatory agencies and ethics committees, Diamyd Medical, Janssen/JDRF (or its designees) will be supplied with the necessary information to break the study blind for individual patients as required for regulatory reporting purposes. However, the investigator, sponsor, and study team will be kept blinded to the treatment allocation.

The study blind will be broken when all patients have completed the study, and all data for this period has been entered into the database and locked. One randomization list is retained by the un-blinded pharmacist. After the database lock, the results will be analyzed and un-blinded where after a 12 month analysis will be performed.

6 Visit Schedule, Study Procedures, and Laboratory Measurements

6.1 Our new-onset type 1 diabetic children are admitted to the hospital, typically at most for two days, for initial glucose management and diabetes education. Diabetes is defined per ADA recommendation [20]. All patients with newly diagnosed type 1 diabetes return for follow up at approximately one month after diagnosis. Prior to this visit subjects may be enrolled if consented and enrollment criteria is met. This will serve as the baseline visit. The second visit should then be scheduled one month from the baseline visit (second injection with GAD-alum/placebo) with a visit window of +/- 7 days. Please note that all visits must be calculated from the baseline visit according to the visit schedule. See table 2 for schedule of patients visits, visit windows and study drug administration.

If patients are febrile when scheduled for an injection, treatment will be postponed until the patient is afebrile.

Table 2 Schedule of Patient Visits, Visit Windows and Study Drug Administration

Study	Intervention				
	Day 1 Baseline	Month 1	Month 5	Month 8	Month 12
	Visit 1	Visit 2 ± 7	Visit 3 ± 7	Visit 4 ± 7	Visit 5 ± 7
GABA/ Placebo	Every day from Day 1 through Month 12				
GAD-alum/ Placebo	Prime: One injection	Boost: One injection			

6.2 Study Events

The treatment therapy consists of oral GABA or placebo given with meals twice daily at breakfast and dinner throughout the study and 2 GAD-alum or placebo injections given at baseline and 1 month later . i.e.1 prime and 1 booster dose.

Study visits for all patients will occur for randomization at baseline and subsequently for the injection, and for medical monitoring.

General assessments include:

- medical history and routine physical exam
- concomitant medications
- adverse events
- Laboratory Assessments (hematology, biochemistry, urine pregnancy test as appropriate, islet antibodies, liver function and urine studies)

Metabolic assessments include:

- Glucose records and reports of hypoglycemia
- Insulin Dose
- HbA1c
- Mixed Meal Tolerance Test

6.2.1 Mixed Meal Tolerance Test

Participants will undergo a mixed meal tolerance test at their outpatient visits as outlined in the instructions below.

- On the evening before the MMTT, subjects will eat a full, standard meal. The will be asked to fast from 9 pm the evening before the test until the MMTT is completed. They should remain hydrated and may consume water during the fast and test.
- Subjects will be instructed to take their long acting insulin the night before the test, but not to administer short-acting insulin for at least 6 hours before the morning MMTT test.

- Participants must have a fasting plasma glucose level in the range of 72-216 mg/dL on the patient's home glucose monitoring device the morning of the test. If for safety reasons, subjects need to eat or take insulin, the visit will need to be rescheduled.
- An Intravenous catheter will be inserted just prior to the test and then GABA or placebo will be given just prior to drinking the Boost High Protein drink (1 cal/mL; 55% carbohydrates, 21% lipids and 24% protein). They will drink 6 ml/kg Boost, up to maximum of 360 ml, to be ingested within 5 minutes. Blood samples from an intravenous catheter are collected 10 minutes prior to the meal (-10), at the time of ingestion (0), and at 15, 30, 60, 90 and 120 minutes thereafter. This schedule will be followed at the baseline and 12 month visits. At 1 month and 5 month visits only a fasting and 90 min sample will be taken. There will not be a MMTT on month 8.
- 3 cc's of urine (if available) will be placed in an aliquot tube and frozen for evaluation of urine C-peptide. The remaining sample will be used for evaluation of microalbuminuria and creatinine levels.

6.2.2 Neurological Assessment

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

The neurological examination includes:

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

These examinations may also be repeated between scheduled visits at the discretion of the investigator. If any signs of neurological dysfunction are detected, the patient should be referred to a pediatric neurologist for further evaluation.

Additional studies will be performed to evaluate the effect of GABA or GABA/GAD on relevant biochemical and hormone concentrations as outlined in the Table below and also to monitor for any possible adverse effects.

Table 3. Schedule of Study Events

	At Baseline	1mo post baseline (+/- 1 week)	5mo post baseline (+/- 1 week)	8mo post baseline (+/-1 week)	12mo postbaseline (+/- 1week)
Informed Consent	X (or before)				
Randomization	X				
Gad-alum or placebo injection[a]	X	X			
Oral GABA or placebo administration starts	X				
Medical History	X				

Vital Signs	X	X	X	X	X
Ht	X	X	X	X	X
Weight	X	X	X	X	X
Physical Exam + Neurological	X	X	X	X	X
Hgb A1C	Done as standard of care at every visit.		Obtain results from chart		
Growth Rate	X	X	X	X	X
Hx of DKA	X	X	X	X	X
Autoimmune antibodies	Done as standard of care at diagnosis. Obtain and document results.		X		X
Mixed Meal Tolerance Test (glucose, glucagon, C peptide,)	X	X (only fasting and 90 min samples)	X (only fasting and 90 min samples)		X
CBC with diff	X		X		X
Hx of Hypoglycemia	X	X	X	X	X
Complete Metabolic Profile	X		X		X
Glucagon(fasting), glucose, C-peptide	X	X	X		X
Urine Pregnancy test (for menstruating females)	X	X	X	X	X
Total Daily Insulin Dose(U/kg)	X	X	X	X	X
Medication Review/ Concomitant Medications	X	X	X	X	X
Cell Activation Markers	X		X		X
Inflammatory Cytokines	X		X		X
Skin exam: *injection site:* [b]	X	X	Ask regarding any issues after last injection. X		
Urinalysis (if available) for microalbuminuria and creatinine and C-peptide	X	X	X	X	X
Adverse Events	X	X	X	X	X
Proinsulin	X		X		X

[a] GAD-alum/Placebo administration: The target date for the second GAD-alum/Placebo administration will be set in accordance with baseline (i.e. the first injection, Visit 1) so that the second dose will be 1 month post first dose.

[b] Inspection of injection site before and 60 minutes after injection by investigator or nurse

6.3 *Patient Compensation*

Children will receive $60 at each visit that requires a mixed-meal tolerance test.

6.4 Patient Management

During the study period, all participants will receive intensive management of their diabetes**.** The goal of treatment will be to keep HbA1c levels within the currently recommended American Diabetes Association age-specific target range in the absence of significant or severe hypoglycemia or diabetic ketoacidosis**.**

All insulin dose adjustments, at either the clinic visit or by phone consultation, are at the discretion of the UAB Pediatric Endocrine Division staff (physicians, diabetes nurse practitioners, diabetic nurse educators) as per our usual type 1 protocol.

6.5 Rescue Therapy:

Hypoglycemia (low blood sugar) is frequently encountered in diabetics, it could potentially occur after starting GABA or GABA/GAD treatment. All diabetic children and/or their parents are routinely trained to treat hypoglycemia by Children's of Alabama diabetes educators. As standard procedure, all patients have glucagon available if a low blood sugar has rendered them unresponsive. The parents and subjects are provided the research teams contact information and there is a doctor on call 24hrs a day.

6.6 Withdrawal of Participant Consent and Discontinuation of Study Drug:

Study participation may be discontinued for any of the following reasons:

a. Subject decision to withdraw consent for study
b. Evidence of allergy to administered products
c. Intolerable adverse event as judged by study investigator and participant

If the PI chooses to remove the participant from study dosing, rationale will be provided to the participant. If the study drug is discontinued, unless the subject withdraws consent, the subject will be followed for the full treatment period and all data will be collected as scheduled. Attempt will be made to schedule an early end of study assessment in the case of study drug discontinuation. If more than one participant have the same SAE that are thought to be related to treatment compound, the PI and Co-Investigators will meet to discuss whether or not a change in protocol is called for. This information will also be assessed by the independent safety monitoring board. This decision would be reported to the IRB.

6.7 Data Collection and Assays:

Subject visits will consist of a baseline visit and subsequent visits as noted above. The data noted in the table above will be collected by study personnel and stored in a locked office and on a password-protected UAB computer. All laboratory specimens will be collected by study personnel, labeled with the study participant's unique identifier and date of collection, and then stored at -80°C in Dr McCormick's lab. Analysis of all laboratory measurements will be completed at the conclusion of the study.

1.) *Hemoglobin A1C:* Measured by latex immunoagglutination inhibition methodology(DCA 2000, Siemens-Bayer)
2.) *Mixed Meal Tolerance Test*: After an overnight fast, the patients will have an IV placed and will drink Boost. They will have C-peptide and glucose and glucagon levels drawn at10 minutes prior to dose (-10), 0, 15, 30, 60, 90, and 120 min. The C-peptide, glucagon, and glucose levels will be assayed by the UAB Metabolism Core of the Clinical Nutrition Research Center (CNRC). At the 1 and 5 month visits, only a fasting and 90 minute sample are obtained. A recent 2013 article confirms that this 90- minute sample alone correlates significantly with the integral of the C- peptide as a function of time curve, oft-called the AUC [21].
3.) *Pancreatic Antibodies*: GAD-65, ICA512 and Anti-Zinc Transporter 8 samples are collected as standard of care at initial diagnosis. We will use these results as our baseline. Follow-up samples will be collected at the visits noted in the Table. Samples will be assayed at ESOTERIX Laboratory Services, Inc..
4.) *CBC with manual differentiation*: Performed at baseline and at the visits noted in the Table, and assayed in the hematology lab of Children's Hospital Alabama

5.) *Complete Metabolic Profile*: CMP will be monitored at the visits noted in the Table, and assayed in the chemistry lab of Children's Hospital Alabama.

6.) *Glucagon*: Glucagon levels will be measured under fasting conditions at the visits noted in the Table, and during the Mixed Meal Tolerance Tests. Assays will be conducted by UAB Metabolism Core of the Clinical Nutrition Research Center (CNRC).

7.) *Height:* Collected at each visit

8.) *Weight:* Collected at each visit to make dosing adjustments in the GABA .

9.) *Medications:* We will review total daily insulin dose and compliance. We will review all other non-study medications which the patients are taking.

10.) *Date of Birth:* Obtained at baseline screening.

11.) *Medical History*: Will be obtained at screening and updated as needed throughout the study.

12.) *Demographics*: Collected at baseline.

13.) *Adverse events/Serious adverse events*: Assessed for adverse events and serious adverse events following the baseline visit.

14.) *Cell Activation and Inflammation Markers:* Since GABA has been shown to possible improve inflammation and cellular activity in animal studies we will evaluate for inflammatory and cell markers at varied points in the study. We propose a detailed immune analysis strategy that has 3 arms: (1) analysis of functional CD4 and CD8 subsets (effector vs regulatory) We will perform immunophenotyping studies of the peripheral CD4 and CD8 subsets at baseline and at the 5 and 12 month study visits with monoclonal antibodies specific for CD3, CD4, CD8, CCR4, CCR5, CCR7, CXCR3, CXCR5, CD25, CD28, CD38, CD39, CD45RA, CD45RO, CD62L, CD127, Helios, Foxp3, and PD-1[BD Biosciences, R&D Systems, eBiosciences] Intracellular cytokine staining of PMA/lonomycin-anti-CD3/CD28, GAD65 peptivator (Miltenyi Biotec), and tetanus toxoid-stimulated cells will also be examined with monoclonal antibodies to IFN-#, TNF-, IL-13, IL-10, and IL-17A (BD Biosciences, eBioscience) We expect to observe a decrease in CD4 and CD8 Tcell effector cell populations secreting IFN-# and a concomitant increase in CD4 Treg cells (CD4+/CD25+/FoxP3+) that secrete IL-10. Human multi-color flow cytometric analysis will be performed as previously described. (2) functional assays of T cell responses after GABA exposure. we will perform T cell proliferation (CFSE dilution or cell counting kit-8 assays and primary recall assays as previously described to determine if GABA treatment has as effect on decreasing effector cytokine production of CD4 and CD8 Tcells and a concomitant increase in Treg cells as described above. Briefly, PBMCs will be purified from fresh blood and stimulated with PMA/lonomycin. Dynal beads conjugated with anti-CD3 and anti-CD28, GAD65 Peptivator peptides and tetanus toxoid. Supernatants will be collected and the levels of cytokines and chemokines produced by activated T cells will be determined with a Milliplex Human Cytokine/Chemokine Magnetic Bead panel (3) monitoring of serum autoantibody expression {both number of autoantibodies expressed, as well as changes in titer} over the course of the study. The prescence and number of positive autoantibodies correlates not only with diabetes diagnosis, but also with degree of beta cell destruction and progression of disease. We are planning subgroup analyses to see if GABA and or GABA/GAD combination have different efficacies based on antibody titers and number of positive antibodies (i.e. participants who are likely to be slow- or fast- progressors). We also want to determine if the number of positive autoantibodies per participant changes, either increases, or decreases, with GBA therapy. Obviously we understand that autoantibodies are not directly pathologic to beta cells, but they may serve as an epiphenomena/surrogate marker for the degree of beta cell destruction. We also propose to add the pro-insulin/C-peptide ratio as a marker of beta cell health. We have arranged with our Metabolism Core Lab at UAB to run the proinsulin levels. For participants who have already completed some study visits, these assessments can still be run from the samples previously collected. HLA (Human Leukocyte Antigens will be performed per Dr. Tse. This will be done on samples already collected for the Cell Activation and Inflammatory Markers. The genes in HLA are reported to account for 40-50% of the familial aggregation of Type 1 diabetes. There are certain genes within the HLA typing that have been shown to cause a rapid decrease in β-cell function, whereas other genes seem to have a more gradual destruction. We feel this information can be most beneficial in our research concerning how Type 1 progresses and does the HLA typing effect how these patients respond to the GABA/GAD therapy. Diamyd agreed to fund the additional cost of the research.

15.) Urine pregnancy test: at each visit for menstruating females.

16.) Skin inspection: : and reports of problems with injection site at baseline and the 1 month visit, as well as the 5 month visit to capture any reports of problems after the second injection.

18.) *Physical examination including neurological assessment and vital signs.*

19.) *Assessment of number of hypoglycemic events and DKA's.*

20.) Urinalysis for microalbuminuria, creatinine, and C-peptide (if available)

7.0 Adverse Event Reporting and Safety Monitoring

7.1 Adverse Events:

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and which does not necessarily have to have a causal relationship with this treatment. An AE 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.

All treatment emergent AEs will be recorded from the baseline visit forward (First dose of study drug) for the duration of the study on source documents (i.e., original documents, data, and records). AEs include those reported spontaneously by the subject and those noted incidentally or as observed by the investigator or study personnel. To avoid vague, ambiguous, or colloquial expressions, the AE will be recorded using standard medical terminology that is as specific as possible, rather than the subject's own words. Whenever the investigator is confident in making a unifying diagnosis, all related signs, symptoms, and abnormal test results should be grouped together and recorded as a single AE (e.g., cough and rhinitis should be reported as an "upper respiratory tract infection").

All clinically significant abnormalities noted upon physical examination, or other diagnostic test results should be reported as an AE, except for baseline measurements that may be considered part of the medical history. In addition, all clinically significant AEs that continue at Study Termination should be followed up by the investigator and evaluated with additional tests if necessary, until the underlying cause is diagnosed or resolved, or is determined to have resolved without sequelae. All AEs will be evaluated for intensity and causal relationship with use of the study medication (or study procedures if applicable) by the investigator.

AEs that occur following completion of study termination/early termination procedures will be recorded on the AE page of the source documents only if the investigator considers the event as clinically significant and as related to study medication or study procedures. All adverse events that are deemed to be serious and meet the definitions provided in section 7.2, for serious adverse events will be reported as¨ SAE's."

7.2 SAEs

Any AE that results in any of the following outcomes will be considered an SAE. The following outcomes are defined according to Code of Federal Regulations (CFR) Title 21 part 312.32.

An SAE is an AE, occurring at any dose that fulfils 1 or more of the following:

- Death
- Life-threatening situation (Subject was at risk of death at the time of the event. This does not refer to an event that might have caused death if it was of greater intensity.)
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability or incapacity
- Congenital anomaly or birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization but may jeopardize the subject and may require medical or surgical intervention to prevent one of the above outcomes (based upon appropriate medical judgment), e.g., allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.3 Methods for Collection and Recording of Adverse Events

All patients are carefully monitored for occurrence of any AE. At each clinic visit/contact after baseline AEs will be collected with a non-leading question such as "Have you had any new or worsening health problems since the previous visit/last contact?" as well as reporting events directly observed or spontaneously volunteered by the patient.

Any abnormal findings in physical examination, vital signs, and clinical laboratory tests must also be reported as AEs.

All reported AEs are to be recorded in the CRF, and an identical entry should be made in the patient's medical record. If no AE has occurred during the study period, this should also be indicated in the CRF/medical records.

7.4 Grading and Attribution

7.4.1 Intensity

The study site will grade the intensity of adverse events experienced by study participant according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI CTCAE manual:

Grade 1 = mild adverse event

Grade 2 = moderate adverse event

Grade 3 = severe and undesirable adverse event

Grade 4 = life threatening or disabling adverse event

Grade 5 = death

All adverse events will be reported and graded whether they are or are not related to disease progression or treatment.

NOTE: A distinction should be drawn between serious and severe AEs. The term "severe" is used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe pain); the event itself, however, may be of relatively minor medical significance (such as severe headache), and does not necessarily need to be considered serious. The term "serious" is used to describe an event based on an event outcome or actions usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

7.4.2 Definition of Attribution

The relationship, or attribution, of an adverse event to an investigational product will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate CRF and/or SAE report form. The relationship of an adverse event to the study treatment will be defined as noted below.

Unrelated Category:

1 Unrelated: The adverse event is clearly not related to the investigational agent.

Related Categories:

2 Unlikely: The adverse event is doubtfully related to the investigational agent.

3 Possible: The adverse event may be related to the investigational agent.

4 Probably: The adverse event is likely related to the investigational agent.

5 Definite: The adverse event is clearly related to the investigational agent.

NOTE: Relationship to study medication will be assessed for the two treatments (GABA and GAD-alum) separately

7. 5 Timelines and reporting of SAE

Reporting timelines are necessary to establish compliance with Regulatory Authority requirements for the reporting of drug safety data

The investigator should complete an SAE report and submit via e-mail or fax to the IRB and Diamyd Medical (or its designee) within 24 hours of the investigator becoming aware of an SAE. Any new information relating to an SAE that subsequently becomes available should also be faxed to Diamyd Medical within 24 hours of the investigator becoming aware of the information.

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

As much information as possible should be supplied at the time of the initial report with at least the following information:

- patient, treatment, and trial identifiers
- investigator's name and address
- a description of the event, action taken, and outcome
- the date of onset and current status
- any suspect (study) drug, with its start date, dose and form of administration
- the reason why it is regarded as serious
- the current assessment/opinion of 'causality'
- any other available diagnostic information which will assist the understanding of the event
- if applicable, whether or not the randomization code has been broken for the SAE

7.6 Pregnancy

Pregnant and breast feeding women are excluded from the study. Menarchal females must have a negative urine pregnancy test prior to randomization and a negative urine pregnancy test at each visit with GAD-alum/placebo administration. Participants and their partners will be strongly advised to avoid pregnancy for one year following the last dose of GAD-alum/placebo and instructed to use adequate form of birth control. A pregnancy occurring during the study must be recorded on the Pregnancy Report Form and no further doses with GAD-alum/placebo will be administered. If a pregnancy is verified the Pregnancy Report Form must be sent to Diamyd within 24 hours of site being aware and a copy of the report must remain on-site in the chart and followed until delivery.

7.7 Safety Monitoring Board:

An independent data safety monitoring board (DSMB) who is unaffiliated with the protocol and with no conflict of interest will be appointed. During the course of the study, the independent safety monitoring board will review cumulative study data twice per year to evaluate safety, study conduct and integrity of the trial. At each visit, vital signs, adverse events/side effects, concomitant medications, and pertinent lab values will be recorded. The independent safety monitoring board may also be requested to review data as needed if stopping criteria are met or if safety issues arise that the Sponsors or Principal Investigator would like the independent safety monitor to address. The independent safety monitoring board will be notified in the case that un-blinding occurs. The independent safety monitoring board will conclude each review with their recommendation to the sponsors as to whether the study should continue without change, be modified, or terminated. The sponsor will submit the written recommendations of the independent safety monitoring board to the IRB upon receipt.

The independent data safety monitoring board consisting of Dr. Brooks Vaughan, UAB Department of Medicine, Alexandra Blumenthal, CRNP, Children's of Alabama, James Woodard, RN, Children's of Alabama, and Ulf Smith, MD, PhD, Department of Molecular and Clinical Medicine, Sahlgrenska University Hospital, Gothenborg, Sweden, will review data after the 6 months visit to assess benefit of the study. The board may suspend the study at any time if felt needed to evaluate safety further.

7.8 Stopping Rules for Premature Termination of the Study

1.) Any death related to the study medication

2.) By request of the independent data safety monitoring board

8.0 *Data Processing and Management:*

All participant data will be obtained and recorded in individual files that will be stored in a secured file cabinet within a locked office. Upon study entry, all participants will be given a unique identifier to be used through the entirety of the study and this too will be maintained in a locked file cabinet in secured office. Following each study visit, the data obtained will be entered into a database using the study participants' unique identifiers.

All other personal data will be omitted from electronic records. At the close of the study, any missing data will be verified with chart review. Only authorized study personnel will have access to patient data. Laboratory results will be transcribed from laboratory printouts to the Access database.

9.0 Data Analysis:

Baseline demographic and other (e.g., weight) characteristics will be compared between the treatment groups using t- and chi-square tests (or their non-parametric equivalents) for continuous and categorical variables, respectively, in order to demonstrate the success of the randomization process. The primary outcome measure is C-peptide level, which is measured at multiple time points during the study. As is standard practice, the post-stimulus area under the curve (AUC) will be calculated in addition to mean and peak values. The main analysis will focus on a comparison of the baseline and the > 12-month post baseline C-peptide measurements. Analysis of covariance will be used to compare changes in C-peptide levels between the treatment groups. For these analyses the 12-month post baseline measurement will serve as the dependent variable; there will be two independent variables: (1) a binary variable for treatment group and (2) the baseline C-peptide measurement. This is a standard analytical approach for study designs of this type. Given that GABA may affect α-cell glucagon secretion, possible changes in the circulating concentrations of this hormone will be appraised. In the unexpected event that the treatment groups are not similar with respect to specific demographic and other characteristics, these measurements will also be considered as potential confounders in the aforementioned analysis. A similar analytical approach will be used for the other study outcomes of interest besides glucagon, i.e., hemoglobin A1C, insulin dose/kg body weight/24 hours. Mixed statistical models will be used to conduct longitudinal analyses of C-peptide and hemoglobin A1C measurements, and daily insulin requirements, incorporating all three measurements. All analyses are conducted on an intention to treat basis.
Adverse events and other safety data will be summarized descriptively.

10.0 Power Calculations:

The sample size for the proposed study is 95 children; 25 in the active GABA/GAD-alum treatment group, 35 in the GABA/Placebo Gad-alum group, and 35 in the placebo group. For the primary comparison of the 12-month post baseline C-peptide measurements between these groups, assuming an α of 0.05 and a mean (SD) C-peptide AUC of 1.0 (0.4) this sample size yields a ~97% power to detect a 50% difference.

11.0 Administrative and Regulatory Procedures

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) must be made available for inspection by the authorities.

The University of Alabama at Birmingham IRB will be utilized for this study.

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

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

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

The patient's completed Consent Forms should be retained by the investigator in strict confidence.

The investigator must keep a confidential screening log, recording all patients who were screened, whether they were enrolled or not, and a separate Patient Identification List showing code numbers, names, and dates of birth to allow unambiguous identification of each patient included in the study.

In order to conduct this study consistent with established research principles and ICH-GC guidelines, there may be site visits conducted during the study to evaluate study conduct. All sites will be monitored for patient enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the case report forms (DRFs) and the occurrence and reporting of AEs and SAEs

12.0 Publication Strategy:

Data obtained from this study will be presented as a poster or oral presentation at the Pediatric Endocrine Society meeting (formerly Lawson Wilkins Society). Thereafter, results will be culminated and submitted for review to a first-tier, peer-reviewed journal.

13.0 References:

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