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Effect of gamma aminobutyric acid (GABA) or GABA with glutamic acid decarboxylase (GAD) on the progression of type 1 diabetes mellitus in children: Trial design and methodology

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ABSTRACT

Background: Evidence suggests that GABA may reduce pancreatic inflammation, protect β-cells from autoimmune destruction, and potentiate the regeneration of new β-cells in the setting of type 1 diabetes mellitus (T1DM). The enzyme GAD, also expressed in human pancreatic β-cells, is an antigenic target of reactive T cells. We hypothesized that treatment of children with recent onset T1DM with GABA or combination GABA with GAD will preserve β-cell function and ameliorate autoimmune dysregulation.

Methods: This is a one-year, prospective, randomized, double-blind, placebo-controlled trial. Ninety-nine patients aged 4–18 years with newly diagnosed T1DM are randomized into three treatment groups: 1) oral GABA twice daily in addition to two injections of recombinant GAD enzyme, 2) oral GABA plus placebo GAD injections, or 3) placebo GABA and placebo GAD. Patients are evaluated at baseline and months 1, 5, 8 and 12. Mixed meal tolerance testing is performed at all but the 8-month visit. Laboratory studies will assess indices of beta and alpha cell function, glycemic control, immunophenotyping, and diabetes-related autoantibodies.

Results: The primary outcome is the effect on pancreatic β-cell function as measured by meal-stimulated c-peptide secretion compared between the treatment groups before and after one year of treatment. Secondary outcomes include: 1) fasting and meal stimulated glucagon and proinsulin levels, 2) response in insulin usage by participants, 3) indices of immune cell function, and 4) effect on autoantibodies GAD65, ICA512, and ZnT8.

Conclusions: This trial will determine the safety and efficacy of GABA and combination GABA/GAD therapy to delay T1DM progression in children.

1. Introduction

The primary defect in autoimmune T1DM involves infiltration of the pancreatic islets by T-lymphocytes, macrophages, and other immune cells and consequent loss of β-cell function [1–3]. At the onset of clinical T1DM > 70% of β-cells are destroyed [4], while the residual β-cells most likely represent the only appreciable reservoir for restoring islet β-cell mass [5]. Immunological abnormalities reported in those with T1DM include autoantibody production (e.g., against the 65-kD isoform of glutamic acid decarboxylase (GAD65), tyrosine phosphatase-related islet antigen 2 (IA2 or ICA512), zinc transporter 8 (ZnT8)) as well as alterations in the capacity of regulatory T cells (Treg) to suppress the action of effector T cells (Teff); the latter population plays a key role in the destructive processes. Therefore, a majority of studies attempting to prevent or reverse this disease have focused on immune suppression [6]. While these effects have shown limited promise, most have imparted multiple adverse effects resulting in questionable value for short term benefits [6–9]. The identification of an agent, especially one administered orally, with an improved safety profile along with the ability to preserve β-cell function could have an inestimable clinical application. We hypothesize that GABA has the potential to reduce inflammation, protect β-cells from autoimmune destruction, and even regenerate new β-cells.

GABA, synthesized from glutamate by the enzyme GAD, is a well-known neurotransmitter in the central nervous system [10] acting mainly through the $GABA_A$ receptor (GABAAR) [11]. GABA is also

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produced by pancreatic β-cells [12]. GABAARs are expressed in various immune cells, including T-cells and a variety of peripheral blood mononuclear cells (PBMCs) 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 acts on GABAARs in α-cells thereby suppressing glucagon secretion [16]. Finally, GABA suppresses inflammation and increases Treg numbers [12]. In vitro assays determined that GABA suppressed both the production of IL-12 by macrophages and production of IFN-γ by CD8 T-cells, the end result of which is that GABA creates an anti-inflammatory environment by reducing the synthesis of these cytokines [12]. In mouse models of T1DM, GABA treatment can prevent and reverse the disease [12]. Soltani, et al. reported that mice with T1DM treated with GABA had reduced islet lymphocytic infiltration, restored β-cell mass and completely reversed hyperglycemia [12]. This was associated with increased serum insulin, decreased glucagon concentrations, and improved glucose homeostasis.

Based on this compelling information, we envisage that administering oral GABA to patients with new onset T1DM may preserve or increase residual insulin production, suppress glucagon release, and decrease pancreatic inflammation. By these mechanisms, GABA may extend β-cell function or even contribute to regeneration of new β-cells. Combining with recombinant GAD-alum injections, aiming to halt the autoimmune attack by inducing tolerance within β-cells, may improve glycemic control even more and significantly reduce the risk of long-term complications in the future [17]. This paper describes the rationale and design of the study in addition to the importance of study outcomes.

2. Methods

2.1. Study design

This is a prospective, randomized, double-blind, placebo-controlled trial to evaluate the safety and effect of GABA and combination GABA/GAD-alum in children with newly diagnosed T1DM. The study consists of 5 clinic visits over the course of 12 months with the intervention being initiated at the baseline visit (Fig. 1). Four of these five visits include a mixed meal tolerance test (MMTT) with laboratory evaluations.

Patients are randomized to one of three study arms in which they receive either: 1) Twice daily oral GABA (NOW Foods, Bloomingdale, IL) plus two separate subcutaneous injections of GAD-alum (Diamyd Medical, Stockholm, Sweden), or 2) Twice daily oral GABA plus two placebo GAD injections, or 3) Placebo GABA plus placebo GAD for a total of 12 months. All patients receive current standard-of-care diabetes management with subcutaneous insulin throughout the study with the goal of maintaining hemoglobin A1c (HbA1c) within the currently recommended American Diabetes Association age-specific target range. Patients are instructed to take their oral treatment daily with breakfast and dinner by opening the capsules, dispensing the contents into liquid and then drinking the solution prior to their meal. Table 1 describes the timeline of visits, all study procedures, and labs obtained.

2.2. Ethics and governance

This trial is listed on Clinicaltrials.gov (NCT02002130) and conforms to all applicable regulatory requirements. The protocol and consent documents were approved by the University of Alabama at Birmingham (UAB) Institutional Review Board (IRB). This trial is managed and conducted according to the latest international (ICH) guideline for Good Clinical Practice. The trial is monitored by an independent Data Safety Monitoring Board (DSMB), the UAB Center for Clinical and Translational Science (CCTS) and also by a sponsor-appointed trial monitor from QA Partners, LLC. Written informed consent and/or assent as applicable was obtained from all participants.

2.3. Participants and eligibility criteria

Patients were screened at the time of diagnosis with T1DM [18] and enrolled from Children's of Alabama (COA), a tertiary care university-associated referral center located in Birmingham, Alabama. All study visits, including baseline, are conducted in the COA pediatric endocrinology outpatient clinic. Participants include males and females aged 4–18 years old who are newly diagnosed (within the past 5 weeks), positive for the GAD65 antibody, and not obese. Table 2 includes all inclusion and exclusion criteria. Between March 2015 to June 2018 we screened 830 patients, and a total of 99 patients were enrolled.

2.4. Recruitment

Recruitment strategies involve broad outreach through emails, internet material, posters and local presentations. All families of children with newly-diagnosed T1DM at COA received an information packet of general resources relevant to the diagnosis, including our study flyer. Formal recruitment discussions were timed for late afternoon or evening, a day or so after admission. This was a more agreeable interval for families and children as the initial trepidation of diagnosis had diminished and the children had recovered from their presenting symptoms. Most often a physician investigator explained the study and answered questions. At discharge families were invited to contact the research coordinator for further information. Out of state participants contacted the research coordinator via clinicaltrials.gov and included 11 patients from 8 states other than Alabama (AZ, GA, MS, MO, NC, ND, TX, VA).

2.5. Randomization

Randomization is to one of the three regimens stratified by age and performed in balanced blocks of three (1:1:1) for the first 75 patients. A second enrollment strategy is utilized for the final 24 participants: randomization 2:1 into the active GABA vs placebo group. A computer-generated randomization list is prepared using randomization.com. At each visit, the study investigator contacts the dedicated research pharmacist with the patient's current height, weight and body surface area, after which the study drug is dispensed based on designated participant number and vial lot. Throughout the study, patients, investigators and study personnel are completely blinded to the treatment assignments. Indeed, GABA and placebo are dispensed as 200 mg indistinguishable capsules, and the GAD-alum versus placebo suspension vials are likewise indistinguishable. Randomization and medication dispensing remain under the stewardship of the COA research pharmacist.

2.6. Mixed meal tolerance testing

MMTTs occur according to the visit schedule outlined in Table 3. Specifically, on the evening before any MMTT subjects eat a full standard meal. They are asked to fast of all food and drink except water from 9:00 p.m. (21:00) that evening until after the MMTT is completed. Subjects are instructed to take their long-acting insulin the evening before the test (or continue their usual basal rate if on an insulin pump), but not to administer any bolus of short acting insulin for at least 6 h prior to the MMTT. Participants must have a fasting plasma glucose between 72 and 216 mg/dL on the patient's home glucometer on the morning of the test. If for safety reasons a subject needs to eat or take short-acting insulin on the morning of the MMTT, the visit is re-scheduled.

For each MMTT, a peripheral intravenous catheter is inserted, and then GABA or placebo is given orally just prior to drinking the Boost® High Protein *Very Vanilla* drink (1 cal/mL; 55% carbohydrates, 21% lipids and 24% proteins). Subjects drink 6 mL/kg of Boost® up to a maximum of 360 mL within 5 min. Blood samples via the catheter are collected 10 min prior to the meal (−10 min time point), at the time of

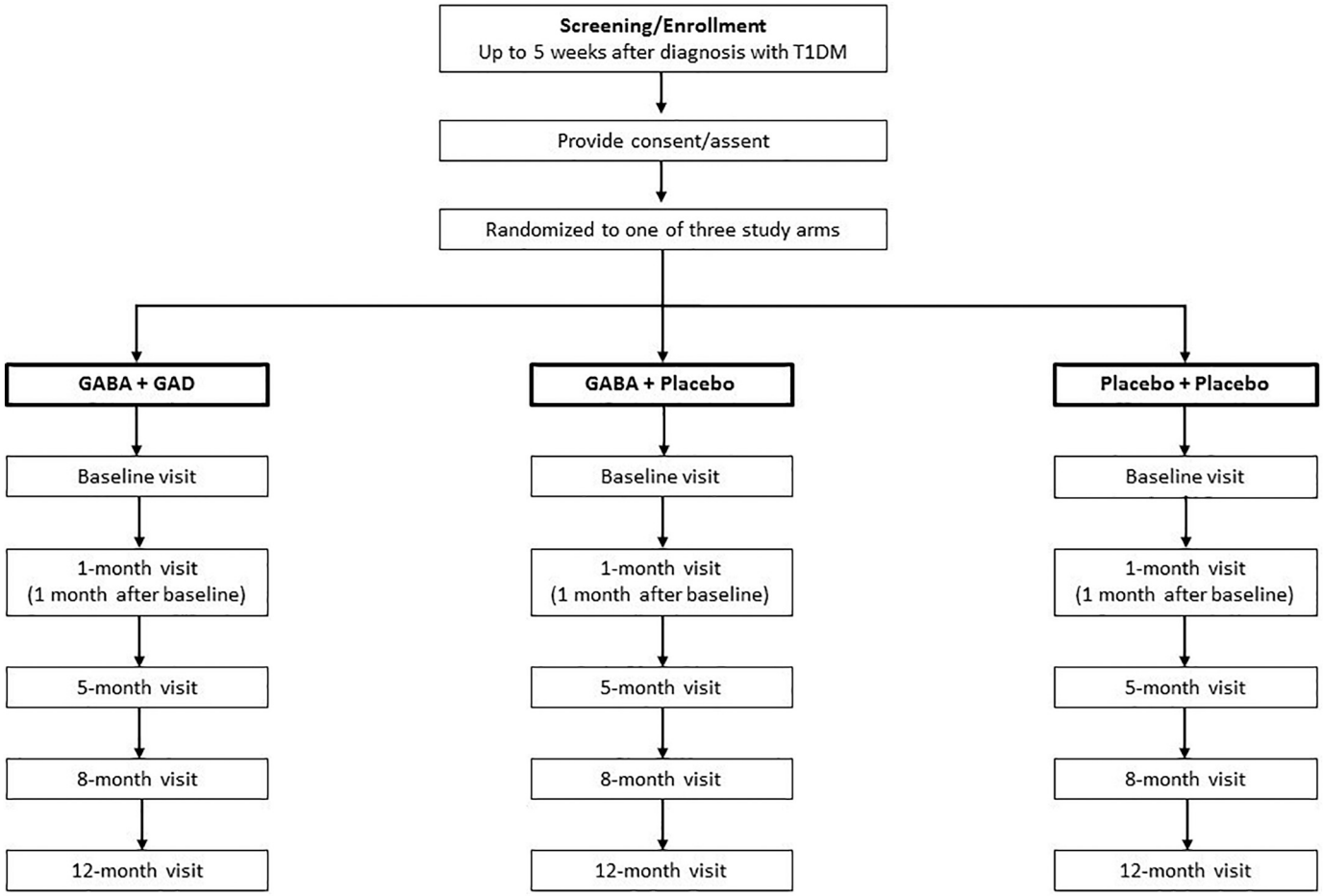

Fig. 1. Study intervention groups and visits.

ingestion (0 min), and at 15, 30, 60, 90 and 120 min thereafter. This schedule is followed at the baseline and 12-month visits. At the 1- and 5-month visits, only fasting and 90-min samples are taken. There is no MMTT at the 8-month visit. A urine sample (if available) is also obtained at each visit for creatinine and microalbumin. Patients receive $60 at each visit that requires a MMTT as compensation.

2.7. Safety/monitoring

Safety assessments include observation of reactions at the injection site, occurrence of all adverse events (AEs)/serious adverse events (SAEs), laboratory measurements, neurological assessments, and physical examinations. All adverse events are collected at each visit and classified by organ system, severity and relatedness to the treatment. For any SAE, an investigator is required to complete and send an SAE report via email or fax to the IRB and Diamyd Medical (or its designee) within 24 h of the investigator becoming aware of the SAE. An independent DSMB reviews cumulative study data twice per year to evaluate safety, study conduct and trial integrity. A sponsor-appointed trial monitor also reviews study conduct and integrity twice yearly.

2.8. Treatment adherence

Treatment adherence of the oral GABA is assessed by capsule count by the research pharmacist at each visit after baseline. Participants are asked whether any capsules have been lost or destroyed to ensure accuracy of the count. Adherence with the subcutaneous GAD-alum injections is ensured as both injections are administered by study personnel.

2.9. Study drugs

GABA and placebo capsules are prepared commercially (NOW Foods, Bloomingdale, IL). The purity of both the GABA and placebo products used in this study was verified by LC/MS/MS prior to beginning trial enrollment. The control GABA for mass spectroscopy analysis was obtained from Sigma-Aldrich Chemical Company (St Louis, MO). The GABA sample is over 85% pure. The placebo is devoid of GABA. GAD-alum injections are prepared by Diamyd Medical (Stockholm, Sweden) as a suspension with recombinant GAD enzyme and the vaccine adjuvant Alhydrogel® (alum).

3. Analysis

Baseline demographics and other characteristics will be compared between groups using T- and Chi-square tests (or their non-parametric equivalents) to confirm the success of the randomization process. Table 4 presents baseline demographic data of participants.

3.1. Primary endpoint

The primary outcome measure is serum c-peptide concentrations which are obtained at multiple time points during the study. The fasting and post-stimulus area under the curve (AUC) will be calculated in addition to mean and peak values. The main analysis focuses on comparison of the baseline and 12-month measurements. Analysis of covariance will be used to compare changes between groups. Longitudinal evaluation of each patient will also be performed to allow for time-point trajectory modeling.

Table 1
Study event schedule.

Study event	Baseline visit	1 month post-baseline	5 month post-baseline	8 month post-baseline	12 month post-baseline
Informed Consent/assent	X (or before)				
Randomization	X				
Autoimmune antibodies	Done as standard of care at diagnosis. Obtain and document results		X		X
GAD-alum or placebo injection	X	X			
Oral GABA or placebo administration starts	X				
Medical history	X				
Medication review/ concomitant medications	X	X	X	X	X
Adverse events	X	X	X	X	X
Vital signs	X	X	X	X	X
Height	X	X	X	X	X
Weight	X	X	X	X	X
Physical Exam + Neurological exam	X	X	X	X	X
Skin exam: injection site	X	X	X		
History of hypoglycemia	X	X	X	X	X
History of DKA	X	X	X	X	X
Growth rate	X	X	X	X	X
Hemoglobin A1c	Done as standard of care at diagnosis and all routine diabetes follow-up visits. Obtain results from chart.				
Mixed meal tolerance test	X	X (only fasting and 90-min samples)	X (only fasting and 90-min samples)		X
CBC with differential	X		X		X
Complete metabolic profile	X		X		X
Glucagon, glucose, c-peptide	X	X	X		X
Pro-insulin	X		X		X
Urine pregnancy test (all menstruating females)	X	X	X	X	X
Cell activation markers	X		X		X
Inflammatory cytokines	X		X		X
Urinalysis (if available) for microalbumin and creatinine	X	X	X	X	X
Total daily insulin dose recorded (units/kg/day)	X	X	X	X	X

3.2. Secondary endpoints

Secondary endpoints include effects of GABA/GAD on: 1) Fasting and meal-stimulated glucose, glucagon, and proinsulin levels, 2) total daily insulin usage by participants, and 3) measures of glycemic control (HbA1c) and residual β-cell function (insulin dose adjusted HbA1c, or IDAA1C). Note that IDAA1C is defined as: HbA1c (percent) + [4 x insulin dose (units per kilogram per 24 h)] [19]. A recently-described mathematical method of estimating stimulated c-peptide concentration, termed CP_{est}, in patients with new onset T1DM will also be evaluated to further elucidate its validity [20]. At each visit, daily insulin usage (units/kg/day) is recorded, while HbA1c is measured at patients' routine diabetes follow-up visits as standard of care. Finally, fasting and stimulated glucose, glucagon, and proinsulin levels are obtained at each visit including a MMTT. Glucagon and proinsulin levels will be analyzed as peak concentration, time to peak, and area under the curve similar to c-peptide.

Table 2
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
(1) Males and Females 4–18 years of age	1. Pregnant and/or breastfeeding
(2) Positive for autoantibody GAD65	2. Chronic systemic steroid use, including inhaled compounds, or any medication which can alter glucose metabolism
(3) Meet the ADA criteria for diabetes: classic symptoms, plus blood sugar > 200 mg/dL or fasting blood sugar > 126 mg/dL or hemoglobin A1c of > 6.5%	3. Treated with any other oral or injectable hyperglycemic medication other than insulin
(4) Enrolled within 5 weeks of diagnosis	4. Obesity, defined as BMI > 95 percentile, or BMI > 27 in adolescents with acanthosis score between 1 and 1.5
(5) 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 are oral contraceptive pills, intrauterine devices and barrier methods	5. History of seizure disorder (epilepsy, head trauma, cerebral vascular accident) or any clinical features of continuous motor unit activity in proximal muscles
(6) Signed informed consent form	6. Patients on medications which may disturb GABA action, such as baclofen, diazepam, acamprosate, gabapentin, or pregabalin
	7. History of alcoholism or any substance abuse
	8. Chronic disease (such as liver, cancer, cystic fibrosis, or renal failure)
	9. Any known chromosome abnormality (such as trisomy 21, Turner Syndrome, etc.)
	10. History of anemia or significantly abnormal hemoglobin results at screening
	11. Clinically significant history of acute reaction to vaccines and other drugs in the past
	12. Known history of HIV or hepatitis
	13. Unwillingness to comply with conditions of the protocol
	14. History of illness, besides diabetes within 2 weeks of first GAD-alum/placebo injection
	15. Any condition that the PI feels would not be beneficial for the subject to be on study

ADA: American Diabetes Association; BMI: body mass index.

3.3. Exploratory endpoints

Given that GABA has been shown to possibly decrease inflammation and alter immune cell function, we have proposed a detailed immune analysis with three arms: 1. analyses of functional CD4 and CD8 subsets (effector and regulatory), 2. Functional assays of T cell responses after GABA exposure or placebo, and 3. Subgroup analyses to evaluate whether GABA or GABA/GAD combination have disparate efficacies based on antibody positivity and titers. The safety of GABA and combination GABA/GAD-alum is also being monitored.

(1) We will perform multi-color flow cytometric analyses of functional CD4 and CD8 T cell subsets (effector and regulatory) at the baseline, 5-month and 12-month visits with monoclonal antibodies specific for: CD3, CD4, CD8, CCR4, CCR5, CCR7, CXCR3, CXCR5, CD25, CD28, CD38, CD39, CD45A, CD45RO, CD62L, CD127, Helios, Foxp3, and PD-1 [BD Biosciences, R&D Systems, BioLegend, eBioscience]. Intracellular cytokine staining of PMA/Ionomycin-, anti-CD3/CD28-, GAD65 Peptivator peptides- (Miltenyi Biotec), and tetanus toxoid-stimulated cells will also be examined with monoclonal antibodies to IFN-γ, TNF-α, IL-13, IL-10 and IL-17A (BD Biosciences, R&D Systems, eBioscience). We expect to observe a decrease in CD4 and CD8 effector T cell populations secreting IFN-γ and a concomitant increase in CD4 Treg cells (CD4 + /CD25 + / Foxp3 +) that secrete IL-10.

(2) Functional assays of T cell responses after GABA or placebo exposure will be assessed. PBMCs will be purified from fresh peripheral blood and stimulated with PMA/Ionomycin, Dyna beads conjugated with anti-CD3/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 Multiplex Human Cytokine/Chemokine Magnetic bead panel. T cell proliferation will be assessed by CFSE dilution staining to determine if GABA treatment has an effect on decreasing cytokine production of CD4 and CD8 cells with a concomitant increase in Treg cells. Additionally, PBMCs will be stimulated with anti-CD3/CD28, and RNA will be extracted from the stimulated cells (Qiagen) for cDNA synthesis (Invitrogen). cDNA is then run with qrtPCR using a Roche Lightcycler® 480 II with Taqman primers to examine the expression of the following genes: *Ifng, Tnf, Cxcl10, Ccl5, Il21, Foxp3, Ctla4, Il10*, and *Bcl6*.

(3) Autoantibodies associated with T1DM (GAD65, ICA512, and ZnT8) are obtained at three time points, and both antibody positivity and concentration will be determined as shown in Table 5. We plan to perform subgroup analyses to evaluate whether GABA or GABA/GAD combination versus placebo have different efficacies regarding antibody positivity and titers.

4. Discussion

4.1. Study design

We believe this study has many distinct advantages. Firstly, GABA is widely considered safe and has very few, if any, side effects [21] and GAD-alum has been studied in previous clinical trials with no safety concerns. Secondly, GABA is sold over-the-counter at many local stores at a reasonable cost ($7–10 per bottle), and GAD-alum is easy to use (subcutaneous injection). Lastly, GABA is an oral treatment and GAD-alum consists of only 2 injections in contrast to many other studies requiring intravenous injections or infusions, even to the point of requiring hospital stays [22]. If the hypothesis is substantiated, the economic, social and health impacts would be profound.

There are several distinguishing features of this trial. To our knowledge, this is the first trial in humans with T1DM using an oral agent with little to no side effects. Our participants are unique in their age distribution; they are all less than or equal to 18 years of age at

Table 3
Treatment groups and visit schedule.

Study group	Day 1- Baseline Randomization Visit #1	Month 1 Visit #2	Month 5 Visit #3	Month 8 Visit #4	Month 12 Visit #5
Group 1: GABA-GAD (GABA + GAD-alum)	GABA twice daily from Baseline to Month 12 visit				
	MMTT GAD-alum 20 μg *prime injection*	MMTT GAD-alum 20 μg *booster injection*	MMTT		MMTT
Group 2: GABA group (GABA + placebo GAD)	GABA twice daily from Baseline to Month 12 visit				
	MMTT placebo GAD *prime injection*	MMTT placebo GAD *booster injection*	MMTT		MMTT
Group 3: Placebo group (Placebo GABA + placebo GAD)	Placebo GABA twice daily from Baseline to Month 12 visit				
	MMTT placebo GAD *prime injection*	MMTT placebo GAD *booster injection*	MMTT		MMTT

MMTT- Mixed meal tolerance test.

treatment initiation and as young as age 4. This is unlike many tertiary intervention studies to date [23]. Moreover, a synergistic combination therapy of two potentially complementary agents, GABA and GAD-alum, is explored in this study. Insofar as GAD-alum may induce immune tolerance, GABA's β-cell preserving actions may be potentiated. Also relevant, the early initiation of GABA/GAD treatment in this pediatric trial (< 5 weeks post diagnosis) as well as the 12-month duration should enhance detection of the proposed actions on β-cell function/survival because the steepest rate of β-cell loss occurs in the first year post-diagnosis [24]. Finally, this trial's five-visit design (unlike infusion trials or those requiring hospitalization) is highly applicable in an outpatient clinical setting.

4.2. MMTT

The use of repeated MMTT allows each patient to be followed longitudinally for time-point trajectory modeling. While the MMTT methodology varies slightly between visits, we used a substantiated abbreviated protocol in which less blood is obtained. Namely, at the 1- and 5-month visits, only fasting and 90-min samples are obtained for c-peptide, glucose, glucagon and proinsulin. A recent article confirmed that the 90-min sample alone correlates significantly with the integral of c-peptide as a function of time (i.e., AUC) [25].

4.3. IDAA1C and CP_{est}

IDAA1C, rather than HgbA1c alone, was selected for assessment as this has been shown to more completely define a period of partial remission (i.e., honeymoon) period in T1DM. The study in 2009 by Mortensen et al. showed that a calculated IDAA1C less than or equal to 9 correlates well with residual β–cell function and stimulated c-peptide concentrations [19]. CP_{est}, is a more comprehensive model proposed to accurately estimate residual β–cell function and stimulated c-peptide concentration without the need for laborious mixed meal tolerance testing [20]. We plan to assess both IDAA1C and CP_{est} against the gold standard MMTT with c-peptide measurement. This direct comparison may mitigate the need for MMTT in future trials if an estimation model can be validated.

4.4. GABA dose

In 2015, a study of GABA pharmacokinetics and pharmacodynamics in healthy subjects found that GABA is absorbed rapidly through the GI tract (in 1–1.5 h) and that the concentration in the blood remains elevated for hours [26]. The T ½ was found to be approximately 5 h. That study, which called for further research including double-blind, randomized, placebo-controlled trials, favored multiple daily dosing in humans as opposed to once daily. In our pediatric trial, twice daily dosing frequency was selected to enhance compliance and forgo the need to dose GABA while at school.

Our dose selection was based on T1DM mouse models [12] and also the healthy human subject data [26]. Specifically, in the study from 2011 by Soltani et al., diabetic mice were injected intraperitoneally with 20 umol of GABA per dose. Using the molecular weight of GABA

Table 4
Characteristics of the patients at baseline.

Age (years)	
Range	4–18
Mean ± SD	10.5 years ± 3.6
4–8	*n* = 31 (31%)
9–13	*n* = 48 (49%)
14–18	*n* = 20 (20%)
Sex	
Male	*n* = 54 (54.5%)
Female	*n* = 45 (45.5%)
Body Mass Index	
Mean BMI percentile ± SD	62.2 ± 28
< 50th percentile	*n* = 29 (29%)
51–85th percentile	*n* = 44 (44%)
> 85th percentile	*n* = 26 (26%)
Race	
Caucasian	*n* = 89 (90%)
African American	*n* = 6 (6%)
Hispanic	n = 3 (3%)
Native American	*n* = 1 (1%)
Insurance	
Government Issued (Medicaid)	n = 20 (20%)
Private	*n* = 79 (80%)

Table 5
Laboratory assay specifications.

Autoantibody	Laboratory	Assay	Intra-assay coefficient of variability	Inter-assay coefficient of variability
Glutamic Acid Decarboxylase-65 kD (GAD65)	Esoterix	Enzyme linked immunoabsorbent assay (ELISA)	6.5%	6.7%
Zinc Transporter 8 (ZnT8)	Esoterix	Enzyme linked immunoabsorbent assay (ELISA)	4.1%	6.4%
Islet Cell Antigen 512 (ICA512)	Esoterix	Immunoprecipitation	2.1%	6.5%
Glucose, serum	Stanbio	Glucose oxidase	1.3%	2.5%
Glucagon, serum	Millipore Corp.	Radioimmunoassay (RIA)	4.6%	1.9%
Hemoglobin A1c (A1c)	Siemens	Antibody agglutination	N/A	N/A
C-peptide	TOSOH Bioscience	Immunofluorescence	1.7%	6.8%

(103 g/mol), the standard body surface area of mice (0.007 m^2) [27], and an applied safety factor, an acceptable starting oral dose in humans was calculated. Our human dose was calculated using body surface area rather than body weight alone. These calculations yielded a final dose of 1 g/m^2/day up to a maximum of 1.5 g per day.

4.5. Study recruitment

Retrospective surveys of large pediatric clinical trials, including T1DM, note factors that dissuade families from participating including perceived risk or lack of benefit, travel constraints, lack of adequate trust in the initial rationale, and poor timing for recruitment [28]. Our single-centered study screened 830 patients and enrolled 99 patients over a 39-month period. At COA, new onset T1DM patients are admitted for initial stabilization, insulin initiation and education. Their inpatient admission gave the study team ample time to engage eligible candidates in meaningful recruitment discussions early in the disease course. This situation is less suitable if initial management of the new diagnosis is outpatient.

4.6. Enrollment retention

Concerning follow up visits, patients had both their study visit and routine diabetes care follow up visits coordinated for the same day whenever feasible. These considerations concerning increasing time efficiency and decreasing the number of travel days is greatly appreciated by participants and also noted in other studies as being relevant for study retention [28]. Thus far, about 15% of the 99 participants enrolled have discontinued prior to the 12-month visit: 2 patients had baseline labs drawn but were disqualified due to GAD antibody negativity, and 11 patients have partial data (did not complete the last 1 or 2 visits). No specific complaint or side effect has been identified in these cases. These patients ultimately have incomplete participation, despite numerous phone calls, emails and letters sent in follow up to encourage involvement. Potential reasons include time constraints, perceived lack of benefit, and inability to comply with twice daily dosing.

5. Conclusions

This prospective pediatric T1DM trial investigates the novel, non-toxic oral agent GABA, with and without GAD-alum, for its potential to both preserve and regenerate β-cells while also favorably altering autoimmune responses. The robust study design takes into account a real-time outpatient setting as well as the challenges of compliance with oral medication dosing. Implications of GABA therapy alone or in combination with GAD-alum for the tertiary prevention of T1DM may likewise apply to primary and secondary prevention trials as well as the efficacy of encapsulated islet cells. Furthermore, the proposed glucagon-inhibitory actions of GABA may have beneficial metabolic actions in both Type 1 and Type 2 diabetes. In sum, this promising single-center pediatric trial is designed to promote our understanding of T1DM and help direct future therapies that might mitigate or prevent diabetes and its serious complications.

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